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Mycoflora and Aflatoxin levels of Left-over Harvest in some Farms, South West of Nigeria

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A B S T R A C T

More than ninety percent of the ruminant livestock in Nigeria lies in the hands of herders who keep them under extensive and semi-intensive management systems, whereby the animals rely only on natural pasture and crop residues for survival. In this work, the mycoflora and aflatoxin levels of ten farms were determined by sampling crop residues on farms grazed by cattle. Samples of the remains of farm harvest were surface-disinfected and cultured using standard microbiological techniques while aflatoxins in the left over harvest were determined using High Performance Liquid Chromatography (HPLC) with fluorescence detection. Fungal counts in leftover harvest ranged from 1.2 x 10^6 to 3.8 x10^6cfu/g. *Aspergillus flavus, A. terreus, A.parasiticus, Rhizopus* sp and a yeast, *Candida* sp were most prevalent on all the investigated crop residues. Aflatoxin B1 (AFB1) on the crop residues ranged between 3.0 and 13.30 µg/Kg, while the levels of AFG1 were between 2.30 and 4.50 µg/Kg. Results of the present study is indicative that the accumulation of these doses of AFB1 can lead to transfer of AFB1 into cattle and subsequently into milk. So there is an urgent need to control the feeding pattern of cattle in order to protect the health of the consuming public.

Introduction

Aflatoxins are toxic secondary metabolites produced principally by *Aspergillus* species, basically *A. flavus* and *A. parasiticus*. Aflatoxins have currently become a global issue. A large fraction of the world's food, including maize, rice, sorghum, barley, rye, wheat, peanut, groundnut, soya, cottonseed and other derivative products made from these primary feedstuffs in low-income countries are contaminated with aflatoxins (Rizzi et al., 2003; Masoero et al., 2007). However, they are most prevalent in latitudes between 40º N and 40º S of the equator, but the greatest health risk lies within developing countries in tropical regions, which rely on these commodities as their staple food source (Strosnider et al., 2006). It has been estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shephard, 2003; Williams et al., 2004).

A 2001 study in Nigeria revealed that blood and semen aflatoxin levels ranged from 700 to 1393 and 60 to 148 ng/ml respectively in infertile men and were significantly higher than that in fertile men (Uriah et al., 2001). The high aflatoxin contents of these Nigerians' body fluids might not be unconnected with their dietary exposure. Nigerian basic staple foods such as garri, beans, yam flour, melon, rice and maize contain high levels of aflatoxins (Ibeh et al., 1991; Jimoh and Kolapo, 2008; Somorin et al., 2012) and it is a common practice for these staples to be eaten with animal protein accompaniments.

Maina (1986) stated that meat constitutes the foremost animal product that is highly explored by the Nigerian households particularly for direct consumption and as such, the ruminants especially cattle, constitutes the major and cheapest source of meat consumption for most households in Nigeria. However, over 90 percent of the ruminant livestock in Nigeria lies in the hands of herders who keep them under extensive and semi-intensive management systems, whereby the animals only rely on natural pasture and crop residues for survival (Lawal-Adebowale, 2012). On the understanding that hay, which contains a large complement of cereal grain infested in the field, could be a source of appreciable aflatoxins (Lizarraga-Paulin et al., 2012), it is possible that animal products , in addition to staple foods might be contributing an appreciable quantum to dietary aflatoxin exposure in Nigeria. Due to the presence of aflatoxin M1 in cows' milk ranging from 9 – 456 ng/L (Oluwafemi et al., 2014), this present study was conducted to determine if crop residues could be source of exposure to aflatoxins by evaluating animal feed intake in the grazing areas.

Materials and Methods

Study Areas

Ibadan and Abeokuta are two important cities in south west Nigeria located in Oyo and Ogun states respectively. They are located in the tropical Rain Forest zone with high humidity and temperature. Cultivation of crops in these suburbs is transforming the vegetation into a wooded savanna, which incidentally favours the herding of cattle by the Fulani Pastorialists in communities within the study areas which feed the cattle forage and left-over harvest of cultivated crops such as maize.

Collection of left over harvest sample

Left over harvest of maize cobs, maize grains, cassava roots, cassava stem, sugar cane, cocoyam, pepper were randomly collected from farms in Abeokuta and Ibadan grazed by cattle of the Fulani herdsmen. The left over harvest were collected into a sterilized polyethylene bag and were taken to the laboratory for analysis.

Coordinates of the collecting points were obtained using a hand-held Global Positioning System (GPS), and the areas are shown in figure 1.

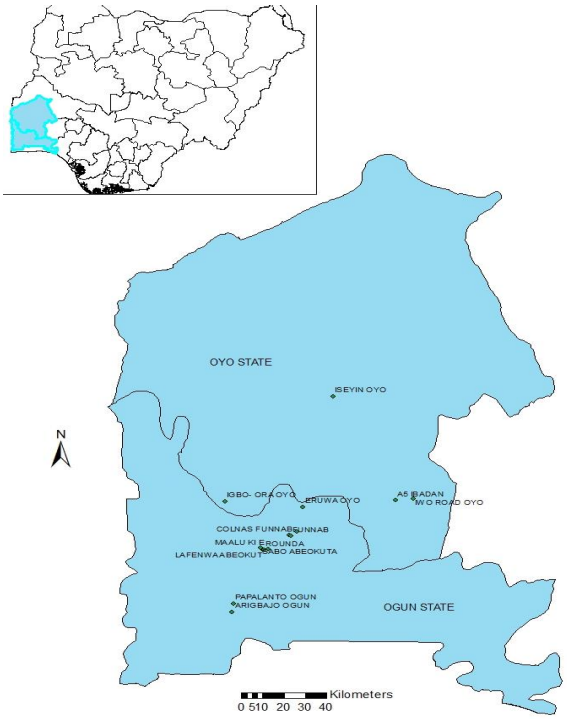

Figure 1 Location of sample collection points.

Isolation and characterization of fungi in left over harvest

Fungi were isolated from the collected left over harvest according to the method described by Klich (2002). The left over harvest were surface disinfected with ethanol. Serial dilution of the samples was done and appropriate dilutions were plated on Sabouraud dextrose agar at 28^{o}C for five days and the fungal counts estimated in cfu/g. Aspergillus isolates were identified using morphological and cultural characteristics as highlighted by Klich (2002).

Chemicals

Standards of aflatoxin B1, B2, G1, and G2 were supplied by Supelco, Bellefonte, Pennsylvania, USA. Solvents such as acetonitrile, methylene chloride, and methanol were of High Performance Liquid Chromatography(HPLC) grade.

Aflatoxin quantification of left over harvest: The method adopted by Oluwafemi et al., (2010) was used, where 50g of each sample was milled with 70% methanol and filtered with Whatman No.1 filter paper. The dried extract was re-dissolved with 1.25-ml of acetonitrile: water (25:75, v/v and was subjected to HPLC.

Table 3 shows the morphological and cultural characteristics of fungal isolates. Isolates were characterized according to the schemes of Klich (2002) and they were *A. parasiticus*, *A. flavus*, *Candida* sp. *A. terreus*, and *A. niger*. Figures 2-4 show chromatograms of both standard and left over harvest AFB1, AFB2, AFG1 and AFG2. Table 4 shows aflatoxin level in left over harvest from the randomly selected farms. AFB1 ranged between 3.3.0 and 13.30µg/Kg, while the levels of AFG1 were between 2.30 and 4.50 µg/Kg.

Results

The geographical region covered by this study showing the coordinates is clearly shown in figure 1 and Table 1. The mean fungal counts and the mycoflora profile of the left over harvest in the randomly selected farms grazed by Fulani Pastorialist in Abeokuta and Ibadan suburbs are presented in Table 1. Results obtained depicted that the fungal counts ranged from 1.20 x 10^6 to 3.80 x 10^6 cfu/g. Moulds such as *Aspergillus flavus, A. terreus, A.parasiticus, Rhizopus* sp and a yeast, *Candida* sp were the most prevalent on all the investigated crop residues. Aflatoxin B1 (AFB1) on the crop residues ranged between 3.0 and 13.30 µg/Kg, while the levels of AFG1 were between 2.30 and 4.50 µg/Kg.

Table 1 Latitude and Longitude of Left-over Harvest Sample Collection Points

Location	Latitude	Longitude
Eruwa Oyo (A)	7.374997	3.496857
Igbo Ora Oyo (B)	7.403869	3.164978
Iseyin Oyo (C)	7.941188	3.623200
Adamasingba Oyo(D)	7.409861	3.888588
A5-Ibadan Oyo (E)	7.395425	3.791685
Papalanto Ogun (F)	6.882528	3.202972
Arigbajo Abeokuta (G)	6.839033	3.196564
Lafenwa Ogun (H)	7.157254	3.325981
Sabo Ogun (I)	7.168428	3.316295
Funaab Ogun(J)	7.227203	3.472481

Discussion

Mycoflora profile of left-over harvest in farms sampled compares favourably with the reports of Gbodi et al. (1986) who isolated *A. parasiticus, A. flavus*, Candida sp, *A. terreus* and *A. niger*. Bankole et al. (2006) and Jimoh and Kolapo (2008) on their separate studies of "Egusi" melon seeds and cereal grains reported these

fungi were found associated with cereal grains due to the humid tropical climate. The presence of fungi in crops in large numbers does not automatically mean aflatoxin contamination (Olojede et al., 1996, but the analysis for the different aflatoxin showed that maximum aflatoxin levels in left-over harvest in this study was 13.3 µg/kg while the lowest level of aflatoxin was 3.3 µg/kg.

In a documentatary by Cornell University (2009), it listed that the maximum permissible worldwide aflatoxin levels in feeds for dairy and finishing beef cattle were 20 and 300 µg/Kg respectively. The concentration levels of aflatoxin levels from this study were lower but consulting earlier studies by Oluwafemi et al (2014) which obtained values between 9.0 – 456ng/L in some grazing areas give an indication that there are some levels of carry-over.

Table 2 Mycoflora and †Fungal counts (cfu/g) of Left-over Maize Harvest on farms grazed by Cattle in Ibadan/Abeokuta Suburbs, South West Nigeria

Farm	Fungal Counts	Fungal Isolates
A	1.20 x 10^6	*Aspergillus flavus, A. niger, A. parasiticus, Rhizopus* sp
B	1.76 x 10^6	*A. flavus, A. niger, A. parasiticus*
C	1.80 x 10^6	*A .terreus, A. flavus, Candida* sp, *Rhizopus* sp
D	3.80 x 10^6	*A .terreus, A. flavus, Candida* sp, *Rhizopus* sp *A. parasiticus*
E	1.83 x 10^6	*A. flavus, Candida* sp, *Rhizopus sp A. parasiticus*
F	2.20 x 10^6	*A. parasiticus, A. niger, A. flavus, Rhizopus* sp
G	5.00 x 10^6	*A. flavus, A. terreus, Rhizopus, Candida* sp
H	3.00 x 10^6	*A. flavus, A. terreus, Candida* sp, *Rhizopus* sp
I	3.40 x 10^6	*A. flavus, A. parasiticus, Candida* sp, *A. terreus*
J	1.80 x 10^6	*A. niger, A. parasiticus, A. flavus, Candida sp*

Table 3 Cultural and morphological characteristics of organism found in raw milk

Isolates	Features		Probable spp
	Microscopic	Macroscopic	
F1	Spores are oval, non septate brown mycelium gives rise to straight sporan-giosphore that terminate with black spo-rangium containing a columella,with a root-like hyphae (rhizoids).	Rapidly growing white coloured fungus swarms over entire plate, aerial mycelium cottony and fuzzy.	*Rhizopus*
F2	Conidia heads are typically radiate, later splitting to form loose column conidiophores arehyaline and coarsely roughened, often more noticed near the vesicles**.**	White colonies becomes yellowish green, grows in a cycle manner.	*A. flavus*
F3	Vesicles are small, dome shaped biseriate, proximal phialides are shorter than distal philiades,smooth, elliptical conidia form long chains, has microconidia borne by short conidiophores which extend laterally from hyphae.	Colonies are velvety, brown with folds, the reverse is white	*A. terreus*
F4	spores are oval, non-septate mycelium give straight spores.	Colonies are greenish- yellow and grows in a trio shape.	*A. parasiticus*
F5	spores are oval, non-septate	Colonies are creamy grows by budding	Candida sp

Table 4 Aflatoxins B_1 and G_1 levels of left over harvest in ten farms

Farm G1(µg/Kg)	Aflatoxin B1(µg/Kg)	Aflatoxin
1	12.0	4.5
2	13.3	2.7
3	3.3	2.3
4	7.4	3.6
5	5.9	3.0
6	3.3	2.4
7	7.0	4.1
8	4.6	4.0
9	5.5	3.2
10	5.5	3.0

Britzi et al. (2013), in their study for low yielding cows reported a value of 1-2%. This observation has an implication for this study as it perfectly agrees with both AFB1 values and the carry-over into milk as reported earlier by Oluwafemi et al., (2014). Nigerian cows according to Shittu et al. (2008) are low yielding producing about 24L of milk per day. In the study by Britzi et al. (2013), the maximum aflatoxin B_1 levels in feed should not exceed 1.4 µg/Kg. Values of AFB1 in all ten farms surveyed exceeded this amount. It has been reported that 40 µg/Kg AFB1 reduces productivity in some animal species (Denli and Okan, 2006).

Apart from the initial concentration of AFB1, other factors that affect carryover rate, include species difference, overall health of the cattle, hepatic biotransformation capacity, rate of ingestion and integrity of mammalian alveolar membranes (Battacone et al., 2003; Fink-Gremmels, 2008).

It is widely acknowledged that in tropical countries, giving an aflatoxin-free feed to animals is practically impossible, therefore the needed efforts to stem the tides of aflatoxicosis in these countries as documented by Jones et al. (1994) and Vincelli et al. (2002), include: gestating, lactating and stressed cattle should not be fed with feeds containing more than 20 µg/Kg; unstressed growing finishing cattle in excess of 400 pounds may be fed diets containing up to 100 µg/Kg of aflatoxins and animals destined for slaughter should receive aflatoxin free diets for at least 3 weeks before slaughter.

As reported earlier, over 90 % of the ruminant livestock in Nigeria are kept under extensive and semi-intensive management system (Lawal-Adebowale, 2012), results of the present study had shown the need to domesticate the provisions of Jones et al. (1994) and Vincelli et al. (2002) in Nigeria and similar countries if the health of the consuming public must be protected.

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