

10.18697/ajfand.75.ILRI06

**ASSESSMENT OF PRE-HARVEST AFLATOXIN AND FUMONISIN
CONTAMINATION OF MAIZE IN BABATI DISTRICT, TANZANIA**

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ABSTRACT

A survey was conducted in 2013 to establish total aflatoxin and total fumonisin in maize, as well as farmers' practices relating to maize cultivation and awareness of mycotoxins, in three villages of Babati District, northern Tanzania. Quantification of 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. The mean aflatoxin was 2.94 $\mu\text{g}/\text{kg}$ and all samples ($n=440$) were within the East African Community (EAC) standard of 10 $\mu\text{g}/\text{kg}$ for total aflatoxin, but the mean fumonisin was 5.15 mg/kg , more than double the EAC standard of 2 mg/kg , and 35% of samples exceeded this standard. Maize samples obtained from farmers in the village in the mid altitude, dry zone had significantly higher mean aflatoxin (3.32 $\mu\text{g}/\text{kg}$) and significantly lower mean fumonisin (3.17 mg/kg) than maize from the other two villages (in the high and mid altitude, high rainfall zones). Most farmers ($n=442$) were male (72%), educated to primary school level (77%) and aware of mycotoxins (62%). As well as participating in a development program, Africa Research in Sustainable Intensification for the Next Generation, most (86%) farmers had experience of working with other development programs. All farmers used flat planting, most used improved seeds (98%), ox ploughing (78%), insecticides (78%) and early planting (36%). Practices associated with mycotoxins were planting time, tillage methods, previous season planted crops, and use of insecticides. Awareness of mycotoxins and climatic conditions were also associated with mycotoxin prevalence. In conclusion, good practices are associated with acceptable aflatoxin levels and should be continued. However, the high level of fumonisins warrants further investigation.

Key words: aflatoxins, fumonisins, at-harvest, maize, Tanzania, mycotoxins, food safety, farming practices, staple food

INTRODUCTION

Maize (*Zea mays* L.) is a major dietary staple in Tanzania, which produced 6.7 million tonnes in 2014 [1]. Among the crops used as food and feed, maize is an especially good substrate for the growth of moulds that produce aflatoxins and fumonisins [2]. Contamination of maize with aflatoxins and fumonisins has been reported in maize samples in Tanzania [3–5].

Aflatoxins, produced mainly by *Aspergillus flavus*, can cause acute and chronic toxicity, immunosuppression, mutagenicity, teratogenicity, genotoxicity and carcinogenicity [6]. The International Agency for Research on Cancer (IARC) classified aflatoxin B1 as a class 1 human carcinogen [7]. Fumonisins are produced by *Fusarium* species, mostly by *Fusarium verticillioides* (previously known as *F. moniliforme*) [6]. Fumonisins have been associated with human oesophageal cancer in South Africa, liver cancer in China and with stunting and, underweight in Tanzania [3, 8, 9]. The IARC classified fumonisin as a group 2B toxin, considered as possibly carcinogenic to humans [7].

Agronomic practices and climatic factors influence contamination of grains by aflatoxins, fumonisins and other mycotoxins. These factors include temperature and humidity [9, 10], soil type and nutrients [10], crop rotation [11], tillage method [12, 13] and time of planting and harvesting [14–16].

Although maize is an important dietary staple in Tanzania, little research has been conducted to establish the levels of contamination of mycotoxins or the relationship between production, handling practices and the occurrence of mycotoxins in maize [3–5]. The aim of this study was to investigate how the presence and level of mycotoxins differed according to agronomic practices and climatic zone.

Farmers in the study area have been working with the Africa Research in Sustainable Intensification for the Next Generation (Africa RISING) program, an initiative of the United States Agency for International Development (USAID)'s Feed the Future program, under the project “*Research in sustainable intensification for the next generation in the sub-humid maize-based cropping systems of Babati: Testing performance of integrated past year best-bet component technologies*”, in collaboration with the International Institute of Tropical Agriculture (IITA), Tanzania. Under this project, farmers were trained on promising crop management technologies, including, integrated approaches to manage maize lethal necrosis disease, fodder and feed for sustainable intensification of crop-livestock systems, strategies for prevention of mycotoxin contamination along food and feed value chains, post-harvest technologies for improving household nutrition and income, and improving productivity of indigenous chicken through better nutrition and management in mixed crop-livestock farming systems. Selected farmers received training and farm inputs (improved seeds, insecticides, inorganic fertilizer) and their farms were used as a demonstration for other farmers. With regards to mycotoxins, awareness was created through fact sheets, village meetings and efforts of extension officers from the district to village levels.

MATERIALS AND METHODS

Study area

The study was conducted between June and July 2013 in three villages participating in a development intervention within the Babati District, Manyara region, Tanzania. The villages were Long, Sabilo and Seloto located in different climatic zones. Long village is in the high altitude, high rainfall zone, between 2150 and 2450 metres above sea level, and has relatively high annual rainfall of 1200 mm. The mid altitude, low rainfall zone (Sabilo village), lies between 1500 and 1850 metres above sea level and has relatively low annual rainfall of 900 to 1100 mm. The mid altitude, high rainfall zone (Seloto village) lies between 1850 and 2150 metres above sea level with a production season characterised by annual rainfall of 1100–1200 mm.

Selection of farmers

A total of 450 farmers (150 from each village) were randomly selected using a list of farmers that had consented to participate previously, provided by the respective village leaders and extension officers. Exclusion criteria were farmers who were both participating in the agricultural intervention (10 per village, or 30 in all) and had good performance (12 out of the 30 participants).

Collection of samples

A total of 442 physiologically mature, ready-for-harvest maize cobs with moisture content around 25% were collected in June and July 2013. Information on crop production practices used by farmers in the three villages was obtained using a semi-structured questionnaire. Questions covered planted variety, previous crops, pest problems in the field, planted and harvested dates, tillage method, planting pattern (flat, on ridges or on mounds), harvested condition (wet or dry), condition of harvested crop (clean or spoiled) and intended use of the harvested crops. Global Positioning System coordinates and basic demographic details of the farmers/producers were also collected.

Samples were taken by walking in two diagonal directions across the farm (most were half to one acre in size) and stopping at regular intervals to pick a maize cob so as to have as representative samples as possible. A total of five stops were made in each field and five maize cobs were sampled at each stop, making a total of 25 cobs. These were then hand-shelled on a clean A1 paper with a separate clean paper used for each sample, producing 4–5 kg of kernels. The shelled kernels were then well mixed and approximately 1 kg was selected. The collected samples were packed in a clean A4 paper bag (envelope) which was then well sealed and transported to the plant pathology laboratory at IITA, Dar es salaam- Tanzania.

Quantification of total aflatoxin and fumonisin

The samples were dried at 65°C for 72 hours in a cabinet drier to reduce the moisture content to 13%. The samples were then 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 g sub-sample was taken from each of the ground samples, extracted with a 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 ($\mu\text{g}/\text{kg}$) and fumonisin (mg/kg) were quantified following the manufacturer's protocol using Reveal AccuScan® III reader (Neogen Corporation, USA), a quantitative enzyme-linked immunosorbent assay (ELISA)-based analytical test designed specifically for either aflatoxins or fumonisins.

The detection limit for total aflatoxin was $2 \mu\text{g}/\text{kg}$ with a quantitation range of 2–150 $\mu\text{g}/\text{kg}$ while the detection limit for total fumonisin was $0.3 \text{ mg}/\text{kg}$ 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 certified total aflatoxin content of $18.1 \pm 3.6 \mu\text{g}/\text{kg}$ and total fumonisin content of $4.2 \pm 0.6 \text{ mg}/\text{kg}$ supplied by Neogen, USA (Neogen Corporation, USA). For the purpose of data analysis, non-detectable levels were based on the limits of detection of the test method for each toxin. Detectable levels were compared to the East African Community (EAC) established maximum tolerable limits.

For technical validation, random subsets of samples were re-analysed using Liquid Chromatography Tandem Mass Spectrometer at the Interuniversity Department for Agrobiotechnology (IFA Tulln, Austria).

Statistical analysis

Data were analysed using SAS 9.4, SAS Institute, Cary, NC. Four models were built: one with all villages, and one for each village. General linear models (PROC GLM) were fitted using backward elimination with mycotoxin contamination as response and the agronomic practices and climatic factors as predictors to identify the factors that were associated with contamination of maize by aflatoxin and fumonisins ($p < 0.05$). Mycotoxin levels were $\log(x + 1)$ transformed to normalize data before analysis. The answers to “yes or no” questions were entered as binomial values and answers to categorical questions were entered as numbers.

RESULTS

Characteristics of the farmers and their farming systems

Maize was collected from 442 farmers, as eight had harvested before the survey. Seventy-two percent of the farmers interviewed in the study area were male and 28% female; most female farmers were also household heads. Seventy-seven percent had completed primary education, while 15% had not completed primary education and 8% had higher than primary education. Sixty-two percent of the farmers were aware of mycotoxins and 2%, both from Sabilo village (lower dry zone), reported being aware that health problems are associated with eating food contaminated with mycotoxins. Eighty-six percent reported to have been previously working with other non-governmental organizations (NGOs) (Table 1).

Farming practices in the villages

Four tillage methods (hand hoe, ox, tractor and combination of hand hoe and ox) and three planting dates (early planting for those who planted maize in November, mid planting in December and late planting in January to early February) were identified in the study area. Farmers mainly planted improved (purchased) maize varieties (98%). Seventy-eight percent of the farmers were found to use insecticides, with Karate® as the most widely used insecticide. Ninety-one percent of farmers were found to have planted maize and beans in the previous season while only 1% planted maize and other crops such as pigeon pea, sunflower and Irish potatoes. The surveyed farming practices in the study area are shown in Table 2.

Total aflatoxin and fumonisin content in maize

A total of 440 samples of maize were analysed (eight farmers had harvested before the survey and two samples were lost during transportation). It was found that 19% and 35% of the maize samples were contaminated with aflatoxin and fumonisin, respectively (Table 3). The highest aflatoxin mean value of 3.32 µg/kg for maize samples was found in Sabilo village (lower dry zone); for fumonisins, the highest means of 6.75 mg/kg in Long village and 6.60 mg/kg in Seloto village were not statistically different (Table 4). Samples from the dry zone (Sabilo) had significantly higher aflatoxins and significantly lower fumonisins than samples from villages in the wet zones.

Farming practices/factors associated with aflatoxin and fumonisin levels in maize

The occurrence of aflatoxin in maize was significantly associated with four practices, namely, the use of insecticides (Karate®), planting time (early planting in November and mid planting in December), tillage methods (hand hoe or ox tillage) and previous planted crops. In addition, less aflatoxin and fumonisin contamination was associated to farmers' awareness on mycotoxin (Table 5 and 6).

DISCUSSION

Total aflatoxin and fumonisin contamination in maize

The overall mean aflatoxin level of 2.94 µg/kg (Table 3) was lower than maximum tolerable limit of 10 µg/kg by EAC standards [17], and lower than those reported from other studies in Africa [18–20]. All samples contained aflatoxin at levels below the maximum tolerable limits and hence were fit for consumption. The intervention by the Africa RISING/IITA project in the study area from the previous year (2012) as well as work by NGOs in the area may have contributed to this.

The overall mean fumonisin level of 5.15 mg/kg (Table 3) was higher than the EAC maximum tolerable limit of 2 mg/kg [17]. Overall, 35% (153/440) of the samples contained fumonisin at levels above the maximum tolerable limit and would, therefore, be considered unfit for human consumption.

Effect of climatic zones on contamination of maize with aflatoxin and fumonisin

In this study, aflatoxins in maize were significantly lower and fumonisins higher in a village in the high rainfall zone (Long village) compared to two villages in dry zones, suggesting that, in this study, rainfall was more important than altitude in predicting mycotoxins. In contrast, in a similar study in Uganda, altitude dominated over rainfall [16, 20]. However, because different criteria were used to distinguish high rainfall and low rainfall and high altitude and low altitude, the studies are not directly comparable. Optimum conditions for aflatoxin production are temperatures of around 33°C and water activity of 0.99, while those for fumonisin production are temperatures of 15–30°C and water activity of 0.9–0.995 [10]. The previously recorded temperature in the study area was found to range from 12°C in Long village to 25°C in Seloto village, so the higher temperature in Seloto may have favoured production of aflatoxin more than production of fumonisins, suggesting temperature was more important than altitude or rainfall [21]. This aflatoxin contamination pattern could be due to the fact that lower altitude areas are usually warmer with high temperatures and humidity compared to higher altitude areas which are usually cooler with low temperatures and humidity.

Effect of farming practices on contamination of maize with aflatoxin and fumonisin

Time of planting

Time of planting had a direct influence on the contamination of grain by aflatoxin. Maize planted in November (early planting) and December (mid planting) had lower levels of aflatoxin contamination compared to maize planted in January (late planting). Several other studies on the effect of planting date reported the same trend [22, 23]. This is due to the fact that early planting reduces the levels of aflatoxin contamination by shifting the period between when the flower is fully open and functional (anthesis) and dough development in maize to a time frame in the growing season when the crop is less susceptible to drought and heat stress as compared to late planting [24].

Method of land tillage

Relative to tractor tillage, hand hoe and ox tillage were associated with lower aflatoxin contamination of maize (Table 5). The method of tillage is known to influence fungal populations on soil surfaces and could contaminate maize ears due to rain splashes or wind [12]. If the fungal population is submerged due to tillage, it will not be able to contaminate the crop; the same also applies to *Fusarium* inoculum which is pushed deeper into the soil and cannot contaminate grains or pods as it cannot reach the soil surface [13].

Use of insecticides

Insect damage is known to influence aflatoxin contamination of pre-harvest maize. Insects play a major role in facilitating spore entry into the cobs and increasing infection by damaging the kernel pericarp [25, 26]. Application of insecticides reduced aflatoxin contamination of maize. It was found that 78% of the farmers used insecticides, with Karate[®], a broad-spectrum insecticide, as the main one used.

Effect of mycotoxin awareness on contamination of maize with aflatoxin and fumonisin

Sixty-two percent of farmers were found to be aware of problems caused by aflatoxin and fumonisin. This result was comparable to the findings from a study conducted in Kenya [27]. Other studies also reported on the importance of farmer awareness of mycotoxins and health problems associated with aflatoxin contaminated crops [28, 29]. The high awareness level in the study area could be attributed to mycotoxin sensitisation and awareness campaigns conducted by Africa RISING/IITA projects in 2012.

CONCLUSION

This study found that aflatoxin levels in maize were generally acceptable but fumonisin levels exceeded the EAC standards in around one-third of samples. Farming practices with regard to timely planting, insecticide application and proper land tillage were generally good and may have helped reduce fungal proliferation and elaboration of aflatoxins in maize. The intervention by Africa RISING/IITA and other NGOs in the three villages probably contributed to this, as 86% of farmers reported experience of working with NGOs. However, control of fumonisins seems much less effective and further research is needed into the extent and management of this mycotoxin.

ACKNOWLEDGEMENTS

The authors are grateful to the Innovative Agricultural Research Initiative, through the support of USAID's Feed the Future program, and IITA, through the support of the Africa RISING eastern and southern Africa project on sustainable intensification of farming systems and USAID's Feed the Future program, for providing technical working facilities and covering the research cost for this study. The farmers who provided maize samples for aflatoxin and fumonisin analysis are highly appreciated.

Table 1: Demographic characteristics of farmers across the three villages

Characteristics	Overall n = 442 (%)	Surveyed villages		
		Long (n=154) (%)	Sabilo (n=145) (%)	Seloto (n=143) (%)
Sex				
Male	318 (72)	134 (87)	71 (49)	113 (79)
Female	124 (28)	20 (12)	74 (51)	30 (21)
Education				
Primary	340 (77)	125 (81)	108 (74)	107 (75)
Secondary	26 (6)	6 (4)	6 (4)	14 (10)
Tertiary	7 (2)	2 (1)	0 (0)	5 (3)
None	69 (15)	21 (14)	31 (22)	17 (12)
Worked previously with NGO?				
Yes	380 (86)	129 (84)	119 (82)	124 (87)
No	62 (14)	25 (16)	26 (18)	19 (13)
Are you aware of aflatoxin/fumonisin?				
Yes	276 (62)	55 (36)	112 (77)	109 (76)
No	166 (38)	99 (64)	33 (23)	34 (24)
Is eating mycotoxin- contaminated food a health problem?				
Yes	10 (2)	0 (0)	10 (7)	0 (0)
No	432 (98)	154 (100)	135 (94)	143 (100)

n: number of farmers visited, which is equal to the number of samples collected

(%): percentage of farmers who responded

Table 2: Farming practices used by farmers across the three villages

Farming practice	Overall n=442 (%)	Surveyed villages		
		Long (n= 154) (%)	Sabilo (n= 145) (%)	Seloto (n= 143) (%)
Planting time				
Early	161 (36)	137(89)	7 (5)	17 (12)
Mid	220 (50)	12 (8)	94 (65)	114 (80)
Late	61 (14)	5(3)	44 (30)	12 (8)
Previous crops				
Maize only	36 (8)	4 (2)	28 (20)	4 (3)
Maize and beans	403 (91)	149 (97)	115 (79)	139 (97)
Maize, beans and other crops	3 (1)	3 (1)	2 (1)	–
Tillage method				
Hand hoe only	28 (6)	4 (3)	5 (3)	19 (13)
Ox only	346 (78)	144 (94)	95 (66)	107 (75)
Hand hoe and ox	57 (13)	4 (3)	42 (29)	11 (8)
Tractor	11 (2)	2 (1)	3 (2)	6 (4)
Seed variety				
Improved seed	432 (98)	152 (99)	139 (96)	141 (99)
Local seed	10 (2)	2 (1)	6 (4)	2 (1)
Planting pattern				
Flat	442 (100)	154 (100)	145 (100)	143 (100)
Insecticide use				
Yes	345 (78)	110 (71)	114 (78)	121 (85)
No	97 (22)	44 (29)	31 (28)	22 (15)

n = number of farmers visited, which is equal to the number of samples collected

(%) = percentage of farmers who responded

Table 3: Prevalence of total aflatoxin and fumonisin in maize samples across the three villages

Mycotoxin	n	Positive samples (%)	Maximum concentration	Mean \pm SE
Aflatoxin ($\mu\text{g}/\text{kg}$)	440	84 (19)	26.2	2.94 \pm 0.28
Fumonisin (mg/kg)	440	153 (35)	46.0	5.15 \pm 0.63

Values are means of total aflatoxin and fumonisin levels in maize samples across the three villages

Positive samples are all analysed samples with value > limit of detection

n: total number of analysed samples

SE: standard error

Table 4: Prevalence of total aflatoxin and fumonisin contamination in maize samples in each village

Village	n	Aflatoxin ($\mu\text{g}/\text{kg}$)			Fumonisin (mg/kg)		
		Positive sample (%)	Range	Mean \pm SE	Positive sample (%)	Range	Mean \pm SE
Long	153	26 (17)	2.10–3.6	2.58 ± 0.08^a	6 (4)	0.90–14.00	6.75 ± 2.41^a
Sabilo	144	40 (28)	2.20–26.2	3.32 ± 0.59^b	65 (45)	0.40–14.00	3.17 ± 0.43^b
Seloto	143	18 (13)	2.10–4.0	2.62 ± 0.11^b	82 (57)	0.40–46.00	6.60 ± 1.08^a

Values are means of total aflatoxin and fumonisin levels of maize samples from each village

Positive samples are all analysed samples with value > limit of detection

n: total number of analysed samples

Means with different superscript letters (by column) are significantly different ($P < 0.05$)

SE: standard error

Table 5: Association of farming practices and aflatoxin contamination in maize (Y) tested across and within three villages in Tanzania

Village/Variable	Regression analysis	Parameter estimate	R ² (F-value)	P value
Across Villages	$Y = 0.60 - 0.73X_1$	0.600	0.12 (4.28*)	<0.0001*
Long Village	$Y = 0.75 - 0.17X_2 - 0.20X_3 - 0.83X_4 - 0.10X_5$	0.7528	0.17 (2.82*)	0.0032*
Sabilo Village	$Y = 0.73 - 0.30X_6 - 0.28X_7$	0.7295	0.16 (2.31*)	0.0128*
Seloto Village	$Y = 0.53 - 0.16X_8$	0.5330	0.10 (1.64)	0.1089
X ₁ Use of insecticides		-0.7353	$t = 3.82^*$	0.0002*
X ₂ early planting		-0.1690	$t = 1.97^*$	0.0423*
X ₃ Mid planting		-0.1987	$t = 2.03^*$	0.0441*
X ₄ Awareness		-0.8250	$t = 2.74^*$	0.0069*
X ₅ Use of insecticides		-0.1014	$t = 3.22^*$	0.0016*
X ₆ Hand hoe tillage relative to tractor		-0.2972	$t = 2.44^*$	0.0160*
X ₇ Ox tillage relative to tractor		-0.2817	$t = 2.74^*$	0.0069*
X ₈ Previous planted crops		-0.1585	$t = 1.99^*$	0.0486*

Y: aflatoxin levels ($\mu\text{g}/\text{kg}$)

*: statistically significant at $P < 0.05$

Table 6: Association of farming practices and mycotoxin awareness associated with fumonisin contamination in maize (Y) tested across and within three villages in Tanzania

Village/Variable	Regression analysis	R ²	Parameter estimate	P value
Across Villages	No variable was statistically significant			
Long Village	No variable was statistically significant			
Sabilo Village	No variable was statistically significant			
Seloto Village	$Y = 0.45 - 0.22X_1$	0.11 (1.86)	0.4529	0.0639
X_1 Mycotoxin awareness		$t = 2.36^*$	-0.2222	0.0200*

Y: fumonisin levels (mg/kg)

*: statistically significant at $P < 0.05$

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