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COMPARATIVE ANALYSIS OF AFLATOXIN CONTAMINATION OF MAIZE IN TWO DIFFERENT PHYSIOGRAPHIC ZONES AND MAIZE SEASONS IN MAKUENI COUNTY, KENYA

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ABSTRACT

***Background*: Aflatoxicosis resulting from consumption of contaminated maize poses a significant public health problem in many countries including Kenya, and many people living in developing countries could be chronically exposed to aflatoxin through their diet. It is caused by Aflatoxins produced by fungus of species *Aspergillus parasiticus* and *Aspergillus flavus* found mainly in cereals and other foodstuffs.**

***Objective*: To determine and compare prevalence of aflatoxin contamination of household maize in two different altitude areas and different maize harvest seasons, in Makueni County in Kenya.**

***Design*: A comparative descriptive analytical study.**

***Setting*:Kibwezi and Kilome sub-counties of Makueni County, Kenya.**

***Subjects*: Four hundred and fifty household heads or their representatives, who had maize in their household stores.**

***Results*: The results of first maize harvest 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 rate of maize contamination was 25% in low altitude area and 4.2% in high altitude area. The results of second maize harvest 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 rate of maize contamination 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. 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 AF B1 followed by AF B2, with maize harvested in low altitude and second 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 second season than maize harvested in first season, in both low and high altitude areas of Makueni County, with low altitude areas having comparatively higher aflatoxin contamination than high altitude areas for maize harvested in second season.**

***Conclusion*: These findings indicate that a sizable proportion of Makueni households mainly relying on maize consumption as their staple foodstuff are likely to consume maize contaminated with aflatoxin. There is therefore need for further public education by local authorities on risks posed by continued consumption of maize contaminated with aflatoxin as well measures to reduce levels of contamination by improving methods of storage. Government authorities need also to periodically sample maize in order to monitor moisture and aflatoxin content of householdstored maize to enable timely interventions should aflatoxin content exceed permissible levels.**

INTRODUCTION

Aflatoxicosis resulting from consumption of contaminated maize poses a significant public health 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. 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 causes liver failure and death in up to 40% of cases in Kenya (3). The eastern region particularly Makueni has been mostly affected by aflatoxicosis outbreaks resulting from consumption of maize contaminated with aflatoxins, having experienced three major outbreaks since 1981 to date (2, 3, 11, 12, 15). The outbreaks, especially the 2004 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 investigations conducted during that time revealed that the 2004 outbreak was the result of aflatoxin poisoning from ingestion of contaminated maize (2). Other aflatoxicosis outbreaks that had occurred previously in the area, and were also acquired from eating contaminated maize attributed to improper drying and storage (12, 14, 15).

Most of the affected local population 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.

This paper therefore reports the findings of investigation conducted to determine the extent and prevalence of aflatoxin contamination in maize as well as the sub-types or strains of aflatoxin, in both lower and higher altitude areas and in different seasons of Makueni County.

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 S02.40157, E037.95143 SW, at an altitude of 916 m above sea level, while Kilome study site is a higher altitude area located at S01.84098, E037.31536NE at an altitude of 1750M above sea level. Makueni county 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 600 m 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.

Study design was comparative analytical study to determine 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 which were purposefully selected for this study for comparison purposes. 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 studied, households storing maize were selected to create a sample which included all types of maize in households representing each study area. Households, which were the sampling units, were then selected at random through systematic random sampling methods using a sampling frame and a table of random numbers. The households' heads who 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 or their representatives within the study area and store home grown maize. It also included, agricultural and public health workers working in the area as well as community informants who consent to participate in the study. The study excluded people below 18 years of age and households which do not 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 225 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 225 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 be selected at fixed nth 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 if he/she consented was then requested to sign the consent form and after which he/she 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.

Data was collected using questionnaires/ interview schedulesfrom 225 households in each study site, Kibwezi and Kilome respectively.They were used to obtain data from respondents either in Kiswahili, kikamba, or English,by face to face interview,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, 2) knowledge and awareness on aflatoxin, 3)maize pre-storage practices (as could be recalled by the household owner),and 4) maize storage practices.

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 first and second season maize harvest, respectively. Collection of data using questionnaires and taking of maize samples were done in November following first season 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 first season maize harvest and in May 2014 after second season maize harvest.

Maize sample analysis: Moisture content was taken at the field during collection of maize samples and was determined using Portable Grain Moisture Tester. Before laboratory analysis, the maize samples were visually inspected for insect/pest infestation, mold or discoloration. 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 with HPLC test, which determined types of aflatoxin and their content/quantities. The procedures for moisture content determination, ELISA test and HPLC test are briefly 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 eight seconds to ease 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 two 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 one drop in three 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 2*10ml 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 1 kg of maize sample and grinding it into flour with a mill and then homogenizing it. Then 20g homogenised sample were taken and weighed. After which 20ml of 70% Methanol were added into the sample. It was then mixed for two hours and filtered using Buchner funnel. The extraction jar was then rinsed with 20 mls 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 were added to 25 mls of 70% methanol. Then 10 mls 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 one drop in three 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 2*10ml 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 percentagesand 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, aflatoxin content and other quantifiable variables between the two study areas and maize harvest seasons. Pearson Correlation coefficient was used to analyse relationships of quantitative variables among different storage and pre-storage practices, and Aflatoxin levels. Tests of significance were at α 0.05 level of significance, and confidence levels at 95%. Quality of data was ensured by proper sampling, collection and analysis at all stages of research through training of research assistants and close supervision.

RESULTS

Household 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 Analysis: Sub-samples of household maize grains were collected from Kibwezi and Kilome for first season maize harvest and second season maize harvest. They were analyzed using ELISA test with cut-off of 1.75μ/Kg. The results showed that out of the 24 maize samples from Kibwezi and 24 maize samples from Kilome for first maize harvest season, 6 (25%) samples from Kibwezi tested positive while one maize sample from Kilome tested positive for aflatoxin.

The maize harvest in first season had higher aflatoxin contamination positivity rate in lower altitude than in higher altitude area. The difference in aflatoxin contamination between high and low altitude area was also significant (P<0.05).

The mean moisture content for first maize harvest season in Kibwezi was 12.78% and Kilome was 12.85%. The mean moisture content was within the recommended limit of 0f 12 to 14.0 %. The 6 samples from Kibwezi and one sample from Kilome which tested positive on ELISA test were further analysed using HPLC quantitative test. The results in Table 1 and 2 below indicate the moisture content and aflatoxin content as well as aflatoxin types/variants for Kibwezi and Kilome, respectively.

Table 1
Aflatoxin and moisture content for maize harvested in first season in Kibwezi

S/No	Moisture	Aflatoxin ppb/μ/Kg				
	Content (%)	AF B1	AF B2	AF G1	AF G2	Total AF
01	12.9	6.0	0.8	0.5	0.5	7.8
02	13.0	3.6	0.2	0.3	0.1	4.2
03	12.8	6.3	0.7	0.5	0.3	7.7
04	12.6	159.5	3.2	1.2	0.3	164.2
05	12.8	2.8	0.5	0.3	0.2	3.8
06	13.1	2.3	0.15	0.05	0.1	2.6
Total	76.8	180.5	5.25	2.85	1.5	190.3
Mean	12.8	30.08	0.88	0.48	0.25	31.71

Table 2
Aflatoxin and moisture content for first maize harvested in first season in Kilome

S/No	Moisture Content %	AF B1	AF B2	AF G1	AF G2	Total AF
01	13.1	1.55	0.1	0.1	0.05	1.8
Total	13.1	1.55	0.1	0.1	0.05	1.8

Sub-samples of household maize grains were also collected from Kibwezi and Kilome for second season maize harvest and analyzed using ELISA test with cut-off of 1.75μ/Kg. The results showed that out of the 24 random maize samples samples from Kibwezi and 24 from Kilome for second maize harvest season, 8 (33.3%) from Kibwezi and 3(12.5%) from Kilome tested positive for aflatoxin. This aflatoxin positivity rate for maize harvest in second season increased in both low and high altitude when compared to maize harvested in first season. This second harvest season also showed a significance difference in aflatoxin contamination between high and low altitude areas (P<0.05). There was also significant interseason increase in aflatoxin contamination of maize (P<0.05). The mean moisture content for second maize harvest season in Kibwezi was 13.63% and Kilome was 13.48%. The mean moisture content was within the recommended limit of of 12 to 14.0 %.

The eight samples from Kibwezi and three samples from Kilome which tested positive on ELISA test were further analysed using HPLC quantitative test.

The results in Tables 3 and 4 below show the moisture content and aflatoxin content as well as aflatoxin types/variants for Kibwezi and Kilome, respectively.

Table 3
Aflatoxin and moisture content for maize harvested in second season in Kibwezi

S/No	Moisture	Aflatoxin ppb/μ/Kg				
	Content (%)	B1	B2	G1	G2	Total
01	13.2	6.8	1.0	0.2	0.2	8.2
02	13.4	1.4	0.3	0.0	0.0	1.7
03	13.8	1.6	0.0	0.0	0.0	1.6
04	15.3	44.9	6.6	0.0	0.5	52.0
05	15.6	99.5	14.8	3.4	6.1	123.8
06	15.8	13.8	1.1	0.0	0.0	14.9
07	14.9	35.5	3.3	0.8	1.6	41.2
08	14.9	9.6	1.3	0.0	0.0	10.9
Total	116.9	213.1	28.4	4.4	8.4	254.3
Mean	38.96	26.64	9.47	0.55	1.05	31.79

Table 4
Aflatoxin and moisture content for maize harvested in second season in Kilome

	Moisture	Aflatoxin ppb(μ/Kg)				
	content (%)	B1	B2	G1	G2	Total
01	13.2	12.9	1.9	0.0	0.0	14.8
02	15.6	5.4	0.0	0.0	0.0	5.4
03	15.3	61.8	14.6	3.2	5.8	85.4
Total	44.1	80.1	16.5	3.2	5.8	105.6
Mean	14.7	26.7	5.5	1.06	1.93	35.2

Comparison of maize analysis parameters for first and second season maize harvest: The moisture content between Kibwezi and Kilome maize harvested in both first and second season was not significant (P<0.05). However aflatoxin positivity rate between Kibwezi and Kilome for maize harvested in both first and second season was significant (P<0.05). Table 5 below shows comparison of Maize analysis parameters for first and second season maize harvest.

Table 5
Comparison of Maize analysis parameters for first and second season maize harvest

	First maize harvest			Second maize harvest		
Parameter of Sample	Kibwezi	Kilome	Sig of difference	Kibwezi	Kilome	Sig of difference
Mean Moisture content (%)	12.78	12.85	$t_{24} = 2.203$ P<0.05	13.6	13.5	$t_{24} = 0.615$ P>0.05
Sample Positivity i.e % of samples with>or=1.75ppm(μ/Kg) aflatoxin content	25.0	4.2	P<0.05	33.3	12.5	P<0.05
Mean Total aflatoxin content(μ/Kg)	31.72	1.80	t_{24} =4.763 P<0.001	31.8	28.4	t_{24} =2.169 <0.05

Aflatoxin Quantitative Analysis: Further analysis of maize harvested in first season using quantitative HPLC method, showed lowlands had one (16.7%) of aflatoxin contamination samples exceeding 10 ppb (μ/Kg), while in highlands none of the maize sample had aflatoxin contamination exceeding 10ppb (μ/Kg). Analysis of maize of second season harvest showed 5 (62.5%) of aflatoxin contaminated samples exceeding 10ppb (μ/Kg) in low altitude area/lowlands while in 2 (66.7%) of samples in high lands exceeded 10ppb (μ/Kg. The overall aflatoxin contamination increased with more maize testing positive for aflatoxin in second maize harvest season than first maize harvest season, while in highlands maize with aflatoxin level exceeding 10ppb (μ/Kg) increased. The overall sample positivity rate also increased in maize harvested in second season. The results indicate that there was higher aflatoxin contamination of maize in second maize harvest season than first maize harvest season in both lowland and highland areas of Makueni.

Overall the results showed that low altitude area had more aflatoxin contaminated maize than high altitude area. Similarly maize harvested in second season had more aflatoxin contaminated maize than maize harvested in first season.

The results further indicated that the most common strain/type of aflatoxin in both study sites and in both maize harvest seasons was AF B1 followed by AF B2. However lowlands had higher AF B1 mean aflatoxin contamination than highlands in first maize harvest, but in second maize season harvest the mean contamination of AFB1 was virtually the same with no significant difference, except for total aflatoxin contamination. Second maize season harvest had slightly higher mean AF B1 as well as total aflatoxin content than maize of first harvest season.

Temperature and Humidity of study sites: Data on temperature and humidity of study sites (in Kilome, a high altitude area, and in Kibwezi, a low altitude area) was collected for several days during maize sample collection period. Temperature and Humidity of the two study sites were taken using hygrothermometer instrument in few households where maize sample was collected for analysis. The recordings were then computed into means for each study site. In the first phase of maize sample collection in October/November the mean temperature was 30.7ºC in Kibwezi and 23.6ºC in Kilome while humidity was 45.8% in Kibwezi and 32.3% in Kilome. In the second phase in /May/June of maize sample collection the mean temperature was 31.6ºC in Kibwezi and 25.4%ºC in Kilome while humidity was 49.9% in Kibwezi and 42.4% in Kilome.

DISCUSSION

The study results have revealed existence significant levels of contamination of household maize with aflatoxin. A considerable proportion of contaminated household maize exceeded permissible levels of 10 μ/Kg set by authorities (10, 6, 7). There was significant variation in aflatoxin contaminated maize between low and high altitude areas of Makueni County. In addition there was significant variation in aflatoxin contamination between maize harvested in season of August/September and maize harvested in second maize season of February/March. The first season maize harvest was more contaminated in higher altitude area than in low attitude area. The difference in aflatoxin contaminated (positivity rate)

between these two areas of different attitudes was quite significant (P<0.05).

The lower altitude maize harvested in second season was more contaminated (higher positivity rate) than maize harvested in higher altitude area, 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 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.

In terms of comparison of maize harvested in first season and those harvested in second season, results revealed significant variation in aflatoxin contamination between the two seasons (P<0.05), implying a higher risk of consuming maize harvested in second season, and particularly those harvested in higher altitude areas.

These levels of aflatoxin contamination of household stored maize were almost consistent with the 25% estimate of aflatoxin contamination of food by Food and agriculture Organization (6), thus indicating continued existence of considerable risk of occurrence of aflatoxicosis condition in humans.

Analysis of first maize season maize harvest using quantitative HPLC method, which previously had tested positive on ELISA test, showed that about 50% of positive aflatoxin contaminated maize samples in lowlands had aflatoxin levels exceeding 10 ppb (μ/Kg), while in highlands none of the maize had aflatoxin contamination exceeded 10ppb (μ/Kg).

The positive samples on ELISA test for maize harvested in second season were further analyzed using quantitative HPLC method. The low altitude samples that showed samples with aflatoxin content exceeding 10ppb (μ/Kg) increased from 1(16.7%) to 5 (62.5 %) , but overall aflatoxin contamination increased with more maize testing positive for aflatoxin in second maize harvest season (33.3%) than first maize harvest season (25.0%). In high altitude area, maize with aflatoxin level exceeding 10ppb (μ/Kg) increased from 0% to 2 (66.7) %. The overall sample positivity rate also increased from 4.2% to 12.5%. These results indicate that there was higher aflatoxin contamination of maize harvested in second season than maize harvested in first season, in both lowland and highland areas of Makueni.

Overall the results showed that low altitude area had more aflatoxin contaminated maize than high altitude area. This could be because lower attitude areas are usually warmer/hotter characterised by higher temperatures and humidity while higher altitude areas are usually cooler characterised by low temperatures and humidity. Similarly maize harvested in second season had more aflatoxin contaminated maize than maize harvested in first season. This could be because second maize harvest season which occurs around February/March has higher temperatures and humidity than first maize harvest season which occurs in August/September. Indeed the mean temperature for first season was 30.7°C in low altitude and 23.6°C in high altitude while in second season the mean temperature was 31.6°C in low altitude and 25.4°C in high altitude. The relative humidity for first season was 45.8% in low altitude and 32.3% in high altitude while in second season the mean relative humidity was 49.9% in low altitude and 42.2% in high altitude.

The results further indicated that the most common strain/type of aflatoxin in both study sites and in both maize harvest seasons was AF B1 followed by AF B2. However lowlands had higher AF B1 mean aflatoxin contamination than highlands in first maize harvest, but in second maize season harvest the mean contamination of AFB1 was virtually the same with no significant difference, except for total aflatoxin contamination. Second maize season harvest had slightly higher mean AF B1 as well as total aflatoxin contamination than maize of first harvest season which was slightly lower.

Similarly the mean aflatoxin of other sub-types of AF B2, AF G1 and AF G2 were higher in maize harvested in second season than maize harvested in first season indicating increased aflatoxin contamination in maize harvested in second season. The levels of these aflatoxin sub-types were also higher in lowlands than in highlands, indicating higher contamination of maize in lowlands. The presence of AFB1 and AFB2 can 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 (4).

The moisture content of maize was slightly higher in maize harvested in lowland area than 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 (9). The moisture content was also higher in maize harvested in second season than in first season in both areas. The increased moisture content could have contributed to increased aflatoxin contamination of maize in second maize harvest season. Similarly the higher moisture content in lowlands than highlands could more likely have contributed to higher aflatoxin contamination in lowlands.

These study findings have showed that levels of aflatoxin contamination were quite high especially in lowland areas and in second maize harvest season, thus exceeding permissible limits of 10 μ/kg (ppb) for humans adopted in Kenya and many other countries for guiding action points (10, 1,7). Maize or foods exceeding permissible limits is not supposed to be

used as food or animal feed (1). As a result of this limit, various efforts have been ongoing focusing on reducing aflatoxin exposure to humans by keeping aflatoxin levels in food as low as reasonably possible and removing those exceeding legal limits not to be used as food. However, enforcing these regulations has been quite challenging especially for home grown maize which are consumed locally. This underscores the need for proper maize harvesting, drying handling and storage. There is also need for authorities to strengthen enforcement of regulatory limits for home grown maize and non-home grown maize.

CONCLUSION

The study findings have shown the prevalence of aflatoxin contamination of maize in both low and high altitude areas of Makueni, with some aflatoxin contaminated maize exceeding permissible levels of 10μ/Kg, thus exposing consumers to high risk of aflatoxicosis. 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. The results of first maize harvest season has shown that the mean moisture content of household maize in high altitude area was slightly lower than that of high altitude maize.

These study findings imply that households of Makueni relying mainly on maize are likely to be exposed to risk of aflatoxin poisoning with the likely risk being higher in lower altitude areas than higher altitude areas, and in maize of second harvest season than first season maize. Hence people in lower altitude areas are consuming more aflatoxincontaminated homegrown maize than people in higher altitude areas. Similarly household people with maize harvested in second season around February and March are likely to consume more aflatoxin contaminated maize than thanhousehold people with maize harvested in first season around August/September.

Several recommendations are made from the findings of this study. The is need for focused interventions targeting aflatoxin prevention in general and specifically in high risk low lying areas, and maize harvesting 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. This can go along way in sensitizing and encouraging people to adopt improved household maize storage methods. There is urgent need for regulatory authorities to periodically monitor aflatoxin contamination levels of household stored maize by random sampling of households for timely interventions, incase they exceed regulatory limits. There is also need for policy makers and implementers to extend enforcement of quality standards tomaize stored in households.

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