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ORIGINAL ARTICLE

Dynamics of mycotoxin occurrence in farm-stored maize in Merawi district, Ethiopia

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ABSTRACT

A study was conducted to investigate the progressive development of mycotoxins in farm-stored maize in Merawi district, Ethiopia. Thirty farming households were randomly selected as sampling units, from which 30 maize samples were collected each month from the last part of the dry season (March 2015) and through the wet season until August 2015. Total aflatoxins (AFT), total fumonisins (FUM), and deoxynivalenol (DON) were measured using lateral flow immunoassays. Results indicated that incidences of AFT, FUM, and DON in the overall samples (N = 180) were 81.1%, 8.9%, and 4.4%, respectively. AFT contamination levels were highest in May- June and lowest in March and July. The highest level of FUM was observed in August. Deoxynivalenol (DON) was detected only in July-August, coinciding with the peak rainfall months. Regardless of the sampling month, levels of AFT, FUM, and DON in positive samples ranged from 2.5 to 29.3 µg/kg, 0.20 to 0.75 mg/kg, and 0.32 to 2.9 mg/kg, respectively. The AFT level showed an increasing trend across sampling months until June and decreased in July-August. The AFT levels correlated positively with the percentage of insect-damaged kernels (IDK) (r = 0.31). The proportion of samples contaminated with at least one mycotoxin was 84.4% (N = 180). The co-occurrence of two or three mycotoxins was observed in 8.9% (N = 180), of which 5.6% (N = 180) were observed in samples collected in July-August. This is the first report of a periodically monitored observation of mycotoxin development in farm-stored maize in Ethiopia. The results demonstrate the need to have a bigger picture of the magnitude of mycotoxins contamination across the storage period in our maize growing area. The finding of insect infestation is useful as a background to implement new intervention measures like hermetic storage during on-farm maize storage to reduce the impact of mycotoxins.

Key words: Farm storage; maize, mycotoxin development; Merawi, Ethiopia.

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1. Introduction

Mycotoxin build-up during prolonged grain storage is influenced by the initial grain quality and the storage environment. Grains that are insect-damaged, mechanically broken, diseased, shriveled, and inadequately or slowly dried ones influence mycotoxin build-up during storage (Fandohan et al., 2003; Magan and Aldred, 2007). Among the agricultural practices leading to poor initial grain quality for subsequent storage are delayed harvest, field drying of whole maize stalks, mechanical damage of grains during and after harvest, and drying of de-husked maize cob on the soil (Fandohan et al., 2003; Hell et al., 2003; Kaaya et al., 2005; Magan and Aldred, 2007). In traditional practices, good initial grain quality can be achieved when the weather condition favors the rapid drying of freshly harvested cobs. Besides, cleaning and sorting practices enhance grain quality (Matumba et al., 2015) and hence contribute to improved storability of grains. Regardless of initial quality, however, the moisture content of grains stored in traditional methods oscillates with the dynamic climatic and weather conditions in the storage environment. Bradford et al. (2018) highlighted the need to control product dryness for the maintenance of the quality of durable foods during storage. The most common method of farm storage of maize in Ethiopia is in woven polypropylene bags (Worku et al., 2019). As a porous container, polypropylene bags can render the grain moister during times of high relative humidity, enabling fungal and insect infestations (Bradford et al., 2018), which can lead to mycotoxin development. Grain moisture content and temperature are among the principal environmental factors that can affect the development of toxigenic fungal species and synthesis of mycotoxins in grains during storage (Mannaa and Kim, 2017). Grain moisture content and temperature of the intergranular gaseous environment can be influenced by climatic conditions and pest infestation levels. In conventional storage methods such as woven polypropylene bags, continued drying or re-wetting of grains can happen depending on the ambient climate (Williams et al., 2014). Maize stored in non-hermetic containers has shown a continuous decrease in moisture content as the rainy season was replaced by a dry season (Chigoverah and Mvumi, 2016). In contrast, a moisture gain was observed in maize stored in woven bags for three months during the wet season (Lane and Woloshuk, 2017). Results of surveys made at a single point in time to determine occurrences and co-occurrences of mycotoxins in stored maize will depend on when sampling was done after storage. Besides, in cross-sectional surveys for mycotoxin occurrences in storage, one cannot distinguish whether the observed occurrences were carried over from the field or produced during storage.

Previous reports from mycotoxin surveys on farm-stored maize in Ethiopia were made based on a one-time sampling of grains with variable grain ages after harvest. In those studies, sampling was done at a single point during the postharvest period, with ages of grains ranging between three and 10 months (Getachew et al., 2017), seven and nine months (Ayalew, 2010), and six to seven months (Tsehaye et al., 2017). Little is known in the country about the dynamics of mycotoxin contamination levels across storage periods of maize. In addressing this gap, this study was conducted to understand the progressive development of different types of mycotoxins and their concentration levels in farm-stored maize from Merawi district, north-western Ethiopia, over six months storage period.

2. Materials and methods

2.1 Description of the study area

The district Merawi was selected for sampling maize because it belongs to the leading maize-producing districts of the country (IFPRI, 2015). Through discussions with district-level crop production experts, *Kudmi* was identified as high maize production *Kebele* (the smallest administrative unit in Ethiopia) of the district. The geographic location of the sampling area is depicted in Figure 1. The altitude of the study area ranges from 1987m to 2001m above sea level.

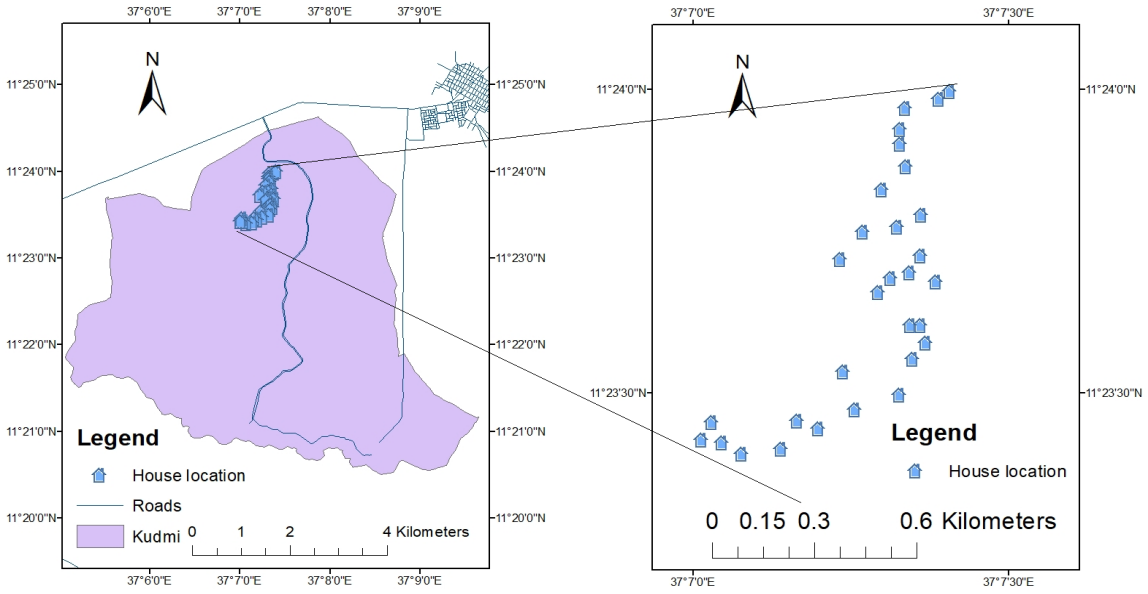

Figure 1: Location map showing farmers' houses in Kudmi Kebele used as maize sampling units.

The study area is characterized by distinct wet and dry seasons; the wet season generally occurs between May and October, and the dry season is between November and April (Yeshaneh et al., 2013). Besides, Gissila et al. (2004) indicated that in north-western Ethiopia, to which the study area belongs, the rainfall pattern is unimodal. In the study site, maize is generally planted in mid-June and harvested from mid-November to the end of December.

After de-husking on the field, maize cobs are transported near the homestead and temporarily stored in drying cribs or put on a platform for three to five months until dry enough for shelling. Shelled maize is then transferred to another store (Boxall, 1998), most commonly woven polypropylene bags (Worku et al., 2019).

2.2 Sample collection

Thirty farmers' households (every third household until a total of 30) in the Kebele were selected as sampling units. Samples were collected from the maize stores of those units, starting from March to August 2015. Maize stores of the same households continued to be sampled during the successive months. However, the maize stocks in the farmers' stores are subject to change because farmers use part of it for sale or household utilization. Besides, re-staking of bags after exposing the grain to the sun, followed by cleaning and reloading, is done as part of traditional pest management. A similar longitudinal sampling approach, where the same source was sampled monthly without ruling out the possibility of re-stocking the maize stores, was reported by Danso et al. (2018). A total of 180 maize samples of each 2 kg size (1kg for mycotoxin and another 1kg for insect data) over the six months were collected. The grain age from harvest to first sampling in March was 5 months. When storage containers were bags, 10 randomly chosen bags were sampled (Boxal et al., 2002). In traditional gotta, however, samples were taken from the top, mid, and bottom of the container and thoroughly homogenized to form a primary sample. Each sample was reduced to 1kg by sub-dividing it through coning and quartering. One kg samples were packed in plastic bags and transported immediately to the food safety laboratory located at Bahir Dar Institute of Technology, Bahir Dar University. Samples were stored at 4^{o}C until used for mycotoxin analysis.

2.3 Data collected

2.3.1 Farmers' postharvest practices for maize

A one-page checklist was prepared to assess farmers' postharvest practices applied to maize of the 2014/15 harvest. The checklist was used to interview farmers (household heads) of the 30 sampling units.

2.3.2 Grain moisture

At each sampling time, a representative sub-sample of about 200 grams of maize was selected, and grain moisture content was measured using the John Deere Moisture Check-Plus Grain Moisture Tester (AHW LLC, Watseka, Illinois, USA). Each sample was measured three times, and the average was recorded (Kalsa et al., 2019a).

2.3.3 Mycotoxin analyses

A representative 500 grams of each sample was ground using a cyclone sample mill (Model: 3010-019, UDY Corporation, Fort Collins, Colorado, USA). Seventy-five percent of the ground sample passed through a 20-mesh screen. The ground sample was thoroughly mixed and sealed in a labeled polyethylene bag at 4°C until needed for mycotoxin analysis.

Analysis of total aflatoxins (AFT): The level of total aflatoxins (aflatoxins B_1, B_2, G_1, and G_2) was quantified based on lateral flow immunoassay on an indirect competitive format (AgraStrip®AFT- COKAS1600WS) developed for

maize matrix by Romer Labs® Inc. (Union, Missouri, USA). The test was conducted according to the manufacturer's instructions (Romer Labs Methods, romerlabs.com), which has been published previously (Danso et al., 2018; Mendoza et al., 2018) and USDA-GIPSA approved for maize matrix. Readings below the limit of detection (LOD) were recorded as zero (Mendoza et al., 2018), and those between the LOD and limit of quantitation (LOQ) were recorded as half the LOQ (Nielsen et al., 2014).

Analysis of total fumonisins (FUM): The level of total fumonisins (Fumonisins B_1, B_2, and B_3) was analyzed using a lateral flow immunoassay on an indirect competitive format (AgraStrip®FUM- COKAS3000A) developed for maize matrix by Romer Labs® Inc. (Union, Missouri, USA). The test was performed according to the manufacturer's instructions (Romer Labs Methods, romerlabs.com), which has been previously published (Danso et al., 2018; Mendoza et al., 2018) and USDA-GIPSA approved for maize matrix. Readings below the LOD and those between the LOD and LOQ were recorded as described above under AFT determination.

Analysis of deoxynivalenol (DON): Determination of DON was carried out by lateral flow immunoassay on indirect competitive format (AgraStrip®DON- COKAS4000A) developed by Romer Labs® Inc. (Union, Missouri, USA). The test was performed according to the manufacturer's instructions (Romer Labs Methods, romerlabs.com), which has been previously published (Alemayehu et al., 2020) and USDA-GIPSA approved for maize matrix. The detection range was 0 to 5 mg/kg, with a limit of detection of 0.19 mg/kg and a limit of quantitation of 0.24 mg/kg. Readings below the LOD and those between the LOD and LOQ were recorded as described above under AFT determination.

Validation of the test methods was carried out for the three analyzed mycotoxins with the determination of recoveries, and the coefficient of variation (CV) was previously reported by Worku et al. (2019). Both the CVs and recovery percentages were consistent with the guideline for the performance criteria of test methods for different mycotoxins (European Union, 2006b).

2.3.4 Storage insect count and types

All of the 1kg maize samples collected from each farmer were subjected to insect count carried out as described by Kalsa (2019b). Insects were morphologically identified to genera and species levels (where possible) using a stereomicroscope, following identification keys described in Reichmuth et al. (2007).

2.3.5 Insect damaged grain

Each maize sample was divided following the coning and quartering technique until a final sample of around 100 grams was obtained. From 100 grams of sound maize, damaged and undamaged kernels were separated and counted. Insect-damaged kernels were visually identified based on holes made by boring insects. Mechanical damages were included in dockages (when it was < 50% of the average size) (Kalsa, 2019b). The percentage of insect-damaged kernels (IDK %) was then calculated based on the following formula:

$$\text{IDK (\%)} \frac{\text{Number of insect damaged kernels}}{\text{Number of kernels in 100 grams of maize}} \text{x } 100$$

2.3.6 Data Analysis

Mycotoxin occurrence data were analyzed using descriptive statistics such as frequency and central tendency measures. One-way analysis of variance (ANOVA) was employed to detect differences among samples across months. When ANOVA was significant ($P < 0.05$), treatment means were compared using Tukey's Honest Significance Test (HSD) test at a 5% level of significance. Where data normality and homogeneity of variance assumptions were not met, the non-parametric Kruskal-Wallis test was used (McKnight and Najab, 2010). Analyses of all data were performed using R version 3.5.0 (R Core Team, 2018). Graphs were plotted using SigmaPlot software Version 12.5 (Anonymous, 2013).

3. Result

3.1 Farmers' postharvest practices for maize

The Farmers responded that all the maize samples were harvested during November 2014 by mowing the stalks and piling (Figure 2) them upward on the field for one month ($N = 15$), two months ($N = 8$), and three months ($N = 7$) before the maize ears were de-husked. The de-husked maize cobs were then allowed to dry near the homestead in an outdoor ventilated crib-like structure (Figure 3), which also serves as temporary storage until the maize cobs were shelled. The duration of keeping the de-husked maize cobs in the ventilated structure was estimated by the farmers to be one month ($N = 7$), two months ($N = 13$), and three months ($N = 10$).

Figure 2: Piles of maize stalks left on the field to dry.

Maize cobs were shelled around the beginning of March and stored in the farmers' residential houses using polypropylene bags ($N = 18$) and gota ($N = 12$). All of the respondents indicated that they separated maize cobs with visible indications of pest attack, both during de-husking and shelling.

Figure 3: Ventilated structure used to store de-husked maize cobs near the homestead.

Farmers' responses on the severity and management practices of insect infestation during maize storage are shown in Table 1. Eighty-seven percent of farmers observed that insect activity at the beginning of shelled maize storage was low. However, 20.0%, 43.3%, and 36.3% of the respondents pointed out that the onset of high insect activity was observed during May, June, and April, respectively. Farmers' mitigation practices were done one month after the first appearance of high insect activity and were mostly conducted in June. Among the practices performed by farmers to tackle insect activity in maize storage, aeration/sunning and sorting out the affected portion by winnowing and screening was the dominant one (Table 1). Part of the grain that was sorted out was destined for the preparation of distilled spirit (*Arekie*) by all households. Besides, 43.3% and 13.3% of households sold the sorted-out grains and used them as livestock feed, respectively.

Table 1: Farmers' response regarding the severity and management practice of insect infestation in shelled maize storage.

Variable	Response	Percentage response (*N* = 30)
Severity of visible insect activity at storage outset	No activity	13.3
	Low activity	86.7
	High activity	0.0
Month of first appearance of high insect activity after shelled maize storage	April	20.0
	May	43.3
	June	36.7
	July	0.0
	August	0.0
Method used to arrest insect activity during storage	Aeration/sunning	80.0
	Sort-out affected portion	80.0
	Apply contact insecticide	53.3
	Fumigate the grain while in the storage	13.3
The fate of sorted out grain portion	Sale the grain	43.3
	Household food preparation	0.0
	Household beer (*Tella*) preparation	0.0
	Household distilled spirit (*Arekie*) preparation	100.0
	Livestock feed	13.3

3.2 Moisture content (m.c.)

The mean m.c. of maize samples ranged from 8.7% in April to 11.0% in August. The sampling month had a significant effect ($F_{5, 174} = 21.9$, $P < 0.01$) on the m.c. of maize. The moisture contents of maize observed in April and May were significantly lower than that of maize in the other months. And for July and August, the grain moisture was higher than in March-May. The stored maize continued to dry in storage from March through May and then started to regain moisture after May (Figure 4).

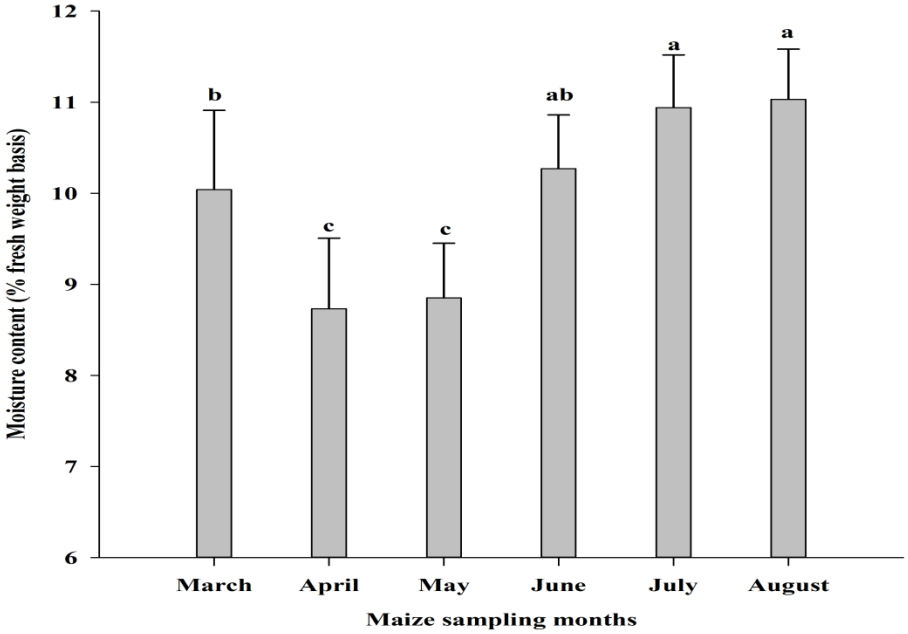

Figure 4: Mean (± *SE*) moisture content of farm-stored maize across months. Means followed by the same letter are not significantly different at Tukey's 5% level of significance.

3.3 Storage insect types, abundance, and insect-damaged kernels (IDK)

Table 2 shows the frequency and density of the most abundant insects detected in samples collected from March to August 2015. *Sitophilus zeamais* was the most frequently encountered species (detected in 93.3% of the overall samples; $N = 180$), followed by *Sitotroga cerealella* (25.6%, $N = 180$) and *Plodia interpunctella* (12.8%, $N = 180$). Significant differences ($P < 0.05$) were detected among samples across sampling months in the density of insects per unit weight of maize. *Sitophilus zeamais* infested at high densities ranging from 10.2 ± 10.9 (in March) to 262.9 ± 302.3 (in August) insects per kg. *Sitotroga cerealella* was the next most abundant species in maize samples collected at different months. The mean density of live adults of *Sitotroga cerealella* ranged from 5.1 ± 15.9 (March) to 25.5 ± 45.3 (April) insects per kg. *Plodia interpunctella* was also detected at densities ranging from 1.5 ± 5.2 (June) to 3.3 ± 10.2 (April) insects per kg.

Table 2. Live insect incidence and mean (± *SE*) of major insects detected (counts per kg of maize)

Month	Total live insects		*Sitophilus zeamais*		*Plodia interpunctella*		*Sitotroga cerealella*	
	Incidence (%)	Mean counts/kg	Incidence (%)	Mean counts/kg	Incidence (%)	Mean counts/kg	Incidence (%)	Mean counts/kg
March	80	15.3 ± 19.9 [a]	80.0	10.2 ± 10.9[a]	0.0	0.0 ± 0.0	30.0	5.1 ± 15.9
April	93.3	121.9 ± 165.7 [bc]	93.3	93.1 ± 153.0[bcd]	20.0	3.3 ± 10.2	73.3	25.5 ± 45.3
May	90.0	23.2 ± 41.4 [a]	90.0	22.0 ± 41.2[ab]	30.0	1.3 ± 2.7	0.0	0.0 ± 0.0
June	100.0	87.7 ± 116.5 [bc]	100.0	78.0 ± 105.4[cd]	26.7	1.5 ± 5.2	33.3	8.2 ± 14.2
July	100.0	27.4 ± 14.5 [b]	100.0	27.4 ± 14.5[c]	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
August	96.7	269.4 ± 303.2 [c]	96.7	262.9 ± 302.3[d]	0.0	0.0 ± 0.0	16.7	6.6 ± 31.0
χ^2		**49.5**		**53.6**				
***P*-value**		**<0.001**		**<0.001**				

Means within a column followed by the same letter are not different at 5% level of significance.

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The lowest and highest values of the percentage IDK (Figure 5) were observed in March (7.2%) and June (23.2%). It increased from March to June, followed by a sharp decrease in July (7.3%) and re-increased in August (20.2%). The mean rank of percentage IDK was significantly affected by the maize sampling month ($\chi^2 = 39.8$, $df = 5$, $P < 0.001$) (Figure 5). Percentage IDK was positively correlated with the live insect population ($r = 0.59$, $df = 178$, $P < 0.001$).

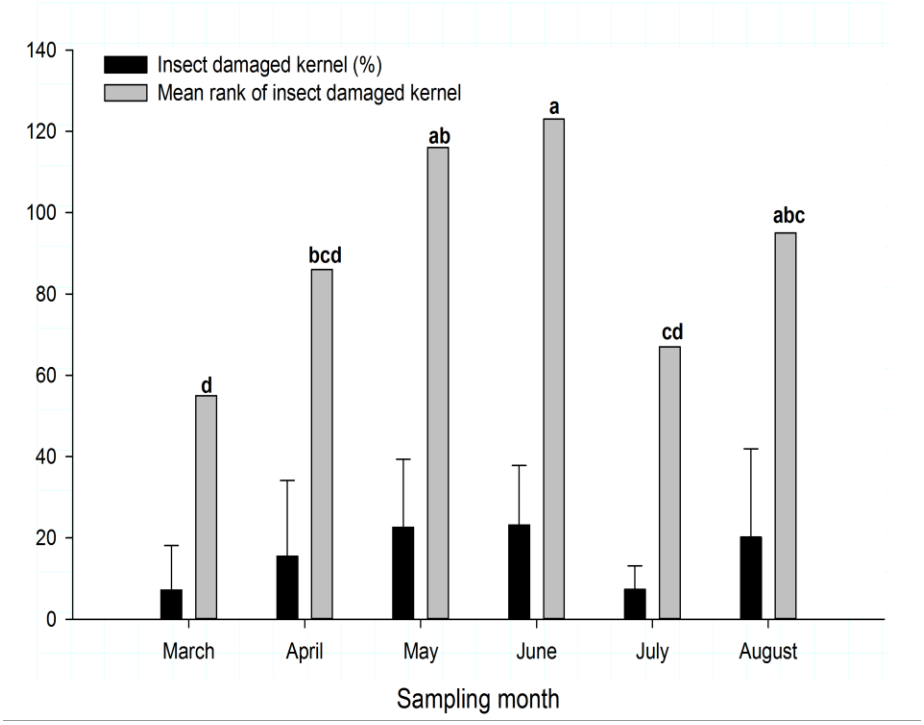

Figure 5: Kruskal-Walis test result for percentage of insect-damaged grains (IDK%) across sampling months. Bars with the same letter are not significantly different at p = 0.05.

3.4 Mycotoxin incidence and levels

Total aflatoxin (AFT) was detected in 81.1% of the overall samples ($N = 180$) with levels ranging from 0.0 to 29.3 µg/kg. Incidences and levels of AFT in maize samples collected monthly from March to August are summarized in Table 3. Regarding monthly incidences, AFT was detected in all maize samples, except in March and July, where 53.3% ($N = 30$) and 33.3% ($N = 30$) of samples were AFT-positive. Kruskal-Wallis test showed a significant effect of sampling month on AFT levels across months, both in all samples and in AFT positive ones (Table 3).

Table 3: Incidences and levels of Aflatoxins (AFT) across sampling months

Month	Incidence (%, N = 30)	Levels in positive samples µg/kg, N = 30)			
		Min	Max	Median	Mean (± *SD*)
March	53.3	2.5	29.3	2.5	5.4 ± 6.6[a]
April	100.0	6.3	22.1	11.8	12.9 ± 3.9[b]
May	100.0	8.5	26.2	16.6	16.7 ± 4.3[c]
June	100.0	7.0	23.2	16.4	16.8 ± 3.7[c]
July	33.3	2.5	7.7	6.5	5.9 ± 1.9[a]
August	100.0	2.5	12.7	6.3	6.8 ± 2.2[a]
χ^2					**96.0**
***P*-value**					**<0.001**

Means followed by the same letter are not significantly different by the Wilcoxon rank-sum test at a 5% level of significance

Ethiopia does not currently have its maximum level (ML) standard set for AFT in maize grain marketed in the country. Therefore the aflatoxin-related food safety status of the contaminated samples in the present investigation was evaluated by the 10 µg/kg ML of the East African Community (EAC, 2013) and the 20 µg/kg ML of the World Food Program (WFP, 2013). The East African Community (EAC) is a potential market for Ethiopian maize during surplus production. Besides, WFP, using its aflatoxin ML, is already involved in maize procurement from the domestic markets (Hodges and Stathers, 2013).

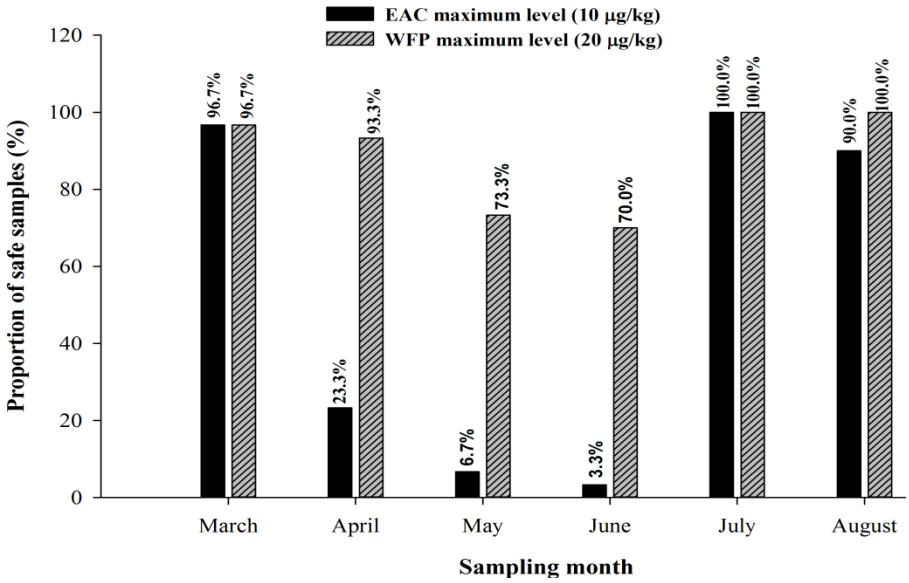

Figure 6: The proportion of samples (N = 30 for each month) with aflatoxin levels of < 10 µg/kg (EAC-ML) and < 20 µg/kg (WFP-ML).

The overall proportion of samples with AFT concentrations below the maximum level was 53.3% and 90.0% (N = 180), when evaluated by EAC (EAC, 2013) and WFP (WFP, 2013) maximum level standards, respectively. By EAC standard, the proportion of samples with AFT levels below the maximum levels declined steadily from March to June. However, it was surprising to observe that maize sampled in the subsequent July was entirely below the maximum level (Figure 6).

Incidences and levels of FUM and DON are shown in Table 4. The overall incidence of total fumonisins (FUM) was 8.9% (N = 180). Among the sampling months, the highest proportion of FUM-positive samples was detected in August (23.3%, N = 30). None of the FUM-positive samples exceeded the 2 mg/kg ML set by EAC for FUM in maize (EAC, 2013). Levels of FUM correlated positively with insect damage (r = 0.18, df = 178, P < 0.05) and grain moisture (r = 0.16, df = 178, P < 0.05).

Table 4: Distribution of incidences and levels of mycotoxins in maize samples across months

Mycotoxin	**Sampling Month**	**Incidence (%, N = 30)**	**Levels in positive samples (mg/kg)**			
			Min	**Max**	**Median**	**Average (± *SD*)**
Total fumonisins (FUM)	March	0.0	0.00	0.00	0.00	0.00 ± 0.00
	April	6.7	0.20	0.60	0.40	0.40 ± 0.28
	May	13.3	0.40	0.45	0.43	0.43 ± 0.03
	June	0.0	0.00	0.00	0.00	0.00 ± 0.00
	July	10.0	0.20	0.56	0.20	0.32 ± 0.21
	August	23.3	0.20	0.75	0.51	0.51 ± 0.17
Deoxynivalenol (DON)	March	0.0	0.00	0.00	0.00	0.00 ± 0.00
	April	0.0	0.00	0.00	0.00	0.00 ± 0.00
	May	0.0	0.00	0.00	0.00	0.00 ± 0.00
	June	0.0	0.00	0.00	0.00	0.00 ± 0.00
	July	13.3	0.60	2.89	0.83	1.29 ± 1.10
	August	13.3	0.32	1.76	0.45	0.75 ± 0.68

The incidence of DON was the least among the three mycotoxins measured in maize samples. Only 4.4% of the total samples (N = 180) were DON-positive. However, DON was detected only in samples collected in July and August. Three of the eight DON-positive samples had levels exceeding the 0.75 mg/kg ML set for cereals intended for direct human consumption in the European Union (European Union, 2006a). Levels of DON correlated positively with m.c. (r = 0.16, df = 178, P < 0.05).

The proportion of samples contaminated with at least one mycotoxin was 84.4% (N = 180). The co-occurrence of two or three mycotoxins was observed in 8.9% (N = 180), of which 5.6% (N = 180) were observed in samples collected in July-August. The binary co-occurrence of mycotoxins (AFT-FUM, AFT-DON, or FUM-DON) was dominant, with only 1.1% (N = 180) of samples contaminated with all three mycotoxins. Mycotoxin concentration in co-contaminated samples varied with months of sampling. The mean (± *SD*) concentrations of AFT and FUM in the AFT-FUM co-contaminated samples in April were 13.7 ± 4.7 µg/g and 0.40 ± 0.28 mg/kg, respectively. Similarly, the concentrations of AFT and FUM in AFT-FUM co-contaminated samples of May were 17.4 ± 3.9 µg/g and 0.43 ± 0.03 mg/g,

respectively. In August, AFT and FUM were 6.7 ± 1.4 µg/g and 0.48 ± 0.20 mg/kg, respectively. In the AFT-DON co-contaminated samples observed in August, the average concentrations of AFT and DON were 6.1 ± 0.2 µg/g and 1.04 ± 1.02 mg/kg, respectively. The AFT-FUM-DON tertiary co-occurrence observed in August had average concentrations of 6.4 ± 0.0 µg/g, 0.60 ± 0.07 mg/kg, and 0.48 ± 0.04 mg/kg for AFT, FUM, and DON, respectively.

4. Discussions

Traditional storage structures used by farmers are highly permeable to the surrounding gaseous environment. Bradford et al. (2018) reported that the moisture content of grains stored in porous containers oscillates with the relative humidity of the ambient air. Hence, stored maize grains can be re-wetted (Lane and Woloshuk, 2017) or dried (Williams et al., 2014) until the equilibrium moisture content reaches. Given that hot and dry conditions prevailed, maize in the present study continued to dry during storage from March to May. Then from June onwards, it regained moisture (Figure 4). The loss and gain of moisture by the stored maize were reminiscent of the trends observed in the external weather and climatic conditions (Figure 7).

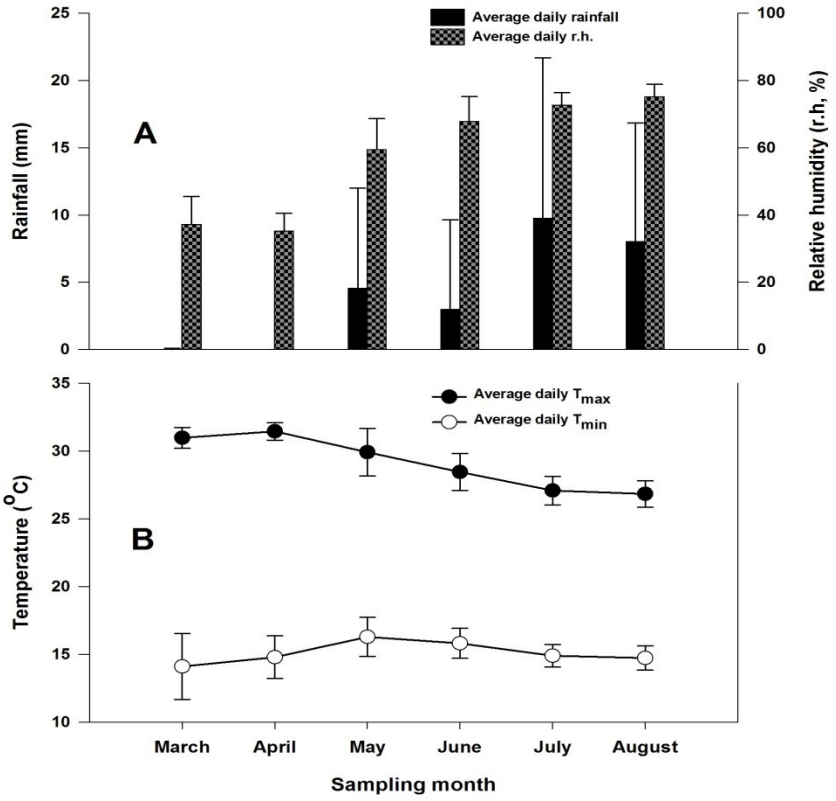

Figure 7: Daily average rainfall and relative humidity (A) and the maximum and minimum daily average temperatures (B) of the study site during the storage period investigated.

In our case, grain moisture content showed a decreasing trend from March to May, while at the same time, the AFT was increasing (Figure 8). This result is not expected, as *Aspergillus spp.* need much higher seed moisture to grow and produce AFT (Sauer and Burroghs, 1980).

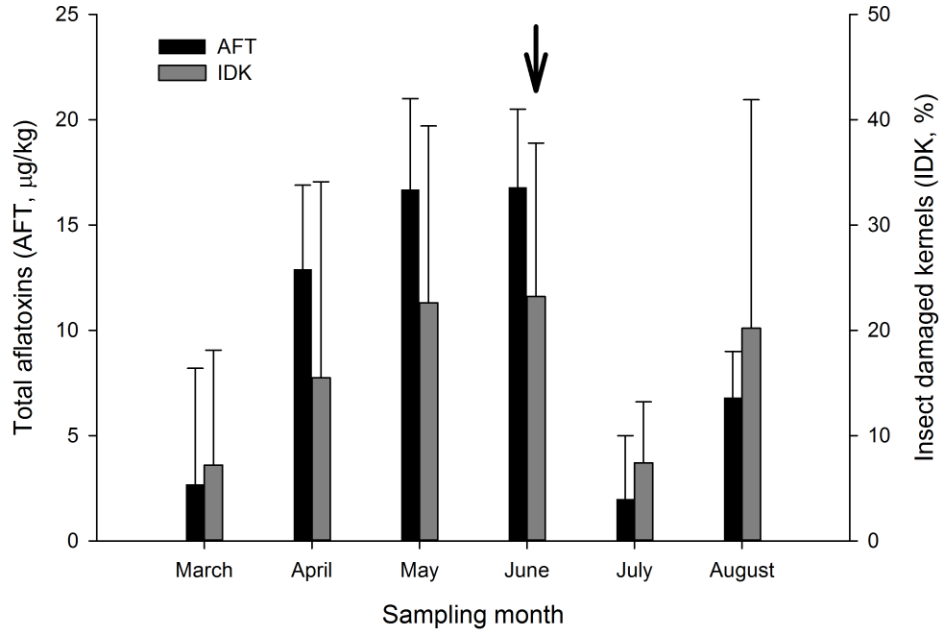

Figure 8: Average (± *SD*) total aflatoxins (AFT) and insect-damaged kernels (IDK) of maize across sampling months. The downward arrow indicates the month when most farmers conducted insect mitigation practices.

The increase in AFT levels from March to May-June (Table 3) may be attributed to a similar increasing trend of insect damage over the same duration (Figure 8). *Sitophilus zeamais* and *Sitotroga cerealella*, two of the most abundant insect species identified in the maize samples, are known to be primary pests capable of boring intact grains (Farrell et al., 2002). The least frequent insect species identified in the maize samples, *Plodia interpunctella,* behaves as a primary pest because it feeds on the embryo of undamaged grains despite its designation as a secondary pest of maize (Farrel et al., 2002).

Overall, the level of AFT was positively correlated with the percentage of insect-damaged kernels ($r = 0.31$, $df = 178$, $P < 0.001$). Insects can facilitate mold infection and mycotoxin accumulation in stored grains by physically damaging and hence predisposing the kernels to mold invasion. Besides, they transport mold spores from grain surfaces to the inside of the open kernels easing fungal infection (Hubert et al., 2018; Avantaggiato et al., 2003). The association of % IDK with AFT contamination in maize is in accordance with results from Hell et al. (2000) and Baoua et al. (2014).

A strong increase in seed moisture, *Aspergillus flavus,* and AFT was found with an artificial infestation of insects by Sinha and Sinha (1992). The assimilation of starch by insects causes production of water, heat, and CO2. This may

explain why *Aspergillus spp*., in our case, could produce AFT even at low average grain moisture in more humid hot spots (Sinha, 1961) of insects without affecting average seed moisture significantly. This could also explain why Danso et al. (2018) and Nganga et al. (2016) got increased AFT at low grain moisture in maize. In Danso et al. (2018) ´s case, a strong association between AFT and insect damage was found. Nganga et al. (2016) did not observe insects but speculated that such an association could be present.

Strong evidence is given in this study that the reduction in AFT during July is attributed to farmers' practices undertaken in response to pest infestation in storage: Aeration/sunning and sorting out the affected part of the stored grain, followed by reloading the store, often after applying contact insecticide with a cleaner portion of the original grain stock. In August, some increase in AFT was associated with increased IDK, indicating that the effects of mitigation practices were not permanent. Compared to the cleaner portion, the affected part collected by the sorting out practice was believed to have a disproportionately higher AFT level. The sorted-out part was utilized within the household or immediately sold (Table 1), while our follow-up sampling continued to be representative of the stored maize stock.

The *Fusarium* mycotoxins FUM and DON were detected at much lesser incidence than the AFT. *Fusarium* spp. is generally considered field fungi because they usually invade the growing plant in the field at higher water activity. The fact that no *Fusarium* mycotoxins were detected in samples of March may indicate that they were not formed in the field. The highest incidence of FUM observed in August could be a consequence of the creation of humid hot-spots (Marin, 1999) by insects. DON was detected only in July-August. This might be attributed to the fact that the period from July to August is part of a year when rainfall is continuous and at its peak, creating a favorable condition for the re-wetting of grains and hence facilitating the development of DON. Homdork (2000) found an increase in DON in wheat with a low incidence of *Fusarium* and storage at high temperatures and humidity.

Mycotoxins can co-exist in foods or feeds because more than one fungus species may colonize a substrate, and/or some fungi species can produce more than one mycotoxin. Although no legislative regulation is known to address co-occurring mycotoxins in foods or feeds, the co-occurrence and subsequent combined exposure are of a public health concern due to synergistic effects (Hove et al., 2016). The incidence of co-occurrence was highest in samples taken in August because of the late appearance of *Fusarium* mycotoxins, and it was less frequent during the earlier and drier months.

5. Conclusions

The present investigation has focused on the dynamic development of mycotoxins in farm-stored maize in Merawi district, Ethiopia, starting from the later part of the dry season and through the rainy season. AFT was the dominant one among those of the investigated mycotoxins. It was also the mycotoxin detected earlier, starting from the first sampling month. The main finding is that the AFT content increases with increased insect damage and is then reduced by the farmers´ mitigation practices. The levels in July- August is below the EAC- toxic limit, and other mitigation

practices would not be needed in this case. However, in years with higher AFT contamination, more intervention actions could be needed to control insects and AFT- contamination. In such situations, other mitigation practices, e.g., hermetic storage, could be recommended (Groote et al., 2013; Williams, 2014). This is the first work done in the country based on longitudinal observation of mycotoxin development in farm-stored maize.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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