Household Maize Storage Practices and Aflatoxin Contamination in Makueni County, Kenya

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Summary

Introduction: 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 of the study: To determine maize storage practices and their association with aflatoxin contamination of maize in Makueni County, Kenya.

Study design: A cross-sectional study design in Makueni County, with comparative assessment of two sites, to determine household maize storage practices and aflatoxin contamination of maize.

Methods: Four hundred and fifty households (225 from each study site) which had maize in household were randomly sampled and household heads or their representatives interviewed using questionnaires. In addition, a sub-sample comprising 10 percent of the sampled households had their maize samples collected for moisture content determination using Portable Grain Moisture Tester, and for aflatoxin determination using Enzyme Linked Immunoassay (ELISA) Test, and for aflatoxin sub-type determination using High Performance Liquid Chromatography (HPLC).

Results: Majority of households stored their maize in bags which were kept on raised platform. This was followed by storing maize in traditional cribs, storing maize with cobs directly on the floor, keeping maize under or on top of roof and storing maize on improved cribs, but none of the households stored their maize in sealed containers. Majority of households stored their maize properly. However, storing maize in grains form in bags/sacks was preferred by most households. Mean length of storage of maize was about two months. The frequency of store cleaning of maize stores was about three times per two months. This study has shown that duration of maize while in storage (storage time) had significant positive correlation with aflatoxin content in maize (P<0.05). Frequency of store cleaning had significant negative correlation with aflatoxin content (P<0.05). Results further indicated significant association between proper maize storage in households and discoloration of maize (P<0.05). Poor maize storage practices was also associated with pests/insects infestation in stored maize (P<0.05). Moreover poor storage was also significantly associated with moldy infestation and aflatoxin content in maize (P<0.05). Moldy condition of maize had significant association with aflatoxin content while maize affected by pests/insects had significant association with aflatoxin content.

Moisture content in maize had positive correlation with aflatoxin content while ventilation of maize store had significant association with aflatoxin content in maize.

Conclusion: **Households store maize using various methods with the main aim being prevention of contamination and spoilage of maize. Some of the maize storage practices were found to be associated with aflatoxin contamination of maize. There is need, therefore, for households to adopt proper maize storage practices. Policy makers and stakeholders should also encourage households to adopt and practice proper maize practices as this can greatly contribute to reduction of moisture content and aflatoxin contamination of maize.**

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 [1]. Aflatoxins are secondary metabolites from mould of the *Aspergillus* family and include among others *Aspergillus parasiticus* and *Aspergillus* flavus and that produce aflatoxins which contaminate cereals and other foods.

In Kenya, the eastern region particularly Makueni has been seriously affected by aflatoxicosis outbreaks resulting from consumption of maize contaminated with aflatoxins, having experienced three major outbreaks since 1981 to date [2, 3, 4, 5]. This has necessitated authorities and stakeholders to undertake various strategies to control aflatoxin problem. One such strategy is improvement of maize storage at household level. Improvement of post-harvest handling of maize including maize storage has been known to reduce or minimize aflatoxin contamination. Moreover this can also greatly reduce maize losses resulting from post-harvest handling inefficiencies and poor storage [6, 7]. Regulatory authorities have also set regulatory limits of aflatoxin contamination in foods including maize to 10ppb in order to control aflatoxin poisoning [8, 9, 10]

Although maize may be stored in various ways, whatever method or practice is adopted, however, should ensure proper storage conditions are maintained to prevent development of aflatoxins. Besides, any storage structure or method should ensure good drying of maize while in storage. Other studies have also found that storage systems such as type of storage structure, hygiene and insect infestation interact and influence fungal infection and mycotoxins contamination [11].

Despite infrastructure and grain storage practices being able to prevent post-harvest development of mycotoxins in developed countries, it has been noted that this aspect still remains a threat in developing countries, especially in tropical areas [12]. A previous study done in the area found out that varying sources of maize, inadequate households with homegrown maize as well as different storage practices of maize were main challenge to effective storage practices [13]. Further this study indicated that most maize found in household was purchased and not home grown thus making it difficult to determine relationship between storage practices and aflatoxin levels.

Since traditional methods of maize storage are culture sensitive, there is need for finding ways of improving these methods. This view is supported by other studies which found that the wide spread and continued use of

traditional practices by small scale and subsistence farmers despite considerable aflatoxin development risk and considerable loses warrants investigation with respect to improved storage [14].

This paper therefore reports the findings of a study conducted to determine the storage practices of household maize and their influence on aflatoxin contamination, in lower and higher altitude areas of Makueni.

Materials and Methods

Description of Study Site

This study was conducted in Kibwezi and Kilome sub-counties of Makueni County. Kibwezi study site is a low altitude area and its estimated midpoint is located at latitude South 02.40157, and longitude East 037.95143 on South West orientation, at an altitude of 916m above sea level. Kilome study site is a higher altitude area and its estimated midpoint is located at latitude South 01.84098 and East 037.31536 on North East orientation, 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 $884,527(11)$, which in 2012 was projected to $922,183$ with estimated annual population growth of 1.4%. Physiographically, Makueni 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. 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. Maize is the main food crop produced and is staple food for residents.

Study design and Setting

This was cross-sectional comparative study to determine household maize storage practices and aflatoxin contamination of maize in Kibwezi and Kilome sub-counties of Makueni County.

Sampling of Households and Subjects

Two geographically and ecologically different zones namely Kibwezi and Kilome 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 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 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 be determined using a formula as used by Fisher *et al.* (1998) which gave a minimum of 225 households for

91

each study site. 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 these 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 the 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 if he/she consented was then requested to sign the consent form and after which he/she was recruited to participate in the study.

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.

Data Collection

Questionnaires/interview schedules, checklists, in-depth interviews schedules and focus group discussion guides were used to collect data from the study sites. Questionnaires/Interview schedules were used to obtain data from respondents in Kiswahili, Kikamba, or English, but the recording was in English. Information collected 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, ,and $3)$ maize storage practices. A checklist was used as a guide for collection of information on visual assessment of condition of maize as observed during storage as well as the condition of the storage structures.

Focus group discussions (FGDs) with key community opinion leaders/informants using FGD guides and in-depth interviews with agricultural and public health workers were also conducted to corroborate information collected using questionnaires/interview schedules.

Maize sampling

Maize samples were obtained from 10 % of sub-sampled households with maize which were selected for study and interviewed using structured interview schedules/questionnaires. A one kg of maize sample was taken from maize found in the sub-sampled household. The sample was taken 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 of maize harvest.

Maize sample analysis

Moisture content was determined in the field during collection of maize samples and was determined using Portable Grain Moisture Tester model GMK303 powered by 9volts battery according to manufacturer's instructions, calibrated for maize, beans, peas, green grams and millet etc. and had accuracy of +or -0.5% with measuring range of $5-35\%$ moisture content.

92

Before laboratory analysis, the maize samples were visually inspected for insect/pest infestation, mold or discoloration. The analysis of maize samples for aflatoxin was done using ELISA test to determine presence of total aflatoxin content and those samples which tested positive were further tested using High Performance Liquid Chromatography (HPLC) method. The procedures for moisture content determination, ELISA and HPLC tests are briefly described here below.

Moisture Content Determination

Moisture content was measured in the field using Portable Grain Moisture Tester/Meter as per manufacturers' instructions.

Determination of Aflatoxin contamination

Determination of Aflatoxin contamination was done using Enzyme Linked Immunoassay (ELISA) test, and for those samples which tested positive in ELISA test determination of aflatoxin sub-types was done using High Performance Liquid Chromatography (HPLC) method.

Enzyme Linked Immunoassay Aflatoxin (ELISA) Test

Extraction of Sample: One Kilogram (Kg) of maize sample was grinded into flour with a mill and then homogenized. Then 20 Grams (g) homogenized sample were weighed and 20ml of 70% Methanol were added into the sample. They were mixed for 2 hours (hrs) 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: Five (5) g in 25 millilitres (mls) (70% methanol) of extract were taken. And 10 % of methanol in Phosphate Buffered Saline (PBS) was prepared. Then 5mls of 10% methanol PBS were passed through without letting it dry. A sample comprising 1ml of extract and 6mls water was then applied and let run slowly at the rate of 1 drop in 3 seconds. Distilled water-15mls was then applied and passed slowly at rate of l drop per second. Then air was passed to dry and the column was put to a receptacle for eluent. One (1) millilitre (ml) methanol (100 %) was then applied and passed slowly into receptacle.

Cleaning up 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 $2*10$ml 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 200 microliters (µl) Trifluoroacetic acid (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 0.2 micrometer (µm) membrane filter into a vial.

Enzyme Linked Immunoassay Aflatoxin (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

93

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 nanometre (nm). Reading was done within 30 minutes after adding stop solution.

High Performance Liquid Chromatography (HPLC) Test: Sample Extraction was done by taking One 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 adding five (5) g of solid extract were 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 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/minute (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 min. Then 800µl Acetonitrile: water (30:70) was added and dissolved using a sonicator. They were then filtered through a 0.2 µm membrane filter 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 were 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, frequency tables, bar charts, and Pie charts. Descriptive statistics such as frequencies and means were applied in order to group and summarize data to facilitate presentation.

Chi–square test for independence was used to determine association of categorical variables such as age, education, occupation, maize storage practices. Pearson Correlation coefficient was used to analyse relationships of quantitative variables among different storage and pre–storage practices, and Aflatoxin levels.

94

Tests of significance were at ?=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.

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. Fifty eight per cent (58%) of respondents were female and 74.2% of them were married. Over half (61.7%) of them had attained primary education. The main occupation of respondents was farming (79.2%) and farming was their main source of income for households (75.0%)) with 68.4% of them earning less than Ksh.5000 (mean income was Ksh.4800), implying that majority of the people were poor. Households had an average of five people.

Maize Storage Methods/ Practices in Households

Maize storage in households can influence contamination of maize with aflatoxin depending on storage methods/practices as well as conditions of storage. Respondents were therefore asked to state where they stored their maize for current and future use. In Kibwezi 73.3% of households stored their maize in bags placed on raised platform, 3.3% stored their maize in bags placed directly on the floor, 15.8% on traditional crib, 6.3% in cobs, directly on the floor, 0.8% under or top of roof of building (arctic) and 0.4% on improved crib. In Kilome 82.5% stored their maize in bags placed on raised platform, 14.6% in bags placed directly on the floor,1.3% on traditional crib, 0.8% on improved crib, 0.4 % on under or on top of the roof of building(arctic) and another 0.4% stored in cobs placed directly on the floor. None of the households in both study sites shelled and stored maize in sealed containers. Table 1 shows types of storage practices/methods in households.

Table 1: Types of storage practices of maize in households

Storage practices	Kibwezi	Kilome
	Frequency (%)	Frequency (%)
In bags placed directly on the floor	8 (3.3)	35(14.6)
In bags placed on raised platform	176 (73.3)	194 (80.8)
Under or on top of the roof of building(arctic)	2 (0.8)	1 (0.4)
In cobs placed directly on the floor	15 (6.3)	1 (0.4)
On traditional cribs	38 (12.8)	5 (2.1)
On improved cribs	1 (0.4)	4 (1.7)
Shelled and stored in sealed containers	0 (0.0)	0 (0.0)
Total	240 (100)	240 (100)

Proportion of Households which stored Maize either properly or improperly

Storage of maize practice was further categorized into proper storage and improper storage. Proper maize storage was maize which was stored either in raised platform, granary, traditional cribs or improved cribs. Improper maize storage was maize which was stored placed directly on the floor regardless of whether in

bags or in cobs form. In Kibwezi 209 (87.3 %) of household maize was stored properly while in Kilome 204 (85.0 %) was stored properly. A total of 21 (12.7%) of sampled households in Kibwezi and 36(15.0%) sampled households in Kilome did not store their maize properly.

Duration of maize in store and frequency of store cleaning

Length of stay of maize in storage could have an effect on occurrence of aflatoxin contamination of maize. Respondents were asked to state how long there maize had been in storage in households. This was then computed into average (mean) duration of storage. In Kibwezi the mean storage duration was 63.5 days (95% Confidence Interval (CI) =54.7 to 72.4) while in Kilome it was 64.9 days (95% CI=58.9 to 70.8).

The importance of maintaining stores clean at all times so that maize is stored in a clean and hygienic environment has influence on aflatoxin contamination. In this study respondents were further asked how often they cleaned their maize stores since they stored maize which was harvested in previous season. This was then calculated to mean frequency of store cleaning. In Kibwezi the mean frequency was 2.5 times (95% CI=2.27 to 2.68) while in Kilome it was 2.8 times (95% CI=2.62 to 3.04). When this frequency is rounded up it turns out to be about 3 time's in terms of frequency of store cleaning.

Insect pests affecting maize in storage

Insect pests may affect maize in storage if precautions are not taken to prevent their infestation. Maize in storage was inspected to observe whether there were any insect pests which had attacked/infested stored maize. In Kibwezi 68(28.3%) had their maize infested with pests/insects while 172(71.7%) were not. In Kilome 98(40.8%) were infested with insect pests while 142(59.2%) were not. Pests infesting maize were mainly weevils.

Measures taken on insect pests affecting maize in household storage

Households whose maize was infested with insect pests took various actions to try to get rid of insect pests. In Kibwezi 75.6% sprayed with pesticides, 4.9% applied herbs, 12.2% did nothing while 3.7% did not know what to do. In Kilome 63.7% sprayed with pesticides, 11.9% applied herbs, 14.0% did nothing while 5.2 % did not know what to do. Other actions taken included sieving and smoking which accounted for 3.7% in Kibwezi and 5.2 % in Kilome. Table 2 shows action taken to remove insect pests affecting maize in household storage.

Table 2: Action taken to remove pests/insects affecting maize in household

Action taken to remove pests affecting maize	Kibwezi	Kilome
	Frequency (%)	Frequency (%)
Sprayed with pesticides	51(75.6)	62 (63.7)
Applied herbs	3 (4.9)	12 (11.9)
Others	3 (3.7)	6 (5.2)
Did nothing	8 (12.2)	13 (14.0)
Don't know	3 (3.7)	5 (5.2)
Total	68 (100)	98 (100)

Conditions of Maize Stores/Storage Facilities

Condition of maize store or storage facility has a bearing on hygienic and safe storage of maize for later use. It is therefore important that these storage facilities are constructed and maintained clean and in good condition. Various storage conditions were therefore observed using a checklist. Regarding cleanliness, 54.2% stores were maintained clean in Kibwezi while 76.2% were maintained clean in Kilome. Regarding ventilation, 37.1% of stores in Kibwezi were properly ventilated naturally while in Kilome it was 69.6%. Moreover, 40.0% of stores in Kibwezi had adequate natural lighting while (72.9%) of stores in Kilome had adequate natural lighting. Besides, 67.9% of stores in Kibwezi were overstocked with other non-food items while in Kilome overstocking of stores with non-food items was 36.3%. Table 3 below shows storage conditions observed.

Table 3: Storage conditions observed

Parameter observed	Status	Kibwezi	Kilome
		Frequency (%)	Frequency (%)
Cleanliness condition of store (%)	Clean	130 (54.2)	183 (76.2)
	Dirty	110 (45.8)	57 (23.8)
Whether maize store is properly ventilated naturally	Yes	89 (37.1)	167 (69.6)
	No	151 (62.9)	73 (20.4)
Whether the store has adequate natural lighting	Yes	96 (40.0)	174 (72.9)
	No	144 (60.0)	65 (27.1)
Whether the store is over stocked (%)	Yes	163 (67.9)	87 (36.3)
	No	77 (32.1)	153 (63.8)

Reasons given for storing maize properly

The respondents were asked to give reasons why maize should be stored properly (i.e. in well ventilated clean store and not placing directly on the floor. They gave various reasons. In Kibwezi 43.8% said they did so to avoid dirt, 40.0% said to avoid rotting/spoilage, 2.9% said to avoid/reduce moisture, 8.8% to avoid aflatoxin contamination, 1.7% said to let maize dry while 2.5% did not know. Similarly, in Kilome 47.5% said they did so to avoid dirt, 19.6% said they did so to avoid/reduce moisture, 19.2 % said to avoid rotting/spoilage, 4.6% to let maize dry while 0.4% did not know. Table 4 shows reasons given for storing maize in well ventilated clean and not placing directly on the floor.

Table 4: shows reasons given for storing maize in well ventilated clean and not directly on the floor.

Reasons for storing maize in well ventilated clean and not directly on the floor	Kibwezi	Kilome
	Frequency (%)	Frequency (%)
To avoid aflatoxin contamination	21 (8.8)	20 (8.3)
To avoid dirt	105 (43.7)	114 (47.7)
To avoid/reduce moisture	7 (2.9)	47 (19.6)
To avoid rotting/spoilage	96 (40.0)	46 (19.2)
To let maize dry	4 (1.7)	11 (4.6)
Others	1 (0.4)	1 (0.4)
Don't know	6 (2.5)	1 (0.4)
Total	240 (100)	240 (100)

Associations/Correlations of Maize Storage Practices with Aflatoxin Contamination Variables

Maize storage practices were analysed with variables related to aflatoxin contamination in maize to determine their associations/correlations using Chi-square test and Karl Pearson's coefficient of correlation. Duration of maize in storage had significant positive correlation with aflatoxin content in maize in Kibwezi (Kcorrelation $r_{,22}=0.411$, P<0.05) but not in Kilome (Correlation $r_{,22}=0.060$), P>0.05). Frequency of store cleaning had significant negative correlation with aflatoxin content in Kibwezi (Correlation $r_{,22}=-0.405$, P<0.05), while in Kibwezi there was correlation but not significant (Correlation $r_{,22}=0.0.105$, P>0.05).

Proper maize storage in households had significant association with discoloration of maize in ($\chi2,_1$ =38.499, P<0.001). Proper maize storage also had significant association with maize affected by pests/insects in storage in both in Kibwezi ($\chi2,_1$ =4.535, P<0.05, and Kilome ($\chi2,_1$ =4.535, P<0.01). It had also significant association with aflatoxin content in Kibwezi ($\chi2,_1$ =3.865, P<0.05), but not in Kilome ($\chi2,_1$ =1.0403, P>0.05). Moldy condition of maize in Kibwezi had significant association with aflatoxin content ($\chi2,_1$ =4.047, P<0.05) but in Kilome association was not significant ($\chi2,_1$ =0.049, P>0.05).

Maize affected by pests/insects in storage had significant association with aflatoxin content in Kilome (Correlation $r_{,22=}$ 0.407, P<0.05), but in Kibwezi association was not significant(Correlation $r_{22}=0.149$, P>0.05). Moisture content in maize had positive correlation with aflatoxin content (Correlation $r_{,22} =$ 0.229, P>0.05).

Aflatoxin Contamination of Maize

Sub-samples of household maize grains were collected from Kibwezi and Kilome analyzed using ELISA test with cut-off of 1.75micrograms per Kilogram (µg/Kg). The results showed that out of the 24 maize samples from Kibwezi and 24 maize samples from Kilome analyzed using ELISA test with cut-off of 1.75 µg/Kg, 6 (25.0 %) samples from Kibwezi tested positive and 1 (4.2%) maize sample from Kilome tested positive for aflatoxin. There was high aflatoxin positivity rate for maize harvested in low altitude area (Kibwezi) than maize harvested in high altitude area (Kilome).

Further analysis using HPLC method for aflatoxin sub-types quantitative determination showed that in Kibwezi

the mean aflatoxin B1 content was 30.08 µg/Kg, aflatoxin B2 was 0.88 µg/Kg, aflatoxin G1 was 0.48 µg/Kg, aflatoxin G2 was 0.25 µg/Kg, and total mean aflatoxin was 31.71 µg/Kg. Whereas in Kilome site the results showed that the mean aflatoxin B1 was 1.55 µg/Kg, aflatoxin B2 was 0.1 µg/Kg, aflatoxin G1 was 0.1 µg/Kg, aflatoxin G2 was 0.05 µg/Kg and total mean aflatoxin was 1.8 µg/Kg.

The mean moisture content in Kibwezi site was 12.78% while in Kilome site it was 12.85%. The low altitude maize had higher moisture content than high altitude maize. However in both areas, the mean moisture content was within the recommended limit of 12 to 14.0 %.

Discussion

Maize Storage Practices

Harvested dried maize intended for later use in the household, is usually subjected to different storage practices which include keeping or preserving them in a storage facility. As noted, maize storage practices and methods should ensure that proper storage conditions are maintained in order to prevent development of aflatoxins [11]. Maize is stored in various ways among the different communities and the form of storage practice or method could greatly determine whether maize will be contaminated or not. Within communities, storage practices vary depending on individual household practices. This study revealed the various maize storage practices used by the households in both high and low altitude areas. These storage practices or methods include storing maize directly on the floor, storing maize in bags on raised platform, storing maize under or on top of the roof of building (arctic), storing maize without removing the outer covering directly on the floor, storing maize on traditional cribs, and storing maize on improved cribs. Although metal or clay silos have been proved to be

viable for maize storage in [15], they were not found to be in use in the area. The storage practices observed were not much different from storage practices in other communities [7].

Analysis of the methods practiced in the area showed that majority of households, stored their maize grains in bags/sacks on a raised platform. A significant number of households stored their maize in bags directly on the floor while few other households stored their maize on traditional cribs. Only very few households stored their maize on improved cribs implying that adoption of this storage method, though highly recommended by authorities, is low. Storage of maize in gunny plastic bags are not appropriate as they do not allow air circulation in maize thus making maize vulnerable to fungal growth and subsequent aflatoxin contamination [6].

The results show that granaries which have traditionally been the main grain storage facilities in households were no longer commonly used nowadays. This could be probably because they are usually located outside the house and thus making maize prone to theft. Results further showed that storing maize on improved cribs which was a recommended method of maize storage by authorities was practiced by few households, and indication that acceptability of this method was low despite public education on the importance of the same. Maize may also be stored in grain form in clay containers or mud silos but these storage methods were not found in Makueni although they are considered unfavorable for proper drying of maize, particularly in humid and semi humid conditions since they do not allow air circulation [16]. This could be because in Makueni especially in low altitude areas the climate is humid, or perhaps these methods were not culturally acceptable, feasible or convenient to household owners.

99

There was no much difference in terms of maize storage practices among the high and low altitude areas. This implied that acceptance and adoption of awareness messages about aflatoxin and storage practices of the two communities were almost similar. This was supported by socio-cultural information which showed that these communities have no much difference in terms of socio-cultural characteristics. This is also consistent with other studies which found the same [17]. However there were more households in high altitude area storing maize in bags directly on the floor than low altitude area. Conversely there were more households in low altitude area storing maize in traditional cribs than households in high altitude area.

Analysis of storage practices showed that majority of households stored their maize properly i.e. they stored their maize either in raised platform, granary, traditional cribs or improved cribs. However a few did not store their maize properly i.e. they stored their maize by placing them directly on the floor either in form of grains packed in bags/sacks or in cob form. Nevertheless there was no significant difference between households in high and low altitude areas (P<0.05).

It has been observed that any proper maize storage structure or method should ensure good drying of maize while in storage [11]. This may be either airtight storage or non-airtight storage, but airtight storage has been found to be superior to non-airtight storage owing to its effectiveness in preventing infestation by insect pests [18]. Proper storage of maize is vital to prevention of mold growth and aflatoxin contamination as previous studies have shown that infrastructure and proper grain storage practices in developing countries can prevent post-harvest development of mycotoxins [12]. For instance methods of drying and storing maize in elevated granaries were found to be protective against aflatoxin development [19]. Granaries being elevated platforms isolate maize from spores and insects on the ground. For this reason, among others, traditional improved granaries which are raised structures that are well ventilated thus promoting proper drying of maize are more appropriate maize storage facilities compared to others mentioned above. It has also been observed that the improved crib in its many forms acts as both a dryer and storage structure [20].

The other aspect of consideration in maize storage is the length of time (duration) of maize while in store as this could have a likely effect of increasing aflatoxin contamination. In this study household maize was found to have been stored for average period of two months and there was no significant difference in storage time between high and low altitude areas. A study done in Uganda found out that aflatoxin levels increased with storage time [5]. The Uganda study also showed that maize stored for more than six months had higher aflatoxin contamination levels than maize stored less than six months.

It is important for maize stores to be maintained clean in order to prevent maize contamination. The results of this study have showed that household owners cleaned their maize stores about three times in each maize storage season in both high and low altitude areas, and there was no significant difference in frequency of cleaning between the two areas. Given that the estimated storage time for maize was two and half months, this implied that cleaning of maize store was done once per month. However, there is need for further studies to be done to determine association of frequency of store cleaning and aflatoxin contamination.

Insects and other pests may affect maize either in field or in storage if proper precautions are not taken to prevent their infestation. About a third of stored maize was found to be infested with pests/insects in varying levels, but infestation was slightly higher in higher

100

altitude than in lower altitude areas. The kind of insect pests infesting maize were found to be mainly weevils.

Household owners whose maize was infested with pests/insects took various measures to try to get rid of them. About three-quarters in low altitude area and nearly two-thirds of households in high altitude area which had their maize infested with insect pests reported that they sprayed with pesticides to get rid of pests while a few said they applied herbs. This implied that majority of households were concerned with pest infestation of maize and at least did something to get rid of pests/insects. However, there were few household which did nothing to get rid of insect pests while few others did not know what to do to get rid of insect pests. According to a recent study, insect damage of host (maize) is major determining factors in mold infestation [21]. Damaged grain is more prone to fungal invasion and subsequent aflatoxin contamination. Study has shown that when grain is attacked by insects, moisture accumulates from insect activities, thus providing ideal conditions for fungal activity [22]. In order to avoid moisture and fungal contamination, insect infestation should be kept to a minimum or eliminated completely.

Physical conditions of household maize stores or storage facilities have a bearing on hygienic and safe storage of maize as a way of preventing or minimizing aflatoxin contamination. It is therefore important that these stores or storage facilities be constructed and maintained in a good and clean condition at all times. This will go a long way in preventing mold growth and consequent aflatoxin contamination of maize. Results of this study have shown that over half of household owners maintained their stores clean with more households in higher altitude area maintaining their stores clean than households in lower altitude area.

Regarding ventilation of maize stores, two thirds of stores in higher altitude area had proper ventilation while only a third in lower altitude area had proper ventilation. Ventilation is important because the rate of uniformity of drying when maize is in storage is controlled by movement of air through the loaded store [20]. On natural lighting, over two thirds of maize stores in high altitude area had adequate natural lighting while in low altitude area slightly over a third had adequate natural lighting. On overstocking of maize stores with non-food items, two thirds of stores in low altitude were overstocked with other items while only a third in low altitude was overstocked. Based on these observations, there is need for advocacy for proper construction of maize stores/granaries as well as maintaining them in a good clean condition and with adequate ventilation and lighting and also not overstocking with other non-food items. Maize cribs, in particular, should be properly designed, constructed and operated/maintained for proper storage of maize.

There were also other extrinsic factors hindering proper storage of maize in households. During FGDs it was reported that theft of maize while in storage in outside granaries discouraged people from storing their maize in outside granary cribs. Improving the general security in the community will therefore go a long way in improving maize storage in households. They also considered costs involved for constructing proper maize storage facilities such as improved cribs as prohibitive, an aspect which needs to be addressed by relevant authorities and stakeholders.

Aflatoxin contamination of household maize

The study results have revealed existence of 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 [8, 9, 10]. There was significant variation in aflatoxin contaminated maize between low and high altitude areas of Makueni County. The prevailing cooler climate in higher altitude areas, which

101

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 could have been unfavorable for fungal growth and aflatoxin development.

The existing levels of aflatoxin contamination of household stored maize found in this study were almost consistent with the 25% estimate of aflatoxin contamination of food by Food and agriculture Organization [9], thus indicating continued existence of likely risk of exposure and likelihood of occurrence of aflatoxicosis condition in humans.

The results further indicated that the most common strain/type of aflatoxin in both study sites was Aflatoxin B1 followed by Aflatoxin B2. However lowlands had higher Aflatoxin B1 mean aflatoxin contamination than highlands. The presence of Aflatoxin B1 and Aflatoxin B2 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 [21].

The moisture content of maize was slightly higher in maize harvested in lowland area than in highland area. 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 [5]. These study findings have demonstrated that levels of aflatoxin contamination were quite high especially in lowland areas, thus exceeding permissible limits of 10 µg/kg or 10 parts per billion (ppb) for humans adopted in Kenya and many other countries for guiding intervention action points [8, 10, 23].

Maize or foods exceeding permissible limits is not supposed to be used as food or animal feed [23]. Owing to this limit, focus has been 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 household maize, thus underscoring the need for proper maize storage at households.

Maize Storage Practices affecting Aflatoxin Contamination of Maize

This study has shown that duration of maize while in storage (storage time) had significant positive correlation with aflatoxin content in maize particularly in lower altitude areas of maize harvest. This finding indicates that the longer the maize is stored the higher its aflatoxin contamination. This was demonstrated by increased aflatoxin content for maize that stayed longer in storage. This is consistent with a study done elsewhere which showed that aflatoxin levels increased with storage time [5].

Frequency of store cleaning had significant negative correlation with aflatoxin content. This implied that the more frequent the store is cleaned the less the likelihood of aflatoxin contamination of maize. The implication of this is that if the store is not cleaned frequently dirt will accumulate thereby creating favorable condition for mold growth and likelihood of aflatoxin production. As a result of this finding there is need for maize stores to be maintained clean by cleaning them more frequently. This will go a long way in minimizing mould growth and subsequent aflatoxin contamination of maize while in storage.

Results further showed significant association between proper maize storage in households and discoloration of maize (ie. maize losing its original color). This means that when maize is stored properly it is less likely to be discolored. This finding thus underscores the need for proper maize storage to minimize discoloration which leads to moldy infestation

102

particularly in lower altitude areas. Proper maize storage was also associated with pests/insects infestation in stored maize in both high and low altitude areas, indicating that maize properly stored maize are more likely to have less insect pests infestation. Moreover proper storage was also significantly associated with moldy infestation and aflatoxin content in maize in lower altitude area, implying that properly stored maize was less likely to develop aflatoxin.

Moldy condition of maize had significant association with aflatoxin content, further confirming that certain type of fungi (mold) when it infests foods/maize produces aflatoxin [21]. Maize affected by pests/insects had significant association with aflatoxin content. The more maize is affected by insect pests the more likely for it to develop aflatoxin. Damage to maize grains by pests/insects render them susceptible to moldy infestation resulting in aflatoxin contamination.

Moisture content in maize had positive correlation with aflatoxin content implying that the increase in moisture content is likely to increase aflatoxin content. In addition, ventilation of maize store had significant association with aflatoxin content in maize in lower altitude areas, thus underscoring the importance of proper ventilation of maize stores in order to minimize or eliminate aflatoxin contamination.

In conclusion, the findings of this study have revealed that despite prevention and control of aflatoxin contamination of maize being a challenge, households were using various storage methods with the main aim of most of them being prevention of aflatoxin contamination and spoilage of their maize. Results have showed that majority of households stored their maize in bags kept which were kept on raised platform. However none of the households stored their maize in sealed containers although they are considered to be better storage methods.

Maize storage conditions should not allow insect pests infestation and moldy growth as this had significant association with aflatoxin content. In addition, moisture content in maize should be kept at $12-13\%$ level as higher moisture content will likely contribute to aflatoxin contamination in maize. Besides maize stores should be properly maintained and ventilated as this had significant association with aflatoxin content in maize especially in lower altitude areas.

From these findings it is recommended that policy makers and stakeholders should promote household positive maize storage practices which include use of properly constructed, ventilated and maintained stores such as improved maize cribs. This will prevent storing maize in environment favorable for mold growth and aflatoxin contamination. Relevant authorities should combine efforts with other stakeholders to guide, and if possible, support households in construction of low cost improved maize cribs. There is also need for experimental studies on specific maize storage methods to determine their effects on aflatoxin contamination.

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