Isolation and Characterization of *Fusarium* Species and Fumonisins Contamination in Maize from Lower Eastern and Rift Valley Regions of Kenya

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Abstract

*Maize serves as a staple food in many Sub-Sahara African (SSA) Countries. It is mostly susceptible to mycotoxins including aflatoxin and fumonisin contamination. Fumonisins are produced by the Fusarium species, predominantly Fusarium verticillioides. Fumonisins' health hazards are documented in many parts of the world. However, few studies exist on fumonisin contamination in maize locally. The presence of Fusarium species and the associated fumonisin contamination of maize grown in Rift Valley and Lower Eastern regions of Kenya were assessed. Maize samples were collected from randomly selected households in three Counties from each of the two regions. Isolation and characterization of Fusarium species was done using Daniel et al., (2011) protocol. Envrologix Quick Tox Kit was used to quantify fumonisin levels. Aspergillus species was the most prevalent fungi species isolated (50.3%) followed by Fusarium species (39.3%) with F. verticillioides accounting for 80.8% of all Fusarium spp. Of the 200 samples analyzed, 133 (65.5%) had fumonisin levels below the level of detection (< 0.1 ppm), 63 (31.5%) had fumonisin level of between 0.1 ppm- 4.0 ppm and 4 (2.0%) sample had fumonisin levels of more than 4.0 ppm. Lower Eastern Region had higher proportion of samples with detectable fumonisin levels compared to Rift Valley Region (55.4% vs 11.1%)***.** *In conclusion Fusarium verticillioides commonly associated with fumonisin contamination of maize was a common fungus isolated in the study regions. It also showed that some of the maize samples consumed by the respondents have fumonisin levels that are above the internationally accepted levels. These results suggest that people are likely to be exposed to fumonisins that has been associated with adverse health hazards.*

Keywords: Fumonisin; *Fusarium*; Maize; Contamination; health hazards

INTRODUCTION

Maize is one of the main food crops consumed in many countries worldwide. It is a staple food in many nations especially in Sub-Saharan Africa (SSA). Among the cereals, maize has been reported to be the most susceptible to mycotoxin contamination (Miller, 2008). It is contaminated by different mycotoxins including aflatoxin, fumonisin, ochratoxin, deoxynivalenol and zearalenone. The mycotoxin existing in maize in a given region depends on the toxigenic profile of the existing population of pathogens in the given area (Stumpf *et al.*, 2013).

Health hazards in both humans and animals associated with fumonisin contamination has been reported world over, and yet there are limited studies in Kenya (Koskei *et al.*,, 2020) with a few researchers having attempted to address the issue (Kedera *et al.*, 1994; Kedera *et al.*, 1999; Kirui *et al.*, 2014). According to the study by Kedera *et al.*, (1994), assessing the incidence of maize ear rot in 21 maize genotypes grown in western Kenya, *Fusarium verticillioides* and *Fusarium graminearum* were the most prevalent species isolated from the asymptomatic and rotted kernels. Kedera *et al.*, (1999) has further reported the incidence of *Fusarium* spp. and fumonisins in maize from western parts of Kenya where *F. verticillioides* was found to be the most common species. A study by Kirui *et al.*, (2014) detected fumonisins in 9.8% of the samples of a local brew made from maize (*Busaa*) in Bomet constituency in Rift Valley, Kenya.

Fumonisins are mycotoxins produced by *Fusarium* species and predominantly by *Fusarium verticillioides* and *F. proliferatum.* The two have been the main isolates in maize samples from different parts world (Bryła *et al.*, 2013). However, the dominance of one of the fungal species over the other has been shown to vary depending on the region and existing environmental conditions (Ferrigo *et al.*, 2016).

There are four main types of fumonisins; FB_1, FB_2, FA_1, FA_2. Among them, FB_1 has been reported to have the highest toxicity and to be produced in largest amounts (Voss *et al.*, 2007). The International Agency for Research on Cancer (IARC) has reported FB_1 to be carcinogenic to humans (IARC, 2002) and has been shown to be a common contaminant of maize grown in Africa, South America, China, USA, Philippines and Thailand (Soriano & Dragacci, 2004; Voss *et al.,* 2007).

Maize and maize products were reported to contain the highest FB_1 concentration in maize from Africa, Central and South America and some countries in the Western Pacific Region when compared to other cereals by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2016 (FAO/WHO, 2002).

Fumonisin B_1 has been detected in the urine of exclusively breastfed infants, hence it is likely that human breast milk might be a source of exposure in children (WHO, 2018). Magoha *et al.,* (2014) reported mother's milk contamination with fumonisin with 58 of the 143 milk samples from mothers containing FB_1 in the range of 0.0066 to 0.4711 mg/kg (0.0066 to 0.4711 ppm) in Tanzania. This means that neonates are exposed to FB_1 with some of them being exposed to levels that were higher than the internationally recommended levels.

In sub-Saharan Africa, maize and its products are consumed on a daily basis. It follows that people may be getting exposed to a high level of fumonisins. In South Africa, Phoku and colleagues found the level of FB_1 in urine to be more than that contained in porridge consumed by the study participants. This was linked to other foods consumed by them which might also be contaminated with or by fumonisin (Phoku *et al.,* 2012). Understanding the diversity of *Fusarium* species and its associated fumonisin contamination of maize in relation to health impacts is vital in informing mycotoxin prevention strategies (Stumpf *et al.,* 2013). Hence, this study was aimed at determining the diversity of *Fusarium* species and fumonisin contamination of maize from Rift Valley and Lower Eastern regions of Kenya.

METHODOLOGY

A cross-sectional study was carried out in Rift Valley Kenya (Bomet, Nakuru and Trans-Nzoia Counties) and Lower Eastern Kenya (Makueni, Machakos and Kitui Counties). Rift Valley region was selected as it is considered the grain basket of Kenya while the Lower Eastern was selected due to the high reported cases of aflatoxin in the region (Daniel *et al*., 2011). Households were randomly selected in the two regions and Maize grains (samples) were collected for laboratory analysis.

Isolation and characterization of *Fusarium* species

Daniel *et al*., (2012) protocol involving seed disinfection method was used. It involves the treatment of maize seeds with 1% sodium hypochlorite (NaOCl) to reduce surface contaminants. Thereafter, the grains were soaked in hydrochloric acid (HCl) for 30 minutes and then soaked in 60ºC hot water for 5-10 minutes. The seeds were then put in lots each of 15g after which each lot was wrapped in 1 to 2 layers of cheesecloth and soaked in sterile distilled water for four hours at room temperature. They were then transferred to a water bath at 60ºC for 5 minutes. From the water bath, they were blotted in a sterile paper in a laminar flow before culture. Culture of disinfected kernel was done on Carnation Leaf-piece Agar (CLA) and Potato Dextrose Agar (PDA). The inoculated CLA plates were incubated for 2-7 days at 27^{o}C ambient air. The plates were examined daily for fungal growth. Suspected Fusarium species colonies were sub-cultured on PDA for purity and sub-sequent morphological and microscopic examination carried out.

Sample preparations and determination of fumonisin levels

Sample preparation for fumonisin quantification was done using Envilogix QuickTox™ Kit (Portland, Maine USA) as per the manufacturer's instructions. A portion of the collected maize samples was ground with a laboratory mill and a sample of 20 grams weighed into sample cups. Equal amount of 50% Ethanol was added to each sample and shaken for 2 minutes. One hundred microliters of the extract were diluted with 100 µl of buffer. Quantification of fumonisin was done using Envrologix QuickScan Reader.

Data management and analysis

Statistical Package for Social Sciences (SPSS) version 23.0 software was used for data analysis. Descriptive statistics were used to analyze *Fusarium* isolates and Fumonisin contamination levels. This included determination of frequencies and proportions of *Fusarium* species isolated and mean concentration levels of fumonisin toxins. The differences in proportion of *Fusarium* isolated between the two regions were compared using Fisher's exact test, while the differences in the level of fumonisins among the two regions and the different counties were assessed using the Mann-Whitney U test and Kruskal-Wallis test respectively. A P-value < 0.05 was considered statistically significant.

Ethics approval and consent to participate

Ethical approval for the study was given by the Institutional Research and Ethics Committee (IREC) of Moi University and Moi Teaching and Referral Hospital, Eldoret approval no (FAN: IREC 1829 of 2nd March 2017). Confidentiality was maintained and the identity of the farmers protected by assigning codes to each sample. Permission was obtained from respective County governments through assistance of National Commission of Science, Technology and Innovation (NACOSTI) Research Clearance permit No 14093.

RESULTS

Aspergillus spp. was the main fungal species isolated in the samples (50.3%), followed by *Fusarium* (39.3%), *Rhizopus* (24.3%), *Penicillium* (23.1%) and *Yeast* (17.9%) in that order. Other species identified included *Mucorales*, *Acremonium*, *Cladosporium* and non-sporulating fungal species. Lower Eastern Region had higher *Fusarium* isolates compared to North Rift Region and the difference was statistically significant (P-value < 0.001) (Table 1).

Table 1: Fungal species isolated from the samples (n = 173)

	Study region			
Variable	Rift Valley	Lower Eastern	Total	Fisher's Exact test P-value
Fungal species	n = 78	n = 95	n = 173	
Fusarium	18 (23.1%)	50 (52.6%)	68 (39.3%)	<0.001
Aspergillus	23 (29.5%)	64 (67.4%)	87 (50.3%)	<0.001
Rhizopus	20 (25.6%)	22 (23.2%)	42 (24.3%)	0.725
Yeast	19 (24.4%)	12 (12.6%)	31 (17.9%)	0.049
Penicillium	18 (23.1%)	22 (23.2%)	40 (23.1%)	>0.999
Mucorales	2 (2.6%)	2 (2.1%)	4 (2.3%)	0.999
Acremonium	5 (6.4%)	0 (0.0%)	5 (2.9%)	0.017
Cladosporium	0 (0.0%)	2 (2.1%)	2 (1.2%)	0.502
Non-sporulating	3 (3.8%)	0 (0.0%)	3 (1.7%)	0.090

*P-value of < 0.05 was taken to represent statistically significant level

Of the *Fusarium* species, *F. verticillioides* was the most predominant (80.8%). Other *Fusarium* species isolated in the study areas included *F. andiyazi* (17.3%) and *F. temperatum* (1.9%) (Table 2).

Table 2: Fusarium species isolated in the study sites

	Region			
Fusarium species	Rift Valley (n=11)	Lower Eastern (n=41)	Total (n=52)	Fisher's Exact test P-value
Fusarium andiyazi	5 (45.5%)	4 (9.8%)	9 (17.3%)	0.014
Fusarium verticillioides	6 (54.5%)	36 (87.8%)	42 (80.8%)	0.025
Fusarium temperatum	0 (0.0%)	1 (2.4%)	1 (1.9%)	>0.999

Fumonisin levels

The median fumonisin levels for Rift Valley and Lower Easter Regions was 0.66 ppm and 0.62 ppm respectively. There was no statistically significant difference in the fumonisin levels distribution between the two regions (z = -0.542, *P- value* = 0.588) (Figure 1).

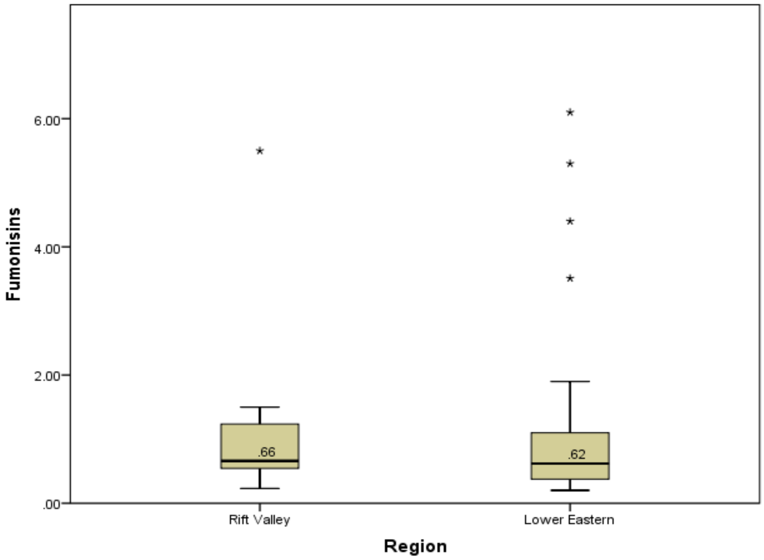

Figure 1: Boxplot of the distribution of fumonisins in the study regions
**Each star represents outlier fumonisins level*
*** The level of fumonisins values are in ppm*

Of the 200 samples tested, fumonisin was undetectable in 133 (66.5%) samples presumably because they were below the level of detection (<LOD). Sixty-three (31.5%) had fumonisin levels ranging from 0.1 ppm to 4.0 ppm while 4 (2.0%) had levels that were greater than 4.0 ppm. Lower Eastern Region had higher proportion of samples with detectable fumonisin levels when compared to Rift Valley Region (55.4% vs 11.1%, *P-Value* <0.05) (Table 3).

Table 3: Fumonisin contamination levels in the study sites

Fumonisin levels	Total (n=200)	Rift Valley (n=99)	Lower Eastern (N=101)	Fishers exact test P value
< LOD	133 (66.5%)	88 (88.9%)	45 (44.5%)	<0.001
0.1- 4.0	63 (31.5%)	10 (10.1%)	53 (52.5%)	<0.001
>4.0	4 (2.0%)	1 (1.0)	3 (3.0%)	<0.001

*Fumonisin values are in ppm

The distribution of fumonisins per county did not show any statistically significant differences (Table 4).

Table 4: Fumonisin contamination levels per county

	Fumonisins levels			
County	<LOD	0.1- 4.0 ppm	> 4.0 ppm	Total
Bomet	35 (26.3%)	7 (11.1%)	0 (0)	42 (21.0%)
Kitui	19 (14.3%)	20 (31.7%)	2 (50.0%)	41 (20.5%)
Machakos	13 (9.7%)	16 (25.4%)	0 (0)	29 (14.5%)
Makueni	13 (9.7%)	17 (27.0%)	1 (25.0%)	31 (15.5%)
Nakuru	27 (20.3%)	0 (0)	0 (0)	27 (13.5%)
Trans Nzoia	26 (19.5%)	3 (4.8%)	1 (25.0%)	30 (15.0%)
Total	133 (100%)	63 (100%)	4 (100%)	200 (100%)

Kruskal-Wallis test showed that there was no statistically significant difference in fumonisin levels between the different counties, $\chi^2(4) = 3.397$, *P-value* = 0.494.

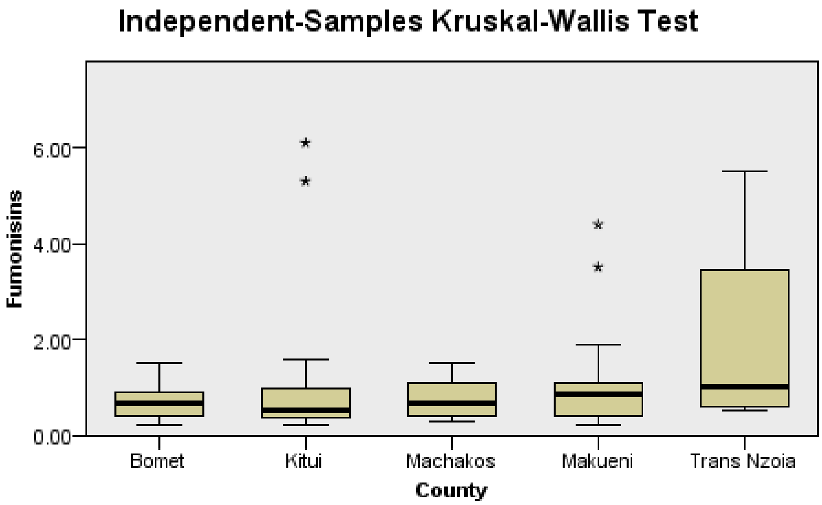

Figure 1: Distribution of Fumonisin Contamination per *County*

DISCUSSION

Fusarium

The main *Fusarium* species isolated in this study was *F. verticillioides*. This agreed with study by Kedera *et al.* (1999) that isolated *F. verticillioides* in 60% of samples collected in western region, Kenya. Similar surveys have showed *F. verticillioides* as the main species isolated from maize in the country (Kedera *et al.*, 1994) and 14% in maize from stalls and roadside traders in Western and Central Kenya (MacDonald & Chapman, 1997).

Fusarium verticillioides has also been shown to be the most common *Fusarium* species in most parts of Africa as was the case in this study. In Ethiopia, *F. verticillioides* was shown to be the most commonly isolated *Fusarium* species associated with maize kernels (42%) (Tsehaye *et al.,* 2017). Studies in South Africa also showed high *F. verticillioides* prevalence as was the case in a study by (Shephard *et al.*, 2013) where all samples tested positive for *F. verticillioides. For example,* study by (Phoku et al., 2012) reported 70.3% of the samples were positive for *F. verticillioides*, while Chilaka et al., (2012) reported 88% *F. verticillioides* isolates in the studied samples in the same country. This was also the case in Nigeria where Adejumo *et al*., (2007) reported 70% of the samples were positive for *F. verticillioides* Bankole & Mabekoje (2004) reported 89.3% of the samples were positive for *F. verticillioides* in the same country. *F. verticillioides* prevalence was 65.9% in Ghana (Kpodo *et al*., 2000) and 61.9%-77.5% in Uganda (Atukwase *et al*., 2012).

Fusarium verticillioides has also been reported to be the most common *Fusarium* species in other regions of the world. It was shown to be the most dominant species in Spain (Aguín *et al.,* 2014). de Oliveira *et al.,* (2011) found *F. verticillioides* to be the predominant *Fusarium* species with a proportion of 96% in maize collected from the different parts of Brazil.

There are studies where *F. verticillioides* and *F. proliferatum* have been reported to occur together as was the case in southern Europe, and Iran (Covarelli *et al*., 2012; Logrieco *et al*., 2002; Rahjoo *et al.,* 2008). However, there are places where other *Fusarium* species have been found to dominate. Studies conducted in Kosovo in 2009 and 2010 found *F. subglutinans* to be the most prevalent *Fusarium* species at 73% and 54% respectively while *F. verticillioides* was the second most prevalent species at 14% and 32% respectively (Shala-Mayrhofer *et al.,* 2013). This was also the case in Canada where *F. subglutinans* was the main species isolated from maize (Tamburic-Ilincic & Schaafsma, 2009). The variations in the prevalence of *Fusarium* species has been attributed to climatic conditions, with species such as *F. graminearum* being highly prevalent in temperate regions while *F. verticillioides* being highly prevalent in warmer regions (Czembor *et al*., 2015).

Fusarium verticillioides has been shown to be the main *Fusarium* species associated with fumonisin contamination. The other species associated with fumonisin contamination are *F. proliferatum*. Hence the presence of *F. verticillioides* as the main *Fusarium* species is a pointer of maize contamination with fumonisin in our study area.

Fumonisin

Most of the samples tested had fumonisin levels within the range of 0.2-4.0 ppm. Codex Alimentarius has set the maximum limit for fumonisins in raw maize grain at 4 mg/kg and 2 mg/kg (4 ppm and 2 ppm) in maize flour and maize mill respectively (Standard, 2015) meanwhile, the Europe commission has set a limit levels of 1 mg/kg (1 ppm) in maize or maize-based products for human consumption (European Commission, 2007). A total of four (2.0%) samples had levels exceeding the set limits of Codex Alimentarius of 4 ppm in raw maize. However, 21 (10.5%) of the samples had fumonisin levels of more than 1 ppm recommended by the European Commission, of which most 17 (80.9%) were from Lower Eastern Region.

In this study, 33% of the samples had levels of fumonisins of 0.2-6.0 ppm while one sample had fumonisin of more than 6.0 ppm. Hence, the proportion of samples with more than 0.2 ppm fumonisin levels was higher than that reported by Kedera and colleagues in a study conducted in western Kenya where 47% of the 197 maize kernel samples had fumonisin B_1 of levels above 0.1 mg/kg (0.1 ppm) with 5% having FB_1 levels of more than 1 mg/kg (1 ppm) (Kedera *et al.,* 1999). The differences observed in the two studies could be due to the differences in climatic conditions and post-harvest practices which have been shown to influence fumonisin levels (Atukwase *et al*., 2012; Tsehaye *et al*., 2017).

Maize is a staple food in Kenya and many other African countries. Its susceptibility to aflatoxin and fumonisin contamination has been widely reported (Kimanya *et al.,* 2008). However, most studies in Kenya have focused more on aflatoxin with little emphasis on fumonisins and other mycotoxins including ochratoxin, deoxynivalenol (DON), nivalenol, zearalenone and ochratoxin A that are also known to be of public health importance. It is therefore likely that people are chronically exposed to fumonisins through the maize and its products with adverse public health implications. High levels of fumonisin were reported in local maize-based brew in Kenya (Kirui *et al.,* 2014), hence a high risk of exposure to humans.

Fumonisin exposure has been associated with poor growth or growth impairment in children (Shirima *et al.,* 2014), high incidences of oesophageal cancer (Chu & Li, 1994; FAO/WHO, 2002; Sydenham *et al.*, 1990), and neural tube defects in foetus (Marasas *et al.*, 2004; Missmer *et al.,* 2005). Exposure to fumonisins in animals has also been associated with porcine pulmonary edema (PPE) syndrome in pigs (Haschek *et al*., 2001), Equine Leucoencephalomalacia (ELEM) in horses, and experimentally, liver cancer in rats (Marasas *et al*., 1984).

In conclusion, there was high incidence of *F. verticillioides*, the main producer of fumonisin in the study sites. Maize being the staple food in the Country, many people could be consuming contaminated maize. Contamination of maize with fumonisin is both a public health threat and poses much risk to food safety and security in the Country.

Fumonisin having been reported to be carcinogenic, there is an urgent need for continued monitoring of maize and maize products and formulation of policies to prevent their contamination. There is also urgent need for more studies on fumonisin and its relationship with cancer to provide the much-needed evidence to inform policy and practice.

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