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Aflatoxins Contamination of Maize at Harvest and during Storage in Dodoma, Tanzania

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Abstract:
This study investigated the level of contamination and factors influencing aflatoxin contamination in maize at harvest and during storage in three major maize producing villages (Manungu, Mlanga and Kongwa) in Kongwa district, Dodoma region, Tanzania in year 2015/2016. The Kibaigwa International Grains market is located in this area. Ninety-one maize samples were analyzed, and 30% (7/23) of the samples were contaminated with aflatoxins (mean 13 µg/kg). Forty one percent of the samples stored for 90 days and 67% of the samples stored for 180 days were contaminated with aflatoxins. Aflatoxins contamination in all maize samples exceeded the East African Standards regulatory limit (10 µg/kg) thus undermining food safety. Factors associated with higher aflatoxin contamination were storage duration and treatment of grains. The storage technique with high risk of aflatoxin development was polypropylene bags without pesticides treatment. Maize stored in polyethylene bags (uncontrolled) for 180 days showed increase in aflatoxin levels with time, mean 19. 06 µg/kg. Low aflatoxin was related to the use of insecticides, sorting and use of improved bags (PICS) with a mean of 5.4 µg/kg at 180 days. Pesticide use decreased pest infestation. Use of improved bags (PICS) reduced the levels of aflatoxin contamination in maize.

Keywords: *Aflatoxin, contamination in stored maize*

1. Introduction

Aflatoxins are naturally occurring my co toxin produced by some strains of moulds such as Aspergillus flavus and Aspergillus parasiticus (Pasone et al., 2010; Sardin et al., 2011). Aflatoxins cause serious problems in many foods but are most abundant in maize and maize products since maize can be infected while in the field under specific environmental conditions (Krnjaja et al., 2013). Contamination of maize depends on the co-existence of susceptibility of hybrids and environmental conditions favorable for proliferation of my cotoxigenic fungi (Blandino et al., 2009).

Maize is one of the most important food crops grown in all regions of Tanzania (Ismail et al., 2015). Maize is cultivated and used as a staple food by the majority of Tanzanians and used as an ingredient for complementary foods. National maize consumption is estimated to be over 3 million metric tons per year, whereas the daily per capita consumption of maize for people in rural areas is estimated to be 450g (Smith and Subandoro, 2012). Maize is the commodity most often contaminated with fumonisins and aflatoxins in Eastern and Southern Africa, as in most of the developing countries (Hell et al., 2005). Aflatoxins contaminate maize from field and during storage, thus making the grains unsafe for consumption. Several outbreaks of aflatoxicosis and associated mortality have been reported in Eastern Africa including Kenya (Moturi, 2008) and in Tanzania in year 2016. These outbreaks have caused a lot of concern because they have worsened the food security and safety status as maize is a major staple food in several households in the country (Smith and Subandoro, 2012). Considering that maize is also a staple food for the majority of Tanzanians, it was necessary to assess storage practices and mycotoxin contamination in stored maize in Tanzania (Shaaban et al., 2015).

The aim of this study was to assess factors influencing aflatoxins contamination in naturally contaminated maize in Kongwa at harvest and during storage as a first and essential step in intervention and selection and management options to mitigate aflatoxins contamination during growth and storage of maize.

2. Materials and Methods

2.1. Study Area

The study was conducted in the three villages of Manungu, Mlanga and Kongwa in Kongwa district, Dodoma, central Tanzania. The district is located between latitude 5˚30' to 6˚0' South and longitude 36º 15' to 36º East. The district borders Kilosa district in the East, Chamwino district in the West, Kiteto district in the North and Mpwapwa district in the South. The elevation of Kongwa district ranges from 900 to 1,000 metres above sea level. Generally, the district lies on the leeward side of Ukaguru Mountains. The Headquarters, Kongwa, bear the same name as the district and is located about 89 kilometres east of the designated capital, Dodoma Municipality. The district covers 4041 km^2 with a total population of 309,973 composed of 149,221 males and 160,752 females (URT, 2012). The district is consisting of three divisions namely Zoissa, Kongwa and Mlali with 14 wards and 67 villages. The main economic activity is farming followed by animal husbandry.

2.2. Selection of Farmers

Ten farmers from each of the three villages were selected to provide samples for aflatoxin analysis representative of those consumed on farm as food and feed from harvest to store. Collection sites were selected based on information provided by village leaders, farmer organizations, District Agriculture and Livestock Development Officer (DALDO) and local extension officers.

2.3. Sample Collection for Aflatoxin Analysis

Ninety-one maize samples were collected, from Mlanga village (32), Kongwa (33) and Manungu (26). A questionnaire was used to collect data on each 1 kg sample collected in each village on day 1 (harvested were properly dried maize ready for storage), after 90 and 180 days in traditional storage and improved storage. The information solicited included; basic demographic details of supplier, processing and storage methods such as use of improved bags namely, Purdue improved crop storage (PICS) bags and traditional storage facility namely, vihenge (cribs) or polypropylene (POP) bags, and knowledge of aflatoxins. The information was catalogued in a database to correlate aflatoxin levels with production, and storage practices.

2.4. Quantification of Total Aflatoxins

Clearly labelled samples were dried to maximum of 13% moisture content to avoid mould growth and subsequent aflatoxin production. Due to the heterogeneous contamination of aflatoxin in grains, sampling is the largest source of variation associated with the analysis of these naturally occurring contaminants (Whitaker, 2004). To address this problem, 1kg samples were milled using the Bunn and Waring grinder® (Mann: Bunn-o-Matic Corporation Springfield, Illinois, U.S.A) and sub sampled in order to produce a blended sub-sample that was representative of the whole sample and cross referenced with codes linking them to accompanying questionnaires. A 50 gm of ground sample was mixed with 50 mL of 65% ethanol (v/v) and shaken vigorously for 3 min using a laboratory shaker (IKA ®. Werker, German). The samples were allowed to settle, and then filtered through Whatman No.1 filter paper (Whatman International Ltd, Maidstone, UK). The extracts were assayed for total aflatoxins using enzyme-linked immunosorbent assay (ELISA) as indicated by the manufacturer's protocol using Reveal Accuscan®III Reader (Neogen, USA). The lower limit of detection (LOD) of the ELISA was 2µg/kg, with a qualitative range of 2-150 µg total aflatoxins/kg. The concentration below the LOD was reported as not detected (n.d.).

Validation was carried out using liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Department of Bioanalyses Laboratory, Ghent University, Belgium, on 30 randomly selected samples previously analysed using Reveal AccuScan ® III reader (Neogen, USA) at the Plant Pathology Laboratory, IITA in Dar es Salaam, Tanzania. There was no significant difference between two methods during this study.

2.5. Statistical Analysis

The data were analysed using Statistical Analysis System (SAS® Version 9.4 (SAS- Institute Incorporation, USA), 2010. A generalized linear model (GENMOD) was run to identify the factors that significantly affected contamination of maize with aflatoxins. The differences in mean total aflatoxins amongst the climatic zones and storage practices were detected using the least square means (LSMEANS). Aflatoxin levels were transformed using the natural log to normalise the data before analysis.

3. Results and Discussion

3.1. Results

3.1.1. Characteristics of Farmers

Overall, 89 % of the farmers interviewed were males. Even though most of the farm workers were female, when requested for an interview, the father of the house was responsible to give information due to local perception. In terms of awareness, only 11% of the farmers had heard of aflatoxins and thought that it was a - "poison found in spoiled maize"-. These insights revealed limited understanding of what aflatoxins were and how they were formed. In terms of formal education, 96% of the farmers had primary school education, 2% had secondary education and 2% had tertiary education (diploma and technical education). (Ref table number)

3.1.2. Harvest and Drying

Harvesting of maize was early August in all three villages. Immediately after harvest, farmers used different means of transport to carry maize to their homes. In all the three villages maize was dried to the safe storage moisture content (13-15%) mainly on the bare ground by 60 %, on platforms by 34% in the field by 6% of the farmers. Some of the maize was stored before it was well dried. Farmers used traditional methods to test moisture content in grains including biting dry grains, sound produced by shaking grains in cans.

3.1.2. Total Aflatoxins Content in Maize

Aflatoxin (mean) levels increased with storage 13.12, 14.75, 19.39 µg/kg, on day 1, 90 and 180, respectively. The observed increase was statistically significant ($p<0.05$) after storage for 180 days (table 3).

3.2. Discussion

3.2.1. Total Aflatoxins in Maize

Aflatoxin contamination in all maize samples was above East African Standards regulatory limit of 10 µg/kg thus posing a health hazard for consumers. This might be due to lack adequate knowledge on the effect of aflatoxin contamination of food, hence there is need to educate farmers on the consequences of aflatoxin contamination in food especially maize, a widely consumed staple and use of best practices along the commodity value chain.

3.2.2. Effect of Storage Structures

Traditional storage techniques used in the three villages were almost similar, and the most commonly used were POP bags stored in the house store. This storage technique was used by 56% of the farmers and the sample collected from it was contaminated with aflatoxins, reported by Mwihia et al., (2008) that nylon sacks and polyethylene sacks maintained moisture and are impervious to free air circulation within the grain store and may promote aflatoxin contamination. Thirty eight percent of the samples stored in improved bags (PICS) were contaminated with aflatoxins although increase was very minimal, Williams et al. (2014) reported that Purdue bags can prevent spoilage by moulds and aflatoxin accumulation and one percent of those stored in cribs in Swahili called "vihenge" were contaminated with aflatoxins. Type of storage used had direct influence on the contamination of grain by aflatoxin. Maize stored in traditional storage structures both POP and cribs had higher levels of aflatoxin contamination compared to the maize stored in improved bags if other variables are constant as shown in table 1.

3.2.3. Effect of Sorting

In the whole population of 30 farmers, 24% of the farmers sorted their maize before shelling and after they were properly dried. The observation was similar to those reported in other studies on the use of sorting to reduce mycotoxin contamination of maize (Afolabi et al., 2006; Kimanya et al., 2009; Matumba et al., (2015). Sorting reduces the level of aflatoxin in maize because it involves removing damaged, discolored and moulded grains that may be heavily contaminated by mycotoxins, reported by Kamala et al., (2015) the likelihood of aflatoxin contamination was more than three times higher for unsorted maize when compared with the likelihood for sorted. Seventy six percent of the farmers did not sort their grains and sold or consumed their grains with bad portions, this may bring heath safety concern.

3.2.4. Use of Insecticides / Pesticides

Infection of stored products by toxigenic fungi and consequent contamination with mycotoxins are generally influenced by many factors including fungal populations, environmental conditions (generally climate, temperatures and humidity, O_2 and CO_2), insects infestation and pre- and post-harvest handling, but in most cases their complex interactions among different factors (Gnonlonfin et al.,2013). In all villages the common pest infesting maize was identified as Sitophillus zeamaiz. It has also been reported that maize weevils and the larger grain borer are the main and most serious pests of stored maize (Holst et al., 2000). Grain treatment with traditional pesticides was related to higher aflatoxin levels compared to farmers who did not apply any insecticides. The effect of the application of insecticides during maize storage reduced aflatoxin contamination was reported in several studies (Hell et al., 2000; Atukwase et al., 2012). Some of the farmers used Chemical pesticide which is 3% Actellic Super a cocktail of 1.6% Pirimiphos-methyl and 0.3% Permethrin. Thirty three percent of the farmers did not use any chemical to store their crops. High levels of insects' infestation were observed in their crops and during test most of the samples were positive in aflatoxin, hence application of insecticides as control measures for aflatoxin in maize should be employed by the farmers. Nyangi et al., (2015) reported that the use of Insecticides reduced aflatoxin contamination of maize. In this study no aflatoxins or lower total aflatoxin levels were found in stored maize that was free of insect damage such as those stored in controlled improved bags (super grain safe bags) as shown in table 2.

3.2.5. Effect of Storage Duration

The influence of storage time on aflatoxin content was noticed for period of 3 – 6 months, which generally resulted in higher aflatoxin content in stored maize samples. Results from analysis indicated that in traditional/no treatment maize as the number of days increased from zero to day 180, there was an increase of total aflatoxin in maize, while those stored in improved bags there was no/or minimal increase in aflatoxin levels. Several authors/investigators have reported an increase in aflatoxins in stored maize, especially in the tropics (Kankolongo et al., 2009: Hell et al., 2000: Tedihou et al., 2012) as shown in table 3.

4. Conclusion

The conclusion drawn from this study was that, storage duration and storage techniques influenced the aflatoxin contamination in maize. Thus, maize grains stored in polyethylene bags (uncontrolled) for 180 days showed increase in aflatoxin levels with storage time. Use of insecticides reduced aflatoxin levels and use of Purdue bags (PICS) was more effective in controlling mycotoxin contamination. An association and interaction between insect and fungi in stored maize was observed.

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Aflatoxins (µg/kg)				
Maize Storage Structure	**Number of Samples**	**Positive Samples (%)**	**Range**	**Means ± SE**
Purdue	34	13(38)	2.30 - 70.40	13.40[a] ± 5.19
POP bags	56	30 (54)	2.70 - 70.50	19.06[ac] ± 2.95
Granaries	1	1 (100)	3.20 – 3.20	3.20[bc]

Table 1: Total Aflatoxins Contamination in Maize Stored in Different Storage Techniques for 180 Days

- Values are means of positive total aflatoxin levels of maize samples stored in different storage structures.
- Means with the different letters (by column) are significantly different (P<0.05).
- Positive samples are all analysed samples with value > limit of detection (LOD)
- POP represents polypropylene bags commonly used for storage.

Parameter	**Estimate**	**Standard Error**	**Chi-Square**	**Pr > ChiSq**
Intercept	0.4452	0.1861	5.7222	0.0168*
Polypropylene bags	0.6142	0.1507	16.6047	<.0001*
Cribs/granaries	1.1895	0.4450	7.1463	0.0075*
Storage at day 180	0.6289	0.1273	24.3964	<.0001*
Chemical pesticides	-0.4363	0.1336	10.6713	0.0011*
Traditional pesticides	0.7222	0.2968	5.9208	0.0150*
6 - 10-year storage facilities	-0.3932	0.1297	9.1937	0.0024*

Table 2: Association of Storage Factors with Aflatoxin Contamination in Maize (Y) Across Three Villages
Aflatoxin $Y = 0.4452 + 0.6142X_1 + 1.1895X_2 + 0.6289X_3 - 0.4363X_4 + 0.7222X_5 - 0.3932X_6$;
** = Statistically Significant at P < 0.05*

Aflatoxin (µg/kg)				
Maize Storage Days	Samples	Contaminated Samples (%)	Range	Means ± SE
Day 0	23	7(30)	3.20-24.50	13.12[ad]±3.56
Day 90	32	13 (41)	2.50 – 43.80	14.75[a] ± 3.08
Day 180	36	24 (67)	2.30 – 70.50	19.39[bc]± 4.25

Table 3: Aflatoxin Content in Maize during Storage.
Values Are Means of Positive Total Aflatoxin Levels of Maize Samples
Means with Different Letters (by Column) Are Significantly Different (P<0.05)

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