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OCCURRENCE OF MYCOTOXINS IN FARM-STORED WHEAT IN ETHIOPIA

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ABSTRACT

In recent years, food safety has become an integral component of ensuring food security. Mycotoxin development in staple cereals is a principal threat to realizing food safety in developing countries. With the objective to investigate incidences and levels of mycotoxins in farm-stored wheat from major growing districts of Ethiopia, a study was conducted based on 179 samples collected from six districts: Wenberma, Merawi, Ofla, Hetosa, Gedeb, and Lemo. Total aflatoxins (AFT), Ochratoxin A (OTA), total fumonisins (FUM), and deoxynivalenol (DON) were determined using lateral flow immunoassays. Results indicated that the most common method used by farmers for storing wheat was polypropylene bag (92.2%, n=179). Incidences of AFT, OTA, FUM, and DON were 60.3%, 20.1%, 16.2%, and 9.5%, respectively. Levels in positive samples ranged from 2.5 µg/kg to 16.7 µg/kg for AFT, 2.1 µg/kg to 148.8 µg/kg for OTA, 0.33 mg/kg to 0.71 mg/kg for FUM, and 0.35 mg/kg to 1.14 mg/kg for DON. Age of wheat grains after harvest had a significant effect ($P < 0.05$) on the incidence of AFT. Less AFT incidence was observed in samples aged 3 months than in the older ones. None of the wheat samples exceeded the 20 µg/kg maximum level set for AFT by the FDA of the USA. However, 50.8% (n=179) exceeded the EU maximum level set at 4 µg/kg for AFT in cereals. Ochratoxin A (OTA) levels in 4.5% (*n*=179) of wheat samples exceeded the 5 µg/kg maximum level set by the EU for unprocessed cereals. Deoxynivalenol levels in 3.4% (*n*=179) of wheat samples exceeded the 0.75 mg/kg maximum level set by the EU. Besides, co-occurrences of mycotoxins were observed, and the binary co-occurrences of AFT-OTA (7.8%, n=179) and AFT-FUM (7.3%, n=179) were the dominant ones. The result of the present investigation underscores the occurrence of mycotoxins in farm-stored wheat in Ethiopia. Appropriate measures need to be taken to enable farmers to use improved grain storage technologies such as the use of multi-layer hermetic bags, which are capable of protecting wheat from physical and biological factors that lead to mycotoxin formation.

Key words: Farm-stored wheat, Mycotoxin co-occurrence, Ethiopia, Grain age, Water activity

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INTRODUCTION

Wheat accounts for 13.8% of African cereal production and Ethiopia ranks first in wheat production among the countries of sub-Saharan Africa [1] with 4.6 million tons produced on 1.7 million hectares during the major season of 2017/18 [2]. The most consumed food groups in Ethiopia are cereals [3], of which wheat is an important grain to the national food supply. On average, wheat accounts for 287 kcal/capita/day, second to maize (401 kcal/capita/day) over the period between 2010 and 2013 [4]. The demand for wheat has been rapidly rising [5]. According to the Central Statistical Agency (CSA) [6], most of the annual production was used for home consumption after milling by local stone mills into whole grain flour, and only about 20% was for sale in the marketing channels. Only part of the 20% of wheat sold is further processed into refined flour using industrial roller milling operations. This suggests that most of the wheat production is stored on-farm for household food supply until the next successful harvest and milling to whole grain flour is the dominant primary processing method.

On-farm grain storage methods in Ethiopia represent poor barriers against environmental and biological deterioration agents, and grains are stored mainly inside the house in porous bags [7]. Dried commodities stored in woven porous bags can gain moisture from an environment of high relative humidity, enabling insect infestation and fungal infections [8].

Traditional storage methods often lead to mould infection and contamination by mycotoxins and toxic secondary metabolites [9] produced by various species belonging to three main genera of fungi (*Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp.) [10]. In Ethiopia, grains are stored in traditional structures such as *gotera*, *gota*, polypropylene and jute bags. *Gotera* is an outdoor storage structure made from mud or cow dung plastered basketwork covered with thatched roofing and raised off the ground with stones or wooden platform. *Gota* is an indoor grain storage bin made of mud plaster mixed with *teff* straw [7] to render the storage structure durability by cementing the mud plaster together. Based on extensive analytical results and detailed information on the distribution of fungi in staple crops, total aflatoxins (AFT), ochratoxin A (OTA), fumonisin (FUM), and deoxynivalenol (DON) have gained global importance [11].

Few reports in the past indicated that wheat from sub-Saharan Africa was infected by the mycotoxigenic fungi and can be contaminated with associated mycotoxins [12, 13]. Although the occurrence of *Fusarium* toxins in wheat from temperate climate is well documented, little has been done in that respect in tropical regions, hence limited occurrence data is available [14].

Therefore, the objective of this study was to investigate incidences, levels, and co-occurrences of AFT, OTA, FUM, and DON in farm-stored wheat samples collected from high wheat-producing areas of Ethiopia. The result will serve as an input to mycotoxin exposure assessment studies in the country. It will also play a crucial role as evidence to encourage concerned stakeholders to devise an appropriate mechanism to protect the grain from mycotoxin contamination during farm storage.

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MATERIALS AND METHODS

Description of study areas

Six wheat-growing districts from four regions of the country, namely Amhara, Oromiya, Southern Nations Nationalities and Peoples Region (SNNP), and Tigray were included in the survey (Figure 1). The six districts were Wenberma, Merawi, Ofla, Hetosa, Gedeb, and Lemo. The latitude, longitude, and altitude range of the study sites were7.093º to 12.553º, 36.905º to 39.491º, and 2046m to2468m, respectively.

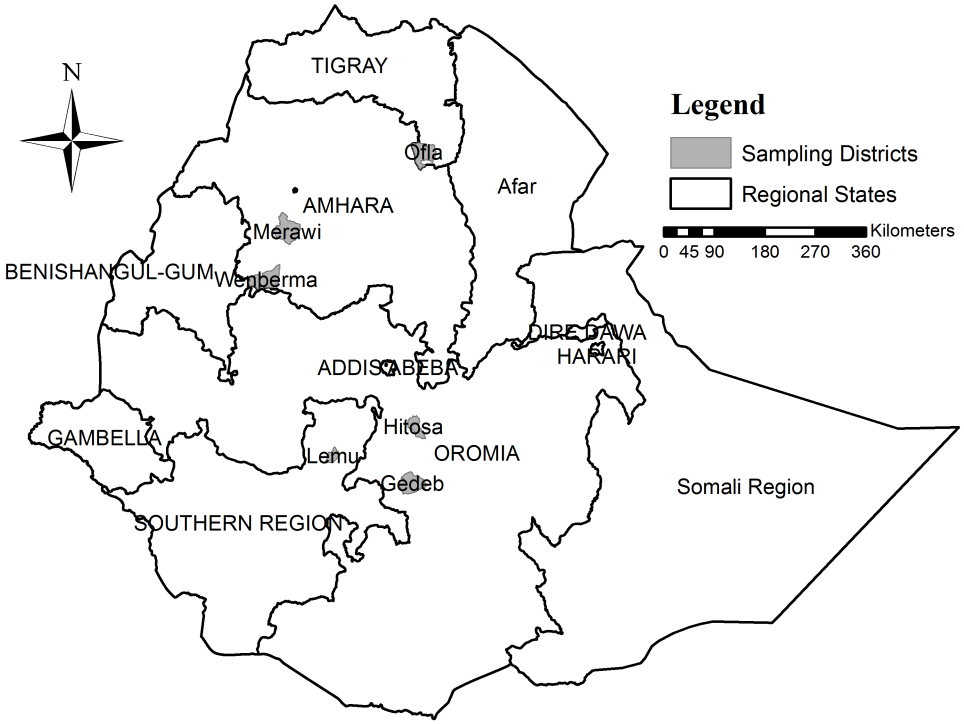

Figure 1: Map of Ethiopia showing wheat sampling districts

Sampling

A total of 179 farm stores in the six wheat-growing districts were selected to collect wheat samples in June 2016. A village with high wheat production was identified through discussions with district-level crop production experts. A total of 30 farmers from each village who grow wheat were randomly selected. Taking 30 samples per district was justified based on the central limit property [15]. Every third household within each village was chosen as a sampling unit. In case of absence of an adult person in a household, the next household was used as a sampling unit. When storage containers were bags, as many bags as possible were sampled depending on the number of available bags [16]. In traditional *gota* and *gotera*, however, samples were taken from the top, mid and bottom of the container and then thoroughly mixed to form a primary sample. Each sample collected was approximately 2 kg. At the time of sampling, information on grain age (duration from harvest to sampling) was recorded. Each primary sample was reduced to 1 kg size by subdividing it through coning and

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quartering. Samples were packed in polypropylene bags and transported immediately to the food safety laboratory of Bahir Dar University. Samples were then stored in a refrigerator at 4ºC until used for analysis.

Water activity

Water activity was measured using the chilled mirror dew point method [17] using an Aqualab 4TE (Decagon Devices, Inc Pullman, Washington, USA) following the manufacturer's instructions. The measurements were conducted at 25°C on randomly taken samples of about 100 wheat kernels from each sample collected in each district. Each 1 kg wheat sample was reduced by coning and quartering method [16] to approximately 125 g sub-sample from which the 100 kernels were taken. Measurements were taken on the same date as sampling of wheat was done.

Mycotoxin Analysis

A representative 500 g of each sample was ground to flour using a cyclone sample mill (Model: 3010-019, UDY Corporation, Fort Collins, Colorado, USA). A 75% portion of the ground sample passed through a 20-mesh screen, indicating that the particle size requirement of the whole ground sample was met. The whole grain flour was then thoroughly mixed and kept sealed in a labelled polypropylene bag and stored in a refrigerator at 4ºC until needed for mycotoxin analysis.

The levels of AFT, OTA, FUM, and DON in wheat samples were quantified using the lateral flow immunoassay principle on indirect competitive immunoassay format according to the protocols of the manufacturer provided in the respective kit (Charm Sciences, Lawrence, Massachusetts, USA). Prior to analysis, the Charm reader (model LF-ROSA-EZ-M) performance was tested daily using the calibration strips provided by the manufacturer. Additionally, the test strip performance was verified weekly by running a negative control and a positive control supplied in each kit by the manufacturer. Further steps were continued only when the kit and reader performances were within specified limits indicating readiness for use for the analysis. Before starting the mycotoxin extraction process, the Charm EZ-M reader was switched on, and the incubator temperature read 45±1°C. Besides, it was ensured that all reagents and the kit components were at room temperature (18-30ºC). Next, 50g ground wheat sub-sample was weighed into a whirl-pack bag and extracted with 100 ml 70% methanol (for AFT, OTA, and FUM) or 250 ml distilled water (for DON) by vigorously shaking for 1 min. About 1.5 ml of the extract was centrifuged in a micro-centrifuge tube for 10 sec to get a supernatant from which 100 µl (50 µl for DON) was pipetted into a new micro-centrifuge tube containing 1.0 ml dilution buffer of the respective mycotoxin, capped and mixed by inverting up and down five times. The diluted extract was further filtered through a Minisart RC15 syringe into a new micro-centrifuge tube to obtain a diluted-filtered extract that was ready for analysis. Next, the Charm EZ-M reader was set up for a test by selecting the mycotoxin of interest and the test type, followed by selecting wheat from a list of grains displayed by pressing the commodity button. Finally, the appropriate dilution (matrix) was selected (Table 1).

A test strip belonging to a specific mycotoxin was then inserted into the slot of the Charm EZ-M built-in incubator. After exposing the sample compartment of the test

strip by peeling the tape, 300 µl of the diluted-filtered extract was pipetted into it, followed by incubating for 5 min (AFT and FUM), 10 min (OTA), and 2 min (DON). The concentration of the mycotoxin was then displayed at the end of the test. Total aflatoxins (AFT) and OTA are expressed in parts per billion (ppb) or microgram per kilogram (µg/kg). However, FUM and DON are expressed in parts per million (ppm) or milligram per kilogram (mg/kg).

Readings below the limits of detection (LOD) were recorded as zero [18]. Readings between LOD and the lower limit of quantification (LOQ) were recorded as LOQ/2 [19]. For readings exceeding the higher limit of quantification of the filtered diluted extract (matrix 00), the filtered diluted extract was further diluted to the 2nd diluted extract by pipetting 300 µL of the filtered diluted extract to a micro-centrifuge tube containing 1.0 ml of dilution buffer of the respective mycotoxin. For the reading of the mycotoxin in the 2nd diluted extract similar procedure was followed except the matrix 01 was selected in the test set up.

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 studied districts or different grain ages after harvest. Where ANOVA was significant ($P < 0.05$), treatment means were compared using Tukey's Honest Significance (HSD) Test at 5% level of significance. Associations between the frequencies of mycotoxin positive samples and district or grain age were carried out using Fisher's exact test [20]. All analyses were performed using R software version 3.5.0 [21].

RESULTS AND DISCUSSION

Storage methods, grain age, and variety

The most common method used by farmers for storing wheat was polypropylene bag (92.2%, n=179) followed by *gota* (6.1%, n=179). *Gotera*, metal silo, and plastic drum were each used by one (0.6%) out of the 179 farmers' stores. The results are in good agreement with a report by Hengsdijk and De Boer [7], who indicated that most cereals in Ethiopia are stored in bags that are placed inside the house. Such storage methods cannot provide good protection against external factors, regardless of initial grain quality. These porous bags allow the grains to exchange moisture and air with the environment and provide access to insects to develop inside [8]. Insect activity and a high relative humidity environment permit dry grains to re-hydrate, thereby increasing the risk of mould growth and mycotoxin development. In developing countries, storage methods generally provide poor protection, and hence losses of grains at this stage of the supply chain are considered most critical [22].

Grain ages (durations from harvest to sampling) were three months (12.8%), seven months (48.8%), and eight months (41.3%) (n=179). Of the 179 wheat samples, the most frequent varieties were Kubsa (28.5%) and Kakaba (26.8%) followed by Danda'a (11.2%), Kingbird (7.3%), Digelu (7.3%), Hidasie (7.3%), Local (3.9%), Ogolcho (2.8%), Galama (2.2%), mixed varieties (2.2%), and Dashen (0.6%).

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Water activity (a_w)

Water activity (a_w) values of wheat samples across districts and grain ages are shown in Table 2. District and grain age had significant effects on a_w of wheat grain samples ($P<0.05$). The lowest mean a_w was recorded in Merawi district and at the age of three months (Table 2). The result agrees with an earlier report on farm-stored maize collected at ages of six and seven months from five districts of Ethiopia [23]. Those authors highlighted that a higher average a_w was exhibited by the seven-month old maize than that exhibited by the six-month old maize. Insect activity and mould growth in stored grain can advance as storage time progresses. The increase in biological activity with storage time results in increased a_w that might lead to mould growth and mycotoxin development. Differences in a_w across districts might be attributed to variations in weather condition and relative humidity and pest infestation levels of stored grains. Farmers' practices, such as harvesting, drying, and storage can influence stored grain's pest infestation and rehydration levels, which can vary from district to district.

Incidences and levels of mycotoxins

The incidences and levels of the analyzed mycotoxins are shown in Table 3. The overall level of AFT in positive samples ranged from 2.5 µg/kg to 16.7 µg/kg. While all samples had AFT levels below the 20 µg/kg maximum level set by the Federal Drug Administration (FDA) of the USA [24], 49.2% (n=179) of samples were below the stricter 4 µg/kg maximum level set by the European Union (EU) [25]. Proportions of positive samples across grain ages and districts are shown in Table 4 and Table 5, respectively. Fisher's exact test revealed that the proportion of AFT- positive samples were significantly ($p<0.05$) influenced by grain age (Table 4) and district (Table 5).

No significant effect of grain age (Table 4) or district (Table 5) was observed on the proportion of OTA-positive samples. About 4.5% of the total samples (n=179) or 22.2% of OTA-positive samples (n=36) exceeded the 5 µg/kg maximum level set by the EU. A significant ($p<0.05$) difference among districts was detected in the proportion of FUM-positive samples (Table 5). However, the proportion of FUM-positive samples was not significantly influenced ($p>0.05$) by the age of grain (Table 4). Incidence of DON was the least of all the mycotoxins' frequencies, and 3.4% of total samples (n=179) or 35.3% of DON positive samples (n=17) exceeded the EU maximum level (0.75 mg/kg) set for cereals intended for direct human consumption. The proportion of DON positive samples was not significantly influenced by grain age (Table 4) or district (Table 5) at a 5% significance level.

The incidence of AFT-positive samples (60.3%) was the highest of the investigated mycotoxins in the wheat samples, followed by OTA (20.1%). All the AFT-positive samples had levels below the FDA maximum level set for human food (20 µg/kg). On the other hand, only 15.7% of the AFT-positive samples (n=108) or 49.2% of total samples (n=179) had AFT levels below the EU maximum level (4 µg/kg). However, the stricter EU maximum level assumes that Aflatoxin contamination of grains is unavoidable and the set maximum can be reasonably achieved by following good agricultural practice [25], a practice that is non-existent in the smallholder-based production system of Ethiopia. Limited information exists on AFT contamination in

wheat from Ethiopia. Ayalew *et al*. [13] reported that Aflatoxin B_1 incidence in wheat collected from West Shewa (Ethiopia) in 1999 was 4.2% (n=120) with median, mean and maximum levels among positive samples of 9.5 µg/kg, 8.7 µg/kg, and 12.3 µg/kg, respectively. The lower incidence rate of Aflatoxin B_1 reported by Ayalew *et al*. [13] compared to the present investigation could be attributed to seasonal variations, sampling location, or the fact that those authors considered only Aflatoxin B_1. Besides, the age of wheat grain samples after harvest might have also contributed to the lower incidence of Aflatoxin B_1. Wheat samples used by Ayalew *et al*. [13] were collected from threshing yards and four to six months later from traditional farm storage structures. This assumption is supported by the result of the present investigation regarding the effect of grain age on AFT incidence (Table 4).

The OTA incidence in the present study (20.1%) agrees with the 23.4% OTA incidence (n= 107) reported by Ayalew *et al.* [13]. Those authors also reported that the median, average, and maximum levels of OTA in the positive wheat samples were 14.9 µg/kg, 19.6 µg/kg, and 66 µg/kg, respectively. The OTA concentration level reported by Ayalew *et al.* [13] is generally higher than that in the present study (Table 3), for some of the reasons discussed above.

The occurrence of *Fusarium* mycotoxins has been associated with a temperate climate. However, recent trends in climate change seem to have exposed the tropics to the toxins [14]. Sub-Saharan Africa is among regions that are vulnerable to climate change impacts because its agricultural production depends solely on weather and climate [26]. A positive association between drought stress and fumonisin production was reported [27, 28]. The present survey showed that levels of the FUM-positive samples were generally low (Table 3), and no sample had FUM levels above 1 mg/kg. A previous report on FUM contamination in wheat samples (n=15) collected from West Shewa, Ethiopia, indicated that no FUM was detected [13]. However, Mashinini and Dutton [12] highlighted that FUM was detected in 4% (n=84) wheat samples from South Africa with levels ranging between 1 mg/kg to 2 mg/kg.

Co-occurrence of mycotoxins

None of the analyzed mycotoxins tested for was detected in 24.6% (n=179) of the samples, while only one of them was detected in 50.3% (n=179) of all the wheat samples. However, 19.6% (n=179) and 5.6% (n=179) of samples were contaminated with two and three mycotoxins, respectively (Figure 2). The co-occurrence of AFT-OTA and AFT-FUM were the dominant ones observed in 7.8% and 7.3% of total samples (n=179), respectively. Incidences of AFT-OTA and AFT-FUM co-occurrences are among the most prevalent mycotoxin co-occurrences reported in the literature [29]. In the AFT-OTA co-contaminated samples, AFT ranged between 2.5 µg/kg and 12.8 µg/kg (mean=8.6 µg/kg) whereas OTA levels ranged between 2.1 µg/kg and 132.2 µg/kg (median=3.7 µg/kg). In the AFT-FUM co-contaminated samples, AFT ranged from 2.5 µg/kg to 10.3 µg/kg (mean= 6.6 µg/kg) while FUM ranged from 0.33 mg/kg to 0.71 mg/kg (mean= 0.44 mg/kg).

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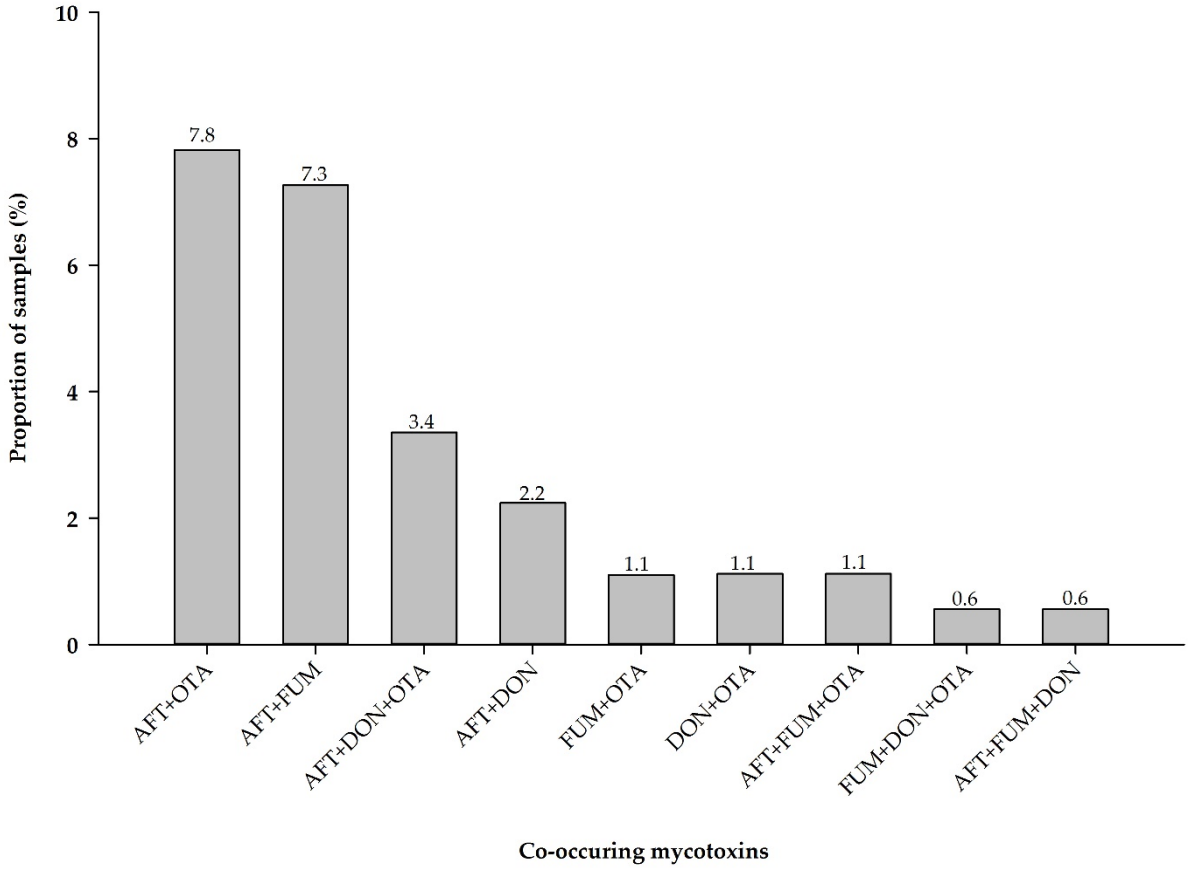

Figure2: Co-occurrence of mycotoxins in stored wheat samples (*n*=179) in Ethiopia
Legend:
AFT= Total aflatoxins, OTA= Ochratoxin A, FUM= Total Fumonisins, DON= Deoxynivalenol

Mycotoxins occur in foodstuffs, seldom alone, and they may have synergistic effects exacerbating the risk of adverse effects on human and animal health. Thus, grains consumed as staple diet could pose an increased risk to human [30]. Total aflatoxins have been classified as known carcinogens to humans [31], while fumonisin B_1 and OTA have been classified as possibly carcinogenic substances to humans [32, 33]. Several studies on experimental animals have revealed increased toxic effects through combined exposure to different mycotoxins.

In commercial milling operations of contaminated wheat into different flour streams, the grain mycotoxins will partition into the different flour streams. Compared to the original level in the whole grain, mycotoxins become concentrated in the non-endosperm streams (bran and germ), with a corresponding reduction in the endosperm flour stream [34]. Such redistribution of mycotoxins, however, cannot be achieved by the usual whole-grain flour production process accomplished using the local stone mills in Ethiopia. Rural households and a significant proportion of the urban ones consume wheat-based foods made from the whole grain. Although it is advisable to continue the consumption of wheat as a nutrient-dense and healthy whole grain product, appropriate

storage management is necessary to prevent mycotoxin formation, thereby minimizing dietary exposure to it.

Overall, the result indicates the need to put in place an appropriate mechanism to minimize mycotoxin related food safety problems in farm-stored wheat. Prevention is a better option for Ethiopia because the majority of wheat production is used for own food security by the smallholder farmer [6]. Hence, it is not practical to control mycotoxin contamination by enforcing maximum level standards in foods that do not pass through the marketing channels. Besides, the country is currently a net wheat importer [35] such that outlawing locally produced wheat by inspection alone can exacerbate shortage of the grain.

Long-term grain storage in hermetic bags is reported to be profitable in areas where grain price fluctuations are predominant [36]. Besides, hermetic bags can fit the practical needs of smallholder farmers in that bags are portable and occupy less space in the house where the grain is better monitored during storage [37]. Hermetic bags were found effective in preventing aflatoxin build-up and maintaining the initial grain quality during storage [1, 38].

CONCLUSIONS

The present investigation revealed the contamination of farm-stored wheat by four important mycotoxins. Regarding incidences, AFT was the most frequent contaminant among the four mycotoxins analyzed while DON was the least. Currently, there is no maximum level set for mycotoxins in cereals marketed in Ethiopia. Hence, the mycotoxin levels of wheat samples in the present study were evaluated using the maximum levels set by other countries. For instance, AFT concentrations of all wheat samples were below the maximum limit set by the FDA, while 49.2% of the samples were safe going by the EU maximum limit. The levels of OTA and DON (regulated in unprocessed cereals in the EU) were generally not high except for a few samples that had unacceptably high concentrations.

The authors recommend encouraging farmers to invest in improved grain storage technologies capable of protecting the grain from physical and biological factors that lead to mycotoxin formation. Hermetic bags are among the storage technologies capable of maintaining the initial quality of dried grains while practical to the needs of the smallholder farmers.

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Conflict of Interest: The authors have no conflict of interest to declare.

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Table 1: Characteristics of Charm Sciences Lateral Flow test kits used in this study to quantify mycotoxins

Channel (Mycotoxin)	Test*	Dilution (matrix)	Quantification Range	Limit of Detection (LOD)
Aflatoxin	AFQ-FAST (5MIN) Wheat	Diluted extract (00)	5-30 (µg/kg)	2 (µg/kg)
		2nd diluted extract (01)	20-100 (µg/kg)	-
Fumonisin	FUMQ-FAST5- Wheat	Diluted extract (00)	0.5-1.5 (mg/kg)	0.25 (mg/kg)
		2nd diluted extract (01	1-5 (mg/kg)	-
Ochratoxin A	OchraQ-Wheat	Diluted extract (00)	5-30 (µg/kg)	2 (µg/kg)
		2nd diluted extract (01)	20-150 (µg/kg)	-
Deoxynivalenol	DONQ2-Wheat	Diluted extract (00)	0.5-5 (mg/kg)	0.1 (mg/kg)
		2nd diluted extract (01)	5-30 (mg/kg)	-

* The test methods have been approved by USDA-GIPSA to quantify the four mycotoxins in wheat grain

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Table 2: Mean (±SD) of water activity of wheat grains across districts and grain ages (n=179)

Variable	n	Water activity (Mean±*SD)*
District		
Gedeb	30	0.690±0.098[a]
Hitosa	30	0.724±0.128[a]
Lemo	30	0.716±0.121[a]
Merawi	29	0.537±0.083[c]
Ofla	30	0.608±0.078[b]
Wenberima	30	0.607±0.062[b]
$F_{5, 173}$		**18.07**
P-Value		**<0.001**
Grain age		
3 months	23	0.532±0.092[c]
7 months	82	0.623±0.093[b]
8 months	74	0.711±0.114[a]
$F_{1, 177}$		**48.12**
P-Value		**<0.001**

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

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Table 3: Proportion of positive samples and levels of mycotoxins in wheat samples

Mycotoxin	Proportion of positive samples (%) (n=179)	Mycotoxin level in positive samples [a]			Concentration category	Number of positive samples per category [b]
		Range	Mean±SD	Median		
Total Aflatoxins	60.3	2.5−16.7	8.4±3.3	9.2	<20	108(100)
					<10	74(68.5)
					<4	17(15.7)
Ochratoxin A	20.1	2.5−148.8	12.1±32.0	3.6	<5	28(77.8)
					<10	33(91.7)
					<100	34(94.4)
Total Fumonisins	16.2	0.33−0.71	0.45±0.08	0.42	<1	29 (100)
Deoxynivalenol	9.5	0.35−1.14	0.64±0.25	0.63	<0.75	11(64.7)
					<1.0	15(88.2)

[a]µg/kg for AFT and OTA, and mg/kg for FUM and DON
[b]Numbers in parenthesis are percentages of positive samples in the concentration category regardless of district

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Table 4: Fisher exact test between the proportion of mycotoxin positive samples and age of grain after harvest (n=179)

Grain age (months)	n	Proportion of positive samples (%)			
		Total Aflatoxins	Ochratoxin A	Total Fumonisins	Deoxynivalenol
3	23	21.7[a]	17.4	17.4	13.0
7	82	73.2[b]	19.5	18.3	12.2
8	74	58.1[b]	21.6	13.5	5.4
	***P*-value**	**<0.01**	**0.90**	**0.75**	**0.25**

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

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Table 5: Incidence and levels of mycotoxins in wheat samples from each district

			Mycotoxin incidences and level in positive samples*											
			Total aflatoxins (µg/kg)			Ochratoxin A (µg/kg)			Total Fumonisins (mg/kg)			Deoxynivalenol (mg/kg)		
District	n	Grain age (months)	Incidence (%)	Range	Mean±SD	Incidence (%)	Range	Mean±SD	Incidence (%)	Range	Mean±SD	Incidence (%)	Range	Mean±SD
Gedeb	30	8	76.7[a]	2.5-13.4	8.5±3.9	20.0	2.1-148.2	27.1±59.3	0.0[a]	0.0-0.0	0.0±0.0	0.0	0.0-0.0	0.0±0.0
Hitosa	30	8	53.3[ab]	2.5-13.5	7.5±3.9	20.0	2.2-32.8	8.9±12.0	13.3[ab]	0.42-0.46	0.44±0.02	10.0	0.42-1.11	0.82±0.36
Lemo	30	7.5	26.7[bc]	2.5-10.7	5.1±3.2	23.3	2.1-4.7	2.9±1.0	33.3[b]	0.36-0.47	0.41±0.03	6.7	0.47-0.77	0.62±0.21
Merawi	29	3.8	24.1[c]	2.5-7.0	4.7±2.1	13.3	2.1-4.9	3.0±1.3	17.2[ab]	0.42-0.71	0.53±0.11	10.3	0.35-0.45	0.41±0.05
Ofla	30	7	80.0[a]	2.5-16.7	10.7±2.0	10.0	2.3-132.8	46.4±74.8	0.0[a]	0.0-0.0	0.0±0.0	3.3	0.62	0.62
Wenberima	30	7	100.0[d]	5.9-13.1	8.9±1.6	33.3	3.2-6.0	4.5±0.9	33.3[b]	0.33-0.62	0.44±0.09	26.7	0.42-1.14	0.67±0.24
***P*-value**			**<0.01**			**0.32**			**<0.01**			**0.34**		

*Means followed by the same superscript letters are not significantly different at P=0.05 by Fisher exact test.

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