# A PRELIMINARY STUDY OF THE FUNGAL FLORA OF ETHIOPIAN CEREAL GRAINS WITH SPECIAL EMPHASIS ON THE PREVALENCE OF TOXICOGENIC GROUPS

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C O N T E N T S

# List of Tables

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## ABSTRACT

Bacteria, moulds and yeasts on surface of cereal seeds ("teff", maize, barley and sorghum) were enumerated. Bacteria were in general found in greater numbers than moulds or yeasts per gram of grain. Teff harbours a higher number of bacteria (100,000 to 10,000,000 per gram) than the three cereal grains. Mould and yeast counts were not appreciably different - these fungi were found to occur in the range between 10,000 and 1,000,000 /gm of grain.

The mycoflora of the four cereal grains was studied, and in the survey 18 genera of fungi were identified. Aspergillus, Penicillium and Fusarium were found to be the most prevalent genera. Teff was found to harbour the least number of fungal genera. Fusarium, Trichoderma, Rhizopus and Mucor were not found from any ofthe teff samples examined. Chaetomium (35%), Penicillium (35%), Aspergillus (25%), Alternaria (15%) and Helminthosporium (15%) were the most common genera in teff. Aspergillus and Penicillium species were found in all grain types in a range of 25-60%. Fusarium was found to be most associated

with maize (62%) and sorghum (50%).

Changes in the fungal flora were found to vary with storage period. Fusarium (80%), Alternaria (53%) and Chaetomium (53%) were the most prevalent genera in maize and sorghum stored for less than 2 months. Aspergillus (100%), Penicillium (42%) and Rhizopus (42%) were found to be dominant from long-term (greater than 6 months) stored grains. On the other hand, Alternaria, Chaetomium and Helminthosporium were the genera absent from all long-term stored samples.

The range 10-14% was the moisture level of most grain samples examined. In general, teff and barley samples contained less moisture than maize and sorghum. The highest moisture content (16-17%) was determined from sorghum samples.

Aspergillus flavus was present in 24% of 110 grain samples examined. It was found to be more prevalent in maize (33%) and sorghum (33%). It was found to be more common in grain samples from Jimma (32%) and Dire Dawa (30%) areas than from Addis Ababa (15%) or Shashemene (15%) samples.

The aflatoxin producing ability of 20 isolates of A.

flavus was investigated. Sixteen isolates (80%) were capable of producing aflatoxin $B_1$ and $G_1$. It was also found that all cereal grains provide a suitable substratum for aflatoxin production in vitro.

Fusarium roseum was found to be most prevalent in maize (35%), rare in barley (5%) and sorghum (7%) while none was isolated from teff. Out of the five Fusarium roseum isolates examined only one showed toxicity by the rabbit skin toxicity test. From other Fusarium spp. 2 isolates showed toxicity out of the seven tested.

Ochratoxin was not detected in any of the Aspergillus and Penicillium isolates examined.

# I N T R O D U C T I O N

## 1.1 Seed deterioration by micro-organisms

Seeds harbour a variety of micro-organisms on their surfaces and in their internal parts. Fungal invasion begins in the field before harvest. This is particularly true of those kinds of seeds that are more or less exposed to contamination by airborne inoculum as are the seeds of cereal grains, with the exception of maize. Fungal invasion also occurs during storage after harvest.

It is very difficult to find seeds that are completely free of fungi (Christensen, 1957). The mycelium is commonly found in the pericarps of seeds and also under the hulls in cereals such as barley (Prado and Christensen 1952). They are rarely found in the embryo or endosperm of the caryopsis.

Christensen (1957) classified fungi of cereal grains into two groups: those which invade grains before harvest and those which invade after harvest. According to him, infection of seeds by storage fungi does not occur prior to harvest because the seeds are attached to the rachis and are surrounded by glumes. The inability

of storage fungi to compete with the field fungi is stated as the possible cause by Agrios (1978), but FAO (1977) states that the organisms cannot develop on a physiologically active substratum.

Fungi in grains utilize carbohydrates, lipids, unsaturated amino-acids and bitamins. They consequently decrease the nutritional value of grains. They cause discoloration of seeds and bring about undesirable odour and taste. The processing quality and the germination ability of seeds are reduced by fungal invasion (copeland, 1976).

Many of the micro-organisms that can be present on the surface of seeds cannot penetrate into the internal parts of the seed. This is due to the protective properties of the grain coating membrans under normal conditions.

Though bacteria may be present on the surface of seeds in huge numbers, they are not important in seed deterioration. However, valuable information on the way in which the grain has been harvested transported and stored can be obtained by enumerating surface

micro-organisms of grains (Lacey et al. 1980).

Although fungal damage of grains has long been recognized as one of the major causes of grain loss, the additional problem of mycotoxin contamination and the possible importance of toxins in the health of humans and animals is a relatively recent finding.

## 1.2 History of Mycotoxicosis

Mycotoxins are secondary fungal metabolites which cause pathological changes or physiological abnormalities in animals. Unlike primary metabolites which are common to most organisms, secondary metabolites are characteristic of particular micro-organisms (Ueno and Ueno, 1978).

Ergotism with its alarming effects of convulsions and gangrene of the extremeties was the first mycotoxicosis to be observed and studied. The toxic alkaloids of the fungus, Claviceps purpurea, had been identified by the 1940's (Austwick, 1975). Mycotoxicosis responsible for the death of thousands of horses in the Ukraine (USSR) was traced to a fungus in the

1930's (Forgacs and Carll, 1962).

From 1943 - 1947, there occurred a widespread outbreak of a fatal haemorhagic disease called Alimentary Toxic Aleukia among the populations of Orenburg district of the USSR. This was found to be due to consumption of bread made from mouldy grain that had overwintered under the snow. Fusarium Sporotrichioides was the etiological agent.

The cause of a disease of sheep in New Zealand known as Fical Eczema was found to be due to a toxin produced by Pithomyces chartrum (Thornton and Percival, 1959).

The 'Red Mould Disease' caused by Fusarium roseum and Fusarium graminearum was known after the disease incidence in 1949 and 1954 in Japan (Ueno, 1977).

The death in 1960 of 100,000 turkeys as a result of consumption of meal containing Brazilian groundnut in England (Sargeant, et al. 1961) may be considered as a milestone in mycotoxin research. The fungal origin of these toxic substances was suspected when toxic meals were found to contain large quantities of fungal hyphae. Isolation of the fungi and subculture of the isolates on

non-toxic substrates resulted in the production of the toxins. The causative fungus was identified as Aspergillus flavus and the toxin produced was named aflatoxin to denote its origin.

## 1.3 Toxicogenic fungi and toxins

The discovery of aflatoxins as contaminants of agricultural products and their potent carcinogenic property has emphasized the potential public health hazard which might arise from ingestion of contaminated products (Wogan and Metales, 1968). Thus an extensive work has been accomplished concerning the prevalence of the causative organism, environmental conditions for toxin formation, the chemistry, toxicology and biosynthesis of toxins.

The chemical characteristics of aflatoxins has been reviewed by Wogan (1966). Two of the aflatoxins fluoresce blue visible light and were therefore, named aflatoxin $B_1$ and $B_2$ while the two which flouresce yellow green were named $G_1$ and $G_2$ . They are extractable with methanol and chloroform. They can also be separated by

chromatographic techniques with a slightly different $R_f$ values. They contain a coumarin skeleton (Yamazaki, 1978). Aflatoxins $G_1$ and $G_2$ were later discovered to be mammalian metabolites of aflatoxin $B_1$ and $B_2$ respectively.

Aflatoxins are known to produce acute necrosis, cirrhosis and carcinoma of the liver in a variety of animal species. According to Aibarao(1978), of all substances known today, both natural and articially synthesized, aflatoxin displays the highest oral carcinogenecity in a wide range of animals. The most common and highly toxic of the aflatoxins is aflatoxin $B_1$ and to a lesser extent $G_1$. Though aflatoxins are toxic to varying degrees on short term and long term basis for all species of animals studied, the carcinogenic effects vary with the animal species and the age and sex of the animal. The young animal is considerably more susceptible to aflatoxins than the adult and the male much more than the female (Bulatao-Jayme et al. 1978).

The prevalence of toxin producing strains is an indespensible condition for the production of aflatoxins. The prevalence of the Aspergillus flavus group and occurence of aflatoxin in corn has been shown to be

in good agreement by Hesseltine et al. (1975). Out of 394 samples containing aflatoxin only seven showed no A. flavus present.

There are reports of claims that aflatoxins are produced by several fungal species. Hodges et al. (1964) have reported production of aflatoxins by Penicillium puberulum. Formation of aflatoxins by Aspergillus Ostianus was claimed by Scott et al. (1967). However, investigations of reported aflatoxin production by fungi outside the A. flavus group were found to be non-reproducible (Wilson et al, 1968). A screening study of 121 fungus isolates, representing 29 species, for aflatoxin synthesis demonstrated this property only in A. flavus and A. parasiticus.

A. flavus and A. parasiticus are similar in morphology. The name A. flavus is used for both the group and for a separate species within the group. In this paper no distinction is made between the two, and A. flavus is considered as possibly including A. parasiticus.

Not all isolates of A. flavus produce aflatoxins. According to Stoloff (1977), a major proportion of the isolates are capable of toxin production. Ayrest (1963)

reported that about 52% of isolates are capable of toxin production. According to Mitales and Wogan (1967) about 10-30% of strains of A. flavus group isolated from agricultural products have the potential to produce aflatoxins.

A. flavus was believed to be a fungus which invades grains in storage only due to faulty storage practices. However, Lillehoj (1976) has demonstrated that the fungus and the toxin can be present at harvest, though the conditions required for aflatoxin production in preharvest maize remained unknown. According to Stoloff (1977), most of the aflatoxin contamination encountered in maize in the United States probably occurs prior to harvest. Insect damage before harvest is a possible factor.

The production of aflatoxin in vitro is temperature dependent. Schindler et al. (1967) showed that aflatoxin is not produced at temperatures of 2, 7, 41, and 52° C. Maximal production of aflatoxin occurred at 24°C. Harry et al. (1967) reported the optimal temperature range for aflatoxin production to be between 20 and 35°C. Only small amounts of aflatoxin were produced at

10 and 40°C.

The optimal water activity for the growth of A. flavus and toxin synthesis is 0.82 to 0.87 and a relative humidity of 85-90%. Moisture content of 15-35% is optimal in cereal grains while only 10% moisture content is needed in fatty substrates.

Aflatoxin in contaminated grains is contained in very small amounts in individual seeds. A feasible method for detoxification of aflatoxins has been attempted by Ciegler (1966). Yeasts, moulds, bacteria, actinomycetes and algae were screened for their ability to degrade aflatoxins. Only one of the bacteria tested, Flavobacterium auriantium removed aflatoxin from solution. A few moulds, notably A. niger transformed aflatoxin $B_1$ to new flouresceing compounds. Penicillium raistrictii gave a partial conversion of aflatoxin $B_1$ to a compound similar to aflatoxin $B_2$.

From indirect evidence, it was hypothesized that aflatoxins may play a part in the etiology of primary liver cancer in human populations of Africa and South East Asia (FAO, 1977). Mycotoxicosis as the possible cause of primary carcinoma of the liver in man was first

hypothesized in South Africa. The hypothesis was based on the observation that liver cancer is rare in dry areas where mould spoilage of foods is minimal. The disease was also found to be rare among tribes which consume a predominantly fresh diet. Thus the hypothesis of mycotoxicosis resulting from spoilage of foods by toxic moulds fitted the epidemiologic pattern of primary carcinoma of the liver in man better than earlier hypotheses regarding the cause of liver cancer in Africa (Oettle, 1965).

Epidemiological studies have been undertaken in areas where there is a high incidence of primary liver cancer. Due to the high prevalence of primary liver carinoma in the Philipines, it was similarly hypothesized to be due to some carcinogenic substance in the environment. Studies of Bulatao-Jayme et al, (1976) showed the existence of a positive correlation between the amount of aflatoxin in the diet and the incidence of primary liver carcinoma in the region. It was specifically associated with the human consumption of corn. A similar result was obtained by Peers and Linsell (1973) in a selected regional study in Kenya.

Chemical and biological methods of aflatoxin detection have been reviewed by Wogan (1966). Methods of aflatoxin production on rice (Shotwell et al. 1966) and on wheat and oats (Stubblefield, 1967) have elucidated the fact that cereal grains vary in their suitability as substrates for maximal production. According to Jarris (1971) in laboratory studies of toxin production neutral substrates such as cereals have proved suitable and the yield of toxin has been superior to that obtained on synthetic media.

Though aflatoxins are considered as the most important mycotoxins due to the implications for human health, Fusarium toxins notably trichothecenes and zearalenone are also important in cereal grains.

The species of Fusarium commonly reported as toxin producers are Fusarium roseum (Giberella zeae), Fusarium graminearum, Fusarium nivale, Fusarium oxysporum and Fusarium moniliforme.

Zearalenone is a toxin which was first detected after an occurrence of an estrogenic syndrome in swine. It is produced by Fusarium roseum (Mirocha et al., 1977).

Trichothecenes are a group of related toxic

substances produced mainly by a few species of Fusarium, including Fusarium roseum. They cause haemorhagic syndrome in cattle.

Fusarium spp. infect a wide variety of hosts causing symptoms of wilt and ear rot in maize. They causes lodging when they attack stalks of maize.

In maize and wheat, Fusarium spp. need a minimum moisture content of 23% before they can develop (Mirocha et al., 1968).

A 1973 survey by the food and drug adminstration for Fusarium toxins reported a 17% incidence of zearalenone contamination in maize (Eppley, 1974).

According to Ueno (1978) the widespread nature of trichothicene producing fungi in cereals and feeds is an indication that they may be the most important of mycotoxins.

Ochratoxin is a toxin which is comparatively rare in cereal grains. The toxin was discovered in South Africa from Aspergillus ochraceus. The toxicity of this toxin is due to the presence of a lactone group in its structure (Purchase, and Nel, 1967). Ochratoxin was detected as a natural contaminant for the first time by

Shotwell et al. (1969).

The work of Hesseltine et al. (1972) to determine whether ochratoxin is produced by species of Aspergillus other than A. ochraceus after growing them on pearled wheat and cracked corn showed that ochratoxin was detected in some strains of A. sulfureus, A. melleus and A. ostianus. Later, it was elucidated that several species of Penicillium have the ability to produce the toxin.

Though toxin production by fungi is commonly found among species of Aspergillus, Fusarium and Penicillium, reports of toxin producing species are found in a few other genera.

Ben Doupnik and Sobers (1968) tested 96 isolates of Alternaria longipes isolated from tobacco and found out that 31 of the isolates were lethal to chicks. Out of 53 isolates of Chaetomium globosum isolated from maize and other products, 25 were lethal to mice (Christensen et al. 1964).

The toxin called sterigmatocystin, which bears a close relationship to aflatoxin $B_1$ , is another mould metabolite which has been shown to be carcinogenic. It is produced by a few strains of Aspergillus versicolor

and A. nidulans. The toxicity and chemical assay has been studied by Holzaptel et al. (1966).

## 1.4 Conditions for mycotoxin formation.

In general, the physical presence of the generating organism in agricultural (food) products is an indespensible factor for toxin production provided that the substrate and the environment are suitable for growth (Mislevic, 1977).

Some materials are much more likely than others to contain appreciable amounts of mycotoxins. In part this may be due to the inherent nature of the material (Mirocha and Christensen, 1974).

The moisture content of the substratum, which is directly related to the relative humidity of the air, is one of the main factors which regulate fungal growth and toxin formation. According to Lacey et al. (1980), water activity is a more useful parameter than water content since it reflects the availability of water for metabolic processes. Different products with the same water activity may have very different water contents.

For example, oil seeds have a higher water activity at a given water content than starchy cereal seeds; 15-35% moisture content is favourable for the growth of most fungi.

Fungi are completely inactive below 62% relative humidity, and there is very little activity below 75% relative humidity. From 75% relative humidity upwards the amount and kinds of fungi increase with relative humidity (Roberts, 1972). Aspergillus flavus for instance, develops well at a relative humidity of 85 to 90%.

The availability of specific minerals in substrates is believed to be a factor for mycotoxin formation (Gerolova, 1980). According to Maggon et al. (1977) the role of trace elements in regulating biosynthesis of mycotoxins is not fully understood. Many secondary fungal products including aflatoxins are zinc dependent for their synthesis. Stoloff (1977) also reports that the limiting essential element for aflatoxin production is zinc.

Crops in tropical and subtropical areas are more subject to mycotoxin contamination than crops in temperate regions, since optimal conditions for growth and toxin

formation are prevalent in areas of high humidity and high temperature (WHO, 1979).

Insect attack of grains can lead to fungal development and mycotoxin formation in two ways. Firstly, the damaged crop is more susceptible to infection by airborne fungi, and secondly the insects themselves act as fungal spore carriers taking the spores into the interior of the damaged crop (WHO, 1977).

## 1.5 Storage and fungal deterioration of Ethiopian cereal grains.

"Teff", maize, sorghum and barley are the most important cereal grains grown in Ethiopia. Teff (Eragrostis teff (Zucc) Trotter) is unique to Ethiopia as a cereal crop. It is ground into flour and the fermented pancake "Injera" is the staple diet of the highland dwelling populations. It is cultivated at altitudes of 1700-2800 meters (Purseglove, 1976). Barley (Hordeum vulgare L.) is grown on the highlands of Ethiopia. It is used for food and for the preparation of local beverages. Maize (Zea mays L.) is a major crop

in the South and South Western regions. The long wet season in these regions with a relatively high temperatures is favourable for maize production. Sorghum (Sorghum bicolor (L.) Moench) is grown mainly in areas that are generally too dry for consistent maize production. It is primarily used for human consumption in both Asia and Africa.

The regions considered for this study show differences in their importance as growing regions of the crops under study. The areas around Addis Ababa are important in teff and barley production. Jimma and Shashemene are in the major maize growing regions while the Dire Dawa area is important in sorghum production. These regions are also different in the amount of annual precipitation, annual average temperatures and other climatic factors.

Storage losses of grains in Ethiopia has been surveyed by Mcfarlane (1969). It is reported that teff is the most unaffected by insects of all cereal grains. It is a grain which can withstand long term storage with no significant damage. Artificially infesting stored teff seed with Sitophylus oryzae resulted in the

death of insects, possibly because of the small size of the teff grains (Gilman, 1968).

Gilman (1968) has surveyed storage structures in common use in Ethiopia. For bulk storage, the conical "gottera", the cylinderical crib and the barrel shaped "gottera" are used. Storage in underground pits is a common form of bulk storage in certain regions. "Dibignet", "Silicha", the jute-sack and metal drums are the common house hold stores.

Niles (1976) studied the mycoflora of sorghum stored in underground pits in Ethiopia and reported that over half of the species isolated belong to the genera Aspergillus, Penicillium and Fusarium. According to Gilman and Boxall (1974) the storage of grain in underground pits has advantages and disadvantages for the small farmer. It is particularly useful in that grain stored in pits is to some extent protected from insect attack because of reduced oxygen levels. Since moisture moves into the grain from the surrounding soil, mould damage to the grain bulk is to be expected in traditional pits.

At present in Ethiopia, there is no micro-biological

evaluation of seeds when they go into storage or when distributed for consumers. The Agricultural Marketing Corporation (AMC) applies the following three criteria before grains go into storage (Ashman, undated). The moisture content, the proportion of foreign matter to the normal grain and the amount of insect and physically damaged seeds are taken into consideration. The acceptible moisture content for cereal grains is 14% for the high lands of Ethiopia. A decrease in the moisture level by 1% is recommended for hot-humid regions.

## 1.6 Aim of the present study

The main objective of this study is to elucidate the mycoflora of Ethiopian cereal grains in order to find the prevalence of important toxicogenic fungi. This was recommended by Gilman (1968) after his observation of mould damaged grains in some market places in Ethiopia. This study is also supposed to provide a general picture of the fungi which may be responsible for deterioration of grains.

The high prevalence of liver diseases in Ethiopia

has been reported by Coady (1965) and has been reviewed by Edemariam (1977). Coady (1965) suggested factors of plant (particularly fungal) origin as possible causes for the high prevalence of liver diseases in Ethiopia. The carcinogenic property of aflatoxins and the positively correlated studies of dietary aflatoxins and liver cancer in some regions in the tropics makes this study essential. Knowledge on the prevalence of the aflatoxin producing fungus (Aspergillus flavus) in Ethiopian cereal grains is believed to provide the background information about the possible problem of aflatoxicosis in Ethiopia. The toxin producing ability of A. flavus isolates and the suitability of grains as substrates for aflatoxin formation is essential in this aspect. In a preliminary study on the aflatoxin content of selected Ethiopian foods, Abraham and Petros (1981) have recommended more investigations to be made on dietary aflatoxins.

The problem of mycotoxicosis in general and aflatoxicosis in particular may show variation with region due to the high variability of environmental conditions in Ethiopia, and in this work is an attempt to identify high risk regions and cereal grains. Such

information should be of value in undertaking epidemiological studies to ascertain the contribution of mycotoxins, particularly aflatoxins, in the etiology of liver cancer in Ethiopia.

# 2.0 MATERIALS AND METHODS

## 2.1 Sample collection

Addis Ababa, Shashemene, Jimma and Dire Dawa areas were selected as study sites. Grain samples were collected from September, 1981 to May, 1982. Collections from Shashemene were made in November, 1981, from Jimma in March, 1982 and from Dire Dawa in May, 1982. Samples from Addis Ababa were collected at different times during the study period.

Samples were collected from stores and market places in and around the towns. A store sample was obtained from as many sacs as possible from a seed lot. The Agricultural Marketing Corporation (AMC) provided the sampling probes used in some of this study. Market samples were bought from individual farmers (sellers) provided that the grain bulk was produced and harvested in the area around the study site. A market sample represents a seed lot.

Time of harvest, period of storage and degree of insect damage were recorded for all samples. Samples

were sealed in plastic bags at the collection site and transported to the laboratory for further studies. Mycological and other examinations were made within 15 days after collection.

## 2.2 Moisture content determination

Moisture content of grain samples was determined according to methods of the International Organization for Standardization (ISO). Determination of moisture content of maize was made by using the routine method on whole grains (ISO, 1980). For the other cereal grains including teff moisture content was measured according to (ISO, (1979).

## 2.3 Enumeration of surface microflora

50 gm of each grain sample was shaken in 450 ml of sterile water with a magnetic stirrer for 15 minutes. The suspension was serially diluted and the appropriate dilutions were plated on Nutrient Agar (Oxoid CH3) and on Yeast Malt Extract Agar (YMEA) (15 gm Malt extract, 3 gm Yeast extract, 5 gm peptone and 15 gm agar per litre).

YMEA was acidified with sterile 10% lactic acid until the pH was lowered to 3.5.

Plates were incubated at 30°C. Bacterial colonies were counted after 24-48 hrs of growth on nutrient agar. Mould and Yeast colonies were counted after 72-96 hrs on the acidified YMEA.

The number of bacteria, moulds and yeasts per gram of grain was determined for the test samples. Identification of surface micro-organisms has not been attempted.

## 2.4 Surface disinfection and plating of seeds.

About 50 gm of each grain sample was surface sterilized in 200 ml 0.1% mercuric chloride solution for 1 minute. It was then rinsed 3 times in 200 ml of strile distilled water. Potato Dextrose Agar (PDA) (500 ml of infusion from 300 gm of potato tubers, 20 gm dextrose and 20 gm agar per litre) and Czapek Dox Agar (Oxoid, CM97) were used.

About 100 seeds were aseptically plated (50 seeds on PDA and the same number on Czapek Dox Agar) from a

sample. Five seeds of maize, 8 seeds of barley, 10 seeds of sorghum and 15 seeds of teff were plated on a 9 cm culture plate.

All agar plates containing seeds were incubated at 28°C for a maximum of 12 days.

## 2.5 Isolation and Identification

Fungi that grew out of plated seeds were subcultured on Czapek Dox Agar to obtain pure cultures. Isolation of Rhizopus and Trichoderma species was made on PDA only since these organisms were not found to develop on Czapek Dox Agar. Colony growth rate, colour of colonies, media pigmentation, and other cultural and morphological characteristics were noted to help in identification of the isolated fungi. Slide cultures were also prepared for microscopic observation of isolates when necessary.

Identifications were made using Gilman's "A manual of soil fungi', Barnet Hunter's 'Illustrated genera of Imperfect Fungi' and other references.

All isolates were identified to the genus level, and to species level only when possible. Isolates were

maintained on PDA slants in the refrigerator at 4°C. Aspergillus species which were found to be similar in morphology with A. flavus were inoculated on Aspergillus Differential Medium (ADM) prepared from 1.5% tryptone, 1.0% Yeast extract, 0.05% Ferric citrate and 1.5% agar per litre. Aspergillus flavus group produces a bright yellow orange pigment on this medium (Bothast and Fennel, 1974).

## 2.6 Toxin production on grains

To test the ability of fungal isolates for the production of toxins in vitro, 50 gm of a normal grain sample was moistened by adding 15 ml of water to the grain in a 500 ml Erlenmeyer flasks. They were kept in a warm water bath for 3 hours. The flasks were sterilized in steam under pressure at 121°C for 15 minutes. This raised the moisture content of grains to about 30-40%.

The inoculum was prepared by adding 3 ml of sterile water to PDA slants containing young cultures. The spores were scrapped loose with sterile loop, and the slant was

shaken to give a uniform suspension of spores. This was used to inoculate the sterile moist grain.

The flasks were incubated at 28°C for 5-7 days and were hand shaken twice a day to prevent clumping of grains. However, it was difficult to prevent moist teff grains from clumping.

## 2.7 Detection of toxins

### 2.71 Aflatoxins

To test the ability A. flavus isolates for the production of aflatoxins Association of Official Analytical Chemists (AOAC, 1975) Method was used. In brief, moldy grain samples prepared by the above procedure, were extracted with chloroform and were eluted with n-hexane, ether and methanol: chloroform (3:97) in a silica gel column containing $Na_2SO_4$. The methanol-chloroform fraction was taken and the solvents were evaporated. The extract was dissolved in 0.2 ml of benzene: acetonitrile (98:2) for thin layer chromatography.

4 to 10 µl of extracts were spotted side by

side with aflatoxin $B_1$ and $G_1$ standards on prepared TLC plates (0.25 mm thickness and 20 X 20 cm dimension). Plates were developed in acetone: chloroform (5:95) in a developing tank for about 1 hr. Dried plates were illuminated on longwave UV light in a cabinet.

Flourescence of standard and sample spots were noted. The $R_f$ values of the standards and samples were calculated. The percentage of toxin producing isolates of A. flavus was determined.

## 2.72 Fusarium toxins

After 7 days of growth of Fusarium isolates on sterile moist grains, the samples were extracted with ether: alcohol (1:1) mixture for 24 hrs by periodic hand shaking. The extract was filtered through filter paper and the solvent was evaporated. The concentrated extract was applied on a shaved rabbit skin twice in 24 hrs. Dermal reactions on treated skin spots were observed on the $4^{th}$-$6^{th}$ days of application.

## 2.73 Ochratoxin

A few common Aspergillus and Penicillium isolates were tested for ochratoxin production. The method used by Scott et al. (1967) for the detection of Ochratoxin A in cereal products was used. In short, the mouldy samples were extracted with 200 ml of methanol: distilled water (55:45) and 50 ml of n-hexane was shaked for 3 hrs. 50 ml of the aqueous methanol was eluted in silica gel column with chloroform : n-hexane (1:1). The solvent mixture was evaporated and sample residue was dissolved in 0.25 ml of chloroform.

About 10 µl of sample extracts were spotted on prepared thin layer chromatographic plates of silica gel G (0.25 mm thick, 20 X 20 cm). Ochratoxin standards were not available and were not spotted.

Plates were developed with benzene: methanol : acetic acid (24 : 2 : 1) and were kept until the solvent front has travelled 13-15 cm above the origin. Plates were observed under both short wave and long wave ultraviolet light simultaneously. Green flourescent spots at an $R_f$ of 0.6 is characteristic of Ochratoxin A.

The same plates were exposed over a concentrated solution of ammonia. The same spots of ochratoxin flouresce blue after this treatment. The change helps to confirm the presence of Ochratoxin A in the sample.

# 3.0 RESULTS

## 3.1 Number of surface micro-organisms

The number of micro-organisms on the surface of cereal seeds is shown in Table 1. More than 10,000 bacteria/ gm of cereal grain was found from all samples.

About 50% of the teff samples examined contained more than one million bacteria /gm of teff, and there were no samples containing less than 100,000 bacteria /gm. Teff harbours the highest number of bacteria / gm among the cereal grains.

The bacterial count of maize seeds was more variable than the counts of the other cereal seeds. Two samples had less than 100,000 bacteria while more than ten million bacteria were enumerated from one of the eleven samples.

Mould counts were in general lower than bacterial counts except in maize where more than 50% of the samples contained more than one million moulds / gm. More than 60% of the teff samples contained less than 10,000 moulds /gm and this is about half the number of bacteria that can be found in the same amount of teff.

For teff and barley, the yeast count was in general greater than the mould count, but maize and sorghum showed mould counts greater than the yeast count.

## 3.2 Prevalence of fungal genera

Table 2 gives prevalence of fungal genera associated with cereal grains. Aspergillus, Penicillium, Chaetomium, Alternaria, Helminthosporium, Trichoderma, Rhizopus, Mucor and Trichothecium were the major genera isolated. Cladosporium, Curvularia, Rhizoctonia, Glioc1adium and Cephalosporium were rarely found. Fungi with sterile mycelia (non-sporulating types) were also isolated, but they were not identified.

Aspergillus, Penicillium and Fusarium species are the three most prevalent organisms in Ethiopia grown (and stored) cereal seeds.

Teff harbours the least kinds of fungal genera. Fusarium, Trichoderma, Rhizopus and Mucor were not isolated from any of the 20 samples examined. Penicillium and Chaetomium are the two genera most often associated with teff. They were isolated from 35% of the teff

T A B L E 1

Range of Microbial Numbers on surface of cereal seeds

| | Number of samples examined | Range of Numbers of bacteria / gm | | | | | |
|---|---|---|---|---|---|---|---|
| | | less than $10^3$ | $10^3$-$10^4$ | $10^4$-$10^5$ | $10^5$-$10^6$ | $10^6$-$10^7$ | greater than $10^7$ |
| Teff | 16 | none | none | none | 8 | 6 | 2 |
| Barley | 12 | none | none | 2 | 2 | 8 | none |
| Maize | 11 | none | none | 2 | 4 | 4 | 1 |
| Sorghum | 9 | none | none | 3 | 4 | 2 | none |
| | | Range of Mould count / gm | | | | | |
| Teff | 16 | none | 10 | 4 | 2 | none | none |
| Barley | 12 | 3 | 8 | 1 | none | none | none |
| Maize | 11 | 1 | 1 | 2 | 2 | 4 | 1 |
| Sorghum | 9 | none | 5 | 2 | 2 | none | none |
| | | Range of Yeast count / gm | | | | | |
| Teff | 16 | none | 4 | 5 | 7 | none | none |
| Barley | 12 | none | 6 | 3 | 3 | none | none |
| Maize | 11 | none | none | 10 | 1 | none | none |
| Sorghum | 9 | 1 | 5 | 3 | none | none | none |

samples. Aspergillus, Alternaria and Helminthosporium were found in 25%, 15% and 15% of the teff samples respectively.

Aspergillus and Penicillium were found in all grain types examined with in a range of 25 - 60% showing that these fungi have the ability to invade cereal grains including teff.

Fusarium is the most prevalent organism in maize. It was isolated from 62% of the samples. It is also one of the most common genera associated with sorghum. It was found from 50% of sorghum samples. It was not isolated from any of the samples of teff examined.

## 3.3 Mycoflora changes due to storage period

Table 3 shows changes in the mycoflora of sorghum and maize stored for different periods. There were not enough teff and barley lots stored for longer than six months. Thus, barley and teff are not included in this comparison.

Aspergillus spp. were found in 100% of stored grain samples while it is only in 13% of the samples

TABLE 2

Prevalence of fungal genera in cereal grains

| Grain type | Number of samples | Asper-gillus | Peni-cillim | Fus-arium | Chaet-omium | Alter-naria | Helmintho-sporium | Tricho-derma | Rhizo-pus | Mucor | Tricho-thecium | Other* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Teff | 20 | 5 (25%) | 7 (35%) | none (0%) | 7 (35%) | 3 (15%) | 3 (15%) | none (0%) | none (0%) | none (0%) | 1 (5%) | 4+ (20%) |
| Barley | 20 | 12 (60%) | 7 (35%) | 5 (25%) | 7 (35%) | 6 (30%) | 5 (25%) | 2 (10%) | 2 (10%) | 5 (25%) | 2 (10%) | 3++ (15%) |
| Maize | 40 | 15 (38%) | 21 (53%) | 25 (63%) | 7 (18%) | 2 (5%) | 1 (3%) | 5 (13%) | 9 (23%) | 7 (18%) | none (0%) | 8+++ (20%) |
| Sorghum | 30 | 15 (50%) | 12 (40%) | 15 (50%) | 9 (30%) | 14 (47%) | 4 (13%) | 7 (23%) | 2 (7%) | 2 (7%) | 2 (7%) | 6++++ (20%) |
| TOTAL | 110 | 48 (43%) | 47 (42%) | 45 (41%) | 30 (27%) | 25 (23%) | 13 (12%) | 14 (13%) | 13 (12%) | 14 (13%) | 5 (5%) | 21 (19%) |

* This is a group consisting of fungi rarely isolated

\+ Sterile fungi

++ *Cladosporium*, *Curvularia* and sterile types.

+++ *Rhizoctonia*, *Gliocladium*, *Cephalosporium cladosporium* and sterile fungi

++++ *Cephalosporium* and sterile fungi

from grains stored for less than 2 months.

Rhizopus spp. were recovered from 42% of samples of long term stored maize and sorghum.

Alternaria, Chaetomium and Helminthosporium species were not isolated from any of the long period stored samples. On the other hand, Fusarium, Alternaria and Chaetomium species are the most prevalent genera in samples of maize and sorghum stored for less than 2 months.

Penicillium spp. were found in 33% of the short-period stored samples and in 42% of the long term stored samples.

## 3.4 Moisture content of Grain samples.

Table 4 presents moisture content of grain samples examined. It was determined according to the methods developed by the International Organization for Standardization.

Moisture content is expressed as a percentage of fresh weight minus dry weight divided by the fresh weight (i.e wet-weight basis).

TABLE 3

Changes in the fungal flora of maize and sorghum due to storage period differences.

| Storage period | Number of samples examined | Number of samples positive for the common genera | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Asper-gillus | Peni-cillium | Fus-arium | Rhizo-pus | Tricho-derma | Alter-naria | Chaet-omium | Helmintho-sporium |
| less than 2 months | 15 | 2 (13%) | 5 (33%) | 12 (80%) | none (0%) | 3 (20%) | 8 (53%) | 8 (53%) | 2 (13%) |
| longer than 6 months | 12 (100%) | 12 (100%) | 5 (42%) | 3 (25%) | 5 (42%) | 2 (17%) | none (0%) | none (0%) | none (0%) |

The moisture content of most grain samples lies between 10-14%. In general, teff and barley contain less moisture than maize and sorghum.

14-16% moisture was recorded in four samples of sorghum, in one sample of maize and none in teff and barley.

Two samples of sorghum were found to contain 16-17% moisture, which was not found in any of the other grain samples examined.

## 3.5 Prevalence of Aspergillus flavus.

Prevalence of Aspergillus flavus in grains irrespective of collection site and period in storage is given in table 5. A. flavus was found in 28 grain samples out of 110 cereal grain samples examined. This makes up about a 24% prevalence of the organism in Ethiopian cereal grains.

Out of the 28 isolates of A. flavus, 24 were recovered from grains which had been stored for longer than six months. The number of samples examined from the two kinds of grains was not appreciably different.

TABLE 4

Percentage moisture content of grain samples.

| Grain type | Number of samples | % Moisture content: less than 10% | 10-12% | 12-14% | 14-16% | 16-17% | greater than 17% |
|---|---|---|---|---|---|---|---|
| Teff | 10 | 1 | 7 | 2 | none | none | none |
| Barley | 10 | 2 | 6 | 2 | none | none | none |
| Maize | 12 | 2 | 4 | 5 | 1 | none | none |
| Sorghum | 12 | none | 2 | 4 | 4 | 2 | none |

A. flavus is more prevalent in maize and sorghum than it is in barley and teff.

The occurrence of A. flavus in cereal grains collected from the four regions is shown in table 6. The number of samples of each grain type from each of the four regions is also indicated. The prevalence of A. flavus from Jimma samples (32%) and Dire Dawa samples (30%) is appreciably higher than in samples from Addis Ababa or Shashemene (15% each).

## 3.6 Aflatoxin producing potential of A. flavus isolates.

The aflatoxin producing potential of twenty isolates of A. flavus from grains is presented in table 7. Sixteen isolates out of 20 examined are capable of producing aflatoxin $B_1$ or $G_1$ . Thus 80% of the isolates are capable of toxin production under the conditions of the experiment. Aflatoxin $B_1$ was detected in 16 samples (80%) while aflatoxin $G_1$ was detected in 11 samples (55%).

It is shown here that all the four grains provide a suitable substratum for aflatosin production including teff

T A B L E 5

Recovery of *A. flavus* from grain samples.

| | Number of samples | *A. flavus* positive samples | % prevalence |
|---|---|---|---|
| Teff | 20 | 3 | 16% |
| Barley | 20 | 5 | 20% |
| Maize | 40 | 12 | 33% |
| Sorghum | 30 | 10 | 33% |

TABLE 6

Recovery of Aspergillus flavus from grains

by region and grain type

| Region | Grain type and number of samples | A. flavus positve samples | % average prevalence |
|---|---|---|---|
| Addis Ababa | 6 samples of teff | 1 | 15% |
| | 6 samples of barley | 0 | |
| | 4 samples of sorghum | 1 | |
| | 4 samples of maize | 1 | |
| Shashemene | 6 samples of teff | 0 | 15% |
| | 4 samples of sorghum | 1 | |
| | 4 samples of maize | 1 | |
| | 6 samples of barley | 1 | |
| Dire Dawa | 4 samples of teff | 1 | 30% |
| | 13 samples of sorghum | 4 | |
| | 13 sample of maize | 4 | |
| | barley* | | |
| Jimma | 4 samples of teff | 1 | 32% |
| | 8 samples of barley | 4 | |
| | 15 samples of maize | 4 | |
| | 12 samples of sorghum | 4 | |

*No samples of barley were examined from Dire Dawa.

in vitro.

When maize isolates MAASIL-H and MSS-7 were inoculated into sterile moist teff they produced aflatoxins as they did on sterile moist maize.

## 3.7 Prevalence of Fusarium spp. and Toxicity Test.

Table 8 shows prevalence of Fusarium roseum and other Fusarium spp. from maize and sorghum. Out of 40 maize samples examined F. roseum was isolated from 14 samples examined. Other Fusarium spp. are common in both maize and sorghum, occurring in about 50% of maize and sorghum samples (see table 2).

Out of the five Fusarium roseum isolates tested by the Rabbit skin toxicity test only one showed a positive detectable reaction to the test. Seven isolates from other Fusarium spp. were also tested, and 2 of the isolates showed a necrotic reaction. Out of the total isolates of Fusarium tested three isolates showed a positive reaction. Identification of the toxin has not been attempted.

# TABLE 7

A flatoxin producing potential of *Aspergillus flavus* isolates on moist sterile grains at 28°C.

| *A. flavus* isolate number | isolated from (grain) | cultivated on (grain) | Aflatoxin detected $B_1$ | $G_1$ |
|---|---|---|---|---|
| SGM-2 | Sorghum | Sorghum | + | + |
| MGM-5 | Maize | Maize | + | - |
| SAASIL-9 | Sorghum | Sorghum | + | + |
| H-17-S | Maize | Maize | + | - |
| TAASIL | Teff | Teff | - | - |
| SSS-1 | Sorghum | Sorghum | - | - |
| BSS-B-1 | Sorghum | Sorghum | - | - |
| MAASIL-H | Maize | Maize | + | + |
| MSS-7 | Maize | Maize | + | - |
| SJM-E | Sorghum | Sorghum | + | + |
| SJS-E | Sorghum | Sorghum | + | + |
| BJS-B | Barley | Barley | + | + |
| SJS-B | Sorghum | Sorghum | + | + |
| BJS-D | Barley | Barley | + | + |
| BJS-E | Barley | Barley | + | - |
| BJS-C | Barley | Barley | - | - |
| MAASIL-H | Maize | Teff | + | + |
| MSS-7 | Maize | Teff | + | - |
| MJS-A | Maize | Maize | + | + |
| MJS-B | Maize | Maize | + | + |

## 3.8 Ochratoxin detection

Four common isolates of Aspergillus including two isolates preliminarily identified as A. sulfureus and A. ochraceus were examined for ochratoxin. In none of the isolates was ochratoxin detected. Four common Penicillium spp. of grains were also examined for production of the toxin and none of them was shown to produce the toxin under the experimental conditions.

# T A B L E 8

Prevalence of *Fusarium roseum** and other *Fusarium* spp. in maize and sorghum and toxicity of some isolates

| Isolate | % Prevalence | | Number of isolates tested | reaction to skin test | |
|---|---|---|---|---|---|
| | Maize | 14 | | positive | negative |
| | (40 samples) | (35%) | 4 | 1 | 3 |
| *Fusarium roseum* | Sorghum | 2 | | | |
| | (30 samples) | ( 7%) | 1 | | 1 |
| | Maize | 21 | | | |
| | (40 samples) | (53%) | 4 | 1 | 3 |
| other *Fusarium* spp. | Sorghum | 14 | | | |
| | (30 samples) | (47%) | 3 | 1 | 2 |

*_Fusarium roseum_ is also called _Giberrela zeae_

## 4.0 DISCUSSION

The number of micro-organisms on the surface of grains show variability even among samples of the same grain type (Table 1). This depends upon the history of the grain lots from which samples were drawn. There are different methods of threshing grains in Ethiopia. If threshing has been done on the ground with cattle trampling, the grain would contain micro-organisms of the soil and the dung of the trampling cattle. The type of storage and duration of storage will also influence microbial numbers. Stores, exposed to the air for a long time would carry the microbial flora of the air.

Teff samples contain a higher number of bacteria than any of the cereal grains examined. Teff is a small sized grain. It contains the highest surface area/unit mass as compared with maize, sorghum, or barley, which are much bigger in size.

Teff is traditionally threshed on a hardened ground and is trampled using cattle while maize and sorghum heads are piled and beaten with sticks and threshing of contamination from dung is limited in the latter two.

The samples examined have not been sieved (cleaned)

as it is normally done before milling grains. Cleaning of grains, as traditionally practiced may give a lower microbial count.

The shaking procedure allows fragmentation of hyphae which may exaggerate mould counts. The other drawback in the method is that yeast colonies appear on nutrient agar plates and this makes counting of bacterial colonies difficult. In general, the number of micro-organisms obtained on a nutrient medium, incubated at a fixed temperature shows lower counts. This is true especially for bacterial counts.

When surface disinfected seeds are plated on appropriate media, the seeds take up water, and the internally existing fungi grow out of the seeds. Potato Dextrose Agar favors the growth of field fungi while Czapek Dox Agar favors the growth of storage fungi (Aspergilli and Penicillia). However, most members of both groups grow on both media. The problem with this method is that fast growing fungi grow over the slow growing types and makes isolation (obtaining pure culture)difficult.

The mycoflora profile of the four grain types shows the following genera with decreasing prevalence (Table 2).

Aspergillus, Penicillium, Fusarium, Chaetomium, Alternaria, Trichoderma, Mucor, Rhizopus and Helminthosporium. However, percentage prevalence of these genera is slightly variable with grain type. The absence of Fusarium, Trichoderma, Rhizopus and Mucor in teff makes it unique. This may be accounted for with the following reasons: teff is unaffected by insects (Gilman, 1968); and seeds are not cracked during harvesting. Teff grains dry more quickly than other grains due to their small size. As shown in table 4 teff has a moisture content (about 11%) which is slightly lower than other cereal grains and this is unfavorable for invasion by most fungi.

Fusarium was found to be highly prevalent in maize and sorghum. It is a facultative parasite which attacks stalks of maize and sorghum. It also causes diseases of the ear of maize and sorghum heads. Since it is common in the soil, lodging encourages invasion by these organisms.

A difference in storage period by six months shows an appreciable difference in the fungal types associated (Table 3). A 100% recovery of Aspergillus in stored samples and 13% recovery in short term stored samples shows that members of this genus are responsible for

deterioration of grains in storage. On the other hand, the complete absence of Alternaria, Chaetomium and Helminthosporium in stored samples demonstrates that these organisms are associated with unstored (freshly harvested) grains. Thus with storage these fungi die out and are replaced by storage fungi especially by species of Aspergillus. Though Penicillium is also classified as a storage mould, there is no appreciable difference in prevalence between the two groups of samples in this study.

Field Fungi invade the developing or mature seed in the field while it is on the plant. According to Agrios (1978), this group of fungi die out after a few months in storage. Christensen (1957) classified Rhizopus with the field fungi. In this study, Rhizopus was not found in any of the unstored samples, but was found in 42% of the stored grain samples.

Thus it seems clear that stored samples are possible sources of Aspergillus and Penicillium toxicosis while Fusarium toxicosis may occur from freshly harvested maize and sorghum.

Invasion of stored grains is influenced by moisture

content. 10-14% was the moisture level of most grain samples examined. This is in general in accordance with the local standards (AMC). The moisture equilibrium of a commodity at a given relative humidity is dependent on its chemical compostion (Christensen, 1972). Higher moisture levels in sorghum and slightly lower level in teff may partially be ascribed to this fact. The RH of the collecting site will also be a contributing factor. According to Forgacs and Carll (1962) at a constant relative humidity, temperatures determined the dominating organisms. For instance, at 80% RH Penicillium is favored at 25°C. Aspergillus flavus is favored at 30°C while A. glaucus group is favored at 35°C.

Though 14% moisture content is the safe maxium moisture level. Aspergillus glaucus and Aspergillus restrictus groups are responsible for deterioration of grains at lower than this level (Christensen, 1957). These Aspergilli, however, are not reported to be toxin producing in the literature.

There seems to be disagreement between the moisture requirements of fungal isolates and moisture levels of grains. Invasion of grains occurs, when the moisture

level is high but the mycelia can exist for sometime in decreased moisture content (Roberts, 1972). Since the moisture content was not determined on the site of collection, loss of moisture may have occured during transportation.

Prevalence of A. flavus (Table 5) was found to be least in teff and highest in maize and sorghum. This may partially or completely be due to the resistance of teff grains to insect infestation (Gilman, 1968 and Macfarlane, 1969). This has been observed during sample collection. Lillehoj (1976) and Stoloff (1977) reported that invasion of maize by A. flavus is encouraged by insect damage before and after harvest. The close association of A. flavus with insect damaged corn has been reported by Zuber (1977). Bulatao-Jayme et al. (1976) reported that A. flavus usually makes an entry when the protective shell, husk or membrane is damaged by insects or develops even the slightest break during harvest. Thus this could be the primary cause for the high prevalence of A. flavus in maize and sorghum and the low prevalence in teff. The higher moisture level in sorghum and maize than in teff may also be a factor.

The high prevalence (32%) of A. flavus from Jimma

samples followed by 30% from Dire Dawa samples demonstrates that environmental conditions play a great role for the prevalence of the organism. Temperature and relative humidity must be the most important factors contributing to the difference in the occurrence of A. flavus by region. Optimal temperature for insect activity lies between 28°C to 34°C and the temperature below which stored seed is safe from their activity is 20°C (Roberts, 1972). The mean annual rainfall in Jimma is 1534.6mm, It is 1128.5 mm in Addis Ababa and 608.9 mm in Dire Dawa (Kebede, 1964). The average monthly temperature in Addis Ababa lies between 15-18°C, 18-22°C in Jimma and 22-28°C in Dire Dawa (Mesfin, 1970).

Reports on the aflatoxin producing ability of A. flavus isolates varies between 30 to 70%, In this work (Table 7), 80% of the isolates were found to be capable of producing aflatoxins ($B_1$ or $G_1$ ). This is a high incidence of aflatoxicogenic isolates in Ethiopian cereal grains.

Thus from the results it can be seen that the grains that may be associated with aflatoxicosis are maize and sorghum. These grains types are highly associated with

A. flavus partly because of their susceptibility to insect infestation. Jimma and Dire Dawa provide environments suitable for insect activity.

The low aflatoxin content of teff samples reported by Abraham and Petros (1981) is in good agreement with the low prevalence of the producing fungus in teff. The low moisture level in teff may be a factor to the low prevalence of the organism.

Though the natural contamination of teff was found to be low, artificial inoculation of A. flavus on sterile moist grains showed that the organism can grow and produce aflatoxins on teff.

The aflatoxin content of a fermented product can be much less than before fermentation (Reiss, 1978).

Fusarium is a genus which is highly associated with maize and sorghum seeds (Table 2). Its prevalence is more in unstored grains than in stored ones (Table 3). Fusarium roseum is more prevalent in maize than in sorghum (Table 8). Out of 40 maize samples examined F. roseum was isolated from 14. This organism is known for the production of zearalenone. Hesseltine (1978) showed a positive correlation between the F. roseum seed infection

and production of zearalenone.

There are different methods of zearalenone analysis (Mirooha et al. 1977). The rabbit skin biossay was chosen in this study due to its simplicity. Out of five F. roseum isolates tested by this method one was found to show necrotic reaction on the shaved skin 5 days after the application of the extract.

Various methods of detection and quantification of trichothecenes are available. Since all trichothecene compounds induce skin necrotization (Ueno, 1977) the rabbit skin test was chosen as for zearalenone.

Among 12 isolates of Fusarium spp. including F. roseum only 3 produced a detectable effect (necrotic). This method, however, cannot differentiate between zearalenone and trichothecenes.

Four isolates of Aspergillus and four common isolates of Penicillium spp. were tested for ochratoxin production. There was no isolate which showed a detactable amount of ochratoxin. Ochratoxin and ochratoxin producing fungi are not common in cereal grains. It is also possible that the isolates need a different condition for toxin production.

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## 5.0 RECOMMENDATIONS

Based on the present study the following recommendations are made:

1. The mycoflora of other grain types and foodstuffs (fermented or unfermented) including local beverages should be extensively investigated.

2. The aflatoxin content of food grains and prepared foodstuffs should be studied. Attention should be given to hot and humid areas. Maize and sorghum, and foodstuffs and beverages made from them should be given the greatest attention.

3. Research on appropriate storage practices in order to develop cheap and feasible methods of protecting grains from mould growth and insect infestation are needed. This is necessary not only because of grain loss but also because the aflatoxin producing fungus and other toxicogenic fungi are associated with inappropriate storage practices and insect damage.

4. Fusarium toxins, notably zearalenone and trichothecenes need to be given attention due to the high prevalence of the causative organisms in maize and sorghum. Maize is the most important crop in this

respect.

5. The possibility of animal health hazards from ingestion of mouldy hay, contaminated cereal stalks etc, should be recognized and research on veterinary mycotoxicosis be encouraged.

6. A relatively long term study should be undertaken to investigate aflatoxins in the diet together with liver cancer cases in selected regions.

7. A food quality (control) laboratory should be established, or existing laboratories should undertake mycotoxin surveillance of agricultural products.

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