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| ARTICLE INFO | ABSTRACT | | | | | |
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| Article History: Received: 15/08/2022 Accepted: 27/09/2022 Available online: 31/12/2022 | Aflatoxins contamination of food is a global human health risk because of its ill health effects; liver cancers, suppression of immune system, teratogenic disorders among others. Its outbreak incidences have led to food spoilage, malnutrition and growth retardation in children. This study aimed to establish the occurrence and prevalence of aflatoxin in stored | | | | | |
| <i>Keywords:</i> Aflatoxin maize, Contamination Prevalence Occurrence Stores | National Cereals and Produce Board stores in Nakuru, Kajiado and Trans- Nzoia Counties. One hundred and forty-seven maize samples were purchased from grain stores, processed, extracted and analyzed for aflatoxins. Analysis and quantification of the samples was performed using high performance liquid chromatography coupled with fluorescence detector. The order of mean aflatoxin contamination in samples by the four strains was AFB1>AFG1>AFB2>AFG2. The most prevalent and dominant was Aflatoxin B1 which contaminated 58.5% of samples above 4 μg/kg limit for human food. The occurrence of aflatoxin contamination by maize samples was 89.93%. It was higher in samples from Trans Nzoia and Kajiado counties than in samples from Nakuru County. Aflatoxin occurrence in the counties varied with the stores; thus in Kajiado 67% of the National Cereals and Produce Board stores, 52% of farmers' in Trans-Nzioa, while 42% of farmers' stores in Nakuru were contaminated. | | | | | |

1. INTRODUCTION

Aflatoxins are chemical compounds derived from difuranocoumarins with a coumarin nucleusbased bifuran group on one side and a lactone ring (Gs) or a pentanone ring on the other (Bs and Ms) (Tola & Kebede, 2016; Bbosa *et al.*, 2013). Consumption of Aflatoxin B₁, B₂, G₁ and G₂ in contaminated food and feeds by humans and animals, metabolize into B_{2A}, M₁, M₂, M_{2A}, G_{2A}, GM₁, GM₂, GM_{2A} B₃ and aflatoxicol compounds (figure 1). The strength of aflatoxins' toxicity depends on the structural nature of terminal furan ring but saturated rings are least toxic compared to the unsaturated. Aflatoxin B that means blue and Aflatoxin G which means green as visualized under UV light. The fluorescent colors are associated with aflatoxin chemical structurals. Both B and G affect cereal grains by discoloration and causing valuable loss of

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nutrients (Suleiman *et al.,* 2013). Aflatoxin B1 is a class 1 human carcinogen associated with hepatocellular carcinomas, liver failure and death (Ostry *et al.,* 2017; World Health Organization, 2015), depending on the ingested dosage, duration of exposure, species, breed, diet or nutritional status to be acute and chronic (Negash, 2018).



Figure 1: Structure of AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2.

Naturally maize growing environmental conditions determine the vulnerability levels to Aspergillus, Fusarium and Penicillium fungi species, which transmit aflatoxins B_1 , B_2 , G_1 and G_2 contaminants(Benkerroum, 2020a; Probst *et al.*, 2014). A combination of physical, chemical, biological, technological, ecological, and environmental factors during growth and development of cereal crops (Dutton, 2009), influence incidences of aflatoxin contamination in food across the value chain, (Zain, 2011). These aflatoxigenic strains have the capability of growing in maize at any stage, from cultivation, harvesting, drying, storage, transportation, and in the market (Mitchell *et al.*, 2017). About 25% of cereal produced globally is affected by different mycotoxins including aflatoxins. Aflatoxins are of great concern due to their high toxicity and prevalence in human food and livestock feeds (Kumar *et al.*, 2017). To control these effects, different countries have fixed maximum allowable total aflatoxin levels for human food and animal feeds. In Kenya the accepted levels are 10 µg/kg and 20 µg/kg respectively (Edition *et al.*, 2014)

The impact of aflatoxins is felt more in developing countries where the health burden is huge, with depressed livelihoods and socio-economic developments. Most of the affected countries are in the tropics and subtropics believed to have favorable conditions for molds growth, propagations and infestation on food materials. Among the affected food materials are cereals, oil seed crops, legumes, and nuts (Benkerroum, 2020b; Jallow *et al.*, 2021). A variety of ailments such as liver cancer, suppression of immune system, teratogenic defects, malnutrition, retarded growth in children and also increased incidences of other chronic diseases are linked to prolonged consumption of aflatoxin contaminated food materials (Benkerroum, 2020b; Negash, 2018). The major pathway of exposure to aflatoxin effects is through ingestion of contaminated foods. The number of people exposed to the contamination annually is about 4.5 billion globally and 1.8 million in Kenya (Obade *et al.*, 2015).

In Kenya, major episodes of aflatoxins contamination have been reported in the past that included Murang'a (Linsell & Peers, 1977), Makueni (Ngindu *et al.*, 1982); Makueni, Kitui, Machakos and Thika counties in 2004 where 317 cases were recorded out which 125 people died. Laboratory reports for the latter recorded samples with Aflatoxins contamination levels as high as 4,400 µg/kg (Lewis *et al.*, 2005). In 2005 and 2006, more than 42 deaths were also reported in Machakos and Makueni counties due to aflatoxin contamination (EAC Policy, 2018). Subsequent studies have reported recurrence of aflatoxins contamination in other parts of Kenya, some with aflatoxicosis levels as high as 58,000 µg/kg (Daniel *et al.*, 2011; Hoffmann *et al.*, 2013; Monda *et al.*, 2020; Mutiga *et al.*, 2017; Nelson & Margaret, 2018; Omara *et al.*, 2021; Sirma *et al.*, 2019). From the findings of different studies, it is clear that the risk due to Aflatoxin contaminations on food substances to the public health is high (Gong *et al.*, 2016).

This study sought to evaluate the prevalence and occurrence of aflatoxin contamination, in maize grain storage facilities in the Rift Valley region, Kenya. The region has a wide range of climatic zones which are modulated by various geographical features. The region has escapements,

valleys, and mountainous backgrounds that include Mau Ranges, Nandi Hills, Cherangany Hill, Ngong Hills, Aberdare Ranges, Mt. Kilimanjoro, Mt. Elgon, intercalated with forests, fresh and salt-water lakes and geothermal fountains (Watene *et al.*, 2021) all of which influence crop production. The Rift Valley region covers a total area of 173,854 km² within 13 counties namely: Turkana, West Pokot, Samburu, Trans-Nzoia, Uasin Gishu, Elgeyo Marakwet, Nandi, Baringo, Laikipia, Nakuru, Kajiado, Kericho and Bomet (MEMD Uganda, 2013). The key economic activities in the region include cereal crop growing, livestock husbandry, horticulture, trade, and tourism (Kajiado, 2018). Among main cereal crops grown in the rift valley is maize which is dominant in 10 of 14 counties which include Trans-Nzoia, Uasin Gishu, Elgeyo Marakwet, Nandi, Baringo, Laikipia, Nakuru, Kajiado, Kericho and Bomet (Masambaya *et al.*, 2018). Kajiado, Nakuru and Trans Nzoia counties were selected to represent the other counties in the Rift Valley Region in the study (Figure 2).

Occurrence of fungus and colonization of maize is predominantly influenced by a number of factors which include: environmental conditions in growing fields, such are humidity and temperature, varieties of maize planting, farmers' agronomic practices and post-harvest management of maize (Daou *et al.*, 2021; Landoni *et al.*, 2020). Kajiado, Nakuru and Trans Nzoia counties experience variant weather conditions marked by sub-temperate cold and wet conditions in the highlands, while the lowlands experience warm and dry conditions that affect precipitation, temperature and soil features. The variances in climatic conditions influence aflatoxin infestation in maize grains after harvest and storing. The variance in planting varieties, farming practices and post-harvest manage also played a key role in the aflatoxin status on the stored maize in the three counties.

Kajiado County is located in arid and semi-arid zone in the southern part of the Eastern Rift Valley. The county is characterized by many hills and plateaus raising from 500 to 2,500 meters above the sea level at Ngong Hills. The county has 21.8% and 78.2 % of land area within semi-arid to arid agri-ecological zones (Chepkoech *et al.*, 2018; Morsch & Bartlett, 2011; Online *et al.*, 2022). The county experiences a bi-modal rainfall of 500 - 750 mm per year, which is influenced by altitudinal changes and seasonality (Kaoga *et al.*, 2018). The short rains come between October and December, while the long rains are between March and May. Monthly rainfall ranges from 300 mm to 1250 mm with the highest experienced in the slopes of Ngong ills and Mt. Kilimanjaro (Kaoga *et al.*, 2018). The mean temperature fluctuates from 10 °C in July and August, to 34 °C in November and April. There is pronounced livestock keeping and overdependence on rain fed agriculture (van der Horst *et al.*, 2022). Due to climate change and variability, the county experiences depressed rainfall, drought incidences and temperature variations (Medina *et al.*, 2015). Occasionally, heat stress incidences and flooding occur affecting crop production (Ongoma, 2013; The Ministry of Agriculture, Livestock and Fisheries (MoALF), Nairobi, 2017). Figure 2 shows Kajiado study site.



Figure 2: The maps of sampling sites: Nakuru, Trans Nzoia and Kajiado of Rift Valley region.

Trans Nzoia County borders Bungoma to the west, Uasin Gishu and Kakamega to the south, Elgeyo Marakwet to the east, West Pokot to the north, the republic of Uganda to North West and Mount Elgon to the west, while elevation varies from 1,400 meters towards the north (Fay, 2018) to 4,313 m in Mt. Elgon region. The county lies in humid and semi-humid to semi-arid agriecological zones (Mutiga *et al.*, 2015). The annual rainfall ranges from 1000 mm to 1700 mm, with long rains occurring between March-May and short rains between October-December (Odwori & Wakhungu, 2021). The long and intermediate rain seasons are reliable for agricultural production (Gnonlonfin *et al.*, 2013; Ongoma, 2013). Climate change and variability has contributed to increased drought incidences, unpredictable rains patterns and floods (Masambaya *et al.*, 2018, World Bank, 2017). Maize is the main crop grown in the county and is strongly influenced by weather conditions affecting grain filling and maturation that lead to increased losses in the crop yield and growth of aflatoxins during storage.

Nakuru County borders seven counties namely; Laikipia to the north-east, Kericho to the West, Narok to the south-west, Kajiado to the South, Baringo to the North, Nyandarua to the East and Bomet to the West. It has four agro-ecological zones based on the rainfall received and elevation. Zone 1 has the lowest mean annual rainfall of 500-800 mm per annum; Zone 2 receives annual rainfall of between 800- 1100 mm, Zone 3 receives rainfall of between 1,100 - 1,400 mm per annum with an altitude of between 1,800-2,300 m, and Zone 4 receives annual rainfall of over 1400 mm with an altitude of between 2300 m and 2700 m above sea level. The county also lies in humid to sub-humid, and semi-arid agri-ecozones (Mutiga *et al.*, 2015). Bimodal rainfall patterns are experienced in the county ranging from 500-1800 mm annually for short rains in October – December, and long rains in March-May (Nakuru County Government, 2013). Climate change and variability influence crop pests and diseases outbreaks which are sometimes linked to growth of aflatoxins (Ongoma, 2013).

2. MATERIALS AND METHODS

A) Study Design

The study targeted four maize stores whose characteristic varied in terms of management and volumes of maize stored. They included farmers stores in farmers homes, retailers store in local trading centers, wholesalers store mostly in large urban centers and National cereals produces board (NCPB) stores in county headquarters. The study design covered; collection of maize grain samples from the storage facilities, sample preparation, and laboratory analysis.

B) Sampling Dry Maize

Dry maize grains samples were sampled from farmers, retailers, wholesalers and National Cereals and Produce Board (NCPB) stores per county. Only maize grains harvested within three months period was sampled to avoid old maize in the store that could have influenced the quality of data.

C) County sampling sites and samples size per store type

Forty-nine maize samples were sampled randomly from selected storage bags in each of the four store types namely farmers, retailers, wholesalers and NCPB stores using a closed sampling spear technique (Fisher *et al.*, 1998). Twelve maize samples weighing a kilogram each were sampled from farmers, retailers and wholesalers' stores. Since only one NCPB store was in the county but with a large volume of maize, a sample was collected for every hundredth bag totaling to thirteen samples. Each sample was composite, made from five 90 kilograms bags that were randomly identified, five equal vertical levels marks made from the bottom to the top in accordance with the European Commission (EC) guidelines no. 178/2010 (EC, 2010). At the marked level, a kilogram of sample was pulled out of the bag, the twenty-five kilograms sample was mixed thoroughly out of which a kilogram was resampled into the sample bag. The sample was coded, maintain a constant moisture content level in the fields and during transportation to the laboratory, a pack of silica gel was added and kept in a Coleman cooler box away from direct sunlight. The samples were transported for laboratory analysis at the University of Nairobi.

Before analysis each sample was divided into two 500 g portions, recoded A and B. Portion A was refrigerated at -20 °C as a backup, while B was processed immediately for aflatoxin contamination analysis and quantification.

D) Number of samples

The minimum maize sample size (n) collected was determined using Fisher *et al.* (1998) formula: N_min = $z^2 \times p \times q / d^2$.

Where N_min was the minimum sample size required, q = (1 - p), z = 1.96 is the standard error, p = prevalence of condition under study, which was aflatoxin contamination of maize grain in the study area, and d = 0.05 is the absolute precession required for the study at 95% confidence level. The mean prevalence rate of aflatoxin contamination at study area was 9.3% and was used to determine the sample size. For q = (1 - p) = 0.907, p = 0.093, and $n = (1.96)^2 (0.093) (0.907) / (0.05)^2 = 129.61$. The minimum sample size was 130 samples but for this study a total of 147 samples were collected.

E) Sample preparation

The sample preparation involved thoroughly mixing independently each of the 147 portion B maize samples for 10 minutes, pulling from it 20g, milling to 0.25mm powder, dividing the resulting flour into three 5g portions and extracting it for analysis. Thirty milliliters solution made by mixing methanol, deionized water and acetonitrile in the ratio 12:2:1 (v/v/v) was used for extraction. The extraction was done by shaking the mixture at 120 rpm for thirty minutes with an orbital shaker (Mxbaoheng MPL-20) and filtering through a filter paper No. 4 (Whatman International Ltd., Maidstone, UK). It was followed by drawing 1 mL supernatant, diluting it to 40mL, with 39 mL of phosphate buffered saline (PBS), adjusting the pH to 7.4, centrifuging it at 3400 rpm and filtering through a 0.45 μ m pore size nylon membrane. Further filtration was done through an immunoaffinity column at a flow rate of 1 drop/second, then washing with 10 mL of methanol at a flow rate of 1 drop/second. Evaporation of eluent to dryness was done under a stream of white spot nitrogen, and reconstituting with the mobile phase solution to 400 μ L made by mixing water/methanol/ acetonitrile at 55/10/35, v/v/v ratio.

F) Method Validation

The validation of the method used conform with SANCO/12571/2013 analytical performances criteria (EC, 2010) for high performance liquid chromatography coupled with fluorescence detector (HPLC) method. The validation involved determining the limit of detection (LOD) and limit of quantification (LOQ), based on the measurement and calibration solutions with lowest concentrations. Test of recovery was done by spiking five different samples 5 g each of maize

blanks of with 20 μ L of aflatoxins with three different concentrations standard solutions 20, 40, and 100 μ g/kg, incubating them overnight in an airtight container at room temperature. Extraction of the blank samples was done by mixing them thoroughly through shaking the same way the actual samples are extracted in triplicates. The percentage recoveries were calculated using the formula: Recovery (%) = (S'-S) 100 % /S spiked (Ata *et al.*, 2015). Where S' is the concentration of the spiked sample, S is the concentration of non-spiked sample and S is the spiked concentration. The linearity was tested using matrix match and solvent standards based on the calibration curves constructed from standard solutions of aflatoxins B1, B2, G1 and G2 with a concentration ranging from 0.1 – 50 μ g/mL of aflatoxin. Daily repeatability was tested by estimating the level of precision and the confidence interval of the mean value at 95% (EC, 2010).

G) Calibration curves for aflatoxins

External standard method was done constructing a calibration curve for aflatoxin B1, B2, G1 and G2, with concentrations range from 0.5–10 ng/g for aflatoxins B2 and G2; and 2–40 ng/g for aflatoxins B1 and G1. Total aflatoxins in the samples was determined by doing summation of aflatoxin G1, G2, B2, and B1 measurements per sample (Np & Medhe, 2003). The analysis methods' performance was tested by plotting different calibration curves for the external standards data measured for Aflatoxin B1, B2, G1 and G2 in the samples. The plotted linear curve obeyed the linear equation y= mx+c. The terms represented y as the response signal, m the gradient of the curve, c constant and y-intercept.

Sample analysis and quantification of aflatoxin.

Analysis of sample extracts for aflatoxin B1, B2, G1 and G2 was done using high performance liquid chromatography (Shimadzu model 10AVP) equipped with a fluorescence detector. This was by injecting 10 μ L of the sample and eluting with acetonitrile/methanol/water (15/30/70 v/v/v) mobile phase in isocratic mode. Analyte separation was done on a genesis reverse-phase C18 analytical column (4.6 × 250 mm, 100 Å, and 5 μ m particle size; Gloucester, UK) at 40 °C using potassium bromide and nitric acid as mobile phase at a flow rate of 0.9 mL min⁻¹. The AFB1 and AFG1 fluorescence activities were enhanced by fitting a UV lamp to a cell consisting of 254 nm and 0.5 mm i.d. x 10 m PTFE tube before the fluorescence detector. The detection of aflatoxin fluorescence was done by operating the detector at 360 and 450 nm wavelengths excitation and emission stages respectively. Aflatoxin identification and quantification was done by comparing retention time and peak area with reference standards. Microsoft excel version 21 and Statistical Packages for Social Sciences (SPSS) version 20 were used in analyzing to the data collected.

3. RESULTS AND DISCUSSION

The limit of detection (LOD) for the method used to analyze aflatoxin was 0.49 μ g/kg for Aflatoxin B1, 0.36 μ g/kg for B2, 0. 39 μ g/kg for G1 and 0.45 μ g/kg for G2. Similarly, it's limit of

quantification (LOQ) for the same was 1.16 μ g/kg for Aflatoxin B1, 1.34 μ g/kg for B2, 1.48 μ g/kg for G1 and 1.10 μ g/kg for G2. The four strains of aflatoxins B1, G1, B2 and G2 had a correlation coefficient (R²) above 0.98 for all calibration curves. The range of recoveries for the spiked maize blanks was 70.59-79.46% for aflatoxin B1, 73.46-85.53% for aflatoxin B2, 76.71-83.80% for aflatoxin G1 and 80.75-100.63% for aflatoxin G2. Table 1 is a summary of validation data for the method used to analyze the maize samples.

| Aflatoxins | Retention Time | Correlation coefficient (R ²) | Recoveries | Accuracy | Precision | RSTD | LOD | DOJ |
|------------|---------------------------------|---|--------------|----------|-----------|-------|---------|---------|
| | (min) | | % | % | % | % | (µg/kg) | (µg/kg) |
| B1 | 4.3 | 0.9871 | 70.59-79.46 | 79.46 | 5.61 | 7.06 | 0.49 | 1.16 |
| B2 | 4.15 | 0.9884 | 73.46-85.53 | 73.46 | 9.12 | 12.42 | 0.36 | 1.34 |
| G1 | 4.18 | 0.9914 | 76.71-83.80 | 80.11 | 5.24 | 6.54 | 0. 39 | 1.48 |
| G2 | 4.03 | 0.9809 | 80.75-100.63 | 80.75 | 8.67 | 10.73 | 0.45 | 1.1 |

Table 1. Summary of validation data for the analysis method

Occurrence and prevalence of Aflatoxin

Kajiado County: The mean total aflatoxin contamination in the 49 samples ranged from <0.36 -22.13 µg/kg. The order of individual aflatoxin contamination in the sampled maize was AFB1>AFG1>AFB2 >AFG2. The mean for individual Aflatoxin strain in respect to the store type was for AFB1: 8.50±0.88 µg/kg for NCPB store, 5.55±0.30 µg/kg for retailers' stores, 4.01±0.41 µg/kg for wholesalers' stores and 2.99±00 µg/kg for farmers stores. In the case of AFB2 the mean contamination measured in the sampled maize was: $0.84\pm0.00 \ \mu g/kg$ for farmers' stores, 1.84±0.01 µg/kg for wholesalers' stores, 2.27±0.00 µg/kg for retailers' stores, and 4.34±0.86 µg/kg for NCPB stores. The mean contamination by AFG1 in the sampled maize was: 5.36±0.34 µg/kg for NCPB store, 4.53±0.51 µg/kg for retailers' stores, 3.03±0.52 µg/kg for wholesalers' stores and 2.55±0.95 µg/kg for farmers' stores. Lastly the mean contamination of sampled maize with AFG2 was: 3.93±0.57 µg/kg for NCPB store, 1.92±0.03 µg/kg for retailers' stores, 1.72±0.21 µg/kg for wholesalers' stores and 1.08±0.19 µg/kg for farmers' stores. Sixty-seven percent of the maize sampled from NCPB stores in Kajiado County had aflatoxin B1 contamination, 37% of the samples from retailers' stores, 32% of those from wholesalers' stores and 45% of the samples from farmers' stores. Fifty percent of all maize sampled from this county were contaminated with aflatoxin B1 above the accepted limit of 4 μ g/kg. The same percentage of samples were contaminated with other strains of aflatoxin B2, G1 and G2. Twenty percent of maize sampled from Kajiado county stores had total aflatoxin contamination above the mean of 22.3 ug/kg.

Eighty percent of samples were below the mean, cumulatively 38% of the samples were contaminated above the accepted limit of 10 μ g/kg for human food (Figure 3).

The National cereals and produce board, wholesaler and retailer stores in Kajiado County buy maize directly from farmers and sometimes from middlemen who source it from any part of the country and even import from other countries. Maize traders at the counties have different postharvest management skills, holding facilities and transportation modalities. The variation might have contributed to the observed aflatoxin contamination trend in the stores. It was observed that maize sampled from farmers stores directly had lower occurrence of aflatoxin contaminations. The reason for the observation probably could be the maize was produced in small scale mostly for household consumption and had fewer contacts with different handlers. Retail stores stocked maize from different small-scale farmers who exchange maize for their basic needs, but the buyers at this level lacked testing capacity for moisture content and aflatoxins contamination level. If any aflatoxin contaminants were in the maize batch, it would be passed to the stock already in the stores. Wholesale and NCPB stores management are endowed with better capacity to control required maize quality in terms of mixture content, storage conditions and type of carrier bags used. Diversities of environmental condition at the farm level, farmers' practice, transportation modalities and nature of maize holding facilities prior to delivery, presented a loophole for contaminating with aflatoxin molds. If these contaminating molds are in delivered maize batch and find favorable condition in the store, they develop fully to the measured aflatoxins contamination.

Trans-Nzoia County: The mean total aflatoxin contamination in 49 samples ranged from <0.36 -63.79 µg/kg. The individual strain of aflatoxin had different contamination levels in samples from this county. Eighty-two percent of the maize sampled from farmers' stores in Trans Nzoia County had aflatoxin B1 contamination with a mean of $35.54\pm4.72 \ \mu g/kg$, 60% for those from retailers' stores with a mean of 15.48 \pm 0.38 µg/kg, 23% for sample from NCPB store with a mean of 9.37 \pm 0.81 µg/kg and 9% for samples from wholesalers' stores with a mean of 6.36 ± 0.87 µg/kg. Similar percentages were observed for Aflatoxin B2, G1, and G2 as those B1 but mean varied for the sampled maize from stores. The observed mean aflatoxin contamination for AFB2 was farmers' stores 13.98 ± 1.66 μ g/kg, 6.64 ± 0.69 μ g/kg for NCPB stores, 5.06 ± 0.98 μ g/kg for wholesalers' stores, and 3.88±0.36 µg/kg for retailers' stores. The mean for aflatoxin G1 contamination in the maize sampled was farmers' stores 16.17 ± 1.84 µg/kg, NCPB stores 8.74 ± 0.74 µg/kg, wholesalers' stores 5.81 ± 1.23 µg/kg, and retailers' stores 4.57 ± 0.58 µg/kg. The observed mean aflatoxin G2contamination in stores was: NCPB stores 5.02±0.49 µg/kg, 4.65 ± 0.73 µg/kg for farmers' stores, 3.53 ± 0.04 µg/kg for wholesalers' stores and 2.95 ± 0.03 µg/kg for retailers' stores. Sixty-five percent of maize sampled from Trans Nzoia County stores were AFB1 contaminated above the accepted limit of 4 µg/kg. Thirty-seven percent of the sampled maize in this county had total aflatoxin contamination above mean 63.79 μ g/kg while 63% of the sample

were below. In total 61% of the maize samples had total aflatoxin contamination above the accepted level of 10 μ g/kg (Figure 3).

Trans Nzoia County is known in Kenya as the grain basket because it produces most of the maize consumed in the country. This implies that the demand for maize is high, to maintain the supply for the cereal, farmers grow maize in large scale. When they harvest the cereal, it is stored in the same stores are used year in year out without fumigation, sometimes same carrier bags are used for storage and disposing the cereal to the market. These practices could probably be the reason for the observed aflatoxin contamination trend. Our study observed high levels of aflatoxin B1 contamination in maize from farmers' stores compared to the maize from the retailers, NCPB and Wholesalers who appeared to control the quality of maize they stocked.

Nakuru County: The mean total aflatoxin contamination in 49 samples ranged from <0.36 - 3.78 µg/kg in Nakuru stores. Maize sampled from stores in Nakuru county had the least contamination levels. Contamination of maize samples with AFB1 was found in all stores but varied in the amount of contamination. It was observed that ninety percent of maize sampled from NCPB stores were contaminated with a mean of 0.02 μ g/kg, 80 % of those from wholesalers' stores were contaminated with a mean of $1.84\pm0.74 \,\mu g/kg$, 67% of samples from retailers' stores were contaminated with a mean of 2.25±0.17 µg/kg and 42% of those from farmers' stores were contaminated with a mean of $2.49\pm0.65 \,\mu$ g/kg. The contamination levels for the other strains AFB2, AFG1 and AFG2 was found to be lower both in proportion and quantity. In specific AFB2 contaminated 32% of samples from farmers' stores with a mean of $0.83\pm0.00 \mu g/kg$, 52% of the samples from wholesalers' stores with a mean of 0.16 \pm 0.03 µg/kg, 70% of maize samples from retailers' stores with a mean of $0.02\pm0.00 \ \mu\text{g/kg}$, and 95% for the samples from CPB stores with of less than 0.02 µg/kg. Eighty-two percent of the maize samples from NCPB stores were contaminated with AFG1 with a mean below 0.02 μ g/kg, 79% of samples from retailers' stores with a mean of $1.17\pm0.36 \ \mu g/kg$, 73 % of samples from wholesalers' stores with a mean of 0.03±0.00 μg/kg and 20% of maize samples from farmers' stores with a mean of 0.46±0.00 μg/kg. Aflatoxin G2 contaminated 62% of maize samples from NCPB stores with a mean of less than 0.02 μ g/kg, 40% of the maize sampled at retailers' stores with a mean of 0.15±0.00 μ g/kg, 25% of the samples from wholesalers stores with a mean of 0.82±0.00 µg/kg and 72% of samples from farmer's stores with a mean of less than 0.02 μ g/kg. One hundred percent of the samples from Nakuru County had aflatoxin contamination levels below 4 µg/kg for aflatoxin B1 and also 10 μ g/kg for total aflatoxins that was 5.02 μ g/kg (Figure 3).

Maize sampled from different store in Nakuru County recorded low Aflatoxin contamination levels. The maize chain players in the county probably observed the required good management practices in handling maize at all levels from farmers, to retailers to wholesalers to NCPB. There

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could also be possibility that the strains of aflatoxins producing fungi are not favored by the climatic and environmental conditions in Nakuru County.

Figure 3. The mean concentration of Aflatoxins in maize samples from Kajiado, Nakuru and Trans Nzoia counties.

Sampled maize from wholesalers' stores had a lower aflatoxin contamination level when compared to the retailers' stores samples. Although the farmers, wholesalers and retailers supply NCPB stores with maize, it was observed that samples from NCPB stores had a lower aflatoxin contamination level overall. Some the reasons for the observation that maize supplied to these stores must meet certain quality requirements like moisture content level of 13% and must be in sisal bags. Another reason that the technical staff is trained in cereals management skilled. Cumulatively, maize sampled from different stores in Trans-Nzoia, Kajiado and Nakuru counties were contaminated with total aflatoxin with a mean of 63.79 ug/kg, 22.3 ug/kg, and 5.02ug/kg respectively. Higher levels of total aflatoxin contamination in sampled maize from Trans-Nzoia and Kajiado stores suggest a possibility of maize consumers being exposed to aflatoxin risks whose consequences are chronic illness. The frequency of contamination for stored maize by

AFB1 was 89.93%. The order of occurrence was retailers' stores>farmers 'stores> wholesalers' stores > NCPB stores. The reason for the observation could be post-harvest management practices which is in agreement with previous studies. The studies focused on farmers' practices, where they concluded that certain farm practices exacerbate aflatoxin contamination in stored cereals (Kang'ethe et al., 2017; Koskei et al., 2020; Probst et al., 2012; Sirma et al., 2016). The dangers associated with aflatoxin occurrence and contamination are further complicated by effects of climate change and variability that make maize production, management and processing expensive. Maize farmers are required to maintain the moisture content of stored maize below 13%, which is difficult for illiterate and semiliterate farmers. In addition, other factors such as cropping systems and microbial profiles in the soil, storage facilities and processing practices, transportation, and packaging conditions may contribute aflatoxin contamination on maize grains. The concentrations of aflatoxin reported in this study were comparable to those reported in Uganda (Echodu et al., 2019), Nigeria (Ifie et al., 2022), Philippines (Benkerroum, 2020b), Vietnam (Do et al., 2020), Brazil (Oliveira et al., 2017), Central and Southern Europe (Rodrigues & Naehrer, 2012), South Africa (Rodrigues et al., 2011), and Oceania (Rodrigues & Naehrer, 2012). However, the levels were lower than those reported in Tanzania (Boni et al., 2021), India (Mohana et al., 2017), Nepal (Joshi et al., 2022), Ghana (Agbetiameh et al., 2018), Latin America (Odjo et al., 2022), South and North America (Rodrigues & Naehrer, 2012), South East, South and West Asia (Rodrigues & Naehrer, 2012). Table 3 gives a summary of previous reports from different studies on occurrence of aflatoxin in maize samples from selected countries in Africa, Asia, America, Europe and Oceania.

| Country | No. of | Aflatoxin | Contaminated | Mean ug/kg | Range ug/kg |
|-----------------|------------------|--------------|--------------|---------------|-------------|
| | samples | type | sample (%) | | |
| Uganda | 105 | Total | 40 | 20.4 | 25.4–75.2 |
| Tanzania | 200 | Total | 49.5 | 158 | 12.1-158.8 |
| Ghana | 326 | Total | 35 | 11 | BDL-341 |
| Nigeria | 120 | Total | 80 | | 12.0 -31 |
| India | | Aflatoxin B1 | 88.2-100 | | 200.5 - 714 |
| Philippines | 1215 | Total | 95 | | 39–76 |
| Nepal | 500 | Total | 78 | 23.04 ± 27.58 | 1.52–91.24 |
| Vietnam | 1572 | Total | 32.5 | | 2.62–66.1 |
| Latin America | 6943 | Total | 23 | | 8.0–1336 |
| Brazil | 148 | Total | 41.6 | | 16.7 -49.9 |
| North America | <mark>375</mark> | Total | 26 | 67 | 2.6-920 |
| South America | 809 | Total | 25 | 7 | 1.0-273 |
| Central Europe | 16 | Total | 31 | 2 | 1.4-3 |
| South Europe | 42 | Total | 36 | 9 | 1.6-44 |
| North Asia | 446 | Total | 12 | 114 | 2.0-4,687 |
| South East Asia | 330 | Total | 71 | 146 | 11.0-6,106 |
| South Asia | 108 | Total | 82 | 240 | 13.0-2,230 |
| South Africa | 77 | Total | 8 | 0.4 | BDL-10.0 |
| Oceania | 11 | Total | 18 | 3 | 2.0-5.0 |

Table 2: Occurrence of aflatoxin in maize from selected countries of Africa, Asia, America, Europe and Oceania

4. CONCLUSION

The occurrence of aflatoxin contamination in maize sampled from stores in Rift valley, Kenya was 89.93%. Aflatoxin occurrence across the counties varied with those in Kajiado recording 67% in the NCPB store, 52% in the Farmers' in Trans-Nzoia and 42% in Farmers' store in Nakuru. Aflatoxin B1 contamination was the most prevalent and dominant in the maize samples than the other aflatoxin strains G1, B2, and G2. Ninety-one percent of maize samples tested were found to be aflatoxin contaminated with 58.5% aflatoxin B1, above the limit of 4 μ g/kg for human food.

Recommendation

Regulatory authorities in the counties should monitor aflatoxin levels regularly in stored maize grains, and ensure strict adherence by maize chain handler to the set guideline limits for aflatoxins contamination levels. A further study is required to determine why Nakuru County

had a variation in occurance and prevalence of aflatoxin in stored maize and if climatic factors modulate the growth of *Aspergillus flavus* and *A. parasiticus* and their colonization of cereals grown in Nakuru county.

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