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FACTORS INFLUENCING MYCOTOXINS PRODUCTION IN MAIZE (*ZEA MAYS* L.) AND GROUNDNUTS (*ARACHIS HYPOGAEA* L.) IN SUB-HUMID, SEMI-ARID AREAS OF EASTERN CENTRAL, TANZANIA

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ABSTRACT

The aim of this study was to assess factors influencing mycotoxins contaminations in maize (*Zea mays* L.) and groundnuts (*Arachis hypogeal* L.) in Kilosa District, Tanzania. The data for the research was collected by use of questionnaires. There were several post-harvest practices that were positively related with mycotoxin contaminations. Mycotoxin development in maize and groundnuts was positively related to shelling of the stored produces by using machinery (p=0.022), insect damage (p=0.012), storing maize and groundnuts in the same storage room from year to year (p=0.006), heaping of maize on the floor in a house (p=0.004) and storing the produce in shelled form (p=0.042). Storage practices associated with lower mycotoxin level were; sorting of damaged spoilt cobs, drying the produce after three (3) weeks and use of traditional storage protectants. Control measures of mycotoxins suggested by this study include early harvesting, rapid drying to the required moisture content, sorting, sanitation, insect control, use of botanicals and synthetic chemicals as storage protectants.

INTRODUCTION

Mycotoxins are secondary metabolites produced by a wide variety of fungal species leading nutritional losses and representing a signi cant hazard to the food chain (Mankeviciene *et al*., 2011).They frequently contaminate the crops in the field and/ or during storage (Smith *et al*., 2012). The most important mycotoxins in maize are the Aflatoxins, Fumonisins, Deoxynivalenol, and Ochratoxin (Kimanya *et al*., 2012). Aflatoxin is a group of mycotoxin produced as secondary metabolites by the spoilage of two fungi species *Aspergillus flavus* and *Aspergillus parasiticus* (Marin *et al*., 2013; Feng *et al*., 2011). Fumonisins are mycotoxins synthesized mainly by *Fusarium verticilloides* and *Fusarium proliferatum* (Garrido *et al*., 2012). Deoxynivalenol (DON) is a common type of mycotoxins produced by pink mould *F. graminerarum* (Garrido *et al*., 2012). Ochratoxin is other types of mycotoxins mostly produced by *Penicillium verrucosum*, *Aspergillus ochraceus*, *Aspergillus niger* species (Lai *et al*., 2014). The Food and Agricultural Organization (FAO) has estimated that one-quarter of the world's food crop is contaminated with mycotoxins (JECFA, 2001).

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The production of mycotoxins depends on various factors, such as poor agricultural and harvesting practices, improper drying, handling, storage conditions, insect damage, drought and inadequate storage conditions, climatic conditions and seasonal variations (temperature, relative humidity) (Miraglia *et al*., 2009; Prandini *et al*., 2009). Mycotoxins contamination attracts worldwide attention due to the huge economic losses incurred and their impact on human, domestic animals and trade (Wu, 2006; Chilaka, *et al*., 2012). Mycotoxin contaminations are also detrimental to the health of humans and animals (Mboya *et al*., 2012; Suleiman *et al*., 2013). Dietary exposure to mycotoxins can result in serious health effect both acute and chronic. Ranging from sudden death to deleterious effects upon the central nervous, induction of hepatocellular carcinoma, effects on the cardiovascular, reproductive, pulmonary, and gastrointestinal systems to mention few (Suleiman *et al*., 2013). Severe health problems and death have occurred from mycotoxin exposure. The ingestion of such mycotoxin-contaminated grains by animals and human beings has enormous public health significance, because these toxins are capable of causing diseases in man and animals (Bhat and Vasanthi 2003). In animals, aflatoxin contaminated feeds have been associated with aflatoxicosis, impaired growth, immunosuppression, liver and kidney tumors in rodents and reduced quality of milk and milk products

because of the presence of aflatoxin M_1, a derivative of aflatoxin B_1 (Lizárraga-Paulín *et al*., 2011).

MATERIAL AND METHODS

Description of the Study Area

Studies were carried out in Mamoyo, Rudewa-Batini, Mkalama and Msingisi villages in Kilosa District between August and October, 2010 (see Table 1). The District is located in semi arid, sub humid parts of Tanzania. The district is divided into three physio-geographic units, which also constitute different agro-ecological zones which contribute to the variation of climatic condition of the district. The highest parts of the district found in the Ukaguru, Rubeho, and Vidunda mountains, which is 2 200 m.a.s.l gets annual rainfall between 1 000 mm –1 600 mm, and the area is characterized by moderately fertile well drained soil, comprising of sandy (clay) loam soil. The lowest parts of the district is found in the central and southern plains, which experience an average rainfall of 800 mm–1 400 mm with poorly drained black clay and loamy soils which is suitable for maize, paddy, sisal, sugarcane and onion cultivation. The annual temperature is between 25°C -30°C. The highlands are characterized by hot climate and short rainy seasons with rainfall deficiencies for crop production. The lowlands experience high annual precipitation and warm climatic conditions during and towards the end of rainy season. Farmers in the study villages grow maize and groundnut. The study obtained lists of farmers who grew and stored maize and groundnuts from village government records. The study then randomly selected the farmers for inclusion into the survey. Residents of Kilosa District rely on subsistence and mixed farming as their major source of livelihoods. Maize is the primary dietary staple and the main crop produced. Groundnut is a nutritious and valuable crop with immense untapped potential to improve food security, nutrition, and raise incomes among smallholders in the study area. Groundnuts provide an important source of protein, fats, vitamins, and minerals for communities that struggle with malnutrition. Groundnut is a valuable commodity, and known as an excellent rotation crop that enriches the soil with nitrogen and greatly increases farmers' yields.

Field survey and Sampling

The study sites were purposively selected because of their high maize and groundnuts growing activity. A list of farmers who grew and stored maize and groundnuts were obtained from village government records. The study then randomly selected the farmers for inclusion into the survey. Eighteen (18) farmers were selected from each village for the interview making a total of seventy-two (72) respondents. The target respondents were maize and groundnuts farmers in the selected study villages. Seventy two maize farmers were purposively selected across the three villages. A structured-questionnaire was administered to the farmers. The basic questionnaire was adapted from a similar study by Kaaya *et al.* (2006) in Uganda. The farmers were asked, among other things, questions on the type of storage protectants, storage practices they used after harvesting, length of maize drying period, farmers' and awareness of the insects problems in storage. The study also took some personal observation to get salient information that would help in identifying problems faced by the farmers.

Data collection and research instrument

After reviewing literature on recommended best post-harvest practices in maize and groundnuts, sets of semi-structured questionnaires were developed to investigate empirically the practices used by farmers in maize and groundnuts storage in the study area. The questionnaire for the farmers sought information on farmer's post-harvests practices (on drying, shelling, type of protectants, insect damage and farmers awareness). Face-to-face interview script was used to solicit responses for the survey questions. The questions were standardized to increase interviewer consistency (Fowler, 2002).

Statistical Data Analysis

Survey data were analyzed using SAS software package version 9.1. Linear multiple regression model shown in Equation 1 was used in the analysis of factors influencing mycotoxins production in the study area. Standardized coefficients of regressions were obtained to determine the factors that pose the greatest risk mycotoxins contamination. The standardized coefficient refers to how many standard deviations a dependent variable will change, per standard deviation increase in the predictor variable. Linear multiple regression model check was conducted to:

- Examine collinearity diagnostics for multicollinearity using tolerance and variance inflation factor (VIF). More checking was done when the tolerance was less than 0.20 or VIF was greater than 5 (Cohen *et al*., 2003).
- Examine residual plots for error variance assumptions (i.e., normality and homogeneity of variance)
- Examine influence diagnostics (residuals, dfbetas) for outliers. If the results showed no standardized Dfbeta values of < -2 or > 2, it was concluded that the dataset does not include outliers or influential cases.
- Examine significance of coefficient estimates to trim (i.e., removing insignificant predictors) the model and revising the model and rerun the analyses based on the results of steps 1-4 and finally, the final regression equation was interpreted using the coefficient estimates. Significance was reported at 95% ($p < 0.05$) confidence interval.

Linear multiple regression model used in the study

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + \varepsilon \ldots (1)$$

Where:

$\boldsymbol{y}$	=	is a continuous variable denoting mycotoxin contaminations in µg/kg
β_0	=	is the intercept (the value of the response variable when the explanatory variable is 0)
β_1-β_9	=	are independent variable coefficients showing the marginal effects of the unit change in the independent variables on dependent variable
$\boldsymbol{X_1}$ $\boldsymbol{X_9}$		are independent variables
ε_i	=	is an error term which represents unobservable factors assumed to be independently distributed over the survey period.

The explanatory variables used in the analysis are;

SORT(X_1)=	Sorting before storage
LAT (X_2) =	Leaving to dry after three (3) weeks
SUM(X_3) =	Shelling using machinery
IND(X_4) =	Insect damage of the grain
SSR(X_5) =	Storing the harvested produce in the same room from one season to another season
HOF(X_6) =	Heaping produce on the floor in a storage room
SPS(X_7) =	Storing the produce in shelled form
FAI (X_8) =	Farmers awareness of insect in storage room
UTP(X_9) =	Use of traditional and botanical grain protectants

RESULTS

Nine major factors affecting mycotoxin contamination of stored maize and groundnuts were identified (Table 2). A step-wise linear regression analysis of the factors affecting mycotoxin contamination gave a coefficient of determination (R^2) of 0.74 an indication that the model used was able to explain 74% of the variation in the dependent variable which were explained by independent variables and this value has a big explanatory power in the model. It appears multicollinearity was not a concern because the VIF scores are less than 3 (Table 3). Similarly, the results showed no standardized Dfbeta values of < -2 or > 2 and it can be concluded that the dataset did not include outliers or influential cases (Table 3).

The factors associated with decreased mycotoxin levels in maize and groundnuts were sorting the produce before storage and was significant (p=0.023), leaving to dry after three weeks (p<0.0001), farmers' awareness of the insects problems in storage (p=0.012), and the use of traditional protectants (p=0.040). Mycotoxin development in maize and groundnuts was positively related to shelling of the stored produces using machinery (p=0.022), insect damage (p=0.012), storing maize and groundnuts in the same room from year to year (p=0.006), heaping of maize on the floor in a house (p=0.004) and storing the produce in shelled form (p=0.042). All of these factors increased mycotoxin development in maize and groundnuts.

For mycotoxin production

$$Y = 2.109 - 0.178X_1 - 0.297X_2 + 0.195X_3 + 0.227X_4 + 0.231X_5 + 0.250X_6 + 0.142X_7 - 0.172X_8 - 0.141X_9 ;$$

where
X_1 represents sorting;
X_2 represents leaving to dry after 3 weeks;
X_3 represents shelling using machinery;
X_4 represents insect damage;
X_5 representing storing produce in the same room year to year;
X_6 represents heaping produce on the floor;
x_7 represents storing the produce in shelled form;
x_8 represents farmers awareness of insect damage and
X_9 represents use of traditional protectants.

Table 1. Description of four surveyed villages in Kilosa District, Tanzania

Location	Latitude (South)	Longitude (East)	Elevation (m. a. s. l)	Average Temperature (^{0}C) (Annual)	Average Relative Humidity (%)	Total Rainfall (mm)
Mamoyo	S06.83447^O	E037.04475^O	506.4	26.5	93.3	1,400
Rudewa-Batini	S06.69351^O	E037.12182^O	428.1	25.4	90.2	1,300
Msingisi	S06.21324^O	E036.86646^O	1,357	31.9	76.5	850
Mkalama	S06.08692^O	E036.85160^O	1,270	30.1	66.2	800

Source: Field Survey, 2010

Table 2. Multiple Regression Analysis showing the Factors Influencing Mycotoxins (fumonisins and aflatoxins) contaminations in Maize and Groundnuts (Y) across four villages in Kilosa District, Tanzania

Regression variables	Standardized β-coefficients	T-value	Test Statistic (p-value)	Tolerance	VIF
(Constant)	2.109	4.739	0.000***		
X_1=Sorting before storage	-0.178	2.337	0.023*	0.631	1.585
X_2=Leaving to dry after 3 weeks	-0.297	3.958	0.0001***	0.651	1.536
X_3=Shelling using machinery	0.195	2.342	0.022*	0.528	1.893
X_4=Insect damage	0.227	2.587	0.012**	0.475	2.105
X_5=Storing produce in the same room year to year	0.231	2.853	0.006**	0.560	1.786
X_6= Heaping produce on the floor	0.250	3.003	0.004**	0.527	1.898
X_7=Storing the produce in shelled form	0.142	2.080	0.042*	0.784	1.275
X_8=Farmers awareness of insect damage	-0.172	2.598	0.012**	0.835	1.198
X_9=Use of traditional protectants (kitchen wood ash and neem leaves)	-0.141	2.095	0.040*	0.811	1.234

Adjusted R^2=0.74, β=Regression coefficients, $\beta_1 ... \beta_9$=Regression coefficients for X_1 to X_9
* Significant at p<0.05, ** significant at p<0.01 and *** significant at p<0.001

Table 3. Descriptive Statistics of Factors Influencing Mycotoxins Contamination in stored Maize and Groundnuts

	N	Minimum	Maximum	Mean	Std. Deviation
Standardized DfBeta SORT	72	0.0000	1.0000	0.1389	0.34826
Standardized DfBeta LAT	72	1.0000	2.0000	1.3889	0.49092
Standardized DfBeta SUM	72	1.0000	2.0000	1.3056	0.46387
Standardized DfBeta IND	72	1.0000	2.0000	1.6389	0.48369
Standardized DfBeta SSR	72	0.0000	1.0000	0.9028	0.29834
Standardized DfBeta HOF	72	1.0000	2.0000	1.1389	0.34826
Standardized DfBeta SPS	72	0.0000	1.0000	0.6389	0.48369
Standardized DfBeta FAI	72	0.0000	1.0000	0.1944	0.39855
Standardized DfBeta UTP	72	0.0000	1.0000	0.3889	0.49092
Valid N (listwise)	72				

Source: Field Data, 2011

DISCUSSION

Parameter estimates from the regression model indicated that leaving to dry the harvested maize and groundnuts (-0.297) was the major factor in reducing mycotoxin levels (fumonisins and aflatoxins). Leaving to dry the harvested produce after three (3) weeks had significant effects on the contamination of maize and groundnuts by fumonisin and aflatoxin. A study by Hell *et al*. (2000) reported that farmers in Benin left maize in the field for 2 to 3 weeks after physiological maturity before harvest and therefore, harvested maize may have low moisture content, thereby making the grains less susceptible to fungal growth and aflatoxin contamination. As Tanboon-ek (1989) proposed, field drying on the stalk before harvest, followed by mechanical drying after shelling, were the most effective ways of reducing fumonisin contamination of maize. Siriacha *et al.* (1989) found that if shelled grain was immediately sun-dried the chance of contamination was reduced as compared with that of undried shelled maize. However, lack of storage space, unpredictable weather, labour constraint, theft of the produce, rodent damage and other animals compel farmers to harvest at inappropriate time (Bankole and Adebanjo, 2003).

Sorting before storage such as sorting out diseased, damaged and discoloured maize and groundnuts kernels as well as cleaning before storage were associated with reduced aflatoxin and fumonisin levels. Similar results were reported by Hell *et al.* (2000) and Udoh *et al*. (2000) in Benin and Nigeria, respectively. Udoh (1995) and Hell (1997) found that sorting bad grains reduced losses due to insect attack and aflatoxin contamination after harvest. Sorting out physically damaged and infected grains from produce can result in 40-80% reduction in a atoxin levels (Park, 2002). Therefore, the removal of mouldy cobs increases the chances of preserving good quality grains in storage. Such practices help to reduce the fungal inocula load and infected substrates. This reduces chances of mould proliferation by infecting health kernels and subsequent production of mycotoxins as confirmed by Martin *et al.* (1999). Kedera *et al*. (1999) reported that poor quality maize grains were correlated with higher levels of fumonisins. Hell and Mutegi (2011) also reported that sorting reduced toxin concentrations to safe levels without the production of toxin degradation products or any reduction in the nutritional value of food. This might also help in the management of mycotoxins. Hell *et al*. (2000) observed that cleaning of stores before loading new produce reduced aflatoxins concentration in Benin. There is a close relationship between storage fungi and insect infestation. Jian and Jayas (2012) reported that some storage fungi attract insects and promote their growth, but other prevent through secretion of toxic metabolites. In connection to this, Bruns (2003) found direct association between insect feeding activity, fungal growth and mycotoxin production. Likewise, Setamou *et al*. (1997), detected low levels of mycotoxin for less damaged maize (2%) than in higher damaged maize.

From the regression model, it was found that the application of traditional storage protectants (using kitchen wood ash and neem leaves) was negatively related to mycotoxin concentrations in the stored maize and groundnuts samples (parameter estimate of -0.141 and p-value of 0.040) (Table 2). This finding is similar to other studies in which plant substances were used in vitro to control growth of *Aspergillus* fungi (Cardwell and Dongo, 1994). It was reported that *Aspergillus* fungi would not grow on medicinal plants, and could not lead to aflatoxins and fumonisins on them (Roy and Kumari 1991). Thus, the mixing of plant substances with stored grains may actually reduce the risk of aflatoxin development and controlling it. Also, plant materials may reduce relative humidity inside the grain store through their biomasses, and consequently reduce fungal growth. Ash is used both as inert filler and for its other negative effects on insects. As inert filler, ash works by filling up the space around the seed and impeding the movement of insects as well as in sealed containers, reducing the volume of air available to the insects for respiration. Ash has been reported to damage the cuticle of insects causing them to dehydrate and to have detrimental effect on egg development (Almekinder, 1999).
Beating cobs on a threshing floor also inflicts physical or mechanical damage to the grain making them prone to fungal invasion and therefore mycotoxin production (Tuite *et al.,* 1985; Bankole and Adebanjo, 2003). Similarly, Dharmaputra *et al*. (1994) reported that mechanical damage during or after harvesting on maize grains can provide entry points to fungal spores. Likewise, Fandohan *et al*. (2006) reported that increases in grain damage and cracking create an opportunity for fungi to grow and penetrate the maize grain. Possibly, the use of hand shellers should be promoted in Kilosa District since grain shelled by this equipment is often clean with no mechanical damage (Kaaya *et al.,* 2006).

Insect damage was observed to increase mycotoxin contamination in the storage (parameter estimate of 0.227and p-value of 0.012) (Table 2). This is substantiated with a study by Mutiro *et al.* (1992) who evaluated insect damage and aflatoxin development on maize in traditional and improved storage structures in Zimbabwe. The grain damage by insects and rodents, as well as birds predisposes the crop to colonization by the fungus and aflatoxin contamination and lead to aflatoxin occurrence in groundnuts and maize (Williams *et al.,* 2004). It is well documented that insects propagate Aspergillus spores in the stores (Lynch and Wilson, 1991). As Wright (1992) revealed, *A. flavus* contamination was strongly correlated with high densities of weevils. Insects play a big role in the vectoring of fungal spores and also provide entry holes to fungal organisms through their tunneling activity, both prior to and after harvest (Hell *et al*., 2000). Studies carried out by Hell *et al*. (2008) in Benin and Shabani *et al*. (2015) in Tanzania, reported that insects and rodents were common maize storage problems. Storage pests, in particular *Cathartus quadricollis* and *Sitophilus zeamais*, play an important role in the contamination of foods with fungi, especially those that produce toxins (Lamboni and Hell, 2009). Pest infestation is largely due to improper post-harvest and storage conditions and the level of insect damage influences the extent of mycotoxin contamination (Atanda *et al*., 2011).

Conclusion

Mycotoxin contamination of maize and groundnuts increased with shelling using machinery, insect damage; storing produce in the same room year to year and heaping produce on the floor and storing the produce in shelled form. Several agricultural practices found to reduce aflatoxin and fumonisin contaminations in maize in the storage were factors that might facilitate reduction of aflatoxin and fumonisins levels in stored maize and groundnuts in the study area were identified. These includes sorting or separating foreign materials and broken corn kernels produced during harvesting from clean maize;

removal of residues from the previous harvest or separate old grain from new grain to avoid contamination and transfer of pests from one lot to another, removal of damaged cobs and pods), and removal of any visible unhealthy crops to protect the remaining healthy one and good crop residue management, leaving to dry after three (3) weeks, farmers awareness of insect damage in the stored produce and use of traditional protectants. Hygiene and sanitation from harvest to storage are key factors in eliminating sources of infection and reducing levels of contamination. There should be extensive awareness programmes across all districts in the country. Awareness of aflatoxin and fumonisin problem and management strategies should be extended to inform farmers, traders, processors, extension officers, other agriculture research partners, private sector, government regulatory agencies and the ministry of agriculture about the risk of mycotoxin contaminations.

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