DISTRIBUTION OF *FUSARIUM* SPECIES AND THE OCCURRENCE OF TOXIGENIC STRAINS OF *FUSARIUM VERTICILLIOIDES* AND *FUSARIUM PROLIFERATUM* IN NANDI COUNTY, KENYA.

by

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DECLARATION

The work described herein was undertaken at the University of Nairobi, Chiromo campus and is my original work as part of an ongoing project. Articles and texts cited have been acknowledged. The contents of this thesis have not been submitted previously to any other University in whole or in part, for the award of any degree or academic titles.

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DEDICATION

This thesis is dedicated to

My beloved mother Phoebe Kiaye

Who went to be with the Lord

In June 2011

You were my main anchor as I studied,

My encourager when I lost courage

And my home when I needed rest

I love you and miss you

And

My dear brother Franklin Kiaye

Who went to be with the Lord

In January 1998

You inspired me never to stop dreaming

To be courageous and adventurous

I love you and miss you

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My deepest gratitude goes to God, my Loving Heavenly Father, in the name of Jesus Christ for His favour, His love, His blessings. I acknowledge that apart from Christ, I can do nothing.

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LIST OF ABBREVIATIONS AND ACRONYMS

CD-ELISA	Competitive direct-enzyme linked immunosorbent assay
CLA	Carnation Leaf Agar
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organization
FB	Fumonisin B analogs
FDA	Food and Drug Administration
FUM1 gene	Fumonisin-producing gene
IARC	International Agency for Research on Cancer
IGS sequences	Intergenic spacer sequences
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KARI	Kenya Agricultural Research Institute
NaOCL	Sodium hypochlorite
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PMTDI	Provisional maximum tolerable daily intake
PPA	Peptone-Pentachloronitrobenzene Agar
ppb	parts per billion
ppm	parts per million
RNA	Ribonucleic acid
rRNA	ribosomal ribonucleic acid
rpm	rotations per minute
SNA	Spezieller Nahrstoffarmer Agar
SPSS	Statistical Package for the Social Sciences

TWA	Tap Water Agar
UV	Ultra violet
WHO	World health organization

ABSTRACT

Maize ear rot is one of the main concerns of maize production in Kenya. There have been numerous reports of fungal ear rot in maize crops, principal of which is *Fusarium* ear rot which results in an estimated 50-80% loss at harvest and mycotoxin contamination. *Fusarium* ear rot contamination can lead to fumonisin contamination in the grain and exposure to fumonisins has been linked to serious negative health effects in both humans and animals. While *Aspergillus* ear rot has received considerable attention, *Fusarium* ear rot and resultant fumonisin toxin have been ignored. Nandi County is one of the major maize-growing regions in Kenya and it has also had a high incidence of esophageal cancer reported. Fumonisins have been linked to high incidences of cancer.

This was a cross-sectional study carried out in Nandi County, one of the major maize-growing regions in Kenya to characterize the species of *Fusarium* found in Nandi County from soil, maize, sorghum, millet and animal feed samples and to identify toxigenic strains of *F. verticillioides* and *F. proliferatum.* Quantification of the amounts of fumonisins present in grain and animal feed samples using ELISA was also done to assess the extent of the fumonisin problem in Nandi County. The farming practices and farmers' perceptions on mycotoxin contamination was also assessed. Grain samples were collected from 255 households sampled and *Fusarium* isolated by direct plating onto *Fusarium*-selective Peptone Pentachloronitrobenzene Agar (PPA) after surface-sterilization. *Fusarium* isolates were sub cultured onto Potato Dextrose Agar (PDA), Spezieller Nahrstoffarmer Agar (SNA) and Carnation Leaf Agar (CLA) for morphological characterization. Isolates identified as *F. verticillioides* and *F. proliferatum* were further confirmed and tested for presence of fumonisin-producing (FUM1) gene using PCR. The maize and feed samples were tested for fumonisins using CD-ELISA method.

The most frequently isolated *Fusarium* species was *F. subglutinans* (24%), followed by *F. verticillioides* (22%), *F. gramminearum* (21%), *F. anthophilum* (17%), *F. proliferatum* (8.4%), *F. solani* (2.5%), *F. lateritrium* (2.5%), *F. oxysporum* (1.8%), and *F. acuminatum* (0.8%). All the 62 isolates morphologically identified as *F. verticillioides* amplified VERTF-1/VERT-R primers while all the 23 *F. proliferatum* isolates amplified Fp3-F/Fp4-R primers. More than half (53%) of *F. verticillioides* isolates from maize and feed samples had potential to produce fumonisins confirmed by amplification of the VERTF-1/VERTF-2 primers and this number was highly significant ($\chi^2_{(1,0.05)}=17.894$, $P<0.05$). Isolates of *F. proliferatum* that tested positive for FUM1 by amplifying Fpro-fum1 forward/Fpro-fum1 primers were fewer (17.6%). Fumonisin levels ranged 0.001 to 35.5 ppm with most samples (74%, n=226) having fumonisin quantities exceeding the joint FAO/WHO set limit of 2 µg/kg per person per day suggesting high exposure levels to humans and animals. While 59% of the fungal isolates from household maize were potential toxigenic strains, the percentage was higher for feed (96%) confirming that poor quality grains go into feed production.

The study concluded that there is widespread occurrence of toxigenic strains of both *F. proliferatum* and *F. verticillioides* in Nandi County and households were extensively exposed to fumonisins and other mycotoxins at high levels. There is therefore great need to give more attention to the fumonisin problem in order to educate farmers on affordable farming, grain-handling and storage practices that can be used to reduce the exposure to dangers posed by fumonisins.

Key Words: *F. verticillioides*, *F. proliferatum,* fumonisins, maize, FUM1 gene

CHAPTER ONE

1.1 INTRODUCTION

Maize, a staple crop in Kenya is grown throughout the country on about 1.4 million hectares of cropland that yield an estimated 28 million tonnes annually (Kedera *et al*., 1999). The tropical highlands of western Kenya, bordered on the west by Lake Victoria and on the east by the Great Rift Valley, are the major high production maize-growing regions. Maize production in developing countries is done mainly by resource-poor, small-holder farmers under low-input conditions where productivity is limited by rainfall and low soil fertility (Kedera *et al*., 1999).

Maize production is hampered by many biotic and abiotic factors both in the field as well as in store (Fandohan *et al*., 2003). Insect, pests and diseses cause an estimated 20 – 40% grain loss nationwide in Kenya (Kedera *et al*., 1999). Fungal contamination both in the field and post-harvest are also an important cause of deterioration and loss of maize; fungi can also cause about 50 – 80% of damage to farmers' maize during the storage period if conditions are favorable for their development (Fandohan *et al*., 2003). Maize ear rots are widespread and occur worldwide wherever maize is grown. One of the main concerns of maize production in Kenya is maize ear rot disease (KARI, 1998). The rots are caused by several fungal pathogens belonging mainly to genera of *Fusarium, Aspergillus, Penicillium, Stenocarpella* (also known as *Diplodia*)*, Nigrospora* and *Macrospora* (Olanya *et al*., 1997; Alakonya *et al*., 2008).

Previous studies on maize from Western Kenya (Kedera *et al*., 1999; Kedera *et al*., 1994) have readily isolated various ear rot fungi, the most frequently isolated being *F. verticillioides, F. gramminearum, F. subglutinans* and other *Fusarium* species. Both symptomless and rotten maize have shown ear rot contamination in these studies. The major and dominant ear rot fungus in Kenyan maize that has been isolated is *F. verticillioides* (Alakonya *et al*., 2008). The predominance of *F. verticillioides* in Kenyan

maize is a cause of concern. Other than fungal ear rots causing yield losses through rotting, they also reduce the grain quality by producing mycotoxins which have been shown to pose both human and animal health risks (Kedera *et al*., 1999). Fumonisins are a group of toxic secondary metabolites known as mycotoxins that are primarily produced by *F. verticillioides* and *F. proliferatum*, among fungi commonly associated with maize. They are considered among the most significant toxins produced by *Fusarium* species on agricultural commodities in the field or during storage (Nayaka *et al.,* 2010). More than ten types of fumonisins have been isolated and characterized; of these, fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin B3 (FB3) are the major types produced in nature. Fumonisin B1 being the most prevalent in maize as well as the most toxic, has been implicated in the cause of equine encephalomalacia in horses, pulmonary edema in swine and life-threatening cancer disease in humans (Alakonya *et al.,* 2008). Fumonisins have also been associated with high incidences of esophageal cancer (Wakhisi *et al.,* 2005) and the International Agency for Research on Cancer (IARC) has designated fumonisin B1 to be possibly carcinogenic as a class 2B carcinogen (IARC, 2002).

In westrn Kenya, the leading maize producing region in the country, farmers habitually leave their maize in the field upon maturity so that it may dry. The harvest season in most instances coincides with the second rains and the result is increased rotting when harvesting is delayed (Alakonya *et al*., 2008). Most small-holder farmers do not have sufficient knowledge regarding fumonisins or what causes fumonisin contamination in maize. Consequently, they do not incorporate any strategies to reduce potential contamination of their grain by fumonisins or other mycotoxins. This has resulted in both humans and animals being exposed to fumonisins from the maize they consume. Despite their importance, fungal ear rots have not received the attention they deserve. Sufficient data is lacking regarding the full extent of delayed harvesting on maize ear rot in the different maize varieties in Kenya, the different environmental factors prevailing in the maize-growing regions and their importance in the development of ear

rot in these areas. Consequently, management strategy and extent fumonisin exposure is not known.

This study was carried out to establish the distribution of *Fusarium* species in Nandi County and the extent to which toxigenic strains of *Fusarium* species, specifically *F. verticillioides* and *F. proliferatum*, are prevalent within the region. The study also assessed the farming practices used, the handling of grain, storage of grain and the individual perceptions on the mycotoxin problem within the households sampled in order to determine how informed the households were on the mycotoxin problem.

1.2 JUSTIFICATION

Maize is a staple food commodity in Kenya. The vast majority of maize consumed by the Kenyan populace is produced in the western region of Kenya of which Nandi County is a part. Maize is also a major component in livestock and poultry feed, and therefore, regular and indirect human exposure may occur through the consumption of animal products that contain mycotoxin residues. There have been extensive reports on aflatoxin outbreaks especially in Eastern and Central Provinces. In the case of fumonisins very little research has been done despite the numerous reports on the occurrence of maize head blight caused by *Fusarium verticillioides* (KARI, 1998) which indicates a likelihood of widespread fumonisin contamination in maize and maize products. Since there have been no reports of acute *Fusarium* toxicity outbreaks in Kenya, little has been done to deal with the long-term, chronic dangers that exposure to fumonisin-producing *Fusarium* poses. Fumonisins have been linked to esophageal cancer. There have been increased incidences of esophageal cancer in Nandi County, which indicate that there is possible chronic exposure to fumonisins in that area (Wakhisi *et al.,* 2005). This project surveyed the prevalence of *F. verticillioides* and *F. proliferatum* and their possible extent of fumonisin contamination Nandi County. In addition, the project also surveyed the distribution of other *Fusarium* species in the area and the farming practices as well as the farmers' perceptions on mycotoxins.

1.3 PROBLEM STATEMENT

Fumonisin contamination is widespread in Nandi County.

1.4 HYPOTHESIS

Toxigenic strains of *Fusarium verticillioides* and *F. proliferatum* occur in soil, grains and animal feed samples from Nandi County.

1.5 RESEARCH OBJECTIVES

The main objective of the study was to characterize *Fusarium* species found in Nandi County from soil, household maize, sorghum, millet, market-bought maize and animal feed samples and to determine the prevalence of toxigenic strains of *F. verticillioides* and *F. proliferatum* within the county in addition to assessing the effect of farming practices on fumonisin contamination and household perceptions on mycotoxins.

The specific objectives were to:

1. Characterize *Fusarium* species from soil, household maize, sorghum, millet, market-bought maize and animal feed in Nandi County.
2. Identify toxigenic strains (presence of FUM 1 gene) of *F. verticillioides* and *F. proliferatum* among the isolates recovered in objective 1 above using polymerase chain reaction (PCR) based assay.
3. Quantify the amount of fumonisins present in maize, sorghum, millet and animal feed samples using competitive direct enzyme-linked immunosorbent assay (CD-ELISA).
4. Assess the effect of farming practices on fumonisin contamination and household perceptions on mycotoxins.

CHAPTER TWO

LITERATURE REVIEW

2.1 IMPORTANCE OF MAIZE

Maize (*Zea mays* L.) is one of the major cereal crops grown throughout the world as a source of human food and component of animal feed and it annually occupies more than 120 million hectares of cropland globally (Munkvold, 2003). As a result of high yield per hectare, its ease in cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics, maize plays an important role in the diet of millions of Africans, particularly in Sub-Saharan Africa (Asiedu, 1989). Demand for maize is projected to increase by 50% globally by the year 2020, including 93% in Sub-Saharan Africa and 46% in East and Southeast Asia (Alakonya *et al*., 2008). Maize was introduced into East Africa more than 300 years ago and has adapted to diverse conditions of soil, climate and altitude (Kedera *et al*., 1999).

In Kenya, maize is a staple crop and is used primarily for direct human consumption. It is grown on about 1.4 million hectares of cropland that yield an estimated 28 million tonnes annually (Kedera *et al*., 1999). Like in other developing countries, much of the maize is grown in Kenya by resource-poor, small-holder farmers under low-input conditions where productivity is limited by rainfall and low soil fertility (Kedera *et al*., 1999). The major growing area is the tropical highlands of Kenya, bordered by Lake Victoria on the West and The Great Rift Valley on the East.

The projected increased demand for maize globally by the year 2020 is alarming given the current shortfall in maize supply due to the constraints that adequate maize supply faces. In the field as well as in store, many pests and parasites attack maize. Insects are most often considered the principal cause of maize losses (Fandohan *et al*., 2003), however, fungal contamination both in the field and post-harvest are also an important cause of deterioration and loss of maize. Fungi can also cause about 50 – 80% of damage to farmers' maize during the storage period if conditions are favorable for their

development (Fandohan *et al*., 2003). In Kenya, pests and diseases cause an estimated 20 – 40% grain loss nationwide (Kedera *et al*., 1999). Despite increasing demand for maize in Kenya, its production is on the decline due to drought, low soil fertility, pests and diseases. One of the main concerns is maize ear rot disease (KARI, 1998).

2.2 MAIZE EAR ROTS CAUSED BY FUNGI

Maize ear rots are widespread and occur worldwide wherever maize is grown. The rots are caused by several pathogens belonging mainly to genera of *Fusarium, Aspergillus, Penicillium, Stenocarpella* (also known as *Diplodia*), *Nigrospora* and *Macrospora* (Olanya *et al*., 1997; Alakonya *et al*., 2008). Most farmers habitually leave their maize in the field upon maturity so that it may dry, since the harvest season in most instances coincides with the second rains, there is increased rotting when harvesting is delayed (Alakonya *et al*., 2008). It has also been shown that delayed harvesting could ruin the grain quality in both susceptible and resistant varieties of maize and lead to increased mycotoxin contamination (Lauren & Smith, 2007).

When maize stovers are left on the soil surface throughout the cropping season for the new crop, there is little chance of breaking the disease cycle. Fungi like those belonging to *Fusarium* species are known to survive and sporulate on field debris and in soil, thus providing source innoculum for the new crop (de Nazareno *et al*., 1992). Ear rot pathogens of maize overwinter to a greater or lesser extent on host residues (Kommendhal & Windels, 1981). The occurrence of ear rot even when maize is harvested early could be due to high innoculum density of the ear rot pathogen in maize fields given that the crop is grown annually and also due to multiple infection routes associated with ear rot fungi (Olanya *et al*., 1997). Varied routes of infection such as through roots, seeds, silk and from kernel damage by insects make control of the disease difficult. Minimum tillage promotes *Stenocarpella* ear rot and stalk rot, however, *Fusarium* ear rot is not affected by tillage practices (Flett & Wehner, 1991).

Fusarium ear rot is a white to pink or salmon colored mold, which occurs anywhere on the ear or on scattered kernels. Infected kernels are often tan or brown, or have white or pinkish streaks or they may be red-brown or grey if badly rotten (Alakonya *et al*., 2008). The fungus enters through the silk, and the disease develops best when warm, wet weather occurs during the second and third week period before silking.

Birigwa *et al*. (2007) found that ear rots caused by *Stenocarpella maydis* were more common in Uganda than those caused by *Fusarium* species. *Fusarium* species were dominated by *F. gramminearum*, exhibiting pink coloration and *F. verticillioides* exhibiting pericarp etching and kernel popping. In addition, *F. gramminearum* was also found to be dominant in all seasons in the higher altitude (above 1800m above sea level) districts. Marasas *et al*. (1997) also found ear rots due to *F. gramminearum* at higher elevations, of 1800m above sea level with high rainfall and cool temperatures. In previous studies on maize from Western Kenya (Kedera *et al*., 1999; Kedera *et al.*, 1994) various ear rot fungi have been isolated, including, *F. verticillioides, F. gramminearum, F. subglutinans* and other *Fusarium* species. Both symptomless and rotten maize have shown ear rot contamination in these studies. The major and dominant ear rot fungus isolated in Kenyan maize is *F. verticillioides* (Alakonya *et al.,* 2008).

2.3 THE GENUS *FUSARIUM*

The genus *Fusarium* is a filamentous fungus that is one of the most ubiquitous and abundant fungal genera found in soil microflora and on freshly harvested and stored agricultural commodities such as cereals (Marasas, 1996). The genus contains many species of environmental, agricultural and human health importance (Maina *et al*., 2009). *Fusarium* species have been found in normal mycoflora or contaminants of commodities, such as cereals, rice, bean, soybean and oilseed (Sopterean & Puia, 2012). Among cereals, maize is the major food commodity in which natural occurrence of *Fusarium* and fumonisins have been reported from different parts of the world (Sreenivasa *et al*., 2006). *Fusarium* species are commonly considered as field fungi

invading more than 50% of maize grains before harvest (Robledo-Robledo, 1991). Most species are common in tropical and subtropical areas.

Fusarium spp. grow rapidly on potato dextrose agar at 25°C and produce woolly to cottony, flat, spreading colonies. The only slow-growing species is *F. dimerum*. From the top the colour of the colony may be white, cream, tan, salmon, cinnamon, yellow, red, violet or pink. From the reverse it may be colourless, tan, red, dark purple, or brown. A sclerotium, which is the organized mass of hyphae that remains dormant during unfavorable conditions, may be observed macroscopically and is usually dark blue in colour. On the other hand, sporodochium, the cushion-like mass of short conidiophores, is usually absent in culture. When present, it may be observed in cream to tan or orange colour, except for *F. solani*, which gives rise to blue-green or blue sporodochia. Hyaline, septate hyphae, conidiophores, phialides, macroconidia, and microconidia are observed microscopically. In addition to these basic elements, chlamydospores are also produced by *F. chlamydosporum, F. oxysporum, F. solani* and *F. napiforme.* Macroscopic and microscopic features such as colour of the colony, length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species. Molecular methods such as 28S rRNA gene sequencing may be used for rapid identification of *Fusarium* strains to species level. Polymerase chain reaction (PCR) protocols based on intergenic spacer (IGS) sequences have been extensively used for the accurate detection of *Fusarium* species (Jurado *et al.*, 2006).

According to Fandohan *et al.* (2003), several phytopathogenic species of *Fusarium* are found to be associated with maize including *F. verticillioides, F. proliferatum*, *F. graminearum* and *F. anthophilum.* Among them, *F. verticillioides* is likely to be the most common species isolated worldwide from diseased maize. In Africa generally, very little information is available on *F. verticillioides* occurrence on maize but there have

been reports that it is the most prevalent fungus on maize (Marasas *et al*., 1988; Allah Fadl, 1998; Kedera *et al*., 1999).

2.3.1 *Fusarium verticillioides*

Fusarium verticillioides is distributed worldwide and is an important plant pathogen with a wide range of hosts such as maize, sorghum, rice, millet, infecting plants in all stages of development, from the early hours of kernel germination to the time of harvest, including post-harvest deterioration of grains (Sreenivasa *et al*., 2008). It is transmitted horizontally and vertically to the next generation of plants via clonal infection of seeds and plant debris. Horizontal infection is the manner in which the fungus is spread contagiously and through which infection occurs from the outside via maize silks from saprophytic colonization of soil debris and insect vectors. The endophytic phase is vertically transmitted to the next generation of plants via clonal infection of seeds and may remain a symptomless biotrophic parasite throughout the entire growing season. The maize plant is therefore subject to infection throughout the entire growing season. Horizontal infection can be reduced by application of certain fungicides and it remains the reservoir from which infection and toxin biosynthesis takes place in each generation of plants (Bacon *et al*., 2001).

Fusarium verticillioides is an endophyte of maize establishing long-term associations with the plant; therefore, symptom-less infection can exist throughout the maize plant in leaves, stems, roots and grains at all stages of plant development, either via infected seeds, the silk channel or wounds. The presence of the fungus is in many cases ignored because it does not cause visible damage to the plant (Munkvold & Desjardins, 1997). This suggests that some strains of *F. verticillioides* produce disease in maize and others do not (Bacon & Williamson, 1992). Symptoms of *Fusarium* species infection are difficult to tell when the crop is still growing until the husks are removed and a white-pink cottony mould on kernels or pink kernel discoloration is seen. However, in some instances, these fungi infect kernels without any visible symptoms (Bigirwa *et al.,* 2007).

When weather conditions, insect damage and the appropriate fungal and plant genotype are present, it can cause seedling blight, stalk rot and ear rot (Sopterean & Puia, 2012). Late planting of maize with harvesting in wet conditions favors disease caused by *F. verticillioides* and the fungus' prevalence is considerably increased with wet weather later in the season. Repeated planting of maize and other cereal crops in the same or nearby fields favors fungal infection by increasing the fungal inoculum and insect population that attack the maize plant (Fandohan *et al*., 2003). *Fusarium verticillioides* appears on the corn ears as a cottony white to light grey filaments between the corn kernels. As the fungus progresses, the kernels turn grey to light brown (Sopterean & Puia, 2012).

In addition to causing plant diseases, infection by *F. verticillioides* can also result in contamination of kernels by fumonisins which can cause food safety problems for humans and animals and these fumonisins cannot easily be detoxified or removed from the grains (Nayaka *et al*., 2010).

2.3.2 *Fusarium proliferatum*

Fusarium proliferatum (Matsuma) Nirenberg occurs worldwide as a moderately aggressive pathogen of multiple plant species. The pathogen can also survive as an endophyte-like organism, without visible disease symptoms in the host. Apart from *F. verticillioides,* the species is considered as the most common maize pathogen, as well as the most effective producer of the polyketide-derived fumonisin mycotoxins (Rheeder *et al*., 2002). *Fusarium proliferatum* have been associated with soybean roots, and can occur on a wide range of economically important vegetable plants as well but its role in disease and relative aggressiveness as root rot pathogens is not known since pathogenicity has not been established for all reported species (Arias *et al*., 2011). Stankovic *et al*. (2007) reported the occurrence of *F. proliferatum* in onion and garlic. These results confirmed *F. proliferatum* is an important pathogen of these plants in Europe and that there is a potential risk of mycotoxin accumulation in contaminated

plants. There is also a possibility of the pathogen activity in other plants, including maize.

Fusarium proliferatum is capable of producing large quantities of mycotoxins. It has been reported to produce fumonisin B1, beauvericin, fusaric acid, moniliformin and fusaproliferin (Stankovic *et al*., 2007). Although *Fusarium* species have been isolated from maize showing typical rot symptoms at ears or stems, no report is available on the symptom development of *Fusarium* species on maize leaves (Nguyen, 2011). While fumonisin production by *F. verticillioides* has been well studied, there is limited data for *F. proliferatum* produced fumonisins (Rheeder *et al*., 2002).

As much as it has been reported that *F. proliferatum* causes plant diseases and produces fumonisins, there is limited data regarding this compared to the data that is available regarding *F. verticillioides.*

2.4 LOSSES CAUSED BY MYCOTOXIN CONTAMINATION

The term mycotoxin literally means poison from fungi. Mycotoxins are secondary metabolites produced by fungi that are capable of causing disease and death in humans and other animals. The most important mycotoxins include: Aflatoxins, Deoxynivalenol, Ochratoxin A, Fumonisins, Zearalenone, Patulin and T-2 Toxin. Since they are produced by fungi, mycotoxins are associated with diseased or mouldy crops, although the visible mould contamination may be superficial. The effects of some food-borne mycotoxins are acute, with symptoms of severe illness appearing very quickly. Other mycotoxins occurring in food have long-term chronic or cumulative effects on health, including the induction of cancers and immune deficiency.

Crops in tropical and subtropical areas are more susceptible to contamination than those in temperate regions because the high humidity and temperature in these areas provide optimal conditions for toxin formation. According to the Food and

Agricultural Organization (FAO) of the United Nations more than 25% of the world's agricultural production is contaminated with mycotoxins. This equates to economic losses estimated at $923 million annually in the US grain industry alone. Most countries have adopted regulations to limit exposure to mycotoxins, this has a strong impact on food and animal crop trade. The presence of mycotoxins is unavoidable and therefore testing of raw materials and products is required to keep food and feed safe. The presence of mycotoxins in food is often overlooked in Africa because of public ignorance of their existence, lack of regulatory mechanisms, dumping of food products, and the introduction of contaminated commodities into the human food chain during chronic food shortage due to drought, wars, political and economic instability.

Most toxigenic fungi survive in crop residue. Mycotoxin contamination in maize depends on the coincidence of host susceptibility, environmental conditions favorable for infection and in some cases, vector activity (Munkvold, 2003). Timing of harvest can have major consequences for the ultimate level of mycotoxin accumulation. In general, earlier harvesting results in lower concentrations of mycotoxins (Jones *et al*., 1981). While grain dries slowly in the field, moisture content remains high enough to allow continual development and toxin production by fungi that infect maize before harvest. Insects may also continue to feed on maize in the field late in the season, enhancing the ability of fungi to attack the kernels. On the other hand, if there is little pre-harvest infection, if insect activity is not a serious problem and if weather conditions are favorable for grain drying, it can be safe to allow field drying to proceed to desirable moisture levels (Munkvold, 2003). The direct impact of mycotoxins on the staple product quality constitutes an important danger for human health and among them, fumonisins produced by toxigenic *Fusarium* species on maize and maize-based foods and feeds increase the risk (Fandohan *et al.*, 2003).

The most important *Fusarium* mycotoxins that can frequently occur at biologically significant concentrations in cereals are fumonisins, zeralenone and trichothenes

(deoxynivalenol, nivalenol and T-2 toxin). These compounds occur naturally in cereals either individually or as specific clusters of two or more depending on the producing fungal species or strain (Sopterean & Puia, 2012). The major fungal species (widely distributed in cereal crops) producing these mycotoxins are:

- *F. gramminearum, F. culmorum* and *F. crockwellense* producing zeralenone, deoxynivalenone and related trichothenes.
- *F. sporotrichioides* producing T-2 toxin.
- *F. verticillioides* and *F. proliferatum* producing fumonisins.

However, other *Fusarium* species that have been reported to produce fumonisins include: *F. nyagami, F. anthophilum, F. dlamini, F. napiforme, F. thapsinum* and *F. globosum* (Sreenivasa *et al*., 2006).

2.5 PRODUCTION OF AND CONTAMINATION BY FUMONISINS

Fumonisins are a group of mycotoxins produced by *Fusarium* species like *F. verticillioides*, *F. proliferatum, F. nygamai* as well as *Alternaria alternata* f sp. *lycopersici*. The most abundantly produced member of the family is fumonisin B1. They are thought to be synthesized by condensation of the amino acid alanine into an acetate-derived precursor. Conditions favoring fumonisin formation are drought stress followed by warm, wet weather.

Maize is the product in which fumonisins are most abundant. The occurrence of fumonisin B1 has been isolated at high levels in maize meal and maize grits. Fumonisins have also been detected, but at lower levels, in, sorghum, rice and spices. Fumonisins can contaminate maize foods and feeds as a result of *Fusarium* invasion both before and after harvest (Doko *et al*., 1995). The contamination of maize by fumonisin-producing strains is not always easily detected in the field because fungal contamination may not cause visible disease symptoms (Bacon & Hinton, 1996) since *Fusarium* species can enter kernels unassisted by insects (Munkvold, 2003).

The fumonisins, a family of food-borne carcinogenic mycotoxins were first isolated in 1988 (Gelderblom *et al.,* 1988) from cultures of *Fusarium verticillioides* (Sacc) Nirenberg (previously known as *Fusarium moniliforme* Sheldon). During the same year, the structures of fumonisins were elucidated (Bezuidenhout *et al.,* 1988).

Fumonisins cause maize kernels to become brittle and crack more frequently than normal. The more the grain is handled, the more cracking and breaking occurs, giving the fungi more host material to grow on (Sopterean & Puia, 2012). According to Fandohan *et al*. (2003), fumonisins can cause disruption of lipid metabolism in animal cells that can lead to cellular deregulation or toxic cell injury and finally death; are phytotoxic and damage a wide variety of plants, including maize; [Although it is phytotoxic, fumonisin B1 is not required for plant pathogenesis (Bennet & Klich, 2003).]; are relatively heat stable and light stable; are also stable in stored products where these are kept airtight at very low temperatures; are unstable in contaminated products over time; and are water soluble.

High levels of fumonisins have been associated with warmer and drier climates and when weather conditions are favorable for *Fusarium* infection. At the same location, fumonisin contamination is not necessarily the same from one year to another (Fandohan *et al*., 2003). Hennigen *et al*. (2000) found that in Argentina, there was a marked difference in terms of fumonisin contamination for the same maize varieties during two consecutive seasons, due to the fact that environmental conditions may differ from one growing season to another. Studying the effect of climatic conditions on *Fusarium* occurrence in freshly harvested maize in different regions of the State of Parana in Brazil, Ono *et al*. (1999) detected higher fumonisin levels in maize samples from the Northern regions compared to the South. The authors suggested that it could be due to the difference in rainfall levels during the month preceding harvest (92.8mm in the South and 202mm in the North). Higher incidences of infections with fumonisins

seemed to have occured after periods of drought followed by heavy rains which caused stress to the plant immune system (Sopterean & Puia, 2012).

Fumonisins affect animals in different ways by interfering with sphingolipid metabolism (Wang *et al.,* 2006). They have been linked with leukoencephalomalacia (hole in the head syndrome) in equines and rabbits (Aziz *et al.,* 2007); pulmonary edema and hydrothorax in swine and hepatotoxic and carcinogenic effects and apoptosis in the liver of rats (Thiel *et al.,* 1991). Fumonisins have also been linked with various diseases associated with liver and kidney toxicity and carcinogenicity and immunosuppression (Wang *et al*., 2006). The occurrence of fumonisin B1 has been correlated with the occurrence of a higher incidence of esophageal cancer in humans (Colvin & Harrison, 1992; Lino *et al*., 2006, Wakhisi *et al*., 2005). Based on toxicological evidence, the International Agency for Research on Cancer (IARC) has designated fumonisin B1 to be possibly carcinogenic as a class 2B carcinogen (IARC, 2002). The United States Food and Drug Administration (FDA) have recommended 2-4 ppm of fumonisins on cleaned maize intended for mass production and processed maize products for human consumption. The recommended fumonisin levels on animal feed range between 10 and 30 ppm for most animals and a maximum of 100 ppm for poultry (FDA, 2001a, b).

Esophageal cancer is the ninth most common cancer in the world, and the fifth most common cancer in developing countries, with approximately 300,000 newly diagnosed patients every year (Parkin *et al*., 1993). A unique epidemiological feature of esophageal cancer is its uneven geographic distribution, with high incidence found within sharply demarcated geographic confines (Ahmed & Cook, 1969). These geographic 'hot spots' include areas in Northern Iran, Kazakhstan, South Africa, Northern China and Northern Italy, where annual incidence rates can exceed 200 per 100,000 (Zhang *et al*., 1990). On the African continent, a number of reports have documented a very high incidence of esophageal cancer in South Africa's Transkei region (Roth *et al*., 1993; Rheeder *et al*., 1992). In Kenya, reports have indicated that in certain regions of Central

and Western Kenya, esophageal cancer ranked as the first or second most common cancer (Ahmed & Cook, 1969; Cook, 1971).

The North Rift Valley region of Western Kenya was previously considered as a low incidence area, but data by Wakhisi *et al.* (2005) suggested that the incidence of esophageal cancer was high and this area and could be a 'hot spot' for esophageal carcinoma. The male to female ratio among esophageal cancer patients was approximately 1.5:1 and suggested that this may be due to the fact that males and females are more or less exposed to the same risk factors in this area. The inhabitants of this region typically consume maize at nearly every meal. Several hypotheses have been raised regarding the link between maize consumption and esophageal cancer. One hypothesis suggests that increased risk is derived from nutritional deficiencies, which are often seen in diets composed mainly of cereal grains. Dietary deficiencies in vitamins A, C, E, selenium and zinc have all been suggested as factors in the development of esophageal cancer. A second hypothesis proposes that the reliance on maize increases exposure to the mycotoxin fumonisin, produced by commonly occurring maize mould *F. verticillioides.* Fumonisins, in association with nitroamines have been shown to be statistically correlated to the prevalence of esophageal cancer in the Eastern Cape, a high incidence area of esophageal cancer in Southern Africa (Sydenham *et al.,* 1990) and China (Chu & Li, 1994). In Western Kenya, maize is the staple diet for this population. They also consume a locally brewed beer called '*busaa*', which is made of maize (with millet and sorghum) that may or may not be free of molds (Wakhisi *et al*., 2005). Most local brewers prefer moldy grain as they say it adds 'flavor' and 'aroma' to the brew and makes it more potent (Bigirwa *et al*., 2007), hence the possibility of increased exposure to fumonisins.

A report from India described an acute but self-limiting food-borne disease outbreak in villages consuming moldy grain containing up to 64.7 mg fumonisins/Kg (Shetty &

Bhatt, 1997). This survey was carried out in fifty villages belonging to six districts of Andra Pradesh in India. Nearly one thousand four hundred and twenty nine people were affected, and the disease was observed only in adults who consumed the mouldy grains of sorghum and not in pregnant women and children who did not consume sorghum. The symptoms caused were: transient abdominal pain, excessive borborygmus and diarrhea. All those affected recovered fully.

Substantial amounts of fumonisins (up to 74%) can be removed by simply washing maize grains, immersing them in water and by removing the upper floating portion, as contaminated grains generally have low density. In addition to immersing in water, removal of toxin is more significant (about 86%) if salt is added to the water during the process (Shetty & Bhatt, 1999). Likewise, sorting and removal of small, broken and visibly contaminated grains during processing can significantly reduce toxin levels (Charmley & Prelusky, 1995). Fermentation of maize does not seem to reduce fumonisin levels (Desjardins *et al*., 2000).

Most *F. verticillioides* strains do not produce the toxin, so the presence of the fungus does not necessarily mean that fumonisin is also present, however, the fumonisin toxin can develop in the kernels even with no apparent outward signs of mold (Sopterean & Puia, 2012). Molecular detection tools have been used to detect *Fusarium* species contaminanation in cereals (Bluhm, 2004; Patino *et al.,* 2004) and the presence of fumonisin-producing genes in these species (Sreenivasa *et al*., 2006). Testing of grains for fumonisin contamination is therefore the only positive method of verifying the presence or the absence of fumonisins.

The fumonisin-producing gene (FUM1 gene) cluster is responsible for the entire fumonisin biosynthetic pathway with the key enzyme, polyketide synthase, encoded by the FUM1 gene. It is responsible for synthesizing the fumonisin backbone subsequently modified by other enzymes (Stepien *et al.,* 2011). Recently, genes and other sequences

directly involved in secondary metabolism gained more attention in phylogenetic studies, as those have the advantage of possible usage in combined approaches to the diagnostics of mycotoxin production abilities (Proctor *et al*., 2009). Therefore, genes from the FUM cluster have been used in investigations as a good additional marker for phylogenetic studies of fumonisin-producing *Fusarium* species (Baird *et al*., 2008; Gonzalez-Jaen *et al*., 2004).

2.6 THE EXTRACTION OF FUMONISINS FROM GRAIN

Unlike most known mycotoxins, which are soluble in organic solvents, fumonisins are hydrophilic making them difficult to study. Usually they are extracted in aqueous methanol or aqueous acetonitrile. High-performance liquid chromatography with fluorescent detection is the most widely used analytical method.

Numerous studies have provided valuable data on the toxicology of purified fumonisins of the FB series, yet the search continues for other toxic fumonisin analogs which may also pose a health risk to humans and animals. Maize and maize-based products have been the main focus of fumonisin research due to the widespread contamination of this food source by relatively high levels of FB1, FB2, and FB3. As additional and new *Fusarium* species with various fumonisin producing capabilities are described from other agriculturally important crops, such as sorghum and millet, it becomes necessary to determine whether fumonisins also occur in these crops and, if so, to determine which analogs occur and at what levels.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study was carried out in 3 locations of Nandi County: Kilibwoni, Kaptumo and Laboret Locations which are located in Kilibwoni, Kaptumo and Kipkaren Divisions respectively (Figure 1).

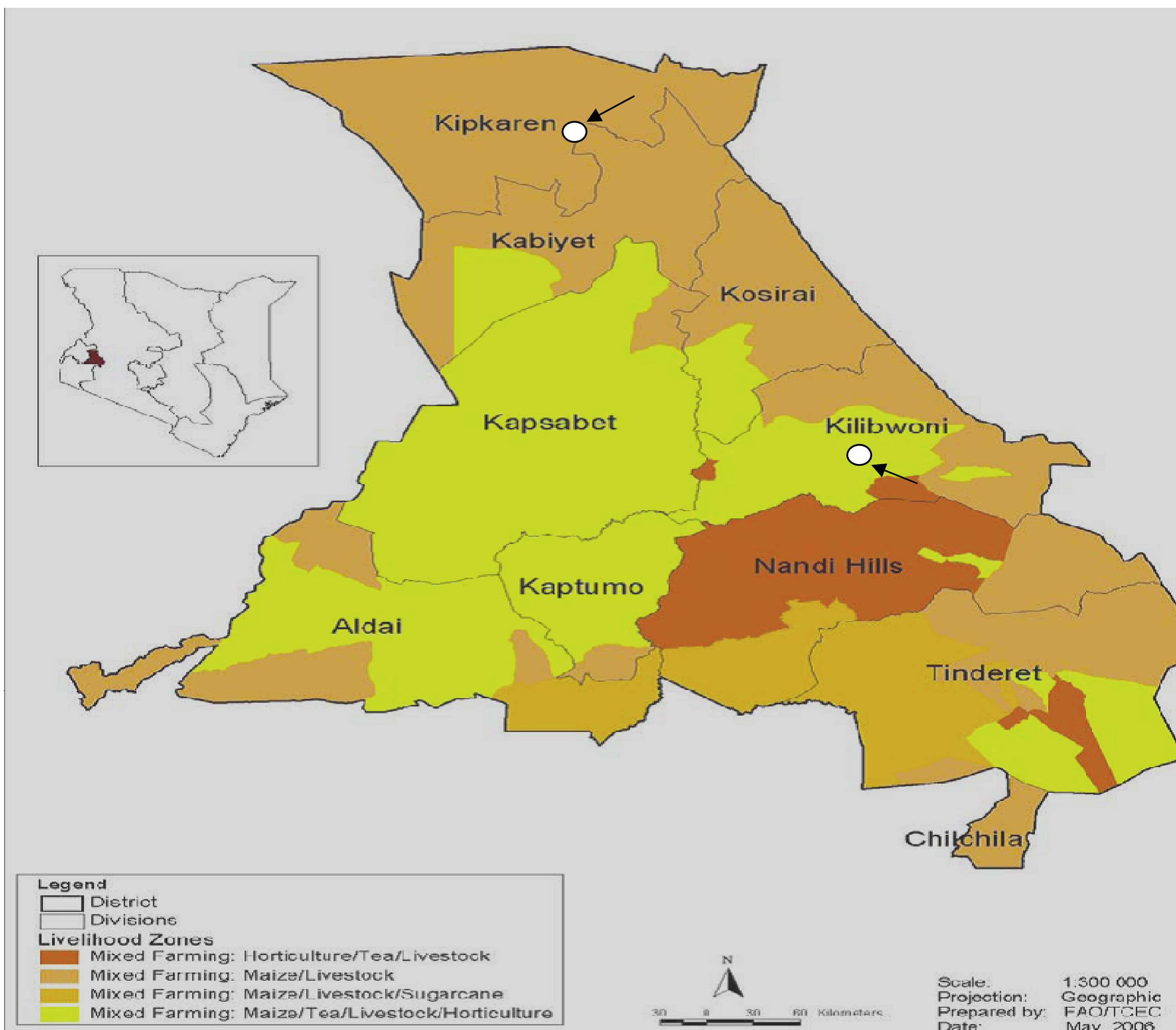


Figure 1: Map of Nandi County livelihood zones

Nandi County is located in the tropical zone between latitude 0° - 2° North and longitude 34° - 36° East at an altitude ranging between 2000m and 3000m above sea level. A vast portion of Nandi County has mountain forests and grassland vegetation and experiences an average of 1200-2000 mm of rainfall annually and an average temperature range of 17.5^o C to 20.0^o C all year. There is one major maize-growing season from the months of April to December. The major agricultural activity in this area is mixed farming with maize, tea, horticulture and livestock rearing.

3.2 STUDY DESIGN

The study was done as part of a larger study dealing with the incidence of mycotoxin contamination in the food and feed chain in Kenya. It was a cross-sectional study through 3 locations of Nandi County. These locations were Kilibiwoni, Laboret and Kaptumo. Nandi County was selected for the study of fumonisins due to the high incidence of esophageal cancer that has been reported. The locations were selected based on the widespread cultivation of maize and its large livestock population. The households sampled within these locations were selected based on the criteria that they were families that cultivated cereal crops, either maize, sorghum, or millet and kept cattle. Households within each selected location that fitted the criteria were listed and then randomized to select the specific households from which soil, household maize, market-bought maize, sorghum, millet and animal feed samples were collected for the study. The households that did not have maize in storage were represented by maize from the market from which they bought their maize. The animal feed samples collected were spoilt grain that was being used as animal feed in most households and did not refer to commercial animal feed.

Kilibiwoni location had a total of 412 households with a population of 1927, Laboret location had a total of 457 households and a population of 4533, and Kaptumo location had a total of 420 households with a population of 1500. Out of these, the qualifying

households that fitted the above-mentioned criteria were 221 households in Kilibiwoni, 351 households in Laboret and 343 households in Kaptumo.

In order to come up with a representative sample size, the formula described by Cochran (1969) used was:

$$n^o = \frac{Z^2pq}{e^2}$$

Where: **n°** is the sample size,

Z is confidence level 1.96,

e is desired level of precision 5%,

p is the estimated proportion of attribute present in the population i.e total number of qualifying households/grand total of households which comes to 0.28,

q is 1-p which is 0.72

This gave a total sample size of 304 households. This total number was corrected using the formula:

$$n^1 = \frac{n}{1 + (n^o - 1) / N}$$

Where: **n¹** is the corrected population figure,

n° is the sample size,

N is the grand total of the households

This corrected figure came to 255 households that were sampled to represent Nandi County. To distribute the 255 households proportionately among the three locations, the qualifying households in each location were divided by the grand total of qualifying households in Nandi County and multiplied by 255. This gave Kilibiwoni 61, Laboret 98 and Kaptumo 96 households.

The assistance of the District and Divisional teams was sought to collect samples and get the required cooperation from the selected households. The district team comprised the District Veterinary Officer, District Agricultural Officer, District Public Health

Officer and staff from the District Officer's office. The Divisional team comprised staff from the veterinary, animal production and agricultural extension offices.

3.3 COLLECTION OF SAMPLES

Samples of soil from current maize farm, stored maize grain samples and *posho*-mill and market maize samples, as well as sorghum, millet and animal feed samples were collected to detect the occurrence of *Fusarium* species. A questionnaire (appendix C) was administered to the selected households to capture the post-harvest storage methods used and their perceptions on mycotoxins.

3.3.1 COLLECTION OF MAIZE, SORGHUM, MILLET AND ANIMAL FEED SAMPLES

Ten maize cobs (from those stored for less than two months) from the farmer's store were collected and from each cob, at least 20 kernels were taken. In cases where the maize was already shelled, the maize was sampled to improve sampling homogeneity by subdividing each storehouse into upper, middle and lower layers, with five sites located diagonally within each layer. Approximately four grammes of maize kernels from each of these sites was collected and mixed into one 60g composite sample. Thus, each composite sample collected consisted of 15 sub-samples (3 layers and 5 points within each layer). The kernels were then collected in sterile brown paper bags. All the samples were stored at 4° C to prevent further post-harvest accumulation of moulds and fumonisins prior to analysis (Cardwell & Cotty, 2002). The collection of sorghum, millet, market maize and animal feed samples was also carried out in the same way that shelled maize samples were collected.

3.3.2 COLLECTION OF SOIL SAMPLES

Four quadrants were laid on the farm where the maize, millet or sorghum that was collected above was planted. Five sub-samples of top soil up to four centimeters deep using a trowel (sterilized with sodium hypochlorite between each sample collected) was

randomly collected from each quadrant in sterile brown paper bags (500g paper bags). The 20 sub-samples were pooled to get a composite soil sample of 50-60g. The soil was then oven-dried for two days at 48° C in an air oven, cooled and hammered to remove clods and homogenized by hand mixing using sterile gloves. They were then stored in sealed plastic bags at room temperature (22-26° C) until used (Cardwell and Cotty, 2002). In cases where maize was in the field at the time of soil sampling, observation was made for those with mouldy growth and then sampled.

3.4 LABORATORY ANALYSIS

3.4.1 ISOLATION AND IDENTIFICATION OF *FUSARIUM* SPECIES FROM SOIL

The soil samples were assayed using serial dilution plating using 0.1% Tap Water Agar (TWA). One ml of the soil suspension that had been diluted from 10^{-2} to 10^{-4} was spread on the surface of *Fusarium*-selective Peptone-Pentachloronitrobenzene Agar (PPA) media with three replicates for each dilution factor was used to recover *Fusarium* isolates from the soil according the method described by Leslie & Summerell (2006). The dilution plates were then incubated at 25˚C for seven days and observed daily for colony counting, after which the resulting *Fusarium* colonies on PPA media were transferred to Potato Dextrose Agar (PDA) media and incubated for three days at 25˚C to regenerate the *Fusarium* colonies.

A very dilute innocula, of scrapped mycelia, from the colonies on PDA was prepared and spread on two percent TWA plates in order to obtain monosporic cultures. Germlings were then subcultured onto Potato Dextrose Agar (PDA) media, Spezieller Nahrstoffarmer Agar (SNA) and Carnation Leaf Agar (CLA) media plates for growth and identification (appendix A). Most of the *Fusarium* species forming sporodochia with robust, uniform macroconidia on the CLA were used for identification (Leslie & Summerell, 2006).

PDA cultures were used to assess pigmentation and gross colony morphology, whereas cultures grown on SNA were evaluated for microconidia which were more abundant and diverse on this medium, and for chlamydospores which were common and more rapidly produced on this media. All the pure isolates subcultured on CLA were incubated for seven to ten days in a sporulation chamber under black light to induce sporodochia formation on the carnation leaves.

Fusarium was identified to the species level where morphological features were used as basis for identification (Nelson *et al*., 1983). After identification the single spore cultures were stored in agar slants of SNA in screw capped bottles at 4ºC and in sterilized soil in screw cap bottles.

3.4.2 ISOLATION AND IDENTIFICATION OF *FUSARIUM* SPECIES FROM GRAIN AND ANIMAL FEED SAMPLES

Kernels from each sample was surface sterilized by immersion in 2.5% NaOCl for one minute, rinsed in three washes of sterile distilled water for 20 seconds, and then five sterilzed seeds were directly plated onto PPA medium in triplicates according to the method described by Castella *et al*. (1999). Fungal growth of colonies on maize kernels were visualized using stereo-binocilar microscope (Magnus M24) counted and identified to genus level. These kernels were incubated at 25ºC for five to seven days, and one colony per kernel was transferred to PDA medium for identification based on morphology by the system of Nelson *et al*. (1983). The colonies were then transferred onto SNA, PDA and CLA from 2% TWA as described for soil samples above.

The isolation frequency (Fr) (Gonzales *et a*.,1995) of *Fusarium* isolates from soil, various grain and animal feed samples in the three locations was calculated as:

$$\mathbf{Fr\ (\%)} = \frac{\textbf{Number of samples infected with } \textbf{\textit{Fusarium}} \textbf{ species}}{\textbf{Total number of samples analyzed}} \times \mathbf{100}$$

3.4.3 IDENTIFICATION OF TOXIGENIC STRAINS BY POLYMERASE CHAIN REACTION

DNA from isolates of *F. verticillioides* and *F. proliferatum* was extracted and the approximate DNA concentration determined using the methods described by Sambrook *et al.* (1988). PCR was then done to confirm the identity and toxigenicity of the isolates.

I) DNA EXTRACTION

To obtain DNA from each of the identified *F. verticillioides* and *F. proliferatum* species, single-spored isolates were grown for six days at 24ºC in 50 ml of Potato Dextrose Broth (PDB) (Difco) on a rotary shaker at 150 rotations per minute (rpm). The mycelia were harvested from the cultures by pouring the liquid media containing fungal growth through a sterile cheese cloth. The culture filtrate was allowed to drain briefly for 30 minutes and the filtered mycelia was then washed twice with sterile distilled water, while still in the filter funnel. The washed mycelia samples were removed from the funnel using a clean sterile spatula then placed on a pad of clean paper towels and blotted dry. The mycelia samples were then placed into sterile two mililitre Eppendorf tubes to which 700µl of CTAB buffer and sterile glass beads were added. The tubes were then put in a Miller at a frequency of 30/sec for 10 minutes. In cases where the milling did not appear fine enough, the tubes were returned to the Miller for a second time.

After milling, the samples were incubated in a Clifton water bath for 1 hour at 65˚C. Six hundred microlitres of phenol was then added to each sample and then shaken by inverting the tubes vigorously. The tubes were then loaded into the Mikro 200R centrifuge and run at 14000 rpm for 20 minutes. Approximately one ml of top aqueous layer was removed and added to a new tube to which 600µl phenol:chloroform was added and centrifuged for 20 minutes. Approximately one ml of the top aqueous layer was removed and transferred to a new tube. To this, 600µl of chloroform was added

and centrifuged for 20 minutes. Approximately one mililitre of the top aqueous layer was removed and transferred to a new tube and the process repeated once more with 600µl chloroform. After centrifuging, 550µl of supernatant was aspirated to a new tube to which 60µl 3M sodium acetate (pH 8) was added. Eight hundred microlitres of ice cold isopropanol was then added to fill the tube. The tube was then inverted a few times and samples incubated overnight at 4˚C. After incubation at 4˚C, the samples were centrifuged at 14000 rpm for 10 minutes at 4˚C. The supernatant was then discarded and the resulting DNA pellet dried in an oven with lids open at 55˚C for 30 minutes.

II) DETERMINATION OF APPROXIMATE DNA CONCENTRATION

The dried DNA pellet was re-suspended in 80µl sterile distilled water and 5µl RNase (1mg/ml) was added to remove any RNA contamination and was incubated overnight at room temperature (25˚ C). To determine the approximate concentration of DNA, one µl of uncut DNA was run on one percent Agarose gel, stained with ethidium bromide and run at 70 volts for 45 minutes and then the gel plate was checked using the UV transilluminator. The amount of fluorescence provided a rough estimate of the DNA concentration. The samples that showed adequate concentration of DNA were stored at -20˚ C until PCR could be carried out.

III) PCR MIXTURE AND CONDITIONS

Polymerase chain reaction was performed using PTC – 100® Peltier Thermal Cycler (Bio-Rad Laboratories, Inc.). The PCR mixture (25µl) contained two µl of DNA sample, 2.5µl 10X PCR buffer, one µl 25mM magnesium chloride, one µl 2 mM dNTPs, 0.5µl 20pmol of each forward and reverse primer and 0.5 µl (3U/µl) of Taq DNA polymerase and topped up to 25µl with double distilled water.

The primers used to confirm the species *F. verticillioides* were VERT-F-1 (forward primer) and VERT-R (reverse primer) and to confirm *F. proliferatum* species, Fp3-F

(forward primer) and Fp4-R (reverse primer) were used. The primers used to determine the IGS sequences for fumonisin production in species of *F. verticillioides* were VERT F-1 (forward primer) and VERT F-2 (reverse primer). In the case of *F. proliferatum* species, Fpro-fum 1 (forward primer) and Fpro-fum 1 (reverse primer) were used.

The forward primer VERTF-1 (5′- GCG GGA ATT CAA AAG TGG CC -3′) designed by Patino *et al.* (2004) was used with VERT-R (5′- CGA CTC ACG GCC AGG AAA CC-3′) to identify *F. verticillioides* strains at the species level. The isolates were tested using the PCR specific assay for potential fumonisin-producing *F. verticillioides* with the set of primers VERTF-1 (5′- GCG GGA ATT CAA AAG TGG CC −3′) and VERTF-2 (5′- GAG GGC GCG AAA CGG ATC GG −3′) as described by Patino *et al*. (2004) to differentiate potential fumonisin producing *Fusarium* species. The expected PCR amplicon sizes were 1016-bp and 400-bp, respectively.

A pair of primers, Fp3-F (5′CGGCCACCAGAGGATGTG 3′) and Fp4-R (5′ CAACACGAATCGCTTCCTGAC 3′) (Jurado *et al.* 2006), specific to *F. proliferatum* was used for species identification. Another set of primers specific to the *fum1* gene (involved in fumonisin biosynthesis), Fpro-fum1 forward (5′-CCATCACAGTGGGACACAGT-3′) and Fpro-fum1 reverse (5′-CGTATCGTCAG CATGATGTAGC-3′) (Bluhm *et al.*, 2004), was used to determine the fumonisin producing ability of *F. proliferatum*. The expected amplicon sizes were 230 bp and 183 bp, respectively.

The PCR conditions for FUM 1 regions in tests for ability to produce fumonisins include 94°C for four minutes for initial denaturation, followed by 35 cycles of denaturation at 94°C for one minute, primer annealing at 58°C for one minute, primer extension at 72°C for one minute. The final extension was set at 72°C for 10 min. Ten µl of the PCR product was electrophoresed on 1.5% agarose gel, stained with ethidium bromide at 70

volts for 45 minutes, illuminated and documented using Biorad UV Transilluminator (Patino *et al.*, 2004).

3.5 ANALYSIS OF FUMONISINS IN SELECTED GRAIN SAMPLES USING COMPETITIVE DIRECT-ENZYME LINKED IMMUNOSORBENT ASSAY (CD-ELISA)

The collected household maize and animal feed samples were tested for the presence of fumonisins.

3.5.1 EXTRACTION OF FUMONISINS

The presence of fumonisins was determined using the CD-ELISA Kit from Thermo Electron Coorporation, China. A negative control (comprising of 'non-infected' maize) and a positive control were included. To prepare the control for fumonisin content pretesting, semi-processed maize grains were fermented in sterile tap water, dried and then ground with a blender to fine powder. A sub-sample (five grammes) was weighed out and extraction of solution for fumonisin testing carried out alongside the samples. Sub-samples of maize samples selected from households were each ground and five grammes weighed into a screw cap glass vial. Then 25 ml of 70% methanol was added, mixed by vortexing for five minutes and then filtered using Whatman No. 1 filter paper. The extract was diluted 1:14 with distilled water.

3.5.2 ELISA ANALYSIS OF FUMONISINS

Quantification of fumonisin B1 was by use of ELISA, using commercial standards (Thermo Electron Coorporation, China). Fumonisin levels were determined at 450 nm as described by Usleber *et al.* (1994) and Gathumbi *et al.* (2001). Fifty µl each of the prepared samples, standard solution and negative control solution (maize without fumonisins), were pipetted into separate duplicate wells. Then 50µl each of enzyme conjugate and anti-fumonisin antibody solution was put into each well. The reagents were mixed gently by tapping the plate and incubated for 30 min in the dark at room

temperature. The plates were washed three times using distilled water. Then 100µl of Substrate/Chromogen was added to each well, mixed by tapping and incubated for 15 min in the dark at room temperature. One hundred microlitres of stop solution was then added into each well, mixed gently by tapping the plate and then absorbance measured at 450nm within 10 minutes of reaction.

An ELISA kit was used for this assay and the sample and controls (positive and negative) were allowed to compete with the conjugate for the antibody binding sites. After the washing step, a substrate was added which reacted with the bound enzyme conjugate to an end produce color. The color intensity was read in a microtitre-plate reader (Biotek Instrument, USA) to yield optical densities. The controls were used to establish the standard curve and the sample optical densities were plotted against the curve to calculate the exact concentration of the toxin in parts per million (ppm). The detection limits for FB1 ranged 0.001 ppm to 5 ppm.

3.6. DATA ANALYSIS

Statistical analysis was done using SPSS. Qualitative and quantitative binary Chi square tests for equality of proportions (Pearson's Chi-squared test) were used to compare frequencies of occurrence of isolates.

CHAPTER FOUR

RESULTS

4.0 FUNGAL GENERA ISOLATED

Seven genera of fungi were isolated from all three locations sampled in Nandi County. Out of all the genera isolated, only *Rhizopus* was isolated from Kaptumo location otherwise all the others were found in all the three locations. The genera isolated include: *Fusarium* (61%, n=704), *Diplodia* [also known as *Stenocarpella*] (12.6%, n=704), *Penicillium* (13.5%, n=704), *Aspergillus* (6.7%, n=704), *Alternaria* (3.7%, n=704), *Trichoderma* (2.2%, n=704) and *Rhizopus* (0.3%, n=704). These were isolated from various grain, soil and animal feed from Nandi County in all the three locations sampled as shown in table 1 below.

Table 1: Frequency of isolation of fungal genera

Fungal Genera	Location in which household is located			Total
	Laboret	Kilibwoni	Kaptumo	
Fusarium	98(23)	160(37)	171(40)	429
Diplodia (Stenocarpella)	5(6)	22(25)	62(69)	89
Penicillium	16(17)	37(39)	42(44)	95
Aspergillus	23(49)	5(11)	19(40)	47
Alternaria	4(15)	13(50)	9(35)	26
Trichoderma	5(31)	6(38)	5(31)	16
Rhizopus	0(0)	0(0)	2(100)	2
Total	151(21)	243(35)	310(44)	704

The values are frequencies while those in brackets are row percentage

The frequencies of isolating different genera varied significantly within locations sampled in Nandi County ($\chi^2(12, 0.0001)=61.805$, $P<0.0001$).

Isolation frequency of fungal genera from Nandi County from a total of 704 isolates is shown in the pie chart, Fig. 2 below. Kaptumo location had the highest frequency at 44%, followed by Kilibwoni location at 35% and then Laboret location at 21%.

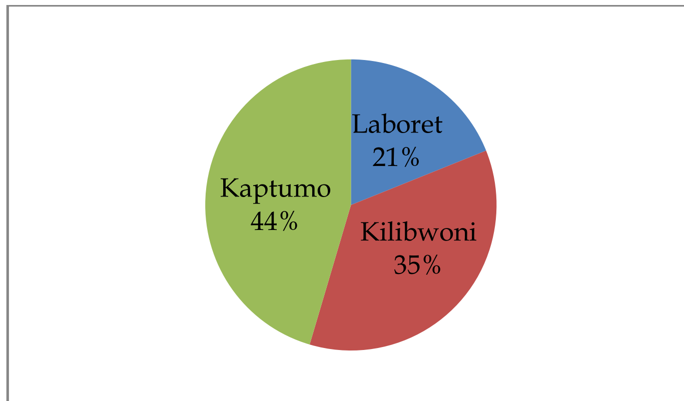


Figure 2: Fungal species isolation from locations sampled

4.1 *FUSARIUM* SPECIES ISOLATED

Fusarium species were isolated from household maize, animal feed, sorghum and millet, market-bought maize and soil samples in Nandi County from the three locations of Kilibwoni, Kaptumo and Laboret. A total of 437 *Fusarium* isolates of various species were isolated from the substrates sampled. The most frequently isolated *Fusarium* species from household maize samples, animal feed samples and market-bought maize samples was *F. subglutinans,* 24% (n=211), 27% (n=71) and 42% (n=19) of all *Fusarium* species isolated from the 3 substrates respectively. The most frequently isolated *Fusarium* species from the other substrates were *F. oxysporum* (41%, n=123) and *F. andiyazi* (38%, n=13) from soil and sorghum and millet substrates respectively. Table 2 shows the species isolated from the different substrates in the 3 locations sampled.

Table 2: Effect of media on isolation of *Fusarium* species from different substrates

Substrate from which *Fusarium* was isolated	*Fusarium* species	Location in Nandi District			Total
		Laboret	Kilibwoni	Kaptumo	
Maize	*F. verticillioides*	3(7)	17(40)	23(53)	43
	F. proliferatum	2(12)	11(69)	3(19)	16
	F. gramminearum	17(37)	21(46)	8(17)	46
	F. subglutinans	15(30)	14(28)	21(42)	50
	F. anthophilum	9(22)	14(33)	19(45)	42
	F. oxysporum	1(50)	0(0)	1(50)	2
	F. lateritrium	6(100)	0(0)	0(0)	6
	F. solani	2(40)	0(0)	3(60)	5
	F. acuminatum	0(0)	1(50)	1(50)	2
	Total	55(26)	78(37)	78(37)	211
Soil	*F. subglutinans*	0(0)	0(0)	3(100)	3
	F. anthophilum	5(70)	1(15)	1(15)	7
	F. oxysporum	11(22)	24(47)	16(31)	51
	F. lateritrium	2(18)	1(9)	8(73)	11
	F. solani	2(22)	3(33)	4(45)	9
	F. acuminatum	0(0)	4(40)	6(60)	10
	F. dimerum	1(10)	2(20)	7(70)	10
	F. equiseti	2(18)	3(27)	6(55)	11
	F. simitectum	2(100)	0(0)	0(0)	2
	F. poae	1(11)	3(33)	5(56)	9
	Total	26(21)	41(33)	56(46)	123
Animal feed	*F. verticillioides*	4(21)	7(37)	8(42)	19
	F. proliferatum	3(42)	2(28.5)	2(28.5)	7
	F. gramminearum	0(0)	10(71)	4(29)	14
	F. subglutinans	3(16)	6(32)	10(52)	19
	F. anthophilum	2(33)	1(17)	3(50)	6
	F. oxysporum	0(0)	0(0)	3(100)	3
	F. lateritrium	1(100)	0(0)	0(0)	1
	F. solani	0(0)	2(100)	0(0)	2
	Total	13(18)	28(40)	30(42)	71

Sorghum and Millet	*F. proliferatum*	0(0)	2(100)	0(0)	2
	F. gramminearum	1(100)	0(0)	0(0)	1
	F. subglutinans	2(50)	2(50)	0(0)	4
	F. anthophilum	0(0)	1(100)	0(0)	1
	F. andiyazi	1(20)	2(40)	2(40)	5
	Total	4(31)	7(54)	2(15)	13
Market-bought maize	*F. verticillioides*	0(0)	2(67)	1(33)	3
	F. proliferatum	0(0)	0(0)	1(100)	1
	F. gramminearum	0(0)	1(33)	2(67)	3
	F. subglutinans	1(12.5)	6(75)	1(12.5)	8
	F. anthophilum	2(67)	0(0)	1(33)	3
	F. solani	0(0)	0(0)	1(100)	1
	Total	3(16)	9(47)	7(37)	19

The values are frequencies while those in brackets are row percentage

Fusarium subglutinans was isolated from all substrates sampled, 24% from household maize (n=211), 3% from soil (n=123), 27% from animal feed (n=71), 31% from sorghum and millet (n=13) and 42% from market-bought maize (n=19). *Fusarium verticillioides* was isolated from household maize (20%, n=211), animal feed (18%, n=61) and market-bought maize samples (16%, n=19); with Kilibwoni (9%, n=301) and Kaptumo (11%, n=301) locations recording the highest numbers of this species. *Fusarium proliferatum* was isolated from market-bought maize (5%, n=19), sorghum and millet (15%, n=13), household maize (8%, n=211) and animal feed (10%, n=71); Kilibwoni (5%, n=314) recorded the highest frequency of this species, while both Kaptumo and Laboret had a 2% (n=314) isolation frequency. There was a significant presence of *F. gramminearum* in isolates from household maize (22%, n=211), animal feed (20%, n=71), sorghum and millet (8%, n=13) and market-bought maize (16%, n=19).

4.1.1 *FUSARIUM* SPECIES ISOLATED FROM HOUSEHOLD MAIZE SAMPLES

A total of 211 isolates of *Fusarium* were recovered from household maize isolates from Nandi County. The most frequently isolated species were *F. subglutinans* (24%), *F.*

gramminearum (22%), *F. verticillioides* (20%), followed by *F. anthophilum (*19%). Other *Fusarium* species isolated were *F. proliferatum* (8%), *F. oxysporum* and *F. acuminatum* (both 1%), *F. lateritrium* (3%) and *F. solani* (2%).

In Kilibwoni and Laboret, *F. gramminearum* was the species most frequently isolated, 27% (n=78) and 31% (n=55) respectively. In Kaptumo, the isolation frequency of *F. gramminearum* was only 10% (n=78). The species most frequently isolated in Kaptumo was *F. verticillioides* (29%, n=78). In Laboret and Kilibwoni, the isolation frequency of *F. verticillioides* was at 5% (n=55) and 22% (n=78) respectively. The isolation frequency of *F. proliferatum* was 4% (n=78) in Kaptumo, 4% (n=55) in Laboret and 14% (n=78) in Kilibwoni. There was a significant difference ($\chi^2(16, 0.05)$=46.236, $P<0.05$) in the isolations of *Fusarium* species from household maize in the three locations sampled.

4.1.2 *FUSARIUM* SPECIES ISOLATED FROM SOIL SAMPLES

A total of 123 *Fusarium* species were recovered from soil samples collected from Nandi County. The most frequently isolated species from soil from all 3 locations was *F. oxysporum*: 42% (n=26) from Laboret, 59% (n=41) from Kilibwoni and 29% (n=56) from Kaptumo locations. The isolation frequency of this species was 41% in Nandi District. There were no species of *F. verticillioides* or *F. proliferatum* isolated from the soil samples in the study area, however, a larger variety of *Fusarium* species was recovered from the soil samples compared to isolations from the other substrates sampled.

Other *Fusarium* species isolated from the soil samples in Nandi District were *F. lateritrium* with 9% isolation frequency. *Fusarium solani* and *F. poae* with 7% isolation frequency. *Fusarium dimerum* (8%), *F. equiseti* (9%), *F. acuminatum* (8%), *F. anthophilum* (6%), *F. subglutinans* (3%) and *F. semitectum* (2%). There was no significant difference ($\chi^2(18, 0.05)$=21.983, $P>0.05$) in the isolations of *Fusarium* species from soil samples in the three locations sampled.

4.1.3 *FUSARIUM* SPECIES ISOLATED FROM ANIMAL FEED SAMPLES

Fusarium isolates from animal feed samples in Nandi County totaled 71. The most frequently isolated *Fusarium* species from animal feed in Laboret were *F. subglutinans* (23%, n=13) and *F. verticillioides* (31%, n=13). in Kaptumo, it was *F. subglutinans* with 33% (n=30) isolation frequency. In Kilibwoni, however, *F. gramminearum* was most frequently isolated with an isolation frequency of 36% (n=28).

In Nandi County, therefore, the most frequently isolated *Fusarium* species from animal feed samples were both *F. subglutinans* and *F. verticillioides* (27%); and *F. gramminearum* (23%). *Fusarium proliferatum* had 10% incidence. Other *Fusarium* species isolated were: *F. anthophilum* (8%), *F. oxysporum* (4%), *F. solani* (3%) and *F. lateritrium* (1%). There was a significant difference ($\chi^2_{(14,\ 0.05)}$=21.983, $P<0.05$) in the isolations of *Fusarium* species from animal feed samples in the three locations sampled.

4.1.4 *FUSARIUM* SPECIES FROM SORGHUM AND MILLET SAMPLES

There were a total of 13 *Fusarium* isolates from sorghum and millet samples from Nandi County. The most frequently isolated *Fusarium* species from sorghum and millet samples from Laboret location was *F. subglutinans* (50%, n=4), from Kilibwoni location, *F. proliferatum, F. andiyazi* and *F. subglutinans* were most frequently isolated at 29% (n=7). In Kaptumo location only *F. andiyazi* (n=2) was isolated.

From the entire county, *F. andiyazi* (38%) and *F. subglutinans* (31%) were the most frequently isolated *Fusarium* species. Other *Fusarium* species isolated, included *F. proliferatum* (15%), *F. gramminearum* (8%) and *F. anthophilum* (8%). There was no significant difference ($\chi^2_{(8,\ 0.10)}$=8.264, $P>0.10$) in the isolations of *Fusarium* species from sorghum and millet samples in the three locations sampled.

4.1.5 *FUSARIUM* SPECIES FROM MARKET-BOUGHT MAIZE SAMPLES

Fusarium species isolated from market-bought maize samples from Nandi County were 19 in total. The most frequently isolated species from market-bought maize samples from Laboret location was *F. anthophilum* (67%, n=3), *F. subglutinans* (67%, n=9) from Kilibwoni location and *F. gramminearum* (29%, n=7) from Kaptumo location. Neither *F. proliferatum* nor *F. verticillioides* was isolated from Laboret and Kilibwoni locations; however, in Kaptumo, both of these species were isolated in same frequency (14%, n=7). The most frequently isolated species from market-bought maize samples in Nandi County was *F. subglutinans* (42%). Both *F. gramminearum* and *F.anthophilum* were isolated at 16%. *Fusarium proliferatum* and *F. solani* were also both isolated at 5% isolation frequency. There was no significant difference ($\chi^2_{(10,\ 0.10)}$=14.451, P>0.1) in the isolations of *Fusarium* species from market-bought maize samples in the three locations sampled.

4.1.6 MORPHOLOGICAL CHARACTERIZATION OF *FUSARIUM* SPECIES ISOLATED

The *Fusarium* species isolated were identified morphologically and microscopically as described by Leslie and Summerall (2006). The primary morphological features used to differentiate between species were the colony colour in reverse and on top on PDA, the presence or absence of diffusion (this refers to the spreading of secretions known as metabolites from the fungus into the agar that changes the colour of the culture media) into the agar and presence of sporodochia on CLA. The key microscopic features used for identification were the presence or absence of macroconidia and microconidia and the shape, size, orientation of both microconidia and macroconidia. The presence and absence of chlamydospores and their orientation was also used for identification. These features were observed at a magnification of ×40.

A) *FUSARIUM PROLIFERATUM*

Fusarium proliferatum is classified in SECTION LESIOLA.

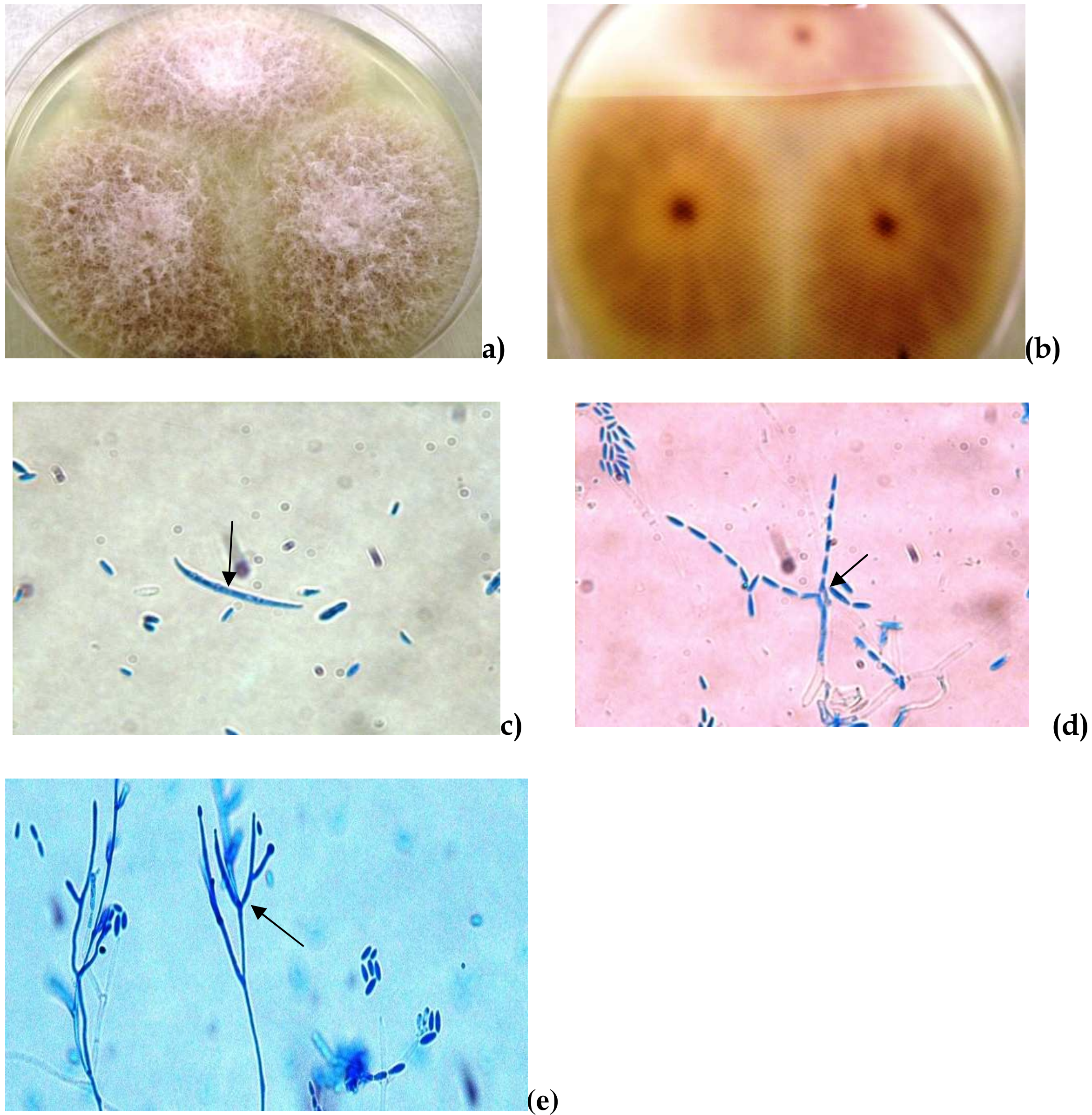


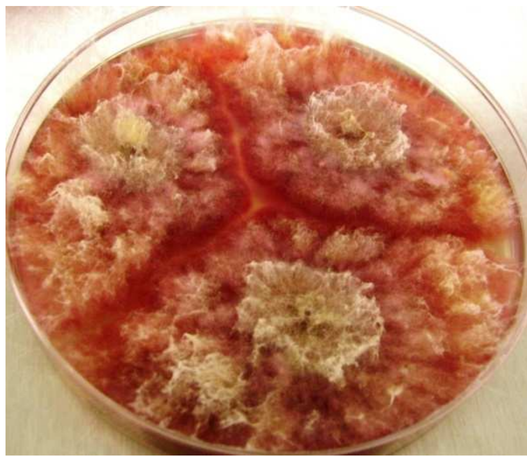
Plate 1: *Fusarium proliferarum:* colony on PDA, top(a) and reverse(b); (c)macroconidia; (d)characteristic V-shaped microconidial chains; (e)branched polyphiliades

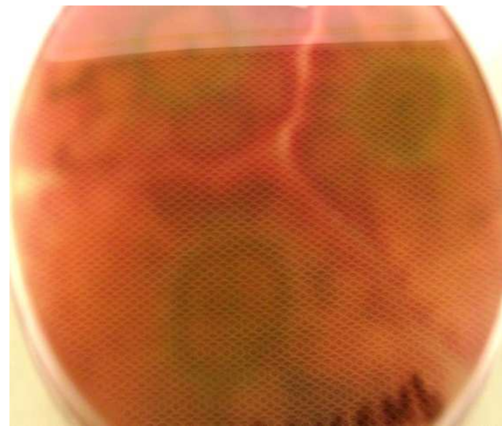
Characteristics on PDA: The colonies formed abundant ariel mycelia that were initially white then became purplish with age. On reverse, the colour was purple. There was diffusion into the agar that was purple to violet in colour.

Characteristics on CLA: Pale orange sporodochia were produced on carnation leaves. Macroconidia were rarely present, when they were present, they were slender, thin-walled and relatively straight. The apical cell was curved and the basal cell had no distinct foot shape. They were usually 3-5 septate. Microconidia were abundant in the sporodochia and were club-shaped with a flattened base without septation. The microconidia were formed in characteristic V-shaped chains that were not too long and formed in pairs from polyphiliades. There were no chlamydospores formed.

B) *FUSARIUM GRAMMINEARUM*

Fusarium gramminearum is classified in SECTION DISCOLOR


(a)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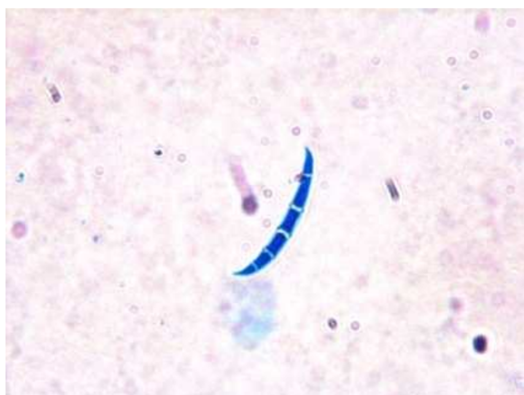
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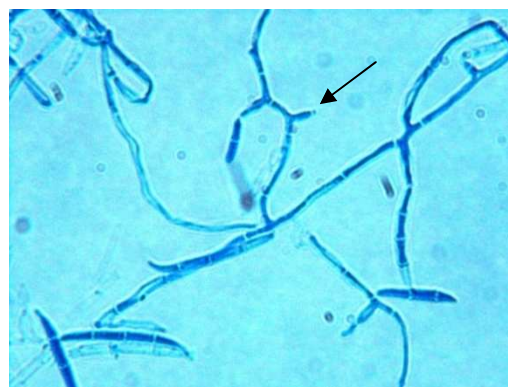

(d)

Plate 2: *Fusarium gramminearum:* colony on PDA, top(a) and reverse(b); (c)macroconidia; (d)macroconidia on monophialides (arrow)

Characteristics on PDA: The colonies grew very rapidly with large amounts of dense mycelia that were predominantly white but occasionally were pale orange or yellow in color. Cultures formed red pigments in agar which was visibly seen on the reverse side of the culture plate.

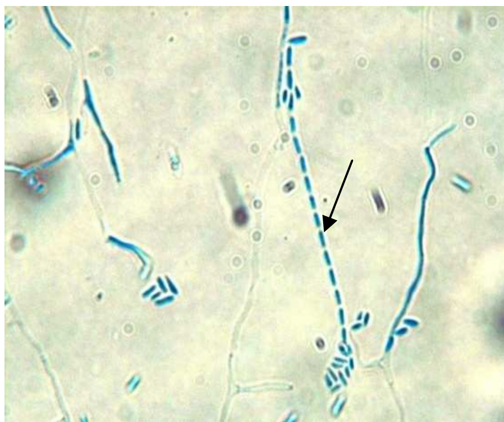
Characteristics on CLA: Pale orange sporodochia were formed on carnation leaves. Macroconidia were very abundant in sporodochia and were uniform in shape and size. They were slender, thick-walled and of medium length. They had a moderate curve and the apical cell was tapered or snout-like in shape. The basal cell had a well-developed foot shape. Macroconidia was septate with 5 to 6 septa. The macroconidia were produced from monophialides on branched conidiophores. This species did not produce microconidia or chlamydospores.

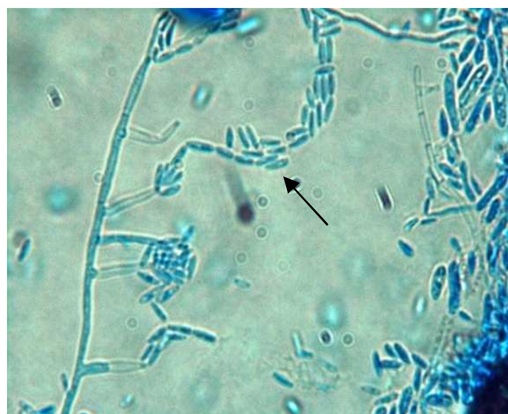
C) *FUSARIUM VERTICILLIOIDES*

Fusarium verticillioides is classified in SECTION LESIOLA

(a)

(b)


(c)


(d)

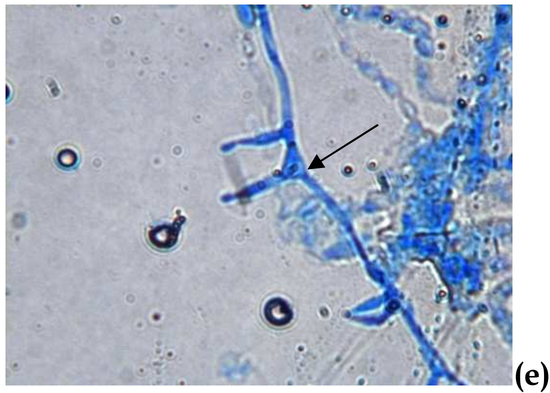

(e)

Plate 3: ***Fusarium verticillioides:*** **colony on PDA, top(a) and reverse(b); (c,d)long microconidial chains from monophialides; (e)monophialides on branched conidiophores**

Characteristics on PDA: The colonies had floccose mycelia that grew relatively fast. The mycelia was initially white and then became violet as the colonies aged. Colonies developed a violet pigmentation in agar that turned dark violet to black the older it got, however, in some cases, there was no pigmentation in the agar or violet coloration in the mycelia.

Characteristics on CLA: Macroconidia were not observed in the pale orange sporodochia that formed on carnation leaves. Microconidia were formed abundantly in relatively long chains from monophiliades on branched conidiophores in the sporodochia. This was a key characteristic of *F. verticillioides* that was mainly used to identify this species. The microconidia were oval to club-shaped with a flattened base and non-septate. Chlamydospores were not produced.

D) *FUSARIUM ANTHOPHILUM*

Fusarium anthophilum is classified in SECTION LESIOLA.

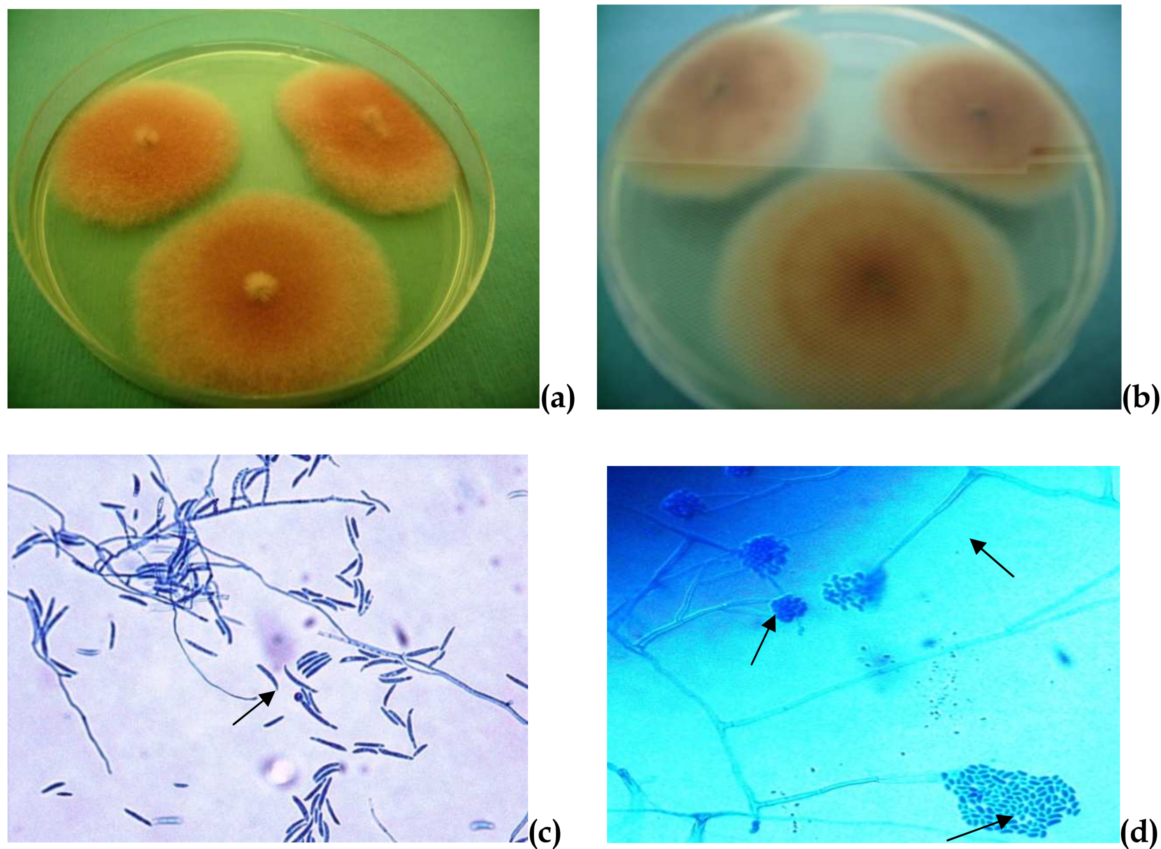


Plate 4: *Fusarium anthophilum:* colony on PDA, top(a) and reverse(b); (c) macroconidia; (d) microconidia on monophialides and polyphialides

Characteristics on PDA: The colonies were rapidly growing, with abundant, white floccose mycelia. The colonies were mainly white in colour with a pale salmon-coloured tinge. Some colonies turned violet as violet pigment was formed in the agar.

Characteristics on CLA: Orange sporodochia were formed on carnation leaves. Macroconidia were present and had thin walls, were relatively slender and had no significant curvature. They were usually 3-septate. The apical cell was curved and the basal cell had a distict or poorly developed foot-shape. Microconidia were abundant and were either globose, pyriform or ovoid with no septation. They were formed on monophialides and polyphialides. Chlamydospores were not present.

E) *FUSARIUM OXYSPORUM*

Fusarium oxysporum is classified in SECTION ELEGANS.

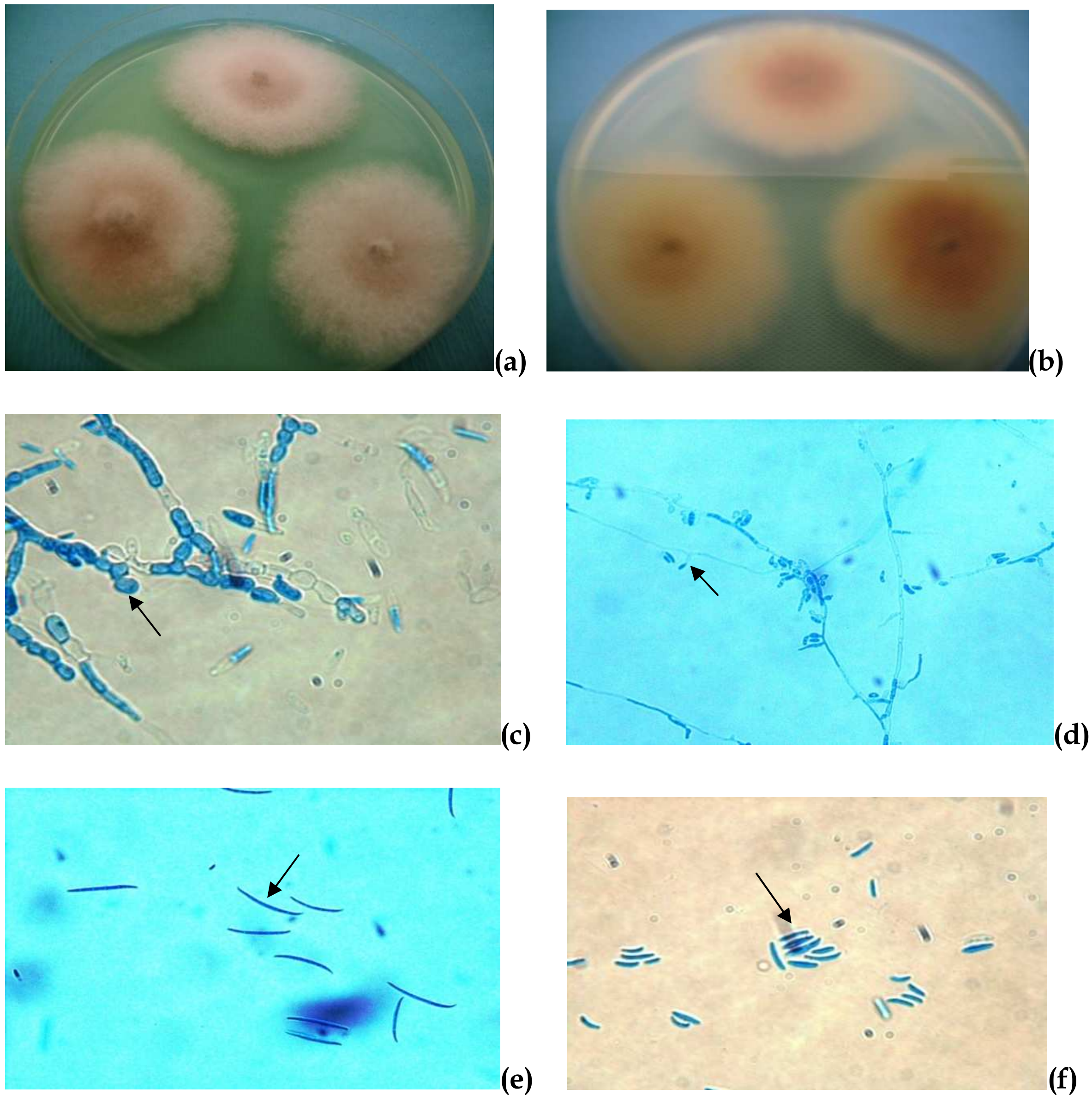


Plate 5: *Fusarium oxysporum:* colony on PDA top(a) and reverse(b); (c)chlamydospores; (d)short monophialides; (e)macroconidia; (f)microconidia

Characteristics on PDA: Colony grew rapidly on PDA and formed abundant, floccose mycelia which varied in colour from white to pale violet. The colony appeared violet on reverse and produced a pale to dark violet pigment in the agar.

Characteristics on CLA: Abundant pale orange sporodochia were formed on carnation leaves. Macroconidia were abundant, short to medium length, slightly curved or straight, relatively slender and thin-walled and usually 3-septate. The apical cell was tapered and curved with a slight hook. The basal cell was either foot-shaped or pointed. Microconidia formed abundantly on short monophialides or on false heads in aerial mycelia. They were oval, elliptical or kidney-shaped and non-septate. Chlamydospores were abundantly formed singly, in pairs or in chains and were smooth-walled.

F) *FUSARIUM SUBGLUTINANS*

Fusarium subglutinans is classified in SECTION LESIOLA.

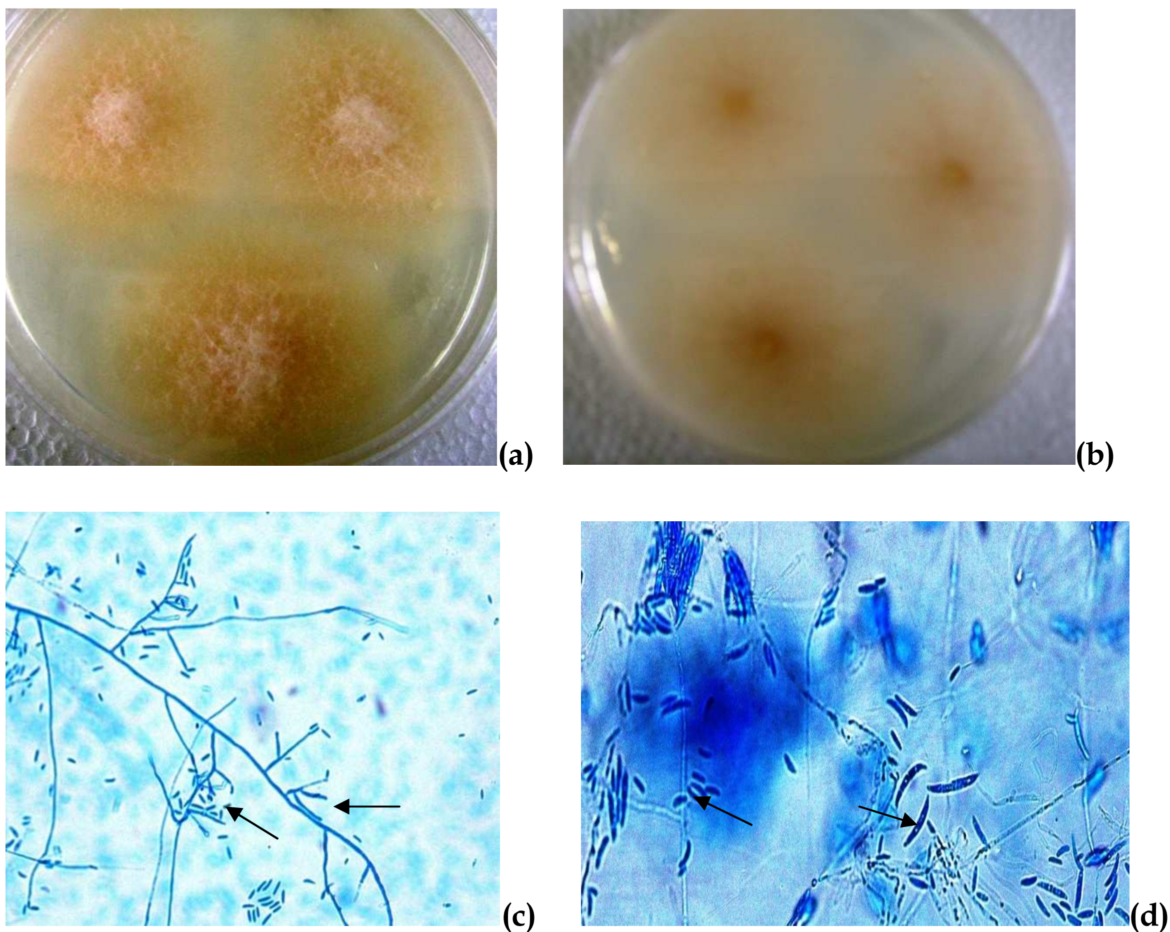


Plate 6: *Fusarium subglutinans:* colony on PDA, top(a) and reverse(b); (c)polyphialides and microconidia; (d) macroconidia and microconidia

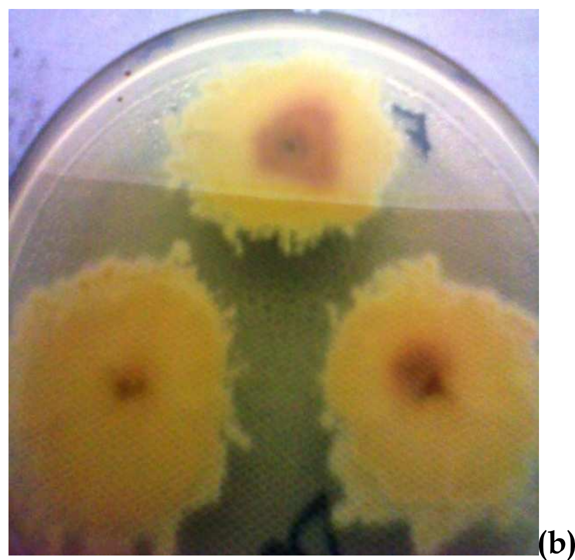
Characteristics on PDA: The colonies were white with flocose mycelia and grew moderately fast. The colonies on reverse were pale orange to salmon in colour.

Characteristics on CLA: Pale orange sporodochia were formed on carnation leaves. Macroconidia from sporodochia were rarely formed. When they formed, they were relatively slender, slightly falcate and thin-walled and 3-septate. The apical cell was curved and the basal cell was poorly developed, having no distinct foot shape. Microconidia were produced abundantly in false heads mainly from polyphialides and were oval or elliptical in shape without septation. Chlamysospores were not produced.

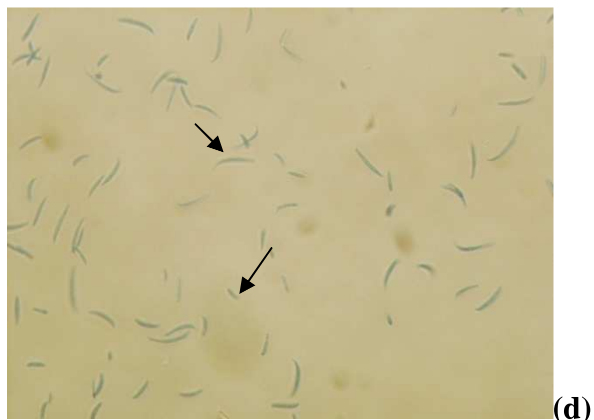
G) *FUSARIUM ACUMINATUM*

Fusarium acuminatum is classified in SECTION GIBBOSUM.

(a)


(b)

(c)


(d)

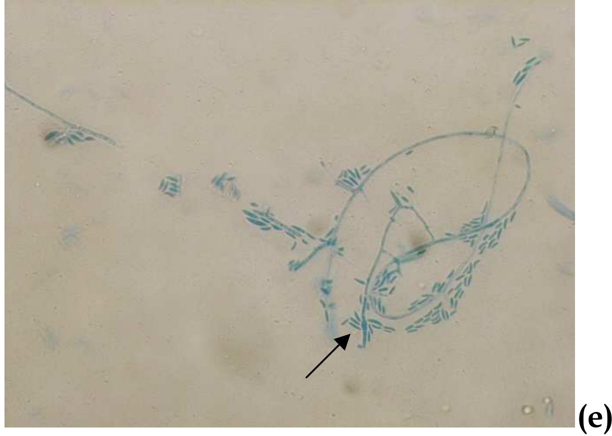

(e)

Plate 7: ***Fusarium acuminatum:*** **colony on PDA, top(a) and reverse(b); (c)macroconidia; (d) microconidia and macroconidia; (e) microconidia**

Characteristics on PDA: The colonies were relatively slow growing, and did not fill the culture plate even after seven days. They had white mycelia that was floccose with rose-colored to burgundy-colored pigmentation that appeared greyish-rose at the periphery.

Characteristics on CLA: Macroconidia were formed abundantly in pale orange sporodochia. The macroconidia were relatively slender, thick-walled and usually 5-septate. The apical cell was long and tapering and the basal cell was distintly foot-shaped. Microconidia were sparse. They were reniform and either had one septa or lacked septation altogether. They formed on monophialides. Did not form chlamydospores although the formation of chlamydospores has been reported to be rare and when they occur, they are present in chains or clusters.

H) *FUSARIUM POAE*

Fusarium poae is classified in SECTION SPOROTRICHIELLA.

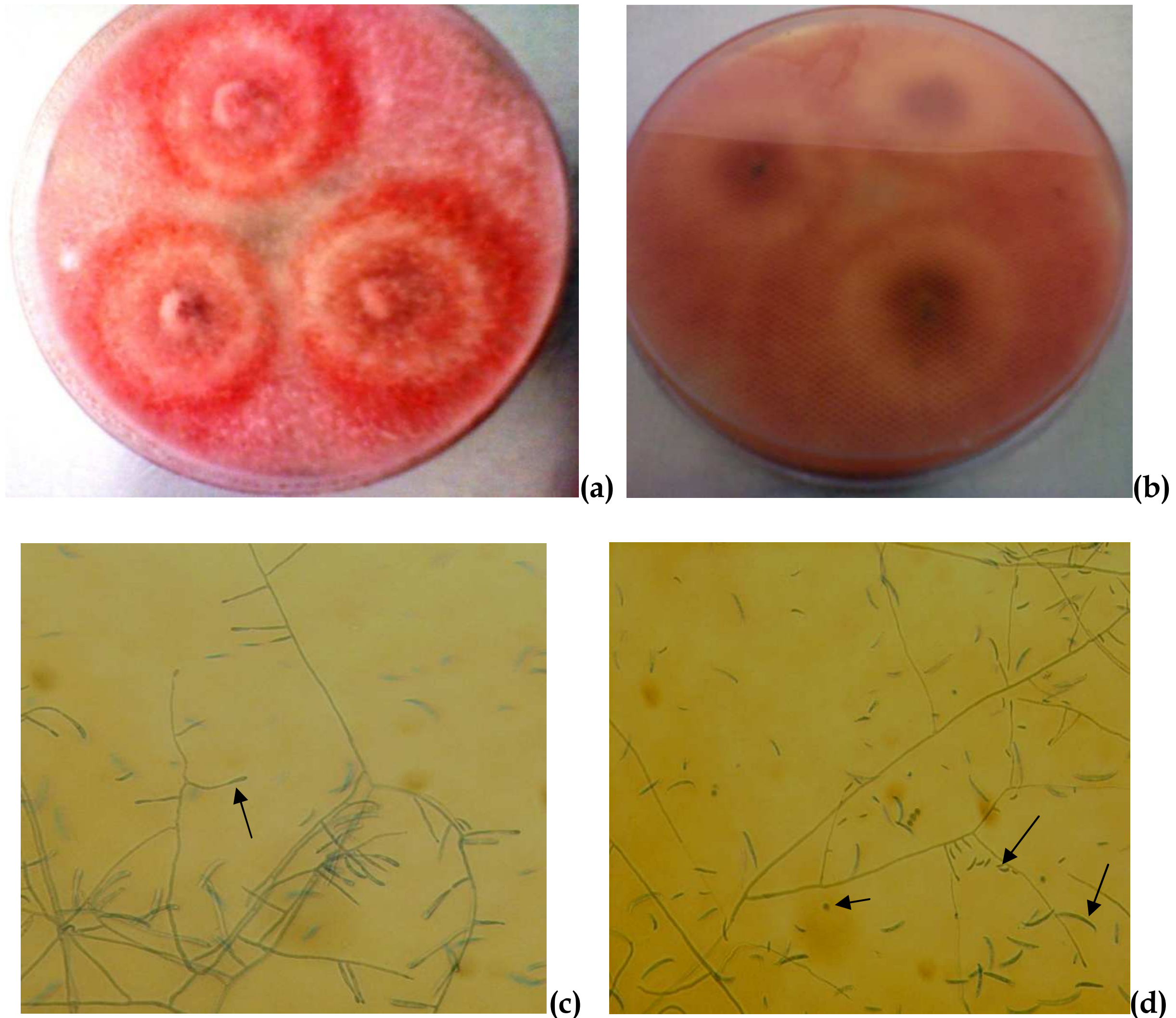


Plate 8: *Fusarium poae:* colony on PDA, top(a) and reverse(b); (c)monophialides; (d)miccroconidia and macroconidia

Characteristics on PDA: The colony grew rapidly and produced abundant mycelia that appeared hairy or felted. Mycelia was initially pale in colour and then darkened to red in colour. Red pigments were produced in the agar.

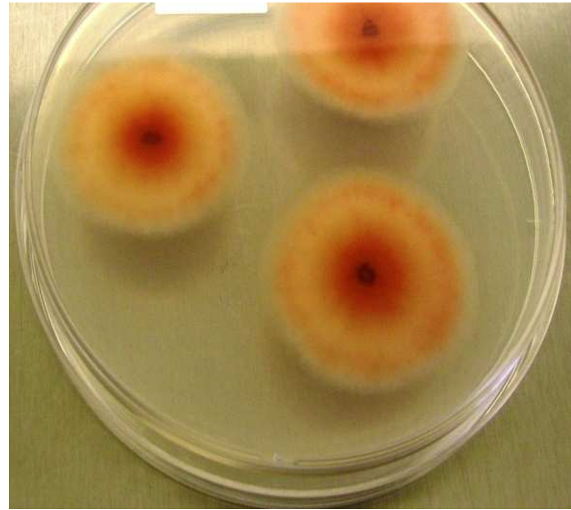
Characteristics on CLA: Sporodochia were formed rarely and macroconidia were scarse. Macroconidia were slender, relatively short and falcate. They were usually 3-septate. The apical cell was curved and the basal cell had a well-developed foot-shape.

Microconidia were produced abundantly in aerial mycelia and were found in clusters. They were globose or napiform with a distinct papilla. Chlamydospores were very rarely formed.

I)*FUSARIUM LATERITRIUM*

Fusarium lateritrium is classified in SECTION LATERITRIUM.

(a)


(b)

(c)

Plate 9: *Fusarium lateritrium:* colony on PDA, top(a) and reverse(b); (c)macroconidia

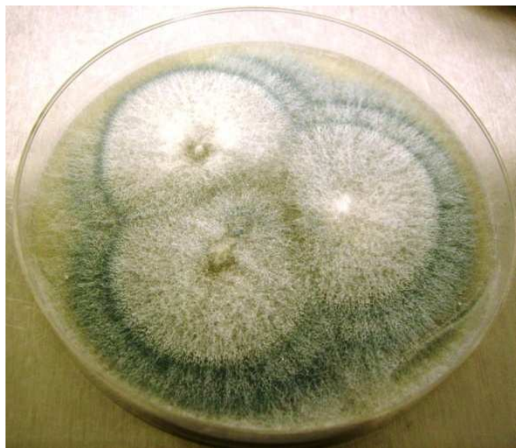
Characteristics on PDA: Colonies of *F. lateritrium* were relatively slow growing, with growth stagnating in culture plate after five days. Mycelia appeared sparse and varied in colour from white, orange or pale pink in colour. Abundant macroconidia were

produced in orange, pink or green sporodochia that spread over the entire surface of the colony, giving it an orange, pink or green colour. Produced a red-orange pigment in the agar.

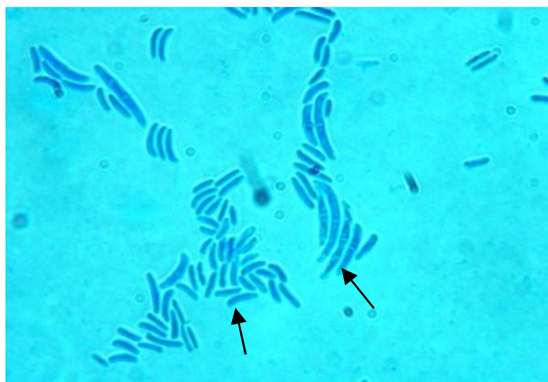
Characteristics on CLA: Abundant pale orange sporodochia were formed on or near the carnation leaves. Macroconidia were medium-long, thick-walled and relatively straight, with parallel walls for most of the spore length. The apical cell had a characteristic hook and the basal cell was notched. They were usually 5-septate. Microconidia were absent. Chlamydospores were not present but have been reported to form quite slowly, appearing singly or in chains.

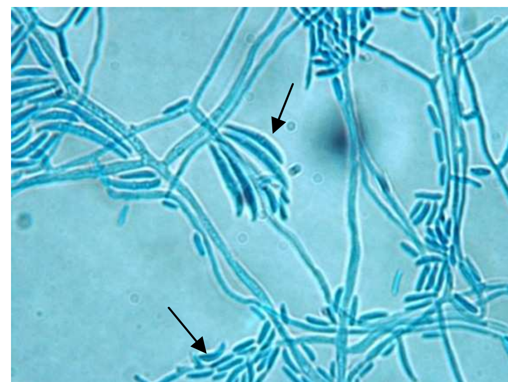
J) *FUSARIUM SOLANI*

Fusarium solani is classified in SECTION MARTIELLA & VENTRICOSUM.


(a)

(b)


(c)


(d)

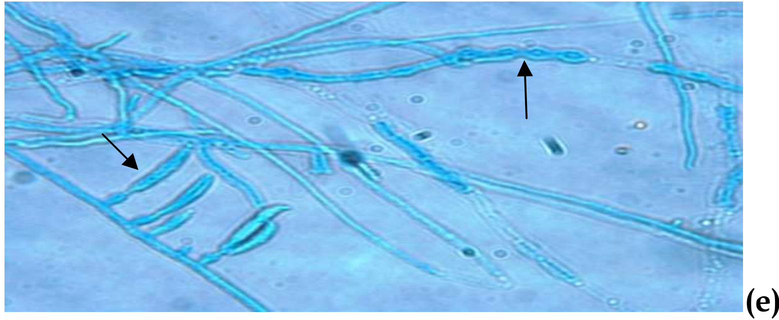

(e)

Plate 10: ***Fusarium solani:*** **colony on PDA, top(a) and reverse(b); (c,d)macroconidia and microconidia; (e)long monophialides and chlamydospores**

Characteristics on PDA: The colony grew moderately fast and produced white to cream sparse mycelia. The colour of the colony changed as abundant macroconidia were produced in cream or bluish-green sporodochia, giving the colony a distinct blue-green appearance. No pigment was produced in the agar.

Characteristics on CLA: Macroconidia were produced abundantly in cream, blue or green sporodochia and were relatively wide, stout, and straight. The apical cell was blunt and rounded and the basl cell had a distinct foot or sometimes was cylindrical with a notched or rounded end. They were usually 5- to 7-septate. Microconidia were abundant in aerial mycelia and present on false heads of long monophialides. They were usually oval, elliptical or reniform in shape with one or two septations. Chlamysospores formed rapidly and abundantly and were present singly, in pairs or in short chains. They appeared globose or oval with smooth or rough walls.

K) *FUSARIUM EQUISETI*

Fusarium equiseti is classified in SECTION GIBBOSUM.

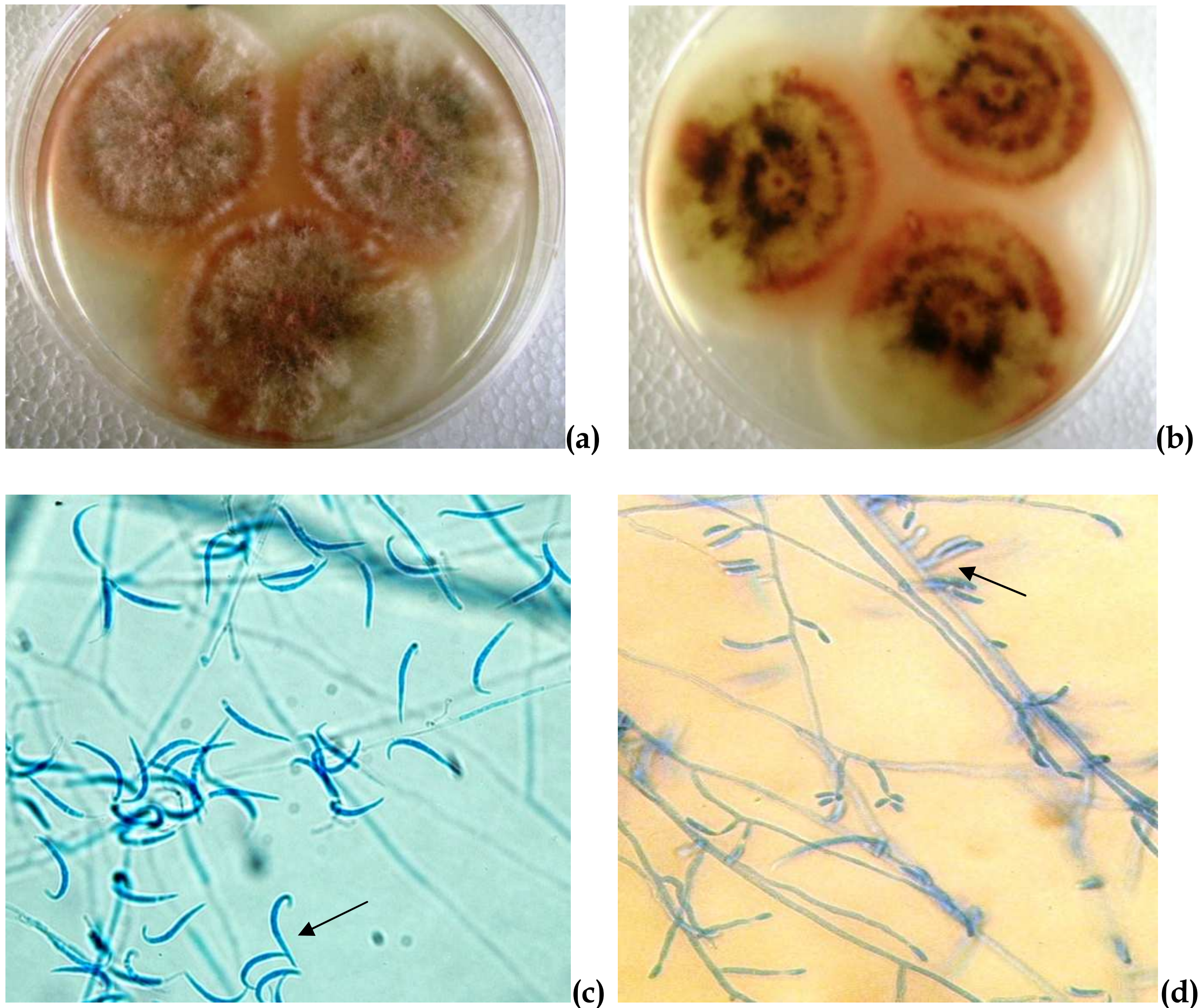


Plate 11: *Fusarium equiseti:* colony on PDA, top(a) and reverse(b); (c)macroconidia; (d)monophialides

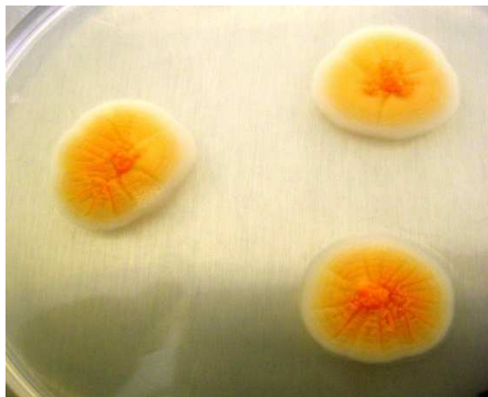
Characteristics on PDA: Colonies were relatively fast-growing and produced abundant white mycelia that became red-brown after 7 days. Dark brown spots or flecks of pigment were usually formed in the agar.

Characteristics on CLA: Orange sporodochia were formed on carnation leaves. Macroconidia formed abundantly in the sporodochia from monophialides on branched conidiophores and were usually uniform in appearance. They appeared long and slender with very pronounced dorsiventrical curvature. The apical cell was tapered

and elongate, appearing whip-like and the basal cell was distinctly foot-shaped and elongate. They usually had 5-7 septations. Microconidia were absent. They have been reported to form chlamydospores abundantly in aerial hyphae that may be found singly, in chains or in clumps which form a brown pigment and thick walls with time.

L) *FUSARIUM DIMERUM*

Fusarium dimerum is classified in SECTION EUPPINOTES.


(a)

(b)

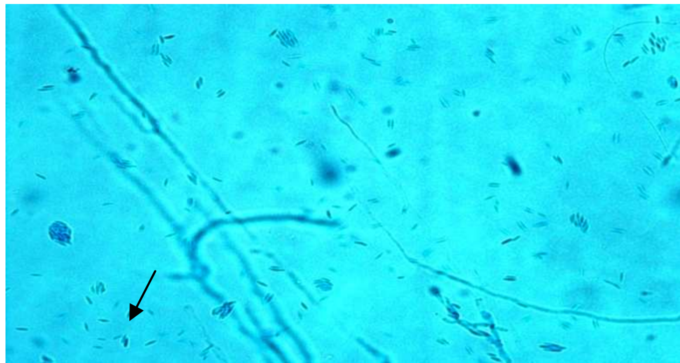

(c)

Plate 12: ***Fusarium dimerum:*** **colony on PDA, top(a) and reverse(b); (c)macroconidia**

Characteristics on PDA: Colonies were extremely slow-growing, with a slimy and yeast-like in appearance. They were white to orange in colour, but mostly orange with no obvious aerial mycelia. No pigmentation was formed in the agar.

Characteristics on CLA: Sporodochia were absent and macroconidia were found across the colony. Macroconidia formed abundantly on monophialides on branched

conidiophores. They were very short with even curvature on both sides and appeared quite wide relative to length. The apical cell was rounded and hooked and the basal cell was blunt or slightly notched. Microconidia were absent. Chlamydospores were not formed although they have been reported to form rarely.

M) *FUSARIUM ANDIYAZI*

Fusarium andiyazi is classified in SECTION LESIOLA.

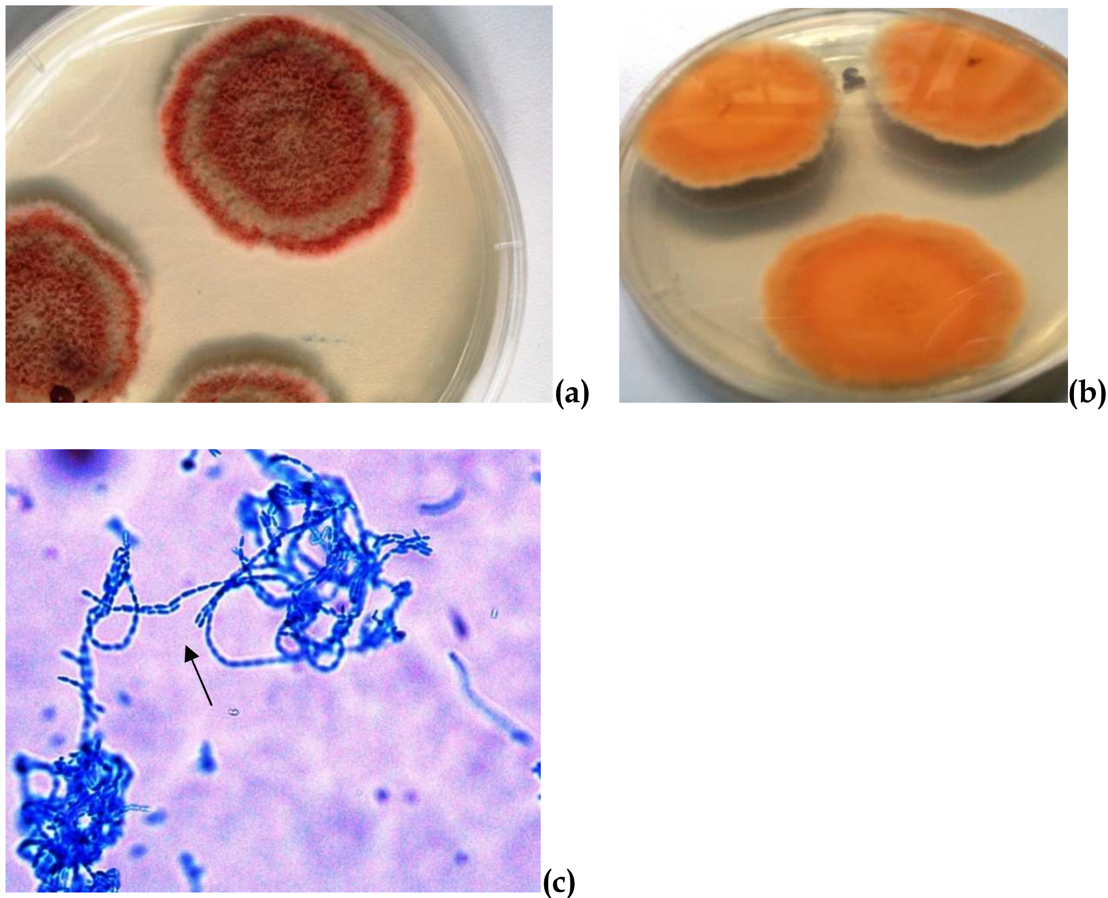


Plate 13: *Fusarium andiyazi:* colony on PDA, top(a) and reverse(b); (c)long microconidial chains

Characteristics on PDA: Colonies of *F. andiyazi* were relatively slow growing with powdery mycelia that were distinctly dark burgundy or violet in colour. They produced pale purple to dark purple pigment in the agar as the colony aged.

Characteristics on CLA: Orange sporodochia formed on carnation leaves. Macroconidia formed abundantly in sporodochia on monophialides or branched conidiophores. They were usually thin-walled and relatively straight. The apical cell was slightly curved and the basal cell was notched. Most of them had 3 septations. Microconidia were abundant in aerial mycelia and formed on characteristic long monophialides in long chains. They were pyriform, globose or ovoid in shape and some were 1-septate. Chlamydospores were absent

N) *FUSARIUM SEMITECTUM*

Fusarium semitectum is classified in SECTION ARTHOSPORIELLA.

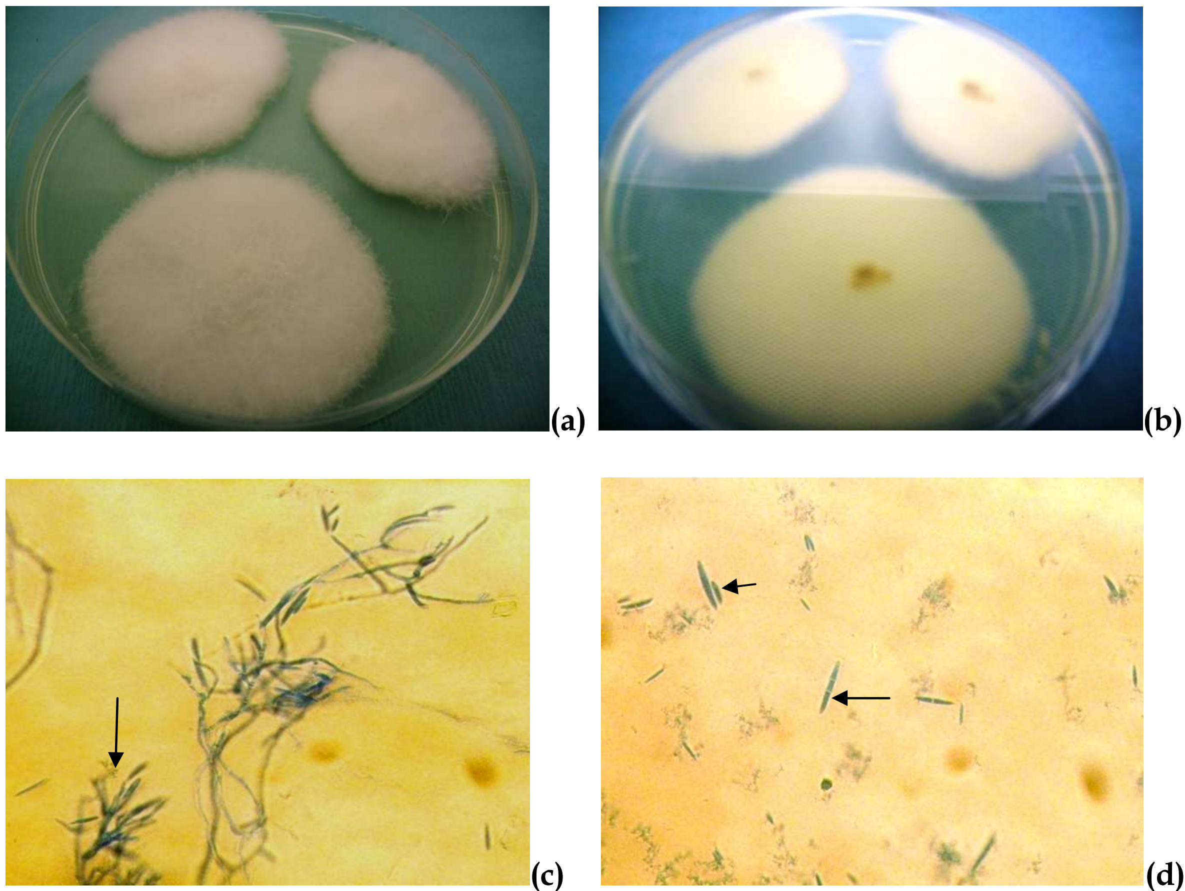


Plate 14: *Fusarium semitectum:* colony on PDA, top(a) and reverse(b); (c)polyphialides with characteristic "rabbit ears"; (d)macroconidia and microconidia

Characteristics on PDA: Cultures grew rapidly and produced abundant dense mycelia. The mycelia were initially white to off-white in color and also appeadred white to off-white in color in reverse.

Characteristics on CLA: Orange sporodochia were produced on carnation leaves. Macroconidia from sporodochia were relatively slender and slightly curved. The apical cell was slightly curved and tapered to a point and the basal cell had a foot shape. They were usually 3- to 5-septate and not very abundant. Microconidia were pyriform to obvate with one septation and were abundant in aerial mycelia. There were 2 spores per polyphiliade which gave the characteristic 'rabbit ear' appearance. Chlamydospores were not observed but have been reported to be present but not common and found in the hyphae both singly and in chains.

4.2 MOLECULAR CHARACTERIZATION OF SPECIES AND PRESENCE OF FUMONISIN-PRODUCING GENE (FUM1 GENE) USING PCR

A total of 85 isolates from household maize and animal feed samples that were morphologically characterized as *F. proliferatum* and *F. verticillioides* were further tested using PCR to confirm the species and test for the presence of the fumonisin-producing gene (FUM1 gene) in these species.

4.2.1 MOLECULAR CHARACTERIZATION OF *FUSARIUM VERTICILLIOIDES* AND *F. PROLIFERATUM* USING PCR

Molecular genotyping results on species identification conformed to morphological identification. Seventy three percent (n=85) of the isolates were *F. verticilloides* and 27% (n=85) of isolates were *F. proliferatum.* The amplification products for VERTF-1 and VERT-R set of primers that verify the species of *F. verticillioides* amplified at 1016 bp while the amplification products for Fp3-F and Fp4-R set of primers that verify the species *F. proliferatum* amplified at 230bp. The gels for these are shown in figure 3 below (Fp3-F and Fp4-R amplification products) and figure 4 (VERTF-1 and VERT-R amplification products).

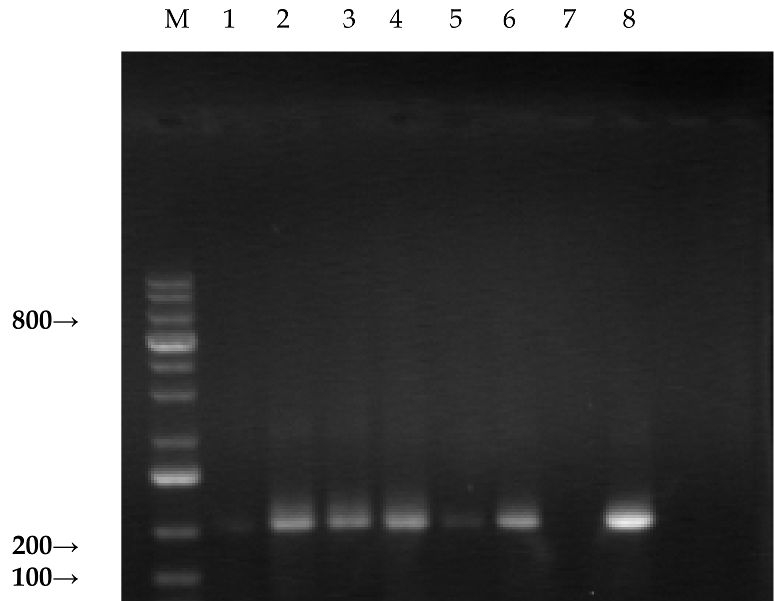


Figure 3: **Agarose gel (1.5%) showing PCR amplification products of Fp3-F/Fp4-R (230 bp) set of primers specific to *F. proliferatum*. Lane M: 1000 bp DNA ladder; lane 1: *F. verticillioides*; lane 2-6, 8: *F proliferatumum*; lane 7: *F. gramminearum* (control)**

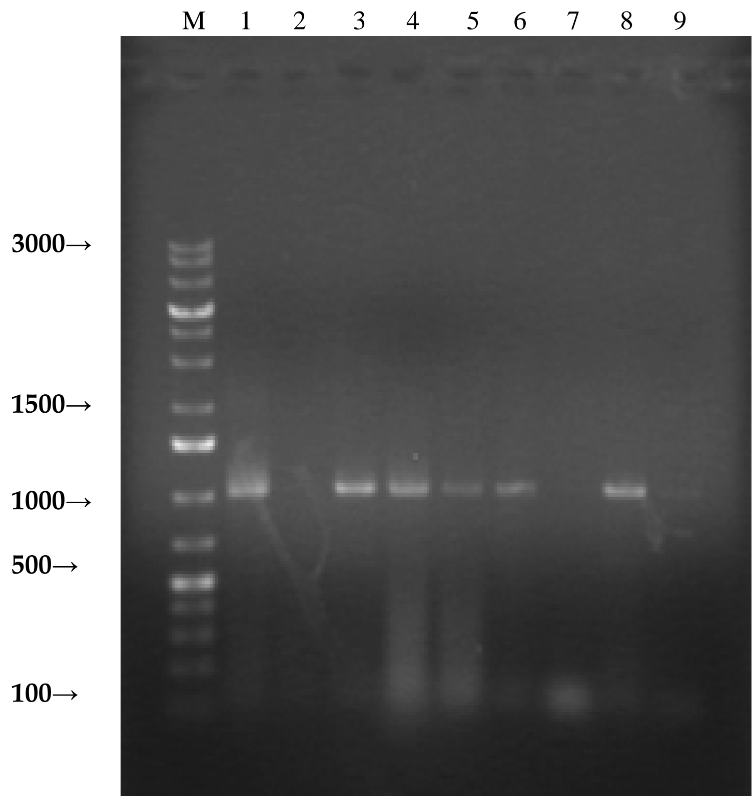


Figure 4: **Agarose gel (1.5%) showing the amplified products of VERTF-1 and VERT-R set of primers specific for *F. verticillioides* Lane M is 3000bp DNA marker; Lane 1,**

3-6, 8: *F. verticillioides*; Lane 2: *F. gramminearum* (control); Lane 7, 9: *F. proliferatum*. Primers amplified at 1016 bp

4.2.2 TOXIGENIC POTENTIAL OF *FUSARUIM VERTICILLIOIDES* AND *F. PROLIFERATUM*

The isolates that were confirmed as *F. proliferatum* and *F. verticillioides* were tested for presence of FUM1 gene. The amplification products to assay the fumonisin-producing potential of *F. verticillioides* with the set of primers VERTF-1 and VERTF-2 amplified at 400bp (figure 5). The amplification products to assay the fumonisin-producing potential of *F. proliferatum* with the set primers Fpro-fum1 forward and Fpro-fum1 reverse amplified at 183bp (figure 6). The gels for these are shown in figure 5 (*F. verticillioides*) and figure 6 (*F. proliferatum*).

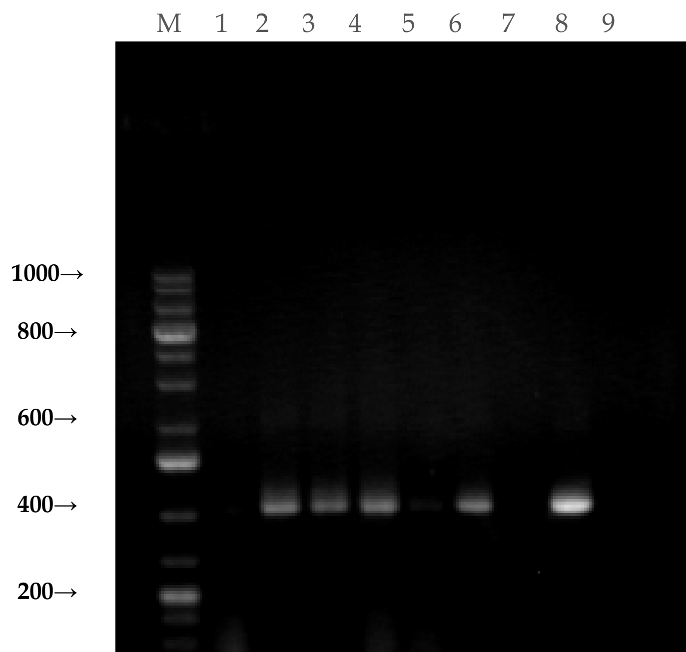


Figure 5: Agarose gel (1.5%) showing the amplified products of fumonisin producing *F. verticillioides* with the FUM1 gene (400 bp) set of primers. Lane M: (1000 bp) DNA ladder; Lane 1-2: *F. verticillioides*; Lane 9: *F. oxysporum* (control)

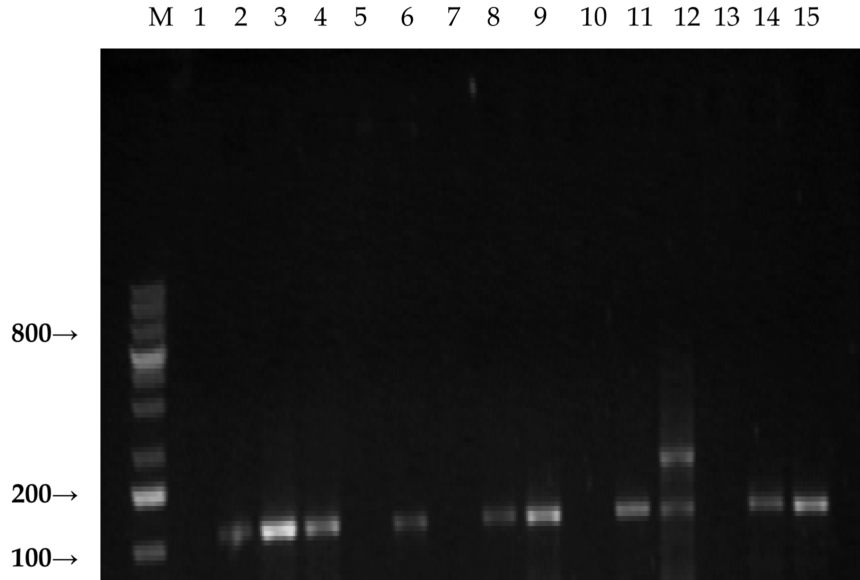


Figure 6: Agarose gel (1.5%) showing the amplified products of fumonisin producing *F. proliferatum* with the FUM1 gene (183 bp) set of primers. Lane M: 1000 bp DNA ladder; Lane 1-4, 6-14: *F. proliferatum*; lane 5: *F. oxysporum* (control)

There was a significant incidence ($\chi^2(1, 0.05)=14.412$, $P<0.05$) of species that were positive for the fumonisin-producing gene in Nandi County. Seventy three percent of *F. verticilioides* and 65% of *F. proliferatum* isolates were positive for FUM 1 gene, Figure 7. This high incidence of potential toxigenic strains was significant ($\chi^2(1, 0.05)=17.894$, $P<0.05$). All the three locations recorded greater positive strains for the FUM1 gene than negative strains, Table 4. Ninety six percent of *Fusarium* strains from feed samples were positive for the FUM1 gene while maize samples that were positive were 59%.

There was also significant presence ($\chi^2(1, 0.05)=12.812$, $P<0.05$) of species that were positive for the fumonisin-producing gene in both animal feed and household maize samples tested which indicates that there in significant exposure of fumonisins to the both human and animal population within Nandi County.

More isolates from each species tested positive for the presence of the FUM1 gene, 62 (73%) were *F. verticilioides,* out of which 73% (n=62) were positive for the presence of

fumonisin-producing gene. Twenty three (27%) were *F. proliferatum* isolates, out of which 65% (n=23) were positive for presence of FUM1 gene.

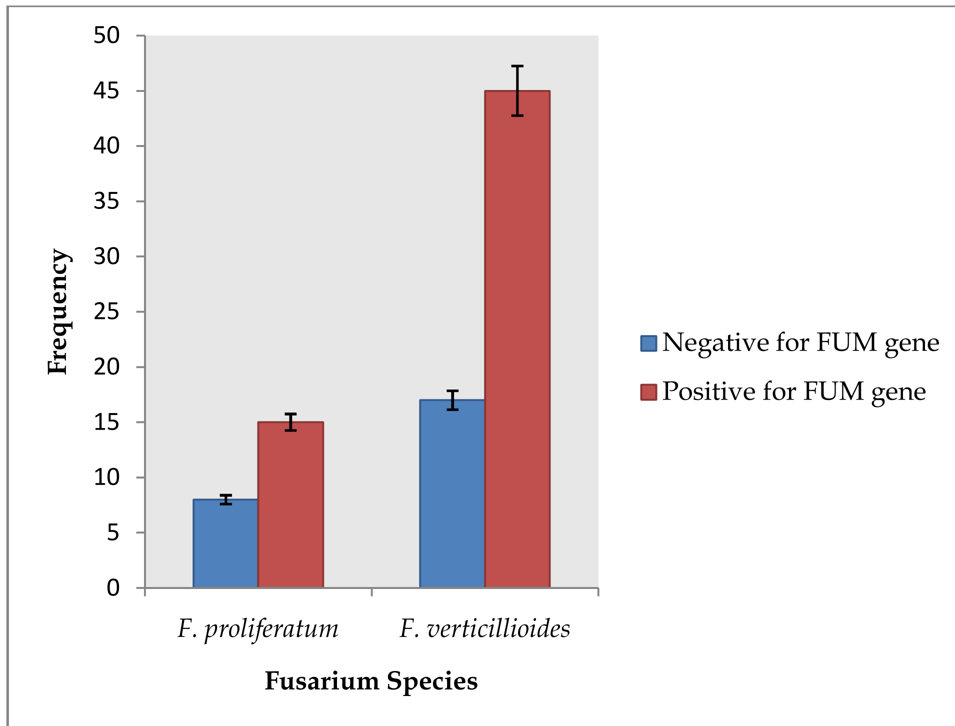


Figure 7: Distribution of potential toxigenic strains of *F. verticillioides* and *F. proliferatum*

Kaptumo location had the highest number (78%, n=37) of isolates that were positive for the FUM1 gene. Kilibwoni location had the highest number (42%, n=36) of isolates that were negative for the FUM1 gene. Kilibwoni (25%, n=85) and Kaptumo (34%, n=85) locations recorded the highest numbers of FUM1 gene positive isolates. All locations had a higher number of FUM1 positive isolates than FUM1 negative isolates (figure 8).

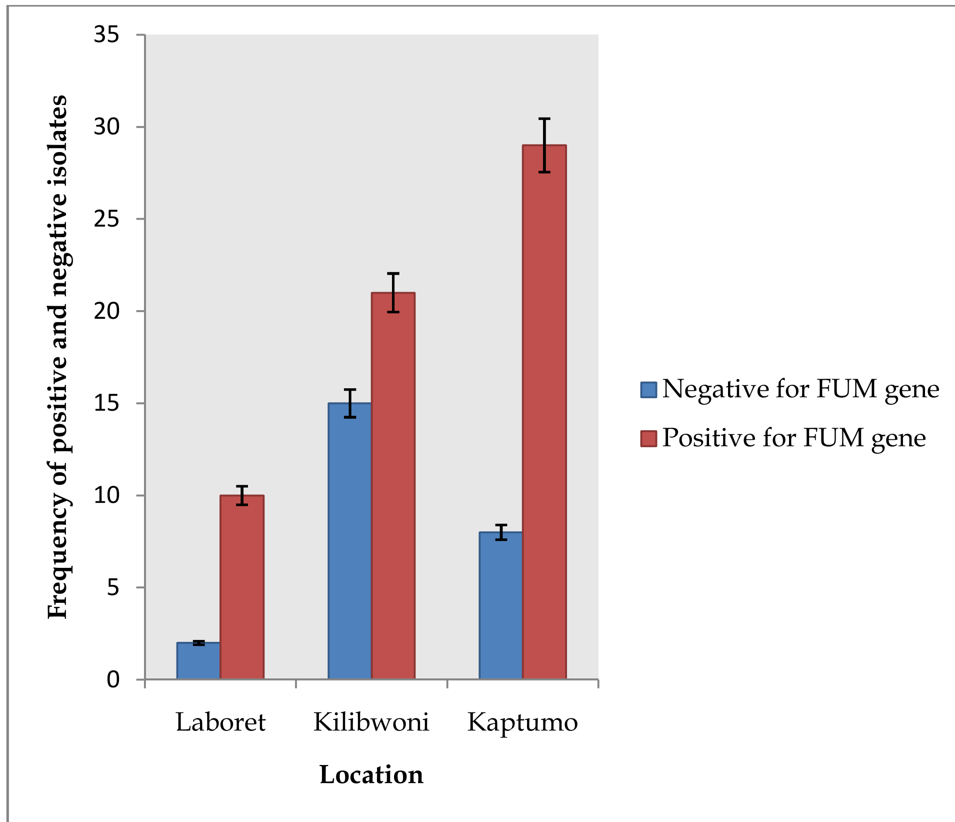


Figure 8: Frequency of isolation of fumonisin-producing isolates in locations of Nandi County

A total of 59 samples from household maize and 26 from animal feed were tested for the presence of FUM1 gene. Both substrates had more isolates positive for the presence of the gene. Household maize isolates had 59% positive isolates and animal feed isolates had 96% positive isolates as shown in figure 9.

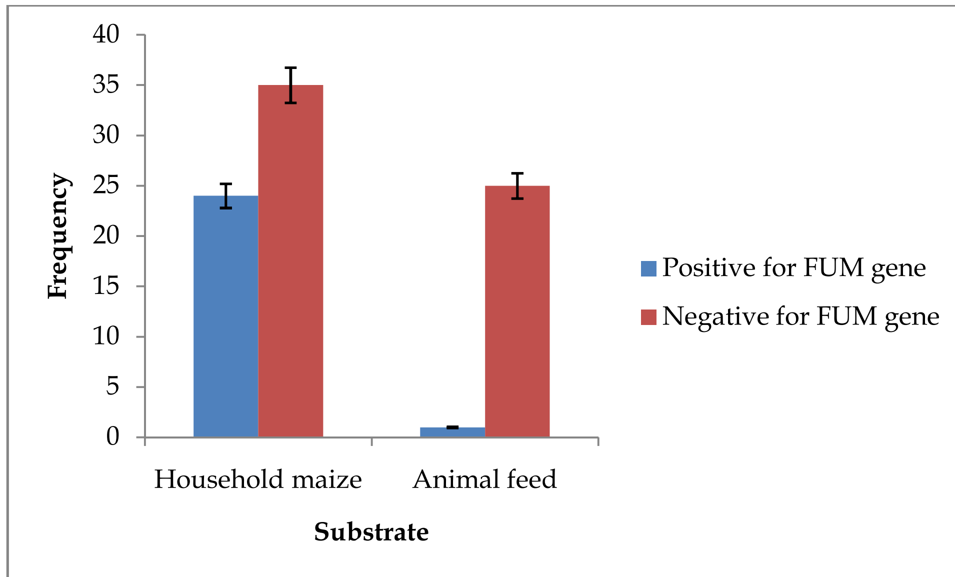


Figure 9: Frequency of isolation of fumonisin-producing isolates in animal feed and household maize samples

4.3 QUANTITY OF FUMONISINS IN HOUSEHOLD MAIZE AND ANIMAL FEED SAMPLES

The quantity of fumonisins found in various grain samples collected was done using CD-ELISA and the amount of fumonisin recorded in parts per million (ppm). A total of 286 grain samples from household maize (219), animal feed (60) and sorghum and millet (7) samples were tested for quantities of fumonisins. The detection limits ranged between 0.001 to 5 ppm. Forty of these grain samples tested for fumonisins had no fungal species isolated from them, 36 out of these 40, however, had fumonisin levels recorded ranging from 0.004-3.9 ppm. Of the other 246 samples from which fungal species had been isolated, 37 samples had fumonisin quantities that were either zero or had levels too low to be detected by CD-ELISA; the rest of the samples recorded fumonisin quantities ranging from 0.002 to 35.5 ppm. Animal feed samples recorded the maximum range in one sample. Five out of the seven sorghum samples recorded fumonisin levels of between 3.8 and 5.5 ppm. Maize samples had the majority of samples with quantities that were not calculable (96%, n=27) with the highest quantity

of fumonisins recorded in these samples being 9.8 ppm. In grain that was intended for household use, 18 samples had quantities of fumonisins higher than 1ppm (1-9.8 ppm). However, most of the samples (74%, n=226) had quantities of fumonisin exceeding the joint FAO/WHO limit of 2 μg/Kg per person per day.

The quantities of fumonisins found in households where neither *F. verticillioides* nor *F. proliferatum* were isolated are shown in appendix B. The fungal species isolated from households are also indicated from samples of maize, animal feed and sorghum and millet.

Forty-nine out of the 85 isolates that were confirmed to be either *F. proliferatum* or *F. verticillioides* (table 6) were from household maize and animal feed samples that were tested for quantity of fumonisins. Out of these 49, 16 were negative for the FUM1 gene and 33 were positive for the FUM1 gene. Sample number D489Af was the one with the highest amount of fumonisins, 35.5 ppm, and from this animal feed sample, *F. verticillioides* was isolated. The remaining 46 of these samples either had no fumonisins or the levels were in quantities that were too low to be detected using CD-ELISA. Nine out of forty-six samples had tested negative for the FUM1 gene and thirty-five out of forty-six were positive for the FUM1 gene. The grain samples with *F. verticillioides* and *F. proliferatum* species that recorded fumonisin levels are in table 3.

Table 3: Fumonisin contamination of household maize and animal feed samples and presence of *F. verticillioides* and *F. proliferatum*

SUBSTRATE	SPECIES	SAMPLE ID	Fum1 gene presence	Quantity of fumonisin in ppm
Household maize	*F. verticillioides*	D444Ma	+ve	2
		D4296Mb	+ve	1.9
		D5482Ma	+ve	0.02
		D5503Ma	+ve	0.024
		D6576Ma	+ve	0.027
		D6579Mb	+ve	0.105
		D6581M	+ve	0.056
		D6655M	+ve	0.024
		D6658M	+ve	0.01
		D6735Mb	+ve	0.145
		D6727Mb	+ve	0.246
		D6736Mb	+ve	0.025
		D6835Mb	+ve	0.008
		D6836Ma	+ve	0.063
		D491M	-ve	0.026
		D5422Ma	-ve	0.011
		D5429Mc	-ve	0.017
		D5437Ma	-ve	0.011
		D5469Mb	-ve	0.002
		D5477M	-ve	0.008
		D5553Ma	-ve	0.192
		D6572M	-ve	0.002
		D6598Ma	-ve	0.003
		D6605Ma	-ve	0.006
	F. proliferatum	D4297M	+ve	2.4
		D5510M	+ve	1.1
		D5517Ma	+ve	0.016
		D5560M	+ve	0.009
		D6708Ma	+ve	0.018
		D4304Mc	-ve	0.019

		D5355M	-ve	0.02
		D5373Mb	-ve	0.158
		D5377Ma	-ve	0.014
		D5511Ma	-ve	0.002
		D6736Ma	-ve	0.002
Animal feed	*F. verticillioides*	D4296SMa	+ve	2.1
		D5467SMb	+ve	0.08
		D6650SM	+ve	2.9
		D6899SMa	+ve	0.399
		D6901SMb	+ve	0.029
		D421Af	+ve	2.7
		D489Af	+ve	35.5
		D5398Af	+ve	0.104
	F. proliferatum	D457SMa	+ve	0.04
		D4332Sma	+ve	0.838
		D6569SM	+ve	4.4
		D6575SMb	+ve	0.479
		D4236Af	+ve	0.379
		D5432Af a	+ve	0.095

In the analysis for FB1 levels, it was found that there was a significant correlation (r=0.305, P=0.05) between the presence of the FUM1 gene and the FB1 levels assayed.

4.4 FARMING PRACTICES

A total of 262 questionnaires (appendix C) were administered. From the questionnaires that were administered, it was found that the farming practices, methods of storage of maize, methods of dealing with spoilt grain and methods used to dry the grain could be contributing factors in the proliferation of fungal contamination. This fungal contamination could lead to the possible exposure to fumonisins to both the human population that consumes the maize and the animals that are fed the grain.

The main source of the maize that is consumed in Nandi County was from the individual household farms (74%, n=262). Maize that was bought from markets

accounted for 4% (n=262) and that was received as gifts was 1% (n=262). The remaining 24% (n=262) was from other sources that were not specified.

4.4.1 CROP ROTATION

Crop rotation was practiced by 44% (n=262) of the respondents in Nandi County while the remaining 55% (n=262) did not practice crop rotation. With only 44% of the households sampled practicing crop rotation, most farmers sampled in the County grow the same crop season after season on the same lot of land which could be a contributing factor to exposure of their maize crop to *Fusarium* ear rot from one planting season to the next. In Laboret location however, 59% (n=97) of the households sampled practiced crop rotation but this was not the case for both Kilibwoni (42%, n=64) and Kaptumo (27%, n=101). There was a significant ($\chi^2(2, 0.001)$=13.86, P<0.001) practice of crop rotation in Nandi County.

Table 4: Practice of crop rotation

Location of the HH	Whether crop rotation is practiced			Total
	No	Yes	No response given	
Laboret	31(32)	57(59)	9(9)	97
Kilibwoni	36(56)	27(42)	1(2)	64
Kaptumo	72(71)	27(27)	2(2)	101
Total	139(53)	111(42)	12(5)	262

Figures in brackets are percentages

4.4.2 QUANTITY OF SPOILT MAIZE IN HOUSEHOLD STOCK FROM HOUSEHOLDS

Each household was asked to give an estimated amount of maize grain that was considered spoilt and this was converted into percentage and grouped as shown in table 5.

Table 5: Percentage quantity of spoilt maize in household stock

Location of the HH	Quantity of spoilt maize in HH stock in percent					**Total**
	0-20%	21-40%	41-60%	61-80%	81-100%	
Laboret	67	26	3	0	1	97
Kilibwoni	32	8	16	1	7	64
Kaptumo	66	6	11	4	14	101
Total	165	40	30	5	22	262

Most households (63%, n=262) had zero to 20% of spoilt grain in their household stock. This was the trend throughout all the 3 locations. There was a significantly large (χ^2(8, 0.001)=47.583, P<0.001) amount of spoilt maize in the household stock within the households sampled. In addition to this significantly large quantity of spoilt maize in household stock, it was found that when correlated with the practice of crop rotation, there was a significant two-tailed negative correlation (r=-0.141, P=0.05) with the quantity of spoilt maize in household stock.

4.4.3 DRYING OF GRAIN

After grain was harvested, it was dried in various ways. In Nandi County, maize was most commonly dried on canvas spread on the ground by 77% (n=262) of households sampled. This method of drying maize was a common trend throughout all the 3 locations sampled within Nandi County as is shown in table 6.

The other methods of drying maize that were still being used, such as drying maize directly on the tarmac roads and drying the maize on the cob in the granary were not very common and were only practiced by slightly less than 1% of the households sampled. Other methods used to dry maize were leaving maize to dry in the field (3%) and drying the maize on the cob directly on the ground (10%). This trend was the same in all the 3 locations sampled within the county.

Table 6: Methods used to dry maize

Method of drying grain	Location of household			Total
	Laboret	Kilibwoni	Kaptumo	
On tarmac (no canvas)	2	0	0	2(1)
On ground with canvas	71	36	94	201(77)
On ground (no canvas)	6	3	5	14(5)
On ground, on cob	8	19	0	27(10)
On farm	5	3	1	9(3)
In granary, on cob	0	2	0	2(1)
No response given	5	1	1	7(3)
Total	97	64	101	262(100)

Figures in brackets represent percentages

4.4.4 GRAIN STORAGE PRACTICES

The traditional methods of storing grain either in cribs or thatched granaries was not widely practiced in Nandi County it was only practiced by 15% of the households sampled as shown in table 7. Thirty-three percent of households sampled stored grain in iron sheet granaries and 49% of the households sampled stored their grain in polypropylene bags which are kept either in a granary or in a room in the house.

There was no significant difference ($\chi^2_{(9,\ 0.05)}$=12.000, $P>0.05$) in the methods used to store maize/grain in Nandi County, which indicated that the storage practices used were generally a matter of convenience for the farmers and were not necessarily related to or specific to any particular location.

Table 7: Grain storage methods within households

Mode of storage	Location of Household			Total
	Laboret	Kilibwoni	Kaptumo	
Crib raised	1	12	5	18(7)
Crib not raised	0	0	3	3(1)
Iron sheet granary, raised	28	12	11	51(19)
Iron sheet granary, not raised	29	1	7	37(14)
Thatched granary, raised	3	7	4	14(5)
Thatched granary, not raised	1	0	3	4(2)
Bag, raised	16	23	31	70(27)
Bag, not raised	18	8	34	58(22)
No response given	3	1	3	7(3)
Total	97	64	101	262(100)

Figures in brackets represent percentages

4.4.5 CRITERIA USED TO DETERMINE MAIZE SPOILAGE

The questionnaire also sought responses on the criteria the households used to determine whether the maize was spoilt. The responses ranged from rotting of grain, colour change in the grain (black, green or red), insect damage, mouldiness, smell, sprouting of grain or bitter taste. These responses are recorded in table 8.

There was a significant difference ($\chi^2_{(14,\ 0.001)}$=63.845, P<0.001) in the criteria used to determine whether the maize is spoilt within households sampled in Nandi County which indicates that there is a widespread lack of knowledge regarding fumonisins and mycotoxins in general.

Table 8: Criteria used by households to determine whether grain is spoilt

Criteria for spoilt maize	Location in Nandi County			Total
	Laboret	Kilibwoni	Kaptumo	
Rotten	9	29	45	83(32)
Colour change	80	18	40	138(53)
Insect damage	1	4	3	8(3)
Mouldy	2	2	5	9(3)
Smell	2	4	4	10(4)
Sprouting	1	3	2	6(2)
Bitter taste	2	3	1	6(2)
No response given	0	1	1	2(1)
Total	97	64	101	262(100)

Figures in brackets are percentages

4.4.6 HOW SPOILT MAIZE WAS DEALT WITH IN HOUSEHOLDS

After establishing that the households had some spoilt maize in their stock, the questionnaire went further to determine how the spoilt maize was dealt with. From the responses given, 64% of the respondents used the spoilt maize as animal feed and this was a common trend throughout all the 3 locations as shown in table 9.

Table 9: How spoilt maize is dealt with in households

Location of the HH	How spoilt maize is dealt with						Total
	Destroy	Sell	Consume	Animal feed	Leave in the shamba	No response given	
Laboret	4	1	6	65	12	9	97
Kilibwoni	3	2	10	38	6	5	64
Kaptumo	4	5	5	64	12	11	101
Total	11(4)	8(3)	21(8)	167(64)	30(11)	25(10)	262(100)

Figures in brackets represent percentages

There was no significant difference ($\chi^2_{(10,\ 0.05)}$=9.840, $P>0.05$) in the way spoilt maize was dealt with wihin households in all the three locations sampled in the county. In Kilibwoni, the second commonest way of dealing with spoilt maize was by consuming

it (16%, n=64), mainly in brewing *busaa* (a locally brewed alchoholic drink). In both Kaptumo and Laboret, the second most common way of dealing with spoilt maize was by leaving it in the field (*shamba*) (12%, n=101 and n=97 respectively) which greatly contributes to the spread of maize ear rot.

CHAPTER FIVE

5.1 DISCUSSION

In this study the most frequently isolated fungal genera in this study were *Fusarium Penicillium* and *Stenocarpella.* Other fungal genera isolated were *Aspergillus, Alternaria, Trichoderma* and *Rhizopus.* These findings are consistent with reports that maize ear rots are caused by several pathogens belonging mainly to genera of *Fusarium, Aspergillus, Penicillium, Stenocarpella* (also known as *Diplodia*), *Nigrospora* and *Macrospora* (Olanya *et al*., 1997; Alakonya *et al*., 2008).

The most prevalent *Fusarium* species in this study from household maize samples was *F. subglutinans* followed by *F. gramminearum* in both maize and animal feed samples this was then followed by *F. verticillioides* isolates which is consistent with previous studies on maize from Western Kenya that have readily isolated various ear rot fungi that include *F. verticillioides*, *F. graminearum, F. subglutinans, S. maydis, Aspergillus flavus* and *A. parasiticus* where both symptomless and rotten maize have shown ear rot contamination (Kedera *et al*., 1999). A field study in Western Kenya also found that mainly, *F. verticillioides, F. subglutinans* and *F. gramminearum* were readily isolated from both rotten and symptomless maize kernels in Western Kenya confirms that maize infection occurs in the field (Olanya, 1997). *Fusarium verticillioides* was not found to be predominant in this study although other studies have shown *F. verticillioides* (82% of isolates from maize) to be the predominant *Fusarium* species in maize in Kenya followed by *F. graminearum* (9% of isolates) and *F. subglutinans* (7% of isolates) (Kedera, 1994). In another study done in Western Kenya that covered nine districts by Kedera *et al.,* (1999), it was found that the prevalence of *F. verticillioides* was 60% overall and that of *F. gramminearum* was 31%. However, in the same study, only 6% of *F. verticillioides* was recovered from Nandi. Maize grain purchased from market stalls and roadside traders in Central and Western Kenya, Macdonald & Chapman (1997) also reported high incidence of *F. gramminearum* (9% of kernels tested) and of *F. verticillioides* (14% of kernels tested).

Isolation of *F. gramminearum* in Nandi in this study was very noticeable because it was higher than that of *F. verticillioides* and *F. proliferatum.* In studies done by both Kedera *et al*., (1999) and Olanya (1997) isolations of *F. gramminearum* were notably significant. Valleti *et al*., (2000) showed that populations of *F. verticillioides* and *F. proliferatum*, the most important fumonisin producers, are markedly reduced by the presence of *F. gramminearum*, and that fumonisins produced by them can be significantly reduced and inhibited as well by the presence of *F. gramminearum.* The altitude range within the study area of Nandi County was recorded to be between 1801m and 2083m above sea level. Bigirwa *et al*. (2007) also found that in districts of higher altitude (above 1800m above sea level) *F. gramminearum* dominated in all seasons.

Some household maize and animal feed samples that had tested negative for the presence of FUM1 gene in isolates of *F. proliferatum* and *F. verticillioides* had fumnisin levels recorded in CD-ELISA analysis. A similar situation was observed in the study where there were fumonisin levels recorded yet there were no *F. verticillioides* or *F. proliferatum* isolated from the grain samples. This discrepancy could be due to the possibility that more than one species of *Fusarium* may exist in the maize sample and the one producing fumonisins is responsible for the recorded fumonisin levels or possibly that there are other *Fusarium* species present in the isolate other than *F. proliferatum* and *F. verticillioides* that have the capability to produce fumonisins. It is also a common characteristic of *Fusarium* species to deteriorate and eventually die off in harvested grain yet leave fumonisin residue in the grain since the *Fusarium* species is endophytic. The presence of the fumonisins in sound-appearing, food-grade maize kernels (Ross *et al*., 1992; Riley *et al.,* 1993) supports the hypothesis that low concentrations of the fumonisins are synthesized by symptomless endophytic hyphae. This hypothesis would also account for the production of the fumonisins in higher concentrations in sound corn when stored under poor postharvest conditions or in kernels and debris from cobs and in other plant parts not showing ear and stalk rot symptoms (Riley *et al.,* 1993; Rheeder *et al*., 1992). In previous studies, it has been

reported that there are up to 15 *Fusarium* species that produce fumonisins. These species include: *F. verticillioides* (Bezunhout *et al*., 1988; Branham & Plattner, 1993); *F. sacchari* (Leslie *et al*., 1992); *F. fujikuroi* (Desjardins *et al*., 2000); *F. proliferatum* (Abbas *et al*., 1999; Cabanes *et al*., 1993); *F. subglutinans* (Leslie *et al.,* 1992); *F. thapsinum* (Klittich *et al*., 1997); *F. anthophilum* (Chelkowski & Lew, 1992); *F. globosum* (Sydenham *et al*., 1997); *F. nygamai* (Magnoli *et al*., 1999); *F. dlamini* (Nelson *et al*., 1992); *F. napiforme* (Nelson *et al*., 1992); *F. oxysporum* (Abbas *et al*., 1995) and *F. polyphialidicum* (Abbas & Ocamb, 1995). Trace amounts of fumonisin were detected in culture material of two recently described species, i.e., *F. andiyazi* and *F. pseudonygamai* (Rheeder *et al*., 2002). The only fungus that does not belong to the genus *Fusarium* that has been reported to produce fumonisins (FB1, FB2, andFB3) in culture is *Alternaria alternata* (Abbas & Riley, 1996; Chen *et al*., 1992). Several endogenous ear rot fungi co-occur on maize kernels from Western Kenya making it a possibility that more than one mycotoxin can be found in a single maize sample (Alakonya *et al*., 2009). In addition, isolation of other *Fusarium* species points to the possibility of the presence of other mycotoxins like moliniformin, deoxynivalenol and zeralenone associated with hyper-estrogenism and infertility (Marasas, 1984).

The most frequently isolated species of *Fusarium* from soil samples was *F. oxysporum*. These findings of *F. oxysporum* as the predominant *Fusarium* species in soil, corresponds to findings previously done that found *F. oxysporum* to be the predominant species isolated from all samples from soil and soil debris obtained from different altitudes in the Republic of Transkei (Jeschke *et al.,* 1990). Siti *et al*. (2012) also found that the highest number of *Fusarium* species isolated from soil was *F. oxysporum*. *Fusarium oxysporum* was the predominant species recovered from soil in Lesotho (Onyike & Nelson, 1993). *Fusarium oxysporum* was also widely distributed in various types of soil, such as those of the Arctic (Kommendahl *et al*., 1988), the desert (Joffe & Palti, 1977), cultivated and temperate soils (McMullen & Stack, 1983; Latiffah *et al*., 2007). *Fusarium oxysporum* was also one of the fungal species isolated from mangrove mud in Kagh

Islands, India (Rai *et al*. 1966). Stover (1955) reported that *F. oxysporum* could survive in submerged anaerobic marsh soils, another extreme environment. It has been reported that *F. oxysporum* can be dispersed through wind, soils, seeds and infected planting material (Garibaldi *et al*., 2004). The survival of *F. oxysporum* in soil and debris has been proven by Vakalounakis & Chalkias (2004), whereby it was found that *F. oxysporum* could survive as a successful soil-inhabiting fungus for more than 13 months. The ubiquitous distribution of *Fusarium* in soil may contribute as saprophytic decomposition in the process of nutrients cycling, while some species such as *F. oxysporum* have been found to beneficial in soil denitrification (Takaya *et al*., 2002; Steven *et al*., 2008).

The absence of both *F. verticillioides* and *F. proliferatum* from soil samples in this study, in spite of their presence in grain that was harvested from the same farm from which the soil samples were collected, is very indicative that these species may have been vertically transmitted. According to Bacon *et. al.* (2001), the endophytic phase is vertically transmitted, this type infection is important because it is not controlled by seed applications of fungicides, and it remains the reservoir from which infection and toxin biosynthesis takes place in each generation of plants. Control, prevention, and detection of the endophytic infections by *F. verticillioides* in maize are difficult, especially because kernels appear to be of sound quality. The intercellular nature of this endophyte makes chemical control highly unlikely. Applications of systemic fungicides are impossible during later stages of plant growth, and because the fungus is a systemic seed-borne infection, conventional fungicides as seed treatments are also ineffective. Vertical transmission is important because it remains the reservoir from which infection of each generation of plants takes place and from which renewed toxin synthesis can take place. Application of fungicides cannot control the fungus during this phase.

The regulations that exist in various nations regarding fumonisin levels are varied. The 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in

2001 recommended a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bodyweight per day (Wu, 2004). In this study, most households (74%) had fumonisin quantities higher than the above recommendation. In sub Saharan Africa and Latin America, the diet is mainly maize based with an average daily intake of 400g per person; in Europe and the United States the daily intake is 10g per person; thus, meeting the PMTDI for fumonisins would be more difficult in Africa and Latin America than in Europe and the United States. In Asia the maximum acceptable limit for FB1 ranges 5 - 35 µg/Kg, Latin America ranges 2 - 35 µg/Kg whereas North America ranges 0 - 5 µg/Kg (Van Egmond *et al*., 2007).

Most households (55%) did not practice crop rotation. When it comes to drying the grain after harvest, 77% of the households dried the maize on canvas spread on the ground. This helped reduce direct contact of the grain to soil-dwelling fungal species. However, as much as 15% of the households still dried their grain directly on the soil, while still on the cob or shelled from the cob which could contribute to additional fungal contamination. The common practice of storing the grain was in polypropylene bags inside iron sheet granaries or inside a room in the house, either directly on the floor (36%) or raised above the floor (46%). Polypropylene bags, though relatively cheaper than sisal and jute bags are known to retain more moisture than sisal and jute bags for storage of grain. In this study, it was found that 63% of the households recorded grain losses of up to 20% in their household stock. Eight percent of the households sampled recorded losses of between 80 and 100%, this kind of loss is very significant especially to small holder farms that depend on the grain harvested both for the household use and also as a source of income. Most households used colour change in the grain (53%) to determine whether grain is spoilt. A previous study found that the visible sorting of grain as a technique to reduce the exposure of subsistence farmers to fumonisins could be successful if there were enough good quality grain available to permit the poor quality grain to be used for another purpose or discarded (Afolabi *et al*., 2006). Sixty-four percent of the households sampled used the spoilt maize as animal

feed. Only 4% destroyed the spoilt grain. Three percent sold the spoilt grain and 8% consumed the spoilt grain either for food or for brewing a local alchoholic drink commonly known as *busaa* which exposes the consumers to fumonisin exposure since fermentation does not reduce fumonisin concentration.

The use of ELISA to screen fumonisin contamination in this study made it possible to screen large samples at lower cost. The CD-ELISA method is an official AOAC method used to detect fumonisins in food and feed, the assay may be used to quantify fumonisins in food and feed within 30 min (Abouzied *et al.* 1996; Bird *et al.,* 2002). Previous studies have detected several mycotoxins in both clean and rotten maize from Kenya among them fumonisins, aflatoxins, moniliformin, deoxynivalenol and zearalenone (Kedera *et al.,* 1999; Claudia *et al.,* 2007). Although Kenya has established the minimum tolerance levels for mycotoxins through the Kenya Bureau of Standards (KEBS) implementation of the standards has not been effectively executed due to lack of resources, manpower and proper legislation.

5.2 CONCLUSIONS

This research has shown that Fusarium species that have been associated with maize ear rot are predominant in Nandi county. *Fusarium subglutinans, F. verticillioides* and *F. gramminearum* were the main fungal species infecting maize and that *F. oxysporum* was the dominant species in soil in Nandi County. Toxigenic strains of *F. verticillioides* and *F. proliferatum* were also reported with high levels of fumonisins recorded from the grains sampled. The seemingly low incidence of toxigenic strains should not be overlooked since *Fusarium* species have been known to die off from the substrate yet leave the fumonisin residue in the substrate. This also indicates that more *Fusarium* species have the ability to produce fumonisins. Fumonisin levels were reported in majority of the grains tested which indicates that households are exposed to fumonisins in large quantities. Fungal infection of maize varied with households and this reflects different farm management practices, some of which contribute to contamination of the

maize. The farming practices employed by the households also help in the proliferation of ear rot causing fungi since crop rotation is not as widely practiced yet it is the easiest way of reducing the spread of ear rot fungi. The practice of delayed harvesting also contributes to the proliferation of these fungal species. The drying and storage practices used within the households encourage moisture retention in the grain; increased moisture content reduces grain quality as it aids in proliferation of fungal species that can and do cause mycotoxin contamination. Consequently, households are exposed to fumonisins and other mycotoxins at alarmingly high levels. Most households in this study were not aware of the dangers posed by mycotoxins and the onus is on the farming extension workers to educate farmers on affordable farming, grain handling and storage practices that can be used to reduce this exposure to the dangers posed by mycotoxins.

5.3 RECOMMENDATIONS

1. The widespread nature of *Fusarium* species associated with maize ear rot warrants the need to carry out further research in order to come up with ways of controlling and managing these species.
2. The high levels of fumonisins found in many of the grain samples warrants the need for better surveillance and monitoring of maize samples for fumonisins and other mycotoxins and re-evaluating the management practices of the crop to reduce the risk of fumonisin contamination.
3. There is clear need for tools to manage contamination of locally produced maize.
4. Given the widespread nature of toxigenic strains, there is need for the control strategies to include field interventions.

REFERENCES:

Abbas H.K., and Ocamb C.M. (1995). First report of production of fumonisin B1 by *Fusarium polyphialicum* collected from seeds of Pinus strobus. *Plant Dis.;* **79**: 642.

Abbas H.K., Ocamb C.M., Xie W., Mirocha C.J., and Shier W.T. (1995). First report of fumonisin B1, B2 and B3 production by *Fusarium oxysporum* var. redolens. *Plant Dis*.**;** **79**: 968.

Abbas H.K., and Riley R.T. (1996). The presence and phytotoxicity of fumonisins and AAL-toxin in *Alternaria alternata*. *Toxicon;* **34**: 133–136.

Abbas H.K., Cartwright R.D., Xie W., Mirocha C.J., Richard J.L., Dvorak T.J., Sciumbato G.L. and Shier W.T. (1999). Mycotoxin production by *Fusarium proliferatum* isolates from rice with Fusarium sheath rot disease. *Mycopathologia;* **147**: 97–104.

Abouzied M.M., Askegard S.D., Bird C.B. and Miller B.M. (1996). Fumonisins Veratox. A new rapid quantitative ELISA for determination of fumonisin in food and feed. *Adv Exp Med Biol*; **392**: 135–144.

Afolabi C.G., Bandyopadhyay R., Leslie J.F. and Ekpo E.J. (2006). Effect of sorting on incidence and occurrence of fumonisins and *Fusarium verticillioides* on maize from Nigeria. *J Food Prot.*; **69**(8): 2019-2023.

Ahmed N. and Cook P. (1969). The incidence of cancer of the oesophagus in West Kenya. *Br J Cancer*; **23**: 302-312.

Alakonya A.E., Monda E.O. and Ajanga S. (2008). Variation in *in vitro* fumonisin B1 production by different *Fusarium verticillioides* isolates in Kenya. *Eurasian J. Agric & Envn. Sci.;* **4**(3): 368-371.

Alakonya A.E., Monda E.O. and Ajanga S. (2009). Fumonisin B1 and aflatoxin B1 levels in Kenyan maize. *J. Plant Pathol.;* **91**(2): 459-464.

Allah Fadl (1998). Occurance and toxigenicity of *Fusarium moniliforme* from freshly harvested maize ears with special reference to fumonisin production in Egypt. *Mycopathologia;* **140**: 99-103.

Arias Diaz M.M., Munkvold G.P. and Leandro L.F. (2011). First report of *Fusarium proliferatum* causing root rot on soybean (*Glycine max)* in the United States. *Plant Disease*; **95**(10): 1316.

Asiedu J.J (1989). Processing Tropical Crops: A Technological Approach. The Macmillan Press, London & Basingstoke. P. 266.

Aziz N.H., El-Far F. M., Shahin A.A.M. and Roushy S.M. (2007). Control of *Fusarium* moulds and fumonisin B1 in seeds by gamma-irradiation. *Food Control.* **18:** 1337-1342.

Bacon C.W. and Hinton D. (1996). Symtomless endophytic colonization of maize by *Fusarium moniliforme*. *Can. J. Bot.;* **74**: 1195–1202.

Bacon C.W. and Williamson J.W. (1992). Interactions of *Fusarium moniliforme*, its metabolites and bacteria with corn. *Mycopathologia;* **117:** 65-71.

Bacon C.W., Yates I.E., Hinton D.M. and Filmore M. (2001). Biological Control of Fusarium moniliforme in Maize. *Environ. Health Pers.;* **109**: (2).

Baird R., Abbas H.K., Windham G., Williams P., Baird S., Ma P., Kelley R., Hawkins L. and Scruggs M. (2008). Identification of select fumonisin-forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production *in vitro. Int. J. Mol. Sci.*; **9**: 554-570.

Bennett J.W. and Klich M. (2003). Mycotoxins. *Clinical Microbiology Reviews*; **16**(3): 497–516.

Bezuidenhout S.C., Gelderblom W.C.A. *et al.* (1988). Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *Journal of the chemical Society;* **Chemical Communications**: 743-745.

Bigirwa G., Kaaya A.N., Sseruwu G., Adipala E. and Okanya S. (2007). Incidence and severity of maize ear rots and factors responsible for their occurance in Uganda. *Journal of Appl. Sci.;* **7**(23): 3780-3785.

Bird C.B., Malone B., Rice L.G., Ross P.F., Eppley R. and Abouzied M.M. (2002). Determination of total fumonisins in corn by competitive direct enzyme-linked immunosorbent assay: collaborative study. *J Assoc Anal Chem*; **85**: 404–410.

Bluhm B.H., Cousin M.A. and Woloshuk C.P. (2004). Multiplex real time PCR detection of fumonisin-producing and trichothecene-producing groups of *Fusarium* species. *Journal of Food Protection;* **67**(3): 536–543.

Branham B.E., and Plattner R.D. (1993). Alanine is a precursor in the biosynthesis of fumonisin B1 by *Fusarium moniliforme*. *Mycopathologia;* **124**: 99–104.

Cabanes, F. J., Abarca M. L., Bragulat M. R., and Castella G. (1993). High incidence of fumonisin-producing strains of *Fusarium moniliforme* isolated in Spain, p. 293–296. In K.

A. Scudamore (ed.), Occurrence and significance of mycotoxins. Central Science Laboratory, Slough, United Kingdom.

Cardwell K.F. and Cotty P.J. (2002). Distribution of *Aspergillus* section *Flavi* among field soils from the four agroecological zones of the Republic of Benin, West Africa. *Plant Diseases;* **86**(4): 434-439.

Castella G., Bregulat R.M. and Cabanes F.J. (1999). Fumonisin production by *Fusarium* species isolated from cereals and feeds in Spain. *J. Food Protection*; **62**(7): 811-813.

Charmley L.L. and Prelusky D.B. (1995). Decontamination of *Fusarium* mycotoxins. *Appl. Environ. Microbiol.*; **1**: 421–435.

Chelkowski J., and Lew H. (1992). *Fusarium* species of Liseola Section— occurrence in cereals and ability to produce fumonisins. *Microbiologie Aliments Nutr.*; **10**: 49–53.

Chen, J.P., Mirocha C.J., Xie W., Hogge L. and Olson D. (1992). Production of the mycotoxin fumonisin B1 by *Alternaria alternata* f. sp. lycopersici. *Appl. Environ. Microbiol.;* **58**: 3928–3931.

Chu F.S. and Li G.O. (1994). Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidence of esophageal cancer. *Appl. Environ. Microbiol.;* **60:** 847-852.

Claudia P., Njapau H. and Cotty P.J. (2007). Outbreak of an Acute Aflatoxicosis in Kenya in 2004: situation Identification of the Causal Agent. *American Society for Microbiology*; pp. 2762-2764.

Cochran W.G. Sampling Techniques. 2nd. John Wiley and Sons Inc.; New York, NY, USA: 1963.

Colvin B.M. and Harrison L.R. (1992). Fumonisins induced pulmonary edema and hydrothorax in swine. *Mycopathologia*; **117**: 79-82.

Cook P. (1971). Cancer of esophagus in Africa. *Brit J. Cancer;* **25**: 853- 880.

deNazareno N.R.X., Lipps P.E. and Madden L.V. (1992). Survival of *Cercospora zeae maydis* in Corn Residues in Ohio. *Plant Dis.;* **76:** 560-564.

Desjardins A.E., Manandhar G., Plattner R.D., Maragos C.M., Shrestha K. and McCormick S.P. (2000). Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. *J. Agric. Food Chem.*; **48**: 1377–1383.

Doko M.B., Rapior S., Viscont A. and Sehjoth J.E. (1985). Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *J. Agric. Food Chem.;* **43:** 3240-3243.

Fandohan P., Hell K., Marassas W.F.O. and Wingfield M.J. (2003). Infection of maize by *Fusarium* species and contamination with fumonisins in Africa. *African Journao of Biotechnology;* **2**(12): 570-579.

Flett B.C. and Wehner F.C. (1991). Incidence of *Stenocarpella* and *Fusarium* in Monoculture Maize under different Tillage Systems. *J. Phytopathol*.; 133:327-333.

Food and Drug Administration (2000a). *Background Paper in Support of Fumonisin Levels in Corn and Corn Products Intended for Human Consumption*. http:// www.cfsan.fda.gov/_dms/fumonbg3.html

Food and Drug Administration (2000b). *Background Paper in Support of Fumonisin Levels in Animal Feed*. http://vm.cfsan.fda.gov/_dms/fumonbg2.html.

Gathumbi J.K., Usleber E. and Martlbauer E. (2001). Production of ultrasensitive antibodies against aflatoxin B1. *Letters in Applied Microbiology;* **32**: 349-351.

Garibaldi A., Gilardi G. and Gullino M.L. (2004). Seed transmission of *Fusarium oxysporum* f. sp. lactucae. *Phytoparasitica*; **32**: 61–65.

Gelderblom W.C.A., Jaskiewicz K., *et. al.* (1988). Fumonisins – Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme. Applied & Environmental Microbiology;* **54:** 1806-1811.

Gonzalez H.H.L., Resnik S.L., Boca R.T. and Marasas W.F.O. (1995). Mycoflora of Argentinian corn harvested in the main production area in 1990. *Mycopathologia;* **130:** 29-36.

Gonzalez-Jaen M.T., Mirete S., Patino B., Lopez-Errasqui and E., Vasquez C. (2004). Genetic markers for the analysis of variability and for prodiction of specific diagnostic sequences in fumonisin-producing strains of *F. verticillioides. Eur J Plant Pathol*.; **110**: 525-532.

Hennigen M.R., Valente Soares L.M., Sanchez S., Di Benedetto N.M., Longhi A., Eyhérabide G., Torroba J. and Zanelli M. (2000). Fumonisin in corn hybrids grown in Argentina for two consecutive seasons. In: De Koe W.J., Samson R.A., van Egmond

H.P., Gilbert J., Sabino M. (Eds.) Proceeding of the Xth international IUPAC symposium on mycotoxins and phycotoxins. **21–25, May 2000**. Guaruja, Brazil; pp. 331–339.

International Agency for Research on Cancer (2002). IARC monographs on the evaluation of carcinogenic risks to humans vol. 82. Some traditional herbal medicines, mycotoxins, naphthalene & styrene. (International Agency for Research on Cancer) Lyon, France; p. 590.

Jeschke N., Nelson P.E. and Marasas W.F.O. (1990). *Fusarium* species isolated from soil samples collected at different altitudes in Transkei, South Africa. *Mycologia*; **82**(6): 727-733.

Joffe A.Z. and Palti J. (1977). Species of *Fusarium* found in uncultivated desert-type soils in Israel. *Phytoparasitica;* **5**: 119–122.

Jones R.K., Duncan H.E. and Hamilton P.B. (1981). Planting date, harvest date, irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology;* **71**: 810–816.

Jurado M., Vázquez C., Marín S., Sanchis V. and González-Jaén M.T. (2006). PCR-based strategy to detect contamination with mycotoxigenic *Fusarium* species in maize. *Systematic and Applied Microbiology* **29**(8): 681–689.

Kedera C.J., Ochor T.E.O., Ochieng J.A.W. and Kamidi R.E. (1994). Maize Ear Rot Incidence in Western Kenya. *Int. J. Pest Manag*. **40**: 117-120.

Kedera C.J., Plattner, R.D. and Desjardins A.E. (1999). Incidence of *Fusarium* spp. and levels of fumonisins in maize in Western Kenya. *Applied & Environmental Microbiology;* **65:** 41-44.

Kenya Agricultural Research Institute (1998). Annual Report Kenya Agricultural Research Institute, Nairobi, Kenya; p. 17.

Klittich, C.J.R., Leslie J.F., Nelson P.E. and Marasas W.F.O. (1997). *Fusarium thapsinum* (*Gibberella thapsina*): a new species in section Liseola from sorghum. *Mycologia*; **89**: 643–652.

Kommendhal T. and Windels C.E. (1981). Root-, Stalk- and Ear-infecting *Fusarium* species in the USA. In: *Fusarium:* Diseases, Biology and Taxonomy. Nelson P.E, Toussoun P.A and Cook R.J (Eds.), Pennsylvania State University, University Park; pp. 94-103.

Kommedahl T., Abbas H.K., Barnes P.M. and Mirocha C.J. (1988). Prevalance and toxigenicity of *Fusarium* species form soils of Norway near the Arctic Circle. *Mycologia;* **80**: 790–794.

Latiffah Z., Mohd Zariman M. and Baharuddin S. (2007). Diversity of *Fusarium* species in cultivated soils in Penang. *Malaysian Journal of Microbiology;* **3**(1): 27–30.
Lauren D.R. and W.A. Smith, (2007). Influence of harvest date and hybrid on the mycotoxin content of maize (*Zea mays*) grain grown in New Zealand. *New Zealand J. Crop and Horticul*. Sci., **35**: 331-340.

Leslie J. F., Plattner R. D., Desjardins A. E., and Klittich C. J. R. (1992). Fumonisin B1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* Section Liseola). *Phytopathology;* **82**: 341–345.

Leslie J.F. and Summerall B.A. (2006). The *Fusarium* laboratory manual. 1st ed. Blackwell, Ames, Iowa. USA.

Lino C.M., Silva L.J.G., Pena A.L.S. and Silveira M.I. (2006). Determination of fumonisins B1 and B2 in Portuguese maize and maize-based samples by HPLC with fluorescence detection. *Anal. Bioanal. Chem*.; **384**: 1214-1220.

Macdonald M.V. and Chapman R. (1997). The incidence of *Fusarium moniliforme* on maize from Central America, Africa and Asia during 1992–1995.*Plant Pathol*.; **46**: 112–125.

Magnoli C. E., Saenz M. A., Chiacchiera S. M. and Dalcero A. M. (1999). Natural occurrence of *Fusarium* species and fumonisin-production by toxigenic strains from poultry feeds in Argentina. *Mycopathologia*; **145**: 35–41.

Maina P.K., Okoth S., Njoroge C.N. and Monda E. (2009). Genetic relatedness among *Fusarium* species isolated from Taita Taveta region, Kenya. *Tropical & Subtropical Agroecosystems;* **11:** 337-345.

Marasas W.F.O., Nelson P.E. and Toussoun T.A. (1984). Toxigenic *Fusarium* species. Identity and mycotoxicology. The Pennsylvania State University Press, University Park, PA, USA.

Marassas W.F.O., Jaskiewicz K. *et al.* (1988). *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei, S. Africa. *Med. J.;* **74:**110-114

Marasas W.F.O. (1996). *Fumonisins*: History, Worldwide Occurrence and Impact. In: Jackson L.S., Devries J. W. and Bullerman L. B. (Eds.), Fumonisins in Food. Plenum press. New York, USA; pp. 1-18.

Marassas W.F.O., kriek N.P.J., Wiggins V.M., Steyn P.S., Towers D.K. and Hastie T.J. (1997). Incidence, Geographic Distribution and Toxigenicity of *Fusarium* species in South African Corn. *Phytopathology*; **69**: 1181-1185.

McMullen M.P. and Stack R.W. (1983). *Fusarium* species associated with grassland soils. *Canadian Journal of Botany*; **61**: 2530–2538.

Munkvold G.P. and Desjardins A.E. (1997). Fumonisins in maize: Can we reduce their occurance? *Plant Dis.;* **81:** 556-565.

Munkvold Gary P. (2003). Cultural and Genetic Approaches to Managing Mycotoxins in Maize. *Annu. Rev. Phytopathol.*; **41**: 99-116.

Nayaka C.S. and Udaya Shankar A.C. (2010). Detection and quantification of fumonisins from *Fusarium verticillioides* in maize grown in Southern India. *World Microbiol. Biotechnol.;* **26:** 71-78.

Nelson P.E., Desjardins A.E. and Plattner R.D. (1983). Fumonisins. Mycotoxins produced by *Fusarium* species – biology, chemistry and significance. *Annual Rev. Phytopathol.;* **31:** 233-252.

Nguyen, Thi Thanh Xuan (2011). Symptoms of *Fusarium proliferatum* on Maize Leaves. Tropentag, Bonn.

Olanya O.M., Hoyos G.M., Tiffany L.H. and McGee D.C. (1997). Waste Corn as a Point Source of Innoculum for *Aspergillus flavus* in Corn agroecosystems. *Plant Dis.;* **81**: 576-581.

Ono E.Y.S., Sugiura Y., Homechin M., Kamogae M., Vizzoni E., Ueno Y. and Hirooka E.Y. (1999). Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the State of Parana, Brazil. *Mycopathologia;* **147**: 139–148.

Parkin D.M, Pisani P. and Ferlay J. (1993). Estimates of worldwide incidence of eighteen major cancers in 1985. *Int. J Cancer***; 54**: 594-606.

Patino B., Mirete S., Gonzalez-Jaen T., Mule G., Rodriguez T.M. and Vazquez C. (2004). PCR detection assay of fumonisin-producing *Fusarium verticillioides* strains. *J. Food. Prot.*; **6**: 1278-1283.

Proctor R.H., McCormick S.P., Alexander N.J. and Desjardins A.E. (2009). Evidence that a secondary metabolic biosynthetic gene cluster has grown by gene relocation during evolution of filamentous fungus *Fusarium. Mol. Microbiol.*; **74**: 1128-1142.

Rai J.N., Tewari J.P. and Mukerji K.G. (1966). Mycoflora of mangrove mud. *Mycopathologia et Mycologia Applicata*; **38**: 17–31.

Rheeder J.P., Marasas W.F.O., Thiel P.G., Sydenham E.W., Shephard G.S. and Van Schalkwyk D.J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology*; **82**: 353–357.

Rheeder, J.P., Marasas, W.F.O. and Vismer, H.F. (2002). Production of fumonisin analogs by *Fusarium* species. *Appl. Environ Microbiol.*; **68**(5): 2101-2105.

Riley R. T., Norred W. P. and Bacon C. W. (1993). Fungal toxins in foods: recent concerns. *Annu Rev Nutr*; **13**: 167–189.

Robledo-Robledo E. (1991). Strategies for the prevention aand control of fungi and mycotoxins in Central and South America. In: Champ B.R., Highby E., Hocking A.D., P.H JI. Eds. Fungi and mycotoxins in stored products. (Proceedings of an international conference) Bangkok, Thailand; **23-26 April, 1991:** pp. 39-46.

Ross P.F., Rice L.G., Osweiler G.D., Nelson P.E., Richard J.L. and Wilson T.M. (1992). A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. *Mycopathologia*; **117**: 109–114.

Roth J.A., Putman Allen S.L. and Aslene A.F. (1993). Cancer: Principles and Practise of Oncology: 4th Edition.

Sambrook J., Frisch E.F. and Manatis T. (1988). Molecular cloning: a laboratory manual, 2nd ed. New York: Cold Spring Harbor Press.

Shetty P.H. and Bhat R.V. (1997). Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in Indian sorghum, maize and poultry feeds. *J. Agric. Food Chem.*; **45**: 2170-2173.

Shetty P.H. and Bhat R.V. (1999). A physical method for segregation of fumonisin contaminated maize. *Food Chem.*; **66**: 371–374.

Siti N.M, Nur Ain Izzati M.Z, Nur Azlin A. and Salleh B. (2012). Diversity of Fusarium species isolated from soil cultivated with cucurbits within East Coast Peninsular, Malaysia. *Pertanika J. Trop. Agric. Sci.*; **35**(2): 381-386.

Sopterean L.M. and Puia C. (2012). The Major Mycotoxins Produced by *Fusarium* Fungi and their Effects. *ProEnvironment*; **5**: 55–59.

Sreenivasa M.Y., Sharmila Dass R., Charith Raj A.P. and Janardhana G.R. (2006). Molecular Detection of Fumonisin Producing *Fusarium* Species of Freshly Harvested Maize Kernels Using Polymerase Chain Reaction (PCR). *Taiwania*; **51**(4): 251-257.

Sreenivasa M.Y., González-Jaen M.T., Sharmila Dass R., Charith Raj A.P. and Janardhana G.R. (2008). A PCR-Based Assay for the Detection and Differentiation of Potential Fumonisin-Producing Fusarium verticillioides Isolated from Indian Maize Kernels. *Food Biotechnology*; **22**: (2): 160-170.

Stankovic S., Levic J., Petrovic T., Logrieco A. and Moretti, A. (2007). Pathogenicity and mycotoxin production by *Fusarium proliferatum* isolated from onion and garlic in Serbia. *European Journal of Plant Pathology*; **118**(2): 165-172.

Stepien Lukasz, Koczyk Grzegorz and Waskiewicz Angieszka (2011). Genetic and Phenotypic Variation of *Fusarium proliferatum* isolates from different host species. *J. Appl. Genet*.; **52**(4): 487-496.

Steven A.W., Rosemary A.W., Lingxiao K. and Paul R.H. (2008). Management factors affecting size and structure of soil *Fusarium* communities under irrigated maize in Australia. *Applied Soil Ecology*; **39**: 201-209.

Stover R.H. (1955). Flood fallowing for eradication of *Fusarium oxysporum* f. cubense: III. Effect of oxygen on fungus survival. *Soil Science;* **80**: 397–412.

Sydenham E.W. and Thiel P.G. (1990). Natural occurance of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei, Southern Africa. *Journal of Agricultural & Food Chemistry;* **38:** 1900-1903.

Sydenham, E.W., Shephard G.S., Stockenström S., Rheeder J.P., Marasas W.F.O. and Van der Merwe M.J. (1997). Production of fumonisin B analogues and related compounds by *Fusarium globosum*, a newly described species from corn. *J. Agric. Food Chem.*; **45**: 4004–4010.

Takaya N., Uchimura H., Lai Y.P. and Shoun H. (2002). Transcriptional control of nitric oxide reductase gene (cryp55) in the fungal denitrifier *Fusarium oxysporum*. *Biosci. Biotech. Biochem*; **66**: 1039-1045.

Thiel P.G., Marassas W.F.O., Sydenham E.W., Shephard G.S., Gelderblom W.C.A. and Nieuwenhuis J.J. (1991). Survey of fumonisin production by *Fusarium* species. *Applied & Environments Microbiology;* pp. 1089-1093.

Usleber E., Margit S. and Gerhard T. (1994). Enzyme immunoassay for fumonisin B1 applied to corn- based food. *American Chemical Society;* **42**: 1392-1396.

Vakalounakis D.J. and Chalkias J. (2004). Survival of *Fusarium oxysporum* f. sp. Radicis-cucumerinum in soil. *Crop Protection*; **23**: 871-873.

Van Egmond H.P., Ronald C.S. and Marco A.J. (2007). induced pulmonary oedema and hydrothorax in swine. *Mycopathologia*; **117**: 79-82.

Wakhisi J., Patel K., Buziba N. and Rotich J. (2005). Esophageal cancer in North rift valley of Western Kenya. *African Health Sciences;* **5**(2): 156-163.

Wang S., Quan Y., Lee N. and Kennedy R.I. (2006). Rapid determination of fumonisin B1 in food samples by enzyme-linked immonosorbent assay and colloid gold immunoassay. *J. Agric. Food Chem.;* **54**: 2491-2495.

Zhang Z.X., Li B.Y. and Jin S.S. (1990). Epidemiologic trends of esophageal cancer in Linxian Shi Guan. *Gan Zhi Yaqn Fiu-Lixian*; **1**: 1-14.

APPENDIX A: Table 1: Identification Table for Isolated *Fusarium* species

FUSARIUM SPECIES	COLONY MORPHOLOGY	TOP COLOUR ON PDA	REVERSE COLOUR ON PDA	DIFFUSION INTO MEDIA	SPORODOCHIA ON CLA	MACROCONIDIA PRESENCE	MACROCONIDIA SHAPE	MACROCONIDIA WALL AND SEPTATION
F. verticillioides	Dense, fast-growing mycelia	White	Violet	Dark violet	Orange	Rarely present	Long, slender, slightly curved	Thin-walled, 3 to 5 septa
F. proliferatum	Abundant aerial mycelia	White	Violet	Violet	Pale orange	Rarely present	Slender, relatively straight	Thin-walled, 3 to 5 septa
F. gramminearum	Very dense, rapid growing mycelia	White or Yellow	Red	Red	Pale orange	Present and abundant	Slender, medium length	Thick-walled, 5 to 6 septa
F. subglutinans	Abundant mycelia	White	Pale orange or Salmon	None	Orange	Present but sparse	Slender, slightly falcate	Thin-walled, 3 septa
F. anthophilum	Rapid growing mycelia	White	Pale orange or Salmon	None	Orange	Present but sparse	Slender, almost straight	Thin-walled, 3 or 4 septa
F. oxysporum	Flocose, abundant mycelia	White	Pale violet	Pale violet	Pale orange	Present and abundant	Short to medium length, almost straight	Thin-walled, 3 septa
F. lateritrium	Slow growing	White to pale orange	White	None	Pale orange	Present and abundant	Medium long, thin, straight parallel walls	Thin-walled, 5 septa
F.solani	Sparse mycelia	Cream	Cream	None	Cream, blue or green	Present and abundant	Wide, straight	Thick-walled, 5 to 7 septa
F. acuminatum	Floccose, slow growing	White	Rose	Burgundy	Pale orange	Present and abundant	Moderate equally curved	Thick-walled, 5 septa
F. dimerum	Very slow growing, yeast-like colony	Orange	Orange	None	Absent	Present and abundant	Very short with even curvature	Thin-walled, 0 to 1 septa
F. equiseti	Abundant mycelia	White	Brown	Brown	Orange	Present and abundant	Long and slender	Thin-walled, 5 to 7 septa
F. semitectum	Abundant, fast growing mycelia	Off-white	Beige to brown	None	Orange	Present but sparse	Curved dorsal and straight ventral surfaces	Thick-walled, 3 to 5 septa
F. poae	Felted abundant mycelia	Off--white	Reddish-brown	Red	Pale orange	Present	Slender, relatively short, falcate	Thick-walled, 3 septa

Table 2: Continuation of Table 1 above

FUSARIUM SPECIES	MACROCONIDIA APEX	MACROCONIDIA BASE	MICROCONIDIA PRESENCE	MICROCONIDIA SHAPE	PHIALIDES	CHLAMYDOSPORE PRESENCE	CHLAMYDOSPORE LOCATION
F. verticillioides	Curved and tapered to a point	Notched or foot-shaped	Present and abundant	Oval with flat base	Long monophialidic chains	Absent	-
F. proliferatum	Curved	No foot shape	Present and abundant	Club-shaped with flat base	V-shaped polyphialides	Absent	-
F. grammineaarum	Tapered	Foot-shaped	Absent	-	-	Present, globose	Singly, in clusters and in chains
F. subglutinans	Curved	No foot shape	Present and abundant	Oval	Extensively proliferating polyphialides	Absent	-
F. anthophilum	Curved	Not clear foot shape	Present and abundant	Pyriform, globose or ovoid	Long monophialides	Absent	-
F. oxysporum	Tapered and curved	Foot-shaped, pointed	Present and abundant	Oval, elliptical or kidney-shaped	Short monophialides	Present and abundant	Singly or in pairs
F. lateritrium	Hook or beak	Foot-shaped or Notched	Absent	-	-	Present	Singly or in chains
F.solani	Blunt and rounded	Notched or rounded	Present and abundant	Oval, ellipsoid, fusiform	Long monophialides	Present	Singly or in pairs, short chains
F. acuminatum	Tapered and elongate	Foot-shaped	Present and abundant	Present but rare	Fusiform or reniform	Present	In chains or clusters
F. dimerum	Rounded and hooked	Blunt or slightly notched	Absent	-	-	Present	Singly, in pairs or chains
F. equiseti	Tapered an elongate, whip-like	Elongate foot shape	Absent	-	-	Present	Singly, in chains or clumps
F. semitectum	Curved and tapering to a point	Foot-shaped	Present but sparse	Pyriform or obvate, 1 septa	Monophialides and polyphialides	Present	Singly or in chains
F. poae	Curved and tapering	Foot-shaped	Present and abundant	Globose or napiform	Urn-shaped monophialides	Present butrare	In clumps or chains

APPENDIX B

Table 3: Quantity of fumonisins and fungal species found in household maize samples without *Fusarium verticillioides* and *F. proliferatum* from Laboret location

Sample Code	Quantity of fumonisins in grain in ppm	Fungal species isolated from the tested sample
D414M	0.012	*F. subglutinans*
D418M	0.002	*F. lateritrium*
D424M	0.053	*F. lateritrium*
D425M	0.016	*F.oxysporum*
D431M	1.1	*F. subglutinans*
D434M	0.001	*F. subglutinans*
D435M	0.035	*F. lateritrium*
D437M	0.017	*F. anthophilum*
D438M	0.013	*F. anthophilum*
D440M	0.755	*F.anthophilum*
D443M	3	*F. anthophilum*
D445M	0.017	*Aspergillus spp.*
D453M	0.034	*F. subglutinans*
D461M	0.251	*F. gramminearum*
D464M	0.293	*F. solani*
D465M	0.088	*Trichoderma spp.*
D485M	0.009	*F. anthophilum*
D487M	0.004	*F. anthophilum*
D489M	0.038	*F. subglutinans*
D4118M	9.8	*Alternaria spp.*
D4119M	0.044	*Alternaria spp.*
D4144M	0.585	*F. subglutinans*
D4145M	2.6	*F. gramminearum*
D4145M	0.006	*F. gramminearum*
D4147M	0.022	*F. anthophilum*
D4147M	0.057	*F. anthophilum*
D4161M	0.017	*F. gramminearum*
D4164M	2.4	*F. gramminearum*
D4168M	0.034	*F. gramminearum*
D4181M	0.049	*Penicillium spp.*
D4218M	0.213	*F. anthophilum*
D4219M	0.087	*F. gramminearum*
D4223M	0.218	*F. subglutinans*
D4243M	0.14	*F. gramminearum*
D4245M	0.003	*F. gramminearum*
D4250M	0.081	*F. gramminearum*
D4251M	0.023	*F. gramminearum*
D4255M	0.004	*F. anthophilum*
D4267Ma	0.026	*F. subglutinans*
D4270M	0.004	*Rhizopus spp.*
D4271Ma	2.3	*F. gramminearum, F. solani*

D4308M	0.261	*F. subglutinans*
D4318M	0.022	*Diplodia spp.*
D4319M	0.216	*Aspergillus spp., Penicillium spp.*
D4331M	0.015	*F. gramminearum*
D4332M	0.112	*F. subglutinans, Alternaria spp.*
D4338M	0.03	*Alternaria spp.*

Table 4: Quantity of fumonisins and fungal species found in household maize samples without *Fusarium verticillioides* and *F. proliferatum* from Kilibwoni location

Sample Code	Quantity of fumonisins in grain in ppm	Fungal species isolated from the tested sample
D5362M	0.029	*F. gramminearum*
D5364M	0.031	*F. subglutinans, Penicillium spp.*
D5376M	0.018	*Rhizopus spp.*
D5380M	0.112	*Rhizopus spp.*
D5400M	0.014	*Diplodia spp.*
D5409M	0.012	*Diplodia spp.*
D5410M	0.053	*F. anthophilum, F. gramminearum*
D5416M	0.015	*F. subglutinans, Diplodia spp.*
D5431M	0.192	*F. anthophilum, F. gramminearum*
D5436M	0.002	*Diplodia spp.*
D5438M	0.064	*F. subglutinans, F. gramminearum*
D5446M	0.001	*F. subglutinans*
D5467M	0.042	*Diplodia spp.*
D5473M	0.009	*F. subglutinans, F. anthophilum*
D5481M	0.03	*F. subglutinans*
D5484M	0.024	*Diplodia spp., Trichoderma spp.*
D5490M	2.4	*F. subglutinans*
D5525Ma	0.017	*F. anthophilum, F. gramminearum*
D5534M	0.031	*F. gramminearum*
D5544M	0.003	*F. gramminearum*

Table 5: Quantity of fumonisins and fungal species found in household maize samples without *Fusarium verticillioides* and *F. proliferatum* from Kaptumo location

Sample Code	Quantity of fumonisins in grain in ppm	Fungal species isolated from the tested sample
D6569M	0.019	*Diplodia*
D6570M	0.019	*F. subglutinans*
D6574M	0.008	*Diplodia spp., F. anthophilum*
D6575M	0.002	*F. gramminearum, Diplodia spp.*
D6577M	0.113	*Diplodia spp.*
D6580M	2.3	*Diplodia spp.*
D6596M	0.043	*Diplodia spp.*
D6600M	0.485	*F. gramminearum*
D6601M	0.066	*Diplodia spp.*
D6613M	0.034	*F. subglutinans*
D6614M	0.048	*Diplodia spp.*
D6616M	0.011	*Diplodia spp.*
D6618M	0.005	*F. subglutinans*
D6650M	0.015	*F. gramminearum*
D6651M	0.012	*Trichoderma spp.*
D6657M	0.007	*Aspergillus spp*
D6658Mb	0.01	*F. subglutinans, Trichoderma spp.*
D6660M	0.002	*F. solani*
D6680M	0.004	*Aspergillus spp.*
D6681M	0.005	*F. anthophilum*
D6683M	0.002	*F. subglutinans*
D6684M	0.064	*F. gramminearum*
D6688M	0.684	*Diplodia, F. subglutinans*
D6689M	0.005	*Diplodia, F. anthophilum*
D6728M	0.004	*Diplodia spp.*
D6729M	0.016	*Diplodia spp.*
D6735M	0.145	*Trichoderma spp.*
D6746M	0.02	*Diplodia spp.*
D6751M	0.012	*F. anthophilum, Diplodia spp.*
D6759M	0.007	*Trichoderma spp.*
D6793M	0.006	*F. anthophilum*
D6796M	0.006	*F. subglutinans, Diplodia spp.*
D6797M	0.007	*F. subglutinans, Diplodia spp.*
D6826M	0.017	*Diplodia spp.*
D6842M	0.016	*Diplodia spp.*
D6851M	0.006	*Diplodia*
D6854M	0.029	*F. anthophilum*
D6855M	0.006	*Penicillium spp.*
D6880M	0.007	*Diplodia spp.*
D6882M	0.02	*F. anthophilum*
D6886M	0.008	*F. gramminearum*
D6891M	0.062	*F. anthophilum*
D6892M	1.4	*Diplodia spp.*
D6894M	0.034	*F. anthophilum*
D6902M	0.038	*F. anthophilum*

Table 5: Quantity of fumonisins and fungal species found in sorghum samples without *Fusarium verticillioides* and *F. proliferatum* from Nandi County

Sample Code	Quantity of fumonisins in grain in ppm	Fungal species isolated from the tested sample
D413So	0.018	*F. subglutinans*
D420So	3.8	*F. andiyazi*
D485w	0.002	*F. gramminearum, F. andiyazi*
D5377w	4.3	*F. andiyazi, F. subglutinans, F. anthophilum*
D5510So	5.5	*F. andiyazi*
D5534So	5.5	*F. subglutinans*

Table 6: Quantity of fumonisins and fungal species found in animal feed samples without *Fusarium verticillioides* and *F. proliferatum* from Nandi County

Sample Code	Quantity of fumonisins in grain in ppm	Fungal species isolated from the tested sample
D460M	2.4	*F. anthophilum*
D484M	0.713	*F. lateritrium*
D487SM	0.005	*F. lateritrium*
D4143SM	0.037	*Aspergillus spp.*
D4192Af	0.046	*F. anthophilum, Penicillium spp.*
D4236Af	0.379	*F. anthophilum*
D4304SM	0.663	*F. subglutinans*
D4318SM	2.4	*Penicillium spp.*
D4319SM	0.094	*F. gramminearum, F. anthophilum*
D4331SM	2.7	*F. subglutinans*
D5377SM	14.5	*F. gramminearum, F. anthophilum*
D5380SM	0.022	*F. gramminearum, F. anthophilum, F. subglutinans*
D5410SM	0.04	*F. subglutinans, F. solani*
D5422SM	0.013	*F. subglutinans, F. solani*
D5423SM	0.095	*F. gramminearum*
D5430SM	0.009	*F. anthophilum, F. gramminearum*
D5431Af	0.776	*F. gramminearum*
D5433Af	0.108	*F. subglutinans, Penicillium spp.*
D5437SM	0.011	*F. gramminearum*
D5446SM	0.038	*Diplodia spp.*
D5450SM	0.013	*Penicillium spp.*
D5476SM	0.005	*F. subglutinans*
D5503SM	0.428	*Penicillium spp.*

D6580SM	0.004	*F. anthophilum*
D6597SM	0.589	*F. gramminearum*
D6600SM	4.5	*F. subglutinans*
D6614SM	0.605	*F. subglutinans, Alternaria spp.*
D6616SM	0.509	*F. subglutinans*
D6617SM	2.9	*F. subglutinans*
D6618SM	2.2	*F. anthophilum*
D6639SM	0.043	*Aspergillus spp.*
D6651SM	1.5	*Diplodia spp.*
D6657SM	0.137	*Diplodia spp.*
D6658SM	0.409	*Diplodia spp.*
D6661SM	2.7	*F. subglutinans*
D6680SM	0.012	*Diplodia spp.*
D6681SM	0.578	*F. anthophilum, F. subglutinans*
D6684SM	0.338	*F. subglutinans*
D6795SM	2.3	*F. oxysporum*
D6796SM	2.4	*Aspergillus spp., Rhizopus spp.*
D6797SM	0.2	*F. subglutinans, F. gramminearum*
D6880SM	0.33	*Diplodia spp.*
D6902SM	0.092	*F. oxysporum*

APPENDIX C

QUESTIONNAIRE

Safe Food Safe Dairy Household Survey

Welcome and introduction: Good morning/afternoon. Thank you for welcoming us today. We want to learn about fumonisins in this community. Your household was selected for this purpose.

You are being asked to be in a research study. It is entirely your choice. If you decide not to take part, you can change your mind later on and withdraw from the research study. The decision to join or not to talk with us today will not cause you to lose any benefits. Your decision to give us samples (maize, sorghum, animal feeds, soil and millet) to take to the laboratory for testing is completely voluntary.

There are no foreseeable risks or discomforts associated with this study. The research team promises to respect your privacy and confidentiality. This information we talk about will be shared with the research team, but we will remove all names so they will not be able to trace back the information to you. If you accept to work with us on this project, we would be grateful if you sign this consent.

Respondent's name ____

Signature __________________________ **Date** ____________________

Q1) District:	3=Nandi North	4=Nandi Central	5=Nandi South
Q2) Division:	4=Kipkaren	5=Kilibwoni	6=Kaptumo
Q3) Location:	4=Laboret	5=Kilibwoni	6=Kaptumo
Q4) Sub-location:	4=Laboret	5=kilibwoni	6=Chepkongony

Q5) Village: ____________________________

Q6) Longitude: ________________ **Q7) Latitude:** _______________

Q8) Elevation: _________________

Q9) What is your main food item?

1=Maize
2=Milk
3=Sorghum

Q10) What is the source of your main food item?

1= Own farm
2= Market
3= Food relief
4= Gift
5= Other

Q11) Do you practice crop rotation?

1= Yes
2= No

Q12) Method of drying grain:

1= On tarmac (on canvas)
2= On ground with canvas
3= On ground, no canvas
4= On ground on cob
5= Electric driers
6= On farm
7= In granary, on cob

Q13) Mode of storage used:

1= Crib, raised
2= Crib, not raised
3= Iron sheet granary, raised
4= Iron sheet granary, not raised
5= Thatched granary, not raised
6= Thatched granary, raised
7= Bag, raised
8= Bag, not raised

Q14) Criteria used for spoilt maize:

1=Rotten
2= Colour change
3= Insect damage
4= Mouldy
5= Smell
6= Sprouting
7=Bitter taste
8= Moist and clumping together

Q15) Quantity of spoilt maize in household stock in percent: ______________________

Q16) How spoilt grain is dealt with:

1=Destroy
2= Sell
3= Consume
4= Animal feed
5= Sell
6= Leave in shamba

Household Samples Collected:

Sample	Status (Yes/No)	Quantity, where applicable
1. Soil		
2. Animal Feed i)		
ii)		
iii)		
3. Maize		
4. Sorghum		
5. Finger millet		
	Date	**Signature**
Entered by:		
Cross-checked by:		