# International Journal of Agriculture (IJA)

OCCURRENCE AND DISTRIBUTION OF AFLATOXIN IN MAIZE FROM SELECTED COUNTIES, EASTERN REGION, KENYA

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## Occurrence and Distribution of Aflatoxin in Maize from Selected Counties, Eastern Region, Kenya

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#### Abstract

**Purpose:** The study aimed to assess the occurrence and distribution of aflatoxin contamination on dry maize in different types of stores in Meru, Embu, Isiolo, Makueni and Machakos Counties of Eastern region of Kenya.

**Methodology:** Automatic spear sampler was used to collect maize samples from each bag at even intervals. 280 maize samples were collected from 29 stores in five Counties. 100 g of each maize sample was ground, resampled into 50g, blended, extracted, centrifuged, filtered and a quantified for Aflatoxin B1, B2, G1 and G2. Samples were prepared and extracted with methanol/water. The bulky of the samples were analyzed with enzyme-linked immunoassay test kits. Confirmation of positive samples was done with high performance liquid chromatography (HPLC) coupled with fluorescence detector. Data analysis was done with SPSS and Microsoft excel.

**Findings:** Maize samples from Counties in eastern region of Kenya had significantly high levels of (93.10%) aflatoxin contamination. The mean values for aflatoxin  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  were:  $50.08\pm4.42$ ,  $17.26\pm1.08$ ,  $30.17\pm2.06$  and  $10.54\pm1.52$  (ng/g) in that order. Only nine samples had total aflatoxin within the accepted limit for human consumption of 15 ng/g. The highest total aflatoxin contamination recorded was 198.45ng/g in Makueni county and the lowest recorded was 8.76ng/g in Embu county. Makueni and Embu had mean values for aflatoxin  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  being  $(83.07\pm7.53,\ 22.15\pm1.36,\ 49.38\pm3.11,\ 20.52\pm0.70\ ng/g)$  and  $(18.71\pm2.63,\ 8.07\pm0.64,\ 17.02\pm1.38,\ 8.86\pm1.62\ ng/g)$ . Makueni NCPB depot had the highest mean contamination with aflatoxin  $B_1$  of  $92.67\pm5.78$  ng/g and Embu had the lowest with  $6.26\pm4.14$  ng/g. All the county markets recorded high aflatoxin  $B_1$  contamination with exception of Embu county which had a mean of  $4.0\pm0.84$ , Makueni  $(83.67\pm10.42\ ng/g)$ , Isiolo  $(51.27\pm32.29\ ng/g)$ , Meru  $(46.02\pm23.88\ ng/g)$  and Machakos  $(36.34\pm26.27\ ng/g)$ . The stores had aflatoxin load varied from on store to the other and county to county.



Unique contribution to theory, policy and practice: The counties in the region had high occurrence and distribution of aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in maize in all stores where samples were picked. Location for maize stores should be in areas with low levels of carbon dioxide because mycotoxins are produced under aerobic conditions. The design for maize threshing machines should not course shocks, breakage and cracks on maize grains to decrease chances of mycotoxins infestation during their storage.

**Key words:** Aflatoxin, distribution, maize, occurrence, stores.

#### 1.0 INTRODUCTION

Kenya is among tropical countries with conducive climatic and environmental conditions favorable for fungal growth. The result has been frequent episodes of mycotoxicosis outbreaks and attacks on cereal grains (Eze & Okonofua, 2015). Among the mycotoxins communities that attack foods crops are aflatoxin, fumonisin, deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZON) (Alexa *et al.*,2013). Aflatoxin and fumonisins contaminate maize and maize products, affecting staple food for majority of Kenyan. They cause a significant challenge to food safety and security. Food safety, because aflatoxin exposure to humans, is associated with weakening of the immune system and different health ailments like cancers (Kitya et al., 2013). Since 1960s, chronic incidences of aflatoxicosis outbreaks have been reported with over 200 deaths and 500 ailments, from among the Makueni, Meru, Isiolo, Embu, Tharaka Nithi, Machakos and Kitui counties (Owuor *et al* 2020; Kang'ethe *et al.*, 2017: Gong *et al.*, 2012). Occurrence of these incidences have been mostly in rural subsistence farming communities who consume homegrown maize (Daniel *et al.*, 2011; Azziz-Baumgartner *et al.*, 2005).

Hot and humid climatic conditions and sporadic droughts with high temperatures and humidity exceeding 28 °C and 79% respectively are experienced in greater parts of Kenya. These conditions have over time worsened because of climate change (Negash, 2018; Ongoma, 2013; Cotty & Jaime-Garcia, 2007). Extreme weather events such as floods, pests and diseases are some of the negative effects of climate change that farmers have faced in the recent years. Most farmers have adopted various adaptation measures to reduce the adverse effects of climate change on their agricultural out puts. However, they have not been able to cope fully with the mycotoxin effects on foods because of its complex nature. According to the Food and Agricultural Organization of the United Nations approximately 25% of the world's agricultural produce is lost to mycotoxins contamination (Okoth *et al.*, 2014). Infestation of agricultural products by toxigenic fungi is influenced by a complex interaction of factors. The factors include agro-ecological zones, variety of crop, altitudes, fungal populations, environmental conditions, insect infestation, relative humidity above 85% or 16% moisture content and pre-and post-harvest management (Xu *et al.*, 2018; Nyangi *et al.*, 2016; Reddy *et al.*, 2014).

Crop commodities grown in hot and humid weather of eastern Kenya, are frequently associated with fungal growth and toxin production. Previous reports on eastern Kenya, have reported presences of L- and S-strain morphotypes of A *flavus*, known to produce high levels of aflatoxin



(Milani, 2013). A wide-range of these toxins occur in cereal crops, oil seed crops, legumes, roots, tree nuts and other domesticated and non-domesticated plants (Olarte *et al.*, 2012).

Eastern Kenya is prone to food shortages linked to unreliable rainfall, high temperatures and drought (Wassmann *et al.*, 2009). A big portion of the population in the region depend on relief food for survival, from charitable organizations, county and national governments. Contamination of maize and other cereals with aflatoxin have increased food shortage burden. Ordinarily, contaminated maize or cereal is condemned as unfit for human consumption by the public health and should be destroyed. The reality is that not all contaminated cereals are destroyed because they are neither noticed by the producer nor consumer. Aflatoxin contaminations in some cases does not have physical notable change. In cases where the producers suspect contamination on a produce, it is often diluted with more of clean produce and sold to unsuspecting consumers or traders. Trend is warrying because the diluted maize or produce circulates with the same people. On the farmers' side a significant loss of food supply and market is experienced. The grassroot population is experiencing an increased disease burden that eat into their incomes because mycotoxin contamination on their diet. Moreover, aflatoxin contamination contribute to food insecurity, food safety and decreased livestock productivity.

Aflatoxin strains that contaminate foods are not known by farmers and many of the actors in maize chain. Safe post-harvest management is a challenge owing to chain actors' mycotoxin knowledge levels. The knowledge of the effects of aflatoxin and control exist with non-functional extensional systems in most counties. The regional occurrence and distribution of aflatoxins statistics vary with years and other underlying environmental factors. Other factors include maize breeds, farm conditions and changing climatic factors, and different agronomic practices (Wu *et al.*, 2010). This study aimed to assess the occurrence and distribution of aflatoxin contamination on dry maize collected from farm stores, NCPB depot, county markets and retail shops Meru, Isiolo, Embu, Makueni and Machakos in Eastern region of Kenya.

The cause of the high levels of aflatoxins in Eastern Kenya is not known, but it could be related to prevailing climatic conditions and different strains of *A. flavus* (Probst, Bandyopadhyay, & Cotty, 2014). High incidences of drought and high temperatures have been reported in Eastern Kenya (Mutunga, Ndungu, & Muendo, 2017) and these parameters have been shown to exert positive impact on establishment and proliferation of *A. flavus* and subsequent aflatoxin contamination (Mahuku *et al.*, 2019). Besides, the region has been reported to have high incidences of aflatoxin producing strain of S-morphotype of *A. flavus* (Probst *et al.*, 2014). Although these studies tend to link Climatic conditions and presence of different strains of *A. flavus* to high prevalence of Aflatoxin in Eastern region, there are still gaps on the distribution of the aflatoxin in the region. The region has variations of other factors like altitudes, rainfall distribution, land fertility, land preparation methods, and soil physical chemical properties which may exert positive impact on establishment and proliferation of fungi and aflatoxin contamination on foods.



#### 2.0 MATERIALS AND METHODS 2.1 Location of Study

Maize samples were collected from randomly sampled county stores in: Meru, Isiolo, Embu, Makueni and Machakos Counties. All the selected counties have had some past reports of acute aflatoxicosis. The region has a high daily maize consumption rate, estimated at 400 g per person (Kang'ethe *et al.*, 2017).

#### 2.2 Sampling

Maize samples were collected from National Cereal and Produce Board (NCPB) Depots, County market Stores, Retail stores and farmers store in Makueni, Machakos, Meru, Isiolo and Embu counties. The counties were selected based on the past record of previous aflatoxin out breaks (Mutegi *et al.*, 2018), microclimatic conditions (Gachara, *et al.*, 2018) and soil physicochemical characteristics (CDC, 2004). Within each county, NPCB depots were selected based on maize catchments areas, maize breed and harvesting seasons. The NCPB Depot represented optimal storage conditions for the grains, deemed unfavorable for growth of mycotoxins. On the other hand, farmers and small-scale maize traders from targeted markets represented wider setting with no uniform and optimal conditions, hence maize harvested in some cases had high moisture content that could promote proliferation of mycotoxins. In addition, farmers had different post-harvest handling methods which included piling in the farms, mechanical shelling, and storage in containers, hanging and cob-stacking in stores, among others. Figure 1 show the sampling sites.

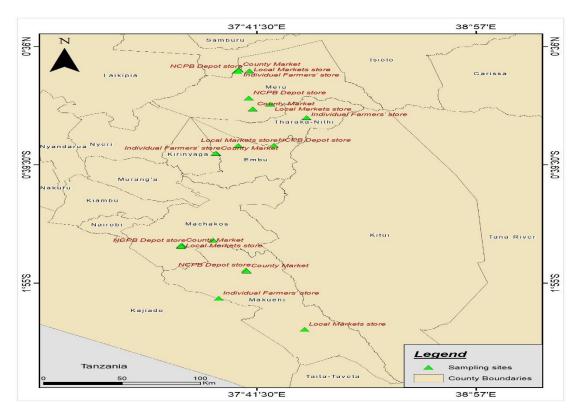




Figure 1: The map of the study area: Isiolo, Embu, Meru, Makueni and Machakos

#### 2.3 Maize Sample Size Selection

The study sample (N=280) comprised of maize from farmer stores, NCPB depots and traders in Makueni, Machakos, Meru, Isiolo and Embu. The minimum sample size (n), was determined using formula:  $n > z^2 \times p \times q / d^2$ 

Where n 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 <math>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% (Lauren *et al.*, 2004; CDC, 2004), and was used to determine the maize sample size. Factoring in the value of q = (1 - p), as 0.907, and p = 0.093, then  $n = (1.96)^2 (0.093) (0.907) / (0.05)^2 = 129.61$ . The minimum maize sample size was approximated to be 130 samples of a composite weight of 1 kg each. The study however picked 280 samples of 1kg each for better representation of the region.

Automatic spear sampler was used to collect maize samples from each bag at even intervals. The samples were placed in self- sealing bags and coded. Silica gel packs were added into the sample bags, to reduce moisture content and stop fungal growth. In the field the samples were held at ambient temperature for a maximum of 72 hours before they were transported to the laboratory for storage at -20 °C until analysis. During analysis, each sample was divided into triplicates for physical- chemical analysis and aflatoxin quantification.

#### 2.4 Maize Sample Preparation

Each maize sample was accurately weighed into 100 g and carefully grounded into 20 mesh size flour with a laboratory scale hammer mill. The flour was then reweighed into two 50 g for aflatoxin extraction. Each of sample was blended for 30 minutes at 120 RPM in the extraction solution (thus 150 mL of 80 ml methanol and 20 ml deionised water, 50 mL of Acetonitrile, 1 g NaCl and 4 g of anhydrous magnesium sulphate) and passed through a filter paper. Twenty (20) mL of the filtrate aqueous layer was diluted into 4:1 with Phosphate Buffer Solution (PBS) of pH=7. 2. The diluent was centrifuged at 3400 revolutions per minute for 15 minutes, then filtered through nylon membrane filter (pore size, 0.45  $\mu$ m). About 10 mL of the filtrate was applied onto the immunoaffinity column and eluted with 2 mL of methanol at a flow rate of 1 mL/min, by gravity. The eluate was incubated at 50 °C and the solvent evaporated using nitrogen gas. It was reconstituted with 200  $\mu$ l trifluoroacetic acid and 800  $\mu$ l methanol- water. From the reconstituted solution 20  $\mu$ l was injected into the HPLC for quantification of Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>.

#### 2.5 Sample Analysis

Aflatoxin quantities of standards and samples were determined using HPLC (Shimadzu, Kyoto, Japan) with a fluorescent detection. The analytical column used was a Symmetry® C-18 3.9x150 mm with 5 μm particle size with a guard column Sil-filter STD C-18 3.0 x10 mm. The fluorescence detection (FLD) was set at an excitation wavelength 360 nm and 440 nm for emission. The column was maintained at 40 °C temperature. Analysis was run at a flow rate of 1 mL/min by an isocratic



mobile phase using a mixture of acetonitrile/methanol/water (15/30/70 v/v/v). An aliquot of a  $10 \mu l$  sample extract was injected into the chromatographic system. Individual aflatoxin retention and total run times in minutes were; AFG<sub>2</sub> 8–9, AFG<sub>1</sub> 10.5–11.5, AFB<sub>2</sub> 13–14 and AFB<sub>2</sub> 16–17 respectively.

#### 2.5 Calibration Curve

An external standard curve was constructed using aflatoxin standards to quantify the aflatoxins concentrations in all the samples. A stock solution was prepared containing 100 ng/mL. The stock solution was serially diluted into seven different concentrations using methanol/water (4:6, v/v), according to AOAC reference method 994.08. The method was validated according to SANCO/12571/2013 which demonstrate the conformity of the analytical performances with criteria established in the European Commission (EC) regulation no. 178/2010 (EC, 2010). This provide guidelines for validation procedure for linearity, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision.

The linearity was tested by external standardization using matrix calibration curve. It was constructed from AFB<sub>1</sub> standard solutions of six different concentrations within the range of 5100 ng/mL (1,5, 10, 30, 50, and 100 ng/g). Analytical curves were established by plotting the peak areas, which were used as the analytical signal response (y) versus the concentration of AFB<sub>1</sub> (x) (Figure 2).

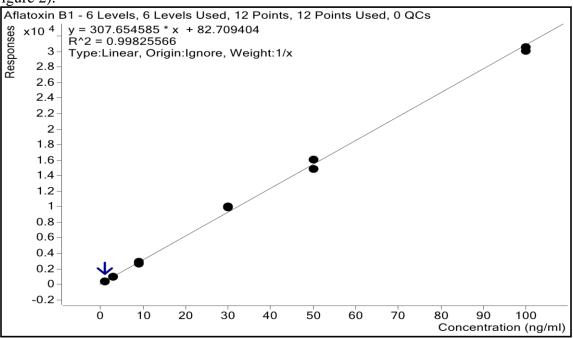


Figure 2: Calibration curve for Aflatoxin B1



#### 2.6 Calculations

The peak area of the aflatoxin standards was plotted against concentration. From the plotted curve the slope (S) was determined as well as the Y-intercept (a). The level of aflatoxins in the sample was calculated using the formula: **Aflatoxin**,  $\mu g/kg = \{(R - a)/S\} \times V/W\} \times F$ .

Where R represent the test solution peak area, V the final volume (mL) of the injected test solution, F the dilution factor and W amount in grams of test sample passed through the immune-affinity column. The total aflatoxin is the sum of the Aflatoxin  $B_1$ ,  $G_1$ ,  $B_2$ , and  $G_2$ .

#### 2.7 Quality Control and Quality Assurance

Aflatoxin standard solution was prepared from Sigma (Germany) with purity of 98%. Standard stock solutions were prepared in acetonitrile according to the modified AOAC method 994.08. Solvents used for the experiments (methanol, acetonitrile and deionized water) were HPLC grade.

Aflatest immunoaffinity columns (IAC) and HPLC column (C<sub>18</sub>) were obtain for the analysis and quantification of sample items.

#### 2.8 Data Analysis

The data was analysed using different programs that included SPSS software and Microsoft Excel Variances (ANOVA) and Fisher's LSD test (p<0.05) to compare significant differences. The data was analysed using mean and standard deviation, confidence, and presented using bar graphs.

## 3.0 RESULTS AND PRESENTATION 3.1 Analysis of Aflatoxin in Maize grains from Eastern region of Kenya

A total of 280 samples were collected from the 5 counties in eastern region of Kenya. Out of the maize samples collected, 93.1% had total aflatoxin load above the maximum guideline limit of  $(15 \, \text{ng/g})$  for human consumption. The highest and the lowest total aflatoxin contamination recorded per county was: Makueni  $(198.45 \, \text{ng/g})$ ,  $136.78 \, \text{ng/g}$ ; Embu  $(192.85 \, \text{ng/g})$ ,  $8.76 \, \text{ng/g}$ , Isiolo  $(188.63 \, \text{ng/g}, 76.13 \, \text{ng/g})$ , Machakos  $(107.91 \, \text{ng/g})$ ,  $86.69 \, \text{ng/g})$  and Meru  $(106.38 \, \text{ng/g}, 81.96 \, \text{ng/g})$ . The study generally calculated central tendencies for the region to figure out the distribution of the aflatoxin contamination. The tendencies were; the mode 86, the median  $104.32 \, \text{and}$  the mean  $108.04 \, \pm 9.08$ .

## 3.2 Analysis and quantification of aflatoxin contamination in maize samples from the five Counties of Eastern Kenya.

Samples were randomly collected from different stores. They included NCPB depots, county markets, retailers and farmers owned across the five counties. They were analyzed and quantified for levels of aflatoxin  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  in terms of mean and standard deviation. The mean aflatoxin  $B_1$  contamination in the stores in Isiolo county were in the order farmers (82.33±2.95 ng/g), retail (60.51±8.48 ng/g), county market (51.27±3.28 ng/g) and NCPB depot (46.23±2.93 ng/g). The mean aflatoxin  $B_1$  contamination in the stores in Meru county were; NCPB depot (55.08±2.91 ng/g), county market (46.02±3.87 ng/g), farmers (41.13±2.15 ng/g) and retail



 $(36.90\pm2.25 \text{ ng/g})$ . The mean levels of  $B_1$  in the stores in Makueni county were; NCPB depot  $(92.66\pm5.78 \text{ ng/g})$ , county market  $(83.67\pm10.41 \text{ ng/g})$ , retail  $(82.91\pm10.56 \text{ ng/g})$  and farmers  $(73.02\pm3.37 \text{ ng/g})$ . For Machakos county stores, the mean aflatoxin  $B_1$  contamination were; Retail  $(54.81\pm1.44 \text{ ng/g})$ , farmers  $(45.62\pm8.41 \text{ ng/g})$ , NCPB depot  $(38.27\pm2.75 \text{ ng/g})$  and county market  $(36.34\pm6.26 \text{ ng/g})$  in that order. The mean aflatoxin  $B_1$  contamination for Embu county was; farmers  $(40.24\pm5.54 \text{ ng/g})$ , retail  $(24.31\pm3.96 \text{ ng/g})$ , NCPB depot  $(6.26\pm0.14 \text{ ng/g})$  and county market  $(4.02\pm0.84 \text{ ng/g})$  respectively.

The mean aflatoxin B<sub>2</sub> contamination was relatively low in the five counties. The stores in Isiolo county had aflatoxin B<sub>2</sub> contamination; NCPB depot (19.49±1.40 ng/g), Retail (12.33±0.56 ng/g), county market (14.64±1.19 ng/g) and farmers (16.86±0.63 ng/g). There results for stores in Meru county had aflatoxin B<sub>2</sub> contamination; Retail (30.21±1.92 ng/g), county market (23.96±2.87 ng/g), farmers (26.01±0.48 ng/g) and NCPB depot (20.16±1.23 ng/g). The results for stores in Embu county had aflatoxin B<sub>2</sub> contamination; farmers (15.53±0.05 ng/g), Retail (13.64±1.93 ng/g), NCPB depot (2.16±0.06 ng/g) and county market (0.95±0.00 ng/g). The results for stores in Makueni county had aflatoxin B<sub>2</sub> contamination; farmers (26.33±1.79 ng/g), Retail (22.23±1.47 ng/g), NCPB depot (20.88±1.15 ng/g) and county market (19.13±1.02 ng/g). For Machakos county stores, the mean aflatoxin B<sub>2</sub> contamination was; farmers (23.33±1.32 ng/g), county market (15.15±0.22 ng/g), retail (12.08±0.63 ng/g) and NCPB depot (9.60±0.79 ng/g) in that order.

The mean aflatoxin  $G_1$  contamination was second highest after aflatoxin  $B_1$  in the five counties. The stores in Isiolo county had aflatoxin  $G_1$  contamination; county market  $(36.25\pm2.41 \text{ ng/g})$ , Retail  $(32.68\pm1.49 \text{ ng/g})$ , NCPB depot  $(26.63\pm1.95 \text{ ng/g})$  and farmers  $(14.71\pm1.45 \text{ ng/g})$ . The results for stores in Meru county had aflatoxin  $G_1$  contamination; retail  $(25.63\pm1.39 \text{ ng/g})$ , county market  $(23.48\pm1.47 \text{ ng/g})$ , farmers  $(22.93\pm1.82\text{ng/g})$  and NCPB depot  $(22.58\pm1.83\text{ng/g})$ . There results for stores in Embu county had aflatoxin  $G_1$  contamination; farmers  $(38.50\pm3.34 \text{ ng/g})$ , Retail  $(20.96\pm1.13 \text{ ng/g})$ , NCPB depot  $(5.19\pm0.56 \text{ ng/g})$  and county market  $(3.41\pm0.49 \text{ ng/g})$ . There results for stores in Makueni county had aflatoxin  $G_1$  contamination; retail  $(53.33\pm1.70 \text{ ng/g})$ , NCPB depot  $(52.12\pm1.25 \text{ ng/g})$  s, county market  $(45.27\pm6.73 \text{ ng/g})$  and farmer  $(46.81\pm2.73 \text{ ng/g})$ . For Machakos county stores, the mean aflatoxin  $G_1$  contamination was; NCPB depot  $(42.62\pm3.55 \text{ ng/g})$ , county market  $(38.22\pm3.16 \text{ ng/g})$ , farmers  $(26.38\pm1.59 \text{ ng/g})$  and retail  $(25.76\pm1.11 \text{ ng/g})$  in that order.

The mean aflatoxin G<sub>2</sub> contamination was relatively low in the five counties. The stores in Isiolo county had aflatoxin G<sub>2</sub> contamination was; retail (12.69±1.77 ng/g), county market (10.95±0.58 ng/g), NCPB depot (10.05±0.20 ng/g) and farmers (5.11±0.11 ng/g). The results for stores in Meru county had aflatoxin G<sub>2</sub> contamination; farmers (8.59±0.96 ng/g), retail (6.38±0.99 ng/g), county market (5.34±0.64 ng/g), and NCPB depot (4.32±0.91 ng/g). The results for stores in Embu county had aflatoxin G<sub>2</sub> contamination; farmers (25.65±4.72 ng/g), retail (4.86±0.52 ng/g), NCPB depot (3.42±0.45 ng/g) and county market (1.51±0.76 ng/g). The results for stores in Makueni county had aflatoxin G<sub>2</sub> contamination; county market (22.67±4.76 ng/g), farmers (21.41±2.93 ng/g), NCPB depot (20.87±1.19 ng/g) and retail (17.15±5.91 ng/g). For Machakos county stores, the mean aflatoxin G<sub>2</sub> contamination was; county market (9.94±0.08 ng/g), NCPB depot



(9.37±1.91ng/g), farmers (5.42±0.81 ng/g) and retail (5.16±0.11 ng/g) in that order. The mean aflatoxin strain contamination on maize samples collected from different stores in selected counties of eastern Kenya are shown in Figure 3.

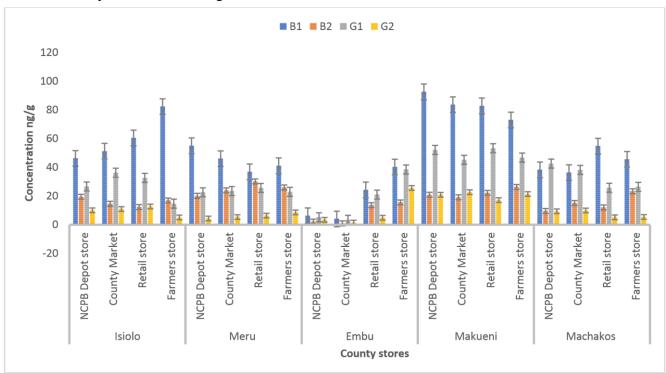


Figure 3. The mean aflatoxin strain contamination on maize samples collected from different stores in selected counties of eastern Kenya

#### 4.0 DISCUSSION, CONCLUSION AND RECOMMENDATION 4.1 Discussion

The total aflatoxin contamination levels were high in eastern region concurring with reports by Yard (2013), who reported that aflatoxin exposure varied from one region to another, with eastern recording the highest levels compared to the other regions. The high levels of aflatoxins in this region could probably be associated with the prevailing conditions which include; presence of different strains of *A. flavus*, climatic conditions, period of storage, and uncoordinated post-harvest management. These findings suggest that a majority of the maize farmers, harvested, shelled and stored the product in their stores and even sold locally through county markets, retail traders and also to NCPB stores.

Daniel *et al.* (2011) in their work reported aflatoxin contamination levels of 48,000 ppb in maize collected from a farmer's store in eastern region of Kenya. According to the study, high levels of aflatoxin contamination occur in any form of storage for maize, this suggests that toxigenic fungi may be either airborne or soilborne. Variations in occurrence of aflatoxin contamination observed



across the counties could be attributed to environmental conditions such as precipitation, relative humidity, temperature and drought. The high prevalence rate is connected with the warmer and dry tropical region microclimate (Hell *et al.*, 2003). In addition, variations in environmental factors may also lead to occurrence of illnesses and emergence of newer strains of diseases (Medina *et al.*, 2014), the respondents and concurred with this fact. Spatial climatic patterns have an impact on food contamination with direct influence on the Aspergillus flavus fungi growth (Cotty & Jaime-Garcia, 2007; Gnonlonfin *et al.*, 2013).

In this study aflatoxin B<sub>1</sub> was high in samples collected from all the sampled farmers' store in the five counties. The situation could be linked to regionals soils, air or the stores hosting toxigenic fungi poles, which sprout to colonize the maize at favourable conditions. In addition, invasion of crops by toxigenic blights may be aided by insect pests' vectors due to climatic changes (Zain, 2011; Paica, *et al.*,2013; Marechera and Ndwiga.,2014). A Few Farmers, fumigate their store before storing new crop. The farmers' livelihoods revolve round selling their farm produce, hence the reason for finding almost similar aflatoxin contamination in all store types (Figure 3). Maize contamination with mycotoxins has been linked to dormant toxigenic fungi poles in the soils, air and stores, which when conditions are favourable, they sprout and colonize the maize in the field and in stores.

Other studies elsewhere have linked aflatoxin development to oxidative stress that result from abiotic and biotic plant stressors (Ren *et al.*, 2020). The fungus Aspergillus flavus usually colonizes grain kernels mostly those that have had suffered stresses. The stress may be experienced during the crop's and development, mechanical injuries and breakages, insect pest damage and transportation shocks from the field to the store. Aflatoxin attack can be minimized through reducing the stressors to the crop (Kumar *et al.*, 2017). Aflatoxins are exceedingly resilient throughout food transportation chain and to food handling, processing and storage conditions.

The temperatures, moisture and humidity in the five counties were optimal, a critical condition for mycotoxin production. Moisture content levels of 25% at 30 °C, and relative humidity that fluctuates between 83 % and 88 % in eastern region favoured the production of aflatoxins in the maize kernels (Kaaya *et al.*, 2006; Houssou *et al.*, 2009). Most of the stores had high levels of carbon dioxide because they were in towns. Probably the reason for high levels of contamination in NCPB depots for they are in towns with high probability of carbon dioxide. There some unconfirmed suggestion that mycotoxins are produced more under aerobic conditions (Kang'ethe *et al.*, 2017; Yard *et al.*, 2013; Gnonlonfin *et al.*, 2013, Zain, 2011; Daniel *et al.*, 2011; Lewis *et al.*, 2005).

The findings show high frequency of aflatoxin incidences in all the four types of stores. This agree with findings from previous studies that most of the eastern region of Kenya is a high-risk area with high number of aflatoxicosis cases. Although these findings are true, they impact negatively for maize and maize of products from the region, in terms of market.



#### 4.2 Conclusion

This study showed that there was aflatoxin contamination occurrence distributed in all the store types where maize was sampled in eastern region of Kenya. The counties of Meru, Isiolo, Embu, Makueni and Machakos had all reported previously aflatoxicosis outbreaks. This study found aflatoxin B1, B2, G1, and G2 in all the maize samples analyzed. The levels of toxin varied from county to county and also from one store type to another.

#### 4.3 Recommendations

- i. Location for maize stores should be in areas with low levels of carbon dioxide because mycotoxins are produced under aerobic conditions.
- ii. The design for maize threshing machines should not course shocks, breakage and cracks on maize grains to decrease chances of mycotoxins infestation during their storage. iii. The government of Kenya through the ministries of agriculture, trade and industries should develop enough capacity to detect, quantify, monitor, and regulate aflatoxin in foods, in all store types at the county level.
- iv. More study is needed to determine real effect of chronic asymptomatic exposure to aflatoxins on human health in eastern region of Kenya.

#### REFERENCES

- Alexa, E., Dehelean, C. A., Poiana, M. A., Radulov, I., Cimpean, A. M., Bordean, D. M., Pop, G. (2013). The occurrence of mycotoxins in wheat from western Romania and histopathological impact as effect of feed intake. *Chemistry Central Journal*, 7(1).
- Azziz-Baumgartner E, Lindblade K, Gieseker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, DeCock K, (2005). Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ Health Perspect.;113:1779–1783.
- Centers for Disease Control and Prevention. Outbreak of aflatoxin poisoning eastern and central provinces, Kenya, January-July 2004. MMWR Morb Mortal Wkly Rep. 2004; 53:790–793.
- Cotty, P. J., & Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology*, 119(1–2), 109–115.
- Daniel J.H, Lewis LW, Redwood YA, Kieszak S, Breiman RF, Flanders WD, Bell C, Mwihia J, Ogana G, Likimani S, (2011). Comprehensive assessment of maize aflatoxin levels in Eastern Kenya, 2005–2007. Environ Health Perspect.119:1794–1799.
- Eze, U. A., and Okonofua, F. E. (2015). High prevalence of male infertility in Africa: Are mycotoxins to blame? *African Journal of Reproductive Health*, 19(3), 9–17.



- 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).
- George Marechera and Josphe Ndwiga (2014). Farmer perceptions of aflatoxin management strategies in lower Eastern Kenya. *Journal of Agricultural Extension and Rural Development*, 6(12), 382–392.
- Gnonlonfin, G. J. B., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D. O., Mensah, G. A., Brimer,
  L. (2013). A Review on Aflatoxin Contamination and Its Implications in the Developing
  World: A Sub-Saharan African Perspective. Critical Reviews in Food Science and
  Nutrition, 53(4), 349–365.
- Gong Y.Y., Wilson, S., Mwatha, J.K., Routledge, M.N., Castelino, J.M., Zhao, B., Kimani, G., Kariuki, H.C., Vennervald, B.J., Dunne, D.W. and Wild, C.P., 2012. Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. Environmental Health Perspectives 120: 893-896.
- Hell K, Cardwell K., Poehling H (2003). Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. J. Phytopath., 151: 690-698.
- Houssou, P. A., Ahohuendo, B. C., Fandohan, P., Kpodo, K., Hounhouigan, D. J., & Jakobsen, M. (2009). Natural infection of cowpea (Vigna unguiculata (L.) Walp.) by toxigenic fungi and mycotoxin contamination in Benin, West Africa. *Journal of Stored Products Research*, 45(1), 40–44.
- Kaaya, A. N., Kyamuhangire, W., & Kyamanywa, S. (2006). Factors affecting aflatoxin contamination of harvested maize in the three agroecological zones of Uganda. Journal of Applied Sciences, 6(11), 2401–2407.
- Kang'ethe, E. K., Gatwiri, M., Sirma, A. J., Ouko, E. O., Mburugu-Musoti, C. K., Kitala, P. M., ... & Joutsjoki, V. (2017). Exposure of Kenyan population to aflatoxins in foods with special reference to Nandi and Makueni counties. *Food Quality and Safety, 1*(2), 131-137. Kitya S., G. D., Lubega, A., Ogwal-Okeng, J., W., W., & B., D. (2013). Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. In *Aflatoxins Recent Advances and Future Prospects*.
- Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., & Kang, S. G. (2017, January 17). Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in Microbiology*. Frontiers Media S.A.
- Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., DeCock, K. and Rubin, C., 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environmental Health Perspectives 113: 1763-1767.



- Mahuku, G., Nzioki, H. S., Mutegi, C., Kanampiu, F., Narrod, C., & Makumbi, D. (2019). Preharvest management is a critical practice for minimizing aflatoxin contamination of maize. *Food Control*, *96*, 219–226. https://doi.org/10.1016/j.foodcont.2018.08.032
- Medina, A., Rodriguez, A., & Magan, N. (2014). Effect of climate change on Aspergillus flavus and aflatoxin B1 production. Frontiers in Microbiology. Frontiers Research Foundation.
- Milani, J. M. (2013). Ecological conditions affecting mycotoxin production in cereals: A review of Veterinarian Medicine 58(8): 405 411.
- Mutegi, C.K., Cotty, P.J. and Bandyopadhyay, R., 2018. Prevalence and mitigation of aflatoxins in Kenya (1960-to date). World Mycotoxin Journal, 11(3), pp.341-357.
- Mutunga E, Ndungu C. and Muendo P. (2017). Smallholder Farmers Perceptions and Adaptations to Climate Change and Variability in Kitui County, Kenya. *Journal of Earth Science & Climatic Change*, 08(03). https://doi.org/10.4172/2157-7617.1000389
- Negash D. (2018). A review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. Journal of Applied Microbiological Research 1: 35-43.
- Nyangi C., Beed, F., Mugula, J. K., Boni, S., Koyano, E., Mahuku, G., Bekunda, M. (2016). Assessment of pre-harvest aflatoxin and fumonisin contamination of maize in Babati district, Tanzania. *African Journal of Food, Agriculture, Nutrition and Development*, 16(3), 11039–11053.
- Okoth, S.; Nyongesa, B.; Ayugi, V.; Kang'ethe, E.; Korhonen, H.; Joutsjoki, V. (**2012**). Toxigenic Potential of Aspergillus Species Occurring on Maize Kernels from Two Agro-Ecological Zones in Kenya. *Toxins*, *4*, 991–1007.
- Olarte, R. A., Horn, B. W., Dorner, J. W., Monacell, J. T., Singh, R., Stone, E. A., & Carbone, I. (2012). Effect of sexual recombination on population diversity in aflatoxin production by Aspergillus flavus and evidence for cryptic heterokaryosis. *Molecular Ecology*, 21(6), 1453–1476.
- Ongoma, V., 2013. A review of the effects of climate change on occurrence of aflatoxin and its impacts on food security in semiarid areas of Kenya. International Journal of Agricultural Science Research 2: 307-311.
- Owuor Lalah, J., Omwoma, S., & A.O. Orony, D. (2020). Aflatoxin B1: Chemistry, Environmental and Diet Sources and Potential Exposure in Human in Kenya. In Aflatoxin B1 Occurrence, Detection and Toxicological Effects.
- Paica, A., Ene, C. I., & Stefan, L. A. (2013). Toxigenic fungi of Aspergillus genus and corn crop vulnerability to infection produced by these. *Romanian Journal for Plant Protection*, 6, 77–81.
- Probst, C., Bandyopadhyay, R., & Cotty, P. J. (2014). Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology*, 174, 113–122. https://doi.org/10.1016/j.ijfoodmicro.2013.12.010



- Reddy, K. R., Seepaul, R., Henry, W. B., Gajanayake, B., Lokhande, S. and Brand, D. (2014). Maize (Zea mays L) yield and aflatoxin accumulation responses to exogenous glycinebetaine application. International Journal of Plant Production 8(2): 1735 6814
- Ren, Y., Jin, J., Zheng, M., Yang, Q., & Xing, F. (2020). Ethanol Inhibits Aflatoxin B1 Biosynthesis in Aspergillus flavus by Up-Regulating Oxidative Stress-Related Genes. *Frontiers in Microbiology*, 10.
- Shephard, G. S. (2008). Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 25(2), 146–151.
- Wassmann, R., Jagadish, S. V. K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., ... Heuer, S. (2009). Chapter 3 Regional Vulnerability of Climate Change Impacts on Asian Rice Production and Scope for Adaptation. *Advances in Agronomy*.
- Wu, F., 2010. The global burden of disease caused by foodborne aflatoxin. WHO Commissioned Report. Geneva: World Health Organization Food Borne Disease Burden Epidemiology Reference Group
- Xu, Y., Gong, Y. Y., & Routledge, M. N. (2018). Aflatoxin exposure assessed by aflatoxin albumin adduct biomarker in populations from six African countries. *World Mycotoxin Journal*. Wageningen Academic Publishers.
- Yard, E.E., Daniel, J.H., Lewis, L.S., Rybak, M.E., Palikov, E.M., Kim, A.A., Montgomery, J.M., Bunnell, R., Abudo, M.U., Akhwale, W., Breiman, R.F. and Sharif, S.K., 2013. Human aflatoxin exposure in Kenya, 2007: a cross-sectional study. Food Additives and Contaminants Part A 30: 1322-1331.
- Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2), 129–144. https://doi.org/10.1016/j.jscs.2010.06.006