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Moulds and aflatoxin contamination of maize and groundnuts in Mayuge and Kumi districts of Uganda

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Abstract

Mycotoxins are produced by specific types of moulds that grow on inadequately processed and stored grain cereals and legumes. While they are known to have serious effects on the health of both human beings and animals, their incidence in Uganda has not been adequately studied. Thus, the objectives of this study were to identify moulds infecting maize and groundnuts in Uganda, and relate the incidence at farm level to levels of aflatoxin contamination in these produce at harvest and under and storage conditions. *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* were the predominant moulds isolated and occurred in more significant quantities in samples stored for five to seven months than in newly harvested samples. In Kumi, 48% of groundnuts stored for up to seven months and 28% of those newly harvested tested positive for aflatoxin with mean levels of 2.96 and 1.83 ppb, respectively. In Mayuge, 50 % of the groundnuts and 40% of the maize stored up to five months were positive fo aflatoxins, with mean levels of 5.38 and 1.64 ppb, respectively. No aflatoxins were found contaminating the newly harvested maize in Mayuge. The aflatoxin levels observed were low compared to those earlier reported in samples from markets, probably because of the low levels of moisture content of the stored kernels and low levels of insect damage. The results of this study indicate that mycotoxigenic fungi and aflatoxin contamination of maize and groundnuts starts at farm level and contamination occurs in both pre and postharvest phases. Since potential mycotoxigenic fungi rather than *Aspergillus* were isolated in significant quantities in this study, the occurrence of mycotoxins other than aflatoxins should be studied.

Key words: Fungi, hygiene, moulds, mycotoxins

Introduction

Maize (*Zea mayis* L.) and groundnut (*Arachis hypogaea* L.) are major staple foods for the majority of people in Uganda (Rwabwoogo, 1997). They are both usually harvested and sun-dried traditionally, and thereafter stored or utilised in different forms.

According to Busolo-Bulafu (1990) groundnuts is the second most widely grown grain legume, after common beans (*Phaseolus vulgaris* L.). Total groundnut production in the country has been estimated at 140,000 tons grown on 175,000 ha, mainly in eastern parts of the country (Busolo-Bulafu, 1990).Although mostly consumed locally, the use of groundnut as a food and cash crop has increased substantially because of an increased awareness of protein shortage in Uganda. The crop is consumed in a roasted form as nuts or ground into a paste. Albeit, the majority of the people consume it as groundnut source which is prepared by boiling a mixture of water and pounded raw nuts.

Maize, is also grown throughout the country but production is most intense in mid-altitude (900 -

1500 metres above sea level) and moist areas representing 75% of the production, dry mid-altitude - (900 - 1500 metres above sea level representing 15% of the production area, and highlands (>1500 metres above sea level) representing 10% of the production area (Kyetere, 1996). The crop is usually consumed as flour either made into a paste commonly known as posho, or into a porridge which is a very good infant food. It is also a good source of animal feed (Sebunya and Yourtee, 1990).

During storage, grain crops may be attacked by storage fungi but this may also occur before havest (Magan and Mercy, 1988). According to Magan and Lacey (1988) storage fungi are present in low numbers pre-harvest, but may develop rapidly under storage when conditions are suitable. The predominant grain storage fungal genera are *Aspergillus, Fusarium* and *Penicillium.*The danger possed by such infection is the production of mycotoxins which is a potentially serious public health issue.

Aflatoxins, an improtant mycotoxin, is produced by *Aspergillus flavus* and a *parasiticus.* These fungi grow on inadequately processed and stored foods like grains and legumes (Munimbazi and Bullerman, 1996). Although mycotoxins pose a serious health hazard to both humans and animals, their incidence in Uganda has not been adequately studied. However, studies conducted during the 1960s (Lopez and Crawford, 1967) on groundnuts sold for human consumption in Uganda showed that the population was exposed to high levels of aflatoxins. Alpert *et. al.* (1971) found that hepatoma frequency in Uganda was associated with aflatoxin content of maize and finger millet. Sebunya and Youtee (1990) also reported that maize, groundnuts and poultry feeds had aflatoxins, with some samples containing levels up to 20 ppb.

These studies concentrated on aflatoxin incidence in stored produce in markets but no studies were conducted on farm-level stored products. Additionally, traditional cereal and groundnuts processing technologies used by farmers such as sun-drying on bare ground, threshing and shelling by manual beating and winnowing, and poor storage conditions creats varied moisture content in dried grains (Odogola, 1994). Furthermore, traditional storage structures do not protect foods against moisture pick-up and put the produce at the risk of mould growth which are the likely sources of aflatoxin production (Odogola, 1994). Therefore, the objectives of this study were to identify moulds infecting maize and groundnuts at farm level in Uganda, determine levels of aflatoxin contaminating in maize and groundnut produce and to establish the influence of grain storage conditions on aflatoxin production.

Materials and Methods

Sample collection

Samples were collected from Kumi and Mayuge districts where both crops are important food crops (Rwabwoogo, 1997). Kumi district lies at an average altitude of 1,036 and 1,127m above sea level, and has high rainfall (1300 mm/year) and high temperature (25-30°C). Mayuge district lies at an altitude of about 1,070 - 1,161m above sea level with annual rainfall ranging between 1,250 and 2200 mm. Temperatures are always almost uniformly high, over 21°C. Samples of unshelled groundnuts were randomly collected twice from farmers in Akalabai and Atuturo villages, Atuturi sub-county, Kumi District on 29 March, and August 31, 2000. One sample was collected per farmer. Twenty five samples that were collected on March 29, 2000 were from crops harvested in July/August 1999. They had therefore been stored for about seven months. This storage period was selected because, a period of two months is long enough for aflatoxin development in *Aspergillus flavus*-infected foods (Sauer, 1987). The 25 samples collected on 31 August 2000 had just becn harvested that day. Farmers in Kumi leave harvested groundnuts in the field for one to two days before plucking the pods from the haulms. Analysis of these nuts would give an idea whether mould infection and aflatoxin development in these nuts start in the field or during storage. *Igola1* (commonly known as India), *Serere Red* (commonly known as *Erudulo arengan*), *Etesot* and *Erudurudu akwangan* are the varieties of groundnuts grown

in Kumi county. In this study, sampling was done irrespective of groundnut variety, although majority of samples collected were of *Igola 1*, the popular variety in this area due to its good yield and drought tolerance.

Twenty samples of unshelled maize and ten samples of unshelled groundnuts were similarly collected from farmers in Bugodi and Musita villages, Baitambogwe sub-county, Mayuge fomerly part of (Iganga district) on May 8, 2000. These samples had been harvested and stored in unshelled form for about 5-6 months. Twenty freshly harvested maize samples that had been dried for 5-6 days were collected from farmers in the same area on September 1, 2000. Analyisis of these samples would give some idea of whether moulds infect and produce aflatoxins prior to storage. Due to the drought that was experienced by farmers in this area during the previous two seasons, fewer groundnut samples were obtained from Mayuge during the first sampling period than in Kumi. During the second sampling period no samples were obtained from Mayuge.

During each season, about 500 g of groundnut pods and five cobs were obtained from each farmer, put in polyethylene bags and transported to the Department of Food Science and Technology, Makerere University, where they were stored at -10°C prior to mould isolation and aflatoxin analysis.

Isolation and identification of moulds

Fifty kernels of maize and groundnuts from each sample were assayed by direct plating technique for internal mould infection (Hocking, 1991; Pitt and Hocking, 1997). Samples were surface sterilised for 1 - 3 minutes with 10% commercial bleach (Jik), washed three times with sterile distilled water and placed directly on the surface of different agar media under recommended conditions. *Aspergillus flavus/A. parisiticus* were identified by direct plating on *Aspergillus flavus* and *A. parasiticus* agar (AFPA) medium (Pitt *et al.*, 1983). The plates were incubated upright at 30°C for 42 - 72 hours and then examined for the characteristic orange reverse colouration of *A. flavus/A. parasiticus*. Species of other *Aspergillus*, *Penicillium*, *Fusarium* and other moulds were isolated on malt salt agar (Tuite, 1969), transferred on acidified PDA several times for purification, and were identified using the mannuals and keys recommended by Tuite (1982) and Pitt and Hocking (1997) .

Determination of moisture content

Each of the collected samples was divided into two portions and their moisture content determined by the standard air oven method (AOAC, 1999). The samples were dried at 100°C to constant weight and the range and mean moisture content were calculated on dry-weight basis.

Determination of insect damage

Maize and groundnut samples were shelled and insect damage on grains assessed using a qualitaive scale of 0 - 4, where 0 = no damage, 1 = low (0 - 10 seeds), 2 (10 - 20 seeds) = moderate, 3 (20 - 30 seeds) = high and 4 = very high (>30 seeds) damage.

Aflatoxin analysis

Each of the samples was divided into two replicate lots and aflatoxins were extracted using methanol-water solution (80:20 vol) and quantified (ppb) using AflaTest Fluorometer according to the manufacturer's instructions (VICAM L. P., 313 Pleasant Street, Watertown, MA 02472, USA). The range and mean aflatoxin content of the samples were computed.

Results and Discussion

Results in Table 1 indicate that a wide range of moulds infect groundnuts and maize during storage in Kumi and Mayuge districts . *Aspergillus, Fusarium, Penicillium* and *Rhizopus* species were the most prevalent fungal genera found contaminating stored kernels in both districts . *Aspergilus flavus, A. parasiticus, A. niger, P. italicum* and *Rhizopus* spp. were isolated from groundnuts and maize. Of these, *A. flavus* and A. *parasiticus* occurred most often, with mean incidence of 7.2% on groundnuts from Kumi, 8.2% on groundnut and 9.6% on maize kernels from Mayuge. No particular species of *Fusarium* was isolated from groundnuts and maize in both districts. For example, *F. graminearum* was isolated from groundnuts in Kumi (1.52%) but not from those in Iganga, and was not found in maize. *F. moniliforme* on the other hand was isolated from groundnuts (0.96%) and maize (2.4%) in Mayuge but not in Kumi.

Identification of a fungus, particularly if frequent in a sample, will probably indcate that the grain or feed sample is potentially toxic (Tuite, 1982). Over 200 diferent mycotoxins have been reported (Cole and Cox, 1981) but only those occurring naturally in foods are of significance in terms of food safety. The mycotoxins are produced mostly by *Aspergillus, Penicillium* and *Fusarium* (Bullerman, 1979) and includes aflatoxins produced by *A. flavus* and *A. parasiticus*, ochratoxins by *A. ochraceous*, fumonisins by *F. moniliforme*, zearalenones by *F. graminearum*, vomitoxins by *F. graminearum*, citrinin by *P. citrinum* and patulin by *P. patulum*. Therefore follwoing previous suggestion the incidence of moulds and levels of mycotoxins in foods and feeds should be frequently and routinely determined by Munimbazi and Bullerman (1996). In this study, two of the important mycotoxin producing fungi *A. ochraceous* and *P. patulum* were detected (Table 1).

Mould growth and spoilage of stored grain is reportedly determined predominantly by the moisture content (or more precisely the availability of water) and the range of contaminating fungi and how they interact with temperature and gas composition (Magan and Lacey, 1988). For both crops studied the recommended safe storage moisture content (wet basis) for maize is about 10 - 14% and for groundnuts 8 - 9% (Odogola, 1994). In this study, the mean percentage moisture content for maize and groundnut kernels from both districts show that the samples were properly dried (Table 2). Conversly, Ssebukyu (2000) reported the presence of 11, 13 and 5 species of *Aspergillus, Fusarium* and *Penicillium*, respectively in markets samples from Kampala, Mpigi, Mubende and Mukono and attributed this to the high grain moisture content. In this study, fewer species of each of the above fungus were found in samples stored for five to six months (Table 1). Although damage and insect infestation have been strongly implicated in promoting fungal attack (Dunkel, 1988), none of the groundnuts and maize samples collected in this study were infested with insects. A combination of adequate storage moisture content and absence of insect infestation of the kernels tested, may thus explain the low levels of mycotoxigenic fungal infestation.

Inspite of the low fungal infestation, 48% of the groundnuts samples from Kumi, 50% of the groundnuts from Mayuge and 40% of the maize samples from Mayuge tested positive for aflatoxin. These results are similart to those of Alpert *et al.* (1971) who reported that 44.9% of maize and 17.8% of groundnuts from Uganda tested positive for aflatoxin content, which range between 1 - 1000 and 1 - >1000 ppb, respectively. Sebunya and Yourtee (1990) indicated that 77% maize and 36% groundnuts from Uganda tested positive for aflatoxin, with 2 samples out of 25 showing up to 20 ppb. Studies by Ssebukyu (2000) revealed that 50% of maize samples from markets in Uganda had 0 - 10 ppb aflatoxin levels.

It is therefore apparent that aflatoxin contamination is prevalent in stored grain produce in Uganda, although the amounts vary. Aflatoxin content in positive samples of groundnuts and maize tested in this study are comparable to those reported before, although the mean aflatoxin levels (Table 2) suggest that the kernels are safe for human and animal consumption (20 ppb and 25 ppb, respectively) as recommended by United States Food and Drug Authority (FDA) and United States Agency for International Development (U.S.A.I.D), respectively (FAO, 1982) . Among the groundnuts samples

Table1. Percentage of mould-infected groundnut and maize kernels collected from farmers in Kumi and Mayuge districts after five to seven months of storage.

Location	No. of samples tested		Moulds	% Mouldy kernels	
	Maize	Groundnuts		Range	Mean[a]
Kumi	NA	25	*Aspergillus* species		
			A. flavus/parasiticus	0 - 40	7.2
			A.candidus	0 - 20	0.32
			A.niger	10 -30	6.64
			A.tamarii	0 - 10	0.24
			spergillus spp	0 - 30	0.72
			Fusarium species		
			F.graminearum	- 40	1.52
			Fusarium spp	0 - 10	0.16
			Penicillium species		
			P.digitatum	0 - 30	1.20
			P.italicum	0 - 20	1.44
			P.citrinum	0 - 20	0.96
			Penicillium spp	0 - 10	0.08
			Rhizopus sp.	0 - 20	0.12
			Other moulds	0 - 10	0.04
Iganga	NA	10	*Aspergillus* species		
			A.flavus/parasiticus	0 - 50	8.2
			A.niger	0 - 30	2.2
			A.tamarii	0 - 10	0.8
			Aspergillus spp	0 - 10	0.2
			Fusarium species		
			F.moniliforme	0 - 30	0.96
			F.graminearum	0 - 20	1.6
			Fusarium spp	0 - 10	0.4
			Penicillium species		
			P.italicum	0 - 20	0.5
			P.xpansum	0 - 10	0.8
			P.citrinum	0 - 30	0.75
			Rhizopus sp.	0 - 20	0.62
			Other moulds	0 - 10	0.04
	20	NA	*Aspergillus* species		
			A. flavus/parasiticus	0 - 30	9.6
			A.oryzae	0 - 20	0.7
			A.candidus	0 - 30	0.3
			A.wentii	0 - 30	0.6
			A.niger	10 - 40	5.5
			Aspergillus spp	0 - 10	0.1
			Fusarium species		
			F.moniliforme	10 - 20	2.4
			Fusarium spp	0 - 10	0.2
			Penicillium species		
			P.digitatum	0 - 30	1.1
			P.italicum	0 - 20	1.9
			P.expansum	0 - 10	1.1
			Penicillium spp	0 - 10	0.8
			Rhizopus sp.	0 - 40	2.1
			Other moulds	0 - 20	0.72

[a] Means are for 50 kernels per sample. NA = Not applicable

from Kumi, only one sample out of 25 had aflatoxin levels of 22 ppb, while for Mayuge, only one out of 10 had levels of 18 ppb. The highest aflatoxin level in maize was 5 ppb, and was observed in only 2 of the 20 samples.

For toxic production by fungi on grains and foodstuffs, environmental and storage conditions must be favourable. The optimal environmental conditions for the growth of *A. flavus* and aflatoxin production are; temperature of about 20 - 35°C, relative humidity of 85% and above, in equilibrium with a moisture content of 14 - 30% in grains (Abate and Gashe, 1985). Since the moisture content of the tested kernels was lower than 14%, this could explain the low levels of aflatoxins although the storage temperatures was within the range of 20 - 35°C.

Table 3 shows the levels of mould infection of the newly harvested maize and groundnut samples. *Aspergillus, Fusarium, Rhizopus, Cladosporium* and *Mucor* species were commonly found on these grains. Compared to results in Table 1, *Aspergillus, Penicillioides, Cladosporium herbarum* and *Mucor* species were isolated in newly harvested produce but not in samples stored for five to seven months.. Although a higher percentage of maize kernels were infected by *F. graminearum* and *F. moniliforme*.there were less *Aspergillus* and *Fusarium* species in the newly harvested samples than in those stored for 5-6 months. *Aspergillus flavus* was only isolated from groundnuts and not maize samples, although it is known that *A. flavus* can grow in maize in the field (Sauer, 1986). *Penicillium* was not isolated from any samples (Table 3). These results support the findings of Lacey (1971) and Flannigan (1978) who reported that at harvest, storage fungi may be present at low levels but their numbers increase during drying or when grain is placed in contaminated storage structures. The rare occurrence of *Penicillium* species has also been reported by other workers such as Malloch (1981) who reported that over 20 species of *Penicillium* occur in maize but only a few (5 - 8 species) were common storage fungi.

Aflatoxin contamination was found only in groundnut but not maize (Table 4). Twenty eight percent of the samples tested positive with mean aflatoxin levels of 1.83 ppb from previous studies reported aflatoxin content of groundnuts from one of the regions in Mozambique at harvest time was in the range of 0 - 1320 ppb with a mean of 750.8 ppb which is much higher than levels observed in Kumi groundnut samples (van Wyk *et al.*,1999).

Table 2. Moisture content, insect damage and aflatoxin contamination of groundnut and maize kernels collected from farmers in Kumi and Mayuge districts after five to seven months of storage

Location	No. of samples tested		Moisture content (%)		Insect damage	Aflatoxin levels (ppb)		Positive samples (%)
	Maize	Groundnuts	Range	Mean		Range	Mean	
Kumi	NA	25	7.05 - 8.09	7.72	0	0 - 22	2.96	48
Iganga	NA	10	7.52 - 9.58	8.89	0	0 - 18	5.38	50
	20	NA	8.69 - 12.31	9.58	1.05	0 - 5	1.64	40

NA Not applicable

Ranked from 0 - 4 (0 = No, 1 = Low, 2 = Moderate, 3 = High and 4 = Very high)

Table 3. Percentage of mould-infected groundnut and maize kernels collected from farmers in Kumi and Mayuge districts at harvest (groundnuts) and after two to five days of drying (maize).

Location	No. of samples tested		Moulds	% Mouldy kernels	
	Maize	Groundnuts		Range	Mean
Kumi	NA	25	*Aspergillus* species		
			A.flavus/parasiticus	0 - 60	2.2
			A.niger	0 - 60	16.6
			A.tamarii	0 - 20	0.16
			A.penicillioides	0 - 60	2.56
			Fusarium species		
			F.graminearum	0 - 60	5.18
			F.moniliforme	0 - 40	3.45
			Rhizopus sp	0 - 60	3.42
			Cladosporium herbarum	0 - 200	18
			Mucor sp	0 - 20	1.41
Iganga	20	NA	*Aspergillus* specie		
			A. niger	0 - 20	0.08
			Fusarium species		
			F.moniliforme	0 - 60	5.4
			Fusarium graminearum	0 - 100	12
			Rhizopus sp	0 - 40	2.1
			Cladosporium herbarum	0 - 10	0.14

[a] Means are for 50 kernels per sample.
NA = Not applicable

Table 4. Moisture content, insect damage and aflatoxin contamination of kernels collected from farmers in Kumi and Mayuge districts at harvest (groundnuts) and two to five days of drying (maize).

Location	No. of samples		Moisture Conten (%)		Insect damage	Aflatoxin levels (ppb)		Positive samples(%)
	Maize	Groundnuts	Range	Mean		Range	Mean	
Kumi	NA	25	24.23 - 41.32	35.41	0	0 - 5	1.83	28
Iganga	20	NA	14.74 - 28.70	18.15	0	NA	0	0

NA = Not applicable
Ranked from 0 - 4 (0 = No, 1 = Low, 2 = Moderate, 3 = High and 4 = Very high)

Conclusion

The results of this study indicate that mycotoxigenic fungi and aflatoxin contamination of maize and groundnuts start at farm level and contamination occurs in both pre and postharvest phases. However, mould incidence and aflatoxin contamination levels were low compared to those reported at market level for the same commodities. This indicates that farmers are at a low risk with regard to consumption of oxic foods compared to consumers who purchase produce from markets. It is recommended that produce should be properly dried and stored both at farm and market levels to control mould and aflatoxin contamination of produce. In order to improve the export potential and generate increased incomes from farm produce, farmers and traders should be educated on management of moulds and mycotoxin contamination. Since potential mycotoxigenic fungi rather than *Aspergillus* were isolated in significant quantities in this study, the occurrance of mycotoxins other than aflatoxins should be studied.

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