

# PREVALENCE OF AFLATOXINS IN STORED MAIZE IN BUSIA AND MIGORI COUNTIES OF KENYA

Nicholas M. Jacob<sup>1,2,</sup> Vincent O. Madadi<sup>1</sup>, Shem O. Wandiga<sup>1</sup>, and David K. Kariuki<sup>1</sup>

<sup>1</sup>Department of Chemistry, Faculty of Science and Technology, University of Nairobi, Kenya <sup>2</sup>Department of Pure and Applied Sciences, School of Science and Technology, Kenya Methodist University, Meru, Kenya

#### mwendanj2014@students.uonbi.ac.ke

ARTICLE INFO	ABSTRACT			
Article History:	Consumption of aflatoxin contaminated maize causes abdominal pains,			
Received: 14/09/2022 Accepted: 27/09/2022 Available online: 31/12/2022 <i>Keywords:</i> Aflatoxin Maize Consumption contamination	vomiting, acute liver damage, and other chronic ailments. This study aimed to determine the impact of post-harvest management practices and environmental conditions on prevalence of aflatoxin strains in stored maize. A hundred and seventy-eight maize grain samples were collected from stores: farmers, retailers, wholesaler, National Cereals and Produce Board in Migori and Busia counties. The samples were processed and analysed for individual aflatoxins B1, B2, G1, G2 and their totals. Samples from Migori stores had contamination between $3.04-23.75 \ \mu g/kg$ of B1 and $5.81-61.37 \ \mu g/kg$ of total aflatoxin. The same contamination in samples from Busia stores was between $0.50-1.74 \ \mu g/kg$ and $0.71-3.67 \ \mu g/kg$ respectively. The aflatoxin prevalence trend was B1>G1>B2>G2. The total aflatoxin contamination trend in stores was farmers>retailers>wholesalers>National Cereals and Produce Board for Migori, and retailers>farmers>wholesalers>National Cereals and Produce Board for Busia. Seventy-four percent of samples from Migori stores were contaminated above the allowed $10 \ \mu g/kg$ limit for total aflatoxin whereas all samples from Busia stores were not contaminated. The National Cereals and Produce Board stores adhered to the set standards for drying,			
	storage and practices to prevent aflatoxin development in stored maize, while other stores did not comply to the same.			

©2022 Africa Journal of Physical Sciences (AJPS). All rights reserved. ISSN 2313-3317

### 1. Introduction and Background

Maize (*Zea mays L.*) is the third world's largest consumed cereal after rice and wheat (Rouf Shah *et al.*, 2016) due to its ability to adapt to a variety of agro-ecological zones (Jonah *et al.*, 2020). Maize is also a raw material for a wide range of industries including animal feeds, starch, syrup, fuel and alcohol (Dabija *et al.*, 2022; Grote *et al.*, 2021; Klopfenstein *et al.*, 2013; Lopes, 2014). The cereal is the main source of livelihood for over 80% of people living in Sub-Saharan Africa (Pardey *et al.*, 2016; Ranum *et al.*, 2014). In Kenya, maize is a staple food and a source of income for 98% of the rural population who cultivate it predominantly in small- scale (Mang'eni, 2022).

Maize is frequently infested by *Aspergillus, Penicillium,* and *Fusarium* fungi in growing fields, during harvesting processes, transportation and storage (Liu *et al.*, 2016; Okoth *et al.*, 2018). These fungi are ubiquitous in the environment mostly in tropical and subtropical regions because of high temperatures and humidity. Fungal growth and aflatoxin contamination of foods are favored by occasional rains during harvesting period, improper harvesting and storage methods, poor aeration in stores and prolonged storage (Shrestha & Control-Nepal, 2019). The most vulnerable foods to aflatoxin contamination are cereals and legumes such as maize and peanuts (Rasheed *et al.*, 2021; Benkerroum, 2020; Gachara *et al.*, 2018). Aflatoxin contamination of cereals and legumes impact negatively to food security, food safety and trade (Achparaki *et al.*, 2012; Kumar *et al.*, 2016). High frequencies of aflatoxin contamination of maize, rice, sorghum, and groundnuts reported occasionally in different parts of Kenya (Ngindu *et al.*, 1982). Just to remember, high prevalence and acute aflatoxicosis were reported in 2001, 2004, 2005 and 2006 in some parts of Eastern Kenya (Lewis et al 2005). Despite extensive consumption of groundnuts and maize products, no incidences of acute aflatoxicosis have ever been reported in Western and Nyanza regions of Kenya. However, no study has even done to ascertain this observation.

In chemical terms, aflatoxin refer to a wide class of natural chemical compounds composed of five-membered cyclo-pentenone ring known as blue series or B- series and the six-membered lactone ring named Green series or G-series (Filazi & Tansel, 2013) when viewed under UV light. The toxicity of these toxins is determined by the position of active sites in their structure. The aflatoxin strains that form epoxidation on the 8, 9-double bond are more potent than those that lack double bond in the position. Common aflatoxin strains when arranged in order from the highest to lowest potency are B1, G1, B2 and G2. When different strains of aflatoxin are consumed in contaminated foods and feeds by animals or birds, various metabolites among them aflatoxin M1 and M2 found in milk after consuming aflatoxin B1and B2 through foods.

Exposure to aflatoxin through consumption of contaminated food materials is linked to a variety of harmful health effects including; liver cancer, immune system suppression, teratogenic effects, infant malnutrition and development retardation, among other chronic ill health ailments ('Kang'ethe *et al.*, 2017; Kiarie *et al.*, 2016; Mutegi *et al.*, 2018; Stepman, 2018). Effort to safeguard both human health and livestock against contamination effects of aflatoxin, has led to various food organizations and governments to establish maximum acceptable limits for aflatoxin B1 and total aflatoxin. In Kenya, the total aflatoxin contamination limits allowed is 10  $\mu$ g/kg and 20  $\mu$ g/kg for human food and animal feeds respectively (Nakavuma *et al.*, 2020; Njugi *et al.*, 2018). Aflatoxin B1 is the number 1 human carcinogen associated with hepatocellular carcinomas, liver failure and death (WHO, 2015), it has a 4  $\mu$ g/kg limit in human food.

Studies conducted by the Kenya demographic and health survey in 2014, reported on average 25.2% and 22.7% of children under 5 years of age were stunted in the Western and Nyanza

regions, respectively. In the same report Busia and Migori counties had 22% and 26.4% as accordingly. According to Mutegi *et al* (2018), post-harvest management and environmental conditions contribute to aflatoxin contamination in peanuts. There are no studies, however, to evaluate the impact of post-harvest management practices and environmental conditions on prevalence and composition of aflatoxin strains in maize from different stores in Migori and Busia counties.

**Busia County** rises from 1,140 to 1,500 meters above sea level. This county has four different Agro-Ecological Zones (AEZs) with good soils and climate favorable for maize growing. The lowest temperature range is from 14 to 22°C and highest range is from 29 to 30°C. The mean temperature is range from 21 to 27°C. The county has a bimodal pattern of rainfall, where the average annual rainfall was between 750 and 2,000 mm (Midega *et al.*, 2015). The distribution was: long rains fall from March - May and the short rains from August – October. Across the county, the lowest rainfall is 760 -1,015 mm in the vicinities of Lake Victoria. The agricultural productivity in the county is influenced by soil types and precipitation patterns (USAID, 2019 & 2017).

**Migori County,** covers an area of 2,597 km<sup>2</sup> of land and 478 km<sup>2</sup> surface water is located between latitude 1°24′S and 1°40′S and longitude 34°50′E. It has undulating hills, plains and ranges rising from 1,135 to 1,700 m above the sea level. The total arable land for the county is approximately 1,919 km<sup>2</sup> spread in six different Agro-Ecological Zones (AEZ). Migori experiences mild inland equatorial type of climate, modified by relief and altitude owing to its proximity to Lake Victoria. The county experiences two rainfalls: March –May long rains and October -November short rains the rainfall ranges between 700 - 1,800 mm annually. The county is humid throughout the year, with the mean annual temperature of 21.2 °C. The county experiences coldest month in July with a mean of 13.3 °C and the hottest months between February-March with a mean of 29.2 °C (GoK, 2013a). The precipitation patterns, climate, biota, relief and age have made this county to have soils of medium fertility (NARIGP *et al.,* 2020), good for growing maize and sugar cane.

Busia and Migori counties share international borders with Uganda and Tanzania, respectively this allows cross border trade among people living on sides of the countries. Agricultural products include maize form part of the accommodates in the trade. The quality of maize in terms of levels of aflatoxin contamination are normally not known. The objective of this study was to determine the impacts of post-harvest management practices and environmental conditions on prevalence and composition of aflatoxin stored maize in Migori and Busia counties.

### 2. Materials and Methods

### A) Study Design

Maize grain samples were collected from different stores: farmers, retailers, wholesalers, and NCPBs in the counties of Busia and Migori (Figure 1). The selected stores differed in terms of source of maize before storage, quality and size of storage facility, management practices in the storage facility that depended on post-harvest management skills employed in the store.



Figure 1: Maize sampling collect site maps for Busia, and Migori Counties

## B) Sampling Dry Maize

A formal sampling protocol was followed to sample dry maize grains from the four different categories of stores in each county (Donnelly *et al.*, 2022). Only maize harvested within three-month period was considered to rule out influence of extended storage period on the maize quality and aflatoxin load.

## C) County Sampling strategy used

In each county the sampling strategy addressed the sites and number of samples per store. The sampling locations were categorized into four store types namely farm, retail, wholesale and NCPB stores. A total of 42 stores equally distributed within the two counties were sampled which were broken down into 22 farm stores, 10 retail stores, 8 wholesale stores and 2 NCPB stores. A total of 89 maize samples were collected from each county constituting of twenty-two samples each from farm, retail, wholesale stores, and twenty-three from NCPB store.

The farm stores were temporary storage facilities for harvested maize based in the farm or farmers homes. Maize grains from these stores were then sold to different distribution channels such as retailers, wholesalers or NCPB stores for storage or distribution to millers. Farm stores are not required to have licenses or permits but also lacked in storage structures for providing absolute protection to stored maize grains from contaminations, moisture and pests' infestation. The retail stores were facilities based in local trading centers that buy or sell small quantities of dry maize from small scale farmers and sell to local consumers, wholesalers and NCPB stores for storage. Expect for a wider source of maize, trading license and location, retail stores shared similar characteristics with farm stores. They lacked in structures, operational technical knowledge and adherent to KEBS set standards for cereal drying, storage and transportation. Wholesale stores were relatively large in terms holding capacity, bought maize from both largescale farmers, retailers and occasionally from NCPB stores within the regions. To some extent these stores adhered to KEBS set standards for cereal drying, storage and transportation. Wholesale stores had other than the requisite trading and operation licenses, but also some structures, building permits, environmental permits and occupancy certificates. They, however, lacked in area of trained personnel to manage the operations of the store. Occasionally, retailers and wholesalers source maize from the neighboring country depending on supply and demand within the counties.

Nation Cereals and Produce Board silos/stores were large conventional stores run by the National government for cereal holding for food security reasons. They mostly bought maize from farmers, wholesalers, retailers and also imported from abroad. The NCPB stores mostly sell their maize grains to the millers for largescale production of maize flour. The Nation Cereals and Produce Board stores by nature of their level adhered to all KEBS set standards for cereal drying, storage and transportation as well as legal regulations for trade and operation. Warehouses owned by NCPB had similar in building designs, aeration provisions, size of pacing racks for placing maize bags, building permits, environmental permit and occupancy certificate. The management personnel in these stores had required technical knowledge for storage and who displayed clear guideline manuals at the entrance. Stored maize in NCPB was maintained at 15% moisture content, 13°C temperature, in dusty free warehouse that were fumigated regularly to controls pests (KEBS, 2019).

Sampling was done following commission regulation guidelines (Commission Regulation (EC) No 401/2006; FSA, MS, 2016) where a maize bag was randomly selected from the store. A sampling spear was used to pull out a kilogram of maize from 5 points on the sides of each 90 kg bag marked from the bottom to the top (Iso *et al.*, 2010). These five different subsamples were thoroughly mixed together and a kilogram of the composite sample collected. The sample was stored in a brown paper bag, a pack of silica gel added to absorb excess moisture, before the

sample was sealed and coded. The samples were kept in Coleman cooler boxes while in the field and during transportation to the laboratory for analysis. For each sample pack two 500 g subsamples A and B were drawn. Set A was refrigerated at -20 °C for backup analysis, while set B was analyzed immediately for aflatoxin presence and composition.

### **D)** Sample Preparation

In preparation, subsample B was mixing thoroughly before drawing three replicates of 20 g portions each, ground to powder and sieved though 65 mesh or 0.25 mm size. The flour potions were subsampled further into triplicates of 5 g each in 50 mL tubes. 30 mL HPLC grade methanol, deionized water, and acetonitrile were added in the ratio 12:2:1 (v/v/v). The mixture was briskly shaken at 120 rpm for thirty minutes using Mxbaoheng MPL-20 orbital shaker to extract aflatoxins. The extract was filtered through a Whatman filter paper No. 4 (Whatman International Ltd., Maidstone, UK). 1 mL of the supernatant of the extract was transferred into an extraction tube, topped to 40 mL with phosphate buffered saline (PBS) pH 7.4 and centrifuged at 3,400 rpm for one minute. The resulting supernatant was filtered through a 0.45 μm nylon membrane filter. All the 40 mL filtrate was passed through immunoaffinity column at a flow rate of 1 drop/second. The column was repeatedly washed with 10 mL of deionized water at a flow rate of 2 drops/second and discarded, then aflatoxins fraction was eluted with 1 mL methanol at a flow rate of 1 drop/second. The eluent was evaporated to dryness under a stream of white spot nitrogen and reconstituted with 400 µL mobile phase (water/methanol/acetonitrile, 55/10/35, v/v/v) into HPLC vial ready for analysis. Quality control blank samples for aflatoxin, free maize was prepared in the same way to represent matrix-blank.

### E) Method Validation

In conformity with SANCO/12571/2013 analytical performances criteria (EC, 2010) validation of HPLC method was done before analysis of the samples. The limits of detection (LOD) and quantification (LOQ) were determined based on the measurement of calibration solutions with lowest concentrations (Shrestha & Control-Nepal, 2019). Recovery experiments were performed by spiking 5 g each of ground maize blanks with 20  $\mu$ L of aflatoxins standards to a concentration of 20  $\mu$ g/g. The samples were incubated overnight at room temperature in airtight containers, followed by shaking with an orbital shaker, extraction and cleaning up in triplicates (Serrano *et al.,* 2012). The linearity was tested by matrix match and solvent standards based on the calibration curves constructed from standard solutions of aflatoxins B1, B2, G1 and G2. The concentration range used was 0.1 – 50  $\mu$ g/mL aflatoxin. The degree of precision was estimated daily and the confidence interval of the mean value at 95% checked (EC, 2010).

### F) Calibration Curves for Aflatoxins

External standard method was used to construct a calibration curve for aflatoxin B1, B2, G1 and G2. Concentration range for aflatoxins B2 and G2 was  $0.5-10 \ \mu g/g$  and for aflatoxins B1 and G1 was  $2-40 \ \mu g/g$ . The total aflatoxin in each sample was determined by summation of

concentrations of G2, G1, B2, and B1 (Np & Medhe, 2003). Aflatoxin method recovery and precision tests were determined by spiking blank samples at three different concentrations of 20, 40, and 100 g/kg in duplicates (Beltrán *et al.*, 2019). The percentage recovery was calculated using the formula: recovery (%) = (S'-S) 100 % /S spiked (Ata *et al.*, 2015). Where S', S and S- spiked is the concentration of the spiked sample, of non-spiked sample. To control interference with the target analyte, the method specificity was assessed by comparing the retention time for aflatoxins in spiked blanks with standards at 100 g/kg of aflatoxin.

Quantification of the Aflatoxin in maize sample was carried out using high performance liquid chromatography (Shimadzu model 10AVP) equipped with a fluorescence detector (FLD), a pump (RF–20A, LC-20AT), an auto sampler system (SIL-20A) and a column oven- thermo-controller (CT 10AS- VP). 10  $\mu$ L of the sample was injected under isocratic elution with a mixture of acetonitrile/methanol/water (15/30/70 v/v/v) as the mobile phase. Separation was carried out using genesis reverse-phase C18 analytical column of dimensions 4.6 × 250 mm, 100 Å pore size, and 5  $\mu$ m particle size; (Gloucester, UK) was set at 40 °C temperature. The mobile phase of potassium bromide and nitric acid was set at a flow rate of 0.9 mL/min<sup>-1</sup>. A KOBRA <sup>®</sup> cell electrochemical post column derivatization system (R-Biopharm Inc., Marshall. MI) was set at 100  $\mu$ A current, consisting of 254- nm UV lamp and 0.5 mm id. x 10 m PTFE tube fitted around the UV lamp where it was applied before the fluorescence detector to enhance the AFB1 and AFG1 fluorescence activity. The detector was operated at 360 and 450 nm wavelengths for detection of aflatoxin fluorescence excitation and emission, respectively. Identification and quantification of aflatoxin was based on retention time and peak areas of the reference standards and calibration curves.

# G) Statistical Analysis

Excel 21 sheets were used to enter data before subjecting to analysis of variance using the general linear model method R 4.1.1 software of August 2021. Tukey's Honestly Significant Difference test was used to post ANOVA treatments and mean comparisons. The homoscedasticity test was done for the two-way ANOVA model.

## 3. Results and Discussion

# A) Method validation

Validation of the method used involved determining the limit of detection, limit of quantification and correlation coefficient for the four strains of aflatoxins. The limit of detection was 0.12  $\mu$ g/kg for Aflatoxin B1, 0.12  $\mu$ g/kg for B2, 0. 21  $\mu$ g/kg for G1 and 0.25  $\mu$ g/kg for G2. The limit of quantification was 0.26  $\mu$ g/kg for Aflatoxin B1, 0.44  $\mu$ g/kg for B2, 0.29  $\mu$ g/kg for G1 and 0.39  $\mu$ g/kg for G2. The correlation coefficients for the four strains of aflatoxins B1, G1, B2 and G2 obtained from the calibration curves were above 0.99 which were considered adequate. The method accuracy determined by calculating spiked blank recoveries for the aflatoxin standards ranged from 66.5 to 85.7% for B1, 78.4 to 89.2% for B2, 85.51 to 89.5% for G1, and 82.4 to 103.4% for G2 were within accepted range of 70-150%, which qualified the analytical method. Other tests carried out to validate the method included precision and the relative standard deviation. The results of method validation are summarized in Table 1.

Parameter	(RT)	R <sup>2</sup>	Accuracy	Precision	RSTD	lod	loq
	(min)		%		%	(µg/kg)	(µg/kg)
B1	4.3	0.9983	77.2	1.28	26.58	0.12	0.26
B2	4.15	0.9943	83.83	0.29	20.81	0.16	0.44
G1	4.18	0.9961	82.52	1.62	25.57	0.21	0.29
G2	4.03	0.9978	88.38	0.47	10.13	0.25	0.39

Table 1: Method Validation Data

### B) Mean Levels of the Individual Strain and Total Aflatoxins in Maize Samples

A total of 178 individual maize samples were analysed and quantified for Aflatoxin B1, B2, G1, and G2, comprised of 89 samples each for Busia and Migori counties. The mean concentration for aflatoxin B1 in all stores in Busia County, were within the accepted limits of < 4  $\mu$ g/kg. On the other hand, the samples from Migori County stores exceeded the set limit except for NCPB stores. The observed difference in aflatoxin B1 levels for the two counties could be due to adapted postharvest management practices by maize chain players. Post-harvest management practices such as harvesting time, drying methods, protection from pests, mechanical injury at the time of harvesting and shelling, transportation and storage methods, sorting of contaminated and damaged maize grains, and the use of clean harvesting equipment depended on individual farmers and traders. Secondly, environmental conditions such as soil type, fungal species, climate, humidity, maturity time of grains, temperature and moisture (Njugi et al., 2018) were different in Busia and Migori counties, these also influenced the quality of maize and aflatoxin development in the store. These results suggested environmental factors experienced in Busia did not favor growth of aflatoxin causing molds. Levels of Aflatoxin B1 in the maize samples were different in the two counties. Thirdly, influence of cross border trade could have affected the two counties differently since Migori and Busia border two different nations with different environmental and climatic regimes, hence the imported maize would be different.

All maize samples collected from different store in Busia County had mean total aflatoxins below the set limit of 10  $\mu$ g/kg. On the other hand, more than 74 % of maize samples collected from

stores in Migori County had total aflatoxin above the same limit. The reason for the observation is stated earlier. The highest mean total aflatoxin measured was  $61.37\pm 5.26\mu$ g/kg recorded in samples from the farm stores, while the least was  $0.71\pm 0.15\mu$ g/kg from NCPB stores (Table 2).

Individual farmer's store hold maize from a particular farm or a set of farms. The findings from this study show that characteristic practices of farmers involved in maize production along the value chain varied in terms of sowing, caring, harvesting, shelling, packaging, transportation and storage. These practices were likely to impact on the aflatoxin contamination, in addition to natural factors such as drought, insect-damage, seasonal changes, temperatures and extreme precipitation patterns that would uniquely influence the quality of produce in different counties. Additional factors included social dynamic like poverty and education levels among the subsistence farmers. The last two factors influence agricultural management practices and noncompliance to guidelines regarding post-harvest handling of the grains. During the study it was observed that some farmers redirected rejected maize due to aflatoxin contamination, discolored grains and insects damaged grains to domestic markets such as local brews and animal feeds, which was also reported in earlier studies (Ambler et al., 2018; Matumba et al., 2016; Misihairabgwi et al., 2019). Retailers, wholesalers and NCPB stores on the other hand, pooled maize from different farmers who followed different management practices in their holding stores that could influence aflatoxin levels. Grain management problems at the storage levels are the main cause of aflatoxin contamination in maize (Agric, 2020). The order of total aflatoxin levels followed was retailers>farmers>wholesalers>NCPB store for Busia and farmers>retailers>wholesalers>NCPB stores for Migori.

STORE TYPE	AFLATOXIN STRAIN	BUSIA	MIGORI
NCPB store (µg/kg)	AFB1	0.53±0.00	3.04±0.79
	AFB2	0.07±0.00	1.05±0.04
	AFG1	0.05±0.00	1.1± 0.33
	AFG2	0.06±0.00	0.62±0.03
	Total	0.71± 0.15	5.81± 1.23
Wholesaler store (µg/kg)			
	AFB1	1.61±0.06	10.2±1.46
	AFB2	0.01±0.00	4.51±0.91
	AFG1	0.01±0.00	6.55±0.37

 Table 2: Individual aflatoxin strains and total aflatoxin in maize samples from Busia and Migori County stores

STORE TYPE	AFLATOXIN STRAIN	BUSIA	MIGORI
	AFG2	0.01±0.00	2.36±0.51
	Total	1.64±0.32	23.62± 1.65
Retailer store (µg/kg)	AFB1	1.74±0.00	16.72±1.30
	AFB2	0.66±0.00	4.82±0.41
	AFG1	$1.06 \pm 0.04$	14.24± 2.14
	AFG2	0.21±0.00	3.46±0.57
	Total	3.67± 1.13	39.24±3.31
Farmer store (µg/kg)	AFB1	1.45±0.50	23.75 ±2.21
	AFB2	0.99±0.00	7.43±0.76
	AFG1	0.15±0.00	21.03± 1.48
	AFG2	0.4± 0.07	9.16±1.39
	Total	2.99± 0.73	61.37± 5.26

### C) Influence of post-harvest management practices on prevalence of aflatoxin in samples

Analysis was based on samples collected per county in NCPB store (23), farmer stores (22), wholesale stores (22) and retail stores (22). The upper, lower and median percentiles were calculated based on characteristics of individual sample lot and average aflatoxin contamination levels in the maize. There was a significant difference between maize samples collected from Busia and Migori county stores in terms of aflatoxin contamination levels at p-value < 0.05%, signifying influence of varying post-harvest management practices in each county. Practices such as roadside spreading, drying in open bags near dusty roads, poorly ventilated stores, reuse of bags or sharing bags for different cereals, and placing maize bags on bear floor would affect development of molds. Hence single or a combination of these factors contributed to the observed variations in the levels of aflatoxin between Busia and Migori Counties.

Figure 2 shows the levels of aflatoxin occurrence in different stores per county. The box symbolized the middle 50% of the measurements for each aflatoxin strain in the maize samples, while the height of the box shows the range of samples with aflatoxin levels. The top and bottom bars represented the 25<sup>th</sup> and 75<sup>th</sup> percentiles of the aflatoxin contaminants in the samples. The 90<sup>th</sup> and 10th percentiles were represented by the vertical whisker line at the top and bottom of the box. The box plots representing analysed data for Busia County maize samples were smaller

compared to those for samples collected from Migori County, implying that samples from Migori County had higher aflatoxin contamination levels. The boxplots for Aflatoxin B1 show that 50% of the samples from Busia County had levels > 0.5 µg/kg for farmer stores, > 0.3 µg/kg for retailer stores and > 1.0  $\mu$ g/kg for wholesaler stores. The range for the same cluster of samples in Migori County for Aflatoxin B1 levels followed farmers' stores (> 2.5 µg/kg), retailer stores (>2.0 µg/kg) and wholesale stores (> 2.0 µg/kg). Migori County samples recorded contamination levels than samples from Busia County. The black dots on the upper and lower side of the box represent the extreme or outlier contaminants recorded in a few samples from different aflatoxin strains. Aflatoxin levels in Migori County samples were in contrast, because more than thirty-three percent of outliers appeared in the lower and upper limits. The levels of aflatoxin in the maize samples from Migori County stores varied from low to high suggesting large differences in postharvest management practices for maize handlers. All samples collected or 74 % of the samples from farm, retail and wholesale stores were all contaminated with aflatoxin above the set limit of 4 µg/kg for AFB1 except for NCPB stores. The findings of this study agreed with previous study by Mahuku et al. (2019) who found high prevalence of aflatoxin with 42.7% of collected samples from Migori County market stores having high levels of aflatoxin B1.



Figure 2: Concentrations of aflatoxin strains in maize samples in various stores in both Counties

### D) Impact of store type on levels of different strains of aflatoxin in maize

The proposed null hypothesis (H<sub>0</sub>) that there was no difference in the mean concentrations attributable to the aflatoxin strains and the type of store was tested by conducting a two-way ANOVA test. Table 3 shows f-values and the corresponding p-values measured for the mean levels of aflatoxin strains, stores and the product of the two. According to the f-values and a corresponding p-value < 0.05%, aflatoxin strain BI, B2, G1 and G2 had an impact on the contamination levels measured in stored maize. Similarly, the store types had impact on the contamination levels in the maize. In addition, product of aflatoxin strains and the store types was significant on the contamination levels in the stored maize at p-value < 0.05%. We therefore accepted the proposed null hypothesis that there was no significant difference in the mean concentrations attributable to the aflatoxin strains and the type of store where maize was collected.

Source of Variation	Df	F value	Pr(>F)
Aflatoxin	3	38.79	<2e-16 ***
Store	3	57.04	<2e-16 ***
Residual Variance	-	-	29.6
Aflatoxin: store	9	4.908	< 2e-06
Residual Variance			28.2

**Table 3:** Analysis of variance for the impact of store type on the concentration of Aflatoxin tested in Busiaand Migori counties

## D) Post-Hoc Test for Significant Parameters

A Tukey Significant Difference test was conducted to compare impact of different aflatoxin strains and stores types to the mean levels of aflatoxin in stored maize (Table 4). The results from Tukey significant difference test of the mean levels of aflatoxin strains G2 and B2 showed p-values < 0.05% for 83.3% of the tests and only 16.3% of the tests > 0.05% for G2 and B2 pair, suggesting a significant difference between the means of the two groups. Further analysis revealed that the store type impacted 100% on the mean aflatoxin contamination in maize. The influence of the store type and aflatoxin strain to contamination in stored maize was significant with p-values < 0.05%.

AFLATOXIN	DF	LOWER	UPPER	P ADJ
AFB2-AFB1	-4.8869	-6.3725	-3.4013	0.0000
AFG1-AFB1	-1.8416	-3.3271	-0.356	0.0080
AFG2-AFB1	-5.3373	-6.8229	-3.8517	0.0000
AFG1-AFB2	3.0453	1.5598	4.5309	0.0000
AFG2-AFB2	-0.4504	-1.936	1.0352	0.8632
AFG2-AFG1	-3.4957	-4.9813	-2.0102	0.0000
STORE TYPE				
NCPB DEPOT-FARM STORE	-7.1847	-8.6623	-5.707	0.0000
RETAIL STORE -FARM STORE	-2.6469	-4.1409	-1.1529	0.0000
WHOLESALE STORE -FARM	-4.8418	-6.3358	-3.3478	0.0000
STORE				
RETAIL STORE -NCPB DEPOT	4.5377	3.0601	6.0154	0.0000
WHOLESALE STORE -NCPB	2.3429	0.8652	3.8206	0.0003
DEPOT				
WHOLESALE STORE-RETAIL	-2.1948	-3.6888	-0.7008	0.001
STORE				

**Table 4:** Impact of the store type and aflatoxin strain to aflatoxin contamination in stored maize usingTukey Significant Difference test

### 4. Conclusion

Aflatoxin contamination was more prevalent in maize samples from Migori than those from Busia. Individual aflatoxin contamination trend was B1>G1>B2>G2 for both counties. The total aflatoxin trend was farmers>retailers>wholesalers>NCPB and retailers>farmers>wholesalers>NCPB for Migori and Busia stores, respectively. Regular checks of moisture content and cereal temperatures, storage aeration and hygiene including pests control varied with the store from farmers to NCPB stores, which influenced prevalence and composition of aflatoxins in stored maize.

### 5. Recommendation

We recommend regular education and training on effective post-harvest management techniques for farmers and traders in both Busia and Migori Counties. The two counties to build adequate capacity in the sector to stop the proliferation of aflatoxin molds into maize cereal

during post-harvest handling. In addition, county public health department should intensify regular inspection and licensing of maize storage facilities to entrench good post-harvest management practices. Further studies are recommended to determine how interaction between soil physical and chemical factors, influence the development of aflatoxin causing contamination of maize in the two counties.

### 6. Acknowledgements

The authors would like to acknowledge Ms. Agrippina from the National Cereals and Produce Board for her assistance during sample collection, guidance to access aflatoxin sampling locations. The maize farmers, traders and all other maize handlers are appreciated for their assistance during maize sample collection from the stores. The technical staff from the University of Nairobi and the Kenya Plant Health Inspectorate Services are acknowledged for their assistance in laboratory analyses. The Erasmus-Mundus Foundation is acknowledged for the exchange program accorded to Mr. Nicholas Mwenda to do part of his research at the University of Koblenz-Landau in Germany, and finally the National Commission for Science, Technology and Innovation for the research grant.

### 7. References

- Achparaki, M., Thessalonikeos, E., Tsoukali, H., Mastrogianni, O., Zaggelidou, E., Chatzinikolaou, F., Vasilliades, N., Raikos, N., Isabirye, M., Raju, D. V., Kitutu, M., Yemeline, V., Deckers, J., & J. Poesen Additional. (2012). We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists TOP 1 %. *Intech*, 13.
- [2] Agric, F. (2020). Prevalence of aflatoxins in dietary staples in the border. *African Journal of Food, Agriculture, Nutrition and Development,* 20(7), 17045–17062.
- [3] Ambler, K., de Brauw, A., & Godlonton, S. (2018). Measuring postharvest losses at the farm level in Malawi. *Australian Journal of Agricultural and Resource Economics*, *62*(1), 139–160. https://doi.org/10.1111/1467-8489.12237
- [4] Ata, S., Wattoo, F. H., Ahmed, M., Wattoo, M. H. S., Tirmizi, S. A., & Wadood, A. (2015). A method optimization study for atomic absorption spectrophotometric determination of total zinc in insulin using direct aspiration technique. *Alexandria Journal of Medicine*, 51(1), 19–23. <u>https://doi.org/10.1016/j.ajme.2014.03.004</u>
- [5] Beltrán, B. G., Martínez-Serrano, I., Ramos-Sanchez, V., Chávez-Flores, D., Nevárez-Rodríguez, M. C., & Suárez-Domínguez, E. A. (2019). Development and Validation of a New Method for Determination of Pb and Cr in Marine Organisms by Total Reflection X-Ray Fluorescence (TXRF) Spectroscopy. *Journal of Analytical Methods in Chemistry*, 2019. https://doi.org/10.1155/2019/8150678
- [6] Benkerroum, N. (2020). Aflatoxins: Producing-molds, structure, health issues and incidence in Southeast Asian and Sub-saharan African countries. *International Journal of Environmental Research and Public Health*, 17(4). <u>https://doi.org/10.3390/ijerph17041215</u>
- [7] Dabija, A., Ciocan, M. E., Chetrariu, A., & Codină, G. G. (2022). Buckwheat and Amaranth as Raw Materials for Brewing, a Review. *Plants*, 11(6). <u>https://doi.org/10.3390/plants11060756</u>
- [8] Donnelly, R.; Elliott, C.; Zhang, G.; Baker, B.; Meneely, J. (2022). Understanding Current Methods for Sampling of Aflatoxins in Corn and to Generate a Best Practice Framework. *Toxins*, *14*, 819. https://doi.org/10.3390/
- [9] EC. (2010). Commission Regulation (EC) No 178/2010 of 2 March 2010 amending regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of

mycotoxins in foodstuffs. Official Journal of the European Union, 2010(178), 32-43.

- [10] Evaluation of certain contaminants in food. (2017). In World Health Organization technical report series (Issue 1002, pp. 1–166).
- [11] Filazi, A., & Tansel, U. (2013). Occurrence of Aflatoxins in Food. *Aflatoxins Recent Advances and Future Prospects, January 2013*. https://doi.org/10.5772/51031
- [12] Gachara, G. W., Nyamache, A. K., Harvey, J., Gnonlonfin, G. J. B., & Wainaina, J. (2018). Genetic diversity of Aspergillus flavus and occurrence of aflatoxin contamination in stored maize across three agro-ecological zones in Kenya. *Agriculture and Food Security*, 7(1). https://doi.org/10.1186/s40066-018-0202-4
- [13] GoK (2013a) Brief of Migori County. Agriculture Sector Development Support Programme (ASDSP), Migori.
- [14] Grote, U., Fasse, A., Nguyen, T. T., & Erenstein, O. (2021). Food Security and the Dynamics of Wheat and Maize Value Chains in Africa and Asia. *Frontiers in Sustainable Food Systems*, 4(February), 1–17. https://doi.org/10.3389/fsufs.2020.617009
- [15] Iso, I. S., European, S., Regulation, C., & Standard, T. I. (2010). *Standard Operating Procedure Sampling instructions for assessing microbiology and / or toxin of stacked ' flour ' foods ( i . e . flours , meal , and FBF )*.
- [16] Jonah K., B., Richard C., C., & Teh Exodus, A. (2020). Mycotoxin Contamination of Stored Maize in Kenya and the Associated Fungi. *Journal of Plant Pathology Research*, 2(1), 7–13. https://doi.org/10.36959/394/620
- [17] Kang'ethe, E. K., Gatwiri, M., Sirma, A. J., Ouko, E. O., Mburugu-Musoti, C. K., Kitala, P. M., Nduhiu, G. J., Nderitu, J. G., Mungatu, J. K., Hietaniemi, V., Joutsjoki, V., Korhonen, H. J., Nairobi, K., & Kang'ethe, E. K. (2017). Exposure of Kenyan population to aflatoxins in foods with special reference to Nandi and Makueni counties The Ministry of Agriculture Livestock and Fisheries, Veterinary Laboratories Kabete, Private Bag Kabete. *Food Quality and Safety*, *1*(2), 131–137. <u>https://doi.org/10.1093/fqs/fyx011</u>
- [18] KEBS (Kenya Bureau of Standards). 2019. https://www.kebs.org
- [19] Kiarie, G. M., Dominguez-Salas, P., Kang'ethe, S. K., Grace, D., & Lindahl, J. (2016). Aflatoxin exposure among young children in urban low-income areas of Nairobi and association with child growth. African Journal of Food, Agriculture, Nutrition and Development, 16(3), 10967–10990. https://doi.org/10.18697/ajfand.75.ILRI02
- [20] Klopfenstein, T. J., Erickson, G. E., & Berger, L. L. (2013). Maize is a critically important source of food, feed, energy and forage in the USA. *Field Crops Research*, 153, 5–11. https://doi.org/10.1016/j.fcr.2012.11.006
- [21] Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., & Kang, S. G. (2016). Aflatoxins: A Global Concern for Food Safety, Human Health and Their Management. *Frontiers in Microbiology*, 7, 2170. https://doi.org/10.3389/fmicb.2016.02170
- [22] Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Misore, A., DeCock, K., Rubin, C., Nyikal, J., Njuguna, C., Langat, A., Kilei, I. K., Tetteh, C., Likimani, S., Oduor, J., Gupta, N. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives*, *113*(12), 1763–1767. https://doi.org/10.1289/ehp.7998
- [23] Liu, Z., Zhang, G., Zhang, Y., Jin, Q., Zhao, J., & Li, J. (2016). Factors controlling mycotoxin contamination in maize and food in the Hebei province, China. Agronomy for Sustainable Development, 36(2). https://doi.org/10.1007/s13593-016-0374-x
- [24] Lopes, C. (2014). We need more agribusiness in Africa. *Africa Renewal*, 27(4), 20–21. https://doi.org/10.18356/f10df8cf-en
- [25] Mahuku, G., Sila, H., Mutegi, C., Kanampiu, F., Narrod, C., & Makumbi, D. (2019). Pre-harvest management is a critical practice for minimizing a fl atoxin contamination of maize. *Food Control*, 96: 219–226. https://doi.org/10.1016/j.foodcont.2018.08.032
- [26] Mang'eni, F. O. (2022). Historical Analys of Declining Maize Production in Kenya; A Case of Trans-Nzoia County. 5(7), 312–316.
- [27] Matumba, L., Monjerezi, M., Kankwamba, H., Njoroge, S. M. C., Ndilowe, P., Kabuli, H., Kambewa, D., & Njapau,

H. (2016). Knowledge, attitude, and practices concerning presence of molds in foods among members of the general public in Malawi. *Mycotoxin Research*, *32*(1), 27–36. https://doi.org/10.1007/s12550-015-0237-3.2017.1357003

- [28] Midega, C. A. O., Bruce, T. J. A., Pickett, J. A., Pittchar, J. O., Murage, A., & Khan, Z. R. (2015). Climate-adapted companion cropping increases agricultural productivity in East Africa. *Field Crops Research*, 180(July), 118–125. https://doi.org/10.1016/j.fcr.2015.05.022
- [29] Misihairabgwi, J. M., Ezekiel, C. N., Sulyok, M., Shephard, G. S., & Krska, R. (2019). Mycotoxin contamination of foods in Southern Africa: A 10-year review (2007–2016). *Critical Reviews in Food Science and Nutrition*, 59(1), 43–58. https://doi.org/10.1080/10408398
- [30] Mutegi, C. K., Cotty, P. J., & Bandyopadhyay, R. (2018). Prevalence and mitigation of aflatoxins in Kenya (1960to date). World Mycotoxin Journal, 11(3), 341–357. https://doi.org/10.3920/WMJ2018.2362
- [31] Nakavuma, J. L., Kirabo, A., Bogere, P., Nabulime, M. M., Kaaya, A. N., & Gnonlonfin, B. (2020). Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. *International Journal of Food Contamination*, 7(1), 1–11. https://doi.org/10.1186/s40550-020-00079-2
- [32] NARIGP, GoK, & World Bank. (2020). Kenya County Climate Risk Profile : Kiambu County. 1–34.
- [33] Ngindu, A., Kenya, P. R., Ocheng, D. M., Omondi, T. N., Ngare, W., Gatei, D., Johnson, B. K., Ngira, J. A., Nandwa, H., Jansen, A. J., Kaviti, J. N., & Arap Siongok, T. (1982). Outbreak of Acute Hepatitis Caused By Aflatoxin Poisoning in Kenya. *The Lancet*, *319*(8285), 1346–1348. https://doi.org/10.1016/S0140-6736(82)92411-4
- [34] Njugi, A., Nyang, J., González-Villa, M., Ahenda, S., Maribel, G.-V., & Author, C. (2018). Aflatoxin Contamination In Maize Used As Animal Feed For Cattle And Its Potential Mitigation Measures, Current Situation In Kenya:A review Antibiotic resistance mechanisms View project Characterization and adsorption of heavy metals in industrial effluent. *IOSR Journal of Environmental Science*, *12*(7), 35–42. https://doi.org/10.9790/2402-1207013542
- [35] Np, G., & Medhe, S. (2003). Determination of aflatoxins B1, B2, G1, and G2 in foodstuffs. *Camag*, 17(2), 1–4. http://www.camag.ch/
- [36] Okoth, S., De Boevre, M., Vidal, A., Di Mavungu, J. D., Landschoot, S., Kyallo, M., Njuguna, J., Harvey, J., & De Saeger, S. (2018). Genetic and toxigenic variability within Aspergillus flavus population isolated from maize in two diverse environments in Kenya. *Frontiers in Microbiology*, 9(JAN), 1–14. https://doi.org/10.3389/fmicb.2018.00057
- [37] Pardey, P. G., Andrade, R. S., Hurley, T. M., Rao, X., & Liebenberg, F. G. (2016). Returns to food and agricultural R&D investments in Sub-Saharan Africa, 1975–2014. *Food Policy*, 65, 1–8. <a href="https://doi.org/10.1016/j.foodpol.2016.09.009">https://doi.org/10.1016/j.foodpol.2016.09.009</a>
- [38] Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences, 1312(1), 105–112. https://doi.org/10.1111/nyas.12396
- [39] Rouf Shah, T., Prasad, K., & Kumar, P. (2016). Maize, A potential source of human nutrition and health: A review. Cogent Food and Agriculture, 2(1). https://doi.org/10.1080/23311932.2016.1166995
- [40] Shrestha, S., & Control-nepal, Q. (2019). Simultaneous Analysis of Aflatoxin B1, B2, G1 and G2 in Maize and Feed of Nepal by HPLC with Fluorescence Detection Method. January.
- [41] Stepman, F. (2018). toxins Scaling-Up the Impact of Aflatoxin Research in Africa. The Role of Social Sciences. https://doi.org/10.3390/toxins10040136
- [42] USAID (2017). Climate Risk Profile. Usaid, April, 1–5.
- [43] World Health Organization. (2015). IARC Monographs evaluate consumption of red meat and processed meat and cancer risk. *International Agency of Research on Cancer, October*, 1–2.
- [44] Food Standards Agency. Mycotoxins Sampling Guidance. 2016. Available online: https://www.food.gov.uk/sites/default/files/ media/document/mycotoxins-sampling-guidance.pdf (accessed

on 20 April 2022).

- [45] Commission Regulation (EC) No 401/2006 of 23 March 2006 Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Mycotoxins in Foodstuffs. 2006. Available online: https://eurlex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32006R0401 (accessed on 18 February 2022).
- [46] KNBS. Kenya demographic and health survey 2014. Rockville, MD, USA: Kenya National Bureau of Statistics, Ministry of Health/Kenya, National AIDS Control Council/Kenya, Kenya Medical Research Institute, National Council for Population and Development/Kenya, and ICF International; 2015. http://dhsprogram.com/publications/publication-fr308-dhs-final-reports.cfm. Accessed 15 May 2017.
- [47] Rasheed, H., Xu, Y., Kimanya, M. E., Pan, X., Li, Z., Zou, X., Shirima, C. P., Holmes, M., Routledge, M. N., & Gong,
   Y. Y. (2021). Estimating the health burden of aflatoxin attributable stunting among children in low income countries of Africa. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-020-80356-4