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Detection of total fumonisins produced by *Fusarium verticillioides* (Sacc) isolates from maize kernels in Ethiopia

Hadush Tsehaye[1,2]*, Leif Sundheim[2,3], Arne Tronsmo[4], May Bente Brurberg[2,3], Dereje Assefa[1] and Anne Marte Tronsmo[2]

[1] Department of Dryland Crop and Horticultural Sciences, P.O. Box 231, Mekelle University, Mekelle, Tigray, Ethiopia (*had031@yahoo.com; hadush.tsehaye@gmail.com).
[2] Department of Plant Sciences, P.O. Box 5003, Norwegian University of Life Sciences, NO-1432 Ås, Norway.
[3] Biotechnology and Plant Health Division, Norwegian Institute for Bioeconomy Research, P.O. Box 5003, NO-1431 Ås, Norway.
[4] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway.

ABSTRACT

Fusarium verticillioides is the most common fungal pathogen of maize in Ethiopia. Many strains of this pathogen produce fumonisin myotoxins that are harmful to human and animal health. This study was conducted to determine the fumonisin- producing ability of isolates of *F. verticillioides* isolated from maize kernels collected from different maize- growing areas of the country. Eighty *F. verticillioides* isolates were grown on autoclaved maize cultures for one month, and the fumonisin content was quantified using Enzyme Linked Immunosorbent Assay (ELISA). All the 80 isolates evaluated were able to produce detectable levels of total fumonisins in the maize culture with values ranging from 0.25 to 38.01 mg of the toxin per kg of culture material (fungal biomass and maize kernels). The mean levels of total fumonisins produced by the *F. verticillioides* isolates were not significantly ($p>0.05$) different among maize- growing areas, however, the total fumonisins levels produced by isolates obtained from the same area as well as agroecological zones were wide-ranging. The results indicate that the majority (57.5%) of the *F. verticillioides* isolates associated with maize grains in Ethiopia produced total fumonisins >4 mg/kg, while 35% of the isolates produced total fumonisins <2 mg/kg. The widespread occurrence of higher fumonisin- producing strains across all maize-growing areas in Ethiopia indicates a possible food safety risk. Thus, efforts should be made to prevent the spread of this fungus with good agronomic practices and to implore all possible ways to avoid maize contamination with fumonisin both in the field and in storage.

Keywords: Agroecological zones, Fumonisin, Maize, Kernel, Food safety, Ethiopia.

1. INTRODUCTION

Maize (*Zea mays*) is one of the major staple foods in Ethiopia. It is second after Teff (*Eragrostis tef* Zucc.) in area coverage (2.12 million ha), and has the highest average yield (3.2 tones/ha) than any other crop in the country (FAO, 2015). The crop is grown in several agroecological zones of Ethiopia, from moisture deficit semi-arid to moisture surplus areas (Mosisa et al., 2012). *Fusarium verticillioides* (Sacc.) Nirenberg is the most common fungal pathogen of maize in Ethiopia (Amare Ayalew, 2010; Hadush Tsehaye et al., 2016). This fungus causes stalk and ear rot in maize and it is a prolific producer of dangerous mycotoxins known as

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fumonisins (Munkvold and Desjardins, 1997; Picot et al., 2010). *Fusarium verticillioides* is the most important fungal pathogen of maize in warmer climatic conditions worldwide (Picot et al., 2010) and high amount of fumonisins are frequently found in maize and maize based products (Bryla et al., 2013; Sundheim and Hadush Tsehaye, 2015). This mycotoxin has also been reported at low levels in several other agricultural commodities such as sorghum, rice, wheat, barley, beans, asparagus and medicinal plants (Bryla et al., 2013; Sundheim and Hadush Tsehaye, 2015). In Ethiopia, previous assessment of maize kernel samples collected from different maize growing areas indicated a widespread occurrence of fumonisin at concentrations ranging from 0.03 to 4.5 mg/kg (Amare Ayalew, 2010; Hadush Tsehaye et al., 2016).

At least 28 different fumonisin molecules, which are extracted from natural samples or produced by fungi in artificial culture media in the laboratory, are known. These are classified into A, B, C and P series based on their chemical structures (Falavigna et al., 2012; Picot et al., 2010). Members of the fumonisin B series (mainly FB_1, FB_2 and FB_3) are the analogues that often occur in great quantities in naturally contaminated maize samples (Bryla et al., 2013; Stępień et al., 2011). Fumonisin B_1 is the most important one, as it accounts for more than 70% of all fumonisins found in naturally contaminated food and feed (Nelson et al., 1991). Among the minor fumonisin analogues, the C series are known to occur on moldy as well as normal non-moldy maize grains, while members of the fumonisin A and P series are secondary metabolites produced in trace amount on artificial culture media in the laboratory (Falavigna et al., 2012). The pure chemical substance of fumonisin is a white hygroscopic material, which is very soluble in water and aqueous solutions of methanol or acetonitrile (Bryla et al., 2013; Picot et al., 2010). Structurally, the fumonisins that belong to the B-series, are characterized by a 20 carbon aminopolyhydroxy-alkyl chain, which is di-esterified with propane-1,2,3-tricarboxylic acid (Bryla et al., 2013). Due to their structural similarity with sphingolipid intermediates (sphinganine and sphingosine), fumonisins can inhibit the enzyme ceramide synthase and disrupt sphingolipid metabolism (Wang et al., 1991). Sphingolipids are major components of the cell membrane and they are important components of many signaling pathways (Wang et al., 1991).

Contamination of agricultural produce by fumonisin is a growing concern globally, as it causes adverse effects on human and animal health (Bryla et al., 2013; Picot et al., 2010). Upon consumption of contaminated feed, fumonisins may cause a fatal brain lesion known as leucoencephalomalacia in horses (Kellerman et al., 1990) and pulmonary edema in pigs (Harrison et al., 1990). Fumonisins are nephrotoxic, hepatotoxic, carcinogenic and embryo-

toxic in laboratory animals (Bryla et al., 2013; Gelderblom et al., 1996). In humans, long-term consumption of fumonisin contaminated food has been linked with high incidence of esophageal cancer and it is considered as a risk factor for liver cancer (Sun et al., 2007). Fumonisin has also been implicated for neural tube defects among populations consuming fumonisin-contaminated maize (Missmer et al., 2006).

The amount of fumonisin produced varies among *F. verticillioides* isolates, and not all isolates produce the toxin (Alakonya et al., 2008; Atukwase et al., 2012; Covarelli et al., 2012). The potential of a strain to synthesize fumonisin has been associated with the presence of the fumonisin biosynthesis gene cluster (*FUM*) (Proctor et al., 2003). Additional genes outside the *FUM* gene cluster are also known to regulate fumonisin biosynthesis, including *FCC1, FCK1, PAC1, ZFR1, GBP1, GBB1, CPP1, AREA, FST1* and *FvVE1* (Picot et al., 2010; Sagaram et al., 2006). Furthermore, fumonisin biosynthesis by *F. verticillioides* strains can be affected by environmental factors. Prevailing temperature, relative humidity, pH and nutrient status of the substrate on which the fungus grows, have been reported to influence the expression of *FUM* genes and fumonisin production by *Fusarium* strains (Picot et al., 2010; Sagaram et al., 2006).

Differences in toxin production profile among *F. verticillioides* isolates could reveal essential differences in the risk for mycotoxin contamination, with potential implication for human and animal health (Proctor et al 2006). Considering the great variation in maize genotypes and agroecological conditions in Ethiopia (Mosisa et al., 2012), *F. verticillioides* populations are expected to show high variation in fumonisin producing ability. At present, little is known about fumonisins production ability of *F. verticillioides* isolates in Ethiopia. To the best of our knowledge, there has been no research done on this issue in the country before. A good information on fumonisin biosynthesis potential of fungal strains coming from different geographic regions and agroecological zones could help predict the risk of contamination by mycotoxins in these areas. Therefore, this study was aimed at examining the total fumonisins (the sum of FB_1, FB_2 and FB_3) production capability of *F. verticillioides* strains isolated from maize kernels produced under different agroecological conditions in Ethiopia.

2. MATERIALS AND METHODS

2.1. Fungal Isolates

A total of 80 *F. verticillioides* isolates, isolated from maize kernel samples collected from 20 major maize growing areas of Ethiopia in 2012, were used in this study (Fig 1). A total of 200 maize kernel samples of 1 kg size were collected from the 20 different maize growing areas, with 10 randomly selected samples from each area. Sampling sites within areas were separated

at least by 1 km and at most by 5 km from each other. The sample collection areas represented seven major agroecological zones of Ethiopia, and their characteristics with respect to elevation, temperature, rainfall and major annual crops are presented in table 1.

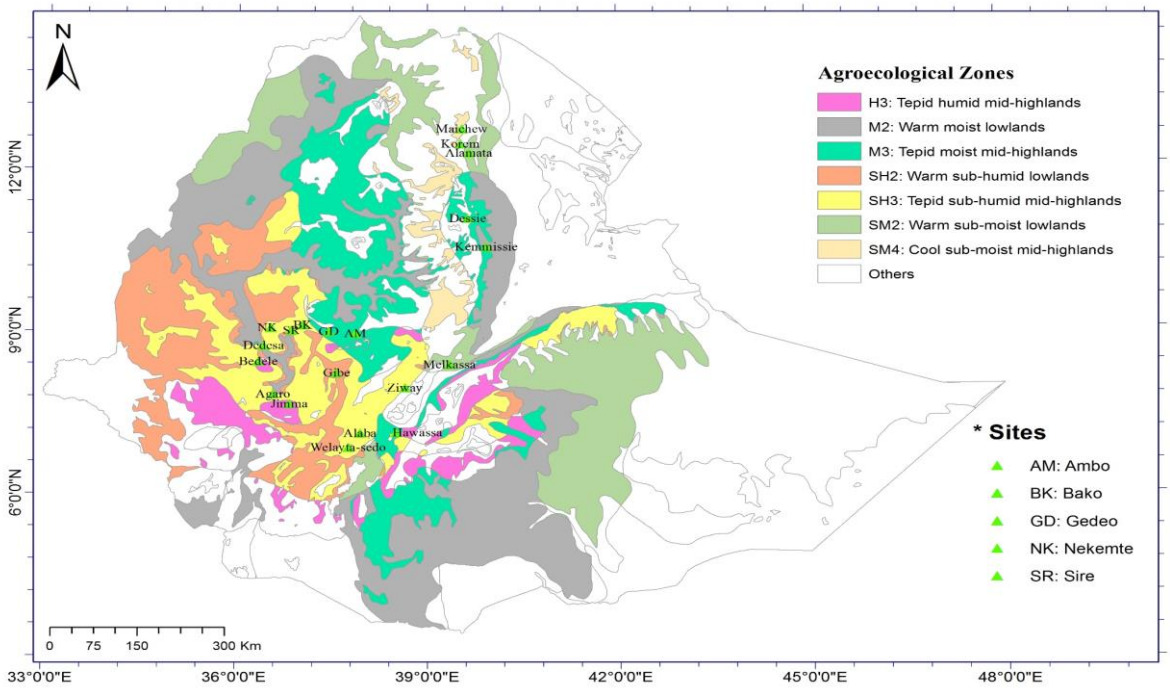

Figure 1. Agroecological zones of Ethiopia where maize kernels were collected, from which the *F. verticillioides* isolates were isolated.

Table 1. Summary of characteristics of the agroecological zones of Ethiopia where maize kernel samples were collected for isolation of *F. verticillioides* strains.

Agroecological zones	***Elevation (masl)*** [a]	***Temp (℃)*** [b]	***Annual Rainfall (mm)*** [c]	***Major crops*** [d]
Tepid humid mid-highlands (H_3)	1600 - 3000	17 - 22.5	1800 - 2200	Co, M, W, Rt
Warm moist lowlands (M_2)	400 - 2000	22.5 - 25	600 - 1400	S, M, T
Tepid moist mid-highlands (M_3)	1000 - 3600	17 - 20	1000 - 1400	W, T, B, M, Pu
Warm sub-moist lowlands (SM_2)	400 - 2000	20 - 25	600 - 1000	S, M, T, Fg
Cool sub-moist mid-highlands (SM_4)	2800 - 4000	15 - 20	600 - 1000	B, W, T, Pu
Warm sub-humid lowlands (SH_2)	400 - 2000	22.5 - 28	1000 - 1800	S, M, Co
Tepid sub-humid mid-highlands (SH_3)	1000 - 3200	20 - 22.5	1400 - 1800	S, M, W, Co, Rt

Note: [a] masl = meters above sea level.
[b] Temp = average temperature (>30 years data).
[c] Annual rainfall, long-term (>30 years data).
[d] Major crops =Coffee (Co), Maize (M), Teff (T), Wheat (W), Sorghum (S), Barley (B), Finger millet (Fg), Pulses (Pu), Roots and tuber crops (Rt) (potato, sweet potato, yams, 'ensete'). (Source: MoARD, 2005).

Maize kernels were disinfected by soaking in 1% sodium hypochlorite (NaOCl) for 2 minutes and washed twice in sterile, distilled water. For isolation of *Fusarium* species, maize kernels (fifty kernels from each sample) were placed on Petri dishes containing a modified

Czapek-Dox Iprodione Dichloran Agar (CZID), where the fungicides propiconazole 0.375 mg/L and fenpropimorph 1.125 mg/L were used (Halstensen et al., 2006). The Petri dishes with maize kernels (five kernels per Petri dish) were incubated for 5-7 days at 25°C in the dark. The identity of *Fusarium* colonies was confirmed by first growing on Spezieller Närstoffarmer Agar (SNA) for 7-10 days at 20°C under alternating near-ultraviolet (NUV)/white fluorescent light (12 h) and dark (12 h). A spore suspension from the resulting colonies was prepared in sterile water and spread onto Water Agar (WA) plates for single spore isolation. The plates were incubated at room temperature (22°C) for 16-20 h and single germinating conidia were removed and transferred to new SNA plates, containing a filter paper, to obtain monosporic cultures (Leslie and Summerell, 2006). Sub-culturing was made on PDA (Difco, Madison, USA) and carnation leaf agar (CLA) plates, incubated at 20°C with alternating photoperiod (12 h near UV/white fluorescent light, followed by a 12 h dark period) for 2 – 6 weeks. The morphological identification was performed according to the procedure described by Leslie and Summerell (2006). For confirmation of morphological identification, sequencing part of the translation elongation factor 1-alpha (*EF-1α*) gene was done for randomly selected (six) isolates, as described by O'Donnell et al. (1998). Sequence data were assembled and analyzed using the CLC Main Workbench software 6.9 (Aarhus, Denmark). The consensus sequences were checked for similarity against the GenBank (NCBI-National Centre for Biotechnology Information) and the *Fusarium*-ID database. The *F. verticillioides* isolate MRC826, obtained from the South African Medical Research Council, known as a high fumonisin producer (Vismer et al., 2004), was used as a reference isolate.

2.2. Preparation of Maize Kernels Substrate

To determine the amount of total fumonisins produced by each isolate, autoclaved whole maize kernels of an improved open pollinated maize variety, Melkassa-4, obtained from the Tigray Seed Agency, was used as cultivation medium. Initially, maize kernels (100 g of kernels and 100 ml of sterile water in 500 ml glass jars) were soaked for one hour and autoclaved at 121°C for one hour (Vismer et al., 2004). The moisture level of the maize kernels was measured with a moisture analyzer (Perten Instruments, Model: AM5100, Sweden) and was approximately 45% at the time of inoculation.

2.3. Inoculum Preparation and Culture Growing Condition

For spore production, each *F. verticillioides* isolate was sub-cultured by transferring a small (6 mm) disc of mycelia onto a petri-dish containing Mung Bean Agar (MBA) (Dill-Macky, 2003). The plates were incubated at 22°C for 7-10 days, with alternating photoperiod (12 h NUV-light

followed by 12 h dark periods). Conidia of the different *F. verticillioides* isolates were suspended in sterile water, and the concentration of the conidia was adjusted to 10^6 conidia/ml after counting the conidial concentration in a Kova glasstic slide spore counting chamber. The conidial suspension (1 ml) was inoculated into the maize cultures in 500 ml glass jars. The cultures were incubated in the dark for 4 weeks at 25°C, with brief manual shaking on daily bases during the first two weeks.

2.4. Extraction and Determination of Fumonisin

After four weeks of incubation, the culture materials (entire fungal mass and maize substrate) were dried in a forced air incubator at 60°C for three days. The dried samples were finely ground to powder using an ultra-centrifugal mill (ZM-200, Retsch GmbH & Co. KG, Germany). The samples were stored at -20°C and thawed at room temperature (22°C) for about 14 hours prior to fumonisins extraction and determination. The extraction was done by thoroughly mixing 5g of the ground samples with 25 ml of 70% methanol using a shaker (1000 rpm) for 3 min. The extract was filtered through a Whatman no. 1 filter paper and the collected filtrate was used in determining the total fumonisins using an Enzyme Linked Immunosorbent Assay (ELISA) kit (RIDASCREEN®fumonisin, R-Biopharm AG, Darmstadt, Germany), according to the manufacturer's instructions. Analysis of the results of RIDASCREEN® enzyme immunoassay was done using RIDA®SOFT Win software (Art. Nr. Z9999, R-Biopharm AG, Darmstadt, Germany). Fumonisins concentrations above the highest standard were further diluted, and the results obtained were multiplied by the dilution factor. The minimum limit of detection for the kit was 0.025 mg/kg, and all the samples were analyzed and recorded in duplicate wells.

2.5. Statistical Analysis

Statistical analysis for comparison of fumonisin levels produced by the *F. verticillioides* isolates per maize growing area and agroecological zone were performed with one-way ANOVA using SPSS (2013) version 22 (IBM SPSS statistics 22, Armonk, New York). One sample T-test was used to compare the mean levels of total fumonisins produced by the test isolates and the South African reference isolate (MRC826). Differences in fumonisin production ability between the test isolates and the South African reference isolate were also computed using Microsoft Excel (2013). Descriptive statistics were used to present the mean, median, quartiles, minimum and maximum fumonisins levels per agroecological zones. All statistical test were performed at a probability level of $P = 0.05$.

3. RESULTS

Total fumonisins produced by *F. verticillioides* isolates from maize kernels grown in Ethiopia are presented in table 2. Fumonisins were detected in cultures of all the 80 *F. verticillioides* isolates evaluated. There was variation in the quantity of total fumonisins produced by the isolates evaluated and ranged from 0.25 to 38 mg of the toxin per kg of culture material (fungal biomass and maize kernels). Isolates collected from the same area as well as from different areas varied in the level of the toxins produced in the culture medium. Isolate SR-6952, collected from Sire area had produced the highest concentration of total fumonisins (38 mg/kg), while isolates SR-3133 and SR-6351 collected from the same area had produced very low levels of total fumonisins (0.66 mg/kg and 1.82 mg/kg, respectively). Similarly, varying ranges in fumonisin concentration were observed among isolates collected from the other areas as well (Table 2).

Table 2. Levels of total fumonisins produced *in vitro* by *F. verticillioides* isolated from maize kernels grown in Ethiopia.

Isolate name	***Source area***	***Fumonisins (mg/kg)*** [a]	***Isolate name***	***Source area***	***Fumonisins (mg/kg)*** [a]
AG-122	Agaro	1.82	AW-412	Hawassa	10.42
AG-131	Agaro	10.29	J-332	Jimma	8.40
AG-411	Agaro	1.26	J-512	Jimma	18.88
AG-431	Agaro	34.86	J-541	Jimma	23.22
AG-6632	Agaro	5.87	J-6811	Jimma	0.71
AB-6921	Alaba	4.61	KM-131	Kemmisse	4.94
AB-6821	Alaba	1.95	KM-142	Kemmisse	2.79
AB-522	Alaba	10.14	KM-313	Kemmisse	27.9
AL-6111	Alamata	35.91	KM-322	Kemmisse	6.83
AL-4432	Alamata	1.87	KM-351	Kemmisse	4.32
AL-3443	Alamata	1.87	KR-231	Korem	24.80
AL-6811	Alamata	1.71	KR-252	Korem	11.87
AL-5843	Alamata	11.23	KR-6743	Korem	3.129
AM-212	Ambo	1.36	MC-212	Maichew	1.38
AM-6552	Ambo	2.73	MC-234	Maichew	12.68
AM-6444	Ambo	24.8	MC-422	Maichew	12.22
AR-212	Dedessa	24.96	MC-112	Maichew	1.95
AR-6441	Dedessa	22.88	MC-431	Maichew	33.81
AR-312	Dedessa	9.60	ML-313	Melkassa	1.71
BK-1122	Bako	0.60	ML-6341	Melkassa	1.82
BK-2734	Bako	8.64	ML-6533	Melkassa	1.97
BK-6521	Bako	31.29	ML-6842	Melkassa	0.25
BK-3741	Bako	11.34	NK-112	Nekemte	9.42
BD-112	Bedele	1.57	NK-242	Nekemte	23.68
BD-331	Bedele	0.95	NK-432	Nekemte	10.24
BD-413	Bedele	1.14	NK-6232	Nekemte	1.62
BD-422	Bedele	1.03	NK-6633	Nekemte	25.60
DS-121	Dessie	0.99	S-3133	Sire	0.66

DS-132	Dessie	2.16	S-322	Sire	22.88
DS-322	Dessie	0.92	S-6351	Sire	1.82
DS-693	Dessie	37.38	S-6952	Sire	38.01
GD-6351	Gedeo	10.27	WS-112	Welayta-Sedo	3.32
GD-6531	Gedeo	2.67	WS-6232	Welayta-Sedo	1.70
G-112	Gibe	7.84	WS-121	Welayta-Sedo	4.56
G-421	Gibe	33.54	WS-6332	Welayta-Sedo	23.20
G-61011	Gibe	30.08	Z-111	Ziway	34.02
G-6331	Gibe	14.09	Z-311	Ziway	1.77
AW-121	Hawassa	18.48	Z-6812	Ziway	4.31
AW-222	Hawassa	34.10	Z-512	Ziway	5.96
AW-231	Hawassa	18.88	MRC-826	South Africa	95.5
AW-314	Hawassa	1.33	*		

Note: [a] fumonisins concentrations presented in mg/kg culture material (fungal biomass and maize kernel). * Fumonisins concentration in the control culture was <0.025 mg/kg.

Table 3. Summary of total fumonisins produced by *F. verticillioides* strains isolated from maize kernels grown in different areas and agroecological zones in Ethiopia.

Isolate source- area	***Agroecological zone*** [a]	***Number of isolates tested***	***Fumonisins levels (mg/kg)***	
			Range	***Mean ± SEM***[b]
Agaro	SH_3	5	1.26 – 34.86	10.8 ± 6.2
Alaba	SH_3	3	1.95 – 10.14	5.6 ± 2.4
Alamata	SM_2	5	1.22 – 35.91	10.5 ± 6.6
Ambo	M_3	3	1.36 – 24.80	9.6 ± 7.6
Bako	SH_3	4	0.60 – 31.29	13.0 ± 6.7
Bedele	H_3	4	0.95 – 1.57	1.2 ± 0.1
Dedessa	M_2	3	9.06 – 24.96	19.0 ± 4.8
Dessie	M_3	4	0.92 – 37.38	10.4 ± 9.0
Gedeo	M_3	2	2.67 – 10.27	6.5 ± 3.8
Gibe	SH_2	4	7.84 – 33.54	21.4 ± 6.2
Hawassa	M_3	5	1.33 – 34.10	16.6 ± 5.4
Jimma	H_3	4	0.71 – 23.22	12.8 ± 5.1
Kemmisse	M_2	5	2.79 – 27.90	9.4 ± 4.7
Korem	SM_4	3	3.13 – 24.80	13.3 ± 6.3
Maichew	SM_4	5	1.38 – 33.81	12.4 ± 5.9
Melkassa	SM_2	4	0.25 – 1.97	1.5 ± 0.4
Nekemte	SH_3	5	1.62 – 25.60	14.0 ± 4.6
Sire	SH_3	4	0.66 – 38.01	15.8 ± 9.0
Welayta-Sedo	SH_3	4	1.70 – 23.20	8.2 ± 5.0
Ziway	SH_3	4	1.77 – 34.02	11.5 ± 7.6
MRC826	-	1	95.5	95.5
Uninoculated control	-	-	<0.025	<0.025

Note: [a] Agroecological zones- H_3: Tepid humid mid-highlands; M_2: Warm moist low-lands; M_3: Tepid moist mid-highlands; SH_2: Warm sub-humid low-lands; SH_3: Tepid sub-humid mid-highlands; SM_2: Warm sub-moist low-lands; SM_4: Cool sub-moist mid-highlands (Source: MoARD, 2005)

[b] SEM: Standard error of means, mean fumonisins levels produced by *F. verticillioides* isolates did not differ statistically ($p>0.05$) between maize growing areas.

Table 3 shows the summary of total fumonisins produced by *F. verticillioides* isolates collected from different areas in Ethiopia. Although it was not statistically significant ($p>0.05$),

the mean fumonisins levels produced by isolates from Gibe, Dedessa, and Hawassa were higher than the levels produced by isolates obtained from the other areas. For isolates from Gibe, the fumonisin levels ranged from 7.84 to 33.54 mg/kg (mean value: 21.4 mg/kg), isolates from Dedessa ranged from 9.6 to 24.96 mg/kg (mean value: 19 mg/kg) and isolates from Hawassa produced 1.33 to 34.1 mg/kg fumonisin, with mean value 16.6 mg/kg. On the other hand, isolates from Bedele and Melkassa produced very low amounts of fumonisins. Fumonisin production by isolates from Bedele ranged from 0.95 to 1.57 mg/kg (mean value: 1.2 mg/kg), whereas those from Melkassa varied from 0.25 to 1.97 mg/kg with mean value of 1.5 mg/kg.

Table 4. Summary of total fumonisins produced by *F. verticillioides* isolates isolated from maize kernels grown in Ethiopia in relation to some critical values set by the Codex Alimentarium Commission (CAC, 2014) and concentrations compared to a reference isolate from South Africa.

Fumonisin levels (mg/kg) [a]	***Number of isolates***	***Proportion from total isolates***	***Differences with reference isolate (%)*** [b]
>20	20	25	60.0 - 76.0
>4	46	57.5	60.0 - 95.5
<2	28	35	98.0 - 99.7
Above detection (>0.025)	80	100	-
P-value			0.001

Note: [a] Fumonisin level 4 mg/kg is the maximum tolerable fumonisins level in unprocessed raw maize grain, and 2 mg/kg is the maximum level in maize flour and meal based on the Codex Alimentarium Commission (CAC, 2014).

[b] Percentage difference in levels of total fumonsins produced by the test isolates compared to the reference isolate (MRC826) computed on Microsoft Excel, while one-sample T-test statistical analysis is performed using SPSS.

Total fumonisins produced by the *F. verticillioides* isolates were compared with some critical values recommended by the Codex Alimentarium Commission in maize for human consumption (CAC, 2014), as presented in table 4. Of all the isolates tested in this study, 57.5% produced total fumonisins in concentrations at >4 mg/kg, which is above the maximum tolerable limit in unprocessed maize recommended by the Joint FAO/WHO Codex Committee on Contaminants in foods (CAC, 2014). The total fumonisins levels produced by some of the *F. verticillioides* isolates was quite low, with 35% of the isolates producing fumonisins levels at <2 mg/kg. Only 25% of the isolates produced fumonisins at >20 mg/kg. One sample T-test analysis revealed that all the *F. verticillioides* isolates showed significantly lower ($p<0.001$) fumonisins production level than the South-African reference isolate (MRC826). Observed differences were in the range of 60%-99.7% compared to the reference isolate (Table 4).

Figure 2 shows total fumonisins levels produced by the *F. verticillioides* isolates collected from different agroecological zones of Ethiopia, presented in box and whisker plot,

depicting the median, mean, quartiles, the minimum and maximum values. When the data were combined and analyzed according to the agroecological zones of Ethiopia, the highest mean total fumonisins production was observed in isolates originating from the warm sub-humid lowlands (21.4 mg/kg), followed by isolates from the warm moist-lowlands (13 mg/kg) (Fig 2). Isolates from the warm sub-moist lowlands produced the lowest amount of fumonisin (mean 6.4 mg/kg).

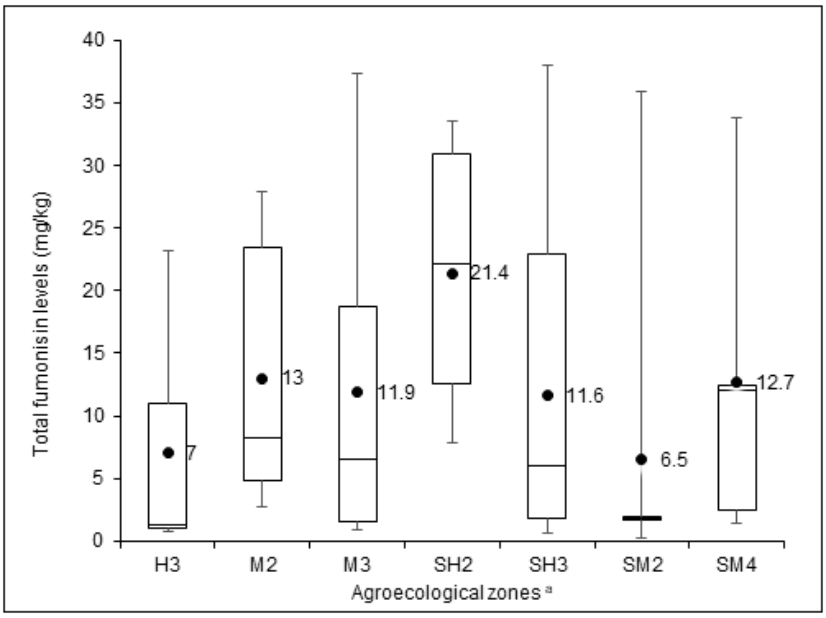

Figure 2. Box and whisker plot showing levels of total fumonisins produced by *F. verticillioides* isolates isolated from maize kernels grown in different agroecological zones of Ethiopia. Boxes represent the interquartile range between the first and third quartiles (25^{th} and 75^{th} percentiles, respectively) and the horizontal line inside denotes the median. Whiskers denote the minimum and maximum observations. Circular dotes denote mean fumonisins values. Data from 20 areas are summarized into their respective agroecological zones.
(***Note***: [a]Agroecological zones- H_3: Tepid humid mid-highlands; M_2: Warm moist lowlands; M_3: Tepid moist mid-highlands; SH_2: Warm sub-humid lowlands; SH_3: Tepid sub-humid mid-highlands; SM_2: Warm sub-moist lowlands; SM_4: Cool sub-moist mid-highlands (Source: MoARD, 2005)).

4. DISCUSSION

The present study revealed a widespread occurrence of fumonisins producing *F. verticillioides* in maize in Ethiopia, as all the 80 isolates evaluated produced fumonisins. These findings agree with the report of Reyes-Velázquez et al. (2011) in Mexico, who stated that all *F. verticillioides* isolates obtained from different maize hybrids able to produce fumonisins. Likewise, all *F. verticillioides* isolates, grown on sterile rice grain, evaluated in Brazil by de Oliveira Rocha et al. (2011) produced fumonisin B_1. In contrast, Covarelli et al. (2012) reported fumonisins producing ability of 25 *F. verticillioides* isolates in Italy and 20% of them did not produce

detectable level of fumonisins, despite of containing the FUM gene cluster. This was explained to mutation or altered expression of the FUM gene clusters. In Kenya, Alakonya et al. (2008) observed that 26% of the *F. verticillioides* isolates did not produce detectable level of fumonisin in maize cultures. However, they were only considered the quantification of fumonisin FB_1 instead of total fumonisins as in the present study.

A wide-range of variation in total fumonisins production among *F. verticillioides* isolates was observed in this *in vitro* study. The detected variation in fumonisin production among isolates could be due to variations in the inherent genetic characteristics of the isolates. Previous molecular studies of *F. verticillioides* isolates revealed a positive relationship between the *FUM* gene cluster and fumonisin production (Picot et al., 2010; Sagaram et al., 2006). The amount of transcription products from the key *FUM* genes, *FUM1*, *FUM6*, *FUM8* and *FUM21* positively correlates with fumonisin accumulation, but disruption of these genes results in significant reduction in fumonisin production (Proctor et al., 2003; Sagaram et al., 2006). Isolates with functionally active *FUM* genes may produce higher total fumonisins (FB_1+FB_2+FB_3) than other isolates that can only produce either of the FB types. A high level of sequence variation inside the *FUM* gene cluster can also explain for inter and intraspecific variation in fumonisin production by *Fusarium* species (Stępień et al., 2011).

The occurrence of high fumonisins concentration producing isolates, compared to others across all the areas investigated, demonstrated that the distribution of fumonisins producing *F. verticillioides* strains is not linked to specific geographic regions. Potent strains appeared widespread and uniformly distributed all over the maize growing areas. The reason for the highest mean total fumonisins observed in isolates originating from the warm sub-humid lowlands may be due to selection of high fumonisin producing strains by chance from this agroecological zone. The observed variation in fumonisin production by *F. verticillioides* isolates obtained from the same geographical areas was in accordance with the results obtained elsewhere (Alakonya et al., 2008; Covarelli et al., 2012; de Oliveira Rocha et al., 2011). The levels of fumonisins observed in this study were lower compared to those reported in Uganda (19.4 – 99.8 mg/kg) (Atukwase et al., 2012), in South Africa (1500 - 4200 mg/kg) (Vismer et al., 2004), in Italy (1 – 115 mg/kg) (Covarelli et al., 2012) and in Mexico (24.5 – 7190 mg/kg) (Reyes-Velázquez et al., 2011). However, Reyes-Velázquez et al. (2011) used higher inoculum concentrations (10^7 spores/ml) compared to the current study.

Depending on the criteria of Nelson et al. (1991), *F. verticillioides* strains can be categorized into three groups, high fumonisins producers (above 500 mg/kg), intermediate (50 to 500 mg/kg) and low producers (trace to 49 mg/kg). Based on the above criteria, all of the

strains isolated from maize kernels and evaluated in this study were probably low fumonisins producers. Total fumonisins levels produced by a substantial proportion of the *F. verticillioides* isolates tested in the current study were above the maximum limit that is considered unsafe for human consumption. The maximum tolerable levels of total fumonisins (FB_1+FB_2+FB_3) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) are 4 mg/kg in raw maize and 2 mg/kg in maize flour and meal (CAC, 2014). The widespread existence of isolates producing fumonisin levels above the maximum tolerable limit indicates a potential risk for food safety. This should not be underestimated taking into consideration the fact that *F. verticillioides* is the most frequently isolated *Fusarium* species in maize kernels (Amare Ayalew, 2010; Hadush Tsehaye et al., 2016). The concentrations of fumonisins produced may become higher when stressful environmental conditions prevail, which are more suitable for fumonisins synthesis. Climate change effects such as increased temperature and limited water availability, may stress host plants and favor growth of mycotoxigenic *F. verticillioides* strains.

5. CONCLUSION

The results revealed that fumonisins producing *F. verticillioides* strains occur across all maize growing areas in Ethiopia. Although the concentrations produced by some of the isolates in the current study were generally low, the widespread occurrence of fumonisins producing strains, capable of producing the toxin above the maximum tolerable levels, in maize intended for human consumption indicates a possible food safety risk. Thus, efforts should be made to prevent the spread of this toxigenic fungus as well as to develop sound management practices including implementation of good agricultural practices and explore all possible ways to avoid maize contamination with fumonisins. It is also very important to prevent introduction of more toxigenic strains with import of germplasms from abroad.

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7. CONFLICT OF INTEREST

There is no conflict of interests.

8. REFERENCE

Alakonya, A., Monda, E & Ajanga, S. 2008. Variation in *in vitro*fumonisin B_1 production by different*Fusarium verticillioides* isolates in Kenya. *American-Eurasian Journal of Agriculture and Environmental Sciences*, **4**: 368-371.

Amare Ayalew 2010. Mycotoxins and surface and internal fungi of maize from Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, **10**: 4110-4122.

Atukwase, A., Muyanja, C & Kaaya, A.N. 2012. Potential for fumonisin production by the strains of *Gibberella fujikuroi* species complex isolated from maize produced in Uganda. *Journal of Biological Sciences*, **12**: 225-231.

Bryła, M., Roszko, M., Szymczyk, K., Jędrzejczak, R., Obiedziński, M.W & Sękul, J. 2013. Fumonisins in plant-origin food and fodder-a review. *Food Additives and ContaminantsPart A*, **30**: 1626-1640.

CAC. 2014. Report of the eighth session of the codex committee on contaminants in foods. Codex Alimentarius Commission (CAC). Geneva, 14 -18 July 2014. Available at: http://www.codexalimentarius.org, Downloaded 13 August 2016.

Covarelli, L., Stifano, S., Beccari, G., Raggi, L., Lattanzio, V.M.T & Albertini, E. 2012. Characterization of *Fusarium verticillioides* strains isolated from maize in Italy: Fumonisin production, pathogenicity and genetic variability. *Food Microbiology*, **31**: 17-24.

de Oliveira Rocha, L.D., Reis ,G.M., da Silva, V.N., Braghini, R., Teixeira, M.M.G & Correa, B. 2011. Molecular characterization and fumonisin production by *Fusarium verticillioides* isolated from corn grains of different geographic origins in Brazil. *International Journal of Food Microbiology*, **145**: 9-21.

Dill-Macky, R. 2003. Inoculation methods and evaluation of *Fusarium* head blight resistance in wheat. In: Leonard K. J. and Bushnell W. R. (Ed.), *Fusarium* head blight of wheat and barley. American phytopathology Society, St. Paul, Minnesota, USA, pp184-210.

Falavigna, C., Cirlini, M., Galaverna, G., Sforza, S., Dossena, A & Dall'Asta, C. 2012. LC/ESI-MS/MS analysis outlines the different fumonisin patterns produced by *F. verticillioides* in culture media and in maize kernels. *Journal of Mass Spectrometry*, **47**: 1170-1176.

FAO. 2015. Food and Agriculture Organization of the United Nations Statistics Division. FAOSTAT crop production data. http://faostat3.fao.org/download/Q/QC/E.

Gelderblom, W., Snyman, S., Abel, S., Lebepe-Mazur, S., Smuts, C., Van der Westhuizen, L., Marasas, W., Victor, T., Knasmuller, S & Huber, W. 1996. Hepatotoxicity and carcinogenicity of the fumonisins in rats. *Advances in Experimental Medicine and Biology*, **392**: 279-296.

Hadush Tsehaye, Brurberg, M. B., Sundheim, L., Dereje Assefa, Tronsmo, A & Tronsmo, A. M. 2016. Natural occurrence of *Fusarium* species and fumonisin on maize grains in Ethiopia. *European Journal of Plant Pathology*, **145**: 1-15.

Halstensen, A. S., Nordby, K. C., Klemsdal, S. S., Elen, O., Clasen, P. E & Eduard, W. 2006. Toxigenic *Fusarium* spp. as determinants of trichothecene mycotoxins in settled grain dust. *Journal of Occupational and Environmental Hygiene*, **3**: 651-659.

Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E & Cole, J. R. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *Journal of Veterinary Diagnostic Investigation*, **2**: 217-221.

Kellerman, T. S., Marasas, W., Thiel, P., Gelderblom, W., Cawood, M & Coetzer, J. A. 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. *Onderstepoort Journal of Veterinary research*, **57**: 269-275.

Leslie, J. F & Summerell, B. A. 2006. *The Fusarium laboratory manual*. Blackwell Publishing, Ames, Iowa, 388p.

Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Alfred, H., Merrill, J. R., Rothman, K. J & Hendricks, K. A. 2006. Exposure to fumonisins and the occurrence of neural tube defects along the Texas-Mexico border. *Environmental Health Perspectives*, **114**: 237-241.

MoARD (Ministry of Agriculture and Rural Development). 2005. *Major Agro-ecological Zones of Ethiopia*. Natural Resource management Department, Addis Ababa, 67p.

Mosisa, W., Legesse, W., Berhanu, T., Girma, D., Girum, A., Wende, A., Tolera, K., Gezahegn, B., Dagne, W & Solomon, A. 2012. Status and future direction of maize research and production in Ethiopia. In: Worku et al. (eds.), Meeting the Challenges of Global Climate Change and Food Security through Innovative Maize Research. CIMMYT, DF, Mexico, pp 17-34.

Munkvold, G. P & Desjardins, A. E. 1997. Fumonisins in maize: can we reduce their occurrence? *Plant Disease,* **81**: 556–65.

Nelson, P. E., Plattner, R., Shackelford, D & Desjardins, A. 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. *Applied and Environmental Microbiology*, **57**: 2410-2412.

O'Donnell, K., Kistler, H. C., Cigelnik, E & Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. National Academy of Sciences*, **95**: 2044-2049.

Picot, A., Barreau, C., Pinson-Gadais, L., Caron, D., Lannou, C & Richard-Forget, F. 2010. Factors of the *Fusarium verticillioides*-maize environment modulating fumonisin production. *Critical Reviews in Microbiology*, **36**: 221-231.

Proctor, R. H., Brown, D. W., Plattner, R. D & Desjardins, A. E. 2003. Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. *Fungal Genetics and Biology*, **38**: 237-249.

Proctor, R. H., Plattner, R. D., Desjardins, A. E., Busman, M & Butchko, R. A. E., 2006. Fumonisin production in the maize pathogen *Fusarium verticillioides*: genetic basis of naturally occurring chemical variation. *J. Agriculture and Food Chem.*, **54**: 2424-2430.

Reyes-Velázquez, W. P., Figueroa-Gómez, R. M., Barberis, M., Reynoso, M. M., Rojo, F. G., Chulze, S. N & Torres, A. M. 2011.*Fusarium* species (section Liseola) occurrence and natural incidence of beauvericin, fusaproliferin and fumonisins in maize hybrids harvested in Mexico. *Mycotoxin Research*, **27**: 187-194.

Sagaram, U. S., Kolomiets, M & Shim, W. 2006. Regulation of fumonisin biosynthesis in *Fusarium verticillioides*-maize system. *Plant Pathology Journal*, **22**: 203-210.

Stępień, Ł., Koczyk, G & Waśkiewicz, A. 2011. *FUM* cluster divergence in fumonisins-Producing *Fusarium* species. *Fungal Biology*, **115**: 112-123.

Sun, G., Wang, S., Hu, X., Su, J., Huang, T., Yu, J., Tang, L., Gao, W & Wang, J.S. 2007. Fumonisin B_1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Additives and Contaminants*, **24**: 181-185.

Sundheim, L & Hadush Tsehaye 2015. Fumonisin in Zambia and neighboring countries in a changing climate. *Advances in Environmental Research*, **39**: 69-84.

Vismer, H. F., Snijman, P. W., Marasas, W. F. O & van Schalkwyk, D. J. 2004. Production of fumonisins by *Fusarium verticillioides* strains on solid and in a defined liquid medium - Effects of L-methionine and inoculum. *Mycopathologia*, **158**: 99-106.

Wang, E., Norred, W., Bacon, C., Riley, R & Merrill, A.H. 1991. Inhibition of sphingolipid biosynthesis by fumonisins: Implications for diseases associated with *Fusarium moniliforme*. *Journal of Biological Chemistry*, **266**: 14486-14490.