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AFLATOXINS AND FUMONISIN CONTAMINATION OF MARKETED MAIZE, MAIZE BRAN AND MAIZE USED AS ANIMAL FEED IN NORTHERN TANZANIA

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ABSTRACT

The objective of this study was to investigate the occurrence of total aflatoxin and total fumonisin in maize and maize-based products in Babati, northern Tanzania. A total of 160 samples were collected in 2013-14. Quantification for total aflatoxin and fumonisin was done using enzyme-linked immunosorbent assay (Reveal AccuScan® Neogen, USA) and the results were confirmed using Liquid Chromatography Tandem Mass Spectrometer. Aflatoxin was detected in 32% of maize samples (mean 3.4 ± 0.3 µg/kg; range 2.1–16.2 µg/kg) and fumonisin in 39% of samples (mean 5.6 ± 1.40 mg/kg; range 0.4–62.0 mg/kg). Among marketed products, maize bran (used for animal feed) was the most contaminated (2.4 µg/kg aflatoxin and 1 mg/kg fumonisin), followed by whole maize in market stalls (1.9 µg/kg aflatoxin and 0.4 mg/kg fumonisin) and then maize flour (1.4 µg/kg aflatoxin and 0.3 mg/kg fumonisin). Un-marketed maize sorted out by farmers as “bad” and intended for animal feeding was the most contaminated (overall mean aflatoxin and fumonisin levels of 1.7 µg/kg and 7.4 mg/kg, respectively). The results indicate that levels of aflatoxin and fumonisin contamination in marketed maize were within tolerable limits.

Key words: Tanzania, aflatoxins, fumonisins, food safety, maize, market, processors, animal feed

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by various fungi, many of which frequently contaminate food and feed worldwide [1]. Their prevalence depends on various factors, such as the commodity, climatic conditions, agricultural practices and storage conditions [1, 2]. Two of the most important mycotoxigenic fungi associated with maize and other crops, fruits and nuts are *Aspergillus flavus*, which produces aflatoxins, and *Fusarium verticillioides*, which produces fumonisins [3–5].

Aflatoxin causes acute and chronic toxicity, depending on the amounts consumed. The compounds are regarded as immunosuppressive, mutagenic, teratogenic genotoxic and carcinogenic [4, 6]. Aflatoxins seriously affect human health by inducing hepatocellular carcinoma [7]. Fumonisins have been linked to oesophageal cancer in South Africa, northern Italy and China [8–10]. Fumonisins were also associated with stunting and underweight in Tanzania [11].

Aflatoxins and fumonisins are not uniformly distributed in maize kernels and higher concentrations tend to be found in germ and bran fractions produced by dry milling due to the presence of the pericarp in these fractions. The pericarp is the first part of the kernel colonized by fungi from the environment because of its peripheral location, and also the part to which kernel dusts adhere [12]. Other studies report that during dry milling of corn, highest amounts of fumonisin B1 were found in the bran fraction used as animal feed, followed by the germ fraction, which is mainly used as animal feed and for oil extraction [13, 14].

Maize is a major staple food in the study sites and Tanzania as a whole. Several studies have been conducted in Tanzania on contamination of harvested, marketed and processed maize with aflatoxin and fumonisin [11, 15, 16] and on contamination of animal products from livestock fed with contaminated feed. The aim of this study was to investigate the level of contamination along the value chain, in different types of maize available in northern Tanzania. The findings will contribute to interventions for subsistence farmers and processors to reduce aflatoxin and fumonisin contamination in the maize and maize products.

MATERIALS AND METHODS

Study area

The study was conducted in three villages (Long, Sabilo and Seloto) in Babati District, Manyara Region, northern Tanzania between August and November 2013. The villages were purposively selected in order to assess mycotoxins in different climatic zones. Villages were located in a priority research area for the United States Agency for International Development (USAID) Feed the Future initiative, where the Africa Research in Sustainable Intensification for the Next Generation (Africa RISING) eastern and southern Africa project on sustainable intensification of farming systems is being implemented. Further details are given by Nyangi *et al.* [17].

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Selection of vendors and processors

The vendors and small-scale mills were selected from only two villages (Long and Seloto), as Sabilo village shared the same market and small-scale mill with Seloto village. In selected villages, five vendors were randomly selected from the village markets and 43 maize grain samples collected. Samples of maize grain (n=29), maize flour (n=24) and maize bran (n=20) were also collected from one of the two small-scale mills in both Seloto village and Long village. Samples of maize sorted out by farmers as bad or mouldy (bad-sorted) (n=41) were collected from households in all three villages when farmers were about to store their maize. All participants had provided their informed consent before participating in the study.

Collection of samples

Most of the samples were collected from 100-kilogram bags of maize. Multiple samples were taken from different parts of one bag or several bags belonging to one vendor and combined to produce a one-kilogram sample for analysis, using the respective vendor's sampling tools (that is, scoops, and locally made probes commonly known in Swahili as 'bambo'). Samples were then placed in a clean A4 envelope, which was sealed, labelled and transported to the International Institute of Tropical Agriculture (IITA) plant pathology laboratory, Dar es Salaam, Tanzania. Samples were then dried in a cabinet drier at 65°C for 72 hours to reach less than 13% moisture content.

Quantification of total aflatoxin and fumonisin

Samples were ground using a Bunn grinder (Man: Bunn-O-Matic Corporation Springfield, Illinois, USA), homogenized and sub-divided to obtain a representative sub-sample for analysis. A 50-gram sub-sample was taken from each of the ground samples, extracted with 250 ml mixture of ethanol/water (65:35, v/v) and shaken vigorously at 150 revolutions per minute for three minutes using a laboratory shaker (IKA® Werke, Germany). Extracts were filtered through Whatman No. 1 filter paper (Whatman International Ltd. Maidstone, UK). Total aflatoxin (µg/kg) and fumonisin (mg/kg) were quantified following the manufacturer's protocol using Reveal AccuScan® III reader (Neogen, USA), a quantitative enzyme-linked immunosorbent assay (ELISA)-based analytical test kits designed specifically for either aflatoxin or fumonisin. The detection limit for total aflatoxin was 2 µg/kg with a quantitation range of 2–150 µg/kg and that for total fumonisin was 0.3 mg/kg with a quantitation range of 0.3–6 mg/kg. The analytical quality of the ELISA methods was assured by the use of certified reference material, a naturally contaminated maize sample with a certified total aflatoxin content of 18.1 ± 3.6 µg/kg and total fumonisin content of 4.2 ± 0.6 mg/kg supplied by Neogen, USA (Neogen Corporation, USA).

For technical validation, a random subset of samples was re-analyzed using Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS) at the Interuniversity Department for Agrobiotechnology (IFA Tulln, Austria).

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Statistical analysis
Data were analysed using Statistical Analysis System (SAS® Version 9.4, SAS Institute Incorporation, USA). The differences between means were detected using least square means to establish differences in mean total aflatoxin and fumonisin among the villages, markets and small scale-mills as well as animal feeds.

RESULTS

Aflatoxins and fumonisins in maize

All 73 samples (whole maize = 29, bran = 20 and flour = 24) from small-scale mills had total aflatoxin and fumonisin levels below the maximum tolerable levels (MTLs) of 10 µg/kg (or 10 ppb) and 2 mg/kg (or 2 ppm), respectively, set by the East African Community (EAC) [18]. They were also below the 10 µg/kg and 1 mg/kg standards set by the European Union [19]. Maize bran had mean fumonisin concentration of 1.0 mg/kg, lower than the MTL for animal feeds of 20 mg/kg [20] (Figures 1 and 2).

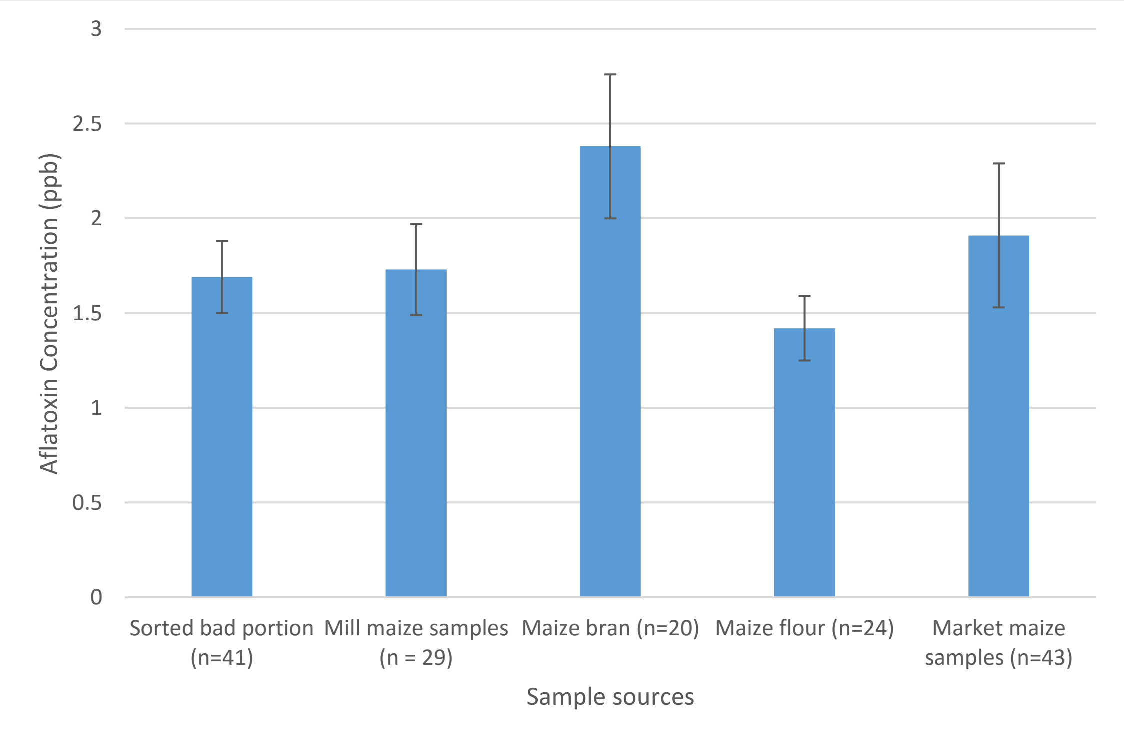

Figure 1: Mean aflatoxin levels with their corresponding standard errors

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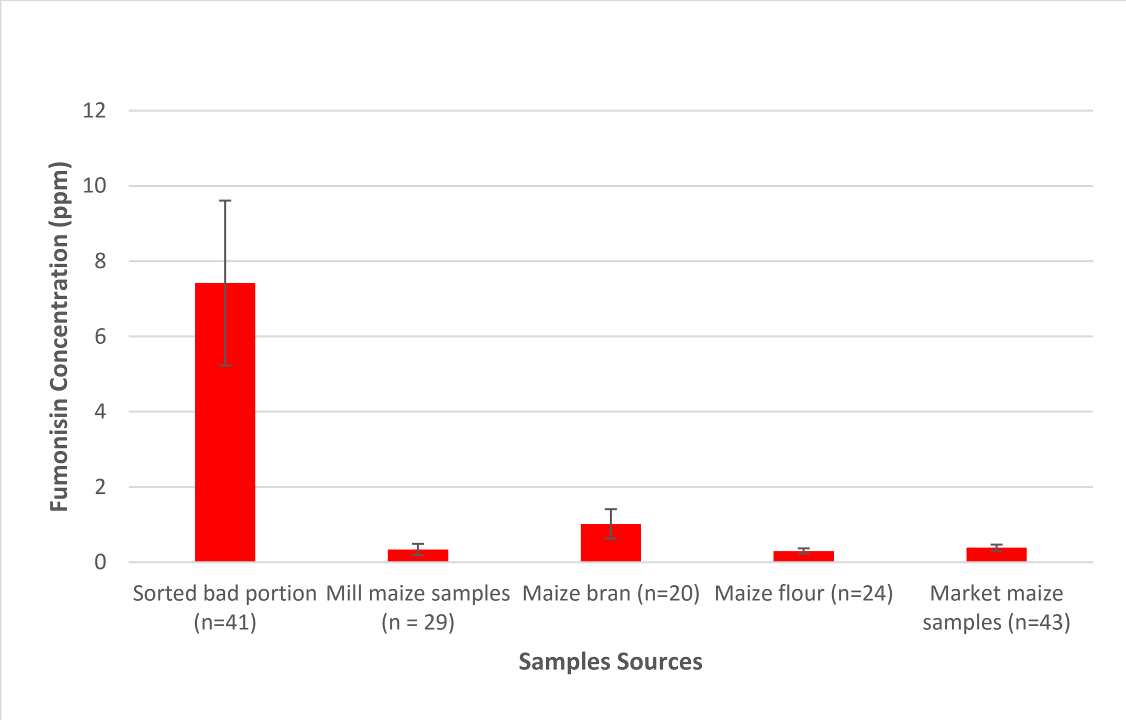

Figure 2: Mean fumonisin levels with their corresponding standard errors

Only 2% of all market maize samples had a total aflatoxin greater than the EAC and EU MTL of 10 µg/kg [18, 19], while all samples had aflatoxin levels below the MTL of 20 µg/kg by USA standards [20]. About 5% of the marketed samples had fumonisin levels above the MTL of 2 mg/kg [18]. All of the maize sorted as bad or mouldy portion collected from farmers' households and maize bran samples from small-scale mills as animal feed had contamination levels below the MTL for animal feeds of 20 µg/kg for aflatoxin and 5–100 mg/kg for fumonisin [21, 19] as indicated in Table 1. Differences between levels of aflatoxin and fumonisin contamination in maize and maize-based products in the three villages and their significance are shown in Tables 3 and 4.

DISCUSSION

Aflatoxin and fumonisin levels in marketed maize and maize products

The mean value for total aflatoxin was lower than those reported in other studies [22]. In Kenya, a very high level of up to 46,400 µg/kg was reported, the result of prolonged drought and food shortages that were followed by off-season rains during harvest, a combination of circumstances that probably favoured the growth of aflatoxigenic *Aspergillus* spp. in household stored maize [7]. The mean concentration of total fumonisin was higher than a mean value of 2.9 mg/kg reported in Burkina Faso [23]. The results from the present study were also lower than those reported in Rwanda, with maximum aflatoxin and fumonisin levels of 154.9 µg/kg and 7.1 mg/kg, respectively [5]. It was found that aflatoxin and fumonisin levels were higher in bran fractions, as expected [12, 13]. The related study of harvested maize in the three villages found that the maize in Sabilo village had significantly higher aflatoxins and significantly lower fumonisins than the other two villages [17]. Selling maize from Sabilo in the same markets as maize from Seloto, therefore, acts to reduce levels of aflatoxin and fumonisin compared to selling the product in separate markets, because there is a diluting effect when more contaminated maize is sold with less contaminated maize. The overall largely satisfactory results may be the effect of good agricultural practices by farmers following their participation in agricultural development programs [17].

Aflatoxin and fumonisin occurrence in non-marketed, sorted maize

The bad-sorted portion was removed from harvested maize before storage. Although aflatoxin and fumonisin levels were below the EAC MTL for animal feeds, some samples contained contamination at levels that were above the EAC MTL for human food. Levels in the bad-sorted maize were higher than reported from a study in Poland [24] and comparable to levels reported from a study in Iran [25].

CONCLUSION

The results from this study showed that most maize products consumed by humans and animals contained aflatoxin and fumonisin at levels below the EAC MTL, which is satisfactory. However, a small proportion of marketed maize was contaminated with mycotoxins at levels that exceeded EAC standards, indicating the need to improve aflatoxin and fumonisin control. Sorting out bad maize by farmers successfully mitigates fumonisin risk. Milling maize and feeding the more contaminated bran to animals also reduces human consumption of mycotoxins.

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Table 1: Incidence of total aflatoxin and fumonisin contamination in maize and maize-based product samples across the three villages

Sample source	n	Aflatoxin (µg/kg)		Fumonisin (mg/kg)	
		Positive sample (%)	Mean ± SE	Positive sample (%)	Mean ± SE
Sorted bad maize	41	12 (29)	3.49 ± 0.28	21(51)	14.45 ± 3.69
Maize from mills	29	10 (34)	3.19 ± 0.32	6 (21)	1.43± 0.56
Maize bran	20	12 (60)	3.26 ± 0.48	12(60)	1.63 ± 0.60
Maize flour	24	3 (13)	2.97 ± 0.12	8 (33)	0.66 ± 0.12
Maize market	43	15 (35)	4.00 ± 0.91	16 (37)	0.96 ± 0.16

Values are means of total aflatoxin and fumonisin.
Positive samples are all analysed samples with value > limit of detection
n: the total number of analysed samples
SE: standard error
Maximum tolerable level of aflatoxin is10 µg/kg by East Africa standards and 20 µg/kg by USA standards and that of fumonisin is 2 mg/kg by East Africa standards
Maximum tolerable level of aflatoxin in animal feed is 20 µg/kg and that of fumonisin is 5–100 mg/kg

Table 2: Overall occurrence/prevalence of aflatoxin and fumonisin in maize, and maize-based products across the three villages

Mycotoxin	n	Positive sample (%)	Range	Mean ± SE
Aflatoxin (µg/kg)	160	51(32)	2.1–16.2	3.4 ± 0.3
Fumonisin (mg/kg)	160	62 (39)	0.4–62.0	5.6 ± 1.4

Values are means for total aflatoxin and fumonisin levels in all analysed samples
Range is only for positive samples
Positive samples are all analysed samples with value > limit of detection
n: total number of all analysed samples
SE: standard error

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Table 3: Aflatoxin contamination in maize and maize-based products in the three villages

Sample type	n	Mean aflatoxin concentration (µg/kg) ± SE			
		Mean overall*	Long village	Sabilo village	Seloto village
Sorted bad portion	41	1.7 ± 0.2[a]	1.4± 0.2[a]	1.9 ± 0.4	1.9 ± 0.4[a]
Maize from mills	29	1.7 ± 0.2[a]	1.2 ± 0.2[ab]	-	2.7 ± 0.4[a]
Maize bran	20	2.4 ± 0.4[ab]	2.2 ± 0.3 [acd]	-	2.6 ± 0.8[a]
Maize flour	24	1.4 ± 0.2[ac]	1.1 ± 0.2 [abeh]	-	1.8 ± 0.3[a]
Maize from market	43	1.9 ± 0.4[a]	1.5 ± 0.2 [abfhi]	-	2.5 ± 0.8[a]

Values are means for total aflatoxin levels in all analysed samples across the three villages
Means with different letters (by column) are significantly different ($P<0.05$)
n: total number of samples analysed
SE: standard error
* represents samples from all three villages including bad sorted portion from Sabilo village

Table 4: Fumonisin contamination in maize and maize-based products across the three villages

Sample type	n	Mean fumonisin concentration (mg/kg) ± SE			
		Mean overall*	Long village	Sabilo village	Seloto village
Sorted bad portion	41	7.4 ± 2.2[a]	0.1 ± 0.1[a]	27.5 ± 7.7	5.2 ± 1.1[a]
Maize from mills	29	0.3 ± 0.2[ba]	0.2 ± 0.1[ac]	-	0.7 ± 0.4[bf]
Maize bran	20	1.0 ± 0.4[a]	0.4 ± 0.1[bde]	-	1.8 ± 0.8[cfg]
Maize flour	24	0.3 ± 0.1[b]	0.2 ± 0.1[acef]	-	0.5 ± 0.1[dfgi]
Maize from market	43	0.4 ± 0.1[b]	0.4 ± 0.1[bdefg]	-	0.4 ± 0.1[efhi]

Values are means for total fumonisin levels in all analysed samples across three villages
Means with different letters (by column) are significantly different ($P<0.05$)
n: total number of samples analysed and (%) for positive samples
SE: standard error
* represents samples from all three villages including bad sorted portion from Sabilo village

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