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RESEARCH PAPER

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The influence of storage practices on aflatoxin contamination of maize in Babati district of Tanzania

Joseph Runyogote, Chacha Nyangi*

Department of Food Science and Technology, College of Agricultural Sciences and Technology Mbeya University of Science and Technology, Mbeya, Tanzania

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Key words: Aflatoxin, Maize, Storage practices, Storage structures

Abstract

Aflatoxin levels were determined in a total of 816 stored maize samples throughout two seasons (576 in season 1 and 240 in season 2) from three villages in Babati District, Northern Tanzania. Questionnaires were used at each sampling unit to evaluate maize storage practices, storage structures, pest problems in storage, and farmer's solutions, including chemical treatments, maize storage form, and duration of storage, sorting practices, and source of samples. Quantification for total aflatoxin was done using an Enzyme-Linked immunosorbent assay (ELISA) (Reveal AccuScan® Neogen, USA), and the results were confirmed using a liquid chromatographytandem mass spectrometer (LC-MS/MS). A total of 38% and 81% of maize samples were positive for aflatoxin from long village in seasons 1 and 2, respectively, while from Sabilo village we had 14% and 89% of positive samples, and from Seloto village 28% and 99% of positive samples from seasons 1 and 2, respectively. Drying maize on a raised platform, sorting out physically damaged and infected grains, storage for 6 months, use of improved bags for maize storage, and application of chemical insecticides during storage were practices found to reduce aflatoxin contamination. The findings from this study suggest that several post-harvest practices can be adopted by farmers to reduce/control aflatoxin development in maize and other crops.

^{*}Corresponding Author: Chacha Nyangi ⊠ nyangichacha@yahoo.com

Introduction

Mycotoxins are toxic secondary metabolites produced by fungi belonging to genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Byssochlamys* in crops, food, and feed products (Aziz *et al.*, 2012; Bosco and Mollea, 2012; Adeyeye *et al.*, 2021). Predominant fungi found in stored maize and maize products are *Aspergillus flavus* and *Aspergillus parasiticus* which produce aflatoxin (Okoth and Kola, 2012; Adeyeye *et al.*, 2021). Aflatoxin causes acute and chronic toxicity, through immunosuppressive, mutagenic, teratogenic, genotoxic, and carcinogenic properties and is widely recognized as a major threat to the public (Villers, 2014; Chhonker *et al.*, 2018).

In the study area maize is generally harvested late and is stored in grain form in wooden granaries, mud silos, or in polypropylene bags. Most of these systems create inadequate storage conditions unfavourable for good drying of maize, particularly in humid and semi-humid zones, subsequently; this promotes fungal infection and production of mycotoxins. The most important mycotoxigenic fungi mostly found associated with stored maize and other products are *Aspergillus flavus* which produces aflatoxins and *Fusarium verticillioides* (previously known as *F. moniliforme*), which produces fumonisins (Okoth and Kola, 2012; Misihairabgwi *et al.*, 2017).

The post-harvest proliferation of aflatoxin can be exacerbated in susceptible commodities under poor storage conditions such as hot and humid storage environment (Njoroge et al., 2019; Muga et al., 2019) If the grain is not properly dried and stored under poor storage conditions (Njoroge et al., 2019); which include high moisture, high air temperature, and high rates of evapotranspiration (Malusha, 2016; Muga et al., 2019) storage time and storage-associated problems such as poor storage, long storage time, high temperature and drought conditions, hygiene and insect infestation (Kahaya and Kyamuhangire, 2006; Sasamalo et al., 2018; Njoroge et al., 2019) as well as the type of storage structure (Maina et al., 2016; Njoroge et al., 2019). All these factors interact and influence fungal infection and proliferation

resulting in mycotoxins contamination that is in turn determined by climatic conditions (Fandohan *et al.*, 2005; Milani, 2013; Bereka *et al.*, 2021).

This study aimed to establish the effect of postharvest storage facilities, storage conditions, and post-harvest practices associated with aflatoxin in stored maize to recommend practices that will reduce contamination levels to smallholder farmers and extension services to improve food safety.

Materials and methods

Study area

The study was conducted in three villages of Long, Sabilo, and Seloto in Babati District, Manyara Region, Tanzania. In the first season, we assessed farmers' maize storage practices and aflatoxin levels in three villages. The villages were purposively selected as they represented different climatic zones. The high altitude high rain zone (Long village) lies between 2150 and 2450 meters above sea level (m.a.s.l), with relatively high annual rainfall of 1200 mm. The mid-altitude low rainfall zone (Sabilo village) lies between 1500 and 1850 m.a.s.l with relatively low rainfall of 900 – 1100 mm, while the mid-altitude high rain zone (Seloto village) lies between 1850 – 2150 m.a.s.l with relatively annual rainfall of 1100 – 1200mm (Fig. 1).

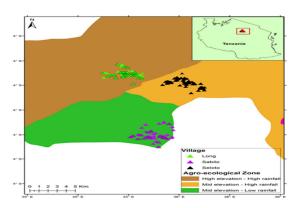


Fig. 1. Location of households where maize samples were collected in three villages in Babati district, Tanzania. They are overlaid on an agro- ecological zone map.

Selection of farmers

Twenty farmers were randomly selected from a list of 150 farmers in each village generated by the respective village's extension officers and previously used in collecting at-harvest maize samples in season 1 (Nyangi *et al.*, 2016). For season 2 only 10 farmers were selected from each village among the twenty farmers who participated in the previous survey, as the previous results indicated low aflatoxin levels and low variation. All farmers agreed to participate in this study after several meetings with the help of the village government and extension officers. Each farmer provided 350 kg of maize to be stored in their household for at least 6 months for both seasons from maize harvested in their respective farms.

Sample collection for aflatoxin analysis

Samples were collected at intervals of 0, 90, and 180 days of storage in both seasons from farmers' traditional storage facilities (i.e., farmers' storage facilities; either granary/cribs or polypropylene bags); improved storage facilities and control (polypropylene bags in which no storage treatment was applied). Farmers were interviewed using a semi-structured questionnaire. Responses were elicited on farmers' storage practices; storage facilities; pests' problem in storage; storage treatment; time/length of storage; source of samples and farmers' solutions to these problems. GPS coordinates and basic demographic details of farmers/producers were also collected.

One sub-sample was drawn from each storage facility, if there was more than one source for the same lot as explained by the interviewee; the sub-samples from each source were mixed to have approximately 1kg of each sample that was representative of the lot. For farmers who sorted their storage lots into suitable for human consumption and bad quality for livestock, two separate samples were taken. The samples were then placed in a clean paper bag (A4 envelope) that was then sealed, labelled and immediately transported to plant pathology laboratory of International Institute for Tropical Agriculture (IITA) in Dar es salaam, Tanzania.

Quantification of total aflatoxin

Aflatoxin was determined in the maize following the method described by Nyangi *et al.* (2016). The

samples were ground using a Bunn grinder (Man: Bunn-O-Matic Corporation Springfield, Illinois, U.S.A), homogenized, and subdivided to obtain a representative sub-sample for analysis. A 50 g subsample was taken from each of the ground samples and extracted with a 250 mL mixture of ethanol/water (65:35, v/v) and shaken vigorously at 150 revolutions per minute (r/min) for 3 min using a laboratory shaker (IKA® Werke, Germany). Extracts were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). Then total aflatoxin (µg/kg) was quantified following the manufacturer's protocol using Reveal AccuScan® III reader (Neogen Corporation, USA), a quantitative ELISA-based analytical test designed specifically for aflatoxin.

The detection limit for total aflatoxin was 2 µg/Kg with a quantitation range of 2 - 150 μg/Kg. The analytical quality of the ELISA methods was assured by the use of certified reference material (CRM), a naturally contaminated maize sample with a certified total aflatoxin content of 18.1 \pm 3.6 $\mu g/kg$ supplied by Neogen, USA (Neogen Corporation, USA). For data analysis, non-detectable levels were based on the detection limits (LOD) of the test method for aflatoxin. Detectable levels were compared to the East African Community (2011) established maximum tolerable limits (MTL) which is similar to that of Tanzania. For technical validation, random subsets of samples were re-analyzed using LC-MS/MS at the Interuniversity Department for Agrobiotechnology (IFA Tulln, Austria).

Statistical analysis

Data were analyzed using SAS 9.4, SAS Institute, Cary NC. Four models were built; one for all villages, and one for each village. A stepwise linear regression in a Generalised linear model (HPGENSELECT) was used to identify factors that significantly affected the contamination of maize with aflatoxin. Aflatoxin levels were $\log (x + 1)$ transformed to normalize data before analysis. The answers to "yes or no" answers were entered as binomial values and answers to categorical questions were entered as numbers.

Results

Farmer's storage practices

Farmer storage practices across all three villages and the proportion of farmers associated with each practice, including the number of maize samples collected are presented in Table 1.

Table 1. Storage practices and demographic characteristics of farmers across three village

Practices	Season 1	Season 2
	Total samples	Total samples
	n = 576 (%)	n = 240 (%)
Storage structures		
Improved bags	179 (31)	77 (32)
Polypropylene (POP)	167 (30)	102 (43)
bags		
Granaries/Cribs	54 (9)	61 (25)
Control (POP) with no	176 (30)	*N/A
treatment		
Storage length/time		
Day 180	174 (30)	90 (37)
Day 90	342 (59)	90 (37)
Day o	60 (11)	60 (26)
Storage pests		
Insects	92 (16)	22 (9)
Insects and rodents	79 (14)	145 (60)
No pests	405 (70)	73 (31)
Remove previous crop		
residue from stores		
Yes	573 (99)	239 (99)
No	3 (1)	1 (1)
Storage of maize with		
other crops		
Yes	270 (47)	127 (53)
No	306 (53)	113 (47)
Stores treatment		
Chemical pesticides	93 (16)	78 (32)
Natural protectants	40 (7)	45 (19)
No stores treatment	443 (77)	117 (49)
Grain treatment		
Chemical pesticides	74 (13)	108 (45)
Natural protectants	39 (7)	2 (1)
No use of pesticides	463 (80)	130 (54)
Drying method		

On ground On platform	568 (99) 8 (1)	186(77) 54 (23)
Sorting Yes No	501 (87) 75 (13)	240 (100) 0 (0)
Storage form	, , , , ,	
Grain	576 (100)	240 (100)
Cobs	0 (0)	0 (0)

n = Number of samples collected, (%) = percentage of farmers responded.

Prevalence and mean total aflatoxin levels in maize
Fig. 2 represents different aflatoxin contamination
from the three villages in season 1, season 2, and both
seasons. Table 2 shows the difference in aflatoxin
prevalence and mean concentration in two
consecutive seasons and post-harvest practices in the
study area. Practices that led to low aflatoxin levels
were sorting, grain treatment with chemical
pesticides, storage for 180 days, and the use of
improved storage bags. The highest aflatoxin levels
were related to maize collected from Seloto village,
drying of maize on bare ground, maize storage with
other crops, and farmers who didn't sort maize.

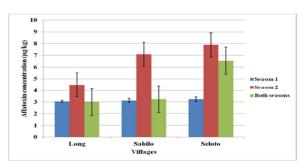


Fig. 2. Mean total aflatoxin levels with their corresponding standard error from three villages in seasons 1, 2, and in both seasons

Table 2. Prevalence and mean aflatoxin levels in maize for each applied post-harvest practice

Variables	Season	Season 1		Season 2	
	Total number of samples (%)	Mean(μg/kg)	Total number of samples (%)	Mean μg/kg)	
Storage structures					
Improved storage bags	179 (43)	3.06	77 (81)	4.42	
Polypropylene bags (POP)	167 (15)	2.95	102 (91)	7.35	
Granaries/Cribs	54 (15)	3.19	61 (98)	7.58	
POP bags without any treatment applied (Control) Storage length in days	176 (28)	3.30	*N/A	*N/A	
o days	60 (13)	4.95	60(98)	7.59	
90 days	342 (22)	2.99	90 (90)	6.67	
180 days	170 (43)	3.06	90 (83)	5.54	
Villages					

Long 197 (38) 3.04 80 (81) 4.48 Sabilo 194 (14) 3.12 80 (89) 7.09 Seloto 186 (28) 3.24 80 (99) 7.90 Storage pest Insects 92 (46) 3.02 22 (68) 3.24 Insects and rodents 79 (39) 3.11 145 (88) 6.57 None 405 (20) 3.18 73 (99) 7.27 Storing maize with other crops Yes 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1 Chemical pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.13 186 (87) 7.53					
Sabilo 194 (14) 3.12 80 (89) 7.09 Seloto 186 (28) 3.24 80 (99) 7.90 Storage pest Insects 92 (46) 3.02 22 (68) 3.24 Insects and rodents 79 (39) 3.11 145 (88) 6.57 None 405 (20) 3.18 73 (99) 7.27 Storing maize with other crops Yes 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method	Long	197 (38)	3.04	80 (81)	4.48
Storage pest 92 (46) 3.02 22 (68) 3.24 Insects and rodents 79 (39) 3.11 145 (88) 6.57 None 405 (20) 3.18 73 (99) 7.27 Storing maize with other crops 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment 58 5.85 5.85 Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 1 0 bare ground 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Sabilo	194 (14)	3.12	80 (89)	
Storage pest 92 (46) 3.02 22 (68) 3.24 Insects and rodents 79 (39) 3.11 145 (88) 6.57 None 405 (20) 3.18 73 (99) 7.27 Storing maize with other crops 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment 585 5.85 5.85 Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 1 0 bare ground 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Seloto	186 (28)	3.24	80 (99)	7.90
Insects 92 (46) 3.02 22 (68) 3.24 Insects and rodents 79 (39) 3.11 145 (88) 6.57 None 405 (20) 3.18 73 (99) 7.27 Storing maize with other crops Yes 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 1 = On bare ground 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Storage pest				
Insects and rodents None None Yes Yes No No 270 (39) 3.11 145 (88) 6.57 Nore Storing maize with other crops Yes No 270 (30) 3.00 127 (83) 5.85 Stores treatment Chemical pesticides 93 (46) 3.05 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.14 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.57	Insects	92 (46)	3.02	22 (68)	3.24
None 405 (20) 3.18 73 (99) 7.27 Storing maize with other crops 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment 5.85 5.85 Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Insects and rodents		3.11	145 (88)	
Storing maize with other crops 306 (24) 3.27 113 (96 7.26 No 270 (30) 3.00 127 (83) 5.85 Stores treatment 78 (81) 4.45 Chemical pesticides 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	None		3.18		
No 270 (30) 3.00 127 (83) 5.85 Stores treatment	Storing maize with other crops		Ü	, , , , , ,	
No 270 (30) 3.00 127 (83) 5.85 Stores treatment	Yes	306 (24)	3.27	113 (96	7.26
Stores treatment 93 (46) 3.05 78 (81) 4.45 Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	No		3.00		
Traditional pesticides 40 (58) 3.20 45 (87) 8.19 No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Stores treatment				
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No pesticides 443 (20) 3.14 117 (97) 7.19 Grain treatment 1= Chemical pesticides 74 (26) 2.89 108 (83) 5.53 2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Traditional pesticides	40 (58)	3.20	45 (87)	8.19
Grain treatment 1= Chemical pesticides 2 = Traditional pesticides 39 (07) 3 = No pesticides 39 (07) 3 - No pesticides 463 (29) 3 - No pesticides 1 = On bare ground 1 = On bare ground 2 = On raised platform 5 - 53 2 - (100) 5 - 60 3 - (100) 5 - 60 3 - (100) 5 - (100)	No pesticides	443 (20)	3.14		7.19
2 = Traditional pesticides 39 (07) 3.13 2 (100) 5.60 3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	Grain treatment				
3 = No pesticides 463 (29) 3.16 130 (94) 7.34 Drying method 1 = On bare ground 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	1= Chemical pesticides	74 (26)	2.89	108 (83)	5.53
Drying method 1 = On bare ground 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	2 = Traditional pesticides	39 (07)	3.13	2 (100)	5.60
1 = On bare ground 568 (27) 3.13 186 (87) 7.53 2 = On raised platform 8 (13) 2.40 54 (98) 6.25	3 = No pesticides	463 (29)	3.16	130 (94)	7.34
2 = On raised platform 8 (13) 2.40 54 (98) 6.25					
	1 = On bare ground	568 (27)	3.13	186 (87)	7.53
	2 = On raised platform	8 (13)	2.40	54 (98)	6.25
Sorting	Sorting				
1 = Yes 501 (27) 3.02 240 (90) 6.57	1 = Yes	501 (27)	3.02	240 (90)	6.57
2 = No 75 (24) 3.95 N/A 00	2 = No	75 (24)	3.95	N/A	00

n = Number of samples collected, (%) = Percentage of positive samples.

Table 3. Storage factors that are significantly associated with aflatoxin contamination in maize (Y) across and within three villages in the first season

Variables	Regression analysis	Estimate	P-value
Across villages	$Y = 0.30 - 0.10X_1 - 0.06X_2 - 0.08X_3 - 0.19X_4 + 0.03X_5$	0.3021	<.0001*
Long village	$Y = 0.41 - 0.08X_6 - 0.08X_7 - 0.17X_8$	0.4118	<.0001*
Sabilo village	$Y = 0.43 - 0.12X_9 - 0.09X_{10} - 0.18X_{11}$	0.4320	0.0002*
Seloto village	$Y = 0.41 - 0.28X_{12} + 0.16X_{13}$	0.4062	<.0001*
X ₁ Maize stored in im	proved bags	-0.1012	<.0001*
X ₂ Maize stored in Po		-0.0551	0.0247^{*}
X ₃ Maize stored in cr	ribs/granaries	-0.0766	0.0282*
X ₄ Maize stored for 6	months	-0.1924	<.0001*
X ₅ Maize stored with other crops		0.0322	0.0461*
X ₆ Maize stored in im	proved bags	-0.0829	0.0070*
X ₇ Maize stored in Po	lypropylene bags	-0.0760	0.0152*
X ₈ Maize stored for 6 months		-0.1681	0.0004*
X ₉ Maize stored in improved bags		-0.1194	0.0002*
X ₁₀ Maize stored in c	ribs/granaries	-0.0905	0.0346*
X ₁₁ Maize stored for 6	months	-0.1829	0.0004*
X ₁₂ Maize stored in improved bags		-0.2781	<.0001*
X ₁₃ Farmers used che	mical insecticides to protect stored maize	0.1628	<.0001*

Y = dependent variable - aflatoxin levels ($\mu g/kg$), X = independent variables (practices), * = statistically significant at P < 0.05.

High aflatoxin levels were only associated with the storage of maize crops. Maize stored in Improved and polypropylene bags from Long Village had a low risk of aflatoxin development. From Seloto village, treatment of stored maize with chemical pesticides did not reduce the risk of aflatoxin development, while maize stored in improved bags had low aflatoxin levels compared to other storage facilities (Table 3).

Surveys in the second season

Across three villages, the aflatoxin development in stored maize was not reduced through store treatment with both chemical and traditional (natural plants) insecticides and storage of maize with other crops. Low aflatoxin levels were related to storage of maize for 6 months (Table 4). Aflatoxin risk increased when maize was stored with other crops in Long Village. In Sabilo village,

the storage of maize for 6 months was related to a decrease in aflatoxin development. In Seloto village, maize stored for 6 months was related to low levels of aflatoxin development and storage problems caused by insects and rodents increasing the risk of aflatoxin contamination (Table 4).

Table 4. Storage factors that are significantly associated with aflatoxin contamination in maize (Y) across and within three villages in the second season

Variables	Regression analysis	Estimate	P-value
Across villages	$Y = 0.64 - 0.39X_1 + 0.08X_2 + 0.20X_3$	0.4003	<.0001*
Long village	$Y = 0.32 + 0.27X_4 + 0.18X_5$	0.5533	0.0002*
Sabilo village	$Y = 0.96 - 0.58X_6 + 0.31X_7$	0.4320	<.0001*
Seloto village	$Y = 0.60 - 0.44X_8 + 0.28X_9$	0.0583	0.0001*
X ₁ Maize stored for (6 months	-0.3876	<.0001*
X2 Use of chemical i	nsecticides to treat stores before storing maize	0.0842	0.0074*
X ₃ Use of the tradition	onal plant to treat stores before storing maize	0.2016	<.0001*
X ₄ Maize stored with	n other crops	0.2662	<.0001*
X ₅ Use of chemical i	nsecticides to treat stores before storing maize	0.1773	<.0001*
X ₆ Maize stored for	6 months	-0.5774	<.0001*
X ₇ Use of chemical i	nsecticides to treat stores before storing maize	0.3078	0.0002*
X ₈ Maize stored for	6 months	-0.4434	<.0001*
X ₉ Insects and roder	nts problems in store	0.2860	0.0005^{*}

Discussion

Post-harvest practices' including storage is a critical stage where infection and mycotoxin accumulation occur. Care must be taken to store grains that are wholesome and healthy; various post-harvest practices were studied over two consecutive seasons. The data from this study support the results from previous studies that reported how the proliferation of aflatoxin interacts with storage factors. It was previously reported that aflatoxin contamination was related to storage length/time (Maina et al., 2016; Ng'ang'a et al., 2016; Nyangi et al., 2016; Sasamalo et al., 2018; Likhayo et al., 2018) storage structures (Maina et al., 2016; Ng'ang'a et al., 2016; Nyangi et al., 2016; Sasamalo et al., 2018; Bereka et al., 2021), and insect infestation (Fandohan et al., 2005; Hell et al., 2003; Nyangi et al., 2016; Sasamalo et al., 2018; Bitu and Gemta, 2022).

The length of storage emerged as the most significant variable explaining the aflatoxin contamination in stored maize in both growing seasons. There was a remarkable decrease in aflatoxin levels from the beginning of storage (day o) to the end of storage (day 180). The decreasing trend was most consistent during the first growing season, this may be attributed to farmers' practices of periodical taking their maize out of storage facilities, then sundry and repacking into storage bags, and this was usually

done monthly and may play a part in control of insect infestation. The higher aflatoxin contamination observed at time zero (o storage day) reveals that the grains were exposed to aflatoxin during pre-storage; this concurs with a study on aflatoxin contamination during harvesting of maize grains in the study area (Nyangi et al., 2016; Sasamalo et al., 2018). The finding from this study also agrees with those reported by Hell et al. (2000) in Benin, Ng'ang'a et al. (2016) in Kenya, and Sasamalo et al. (2018) in Tanzania, that higher aflatoxin levels were associated with short storage period (3 - 5 months) and lower levels in longer storage duration (8 - 10 months). However, our results contrast with Hell et al. (2003) and Likhayo et al. (2018) who stated a higher incidence of aflatoxin contamination in maize stored for 6 months compared to the freshly harvested maize (o months of storage) in Benin. Moreover, the results from this study also disagree with previous findings by Liu et al. (2006) in China, Fandohan et al. (2005) from Benin, and Likhayo et al. (2018) from Kenya who reported an increase in aflatoxin levels in storage systems throughout the storage period.

The decreasing trend of aflatoxin levels with the length of storage suggests that the treatment methods applied by farmers were partly effective in reducing the mycotoxin prevalence in stored maize. Postharvest practices such as the application of chemical

pesticides had a considerable effect on reducing aflatoxin prevalence, common insecticides used were Actellic® (pirimiphos-methyl) and Bami force® (Permethrin and Malathion) and natural plant protectants. The results are comparable to Sasamalo et al. (2018) and Kebede et al. (2020), who reported that the application of chemical pesticides has a direct effect on reducing aflatoxin contamination in maize grains and the conclusion was that aflatoxinreducing effect of insecticides has an indirect effect through the reduction of insect infestation that is known to increases susceptibility to invasion by mycotoxin forming fungi. Berega et al. (2021) also reported that grains damaged by insects are susceptible to mould infection and mycotoxin development, as insect infestation produces a microclimate propitious for the development of storage fungi.

Aflatoxin levels in both seasons were lower in Long village which was located in a high-altitude and higher rainfall zone and higher in Seloto village located in a mid-altitude high rain zone (section 2.1). The climatic condition was the major factor for this trend in results observed from this study, as the optimum conditions for aflatoxin production is a temperature of 33°C and water activity of 0.99 while that for growth is 35°C and water activity of 0.95 (Bereka et al., 2021). Therefore, Aspergillus flavus and aflatoxin are more likely in maize and crops grown in the heat and drought stress associated with warmer climates (Likhayo et al., 2018; Benkerroum, 2020; Biru and Gemta, 2022) and storage environment which is humid and warm for the contamination of stored products (Okoth and Kola, 2012; Likhayo et al., 2018). The previously recorded temperature in the study area was found to range from 12°C in Long village to above 25°C in Seloto contamination levels village. Aflatoxin significantly higher in season 2 which was generally humid and warmer compared to season 1.

All farmers visited during this study were found to store their maize in grain form with the majority of farmers sorting their maize before storage. This could also be a reason for low aflatoxin levels as reported by Malusha (2016), Kumar & Kalita (2017), and Bereka et al. (2021), and that storage of maize in grain form should be encouraged owing to the prevention of contaminants especially when sorting is done and the outer covering is removed. Almost all farmers in the study area cleaned their stores and removed the residue from the previous harvest before loading the new harvest. This might also help in the control of mycotoxins. Aflatoxin contamination was higher in maize stored with other crops in both seasons; the most common crops that are usually stored alongside maize were beans, wheat, sunflower and pigeon pea. These other crops may become infected with A. flavus in the field and lead to aflatoxin development during storage (Nyangi et al., 2016; Sasamalo et al., 2018).

Drying maize on bare ground was found to have higher levels of aflatoxin contamination compared to drying on top of platforms/mats. This finding is comparable with other studies that reported on the relationship of drying maize on bare ground with aflatoxin contamination (Kaaya and Kyamuhangire, 2006; Bereka *et al.*, 2021). Atukwase *et al.* (2009) reported that drying maize on bare ground was found to be positively associated with mycotoxin contamination; this may be attributed to drying harvested maize without husks.

This practice brings maize grains into direct contact with soil which is a primary source of mould spores, in addition, drying maize on the bare ground may cause an increase in the water activity of the grains due to the absorption of moisture from the soil and re-wetting by rain (Kaaya et al., 2006; Kinyungu et al., 2019), which lead to high water activity that creates a favourable condition for fungal growth and aflatoxin production. Maize cobs that are dried on bare ground are therefore vulnerable to fungal infection and subsequent contamination with mycotoxins (Atukwase et al., 2009; Kinyungu et al., 2019). It is therefore suggested that farmers should quickly dry their maize to a moisture content that is unfavourable for fungal growth and avoid drying maize on bare ground. This reduces the free water

required for fungal development and aflatoxin production (Lanyasunya *et al.*, 2005; Likhayo *et al.*, 2018). Aflatoxin contamination was found to increase 10-fold in a day, especially when field-harvested maize is stored with high moisture content (Hell *et al.*, 2005; Likhayo *et al.*, 2018). Thus, proper dying technology should be developed and adopted by farmers.

Sorting out physically damaged and infected grains (based on their colouration, odd shapes, shrivelled, and reduced size) from the intact commodity was found to reduce aflatoxin levels in season 1. The results are comparable to Kumar and Kalita (2017) who reported that sorting alone reduces aflatoxin levels by 40-80%. Nji et al. (2022) found that stored unshelled peanuts were found with reduced levels of aflatoxin contamination compared with stored shelled peanuts. Physical methods can also involve basic sanitation measures such as removal and destruction of debris from previous harvests both in the field and store which would help in minimizing infection and infestation of produce both in the field and storage (Hell et al., 2005; Nji et al., 2022). All farmers (100%) in the study area cleaned their stores and 99% of farmers removed the residue from the previous harvest before loading the new harvest, this practice can reduce dirty and other contaminants from getting to maize and creating an unfavourable storage environment for mould growth and aflatoxin contamination. This practice helps management of aflatoxin as the crop residue from the previous harvest may harbour fungi that can contaminate newly stored crops and produce aflatoxin (Summer and Lee, 2009; Nji et al., 2022).

Conclusion

Several post-harvest practices that may help to reduce aflatoxin levels in stored maize were identified in this study: control of storage insects that increase susceptibility to invasion of grains by mycotoxins-producing fungi, sorting to remove fungi-infested and damaged cobs/grains, use of improved bags, and removal of previous year's residues as well as cleaning of stores. Moreover, the application of genetic

recombination in *A. flavus* and other species is being investigated for its potential to mitigate aflatoxins to ensure the safety and quality of food. Further research is required to show how shelling, drying, insect infestation, storage form, awareness, and storage structures influence aflatoxin levels in different agro-ecological zones in Tanzania and intervention strategies to mitigate mycotoxins.

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