ISOLATION AND IDENTIFICATION OF MYCOTOXIGENIC FUNGI IN MAIZE IN NAROK TOWN, KENYA

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SCHOOL OF SCIENCE

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DECLARATION

Candidate's declaration

I declare that this work is my own and has not been previously presented for the award of degree in any other university

Sign _____ Date _____

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DEDICATION

This work is dedicated to my family (my father Gibson, my mother Gladys and my siblings Sharon, Sabrina and Brawin) for their love, prayers, encouragement, patience and the immeasurable support during the entire period of my studies. God bless you all.

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I wish to acknowledge the following for making my research project a success. The Maasai Mara University for giving me the opportunity to advance my education. My sincere thanks to my supervisors, Dr. Eric Eshitera and Ms. Elizabeth Siameto for their immeasurable contribution to ensuring this piece came out the way it did. I wish to extend my gratitude to the entire staff in the Department of Biological Sciences: Simon Kirui, David Adhiambo and Doris Ndubi for assisting me in data and laboratory analysis respectively. Not also forgetting all my classmates who were always ready to extend a helping hand, this is for you: Thank you!

May our Heavenly Father bless you all abundantly.

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Abstract

The aim of this study was to identify the occurrence of fungal contamination in different samples of corn (*zea mayes*) grains in Narok Town. A total of 30 samples were analyzed by direct plating method on a quarter strength potato dextrose agar and focusing on mycotoxigenic fungi.pure cultures of *Aspergillus* isolates were sub cultured and transferred onto differential media; malt extract agar, czapek yeast extract and czapek dox agar for species identification. The colony were purified and identified using colony growth characteristics, colour on potato dextrose agar and microscopic characterization. The isolated mould included *Aspergillus, Fusarium, Penicillium* and *zygomycete*s. Significant difference was observed between the frequency of fungal isolates from different samples of maize grains. The results indicate possible health hazards in the long run for humans and animal consumption of such contaminated maize grains by mycotoxigenic fungi.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Corn is the world's third most important crop after rice and wheat. About half of this is grown in developing countries where corn flour is a stable food for most third world countries and corn stalks used as animal feed during the dry season (Roige *et al.,*2009). In Kenya, it is the stable food for most Kenyans both in rural and urban areas with an estimated consumption of 125kg per household. (Pingal 2001; Kimanya et al., 2008).

Mycotoxins are secondary metabolites produced by filamentous fungi growing on food crops. When ingested by humans or animals, the mycotoxins can cause detrimental effects to their health and in some cases death depending on the level and duration of exposure. Exposure could be through ingestion, skin contact or inhalation of spore borne toxins. They are odorless, tasteless, and colorless to the naked eye. The poisonous chemical components are capable of causing acute or chronic effects on human beings and these include the induction of cancer, birth effect, digestive system disorders, immune suppression, and impediment of the liver metabolism, reproductive dysfunction, liver cirrhosis and premature puberty in girls. Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha & Muthomi 2008). Mycotoxin producing fungi are ubiquitous (Murphy *et al.,* 2006) and can infect grains at all levels from production to processing and supply chains. A significant level of grain mould infection in the field is accelerated by conditions such as dampness during harvest, insect infestation, delayed harvest as well as improper post-harvest handling, transporting, drying and storage (Reddy *et al.,* 2013, Tiffany 2013).

Mycotoxigenic fungi are also commonly associated with oil seeds as groundnuts, soybeans, cowpea, as well as various cereals such as maize, wheat, barley, sorghum, oats and rye which are the main staple food for humans and raw materials in livestock feed. Sorghum *(Sorghum bicolor L. Moench*) ranks the fifth in the world's economic importance among cereal crops with an annual production of 60 million tons.

Moreover, like other agricultural products, cereals are naturally vulnerable to fungal growth that can affect the quality, aesthetics and technological properties as raw materials. This production and accumulation of mycotoxins causes economic losses to the country (Bennett *et al,* 2003).

Fungi exhibit greater species richness than most other organisms and are thus of significant environmental and economic importance (Varga *et al.,* 2011; Blackwell, 2011). Recent predictions based on molecular methods have suggested that there are 5.1 million fungal species (O'Brien *et al.,* 2005); however, only about 5% of the predicted filamentous fungal species have been described (Hawksworth, 1991).

Fungal growth is one of the main causes of food spoilage resulting mainly in economic losses and contamination through the synthesis of mycotoxins. Some species of the genera *Penicillium* and *Aspergillus* are known to form ochratoxin, but few of them are known to contaminate foods with this mycotoxin. Ochratoxin A (OTA) has been detected in food products such as wine, beer, grape juice, dried fruit, meat, figs, coffee and cereals (Bayman *et al.,* 2002; Cabanes *et al.,* 2002; Creppy., 2002; Gareis and Scheurer., 2000; Hussein & Brasel., 2001; Stefanaki *et al.,* 2003;

Taniwaki *et al*., 2003; Visconti *et al*., 2000). This mycotoxin has been shown to be a nephrotoxic, hepatotoxic, teratogenic and possibly carcinogenic for humans.

This study was aimed at evaluating the diversity of filamentous fungi and to investigate the presence of potentially mycotoxigenic fungi in samples taken from maize collected from various poshomills across Narok town.

1.2 Problem statement

Mycotoxins can adversely affect animal and human health and the impact is greatly felt in developing countries, they are able to provoke various disease symptoms (Varga *et al*., 2009). Aflatoxins are the most studied and widely distributed of the mycotoxins, They have been linked to both toxic and carcinogenic properties, posing serious threats to both animal and human health (Bennett and Klich 2003) and are of significant environmental and economic importance especially in Asia and sub-Saharan Africa (Groopman *et al.,* 2005).

Human deaths have occurred as a result of aflatoxicosis (Nyikal *et al.*, 2004). As a result of its toxicity, over 100 countries restrict the content of aflatoxins in the food and feed supplies (van Egmond *et al.,* 2007) and in turn restricting trade between countries.

1.3 Justification

No region of the world escapes the problem of mycotoxins and their fungi (Lawlor and Lynch 2005). The Food and Agricultural Organization (FAO), estimates that between 25% and 50% of agricultural crops worldwide is contaminated by mycotoxins (Wagacha and Muthomi, 2008). . Several surveys indicated the association of different species of *Aspergillus, Alternaria, Fusarium, Rhizopus, Penicillium, Rhodotorula, Heminsthoporium, Clasporium* etc. with the contamination of polished rice, wheat bread, tea, and nuts such as peanuts, pistachios and walnuts consumed in Africa and Asia (Kazemi et al., 2014).

In recent years, data on mycotoxins of maize in Africa have begun to accumulate with reports, for instance, from Benin, Kenya and Nigeria.

Within the vicinity of Narok town there are vast plantations of wheat as well as pockets of maize farms. A number of studies have been conducted on the mycotoxigenic contamination of the wheat produce in Narok but no data exists on fungal contaminants of maize, a national staple food, from the same region. Owing to the close proximities of the maize and wheat farms, mechanization in farming and detection of mycotoxins in wheat, there is the need to determine and characterize the presence of these fungal contaminants in maize available for consumption in Narok town.

1.4 Hypothesis

H1: Mycotoxigenic fungi is present in stored maize kernels in Narok Town

H0: Mycotoxigenic fungi not present in stored maize kernels in Narok Town.

1.5 Objectives

1.5.1 General objective

Investigate the presence and diversity of mycotoxigenic fungi in maize kernels in Narok Town.

1.5.2 Specific objectives

i) To determine the presence of mycotoxigenic fungi in maize kernels for sale in Narok Town

ii) To determine the diversity of filamentous fungi in maize kernels for sale in Narok Town

CHAPTER TWO: LITERATURE REVIEW

2.1 Fungal growth

Cereals are commonly spoiled by moulds of the genera *Aspergillus, Eurotium, Penicillium, Fusarium, Alternaria* and *Clasdosporium,* which are filamentous fungi (Filtenborg *et al.,* 1996). Moulds have often been associated with spoilage of dry foods and especially those that are stored under dump comditions (Parry and Pawser, 1995). These filamentous fungi are aerobic, and cause spoilage beginning from the surface with eventually mycelium penetrating deep into the food (Parry and Pawsey, 1995). These have a unique mode of growth characterized by extension of the hyphal tip (apical extension). Through apical extension, filamentous fungi can grow towards nutrients and penetrate solid substrates (Sietsma *et al.,* 1995; Wessels, 1993; Wessels, 1994). Enzymes excreted at the hyphal tip degrade substrate polymers such as starch and proteins. The uptake of organic nutrients is an energy dependent process that occurs close to the apex.

Fungal mycelium can be thought of as a "tube" with a rigid wall consisting of polysaccharides (Heath, 1995; Sietsma, *et al.,* 1995), small amounts of proteins, minor amounts of lipids, and a cytoplasm that is rich in proteins, lipids, and nucleic acids (Bartnicki-Garcia and Lippmann, 1982). The hyphal tip is initially highly plastic, but matures into a rigid wall less than 1 mm behind the tip (Sietsma, *et al.,* 1995; Wessels, 1986).

Filamentous fungi are more motile than unicellular bacteria and yeast. The effects of this motility are further enhanced by the ability of the organism to translocate cytoplasm, water and nutrients from older parts of the mycelia, leaving empty hyphae behind (Schneider and Paustian,

1986). The most important genera as regards to grain spoilage fungi are found among ascomycetes and deuteromycetes, the latter producing only asexual spores.

2.2 Factors affecting fungal growth

There are major factors affecting the growth of moulds and include temperature, nutrients, light, water and pH according to Moses et al., (2005) and they vary according to the type of fungal growth.

2.2.1 Nutrient

Nutrient requirement for various fungi vary, while some prefer simple sugars, others have the capacity to utilize complex sugars. Some thrive well on substrates with high sugar or salt content (Moses, 2005). Fungi that are found growing on natural substrate like grain or dung and the media is referred to as natural media. Synthetic media is commonly used for culturing of fungi in the laboratory and its ingredients are known (Warris and Somers, 2008)

2.2.2 Temperature

Most moulds are mesophilic thriving at temperatures of between 10^0C to 35^0C (Moses, 2005). Some are thermophilic growing at temperatures of above 45^0C while some are pyschrophilic and unable to grow above 20^0C. A few significant number are pyschotolerant and are able to grow both at freezing point and room temperature (Pin et al., 2009). As temperatures increase enzyme activity increases thereby promoting fungal growth but are inactivated by low temperatures (Poema, 2007).

2.2.3 pH.

Fungal growth is pH dependent, requiring a range of pH for optimal metabolism and growth. Most grow at a pH ranging between 3 to 7. pH affects the solubility of ions like magnesium and phosphate that coexist at a free state at low pH (REF). At higher pH they form an insoluble complex which reduces their availability to fungi. At low pH, permeability of fungal cell membranes especially to ionizing compounds is affected because the protoplasmic membrane becomes saturated with hydrogen ions thereby impairing the passage of cations (Panasenko, 2007).

2.2.4 Water

All moulds require water to grow but the amount required varies. Some require little amounts of moisture and are called xerophilic while those that require large amounts of water are called hydrophilic (Ruhlmann, 2007).

2.2.5 Light

While most moulds prefer or grow well in dark places, some alternate between light and darkness to produce spores. Light affects the growth rate, synthetic capability and formation of reproductive structures. It also affects the growth and movement and spores are released in response to light in some fungi (Shapton and Shapton, 2007).

2.3 Fungal metabolism

Primary metabolites are produced from metabolic pathways involved in the essential life processes of fungi (Campbell, 1984). These metabolic pathways such as glycolysis and the citric acid cycle are found in all eukaryotes. Fungal secondary metabolites have a more restricted

distribution and are often specific for individual genera, species, or even strains (Campbell, 1984; Larsen, 1994). Secondary metabolism is not directly involved in normal growth and is, thereby, regarded as non-essential for the survival of the fungus (Campbell, 1984). Examples of fungal secondary metabolites are antibiotics commonly used in medicine, such as penicillin and griseofulvin, as well as mycotoxins (Bennett, 1995). Mycotoxins constitute a diverse range of compounds from different precursors and pathways that are grouped together based on their toxicity to higher animals and humans. Some mycotoxins are produced by only a few fungal species, while others are produced by a large range of species from several genera (Smith *et al.*, 1984).

2.4 Mycotoxin production in cereals

The fungi commonly associated with grains are commonly divided into field and storage fungi (Lacey and Magan, 1991; Pitt and Hocking, 1997). The most important species under field conditions are *Alternaria alternata, Aspergillus flavus, Fusarium-* and *Cladosporium-species,* while *P. viridicatum, P. verrucosum, P. hordei, A. candidus, A. flavus* and *Eurotium* species are important storage moulds (Frisvad, 1994; Pitt and Hocking, 1997; Samson *et al.*, 1996). The factors that influence fungal growth, such as substrate composition, temperature, pH, atmosphere, redox potential, and microbial competition, also influence mycotoxin production (Frisvad and Samson, 1991). For many fungi the conditions that are required for mycotoxin production are more limiting than the range over which growth can occur (Frisvad and Samson, 1991; Lacey and Magan, 1991).

Penicillium-species are more common in temperate climate zones, such as that of Scandinavia, while Aspergillus-species prefers tropical climates (Frisvad and Samson, 1991; Pitt and Hocking,

1997). *A.flavus, A. parasiticus*, and possibly *A. nomius* are capable of producing aflatoxins, cyclopiazonic acid, and even maltoryzin and 3-nitropropionic acid (Frisvad, 1994). In temperate climates ochratoxin A from *P. verrucosum* and the trichothecenes from various *Fusarium* species are the most important mycotoxins (Frisvad, 1994). Ochratoxin A has been detected both in grain samples and in swine and human blood (Breitholtz *et al.,* 1991; Holmberg et *al.,* 1991; Holmberg *et al.,* 1990; Olsen *et al.,* 1993). Ochratoxin A contamination of grain has been observed to have both seasonal and geographical variations (Breitholtz, *et al.,* 1991; Holmberg, *et al.,* 1991; Holmberg, *et al.,* 1991). The same contamination is known to occur naturally in barley, rye, wheat, oats, rice, and corn in countries with temperate and hot climate in Europe, Canada, USA, Japan, and Australia (Frisvad, 1994). Ochratoxin A is known to have immunotoxic, nephrotoxic, teratogenic, and carcinogenic properties (Krogh, 1987; Kupier-Goodman & Scott, 1989). Some of the fungi commonly associated with ochratoxin A are *P. verrucosum* and *A. ochraceus* (Frisvad, 1994; Pitt and Hocking, 1997; Samson, *et al.,* 1996).

The trichothecene mycotoxin DON is produced by several fungal genera, but the genus *Fusarium* being the most significant (Smith, *et al.,* 1984). Eriksen and Alexander (1998) reported the field fungi *F. culmorum* and *F. graminearum* to be the most prominent DON producing *Fusarium* species. The relative dominance of these two species was found to br dependent on the temperature. The trichothecenes are cytotoxic, phytotoxic, and antifungal, in addition to their insecticidal properties as reported by Ciegler (1979).

Mycotoxin formation has been correlated with the presence of certain fungal volatiles. Zeringue *et al.* (1993) studied headspace volatiles from aflatoxigenic strains and nonaflatoxigenic strains

of ***A. flavus*** and found that the aflatoxigenic strains produced several $C_{I5}H_{24}$ compounds that the nonaflatoxigenic strains did not. The synthesis of trichothecenes and trichodiene, and other volatile sesquiterpenes, was correlated in both wheat kernels inoculated with *Fusarium* species (Jelen *et al.*, 1997; Jelen *et al.*, 1995) and in incubated grain spikes with natural *Fusarium* head blight infestation (Jelen *et al.*, 1997). Pasanen *et al.* (1996) used toxigenic and nontoxigenic strain of *P. verrucosum* and found that ketones consisted of more than half of the microbial volatiles produced by the toxigenic *P. verrucosum* strain, whereas more alcohols were formed by the nontoxigenic strain.

2.5 Global importance of maize

Maize is the most important cereal crop and staple food in sub-Saharan Africa and Latin America .All parts of the crop can be used for food and non-food products. Worldwide production of maize is approximately 785 million tons, with the largest producer being the United States, producing 42%. Africa produces 6.5% where the majority of maize production is rainfall dependent

The crop is one of the most important staple food and feed crops in the world. In developing countries it contributes directly to the enhancement of household food and nutrition security. It provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries. In parts of Africa and Mesoamerica, maize alone contributes over 20% of food calories (Gitu, 2006).

Currently, the demand for maize as livestock feed has grown tremendously. This has largely been driven by rapid economic growth in highly populated regions in Asia, the Middle East and

Latin America leading to increased demand for poultry and livestock products from more affluent consumers (Delgado, 2003). Maize grain has now become a crucial component in animal feed, and this added demand has driven up prices of maize grain and made it less affordable for poor consumers in several regions of the world. The maize feed market is growing especially in countries such as China and India, where economic growth is enabling many to afford milk, eggs, and meat. Rapid development in these countries is also driving up demand for maize as an industrial raw material while maize is a crucial ingredient in the bioethanol program in the USA (Delgado, 2003).

Maize is the foundation for food security in some of the world's poorest regions in Africa, Asia, and Latin America. This crop currently covers 25 million hectares in Sub-Saharan Africa, largely in smallholder systems that produce 38 million metric tons, primarily for food. Additionally 2.8 million ha is grown in South Africa, mainly in large-scale commercial production, much of it for animal feed (Smale et al., 2011).

Yields in low-productivity rain-fed environments are severely limited by an array of factors, as well as abiotic and biotic stresses. Losses due to abiotic stresses are frequently compounded by a high occurrence of diseases, insect pests and weeds, which on an average can reduce yields by more than 30 percent (Oerke, 2006). Six Maize diseases of global and regional significance include southern corn leaf blight *(Bipolaris maydis),* southern rust *(Puccinia polysora),* northern corn leaf blight *(Exserohilum turcicum),* common rust *(Puccinia sorghi),* gray leaf spot *(Cercospora* species), stalk and ear rots caused by *Diplodia*. Maize is susceptible to *Fusarium,* and kernel and ear rots caused by several *Fusarium* and *Aspergillus* species of fungi which also

contaminate grain with mycotoxins thereby reducing grain quality and safety. An estimated 54 percent of attainable yield is lost annually to diseases (16%), animals and insects (20%) and weeds (18%) in Africa (Oerke, 2006).

2.6 Importance of maize in Kenya

Maize is the most important staple crop in Kenya contributing 3% of Kenya's Gross Domestic Product (GDP) According to Kenya Maize Development Program (KMDP), maize is the primary staple food crop in the Kenyan diet with an annual per capita consumption rate of 98 kilograms contributing about 35percent of the daily dietary energy consumption. Maize in Kenya plays an integral role in national food security. It is the staple food crop in Kenya for both urban and rural areas with an estimated 1.6 million hectares under cultivation. Small-scale farmers in Kenya contribute 75% of the total maize produced in the country

The quantity of maize consumed in Kenya per person per year is high, resulting in greater possibilities of higher doses due to chronic exposure. The exposure can be associated to frequent outbreaks of aflatoxin contamination in maize. Fungal metabolites contaminating maize have been implicated in deadly epidemics in Kenya since 1981. *A. flavus* and *A. parasiticus* are most commonly implicated as causal agents of aflatoxin contamination (Probst *et al.*, 2007). Each family in Kenya has a garden if not a farm where they grow maize.

CHAPTER THREE: METHODOLOGY

3.1 Study design

A cross sectional study was done to determine the diversity of mycotoxigenic fungi in maize kernels obtained from maize stores in Narok town between the months of December 2015 to February 2016.

3.2 Study area

The study was carried out in Narok town, situated west of Nairobi on latitude $01^{0}05^{'}$S $35^{0}52^{'}$E. The town is in Narok County which supports Kenya's economy through its bulk production of cereals in the south west of the country, along the Great Rift Valley. Being the headquarters of Narok County, Narok town stands as the major centre of commerce, services, business and finance with a population of over 40,000 people, mostly Maasai community. The elevation of Narok is 1827 metres (5997 feet) in altitude and a temperature range between 8°C and 28°C. The County receives two rainy seasons with annual rainfall range from 500 mm to 1000 mm, with up to 1800 mm in higher altitudes (Gamba *et al*., 2012; Ministry of Agriculture, 1987). Agropastoralism is one of the main economic income activities around Narok town with most wheat and maize, done both in large and small scale. Maize is also grown within the same agro-ecological zones as wheat which is lower highland 3 (LH3) and lower highland 2 (LH2) (Ministry of Agriculture, 1987).

3.3 Survey and sample collection

A field survey of the town was conducted and it was determined that there were 11 poshomills around Narok Town. The larger Town was sub-divided into three sectors and randomly 1 poshomill was sampled from each sector.

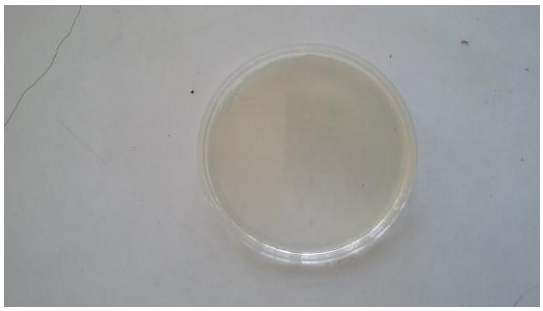
In total 30 batches of maize kernels were collected into polypropylene bags, sealed separately, labeled and transported to the analysis laboratory at Maasai Mara University. The samples were stored at room temperature until subjected to mycological analysis. In addition, unstructured interviews (Mugenda and Mugenda, 2003) were carried out to determine the origin, the mode of storage, hygiene and methods used to ensure that the maize did not deteriorate.

3.4 Assessment of mycotoxigenic incidence in maize kernels

3.4.1 Isolation using direct plating

Presence of fungal pathogens in the samples was examined by the direct plating technique. For each sample, five maize kernels were surface sterilized for 1 minute in 2.5% sodium hypochlorite (NaOCl) (Pitt and Hocking, 2009). After sterilization they were rinsed in changes of sterile distilled water for a minute in each change. The pieces of kernels were then inoculated aseptically onto a quarter strength potato dextrose agar (PDA) amended with 2 ml of lactic acid to suppress bacterial contamination and replicated four times for each. The plates were then incubated at a temperature of 31^0C for three days with daily monitoring. They were then observed for the presence of any fungal growth. From the fungal growth on the primary cultures, fungal isolates were then sub-cultured onto fresh PDA to generate pure colonies.

Taxonomic identication of the genus was thereafter carried out according to macro and microscopic characteristics of the colonies using identification keys by Barnett and Hunter (1998), Pitt and Hocking (1997) and Samson *et al.*,(1996).

a)  b) 

c)  d) 

Figure 3.1: Isolation of fungus from maize kernels. **a**) Petri dish with PDA. **b**) The inoculum on PDA **c**) Primary cultures **d**) The pure culture.

3.4.2 Identification of fungal isolates.

The obtained pure isolates were then inoculated into Czapek Yeast extract Agar, Czapek Dox Agar, PDA and Malt Extract Agar media. For best colony development and sporulation, the

mould being inoculated from the pure culture was placed at the centre. They were incubated at 30^0C for 7 days with constant monitoring.

Slides for light microscopy were prepared by spreading pure fungal isolates using a sterile inoculating in a drop of 95% alcohol previously applied on the slide. The alcohol in the smear was allowed to evaporate before staining the fungal mount with lactophenol blue. A drop of lactophenol blue was added to the slide and a coverslip carefully placed on the smear without allowing air bubbles to form. The excess stain around the slide was removed using blotting paper and the slide viewed under the microscope to observe spores of fungi, shape of vesicles and other microscopic characteristics of the isolates (Wheater, 2011). The characteristics of the isolates and other features were observed and recorded.

Species level identification was based on cultural and morphological characteristics like mycelia color, colony pigmentation, reverse colony color, spore shape, septation and sporophores (Klich, 2002). Morphological features were identified under the microscope and major remarkable microscopic features considered mainly the shape of vesicles (Larone, 1995). Contemporary diagnosis of *Aspergillus* species was based on the descriptions and keys of Klich (2002).

3.4.3 Data Analysis

The survey data was reported as percentage and presented in charts. Data obtained from isolations was subjected to Analysis of Variance (ANOVA) using the R statistical software and MS Excel (2007).

CHAPTER FOUR: RESULTS

4.1 Fungal distribution

A total of 368 fungi of different species were isolated from the 30 samples collected with a mean number of fungi in the area was found to be 3 with a standard error of 0.056 (2.89 to 3.11 at 95% confidence interval). The distribution of the fungi isolated from samples obtained from posho mills is as shown in Figure 4.1.

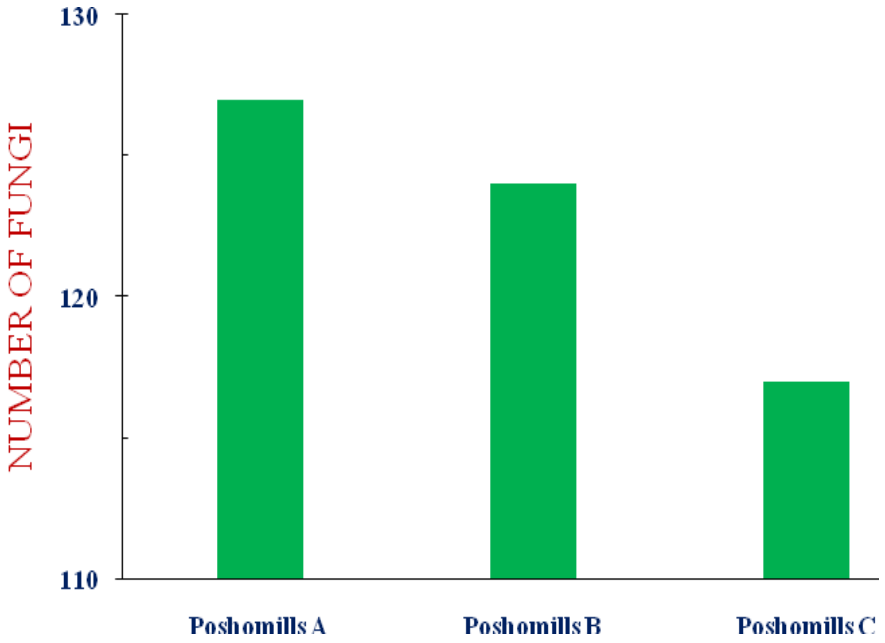


Figure 4.1: Number of fungi isolated from the sampled posho mills

It can be observed from the chart above that the number of fungal isolates from Posho-mill A was the highest, followed by posho-mill B and lastly posho-mill C. The test for the difference in the distribution of fungi in the three posho-mills showed that there is no significant difference in the distribution of fungi in the three posho-mills (F=1.7834, Df=2, P-value=0.1727)(Table 1).

Analysis of Variance Table

Response: Fungal

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Poshomill	2	1.317	0.65833	1.7834	0.1727
Replicates	3	2.067	0.68889	1.8661	0.1393
Residuals	114	42.083	0.36915		
Total	121	45.467			

Table 1: ANOVA analysis of difference of distribution of number of fungi isolated from the three posho-mills.

This shows that the posho-mill do not influence the distribution of fungal despite the fact that the number of fungal in the posho mills differ. There is no sufficient evidence for the difference of the same.

4.2 Distributions in terms of genus

Fungal pathogens isolated from sampled maize kernels were *Fusarium* spp., *Aspergillus* spp., *Trichoderma* spp., *Penicillium* spp.,and *Zygomycetes* spp. (Table 2).

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Genus	***Number***	***% Distribution***	***Average***	***Variance***
Penicillium	110	30.05	36.667	16.333
Aspergillus	84	22.95	28.000	43.000
Fusarium	130	35.52	43.333	201.333
Zygomycetes	40	10.93	13.333	2.333
Trichoderma	2	0.55	0.667	1.333
Total	**366**	**100%**		

Table 2: Distribution of isolated fungi in terms of genus

It was determined that the number of isolates of the Genus *Fusarium* (35.52%) had the highest frquency in the area, followed by *Penicillium* and *Aspergillus* (30.05% and 22.95%) respectively. *Trichoderma* spp. had the lowest frequency. Findings from the genera distribution was further subjected to ANOVA test for the existence of significant difference in the distribution. It was determined that at p= 0.00018, there was significant difference in the number in the fungal isolates based on the genus (F=17.1324, Df=4) (Table 3).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Genus	3622.93333	4	905.73333	17.13241	0.00018	3.47805
Error	528.66667	10	52.86667			
Total	4151.6	14				

Table 3: ANOVA analysis for existence of significant difference in genus distribution

This shows that the distribution of the fungal isolates based on different genus do significantly differ within the study area. Therefore, there does exist conditions within the area that favor the existence of fungus of certain genus and not others.

Further analysis on the identified genus distribution was done in relation to the three surveyed posho-mills as shown in Figure 4.2 below.

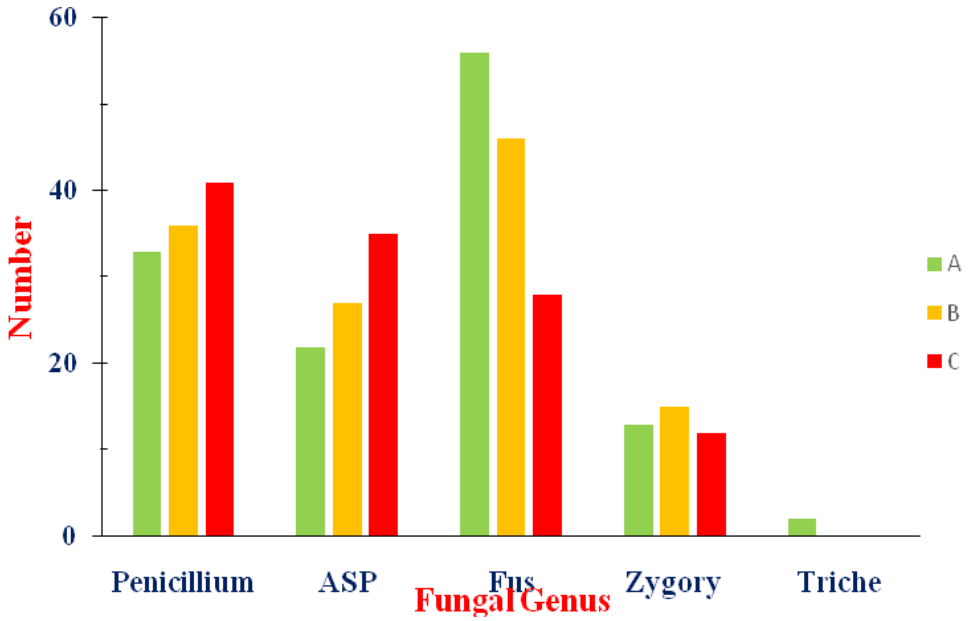


Figure 4.2: Genus distribution of isolated fungi according to surveyed posh mills

From the analysis, it was observed that: samples from posho-mill C had the highest number *Penicillium* and *Aspergillus* isolates while those from posho-mill B had the majority of *Zygomycetes* isolates. Posho-mill A was noted to have the highest number of isolates of the genus *Fusarium*. *Trichoderma* was only found in posho-mill A.

At p-value = 0.984, the test for existence of a difference between the number of fungal genus in the three posho-mills shows that there was no significant difference in the distribution of genus of fungal isolates the three posho-mills (F=0.01623, Df=2, P-value=0.984) (Table 4).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Poshomill	11.2	2	5.6	0.01623	0.983922	3.885294
Error	4140.4	12	345.0333			
Total	4151.6	14				

Table 4: Test for existence of difference between the number of fungi genus in the three posho-mills

This shows that there doesn't exist any suitable conditions within the posho-mills that might have caused the difference in the distribution of the different genus of fungal in the posho-mills. It is likely the sampled maize kernels were contaminated either at the fields or the storage area before they are brought to the posh mill for milling.

4.3 Distribution of fungal isolates based on species

The distribution of the fungal in terms of species was as shown in the chart below (Figure 4.3);

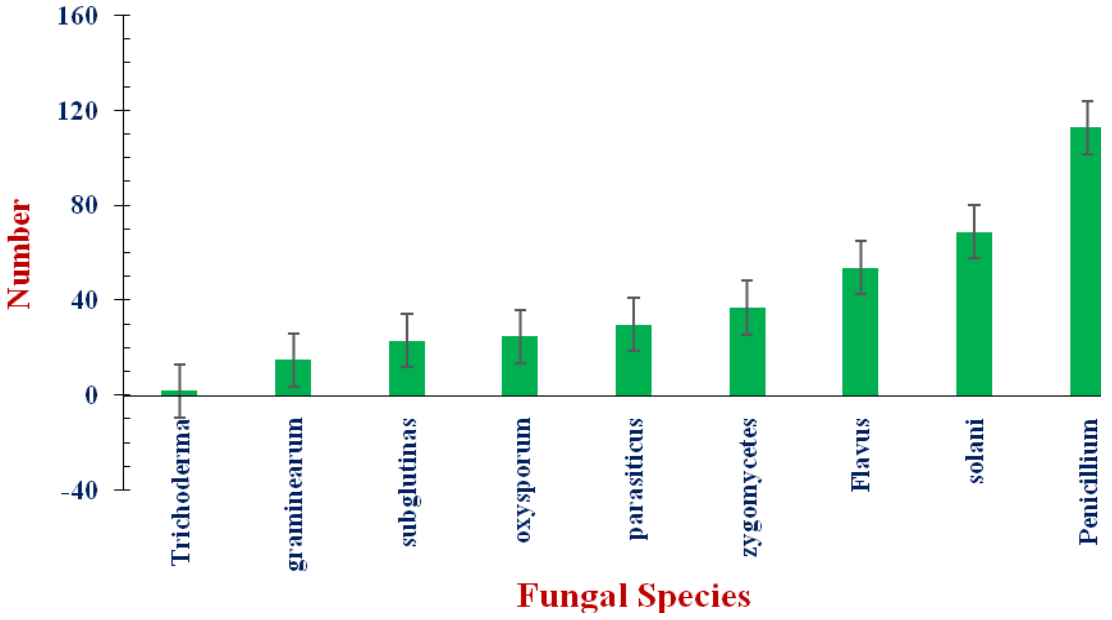


Figure 4.3: Species distribution of the inoculated fungal isolates

From the distribution chart in Figure 4.3, it was observed that *Penicillium* species were the predominant species at 30.71% followed by *F.solani* (18.75%) then the *A.flavus species* (14.84%). *Trichoderma* species were the fewest in terms of distribution in the area at 0.54%. In general the the four species of *Flavus* identified together take up the largest portion of 35.87%. An ANOVA analysis showed there was a significant difference in the distribution of fungal species identified from the cultured samples as shown in Table 5 (F=24.921, Df=8, P-value <0.001)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Species	75.42	8	9.428	24.921	2.051E-35	1.947
Error	405.18	1071	0.378			
Total	480.61	1079				

Table 5: ANOVA analysis in the distribution of the species of cultured fungal isolates

There is thus likelihood there exists conditions in the sampled areas that favor the growth of certain species of fungi more than others. The descriptive statistic for the same is summarized in the Table 6 below.

Species	***Number***	***% Distribution***	***Variance***
A.flavus	54	14.84	0.603
A.parasiticus	30	8.15	0.273
F.oxysporum	25	6.79	0.318
F.subglutinas	23	6.25	0.223
F.solani	69	18.75	0.683
F.graminearum	15	4.08	0.144
Penicillium	113	30.71	0.694
Zygomycetes	37	10.05	0.450
Trichoderma	2	0.54	0.017
Total	**368**	**100%**	

Table 6: Species distribution of the cultured isolates

The identified species were further analyzed for their distribution in accordance to the three sampled posho-mills as shown in Figure 4.4.

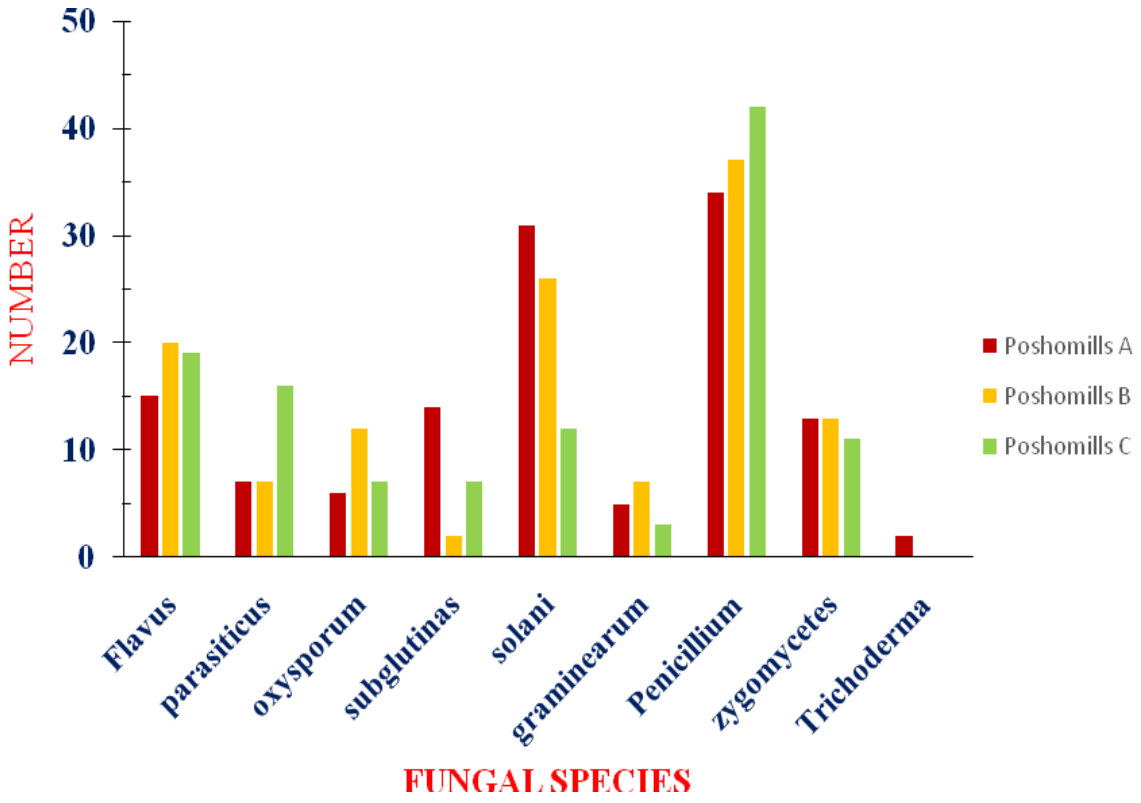


Figure 4.4: Fungal species distribution in the sampled posho-mills

Interestingly, posho mill A samples produced the most abundant of *F.subglutinas* and *F.solani* species of isolates. Similarly, *A. flavus*, *F.oxysporum* and *F.graminearum* species were the most from samples from poshomill B. Posho mill C samples has the biggest fractions of *A.parasiticus*, and *Penicillium* that were isolated and identified. The only incidence of the *Trichoderma* species observed were from samples obtained from poshomill A. However on further analysis by ANOVA, there was no significant differences in the distribution of the number of fungal species in the three posho mills at p=0.98 (F=0.0206, Df=2, P-value=0.98) (Table 7). This indicates that the poshomills do not influence the distribution of the species of mycotoxigenic fungus.

ANOVA

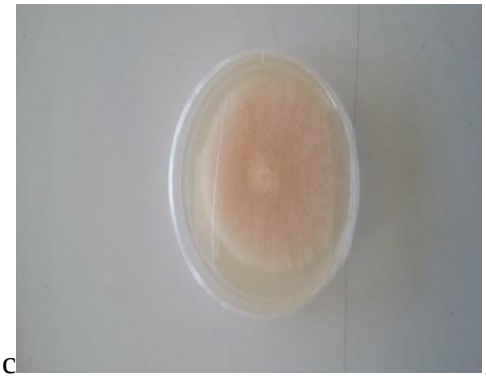
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Poshomills	5.85	2	2.93	0.0206	0.980	3.403
Error	3412.44	24	142.19			
Total	3418.30	26				

Table 6: ANOVA analysis of fungal species distribution in the three posho mills

.

a 

b 

c 

d 

e 

f 

g 

a) *F.oxysporum*

b) A. flavus

c) F. subglutinas

d)Penicillium

e)F. solani

f) zygomycetes

g) A.parasiticus

CHAPTER FIVE: DISCUSSION

Results from this study indicated that mycotoxigenic fungi are present in maize consumed in Narok Town, with eight significant isolates associated with maize kernels. These findings imply that consumption of maize contaminated with the identified fungal pathogens could pose serious health problems to the consumers. Several species of yeast and *Aspergillus* produce toxic substances which if consumed will cause health problems (Wood, 1992).

Fusarium spp.; *Aspergillus* spp. and *Penicillium* spp. were the most prevalent fungal pathogens isolated from maize kernel sampled. Other types of fungus like *Zygomycetes* spp. and *Trichoderma* spp. were also isolated, though *Trichoderma* spp, was the fewest and only localized in one sampled posho mill. The isolation frequency of these fungal pathogens in the sampled maize grain had a significant variation in their distribution across the three sampling areas in Narok town.

Thirty two per cent of Narok farmers rotate wheat and maize or grow the maize side-by-side with (Njeru *et al.,* 2016). Studies by Muthomi *et al.,* (2012) demonstrated a high incidence of *Fusarium* infection in wheat sown in the farms in Narok. Since all *Fusarium* spp. that cause Fusarium Head Blight (FHB) in wheat are capable of surviving as saprophytes on crop residues left in the farms (Parry *et al.,* 1994) and the crop rotation practices, it is conceivable that there is a link between the wheat crop disease and observed *Fusarium* contamination of maize across the town. Therefore, the reservoir for primary inoculum of *Fusarium* infection and contamination can be compounded by rotation of wheat crop with maize, a common practice in the study area.

A previous study in Kenya by Muthomi *et al.,* (2002) showed that most wheat cultivars available are susceptible to FHB. Susceptibility of wheat to FHB together with cropping systems employed by the small scale farmers could provide the fungus with host and inoculum that accounts for the predominant distribution of *Fusarium* spp. on maize from the three posho mills (35.87% of total fungal isolates). This predominance mirrors the aggressiveness of the fungus in colonizing and contaminating cereals in Narok farms.

A complex of 4 *Fusarium* spp were isolated and identified from the maize kernels with *F. solani* being the most common species. The *Fusarium* spp. isolated in this study were consistent with some that Njeru *et al.,* (2016) isolated from crop residues and soil sampled from wheat fields of different agro-ecological zones across Narok County. This suggests a persistent presence of the particular fungi species in the area or cross-contamination of the maize by the wheat crop, wheat plant residues or soil.

One of the species isolated from the maize samples, *Fusarium graminearum* has been noted to produce a variety of potent mycotoxins including deoxynivalenol (DON), zearalenone (ZEA) and fusarin C (Bhat and Miller, 1989; Keller, 2011). Based on the spectrum of *Fusarium* spp. identified in this study, there is a possibility of contamination of maize grains and grain-based foods with other toxins such as, nivalenol, T2-toxin, HT-2 toxin, Fusarenone-X, Diacetoxyscirpenol, enniatins, fusarin, moniliformin, which have been associated with human and animal toxicoses (Desjardins, 2006 and Speijers, 2004).

Penicillium and *Trichoderma* are prevalent fungal genera in soil, stems, heads, straw and grains (Muthomi et al., 2012). The sampled kernels are likely to have been contaminated with the *Penicillium* during drying process. The most affordable and commonly used method in drying is spreading the maize on a tarp in open spaces under direct sunshine. This method leaves the maize prone to exposure to soil and subsequent contamination by *Penicillium. Penicillium* is a producer of various mycotoxins such as patulin, mycophenolic, penicilic acid, roquefortins, marcfortine A, andrastin, gliotoxins and toxins of verruculogen/femitremorgen group (Garon *et al.,* 2006; O'Brien *et al.,* 2006). Therefore, there is a likelihood of a wide spectrum of mycotoxins contaminating maize in the sampled mills translating to potential health implications on human and who are consumers of the grain.

Overall results showed that at 22.99%, the *Aspergillus sp*. were the third most isolated species of mycoflora contaminant of the maize kernels. The *A. flavus* and *A. parasiticus* isolated in this study are most commonly implicated as causal agents of aflatoxin contamination (Probst *et al.,* 2007). Maize samples collected from agricultural markets and storage facilities from affected areas during the 2004 outbreak in Kenya (Muture and Ogana, 2005) were tested and determined to have *A. flavus and A. parasiticus* (Probst *et al.*,2007). The maize contamination event in 2004 led to an outbreak of aflatoxicosis and the subsequent death of more than 100 people. The presence of the two Aspergillus species of fungus in all the three posho mills tested could be an indicator of potential widespread cases of aflatoxicosis across the town. This would likely to be more common among consumers using posho mill C which had the most incidence of Aspergillus contamination (Figure 4.2).

Aspergillus flavus is the most frequently implicated causal agent of aflatoxin contamination of maize (Klich, 2007). The dominant prevalence of *A. flavus* was confirmed by the findings in this study. Fungal communities in Kenya associated with severe maize contamination and deaths in human populations have atypical structures, with the S strain of *A. flavus* highly dominant, and increasing incidence of the S strain associated with increasing contamination levels (Probst et al., 2010).

Aspergillus spp. can attack various commodities including groundnut, maize and spices amongst many others agricultural products. Affected grains may not appear overtly mouldy and producing fungus proliferates mostly in improperly stored grains of moisture content greater than 14%, relative humidity greater than70%, pH 4-6 and temperature 30-40^{o}C (Greekmore, 2012; Whitlow and Hagler, 2013). It is likely that the maize samples in the posho mills had been subjected to improper storage conditions that were conducive the proliferation of the fungi. Maize is also prone to field infections by *Aspergillus*; and crop debris have been noted as the primary source of inocula in the USA (Enyiukwu et al., 2014). The fungal sclerotia survive many years in the soil, germinate and produce numerous conidia during silking. Contaminations generally are more frequent in tropical zones with inclement weather situations; in such zones abound with poor traditional storage facilities (Miller, 1999) as is the case in Kenya. The amount of aflatoxin deposit in corn is influenced by high temperatures, high grain moisture content (15-18%), pH and nitrogen deficiency (John and Steve, 2010; Tiffany, 2013). Aflatoxins are heat stable and are known to target the liver but may target other organs based on their reactivity with DNA, RNA, enzymes and proteins. If ingested, aflatoxin contamination results diminished health of humans and domestic animals that consume the contaminated crops (Wu, 2010). The quantity of ingested

aflatoxins determines whether health effects are chronic (e.g., immune suppression, impaired child growth, abnormal fetal development, and cancer) or acute (e.g., hepatitis and jaundice, abdominal swellings, and death) (Cardwell and Henry, 2006; Gong *et al.*, 2004, Jiang *et al.*, 2008, Williams *et al.*, 2004).

The presence of the isolated species of mycotoxigenic fungi is indicative of factors that can lead to incidences of severe grain rot and in some cases poisoning. The significant level of grain-fungus infection observed can occur in the field being encouraged by damp conditions at time of harvest, insect infestations, delayed harvesting as well as improper post-harvest handling, transporting, drying and storage.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Farming practices have been shown to cause increase in the level of primary fungal inocula and contamination incidence and severity. Farmers in Narok County practiced: rotation of wheat with maize, growing wheat side by side with wheat, leaving wheat residues as standing hay for livestock, planting uncertified seeds and using simple land preparation methods that did not burry previous crop residues. These practices are known to contribute to the buildup of *Fusarium* spp that are the FHB primary inocula and lead up to high FHB prevalence and incidence. The continued presence of FHB in the wheat crop and persistent inocula in the farms could later lead to maize contamination. However, the posho mills sampled here was a quarter of the total number within the town thus the observed incidences of mycotoxigenic fungi were relatively low. This study therefore provides baseline information on the incidence and severity of mycotoxigenic fungus in maize kernels available in posho mills in Narok town.

Diverse *Fusarium* spp. were isolated from the maize kernels with *F. solani* and *F. subglutinas* being dominant in posho mill A while *F. oxysporum* and *F. graminearum* were abundant in posho mill B samples. The contamination with the Fusarium spp may suggest that farmers here grow wheat side by side with maize. As for *Aspergillus*, posho mill B samples were rife with *A. flavus* while *A. parasiticus* was dominant in samples from posho mill C. *Penicillium* spp were very common in posho mill C samples implying that the maize kernels had been contaminated with soil at some point.

6.2 Recommendations

Based on the findings of this study, the following are recommended:

i. The use of good agricultural practices that would discourage fungal growth and mycotoxin production would be necessary to reduce mycotoxin level in maize grains

ii. Drying of corns and cleaning of stores at the end of each season to reduce chances of infection and mould growth.

iii. Drying maize on mats and polythene sheets to avoid contact with soil surface (Muthomi *et al*., 2009)

iv. Avoid intercropping maize with other crops that are prone to mycotoxigenic contamination easily as this may spread its spores to maize leading to its contamination

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