Research Article

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Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia

Muimba A Kankolongo,[a]* Kerstin Hell[b] and Irene N Nawa[c]

Abstract

BACKGROUND: Maize constitutes the main staple food and most important crop grown in Zambia. However, maize incurs considerable losses both in field and storage due to pathogens and insects. Some of the pathogens and resultant mycotoxins reduce the nutritional quality of the product. Mycotoxins are toxigenic fungal compounds that can cause cancer and suppress growth. In spite of this health hazard, there has been very little research to document their occurrence. Maize grains stored for human consumption were sampled from different agro-ecosystems (forest, valley and plateau areas) of three agroecological zones (high, mid and low altitude).

RESULTS: Several fungal genera were recovered among which *Aspergillus flavus, A. niger, Fusarium verticillioides, F. solani, Rhizopus stolonifer* and *Penicillium* spp. were prevalent. The weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncatus* were the most damaging. Enzyme-linked immunosorbent assay (ELISA) tests yielded fumonisins and aflatoxins ranging between 0.02 and 21.44 ppm, and 0.7 and 108.39 ppb in 96.4% and 21.4% of samples, respectively. Fumonisin was more pronounced in villages in forest areas whereas aflatoxin was highest in valley and forest areas in Zone II.

CONCLUSION: Strategic interventions to curtail fungal, mycotoxin and insect contamination should be directed towards improved agronomic and post-harvest practices of maize from fields to consumers.

Keywords: *Zea mays* L.; *Aspergillus flavus*; *Fusarium verticillioides*; *Prostephanus truncatus*; *Sitophilus zeamais*; mycotoxins; post-harvest losses

INTRODUCTION

Agricultural production in Zambia is mainly rain-fed and government policies focus on ensuring food security mainly through production of maize (*Zea mays* L.) in the whole country.[1] Zambia is one of the countries that have been repeatedly hit by drought during the past few years often causing considerable food shortages because of production failure of this primary staple. As a result of maize shortages many communities are exposed to famine and malnutrition. In addition, most rural households are forced to sell good-quality produce to millers and traders to earn money, keeping low quality and poorly dried grains for home consumption. Compounded by the lack of systematic efforts in monitoring mycotoxins in foods in the country,[2] there is a high possibility of the development of mycotoxins in commodities, especially the most potent aflatoxins, which are known to have serious health effects on livestock and humans.[3]

Aflatoxins and fumonisins are most frequently implicated in mycotoxin contamination of maize. Both are produced by a variety of fungi mainly in the genera *Aspergillus* and *Fusarium*, respectively.[4–6] Aflatoxin B1 is a human carcinogen and has been classified as a group 1 carcinogen by the International Agency for Research on Cancer.[7] Similarly, fumonisins constitute a major health concern for both animals and humans. They have been reported to lead to equine leukoencephalomalacia in horses and pulmonary oedema in swine.[8] In humans, ingestion of fumonisins has been linked to high incidences of oesophageal cancer[9] and neural tube defects.[10] The two most prevalent aflatoxin-producing fungi in nature are *Aspergillus flavus* and *A. parasiticus* with the first one being by far the dominant species. The growth of *A. flavus* and *A. parasiticus*, and subsequent aflatoxin production in storage, are favoured by high humidity (>85%), high temperature (>25 °C) and insect or rodent activity,[11] and all these conditions are prevalent in the humid tropics including Zambia.

Other maize pathogens such as stalk and ear rot fungi including *Colletotrichum* and *Diplodia* spp. have been reported from southern Africa including Zambia.[12] Chiarappa[13] gave accounts of about 60% of maize being lost in the country in 1974 due to infection by stalk rotting fungi, namely *Diplodia*

* *Correspondence to: Muimba A Kankolongo, Copperbelt University (CBU), School of Natural Resources, P.O. Box 21692, Jambo Drive Riverside, Kitwe, Zambia.*
E-mail: Kankolongo@cbu.ac.zm or ambayeba@yahoo.com

a *Copperbelt University (CBU), School of Natural Resources, P.O. Box 21692, Jambo Drive Riverside, Kitwe, Zambia*

b *International Institute of Tropical Agriculture (IITA), Cotonou, Benin*

c *University of Zambia (UNZA), School of Agricultural Sciences, Lusaka, Zambia*

spp., *F. graminearum* and *F. verticillioides*, as a result of prolonged drought. He reported that the remaining 40% of maize harvest contained high levels of mycotoxins, were declared unfit for both human and livestock consumption and destroyed. During the same year food emergency campaigns with assistance from international organisations were necessary to sustain the population's nutrition.[2] Recurring situations of food insecurity in Zambia demonstrate the urgency of implementing early monitoring programmes and elaborating protocols for detection of pathogens and mycotoxins. Next to fungal pathogens, insects also pose a great risk for crop spoilage. Insects both vector fungi and cause damage that allows fungi to gain ingress to crop tissues thus increasing chances of mycotoxin contamination.[14,15] Several reports are now available regarding mycotoxin contamination of maize and other commodities in Zambia.[16] Marasas and Smalley[17] reported the occurrence of mycotoxicoses in animals. In 1977, Lovelace and Nyathi[18] found zearalenone in maize malt and traditional beer and Marasas *et al*.[19] detected ochratoxin, zearalenone and deoxynivalenol in moldy maize and animal feeds. Similarly, Njapau and Muzungaile[20] recorded aflatoxin during an analysis of feed samples and Njapau *et al*.[21] reported that substantial amounts of aflatoxin were detected in processed food from maize and peanuts obtained from a farmer and a supermarket in Central and Lusaka Provinces. Investigations on some samples of grains from several maize hybrids have also revealed high levels of mycotoxins especially fumonisin B_1 and fumonisin B_2.[22]

Worldwide, however, most countries control mycotoxins in food and feed through policy regulations and regular monitoring for acceptable limits (http://www.fao.org/docrep/007/y5499e/y5499e07.htm#bm07). In Zambia, the requirements for the Food Reserve Agency (FRA), a Zambian government agency which assures strategic reserves of crop harvests for the whole nation, to purchase maize from farmers and other traders include maximum aflatoxin content of 10 ppb[23] although the Zambian standard (ZS 186: 2004) for cereals and cereal products only stipulates that mycotoxins in maize grains should not be harmful to human health.[24] Considering the place maize occupies in the diet of the population of Zambia, representing 68% of kcal intake estimated at 3600 kcal kg^{-1} energy value[25] and its importance for smallholder farmers, accounting for about 76% of their total income value,[26] it is necessary to do everything that is possible to minimise the risk of fungal contamination and guarantee its safety during post-harvest handling and storage before processing into food. Food safety has increasingly become a major issue in global trade relations[8] but the biggest challenges in food safety and agricultural health in Zambia relates mostly to domestic production and the domestic market.[27]

In spite of the reported presence of these toxigenic fungi in some commodities and the existence of ample information that exposure to mycotoxins constitutes a serious threat to human and animal health, very little research has been conducted on the occurrence of these toxins in different agroecological zones and ecosystems of Zambia. We undertook the present study countrywide to evaluate the quality of stored maize and obtain information on post-harvest practices and factors affecting maize quality in small-holder farming systems in order to develop field and storage management practices that reduce potential toxin risk.

MATERIALS AND METHODS

Sampling, study sites and households

The districts for the study were selected on basis of their ecosystem types, climatic variation, and level of poverty and observed food insecurity patterns. Areas that have been severely hit by drought in the past, those continuously receiving excessive rainfall and experiencing flooding, and where maize is not a major food crop were also included to capture the diversity in maize production systems in Zambia and generate background information to build on for future studies. The sites were Gwembe and Choma Districts in Southern Province in Agroecological Zone I; Kapiri-Mposhi and Mkushi in Central Province, and Chongwe and Luangwa in Lusaka in Zone II; Lufwanyama and Masaiti/Mpongwe on the Copperbelt in Zone III, and Mwinilunga and Solwezi Districts in Northwestern Province also in Zone III. The two districts selected per zone were used as replications. They were selected to cover all the three agroecologies of the country which are defined by rainfall patterns, vegetation types and soil quality.[28] Zone I is a semi-arid area characterised by a hot and dry climate with rainfall of less than 800 mm per annum. Zone II covers the sandy central plateau zone with an annual rainfall of about 800 to 1200 mm, whereas Zone III covers the northern location and has a rainfall of above 1200 mm. In total, samples were obtained from Zone I (Southern), IIa (Central: Kapiri and Mkushi), IIb (Lusaka: Lwangwa and Chongwe), IIIa (Copperbelt) and IIIb (Northwestern) making a total of five ecozones. In each district, three villages were identified based on their proximity to the various ecosystems, namely closed forests, valleys and savannah grasslands as described by Chidumayo[29] and within each village, five households stratified per age groups as young (16–40 years of age), middle age (41–59 years) and old (60 years and above) were randomly selected between July 2005 and February 2006 for maize sample collection. In the village, a transect line was made from the house of the village Headman in order to identify households. Then from the initial point of entry into the village from the transect line, a walk was made along the line while selecting each fourth household for interview and maize collection. If the age of selected farmers did not conform to the age group criteria, the process continued until they were within the age groups set.

Collection of maize grains from storage facilities

Samples of maize grains were taken from each of the household's maize storage facility for further studies. Precautions were taken to obtain as many grains as possible in the middle of the store for representativity. A small grain sampler was used to randomly perforate bags and grains were also collected by introducing the arm into other types of stores and getting random samples. Additional samples were obtained from the State semi-autonomous corporate FRA and maize growers' cooperative satellite depots that store only good quality grains based on established control standards. For each household in each ecosystem and depot per district, maize grains were placed in a paper bag and labelled; samples were then stored in a refrigerator until further analysis.

Sample processing and data recording

Due to a small number of maize grains obtained from some households, especially in the southern part of the country, most hit by food shortage due to drought, only 50 maize seeds were randomly taken from each of the five household samples per village and pooled into another big envelope to have three subset samples

of 250 maize grains each for forest, valley and savannah ecosystems per district. The remaining grains were stored for mycotoxin analysis. The pooled grains were then used to determine levels of grain quality per ecosystem in a district using the protocol described by FRA: namely % grain discolouration based on the grain colour deviation from the normal white maize (e.g. greenish, blackish, pinkish) often as a result of fungal contamination; % insect-damage based on number of exit holes where 0% = no damage, 1–20% = up to 20% seed area damaged, 21–40% = up to 40% area damaged, and more than 50% area damaged; and % diseased grains recognised based on visual signs of fungi on the grain surface.[23] A larger quantity of maize grains was obtained from FRA or cooperative satellite depots in Chongwe, Gwembe, Kapiri Mposhi, Masaiti, Mwinilunga and Solwezi, and 100 grains from different envelopes were used to have a subset sample of 500 grains per depot. Percentage values were then calculated for each class category from the total of grain sub-samples used.

Fungal isolation and identification

Twenty maize kernels from each sample were randomly selected; individually surface sterilised in 10% sodium hypochlorite for 2 min and then rinsed twice in sterile distilled water. Surface sterilisation was necessary to curtail the development of only potential contaminants which would affect the recovery of moulds. On potato dextrose agar (PDA) amended with 0.1% streptomycin, 10 grains were plated and incubated at 25–27 °C in the dark for 7 days. Fungal colonies were identified to species level where possible under a stereo-microscope using conidial and/or spore structures and mycelia characteristics.[30–33] Grains showing fungal infection were recorded as % mouldiness of the total sample and the distribution of each genus was calculated across agroecological zones and per ecosystem surveyed.

Extraction and analysis of mycotoxins

Enzyme-linked immunosorbent assay (ELISA) tests were conducted to assess maize aflatoxin and fumonisin levels. The test kits were USDA/GIPSA (Grain Inspection, Packers and Stockyards Administration) approved and manufactured by Strategic Diagnostics, Inc. (MycoChek® Aflatoxin Test Kit, MycoChek® Fumonisin Test Kit, Strategic Diagnostics, Inc., Newark, DE, USA). Mycotoxins were extracted from their maize matrix with methanol by blending 50 g ground maize with 250 mL of 70% methanol solution. The slurry was shaken on a rotary shaker for 2 min and allowed to settle for an additional 2 min. After settling, the supernatant was filtered through Whatman No. 1 filter paper and subjected to competitive ELISA analysis according to the manufactures instructions (http://www.sdix.com). Briefly, the supernatant from each sample was mixed individually with an enzyme–toxin conjugate containing a known amount of the mycotoxin (aflatoxin or fumonisin). Extracts were added individually to ELISA plate wells coated with toxin specific antigen. After washing a colorimetric substrate was added and allowed to react for 5 min. A stop solution was added to each well and the colour intensity was measured with a microplate reader (ELX800, BioTek Instruments, Inc., Winooski, VT, USA) at 650 nm and mycotoxin values were calculated with a standard curve.

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) using SAS JMP IN Version 4.0.4., Academic PROCANOVA procedure (Statistical Discovery Software, SAS Institute, Inc., NC, USA). Fisher's protected least significant difference (LSD) test was used to compare treatment means in case the *F*-statistic was significant. Before analysis, % values were transformed using arcsine square root transformation to provide for a normal distribution and stabilised error variances.[34]

RESULTS

Visual observation of maize storage facilities

Our visual observation indicated that nearly all farmers' storage facilities were in a poor state (Fig. 1); hence conditions were conducive for insect infestation and fungal contamination of the stored grains. All respondents indicated that they store maize after harvest first in a temporary structure that is made from locally available materials in the village (Fig. 1E) before transferring it to the final storage, often in polypropylene bags inside the house. Storage in bags is preferred for keeping maize for consumption, marketing and planting. The observed storage structures included open-air or roofed cribs constructed with tree poles and woven twigs or bamboos from forests (Fig. 1A and D); and raised platforms (about 1.5–2.0 m above the ground) suspended on four posts also constructed with tree shrub, bamboo, teak poles, and other local materials such as thatch grasses (Fig. 1E). These structures served the dual purpose of continuous drying and storage of the crop; other observed types were roofed iron drums enclosed with mud (Fig. 1C) and storage of shelled grains in bags that are directly kept in homes, often to avoid theft (Fig. 1B), these are usually used for long-term storage of well dried grains. Figure 1F represents one of the FRA satellite depots where small-scale farmers take their harvests for sale on a yearly basis.

Maize quality

ANOVA of level of grain discolouration, number of exit holes due to insect damage and % number of grains with signs of fungal colonisation are presented in Table 1. Both agroecological zone and ecosystem type had a statistical significant effect ($P \leq 0.05$) on grains discolouration, but not their respective interactions. However, significant differences were observed only between agroecological zones, although to a small extent ($P = 0.10$), for maize spoilage as a result of either insect damage or fungal colonisation. For the different tested variables neither the ecosystems surveyed nor the interaction with agroecological zones were not statistically significant ($P = 0.05$) (Table 1). Maize grains from Masaiti/Mpongwe in Copperbelt Province situated in the Agroecological Zone III, and those from Luangwa District in Lusaka Province and Gwembe District in Southern Province in Zone I were considerably more discoloured with distinct signs of fungal infection and insect damage (Table 2). The grain weevil *Sitophilus zeamais,* which was recovered from all the surveyed locations, and the larger grain borer, *Prostephanus truncates,* mainly found in maize samples from Luangwa, Gwembe and Kapiri Mposhi Districts were the most damaging insects. Overall, maize grains from Luangwa District valley (80% insect infestation) and Chongwe District savannah (36% infestation) in Zone II, Gwembe District valley (24% infestation) in Zone I and Kapiri Mposhi District forest (26% infestation) also in Zone II were severely attacked, hence of very poor quality (Fig. 2).

Fungal colonisation of maize grains

Various fungi were recovered from maize samples across the surveyed sites and it was evident that maize spoilage during storage is common throughout the sampled areas (Fig. 2). The

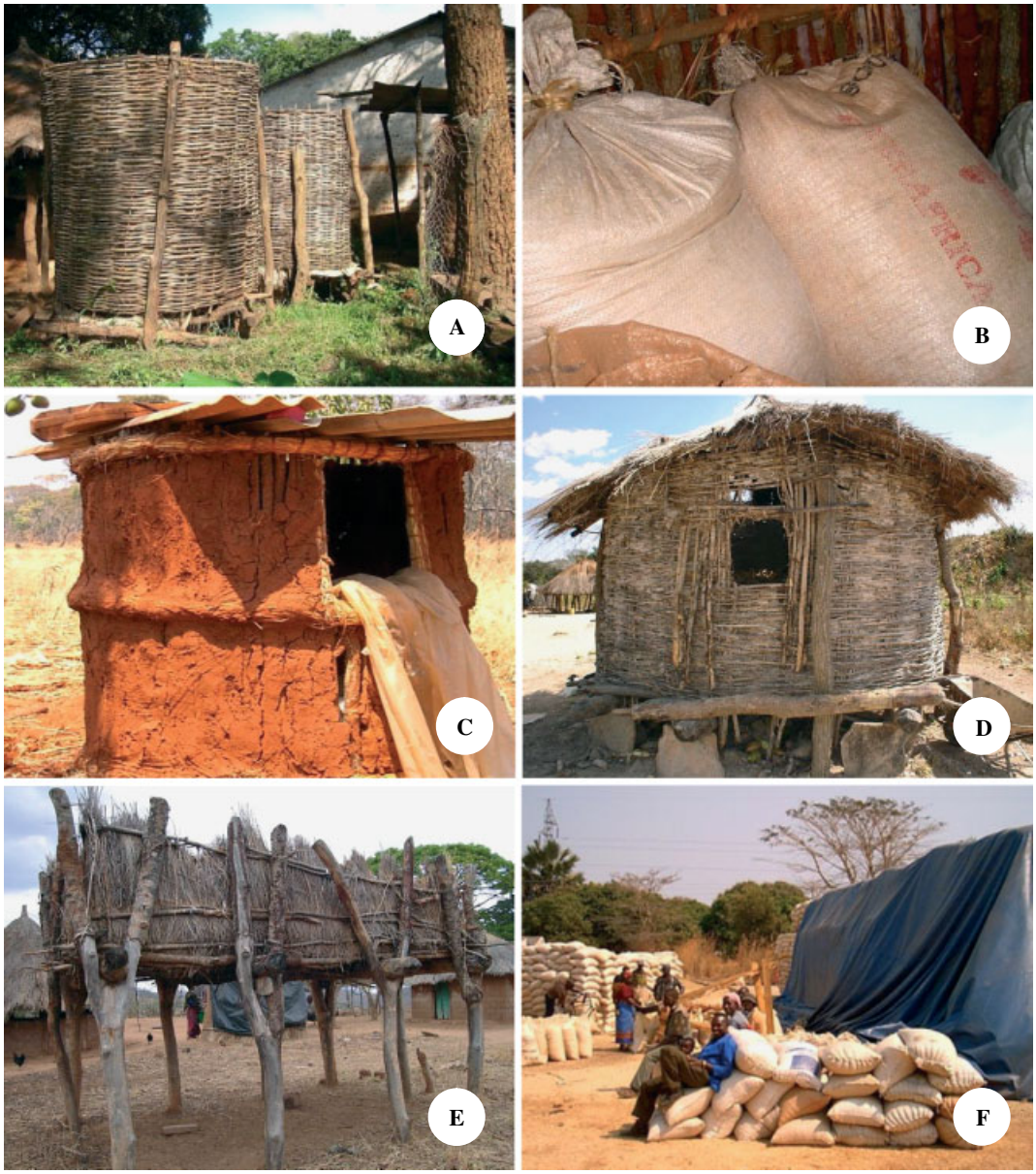

Figure 1. Various types of maize storage structures encountered in different locations surveyed in Zambia, 2006. (A) Open-to-air cribs constructed with tree poles or woven twigs or bamboos from forests; (B) shelled maize grains in bags kept in a corner in a house (hut); (C) a roofed iron drum enclosed with mud; (D) a roofed crib also constructed with tree poles or woven twigs or bamboos from forests or thatch grasses; (E) raised platforms suspended on four posts at about 1.5–2.0 m from the ground also constructed with shrub trees, bamboos, teak poles, and thatch grasses; and (F) FRA satellite depots where small-scale farmers bring their harvests for sale each year.

most prevalent fungi included *Aspergillus flavus, A. niger, Aspergillus* spp., *Fusarium verticillioides, F. solani, Fusarium* spp., and *Penicillium* spp. (Table 3). In samples from the Copperbelt in Zone III and Lusaka and Central Provinces in Zone II, incidence of recovery of *F. verticillioides* ranged from 30 to 66.7% (Table 3). Overall, prevalence of *A. flavus* was very low (0–25%) and the fungus was not found in maize collected from Central Province. However, ANOVA of percent mould colonisation of maize revealed a significant variation ($P \leq 0.10$) in the extent of grain spoilage between agroecologies (Table 1). Generally, maize samples collected from low altitude areas comprising Luangwa District in Lusaka in agroecological Zone II and Gwembe District in Southern Province in Zone I, yielded very limited maize fungal colonisation as compared to the other locations (Table 2).

Mycotoxin contamination

ANOVA of mycotoxin content revealed no significant differences ($P \leq 0.05$) for both aflatoxin and fumonisin contamination of maize across the various agroecological zones, the ecosystems surveyed and their interactions. However, maize samples collected from the valley ecosystem in Kapiri-Mposhi, Central Province, had very high levels of aflatoxin (108.39 ppb) followed by samples from the forest areas in Chongwe District, Lusaka Province with 73.65 ppb. The distribution of both aflatoxin and fumonisin levels among the different agroecological zones and ecosystem types is shown in Fig. 3. Fumonisin contamination was, on the other hand, highest in samples from the forest areas in Luangwa District in Lusaka (10.07 ppm) followed by samples from the forest areas in Lufwanyama in the Copperbelt (5.18 ppm), Solwezi in

Table 1. ANOVA of data for discolouration, insect damage and fungal colonisation of maize from different agroecological zones and ecosystems of Zambia, 2006

		% Discoloured grains		% Insect-damaged grains		% Grains with fungal contamination	
Source of variation	*df*	Sum of squares	Prob.[a] >*F*	Sum of squares	Prob.[a] >*F*	Sum of squares	Prob.[a] >*F*
Replication	1	249.52	0.0366***[b]	12.87	0.7506	142.22	0.1811
Agroecological zone	4	561.43	0.0654**	2192.93	0.1560*	642.19	0.1866*
Rep × Agro. zone (Error A)	4	296.15	0.1187	1216.82	0.2562	350.44	0.3048
Ecosystem	2	91.60	0.1741**	57.35	0.7720	31.75	0.6875
Agro. zone × Ecosystem	8	246.43	0.2605	967.77	0.5187	387.14	0.4850
Rep × Agro. zone × Ecosyst (Error B)	8	186.45	0.3258	851.99	0.5600	446.68	0.4408
Total	27	1631.58	–	5299.73	–	2000.42	–

[a] Prob., probability to have values greater than *F*-statistics.
[b] *, **, ***: Values significantly different from zero at $P \leq 0.10$, $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2. Quality of maize grains from traditional storage structures in various districts from different agroecological zones and ecosystems of Zambia, 2006

Agroecological zone	District	Villages in ecosystem	No. sample grains	Discoloured grains (%)[a]	Insect-damaged grains (%)[b]	Grains with sign of fungal infection (%)[c]
Zone I	Gwembe	Forest	250	0.0	0.0	10.0
		Savannah	250	2.0	22.0	6.0
		Valley	250	2.0	24.0	8.0
	Choma	Forest	250	8.0	0.0	6.0
		Savannah	250	4.0	0.0	4.0
		Valley	–	–	–	–
		Mean		3.2	9.2	6.8
Zone II	Mkushi	Forest	250	0.0	6.0	0.0
		Savannah	250	0.0	2.0	0.0
		Valley	250	2.0	4.0	2.0
	Kapiri Mposhi	Forest	250	2.0	26.0	0.0
		Savannah	250	2.0	10.0	2.0
		Valley	250	2.0	20.0	2.0
	Luangwa	Forest	250	2.0	20.0	16.0
		Savannah	250	6.0	20.0	12.0
		Valley	250	2.0	80.0	6.0
	Chongwe	Forest	250	2.0	10.0	14.0
		Savannah	250	0.0	36.0	0.0
		Valley	250	1.0	15.0	12.0
		Mean		1.8	20.8	5.5
Zone III	Masaiti/Mpongwe	Forest	250	10.0	2.0	0.0
		Savannah	250	2.0	4.0	0.0
		Valley	250	0.0	0.0	2.0
	Lufwanyama	Forest	250	18.0	2.0	0.0
		Savannah	250	20.0	6.0	14.0
		Valley	250	8.0	6.0	6.0
		Mean		9.7	3.3	3.7
LSD ($P \leq 0.05$)[d]				5.7	13.4	3.1
LSD ($P \leq 0.05$)[e]				1.3	NS[f]	NS

[a] Grains discoloured (pinkish, reddish, yellowish, greenish and darkish) due to deterioration as described in the FRA chart for grain quality.
[b] Grains with insect exit holes in the different classes ranging from 1 to more than 50% areas perforated.
[c] Grains showing signs of fungal infection.
[d] LSD ($P \leq 0.05$) for comparison of the differences between agroecological zones.
[e] LSD ($P \leq 0.05$) for comparison of the differences between various ecosystems.
[f] NS stands for *F* for comparison of ecosystems not statistical different from zero at $P \leq 0.05$.

Figure 2. Insect and fungal deterioration of maize grains in smallholder farmers' storage facilities in Zambia, 2006. (A) Poor maize quality being dried on the ground before shelling for storage in Mwila village in Lufwanyama District forest, Zone III; (B) Maize husks from which grains have been shelled for storage in Lufwanyama District savannah in Copperbelt Province, Zone III, showing signs of mould infection; (C) Damaged maize as a result of the larger grain borer *Prostephanus truncates* infestation in Luangwa District savannah in Lusaka Province, Zone II and (D) Discoloured maize grains from a farmer's store in Mpongwe District forest in Copperbelt Province, Zone III.

Table 3. Various mouldy fungi recovered from maize collected in different small-scale farmers' storage from various provinces of Zambia, 2006

	% Infected samples in[b]						
					FRA[c]		
Fungal species[a]	Copperbelt	Lusaka	Central	Southern	Lusaka	Southern	Type of mycotoxins they produce
Aspergillus flavus Link[d]	25.0	10.0	0.0	20.0	20.0	10.0	Aflatoxins B1 and B2
Aspergillus niger V. Tiegham	41.7	10.0	0.0	15.0	0.0	0.0	Ochratoxin A
Aspergillus spp.	16.7	10.0	33.3	10.0	0.0	0.0	Ergot, ochratoxins, patulin
Curvularia lunata Walker	0.0	10.0	0.0	0.0	0.0	0.0	–
Fusarium solani (Mart.) Sacc.	8.4	–	0.0	0.0	0.0	10.0	–
Fusarium spp.	25.0	20.0	50.0	20.0	10.0	0.0	Fumonisins, deoxynivalenol, trichothecenes, T2 and HT2 toxins
Fusarium verticillioides	41.6	30.0	66.7	10.0	0.0	0.0	Fumonisin B and B2, zearalenone, deoxynivalenol
Helmithosporium carbonum	0.0	10.0	0.0	10.0	10.0	0.0	–
Gleocladium spp.	8.4	–	0.0	0.0	–	–	–
Penicillium spp.	16.7	20.0	50.0	50.0	10.0	10.0	Ergot, ochratoxins, patulin
Rhizopus stolonifer	16.7	0.0	0.0	10.0	–	–	Ergot
Average	18.2	13.3	18.8	13.2	5.5	3.3	–

[a] Fungal species recovered by plating maize grains directly on PDA and cultures incubated at 25–27 °C in the dark for 7 days.
[b] % fungal genera recovery from maize stored at households across ecological zones and ecosystems in Copperbelt and Central Provinces out of number of samples tested.
[c] % fungal genera recovered in FRA satellite depots visited in Chongwe, Lusaka Province and Gwembe, Southern Province.
[d] 10 maize grains sample were plated per each Petri dish with PDA for recovery of moulds.

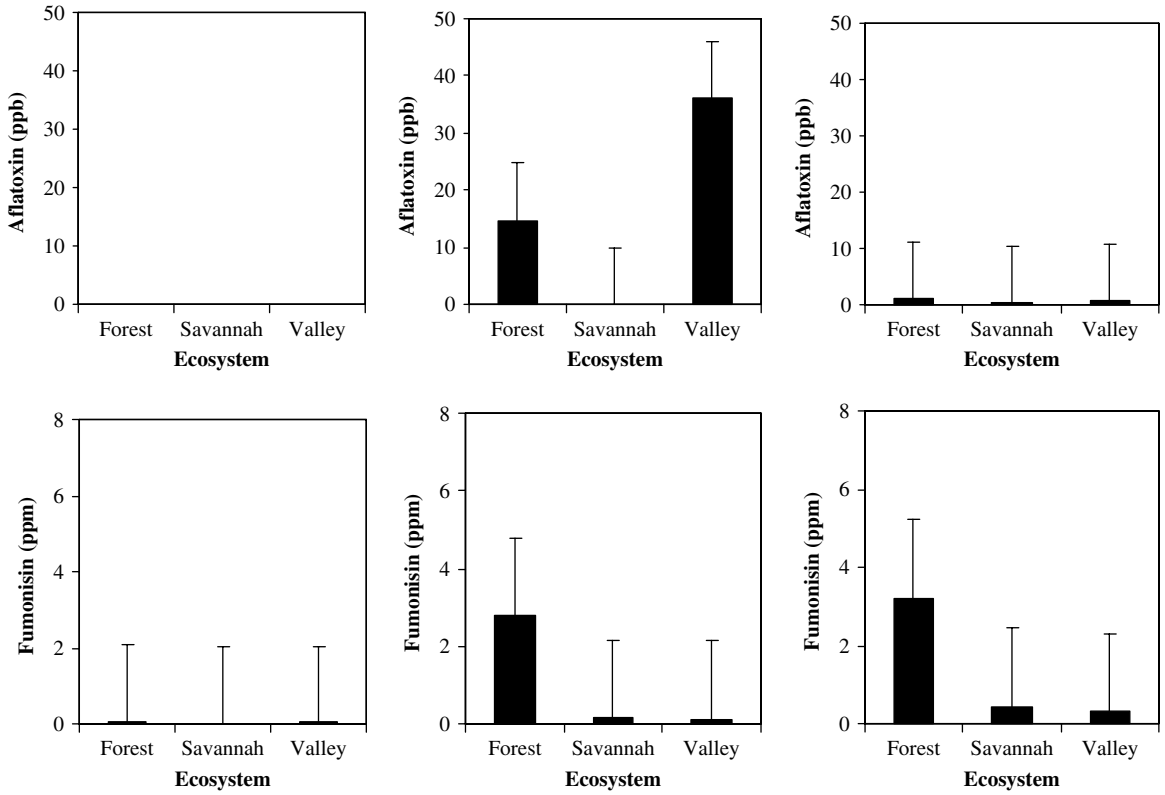

Figure 3. Mycotoxin content in maize grains stored for food for human consumption across the different agroecological zones and ecosystem types of Zambia, 2006. Aflatoxin (above) and fumonisin (below) contents of stored maize in villages in forest, savannah and valley areas in Agroecological zone I (left), zone II (middle) and zone III (right). Vertical lines represent standard errors of the observations made at each location.

Northwestern Province (4.15 ppm) and Mkushi in Central Province (3.48 ppm). Overall, highest contamination of grains by aflatoxin was recorded from villages in valley areas followed by villages near forest areas and no aflatoxin was recorded in the savannah ecoregions which is agroecological zone I. Higher fumonisin content of stored maize was found in forest areas as compared to the other ecosystems of the country (Fig. 3).

Quality of maize from FRA and other commercial depots

Samples obtained from FRA and cooperative satellite depots, which were initially collected to serve as controls because of stringent buying requirements that control maize quality, and fungal and insect infestation, were however highly colonised by various moulds especially the samples from Chongwe and Luangwa Districts in Lusaka Province (Zone II) and in Mwinilunga in Northwestern Province in Zone III (Table 4). There were also many visibly discoloured grains often contaminated with mycotoxins, particularly fumonisin (Table 4). Maize from the FRA depot in Chongwe District, had higher fumonisin levels of 21.44 ppm, than maize in other depots. No aflatoxin was recorded in any of the samples that were obtained from the FRA depots.

DISCUSSION AND CONCLUSION

Most of the households surveyed used bags to store maize often with no use of chemical insecticides, even though it is recommended for maize storage in Zambia,[1] and *Prostephanus truncatus* the most serious pest of maize is endemic.[1] Maize was typically stored either inside the houses or in various structures outside the homestead or in the field. During the survey it was observed that structures used for maize storage by small-scale farmers varied considerably across locations, but nearly all of them were in a poor state of maintenance and hygiene. The most common storage structures were open-air cribs made of tree poles and covered with a thatch roof allowing access to insects and rodents. Hence, these structures were prone to grain spoilage caused by insect and microbial contamination. It has been reported previously that storage structures differ in their ability to protect grains from fungal and insect infestation. Hell *et al.*[35] in a study in West Africa found that some types of farmers' storage structures also provided conditions that were more conducive to fungal infection and aflatoxin development than other types of stores.

Maize grains collected during this study were of poor quality often displaying severe insect damage and a range of discolourations. Grains from certain districts like Gwembe valley and savannah, Luangwa valley, Chongwe savannah and Kapiri Mposhi forest, with 24%, 22%, 80%, 36% and 26% of insect damage, respectively, were severely deteriorated and often had profound discolouration with at times prominent signs of fungal infection. This might have resulted from accelerated insect-mediated spoilage of maize grains in storage as has been reported by others.[36,37] Insects play a big role in the vectoring of fungal spores and also provide entry holes to fungal organisms through their tunnelling activity, both prior to and after harvest.[36,38] However, whether the presence of insects had also mediated maize fungal contamination in our study could not be sufficiently assessed and this warrants further investigations. High insect and fungal infestation was also prominent in maize samples from FRA satellite

Table 4. Quality and mycotoxin contamination of maize grains in Food Reserve Agency and farmers' cooperative depots in Zambia, 2006

	Grain quality[c]			Mycotoxin content[e]	
Depot	% Discoloured	% Insect damaged	% With fungal contamination[d]	Aflatoxin (ppb)	Fumonisin (ppm)
FRA[a] Masaiti	4.8	1.6	4.8	– [f]	–
ZCCM[b] Settlement Cooperative	4.0	0.2	2.0	0.0	1.22
Research Unit (Mkushi)	2.8	3.0	0.8	–	–
FRA Kapiri Mposhi	0.0	0.0	0.0	–	–
FRA Chongwe	3.6	16.0	25.0	0.0	21.44
FRA Solwezi	2.0	10.0	6.0	–	–
FRA Mwinilunga	54.0	100.0	84.0	0.0	0.06
FRA Gwembe	4.0	12.0	10.0	–	–
Average	9.4	17.9	16.6	–	–

[a] Government of the Republic of Zambia own Food Reserve Agency satellite depots.
[b] Cooperative of retirees from Zambia Consolidated Copper Mines in Lufwanyama District, Copperbelt Province.
[c] Grain quality estimated on basis of 500 maize grains.
[d] Maize grains with signs of fungal colonisation.
[e] A 50 g maize powder sample was used for analysis of mycotoxins.
[f] Samples not available for mycotoxin analysis.

depots in Chongwe, Solwezi and Mwinilunga. This is surprising since FRA standards necessitate the purchase of maize grains from cooperatives and farmers of high quality with at most a maximum of 3% insect infestation.[23] The most prevalent insects affecting the crop in storage included the weevil *S. zeamais* and the larger grain borer *P. truncatus* which have been also identified as major causes of maize yield losses in storage in other African countries.[37,38]

Consistently in our study, several mouldy fungi including *A. flavus* and *F. verticillioides* were isolated from a great number of maize samples. These two toxigenic fungi[3] were more prevalent across the different surveyed agroecological zones and accounted for more than 95% of the fungal population isolated from the samples. However, *F. verticillioides* was more prevalent in high rainfall zones II and III, whereas *A. flavus* was recorded in all the three agroecological zones of Zambia, but at a lower recovery rate than *F. verticillioides*. The low rate of recovery might be due to sample sterilisation that could have lowered the fungal population on the grain surface. Ecologically, *Aspergillus* spp. are epiphytic fungi whereas *Fusarium* spp. are endophytes.[39] As indicated earlier, surface sterilisation was necessary to hinder the development of only potential contaminants such as *Rhizopus* and *Bacillus* spp. which would affect the recovery of moulds of interest. *A. flavus* is known to synthesise aflatoxins[4,5] whereas fumonisins constitute one of the secondary toxic metabolites of *F. verticillioides*.[5] There have been previous reports on aflatoxins contaminating staples in Zambia.[19,20,22] The results of the current study are consistent with these findings, but they confirm for the first time the occurrence of high levels of both aflatoxin and fumonisin in maize stored by small-scale farmers and in satellite depots by the FRA and large farmers' cooperatives destined for human consumption across wider agroecological zones and ecosystems of Zambia. Reasons why mycotoxins, particularly fumonisins, occurred in large amounts only in forest areas remain unclear. Generally, in this study, maize obtained from households living in or nearest forest areas was highly contaminated with mycotoxins, results which conflict what has been previously reported from West Africa, where contamination rates in coastal forest areas were lower than those observed in the savannah.[35]

Due to the higher levels of toxicity and health hazard properties of aflatoxins and fumonisins, allowable tolerance limits in foods for human consumption have been regulated at varying levels in different countries. However, Sibanda *et al*.[4] reported that there is no statutory requirement for mycotoxins in Zambia and up to 2006, Zambia was still among many countries in Africa without regulatory limits for various mycotoxins in food and feed.[27] Our findings conclusively revealed mycotoxin contamination levels ranging between 0.7 and 108.39 ppb for aflatoxins in 21.4% of samples and 0.02–21.44 ppm for fumonisins in 96.4% of the samples, which are in some cases above the maximum limits acceptable for commodities destined for human consumption. There were no significant statistical differences ($P \leq 0.05$) for both aflatoxin and fumonisin contamination of maize across various agroecological zones and ecosystems surveyed, suggesting that mycotoxins are common and prevalent in maize stored for food consumption throughout Zambia with no particular agroecozone being more or less at risk. In our study, we used the commercial immunological assay ELISA to quantify mycotoxins in maize. Whether our results would compare perfectly with others using more sensitive methods such as HPLC is not known. Recently, Michelangelo and Visconti[40] stated that immunoassays often provide fast and inexpensive screening approaches and some are now adopted by the AOAC International as official methods to determine mycotoxins. FRA has a maximum limit of 10 ppb aflatoxin content when purchasing maize from farmers.[23] Whether small-holder farmers in Zambia comply with this grain quality requirement or the implementing agency monitors and reinforces the standards is unclear, and this study rather showed that maize in FRA depots presented insect infestation above the requirements and the observed mycotoxin levels gave rise to concern. Several stakeholders have, however, reported that often the Zambia Bureau of Standards has not fulfilled its mandate and that most domestic products comply only with rudimentary standards often dealing with surface imperfection and product size.[27,41]

In our present survey, we constantly observed that most maize grains were stored in polypropylene bags inside the houses where producers live and such grains are used to make 'Nshima', a stiff maize porridge which is the daily staple for the majority of the population. Judging from the observed high fungal and mycotoxins presence in these grains we believe that the Zambian population has a high likelihood of being continuously exposed

to mycotoxins both by fungal spores that are discharged from the bags risking pulmonary infection[42,43] and/or through chronic dietary exposure to mycotoxins from contaminated food which could potentially lead to aflatoxicosis as it has been reported in Kenya,[44] South Africa,[11,45] West Africa[46] and other developing countries.[47] Grains with mycotoxins above acceptable limits are unfit for human consumption and for export because they constitute a serious health hazard. In conclusion, the extent of mycotoxin contamination of stored maize recorded in our study demonstrates that a concerted effort is needed to ensure that improved pre- and post-harvest handling of maize in Zambia, including at FRA and other cooperative depots, for safer storage of the produce is implemented so that good quality food is available for the population.

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