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Occurrence of Mycotoxins in Stored Maize in Ethiopia

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በእህል ላይ በሚከሰቱ ሻጋታዎች ምክንያት የሚፈጠሩ መርዛማ ኬሚካሎች ለምግብ ደህንነት፣ ለምግብ አቅርቦትና ለድንበር ተሻጋሪ የንግድ ሥርዓት ሰጋት በመሆናቸው አለምአቀፍ ትኩረት እየሳቡ ይገኛሉ። በአገራችን የበቆሎ ምርት ላይ ያለውን የእነዚህ መርዛማ ኬሚካሎች ክስተት ለማጥናት ከአምስት የበቆሎ አምራች ወረዳዎች ከተውጣጡ አርሶ አደሮች ቤቶች የተሰበሰቡ 150 ናሙናዎች ላይ ፍተሻ ተደርጓል። በጥናቱ አራት ዓይነት ሻጋታ ወለድ መርዛማ ኬሚካሎች የተገኙ ሲሆን ከእነዚህ ውስጥ አፍላቶክሲን የተባለው መርዛማ ኬሚካል መጠኑ ቢለያይም በሁሉም ናሙናዎች ላይ ተገኝቷል። ስለሆነም የእነዚህን ሻጋታ ወለድ መርዛማ ኬሚካሎች ክስተት ለመከላከል በአርሶ አደሮች ደረጃ አስፈላጊው የድህረ ምርት ክብካቤ እርምጃ መወሰድ አለበት።

Abstract

Mycotoxins are attracting worldwide attention because of their implications for food safety, food availability, and international trading. A study was conducted to determine mycotoxin occurrences in stored maize in 150 samples collected from five major maize growing districts of Ethiopia: namely, Mer-Awi, Wenberma, Chelia, Halaba, and Toke Kutaye. Total aflatoxins (AFT), total fumonisins (FUM) and deoxynivalenol (DON) were measured using lateral flow immunoassay whereas the Enzyme-Linked Immunosorbent Assay was used for ochratoxin A (OTA). Results indicated that incidences of AFT, FUM, OTA, and DON were 100, 32.7, 24, and 7%, respectively (N=150). The average level of AFT was 14.7±11.6 ng/g ranging between 6.3 ng/g to 150 ng/g. Incidences of safe levels of AFT were 94.6%, 11.3% and 0% when evaluated by the maximum tolerable level (MTL) of Food and Drug Administration (FDA), East African Community (EAC), and the European Commission, respectively. The average level of FUM in positive samples was 0.68±0.94 µg/g with a range between 0.2 µg/g and 6.52 µg/g. OTA positive samples had an average level of 8.2±30.6 ng/g and a range between 2 ng/g and 186.5 ng/g. Likewise, the average and range of DON in positive samples were 0.65±0.46 µg/g and 0.27−1.98 µg/g, respectively. Co-occurrences of mycotoxins were observed, and AFT-FUM co-occurrence incidence was the highest of all others (32.7%, N=150). The occurrence of mycotoxins in stored maize in Ethiopia is evident from the survey result.

Introduction

The common traditional grain storage methods in Ethiopia are *gotera*, *gotta*, polypropylene and jute bags. *Gotera* is an outdoor storage structure made from mud or cow dung plastered basket work covered with thatched roofing and raised off the ground with stones or wooden platform; *Gotta* is an indoor grain storage bin made of mud plaster mixed with *teff* straw (Hengsdijk and De Boer, 2017). Maize belongs to grains known to be susceptible to toxigenic mold infection and associated mycotoxin contaminations (Wagacha and Muthomi, 2008).

Several reports indicated that mycotoxins are common contaminants of stored maize in Sub-Saharan Africa (Kimanya *et al*., 2008; Probst *et al*., 2014). Proper initial drying and subsequent moisture-proof storage are crucial to minimizing the growth of toxigenic fungi and toxins development (Bradford *et al*., 2018). In Africa however, many factors including environmental conditions, pest-infestation, pre and postharvest handling, influence infection of stored products by toxigenic fungi and contamination of maize by mycotoxins (Gnonlonfin *et al*., 2013). Because of the traditional post-harvest practices and the prevailing environmental conditions in Ethiopia, the risk of maize grain contamination is expected to be high. Still, available information regarding occurrences of mycotoxins in maize produced in the country is fragmented: they vary in age of grain (duration from harvest to sampling), targeted stage of production and supply chain for sampling, coverage of high producing areas, or number and type of mycotoxins investigated (Chauhan *et al.*, 2016; Getachew *et al.*, 2017; Probst *et al.*, 2014; Tsehaye *et al.*, 2017). Besides, no information is available regarding the occurrence of OTA contamination and co-occurrences of mycotoxins in maize grain from Ethiopia.

This study was conducted to investigate the occurrences of AFT, FUM, OTA, DON and their co-occurrences in farm-stored maize grain in high production areas of Ethiopia.

Materials and Methods

Sample collection

Five major maize growing districts with long-term storage practices were purposively selected from three regional states (Oromia, Amhara, and SNNPR) of Ethiopia. The districts are Mer-Awi and Wenberima (Amhara), Chelia and Toke Kutaye (Oromia), and Alaba (SNNPR). Elevations in the maize sample collection areas of the districts vary between 1710 m and 2342 m; all are located in the mid-altitude range and have a similar seasonal pattern characterized by one growing season. The districts have a major rainy season (June to September) and a longer dry season (October to May), although smaller precipitation amounts are common between March and May. The annual average rainfall of these districts ranges between 1173 mm and 1469 mm, with peak level occurring between July and August (The World Bank Group, 2018). They follow similar trends in monthly average temperatures with higher values exhibited between February and June. In all sample collecting districts, maize is harvested between November and December, when average temperature and precipitation are lowest in the year.

A total of 150 maize samples, 30 from each district, were collected from farmers' stores during late June 2016. A *Kebele* with high maize production was identified through discussion with district-level crop production experts. Every third household within each *kebele* was selected as a sampling unit. In case of absence of an adult person in a household, the next one was used as a sampling unit. When storage containers were bags, as many as possible bags were sampled depending on the number of available bags. In *gotta/gotera*, however, samples were taken from the top, mid and bottom of the container and thoroughly homogenized to form a primary sample. At the time of sampling,

information on grain age (duration from harvest to sampling) and insecticide application (insecticide applied or not) was recorded. Focus group discussions including development agents and model farmers were organized to understand weather conditions at harvesting. Each primary sample was reduced to 1kg size by sub-dividing it through coning and quartering. Submitted samples were packed in plastic bags and transported immediately to the food safety laboratory located in Bahir Dar Institute of technology, Bahir Dar University. Samples were then stored at 4 ^{0}C until used for mold infection and mycotoxin analysis.

Water activity (aw)

Water activity (a_w) was measured using the chilled mirror dew point method using an Aqualab 4TE (Decagon Devices, Inc Pullman, WA, USA) following the manufacturer's instructions. The measurements were conducted at 25°C on three replicates of randomly taken sub-samples of 10 kernels (Maiorano *et al*., 2010).

Assessing kernel mold infection incidence

Randomly selected 50 maize kernels per sample were surface sterilized by soaking in 1% sodium hypochlorite solution for 2 minutes and rinsed twice with sterile distilled water (Tsehaye *et al.*, 2017). Sterilized kernels were placed in Petri Dishes containing Potato Dextrose Agar (Hi-Media Laboratories, Mumbai, India) with 0.1% streptomycin. Plates were incubated at 25 ^{0}C for 7 days. Percentage of kernel mold infection was calculated by counting the kernels with visible fungal growth and dividing it with a total number of maize kernels placed on Petri Dish, and multiplied by 100. Morphological identification of fungal genera of infected maize kernels was carried out according to Warham *et al.* (1996) after sub-culturing from the infected kernel into a PDA plate as described by Adejumo *et al*. (2009).

Mycotoxin Analyses

Sample preparation

A representative 500 grams of each sample was ground to flour using a cyclone sample mill (model: 3010-019, UDY Corporation, Fort Collins, Co, USA). Seventy-five percent of the ground sample had passed through a 20-mesh screen (850µm pore size).

Total aflatoxins (AFT) analysis

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) approved for maize. The test procedure followed was the manufacturer's instruction (Romer Labs Inc. Union, MO, USA). Briefly, a10 g ground sub-sample was mixed with 30 ml distilled water after adding an extraction buffer bag. In a dilution tube, 50 µl sample extract was diluted with a1000 µl dilution buffer. Microwells containing lyophilized antibody conjugate were placed inside the incubator (EQASR1500) which was preheated to 45 ^{0}C. A 100 µl of diluted sample extract was pipetted into a microwell followed by inserting the AFT test strip into the microwell for three minutes incubation. The strip was then inserted into the strip holder of the AgraVision™ Reader (Romer Labs). AFT concentration on test strip was immediately read using the AgraVision reader (Model EQASR1000) after scanning the barcode found on the AFT microwell tube.

Results were interpreted using a built-in calibration curve supplied by the manufacturer and associated with each test kit lot. The range of detection for aflatoxin was 0 to 100 ng/g with a limit of detection of 3.3 ng/g and a limit of quantitation of 5.0 ng/g. Readings below the limit of detection were recorded as zero (Mendoza *et al*., 2018).

Readings between the limit of detection and limit of quantitation were recorded as half the limit of quantitation (Nielsen *et al*., 2014). For samples with readings above the higher limit of quantitation, extracts were diluted until readings within the range of quantitation were obtained; and concentration was recorded after applying the appropriate dilution factor (Mendoza *et al.*, 2018).

Total fumonisins (FUM) analysis

FUM (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 (Romer Labs Inc. Union, MO, USA). Analysis of FUM was carried out according to the manufacturer's instruction (Romer Labs Inc. Union, MO, USA). Briefly, a 10 g sub-sample of the ground maize was extracted in 70% methanol. A 50 µl aliquot of the sample extract was diluted with 1950 µl of dilution buffer (AgraStrip® Fumonisin Dilution Buffer). AgraStrip fumonisin conjugate well was placed in the incubator (Model: EQASR1500) set at 35 ^{o}C. Then, 50 µl of the AgraStrip® FUM assay buffer was added into the conjugate wells and incubated for 30 seconds followed by adding 50 µl sample extract. Next, the FUM strip was inserted into the microwell and incubated for three minutes. Reading was carried out by AgraVision™ Reader (Romer Labs) after scanning the barcode found on the FUM microwell tube, and the result interpreted as in procedure described for the AFT. The detection range was 0 to 5 µg/g with a limit of detection of 0.3 µg/g and a limit of quantitation of 0.4 µg/g. Readings outside the range were treated like it was done for AFT as indicated above.

Deoxynivalenol (DON) analysis

Determination of DON was also carried out by lateral flow immunoassay on indirect competitive format (AgraStrip®DON- COKAS4000A) developed for maize matrix and performed according to the manufacturer's instruction (Romer Labs Inc. Union, MO, USA). A 10 g sub-sample of the ground maize was mixed with 40 ml distilled water and vigorously shaken for 1 minute before allowed to settle for five minutes to get supernatant. AgraStrip DON conjugate well was placed in the incubator set at 35^{o}C. Then, 50 µl of the AgraStrip® DON assay buffer was added into the wells and incubated for 30 seconds followed by adding 50 µl sample extract. The FUM strip was then inserted into the microwell and incubated for three minutes. Reading was carried out by AgraVision™ Reader after scanning the barcode found on the DON microwell tube, and the result interpreted as in procedure described previously for the AFT and FUM. The detection range was 0 to 5 µg/g with a limit of detection of 0.19 µg/g and a limit of quantitation of 0.24µg/g. Readings outside the quantitation range were treated like it was done for AFT and FUM.

Ochratoxin A (OTA) analysis

OTA was analyzed by a competitive direct ELISA method (AgraQuant® OTA-COKAQ2000) according to the manufacturer's instruction (Romer Labs Inc. Union, MO, USA). In short, a 20 g sub-sample of maize was mixed with 100 ml 70% methanol. After shaking for three minutes, the extract was allowed to settle so that the top layer was filtered through Whatman no1 filter paper to collect the filtrate. A 200 µl conjugate solution was mixed with 100 µl extract in a dilution well. A 100 µl of this conjugate-extract mixture was transferred into the antibody-coated microwell and incubated at room temperature for 10 minutes. The microwell was then washed five times with distilled water and tapped to dry on absorbent paper. A100µl of substrate solution was added into the microwell and further incubated at room temperature for 5 minutes. Next, 100 µl of stop solution was added. The optical density of each microwell was read by a Stat Fax Model 4700 microstrip reader (Awareness Technology, Inc. Palm City, FL, U.S.A) using a 450 nm filter and a 650 nm differential filter. The reader interpreted the result by constructing a standard curve made from five AgraQuant® OTA standard solutions (0, 2, 5, 20, and 40 ng/g) supplied by the manufacturer. The quantitation range was 2 ng/g to 40 ng/g with a limit of detection of 1.9 ng/g and a limit of quantitation of 2 ng/g. Readings outside the quantitation range were treated as in the case for AFT, FUM, and DON.

Validation of the test methods was carried out for the four mycotoxins with the determination of recoveries and the coefficient of variation (CV) (Table 1). Both the CVs and recovery percentages were consistent with the guideline for performance criteria of test methods for different mycotoxins (European Union, 2006b).

Table 1: Validation data for the mycotoxin test methods

Mycotoxin	Level in naturally contaminated maize [a, b]	Recovery (%)	Coefficient of Variation (CV%)
Total Aflatoxin (AFT)	5.9	97	16.3
	18.3	109	12.3
	103.4	107	11.0
Total Fumonisin (FUM)	1.0	108	12.1
	5.0	94	3.6
Deoxynivalenol (DON)	0.569	109	12.0
	1.971	98	5.5
	5.13	107	8.6
Ochratoxin A (OTA)	7.0	104	4.0
	30.0	100	5.2

[a] *Three replicates at each level.*
[b] *ng/g for AFT and OTA, and µg /g for FUM and DON*

Data Analyses

Mycotoxin occurrence data were analyzed using descriptive statistics such as frequency and central tendency measures. One-way analysis of variance was employed to detect differences among samples across studied districts or different storage methods. Means were subject to multiple comparisons using Tukey's HSD test at 5% level of significance where analysis of variance affirmed significant differences. Means of mycotoxin levels

were compared using independent sample t-test. One sample t-test was also performed to compare AFT mean level against the MTL stated in the East African Community standard for AFT in maize for human consumption (10 ng/g). Associations between the frequencies of mycotoxin-positive samples and district, storage method, or insecticide application were done using Fisher-exact test. Where data normality and equal variance assumptions were not met, the non-parametric Kruskal-Wallis and Spearman rank correlation (r_s) tests were used. Analyses of all data were performed using R version 3.5.0.

Results and Discussions

Storage methods, grain age, and insecticide application

The most frequent method of storage used for maize was polypropylene bag (77.3%) followed by *gotta* (12.0%) and *gotera* (10.7%, *N*=150). This finding agrees with an earlier report from Kenya suggesting that farmers tend to store shelled maize in polypropylene bags in the house to prevent theft (DeGroote *et al.*, 2013). As recorded during sampling, the ages of maize grains were six months (70%) and seven months (30%, *N*=150). Moreover, samples were grouped by whether they received insecticide treatment during storage (86%) or not (14%, *N*= 150).

Water activity (a_w) and kernel mold infection

Water activity values of maize samples across districts are summarized in Table 2. A Kruskal-Wallis test for a_w showed a significant effect (X^2 =19.19, *DF*=4, *P*< 0.05) among the mean ranks of districts. *Toke Kutaye* stood last in the ascending mean rank order of a_w whereas *Mer-Awi* was on the top of the rank order (Table 2). Independent sample t-tests were conducted to compare mean values of a_w and percentage kernel mold infection in samples grouped by grain age and insecticide application. A significant difference was observed in both a_w (*P*<0.001, t=3.57) and percentage kernel mold infection (*P*<0.001, t=-3.54) of samples aged six and seven months. Samples with seven months of age had a higher mean a_w (0.696±0.108) than those aged six months (0.636±0.082). Likewise, samples aged seven months had a higher mean percentage kernel mold infection (62.1±31.4) than those aged six months (42.4±30.9). Also, a_w was positively correlated with percentage kernel mold infection (r_s=0.71, *N*=150, *P*<0.05). However, both a_w and percentage kernel mold infection were not significantly influenced by insecticide application (p>0.05). From our observations, farmers apply insecticides when they see visible signs of insect infestation. The experiment showed that *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., occurred in 91.3%, 14.7%, and, 28.0%, respectively.

The observed increase in a_w over time could arise from biological activities initiated by inadequately dried grain stored under a poorly ventilated condition that could lead to hotspot formation (Fleurat-Lessard, 2017). Besides, increased activity of insects might also have increased the relative humidity in the inter-granular space, contributing to an increase in a_w (Gnonlonfin *et al.*, 2013). Furthermore, farm-stored maize could be exposed to the limited rainfall that occurs during March and April leading to increased a_w and contributes to pest infestation.

Table 2: Kruskal-Wallis test for a_w values of maize samples (*N*=150) across districts

District	N	Water activity (a_w)			
		Range	Mean ± *SD*	Median	Mean rank
Toke Kutaye	30	0.518−0.792	0.675±0.068	0.675	93.0[a]
Chelia	30	0.516−0.928	0.705±0.126	0.716	92.7[ab]
Halaba	30	0.510−0.959	0.664±0.121	0.636	74.5[abc]
Wenberima	30	0.529−0.765	0.617±0.051	0.612	60.4[bc]
Mer-Awi	30	0.511−0.741	0.614±0.052	0.604	56.9[c]

Means followed by the same superscript letters are not significantly different at P=0.05

Incidences and levels of mycotoxins contamination in maize samples

Total Aflatoxins (AFT)

Total aflatoxin was detected in all samples (*N*=150) (Table 3). Since an MTL for AFT is not in place in Ethiopia, levels were compared with that of Federal Drug Administration (USA), European Commission regulation, and the East African Community standard. Only 5.4% of the maize samples exceeded the 20 ng/g MTL of the Federal Drug Administration (NGFA, 2011), while none complied with that of the European Commission (4 ng/g) (European Union, 2006a). A one sample t-test (*DF*=148) revealed that the average AFT level (13.8±3.2 ng/g) was significantly higher (t=14.32, P<0.001) than the MTL of 10 ng/g set by the EAC standard for maize (EAC, 2013). Only 11.3% (*N*=150) of samples had AFT levels below this MTL.

Table 3. Incidences and levels of mycotoxins in maize samples (N=150)

Mycotoxin	Number of positive samples (%)	Mycotoxin level in positive samples(µg/g)		
		Range	Mean±*SD*	Median
AFT	150(100)	6.3−150	14.7±11.6	13.5
FUM	49(32.7)	0.2−6.52	0.68±0.94	0.45
OTA	36(24)	2−186.5	8.2±30.6	2.9
DON	11(7.3)	0.27−1.98	0.65±0.46	0.53

A report by Chauhan *et al*. (2016) in maize samples (*N*=150) collected from markets within *Gedio* zone in southern Ethiopia indicated a 100% AFT incidence with an average level of 52.1ng/g. Another study conducted by Probst *et al.* (2014) in 81 maize samples from farmers' fields and small markets in Ethiopia, reported an average AFT level of 3 ng/g ranging between 0 and 23 ng/g. A recent report by Getachew *et al.* (2017) suggested only 8% AFT incidence and a maximum AFB_1 level of 513 ng/g in maize samples (*N*=100) collected from farmers' stores during the dry season from the south and southwest Ethiopia. The differences in the previous studies with the present study might be due to variation in grain age at sampling, production location, sample sizes (number of samples), or extent of insect damage.

A significant effect ($F_{4, 144}$=6.07; P<0.05) was observed across studied districts regarding AFT levels. Samples from *Mer-Awi* had significantly higher mean AFT level compared to that of other districts (Table 4). However, the type of storage container did not show a significant influence on AFT levels irrespective of the district (Table 4). AFT levels were significantly associated with a_w (r_s=-0.32, *N*=150, P<0.001) and with percentage kernel

mold infection (r_s=-0.31, N=150, P<0.001). The higher mean AFT level in Mer-Awi might be attributed to the slow drying rate associated with the traditional postharvest drying practice of maize in the area. Although maize is a rain-fed crop, many smallholders in the Mer-Awi are benefited with irrigation scheme for production of other crops during the dry season. Consequently, harvest ready maize stalks (with cobs attached) are cut and piled to dry out in the field to free the land for the next planting. The maize stalk piling practice by itself and the fact that it is performed alongside irrigation activity could reduce the drying rate.

Table 4. Mean (± *SD*) of AFT and proportion of safe maize samples by district and storage method

Variable		Number of samples (*N*=149)	AFT(ng/g)	Number of safe samples by EAC standard (%)
District	*Mer-Awi*	30	16.0±3.4[a]	1(3.3)
	Halaba	30	13.8±2.8[b]	1(3.3)
	Wenberima	30	13.5±2.4[b]	2(6.7)
	Chelia	29	13.3±3.8[b]	7(24.1)
	Toke Kutaye	30	12.3±2.6[b]	6(20.0)
	$F_{4,144}$		6.07	
	P-value		<0.001	
Storage method	*Gotta*	18	14.7±2.8[a]	0(0.0)
	Polypropylene bag	116	13.7±3.4[a]	16(13.8)
	Gotera	15	13.1±2.3[a]	1(6.7)
	$F_{2, 146}$		1.0	
	P-value		0.37	

Means followed by the same superscript letters are not significantly different at P=0.05

Total fumonisins (FUM)

A 32.7% (*N*=150) of the samples had FUM ranging from 0.2 to 6.5 µg/g (Table 3). Only one sample exceeded the 4.0 µg/g advisory maximum limit of Federal Drug Administration (USA) for human consumption (NGFA, 2011), and that of the European Commission MTL (European Union, 2006a). Two samples exceeded the 2.0 µg/g MTL of East African Community (EAC, 2013).

A recent report on stored maize from Ethiopia indicated a 77% (*N*=200) FUM incidence with a mean (0.258 µg/g), median (0.348 µg/g), and maximum (4.5 µg/g) values (Tsehaye *et al.*, 2017). The high incidence rate reported by Tsehaye *et al.* (2017) compared to the present study could be attributed to the low limit of detection (25 µg kg^{-1}) of the test method used by the authors. Besides, contamination of grains with fungi and mycotoxins could differ with seasonal variations in environmental conditions, agronomic practices or postharvest handling activities employed by farmers.

Fisher-Exact test of independence revealed significant effects (P<0.05) of districts concerning incidences of FUM positive samples (Table 5). The highest FUM detection rate was recorded in samples from *Mer-Awi* (60%) while it was lowest in samples from *Chelia* (13.3%). However, storage method or insecticide application did not cause a significant effect in FUM positive incidences (Table 5). The higher incidence of FUM

in maize from *Mer-Awi*, which was drier compared to the other districts (Table 2) could be due to high contamination in the field (pre-harvest condition).

Table 5. Fisher exact test between frequency (proportion) of FUM/OTA positive samples and district, storage method, and insecticide application

Variable		N	Number of positive samples (%)	
			FUM	OTA
District	*Chelia*	30	4(13.3)[a]	9(30.0)[a]
	Toke Kutaye	30	5(16.7)[a]	7(23.3)[ab]
	Halaba	30	11(36.7)[ab]	10(33.3)[a]
	Wenberima	30	11(36.7)[ab]	1(3.3)[b]
	Mer-Awi	30	18(60.0)[b]	9(30.0)[a]
		P-value	0.001	0.020
Storage Method	*Gotera*	16	3(18.8)[a]	4(25.0)[a]
	Gotta	18	9(50.0)[a]	3(16.7)[a]
	Polypropylene bag	116	37(31.9)[a]	29(25) [a]
		P-value	0.151	0.797
Insecticide application	Insecticide applied	129	39 (30.2)[a]	34(26.4)[a]
	Insecticide not applied	21	10(47.6)[a]	2(9.5)[a]
		P-value	0.479	0.107

Numbers followed by the same superscript letters are not significantly different at P=0.05

Ochratoxin A (OTA)

A 24% (*N*=150) of the samples had found containing OTA with levels ranging from 2-186.5 ng/g (Table 3). Three of the 36 positive samples exceeded the 5 ng/g European Commission MTL for OTA (European Union, 2006a). The frequency of OTA positive samples was significantly affected by district ($P<0.05$). Samples from *Wenberima* exhibited less frequent OTA incidence compared to those from other districts. However, the frequency of OTA positive samples was not significantly influenced by the method of storage or insecticide application (Table 5). To the best of our knowledge, there is no previous published report of OTA occurrence in maize from Ethiopia. Adetuniji *et al.* (2014) pointed out that only a few reports of OTA occurrences in maize were available in Sub-Saharan Africa. A report by Makun *et al.* (2013) indicated that maize (*N*=17) marketed in Nigeria in 2010 was 94.1% contaminated with OTA at an average level of 26±35.39 ng/g. In South African commercial maize (*N*=40), 68% incidence of OTA contamination was reported with levels ranging between 0 and 194 ng/g (Chilaka *et al.*, 2012).

Deoxynivalenol (DON)

The result of DON occurrence is summarized in Table 3. DON was detected only in 7% of all samples (*N*=150). Two of the 11 positive samples exceeded the 0.75 µg/g European Commission MTL in cereals intended for direct human consumption (European Union, 2006a). Seven of 11 positive samples (63.6%) were obtained from *Wenberima* district where unseasonal rains were experienced during maize harvesting. The focus group discussion conducted during the survey revealed that there was heavy rain during maize harvesting in December 2015. A recent study conducted in maize from the south and southwestern Ethiopia indicated a 42% DON incidence (*N*=100) with a mean, median,

and maximum levels of 0.227 µg/g, 0.221 µg/g, and 0.595 µg/g, respectively (Getachew *et al.*, 2017). The high DON incidence reported by Getachew *et al.* (2017) might be attributable to a higher sensitivity of the test method used. However, higher mean DON concentration was observed in the present study relative to those reported by Getachew *et al.* (2017).

Co-occurrence of mycotoxins

Because there was a 100% AFT incidence, the other detected mycotoxins were found to co-contaminate the maize samples. Among the tested mycotoxins, FUM co-occurred with AFT at the highest incidence (32.7%) followed by OTA (26.2%) (Figure 1). In the AFT-FUM co-contaminated samples, AFT ranged between 7.4 ng/g and 21.7 ng/g (mean=14.0 ng/g) whereas FUM levels ranged between 0.4 µg/g and 6.52 µg/g (median= 0.450 µg/g). In the AFT-OTA co-contaminated samples, AFT ranged from 7.9 ng/g to 21.7 ng/g (mean=14.7 ng/g) while OTA had ranged from 2.0 to 186.5 ng/g (median= 2.9 ng/g).

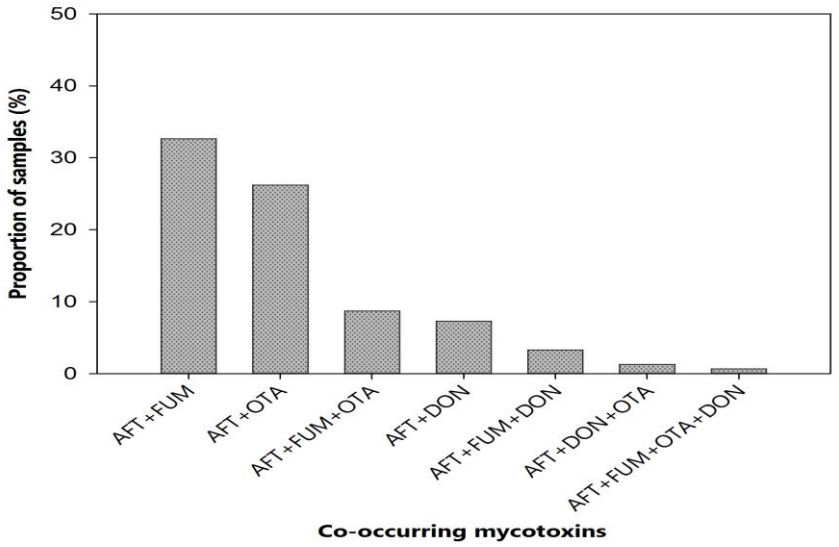

Figure 1: Co-occurrence of mycotoxins analyzed in stored maize samples (N=150) in Ethiopia in 2016

Natural mixtures of AFT have been classified as known carcinogenic to humans, while fumonisin B_1 and OTA have been classified as possibly carcinogenic substances to humans (IARC, 2012). Several studies on experimental animals have revealed increased toxic effects through combined exposure to AFB_1 and fumonisin B_1. Thus, maize consumed as staple diet could pose an increased risk to human (Hove *et al.*, 2016).

Although this study was the first report generated on mycotoxin co-occurrence in grains from Ethiopia, AFT and FUM co-occurrences have been observed by several authors from other parts of the world (Hove *et al.*, 2016). In Tanzanian rural home stored maize, the co-occurrence of AFT and FUM was10% (*N*=120) with AFT levels ranging between 1

ng/g and 151 ng/g (mean= 44ng/g) and FUM ranging between 0.11 and 11.048 µg/g (Kimanya *et al.*, 2008).

In conclusion, AFT was the most frequent contaminant among the four mycotoxins tested on maize grains in Ethiopia. Still, most of the samples were found to comply with the FDA's MTL for AFT. However, this relaxed limit is of little significance in indicating the health and economic implications of AFT contamination for Ethiopia where maize is a staple food. Extent of food (maize in this case) consumption and hence exposure assessment of a given population for a given mycotoxin is among the factors affecting the level of MTL. Thus, FDA's MTL (20 ng/g) is meant for consumers in the USA where food is generally diversified, and maize consumption extent is much lower than that in Ethiopia. Therefore, the relaxed FDA-MTL might underestimate the adverse effect of AFT of maize in Ethiopia. Ethiopia does not have its own MTL for maize, but it can use that of EAC (10 ng/g) since maize consumption rate in the EAC is relatively comparable with that in Ethiopia. Besides, EAC is a potential market for Ethiopian maize during surplus seasons, and hence meeting the MTL of the region would be more important than that of the FDA.

Incidences of FUM and OTA were lower relative to AFT. Also, their levels were generally complying with MTLs suggesting minimal health and economic impact they could pose individually. However, their co-exposure with AFT could present an increased risk to human and animal health.

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