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COMMON TOXIGENIC *FUSARIUM* SPECIES IN MAIZE GRAIN IN ETHIOPIA

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ABSTRACT: Prevalence of toxigenic species of *Fusarium* in maize samples collected in Ethiopia was investigated. The three toxigenic species of *Fusarium* most often associated with Ethiopian maize grain were *Fusarium verticillioides* [= *F. moniliforme*] (51.7%), *Fusarium subglutinans* (24.2%) and *Fusarium graminearum* (13.9%). Other *Fusarium* species contributed 10.2% of the total species recovered. A large number of strains *of F. verticillioides*, *F. subglutinans* and *F. graminearum* are known to produce toxic secondary metabolites. The incidence of *Fusarium* species and the mycotoxins they produce have been positively correlated with numerous toxicoses of man and animals. Thus, the prevalence rate of these toxigenic *Fusarium* species in Ethiopian maize, destined for human consumption, suggests the possible contamination of maize and its products by *Fusarium* mycotoxins.

Key words/phrases: Ethiopia, *Fusarium*, maize, malt, mycotoxins

INTRODUCTION

Maize has been known to Ethiopia for the last 500 years. It grows in all parts of the country from sea level to over 2400 m above sea level and is a staple food crop in some parts of the country (Kebede Mulatu *et al.*, 1993). According to International Maize and Wheat Improvement Centre (cited in CIMMYT, 1992), per capita total maize consumption was 38 kg yr^{-1} in 1982 and the consumption

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rate is continually increasing. In Ethiopia maize is consumed directly as bread, porridge, *injera*, and *nifro*. Moreover, malted maize is used for the preparation of local drinks, *tela*, *borde* and *areke*. Almost all maize produced is directly used for human consumption (Kebede Mulatu *et al.*, 1993).

One of the problems of maize grain is its susceptibility to mould invasion. Fungal invasion brings about lower germination capability, grain discoloration, unpleasant taste, lower nutritional value and subsequent accumulation of mycotoxins hazardous to man and animals (White, 1999). Several species of *Fusarium* are known to infect maize grain in the field before harvest and during storage, and produce mycotoxins.The important *Fusarium* mycotoxins most frequently encountered in maize include: trichothecenes (deoxynivalenol and nivalenol), zearalenone (Patey and Gilbert, 1989), fumonisins (Desjardins and Plattner, 1998; Gelderblom *et al.*, 1988), moniliformin (Marasas *et al.*, 1986) and fusarin C (Farber and Scott, 1989).

About 24 toxigenic species of *Fusarium* comprising more than 200 toxigenic strains that produce one or more of these toxins were identified. These toxigenic *Fusarium* species include: *F. graminearum* Schwabe, *F. verticillioides* (Sacc.) Nirenberg [= *F. moniliforme* Sheldon], *F. equiseti* (Corda) Sacc. sensu Gordon, *F. poae* (Peck) Wolenw., *F. sporotrichioides* Sherb, *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas Comb. nov., and *F. proliferatum* (Matsushima) Nirenberg. Mixed infection of maize grain by more than one toxigenic *Fusarium* spp. and the co-occurrence of their mycotoxins is well documented (Blaney, 1992; Munkvold and Desjardins, 1997).

The mode of action of the most important *Fusarium* mycotoxins is well known. Trichothecenes inhibit protein synthesis in eukaryotic organisms and exhibit a wide range of toxicity to vertebrate animals. Zearalenone is known to cause oestrogenic syndrome in pigs and other animals (Cole and Cox, 1981). Fumonisins exhibit hepatotoxic and carcinogenic properties (Gelderblom *et al.*, 1996). Fumonisin B1 has been shown to cause equine Leucoencephalomalacia (ELEM) in horses and pulmonary edema syndrome in pigs (Miller, 1992).

Grain and foodstuffs contaminated by *Fusarium* species and their mycotoxins have been responsible for a number of large-scale human toxicoses. Alimentary toxic aleukia (ATA) in Russia and red mould poisoning in Japan are among the consequences of consumption of cereal grains contaminated by *Fusarium* mycotoxins (Smith and Moss, 1985). A large population of the Kashmir valley, India, was affected by deoxynivalenol (DON) toxicoses in 1987 caused due to the consumption of wheat and wheat products contaminated with DON (Ramakrishna *et al.*, 1989).

High prevalence of *F. verticillioides* and significantly higher concentration of fumonisins were detected from maize destined for human consumption in Southern Africa and China. Both the prevalence of the fungus and its mycotoxins were significantly higher from high-oesophageal cancer prevalent areas than from the low incidence areas (Rheeder *et al.*, 1992). Moreover, frequent occurrence of trichothecene mycotoxins, DON and 15-acetyl DON, in staple foods was found to be associated with high incidence of human oesophageal cancer (Blaney, 1992).

Knowledge of the fungi that contaminated maize grain is important in assessing the likelihood of mycotoxin contamination. This paper, therefore, reports on the prevalence of toxigenic species of *Fusarium* in maize samples collected in Ethiopia in view of a possible human health hazard.

MATERIALS AND METHODS

Sample collection

Shelled maize grain samples were collected from farmer stores and market places in Shashemene and Alemaya regions (altitudinal range of 1900–2000 m.a.s.l.) in 1995. In this study maize seeds and malted samples were investigated. Grain lots were rated as damaged and normal based on the following criteria:

Samples with greater than 50% kernel discoloration, greater than 40% of wrinkled seeds and greater than 30% of the kernels floating in water, and/or above 50% of kernels attacked by insects were designated as damaged (D). The damage is visually clear and is known locally as *yetela ihele* in some areas. Samples with less than 50% kernel discoloration, less than 40% wrinkled seeds and less than 30% of kernels floating in water and below 50% of kernels showing insect attack were considered to be normal samples (N). Malted maize (M), *bikil*, prepared for making local beer, *tela*, and other traditional brews were also collected from different markets. A total of 36 samples, 12 from each group, were collected and the samples were placed in bags of cotton cloth and kept in a refrigerator until used for mycoflora investigation.

Isolation of seed mycoflora

Aliquots of 50 g of each of the normal and damaged samples were surface sterilised in 0.1% mercuric chloride solution for 1 min. However, samples of malted maize were surface sterilised in 0.5% mercuric chloride for 5 min. In all cases, the samples were rinsed three times with sterile distilled water. A total number of 100 kernels were plated for each sample. Five kernels were placed on Petri dishes of Potato Dextrose Agar (PDA) containing dextrose, 20 g; agar, 20 g; potato extract obtained from 200 g of potatoes boiled in 1000 ml of water, filtered through cheese cloth and diluted with water to make 1000 ml. The same number of kernels were also placed on modified Czapek Dox agar, mCDA, (dextrose, 20 g; KH_2PO_4, 0.5 g; $NaNO_3$, 2 g; $MgSO_4$, 0.5 g; yeast extract, 1 g; 10 mg $FeSO_4$; agar, 20 g; distilled water, 1000 ml).

All plates were incubated in the dark at 25° C for 7 days and fungal colonies that developed from the kernels were counted. Each colony was transferred to plates of PDA for identification and on agar slants of the same medium for maintenance.

Identification of Fusarium *isolates*

The species of *Fusarium* were further characterised using the manual of Nelson *et al.* (1983). Spore morphology is an important characteristic for identification of species of *Fusarium*. To facilitate sporulation, pure cultures were also transferred to KCl medium (KCl, 4 g; agar, 20 g; distilled water, 1000 ml) according to Nelson *et al.* (1983). A modified medium S1 (KNO_3, 1 g; KH_2PO_4, 1 g; $MgSO_4$, 0.5 g; KCl, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; corn grit, 20 g; agar, 20 g; distilled water, 1000 ml) was also used. Cultures on PDA were incubated in the dark at 25° C while those on KCl and medium S1 were incubated at room temperature under diffused day light.

The isolates were determined to the genus level depending on the presence of macroconidia and/or microconidial chains. Macroconidia production was observed on water mount microscopic preparations from PDA and medium S1 cultures while microconidial chain formation was monitored under low power objective from cultures on KCl and S1 medium.

Isolates of *Fusarium* were identified to species level based on gross and microscopic morphological characters. Information used were: presence or absence of microconidial chains; size and shape of macroconidia and microconidia; colony colour (obverse and reverse plate agar) and growth rate of isolates on PDA plates after dark incubation at 25° C for 10 and 3 days, respectively. For chlamydospore formation, a small piece of PDA agar culture was placed on sterile distilled water and incubated for 15 days. Spore size was measured using an eyepiece micrometer fitted into the microscope (Francis, 1991). The identity of the common isolates of *Fusarium* species was confirmed by the German Collection of Microorganisms and Cell Culture (DSMZ), Braunschweig, Germany.

Statistical analysis

Data was analyzed using descriptive statistics (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Mycological screening of maize samples, normal (N), damaged (D) and malted (M), revealed *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. to be common contaminants of Ethiopian maize. Species of *Fusarium* were recovered from 80.5%, *Penicillium* from 72.2% and *Aspergillus* from 66.7% of the total samples examined (Fig. 1). Among these toxigenic genera, *Fusarium* was found to be the most common genus comprising 17.5% of the total fungi isolated from all three groups of samples.

Species of *Fusarium* were recovered from 91.7% of both damaged and normal maize samples. A lower prevalence (66.7%) of the fungi was obtained from malted grain samples. Mean percentage of kernel infection by *Fusarium* spp. was highest in damaged (18.9%) grain followed by malted (9.1%) and normal

(6.83%) samples showing that the fungus is associated with grain deterioration (Fig. 1 and Table 1). The most frequently isolated *Fusarium* species were identified and confirmed to be *Fusarium verticillioides* (Sacc.) Nirenberg, *Fusarium subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas comb. nov., and *Fusarium graminearum* Schwabe (Table 2).

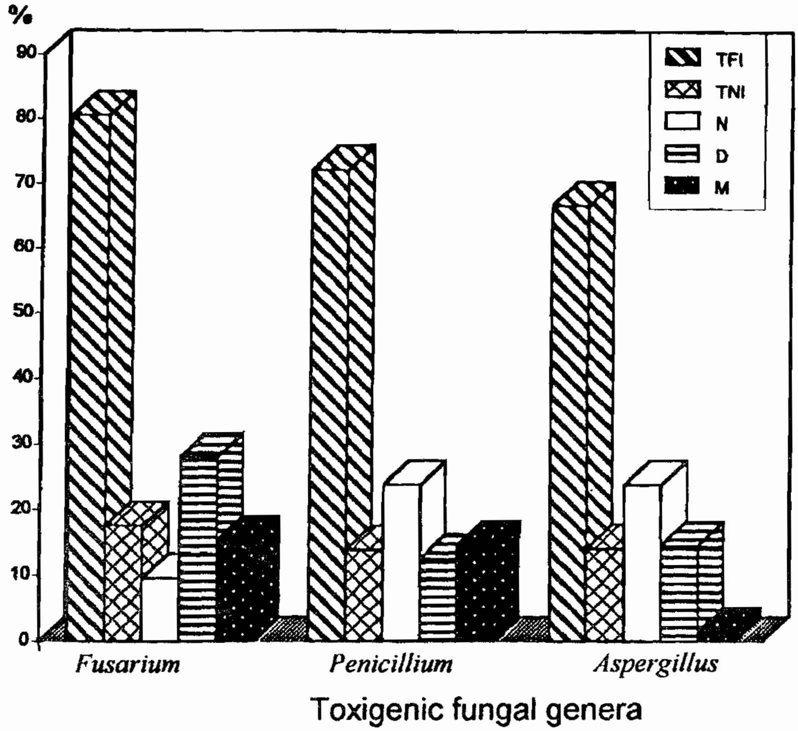

Fig. 1. Percentage values of, Total Frequency of Incidence (% TFI) [a] and Total Number of Incidence (% TNI) [b] of the three toxigenic fungal genera *Fusarium*, *Penicillium*, and *Aspergillus* in the three sets of samples and their percentage distribution in normal (N) damaged (D), and malted (M) samples.

[a] Percentage of total frequency of incidence (% TFI) of the three toxigenic fungal genera was calculated as the ratio of number of samples contaminated by species of the genera to the total number of samples investigated.

[b] Percentage of total number of incidence (% TNI) of each fungal genera was calculated as a ratio of the number of isolates of the genus to the total number of fungi isolated from the samples investigated.

Table 1. Incidence of *Fusarium* spp. and germination of kernels from normal (N), damaged (D), and malted (M) maize samples.

Isolates	Normal looking samples (N)		Damaged samples (D)		Malted samples (M)	
	Samples infected (%)	Kernels infected (mean %) [b]	Samples infected (%)	Kernels infected (mean %)	Samples infected (%)	Kernels infected (mean %)
Fusarium verticillioides	91.7	5.5	83.3	6.9	66.7	5.6
Fusarium subglutinans	25	1.08	75	4.7	50	2.6
Fusarium graminearum	--	--	50	4.8	--	--
Fusarium spp.	16.7	0.25	58.3	2.4	33.3	0.9
Total *Fusarium* [a]	91.7	6.83	91.7	18.9	66.7	9.1
Total isolates [c]	71.5		67.5		59.3	
Seed germination (%)	49.6		40.6			

(a), Mean of the total number of *Fusarium* colonies isolated from plated kernels.
(b), Values represent mean of 1200 surface sterilised maize kernels.
(c), Mean of the total isolates from plated kernels (some kernels were infected by more than one species of fungi).

Table 2. Common morphological and cultural characteristics of the common toxigenic *Fusarium* species.

Toxigenic	Colony			Macroconidia			Microconidia			Chlamy-dospore
species	Type	Colour	Diameter (cm)	No of septa	Size (cm)	Shape	Produc-tion	Size (cm)	Shape	produc-tion
Fusarium verticillioides	Aerial mycelium dense, floccose	Obverse white to light violet Reverse Creamish to reddish brown, creamish with green shades	3.8	3–6	26–40x2-3	Slightly Curved	Domi-nantly in chain and also in false heads.	0–1 septated 5-11x2-4	mostly obovoid with a truncate base	absent
Fusarium subglutinans	Aerial mycelium dense and flat	obverse White to dark violet Reverse Paint orange to light brown pigment dif-fusing into agar	3.7	3–5	26-66x2-5	Shorter thick, sligh-tly Curved to Straight with distinct foot-shaped basal cells	on false heads	0-2 septated 4-18x3	obovoid, oval to ellipsoi-dal in shape	absent
Fusarium graminearum	Aerial mycelium dense and floccose	Obverse Yellowish to orange at the centre surrounded by white, long & dense hyphae Reverse Light to dark rose-red or carmine-red pig-mented.	5.4	3–7	36-53x4-5	curved with distinctly foot -shaped basal cells.	absent	absent	–	absent

F. verticillioides was found to be the most common (80.5%) in all the sample types. The highest infection was observed in normal samples (91.7%) than other samples showing that the infection occurs mostly in the field. The second most common *Fusarium* species, *F. subglutinans*, is more associated with (28.5%) malted samples. This species were isolated from 75% of damaged, 50% of malted and 25% of normal samples. Mean percentage of kernel infection by this group of *Fusarium* species was 4.7% in damaged, 2.6% in malted and 1.06% in normal samples. *F. graminearum* (13.9%) is the third most common *Fusarium* in maize, though it was isolated only from mouldy samples. In damaged samples, it was the second prevalent, represented by 25.5% of the *Fusarium* isolates in 50% of the samples. The mean percentage of kernel infection of damaged samples by *F. graminearum* isolates was 4.8%. Other unidentified isolates comprised 10.2% of the total *Fusarium* isolates (Fig. 2 and Table 1).

The genus *Fusarium* has been commonly associated with maize ear rots as well as damaging stored grains world-wide (Munkvold and Desjardins, 1997; White, 1999). Our result is similar with a previous preliminary survey of Dawit Abate (1982), where *Fusarium* was reported to be the most common genus in maize grain. During the study it was observed that farmers select deteriorated grain during harvest and from store. Such low quality grain is available for sale and is used in the preparation of local alcoholic beverages. *F. verticillioides* and *F. graminearum* are known to cause maize ear rot in Ethiopia (Teklemariam Woldekidan, 1985) showing that the problem starts as a field infection. In addition to field infection, poor methods of traditional storage may also contribute to the high prevalence of the genus *Fusarium* in damaged samples. The traditional malting process seems to favour invasion of grain by the fungus due to increased grain moisture content and grain contact with the soil.

F. verticillioides, which belongs to *Gibberella fugikuroi* (Sawada) Wollenw. mating population A (Munkvold and Desjardins, 1997), is the most widely reported *Fusarium* species in maize kernels in most maize producing countries of the world (Abbas *et al.*, 1988; Blaney *et al.*, 1986; Desjardins and Plattner, 1998; Munkvold and Desjardins, 1997). It is among the most common fungi found colonizing symptomless maize grains (Munkvold and Desjardins, 1997) and the highest frequency of strains of this species in normal maize samples (91.7%) might be due to its endophytic nature.

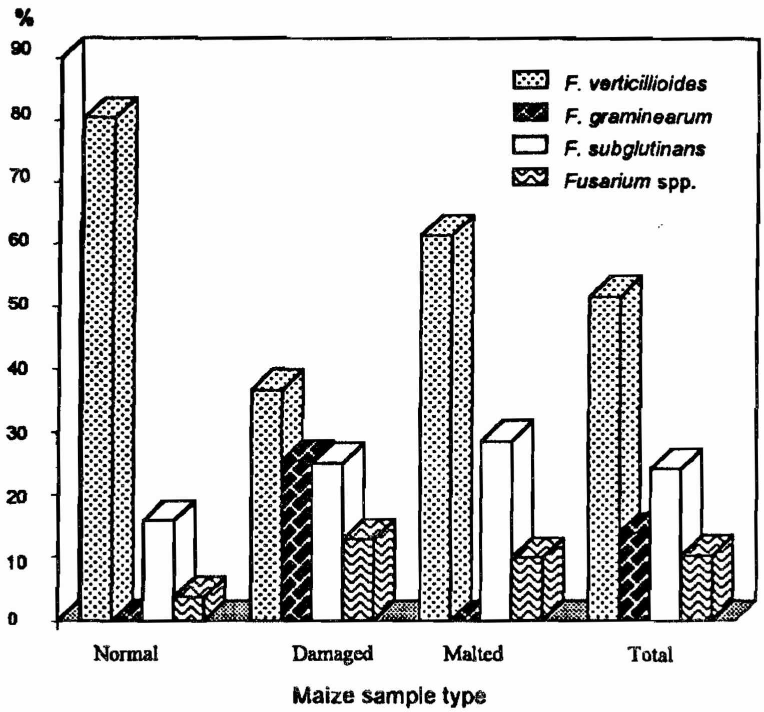

Fig. 2. Percentage distribution of *F. verticillioides*, *F. subglutinans*, *F. graminearum*, and *Fusarium spp.* in normal, damaged, malted and the three set of samples (total).

The highest prevalence of *F. verticillioides* and *F. graminearum* in damaged samples and the report that the two *Fusarium* species are responsible for maize ear rot in Ethiopia (Teklemariam Woldekidan, 1985), shows the persistence of the infection in the grain, though, there exists some variation in the occurrence of each species depending on the specific geographic area (Marasas *et al.*, 1979). In North Queensland, *F. verticillioides* and *F. graminearum* were frequently isolated from damaged maize kernels. Although *F. verticillioides* was the most frequent species, *F. graminearum* was most frequently isolated from kernels with a purple discoloration (Blaney *et al.*, 1986). In Minnesota, USA, *F. graminearum* (30%), *F. subglutinans* (23%) and *F. verticillioides* (20%) were shown to be the first three prevalent *Fusarium* species (Abbas *et al.*, 1988). In South African maize, *F. subglutinans* was reported to be more prevalent than *F. verticillioides* and *F. graminearum* (Marasas *et al.*, 1979).

A number of isolates of *F. verticillioides*, *F. subglutinans* and *F. graminearum* are known to produce a variety of mycotoxins. Zearalenone and trichothecenes (deoxynivalenol and nivalenol), are dominantly produced by *F. graminearum* (Blaney, 1992). Strains of *F. verticillioides* are capable of producing a group of mycotoxins, the fumonisins (Desjardins and Plattner, 1998; Gelderblom *et al.*, 1988; Munkvold and Desjardins, 1997; Nelson *et al.*, 1991). Moniliformin is mainly produced by *F. subglutinans* (Marasas *et al.*, 1979) and some strains of *F. verticillioides* (Burmeister *et al.*, 1979) while, fusarin C is produced by *F. verticillioides* and *F. graminearum* (Farber and Scott, 1989). Natural occurrence and co-occurrence of two or more of these mycotoxins in maize and maize based products have been reported (Hopmans and Murphy, 1993; Munkvold and Desjardins, 1997; Ramakrishna *et al.*, 1990). Moreover, production of *Fusarium* mycotoxins could occur during processing such as malting and most of them are very durable in food processing (Munkvold and Desjardins, 1997; Patey and Gilbert, 1989).

In this study it was learned that damaged grain in malted and unmalted form is consumed in different ways in Ethiopia. In some regions, mould damaged maize is particularly used for the preparation of local drinks such as *tela*, *areke* and *borde*. Such grain is also consumed during periods of grain shortage mixed in some proportion with normal maize grain. Malted maize, *bikil*, however, is solely used in local beverage preparation. The results of this study revealed that *Fusarium* spp., known to produce a variety of mycotoxins, *F. verticillioides*, *F. subglutinans* and *F. graminearum*, are common contaminants of maize in Ethiopia. The high degree of infestation of maize by these toxigenic *Fusarium* species suggests the possible contamination of maize grain and maize- based products by *Fusarium* mycotoxins. Thus products prepared from maize grain infested by toxigenic *Fusarium* species are potential risk to human health.

Researchers and health workers strongly believe that maize heavily contaminated by *Fusarium* should no longer be acceptable for human consumption or animal feed (Blaney, 1992; Hopmans and Murphy, 1993; Munkvold and Desjardins, 1997). Thus widespread infection of maize by toxigenic *Fusarium* species and the subsequent risk on human and animal health has increasingly become a world-wide concern. In Ethiopia, however, the level of awareness on health risk of mycotoxins from consumption of mouldy grain and food is very low.

In Ethiopia, preliminary work previously done on *Fusarium* mycotoxins by Eshetu Bekele (1993) and Tesfaye Wubet (1997) indicated production of zearalenone and trichothecene mycotoxins by Ethiopian isolates of *F. graminearum*. However, more data is required on the mycotoxin producing potential of *Fusarium* species. Moreover, a correlation study between disease symptom in a population with mycotoxin levels in foods has never been done. Such a study was undertaken in South Africa and China, where consumption of *F. verticillioides* contaminated maize and the level of fumonisins have been positively correlated with the oesophageal cancer rates (Blaney, 1992; Rheeder *et al.*, 1992; Munkvold and Desjardins, 1997).

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