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Aflatoxin and Fumonisin Contamination of at-harvest and Storage Beans in Babati District, Northern Tanzania

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ABSTRACT

The natural occurrence of total aflatoxin and fumonisin was determined in 38 bean at-harvest samples and 106 storage beans samples in the year 2013 in Babati District Northern Tanzania. Quantification for total aflatoxin and fumonisin was done using Enzymes Linked Immunosorbent Assay - ELISA (Reveal AccuScan® Neogen, USA), and the results were confirmed using Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS). Eighteen percent (7/38) of bean at harvest samples were contaminated with up to 3 µg/kg aflatoxins and no bean sample was contaminated with fumonisins. There was a significant correlation ($p < 0.05$) between the concentration of aflatoxins and fumonisins with climatic zones and agronomic practices. Only bean samples from the high altitude high rain zone were contaminated with aflatoxins (mean level of 1.53 µg/kg). For storage beans, samples from Seloto village were associated with higher aflatoxin concentration (mean of 3.74 µg/kg) and those from Long village were associated with higher fumonisin levels (mean of 9.0 mg/kg). These results indicate that beans consumers in the study area are exposed to the danger of chronic exposure to aflatoxin and fumonisin poisoning. Thus, those practices that reduce contamination should be adopted by all farmers in the study area to reduce the health hazards associated with consumption of contaminated beans. This also calls for further research to investigate human exposure to aflatoxin and fumonisin in the study area.

1. INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) is major dietary food in most part of Tanzania, with a production of 1 150 000 MT (FAO, 2013). Aflatoxins and fumonisins have been reported to occur in low concentrations in beans (Aiat, 2006; Nyinawabali, 2013). The information on mycotoxin contamination of beans is still scanty in Tanzania.

Aflatoxins, produced mainly by *Aspergillus flavus,* can cause acute and chronic toxicity, immunosuppression, mutagenicity, teratogenicity, genotoxicity and carcinogenicity (Omari, 2013). The International Agency for Research on Cancer classified aflatoxin B_1 as a highly poisonous toxic, class 1 human carcinogen (IARC, 1993). Fumonisins are produced by *Fusarium* spp. mostly by *Fusarium verticillioides* (previously known as *F. moniliforme*) (Omari, 2013; Nyinawabali, 2013). Fumonisins have been associated with human oesophageal cancer in South Africa and in addition to liver cancer in China and stunting and underweight in Tanzania (Marasas et al., 2008, Ueno et al., 1997; Kimanya et al., 2010). IARC classified fumonisin as a group 2B toxin, considered as possibly carcinogenic to humans (IARC, 1993).

Several agronomic practices and factors have direct influence on the contamination of grains by aflatoxins, fumonisins and other mycotoxins. These include; temperature and humidity (Milani, 2013; Atanda et al., 2013). Soil types and nutrients supply (Atanda et al., 2013), tillage method (Janusauskaite et al., 2013; Gil-Sotres et al., 2005), time of planting and harvesting (Abbas et al., 2007; Bruns, 2003; Kahaya et al., 2006) and crop rotation (Atanda et al., 2013).

Although common beans is an important dietary staples in many African countries, including Tanzania, no research has been conducted in Tanzania to establish the relationship between production, handling and storage practices and the occurrence of total aflatoxin and fumonisin in beans. The aim of this study is to determine the effect of pre-harvest field management (agronomic) and post harvest practices on contamination of at harvest and storage beans with aflatoxin and fumonisin.

2.0 MATERIALS AND METHODS

2.1 Study area

The study was conducted in three villages in Babati district, Manyara region, Tanzania, namely, Long, Sabilo and Seloto, representing different climatic zones in the year 2013. The high-altitude high-rain zone represented by Long village, lies between 2150 and 2450 metres above sea levels (m.a.s.l) with a relatively high annual rainfall of 1200 mm. The mid-altitude low-rainfall zone represented by Sabilo village, lies between 1500 and 1850 m.a.s.l and with a production season characterised by relatively low rainfall of 900 to 1100 mm, and the mid-altitude high-rain zone represented by Seloto village that lies between 1850 – 2150 m.a.s.l with a production season; characterised by annual rainfall of 1100 – 1200 mm. The previously recorded temperature in the study area ranged from 12^{o}C in Long village to 25^{o}C in Sabilo village. The selected villages were under the Africa RISING Eastern and Southern Africa project on Sustainable Intensification of Farming Systems supported by USAID's Feed the Future program. Maize and common beans were also the major staple foods.

2.2 Selection of farmers

The farmers who participated in the study were randomly selected using a list provided by the respective village leaders and extension officers. A total of 38 farmers were selected for at harvest beans sampling and 60 farmers for storage beans sampling in three villages.

2.3 Sample collection

A total of 38 bean samples were collected at harvest in March, 2013 and 106 from storage bean samples between March and September, 2013. Information on production practices used by farmers in the three villages was obtained using a semi-structured questionnaire. Responses were elicited on farmers' planted variety, previous crops, pest problems in the field, planted and harvested date, tillage method, planting pattern (flat, on ridges, on mounds), harvested condition (wet or dry), condition of harvested crop (clean or spoiled) and intended use of the harvested crops. GPS coordinates and basic demographic details of farmers/producer were also collected. Responses from the farmers were used to evaluate farming practices. For storage samples, samples were collected at an interval of 0 and 90 days from farmers' traditional storage facilities (i.e., farmers' own storage facilities, either granary or polypropylene bags); The farmers were interviewed using a semi-structured questionnaire. Responses were elicited on farmers' storage practices, storage structures, pest problems in storage, storage treatment, length of storage and farmers' solutions to these problems. GPS coordinates and basic demographic details of farmers/producer were also collected. Responses from the farmers were used to evaluate storage practices and handling techniques.

Samples in the field were taken by walking in two diagonal directions and stopping at regular intervals to pick a sample so as to have a good representative samples. A total of five stops were chosen in each field and 50 pods collected per field, these were then hand shelled, well mixed and approximate 1kg sample was randomly selected. The collected samples were packed in a clean A4 envelope and transported to the Plant

Pathology Laboratory at International Institute of Tropical Agriculture (IITA), Tanzania.

2. 4 Quantification of total aflatoxin and fumonisin

Quantification for total aflatoxin and fumonisin was done according to Nyangi et al. (2016)

2.5 Statistical analysis

Data were analysed using Statistical Analysis System (SAS® Version 9.4, SAS Institute Incorporation, USA). A generalized linear model (GENMOD) was run to identify the factors that significantly affect contamination beans with aflatoxin and fumonisins. The differences between means were detected using least square means (LSMEANS) to establish differences in mean total aflatoxin and fumonisin amongst the climatic zones (Villages) and agricultural as well as storage practices.

3.0 RESULTS

3.1 Total aflatoxin and fumonisin content in beans

Eighteen percent of bean samples were contaminated with aflatoxin (Table 1).

Table 1: Incidence of total aflatoxin and fumonisin in bean samples across three villages

Beans	N	Positive samples (%)	Maximum concentration	Mean ±SE
Aflatoxin (µg/kg)	38	7 (18)	3.0	2.49 ± 0.11
Fumonisin (mg/kg)	38	n.d	n.d	n.d

- Values are means for total aflatoxin and fumonisin levels of beans samples across three villages.
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n = total number of analysed samples
- n.d = fumonisin levels were below LOD
- SE = Standard error

The highest aflatoxin mean value for beans was only found in Long village, with no aflatoxin and fumonisin level detected in Sabilo and Seloto village (Table 2).

Table 2: Incidence of total aflatoxin and fumonisin contamination in bean samples in each village

		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
Village	N	Positive sample (%)	Range	Mean ± SE	Positive sample (%)	Range	Mean ± SE
Long	13	12 (92)	2.0 - 2.4	1.53 ± 0.15	n.d	n.d	n.d
Sabilo	13	n.d	n.d	n.d	n.d	n.d	n.d
Seloto	12	n.d	n.d	n.d	n.d	n.d	n.d

- Values are means of total aflatoxin and fumonisin levels of beans samples from each village.
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n = Total number of analysed samples
- Means with different letters (by column) are significantly different ($p < 0.05$)
- n.d = aflatoxin and fumonisin levels were below LOD
- SE = Standard error
-

3.2 Agronomic practices/factors associated with aflatoxin and fumonisin levels in beans

For aflatoxin and fumonisin contamination in beans, the results indicated that no climatic zone or agronomic practice was statistically significant for effects on aflatoxin or fumonisin levels.

3. 3 Storage practices associated with aflatoxin and fumonisin levels in beans

3.3.1. Storage structures

Traditional storage structures in all three villages were almost similar, the commonly used traditional storage being polypropylene bags and locally made granaries known as *'Vihenge'* in Kiswahili. '*Vihenge*' are made of wooden and woven with twigs or bamboo from surrounding forests and covered with thatch grass or iron sheets and sometimes kept inside a house.

3.3.2. Drying

Drying of beans was mainly done on bare ground and on platform, few farmers were drying their crops on mats/floor. The beans is usually harvested when physiologically mature and transported to farmers' houses for further drying . The raised platform was constructed with medium sized pieces of trees and at a height of approximately one metre above the ground, constructed outside farmer's houses and well protected against animals.

3.3.3. Stores treatment

Farmers were treating their stores against insects' infestation before introducing crops to be stored. Store treatment was done by using either chemical pesticides or natural protectants (plant). Chemical pesticides were sprayed in the store especially on walls, floor and ceiling before introducing crops to be stored. Common pesticides used were Actellic® (pirimiphos-methyl) and Bami force® (Permethrin and Malathion). An alternative treatment involved the use of traditional plants comprised a mixture of dried, ground plant leaves combined with burnt cow dung and sometimes ashes to treat traditional storage facilities (Cribs/Granaries).

3.3.4. Grain treatment

Farmers used chemical pesticides which were specific formulation for stored grains such as Super Shumba® (pirimiphos-methyl and permethrin), Actellic® (pirimiphos-methyl), Bami force® (Permethrin and Malathion) or Zinc phosphate®. Few farmers applied traditional plants as storage protectants; this involves dried and ground plant leaves mixed with burnt cow dung and ashes. The common pest infesting maize was identified as *Sitophillus zeamaiz*. Farmers also complained of rodents as being a constant storage problem.

3.3.5. Storage with other crops

The most common crops that are usually stored alongside maize were beans and few farmers stored maize with wheat, sunflower and pigeon pea. All farmers in the three villages usually cleaned their stores and removed all previous crop residues from the store before introducing new harvest.

3.3.6. Total aflatoxin and fumonisin content in beans

Prevalence, range and mean total aflatoxin and fumonisin beans in each village is reported in Table 3. The highest aflatoxin mean value of 3.74 µg/kg was found in Seloto village, and for fumonisin the highest mean value of 9 mg/kg was found in Long village.

Table 3: Prevalence, range and mean total aflatoxin and fumonisin content in beans in each village

Village		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
	n	Positive samples (%)	Range	Means ± SE	Positive samples (%)	Range	Means ± SE
Long	36	36 (100)	0.4 – 4.6	2.22 [a] ± 0.21	1(3)	0.90 – 9.00	9.0[a] ± 0.25
Sabilo	37	10 (27)	2.10- 3.00	2.64 [a] ± 0.09	27 (57)	0.00 – 0.2	0.08 [a] ± 0.01
Seloto	33	11 (33)	2.10 – 14.2	3.74 [b] ± 1.07	6 (18)	0.40 – 7.90	2.95 [a] ± 0.31

- Values are means of positive total aflatoxin and fumonisin levels of beans samples from each Village.
- Means with different letters (by column) are significantly different ($P<0.05$).
- Positive samples are all analysed samples with value > Limit of detection (LoD)
- n is the total number of analysed samples

Bean samples collected from polypropylene bags had aflatoxin level with a mean value of 3.34 µg/kg, and fumonisin mean value of 3.81 mg/kg (Table 4)

Table 4: Prevalence, range and mean total aflatoxin and fumonisin content in maize and beans stored in different storage structures across three villages

		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
Bean storage structure	**n**	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
POP bags	106	36 (34)	2.10 – 14.2	3.34 ± 0.34	7 (7)	0.40 – 9.00	3.81 ± 1.47

- Values are means of positive total aflatoxin and fumonisin levels of beans 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 as a storage facility.
- n is the total number of analysed samples

The results from the storage time for bean indicated that the mean aflatoxin levels increased from day 0 to day 90. The observed increase was statistically significant at day 90 from the rest of the storage period (P< 0.05). The mean fumonisins level decreases during the entire storage period (day 0 to day 90) and the decrease was not statistically significant (Table 5).

Table 5: Prevalence, range and mean total aflatoxin and fumonisin content in beans during storage across three villages

		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
Storage days	**n**	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
Day 0	55	7 (13)	2.1–4.50	2.73^{a} ± 0.32	4(7)	0.40 – 7.90	3.93^{a} ± 1.99
Day 90	51	29 (57)	2.1–14.2	3.49^{b} ± 0.41	3(6)	0.90 – 9.00	3.67^{a} ± 2.67

- Values are means of positive total aflatoxin and fumonisin levels of beans samples
- Means with different letters (by column) are significantly different (P<0.05).
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n represents total number of all analysed samples

4. DISCUSSION

4.1 Total aflatoxin and fumonisin contamination in at harvest beans

The maximum concentration of 3 µg/kg total aflatoxin (Table 1) was lower than MTL of 10 µg/kg set by East African Community for dry beans and 4 µg/kg set by European Union regulations (EAC, 2011; EC, 2010). This maximum concentration was also lower than 1463 µg/kg one of the highest levels recorded and reported from Egypt, and 154.9 µkg/kg reported from Rwanda (Aiat, 2006; Nyinawabali, 2013).

The fumonisin concentration for all bean samples were below the LOD of 0.3 mg/kg (Table 1), lower than MTL of 2 mg/kg for East African Standards

(EAC, 2011). This maximum concentration was also lower than 7.1 mg/kg reported from Rwanda (Nyinawabali, 2013). All bean samples were considered fit for human consumption because they contained aflatoxin and fumonisin below the permissible levels.

The low aflatoxin and fumonisins levels could be attributed to environmental characteristics of the different climatic zones and different agricultural practices which have shown to have an impact on aflatoxin development. It was reported that soy bean seed coat and integrity acts as a barrier against fungal attack and hence mycotoxins contamination (Stössel, 1986). Other factors being constant, this might be the reason for low levels of aflatoxin and fumonisin reported in beans samples from this study.

4.2. Total aflatoxin and fumonisin contamination in storage beans

Total aflatoxin and fumonisin was quantified in 106 beans samples collected in the year 2013 (Table 3). The highest concentration for total aflatoxin was 14.2 µg/kg; only one sample had aflatoxins levels above permitted levels by East Africa Community standards of 10 µg/kg (EAC, 2011). The highest level observed in this study was lower than 21.48 µg/kg reported by Tseng et al. (1995) in Ontario, Canada and Taiwan; 154.9 µg/kg reported by Nyinawabali (2013) in Rwanda and 0.02 µg/kg reported by Aiat (2006) in Egypt.

The observed maximum concentration for total fumonisin in beans was 9 mg/kg (Table 3), this was higher than the limit of 2 mg/kg set by EAC standards (EAC, 2011). The observed maximum fumonisin level was higher than 1.8 mg/kg of fumonisin B_1 reported by Tseng et al. (1995) in Ontario, Canada and Taiwan; and 7.1 mg/kg reported by Nyinawabali (2013) from Rwanda.

The low aflatoxin levels could be attributed to different agricultural practices that reported to have influence on aflatoxin and fumonisin development (Milani, 2013). Stössel (1986) reported that soy bean seed coat and integrity acts as a barrier against fungal attack and hence mycotoxins contamination, other factors being constant, this might be the reason for low levels of aflatoxin and fumonisin reported in beans samples from this study.

The data from this study support the results from previous studies that reported how the proliferation of aflatoxin and fumonisin interact with storage factors. It was previously reported that aflatoxin and fumonisin was related to storage structures (Hell et al., 2010; Fandohan et al., 2005), insect infestation (Udoh et al., 2000; Hell et al., 2000; Fandohan et al., 2005), length of storage time (Orsi et al., 2000; Egal et al., 2005), climatic conditions (Kaaya and Kyamuhangire, 2006; Milani, 2013), sorting (Hell and Mutegi, 2011), Drying methods (Atukwase et al., 2009).

5. CONCLUSIONS

The results indicated that some of the production practices used by farmers and the environmental conditions that prevailed in the production area predisposed beans to contamination with aflatoxin and fumonisin. As control of the environmental conditions is difficult, farmers should adopt good agricultural practices such as timely planting, fertilizer applications and proper land tillage in order to reduce fungal proliferation and elaboration of mycotoxins in maize and common beans.

Several storage factors that may help to reduce aflatoxin and fumonisin levels in stored beans in the study area were identified. These included control of storage insects and mycotoxins levels by application of insecticides; treating storage structures with pesticides; hygiene and sanitation by removing previous year residues and awareness creation to farmers of the risk of aflatoxin and fumonisin to their crops and health. Further research are required to show how shelling, drying, insects infestation, storage form and storage structures influences aflatoxin and fumonisin levels in different agro-ecological zones in Tanzania and intervention strategies to mitigate mycotoxins.

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