DETERMINATION OF MYCOTOXIN CONTAMINATION OF CEREAL GRAINS IN JUJA STORES AND POSHOMILLS

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Abstract
The survival of micro-organisms in grains mostly depends upon moisture and temperature. Most cereals supplied in various towns are normally stored in conditions that are favourable for fungal growth. Fungal growth in cereals may expose various individuals to fungal infections or any chronic diseases associated with mycotoxin contamination. This study aimed at identifying the mycotoxin and microbial contamination in cereals mainly maize in the cereal stores and several posho meals in Juja town. Twenty cereal stores and poshomills were randomly selected from Muchatha and Gachororo and samples were collected. Questionnaires were then administered in each store and poshomill in order to obtain information concerning the storage conditions of grains. The food homogenate was made using peptone water and then cultured on corn meal agar, sabouraud's dextrose agar, brain heat infusion agar and potato dextrose agar. The colonies formed were stained using lactophenol cotton blue stain and identified. The fungi isolated in this study included: *Fusarium spp., Alternaria spp., Chrysosporium spp, Trichophyton tonsurans, Aspergilus flavus, Acremonium and Penicillium spp. Aspergillus flavus* was isolated in most cereal samples (24.5%) this is an indication that some of the cereals supplied by Juja stores and poshomills may be contaminated with aflatoxins. Since majority of the respondents claimed to store their cereals in good conditions, contamination of cereals with mycotoxins may be as a result of poor harvesting and handling of cereals before being transported to the stores and poshomills. This is a challenge to the ministry of Public health and other stake holders to create awareness on prevention of mycotoxin contamination as it poses a health risk to the consumers of the grains.

Key words: Fungi, aflatoxin, Public health

1 Introduction

Mycotoxins refer to the toxic chemical products produced by fungi that readily colonize crops. One mold species may produce many different mycotoxins. Mycotoxicoses is the term used for poisoning associated with exposures to mycotoxins. The symptoms of a mycotoxicoses depend on the type of mycotoxins, the concentration and length of exposure; as well as age, health, and sex of the exposed individual. Mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. These toxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin (Turner G. *et al.,* 2005).

Although only recognized in recent times as a source of ill-health in humans, the agricultural problems associated with contamination of crops by fungal and bacterial action have been noted for over two millennia (Campbell, 1996). Consequently, human mycotoxicoses have probably also existed since the development of settled agricultural communities reliant on grain stores. Recent attempts to interpret the Biblical plagues of ancient Egypt over three millennia ago have suggested that the tenth plague, mentioned in the Book of Exodus and involving the death of the eldest sons, was due to *macrocyclic trichothecene* mycotoxins (Marr and Malloy, 1996). During the middle Ages in Europe a common affliction known as St Anthony's fire was prevalent and caused thousands of deaths over a period of many centuries (Marasas and Nelson, 1987). More recent understanding has identified this condition as ergotism, produced by the ingestion of the ergots of *Claviceps purpurea*, a fungus occurring on staple food grains such as rye and wheat. It has also been suggested that the 'bewitchment' displayed by persons in the then British colony of Massachusetts in North America in 1692 and which lead to the Salem witchcraft trials and executions were a result of convulsive ergotism (Woolf, 2000).

The deaths during the Second World War of thousands in the former Soviet Union from the haemorrhagic syndrome known as alimentary toxic aleukia, caused primarily by T-2 toxin produced by *Fusarium sporotrichioides* and *F. poae* contaminating cereal overwintered in fields (Marasas & Nelson, 1987), and the discovery in 1960 of aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*, focused attention on the adverse human health implications of the secondary metabolites of fungi.

In 2004 in Kenya, 125 people died and nearly 200 others were treated after eating aflatoxin contaminated maize. The deaths were mainly associated with homegrown maize that had not been treated with fungicides or properly dried before storage. Due to food shortages at the time, farmers may have been harvesting maize earlier than normal to prevent thefts from their fields, so that the grain had not fully matured and was more susceptible to infection. (Howlett, 2008). The presence of fungal growth in stored cereal is a good indication that the cereal is contaminated with mycotoxins. The aim of this study was to identify mycotoxin and microbial contamination in cereals that were stored in several poshomills and stores.

2 Materials and Methods

The study was conducted in Muchatha and Gachororo, situated in Juja constituency. Juja constituency is largely a business zone as most of its bigger part is dry and not conducive for farming. Quarries and

coffee estates dominate the bigger part of the constituency. The main economic activity is subsistence farming and livestock rearing. Twenty cereal stores and poshomills were randomly selected from Muchatha and Gashororo and samples were collected. Questionnaires were then administered in each store and poshomill in order to obtain information concerning the storage conditions of grains. The food homogenate was made using peptone water. One milliliter of the food homogenate was then cultured on corn meal agar, sabouraud's dextrose agar, brain heat infusion agar and potato dextrose agar. The colonies formed were stained using lactophenol cotton blue stain and identified. The data from the questionnaires were analyzed using excel data spreadsheet.

3 Results

3.1 Identification of Organisms

The organisms that were identified in the study were *Fusarium spp, AIternaria spp, Acremonium spp, Trichophyton tonsurans, penicillium spp, Aspergillus flavus* and *chrysosporium spp.* Aspergillus spp were identified in most of the cereal samples (24.53%) as shown in Figure 1. Colonies of *Fusarium spp* were isolated from maize samples collected at poshomill 3 as shown in Figure 2. The colonies observed were whitish, yellow and brownish. Colonies of *AIternaria spp* were identified from samples that were obtained from poshomill 8 (Figure 3). The isolated colonies were grayish, white and cottony. The maize samples from poshomill 12 were found to be contaminated with *Acremonium spp* (Figure 4). The samples formed colonies which were white, rugged with capitate topography and rugged edges. Colonies of *Trichophyton tonsurans* were isolated in maize samples collected at poshomill 13 (Figure 5). The colonies were white granular and powdery. Colonies of *Penicillium spp* were isolated from maize samples collected at poshomill 16 (figure 6). The colonies had shades of green and sometimes white or yellow. Colonies of *Aspergillus flavus* were isolated from maize samples sampled at poshomill 17 (Figure 7). The colonies were grey, white with capitates topography and rugged edges. Colonies of *chrysosporium spp* were isolated from the maize samples collected at poshomill 19 (FIgure 8). The colonies were mucoid, cream and white with cottony parts.

3.2 Storage of Cereals

Figure 9 shows that 60% of the respondents agreed that they sometimes store their cereals at a maximum temperature of 50-70 degrees Celsius, 25% agreed that they store their cereals in the same condition at all times and 15% disagreed that they have been storing their cereals in the same condition. Majority of the respondents (90%) agreed to store their cereals in a dry condition as shown in Figure 10. 45% of the (90%) were certain that their stores were always dry. The other 45% agreed that they sometimes ensure that their stores are dry. About (30%) of the respondent agreed that they occasionally store their cereals in a room with proper ventilation, 50% of the same respondents agreed that they always keep their cereals in that condition while the rest disagreed. The above figures indicate that most cereals in several stores and poshomills around Juja are stored in good conditions. A quater (25%) of the respondents agreed that at times, they have been burning contaminated cereals from their stores. 10% of them were certain that they always do the same while the rest (65%) disagreed. There was however no significant statistical relationship between burning of contaminated cereals and fungal contamination of the stores ($p>0.05$).

4 Discussion and Conclusion

A number of moulds were isolated in this study. These include: *Fusarium spp., Alternaria spp, Chrysosporium spp, Trichophyton tonsurans, Aspergilus flavus, Acremonium and Penicillium spp.* This result correspond the study of P. Michael *et al*., (2005) who was able to obtain *Alternaria spp, Fusarium spp, Penicillium spp, and aspergillus spp* under Lithuanian climatic condition. Abramson *et al.,* (1987), (prior, 1981), Scott et al (1972) and (Young, 1982) were also able to show that the main toxigenic fungi associated with cereal grains are *caviceps spp, Fusarium spp, Penicillium spp, and apergillus spp.*

In this study, *Aspergillus spp* was isolated in most samples (24.53%). This is different to the study done by P. Michael et al who found out *Fusarium spp* as the common fungi isolated from wheat and barley (92.1%). Reports from various countries show that *Aspergillus spp* is the most common post harvest fungi. Saponaro and Madaluni (1960) reported the presence of *Aspergillus spp* in stored wheat grains in Italy, Wallace and Sinha (1962) in Canada, Kurata *et al.,* (1968), Tsunado (1970) and Tsuruta (1970) in Japan. These studies coincide with the one carried in Juja.

Aspergillus spp and *Penicillium spp* are more often considered as 'storage fungi'. They are known to form mycotoxins in stored grains and are usually not regarded as fungi that can produce mycotoxins before harvest (Frisvad, 1995; Wicklow, 1995; Hockings, 2003). Conversely *Aspergillus spp* and *Penicillium spp* are more often considered as 'storage fungi'. They are known to form mycotoxins in stored grains and are usually not regarded as fungi that can produce mycotoxins before harvest (Frisvad, 1995; Wicklow, 1995; Hockings, 2003).

In a study done by (Sauer and Borroughs, 1980) in USA, it was found out that *Alterneria spp* was the most common field fungus in Cereals, followed by *Fusarrium*. Both were present in most samples, and often in the same kernel. *Cladosporium, Epicoccum*, and *Helminthosporium* occurred less frequently. *Fusarrium moliniforme* was the most common field fungus in corn samples. Others were *Cephalosporium, Cladosporium, Trichoderma, Nigrospora, Alternaria, Mucor, Rhizopus, Chaetomium, Diplodia and Epicoccum*. The result of this study contradicted with the study above because there is a big difference in climatic conditions between USA and Kenya which determines the type of fungi you are likely to isolate in cereals.

Fusarium spp, mainly invades and develop on grains and other plant parts in the field. *Penicillium spp* and *Aspergillus spp* mainly invades and develop on grains after it has been harvested. Considerable efforts should be devoted to determine the identity, occurrence and development of *Fusarium, Penicillium* and *aspergillus* derived toxins on cereal grains in Kenya. Since most of poshomills and stores stored their cereals in good condition, contamination of cereals may be as a result of poor harvesting and management of cereals. This occurs mostly when the harvested maize is not dried properly before being transported to the store. *Aspergillus flavus* was isolated in some cereal samples (24.5%). This is an indication that some of the cereals supplied by Juja stores and poshomills may be contaminated with aflatoxins. This is a challenge to the ministry of Public health and other stake holders to create awareness on prevention of mycotoxin contamination as it poses a health risk to the consumers of the grains.

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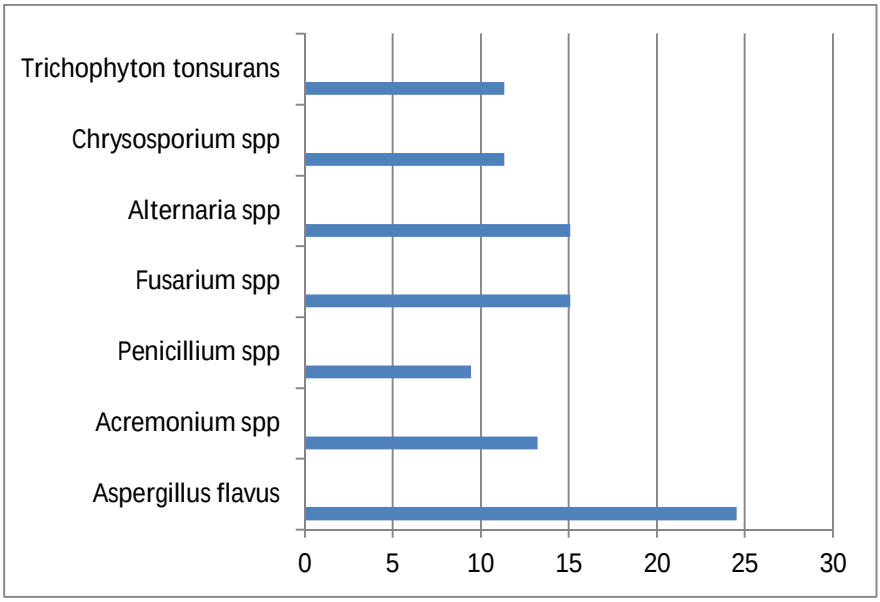
Appendix



X axis- Percentage of organisms

Y axis- Organisms isolated

Figure 1: Percentage of different organisms that were isolated

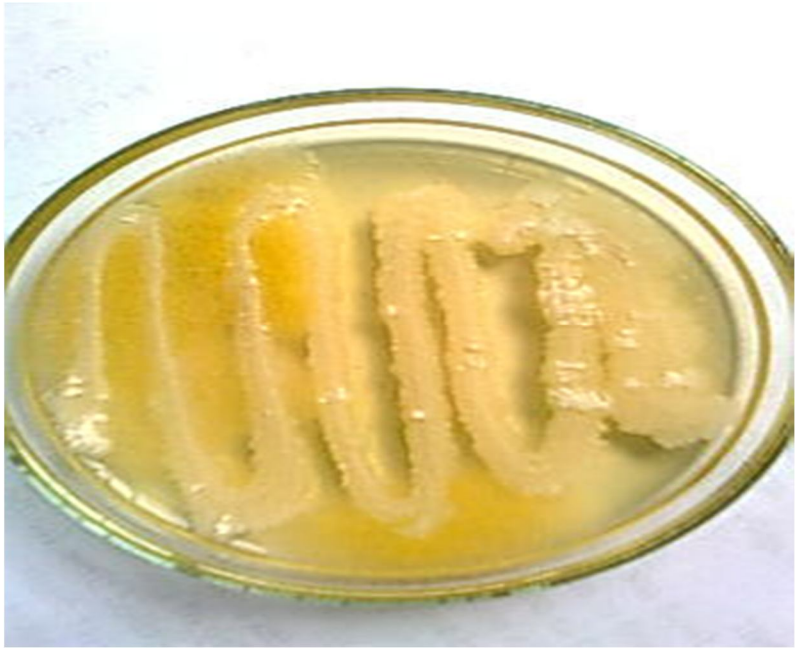


Figure 2: A surface culture growth of Fusarium spp, isolated from poshomill 3 maize samples. The colonies were whitish,yellow, brownish, pink or reddish

Figure 3: A surface culture growth of Alternaria spp isolated from poshomill 8 maize samples. The colonies were grayish, white and cottony

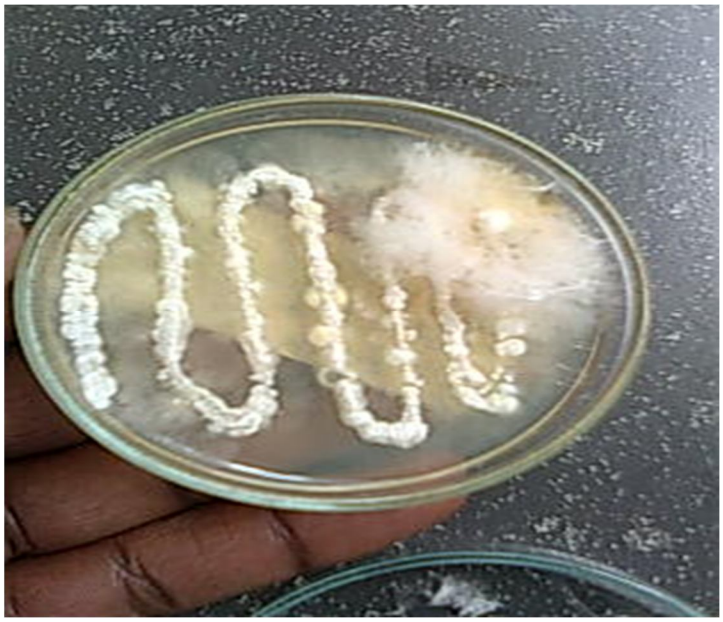


Figure 4: A surface culture growth of Acremonium spp isolated from poshomills 12 maize samples. The colonies were cottony white with capitate topography and rugged edges

Figure 5: A surface culture growth of Trichophyton tonsurans that was isolated from poshomill 13 maize samples. The colonies were white granular and powdery

Figure 6: A surface culture growth of Penicillium spp isolated from poshomill 16 maize samples. The colonies had shades of green and sometimes white or yellow

Figure 7: A surface culture growth of Aspergillus flavus isolated from the maize that was sampled at poshomill 17. The colonies were gray, white with capitates topography and rugged edges

Figure 8: A surface culture growth of Chrysosporium spp that was isolated from the maize sampled at poshomill 19. The colonies were mucoid, cream and white with cottony parts