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Isolation of Mycotoxigenic Moulds Contaminating Maize and Groundnuts in Selected Districts of Kenya

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ABSTRACT

Moulds damage foods, feeds and other agricultural commodities in the field and storage, leading to postharvest losses. In addition, moulds produce mycotoxins that cause illness or even death to consumers. This study evaluated maize and groundnuts from selected districts in Kenya for mycotoxigenic moulds. Both good and mouldy maize and groundnuts were obtained from randomly selected rural households and markets in Trans Nzoia, Kakamega Central and Kuria West districts in Kenya and analysed for moulds using the direct plating method. All the samples were positive for moulds and contained at least one genera of mycotoxigenic fungi; *Fusarium*, *Aspergillus* and *Penicillium*. The major moulds isolated in order of frequency were *Aspergillus* (91.3%), *Penicillium* (86.2%) and *Fusarium* (79.3%). Fifteen species of *Aspergillus*, eighteen of *Fusarium* and fifteen of *Penicillium* were isolated. Most of the samples (86.2%) contained more than two types of moulds. Some of the samples categorized as 'good' based on visual observation were highly contaminated by more species of moulds than samples categorized as mouldy. The highest occurring mould species were *A. flavus* (56.9%), *A. niger* (27.6%), *A. parasiticus* (25.9%), *A. ochraceus* (24.1%), *F. subglutinans* (29.3%), *F. oxysporum* (19%), *F. graminearum* (17.2%), *P. rubrum* (41.3%), *P. cyclopium* (34.5%) and *P. rugulosum* (22.4%). Seventy five percent of the moulds isolated in this study are known to be mycotoxigenic or produce other harmful compounds. The high occurrence of mycotoxigenic moulds in maize and groundnuts points to the great danger posed to human and animal health from consumption of contaminated grains and their associated mycotoxins.

Key words: *Aspergillus*, *Fusarium*, mycotoxigenic fungi, occurrence, *Penicillium*

INTRODUCTION

Moulds cause a high degree of deterioration in foods, feeds and other agricultural commodities and are responsible for considerable economic losses (De Souza *et al*., 2005). They destroy about 30% of crop yields and damage more than 30% of perishable crops in developing countries by lowering their quality and quantity. Furthermore, moulds produce mycotoxins which are potentially poisonous to consumers and can cause illness and death (Azziz-Baumgartner *et al*., 2005). The ubiquitous nature of moulds, their ability to colonize diverse substrates and lack of effective control measures has contributed to the high incidences of mould and mycotoxin

contamination in foods and feeds. Mycotoxins are toxic secondary metabolites produced in agricultural commodities by some fungi either in the field, during harvesting, transportation, storage or food processing (Bennett and Klich, 2003; Wagacha and Muthomi, 2008). According to the Food and Agricultural Organization (FAO) estimates, mycotoxins contaminate about 25% of the world's food crops (USDA, 2003) and are a source of morbidity and mortality (Smith *et al*., 1994). They contaminate foods and feeds throughout the world, thereby threatening food safety (Schmale and Munkvold, 2009). Mycotoxins cause diseases referred to as mycotoxicoses in humans and animals (Agrios, 1997). Most mycotoxicoses are caused by the common and widespread moulds, namely *Aspergillus, Penicillium* and *Fusarium*. They cause acute liver damage, induction of tumors, attack on the central nervous system, skin disorders and hormonal effects (Agrios, 1997; Oguz *et al*., 2003). High level exposure may cause instant death while long-term chronic effects include cancer, mutagenicity and nervous disorders (Kephis, 2006).

Aspergillus and *Penicillium* produce their toxins mostly in stored seeds and hay but also on commercially processed foods and feeds including meats, cheese, spices etc. *Fusarium* produces its toxins primarily on maize or other grains infected in the field or in storage (Agrios, 1997). Many other common moulds infect agricultural commodities or contaminate food produce with several mycotoxins. Some moulds also cause allergic reactions and respiratory problems. Production of mycotoxins is influenced by the surrounding environment while the effects of the toxins depend on the type, susceptibility and defense mechanism of the infected organism (Mazid *et al*., 2011). Mycotoxins get into the food chain either through direct consumption of infected crops and produce by humans or livestock. Due to their ability to resist decomposition or digestion, mycotoxins remain in the food chain in meat and dairy products. Furthermore, mycotoxins are not destroyed even by processes involving high or low temperatures such as cooking or freezing. Ochratoxins, can persist in meat of animals fed on contaminated feed and can be transmitted to humans through the food chain (Lanyasunya *et al*., 2005).

Consumption of mycotoxin contaminated grains kills hundreds of people every year in developing countries (Kung'u, 2005). The most important mycotoxins in terms of economic importance, incidence and toxicity in agricultural products are aflatoxins, deoxynivalenol, fumonisins, ochratoxins and zearalenones produced by *A. flavus, A. parasiticus, F. moniliforme, A. ochraceus* and *F. graminearum,* respectively (FAO, 1999). Acute aflatoxicosis poisoning occurs in Africa and Asia leading to death of several hundred people (Varga *et al*., 2009). In Africa where vulnerable crops such as groundnuts and maize are important staples, aflatoxin poisoning is a major health concern (Shephard, 2003). Kenya experienced dramatic outbreaks of aflatoxicosis in 1981, 2001, 2004, 2005, 2006, 2007 and 2008 resulting in sickness and loss of lives (Bennett and Klich, 2003; Kephis, 2006; MOA, 2008; Probst *et al*., 2007; Shephard, 2008). In 2004 an acute aflatoxicosis outbreak occurred in Machakos district resulting in 317 cases of acute hepatic failure and 125 deaths (Azziz-Baumgartner *et al*., 2005). In the neighbouring Uganda, cases of liver cancer have been linked to high levels of aflatoxins in the country's foods (Kaaya *et al*., 2006). Maize and groundnuts, which are some of the major staple foods in the region, are important sources of human exposure to mycotoxins because of their greater susceptibility to contamination by mycotoxigenic moulds and frequent consumption. The aim of this study was, therefore, to isolate and identify the mycotoxigenic moulds associated with maize and groundnuts from rural households and markets in selected districts of Kenya.

MATERIALS AND METHODS

Sample collection: A total of 58 samples visually categorized as good and mouldy maize and groundnuts were obtained from randomly selected rural households and markets in Trans Nzoia,

Kakamega Central and Kuria West districts in Kenya. The samples were collected in properly labelled khaki study bags to minimize further moulding and saprophytic fungal contamination. The samples were carried to the laboratory in a cool box and stored at -20°C to prevent further accumulation of moulds until analyses.

Isolation and Identification of moulds from maize and groundnuts: Moulds were isolated from maize and groundnut samples using the direct plating technique. Grains in each sample bag were thoroughly mixed, a handful of grains drawn out and five grains picked at random. The process was repeated four times until a composite of twenty grains was obtained. The grains were surface-sterilized in 2.5% sodium hypochlorite for 30 sec and rinsed in three changes of sterile distilled water. The grains were blotted with sterile filter paper and plated (five grains per plate, well spaced) on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czapek Dox Agar (CZ) media containing 133 mg streptomycin sulphate (for 1 litre of media). The plates were incubated at 25°C and monitored daily for fungal growth for 7-10 days. Single spore cultures of the moulds were prepared on PDA, MEA, CZ, Synthetic Nutrient Agar (SNA) and Czapek Yeast Agar (CYA) and species identification was based on cultural and morphological characteristics using taxonomic keys (Pitt, 1979; Nelson *et al.*, 1983; Kozakiewicz, 1989; Seifert, 1996; Klich, 2002). The number of grains per plate showing growth of a particular fungal species were recorded. For identification of *Fusarium* species, the isolates were grown on PDA for cultural characterization and Synthetic Nutrient Agar (SNA) for characterization of conidia and conidiogenous structures. Cultures were examined for growth rate, colony reverse colour, surface texture, colour and shape of aerial mycelium and the development of pigments in medium (Seifert, 1996). Microconidia and macroconidia were observed in 10 day old cultures based on colour of conidial masses, shape, septation and basal and apical cell of macroconidia, shape of microconidia, conidiophores in the aerial mycelium and presence or absence of clamydospores according to Seifert (1996). For identification of *Aspergillus* species, isolates were grown on Czapek Dox Agar (CZ) medium amended with 50 mg of streptomycin and 50 mg of penicillin and identified based on cultural and morphological characteristics according to the descriptions and keys by Klich (2002). Some of the characteristics used were colony diameter, colony colour on agar and reverse, colony texture and zonation. The major morphological features studied under the microscope were conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles. *Penicillium* species were cultured on Czapek Yeast Agar (CYA) and Malt Extract Agar (MEA) and species identification based on cultural and morphological characteristics as per descriptions by Pitt (1979).

Data collection and analysis: Total number of *Aspergillus*, *Fusarium* and *Penicillium* species isolated from each grain sample and the percentage occurrence of each genus and species was determined and compared. In addition, the total number of mould species isolated from each district and their percentage occurrence were also determined and compared.

RESULTS

Each of the 58 samples of maize and groundnuts analyzed contained moulds from at least one of the common genera; *Fusarium*, *Aspergillus* and *Penicillium* (Table 1). The major mould species isolated in order of frequency were *Aspergillus* (91.3%), *Penicillium* (86.2%), *Fusarium* (79.3%), *Rhizopus* (36.2%) and *Mucor* (33.3%). Eighty six percent of the samples contained more than two types of moulds, 10.2% contained two types and only 3.4% had one type of mould. Some of the

Table 1: Moulds isolated from maize and groundnuts collected from Trans Nzoia, Kakamega Central and Kuria West districts in Kenya

		No. of mould species isolated from each grain sample			
Sample No.	Type of grain	*Aspergillus*	*Fusarium*	*Penicillium*	Total No. of mould species isolated
Trans Nzoia					
KEKIM 1	Good maize	4	4	1	9
KEKIM 2	Mouldy maize	4	2	0	6
KEKIM 3	Good maize	4	3	2	9
KEKIM 4	Good maize	1	3	2	6
KEKIM 5	Mouldy maize	4	2	1	7
KEKIM 6	Mouldy maize	2	4	1	7
KEKIM 7	Mouldy maize	3	6	3	12
KEKIM 8	Good maize	1	4	3	8
KEKIM 9	Good maize	1	3	5	9
KEKIM 10	Mouldy maize	1	5	1	7
KEKIM 11	Good maize	1	0	3	4
KEKIM 12	Mouldy maize	1	2	1	4
KEKIM 13	Good maize	2	2	3	7
KEKIG 46	Good groundnuts	0	4	2	6
KEKIG 47	Good groundnuts	4	1	3	8
KEKIG 57	Good groundnuts	2	1	3	6
KEKIG 58	Good groundnuts	3	2	2	7
Kakamega Central					
KEKAG 14	Good groundnuts	4	0	3	7
KEKAG 15	Good groundnuts	1	0	2	3
KEKAG 16	Mouldy groundnuts	4	2	1	7
KEKAG 17	Good groundnuts	4	1	2	7
KEKAM 18	Good maize	1	3	1	5
KEKAM 19	Good maize	2	5	0	7
KEKAM 20	Good maize	2	0	3	5
KEKAM 21	Good maize	3	3	3	9
KEKAM 22	Good maize	0	1	0	1
KEKAM 23	Good maize	1	3	1	5
KEKAM 24	Good maize	1	0	1	2
KEKAM 25	Good maize	1	1	1	3
KEKAM 26	Good maize	0	1	1	2
KEKAM 27	Mouldy maize	1	0	0	1
KEKAG 48	Good groundnuts	6	1	2	9
KEKAG 49	Good groundnuts	4	3	4	11
KEKAG 50	Good groundnuts	3	3	4	10
KEKAG 51	Good groundnuts	4	4	4	12
KEKAG 52	Good groundnuts	3	2	1	6
KEKAG 53	Good groundnuts	4	3	2	9
KEKAG 54	Good groundnuts	4	2	3	9
KEKAG 55	Good groundnuts	3	2	2	7
KEKAG 56	Good groundnuts	5	2	2	9
Kuria West					
KEKUG 28	Good groundnuts	3	4	2	9
KEKUG 29	Mouldy groundnuts	1	1	0	2
KEKUG 30	Good groundnuts	4	2	2	8
KEKUM 31	Mouldy maize	2	1	1	4

Table 1: Continue

		No. of mould species isolated from each grain sample			
Sample No.	Type of grain	*Aspergillus*	*Fusarium*	*Penicillium*	Total No. of mould species isolated
KEKUM 32	Good maize	2	1	1	4
KEKUM 33	Good maize	3	2	2	7
KEKUM 34	Good maize	2	0	2	4
KEKUM 35	Good maize	1	1	2	4
KEKUM 36	Mouldy maize	2	0	1	3
KEKUM 37	Mouldy maize	1	1	0	2
KEKUM 38	Good maize	5	2	0	7
KEKUM 39	Mouldy maize	0	1	1	2
KEKUM 40	Good maize	1	0	2	3
KEKUM 41	Mouldy maize	3	0	1	4
KEKUM 42	Mouldy maize	0	0	2	2
KEKUM 43	Good maize	2	0	2	4
KEKUM 44	Mouldy maize	2	0	1	3
KEKUM 45	Good maize	3	1	0	4
Frequency (%)		91.3	79.3	86.2	

Table 2: Some of the *Aspergillus*, *Fusarium* and *Penicillium* species isolated from maize and groundnut samples collected from Trans Nzoia, Kakamega Central and Kuria West districts in Kenya

Mould	Occurrence (%)	Mould	Occurrence (%)	Mould	Occurrence (%)
F. solani	15.5	*A. flavus*	55.2	*P. citrinum*	20.7
F. graminearum	17.2	*A. parasiticus*	25.9	*P. rubrum*	41.3
F. oxysporum	19.0	*A. ochraceus*	20.7	*P. viridicatum*	10.3
F. avenaceum	15.5	*A. niger*	27.6	*P. rugulosum*	22.4
F. moniliforme	1.7	*A. fumigatus*	22.4	*P. cyclopium*	34.5
F. scirpi	19.0	*A. wentii*	13.8	*P. islandicum*	1.7
F. proliferatum	15.5	*A. flavipes*	8.6	*P. expansum*	15.5
F. tricinctum	8.6	*A. versicolor*	19.0	*P. soppi*	1.7
F. subglutinans	29.3	*A. ustus*	8.6	*P. claviforme*	5.2
F. poae	8.6	*A. nidulans*	8.6	*P. paxilli*	3.4
F. nivale	13.8	*A. humicola*	1.7	*P. ochraceum*	6.9
F. chlamydosporum	22.4	*A. tamarii*	7.0	*P. purpurogenum*	5.2
F. culmorum	15.5	*A. terreus*	5.2	*P. digitatum*	6.9
F. sporotrichoides	6.9	*A. sparsus*	3.4	*P. patulum*	3.4
F. lateritium	27.6	*A. ruber*	1.7	*P. wortmanni*	1.7
F. merismoides	8.6				
F. semitectum	3.5				
F. crookwellence	1.7				

samples categorized as 'good' based on visual observation were highly contaminated by more species of moulds than samples categorized as mouldy. For example, two samples of good groundnuts from Kakamega (KEKAG 49 and KEKAG 51) had as high as 11 and 12 different types of moulds, respectively (Table 1). Fifteen species of *Aspergillus*, 18 of *Fusarium* and 15 of *Penicillium* were isolated (Table 2). The average number of mould species isolated from maize were five whereas, that isolated from groundnuts were eight. The most frequently isolated *Aspergillus* species in all the three districts were *A. flavus*, *A. niger*, *A. parasiticus*, *A. fumigatus* and *A. ochraceus* with

Table 3: Occurrence and number of *Aspergillus*, *Fusarium* and *Penicillium* species isolated from Trans Nzoia, Kakamega Central and Kuria West districts

	Aspergillus species		*Fusarium* species		*Penicillium* species	
District	No. of species	Occurrence (%)	No. of species	Occurrence (%)	No. of species	Occurrence (%)
Trans Nzoia	10	94.1	15	94.1	12	94.1
Kakamega	13	91.3	14	78.3	12	86.9
Kuria	12	88.9	8	61.1	4	77.8

occurrence of 56.9, 27.6, 25.9, 22.4 and 22.4%, respectively. The less prevalent *Aspergillus* species were *A. ruber* (1.7%), *A. humicola* (1.7%) and *A. sparsus* (3.4%). The number of *Aspergillus* species isolated was higher in Kakamega district (13 species) than in Kuria (12 species) and Trans Nzoia (10 species) districts (Table 3). However, Trans Nzoia had the highest occurrence of the *Aspergillus* species at 94.1%. In Trans Nzoia district, *A. flavus* was the predominant species at a frequency of 64.7% followed by *A. parasiticus* at 41.2%. Similarly, in Kuria and Kakamega districts, *A. flavus* was the most frequently isolated at 52.2 and 55.6%, respectively.

The number of *Fusarium* species isolated was higher in Trans Nzoia district (15) than in Kakamega (14) and Kuria (8) districts (Table 3). Only four *Penicillium* species were isolated from samples from Kuria district whereas 12 species were isolated each from Kakamega and Trans Nzoia districts. Trans Nzoia also had the highest occurrence of *Fusarium* and *Penicillium* species at 94.1% followed by Kakamega, while Kuria had lower at 61.1 and 77.8%, respectively. The most frequently occurring *Fusarium* and *Penicillium* species were *F. subglutinans* (29.3%), *F. oxysporum* (19%) and *F. graminearum* (17.2%), *P. rubrum* (41.3%), *P. cyclopium* (34.5%) and *P. rugulosum* (22.4%). Some of the important mycotoxigenic moulds frequently isolated were *A. flavus* (55.2% frequency), *A. niger* (27.6%), *F. subglutinans* (29.3%), *F. graminearum* (17.2%), *P. citrinum* (20.7%) and *P. expansum* (15.5%) (Table 4).

DISCUSSION

All the maize and groundnut samples collected in the three districts were positive for moulds and were contaminated with at least one of the known genera of mycotoxigenic fungi, namely *Fusarium*, *Aspergillus* and *Penicillium*. The major mould species isolated in order of frequency were *Aspergillus*, *Penicillium*, *Fusarium, Rhizopus* and *Mucor*. These are among the most common moulds as reported by Ryan and Ray (2004). The isolated moulds may have contaminated the maize and groundnuts either in the field or during storage. Toxigenic fungi can be divided into three groups, field fungi namely, genus *Fusarium*, for example *F. moniliforme*, *F. roseus*, *F. trincinctum* and *F. nivale*, storage fungi which include the genus *Aspergillus* and *Penicillium*, for example *A. flavus* and *A. parasiticus* and advanced deterioration fungi which normally do not infect intact grains but easily attack damaged ones and require high moisture content. Examples of the third group are *A. clavatus, A. fumigatus, Chaetomium, Scopulariopsis, Rhizopus, Mucor* and *Absidia* (Hussaini *et al.*, 2009).

Both the good and mouldy grains were contaminated by high numbers of the different moulds. A total of 44 mould species were isolated from the samples; 18 species of *Fusarium,* 15 of *Aspergillus* and 15 of *Penicillium*. Some of the samples categorized as 'good' based on visual observation were highly contaminated by more species of moulds than samples categorized as

Table 4: Some of the mycotoxigenic *Aspergillus*, *Fusarium* and *Penicillium* species isolated from the maize and groundnut samples

Sample	*A. flavus*	*A. parasiticus*	*A. ochraceus*	*A. niger*	*F. graminearum*	*F. oxysporum*	*F. avenaceum*	*F. subglutinans*	*P. citrinum*	*P. rubrum*	*P. cyclopium*	*P. expansum*
KEKIM 1	+	+	-	-	-	+	-	+	-	+	-	-
KEKIM 2	+	+	-	+	-	-	-	+	-	-	-	-
KEKIM 3	+	+	+	+	-	-	-	+	-	-	-	-
KEKIM 4	+	-	-	-	-	-	-	+	-	-	-	-
KEKIM 5	+	-	+	+	-	-	-	+	-	+	-	-
KEKIM 6	+	-	-	-	-	-	-	-	-	+	-	-
KEKIM 7	-	+	+	-	-	+	-	+	-	+	+	-
KEKIM 8	+	-	-	-	-	+	-	+	-	+	+	-
KEKIM 9	-	+	-	-	+	-	-	+	+	+	+	+
KEKIM 10	-	+	-	-	+	+	-	-	-	-	-	+
KEKIM 11	-	-	-	-	-	-	-	-	-	-	+	+
KEKIM 12	-	-	-	-	-	-	+	+	-	-	-	+
KEKIM 13	+	-	-	-	-	-	+	-	+	+	+	-
KEKIG 46	-	-	-	-	-	-	+	-	-	-	-	-
KEKIG 47	+	-	-	-	-	-	-	-	-	-	-	-
KEKIG 57	+	-	-	-	-	-	-	-	-	-	-	-
KEKIG 58	+	+	-	-	-	-	+	-	-	-	-	-
KEKAG 14	+	-	-	-	-	-	-	-	+	+	+	+
KEKAG 15	+	-	-	-	-	-	-	-	+	-	+	-
KEKAG 16	+	+	+	-	-	+	-	-	-	-	+	-
KEKAG 17	+	+	-	-	-	-	-	-	-	+	-	-
KEKAM 18	-	-	-	-	-	+	-	-	-	-	+	-
KEKAM 19	+	-	-	-	-	+	-	+	-	-	-	-
KEKAM 20	+	-	-	+	-	-	-	-	-	-	+	-
KEKAM 21	+	-	-	+	+	-	-	+	+	-	+	-
KEKAM 22	-	-	-	-	-	-	-	-	-	-	-	-
KEKAM 23	-	-	-	-	+	+	-	-	-	-	-	-
KEKAM 24	-	+	-	-	-	-	-	-	-	+	-	-
KEKAM 25	-	+	-	-	+	-	-	-	-	-	-	-
KEKAM 26	-	-	-	-	-	-	-	+	-	+	-	-

Table 4: Continue

Sample	*A. flavus*	*A. parasiticus*	*A. ochraceus*	*A. niger*	*F. graminearum*	*F. oxysporum*	*F. avenaceum*	*F. subglutinans*	*P. citrinum*	*P. rubrum*	*P. cyclopium*	*P. expansum*
KEKAM 27	+	-	-	-	-	-	-	-	-	-	-	-
KEKAG 48	+	-	-	+	-	-	-	+	-	+	-	-
KEKAG 49	-	-	+	-	-	-	+	+	+	-	-	-
KEKAG 50	-	-	-	+	+	-	-	+	+	+	-	+
KEKAG 51	-	-	-	+	-	-	+	+	-	+	-	+
KEKAG 52	+	-	+	-	-	-	-	+	-	-	-	-
KEKAG 53	-	+	-	-	+	-	+	-	-	-	-	-
KEKAG 54	+	-	-	-	-	-	+	-	+	-	-	-
KEKAG 55	-	-	-	+	-	-	-	-	-	-	-	+
KEKAG 56	+	+	+	+	-	+	+	-	+	-	-	-
KEKUG 28	+	-	+	+	+	+	-	-	-	+	-	-
KEKUG 29	+	-	-	-	-	-	-	-	-	-	-	-
KEKUG 30	+	-	-	-	-	+	-	-	+	-	+	-
KEKUM 31	+	-	-	+	+	-	-	-	-	-	+	-
KEKUM 32	+	-	+	-	-	-	-	-	-	-	+	-
KEKUM 33	-	+	+	-	-	-	-	-	+	-	+	-
KEKUM 34	+	-	-	+	-	-	-	-	+	+	-	-
KEKUM 35	-	-	-	-	-	-	-	-	-	+	+	-
KEKUM 36	-	-	-	+	-	-	-	-	-	+	-	-
KEKUM 37	-	-	-	+	-	-	-	-	-	-	-	-
KEKUM 38	+	-	+	-	+	-	-	-	-	-	-	-
KEKUM 39	-	-	-	-	-	-	-	-	-	+	-	-
KEKUM 40	-	-	-	-	-	-	-	-	-	+	+	-
KEKUM 41	-	-	+	-	-	-	-	-	-	+	+	-
KEKUM 42	-	-	-	-	-	-	-	-	-	+	+	-
KEKUM 43	-	-	-	+	-	-	-	-	-	+	+	-
KEKUM 44	+	+	-	-	-	-	-	-	-	+	-	-
KEKUM 45	+	-	-	-	-	-	-	-	-	-	-	+
Occurrence (%)	55.2	25.9	20.7	27.6	17.2	19	15.5	29.3	20.7	41.3	34.5	15.5

+: Mould species present in the sample,-: Mould species not present in the sample

mouldy. Most of the samples analyzed (86.2%) contained more than two types of moulds and only 3.4% of the samples contained one type of mould. This high contamination levels by various moulds may be attributed to a number of factors, including the surrounding environments, moisture content and plant susceptibility to fungal infestation. In addition, poor preharvest and postharvest handling, physical damage of grains due to pests, improper drying methods and poor storage of the grains are possible contributing factors. The warm and humid conditions prevailing in the three districts predisposed the grains to moulds infection in the field and also in storage. Environmental factors, particularly high humidity and temperature are known to promote fungal growth in foods and feeds (Wagacha and Muthomi, 2008). In the current study, farmers and traders were found to be drying their grains on bare grounds and such traditional drying methods have been reported to be a major source of fungal contamination (Okello *et al*., 2010). Poor storage practices were evident in the sample collection sites where some households stored grains inside damp and poorly ventilated rooms. Therefore, a combination of these factors may have contributed to the high levels of moulds contamination. Unfavourable conditions during transportation and marketing are also possible contributing factors to fungal growth and mycotoxin production (Bhat and Vasanthi, 2003; Wagacha and Muthomi, 2008). In addition, mechanical damage during and after harvest may have offered entry to the fungal spores either in maize cob or grains and predisposed the maize to moulds attack as earlier reported by Fandohan *et al*. (2003). Insect damage which was evident in some of the maize samples that were observed to be infested by weevils may also have aggravated the moulding. Insects attack maize in the field as well as in storage and play an important role in infection of the grains by moulds, either by wounding the plants or acting as vectors.

Overall, contamination by genus *Aspergillus*, *Fusarium* and *Penicillium* in Trans Nzoia district was substantially higher than in samples collected from Kuria and Kakamega districts. The variation in occurrence of moulds in the three districts could be attributed to the differences in prevailing weather conditions, cropping history, storage, planting dates and crop varieties planted in the three districts. Variations in cropping history, tillage, planting dates, soil types or plant varieties cultivated can influence the level of mould and aflatoxin contamination in different fields (Munkvold *et al*., 2009). Trans Nzoia is the grain basket of Kenya and large quantities of maize are produced, whereby the greatest challenge is improper drying and storage of the product. This is as compared to Kakamega and Kuria districts where less maize is harvested that can be relatively better dried and stored. The observation that groundnuts contained more moulds than maize was unexpected because most of the groundnut samples collected from farmers were unshelled and this should protect them from moulding. It is reported that groundnut shells protect the kernels from mould infection and storing groundnuts in shells/pods is therefore, recommended (Okello *et al*., 2010). The shelled groundnut kernels deteriorate faster in storage because they easily pick-up moisture and are readily exposed to damage by moulds, insects and rodents.

Among the three main genera of moulds isolated in this study, *Fusarium* species were the most abundant and most of the species isolated are pathogenic to maize. This is in line with observations by Munkvold (2003) and Leslie and Summerell (2006) that *Fusarium* is the most common pathogen on maize. The most frequently occurring pathogenic species were *F. subglutinans* (29.3%), *F. oxysporum* (19%) and *F. graminearum* (17.2%). *Fusarium* species are destructive pathogens on cereal crops and other commodities and produce mycotoxins before, or immediately after harvest (Pitt, 2000). They are field fungi reported to infect more than 50% of the maize before harvest (Robledo-Robledo, 1991). Generally when *Fusarium* species invade maize in the field they cause diseases like seedling blights, kernel root, seed stalk and ear rots. For example, *F. subglutinans* and

F. moniliforme cause *Fusarium* ear rot and stalk rots. *Fusarium moniliforme* also causes many other diseases like kernel and root rot, seed rot and seedling blight (De Leon, 2004). *Fusarium graminearum* attack maize and cause stalk, cob and root rots. Most of the maize analyzed in this study may have been infected in the fields and moulding continued in storage.

A large number of *Aspergillus* and *Penicillium* species was also isolated from the samples and their high occurrence could be attributed to poor grain storage conditions. *Aspergillus* species are more commonly associated with cereals during drying and storage. *Aspergillus flavus* and *A. parasiticus*, which were among the most frequently isolated species have a particular affinity for cereals (Varga *et al*., 2011). They can be recognized by yellow green or grey green colour on maize kernels in the field and in storage. Pre harvest invasion is partly dependent on insect damage to cobs but the fungi can also invade down the silks of developing ears and they cause diseases like maize ear and kernel rot. Invasion is primarily due to inadequate drying and improper storage (Pitt, 2000). In the present study, there is a possibility that invasion started in the field and continued in storage, thus maize samples collected from markets and various rural households had already accumulated high levels of the moulds.

Seventy five percent of the mould species isolated in this study are known to be mycotoxigenic or produce secondary metabolites that are harmful to humans and animals. Seven of the *Aspergillus* species isolated (*A. flavus, A. parasiticus, A. ochraceus, A. niger, A. fumigatus, A. wentii, A. ruber*) produce important mycotoxins including aflatoxins, ochratoxins, fumonisins, gliotoxin, aspertoxins as well as other harmful secondary metabolites (Schmale and Munkvold, 2009). Thirteen of the *Fusarium* species isolated (*F. solani, F. graminearum, F. oxysporum, F. avenaceum, F. moniliforme, F. proliferatum, F. tricinctum, F. subglutinans, F. poae, F. nivale, F. chlamydosporum, F. culmorum, F. sporotrichioides*) are mycotoxigenic, producing important mycotoxins such as fumonisins, zearalenones, trichothecenes, deoxynivalenol, nivalenol etc., (Desjardins and Proctor, 2007). Among mycotoxigenic species of *Penicillium* isolated were *P. citrinum, P. rubrum, P. viridicatum, P. rugulosum, P. expansum* and *P. cyclopium* reported to produce harmful mycotoxins and secondary metabolites such as ochratoxins, rubratoxins, patulin, citrinin and cyclopiazonic acid (Frivsad, 1985). Stress conditions such as drought, very high temperatures, poor nutrition, overcrowding, competition from weeds, insect damage and plant diseases predispose maize to infection by mycotoxigenic fungi (Laura and Allen, 2009). Consumption of the mouldy grains, exposes the consumers to the mycotoxins and their associated health complications.

At the farm level, the real problem is that mycotoxin contaminated maize and groundnuts may appear just like the normal grains without any outward physical signs of fungal infection. Mycotoxins, especially aflatoxin are silent killers and there is, an urgent need to make Kenyan food grains safe from the deadly mycotoxins. Possible intervention strategies include good agricultural practices like early harvesting, proper drying, sanitation, proper storage and insect management, breeding for resistance, use of antimicrobial agents to protect the grains, surveillance and awareness creation. Policy makers should establish and enforce quality standards and regulations related to moulds and mycotoxin contamination across the region to minimize health hazards related to consumption of contaminated grains and their products. The results of this study showed that maize and groundnuts that appear good and clean to the eyes are heavily contaminated with various species of mycotoxigenic moulds. This poses a serious danger to human and animal healthy and calls for immediate interventions to avert mycotoxicoses outbreaks, especially considering that one of the districts (Trans Nzoia) where the samples were collected is the grain basket of Kenya.

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REFERENCES

Agrios, G.N., 1997. Plant Pathology. 4th Edn., Academic Press, New York, USA., Pages: 635.

Azziz-Baumgartner, E., K. Lindblade, K. Gieseker, H.S Rogers and S. Kieszak *et al.*, 2005. Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ. Health Perspect., 113: 1779-1783.

Bennett, J.W. and M. Klich, 2003. Mycotoxins. Clin. Microbiol. Rev., 16: 497-516.

Bhat, R.V. and S. Vasanthi, 2003. Food safety and food security and food trade-mycotxin food safety risk in developing countries. International Food Policy Research Institute.

De Leon, C., 2004. Maize Diseases: A Guide for Field Identification. 4th Edn., CIMMYT, Mexico, ISBN-13: 9789706481092, Pages: 119.

De Souza, E.L., E. de Oliveira Lima, K.R. de Luna Freire and C.P. de Sousa, 2005. Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Braz. Arch. Biol. Technol., 48: 245-250.

Desjardins, A.E. and R.H. Proctor, 2007. Molecular biology of Fusarium mycotoxins. Int. J. Food Microbiol., 119: 47-50.

FAO, 1999. Preventing Mycotoxin Contamination. Food, Nutrition and Agriculture. FAO Food and Nutrition Division, Rome.

Fandohan, P., K. Hell, W.F.O. Marasas and M.J. Wingfield, 2003. Infection of maize by *Fusarium* species and contamination with fumonisins in Africa. Afr. J. Biotechnol., 2: 570-579.

Frivsad, J.F., 1985. Creatine sucrose agar, a differential medium for mycotoxin producing terverticillate *Penicillium* species. Lett. Applied Microbiol., 1: 109-113.

Hussaini, A.M., A.G. Timothy, H.A. Olufunmilayo, A.S. Ezekiel and H.O. Godwin, 2009. Fungi and some mycotoxins found in mouldy Sorghum in Niger State, Nigeria. World J. Agric. Sci., 5: 5-17.

Kaaya, A.N., W. Kyamuhangire and S. Kyamanywa, 2006. Factors affecting aflatoxin contamination of harvested maize in the three agroecological zones of uganda. J. Applied Sci., 6: 2401-2407.

Kephis, 2006. Mycotoxins and food safety. Kenya Plant Health Inspectorate Service (KEPHIS) Headquarters, Nairobi, Kenya.

Klich, M., 2002. Identification of Common *Aspergillus* Species. ASM Press, Washington, DC., USA., pp: 116.

Kozakiewicz, Z., 1989. *Aspergillus* species on stored products. Mycol. Pap., 161: 1-188.

Kung'u, J., 2005. Mould and food spoilage. Mold and Bacteria Consulting Laboratories (MBL) Inc., Mississauga, Canada, July 8, 2005.

Lanyasunya, T.P., L.W. Wamae, H.H. Musa, O. Olowofeso and I.K. Lokwaleput, 2005. The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. Pak. J. Nutr., 4: 162-169.

Laura, E.S. and J.W. Allen, 2009. Aflatoxin in corn. University of Missouri-Delta Research Centre, USA.

Leslie, J.F. and B.A. Summerell, 2006. The Fusarium Laboratory Manual. Blackwell Publishing, State Avenue, Ames, Iowa, USA.

MOA, 2008. The role of post harvest in the control of aflatoxins in cereals and pulses. Ministry of Agriculture Headquarters, Nairobi, Kenya.

Mazid, M., T.A. Khan and F. Mohammad, 2011. Role of secondary metabolites in defense mechanisms of plants. Biol. Med., 3: 232-249.

Munkvold, G.P., 2003. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur. J. Plant Pathol., 109: 705-713.

Munkvold, G.P., C. Hurburgh and J. Meyer, 2009. File: Pest management 2-5. Iowa State University, USA.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. Fusarium Species. An Illustrated Manual for Identification. The Pennysylvania State University Press, USA., Pages: 193.

Oguz, H., H.H. Hadimli, V. Kurtoglu and O. Erganis, 2003. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Revue de Medicine Veterinaire, 7: 482-486.

Okello, D.K., A.N. Kaaya, J. Bisikwa, M. Were and H.K. Oloka, 2010. Management of Aflatoxins in Groundnuts: A manual for Farmers, Processors, Traders and Consumers in Uganda. National Agricultural Research Organisation, Entebbe, Uganda, ISBN: 978-9970-401-00-0, Pages: 38.

Pitt, J.I., 1979. The Genus *Penicillium* and its Teleomorphic States: *Eupenicillium* and *Talaromyces*. Academic Press, London, UK., ISBN-13: 9780125577502, Pages: 634.

Pitt, J.I., 2000. Toxigenic fungi and mycotoxins. Br. Med. Bull., 56: 184-192.

Probst, C., H. Njapau and P.J. Cotty, 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. Applied Environ. Microbiol., 73: 2762-2764.

Robledo-Robledo, E., 1991. Strategies for the prevention and control of fungi and mycotoxins in Central and South America. Proceedings of the International Conference on Fungi and Mycotoxins in Stored Products, April 23-26, 1991, Bangkok, Thailand, pp: 39-46.

Ryan, K.J. and C.G. Ray, 2004. Sherris Medical Microbiology. 4th Edn., McGraw Hill, New USA., ISBN: 0-8385-8529-9.

Schmale, D.G. and G.P. Munkvold, 2009. Mycotoxins in crops: A threat to human and domestic animal health. Plant Health Instructor, 3: 340-353.

Seifert, K.A., 1996. FusKey: Fusarium Interactive Key. Agriculture and Agri-Food Canada, Research Branch, Eastern Cereal and Oilseed Research Centre, Ottawa, Canada, ISBN: 0-662-24111-8, Pages: 65.

Shephard, G.S., 2003. Aflatoxin and food safety: Recent African perspectives. J. Toxicol., 22: 267-286.

Shephard, G.S., 2008. Risk assessment of aflatoxins in food in Africa. Food Addit. Contam., 25: 1246-1256.

Smith, J.E., G.L. Solomons, C.W. Lewis and J.G. Anderson, 1994. Mycotoxins in human health. European Commission, Brussels, Belgium.

USDA, 2003. Food safety and inspection service fact sheets. United States Department of Agriculture, USA.

Varga, J., J.C. Frisvad and R.A. Samson, 2009. A reappraisal of fungi producing aflatoxins. World Mycotoxin J., 2: 263-277.

Varga, J., J.C. Frisvad and R.A. Samson, 2011. Two new aflatoxin producing species and an overview of *Aspergillus* section Flavi. Stud. Mycol. J., 69: 57-80.

Wagacha, J.M. and J.W. Muthomi, 2008. Review on mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol., 124: 1-12.