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PAPER ON INFLUENCE OF DIFFERENT ALTITUDES AND MAIZE HARVEST SEASONS AFLATOXIN CONTAMINATION OF MAIZE IN MAKUENI COUNTY, KENYA

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ABSTRACT

Consumption of contaminated maize poses a significant aflatoxin problem in many countries including Kenya, and many people living in developing countries may be unknowingly chronically exposed to aflatoxin through diet from maize and other cereals. The objective of this study was to determine and compare aflatoxin levels of maize stored households in different altitude areas and in different maize harvest seasons, in Makueni County in Kenya. Design of the study was comparative cross sectional analytical study and setting was in Kibwezi and Kilome sub-counties of Makueni County, Kenya. The study comprised four hundred and eighty households sampled from different regions within the county. The results of maize harvested in August/September season indicated that the mean moisture content of household maize was 12.78% in high altitude area which was slightly lower than in high altitude which had 12.85%. The aflatoxin positivity of maize contamination during this season was 25.0 % in low altitude area and 4.2% in high altitude area. The results of maize harvest in February/March season indicated that the mean moisture content of household maize was 13.48 % in high altitude area which was slightly lower than in high altitude area which was 13.63 %. The aflatoxin positivity of maize contamination during this season was 33.3 % in low altitude area which was higher than high altitude which had 12.5%. The findings show that the low altitude maize had higher moisture content and aflatoxin contamination than high altitude maize, indicating that altitude had an influence. The results further indicated that the most common strain/type of aflatoxin in both low and high altitude areas, and in both maize harvest seasons, was aflatoxin B1 followed by aflatoxin B2, with maize harvested in low altitude and February/March season having higher quantities of these aflatoxin sub-types. These study findings indicate that there was higher aflatoxin contamination of maize, in both sub-types, for maize harvested in February/March season than maize harvested in August/September season, in both low and high altitude areas, with low altitude areas having comparatively higher aflatoxin contamination than high altitude areas for maize harvested in February/March season. These findings indicate that people living in higher altitude consuming maize as their staple foodstuff are more exposed to aflatoxin than those living in lower altitude. Likewise people consuming maize harvested in February/Marchseason were more exposed to aflatoxin than those in consuming maize harvested in August/September season. These findings indicate that climate change phenomenon being experienced had effects on aflatoxin production in maize. There is therefore need for sustained public education onaflatoxin risks particularly from maize grown in higher altitude areas and those harvested in August/September seasons, as well as the need for preventive precautions on the same.

INTRODUCTION

Consumption of maize contaminated with aflatoxin, causing aflatoxicosis, poses a problem in many countries including Kenya. It is estimated that 4.5 billion people living in developing countries could be chronically exposed to aflatoxin through their diet of maize and other cereals (3).

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Aflatoxins are secondary metabolites from mould of the *Aspergillus* family and include among others *Aspergillusparasiticus* and *Aspergillusflavus*. Aflatoxins are also of various types which include aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2, out of which B1 is the most common, most toxic and the most potent in terms of causing liver cancer in humans (1). Fungal spoilage and aflatoxin contamination have been known to be of major concern in cereals and other foodstuffs. Acute aflatoxin poisoning is a serious health problem. It causes liver failure and death which

in Kenya it is estimated to be up to 40% of cases (3). The eastern region of Kenya particularly Makueni has been mostly affected by aflatoxicosis outbreaks resulting from consumption of maize contaminated with aflatoxins, the region having experienced three major outbreaks since 1981 to date (2, 3, 15, 12, 11). The outbreaks, especially the 2004 outbreak in Kenya which was the most severe, caused significant human mortality and morbidity resulting in 317 cases and 125 deaths (2) as well as causing widespread socio-economic impact (12,16). Epidemiologic investigation revealed that the outbreak was as a result of aflatoxin poisoning from ingestion of aflatoxin contaminated maize (2). Previous studiesalso revealed that other aflatoxicosis outbreaks that had occurred previously in the area was also acquired from eating contaminated maize which was attributed to improper drying and storage (14, 12, and 15).

Studies further showed that most of the affected population in Kenya engaged in small-scale, subsistence mixed farming that included some livestock with maize being the primary dietary staple and the main crop produced. At harvest, majority of farmers store most of their maize for household consumption while selling some to meet other household needs. Besides many factors contributing to occurrence of aflatoxin contamination in maize and other susceptible foods, different altitudes as well as different seasons leading to climatic variations could also influence disease occurrences including fungal development and aflatoxin production. This paper therefore reports the findings of study conducted to determine influence of altitude and maize harvest seasons on aflatoxin contamination as well as aflatoxin sub-types or strains, in Makueni County, Kenya.

MATERIALS AND METHODS

This study was conducted in Kibwezi and Kilome sub-counties of Makueni County. Kibwezi study site is a lower altitude area located at an altitude of 916m above sea level, while Kilome study site is a higher altitude area located at an altitude of 1750M above sea level. Makueni County, in which the study sites are located, covers an area of 8,034.7 square Km and according to 2009 population census it had a population of 884,527(8), which in 2012 was projected to 922,183 with estimated annual population growth of 1.4% (13). Physiographically, the land rises from 600m above sea level at the southern parts of the county which include Kibwezi and Makindu which are low-lying areas, to 1900 m above sea level in northern highest parts of the county which include Kilome and Kilungu hilly areas (8). Due to change in altitude, the county has climatic variations and extreme differences in temperatures. The northern part is usually cool while the southern part with low-lying areas is usually hot. The mean temperatures in this area range from 20.2 to 24.6 degrees centigrade. The county experiences two rainy seasons, namely: the long rains season occurring in March/April and the short rains season occurring in November/December. The main food crops produced are maize, beans, cow peas and pigeon peas in that order, with maize being a staple food. The study design was cross sectional comparing different altitudes and maize harvest seasons to determine their influence on aflatoxin contamination of household maize. Study setting was in Kibwezi and Kilome sub-counties of Makueni County. Sampling was conducted in two geographically and ecologically different zones namely Kibwezi and Kilome sub-counties which were randomly selected for this study. In each zone, one geographical location was randomly selected. Representative sample of households was then selected from Sub-locations/cluster based on method of probability proportional to size (pps).

At each of the two zones, households which were sampling units were selected at random through systematic random sampling methods using a sampling frame and a table of random numbers. The households' that consented to participate in the study were then recruited. The study and target populations comprised people in households in study areas who store home grown maize in their households. It included all adults (above 18 years of age) who are household heads within the study area and store home grown maize. The sample size for households selected was determined using a formula as used by Fisher *et al.* (1998) which gave a minimum of 240 households for each study site. Owing to limitation in resources which could enable analysis of all household maize samples, a further sub-sample of 10% of the 240 households in each study site with stored home grown maize was selected and maize samples were collected for analysis from sample households. Sampling was achieved by first getting the random starting household in which random numbers were used to pick up the starting household. The remaining households of the sample were then selected at fixed n^{th} intervals determined by dividing total number of households by sample size. After selecting the household, the purpose, nature, procedure and expected benefits of study were explained to the household owner/head after which consent was sought, and upon consenting he/she was requested to sign the consent form and after which was recruited to participate in the study. Particulars of household head such as household number, name, contact address, phone number, sex, age, education level, religion etc. were recorded in the register for the follow up visit in the next phase/season of data and sample collection.

All Sampled households owners/heads with home grown maize were administered a face-to-face interview using structured interview schedule. A representative sub-sample comprising 10% of 450 households sampled were drawn through systematic random sampling method. These households were requested to provide samples of their stored home grown maize for moisture content determination and aflatoxin analysis. The sub-sampled households were later followed for collection of samples in the next seasons harvest in order to take into account seasonal variations. In addition to maize sampling and analysis for aflatoxin, data was also collected using questionnaires/interview schedules from 240 households in each study site. They were used to obtain data from respondents either in Kiswahili or English through face to face interviews, and information was then translated and recorded in English. Information collected from study participants using questionnaires/interview schedules included 1) socio-demographic information such as sex, age, marital status, religion, level of education, occupation, economic status. Temperature and humidity of the two study sites was measured using hygrothermometer at the two study sites during the Month of November 2013 and during the Month of

May 2014) after each maize season harvest. Collection of data using questionnaires and taking of maize samples were done in November following August/September season of maize harvest.

Maize sampling was done by obtaining samples from 10 % of sub-sampled households which had maize and were selected for study. A one kg of maize sample was taken from maize found in the sub-sampled household. The sample was taken from top,middle and bottom of the container then mixed in such a way that it was a representative of the lot. Samples were collected from maize intended for human consumption found in the household. In case of maize packed in small volumes in different bags, multiple samples were taken from different parts of one bag or several bags belonging to one household and combined to produce a one kg sample for analysis. The maize samples were collected using sampling tools such as scoops/probes and put in paper bags, and carried and stored in paper bags while awaiting analysis. Each sample had a sampling form filled with specific identification information pertaining to the sample. Maize samples were collected in households in November 2103 after August/September maize harvest season and in May 2014 after February/March season of maize harvest.

***Maize sample analysis*:** Moisture content was determined in the field during collection of maize samples using Portable Grain Moisture Tester. The analysis for aflatoxin was done in two stages. The first stage entailed screening of the samples to determine presence of total aflatoxin using ELISA test for total aflatoxin content. The second stage involved analyzing the samples which tested positive in ELISA aflatoxin test using HPLC test procedure, which determined types of aflatoxin and their content/quantities. The procedures for moisture content determination, ELISAtest and HPLC test are described here below.

Moisture Content Determination: Moisture content was measured in the field using Portable Grain Moisture Tester/Metre. Nine (9)v battery was put in the moisture tester and P button pressed for 1 second to check if it was working, after the previous data of moisture content reading has been erased by Pressing F button constantly. Seventy (70) g of maize sample was then taken, well shaken and filled into moisture device to flash level and corked tightly. The moisture Tester device was then powered on by pressing P button. Appropriate scale depending on grain, was then chosen and this case since the grain was maize the scale was 1-16. The sample was then allowed to run in the device for one minute and then the moisture content was read, after which the F key button was pressed to save the reading. A new test for a different sub-sample from the same sample was repeated to give an average reading for the sample, which was then recorded. After each reading was noted, the F button was constantly pressed for 8 Seconds to erase previous reading from memory before doing a new test. For each different sample taken the same procedure was followed. *Determination of Aflatoxin contamination:* Determination of Aflatoxin contamination was done using Enzyme Linked Immunoassay (ELISA) and High Performance Liquid Chromatography (HPLC) tests.

Enzyme Linked Immunoassay (ELISA) test: Extraction of Sample was done by taking 0ne kg of maize sample and grinding it into flour with a mill and then homogenizing it. Then 20g homogenized sample were weighed and 20ml of 70% Methanol were added into the sample. They were mixed for 2 hours and filtered using Buchner funnel. The extraction jar was then rinsed with 20mls of extraction solution. The total volume of the extract was then measured and recorded. Column Preparation was done by taking five (5) g in 25 mls (70% methanol) of extract and adding 10 % of methanol in prepared Phosphate Buffered Saline (PBS). Then 5ml of 10% methanol PBS were passed through without letting it dry. A sample comprising 1ml of extract and 6ml water was then applied and let run slowly at the rate of 1 drop in 3 seconds. Distilled water-15ml was then applied and passed slowly at rate of I drop per second. Then air was passed to dry and the column was put to a receptacle for eluent. One (1) ml methanol (100 %) was then applied and passed slowly into receptacle. Cleaning up was done with Acetonitrile. Nine (9)mls of sample extract were taken and evaporated to dryness with nitrogen/rotavapour. It was then diluted with PBS buffer to 10mls (the amount of organic solvent did not exceed 5% of solution). The extract solution was then filtered and dropped off onto the immumo-affinity column at the rate of 1-3ml/min. The Immuno-affinity column was washed with 20ml water and the water dropped through the column with gravity. The column was dried to ensure total Aflatoxins recovery. Derivatisation was done by evaporating all samples to dryness and then 200ul TFA were added and incubated at room temperature for 40 minutes, after which 800 µl Acetonitrile: water (30:70) was added and dissolved using a sonicator. They were then filtered through a membrane filter (GHP 0.2um) into a vial.

Enzyme Linked Immunoassay ELISA Analysis: A sufficient number of micro-titer wells were inserted into the microwell holder for all standards and samples run in duplicate. Standard and sample positions were recorded. Then 50 µl of the standard solutions or prepared sample were added to separate duplicate wells, and 50 µl of the enzyme conjugate were then added to each well. Then 50 µl of the antibody solution were added to each well and mixed gently by shaking the plate manually and incubating for 30 minutes at room temperature (20-25 C). The liquid was then poured out of the wells and the microwell holder tapped upside down vigorously (three times in a row) against absorbent paper to ensure the liquid from the wells was removed completely. All the wells were filled with 250 µl washing buffer 10.1 and the liquid poured out again. The the washing procedure was repeated two times. After which 100 µl of substrate/chromogen (brown cap) were added to each well and mixed gently by shaking the plate manually and incubating for 15 minutes at room temperature (20-25 C) in the dark. Then 100 µl of the stop solution were added to each well and mix gently by shaking the plate manually and the absorbance measured at 450 nm. Reading was done within 30 minutes after adding stop solution.

High Performance Liquid Chromatography (HPLC) Test: Sample Extraction was done by taking 0ne kg of maize sample and grinding it into flour with a mill and then homogenizing it. Then 20g homogenized sample were taken and weighed. After which 20ml of 70% Methanol were added into the sample. It

was then mixed for 2 hours and filtered using Buchner funnel. The extraction jar was then rinsed with 20mls of extraction solution. Then the total volume of the extract was measured and recorded. Column preparation was done by addingfive (5) g of solid extract added to 25 mls of 70% methanol. Then 10mls of 10 % of Phosphate Buffered Saline (PBS) were added, and then 5ml of 10% methanol PBS passed through the column without letting the column to dry. A sample consisting of 1ml extract and 6ml water was applied, and let Let run slowly at the rate of 1 drop in 3 seconds. Distilled water (15ml) was then applied and passed slowly at rate of I drop per second, and then air was passed to dry the column. After which a column was put to a receptacle for eluent and then 1 ml methanol (100 %) was applied and passed slowly into receptacle.

Cleaning up was done with Acetonitrile**.** Nine (9) mls of sample extract were taken and evaporated to dryness with nitrogen/rotavapour. It was then diluted with PBS buffer to 10mls (the amount of organic solvent did not exceed 5% of solution). The extract solution was then filtered and dropped off onto the immumo-affinity column at the rate of 1-3ml/min. The Immuno-affinity column was washed with 20ml water and the water dropped through the column with gravity. The column was dried to ensure total Aflatoxins recovery. Derivatisation was done by evaporating all samples to dryness and 200ul TFA were then added and incubated at room temperature for 40 minutes. Then 800ul Acetonitrile: water (30:70) was added and dissolved using a sonicator. They were then filtered through a membrane filter (GHP 0.2um) into a vial. Analysis entailed adding twenty (20) ml of the filtrate into the HPLC. Then a calibration curve of aflatoxin B1, B2, G1, G2 was run and results quantified as ug/kg. The fluorescence detector was set at Gain X1, Excitation λ=363nm, Emission λ=440nm and Column oven temperature of 35˚.

Data Management and Analysis: Data collected from questionnaires was cleaned, coded and entered in MS Windows Excel software and then transferred to SPSS for Window version 17.0 (SPSS Inc., Chicago, Illinois) for Statistical analyses. Analysed data (results) are presented using percentages and frequency tables. Descriptive statistics such as frequencies and means were applied in order to group and summarize data to facilitate presentation. On statistical test, Student t-test was used to compare means of moisture content and aflatoxin content as well as other quantifiable variables. Pearson Correlation coefficient was used to analyse relationships of quantitative variables. Tests of significance were at 0.05 level of significance, and confidence levels at 95%.

RESULTS

Household respondent's socio-economic and demographic characteristics: The household respondents who were heads of households or their principal representatives had mean age of about 47 years in both study sites. Majority of respondents were female (58%) and most of them were married (74.2 %). Majority of them had attained primary education (61.7%). The main occupation of respondents was farming (79.2%) and farming was their main source of income for households (75.0%)) with majority of them (68.4%) earning less than Ksh.5000 (mean income was Ksh.4800), implying that majority were poor. Households had an average of six people.

Maize sample analysis results for aflatoxin and moisture content

Aflatoxin levelsand moisture content for maize harvested in August/Septembert season. Regarding moisture content for maize harvested in August/September, for Kibwezi, a low altitude area,the mean moisture content was 12.78% (95% CI=12.68 to 12.88) (n=24) and for Kilome, a high altitude area, the mean moisture content was 12.85% (95% CI=12.75 to 12.95) (n=24).The ELISA test resultsof 24 random maize samples gave 25.0% and 4.2 % aflatoxin positivityfor Kibwezi and Kilome respectively at 1.75 µg/Kg cut –off detection point (Table 1).

Aflatoxin sub-types and quantities for maize harvested in August/September season

The maize samples that were positive for aflatoxin when analyzed using HPLCtest showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 30.08 µg/Kg, 0.88 µ/Kg, 0.48 µg/Kg, and 0.25 µg/Kg respectively, for Kibwezi (n=6). AFB1 was most abundant (30.08µg/Kg) while AFG2 was the least abundant (0.25 µg/Kg). For Kilome, maize samples which were positive for aflatoxin when analysed using HPLC test showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 1.55µg/Kg, 0.1 µ/Kg, 0.1 µg/Kg, and 0.05 µg/Kg respectively (n=1). AFB1 was also most abundant at 1.55µg/Kg while AFG2 was the least at 0.05µg/Kg. The high aflatoxin B1 in one of the samples could be attributed to high level of contamination due to poor storage conditions as the lot sampled was placed directly on the floor (Table 2).

Aflatoxin levelsand moisture content for maize ofFebruary/March season

Regarding moisture contentfor maize harvested in February/March season, for Kibwezi, a low altitude area,the mean moisture content was 13.63% (95% CI=13.23 to 14.03) (n=24) and for Kilomea high altitude area, the mean moisture content was 13.48% (95% CI=13.17 to 13.79) (n=24). The ELISA tests results of the 24 random maize sub-samples for maize harvest in February/March season gave 33.3% and 12.5% aflatoxin positivity for Kibwezi and Kilome respectively at 1.75µg/Kg cut-off detection point (n=24) (Table 3).

Aflatoxin Sub-types and quantities for maize harvested in February/March season

The samples that were positive for aflatoxin when analysed using HPLC test, showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 26.64 µg/Kg, 9.47 µg/Kg, 0.55 µg/Kg, and 1.05 µg/Kg respectively, for Kibwezi (n=8). AFB1 was most abundant (26.64 µg/Kg) while AFG2 was the least abundant (1.05 µg/Kg). For Kilome, maize samples which were positive on ELISA test when analyzed using HPLC test showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 26.7 µg/Kg, 5.5 µg/Kg, 1.06 µg/Kg, and 1.93 µg/Kg respectively (n=3). AFB1 was also most abundant at 26.7µg/Kg while AFG2 at 1.93 µg/Kg, was the least (Table 4).

Table 1. Aflatoxin sub-types and quantities for maize harvested in February/March season

Study sites	Maize Samples with Moisture content above 13%		Maize Samples with Aflatoxin detected cutt-off point 1.75ug/Kg		Maize Samples with Aflatoxin levels above 10 ug/Kg	
n=24	No of samples	%	No of samples	%	No of samples	%
Kibwezi	4	16.7	6	25.0	1	4.2
Kilome	4	16.7	1	4.2	0	0

Table 2. Aflatoxin sub-types and quantities for maize harvested in August/September season

S/No	AF B1(µg/Kg)		AF B2(µg/Kg)		AF G1(µg/Kg)		AF G2(µg/Kg)	
	KBZ *(n=6)	KLM (n=1)	KBZ (n=6)	KLM n=1	KBZ (n=6)	KLM (n=1)	KBZ (n=6)	KLM (n=1)
01	6.0	1.55	0.8	0.1	0.5	0.1	0.5	0.05
02	3.6	-	0.2	-	0.3	-	0.1	-
03	6.3	-	0.7	-	0.5	-	0.3	-
04	159.5	-	3.2	-	1.2	-	0.3	-
05	2.8	-	0.5	-	0.3	-	0.2	-
06	2.3	-	0.15	-	0.05	-	0.1	-
Mean	30.08	1.55	0.88	0.1	0.48	0.1	0.25	0.05

*n is the number of maize samples which tested positive on ELISA test

Table 3. Aflatoxin levels and moisture content for maize harvested in February/March season

Study sites	Maize Samples with Moisture content above 13%		Maize Samples with Aflatoxin (detection cut-off point 1.75ug/Kg)		Maize Samples with Aflatoxin levels above 10 ug/Kg	
n=24	No of samples	%	No of samples	%	No of samples	%
Kibwezi	15	62.5	8	33.3	5	20.8
Kilome	14	58.3	3	12.5	2	8.3

Table 4. Aflatoxin sub-types and quantitiesfor second season maize in Kibwezi

S/No	AFB1(µg/Kg)		AFB2(µg/Kg)		AFG1(µg/Kg)		AFG2(µg/Kg)	
	KBZ *(n=8)	KLM (n=3)	KBZ (n=8)	KLM (n=3)	KBZ (n=8)	KLM (n=3)	KBZ (n=8)	KLM (n=3)
01	6.8	12.9	1.0	1.9	0.2	0.0	0.2	0.0
02	1.4	5.4	0.3	0.0	0.0	0.0	0.0	0.0
03	1.6	61.8	0.0	14.6	0.0	3.2	0.0	5.8
04	44.9	-	6.6	-	0.0	-	0.5	-
05	99.5	-	14.8	-	3.4	-	6.1	-
06	13.8	-	1.1	-	0.0	-	0.0	-
07	35.5	-	3.3	-	0.8	-	1.6	-
08	9.6	-	1.3	-	0.0	-	0.0	-
Mean	26.64	26.7	9.47	5.5	0.55	1.06	1.05	1.93

*n is the number of maize samples which tested positive on ELISA test

Temperature and Humidity of Kibwezi and Kilome study sites

Temperature and Humidity of the two study sites were taken and recorded in sampled households using hygrothermometer instrument. The recordings were then computed into means for each study site. In the first phase of August/September season of maize harvest, the mean temperature was 30.7^{o}C (95% CI=30.47 to 30.91) in Kibwezi, and 23.6 ^{o}C (95% CI=23.24 to 23.81) in Kilome, while humidity was 45.8% (95% CI=45.14 to 46.55) in Kibwezi and 32.3% (95% CI=31.84 to 32.78) in Kilome. In the second phase for maize harvested in February/March season, the mean temperature was 31.6^{o}C (95% CI=30.26 to 31.18) in Kibwezi and 25.4% ^{o}C (95% CI=25.02 to 25.71) in Kilome, while humidity was 49.9% (95% CI=48.85 to 50.99) in Kibwezi, and 42.4% (95% CI=40.02 to 44.83) in Kilome.

DISCUSSION

The study results have revealed existence of significant levels of contamination of household maize with aflatoxin with some exceeding permissible levels in different maize growing altitudes as well as in different harvest seasons. A considerable proportion of contaminated household maize exceeded permissible levels of 10 µg/Kg set by authorities 10, 6,7). Previous studies done in the area also found high levels of aflatoxin contamination in maize (17, 18, 12). There was significant variation in aflatoxin contaminated maize between low and high altitude areas, with low altitude areas having higher contamination. In addition, there was significant variation in aflatoxin contamination among different seasons of maize harvest. Maize harvest in August/September season had more contamination than maize harvested in February/March season in both high and low altitude areas, with low attitude area having relatively higher contamination. The difference in aflatoxin contamination positivity between these two areas of different attitudes was significant (P<0.05). This could be attributed to the fact that February/March season was more warm and humid than August/September season. This is consistent with other studies which showed that aflatoxin is affected by changes in weather conditions in that maize harvested in more warm and humid seasons had more aflatoxin contamination than maize harvested in less warm and

humid conditions (19, 20). Further, lower altitude maize harvested in February/March season had higher aflatoxin positivity than maize harvested in higher altitude area in the same season, and the variation between these two different areas in altitude was significant ($P<0.05$). The prevailing cooler climate in higher altitude areas, which their altitude is about two times higher than the altitude of lower areas, could have likely contributed to low aflatoxin contamination of maize since conditions of cooler areas are comparatively unfavorable for fungal growth and aflatoxin development. This is consistent with a study by (20), in Uganda which found higher aflatoxin contamination in mid-altitude maize than highland maize. In terms of comparison of maize harvested in August/September season and those harvested in February/March season, results revealed significant variation in aflatoxin contamination between the two seasons ($P<0.05$), implying a likelihood of higher risk of consuming maize harvested in February/March season, and particularly those harvested in higher altitude areas. This is consistent with likely effects of changes in weather conditions on mould growth and aflatoxin production (19). Thus, this finding point to a more likelihood of exposure to aflatoxin for people consuming maize harvested in February/March season.

The existing levels of aflatoxin contamination of household stored maize found in this study were almost consistent with estimated levels of aflatoxin contamination in foods by Food and agriculture Organisation (6), thus indicating continued existence of considerable risk of exposure and likelihood of occurrence of aflatoxicosis in humans. Analysis of samples of maize harvested in August/September season showed that 50% of positive aflatoxin contaminated maize samples in lowlands had aflatoxin levels exceeding 10µg/Kg, while in highlands none of the maize had aflatoxin contamination exceeded 10 µg/Kg. Further analysis of positive samples on ELISA test for maize harvested in February/March season using quantitative HPLC method showed varying aflatoxin levels. The low altitude samples that showed samples with aflatoxin content exceeding 10 µg/Kg increased from 1(16.7%) to 5 (62.5 %) , but overall aflatoxin contamination increased with more maize testing positive for aflatoxin for maize harvested in February/March season (33.3%) than maize harvested in August/September season (25.0%). In high altitude area, maize with aflatoxin level exceeding 10 µg/Kg increased from 0% to 2 (66.7) %. The overall sample positivity also increased from 4.2% to 12.5%. These results indicate that there was higher aflatoxin contamination of maize harvested in February/March season than maize harvested in August/September season, in both lowland and highland areas. However, lowlands had higher levels of aflatoxin contamination than highlands, and this finding is consistent with other studies done elsewhere (20, 21). Overall the results showed that in both maize harvest seasons, low altitude areas had more aflatoxin contaminated maize than high altitude areas. This could be because lower attitude areas are warmer /hotter, characterized by higher temperatures and humidity, while higher altitude areas are cooler, characterized by low temperatures and humidity. Similarly maize harvested in February/March season had more aflatoxin contaminated maize than maize harvested in August/September season. This could be because February/March harvest season had higher temperatures and humidity than August/ September maize harvest season. Indeed study revealed that the mean temperature for August/September season was 30.7°C in low altitude and 23.6°C in high altitude while in February/March season the mean temperature was 31.6°C in low altitude and 25.4°C in high altitude. The relative humidity for August/September season was 45.8% in low altitude and 32.3% in high altitude while in February/March season the mean relative humidity was 49.9% in low altitude and 42.2% in high altitude. Warm and humid conditions are favorable and ideal for growth of Aspergillus species resulting in the production of aflatoxins (19,22). These warm and humid conditions are influenced altitude and seasons which in turn influence climate (23). Regarding aflatoxin sub-types, results further indicated that the most common strain/type of aflatoxin in both study sites and in both maize harvest seasons was AFB1 followed by AFB2. However lowlands had higher AF B1 mean aflatoxin contamination than highlands in August/September maize harvest, but in February/March maize season harvest the mean contamination of AFB1 was virtually the same with no significant difference, except for total aflatoxin contamination of all sub-types. February/March maize season harvest had slightly higher mean AFB1 as well as total aflatoxin contamination than maize of August/September harvest season which was slightly lower.TheAFB1 aflatoxin sub-type was implicated with previous occurrences of aflatoxicosis (18). Furthermore, in both areas, the mean aflatoxin concentrations of other sub-types of AFB2, AFG1 and AFG2 were higher in maize harvested in February/March season than maize harvested in August/September season indicating increased aflatoxin contamination in maize harvested in February/March season. The levels of these aflatoxin sub-types were also higher in lowlands than in highlands, indicating higher contamination of maize in lowlands.

Predominance of AFB1 sub-type is consistent with findings from other studies done elsewhere (4, 20). The presence of AFB1 and AFB2 types could probably be attributed to sporadic occurrences of aflatoxicosis cases in the area as these aflatoxin sub-types have been implicated as the cause of aflatoxin poisoning and they are portent carcinogenic substances (Cornnel University, 2014). Indeed previous occurrences of aflatoxicosis in Kenyan eastern region were associated with AFB1 sub-type (18). Regarding moisture content of maize which is a contributing factor to mould growth and aflatoxin production, maize harvested in lowland area had slightly higher moisture content than maize harvested in highland area in both seasons of maize harvest. This finding is consistent with the results of a study which found out that mean moisture content was significantly lower in highland maize kernels than in mid-altitude (20). The moisture content was also higher in maize harvested in February/March season than in August/September season in both areas. The increased moisture content could have contributed to increased aflatoxin contamination of maize in February/March maize harvest season. Similarly, the higher moisture content in lowlands than highlands could more likely have contributed to higher aflatoxin contamination in lowlands.

Furthermore, results of this study have revealed that some maize had moisture content above recommended level of 13%. This could have been attributed to inadequate drying and improper storage conditions. Maize of moisture content above 13 % is likely to be attacked by pests and molds which are predisposing factors to aflatoxin development (24). These study findings have demonstrated that levels of aflatoxin contamination were quite high especially in lowland areas and in February/March maize harvest season, thus exceeding permissible limits of 10 µg/kg for humans adopted in Kenya and many other countries for guiding intervention action points (10,1,7). Maize or foods exceeding permissible limits are not supposed to be used as food or animal feed.

Conclusion

The study findings have shown the prevalence of aflatoxin contamination of maize in both low and high altitude areas, with some aflatoxin contaminated maize exceeding permissible levels of 10µ/Kg, thus exposing consumers to aflatoxicosis risk. The results have also shown significant variation in aflatoxin contamination between low altitude and high altitude areas as well the first maize harvest season of August/September and second maize harvest season of February/March. These study findings imply that householdsrelying mainly on maize are likely to be exposed to risk of aflatoxin poisoning with the likely risk being higher in lower altitude areas than in higher altitude areas, and in maize harvest February/March season than maize harvested in August/September season. The is need for focused interventions targeting aflatoxin prevention in general and specifically in high risk low lying areas, and maize harvested in February/March seasons so as to minimize or eliminate aflatoxin contamination in maize. There is urgent need for continued public education on likely risk posed by consumption of aflatoxin contaminated maize, as well as the preventive measures to be undertaken.

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