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Full Length Research Paper

Mycotoxigenic fungi, distribution and infestation of maize in selected sites- Kenya

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Mycotoxin producing moulds are of great significance to food safety and food security in the world as well as Kenya. Fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium* are of public health importance since they produce potent mycotoxins with adverse health effects to humans and animals. Therefore this study was conducted to determine the distribution and incidence of the mycotoxigenic moulds in maize. A Laboratory based cross-sectional study was carried out at the Mycology Laboratory in Kenya Medical Research Institute, Nairobi, Kenya. A total of 138 maize samples were obtained from farmers in Kitale, Machakos, Nairobi, Mombasa and Kisumu and subjected to mycological analysis. Laboratory analysis of maize samples involved culture on Sabourauds dextrose agar (SDA) and incubation at 30°C for 72 hours. Microscopic identification of fungal growth was done by morphological characteristics. Fungal incidence on maize from each region were scored in different categories and compared using ANOVA. Mycotoxigenic fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium* were isolated from maize samples obtained from the five regions. Members of the *Aspergillus spp* and *Fusarium spp* were 38% and 42% respectively in Kitale. While *Penicillium spp* was higher in Mombasa (29%) and Kisumu (24%). In Machakos, *Aspergillus spp* contamination was also higher (26%) compared to other regions while contamination by *Penicillium spp* was the lowest at (5%). Maize samples from Mombasa had the lowest infestation of *Fusarium spp* (4%). Generally 47% and 41% of samples from Kitale and Machakos were infested by different fungal species while those from Nairobi had a low infestation rate of 32%. Conclusion: The maize samples tested from the five regions were infested by distinct fungal genera. The varied climatic conditions in the five regions could favor the development of a certain fungal species and hence a specific mycotoxin would be of great significance.

Keywords: Maize, Mycotoxigenic fungi, Infestation and distribution

INTRODUCTION

Mycotoxin contamination in agricultural food products is a threat to food security and food safety in many countries in the world. Mycotoxins are secondary metabolites of fungi mostly found as food contaminants affecting a wide variety

of cereals (Reddy et al., 2010). Majority of mycotoxins are produced by species in the genera of *Aspergillus*, *Fusarium* and *Penicillium* are of most concern due to their effect on humans and animals. These fungi are found in major food crops pre and post-harvest. Mycotoxigenic fungi produce mycotoxins in food grains under favorable conditions. Mycotoxins may also be found in milk and meat

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products from animals that have consumed contaminated feed.

Controlled experiments and those conducted in the field environment have revealed that increased environmental temperature contributes to the infection of maize by *Aspergillus flavus* and as a result, high levels of aflatoxins are generated in agricultural products (Jones *et al.*, 1980; Payne *et al.*, 1985). High humidity increases the moisture content in the maize grains therefore encouraging the growth of toxigenic fungal species in the food product.

Mycotoxin contamination of food products is dependent upon climatic conditions. Certainly, the ability of fungi to produce mycotoxins is mainly influenced by temperature, relative humidity, insect attack, and stress conditions of the plants (Miraglia *et al.*, 2009). On the other hand, extreme rainfall and drought events would favour formation of DON and fumonisin, respectively (Miller, 2008).

Fusarium species are ubiquitous and are found mainly in the soils from where they infect the maize crop plants. They are usually considered as field fungi and they affect more than 50% of maize grains before harvest (Reddy *et al.*, 2009). Fumonisin, a group of mycotoxins mainly produced by *Fusarium verticillioides* and *F. proliferatum*, are the major contaminants in maize and maize based food products. Consumption of fumonisin contaminated food has been associated with an increased risk of esophageal cancer in humans in South America, Asia, Africa and the African American populations, South Carolina (Marasas *et al.*, 1996). Fumonisin, are also known to cause equine leukoencephalomalacia and porcine pulmonary oedema including a variety of adverse effects in numerous animal species. Animals affected by fumonisin toxicity may have reduced weight gain and productivity as well as immunological impairment (Wu and Munkvold 2008). In Sub Saharan Africa findings from epidemiologic studies have revealed an association between fumonisin exposure and increased susceptibility to HIV infection (Williams *et al.*, 2010). Exposure to mycotoxins and the symptoms depend on the type of mycotoxin, the concentration and length of exposure as well as age, health status, and gender of the exposed individual. Maize is a critical food product for many countries and in Kenya more 90% of the population is dependent on it. Therefore the aim of the study was to establish the distribution and infestation of maize by mycotoxigenic maize in five agro-ecological zones in Kenya.

MATERIALS AND METHODS

Study design and sample collection

This was a laboratory based cross-sectional study that involved the collection of 138 maize samples distributed among five study sites in Kenya. The sites were Machakos, Nairobi, Mombasa, Kitale and Kisumu. These regions

represent different agro-ecological zones where various maize varieties are grown. The samples were packaged and transported to Kenya Medical Research Institute, Centre for Microbiology Research, Mycology Laboratory where they were processed for mycological analysis.

Inoculation of maize samples

Clean maize kernels were picked from each sample, surface sterilized with 70% ethanol and plated on Sabourauds dextrose Agar. Four kernels were plated on each plate and incubated at 30° c and growth of fungi on the maize was assessed after 72 hours. Fungal infestation on the culture plates for each region was scored and compared. Where all the four maize kernels had visible growth of fungi, this was scored as 100% infestation and if three of the four kernels in that plate were infested, this was scored as 75% while two infested kernels was scored as 50% infestation. In a case where only one grain out of the four plated kernels was infested, the score was 25%.

Identification of fungal Isolates

Moulds growing on the plates were sub cultured onto SDA and identified according to their morphological characteristics (Larone *et al.*, 1995). Cultural characterization was based on the rate of growth, presence of aerial mycelium, colour of aerial mycelium as well as colour on the obverse and reverse of the plates. Microscopic identification was based on spore and conidiophore morphology.

Data Analysis

The isolation frequency (Fq) of each fungal genus from the five regions was calculated according to the formula by Gonzalez *et al.* 1999. This was used to determine the distribution of the mycotoxigenic fungi in the five regions.

$$Fq (\%) = \frac{\text{Number of isolates of a genus} \times 100}{\text{Total number of fungi or genus per region}}$$

Statistical Package for Social Sciences (SPSS) software version 21 was used to compare the infestation rates of maize among the five sites.

RESULTS

The pie charts show the distribution of *Aspergillus* spp, *Penicillium* spp, and *Fusarium* spp isolated from maize in the five regions.

Table 1 shows the general infestation of maize samples in different categories for each region. Maize samples from Kitale had the highest infestation at 100% (n=65) while Nairobi had the lowest number of samples in this category

Table 1. Infestation of maize from selected regions in different categories

Infestation on maize	Categories	Selected Regions				
		Kitale	Machakos	Mombasa	Kisumu	Nairobi
0	% within region	10% n=14	6% N=8	16% N=22	14% N=19	11% N=15
25	% within region	14% N=19	14% N=19	19% N=26	8% N=11	22% N=30
50	% within region	12% N=17	12% N=17	19% N=27	15% N=21	18% N=25
75	% within region	17% N=23	27% N=37	9% N=12	24% N=33	17% N=23
100	% within region	47% N=65	41% N=57	37% N=51	39% N=54	32% N=45
Total	% within region	100% n=138	100% N=138	100% N=138	100% N=138	100% n=138

The pie charts show the distribution of *Aspergillus flavus*, *Penicillium spp*, and *Fusarium spp* isolated from maize in the five regions

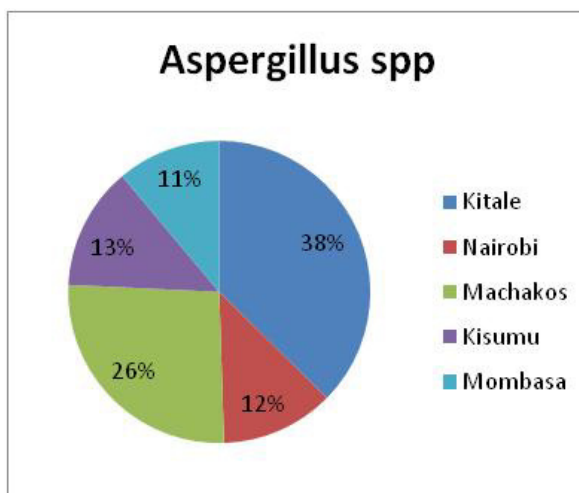


Figure 1. Distribution of *Aspergillus flavus* in maize from the five regions

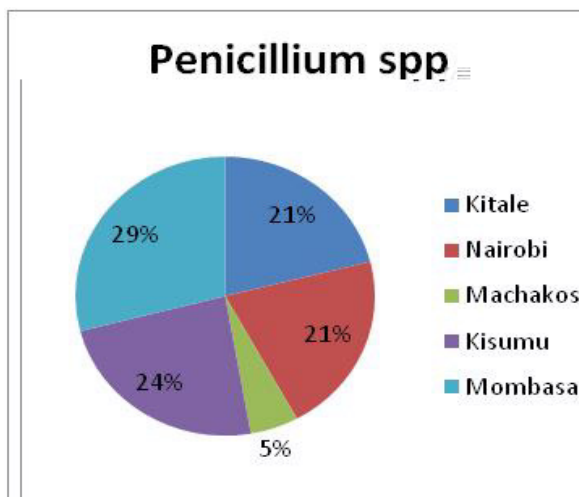


Figure 2. Distribution of *Penicillium spp* in maize from the five regions

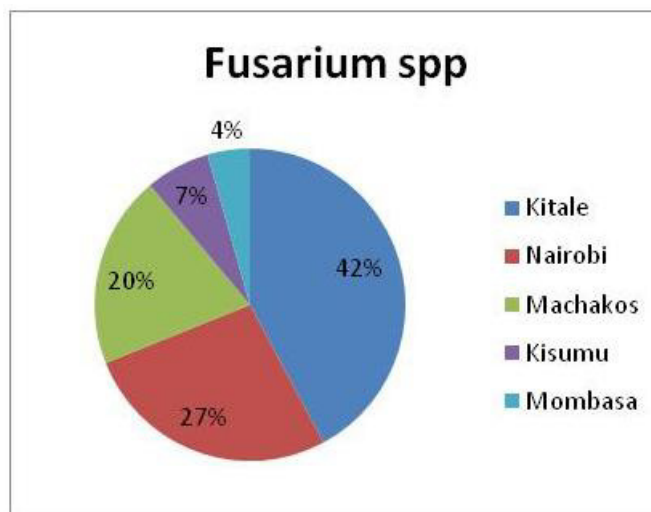


Figure 3. Distribution of infested maize samples in the five regions

(n=45). Machakos had the lowest number of samples that were not infested (n=8) while those from Kitale, Kisumu and Nairobi were (n=10, n=19 and n=15) respectively. Mombasa had the highest number in this category (n=22).

DISCUSSION

Contamination of maize by mycotoxin producing fungi is a significant health and economic problem in the world (Miller, 2008). Kenya has experienced serious aflatoxicosis outbreaks associated with maize which has claimed lives as well as maize yield losses. Findings from the five study sites revealed that, potentially mycotoxigenic fungal isolates were found on maize samples. The distribution of the fungi in the five regions was significantly different $p=0.54$. Maize samples from each region were more infested by a specific fungal genera. Maize grains from Kitale were heavily contaminated by *Aspergillus* spp (38%) **Figure 1** while those collected from Mombasa were frequently contaminated with *Penicillium* spp (29%) **Figure 2**. This possibly shows that the *Penicillium* spp is favoured more than the other fungal isolates in Mombasa due to its high frequency of isolation in this region. The agro-ecological conditions prevailing in each region could favor the development of a certain fungal genera as opposed to another. This concurs with other study findings where it has been reported that, high soil temperature and drought are associated with increased aflatoxin contamination and incidence of aflatoxigenic strains or species (Jaime *et al.*, 2010). Environmental conditions favorable to mycotoxin producing moulds differ, *A. flavus* is known to compete poorly under chilly conditions while their occurrence is higher in warmer environments (above 25°C) than cooler environments (20 -25 °C) (Shearer *et al.*, 1992).

The *Aspergillus* spp were found at high levels in maize samples from Machakos and Kitale at (29%) and (38%) respectively. In Machakos several incidences of aflatoxicoses have occurred (Ngindu *et al.*, 1982). The largest outbreak was reported in 2004 where residents consumed aflatoxin contaminated maize which resulted in hospitalization, death and loss of tons of maize yield (Holbrook *et al.*, 2004). Aflatoxin producing fungi usually thrive in warm arid, semi-arid, and tropical regions with changes in climate resulting in large fluctuations in the quantity of aflatoxin producers (Shearer *et al.*, 1992). Drought conditions are more likely to occur in Machakos, which stresses plants making them more susceptible to contamination by *Aspergillus* spp. (Robertson 2005; Alakonya *et al.*, 2008). In Kenya, Kitale is the leading producer of maize where it is grown on large scale (Alakonya *et al.*, 2008). From the current study, fungi belonging to the genera *Fusarium* were isolated in maize samples from Kitale and Nairobi at 42% and 27% respectively (Figure 3). From the two regions, the frequent isolation of the species shows the possibility of maize contamination by Fumonisin produced by *Fusarium verticilloides*. In Kitale, farmers are known to habitually leave their maize yields in the field upon maturity to allow drying (Alakonya *et al.*, 2008). In addition, the maize harvest coincides with second rains which increase rotting and infestation by moulds. This may be a reason for the frequent occurrence of *Fusarium* species in these maize samples. Findings from other studies on maize from western Kenya have isolated a wide variety of *Fusarium* spp and *Aspergillus* spp consistent with findings from this study. The mould species included *F. verticilloides*, *F. graminearum*, *F. subglutinans* as well as *A. flavus* and *A. parasiticus* known to produce varied toxins (Kedera *et al.*, 1999).



Plate 1: Maize infested by *A. flavus*



Plate 2: Maize infested by *A. niger*

Figure: 4 Culture plates showing the infestation on some of the maize samples analysed.

In this study maize samples from Mombasa and Kisumu had the lowest infestation by *Fusarium* moulds (4% and 7% respectively as shown in Figure 3) compared to Kitale and Machakos that had (42% and 20%) respectively. This could have been as a result of the different maize varieties grown in these regions as well varied climatic conditions that exist which may not be favorable to the *Fusarium* mould. From other study findings, the major factors that influence the risk of *Fusarium* infection and Fumonisin contamination are temperature, insect injury, and drought stress and water activity (Bush *et al.*, 2004). *Fusariums*, such as *F. graminearum*, is predominant in temperate environments, while *F. verticillioides* and *F. proliferatum* and fumonisins are more widely spread in tropical and subtropical environments (Miller, 2001). The most favorable temperature conditions for *F. graminearum* is between 24-28 °C and consequently above this temperature range *F. verticillioides* proliferates more than *F. graminearum* (Miller, 2001; Reid *et al.*, 1999). Rising temperatures within maize growing regions would change the geographical distribution and predominance of *F. verticillioides*, mostly in currently cooler regions where it will replace *F. graminearum*. A shift in *Fusarium* spp may cause a change in mycotoxins from deoxynivalenol and zearalenone (produced by *F. graminearum*) to fumonisin (produced by *F. verticillioides*). The occurrence of *F. verticillioides* and consequent fumonisin contamination due changing weather patterns has been reported in

Guatemala, Mexico, Zimbabwe and Kenya (Torres *et al.*, 2007).

CONCLUSION

In conclusion, it was important to determine the distribution and Incidence of fungi that exist in maize from different regions. Climatic factors have different effects on the different mycotoxin-producing fungi.

Therefore, climate change affects the existence of mycotoxigenic fungi in maize and this may affect future food security and health in Kenya. Maize samples from the five regions tested were infested by different mycotoxigenic fungi. Generally, maize from Kitale and Machakos were totally infested by different mould genera. The coexistence of moulds on the maize samples shows the possibility of occurrence of more than one mycotoxin in grains. The current study also highlights the importance of future work that will seek to determine shifts in pathogen populations in different regions.

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