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Aflatoxin Contamination in Cereals and Animal Feeds in Bomet County, Kenya: A Food Safety and Public Health Concern

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aflatoxin is a type of mycotoxin originating from fungi that contaminates human food and animal feeds, causing serious health effects in humans and animals. The study aims to determine fungal infestation and mycotoxin contamination of human food and animal feeds for public health initiatives in Bomet County. A quarter of a kilogram of ninety-one samples was taken randomly from each household and agro vets shops sampled during the study in brown bags to Kenya Medical Research Institute (KEMRI) laboratory in a deep freezer before laboratory investigation by culturing on mycological media. Fungi classification was based on morphological features. Mycotoxin detection was done using the ELISA-based EnviroLogix QuickTox Kit and the positive samples, was subjected to High-performance Liquid Chromatography analysis for QC

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purposes. *Fusarium* spp., *Aspergillus* spp., *Alternaria* spp., *Mucor* spp., *Rhizopus* spp., *Penicillium* spp. and, *Xeromyces* spp. were isolated from cereals and animal feed. Of all the samples analyzed 34.1% were positive for aflatoxins and 17% of the samples analyzed were exceeding the acceptable levels. AFB1 and AFB2 were the most commonly identified, the largest proportion of Aflatoxins ranges from 0 µg/kg to 480 µg/kg. According to the European Union's acceptable limits, amounts of aflatoxin detected in some foods were unsafe and not fit for human consumption. The presence of aflatoxins in staple foods poses significant health risks, including hepatocarcinogenic effects, and has economic implications for food security. Pre-harvest and post-harvest practices can be applied to prevent or minimize aflatoxin levels in food, such as Crop protection, drying of cereals, sorting moldy or damaged Kernels, storing food in a dry place, use of fungicides and even use of gamma rays to radiate crops.

Keywords: Aflatoxin; maize; food safety; cereals.

1. INTRODUCTION

Maize is a staple foods crops in Kenya, most of the African population and some part of the world (Manjula et al., 2009). Maize contribute about 20% and 15% energy and protein intakes respectively for more than 200 million globally (Emily and sherry, 2010). Maize and other cereals are very good substrate for fungal growth and toxigenesis. Many surveys conducted worldwide indicates that maize can be contaminated by mycotoxins such as aflatoxins (Zinedine et al., 2007), ochratoxin A (Ashiq, 2015), trichothecenes (Pietri et al., 2004), and fumonisins (Nikiema et al., 2004; Arino et al., 2007, AESAN Scientific Committee).

These fungal metabolites have been shown to be toxic to humans and are responsible for toxic incidences in farm animals when concentrations in feeds are high (Bennett and Klich, 2003). More frequently, they are responsible for a decrease in breeding performance and subsequent economic losses for farmers (Hussein and Brasel, 2001). The presence of such fungal metabolites may also be of public health concern. Aflatoxin B1 (AFB1) has been classified as carcinogenic in humans and is a serious risk factor leading to the appearance of hepatocarcinoma (Ashiq, 2015). Other mycotoxins such as fumonisin B1 (FB1) and ochratoxin display carcinogenic properties in laboratory animals and have therefore been classified by IARC in the group 2B of molecules that are carcinogenic in animals and possibly carcinogenic in humans (Ashiq, 2015; Pfohl-Leschowicz and Manderville, 2007).

They over 400 known mycotoxins, however aflatoxins, fumonisins, zearaleunone, trichothecenes and ochratoxins are most studied (Ediage et al., 2011). Aflatoxins contaminate wide range of farm inputs e.g. rice, millet, peas,

beans, groundnuts, sorghum, maize and cassava, they are produced by *Aspergillus* species (*A. flavus*, *A. nomenus* and *A. parasiticus*) (Magan et al., 2011). According to green and blue fluorescence as per UV light on Thin Chromatography plates aflatoxin are classified as B1, B2, G1 and G2 (Sweeney and Dobson, 1998). Since its development Thin layer chromatography (TLC) is the most used method for separation and quantification technique and recommended by AOAC for aflatoxin analysis (AOAC, 2000, Trucksess, 2000). The main advantage be the lost cost, in countries which lack expensive instruments and immunoassay infrastructure are not available visual thin layer chromatography is method of choice (Shantha, 1994). Poor repeatability is the main disadvantage of TLC (Coker, 1984). Rapid test kits e.g. ELIZA does not allow for simultaneous monitoring of aflatoxin (Anklam, 2000, Stroka, 1999).

Mycotoxins especially Aflatoxin does not only have adverse health impact to the consumer health but also to the economy in Kenya and other African countries. In terms of losses of contaminated farm produces leading to destruction of the contaminated food. The aim of this study was conducted to determine aflatoxin contamination levels in maize and animal feeds sold in markets and shops in Bomet county, Kenya.

2. MATERIALS AND METHODS

2.1 Sample Collection

Maize and animal feed samples were collected from all the sub-counties in Bomet County namely; Bomet Central, Bomet East, Chepalungu, Sotik, and Konoin. A quarter of a kilogram was taken from each market vendor

and agro vets shops sampled during the study in brown bags to Kenya Medical Research Institute (KEMRI) laboratory in a deep freezer before laboratory investigation for culture and aflatoxin analysis using High-performance Thin layer chromatography (HPTLC).

2.2 Sample Preparation

Before analysis, the ground maize and feeds were mixed using a mixer to obtain a homogeneous sample. The sample was then sieved into a bottle using a 1.00 mm sieve.

2.3 Culture

The fungal infestation was carried out to determine the species type and colony-forming units of mycotoxin-producing fungi in each sample. Briefly, 1 gram of the ground sample was suspended in 10 ml of sterile water and vortexed for 1 minute. One milliliter of the sample was inoculated onto Yeast Potato Dextrose Agar (Oxoid) supplemented with chloramphenicol to inhibit bacterial contamination. Each samples were inoculated in two yeast potato Dextrose agar plates for quality control. The plates were incubated at 25°C for 72 hours then, CFU was determined. Different colonies were sub cultured onto new plates for purity. Macro and micromorphological features on Lactophenol cotton blue were done to key out the identities of the positive samples. Aflatoxin kits were then used to determine if the isolated species was producing mycotoxins.

2.4 Extraction and Analysis of the Sample

Twenty grams of grounded maize and feed were weighed to the nearest 0.01g and were extracted using a mixture of 20 ml distilled water and 200 ml chloroform in a colored volumetric flask measuring 500 ml. Using a mechanical shaker,

the mixture was homogenized (Amar Ltd, India) and left for 30 minutes to facilitate aflatoxins extraction. The water and chloroform in the mix were evaporated at 40°C. Then 0.2ml of the chloroform was used to dissolve the dry extract before High-performance Thin layer chromatography (HPTLC) spotting. The 20 µl sample extract, blank solution of methanol and chloroform mixture, and B1, B2, G1, and G2 standard aflatoxin solution were injected into HPTLC and were spotted on High-performance Thin layer chromatography (HPTLC) by TLC sampler.

Thereafter to visualize, the plate was moved to the TLC visualizer after spot visualization the plate was further moved to the HPTLC development chamber to develop the sport, then moved again to the TLC scanner in another HPTLC compartment to scan the results at 360 nm. Each aflatoxin evaluated was displayed on the screen. For HPLC the following quality control was done to ensure accuracy i.e. Proper sample preparation, calibration using standards, stable temperatures were maintained and the right column selection was made.

3. RESULTS

For all the samples analyzed 34.1% were positive of aflatoxins and 65.8% were negative. Distribution of aflatoxin per sub-counties is shown in Table 1.

A total of 91 animal's feeds were analyzed for aflatoxins and the highest reading detected were from Maize sample of 480 µg/kg, follows by dairy meal at 59 µg/kg. The lowest been zero for calf meal (Table 2).

The mean Aflatoxin levels were 10.7322 while for Ochratoxin were 1.55714 with the standard deviation of 14.7 and 216. Variance for Aflatoxin.

Table 1. Prevalence aflatoxin in maize per sub-counties

Sub-counties	Sample size n=85	Positive sample (%)	Negative sample (%)
Bomet Central	17 (20%)	6 (35.3%)	11 (64.7%)
Bomet East	17 (20%)	7 (41.2%)	10 (58.8%)
Chepalungu	17(20%)	5 (29.4%)	12 (70.5%)
Sotik	17 (20%)	2 (11.8%)	15 (88.2%)
Konoin	17 (20%)	9 (52.9%)	8 (47%)
Total (overall)	85 (100%)	29 (34.1%)	56 (65.8%)

Table 2. Aflatoxin levels in cattle feeds

No.	Feed ingredient	No. of samples tested	Level of aflatoxins	
			Range ($\mu\text{g/kg}$)	Mean+ -
1	Maize	29	5-480	12.2
2	Barley Germ	13	2-35	2.1
3	Dried Fish	13	0-32	1.8
4	Rice Bran	14	1-34	2.9
5	Dairy meal	15	0-58	4.3
6	Calf meal	7	0-18	0.8
	Total	91		

Table 3. Aflatoxins contamination incidence in feeds and maize flour

Mycotoxins	n	Mean	Std. Deviation	Variance
Aflatoxin	91	10.7322	14.70586	216.262
Ochratoxin	91	1.55714	3.32609	11.063
Valid n(listwise)	91			

Table 4. Number (CFU/g) of fungal isolates from the sub-counties

Isolates	Sub-counties				
	Bomet Central	Bomet East	Chepalungu	Sotik	Konoin
<i>Alternaria</i> spp.					
<i>Fusarium</i> spp.	17 $\pm$ 0.1	6 $\pm$ 0.3	20 $\pm$ 0.2	11 $\pm$ 0.2	9 $\pm$ 0.1
<i>Mucor</i> spp.	23 $\pm$ 0.1	10 $\pm$ 0.2	9 $\pm$ 0.3	17 $\pm$ 0.3	19 $\pm$ 0.2
<i>Aspergillus</i> spp.	19 $\pm$ 0.3	14 $\pm$ 0.2	44 $\pm$ 0.2	9 $\pm$ 0.1	10 $\pm$ 0.1
<i>Rhizopus</i> spp.	20 $\pm$ 0.1	13.7 $\pm$ 0.1	6 $\pm$ 0.2	28 $\pm$ 0.2	8 $\pm$ 0.1
<i>Penicillium</i> spp.	56 $\pm$ 0.1	26 $\pm$ 0.2	15 $\pm$ 0.2	17 $\pm$ 0.2	19 $\pm$ 0.3
<i>Xeromyces</i> spp.	18 $\pm$ 0.1	4 $\pm$ 0.1	13 $\pm$ 0.1	21 $\pm$ 0.2	16 $\pm$ 0.1

The fungal isolates from Bomet central varied from 17 $\pm$ 0.1 CFU/g to 56 $\pm$ 0.1 CFU/g, Bomet East 4 $\pm$ 0.1 CFU/g to 14 $\pm$ 0.2 CFU/g, Chepalungu 6 $\pm$ 0.2 CFU/g to 44 $\pm$ 0.2 CFU/g, Sotik from 9 $\pm$ 0.1 CFU/g to 28 $\pm$ 0.2 CFU/g, and for Konoin 8 $\pm$ 0.1 CFU/g to 19 $\pm$ 0.3 CFU/g.

4. DISCUSSION

Maize is a staple food in Kenya hence its safety is a concern. They analyzed maize for aflatoxin, to determine its safety in Bomet county. Maize cultivation in sub-Saharan Africa has risen to 60% (Santpoort et al., 2020). The study results showed that 34.1% of the samples were positive for aflatoxin. However, the aflatoxin levels of most samples were found to be below the allowable limit as per the European Commission (AESAN, 2021). The study's slightly high prevalence may be due to the low sample size. Kortei et al., (2022) reported an aflatoxin prevalence of 46.4% in food samples in Ghana markets which is higher than what the study found.

AESAN. (2021). Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) concerning the effect on the Spanish population of the derogation of national regulation on maximum allowed limits for aflatoxins B1, B2, G1 & G2 in food (pp. 1-16). The study reports 119PPb as the highest level of aflatoxin detected in Maize, which is within the range reported in other African Region of 110ppb in Togo and 120ppb in Benin (Hell et al., 2000, James et al., 2007). While 770ppb (Nigeria), 490ppb (Ghana), and 162.4ppb (Tanzania) aflatoxin levels from Maize and Corn (Owekisha et al., 2024) which is higher compared to what this study reported. The levels are way high as recommended by FDA of 20ppb for food (<https://www.aflatoxinpartnership.org/wp-content/uploads/2021/05/NGFAComplianceGuide-FDARegulatoryGuidanceforMycotoxins8-2011.pdf> and EU guidelines (<https://www.efsc-gtp.eu/data/1433337461EFISC-%20Code%20of%20good%20practice%20aflatoxin%20monitoring%20version%201.1%20clean.pdf>). In sub-Saharan Africa Maize is one of the crops commonly contaminated by aflatoxin, with

some research reports up to 1000 ppb of aflatoxin (Nakavuma et al., 2020). The aflatoxin prevalence in maize caused a reduction in economic profits and yield.

The high aflatoxin levels found in some cereal may be due to some abiotic and biotic factors e.g. humidity, temperature, insect attack, and storage duration. Simsek et al. (2002) showed that humidity of 97-99% and temperature of 25-30°C are favorable for aflatoxin production. *Aspergillus flavus* can produce aflatoxin within 48 hrs. when the moisture content is at 16-24% and temperature at about 20-38°C according to Silvia et al. (2020). Water activity in the environment, pressure and temperature are some of the factors for AF accumulation and fungal growth (Pradeep et al., 2022). High moisture content in the cereal used in the study may be responsible for the fungi growth and subsequent aflatoxins production since Kenya is located in tropical regions of the world. Aflatoxin control is difficult because of climatic conditions specifically high moisture and high temperature (Awuchi et al., 2020), the same factors fueling high aflatoxin levels in Bomet County hence small-scale farmers in Bomet County are at risk of exposure to aflatoxin. A similar finding was reported by WHO (2015). A survey done in West Africa showed food collected during the rainy season had more aflatoxin than those collected during the dry season (Benkerroum, 2020).

The consumer's health is severely endangered due to fungal infestation and mycotoxins in agricultural products and crops. The most affected consumers are those buying unprocessed farm products for consumption from the farm or markets (Oyedele et al., 2017). Because there is no aflatoxin regulation at this level. In Kenya government agencies responsible mostly sampled processed foodstuffs from the supermarkets instead of the raw farm produce. Aflatoxin development, fungal infestation, and growth are linked to water activity in food/feeds. This is connected with inadequate drying of cereals predisposing them to mycotoxigenic fungi which is inferred to increase as storage time increases (Temba et al., 2017, Reiter et al., 2010, Sarker et al., 2015). Fungal infestation and metabolite accumulation could be elevated in crops harvested at the end of the rainy season also poor post-harvest crop handling e.g. bare ground drying has been linked to contributing to soil contamination by fungus (Okello et al., 2010). In animals aflatoxicosis alter hemato-biochemical parameters, changes in vital organs,

and immunosuppression (Amanda et al., 2020). Poor and inadequate storage conditions result in aflatoxin contamination and fungal attacks (Singh et al., 2019). Fungal growth in grains is also triggered by variables like temperature and humidity (Mtega et al., 2020).

According to Kilonzo et al (2014), the maize consumption rate for the Kenyan population is 400g per person per day, thus exposure to aflatoxin range from 4.3 -554ngkg⁻¹ bw day⁻¹ whereas exposure to 0.8ng and 0.26ngkg⁻¹ bw day⁻¹ for Australians and Americans respectively (Wambui et al., 2017). Therefore, Kenyans are exposed to high levels of Aflatoxin and hence are at a higher risk of developing health complications associated with aflatoxins such as neonatal mortality, still-birth and immunosuppression with predisposing individuals to infectious diseases such as HIV/AIDS, pneumonia, and stunting of growth (Chhonker et al., 2018). Of great public health concern is the frequent intake of such levels of aflatoxin, which can work in synergy with some carcinogens e.g. hepatitis B virus to aggravate liver cancer (Ghouri et al., 2017). It's worth noting exposure to any level of aflatoxins is harmful (Dieter et al., 2020, Naik et al., 2022). Small-scale farmers in Kenya commonly use manual sorting to sell healthy kernels and retain discolored kernels for consumption (Bandyopadhyay et al., 2016). This practice increases exposure to aflatoxin in homes. For industries after sorting kernels automatically, the unhealthy ones are used in manufacturing animal feed which eventually gets into the food chain and humans are exposed after consuming animal products e.g. milk or meat.

5. CONCLUSION

Food/ feeds analyzed in Bomet County, Kenya showed up to 34.1% contamination with aflatoxin. Aflatoxin in food and animal feeds causes adverse health effects in humans and animals. Aflatoxin contamination in most cereal and animal feeds was due to long storage of food and feeds, low education status among farmers, and mixing of different concentrates as animal feed. Hence Farmers should have been educated about physical detection of maize/feeds contaminated with aflatoxin and prevention mechanisms.

6. RECOMMENDATION

The application of novel Agricultural practices to manage and mitigate contamination of feed and

food along the chains is highly recommended. Also, a modeling approach, further research, and the use of biological control such as the use of atoxigenic *Aspergillus flavus* strain to displace fungal toxigenic strain in the population should be applied.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

The study was approved by the University of Kabianga institutional ethics and review committee. (IERC/2020/003).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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