Fusarium Species and Associated Fumonisin Contamination in Maize as Influenced by Sample Types in Eastern Ethiopia

Dawit Getahun[1], Mashilla Dejene[1], Habtamu Terefe[1], Wassu Mohammed[1]*
[1]School of Plant Sciences, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia
[*]Corresponding author: dawitgetahun@gmail.com; Cell Phone: 251 9 49 32 93 96

Abstract

Fusarium species are the most toxigenic fungal pathogens responsible for various diseases in maize and other food grains. *Fusarium* infection in maize contaminates harvested grain with mycotoxins such as Fumonisins. Fumonisins contamination in maize can cause yield loss and health problems in humans and animals. The study aims to assess *Fusarium* species and fumonisin contamination in fresh harvested and three-month stored maize samples collected from five major maize growing districts of eastern Ethiopia, during the 2017/18 cropping season. *Fusarium* species were isolated and identified using direct plating technique, while Fumonisin concentration was analyzed by ELISA protocols. Higher numbers of *Fusarium* isolates were recorded in fresh harvested (2376 isolates) than in three-month stored maize samples (1897 isolates). The isolates were recorded in 97 and 90% of fresh harvested and three-month stored maize samples, with an average kernel contamination of 62 and 50%, respectively. A total of seven *Fusarium* species were identified from the samples. The most prevalent species was *F. verticillioides* in both samples, followed by *F. proliferatum* and *F. subglutinans* in fresh harvested and three-month stored samples, respectively. All fresh harvested and 92% of three-month stored maize samples were found fumonisin-positive (ranging from 105 to 5,460 μg kg^{-1}). In all districts fumonisin concentration recorded in fresh harvested samples (2,509 μg kg^{-1}) was higher than that of three-month stored maize samples (1,668 μg kg^{-1}). Prevalence of different *Fusarium* species could indicate the possibility of maize contamination by other mycotoxins. Therefore, further studies are needed considering different factors over different cropping years. Findings of the study could serve as foundation for any other study on *Fusarium* species and fumonisin contamination in maize in Ethiopia to design appropriate management strategies.

Keywords: Districts, Fresh harvested sample, Kernels, *Fusarium* isolate, Three-month stored sample

Introduction

Maize (*Zea mays* L.) is among the most important food crops in Africa, produced and consumed directly by smallholder farmers (Keno *et al.*, 2018)-. Maize is the second extensively cultivated crop under diverse agro-climatic and socioeconomic conditions, and is consumed as food, both as green and dry grain in Ethiopia (Abate *et al.*, 2015). All maize produced in Ethiopia is (Abate *et al.*, 2015). The crop accounts for 29% of the national calorie intake (Berhane *et al.*, 2011). Maize is also considered as a source of income, means of employment for all producers to business communities, while the stalk serves for feed and fuel (Keno *et al.*, 2018). During the 2017/18 cropping season, total maize production was

8.38 million tons, harvested from 2.13 million ha of land [Central Statistical Agency (CSA), 2018]. Oromia National Regional State of Ethiopia produced maize on 1,146,899.78 ha of land with an average productivity of 4.1 t ha^{-1} and it was the leading maize producer in the country in the 2017/18 cropping season (CSA, 2018). Among Oromia region, maize covered 49,980 and 42,044 ha of land in Eastern and Western Hararghe areas of eastern Ethiopia, respectively, while the average productivity was 2.7 and 2.3 t ha^{-1} in that order in the 2017/18 cropping season (CSA, 2018).

However, maize production is affected by various diseases worldwide. Among these diseases, ear rot is one of the most important diseases wherever maize is growing (Gxasheka *et al.*, 2015). The common ear rot diseases of maize are *Aspergillus* and *Fusarium* ear rot/kernel rot (Mesterházy *et al.*, 2012; Gxasheka *et al.*, 2015). Two types of maize ear rot are associated with *Fusarium* species, namely *Fusarium* ear rot, caused by *F. proliferatum*, *F. subglutinans*, and *F. verticillioides*, and Gibberella ear rot caused by *F. avenaceum*, *F. cerealis*, *F. culmorum*, and *F. graminearum* (Mesterházy *et al.*, 2012).

Fusarium kernel rot development in maize lowers yield and grain quality by contaminating harvested maize grains with their secondary metabolites, mycotoxins (Kamala *et al.*, 2016). *Fusarium* species contamination in maize produces mycotoxin such as fumonisins, zearalenone and trichothecenes (T2, TH2 and deoxynivalenol). Fumonisins are mostly produced by *F. verticillioides* and *F. proliferatum* in maize (Sundheim and Tsehaye, 2015) wherever maize is growing (Waśkiewicz *et al.*, 2012; Tsehaye *et al.*, 2017). Consumption of fumonisins contaminated maize grains can cause several health problems in humans and animals (Waśkiewicz *et al.*, 2012). In human beings, fumonisin B_1 is a potent cancer promoter associated with child stunting and may contribute to birth defects (Kimanya *et al.*, 2010), and is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2002). Also, Exposure to fumonisin could lead to increased mortality and morbidity [Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2017].

High levels of maize ear rot and kernel infection by several *Fusarium* species have been reported in different parts of the globe (Ncube *et al.*, 2011; Reyes-Velázquez *et al.*, 2011). Similarly, occurrences of different Fusarium species in storage maize have been reported in Ethiopia (Ayalew, 2010; Tsehaye *et al.*, 2017). However, *Fusarium* spp. and fumonisin occurrence in maize varies between cropping years and maize growing areas depending on environmental conditions, insect pest infestation and poor agronomic practices such as choice of cultivars, crop residue management and others (Goertz *et al.*, 2010; Ncube *et al.*, 2011; Pitt *et al.*, 2013). *Fusarium* species are commonly considered as field fungi (Maina et al., 2009) and produce fumonisins on maize kernels in the field prior to harvesting (Kimanya *et al.*, 2014). In this regards, all available information generated at certain period in Ethiopia was mainly focused on stored maize samples without giving due emphasis to pre-harvest contamination and, the information not considered sufficient to design management strategies and reduce public health hazards due to grain contamination by fumonisin.

Despite the importance of maize in Ethiopia, particularly in eastern Ethiopia, and high risk of fumonisin to human and the legislated regulation of maximum acceptable levels of fumonisins (EU commission, 2006; US Food and Drug Administration, FDA, 2001; East African

Community, EAC, 2013), very little is known and documented about the natural occurrence of *Fusarium* species and fumonisin contamination levels in maize grains produced in eastern Ethiopia. Hence, the objective of the present study was to determine the natural occurrence of *Fusarium* species and associated fumonisin contamination in fresh harvested and three-month stored maize samples in major maize-growing areas of eastern Ethiopia.

Materials and Methods

Description of the study areas

Maize samples were collected from five major maize-producing districts (Girawa, Goromuti, Haramaya, Meta, and Tullo) of Eastern Ethiopia, during the 2017/18 cropping season. Girawa, Goromuti, Haramaya, and Meta districts are located in Eastern Hararghe, while Tullo district is located in Western Hararghe of eastern Ethiopia (Figure 1). Eastern and Western Hararghe are located between 8°90′–9°90′ N latitude and 41°12′–42°53′ E longitude and 7°32′–9°47′ N latitude and 41°24′–43°48′ E longitude, respectively, at an altitudinal range of 500 to 3600 meter above sea level (m.a.s.l.). All districts are characterized by bimodal rainfall distributions, where the short-rainy season extends from March to June, and the main-rainy season occurs from mid-June to October (Tolossa and Tafesse, 2008). Geographical location and mean weather variables for the surveyed districts are organized from Ethiopian Meteorological Agency and presented in Table 1.

Table 1. Means of agro-meteorological features of the five districts of eastern Ethiopia during the 2017/18 cropping season.

District	Latitude (N)	Longitude (E)	Altitude (m.a.s.l.)	Rainfall (mm)	Max Temp (°C)	Min Temp (°C)	RH (%)
Girawa	09°10'51"	41°47'29"	500–3230	1156	20	10	81
Goromuti	09°12'50"	41°34'7"	2105–2584	1034	21	11	80
Haramaya	09°24'10"	41°59'58	1400–2340	826	25	8	79
Metta	0 9°0'31"	41°0'44"	2120–2380	1015	25	7	78
Tullo	09°09'60"	41°00'0"	1600–2700	549	27	7	74

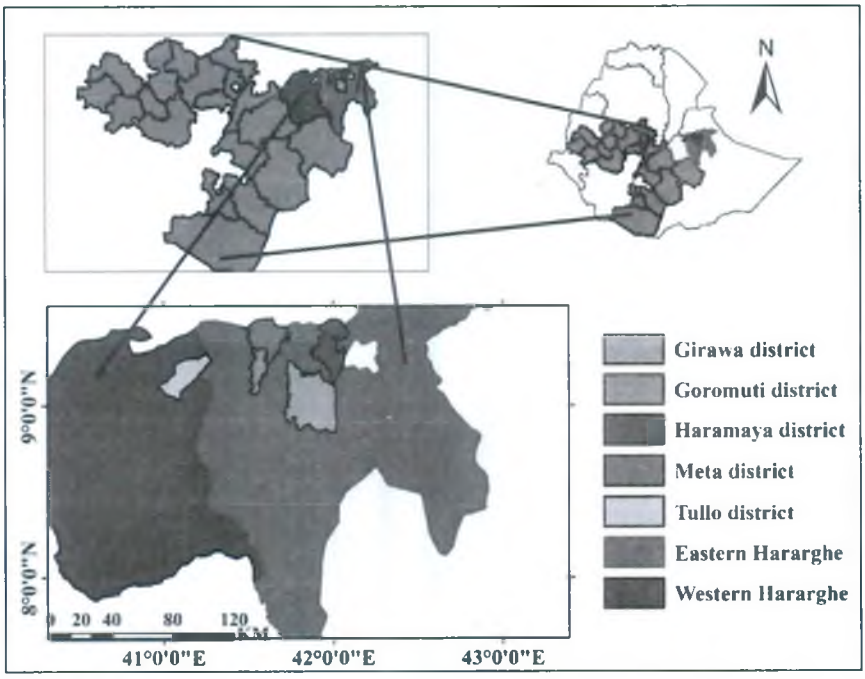

Figure 1. Map showing five major maize growing districts in Eastern and Western Hararghe of Eastern Ethiopia.

Sample collection procedures

Fresh harvested maize samples were collected from five major maize producing districts in the weeks of harvest, during November to December 2017, in the field from harvested heaps, while three-months later stored maize samples were collected from the same farmers following the procedure of Kimanya *et al.* (2009). The districts were selected based on their current maize production status. From each district, five Farmers' Associations (FAs) were selected in consultation with the respective districts' agricultural office crop production experts. Five to seven relatively large producing-farmers were purposively selected from each FA confirming that they were willing and could store maize at least for more than three months. Thus, 127 fresh harvested maize samples (25 samples each from Girawa and Goromuti districts, 27 samples from Haramaya, 26 samples from Meta, and 24 samples from Tullo districts) were collected in the field immediately at harvest from heaps, and another 127 samples were taken from the same farmers' stores after three-months of storage.

For fresh harvested maize samples, 10–30 maize cobs were collected at each selected farm depending on the size of harvested heaps (locally *kusa* in Afaan Oromo or *kimir* in Amharic). Samples were manually taken from all directions of the heaps. Similarly, 10–30 maize cobs, if it was stored as cobs or 1.5 kg, if it was stored in grains, were taken three-months later for storage samples. The maize cobs were hand shelled to get approximately 1 kg grains and transported to Haramaya University Plant Protection Laboratory, and stored in a cold room at 4 °C until further analyses were commenced.

Measurement of moisture content

The moisture content (%) of bot fresh harvested and three-month stored maize grain samples were determined immediately after samples reached at Haramaya University Plant Protection Laboratory using Draminskigmm mini moisture tester.

Preparation of culture media

Malachite Green Agar (MGA) and Carnation Leave Agar (CLA) were used for isolation and identification of *Fusarium* spp. associated with maize grain samples. Malachite Green Agar medium was prepared as per the procedures suggested by Bragulat *et al.* (2004), while CLA was prepared as defined by Fisher *et al.* (1982). After preparation, both media were sterilized by autoclaving at 121 °C for 15 minutes and were allowed to cool to about 45 °C. The cooled media were aseptically poured into Petri dishes in a laminar flow and allowed to solidify. The broad-spectrum antibiotic, chloramphenicol, was applied at a rate of 250 mg L^{-1} agar to both media to inhibit bacterial growth (Leslie and Summerell, 2006).

Isolation and identification of *Fusarium* spp.

For this assay, random sub-samples of maize kernels from each sample type were surface-sterilized with household chlorine bleach (NaOCl) for 3 minutes, rinsed twice with sterile distilled water and dried in a laminar flow cabinet. Thirty maize kernels were plated twice at the rate of 15 seeds per Petri plate on MGA, which is a potent selective medium for the isolation of *Fusarium* species (Bragulat *et al.* 2004). Then the Petri plates were incubated at 28 ± 2 °C for seven days. Following incubation, an agar block containing actively growing *Fusarium* culture was aseptically cut and placed adjacent to the leaf piece of CLA and incubated again at 28 ± 2 °C for seven days. The fungus was allowed to grow on the carnation leaf pieces to form sporodochia over an open area of water agar where the conidiophores producing micro-conidia.

Direct microscopic examination of the culture grown on CLA plates was done at 100x and 400x magnifications seven days after incubation to observe micro-morphological fungal features, such as macro-conidia and micro-conidia and as well as conidial arrangement (shape, size and formation), conidiogenous cell formation (mono- or poly-phialides), and the formation and arrangement of chlamydospores (Leslie and Sumerrell, 2006). Percentage of infected kernels, frequency (percent of infected samples), and relative frequency was calculated according to the formulae suggested by Ghiasian *et al.* (2004) as follows:

$$\text{Maize kernel infection (\%)} = \frac{\text{Number of kernels infected with } \textit{Fusarium} \text{ spp.}}{\text{Total number of kernels analyzed}} \times 100$$

$$\text{Frequency (\%)} = \frac{\text{Number of samples with } \textit{Fusarium} \text{ spp.}}{\text{Total number of samples analyzed}} \times 100$$

$$\text{Relative frequency (\%)} = \frac{\text{Number of individual } \textit{Fusarium} \text{ spp. isolated}}{\text{Total number of } \textit{Fusarium} \text{ isolates recovered}} \times 100$$

Fumonisin extraction and analysis

Total fumonisin extraction was done following the manufacturer's instructions of ELISA kit (Helica Biosystems Inc, Santa Ana, CA). Representative sub-samples of maize grains, 200 g each, were ground to the particle size of fine instant coffee (95% passes through a 20-mesh screen), and extraction solution was prepared by adding 4 mL of distilled or deionized water to 36 mL of methanol (90% reagent grade) for each sample tested (Njeru *et al.*, 2019). A 20 g sub-sample was weighed for each sample and extracted with 40 mL of 90% methanol (the ratio of samples to extraction solvent was 1:2 w/v). The samples were mixed by shaking in sealed containers for one minute, after which the particulate matter was allowed to settle. The extracts were filtered through Whatman no. 1 filter paper and the filtrate was collected for testing. The sample extracts were further diluted with distilled water in the ratio of 1:20 (Njeru *et al.*, 2019).

From the extracted filtrate, fumonisin levels were quantified by direct competitive ELISA. First, reagents were brought to room temperature and the PBS-Tween packet content was washed out with distilled water into 1 L container. All dilution well for each standard, all samples to be tested, and antibody-coated microtiter wells were inserted into their microwell holder. Using pipette, 100 µL of conjugate solution A (Green) was dispensed into appropriate dilution well, followed by 100 µL conjugate solution B (clear). Exactly 100 µL of each standard and samples were added into dilution well-containing conjugate mixture and mixed by priming pipettor three times. Similarly, 100 µL of contents were transferred into the corresponding antibody-coated microtiter wells and incubated for 10 minutes at room temperature. After incubation, the contents in the microwells were decanted and microwells were washed by PBS-Tween wash buffer repeatedly for 5 times.

Then, the microwells were tapped face down on an absorbent towel to remove residual buffer. From 1 mL/strips volume of substrate reagent, 100 µL was added to each microwell, covered with aluminum foil to avoid direct light, and incubated at room temperature for 10 minutes. The, 100 µl of stop solution was added to each microwell in the same sequence and the same pace as substrate was added. Using a 450 nm filter the optical density of each microwell was recorded. Finally, the results were interpreted by constructing a dose-response/standard curve using optical density value expressed as a percentage of the optical density of zero standards against fumonisins content of the standard. The fumonisins contents of the samples were calculated from the standard curves. The lower and the upper limits of detection for fumonisin test ELISA kits were 100 and 6,000 µg kg^{-1}, respectively.

Data analyses

Grain moisture content, frequency of *Fusarium* species and *Fusarium* kernel infection were expressed in percentage, while level fumonisin contamination in maize samples was described in the mean. Variation in number of *Fusarium* species isolate, fumonisin concentrations, and *Fusarium* contaminated kernels per district in both sample types were compared using the nonparametric Kruskal-Wallis one-way ANOVA (Kruskal-Wallis, 1952). Frequency of *Fusarium* spp. and relative frequency in maize growing areas in both sample types were pooled to illustrate an occurrence of different *Fusarium* species in fresh harvested and three-month stored samples in eastern Ethiopia. Variation in fumonisin concentrations, and proportion of *Fusarium* contaminated kernels between sample types were compared using an independent sample t-test. Pearson's correlation coefficient was used to evaluate the relationships between *Fusarium* species kernel infection, fumonisin concentrations, and moisture content of the maize grain samples (Tsehaye *et al.*, 2017). Data analyses were performed using IBM SPSS statistics 20 (SPSS version 20) and all tests were performed at 0.05 probability level.

Results

Moisture contents of maize grain samples

This study found that fresh harvested maize samples had significantly ($P \leq 0.001$) high average grain moisture content (14.96%) ranging from 12.02 to 16.96% as compared to three-month stored maize samples (13.16), which ranged from 10.16 to 15.75%, t (252) =13.25. In both fresh harvested and three-month stored maize samples, there was no significant difference among districts ($P>0.05$) in average moisture content of maize grain (Table 2).

Table 2. Moisture content (%) of maize grain samples collected from five districts of eastern Ethiopia, during the 2017/18 cropping season.

District	Maize sample types					
	Fresh harvested samples (n = 127)		Three month stored samples (n = 127)		t–value	p–value
	Mean	Range	Mean	Range		
Goromuti	15.19	14–17	13.09	10–15		
Girawa	15.25	14–17	13.53	12–16		
Tullo	14.80	12–17	12.91	11–15		
Haramaya	14.56	12–17	12.99	10–15		
Meta	15.04	14–16	13.26	11–15		
Total	14.96	12–17	13.16	10–16	13.25	<0.001

Fusarium contamination in maize samples

A total of 127 fresh harvested and 127 three-month stored maize samples collected from five maize producing districts of eastern Ethiopian were analyzed for *Fusarium* occurrence. For isolation, 7620 maize kernels were plated on MGA and CLA media, and 4273 white colony form of *Fusarium* genus were isolated, infecting 97% of fresh harvested samples and 91% of three-month stored maize samples with average maize kernel infection of 62% and 50%, respectively (Table 3).

There was no significant difference between districts in fresh harvested maize samples for *Fusarium* maize kernel infection [χ^2 (4) = 6.80, P = 0.147]. However, higher *Fusarium* maize kernel infection in fresh harvested maize samples was recorded in Haramaya district (71%), while the lowest

kernel infections were recorded in Goromuti district (57%) compared with districts considered in the assessment. Conversely, there was significant [χ^2 (4) = 15.64, P = 0.004] variation in *Fusarium* maize kernel infection between districts in three-month stored maize samples, with Meta district had the highest *Fusarium* infected kernels (61%) when compared with Girawa (43%) and Goromuti (42.5%) districts (Table 3).

Table 3. Kruskal-Wallis test for *Fusarium* maize kernels infection (%) in five districts of Eastern Ethiopia.

District	TNS[a]	TNKA[b]	Fresh harvested sample				Three-month stored sample			
			TNFI	Mean	Range	Mean ranks	TNFI	Mean	Range	Mean ranks
Goromuti	25	750	424	56.6	20–100	54.9[a]	319	42.5	17–93	50.3[a]
Tullo	24	720	512	71.2	30–100	77.8[a]	395	54.8	37–90	73.6[ab]
Girawa	25	750	439	58.5	27–100	55.5[a]	322	43	27–70	51.5[ac]
Meta	26	780	497	63.7	33–100	69.0[a]	473	60.7	40–97	83.6[bd]
Haramaya	27	810	504	62.1	27–100	63.3[a]	388	47.9	17–80	61a[bcd]
Total	127	3810	2376	62.3	20–100		1897	49.8	17–97	
χ^2-value						6.80				15.64
P-value						0.147				0.004

[a]TNS = Total number of samples studied. [b] TNKA = Total number of kernels analyzed. [c] TNFI = Total number of Fusarium isolate. Mean ranks with the same letter(s) are not significantly different at P≤0.05. Note that zero kernel infection was not included in the range.

Identification of *Fusarium* species associated with maize samples

Seven *Fusarium* species that are morphologically different (Figure 2 and Table 4) were identified from both sample types. The most frequently isolated *Fusarium* species were identified and confirmed to be *F. verticillioides*. Prevalence of all *Fusarium* species varied between sample types. In fresh harvested samples, *F. verticillioides* was the most prevalent species representing 56% of the total isolates recovered, followed by *F. proliferatum*, *F. subglutinans* and *F. graminearum*. In three-month stored maize samples, *F. verticillioides* represented 48% of total isolates, followed by *F. subglutinans*, *F. proliferatum*, *F. graminearum*, *F. oxysporum F. solani* and *F. andiyazi* (Table 4).

The results noted that frequency and incidence of *F. verticillioides* and *F. proliferatum* recorded in fresh harvested maize samples were higher than that recoded in three-month stored maize samples (Table 4 and Figure 3). *Fusarium verticillioides* were isolated from 86 and 68% of fresh harvested and three-month stored maize samples with average kernel infection of 35 and 24% respectively. Marked and significant [t (252) = 4.19; P<0.0001] difference observed between sample types in average maize kernel infected by *F. verticillioides*. The second most prevalent species, *F. proliferatum*, was recorded in 64 and 47% of fresh harvested and three-month stored maize samples infecting 13.17 and 7 % kernels, respectively. Significant [t (252) = 4.33; P<0.0001] difference in average maize kernel contaminated by *F. proliferatum* was observed between fresh harvested and three-month stored kernels (Table 5). On the

contrary, frequency and incidence of *F. oxysporum* and *F. solani* were higher in three-month stored maize samples than in fresh harvested maize samples over districts, while *F. graminearum and F. subglutinans* occurrence did not significantly change with storage. *Fusarium subglutinans* was recorded in 60% of both samples with only 8% average kernel infection (Table 4 and Figure 3).

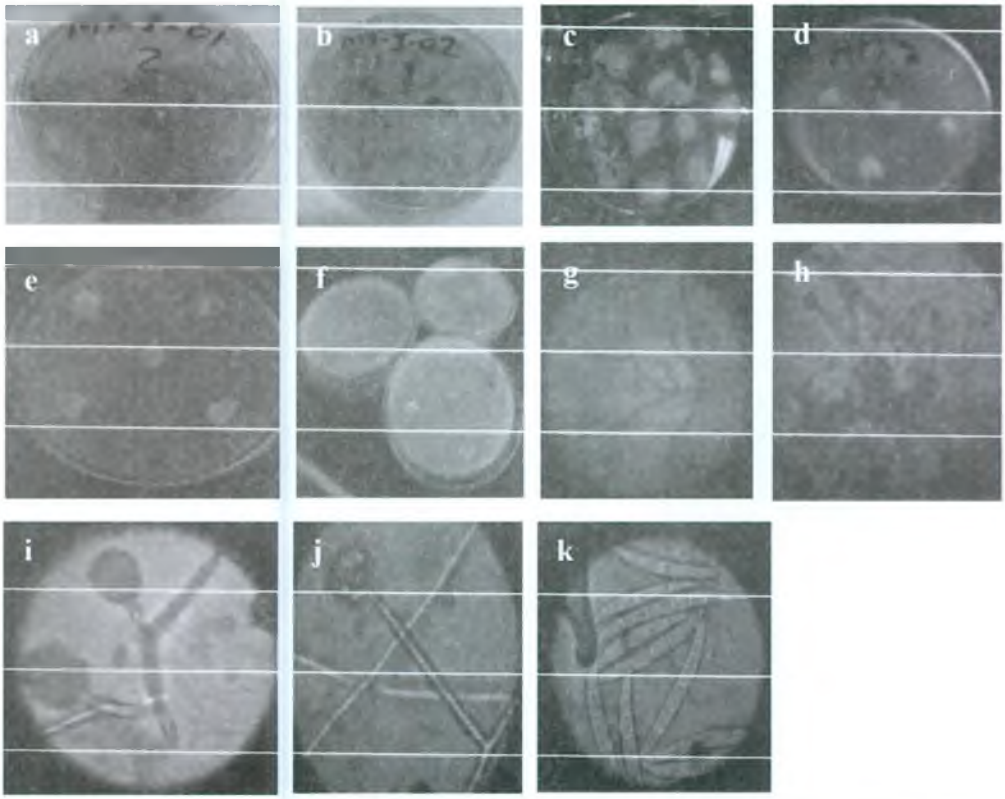

Figure 2. Morphology of *Fusarium* species on MGA and CLA media. *Fusarium* colony grown on maize kernel plated on malachite green agar (MGA2.5) after seven-days incubation at 28 ± 2°C (a–c); *Fusarium* colony grown on CLA observed after seven-days of incubation at 28 °C (d–f); microscopic *in situ* structure of *Fusarium* spp. observed under compound microscope at 400x (g–k) indicating micro-conidia chain of *F. verticillioides* (g), poly-phialides and short chain of *F. proliferatum* (h), false heads of *F. oxysporum* on short mono-phialides (i), false heads of *F. solani* on relatively long mono-phialides (j), and macro-conidia of *F. graminearum* (k).

Table 4. Incidence of *Fusarium* species in fresh harvested and three-month stored maize samples in Eastern Ethiopia.

Fusarium spp.	Maize sample types [a]				*t* –value
	Fresh harvested samples		Three-month stored samples		
	FIC	Incidence (%)	FIC	Incidence (%)	
F. verticillioides	1332	34.9	912	23.9	4.19**
F. proliferatum	503	13.2	252	6.6	4.33**
F. oxysporum	20	0.53	124	3.3	–4.05ns
F. solani	30	0.8	65	1.7	–2.3ns
F. graminearum	167	4.4	193	5.1	–0.79ns
F. subgulitnans	256	7.677	310	8.2	–1.31ns
F. andiyazi	68	1.8	41	1.1	1.68ns
Total	2376	62.3	1897	49.8	4.49**

[a] *Total number of kernels analyzed were 3810 for each maize sample type studied and FIC =* Fusarium *species isolate count as recovered from the samples.* **, * *and* ns *refer to level of statistical significance at P≤0.01; P≤0.05 and no significance at P≤0.05.*

Fusarium species composition and occurrence

Fusarium species isolated from fresh harvested and three-month stored maize samples were varied in composition and prevalence across districts (Table 5). Six *Fusarium* species were isolated in both maize sample types collected from Girawa and Goromuti districts, five species in fresh harvested samples from Haramaya, six from Meta and Tullo districts each. Likewise, seven *Fusarium* species were isolated in three-month stored maize samples from Haramaya, Meta and Tullo districts (Table 5). In all districts, *F. verticillioides* were the most prevalent *Fusarium* species, followed by *F. proliferatum* in fresh harvested maize samples and *F. subgulitnans* in three-month stored samples (Table 5). There was variation between districts for frequency and incidence of individual *Fusarium* species in both maize sample types (Figures 3 and 4). *F. verticillioides* were isolated from 84, 76, 93, 85 and 92% of fresh harvested and; 60, 40, 74 ,77, and 88% of three-month stored maize samples collected from Girawa, Goromuti, Haramaya, Meta and Tullo districts studied, respectively.

Table 5. Total number of *Fusarium* species isolated from maize samples collected from five major maize growing districts of Eastern Ethiopia.

Maize sample types	*Fusarium* spp.	Districts considered in the study [a]					
		Goromuti	Girawa	Tullo	Haramaya	Meta	Total
Fresh harvested sample	*F. verticillioides*	184(43)	189(43)	313(61)	339(67)	307(62)	1332(56)
	F. proliferatum	111(26)	143(33)	83(16)	75(15)	91(18)	503(31)
	F. oxysporum	15(4)	2(0)	0(0)	0(0)	3(1)	20(0.84)
	F. solani	18(4)	10(2)	2(0)	0(0)	0(0)	30(1.26)
	F. graminearum	38(9)	48(11)	28(5)	29(6)	24(5)	167(7)
	F. subglutinans	58(14)	47(11)	63(12)	40(8)	48(10)	256(11)
	F. andiyazi	0(0)	0(0)	23(4)	21(4)	24(5)	68(3)
	Total	424	439	512	504	497	2376
Three-month stored sample	*F. verticillioides*	101(32)	116(36)	236(60)	208(54)	251(53)	912(48)
	F. proliferatum	49(15)	57(18)	31(8)	41(11)	74(16)	252(13)
	F. oxysporum	13(4)	33(10)	21(5)	23(6)	34(7)	124(7)
	F. solani	18(6)	10(3)	14(4)	17(4)	6(1)	65(3)
	F. graminearum	62(19)	38(12)	21(5)	39(10)	33(7)	193(10)
	F. subglutinans	76(24)	68(21)	53(13)	51(13)	62(13)	310(16)
	F. andiyazi	0(0)	0(0)	19(5)	9(2)	13(3)	41(2)
	Total	319	322	395	388	473	1897

[a] *Values in each column and row refer to* Fusarium *species isolate count recovered from each maize sample type, while the values in parentheses indicate the relative prevalence of each respective isolate in the tested samples.*

In fresh harvested maize samples, significantly the lowest *F. verticillioides* kernel infections were recorded in Girawa and Goromuti districts with 25% kernel infection each, followed by Meta (39%), Haramaya (42%) and Tullo (44%) districts. In three-month stored maize samples, the lowest *F. verticillioides* kernel infection was recorded in maize samples collected from Goromuti (13%), followed by Girawa with 15%, Haramaya with 26%, Meta with 32%, and Tullo with 33%. *Fusarium proliferatum* were isolated from 92, 60, 37, 65 and 67% of fresh harvested and 40, 44, 30, 62 and 29% of three-month stored maize samples collected from Girawa, Goromuti, Haramaya, Meta and Tullo districts respectively (Figure 3). In fresh harvested maize samples, the lowest average kernel infection by *F. proliferatum* were recorded in Haramaya district (9%), while the highest (19%) infection was recorded in Girawa district (19%) as depicted in Figure 4. In three-month stored maize samples, the lowest (8%) average kernel infections by *F. proliferatum* were recorded in Tullo (4%), while the highest infections were recorded in Goromuti district

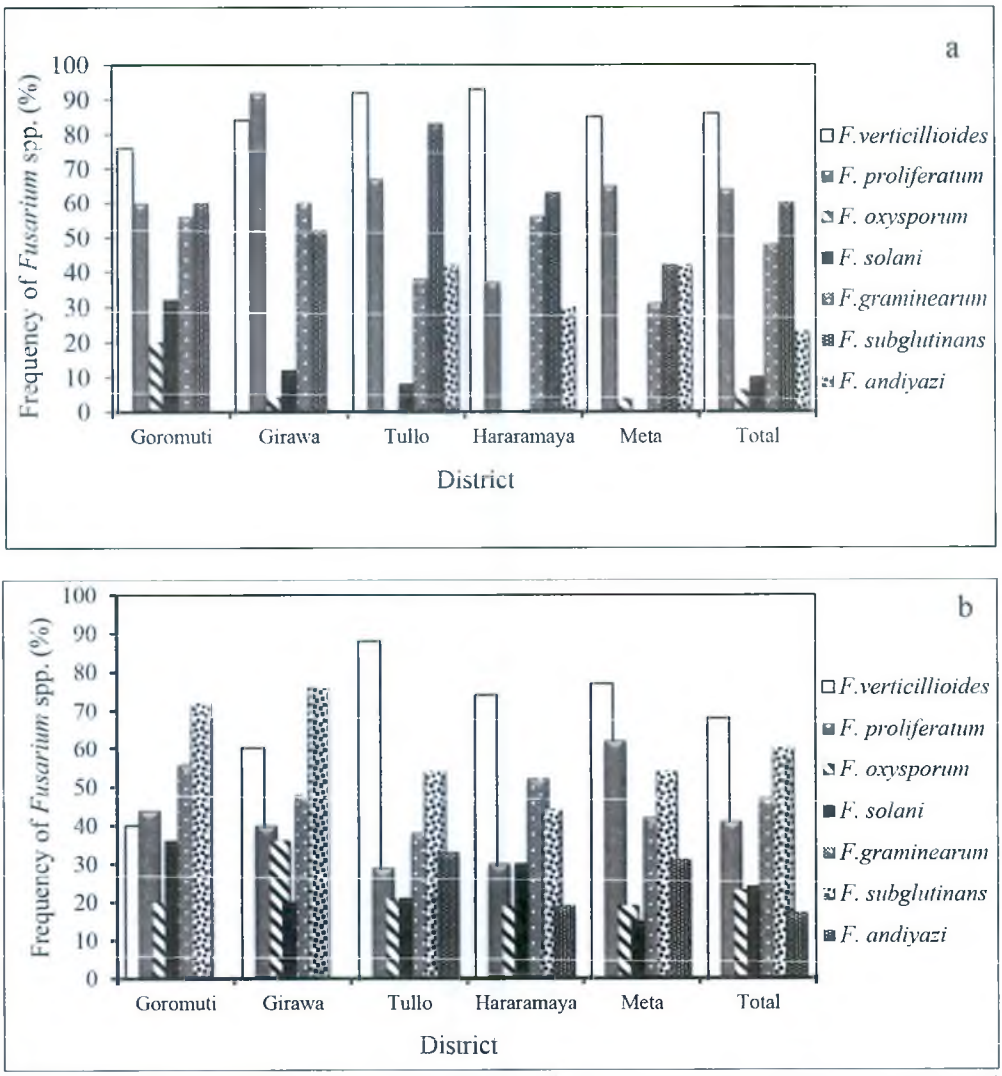

Figure 3. Frequency of *Fusarium* species infecting fresh harvested (a) and three-month stored maize samples (b) in five major maize growing districts of Eastern Ethiopia, during the 2017/18 cropping season.

F. subglutinans were isolated from 52, 60, 63, 42 and 83%, and 76, 72, 44, 54 and 54% of fresh harvested and three-month stored maize samples collected from Girawa, Goromuti, Haramaya, Meta and Tullo districts, respectively. Maximum (10%) average maize kernel infection *F. subglutinans* were recorded in three-month stored maize samples collected from Goromuti districts. *Fusarium graminearum* were also isolated from 60, 5 6, 56, 31and 38%, and 48, 56, 52, 42 and 38% of fresh harvested and three-month stored maize samples collected from Girawa, Goromuti, Haramaya, Meta and Tullo districts in that order with maximum (6%) average kernel

infections noted in Girawa district in both sample types (Figure 4).

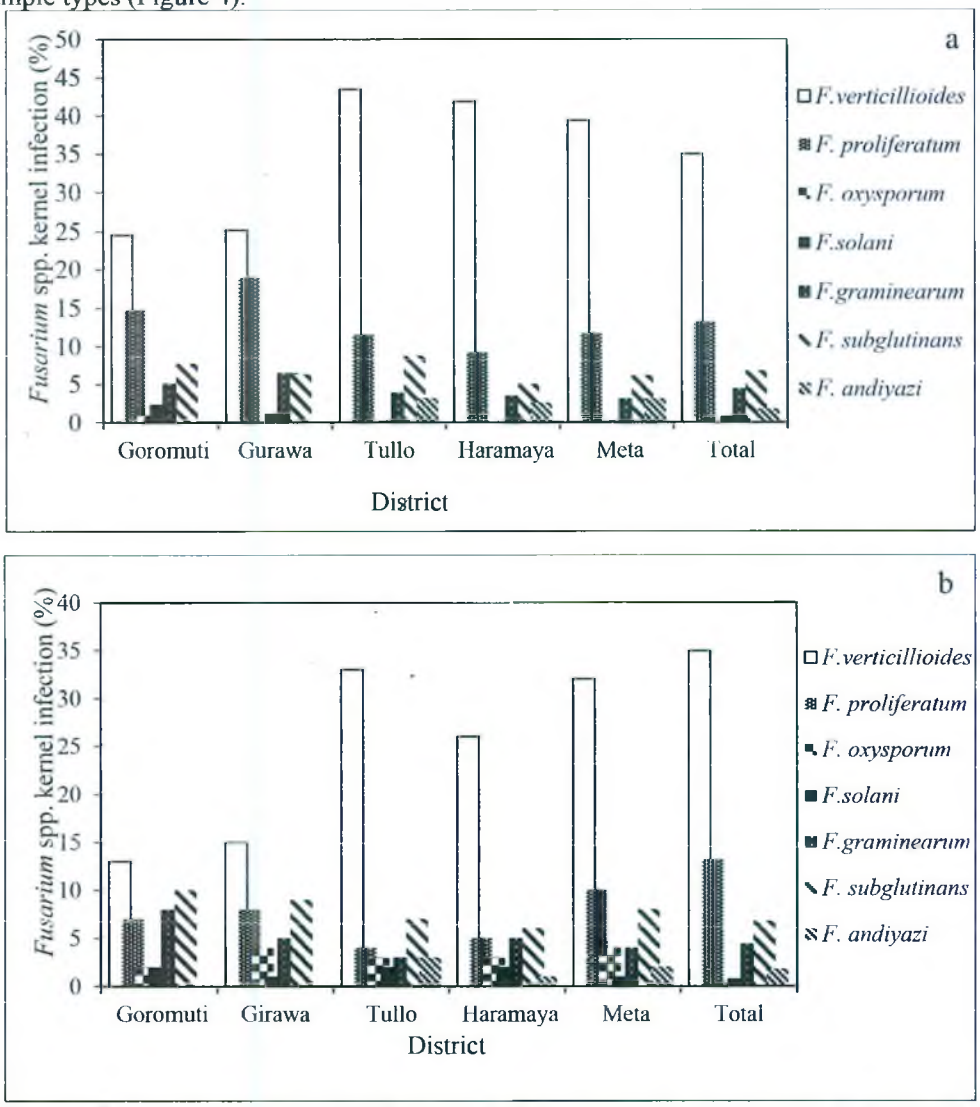

Figure 4. *Fusarium* species kernel infection in fresh harvested (a) and three-month stored maize samples (b) in five major maize growing districts of Eastern Ethiopia, during the 2017/18 cropping season

Fumonisin contamination in maize samples

In the study, all fresh harvested and 92% of three-month stored maize samples were found fumonisin-positive, above the detectable limit of the kit, that ranged from 294 to 5,396 µg kg^{-1} µg kg^{-1} and 105 to 5,460 µg kg^{-1}, respectively. Fumonisin concentration recorded in fresh harvested maize samples (mean: 2,509 µg kg^{-1} and median: 1,920 µg kg^{-1}) was higher than that of three-month stored maize samples (mean: 1668 µg/kg and median: 1,499 µg kg^{-1})

(Table 6). There was no significant (χ^2 (4) = 12.51; n = 127; P>0.05) difference between districts in fumonisin concentration detected in fresh harvested maize samples. However, lower mean ranks for fumonisin concentrations were recorded in fresh harvested samples collected from Goromuti (mean: 2,042 μg kg^{-1}) and Haramaya (mean: 2,072 μg kg^{-1}) than Meta (mean: 2,889 μg kg^{-1}), Girawa (mean: 2,790 μg kg^{-1}) and Tullo (mean: 2,781 μg kg^{-1}) districts in increasing order of their mean ranks (Table 6). Conversely, there was a significant [χ^2 (4) = 27.455; n = 127; P<0.05] difference between districts in fumonisin concentration detected in three-month stored maize grains. Three-month stored maize samples collected from Goromuti (with mean 994 μg/kg) and Haramaya (with mean of 1,082 μg kg^{-1}) had significantly lower mean fumonisin concentration as compared to Meta (with mean of 1,949 μg kg^{-1}), Tullo district (with mean of 2,224 μg kg^{-1}) and Girawa (with mean of 2,150 μg kg^{-1}) districts in order of their mean ranks (Table 6).

Table 6. Fumonisin concentration in maize samples collected from five major maize growing districts of Eastern Ethiopia, during the 2017/18 cropping season.

Maize sample types	District	Fumonisin concentration (μg kg^{-1})		
		Mean	Range	Mean ranks
Fresh harvested samples	Goromuti	2042	316–5102	50.56[a]
	Girawa	2790	1475–3586	73.40[a]
	Tullo	2781	918–5296	77.63[a]
	Haramaya	2072	294–4850	50.39[a]
	Meta	2889	827–5394	69.44[a]
	Total	2509	294–5396	
	χ^2-value		12.54	
Three-month stored samples	Goromuti	994	<LoD–3972	41.22[a]
	Girawa	2150	<LoD–5460	80.46[b]
	Tullo	2224	<LoD–5146	79.38[bc]
	Haramaya	1082	<LoD–2830	45.93[ad]
	Metta	1949	<LoD–5043	74.65[bce]
	Total	1668	<LoD–5460	
	χ^2-value		27.46	

[a]*LoD = Lower limit of detection for fumonisin concentration. Mean ranks of districts were compared according to Kruskal-Walls one-way ANOVA at P≤ 0.05.*

Correlation between *Fusarium* species, kernel infection, fumonisin, and grain moisture content

In fresh harvested maize samples, fumonisin concentration had a positive significant (P<0.001) correlation with moisture contents of maize grain, *Fusarium* maize kernel infection, incidence of *F. verticillioides*, as well as with incidence of *F. proliferatum*, and *F. graminearum* (P<0.05). Similarly, a highly significant (P<0.001) and positive correlation was observed between fumonisin concentration and moisture contents of maize grains, *Fusarium* maize kernel infection, incidence of *F. verticillioides*, and incidence of *F. proliferatum*, in three-month stored maize

samples. Total *Fusarium* maize kernel infection was highly and significantly ($P<0.001$) correlated with moisture contents of maize kernels, the occurrence of *F. verticillioides* and *F. proliferatum*, *F. graminearum* and *F. subgulitnans* in fresh harvested maize samples. Similarly, highly significant ($P<0.001$) relationship was observed between *Fusarium* maize kernel infection and moisture contents of maize kernels, occurrence of *F. verticillioides*, *F. proliferatum*, *F. Subgulitnans*, incidence of *F. oxysporum*, in three-month stored maize samples (Table 7).

Table 7. Correlation coefficients (r) between grain moisture content, *Fusarium* spp., occurrence, *Fusarium* kernel infection and fumonisin contamination in fresh harvested and three-month stored maize samples.

Variable	Maize sample types [a]			
	Fresh harvested samples		Three-month stored samples	
	Fumonisin ($\mu g\ kg^{-1}$)	*Fusarium* kernels infection (%)	Fumonisin ($\mu g\ kg^{-1}$)	*Fusarium* kernels infection (%)
Moisture content (%)	0.383^{**}	0.490^{**}	0.587^{**}	0.289^{**}
F. verticillioides	0.319^{**}	0.607^{**}	0.402^{**}	0.551^{**}
F. proliferatum	0.256^{**}	0.343^{**}	0.230^{**}	0.366^{**}
F. oxysporum	-0.06^{ns}	0.018^{ns}	-0.1^{ns}	0.297^{**}
F. solani	0.007^{ns}	0.024^{ns}	-0.123^{ns}	-0.025^{ns}
F. graminearum	0.199^{*}	0.290^{**}	-0.147^{ns}	0.215^{*}
F. subgulitnans	0.145^{ns}	0.273^{**}	-0.023^{ns}	0.408^{**}
F. andiyazi	-0.04^{ns}	-0.047^{ns}	0.122^{ns}	0.203^{*}
Kernels infection (%)	0.576^{**}		0.361^{**}	

[a] ***Correlation coefficients highly significant at $P<0.01$; *Correlation coefficients significant at $P<0.05$ and ns = not significant at $P\leq0.05$.*

Discussion

The present study compared occurrence of *Fusarium* species and its subsequent fumonisin contamination in fresh harvested and three-month stored maize samples. The results showed that maize grown in five districts of Eastern Ethiopia were highly contaminated by *Fusarium* species and their associated fumonisins. Higher number of *Fusarium* isolates were recovered from fresh harvested than three month stored maize samples suggesting that most of the maize s by *Fusarium* species occurred in the field. Angeline *et al.* (2016) stated that *Fusarium* species are mainly considered as field fungi and thus their high occurrence in maize obtained at harvest could be attributed to pre-harvest infections, improper drying as well as unhygienic conditions during handling. In this study, *Fusarium* infected 97 and 90% of fresh harvested and three-month stored maize samples along with average kernel contamination of 62 and 50%, respectively. The high *Fusarium* occurrence in maize samples observed in this result is in agreement with the results of Ayalew (2010) who reported that *Fusarium* were occurred in 76.5% of the storage maize samples. Wubetu and Abate (2000) also noticed that *Fusarium* species were recovered from 91.7% of both damaged and normal maize samples. Similar reports have been documented from other Africa countries

such as Tanzania (Degraeve *et al.*, 2016) and South Africa (Ekwomadu *et al.*, 2017).

The present study identified seven *Fusarium* species, namely *F. andiyazi, F. graminearum, F. oxysporum, F. proliferatum, F. solani, F. subglutinans*, and *F. verticillioides* from both fresh harvested and three-month stored maize samples. Similarly, Wubetu and Abate (2000) identified *F. graminearum, F. subgulitnans, F. verticillioides* and other unidentified *Fusarium* species from maize samples in other parts of Ethiopia. In a relatively recent study, Tsehaye *et al.* (2017) identified *F. brevicatenulatum, F. equiseti, F. graminearum, F. incarnatum, F. lacertarum, F. oxysporum, F. pseudoanthophilium, F. subglutinans, F. temperatum, F. verticillioides* and other unidentified *Fusarium* species from storage maize samples in other parts of Ethiopia. These *Fusarium* species were also identified from maize grains in different parts of the world (Sinai *et al.*, 2015; Angeline *et al.*, 2016).

Out of seven *Fusarium* species identified, *F. verticillioides* was the most prevalent and found in all areas inspected, and higher infection proportion was observed on fresh harvested maize samples and kernels than three-month stored maize samples and kernels. Also, it was prevalent in the relatively medium to low altitude areas like Meta district to low altitude areas like Haramaya and Tullo as compared to Goromuti and Girawa districts with high altitude range and low temperature (Table 1). Such results agreed with the findings of Tsehaye *et al.* (2017) who reported that *F. verticillioides* was the most prevalent in the low altitude and high temperature areas. The consistent recovery of *F. verticillioides* throughout all maize growing areas and sample types could indicate its intimate association with the maize and its adaptation to the tropical climate (Tsehaye *et al.*, 2017). Moreover, Ayalew (2010) reported that, 99% of the *Fusarium* species isolated on the internal and external surface of storage maize kernels was *F. verticillioides*. The dominant prevalence of *F. verticillioides* in maize kernels is also common in South Africa (Ncube *et al.*, 2011), Mexico (Reyes-Velazquez *et al.*, 2011) and Kenya (Bii *et al.*, 2012).

Fusarium proliferatum was the second most prevalent *Fusarium* species recorded in fresh-harvested samples following *F. verticillioides*. In contrast to *F. verticillioides*, which were the most prevalent in the relatively low altitude areas, *F. proliferatum* was prevalent in samples collected from relatively high altitude areas, such as Goromuti and Girawa districts (Table 1). Gheysens (2015) also reported that *F. verticillioides* is common in warmer areas with high humidity and temperature above 28 °C, while *F. proliferatum* inhabit relatively cooler areas. Such co-occurrence of *Fusarium* spp. may be attributed to having similar optimum growth conditions, and they may not have apparent antagonism among each other. Similar to the present findings, co-occurrence of *Fusarium* specie in the same samples and kernels was also reported in different regions of the world (Ncube *et al.*, 2011; Reyes-Velazquez *et al.*, 2011), such as Kenya (Bii *et al.*, 2012), Iraq (Sinai *et al.*, 2015) and Nigeria (Egbuta *et al.*, 2015). Moreover, these two *Fusarium* species are considered as among the most fumonisin producer on agricultural commodities in the field or during storage (Nayaka *et al.*, 2010).

In the present study, all fresh harvested and 92% of the total maize grain samples were found to be fumonisin-positive. Reports of Tsehaye *et al.* (2017) confirmed that about two-third (77%) of the maize samples from

other parts of Ethiopia were contaminated with fumonisin. However, the overall mean levels of total fumonisin contamination recorded in both fresh and three-month stored maize samples were far higher than over all mean concentration of 925, 348, 925 and mean level of FUM B_1 concentration of 606 µg/kg reported by Ayalew (2010), Tsehaye *et al.* (2017), Worku *et al.* (2019) and Getachew *et al.* (2017), respectively.

The high levels of maize contamination by fumonisins could be due to high level occurrence of *F. verticillioides* and *F. proliferatum* recorded in the studied maize samples, climatic characteristics of eastern Ethiopia, which is characterized by bimodal rainfall distribution and typical sub-humid agro-climates (Tolossa and Tafesse, 2008). Agro-climatic characteristics, such as drought stress, humidity, rainfall and temperature are among the important factors that favour colonization of maize ears by pathogenic *Fusarium* species and subsequent fumonisin contamination (Goertz *et al.*, 2010; Bii *et al.*, 2012). The other factor could be agricultural and post-harvest practice commonly performed by maize growers in the study areas. In this regard, Ncube *et al.* (2011) and Pitt *et al.* (2013) indicated that agricultural practices, such as inappropriate crop rotation, poor insect pest management, choice of cultivars, harvesting dates, crop residue management, and post-harvest handling practices may have great impact on maize kernel contamination by *Fusarium* species and subsequent fumonisin contamination.

The current study also showed that occurrence and incidence of total *Fusarium* species isolates, *F. proliferatum* and *F. verticillioides*, as well as levels of total fumonisin contaminations were higher in fresh harvested than three-month stored maize samples in all districts. The highest level of contamination of fresh harvested maize samples could be explained by prevailing environmental conditions and initial *Fusarium* infection of maize grains prior to harvest. In addition, post-harvest practices, such as sorting molded maize ear and grain, proper drying before storage, types of storage structure used and applying storage pesticide(s) could be other factors that would reduce fumonisin contamination in stored maize grains. Sorting out the discoloured and damaged maize is a common practice and the majority of farmers in the study areas dried their maize before storage, and used to store their maize in polyethylene bags, sisal bags and underground pits. In a related study, Kimanya *et al.* (2009) reported that, five months after sorting and storage, the prevalence of contaminated maize was significantly lower than the prevalence in maize at the harvesting stage – before sorting.

On the other hand, lower maize grain moisture content observed in three-month stored maize samples could be the other attributing factor in reducing occurrence of *Fusarium* species and fumonisin levels in three-month stored maize samples. With regard to it, Temba *et al.* (2016) indicated that moisture content below 13% (v/v) had less effect on fumonisin contamination during storage, while the fumonisin contamination occurs in the field when the grain had moisture content above 13% (v/v) and high relative humidity. Also, Kaaya *et al.* (2005) implied that moisture content below the maximum (13%) is recommended for grain storage in the tropics, and below 15% is favourable for growth and toxin production by *F. verticillioides*. In the current study, the average grain moisture content of fresh-harvested maize samples was 15% (12.02 to 16.96%), while that of

the three-month stored maize samples was 13% (10.16 to 15.75%). In contrast to our results, Domenico *et al.* (2016) found that population of *Fusarium* species in maize increased from harvest to sampling three-months after storage depending on storage systems used, which could be related to storage structures.

There was a positive significant correlation between the total *Fusarium* kernel infection, occurrence of *F. proliferatum, F. verticillioides* and moisture contents of maize samples with fumonisin levels in both fresh harvested and three-month stored maize samples. A comparable result was also previously observed in Ethiopia by Tsehaye *et al.* (2017) who reported significantly a strong positive correlation between the incidence of *F. verticillioides* and fumonisin concentrations in maize samples. The significant correlation recorded between fumonisin levels and *Fusarium* species in the current findings also agree with observations made in other African countries, such as South Africa (Ncube *et al.*, 2011) and Kenya (Angeline *et al.*, 2016). Angeline *et al.* (2016) suggested that the positive correlation could be a result of initial contamination of maize by *Fusarium* species, favourable environmental and poor storage conditions, which promote *Fusarium* species growth and fumonisin contamination.

Conclusions and Recommendations

In the study, it is clear that different *Fusarium* species infect maize kernels both in fresh harvested and three-month stored maize samples in eastern Ethiopia, and their occurrence differs depending on the study areas and samples types. In all areas studied, it was confirmed that *F. proliferatum* and *F. verticillioides*, the main fumonisin producers, are predominantly occurred in maize. High *Fusarium* kernel infection and fumonisin contamination could indicate risk of fumonisin accumulation and exposure as almost all maize produced in the study areas are used for human consumption. The observed prevalence of the other *Fusarium* species, *F. subglutinans, F. graminearum, F. oxysporum, F. solani* and *F. andiyazi,* indicate the possibility of contamination of maize kernels by several other mycotoxins. The findings could serve as foundation for other studies on *Fusarium* species and fumonisin contamination in maize in Ethiopia for designing *Fusarium* species and fumonisin management strategies. However, more studies are needed to identify *Fusarium* species and quantify fumonisin contamination in relation to different field and storage environmental factors, agronomic and storage practices over different years.

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