FUMONISIN CONTAMINATION OF MAIZE (*Zea mays*) IN AFLATOXIN 'HOT' ZONES IN EASTERN PROVINCE OF KENYA

Bii, F[1]., Wanyoike, W[2]., Nyende, A. B.,[1] Gituru, R. W.[2] Bii, C[3]

[1]*Institute for Biotechnology Research- Jomo Kenyatta University of Agriculture and Technology.*
[2]*Botany Department- Jomo Kenyatta University of Agriculture and Technology.*
[3]*Centre for Microbiology Research-Kenya medical Research Institute.*

***Corresponding author**: biichristinec@gmail.com, kiptobi@yahoo.com*

SUMMARY

Natural *Fusarium* and fumonisin contamination were evaluated in 86 stored maize samples and correlated to damaged kernels (%). Maize samples were collected from selected farmers in Aflatoxin 'hot' zones of Eastern province. Samples were collected from Kitui and Kibwezi districts in May to June 2008. *Fusarium* species were isolated and identified using morphological characteristics at Mycology Laboratory, Kenya Medical Research Institute. Fumonisin quantification was done using ELISA (RIDASCREEN® ELISA test kit (Art. No.: R3401) at Bora Biotech, Nairobi.
Colony Forming Unit (CFU) counts indicated that apart from Aspergillus a common contaminant in maize, *Fusarium* species infestation was also high. The most common species being *F. verticillioides* isolated at (39.9 %) in the two districts. Other isolated *Fusarium* species included, *F. proliferatum* (15.1 %), *F. solani* (9.0 %), *F. anthophilium* (9.0 %), *F. oxysporium* (15.1 %), and *F. Lateritium* (12.1 %). Damaged kernels analysed in this study included insect infestation, mouldy kernels, and off coloured kernels. Results showed up to 20 % of the grains were damaged in some samples. Contamination with fumonisin toxin was observed to be high. Most of the samples exceeded 1 mg / kg the maximum tolerable levels recommended by the European commission. *Fusarium* species count and fumonisin levels showed positive correlation ($p < 0.05$). In addition, there was a positive correlation between damaged kernels (%) and *Fusarium* species count ($p < 0.05$). Positive correlation was also observed between damaged kernels (%) and *Fusarium* species count ($p < 0.05$). In general, *F. verticillioides* and *F. proliferatum* were isolated in samples with the highest percentage of kernel damage and highest fumonisin concentrations.
These findings indicate wide spread infestation and contamination of maize by *Fusarium* species and fumonisin toxins. It is apparent that apart from aflatoxins contamination there is also high level of fumonisin exposure in the high risk population necessitating urgent intervention measures to curb the long term health consequences in the population.

***Key words:** Fusarium spp, Mycotoxin, Fumonisin, Zea maize, Aflatoxin 'Hot' zones.*

[Afr J Health Sci. 2012; 20:28-36]

Introduction

Maize (*Zea mays* L.) is the most important staple food crop in Kenya. It is the key food crop constituting 3 % of Kenya's Gross Domestic Product (GDP), 12 % of the agricultural GDP and 21 % of the total value of primary agricultural commodities (Government of Kenya, 1998). Maize is both subsistence and a commercial crop, grown on an estimated 1.4 million hectares both by large-scale farmers (25 %) and smallholders (75 %).

Demand for maize grain is projected to increase by 50 % globally by 2020, including 93 % in Sub Saharan Africa, 92 % in South Asia, 62 %, in Latin America and 46 % in East and South East Asia (Pingali and Pandey, 2001). It is therefore alarming that despite the existing shortfall in maize demand, maize diseases still rank highly as a maize production constraint. Among the diseases at least as attested by the considerable research carried out, the maize ear rots, have not received the attention they deserve (Pingali and Pandey, 2001). Maize ear rots result in maize kernel damage.

Damaged kernels are of great concern due to the loss of corn quality and the potential occurrence of mycotoxins. As the amount of damaged kernels increases, the discounts are greater and sometimes all the lot could be destroyed (Ono *et al*., 2006). The Food and Agricultural Organization (FAO) estimated contamination with mycotoxins in 25 % of the world's crop, and the main fungi involved belonged to the *Fusarium, Aspergillus* and *Penicillium* genera (Pitt *et al*., 2000).

Several phytopathogenic species of *Fusarium* are found to be associated with maize including *F. verticillioides*, *F. proliferatum*, *F. graminearum* and *F. anthophilium*

(Fandohan *et al*., 2003). Among them, *F. verticillioides* is probably the most common species isolated worldwide from diseased maize (Fandohan *et al*., 2003), and have been known to cause root, stalk, ear and kernel rots (Baird *et al*., 2008). Recently, *Fusarium* species have emerged as human pathogens where they are associated with deeply invasive infections in immunecompromised patients (Nucci and Anaissie, 2002 and Summerbell, 2003).

Fumonisins are produced by several closely related species of *Fusarium* that can grow within maize tissues without causing visible symptoms of disease. Interest in fumonisins is due primarily to the discovery that they are potent inhibitors of sphingolipid biosynthesis and that they can impair animal health (Nelson *et al*., 1993). Consumption of fumonisins has been shown to cause leucoencephalomalacia in horses (Kellerman *et al*., 1990), pulmonary edema in swine (Harrison *et al*., 1990), and experimental liver and kidney cancer in rodents, and has been epidemiologically associated with esophageal cancer in humans (Marasas, 2001). Other epidemiological studies available demonstrate only inconclusive associations between fumonisins and human esophageal cancer (Sydenham *et al.,* 1990 and Chu and Li, 1994).

In April 2004, an outbreak of acute hepatotoxicity was identified among people living in Kenya's Eastern Province. Epidemiological investigations determined that the outbreak was the result of aflatoxin poisoning from ingestion of contaminated maize (corn). The outbreak covered more than seven districts encompassing an area approximately 40,149 km^2 (15,502 mile^2). Of the 317 case-patients, 89 % resided in four districts (Makueni, Kitui, Machakos, and Thika) (Lewis *et al.,* 2005). The 2004 outbreak of acute aflatoxicosis in Kenya was one of the most severe episodes in history of human aflatoxin poisoning recorded. Many of these and other reported cases have been related to aflatoxin poisoning. These areas have become the aflatoxin 'hot' zones in Kenya. Aflatoxin has powerful teratogenic, mutagenic and hepatocarcinogenic effects (Wang *et al.*, 2001). There are no confirmed records of acute fumonisin toxicity in human. Despite these reports, the co-occurrence of fumonisin with aflatoxin B1 (AFB1) is presumed to play a crucial role in the promotion of carcinogenesis (Ueno, 2000).

The presence of mycotoxins in food is often overlooked in Africa due to public ignorance about their existence, lack of regulatory mechanisms, dumping of food products, and the introduction of contaminated commodities into the human food chain during chronic food shortage due to drought, wars, political and economic instability (MERCK, 2006).

In view of these reports, we considered of importance to study *Fusarium* species diversity and fumonisin contamination of maize grains from the Aflatoxin 'Hot' zones in Kenya.

In this study, natural *Fusarium* and fumonisin contamination were evaluated in 86 stored maize samples and correlated to percent damaged kernels.

Materials and Methods

Study site

The study areas are sites previously studied for outbreaks of acute aflatoxicosis in the high risk communities in Makueni district (Kibwezi, Makindu, Mtito-Andei locations), and Kitui District (Mutha, Mutomo, Ikutha, Central and Kyuluni locations) in Kenya's Eastern Province (Fig 1). Outbreaks occurred among subsistence farmers following the major harvest in 2004 and again in 2005.

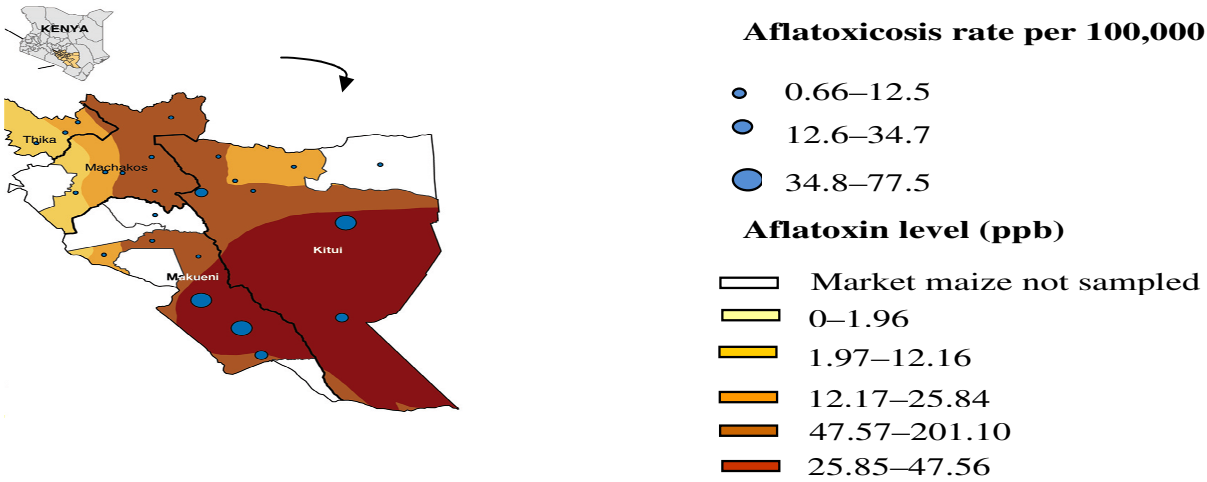

Fig.1. Areas identified as risk zones due to aflatoxin contamination, and used as target zones in this study, Lewis *et al*., 2005.

Sample collection and preparation

Maize samples were collected directly from the farmers' stores during the 2008 crop harvest. Random sampling procedure was used to select 86 farmers, 44 from Makueni and 42 from Kitui districts of Eastern province. The selection was made from a compilation of farmers list prepared by government extension workers. The farmers had previously been affected with the aflatoxin outbreak in 2004. For each farmer, a sample of 500 g of shelled maize was taken randomly. This involved taking 10 cobs from a maize store randomly and shelling off 20 maize grains from each of the cobs and thoroughly mixing till we had a laboratory sample of 500g from the bulk samples. Where the maize had already been shelled, grains were taken from different parts of the bag randomly and pooled together to the required laboratory sample. The samples were immediately sent to the laboratory and maintained at 4 ^{0}C in the collection paper bags for mycological investigations and mycotoxin analysis.

Analysis of Kernel characteristics

Damaged kernels were determined and calculated as a percentage. The damaged kernel characteristics determined during this study include: moldy kernels, insect infestation, and off colour kernels.

Mycological determination

The samples were milled using a commercial blender. One g was transferred into clean sterile bottles containing 5 ml of sterile distilled water and blended for 3 minutes with a shaker.

Fifty micro litres (50 µl) was cultured in dilutions of 10^{-2} dilution plating technique on SDA plates, in three replications, and incubated for 4 days at 28 ^{0}C. Colony Forming Units (CFUs) of *Fusarium* and other fungi in the samples were recorded. Sub-cultures of pure *Fusarium* isolates were incubated at 28 ^{0}C for 4 days. Macroscopic and microscopic features were evaluated on Sabouraud Dextrose agar (SDA) according to procedures by Nelson *et al*. (1983).

Quantification of mycotoxin

Fumonisin toxins were quantified by ELISA according to the procedures provided by RIDASCREEN® ELISA test kit (Art. No.: R3401). The competitive direct enzyme – linked immunosorbent assay was done on a 5g sub sample taken from 500g milled maize sample. In the ELISA test, free fumonisin in the sample competes with added enzyme- labelled fumonisin (conjugate) for antibody binding site. In each run, 6 standards (0.025 ppm, 0.074 ppm, 0.0222 ppm, 0.666 ppm, 2 ppm) are included. The results of absorbance of the standards and unknowns were measured at 450 nm against a blank and read 10 minutes after addition of the stop solution. RIDA®SOFT Win (Art. No. Z99999) software was used to calculate the fumonisin concentration.

Statistical analysis

The Pearson correlation for the variables *Fusarium* species count, fumonisin levels, and damaged kernels were analyzed by the t- test. Total fungal colony count, *Fusarium* species count, fumonisin levels, damaged kernels and moisture content were analysed using ANOVA followed by Turkey's multiple comparison test ($p<0.05$). Mean separation was performed using Statistical Analysis Software SAS and SPSS for windows version 12.

Results

Analysis of kernel damage

The weight of moldy grain and insect infested kernels were compounded to mean the weight of damaged kernels. The weights were analyzed per sample and compared against isolated *Fusarium* species in the same sample, and also against sample site (Kibwezi and Kitui districts) (Table. 1). The amount of mean percent damaged kernels compared against each isolated fungi (Table 1) showed that the difference of degree of damaged kernels varied significantly ($p < 0.05$) against each isolated species. *F. verticillioides* and *F. proliferatum* were isolated in kernels with the highest degree of damage while the samples where no *Fusarium* species was isolated had the lowest degree of damage (Table 1). Makueni district recorded the highest percent of damaged kernels.

Table 1. Mean (±SE) of percent kernel damage in Makueni and Kitui districts.

Isolated *Fusarium* species	Kitui District	Makueni District
	% damaged kernels	
F. anthophilium	5.63 ± 0.86ab	14.22 ± 0.41a
F. lateritium	3.75 ± 0.44b	6.08 ± 0.32b
F. verticillioides	6.68 ± 1.14ab	13.30 ± 0.99a
F. oxysporium	7.62 ± 0.37ab	5.89 ± 1.36b
F. proliferatum	10.29 ± 2.21a	13.58 ± 0.74a
F. solani	10.92 ± 2.01a	3.70 ± 0.042b
ND	3.17 ± 0.34b	0.00 ± 0.00c
Grand mean	**6.62**	**10.95**
$LSD_{(0.05)}$	**3.71**	**5.04**

ND- No detected *Fusarium* species

Fungal mycoflora

A total of 207 CFU of fungal contaminants belonging to different fungal genera were isolated. *Aspergillus* species was the most frequently isolated fungi, in all maize samples (35.8 %), irrespective of their geographic origin. Others isolated fungal species belonged to the genera: *Fusarium* species (15.5 %), *Penicillium* species (9.2 %), *Rhizopus* species (5.3 %) and other contaminations observed constituted 34.4 %.

Contamination of maize with *Fusarium*

A total of 32 *Fusarium* isolates were recovered from 30 samples of the 86 maize grain samples in which six *Fusarium* species were identified namely, *F. verticillioides* (39.9 %), *F. proliferatum* (15.1 %), *F. lateritium* (12.1 %), *F. anthophilium* (9.0 %), *F. oxysporium* (15.1 %) and *F. solani* (9.0 %). Mean CFU of each isolated species (Table 2) was calculated and compared. The means show that *F. verticillioides* had the highest total mean CFU compared to the other *Fusarium* species, while *F. solani* had the lowest total mean CFU. The means varied significantly ($p<0.05$).

Table 2. Total mean (±SE) of CFU counts of *Fusarium* in the samples from Makueni and Kitui

	Makueni District	Kitui District
Isolated *Fusarium* species	**CFU**	
F. anthophilium	19.67±2.028a	1.11±.389de
F. lateritium	14.17±5.082a	1.33±.373de
F. verticillioides	17.42±3.555a	16.33±4.364a
F. oxysporium	16.56±6.629a	14.50±5.143ab
F. proliferatum	17.00±3.464a	10.00±2.147bc
F. solani	8.00±1.732a	6.20±1.458cd
Grand mean	**15.47**	**8.25**
$LSD_{(0.05)}$	**21.69**	**6.12**

Means with the same letter (by column) are not significantly different ($p < 0.05$)

Fumonisin Contamination

Fumonisin profiles of maize samples were analyzed and compared in this study, where a widespread contamination and occurrence of fumonisins in maize samples was observed. The results indicated that all the samples tested were highly contaminated at various levels. A high level of mean fumonisin contamination was detected in the maize samples from Makueni (1.17 ± 0.085), compared to Kitui (0.912 ± 0.134) (Table 3). Fumonisin levels detected varied significantly ($p<0.05$) from one sample to another in the two sites. Regardless of the levels of fumonisin at each site, comparison of fumonisin means against samples of isolated *Fusarium* species, results indicated that a high mean fumonisin level was detected in the samples where the isolated *Fusarium* species is a fumonisin producer (Table 3)(*F. verticillioides*, *F. proliferatum, F. oxysporium and F. anthophilium*).

Table 3. Mean (±SE) total fumonisin level in Makueni and Kitui compared against isolated *Fusarium* species

	Fumonisin means (mg/kg)	
***Fusarium* species**	**Kitui**	**Makueni**
Blank Plate	0.50 ± 0.14c	0.00 ± 0.00d
F. anthophilium	0.00 ± 0.00	1.44 ± 0.004a
F. lateritium	0.50 ± 0.37b	0.61 ± 0.003b
F. verticillioides	1.40 ± 0.35a	1.37 ± 0.057a
F. oxysporium	0.89 ± 0.04ab	0.13 ± 0.007c
F. proliferatum	1.14 ± 0.003ab	1.32 ± 0.004a
F. solani	0.00 ± 0.00	0.00 ± 0.00
Grand Mean.	**0.91**	**1.17**
$LSD_{(0.05)}$	**0.92**	**0.43**

Means with the same letter(s) (by column) are not significantly different ($p < 0.05$).

A trend was observed in the two districts where high quantities of fumonisin were recorded, and the isolated *Fusarium* species is a high fumonisin producer (*F. verticillioides* and *F. proliferatum*). Similarly, no fumonisin was observed in the plates where *F. solani* was isolated (*F. solani* is a non – fumonisin producer). Major difference though, was the finding of fumonisin toxin in plates with *F. lateritium* (a non fumonisin producer) in both locations and in sample plates where there were no contaminations in Kitui. Generally there were significant difference ($p=0.05$) in the levels of fumonisin production when individual species are compared in the two districts.

Correlation analysis

In an attempt to evaluate the interactive effects between colony counts (CFU), fumonisin level ($mg.kg^{-1}$) and percent kernel damage, all the samples were analysed and values presented as linear regression where standard curves were generated (Figures 2, 3 and 4).

The correlation between fumonisin content (mg / kg) and *Fusarium* species CFU was not as strong as that noted in the relationship between percent kernel damage and CFU, but was significant (Fig. 2). The correlation coefficients were $R^2 = 0.015$ were $p = 0.121$. The CFU count reported in this study refer to visible and viable mould propagules.

Non-significant correlation between occurrence of *Fusarium* ear rot (Damaged kernels) and fumonisin content analysed was noted. The correlation coefficients were $R^2 = 0.279$ and $p = 0.528$ (Fig. 3).

There was a significant ($p = 0.05$) relationship between percent kernel damage and *Fusarium* CFU (Fig. 4) indicating occurrence of *Fusarium* species infection is directly related to the severity of maize ear rot (Damaged kernels). The correlation coefficients were $R^2 = 0.348$ and $p = 0.59$ (Fig. 4). Any increase in the level of damaged kernels is increases the number of mould infestation.

These R squared (R^2) and Pearson's correlation values (p) generated may be used a predictor of grain storage quality but, conclusion in terms of levels or types of moulds or mycotoxins present may have to be derived by assessing individual mould.

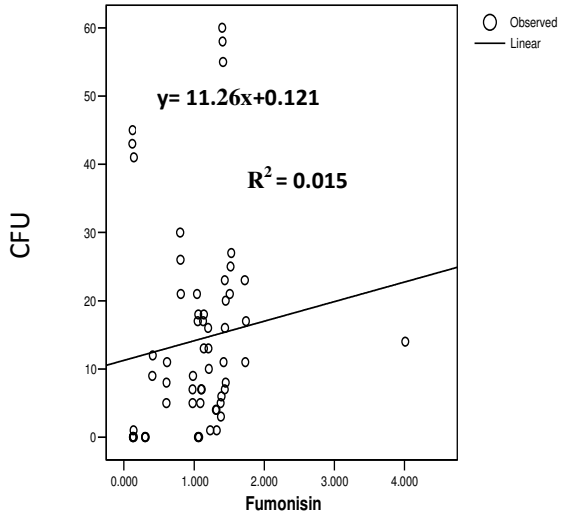

Figure 2: Linear Regression curve of CFU against Fumonisin (mg/kg)

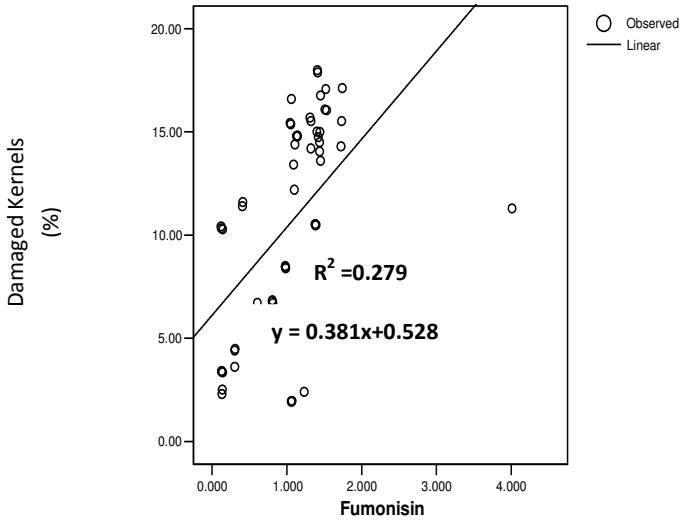

Figure 3: Linear Regression curve of percent damaged kernels against Fumonisin (mg / kg)

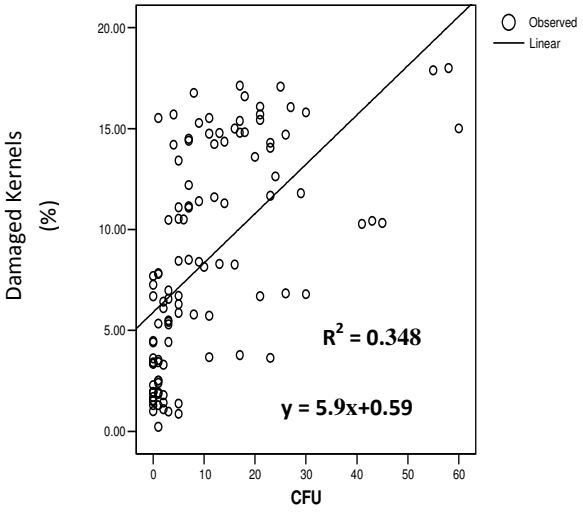

Figure 4: Linear Regression curve of percent damaged kernels against CFU

Discussion

Maize kernel damage

Fusarium species is commonly associated with maize kernels and, under favourable conditions, causes a kernel decay known as *Fusarium* ear rot or maize ear rot.

Summarizing the results of *Fusarium* kernel damage, *Fusarium* species isolated in kernels with the high degree of damage (Table 1) also had the high colony count (CFU) in both Makueni and Kitui (Table 2). Makueni had a high percent of damaged kernels (10.95 ± 0.78) when compared to Kitui (6.62 ± 0.57) (Table 1). The highest mean percent of kernel damage was seen in samples where *F. verticillioides* and *F. proliferatum* were isolated (Table 1). Basied on the total means, it can be concluded that the two *Fusarium* species were partly responsible for the high percentages of kernel damage compared to other *Fusarium* species.

The parameters used here to assess the level of damage were considered sufficient in that, physical symptoms of infection (mouldy kernels and insect infestation) were taken into account. This in part may explain why the occurrene and severity scores were low. Symptomless infections have been reported to exist throughout the plant (Munkvold and Desjardins, 1997). A complication with symptomless infection is that most screening relies on visual ratings and thus infection cannot be completely detected. To overcome the bias of visual appraisals to measure degree of kernel damage, assessment of the fungal mass and propagules within a given amount of grain tissue may provide accurate information on the amount of the fungi within the grain at assessment time which considers all fungal growth events that had taken place. Fungal biomass in maize grain can also be estimated by measuring the concentration of ergosterol, a sterol produced by fungi but not by plants. In this study, it was observed that kernel damage was correlated to CFU count and fumonisin concentration (Figure 3 and 4). This implies increase in kernel damage is likely to increase fungal and fumonisin contamination.

Contamination of maize with *Fusarium*

Fungi in the genus *Fusarium* are common contaminants of maize and other cereals. *Fusarium* species can survive well on maize crop residues, which remain after the harvest. Crop residues as well as non-harvested plants can be a source of inoculum for infection of soil, seed, root, stalk or silk of plants. Fungal structures, like mycelium, chlamydospores (*F. graminearum*) and thickened hyphae (*F. verticillioides*), can survive unfavourable environmental conditions for a longer period (Munkvold, 2003), and allow the proliferation of the fungus and thus is the reason they are continued to be isolated after the crop has been removed from the field. *F. verticillioides, F. oxysporium* and *F. proliferatum* were the three dominant *Fusarium* contaminants isolated from the maize samples during the survey basing on the mean CFU counts (Table 2). The other isolated species were *F. anthophilium*, *F. lateritium* and *F. solani. F. verticillioides* and *F. proliferatum* co-occur worldwide on maize (Leslie *et al.,* 1995) probably they have similar optimum growing conditions and they do not apparent antagonism when growing together (Logrieco and Moretti, 1995). Of the six isolated species in this study, three belong to the section *Liseola,* whose members have teleomorphs in the *Gibberella fujikuroi* species complex. This complex has been divided into at least eight biological species, also termed mating populations, and identified by the letters "A" through "H" (Leslie, 1995; Leslie *et al*., 1996 and Klittich *et al*., 1997). Two of these mating populations, A and F, until recently shared the *Fusarium moniliforme* (= *F. verticillioides*) anamorph. These group also isolated here (comprising 62.5 % of the total isolates) are known to be the most commonly associated with maize diseases, and other field plants have been reported to be infected with up to five different strains of one species of *F. verticillioides* (Kedera *et al*., 1994). The high frequency of isolation in this study compared to other species may support previous work suggesting that there may be differences in ability of *Fusarium* species to be pathogenic to their preferred hosts (Jardine and Leslie, 1999), even though most strains of all species in this section and other sections are regarded as potential pathogens of maize.

Fumonisin analysis

Fumonisin are produced by various *Fusarium* species and have been documented to have human adverse health implications by various authors in past research. Fifteen *Fusarium* species have been reported to produce fumonisins. Eight of these are in the Section *Liseola* (Desjardins *et al*., 1994 and Castella *et al*., 1999). The remaining two fumonisin-producing *Fusarium* species are one species in Section *Elegans*, i.e., *F. oxysporium* Schlecht. emend. Snyd. Et Hans (Seo *et al*., 1996 and Seo *et al*., 1999) and one in Section *Arthrosporiella*, i.e., *F. polyphialidicum* (Abbas and Ocamb, 1995). Among the isolated species, all the species produce fumonisin with the exception of *F. lateritium* and *F. solani* which are known not to produce any fumonisin (Jimenez *et al*., 1997). A high fumonisin concentration was observed in samples where *F. verticillioides* and *F. proliferatum* were isolated (Table 3). Several surveys carried out in many parts of the world have revealed that these two species are the fumonisin producing species most frequently isolated in maize in tropical and subtropical zones (Shephard *et al.,* 2000).

Although the detection of the toxigenic fungi and in particular the species in the Liseola section, known to be highly toxigenic, it does not necessarily indicate that toxins naturally occur in the field,. Some strains of these fungi are not toxigenic, therefore it only serves to alert to the potential risk of mycotoxin contamination. However, the results of this survey indicate that the maize samples and local populations in the surveyed areas in Eastern province are exposed to unacceptable levels of fumonisin contamination (Table 3). From the results, high fumonisin contents (> 1 mg / kg) in both Makueni and Kitui districts were seen in samples with isolated *F. proliferatum* and *F. verticillioides* (Table 3). All the other samples had lower contents of fumonisin (< 1 mg / kg). Fumonisin contents were correlated to fumonisin colony counts and percent damaged kernels (Figures 2 and 3). However, the severity of infection shown by *Fusarium* CFU count was poorly correlated to fumonisin content (Figure 2). This suggests visual evaluation of samples could not be used to determine the risk of fumonisin contamination. This is in agreement with the work of Ono *et al.,* (2006). However, Schaafsma *et al.* (1993) proposed the severity of ear rot to be a useful indicator of ear contamination by mycotoxins.

Fumonisin are known to be heat stable (Dupuy *et al*., 1993 and Howard *et al*., 1998), light stable (IARC, 1993), poorly absorbed, poorly metabolized and rapidly excreted by animals, most fumonisin will eventually end up being recycled into the environment in a manner that will concentrate its spatial distribution. The amount that enters the environment may be quite large, than that estimated. Their continued accumulation may pose more danger to human who continually use products from the environment in addition to the plant and animal products.

Mycotoxins particularly aflatoxins and fumonisins are known to have adverse health implications on humans. Based on these results, it is necessary that future research should determine which analogs occur on which *Fusarium* species and at what levels. The legislation in Europe, which became effective as October 2007, has set a limit for fumonisin B1 +B2 of 1mg/kg in maize intended for direct human consumption (EC, 2007), and several of the samples analyzed in this survey exceed this limit (Table 2). In addition, the daily intake per capita in rural areas of the country (400 g average daily intake per person) is higher than the European consumption (10 g per person) (Alakonya *et al*., 2008).

Because of the immunosuppressive, carcinogenic, and nutritional deficiency effects of some of these mycotoxins, it is possible that it is a significant driver of cancer and other infectious diseases including AIDS in sub-Sahara Africa. Research should particularly target the effects of mycotoxin exposure and the severity of some of the infectious conditions in our population. Contamination of other cereal crops such as sorghum and millet should be investigated. It is essential that health related hazards associated with the consumption of moldy grain by animals and humans be emphasized. Public health awareness, monitoring and education on good storage and agricultural is paramount to prevent the entry of these toxic substances into the food chain.

Conclusion

The present study only showed to reiterate the diversity of *Fusarium* populations in maize grains, as evidently isolated from the study area and could be potential inoculum to infect other agriculture crops. The ecological significance and genetic diversity of each *Fusarium* species need to be further investigated as the study will give more information on the distribution and population structure and toxigenic potential of each species.

Acknowledgements

This work was supported by resources at the Centre for Microbiology Research - Kenya Medical Research Institute, and the authors, convey their utmost sincere appreciation.

References

1. Abbas, H. K. and Ocamb, C. M. (1995). First report of production of fumonisin B1 by *Fusarium polyphialidicum* collected from seeds of *pinus strobus. Plant Dis*. **79**: 642.
2. Alakonya, A. E., Monda, E. O. and Ajanga, S. (2008). Effect of delayed harvesting on maize ear rot in western kenya. *American-Eurasian J. Agric. and Environ. Sci*., **4 (3)**: 372-380.
3. Baird, R., Abbas, K. H., Windham, G., Williams, P., Baird, S., Peter, M., Kelley, R., Hawkins, L., and Scruggs, M. (2008). Identification of select fumonisin forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production *in vitro. Int. J. Mol. Sci* **9**: 554-570.
4. Castella, G., Bragulat, M. R. and Cabañes. F. J. (1999). Fumonisin production by *Fusarium* species isolated from cereals and feeds in Spain. *J. Food Prot* **62**:811–813.
5. Chu, F. S. and Li, G. Y. (1994). Simultaneous occurrence of fumonisin B1 and other mycotoxins in mouldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol* **60**: 847-852.
6. Desjardins, A. E., Plattner, R. D. and Nelson. P. E. (1994). Fumonisin production and other traits of *Fusarium moniliforme* strains from maize in

northeast Mexico. *Appl. Environ. Microbiol* 60: 1695–1697.
7. Dupuy, J., Le Bars, P., Boudra, H. and Le Bars, J. (1993) Thermostability of fumonisin B1, a mycotoxin from *Fusarium moniliforme* , in corn. *Appl Environ Microbiol* **59**: 2864-2867.
8. European Commission (EC). (2007). Commission Regulation (EC) No 1126/2007 of 28th September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Official Journal of the European Union.* L**255**: 14-17.
9. Fandohan, P., Hell, K., Marasas, W.F.O. and Wingfield, M. J. (2003). Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. *African Journal of Biotechnology* **2 (12):** 570-579.
10. Government of Kenya. (1998). (Various issues). Statistical Abstracts. Nairobi: Central Bureau of Statistics.
11. Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E. and Cole, J. R. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme. J. Vet. Diagn. Invest* 2: 217-221.
12. Howard, P. C., Churchwell, M. I., Couch, L. H., Marques, M. M. and Doerge, D. R. (1998). Formation of *N*-(carboxymethyl)fumonisin B1, following the reaction of fumonisin B1 with reducing sugars. *J Agric Food Chem* **46**: 3546-3557.
13. International Agency for Research on Cancer (IARC). (1993). Toxins derived from *Fusarium moniliforme*: Fumonisins B1 and B2 and fusarin C. In: Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. *Lyon, International Agency for Research on Cancer* **56**: 445-466.
14. Jardine, D. J. and Leslie, J. F. (1999). Aggressiveness to mature maize plants of *Fusarium* strains differing in ability to produce fumonisin. *Plant Dis* **83**: 690-693.
15. Jimenez, M. T., Huerta, T. and Mateo, R. (1997). Mycotoxin production by *Fusarium* species isolated from bananas. *Appl. Environ. Microbiol* **63**: 364–369.
16. Kedera, C. J., Leslie, J. F. and Claflin, L. E. (1994). Genetic diversity of *Fusarium* section *Liseola* (*Gibberella fujikuroi*) in individual maize stalks. *Phytopathology* 84: 603-607.
17. Kellerman, T. S., Marasas, W. F. 0., Thiel, P. G., Gelderblom, W. C. A. and Coetzer, J. A. W. (1990). Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. *Onderstepoort J. Vet. Res* **57**: 269-275.
18. Klittich, C. J. R., Leslie, J. F., Nelson, P. E. and Marasas, W. F. O. (1997). *Fusarium thapsinum* (*Gibberella thapsina*): A new species in section *Liseola* from sorghum. *Mycologia* **89**: 643-652.
19. Leslie, J. F. (1995). *Gibberella fujikuroi*: Available populations and variable traits. *Can. J. Bot* **73**: S282-S291.
20. Leslie, J. F., Marasas, W. F. O., Shephard, G. S., Sydenham, E. W., Stockenström, S. and Thiel, P. G. (1996). Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and F mating populations of *Gibberella fujikuroi* (*Fusarium moniliforme*). *Appl. Environ. Microbiol* **62**: 1182-1187.
21. Leslie, J. F., Pearson, C. A. F., Nelson, P. E. and Toussoun, P. A. (1995). Fusarium sp. from corn, sorghum and soybean fields in the central and eastern United States. *Phytopathology* 80: 343 – 350.
22. Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Misore, A., DeCock, K., Rubin, C. and the Kenya Aflatoxicosis Investigation Group. (2005). Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspectives* **113**: 12.
23. Logrieco, A. and Moretti, A. (1995) Occurence and toxigenicity of *F. proliferatum* from preharvest maize ear rot and associated mycotoxins in Italy. *Plant Dis* **79**: 727 – 731.
24. Marasas, W. (2001). Discovery and occurrence of the Fumonisins: A historical perspective. *Environ. Health Perspect* **109**: 239-243.
25. Medical Research Council (MERCK). (2006). Aflatoxin in peanut butter. Science in Africa.
26. Munkvold, G. P. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *Eur J Plant Pathol* **109**: 705-13.
27. Munkvold, G. P. and Desjardins, A. E. (1997). Fumonisins in maize, can we reduce their occurrence? *Plant Dis* **81**: 556-565.
28. Nelson, P. E., Desjardins, A. E. and Plattner, R. D. (1993). Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. *Annu. Rev. Phytopathol* **31**: 233-252.

29. Nelson, P. E., Toussoun, T. A. and. Marasas, W. F. (1983). *Fusarium* Species*:* An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
30. Ono, E. Y. S., Marcelo da Silva, L. B., Vizoni, E., Sugiura, Y., Ueno, Y. and Elisa, H. Y. (2006) Fumonisins in Corn: Correlation with *Fusarium* sp. Count, Damaged Kernels, Protein and Lipid Content. *Brazilian Archives of Biology and Technology* **49**: 63-71.
31. Pingali, P. L. and Pandey, S. (2001). Meeting world maize needs: Technology opportunities and priorities for the private sector. In: P. L. Pingali (ed.) *CIMMYT 1999–2000 world maize facts and trends. Meeting world maize needs: Technology opportunities and priorities for the private sector*. CIMMYT, Mexico City.
32. Pitt, J. I., Basílico, J. C., Abarca, M. L. and Lopez, C. (2000). Mycotoxins and toxigenic fungi. *Medical Mycology* **38**: 41-46.
33. Schaafsma, A. W., Miller, J. D., Savard, M. E. and Ewing, R. J. (1993). Ear rot development and mycotoxin production in corn in relation to inoculation method, corn hybrid, and species of *Fusarium*. *Canadian Journal of Plant Pathology* 15: 185-192.
34. Seo, J.-A., Kim, J.-C. and Lee, Y.-W. (1996). Isolation and characterization of two new type C fumonisins produced by *Fusarium oxysporum*. *J. Nat. Prod* **59**: 1003–1005.
35. Seo, J.-A., Kim, J.-C. and Lee, Y.-W. (1999). N-acetyl derivatives of type C fumonisins produced by *Fusarium oxysporum*. *J. Nat. Prod* **62**: 355–357.
36. Shephard, G. S., Marasas, W. F. O. And Leggott, N. L. (2000). Natural occurrence of fumonisins in corn from Iran. *J.Agric. Food Chem* **48**: 1860 – 1864.
37. Summerbell, R. (2003). An introduction to the taxonomy of zoopathogenic fungi. In: Pathogenic fungi in humans and animals (ed. D.H. Howard). Marcel Dekker, Inc., New York: 394-401.
38. Sydenham, E. W., Thiel, P. G., Marasas, W. F. O., Shephard, G. S., Van Schalkwyk, D. J. and Koch, K. R. (1990). Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. *J. Agri. Food. Chem* **38**: 1900 -1903.
39. Ueno, Y. (2000). Risk of multiple exposure to natural toxins. *Mycotoxins* **50**: 13 – 22.
40. Wang, J.S., Huang, T. And Liang, F. (2001).Hepatocellular carcinoma and aflatoxin exposure in Zhyqing Village, Fusui County, People's republic of China. *Cancer Epidemiol. Biomark* **10**: 143 – 146.