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Post-harvest Practices Associated with Aflatoxins Contamination of Complementary Flours in Bahi District, Dodoma,Tanzania

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Abstract

Aflatoxins are secondary metabolites produced by several species of *Aspergillus* fungi, which occur in food crops due to exposure of pre-harvest and post-harvest conditions. Complementary foods are considered an important source of energy, protein and fat for children aged between 6-24 months. The study was carried out to explore the association between post-harvest handling practices and aflatoxins contamination in maize-based complementary foods. Complementary flour samples were collected from randomly selected household and analyzed by using HPLC. The presence and concentration of aflatoxins B1, B2, G1, G2 and total AFs was detected. About 48.95% of all samples were found to be contaminated with aflatoxins.

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A stepwise linear regression in generalized linear model was used to identify factors that significantly affected contamination of complementary food with aflatoxins. The analyzed sample was found to be less contaminated with aflatoxins B1 across all six villages with range of 0.24-1.39 µg/kg, with mean value of 0.67µg/kg and total mean aflatoxins were found to be 4.79µg/kg. Results indicated that some of the post-harvest handling practices used by parents/caregivers to agricultural produce used to prepare complimentary food are highly associated with aflatoxins contamination ($p<0.05$). The occurrence of total aflatoxin levels in complimentary flour composite across the six villages was significantly associated with insect infestation, maize/cereals stored for more than 12 months, drying on bare ground, uses of pesticides to protect stored maize/cereals ($p<0.05$). Estimate from the linear regression model indicated that, insect infestation (0.3870), and drying on bare ground (0.0856) were positively associated with aflatoxins contamination. This study recommends education and awareness campaign are needed to inform farmers, traders, processors about the risk of fungal growth and toxins contamination.

Keywords: Post harvest practices; Aflatoxins; Complementary flour; Parents; Mycotoxins.

1. Introduction

Aflatoxins are secondary metabolites of fungi, which are produced under exposure of pre-harvest and post-harvest conditions such as high moisture content, high humidity and temperature. Aflatoxins are produced by several species of *Aspergillus* fungi and occur in food crops such as maize, rice, wheat, groundnuts, and oil seeds. Aflatoxins B1 (AFB1), the most potent of the aflatoxins, is classified as a human carcinogen [1, 2] and has been associated with child growth impairment [3-6], suppressed immune function [7] and death due to acute poisoning [5, 8,9]. Children are at higher risk than other populations related to lower body weight, less acidic stomachs, under developed immune systems, and lack of control in food preparation [10].

Complementary foods are non-human-milk sources of food that are given to infants and young children when breast milk alone is no longer sufficient to meet the nutritional requirements [11] and are considered to be an important source of energy, protein and fat for children aged between 4-24 months [12]. In resource-poor communities complementary foods are based on cereals and mostly maize [13]. In many parts of Tanzania for instance, maize forms the main proportion of cereals used in complementary foods together with other cereals like sorghum, rice and finger millet [14]. Maize contains high concentrations of energy sources like fermentable carbohydrates and proteins. However, there is a significant association between consumption of a combination of thin maize porridge and stiff porridge "*Ugali*" with high mycotoxins exposure [15].

Daily consumption of foods contaminated with low levels of aflatoxins can result in chronic exposure associated with impaired growth and kwashiorkor in children, immune suppression, cancer and reduced life expectancy [16-18]. Acute toxicity termed aflatoxicosis with lethal effects can occur when exposed to large doses. The diets of infants in resource constrained area are often cereal-based complementary food that do not provide adequate amount of essential nutrients. Acute and chronic exposure to mycotoxins may cause various human health effects [1,29,20], because these natural contaminants are prevalent in food crops such as maize and related cereals that form basic ingredients of complementary foods to breastfeed, children are at high risk of exposure to

these contaminants by consuming such foods. Apart from the dietary intake, mycotoxins exposure may also occur in utero and through breastfeeding, predisposing children to the risk of chronic exposure from a very early stage of life [20]. Food contaminated by mycotoxins has been recognized as a public health threat [21]. Mycotoxins have been included as priority food contaminants by the Global Environment Monitoring System/Food Contamination Monitoring Assessment Programme [22]. Mycotoxins contamination of maize can occur at post-harvest stages. Post-harvest practices are those practices following harvest and leading up to primary processing such as milling. In food safety trained communities, these processes includes; rapid drying on platforms to avoid direct contact with soil and proper shelling methods to reduce grain damage. Dehulling of maize prior to milling was also found to remove significant amounts of aflatoxins in maize and maize products, with a reduction of up to 92% aflatoxins [23] in addition to sorting to remove bad/molded grain from the lot, use of clean and aerated storage structures, controlling insect damage, good transportation practices and avoiding long storage periods (8-10 months) [24]. Therefore, this study was performed to explore the association between post-harvest handling practices and aflatoxins contamination in maize-based complementary foods in a community in Tanzania where maize is a staple food and used as a main ingredient in preparation of complementary food.

2. Materials and Methods

2.1 Study area

The study was conducted in six villages of Bahi district named; Matitu, Nyerere, Lugongo, Sokoni, Mbuyuni and Miembeni. Bahi district is one of the seven districts of Dodoma region of Tanzania. It is located at 05°57′10″S and 35°18′43″E. The district is part of the semi-arid central zone of Tanzania, which experiences low and short rainfall often erratic, with fairly widespread drought in a year. Bahi district has an annual average rainfall of about 500 to 700 mm and annual average temperature of about 22.6°C, with high geographical, seasonal and annual variation. There are two rather well defined seasons, the short rainy season during the months of December to March or April and the long dry season from April to November. Bahi is among districts producing maize and cereals in the region.

2.2 Study design

A cross-sectional study design was followed by purposive selection of 96 households with infant and young children aged between 6-24 month and have been introduced to complementary food. It has been reported that one in three children under five are stunted, or too short for their age. Stunting is an indication of chronic under nutrition due to consumption of contaminated food supplements [25].

2.3 Household interviews and collection of complementary flour samples

A total of 96 households were interviewed randomly to examine food systems and post-harvest handling of main ingredients used in preparation of complementary flour. Information concerning breastfeeding, complementary feeding and their pre-processes and preparation as well as storage was captured. Complementary flour samples were collected from each household into the paper khaki bags and transported to

laboratory for analysis of aflatoxins.

2.4 Determination of aflatoxins in complementary flour and investigation of post harvesting handling practices of associated ingredients

Aflatoxins were determined in the ready-to-cook complementary flour in accordance with the method described with a slight modification as per Romar's all-purpose laboratory analysis [26].

2.4.1 Extraction and clean-up procedures

25 g of each sample was weighed and mixed with 100 ml of extraction solution of methanol: water (60:40 v/v) into a blender jar.. The contents were mixed at high speed for 3 minutes followed by filtering the slurry using a funnel and Whatman filter number 1 into a 250 ml Erlenmeyer flask. 4 ml of the extract was diluted with 8 ml of phosphate buffer saline (PBS) at pH 7.4. The diluted extract was loaded onto the immunoaffinity column using a syringe and allowed to pass through at a flow rate not exceeding 3 ml/min. The rinsing of the column with 10 ml of distilled water was done twice. After rinsing, the adsorbed aflatoxins were eluted with 1 ml of HPLC grade acetonitrile by passing it through the column into glass vials.

2.4.2 Derivatization of aflatoxins.

To enhance detection and recovery of aflatoxins pre-column derivatization was done by mixing 400µL of the eluent with 600µl of derivatizing reagent (70:20:10 v/v/v of H_20: Trifluoroacetic acid (TFA): Acetic acid). The mixture was vortexed for 20 seconds and then conditioned at 65 $^{\circ}$C for 15 minutes. Afterwards, the mixture was allowed to cool before being injected into HPLC**.**

2.4.3 HPLC analysis

HPLC system (1200 Series Agilent Technology) coupled with fluorescent detector Eclipse XDB-C18 set at the excitation and emission wavelengths of 365 and 450 nm, respectively, was used. The mixture of Water/Methanol/Acetonitrile 50/40/10 v/v/v was used as a mobile phase at a flow rate of 0.8 ml/min. The injection volume of the eluent was 20µL into a C_{18} column,(4.6×150mm, 5µm) set at 40 ˚C.

2.4.4 Validation of the analytical method

The method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), selectivity, precision and recovery.

The selectivity, recovery and precision of the method were evaluated by triplicate spiking of aflatoxins at levels of 1, 5 and 10µg/kg to blank samples. Then these were followed by extraction and quantification by HPLC-FLD using Chemistation Software with the corresponding peaks and concentrations identified in the chromatogram.

2.5 Statistical Analysis

The collected data was analysed using SAS 9.4 from SAS Institute, Cary NC. Seven models were built; one with all villages, and one for each village. A stepwise linear regression in Generalised linear model (HPGENSELECT) was used to identify factors that significantly affected contamination of complementary food with aflatoxin. Aflatoxin levels were log (x + 1) transformed to normalize data before analysis.

3. Results

3.1 Method of Validation

Linearity of the method was evidenced in the individual calibration curves for each aflatoxins presented in figure 3 with coefficient of determination >0.99. With selectivity, the method was considered selective as there were no interfering peaks coinciding with the retention times of the aflatoxins.

The mean recovery of the method were 98%, 102% and 105.5% spiked at levels of 1 ppb, 5 ppb and 10 ppb, respectively. The limits of detection of the analytical method (also defined as the mean value of the lowest concentration plus three standard deviations) of aflatoxins in µg/kg were G1=0.1, B1=0.2, G2=0.25, B2=0.25 while limit of quantification in µg/kg were G1=0.2, B1=0.3, G2=0.4, B2=0.3

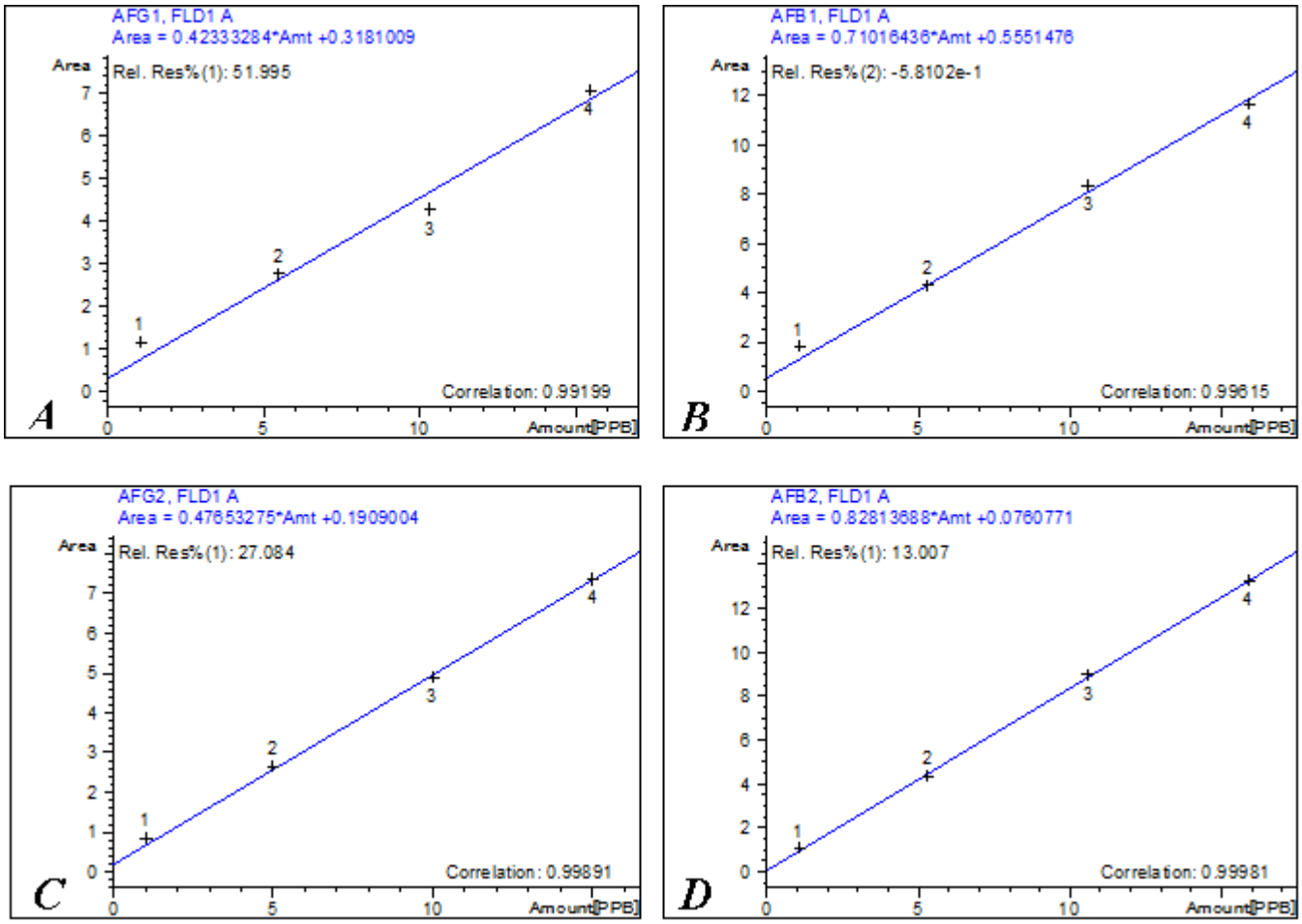

Figure 2: Linearity calibration curves for (A) AFG_1, (B) AFB_1, (C) AFG_2 and (D) AFB_2.

3.2 Aflatoxin contamination of complimentary flour across six villages

A total of 96 complementary flour samples were analysed for aflatoxins contamination and results are presented in (table 1) the samples were found to be less contaminated with aflatoxins B1 across all six villages with rangeof 0.24-1.39 µg/kg, and mean value of 0.67 µg/kg.

The highest aflatoxin mean value was found for aflatoxin G2 (3.76 µg/kg). Total mean aflatoxin was found to be 4.79 µg/kg which is below the regulatory limit of 10 µg/kg total AFs for Tanzania.

Table 1: Occurrence of aflatoxins in complimentary flour across six villages

Aflatoxin	**N**	**Positive samples (%)**	**Range**	**Mean ±SE**
Aflatoxin B1 (µg/kg)	96	4 (4)	0.24 - 1.39	0.67± 0.25
Aflatoxin B2 (µg/kg)	96	19 (20)	0.22 - 3.30	1.17± 0.22
Aflatoxin G1 (µg/kg)	96	11 (11)	0.08 - 4.75	1.14± 0.42
Aflatoxin G2 (µg/kg)	96	25(26)	0.11 - 13.39	3.76± 0.62
Total Aflatoxin (µg/kg)	96	13(14)	1.02 - 13.39	4.79± 0.90

- Values are means of aflatoxin levels of complimentary food sample for positive samples across six Villages.
- Positive samples are all samples with aflatoxins content > Limit of quantification (LOQ)
- n is the total number of samples analysed.

3.3 Aflatoxin levels as relate to post harvest handling practices

In this study mean aflatoxins levels was related to post-harvest practice like storage length, storage facilities, sources of ingredient used to prepare complementary food and drying method.

The occurrence of total aflatoxin levels in complimentary flour composite across the six villages was significantly associated with insect infestation, maize/cereals stored for more than 12 months, drying on bare ground, uses of pesticides to protect stored maize/cereals. Also it was found that the use of molded cereals lead to aflatoxins contamination as were used to feed animals (Table 2).

3.4 Post-harvest handling practices with aflatoxins contamination

Estimate from the linear regression model indicated that, insect infestation (0.3870), and drying on bare ground (0.0856) were positively associated with aflatoxin contamination.

Other practices such as use of pesticides to protect stored maize /cereals (-0.1362), storage for more than 12 months (-0.1924), and aflatoxin awareness (-0.0843) were all found to be negatively associated with aflatoxin

contamination (Table 3).

Table 2: Mean aflatoxin levels as relate to post harvest handling practices

Variables	Mean				
	AFB1	**AFB2**	**AFG1**	**AFG2**	**Total aflatoxins**
Source of maize for your child's food					
Home grown	0.04	0.35	0.06	1.44	1.89
Market	0.02	0.15	0.18	2.39	2.74
Storage problems for crops					
Insect infestation	0.02	0.28	0.07	3.27	3.65
No	0.04	0.18	0.14	1.40	1.76
Storage length					
1 month	N/A	N/A	N/A	N/A	N/A
2-6 months	N/A	N/A	N/A	N/A	N/A
7-12 months	0.03	0.22	0.11	2.16	2.53
More than 12 months	0.00	0.33	0.29	0.45	1.07
Drying of crops					
On bare grounds	0.04	0.17	0.16	1.60	1.97
On roofs	0.00	0.38	0.01	1.36	1.75
On top of platform	0.16	0.33	0.09	0.14	1.69
Storage of crops					
Traditional granaries	0.05	0.54	0.06	1.22	1.87
Roof	N/A	N/A	N/A	N/A	N/A
Bags	0.03	0.25	0.13	2.06	2.45
On the floor	0.00	0.09	0.12	1.45	1.67
Intended use of crops					
Food	0.04	0.26	0.12	1.74	2.15
Market	N/A	N/A	N/A	N/A	N/A
Food and marketing	0.12	0.17	0.15	2.70	3.02

n = number of respondents

Table 3: Association of post-harvest handling practices with aflatoxins contamination of complimentary food (Y) across six villages.

Village/Variable	Regression analysis	Estimate	P value
Across Villages	$Y = 0.64 + 0.39X_1 - 0.19X_2 + 0.10X_3 - 0.14X_4$	0.4003	<.0001*
Matitu Village	$Y = 0.42 - 0.10X_5 - 0.06X_6 + 0.10X_7$	0.1041	<.0001*
Lugongo Village	$Y = 0.41 - 0.28X_8 - 0.08X_9$	0.3253	<.0001*
Nyerere Village	$Y = 0.43 - 0.12X_{10} + 0.19X_{11}$	0.1236	0.0242*
Sokoni Village	$Y = 0.41 - 0.28X_{12} + 0.18X_{13}$	0.3652	<.0001*
Mbuyuni Village	$Y = 0.41 - 0.18X_{14} - 0.19X_{15}$	0.4124	<.0001*
Miembeni Village	$Y = 0.34 - 0.09X_{16} - 0.18X_{17}$	0.4265	<.0001*
X_1 Insect infestation		0.3870	<.0001*
X_2 Maize stored for more than 12 months		-0.1924	<.0001*
X_3 Drying on bare ground		0.0856	0.0024*
X_4 Farmers used chemical pesticides to protect stored maize		-0.1362	<.0001*
X_5 Aflatoxin awareness		-0.0843	0.0143*
X_6 Drying on bare ground		0.0630	0.0248*
X_7 Insect infestation		0.0971	<.0001*
X_8 Maize stored for more than 12 months		-0.2824	<.0001*
X_9 Maize stored on polypropylene bags		0.0231	0.0351*
X_{10} Farmers used chemical pesticides to protect stored maize		0.0624	<.0001*
X_{11} Drying on bare ground		0.1867	0.0024*
X_{12} Maize stored in Polypropylene bags		0.1542	0.0352*
X_{13} Insect infestation		0.1751	0.0024*
X_{14} Farmers used chemical pesticides to protect stored maize		-0.1788	<.0001*
X_{15} Maize stored for more than 12 months		-0.1883	<.0001*
X_{16} Drying on top of platform		-0.0876	<.0001*
X_{17} Maize stored for more than 12 months		-0.1766	<.0001*

Y = Total aflatoxin levels (µg/kg

X = independent variables (practices)

* = Statistically significant at $P < 0.05$.

4. Discussion

This study investigated the influence of post-harvest handling of ingredients used in complementary food on aflatoxins contamination. Most of the flour used for complementary food given to children was found to be contaminated with relatively low levels of aflatoxins G_1, B_1, G_2 and G_2. The literature revealed that, the impact of mycotoxins on human and animal health depends on the gender, age, and length of exposure, dose, and the

amount taken [27,28]. Human intoxication by mycotoxins may occur via dermal contact, ingestion and inhalation. Ingestion through consumption of contaminated food is the most likely and relevant route [29,30].

During preparation of complementary food, different processes were employed, the methods of preparation included, dehulling, sorting, winnowing, roasting and boiling. Majority of respondents reported to sort (81%) and dehull (17%) the cereals before milling, practices which may have contributed to the low levels of aflatoxins contamination of complementary flour. These practices have been reported to significantly reduce aflatoxins in cereal meals [31]. Only few parents (2%) prepared complementary flour from undehulled cereals. The study done in Zimbabwe revealed that undehulled crops had higher percentage of aflatoxin levels when compared to the dehulled crops [32]. In support with previous findings, these results emphasize sorting and dehulling of cereals crops before food preparation and consumption. In Benin-West Africa, women use some unit operations like sorting, winnowing, washing, crushing, and dehulling to remove significant amounts of aflatoxins in agricultural produce used in preparation of complementary food [33. Total mean aflatoxin was found to be 4.79 µg/kg which is below the regulatory limit of 10 µg/kg total AFs in Tanzania and according to the East African Community standards. Several countries have set maximum permissible limits in commodities of food and feeds. These limits are not universal to all the countries. On the other hand out of 96 complementary flour analysed, positive samples were all samples with value above the limit of quantification (LOQ) of 0.25 µg/kg where 48.95 %as found to be contaminated with either aflatoxins B1, B2, G1, and G2 while 51.05%were not detected with any aflatoxins. The sample analysed was found to be less contaminated with aflatoxins B1 across all six villages with mean value of 0.67 µg/kg. The low aflatoxin levels could also be attributed to post - harvest handling practices such as use of pesticides, cereals stored for less than 12 months, well drying of the cereals, harvesting on time and some processes used during preparation of complementary flour like sorting and dehulling. Parameter estimates (coefficient) from the regression model used indicated that, insect infestation was one of the practices that increased total aflatoxins levels across all six villages with a parameter estimate of 0.3870. From Matitu village the parameter 0.0971 in complimentary food (Table 3) means that, for each one unit change in the predictor/independent variable (insect infestation) there was an increase in response/dependent variable (total aflatoxin levels) by -0.241. Insect feeding activity has been found to be associated with fungal infection of maize grain and the subsequent mycotoxins production [31,,34. *Aspergillus flavus* have been known to be facilitated in their infection process of maize and other cereals grain by insect feeding [35]. Poor drying method is one of the practices that can predispose maize and other cereals to fungal infection and aflatoxin contamination. Results from regression model indicated that, drying of maize on ground increases the levels from all the six villages with a parameter estimate of 0.0856, Matitu village (0.0630) and Nyerere village (0.1867). While drying on top of platform was related to low aflatoxin levels at -0.0876. The results from this study are comparable to those reported from Uganda [36] who reported that, drying of maize on bare ground was found to be positively associated with mycotoxin contamination and this may be accelerated where harvested maize are dried without husks. This practice brings maize grains into direct contact with soil which is a primary source of mould as reported [37]. Another reason for having mycotoxin problem when drying on bare ground is that, this may cause an increase in water activity of the grains due to absorption of moisture from the soil and re-wetting by rain [38]. Storage in polypropylene bags was associated with the increase in aflatoxin levels of maize and other cereals with a parameter estimate 0.0231 from the regression

model (Table 3). The parameter estimate from the regression model indicated that, maize storage for less than 12 months had lower total aflatoxin level with the parameter estimate across six villages -0.1924, while it was -0.2824 for Lugongo, -0.1883 Mbuyuni and -0.1766 Miembeni villages (Table 3). This indicates that, storage of maize and other cereals for shorter period of time reduce the level of aflatoxin as reported [18] in Benin that, lower level of aflatoxins were observed with shorter stored grain with duration of 8 - 10 months. The results of this study were contrary to findings [39] in China and [40] who reported a significant increase in aflatoxins with storage duration, from 0.84 µg/kg in 12 months to 1.17 µg/kg in 24 months. Another finding [41] reported that, aflatoxins contamination was facilitated by long-term storage under unhygienic and non-ventilated conditions in Benin and Togo. Farmers from the study area were using pesticides to treat their maize/cereals before storage, with regression model, this showed that treatment of grain with pesticides were found to significantly reduce aflatoxin levels across six villages (-0.1362) and from Mbuyuni village (-0.1788), while from Nyerere village grain treatment was found not to reduce levels of aflatoxins (0.0624). It has been reported that in sub-Saharan Africa, approximately 50 % of the 4.5 million deaths of children under the age of five are associated with under nutrition and growth impairment in which aflatoxin contamination seemingly the main contributor [42].

5. Conclusion and Recommendation

Maize is the main cereal ingredient used for complementary feeding in Tanzania, and is highly susceptible to aflatoxins contamination when compared to other cereals. Most of the parents in this study area are engaged mixed farming like livestock, and thus they found difficult to practice improved post –harvest handling and storage technologies for maize/cereals due to large capital investments. Results indicated that some of the post-harvest handling practices used by parents/caregivers to agricultural produce used to prepare complimentary food are highly associated with aflatoxins contamination. These include, storage for more than twelve months, storage facilities in polyethylene bags, drying on bare ground, and insect infestation. These practices if are well practiced can reduce fungal colonization and mycotoxins contamination. Also sorting and dehulling can always be used before milling of complementary flour as it reduces the amount of toxins. Mycotoxin problems in developing countries can only be handled when the overall food safety, health and agricultural issues are considered together. Therefore this study recommends education; training and awareness campaign are needed to inform farmers, traders, processors, and agricultural officer about the risk of fungal growth and toxin contamination.

6. Limitation of the study

The high costs of sample collection and analysis limited the number of samples that could be collected and analysed. The sample size therefore might not be representative of the actual situation on the ground. However, as a preliminary study, this serves an important purpose as the findings presented here may be taken to the next level in subsequent studies and sample size may be increased to cover a large area.

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Competing interest

The authors declare that they have no competing interests.

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