INCIDENCE OF *FUSARIUM* SPECIES AND LEVELS OF FUMONISIN B1 IN SORGHUM AND FINGER MILLET IN WESTERN KENYA

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I56/CE/15610/05

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE (PLANT PATHOLOGY) OF KENYATTA UNIVERSITY

June 2010

DECLARATION

Candidate

I declare that this thesis is my original work and has not been presented for award of a degree in any other university or any other award

Signed……………………….. Date…………………

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Declaration by Supervisors

This thesis has been submitted with our approval as the university supervisors.

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DEDICATION

This work is dedicated to my daughter Kimberly Nakhone, my wife Roselyn Masombo and my all loving dad Maurice M. Kisaka for their love, prayers and encouragement during the entire period of my studies.

ACKNOWLEDGEMENTS

Am greatly indebted to Dr. Ethel O. Monda for financial, material and professional guidance towards this project. Much gratitude also goes to Dr. Ruth L. Amata who provided the guidance at every stage of the project. My sincere thanks to senior technician Lawrence Alaro for the data and statistical analyses. I also appreciate the assistance of Dr. Gathumbi in mycotoxin analyses

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB1	Aflatoxin B1
a.s.l	Above sea level
ANOVA	Analysis of variance
CLA	Carnation Leaf-Piece Agar
CSA	Central Statistical Authority
DON	Deoxynivalenol
ELEM	Leukoencephalomalacia
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FB1	Fumonisin B1
FDA	Food and Drug Administration
GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
HIV	Human Immune Deficiency Virus
HSD	High significant difference
ICRISAT	International Crops Institute for the Semi-Arid Tropics
KARI	Kenya Agricultural Research Institute
LH	Low Highland
µm	Micrometers
NaOCl	Sodium hypochlorite
nm	Nanometre
PCNB	Pentachloronitrobenzene
PDA	Potato Dextrose Agar
PPA	Peptone Pentachloronitrobenzene Agar
ppm	Parts per million
rpm	Revolutions per minute
SNA	Spezieller Nährstoffarmer Agar
UL	Upper Lowland
UM	Upper Midland
WA	Water Agar

ABSTRACT.

This study was carried out to determine the incidences of *Fusarium* species, fumonisin B1 and aflatoxin B1 production in sorghum and finger millet grains collected from selected districts of Western Kenya region. The grains were collected randomly from five locations per district in Nyamira, Kakamega, Busia and Teso districts respectively. Ten farmers per location were randomly selected and samples of 0.25 kg of grains were picked per variety. A total of 16 sorghum varieties and 14 finger millet varieties were collected from the four districts. There were 19 morphologically distinct *Fusarium* species isolated from sorghum and finger millet grains. These included; *Fusarium compactum, F. equiseti, F. thapsinum, F. verticillioides, F. longipes, F. andiyazi, F. nygamai, F. pseudonygamai, F. brevicatenulatum, F. chlamydosporum, F. heterosporum, F. napiforme, F. graminearum, F. pseudograminearum, F. oxysporum, F. solani, F. subglutinans, F. semitectum* and *F. proliferatum*. *Fusarium compactum* and *F. equiseti* were the most isolated species in all the districts in both grains while *F. thapsinum* and *F. verticillioides* were isolated in sorghum varieties from all the districts. Sorghum had significantly higher percentage incidences of *Fusarium* species than finger millet. Nyamira district had the highest incidences at 65.47% and Kakamega had the lowest at 11.12%. Wagiita variety of sorghum had the highest incidences at 58.95% while Esila had 0% isolation of *Fusarium* species. All finger millet varieties had low incidences of *Fusarium* of less than 8%. The levels of fumonisin B1 detected in sorghum grains (48.81 μg/g) were significantly higher than those in finger millet grains (1.13 μg/g). Sorghum from Nyamira district had the highest fumonisin B1 levels at 76.63 μg/g while low levels of 17.59 μg/g were detected in Busia. Ochuti and Wagiita varieties of sorghum had the highest fumonisin B1 levels, while no fumonisin B1 was detected in KARI Mtama-1 and Esila. Ikhumba variety had levels within the detection limits of 1.75 μg/g. Some finger millet varieties had the lowest levels of fumonisin B1 which were at the detection limit levels of 1.75 μg/g each while some had no fumonisin B1 detected. The aflatoxin B1 in sorghum from Busia and Teso districts had levels below the detection limit of 2 ppb. From the results, finger millet grains are safe for consumption especially when weaning children but sorghum might cause mycotoxicoses. Results also shows that farmers should be sensitized on the dangers of fumonisin B1 levels in sorghum and be advised to adopt varieties like KARI Mtama-1, Esila (with no fumonisin B1 detected) and Ikhumba that had very low levels of fumonisin B1. Some of the fumonisin levels found in this study coincide with levels associated with risks to humans and animals reported in other countries.

Key words: *Fusarium* species, fumonisin B1, sorghum, finger millet, Western Kenya

CHAPTER ONE

INTRODUCTION

1.1 Background

Sorghum (*Sorghum bicolor* (L.) Moench), finger millet (*Eleusine coracana* (L.) Gaertn) and maize (*Zea mays* L.) comprise the major components of human diet in Africa. Other crops such as wheat, groundnuts, pulses (cowpeas and beans) and bananas are also important food crops but to a lesser extent (Siame *et al*., 1998). Sorghum and finger millet are important components in traditional farming systems in the semi-arid tropics of Africa and Asia (FAO, 1999).

Sorghum is a globally cultivated cereal, which is unique due to its tolerance to drought, water logging and saline-alkali or infertile soils and high temperatures (FAO, 2004). The crop provides food security and is becoming a suitable alternative in many places where maize crop fails (FAO, 1999). In Kenya sorghum is grown principally/mostly in the often drought-prone marginal agricultural areas of Western, Eastern and Coast provinces. The crop performs well in areas between 500 m and 1700 m altitude and rainfall of 420 to 630 mm (Kamau *et al.*, 2005; FAO, 2004). From planting to harvesting the crop takes about three to four months (Kenya Seed Company, 2005). The most commonly grown varieties in Kenya include Seredo, Serena, IS8193, KARI Mtama-1, AF28, E525HR, E6518, IS76, I129 and Wagiita and traditional varieties suitable to different regions. The early maturing varieties (3-4 months) include Serena, Seredo and E525 while late maturing (5-6 months) includes E6518. The rest are medium maturing varieties (Kute *et al.*, 1999). The production for some varieties like Seredo is 2.2 tons/ha, KARI Mtama-1 is 2.5

tons/ha (Kamau *et al.,* 2005; ICRISAT, 1988) while according to Kenya seed (2005), Seredo can give yields of up to 5.0 tons/ha and the red seeded I129 can yield up to 6.0 tons/ha. In Kenya the total production in the year 2003 was 126,443 metric tons (FAO, 2004). In Ethiopia the area covered by sorghum is estimated at 995,000 hectares and the national average yield is 1.2 tons/ha (CSA, 2000).

Sorghum is cultivated world wide for fodder, grain and syrup production. Nutritionally sorghum contains higher protein levels than maize. Sorghum grain is ground and used as porridge and the flour is mixed with wheat flour for bread, the flour is also mixed with maize or finger millet flour and used in preparation of *ugali* (ICRISAT, 2004). The starch from waxy sorghums is used in adhesives and sizing paper fabrics. According to Khan *et al.* (1998), sorghum has been identified as a trap plant in push-pull strategy against stem borers and as reservoirs for their natural enemies. Sorghum makes excellent brew for beer and other alcoholic beverages and is also used as animal fodder after harvest and the straw is often used for fencing and building material for huts. Sweet sorghum is used for production of ethanol which is used as a bio-fuel. Sorghum is also grown as a cover crop or green manure (ICRISAT, 2004).

Sorghum plants on the other hand are known to have poison called prussic acid, which develops especially in crops affected by drought when they are young. Prussic acid cause digestion disorders when fed to livestock (Morton, 1978). Sorghum also contains hydrocyanic acid which makes it not prone to attack by locusts that may be destructive to maize. The acid content reaches maximum levels prior to ear emergence and generally declines to insignificant levels as the grain develops and ripens (Kamidi and Chirchir, 1999).

Finger millet (*Wimbi* in Kiswahili) is an important subsistence and food security crop in Eastern Africa especially in the Western and Lake Basin regions of Kenya (Mitaru *et al.*, 1993; Pande *et al.*, 1994). Millet production is mostly concentrated in the drier parts of the country owing to its drought tolerance. It is mainly grown in Western, Central, South Rift and some Coastal areas of Kenya (FAO, 2004). The yields of finger millet range from two to three tons per hectare. In Kenya the total production in the year 2003 was 50,000 metric tons from 115,000 hectares (FAO, 2004).

Finger millet plays an important role in the diet of the people in the region (Makini, 1999). It is an important component of the diets of pregnant and lactating women, infants and children (Gomez, 1993) as well as recuperating and sick patients (Julian *et al.*, 1996; Makini, 1998). Finger millet is traditionally utilized in several ways mainly to prepare thin porridge (*uji*), stiff porridge (*ugali*) and alcoholic drinks (Mbugua *et al.*, 1992). Other products made from finger millet include; leavened breads and non-alcoholic beverages. In some cases flour from finger millet is blended with flours from sorghum, maize and cassava and used in food preparation (Julian *et al.*, 1996). The crop is also used for medicinal purposes such as treating measles, colds, anaemia and diarrhoea (Makini, 1998). The straw may be used for thatching and weaving for example traditional baskets for serving *ugali* (Mulatu *et al.*, 1993). The straw and silage can also be fed to livestock (Julian *et al.,* 1996).

Fusarium species are recognized as a major agricultural problem as the species are world wide on a variety of plant hosts and primarily on cereal grains (Onyike *et al.*, 1993). Several species in the genus *Fusarium* can cause stalk rots, ear rots and grain mould resulting in serious production losses in both sorghum and maize (Frederiksen and Odvody, 2000; White, 1999). Some of the diseases caused by *Fusarium* species on cereal crops include grain mould, stalk and root rots of pearl millet caused by *Fusarium graminearum* and *F. subglutinans* respectively. Sorghum stalk and root rot caused by *F. thapsinum* (Leslie *et al.*, 2002; Summerell, 2003).

Fusarium species also produce mycotoxins that are harmful to both humans and domesticated animals (Frederiksen and Odvody, 2000). The mycotoxins include fumonisins which cause Equine leukoencephalomalacia (ELEM) in horse porcine pulmonary oedema in pigs and cancer in rats. Fumonisins have also been linked to oesophageal cancer in humans (Won-Bo and Charles, 1999). The target organs for fumonisin are the brain, lungs, liver and kidneys (European commission, 2000). Other mycotoxins produced by *Fusarium* species include zearalenone which can cause infertility and cause estrogenic syndrome in pigs. Trichothecene inhibits proteins synthesis and therefore causes impairment of the human immune function (Wang *et al.*, 2006). Moniliformin causes myocardial degeneration and necrosis in experimental animals.

Attempts to control *Fusarium* in sorghum and finger millet have not been consistent as it has been met with limited success. The difficulties in the control are due to the emergence of different *Fusarium* species and lack of detailed knowledge and understanding of the species (Juliano *et al.*, 2005). In Kenya there is also limited information on *Fusarium* species associated with sorghum and finger millet and their potential to produce mycotoxins (Amata *et al.*, 2006).

1.2 Problem statement

Fusarium species produce a variety of mycotoxins with widely divergent and toxicological effects in animals and humans on consuming contaminated sorghum and finger millet grain. The consumption of contaminated sorghum and finger millet with *Fusarium* species exposes animals and humans to mycotoxins that have a potential to cause a variety of diseases related problems. Management of *Fusarium* in pre and post harvest sorghum and finger millet is therefore of paramount importance. There is need to conduct a biological investigation on *Fusarium* species associated with sorghum and finger millet grain and fumonisin B1 contamination in Western Kenya. In Western Kenya sorghum and finger millet consumption is very high and the two cereals are mainly used as weaning food for children through preparation of porridge that may pose fumonisin related problems to children.

1.3 Research questions

i) Which *Fusarium* species are found in sorghum and finger millet grains?

ii) What is the incidence of *Fusarium* species in different varieties of sorghum and finger millet grains?

iii) What are the levels of fumonisin B1 in sorghum and finger millet grains?

iv) What is the incidence of *Fusarium* species in different varieties of sorghum roots and stems from Busia and Teso districts?

v) What are the levels of aflatoxin B1 in sorghum from Busia and Teso districts?

1.4 Objectives

1.4.1 General objective

To identify *Fusarium* species in different varieties of sorghum and finger millet and evaluate fumonisin B1 levels.

1.4.2 Specific objectives

i) To identify *Fusarium* species associated with sorghum and finger millet grain

ii) To determine frequency of occurrence of *Fusarium* species in sorghum and finger millet in selected districts in Western Kenya

iii) To evaluate fumonisin B1 levels in sorghum and finger millet from selected districts in Western Kenya

iv) To determine the incidences of *Fusarium* species in sorghum roots and stems from Busia and Teso districts

v) To evaluate aflatoxin B1 levels in sorghum varieties from Busia and Teso districts

1.4.3 Hypotheses

i) *Fusarium* species are not associated with sorghum and finger millet grains

ii) Incidences of *Fusarium* species associated with sorghum and finger millet grains in Western Kenya do not differ

iii) There is no fumonisin B1 in sorghum and finger millet grains

iv) There are no *Fusarium* species in sorghum roots and stems from Busia and Teso districts

v) There is no aflatoxin B1 in sorghum from Busia and Teso districts

CHAPTER TWO

LITERATURE REVIEW

2.1 *Fusarium*

The genus *Fusarium* is one of the most economically important genera of fungi and includes many pathogenic species that cause a wide range of plant diseases (Nelson *et al.*, 1981). It also includes endophytic and saprophytic species found in association with plants in agricultural and natural ecosystems (Leslie, 2004; Summerell *et al.*, 2003). The genus has widespread distribution and representatives occur in all major regions of the world (Burgess, 1981). Some species have a cosmopolitan geographic distribution whereas others tend to occur predominantly in tropical and subtropical regions or cool to warm temperate regions. Many *Fusarium* species are particularly common in soil, and persist as chlamydospores or as hyphae in plant residues and organic matter. Several species produce airborne conidia and are common colonisers of stems, leaves and floral parts (Burgess, 1981).

The members of this genus can incite diseases directly in plants, humans and domesticated animals (Boonpasart *et al.*, 2002; Martino *et al.*, 1994; Krcmery *et al.*, 1997; Vismer *et al.*, 2002). The mortality rate for human patients with systemic *Fusarium* is greater than 70% (Krcmery *et al.*, 1997), and HIV-infected patients are susceptible to such *Fusarium* infections as well (Eljaschewitch *et al.*, 1996; Guaro *et al.*, 2000; Mselle, 1999). In addition *Fusarium* species produce an intriguing array of secondary metabolites that are associated with plant disease as well as with cancer and other growth defects in humans and domesticated animals. Some of these secondary metabolites are used commercially either directly or as the starting material for chemical syntheses of plant and animal growth promoters in both first world and third world settings (Shukla *et al.*, 2003; Romaine *et al.,* 1997). Reports on the use of mycotoxins produced by some of these fungi as biological weapons also have been made (Heyndrickx *et al.,* 1989; Rynkiewicz *et al.,* 2001; Mirocha *et al.,* 1983). Naturally occurring outbreaks of *Fusarium* mycotoxin poisoning affecting humans have occurred historically for example, in Athens in the 5th century B.C (Schoental, 1994), and the Soviet Union during the World War II (Gajdusek, 1953).

As social phenomena *Fusarium* plant diseases have had several major impacts, for example the near devastation of the commercial banana industry in the 1960s by panama wilt caused by *Fusarium oxysporum* f.sp. *cubense* (Plattner *et al.*, 1996). The recent loses of several billion dollars by many wheat and barley farmers to *Fusarium* head scab in the upper Midwest of the United States has shifted cropping strategies and bankrupted farmers in the region (Windels, 2000). At the same time, the causal agent of *Fusarium* head scab can be used in commercial fermentations to produce a precursor for one of the most widely used commercial cattle growth promoters (Hidy *et al.,* 1977). Recent problems caused by strains of *Fusarium* which may have originated from endophytes or pathogens of native *Gossypium* species are threatening the future of the cotton industry in Australia while simultaneously demonstrating the relatedness of native and agricultural populations and suggesting new avenues for understanding how these fungi evolve (Wang *et al.,* 2004).

Many plants have at least one *Fusarium*-associated disease. The American Phytopathological Society has listed over 81 of the 101 economical plants on the list had at least one associated disease. The types of diseases induced are quite varied as is their severity, and may include root or stem rots, cankers, wilts, fruits or seed rots, and leaf diseases (Leslie *et al.*, 2006).

Fusarium species can cause diseases like stalk rots ear rots and grain moulds resulting in serious production losses in both maize and sorghum (Frederiksen and Odvody, 2000; White, 1999) and produce mycotoxins that are harmful to both humans and domesticated animals (Agrios, 1998). Species recognized as plant pathogens including *Fusarium verticillioides* and *F. subglutinans* which cause ear, cob, and root and stalk rots in maize were recovered in Kisii, Lugari and Mbeere districts in Kenya (Amata et *al.*, 2006). Not all species of *Fusarium* produce mycotoxins and those that do often produce no more than one or at most a few. Strains that are virulent plant pathogens may be atoxogenic (Leslie, 2006). Risks associated with *Fusarium* toxins are assessed based on the *Fusarium* species present as not all species produce all kinds of toxins. Important toxins produced by these fungi include the fumonisins (Gelderblom *et al.*, 1988; Leslie, 2004) and moniliformin (Marasas, *et al.*, 1986); others include zearalenones and trichothecenes (Summerell *et al*., 2003). In African countries such as Lesotho, Nigeria and Zimbabwe, *Fusarium verticillioides* is common on maize, millet and sorghum (Onyike *et al.*, 1993). *Fusarium* species produce a variety of mycotoxins with widely divergent and toxological effects in animals and humans consuming the contaminated commodities (Siame *et al.*, 1998).

2.1.1 *Fusarium* associated with sorghum and finger millet

Fusarium species from sorghum and finger millet have only recently become the subject in depth research and often were included only as isolates of peripheral interest in earlier studies. Modern work on the taxonomy of the genus *Fusarium* began with Wollenweber and Reinking (1935), who established the section *Liseola* within the genus *Fusarium* for species that produce micro-conidia in chains and/or false heads but do not produce chlamydospores. Gerlach and Nirenberg (1982) expanded the number of taxa in the section *Liseola* to ten and pointed out that based on priority the correct name for *Fusarium moniliforme* is *F. verticillioides* (sacc). Nelson *et al*. (1983) accepted four taxa in the section *Liseola* including *F. verticillioides* and other chain forming species such as *F. proliferatum.*

In the last 20 years five chain forming *Fusarium* species in the section *Liseola* have been described, *Fusarium andiyazi* (Marasas *et al.,* 1991), *F. globosum* (Rheeder *et al.,* 1996), *F. miscanthi* (Gams *et al.,* 1999), *F. nisikadoi* (Nirenberg and O'Donnell, 1998) and *F. thapsinum* (Klittich *et al.,* 1997). The taxonomic difficulties are further complicated by chain forming *Fusarium* species that resemble those in the section *Liseola* but also produce chlamydospores example *F. nygamai* (Burgess *et al.,* 1986) and *F. napiforme* (Marasas *et al.,* 1985) were placed in the section *Dlamina* by Kwansa *et al.* (1991). This new section has not been widely used and instead many members of both sections *Liseola* and *Elegans* are now referred to as part of the '*Gibberella fujikuroi'* species complex (Leslie, 1991, 1995 and 1999).

Recent studies have shown that sorghum hosts a number of *Fusarium* species, the most common of which are *Fusarium andiyazi* and *Fusarium thapsinum* and is occasionally colonized by members of *F. verticillioides* found on maize (Leslie, 2006). There are other yet undescribed species in the *Gibberella fujikuroi* species complex from African sorghum whose toxin profiles are not known. From finger millet grown by subsistence farmers in Uganda, a large number of *Fusarium* species, many not yet described were isolated. This species include *F. verticillioides* suggesting that there is potential for fumonisin associated problems especially when this grain is used as a weaning food (Leslie, 2006).

2.2 Mycotoxins

Mycotoxins are ‘fungal metabolites’ which when ingested, inhaled or absorbed through the skin, cause lowered performance, sickness or death in animals or humans. Despite efforts to control fungal contamination, toxigenic fungi are ubiquitous in nature and occur in world wide food supplies due to mould infestation of susceptible products such as cereal grains, nuts and fruits (Patricia *et al.*, 2006). The natural fungal flora associated with foods is dominated by three genera; *Aspergillus, Fusarium* and *Penicillium.* Examples of mycotoxins include patulin produced by *Penicillium*, ochratoxin produced by *Penicillium* and *Aspergillus,* zearalenone, produced by *Fusarium,* aflatoxin produced by *Aspergillus* and trichothecenes and fumonisins both produced by *Fusarium.* Mycotoxins have plagued man since the beginning of organised crop production. For example, ergotism (St. Anthony fire) caused by consumption of rye contaminated with ergot alkaloids produced by *Claviceps purpurae* is recorded in the Old Testament and European epidemics date back as far as 857 A.D (Bove, 1970).

2.2.1 Mycotoxins associated with *Fusarium* species

The most common secondary metabolites produced by *Fusarium* species include trichothecenes (DON, Nivalenol, T-2 toxin, HT-2 toxin), moniliformin, zearalenone, fumonisins and enniatins (Thrane, 2001, 2004; Miller and Mc Kenzie, 2000). The most common trichothecene is deoxynivalenol (DON), which is present in foods and inhibits protein synthesis once ingested (Ehrlich and Daigle, 1987), thus causing impairment of the human immune function. Trichothecenes are produced by *F. graminearum* (Wang *et al.,* 2006; Muthomi *at al.,* 2002), *F. verticillioides* (Gonzalez *et al.*, 2008) and *F. acuminatum* (Leslie and Summerell, 2006).

Zearalenone have the potential to disrupt sex steroid hormone functions and can cause infertility. The estrogenic compound can induce chromosomal anomalies in some lymphocytes, oocytes and kidney cell cultures when present within a range of 0.1-20 µm (Stopper *et al.,* 2005). They cause hyper estrogenic syndromes in pigs like swollen vulva, enlarged mammary glands and nipples while the males show feminine features. The mycotoxin is produced by *F. graminearum* (Akinsanmi *et al*., 2006; Wang *et al*., 2006) and *F. culmorum* (Leslie and Summerell, 2006).

Moniliformin is a toxin produced by several *Fusarium* species. Moniliformin (Sodium or potassium salt of 1-hydroxycyclobut-1-ene 3, 4) is a highly toxic compound that was first isolated in 1973. This compound causes rapid death and pathological lesions including myocardial degeneration and necrosis in experimental animals (Nelson, 1973). *Fusarium verticillioides* is a weak producer of the toxin (Marasas *et al.,* 1986). It is also produced by other *Fusarium* species like *F. concolor, F. equiseti, F. oxysporum, F. semitectum, F. chlamydosporum, F. sporotrichioides, F. culmorum* and *F. reticulatum* (Leslie and Summerell, 2006).

2.2.2 Fumonisins

Fumonisins are produced by *F. verticillioides* and *F. proliferatum* and at very low levels by *Alternaria* (Seefelder *et al.,* 2002). At least 15 fumonisin compounds have been identified with the fumonisin B (FB) being predominant (figures 1-3). Fumonisins are highly water soluble because they do not have an aromatic structure. They are primary amines with two tricarballic groups, which contribute to their solubility. Fumonisin B1 is the best known and studied of the fumonisins, but other derivatives are known to occur naturally as well (Mc Kenzie, *et al.,* 1998; Sewram *et al.,* 2005). Other mycotoxins produced by *Fusarium* species include; beauvericin, fusaproliferin, fusarin, acuminatopyrone, chlamydosporol, steroid and acuminatin (Leslie and Summerell, 2006).

Fumonisin B1 disrupts sphingo-lipid metabolism (Shephard *et al.*, 1996) and cause leukoencephalomalacia in horses (Marasas *et al.,* 1988; Chu and Li, 1994), pulmonary oedema syndrome in pigs (Haschek *et al.,* 2001; Palysik and Moran, 1994), liver cancer (Voss *et al.,* 2001) and liver and kidney toxicity in rats (Voss *et al.,* 1998), neuro-degeneration in mice (Osuchowski *et al.,* 2005), and apoptosis in many types of cells.

Fumonisins are phytotoxic, but their roles in plant diseases are mainly caused by *F. verticillioides* (Abbas *et al.*, 2000; Desjardins *et al.*, 2002). Fumonisins also have some antifungal activity (Keyser *et al.*, 1999). Fumonisins have been implicated in human oesophageal cancer (Marasas *et al.*, 1988) and in birth defects in humans (Marasas *et al.*, 2004).

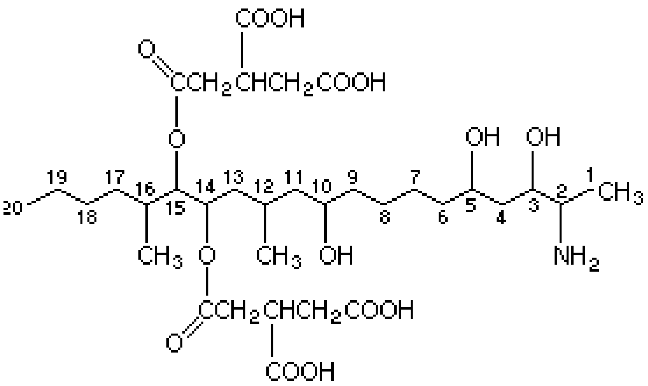

Figure 3 Fumonisin B1

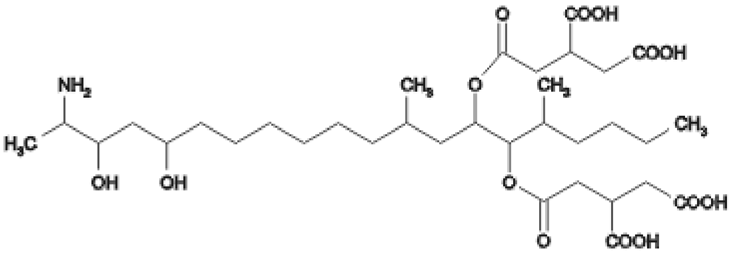

Figure 4 Fumonisin B2

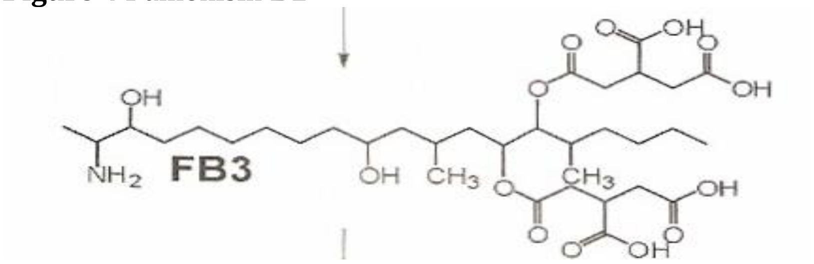

Figure 3 Fumonisin B3

2.3 Factors influencing the accumulation of fumonisins

The infection of cereals with *Fusarium* species and its contamination by fumonisins are generally influenced by many factors including environmental conditions, insect infestation and pre and post harvest handling (Fandohan *et al.,* 2003).

2.3.1 Environmental factors

Worldwide surveys showed high levels of fumonisins associated with warmer and drier climates (Shepherd *et al.,* 1996) and when conditions are favourable for *Fusarium* infection (Marasas *et al.,* 2001). At the same location fumonisin contamination is not necessarily the same from one year to another. Hennigen *et al.* (2000) found in Nigeria a marked difference in terms of fumonisin contamination for the same maize varieties during two consecutive growing seasons due to the fact that environmental conditions may differ from one season to another. Physiological stress during the period just preceding cereal harvest due to drastic oscillations in rainfall and relative humidity is likely to create favourable conditions for fumonisin production (Visconti, 1996). Shelby *et al.* (1994) suggested that dry weather at or just prior to pollination of cereals might be an important factor for fumonisin production (Fandohan, *et al.,* 2003). Munkvold and Desjardins (1997) reported that *F. verticillioides* generally grows in grain when moisture content is more than 18-20%

2.3.2 Agricultural practices

It has been reported that late planting of cereals with harvesting in wet conditions favours *Fusarium* growth (Bilgrami and Choudhary, 1998) and the prevalence of the fungus is considerably increased with wet weather later in the season (Al-Heeti, 1987). Repeated planting of the same cereal crop and related crops in the same or nearby fields favours fungal infection by increasing the fungal inoculum and insect population that attacks the grains (Bilgrami and Choudhary, 1998). Weed control also affects fungal infection because it helps to eliminate nonhost weeds on which *Fusarium* can also be found (Bilgrami and Choudhary, 1998).

2.3.3 Cereal characteristics

The type of cereal cultivar and grain characteristics such as colour, chemical composition and stage of development may influence fungal infection and subsequent fumonisin production (Fandohan *et al.,* 2003). Late maturing cereals cultivars in which grain moisture content decreases slowly below 30% are most susceptible to *Fusarium* disease and fumonisin production (Manninger, 1979).

2.3.4 Post harvest operations

Post harvest handling and processing like sorting, milling, fermentation and cooking favourably or unfavourably affect fungal infection and fumonisin production in cereals. Mechanical damage during and after harvest may offer entry to the fungal spores in the grains (Fandohan *et al.,* 2003). Small, broken and visibly contaminated grains during processing can significantly increase the toxin levels (Charmley and Prelusky, 1995).

2.3.5 Storage insects

The insects act as wounding agents or as vectors spreading the fungus from origin of inoculum to plants (Dowd, 1998). The wounding by insects may provide an opportunity for the fungus to circumvent the natural protection of the integument and establish infection sites in the vulnerable interior (Bilgrami and Choudhary, 1998). Borers and insects of the family Nitidulidae are most often cited as favouring cereal infection by *Fusarium* species (Ako *et al.,* 2003). In South Africa, Flett and Van Rensburg (1992) showed that *Busseola fusca* infestation significantly increased the incidence of *F. verticillioides* in maize cobs irrespective of whether the cobs are artificially inoculated with the fungus or not.

2.3.6 Fungal interactions

The harvested maize grains in the tropical zones contain mycelium and spores of several fungal species including mainly *Aspergillus* and *Penicillium* that can come into contact, grow and compete for food if environmental conditions are favourable. Velluti *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. graminearum*, and fumonisin B1 production by them can be significantly inhibited as well.

2.4 Mycotoxin control strategies

2.4.1 Good agricultural practices (GAPs)/good manufacturing practices (GMPs)

The first line of defense against the introduction of mycotoxins is at the farm level and starts with implementation of good agricultural practices to prevent infection. Preharvest strategies include maintenance of proper planting/growing conditions (for example, soil testing, field conditioning, crop rotation, irrigation), antifungal chemical treatments (for example, propionic and acetic acids), and adequate insect and weed prevention (Patricia *et al.,* 2006). Harvesting strategies include use of functional harvesting equipment, clean and dry collection/transportation equipment, and appropriate harvesting conditions (Patricia *et al.,* 2006). Postharvest measures include use of drying as dictated by moisture content of the harvested grain, appropriate storage conditions, and use of transport vehicles that are dry and free of visible fungal growth (CAC, 2003; Quillien, 2002).

2.4.2 HACCP

In the food industry, postharvest control of mycotoxins has been addressed via HACCP plans, which include use of approved supplier schemes. Such action provides a critical front-line defense to prevent introduction of contaminants into the food and feed supplies (Patricia *et al.*, 2006). Preharvest HACCP programs have been documented for controlling aflatoxin in corn and coconuts in Southeast Asia, peanuts and peanut products in Africa, nuts in West Africa, and patulin in apple juice and pistachio nuts in South America (FAO/IAEA, 2001).

2.4.3 Biological control measures

The potential for using microorganisms to detoxify mycotoxins has shown promise (Patricia *et al.,* 2006). DON levels have been shown to be reduced in the field and in storage without intervention, as reported by Karlovsky (1999) where he suggested the possibility that the mycotoxin may be metabolized by maize enzymes. Exposure of DON to microbes contained in the contents of the large intestines of chickens completely transformed it in vitro to de-epoxy-DON (He *et al.,* 1992), which is 24 times less toxic than DON itself (Eriksen, 2003). Similar findings were demonstrated with the microflora of cow intestines (Binder *et al.,* 1998).

2.4.4 Transgenic approaches

Genetic modification of mold-susceptible plants holds great promise for controlling this food safety issue (Patricia *et al.,* 2006). Articles by Karlovsky (1999), Duvick (2001), and Munkvold (2003) reviewed a variety of approaches that are being or have been pursued. One such approach involves increasing production of compounds (for example, antifungal proteins or secondary metabolites, such as hydroxamic acids, phenolics, stilbenes) that reduce infection by the microorganism. This may be accomplished by introducing a novel gene to express the target compound. Another option is to enhance expression of such a compound by the existing gene, thereby capitalizing on the plant's own defense mechanisms. For example, enzymes that catalyze production of antifungal could be targeted for expression. Alternatively, genetic engineering methods to increase production of enzymes that degrade mycotoxins are also being pursued (Duvick, 2001, Munkvold, 2003). Transgenic maize has been patented for fumonisin-degrading corn for swine consumption (Duvick and Rood 1998). Efforts are also under way to engineer plants to produce compounds that disrupt mycotoxin synthesis. For example, enhanced expression of an α-amylase inhibitor in *Aspergillus* species could result in significantly reduced aflatoxin levels (Duvick 2001, Munkvold 2003). Another avenue for reducing mycotoxin levels would be to reduce insect injury to plant kernels. Insects play an important role in the proliferation of mold growth in the field and in storage (Patricia *et al.,* 2006).

2.4.5 Bioterrorism

Grain storage and delivery systems, as well as food manufacturing plant security systems, deserve attention and crisis plans should be in place to deal with possible biological and chemical terrorism incidents. Where appropriate, these efforts should include mycotoxins (Patricia *et al.,* 2006).

2.4.6 Risk assessment

Kuiper-Goodman *et al.* (1996) performed a risk assessment for fumonisins based on human exposure data from Canada and the extant toxicity literature on these mycotoxins. Because fumonisins are almost exclusively found in corn, this is a somewhat simpler case than for most other mycotoxins. Based on 361 corn food samples analyzed over 4 y, human fumonisin intake was estimated to be <0.089 μg/kg bw/d. This dose was 1700-fold less than the lowest NOEL obtained from animal studies (a 4.4-y study of nine vervet monkeys showing a no-observed effect level (NOEL) of 0.15 mg/kg bw/d). The authors concluded that human fumonisin intakes in Canada were very unlikely to pose health risks. A more recent 2-y rodent carcinogenicity study (NTP, 2001) confirms this finding, with a NOEL of >0.2 mg/kg bw/d with altered sphingosine/sphinganine ratios as a toxic endpoint. The NOEL in this study for cancer (renal carcinogenesis in male rats only) was 0.6 mg/kg bw/d.

2.4.7 Food safety regulations

Public awareness of issues surrounding mycotoxins is increasing (Patricia *et al.,* 2006). Karlovsky (1999) provides three explanations for this phenomenon. First, analytical chemistry is increasingly able to quantify the presence of toxins in a growing number of food commodities. Second, new and improved bioassays for toxicological studies on specific targets have surpassed the abilities of less sophisticated methods and allowed identification of negative health effects where previously none had been found. Third, the availability of routine testing methods that are both efficient and affordable has allowed for in-house monitoring, resulting in greater numbers of identified contaminations Karlovsky (1999) .

2.5 Sorghum

Sorghum is a common name for corn-like grasses native to Africa and Asia where they have been cultivated since ancient times (William, 2003). *Sorghum bicolor* was domesticated in Sudan about 1000 BC probably from S*orghum arudinaceum*, which was later called *Sorghum sudanese* (ICRISAT, 2004). The cultivation of sorghum for grain began in Egypt thousands of years ago. Today, sorghums are the staple food for millions of people in China, India and Africa (William, 2003).

Four of the five major races of sorghum of Africa are in Ethiopia. The seeds of the crop may be white, yellow, light brown, dark brown, red, purple or black. Compared to other cereal grains seeds have high nitrogen, iron, thiamine and riboflavin. The varieties with dark seeds are resistant to damage by birds, insects and fungi (ICRISAT, 2004).

The leaves of sorghum are relatively broad, have numerous but small stomata and are covered with a waxy bloom. Under moisture stress the leaves tend to roll along the midrib. These features afford the plant greater drought resistance and water use efficiency than most other crops (FAO, 1999). In semi-arid Kenya the farmers mostly grow traditional varieties; however, hybrid cultivars have superiority over traditional cultivars. Hybrid production and successful marketing requires skilled labour, an effective seed industry, good infrastructure and a sufficient income for the farmers to be able to afford the costly seed (Haussmann *et al.*, 2000). Globally sorghum is the fifth largest food crop after maize, wheat, rice and barley (ICRISAT, 2004).

Sorghum requires fine seedbed for better seedling establishment. If tractor or oxen plough is used to open up the land then it is advisable to harrow it after the ploughing. Planting is by drilling or hole planting where early or dry planting is highly recommended. Depth of planting on the onset of rains should be 2.5-4 cm and when dry planting it is 5.0 cm. The recommended seed rate is 7-10 kg/ha with an optimum spacing of 75 cm x 20 cm giving a plant population of 6600 plants/ha (Conley, 2003). The recommended fertilizer is NPK (20:20:0) with two (50 kg) bags per acre. Sorghum can be planted as a mono crop or as an intercrop with beans, pigeon pea, cowpea and green gram. Weeding is done 2-3 weeks after emergence and thinning should be done after the first weeding to leave one seedling per hole (KARI, 2006; Hillocks, 2000).

There are biotic and abiotic constraints which affects the yield of sorghum. Some of the constraints include; soil water deficit, weeds especially *Striga asiatica* and *hermotheca*, birds especially *Quelea,* shoot fly and phosphorous deficiency (Wortman *et al.,* 2007). Some field fungi found in sorghum grain include; *Alternaria alternata, Cladosporium cladosporioides, Fusarium verticillioides* and *F. thapsinum.* The most prevalent storage fungi detected in association with the grain include; *Aspergillus candidus, A. flavus, A. niger,* and *Penicillium aurantiogriseum* (Dejene, 2004).

Warm conditions prevalent in many parts of Africa are ideal for fungal proliferation. Poor harvesting, handling and storage practices also contribute to fungal growth and mycotoxin production (Siame *et al.,* 1998). *Fusarium* species can cause stalk rots, ear rots and grain mould resulting in serious production losses both in maize and sorghum. *Fusarium* species also produce mycotoxins that are harmful to both humans and domesticated animals (Frederiksen and Odvody, 2000; White, 1999). Some of the diseases caused by *Fusarium* on cereal crops include grain mould, stalk and root rots of pearl millet caused by *Fusarium graminearum* and *F. subglutinans* respectively. Stalk and root rot and ear rot of maize are caused by *F. verticillioides, F. subglutinans, F. proliferatum* and *F. graminearum.* Sorghum stalks and root rot caused by *F. thapsinum* (Leslie *et al.,* 2002; Summerell, 2003; Krauz, 1996).

2.6 Millets

Millets are small seeded members of the family *Poaceae.* They include five genera namely; *Pennisetum, Panicum, Setaria, Echinochloa* and *Paspalum* in the tribe Paniceae and two genera, *Eleusine and Eragrostis* in the tribe Chlorideae (Peterson *et al.,* 1997). Finger millet, pearl millet and teff are examples of C4 plants (Chapman and Peat, 1992).

A number of wild millets such as *Eleusine indica* subsp. *africana* and subsp. *indica* are serious weeds in cultivated crops in tropical regions due to their rapid growth and high water use efficiency at high temperatures (Jones, 1985).

2.6.1 Origin, history and nature of finger millet

Finger millet (*Eleusine coracana* subsp. *coracana*) is considered by Hilu and de Wet (1976) to have originated in the East African highlands while other authors suggested the covering of Eastern Sudan, Ethiopia or Uganda as the likely area of finger millet domestication in the fourth millennium BC (Adipala, 1993; Engels and Hawkes, 1991; Mehra, 1991). Archaeological findings dating back to the third millennium provide evidence that *E. africana* is the progenitor of finger millet (Hilu and de Wet, 1979). *E. africana* has not been reported in India (Mehra, 1991).

There has been an increase in the consumption of finger millet products in Kenya with the grain being milled together with other millets, sorghum and high protein pulses such as Soya beans. In Kenya, finger millet is grown in areas receiving 300-2000 mm annual rainfall and in a wide range of altitudes (0-2400 m a.s.l) (Mandu *et al.,* 1999). According to the Ministry of Agriculture, annual reports for the years 1999 to 2003, key centres of production lie in the western parts of the country and include Kisii, Nyamira, Gucha, Busia and Kakamega districts. Most homesteads produce a crop every season or once a year. The crop is also grown in the Rift valley, Central, Eastern and Coast provinces of the country (Jaetzold and Schmidt, 1983; Muyanga, 1995). Due to financial constraints most farmers do not plant certified seed, but buy their seed from market places and occasionally use fertilizers. Other countries that grow finger millet include Uganda, Tanzania, Ethiopia, Sudan and some countries in West Africa. In Asia, it is grown in India, Nepal, Sri Lanka, Malaysia, China and Japan (Rathore, 1999).

Weeding is undertaken at least twice in a season (Anon, 1995). Farmers grow finger millet either as an intercrop or pure stand (Makini, 1998; Oduori, 1993). It is harvested when the ears become brown in colour and threshing after sun drying separates the grains. Recommended agronomic practices include planting early in the season and aim to achieve a final spacing of 30 x 10 cm resulting in a plant population of 231,486 per hectare. In addition 20 kg/ha of phosphorous at planting and 20 kg/ha of nitrogen four weeks after planting is recommended (Oduori, 1993).

Finger millet is highly nutritious as it is a source of methionine, an amino acid lacking in the diet of millions of people living on foods that are mainly starchy such as cassava, plantain, polished rice and maize meal (Esele, 2002; Rathore, 1999). It also contains calcium, iron and magnesium (Esele, 2002; Mallesh and Hadimani, 1994; Rathore, 1999), and is useful for pregnant women, lactating mothers, infants and children; recuperating and sick patients (Esele, 2002; Makini, 1999).

Areas of research on this crop have been directed mainly towards breeding for high yielding varieties and for resistance against blast disease caused by *Pyricularia grisea,* a disease that has been reported to be a serious leaf and inflorescence pathogen in most growing areas (Esele, 2002; Makini, 1999; Rathore, 1999). However little attention has been given to diseases and losses that may be attributed to pathogenic members of the genus *Fusarium* (Amata *et al.,* 2006)

2.6.2 *Fusarium* species reported on millets

Onyike *et al.,* (1991) reported various *Fusarium* species associated with pearl millet, proso millet and fox tail millet in Nigeria, Lesotho and Zimbabwe. Species isolated from pearl millet in Zimbabwe included *Fusarium equiseti, F. moniliforme, F. semitectum, F. nygamai, F. chlamydosporum, F. oxysporum* and *F. napiforme.* Those isolated from pearl millet grain in Nigeria included *F. moniliforme, F. nygamai, F. equiseti, F. chlamydosporum, F. semitectum, F. subglutinans* and *F. napiforme. Fusarium equiseti* was the only species recovered from fox tail, proso and pearl millet and was the only species recovered in all three countries.

In 1987, Marasas *et al.* described *F. napiforme* from pearl millet (*Pennisetum typhoides*) grain from Namibia. This species has also been recovered from sorghum in South Africa and from soil debris from grassland site near Emerald, Queensland, Australia. *Fusarium pseudonygamai,* a species with characters that are very close to those of *F. nygamai* (Burgess and Trimboli, 1986), was isolated from pearl millet in Nigeria (Nirenberg and O'Donnell, 1998).

Fusarium graminearum has been reported to be associated with head blight and grain mould in pearl millet (Onyike *et al.,* 1991). *F. graminearum* also causes head blight of wheat, barley and oats, and stalk and cob rot of maize (Burgess *et al.,* 1981). *Fusarium* *subglutinans* has been associated with seedling mould and root and stalk rot in millets (Onyike *et al.,* 1991). *Fusarium verticillioides* has been reported to be the cause of root and stalk rots and ear rot in maize whereas *F. thapsinum* has been associated with rots in sorghum. The presence and impact of *Fusarium* species on finger millet is not well documented despite the ability to cause disease and produce mycotoxin in other crops (Amata *et al.,* 2006).

2.7 Aflatoxin B1

Aspergillus flavus grain mould on corn is often characterized by visible light green mould on the surface of the kernels. This surface mould can develop anywhere on the ear, but is most often observed at the base of the ear (Cotty, 1989). Visible mould growth is not always evident on colonized kernels and not all colonized kernels will be contaminated with aflatoxin. However, colonized kernels with no visible mould may contain aflatoxin ((Klich and Pitt, 1988).

The disease caused by aflatoxin is called aflatoxicosis. Aflatoxicosis is neither infectious nor communicable; it cannot be spread from one animal to another or from one human to another. The primary target of aflatoxin is the liver. Depending on the duration of feeding on contaminated grain or food products and the amount of aflatoxin ingested, the liver may fail to function or liver cancer may develop. Recovery from liver failure depends on the extent of damage. If damage is not too extensive, full recovery can be expected if the contaminated feed or food products are removed from the diet (Stoloff, 1977, 1983).

In addition to the production of aflatoxin, *Aspergillus* species of mould can affect humans or animals in two other ways. Some people and animals are allergic to *Aspergillus* species and exhibit either acute or chronic reactions to the mould itself. *Aspergillus* moulds can infect animals, including humans, with inadequate immune system function causing a disease called aspergillosis. It is an invasive disease of the lungs, although colonization of other organs can occur. Aspergillosis is a serious disease that is often fatal. Dust masks or respirators should be worn by grain handlers to minimize exposure to these fungi and to aflatoxin contaminated dust (Stack and Carlson, 2006).

The other effects of aflatoxins are of economic and health importance because of their ability to contaminate human food and animal feeds, in particular cereals, nuts and oilseeds (Arim, 1995).The economic impact of aflatoxins is derived directly from crop and livestock losses due to aflatoxins and directly from the cost of regulatory programs designed to reduce risks to human and animal health. Aflatoxin losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (Vincelli *et al.,* 1995). Other adverse economic effects of aflatoxins include lower yields for food and fibre crops (Sétamou *et al.,* 1997). Nevertheless, aflatoxins reputation as a potent poison may explain why it has been adopted for use in bioterrorism (Bennett and Klich, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 Description of sampling sites

The samples were collected from Nyamira, Kakamega, Busia and Teso districts of Western Kenya region. These districts grow large amounts of sorghum and finger millet after maize as their major source of food. Nyamira district is one of the districts in Nyanza province. It has a total of four divisions and 14 locations with total arable land of about 1404 hectares. The topographic zones in the district lie between 1250 m and 2100 m above sea level. The district has a bimodal pattern of annual rainfall that is well distributed, reliable and adequate for a wide range of crops. Annual rainfall ranges between 1200 mm to 2100 mm per annum. Long and short rain seasons start from December to June and July to November respectively, with no distinct spell separating them. The maximum day and minimum night temperatures are normally between 28.7˚C and 10.1˚C respectively, resulting to an average normal temperature of 19.4˚C which is favourable for both agricultural and livestock production. It is divided into two major agro-ecological zones; the highland (LH1 and LH2) and the upper midland zone (UM1, UM2 and UM3). The major soils are red volcanic (Nitosols) that are deep, fertile and well drained (Jaetzold *et al.,* 2005).

Kakamega district is in Western province and has a high rainfall averaging between 1000 mm and 1800 mm per annum. In the North-eastern areas the generally humid climate is interrupted by four semi-arid months (November to February) restricting cultivations of important perennial crops like bananas. The rainfall expectation is high at least 500-

100mm during the first rainy season and 450-850 mm during the second rainy season in 10 out of 15 years. In the Eastern part of the district (UM0, 1, 2 and UM 3-4), the annual mean temperature is about 18-21˚C and in the rest of the district it is higher than 21˚C (LM1-3). In the North-Eastern area of the district there is a plateau with moderately deep soils. The dominant soils of the district are found on upper middle level uplands (UH, UM and UL), they are poor in plant nutrients. On plateaus and higher level structural plains, low natural fertility is found. Valley bottom soils with water logging can be seen in some places in the Northern, Western ant North-Eastern part of the district (Jaetzold *et al.,* 2005).

Busia and Teso districts lie on the eastern part of Lake Victoria. The areas receive rainfall of between 900-1500 mm annually. During the long rains they receive between 400-900 mm and 150-800 mm during the short rains. The annual average temperature is between 21.0˚C and 22.7˚C. Humidity of the air is relatively high due to the lake. The potential evapotranspiration is 1800-2030mm per year for both districts. The majority of the soils are moderately deep (soil depth of 50-80 cm to murram or parent materials) and have low fertility. Soils on the hills are shallow with low natural fertility. In the flood plains soils there are complexes of unsuitable and suitable soils. Agriculturally, smallholder farmers cultivate sugarcane, cotton and tobacco as their major cash crops (Jaetzold *et al.,* 2005).

3.1.1 Sampling

Five locations per district and ten farmers per location were randomly selected as sampling areas. Quarter kilograms sorghum and finger millet grains per farmer were collected for each variety (12.5 kg per district). In Nyamira district, the samples were picked from Ekerenyo, Kiabonyoru, Bonyamatuta, Bosamaro and Keera locations. The sorghum varieties collected were Wagiita (plate 6), Ochuti, Gopari and Migogo. Most farmers preferred growing Wagiita because it is high yielding, not easily damaged by birds and it is palatable. The finger millet varieties included Enyaikuro, Enyankundi, Mokomoni and P224 (plate 5). The most preferred variety by the farmers was Enyaikuro because it makes good *ugali* and porridge. Samples collected from Nyamira in November 2007 had been planted in the long rains of January to July 2007.

In Teso district, the samples were collected from Asing'e, Apegei, Ang'orom, Ochude and Okame locations. The sorghum varieties collected were IS8193, Wagiita (plate 6), Nakhadabo (plate 3) and KARI Mtama-1 (plate 1). The most preferred variety was IS8193 because it's good for *ugali* and alcohol brew (mwenge). The finger millet varieties included Ikhulule (plate 4), P224 (plate 5), Aran and KNE 688. Most farmers preferred Ikhulule because of its marketability, suitability for porridge and brew, and also treatment of diabetes. Sorghum and finger millet in Teso district are grown once in a year during the long rains. The samples were picked in March 2008 from the 2007 crop season (February to July). In Busia district, the samples were collected from Bumala, Nambuku, Township, West Bukhayo and Lwanya locations. Sorghum varieties collected were Seredo, AF28 and Esila (plate 2) while finger millet varieties included Gulu E, Namadimwa (P224) and U15. The samples were picked in March 2008 from the 2007 crop season. In Kakamega district the samples were collected from Khayega, Shibuye, Bukura, Bukhungu and Shieywe locations. Sorghum varieties collected were Ikhumba, Livoywa, Essuti, AF28 and IS8193. The most preferred variety was Livoywa because of its good brew and *ugali*. Finger millet varieties included Ikhulule, Nafusi and Agriculture (P224). The samples were picked in May 2008 from the 2007 season crop.

The samples that were picked from the farmers had been stored for two months or more. Grains collected from the same district were mixed thoroughly and bulked together according to their respective varieties. They were then divided into two equal parts where one portion was used for culturing *Fusarium* species and the other portion for fumonisin B1 and aflatoxin B1 analyses at the University of Nairobi. The samples were put in paper bags then taken to the laboratory and stored at 4°C until required.

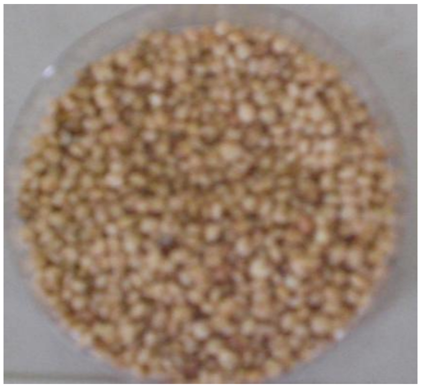

Plate 1: KARI Mtama-1 (sorghum)
Collected March 2008

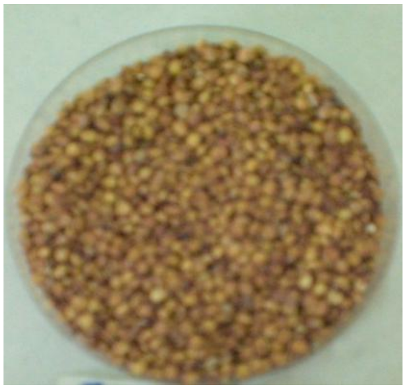

Plate 2: Esila (sorghum)
Collected March 2008

Plate 3: Nakhadabo (sorghum)
Collected March 2008

Plate 4: Ikhulule (finger millet)
Collected March 2008

Plate 5: P224 (finger millet)
Collected March 2008

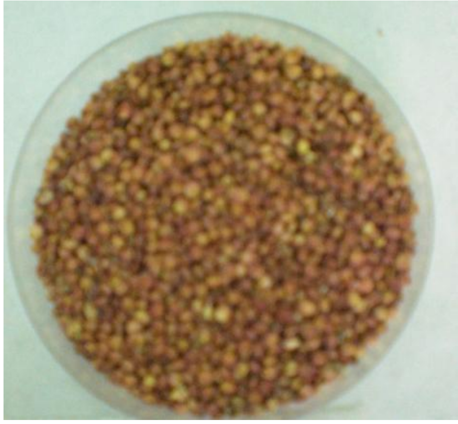

Plate 6: Wagiita (sorghum)
Collected November 2008

3.2 Isolation of *Fusarium* species

The sorghum and finger millet seeds were dipped in 70% alcohol, there after sterilized in 0.5% sodium hypochlorite for two minutes for sorghum seeds and one minute for finger millet seeds. The sterilized seeds were washed in three changes of sterile distilled water and dried on sterile filter paper. Ten grains of each variety were cultured on Peptone PCNB Agar (PPA)/Snyder and Nash medium (1962), then the plates were incubated at alternating temperature regime of 25˚C day and 20˚C night with a 12 hour photoperiod (Burgess *et al.,* 1994). *Fusarium* colonies developed on the selective medium within 5-7 days.

3.3 Purification of *Fusarium* species

The colonies on PPA were subcultured onto CLA plates and incubated as indicated in section 3.3 for 10 days. The cultures were purified using the single spore isolation procedure (Leslie and Summerell 2006). In this procedure, 10 ml of sterile distilled water was prepared in a test tube. A small scrape of macroconidia from the sporodochia was added to the sterile water, and then a drop of the suspension viewed under the low power (100x). Dry WA plates (at least seven days old) were seeded by pouring 1ml of the conidial suspension over the surface. The plates were incubated right side up at 25˚C for 24 hours.

Germinated spores (germlings) were identified by examination using a dissecting microscope at 40x. Individual germlings were excised with a small sterile ‘spatula’ made from nichrome wire mounted on a standard needle holder.

3.4 Identification of *Fusarium* species

Fusarium species were identified according to Leslie and Summerell (2006) and Nelson *et al.* (1983). As the CLA plates were poured the leaf pieces floated to the side of the plate. The cut agar block containing *Fusarium* culture was aseptically placed adjacent to the leaf piece. This allowed the fungus to grow on the leaf pieces where sporodochia were formed and over an open area of water agar where the conidiophores producing microconidia proliferate. *Fusarium* species sporulated on CLA in 6-10 days. Direct microscopic examination (*in situ*) of the plates was done to see how the conidia were borne on conidiophores at 100x and 400x. Microscopic slide preparations were done for detailed examination of macroconidia formed in sporodochia for identification as the macroconidia were more consistent in shape and length. The shape of the macroconidia was recorded according to Leslie and Summerell (2006).

Another agar block was aseptically cut and put on filter papers on SNA. This medium promoted sporulation and good conidogenous development but macroconidia were not morphologically uniform as on CLA. Microconidia morphology and the presence of chlamydospores were examined after 10-14 days. Since SNA was transparent, *in situ* examinations were done using a compound microscope at 100x. Small agar blocks were also cut and mounted on a slide with a drop of water and covered with a cover slip for observation at higher magnifications. The following were recorded; presence or absence of microconidia, shape of the microconidia, and nature of conidogenous cell bearing microconidia and presence or absence of chlamydospores.

The last agar block was aseptically put at the centre of PDA plate. Conidia formed on PDA were not consistent in either size or shape as those formed on CLA or SNA, and thus much less reliable for identification purposes. The culture incubated for 14 days at alternating day and night temperatures of 25˚C and 20˚C respectively and a 12-hour photoperiod. The colour of the PDA culture and the pigments produced in the agar were recorded.

3.5 *Fusarium* preservation

The *Fusarium* cultures were preserved in soil. The soil was sterilized in an oven at 180˚C for two hours then allowed to cool. This was repeated once. A drop of prepared inocula from *Fusarium* was aseptically put into the sterile soil. Other cultures were preserved on a slant of PDA in universal bottles then kept at 4˚C.

3.6 Incidence of *Fusarium* species in sorghum and finger millet

The sorghum and finger millet grains from the four districts were sterilized as earlier described in section 3.3. Ten grains were cultured on PPA and then incubated at alternating day and night temperatures of 25˚C and 20˚C respectively for seven days. The number of grains growing with *Fusarium* species was counted and the percentage of incidence computed as follows;

$$\text{Incidence of } \textit{Fusarium} = \frac{\text{Number of grains with } \textit{Fusarium}}{10} \text{ X } 100$$

There were five replicates per treatment arranged in a randomized block design.

3.7 Incidence of *Fusarium* species in sorghum roots and stem from Busia and Teso districts

The roots and stems (the cuttings were obtained from the first three nodes from the ground) were washed under running water then cut into 1cm pieces. The pieces were sterilized as earlier described in section 3.3. Five pieces were cultured on PPA and then incubated at alternating day and night temperatures of 25˚C and 20˚C respectively for seven days. The numbers of pieces growing with *Fusarium* species were counted and the percentage of incidence computed as follows;

$$\text{Incidence of } \textit{Fusarium} = \frac{\text{Number of pieces with } \textit{Fusarium}}{5} \text{ X } 100$$

There were five replicates per treatment arranged in a randomized block design.

3.8 Other fungi associated with sorghum and finger millet grains

The sorghum and finger millet grains from the four districts were sterilized as earlier described in section 3.3. Ten grains were cultured on PDA and then incubated at alternating day and night temperatures of 25˚C and 20˚C respectively for seven days. The fungal scrap was put on a slide then observed under the microscope at 100x- 400x. Identification was done according to Navi *et al.*, (1999) and Agrios (1988).

3.9 Mycotoxin analysis

Fumonisin B1 and aflatoxin B1 analyses were done at the University of Nairobi, at the School of Agriculture and Veterinary Science, Kabete.

3.9.1 Sample preparation

The samples had been stored in a cool place (4˚C). All samples were dried to a moisture level of 13%. The samples of each variety from the same district were thoroughly mixed and a representative sample of 500 g taken. The samples were ground to flour using the Romer mill (Union, 1L, USA) then thoroughly mixed again.

3.9.2 Sample extraction

Five grams of the ground sample were weighed into a 20 ml tube, 25 ml of 70% methanol (70 ml methanol + 30 ml distilled water) was added and the mixture was then vortexed at high speed for two minutes in a vortex machine. This was followed by centrifugation at 10000 rpm for 15 minutes. The sample extract was diluted 1:14 (100 µl extract + 1.3 ml water) with distilled water and 50 µl of the dilute extract put in 10 ml tubes then coated with aluminium foil since fumonisin is light sensitive. This amount was used per well in the test.

3.9.3 Preparation of chemicals

The test kit was provided which contained the following; microtiter plates coated with capture antibodies, fumonisin standard solutions of 0 ppm (zero standard), 0.025 ppm, 0.074 ppm, 0.222 ppm, 0.666 ppm and 2 ppm fumonisin in methanol/water ready to use, conjugate (peroxidase conjugated fumonisin), anti-fumonisin antibody, substrate/chromogen and stop solution (1 M sulphuric acid).

3.9.4 Test principal for fumonisin B1

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-fumonisin antibodies. Fumonisin standards or sample solutions, fumonisin enzyme conjugate and anti-fumonisin antibodies are added. Free fumonisin and fumonisin enzyme conjugate compete for the fumonisin antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-fumonisin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. When substrate/chromogen is added to the wells, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a colour change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the fumonisin concentration in the sample.

3.9.5 Fumonisin B1 analysis

Direct competitive Enzyme Linked Immunosorbent Assay (ELISA) method using microtiter plates as described by Usleber *et al.* (1994) for fumonisin B1 analysis was used. The microtiter plates were coated overnight at room temperature with 100 µl of anti-FBI antiserum. The wells were then inserted into the microwell holder for all the standards and samples run in duplicate. The standard and sample positions were recorded. An addition of 50 µl of the standard solutions was made and 50 µl of the prepared samples was done in separate duplicate wells. This was followed by an addition of 50 µl of the enzyme conjugate in each well. Lastly, 50 µl of anti-fumonisin antibody solution was added to each well.

The contents were mixed by gently shaking the plate manually then incubated for 30 minutes at room temperature in the dark (25˚C). The liquid was poured out of the wells and the microwell holder tapped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. The wells were filled with 250 µl of distilled water and the liquid poured out again. The washing procedure was repeated twice. Chromogen was added (100 µl) to each well. The contents were mixed gently by shaking the plate manually then incubated at room temperature for 15 minutes in the dark. Lastly, 100 µl of the stop solution was added to each well then mixed gently by shaking the plate. The absorbance of the resultant colour was measured at 450 nm using a Uniskan II microplate reader (Labsystems, Finland). The readings were taken within 10 minutes after addition of the stop solution. The detection level was at 1.75 µg/g

3.10 Aflatoxin B1 analysis

3.10.1 Sample preparation

The samples had been stored in a cool place (4˚C). All samples were dried to a moisture level of 13%. The samples of each variety from the same district (Busia and Teso) were thoroughly mixed and a representative sample of 500 g taken. The samples were ground to flour using Romer mill (Union, 1L, USA) then thoroughly mixed again.

Five grams of the ground sample was weighed on a piece of aluminium foil and transferred into the tube where 50 ml methanol and water (55:45) were added and vortexed at a high speed for three minutes. The mixture was then centrifuged at 5000 rpm for 10 minutes and recovery of four millilitres of the supernatant. Four millilitres of hexane were added to the supernatant then vortexed for 30 seconds. Transfer of 200 µl of the extract into a 10ml test-tube was done. PBS of 800 µl and 3000 µl methanol with PBS (10:90) were added and mixed thoroughly on a vortex.

3.10.2 ELISA analysis

Direct competitive Enzyme Linked Immunosorbent Assay (ELISA) method using microtiter plates as described by Hongyo *et al.,* (1992) for aflatoxin analysis was used. Aflatoxin standards were prepared (1000 ppt, 333 ppt, 111 ppt, 37 ppt, 12 ppt, and 0 ppt) by diluting the calibrated standards then marked as N, S1, S2, S3, S3, S4, S5 and S6. An addition of 1000 µl of methanol: PBS (1:10) was put in N, S2, S3, S3, S4, S5, S6 and 2000 µl into tube S1. Ten micro-litres of the aflatoxin standard were added to tube N (neat) and mixed vigorously on the vortex. Transfer of 20 µl in tube N was put in tube S1 and mixed vigorously. Serial dilution of 500 µl of solution in tube S1 (1 ppb) into tubes S2, S3, S4 and S5 was done and mixed thoroughly. Nothing was added to tube S6. The coated plates were washed twice with wash solution (NaCl-Tween) and dried with blotting paper. This was followed by pipetting 50 µl of standard solutions in tubes S1-S6 and 50 µl sample extract solutions into wells of coated ELISA plates. The enzyme conjugate of 50 µl was then pipetted into the wells of the coated plates. The ELISA plates were then incubated for 30 minutes at room temperature and covered with aluminium foil to protect from light. The wells were then removed and washed three times with wash solution then semi-dried on blotting paper. This was followed by addition of 100 µl substrate solution into each well, and then left for 10 minutes to enhance colour development. Colour development was stopped by addition of 100 µl sulphuric acid solution in each well.

The absorbance was read in each microplate well. The average absorbance values for each aflatoxin standard, sample extract (B) and the blank (Bo) were calculated. These values were used for calculating the percentage inhibition values (B/Bo %). Aflatoxin standard was plotted on X-axis while percentage inhibition on Y-axis. Best-fit line was drawn between the points on a graph paper. Aflatoxin concentrations of each sample were determined by drawing a line of abscissa from its percentage inhibition value on the Y-axis to the curve and a line of ordinate from intersect on the curve to the X-axis. The values obtained were multiplied by the dilution factor of a half (½). The detection levels were at 2 ppb.

3.11 Data analysis

The data findings were statistically analysed using MINITAB 13.0 statistical package. Data collected for percentage incidences was transformed by use of square root before analysis. To establish the variations in the incidences of *Fusarium* species of fungi, ANOVA was done on the data. Mean separations were done where there were significant differences using Tukeys HSD. The findings were presented using measure of central tendency in form of frequencies, percentages, graphs, tables and charts.

CHAPTER FOUR

RESULTS

4.1 *Fusarium* species infecting sorghum and finger millet grains

There were 275 *Fusarium* species isolated from 800 sorghum grains while 38 isolated from 700 finger millet grains. Sorghum had higher isolations of *Fusarium* with Teso district having the highest isolation of 96 isolates followed by Nyamira district with 90 isolates, Busia district with 56 isolates, and Kakamega district with 33 isolates (Table 1). Nyamira and Teso districts had the highest isolation in finger millet with 11 isolates each, followed by Kakamega and Busia had 08 isolates each (Table 2).

Table 1 sorghum grains cultured and infected from the sampled districts

District	**variety**	**Total grains cultured**	**Infected grains**
Nyamira	Wagiita	50	31
	Ochuti	50	25
	Migogo	50	19
	Gopari	50	15
Busia	Seredo	50	28
	AF28	50	28
	Esila	50	00
Teso	Wagiita	50	28
	IS8193	50	27
	KARI Mtama-1	50	21
	Nakhadabo	50	20
Kakamega	IS8193	50	14
	AF28	50	08
	Essuti	50	07
	Ikhumba	50	03
	Livoywa	50	01
Total		800	275

Table 2 finger millet grains cultured and infected from the sampled districts

District	**Variety**	**Total grains cultured**	**Infected grains**
Nyamira	Enyaikuro	50	04
	Mokomoni	50	04
	Enyankundi	50	01
	P224	50	02
Busia	GuluE	50	02
	U15	50	03
	P224	50	03
Teso	Ikhulule	50	02
	Aran	50	02
	KNE688	50	03
	P224	50	04
Kakamega	Nafusi	50	03
	Ikhulule	50	03
	P224	50	02
Total		700	38

Nineteen morphologically distinct *Fusarium* species were isolated from sorghum and finger millet grains including *Fusarium compactum, F. longipes* (plates 15 and 16), *F. equiseti*, *F. thapsinum* (plates 11 and 12), *F. heterosporum, F. chlamydosporum, F. subglutinans, F. semitectum, F. verticillioides* (plates 7 and 8), *F. brevicatenulatum* (plates 17 and 18), *F. proliferatum, F. oxysporum* (plates 9 and 10), *F. nygamai* (plates 13 and 14), *F. pseudonygamai, F. solani, F. andiyazi, F. graminearum, F. napiforme,* and *F. pseudograminearum.* The characters of each *Fusarium* species were summarized in Table 3

Fusarium compactum and *F. equiseti* were isolated from both sorghum and finger millet in all four districts.

Fusarium compactum, F. equiseti, F. thapsinum, and *F. verticillioides* were isolated from sorghum in all four districts. *Fusarium heterosporum* was isolated in Teso, Busia and Nyamira districts while *F. longipes* was isolated in Teso, Nyamira and Kakamega districts. *Fusarium andiyazi* was isolated from Busia, Nyamira and Kakamega districts while *F. graminearum* was isolated from Nyamira and Kakamega districts while *F. brevicatenulatum* was isolated from Teso and Nyamira districts. *Fusarium chlamydosporum, F. subglutinans* and *F. semitectum* were isolated from Teso district only while *F. proliferatum, F. oxysporum F. nygamai* and *F. pseudonygamai* were recovered from Busia district. *Fusarium solani and F. napiforme* were isolated from Nyamira district only while *F. pseudograminearum* was isolated from Kakamega district only (Table 4).

In finger millet, *F. compactum* and *F. equiseti* were isolated in all the districts. *Fusarium oxysporum* was isolated in Teso and Kakamega districts while *F. graminearum* and *F. heterosporum* were isolated in Busia district. *Fusarium solani* was isolated from Kakamega district only (Table 5).

Table 3 Morphological characteristics of *Fusarium* species isolated from sorghum and finger millet grains

***Fusarium* Species**	**Microconidia on CLA**			**Phial-ides**	**Chlamy dospore**	**Macroconidia Shape on CLA**	**Pigment on PDA**	**Mycelia Colour on PDA**
	+/-	**Head/ Chain**	**Shape**					
F. compactum	-	-	-	-	+	Needle-like apical, 5-septa	Brown	White brown
F. longipes	-	-	-	-	+	Long, whip-like apical, 5 septa	Dark red	Greyish-rose
F. equiseti	-	-	-	-	+	Tapered apical, 5-7 septa	Dark brown	Brown
F. thapsinum	+	Long, false	Club-shaped	Mono	-	Slender, thin wall, 3-5 septa	Yellow, colorless	White-violet
F. heterosporum	-	-	-	-	-	Thin wall, slender, 4 septa	colorless	Pinkish-white
F. chlamydosporum	+	False	Oval	Poly	+	Pointed apical	Pale-brow	white
F. subglutinans	+	False	Oval	Poly Mono	-	Slender, thin wall, 3-septa	Dark purple	violet
F. semitectum	+	False	Obovoid 0-3septa	Poly, mono	+	Slender, curved apical, 3-5septa	Brown	Beige
F. verticillioides	+	Long	Oval, flat base	Mono	-	Long and slender, 3-5 septa	colorless	violet
F. brevicatenulatum	+	False, short	Obovoid	Mono	-	Slender, thin wall, 5 septa	Blue-grey	Pale-orange
F. proliferatum	+	False, Short	Club-shaped	Poly, Mono	-	Slender, thin wall, 3-5 septa	colorless	Violet
F. oxysporum	+	False	Oval	Mono	+	Slender, thin wall, 3-septa	colorless	White-violet
F. nygamai	+	False, Short	Oval	Mono, Poly	+	Slender, hyaline, 3-5 septa	Violet	Violet
F. pseudonygamai	+	False, Short	Obovoid	Mono, Poly	-	Fusoid, 3-5 septa	Violet	White
F. solani	+	False	Oval	Mono	+	Wide, 5-7 septa	colorless	white
F. andiyazi	+	False, Long	Obovoid	Mono	Pseudo	Hyaline, thin wall, 3-septa	Dark purple	violet
F. graminearum	-	-	-	-	+	Thick wall, 5-6 septa	Red-yellow	Pale-orange
F. pseudograminearum	-	-	-	-	+	Slender, 5-6 septa	White-yellow	Red
F. napiforme	+	False, Short	Obovoid	Mono	+	Long, hyaline, 5-septa	purple	White

-Indicates absence of character; + indicates presence of character

Table 4 *Fusarium* species isolated in sorghum grains from Nyamira, Kakamega, Busia and Teso districts

***Fusarium* species**	**Nyamira**	**Kakamega**	**Busia**	**Teso**
F. compactum	+	+	+	+
F. equiseti	+	+	+	+
F. longipes	+	+	-	+
F. thapsinum	+	+	+	+
F. verticillioides	+	+	+	+
F. proliferatum	-	-	+	-
F. nygamai	-	-	+	-
F. pseudonygamai	-	-	+	-
F. graminearum	+	+	-	-
F. pseudograminearum	-	+	-	-
F. andiyazi	+	+	+	-
F. heterosporum	+	-	+	+
F. solani	+	-	-	-
F. semitectum	-	-	-	+
F. subglutinans	-	-	-	+
F. oxysporum	-	-	+	-
F. napiforme	+	-	-	-
F. chlamydosporum	-	-	-	+
F. brevicatenulatum	+	-	-	+

- Indicates absence of *Fusarium* species: + indicates presence of *Fusarium* species

Table 5 *Fusarium* species isolated in finger millet grains from Nyamira, Kakamega, Busia and Teso districts

***Fusarium* species**	**Nyamira**	**Kakamega**	**Busia**	**Teso**
F. compactum	+	+	+	+
F. equiseti	+	+	+	+
F. oxysporum	-	+	-	+
F. graminearum	-	-	+	-
F. heterosporum	-	-	+	-
F. solani	-	+	-	-

- Indicates absence of *Fusarium* species: + indicates presence of *Fusarium* species

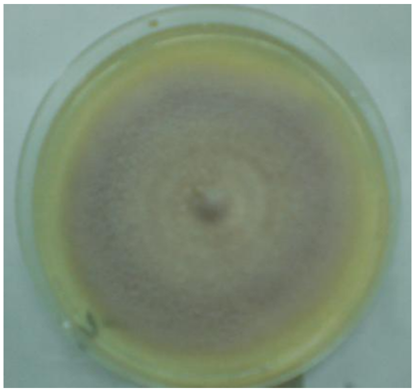

Plate 7: Mycelium of *Fusarium verticillioides* on PDA

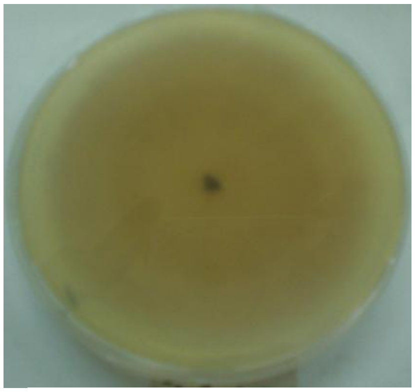

Plate 8: Pigmentation in agar of *F. verticillioides* on PDA

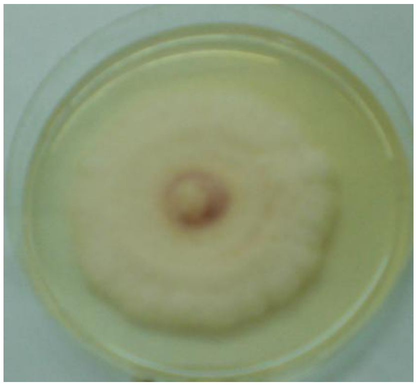

Plate 9: Mycelium of *F. oxysporum* on PDA

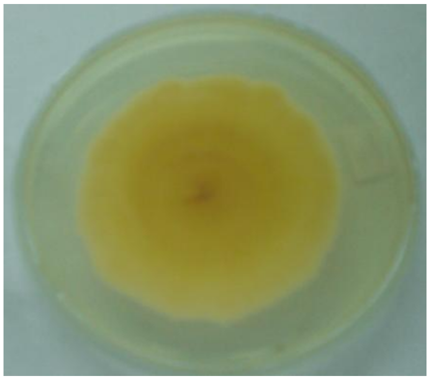

Plate 10: Pigmentation in agar of *F. oxysporum* on PDA

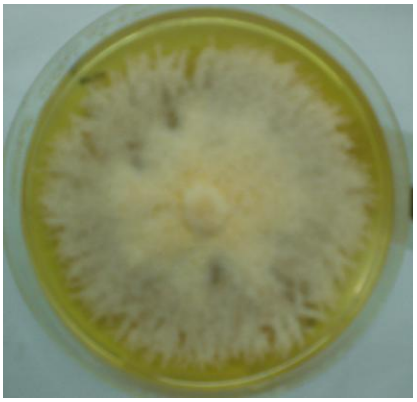

Plate 11: Mycelium of *F. thapsinum*

on PDA

Plate 12: Pigmentation in agar of *F. thapsinum*

on PDA

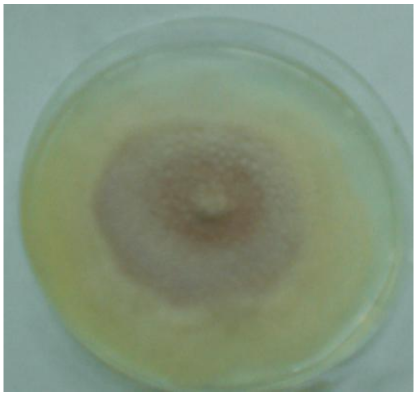

Plate 13: Mycelium of *F. nygamai* on PDA

Plate 14: Pigmentation in agar of *F. nygamai* on PDA

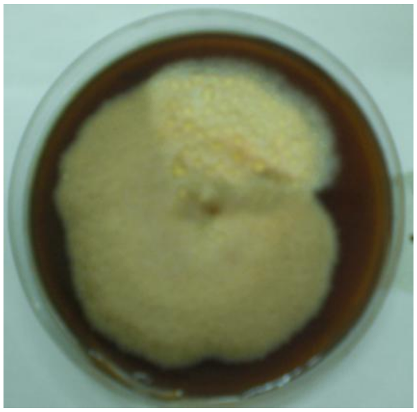

Plate 15: Mycelium of *F. longipes* on PDA

Plate 16: Pigmentation in agar of *F. longipes* on PDA

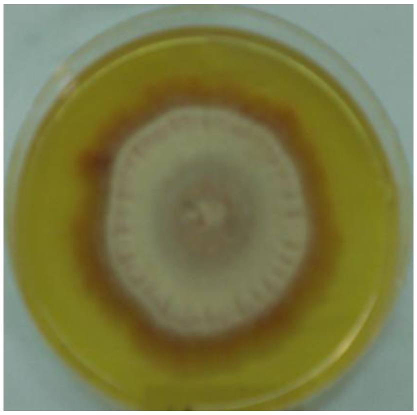

Plate 17: Mycelium of *F. brevicatenulatum* on PDA

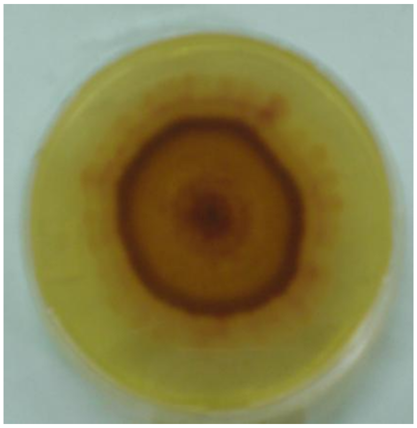

Plate 18: Pigmentation in agar of *F. brevicatenulatum* on PDA

4.2 Incidences of *Fusarium* in sorghum and finger millet grain from the sampled districts in Western Kenya

In the four districts, the study established there was a significant difference ($P < 0.05$) between the incidences of *Fusarium* in sorghum and finger millet. Sorghum had a higher mean at 29.66% relative to finger millet at 4.87%. The general incidences of *Fusarium* for both sorghum and finger millet from the districts sampled showed a significant variation ($P < 0.05$). There was a higher incidence of *Fusarium* species (both sorghum and finger millet combined) in Teso district at 21.16% than in Nyamira district at 19.68% while Busia had 15.37% and Kakamega had the lowest incidence at 8.71% (Figure 4).

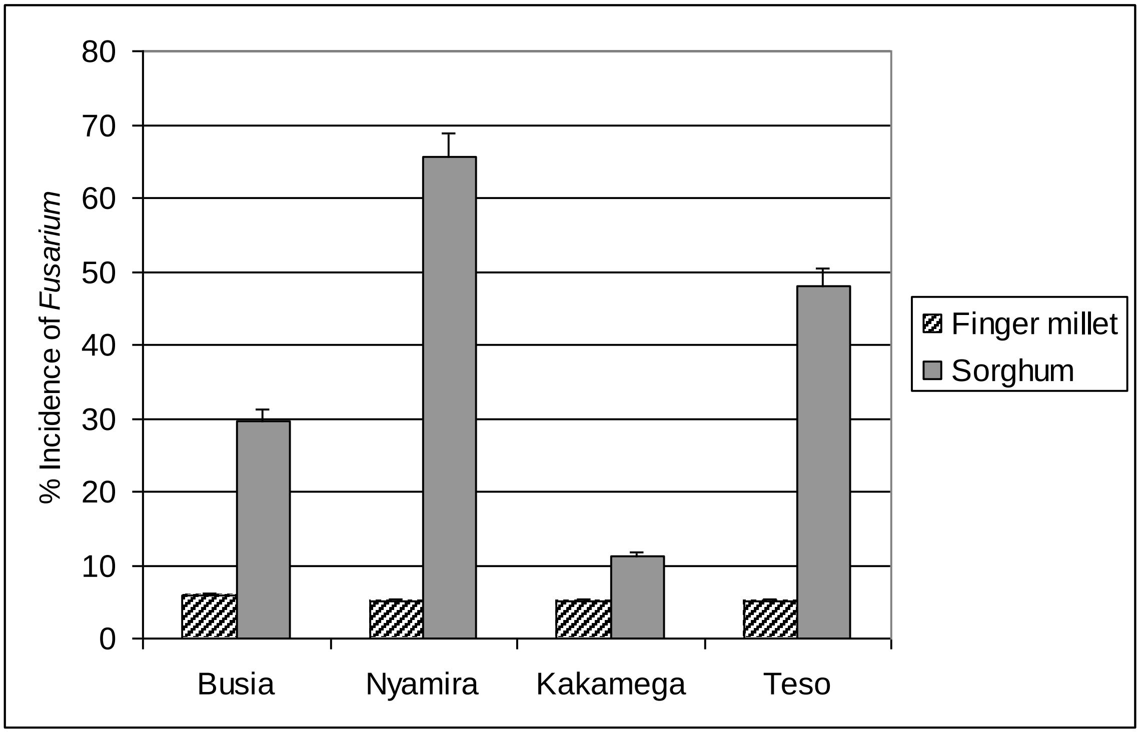

Figure 4 Incidences of *Fusarium* in sorghum and finger millet grains from four districts of Western Kenya

4.3 Incidences of *Fusarium* in sorghum grains from four districts in Western Kenya

The study established that there was a significant difference ($P < 0.05$) in the incidences of *Fusarium* species in the sampled districts. There was a higher incidence in Nyamira district (Wagiita, Ochuti, Gopari and Migogo) with a mean of 65.47% than in Teso (Wagiita, IS8193, KARI-Mtama-1 and Nakhadabo) at 47.89%, Busia (Seredo, AF28 and Esila) with 29.59% and Kakamega (IS8193, AF28, Essuti, Livoywa and Ikhumba) relatively lower mean at 11.12% (Figure 5).

Considering the various sorghum varieties, the study established a significant difference in the incidence of *Fusarium* species ($P < 0.05$). Sorghum variety, Wagiita had the highest incidence at 58.95% followed by Seredo with 55.86% while none was isolated from Esila (Table 6).

4.3.1 Incidence of *Fusarium* in sorghum from Nyamira district

Sorghum varieties sampled from Nyamira district were; Wagiita, Gopari, Migogo and Ochuti. Incidences of *Fusarium* in the respective varieties of sorghum had no significant difference. However, the study established that *Fusarium* isolated from Wagiita variety had a mean of 60.93%, Ochuti had 49.31%, Migogo with 37.58% and Gopari had 29.18% (Figure 6).

Incidence of *Fusarium* species in sorghum differed significantly in Nyamira district ($P < 0.05$). *Fusarium andiyazi* had mean percentage incidence at 17.99% while *F. solani* had 3.3% (Figure 7)

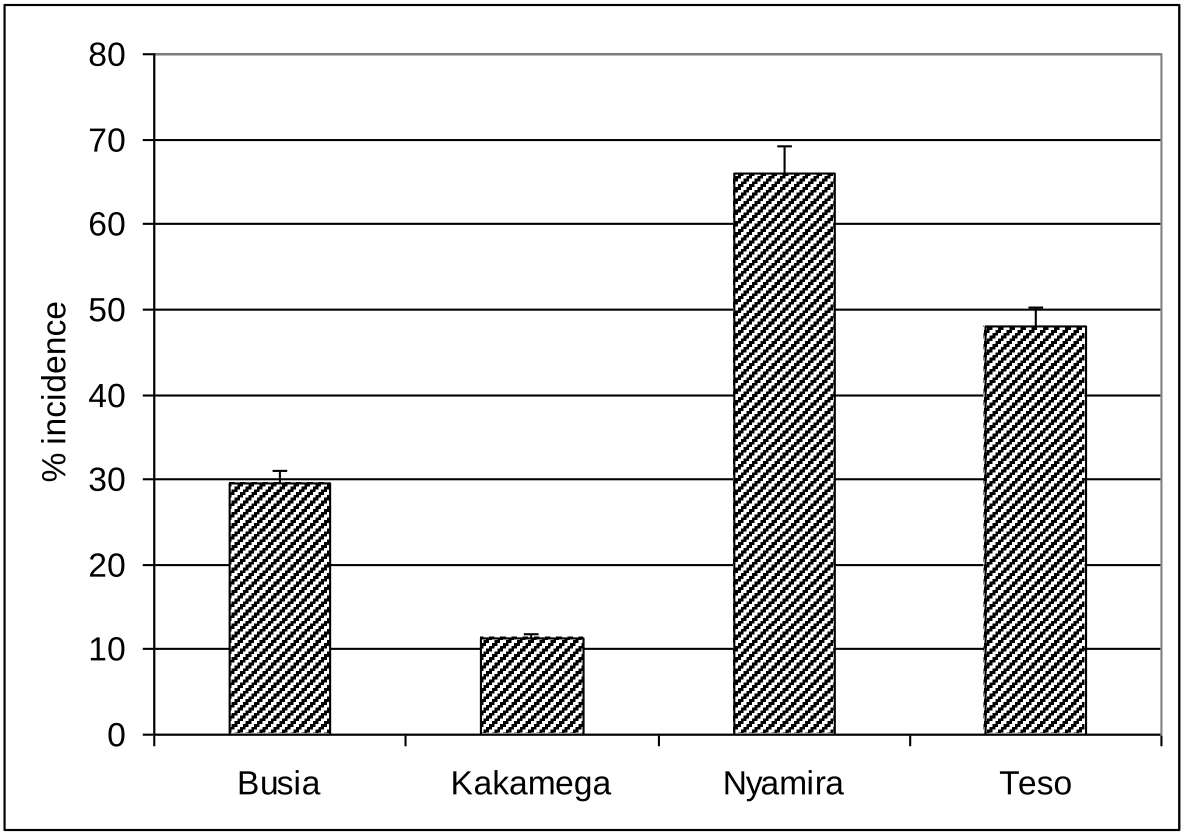

Figure 5 Incidence of *Fusarium* in sorghum from four districts of Western Kenya

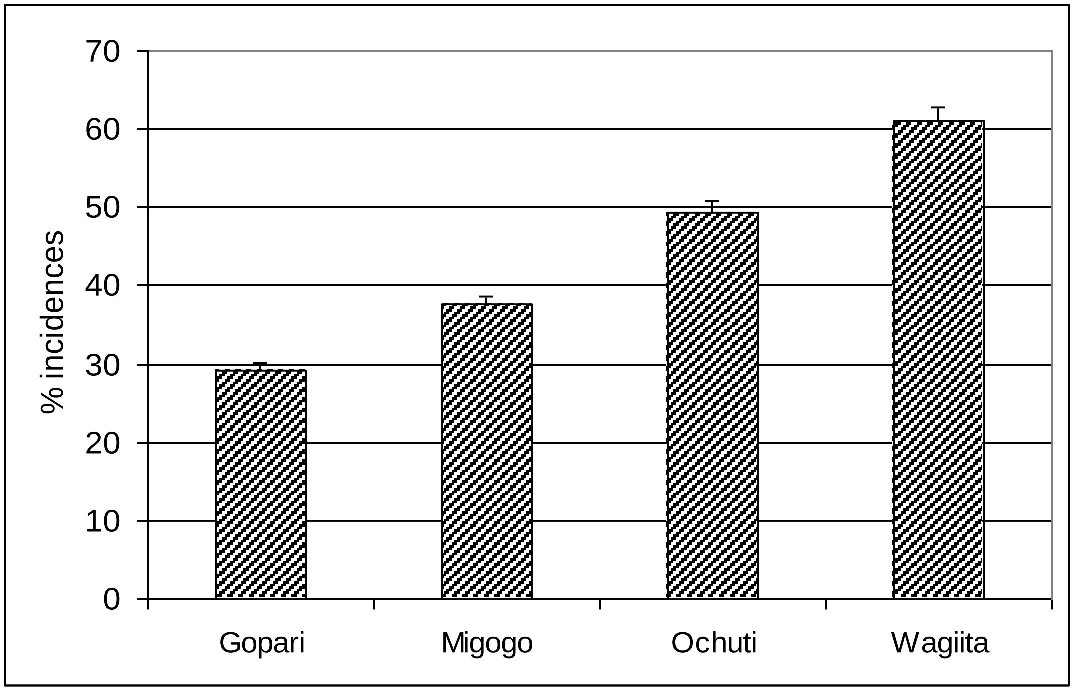

Figure 6 Incidences of *Fusarium* in the varieties of sorghum from Nyamira district

4.3.2 Incidence of *Fusarium* in sorghum from Busia district

Sorghum varieties sampled from Busia district were; Seredo, AF28 and Esila. Incidence of *Fusarium* species in the sorghum varieties significantly differed ($P < 0.05$) in Busia district. Percentage *Fusarium* incidence in Seredo had a mean percentage incidence at 55.86% and was higher than in variety AF28 which had 55.18% while Esila had 0% (Table 6).

Incidence of specific *Fusarium* species in sorghum significantly differed ($P < 0.05$) in Busia district. *F. compactum* had mean incidence at 37.54% which was the highest while *F. proliferatum* with 1.91% was the lowest (Table 7).

4.3.3 Incidence of *Fusarium* in sorghum from Kakamega district

Sorghum varieties sampled from Kakamega district were; Ikhumba, Livoywa, Essuti AF28 and IS8193. Incidence of *Fusarium* species in these sorghum varieties significantly differed ($P < 0.05$). Percentage *Fusarium* incidence for IS8193 had mean at 28.58% which was the highest followed by variety AF28 that had 15.01% while Essuti had mean at 12.96% and Ikhumba had mean of 5.23% then the lowest was Livoywa with incidence at 2.77% (Table 8).

In Kakamega district incidences of specific *Fusarium* species in sorghum significantly differed ($P < 0.05$). *Fusarium compactum* had the highest percentage incidence at 18.47% and the lowest was *F. pseudograminearum* with 1.10% (Table 9).

4.3.4 Incidence of *Fusarium* in sorghum from Teso district

Sorghum varieties sampled from Teso district were; IS8193, Wagiita, Nakhadabo and KARI Mtama-1. Incidence of *Fusarium* species in sorghum varieties did not significantly differ in Teso district ($P < 0.05$). It was noted however that percentage incidence of *Fusarium* in Wagiita had a mean of 56.97%, IS8193 had 54.55%, KARI Mtama-1 with 41.53% while Nakhadabo variety had 39.69% (Figure 8).

Incidences of *Fusarium* species in sorghum from Teso district significantly differed ($P < 0.05$). *Fusarium compactum* was the highest with a mean incidence at 44.25% while *F. heterosporum* was the lowest with 2.71% (Table 10).

Table 6 Incidences of *Fusarium* in the various varieties of sorghum from Busia district

Sorghum variety	**Mean percentage incidences**
Seredo	55.86a
AF28	55.52a
Esila	0b

*Numbers in the table denoted by the same letter are not significantly different at p<0.05

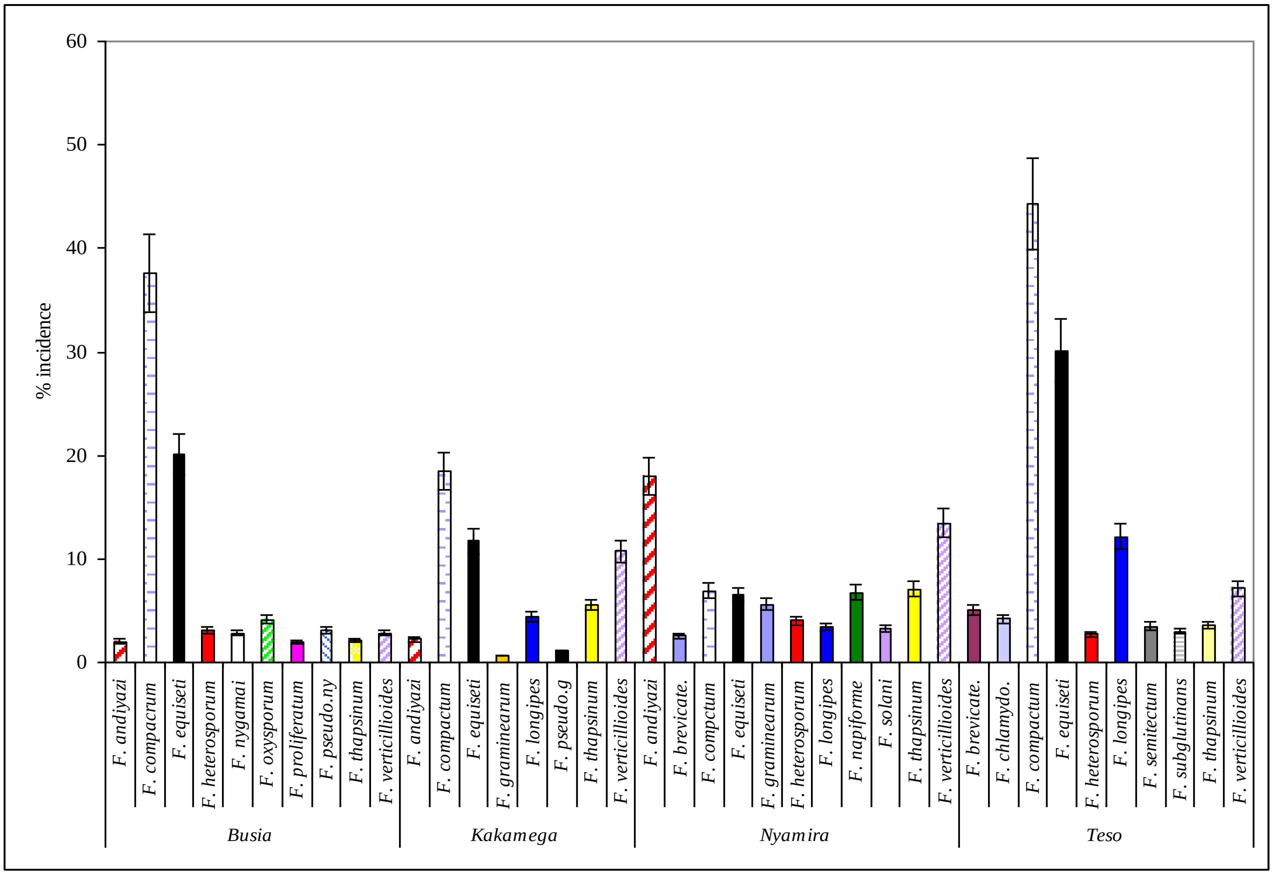

Figure 7 Incidences of various *Fusarium* species in Busia, Kakamega, Nyamira and Teso districts isolated from sorghum

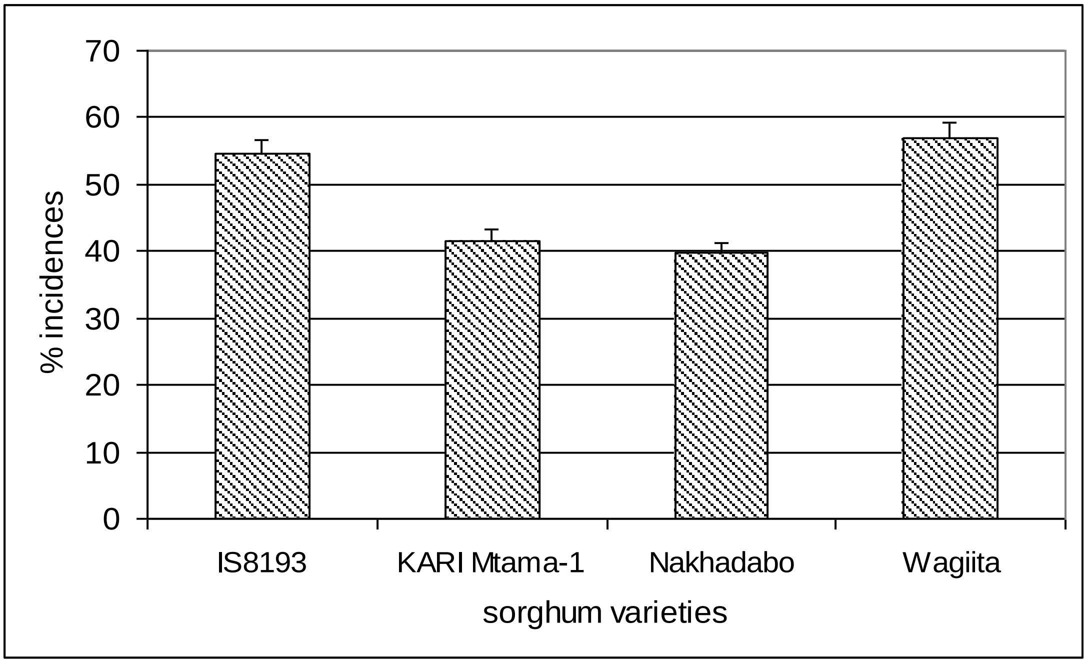

Figure 8 Incidences of *Fusarium* in the various varieties of sorghum from Teso district

Table 7 Incidences of *Fusarium* species in sorghum from Busia district

***Fusarium* species**	**Mean percentage incidence**
F. compactum	37.54a
F. equiseti	20.10ab
F. oxysporum	4.10b
F. heterosporum	3.16b
F. pseudonygamai	3.10b
F. verticillioides	2.86b
F. nygamai	2.86b
F. thapsinum	2.11b
F. andiyazi	2.01b
F. proliferatum	1.91b

*Numbers in the table denoted by the same letter are not significantly different at $p<0.05$

Table 8 Incidences of *Fusarium* in the various varieties of sorghum from Kakamega district

Sorghum variety	**Mean percentage incidences**
IS8193	28.58a
AF28	15.01ab
Essuti	12.96ab
Ikhumba	5.23b
Livoywa	2.77b

*Numbers in the table denoted by the same letter are not significantly different at $p<0.05$

Table 9 Incidences of various species of *Fusarium* in sorghum from Kakamega district

***Fusarium* species**	**Percentage incidence**
F. compactum	18.47a
F. equiseti	11.71a
F. verticillioides	10.25a
F. thapsinum	5.56b
F. longipes	4.43b
F. andiyazi	2.22b
F. pseudograminearum	1.10b
F. graminearum	0.65b

*Numbers in the table denoted by the same letter are not significantly different at $p<0.05$

Table 10 Incidences of various species of *Fusarium* in sorghum from Teso district

***Fusarium* species**	**Mean percentage incidence**
F. compactum	44.25a
F. equiseti	30.12ab
F. longipes	12.15b
F. verticillioides	7.12b
F. brevicatenulatum	5.11b
F. chlamydosporum	4.18b
F. semitectum	3.51b
F. thapsinum	3.60b
F. subglutinans	3.00b
F. heterosporum	2.71b

*Numbers in the table denoted by the same letter are not significantly different at $p<0.05$

4.4 Incidences of *Fusarium* in finger millet grains from four districts in Western Kenya

In this study, it was established that there was no significant difference in the incidence of *Fusarium* in finger millet from the sampled districts. Finger millet from Busia district had a mean incidence at 5.76%, Kakamega district had 5.20% while Nyamira district at 5.20% and Teso district had 5.19% (Figure 9).

When considering the specific *Fusarium* species in finger millet, the study established no significant difference in the incidence of *Fusarium* species in the finger millet varieties.

4.4.1 Incidence of *Fusarium* in finger millet from Nyamira district

Finger millet varieties sampled from Nyamira district were; Enyaikuro, Enyankundi, Mokomoni and P224. Incidences of *Fusarium* in finger millet varieties did not significantly differ in Nyamira district. Enyaikuro and Mokomoni variety had a mean of 7.71% each; P224 had 4.65% while Enyankundi had 0.5% (Table 11).

Incidences of *Fusarium* species in finger millet in Nyamira district did not significantly differ. *Fusarium equiseti* had 1.44% while *F. compactum* had 0.91% (Table 12).

4.4.2 Incidence of *Fusarium* in finger millet from Kakamega district

Finger millet varieties sampled from Kakamega district were; Ikhulule, Nafusi and P224. Incidences of *Fusarium* species in finger millet varieties did not significantly differ in

Kakamega district. However, for Nafusi variety the mean was 6.48%, Ikhulule had 5.70% while P224 had 3.65% (Figure 10).

Incidences of specific *Fusarium* species in finger millet in Kakamega district did not significantly differ ($p<0.05$). *Fusarium compactum* had mean of 1.86%, *F. oxysporum* with 1.68%, *F. equiseti* had 1.42% while *F. solani* had 0.93% (Table 12).

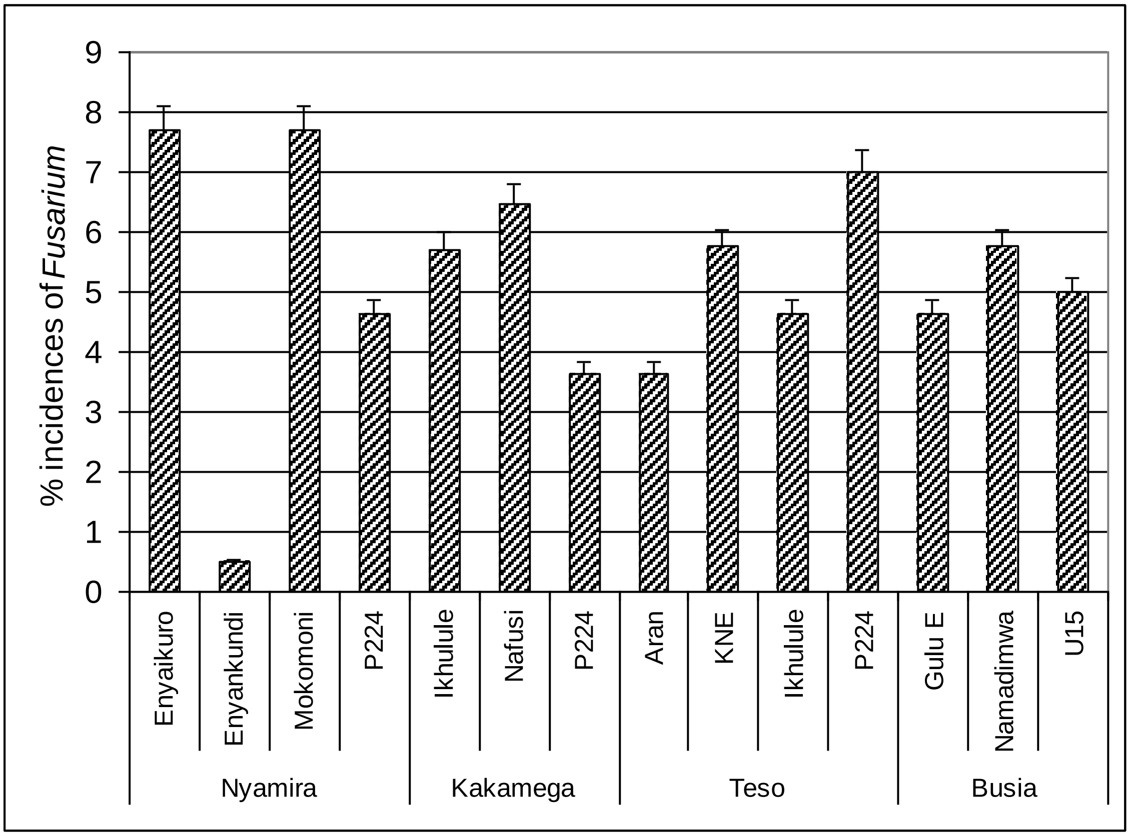

Figure 9 Incidences of *Fusarium* in different varieties of finger millet from four districts in Western Kenya

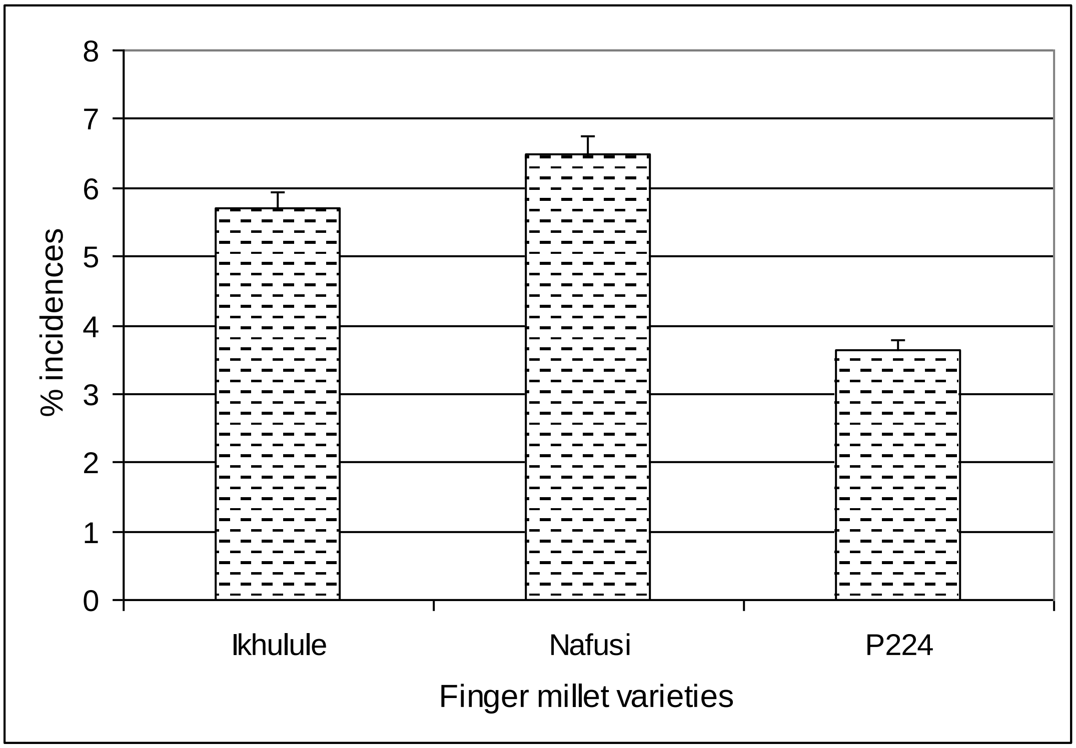

Figure 10 Incidences of *Fusarium* in the varieties of finger millet from Kakamega district

4.4.3 Incidence of *Fusarium* in finger millet from Teso district

Finger millet varieties sampled from Teso district were; Ikhulule, P224, Aran and KNE 688. Incidences of *Fusarium* in finger millet varieties did not significantly differ in Teso district. P224 variety had a mean of 7.00% while Aran had 3.65% (Table 11).

Incidences of various *Fusarium* species in finger millet in Teso district did not significantly differ. *Fusarium graminearum* had a mean of 2.09% while *F. equiseti* had mean of 0.69% (Table 12).

Table 11 Incidences of *Fusarium* in the various varieties of finger millet from Nyamira and Teso districts

District	**Sorghum variety**	**% incidence**
Nyamira	Enyaikuro	7.71
	Mokomoni	7.71
	P224	4.65
	Enyankundi	0.5
Teso	P224	7.00
	KNE 688	5.76
	Ikhulule	4.65
	Aran	3.65

*Means were not significantly different at $p<0.05$

4.4.4 Incidence of *Fusarium* species in finger millet from Busia district

Finger millet varieties sampled from Busia district were Gulu E, Namadimwa and variety U15. Incidences of *Fusarium* species in the finger millet varieties did not significantly differ in Busia district. Namadimwa variety had a mean of 5.76 while Gulu E had 4.65% (Figure 11).

Incidences of various *Fusarium* species in finger millet in Busia district did not significantly differ. *Fusarium equiseti* had mean of 2.33%, *F. graminearum* had 1.44% and *F. heterosporum* also had 1.44% while *F. compactum* had a mean of 0.76% (Table12)

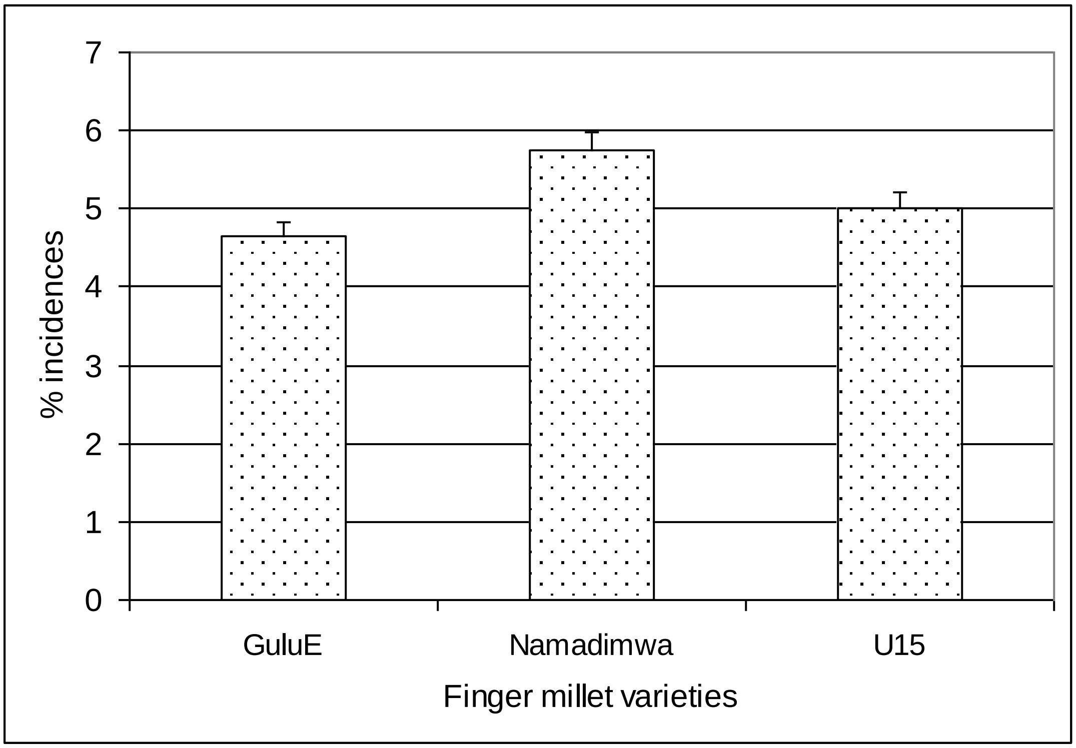

Figure 11 Incidences of *Fusarium* in the varieties of finger millet from Busia district

Table 12 Incidences of *Fusarium* species in finger millet in the sampled districts of Western Kenya

***Fusarium* species**	**% incidence in**			
	Nyamira	**Kakamega**	**Busia**	**Teso**
F. compactum	0.91	1.86	0.76	1.44
F. equiseti	1.44	1.42	2.33	0.69
F. oxysporum	-	1.68	-	1.16
F. solani	-	0.93	-	-
F. graminearum	-	-	1.44	2.09
F. heterosporum	-	-	1.44	-

*Means were not significantly different at $p<0.05$

4.5 Other fungi isolated from sorghum and finger millet grains

A total of eight fungal species were isolated from sorghum while two were isolated from finger millet. *Alternaria alternata, Phoma sorghina, Aspergillus flavus, Curvularia lunata, Rhizopus colonizer, Cladosporium, Penicillium citrinum* and *Colletotrichum graminicola* were recovered from sorghum. The fungi were identified according to Navi *et al.,* (1999) and Agrios (1988). *Alternaria alternata* had dark brown septate mycelium. The spores were club-shaped and appeared singly or in long chains. The conidia were dark coloured and septate. *Phoma sorghina* had black pycnidia which were scattered. They produced hyaline pycnidiospores that were one celled.

Aspergillus flavus appeared green with black spore mass bearing distinct spores. The spores were sub-globose in shape and one celled. *Curvularia lunata* appeared as shiny, velvety and fluffy black growth. The spores were dark and slightly curved with 3-5 septa. The spores appeared in isolation or in clusters of 3-10. *Cladosporium* species had branched septate mycelium which was hyaline at first but later darkened. The spores were dark, oval and two celled and were produced in chains at the tips of the conidiophores where they balled up in water droplets. *Penicillium citrinum* appeared blue green to olive in colour and were produced in long chains from tapered tips. The conidia were light coloured, elliptical, smooth and one celled. *Colletotrichum graminicola* produced black sclerotia which were septate. The conidia were hyaline, curved and one celled.

Pyricularia grisea and *Bipolaris nodulosa* were isolated from finger millet grains. *Pyricularia grisea* produced effuse growth of whitish grey mycelium. Conidiophores arose singly or in groups and covered usually part of the seed and in a few cases the whole seed. Conidiophores were slender, straight, and greyish or pale brown and bore clusters of conidia. Conidia were typically obclavate, hyaline, and two septate, with the central cell larger than the two terminal cells. *Pyricularia grisea* also produced chlamydospores.

Bipolaris nodulosa produced greyish to dark grey to black or brown colonies on the seed surface. Colonies frequently covered the whole seed and extended to the media. Mycelium was scanty. The brown conidiophores were straight or flexuous and bore dark brown to brown conidia. Conidia were generally four to nine septa (six septa were most common), sub-cylindrical, and slightly curved or straight, they tapered toward the rounded ends.

4.6 Incidences of *Fusarium* in roots and stems of sorghum from Busia and Teso districts

Further analysis of incidences of *Fusarium* was done on roots and stems of sorghum from Teso and Busia districts. Incidences of *Fusarium* were higher in sorghum roots with a mean of 34.06% than in stems with mean of 29.27%. The result showed that there was no significant difference in the incidences of *Fusarium* from roots of sorghum varieties. Roots of Wagiita variety had 48.86% while IS8193 had 18.26% (Table 13).

There was no significant difference in the incidence of *Fusarium* isolated from the stems of different sorghum varieties. Wagiita had incidences at 48.53%, Seredo had 36.75% while IS8193 had 12.72% (Table 13).

The study further established that there was a significant variation in the specific *Fusarium* species that attacked the roots. *Fusarium compactum* with mean of 42.32% was significantly ($P < 0.05$) higher in the roots while *F. equiseti* was the lowest at 3.11% (Figure 12)

Table 13 Incidence of *Fusarium* on sorghum roots and stems from Busia and Teso districts

Sorghum variety	**Percentage *Fusarium* incidence**	
	Roots	**Stem**
Wagiita	48.86	48.53
Esila	44.84	22.79
KARI-Mtama-1	33.25	32.26
Seredo	29.53	36.75
IS8193	18.27	12.72

*Means were not significantly different at $p<0.05$

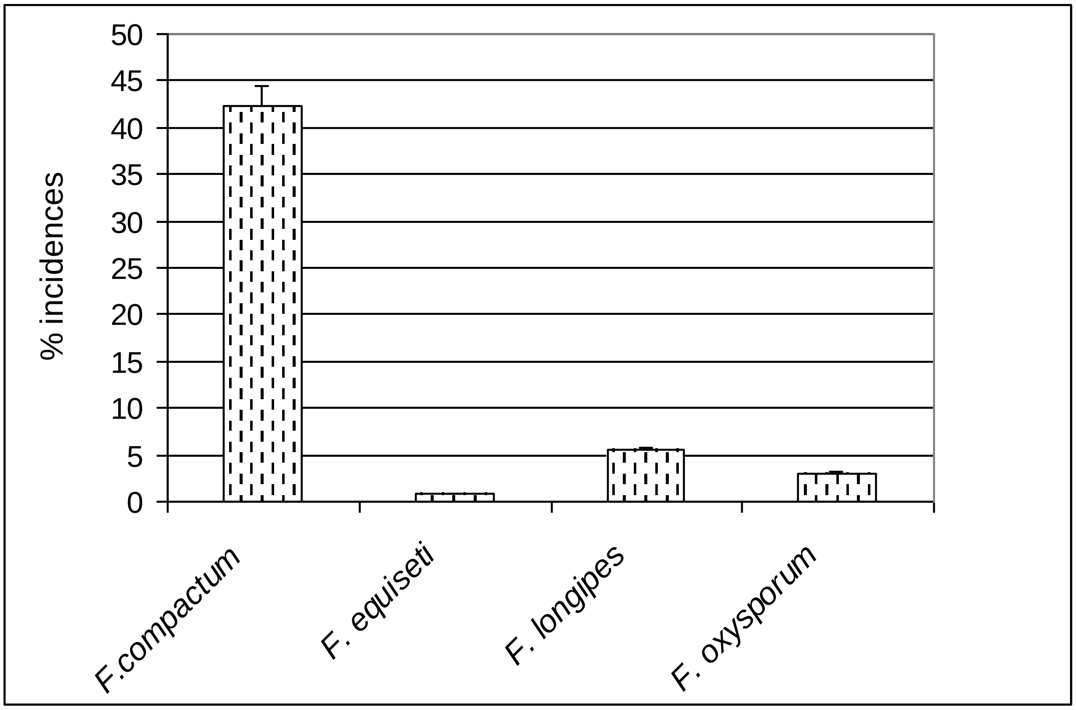

Figure 12 Percentage incidences of various *Fusarium* species on sorghum roots

4.7 Correlation between incidences of *Fusarium* on sorghum roots and stem with incidences on seed grains from Busia and Teso districts

On comparing incidences of *Fusarium* from roots and grains from Busia and Teso districts it was established that there was no significant correlation in the incidences of *Fusarium* species on roots and on the grains of Esila, IS8193, KARI Mtama-1, Seredo and Wagiita varieties (r = -0.413, p< 0.05). However, an increase in *Fusarium* in incidences on roots resulted in a decrease on grains (Table 14)

Incidences of *Fusarium* on grains were also not significantly correlated to those in the stem (r = 0.347, p< 0.05). Increase in *Fusarium* in stems tended to similarly increase in grains (Table 14).

Table 14 Correlation of incidences of *Fusarium* in grains with roots and stems of sorghum from Busia and Teso districts

Sorghum variety	**% *Fusarium* incidence**		
	Roots	**Grain**	**Stem**
Esila	44.84	0	22.79
IS8193	18.27	54.55	12.72
KARI-Mtama-1	33.25	41.53	32.26
Seredo	29.53	55.86	36.75
Wagiita	48.86	56.97	48.53
	r-value = -0.413		
		r-value = 0.347	

4.8 Levels of fumonisin B1 in sorghum and finger millet grains

Levels of fumonisin were determined from sorghum and finger millet from the sampled districts. It was determined that there was a significantly higher ($P < 0.05$) fumonisin B1 levels in sorghum grains with a mean of 48.81 µg/g than in finger millet grains at 1.13 µg/g.

In the two crops from the four districts, this study established that there was significantly higher fumonisin B1 in samples from Nyamira district at 38.75 µg/g while Busia had the lowest levels at 9.38 µg/g fumonisin B1 detected (Figure 13).

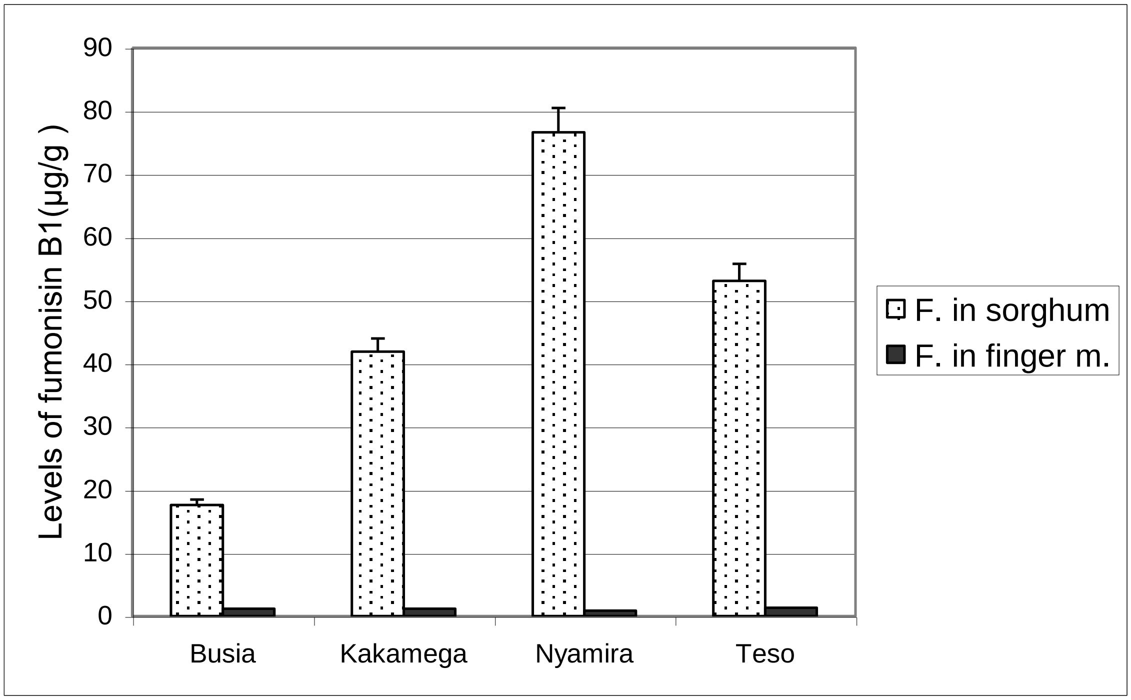

Figure 13 Fumonisin B1 levels in Sorghum and finger millet grains in the four districts

4.8.1 Fumonisin B1 in various sorghum varieties

The study established no significant difference in the levels of fumonisin B1 in sorghum varieties from the respective districts sampled. However, fumonisin B1 was higher in sorghum from Nyamira district with mean of 76.63 µg/g than in sorghum from Kakamega (41.86 µg/g) Teso (53.11 µg/g) and Busia (17.59 µg/g). There was significant difference in the individual sorghum varieties ($P < 0.05$); Ochuti with mean of 129.92 µg/g and Wagiita 128.68 µg/g had the highest levels while Ikhumba with a mean of 1.75 µg/g had the lowest at the detection levels. Fumonisin B1 was not detected in KARI Mtama-1 and Esila varieties (Table 15).

4.8.2 Fumonisin B1 in finger millet

For finger millet samples, there was no significant difference in the incidence of fumonisin B1 in the various districts ($P < 0.05$). Fumonisin B1 from Teso had a mean of 1.313 μg/g, Nyamira and Kakamega each had 1.167 μg/g. Finger millet from Busia had fumonisin B1 of 0.875 μg/g (Table 16).

The different finger millet varieties were detected to have different levels of fumonisin B1. The levels were higher in Aran, Enyaikuro, Enyankundi, Gulu E, KNE 688 and Namadimwa variety with a minimal FB1 mean of 1.75 μg/g. Fumonisin B1 was not detected in Mokomoni, Nafusi and U15 varieties (Table 16).

4.9 Aflatoxin B1 content in sorghum from Busia and Teso districts

AFB1 analysis carried out on sorghum from Busia (AF 28, Seredo and Esila) and Teso (IS8193, Nakhadabo, KARI Mtama-1 and Wagiita) districts had very low amounts of aflatoxin B1 that were not detectable that is below 2 ppb which was the lowest detectable level.

Since no *Aspergillus* species was isolated from the finger millet grains no further aflatoxin analysis was carried out on grains from the sampled districts.

Table 15 Levels of fumonisin B1 in the sorghum varieties from Nyamira, Kakamega, Teso and Busia districts

Sorghum Variety	**Fumonisin B1 in µg/g**	**District**
AF28	25.89b	Kakamega and Busia
Esila	Not detected	Busia
Essuti	31.41b	Kakamega
Gopari	81.15ab	Nyamira
Ikhumba	1.75b	Kakamega
IS8193	66.48ab	Kakamega and Teso
Livoywa	26.44b	Kakamega
Migogo	38.11b	Nyamira
KARI Mtama-1	Not detected	Teso
Nakhadabo	2.83b	Teso
Ochuti	129.92a	Nyamira
Seredo	25.54b	Busia
Wagiita	128.68a	Nyamira and Teso

*Numbers in the table denoted by the same letter are not significantly different at $p<0.05$

Table 16 Levels of fumonisin B1in the finger millet varieties from Nyamira, Kakamega, Teso and Busia districts

Finger millet variety	**Fumonisin B1 in µg/g**	**District**
Aran	1.75	Teso
Enyaikuro	1.75	Nyamira
Enyankundi	1.75	Nyamira
Gulu E	1.75	Busia
Ikhulule	1.75	Teso
KNE 688	1.75	Kakamega
Mokomoni	Not detected	Nyamira
Nafusi	Not detected	Kakamega
Namadimwa	1.75	Busia
P224	1.167	Teso, Kakamega
U15	Not detected	Busia

CHAPTER FIVE

DISCUSSIONS

Both *Fusarium compactum* and *F. equiseti* were isolated in all the districts from sorghum and finger millet grains. However sorghum had higher incidences of the two species than finger millet. Sorghum from Teso had the highest incidence of *F. compactum* and *F. equiseti* where as Nyamira and Kakamega had the lowest. *Fusarium compactum* has also been reported on sorghum grains in Nigeria, Lesotho and Botswana at 1.8% (Onyike *et al.,* 1992, 1993). The high isolation of the species in Teso district could be due to the high temperatures in the area of about 21˚C to 22.7˚C as *F. compactum* is generally found in hot arid to semi-arid climates. *F. compactum* may produce enniatins and trace amounts of trichothecenes (Desjardins, 2006) which do not affect man and domesticated animals. *Fusarium equiseti* has been reported on sorghum grains in Argentina (Gonzalez, 1997) and finger millet stems in Kisii, Kenya (Amata *et al.,* 2006). Despite being reported on finger millet stems in Kisii (Amata *et al.,* 2006) this study found low incidences in the grains. This could be possibly attributed to the hard seed coat that may not be easily penetrated by the fungus. *Fusarium equiseti* produce mycotoxins like equisetin, moniliformin and zearalenone (Hertzberg *et al.,* 2002; Langseth, 2001; Thrane, 2000)

Fusarium verticillioides and *F. thapsinum* were isolated in sorghum seeds from all four districts. *Fusarium verticillioides* had the highest incidence in Nyamira sorghum. It has also been isolated on sorghum grains in Brazil (Josefa *et al.,* 2004), USA (Erpelding and Prom, 2006), Botswana at 65.2% (Onyike *et al.,* 1992) and Guatemala (Torres *et al.,* 2007). The isolation of *F. verticillioides* from sorghum in all the districts poses a great danger for fumonisin related problems to man and livestock as the fungus primarily produces fumonisin B1 that has been associated with human and animal toxicoses (Marasas, 2001). It has also been associated with outbreaks of leukoencephalomalacia in horses and pulmonary oedema in swine world wide (Haschek *et al.*, 2001; Marasas, 2001; Wilson *et al.*, 1990).

The highest incidence of *F. thapsinum* was in Nyamira district. It has also been isolated from sorghum grains in USA (Isakeit *et al.*, 2008; Erpelding and Prom, 2006) and Australia (Huang and Backhouse, 2004). The fungus produces trace amounts of fumonisins (Leslie and Summerell, 2006) thus it might cause mycotoxicoses if consumed in large amounts. *Fusarium thapsinum* is a pathogen of sorghum and causes stalk rot and grain mould (Frederiksen and Odvody, 2000). The occurrence of *F. verticillioides* and *F. thapsinum* in sorghum could cause health risk due to fumonisin production.

Fusarium heterosporum, F. longipes and *F. andiyazi* were isolated from sorghum grains in three districts. *Fusarium heterosporum* was isolated in Nyamira, Busia and Teso districts but the incidence was highest in Nyamira district lower incidences of *F. heterosporum* were found in finger millet grains from Busia. The isolation of *F. heterosporum* is in tandem with that in sorghum in Argentina (Gonzalez, 1997). The isolation of *F. heterosporum* is not a surprise in both sorghum and millet grains as it has been shown to be associated with head blight of millets and other grasses especially in Africa (Leslie and Summerell, 2006). The infection of *F. heterosporum* on the inflorescence of sorghum and millet crops makes it possible to penetrate the grains. The fungus possibly does not pose danger to man and livestock as it produces small amounts of fusaric acid (Desjardins, 2006). *Fusarium longipes* was recovered in Nyamira, Kakamega and Teso districts with the highest incidence in Teso district. It has also been recovered in sorghum seed in Pakistan (Nisa, 1998). This is a tropical species that causes crown rots in cereals and therefore its isolation was expected. The species may produce beauvericin which is not a big risk to animals consuming contaminated feeds (Leslie and Summerell, 2006).

The highest incidence of *F. andiyazi* was from sorghum in Nyamira but was also isolated in Busia and Kakamega districts. It has also been isolated in sorghum grains from Nigeria (Marley *et al.*, 2004) for the first time and from South Africa (Leslie *et al.,* 2005), in Australia, Ethiopia, and The United States (Marasas *et al.,* 2001). This is the first time that *F. andiyazi* is recovered in crops in Kenya as previous researches have not been able to isolate the species from either the grains, stem, roots or flowers. The absence of the fungus in finger millet grains is in agreement with Amata *et al.,* (2006) where no *F. andiyazi* was isolated from the finger millet stem. The species produces trace mounts of fumonisins (Rheeder *et al.,* 2002).

Fusarium graminearum and *F. brevicatenulatum* were isolated from sorghum grains in two districts each. *Fusarium graminearum* was isolated from Nyamira and Kakamega districts with the highest isolation from Nyamira district. It has also been reported on sorghum and finger millet grains in Australia (Loughman *et al.,* 2004) and in India (Bandyopadhyay *et al.,* 2000). In Nigeria, *F. graminearum* was implicated in head blight and head mould of millet (Onesirosan, 1975). The isolation of the fungus although in low incidences is a caution to farmers because it might cause mycotoxicoses. It has been associated with human and animal toxicoses by producing mycotoxins like zearalenone, nivalenol and deoxynivalenol (DON) (Marasas *et al.,* 1984).

Fusarium brevicatenulatum was isolated from Nyamira and Teso districts with the highest incidence in Teso district. It has also been isolated from *Striga asiatica* (witch weed) and is therefore associated with maize, sorghum and millets in Africa (Leslie and Summerell, 2006). It was also isolated in finger millet stems from Kisii, Kenya (Amata *et al.,* 2006). *Striga asiatica* is a parasitic angiosperm of economically important crops including sorghum, maize, millet and rice. Therefore there is possibility of the weed being a source of inoculum. Management of the weed could play a role in reduction of the inoculum. Despite high recovery of this fungus in Kisii from the finger millet stems (Amata *et al.,* 2006), completely none was isolated from the finger millet grains. It is possible that the grains have a hard seed coat that is resistant to the pathogen to penetrate.

Fusarium chlamydosporum, F. semitectum and *F. subglutinans* were isolated from Teso district only. *Fusarium chlamydosporum* had 4.18% isolation and has also been isolated in sorghum from USA (Isakeit *et al.,* 2008), Lesotho (Onyike *et al.,* 1993) and Nigeria at 4.5% (Onyike and Nelson, 1992). The fungus is common in warmer areas of the world and its isolation in Teso district was expected as the area receives about 900-1500 mm rainfall and temperatures range between 21˚C to 22.7˚C which makes it relatively hot. Despite its isolation in Teso district, it was not isolated in sorghum from Busia district which has similar climatic conditions but this could be due to the differences in the varieties as the varieties sampled from the two districts were different. The fungus has been implicated in human mycoses especially immunocompromised patients (Segal *et al.*, 1998) due to production of moniliformin (Nicholson *et al.,* 2004).

Fusarium semitectum had 3.15% and has also been reported on sorghum grains from Nigeria (Onyike and Nelson, 1992; Marley and Malgwi, 1999) and USA (Isakeit *et al.*, 2008; Erpelding and Prom, 2006). It has also been associated with maize grains and peanuts in South Africa (Rensburg, 1986). The fungus is commonly found in warmer regions associated with head mould of sorghum and finger millet, therefore its recovery in both grains was expected but its absence in finger millet could be due to varieties sampled. *Fusarium subglutinans* had 3.00% isolation and has also been reported on sorghum grains in Argentina (Acuña *et al.,* 2005), Nigeria (Marley and Malgwi, 1999) and finger millet stems in Kisii, Kenya (Amata *et al.,* 2006). In Kisii it was isolated in finger millet stems but in this research it was not recovered in grains may be due to hard seed coat that may prevent fungal penetration. The fungus produces some fumonisins and large amounts of moniliformin (Morreti, 1996; Srdic *et al.,* 1993) thus it is dangerous to both man and livestock.

Fusarium proliferatum, F. oxysporum, F. nygamai and *F. pseudonygamai* were isolated from sorghum sampled from Busia district only. *Fusarium proliferatum* has also been reported on sorghum grains in USA (Isakeit *et al.,* 2008), Nigeria (Onyike and Nelson, 1992) and Australia (Huang and Backhouse, 2004). The isolation of this fungus from sorghum could be of concern as it produces high levels of fumonisins among other metabolites. It has been isolated from feed samples associated with leukoencephalomalacia in horses and pulmonary oedema in swine (Ross *et al.,* 1990).

Fusarium oxysporum was isolated in finger millet grains from Kakamega and Teso districts. The fungus has also been reported on sorghum grains in Nigeria at 4.5% incidence (Onyike and Nelson, 1992) and on finger millet stems in Kisii, (Amata *et al.,* 2006). The isolation of this fungus is surprising as the pathogen is believed to be a soil saprophyte and affects the vascular system by causing wilts. The fungus might reach the chalazae serving the inflorescence with water and mineral salts thus infecting the grains. Strains of *F. oxysporum* species complex might produce enniatins and fusaric acid, (Thrane, 2001; Marasas *et al.,* 1984) but most of them are non-toxigenic therefore do not pose any danger to man and livestock.

Fusarium nygamai had an incidence of 2.86% isolation and has also been reported on sorghum in South Africa (Leslie *et al.,* 2004), Nigeria, Lesotho and Zimbabwe at 7.5% (Klaseen and Nelson, 1998). The fungus is found in hot/arid regions and therefore the isolation of the species from sorghum in Busia district is concurrent with the climatic conditions of the area as being a dry area with temperatures of between 21˚C and 22.7˚C. Despite the isolation of the species in North West Australia in wild millet species stems (Amata *et al*., 2006) none was isolated from the finger millet grains. Some strains of *F. nygamai* produce high levels of fumonisins (Leslie *et al.,* 2005) and can cause systemic infections in humans (Krulder *et al.,* 1996) thus the species is dangerous to both man and livestock.

Fusarium pseudonygamai had 3.10% isolation and has been isolated from sorghum grains in USA (Glenn, 2007), South Africa (Leslie *et al.,* 2004) and on pearl millet in USA (Jurjevic *et al.,* 2005). It's surprising that the species was isolated from sorghum and not from finger millet grains although it has been recovered mostly in pearl millet. This could be due to the finger millet varieties sampled as most of them are traditional varieties that may resist penetration of the fungus. The fungus is reported to produce little fumonisins among other metabolites (Fotso *et al.,* 2002).

Fusarium solani and *F. napiforme* were isolated from Nyamira sorghum grains. *Fusarium solani* was also isolated from finger millet grains from Kakamega. The fungus has also been reported on sorghum grains in Nigeria at the incidence of 18% (Onyike and Nelson, 1992; Marley *et al.,* 2004), Brazil (Fronza *et al.,* 2004) and on finger millet stems in Kisii, Kenya (Amata *et al.,* 2006). The fungus is cosmopolitan on a range of substrates. It is found in numerous native soils in the tropics. In humans it causes keratitis which makes patients to be susceptible to HIV infection and also shown to cause allergic reactions (Mselle, 1999; O'Neil *et al.,* 1986). *Fusarium napiforme* had incidences at 5.05% and its isolation is in agreement with that of Marasas *et al.,* (1987) in South Africa, Onyike and Nelson, (1992) in Nigeria and Zimbabwe, Gonzalez, (1997) in Argentina and Melcher *et al.,* (1993) in USA. The fungus has also been isolated from soil, and poultry feed in Africa, Argentina and Australia (Leslie and Summerell, 2006). It has been associated with human cases of hypersensitivity pneumonitis (Lee *et al.*, 2000). Strains of *F. napiforme* can produce moniliformin (Marasas *et al.*, 1991) and fumonisins (Nelson *et al.*, 1992), this implies that if the species is in high amounts then it may cause mycotoxicoses in livestock and man.

Fusarium pseudograminearum was isolated in Kakamega district only. The fungus has also been reported on sorghum grains in Australia (Quazi *et al.*, 2006) and on finger millet and sorghum grains in South Africa (Glenn, 2007). The species is reported to be prevalent in drier areas and therefore its recovery in Kakamega district is surprising as the area is relatively wet with 1000-2200 mm rainfall per year and temperatures of about 18-21˚C.

Apart from *Fusarium* species, other fungi that were isolated in sorghum were *Alternaria alternata, Phoma sorghina, Aspergillus flavus, Penicillium citrinum* and *Colletotrichum graminicola. Alternaria alternata* has also been isolated in sorghum grains in Argentina (Broggi *et al.*, 2007) and in Pakistan (Fakhrunnisa and Ghaffar, 2006). The fungus may produce mycotoxins such as alternariol and fumonisins (Lawley, 2005). *Alternaria* toxins have been implicated in animal and in human health disorders. During investigation into outbreaks of suspected mycotoxicoses, it was shown that cereal samples collected from affected farms in Germany were more frequently contaminated with *Alternaria* mycotoxins than samples from farms with healthy animals. Cases of death in rabbits and poultry have been reported as a result of toxic action of *Alternaria* species found in the fodder and feed. *Alternaria* species were also detected in cereal samples in which

Fusarium species were implicated as the likely cause for the outbreak of alimentary toxic aleukia in Russia (Lawley, 2005).

Phoma sorghina was isolated in sorghum grains and the same has been reported in Samaru, Nigeria where insect damage increased the incidences of *P. sorghina* associated with sorghum grains (Marley and Malgwi, 1999). It was also reported in Argentina (Perello and Moreno, 2005) and Nigeria (Amusa and Falola, 2001). The fungus produces phytotoxins which are non-specific toxins (Venkatasubbaiah, *et al.,* 1992). In humans it causes onyalai disease that causes haemorrhaging lesions in the mouth (Pitt, 1997).

Aspergillus flavus was the most isolated in sorghum grains and has also been reported in Brazil (Josefa *et al.,* 2005), Argentina, where it was the second highest isolated (Broggi, *et al.,* 2007) and Nigeria (Amusa and Falola, 2001). The fungus produces aflatoxins B1 and B2 (Pitt, 1997) which are carcinogenic and hepatotoxic. *Curvularia lunata* has been isolated in sorghum grains in many regions including, USA (Prom *et al.,* 2003), Pakistan where it was the highest recovered fungi (Fakhrunnisa and Ghaffer, 2006; Navi *et al.,* 2005) and in Nigeria (Amusa and Falola, 2001). The fungus produces toxins such as cytochasin-B, (McCloud, 1995), radicinin (Strijewski *et al.,* 1982) and curvularin which causes hepatic necrosis (Rout *et al.,* 1989).

Penicillium citrinum has been reported on sorghum grains in Pakistan (Navi, 2006) and Argentina (Saubois *et al.,* 1999). The fungus produces patulin and citrinin toxins (Harwig *et al.,* 1979). *Colletotrichum graminicola* was also isolated in sorghum grains in Nigeria

(Marley and Malgwi, 2002) and USA (Ali and Warren, 1987). It produces phenols (Cantone and Dunkle, 1990). *Rhizopus stolonifer* was the most common fungus isolated from sorghum grains in Botswana (Nkwe *et al.,* 2005) and also isolated in Pakistan (Navi, 2006). The fungus is generally saprophytic but is also considered mildly pathogenic. The hyphae secrete pectinolytic enzymes and cellulase that break down substances holding the host plant cells in place, causing a loss of cell cohesion (Agrios, 1997). The fungus produces ergoline alkaloids (Krikštaponis *et al.,* 2001).

Pyricularia grisea has been isolated from finger millet grains in Kisii, Kenya (Makini, 1999), India (Ghodke *et al.,* 2000) and Uganda (Pande *et a.,* 1994). The fungus causes blast disease in millets and produces a toxin called pyricularin (Krikštaponis *et al.,* 2001). *Bipolaris nodulosa* has previously been isolated in finger millet grains from India (Ghodke *et al.,* 2000) and Uganda where it was observed that the fungus is seed-borne and causes blight disease in millets (Pande *et a.,* 1994). It produces a fungal metabolite referred to as curvularin (Bettina 1993).

The incidences of *Fusarium* species recovered in sorghum and finger millet grains showed that sorghum had a higher percentage at 29.66% as compared to finger millet with incidence at 4.87%. This could be because finger millet contains chemicals such as polyphenols that inhibit the penetration of *Fusarium* species as compared to sorghum (Harris and Burns, 1973). Finger millet grains also have a relatively hard seed coat that can prevent the growth of fungi (Chandrashekar and Satyanarayana**,** 2006). Finger millet grains could also be having mycoflora around them that prevents *Fusarium* growth and subsequent fumonisin B1 production. Velluti *et al.* (2000), showed that populations of *F. verticillioides* and *F. proliferatum* the most important fumonisin producers are markedly reduced by the presence of other fungi like *F. graminearum* and that fumonisin B1 production by them can significantly be inhibited as well.

There was significant difference in the incidence of *Fusarium* species from the four districts for both cereals. The combined incidences for both sorghum and finger millet were highest in Nyamira district while Kakamega had the lowest. The high incidences in Nyamira district could be due to high moisture content in the region because of high rainfall (1200-2100 mm) that facilitates more growth of fungi than other districts which receive less rainfall like Busia and Teso (900-1500 mm). The high incidences could also be due to large number of farmers growing finger millet and sorghum in Nyamira district than others thereby increasing the inoculum. The varieties grown in Nyamira could also be susceptible to fungal attack as compared to those in other districts

Wagiita variety grown in Nyamira had the highest incidences of *Fusarium* species in all the four districts followed by Seredo (Busia) and Ochuti (Nyamira) while Livoywa (Kakamega) and Esila (Busia) had the lowest incidences. The lowest incidences in Livoywa and Esila could be due to the presence of mycoflora that may inhibit the growth of *Fusarium* species. From Nyamira district, Wagiita variety had the highest incidences at 60.93% while Gopari had lowest incidence at 29.18%. Wagiita was the most preferred variety in the district by farmers for food consumption and with the high incidence of *Fusarium* species it could pose a problem in causing mycotoxicoses. Species wise in

Nyamira district, *F. andiyazi* had the highest incidence while *F. solani* had the lowest. The presence of *F. verticillioides* and *F. andiyazi* indicates the production of fumonisins as these species are known to be high fumonisin producers (Marasas, 2001) and therefore farmers are to be advised appropriately.

From Busia district, there was significant difference in the incidences of *Fusarium*. Seredo had the highest incidence of *Fusarium* while Esila was lowest with 0%. This implies that it is safer for farmers to use Esila as food since it has no *Fusarium* species thus reducing the danger of mycotoxin contamination. The *Fusarium* species recovered from Busia had *F. compactum* with the highest incidence followed by *F. graminearum* and lowest was *F. verticillioides*. The low incidences of *F. verticillioides* and *F. nygamai* which are fumonisin producers mean that the varieties might not pose a problem with fumonisin contamination but the presence of *F. proliferatum* (1.91%) could increase the risk of fumonisin related problems as it produces large amounts of fumonisins.

Teso district had Wagiita variety with the highest incidence of *Fusarium* while Nakhadabo had the lowest. The incidences in the samples were relatively high which might pose a danger of fumonisin related problems. Basing on the species recovered, *F. compactum* had the highest incidence while *F. verticillioides* and *F. andiyazi* had the lowest incidences. The low incidences of *F. andiyazi* and *F. verticillioides* may imply that there are low fumonisin productions in some varieties as they have more *F. compactum* (44.25%) and *F. equiseti* (30.12%) which are non-fumonisin producers.

From Kakamega district, IS8193 had the highest incidences while Livoywa had the lowest incidence. The relatively low levels of incidences may imply that there is low fumonisin contamination but farmers are advised to use Livoywa (2.77%) and Ikhumba (5.23%) that had the lowest incidences. The incidences of *Fusarium* species significantly differed with *F. compactum* having the highest incidence while *F. graminearum* had the lowest incidence. The low incidences of *F. andiyazi* (2.22%) and *F. verticillioides* (10.25%) may imply that there are low levels of fumonisins in the varieties when more saprophytes of *F. compactum* are present. The presence of *F. graminearum* will automatically reduce fumonisin production as its presence inhibits the ability of *F. verticillioides* and *F. andiyazi* to produce fumonisins.

In finger millet there was no significant difference in *Fusarium* incidences between districts and varieties which implies that the growth of *Fusarium* species on the grains was not affected by geographical or varietal differences. The grains had very low incidence of below 10%. The low incidences suggest that the sampled varieties were most favourable for human and animal consumption with little danger of fumonisin contamination as compared to sorghum varieties. The species recovered in high amounts are known to be non-fumonisin producers especially *F. compactum* and *F. equiseti*.

The incidences of *Fusarium* on roots and stems were not significantly different but were higher in roots than stem. From the roots, Wagiita variety had an incidence at 48.86% while IS8193 had an incidence at 18.26%. The incidence of *Fusarium* species in Wagiita from roots and stems was positively correlated with the high incidence in the Wagiita grains. There was however a negative correlation in Esila with root incidence of 58.95% and respective grain incidence of 0% (r = -0.413). This is because the inoculum could be entering through the roots and the tillers then isolated in the roots and stem but the grains could be having a harder seed coat that prevents the penetration of *Fusarium* species. From the stem, Wagiita had the highest incidence of 48.53% and IS8193 had the lowest of 12.73%. There was a positive correlation for incidences for most of the grains and stems except for grains of Esila variety (r = 0.347).

The fumonisin B1 content varied considerably between sorghum grains and finger millet with sorghum having higher concentration. This could be due to the differences in *Fusarium* species present in the two grain types. Sorghum grains had high recovery of *Fusarium* species which have been linked with fumonisin production like *Fusarium verticillioides, F. proliferatum, F. nygamai, F. napiforme* and *F. thapsinum* (Marasas, 2001). Trace amounts of fumonisins have been detected in culture materials of *F. andiyazi* and *F. pseudonygamai*. Amongst these species, *F. verticillioides* and *F. proliferatum* are by far the most prolific fumonisin producers (Marasas, 2001). In finger millet none of the fore mentioned fumonisin producing *Fusarium* species isolates was isolated and this could be the reason for the very low fumonisin B1 levels. According to previous research by Bacon and Nelson, (1994) and Sala *et al.,* (1994), they found out that strains of *F. verticillioides* isolated from agricultural products like wheat, barley and sorghum were shown to produce fumonisin B1 in laboratory cultures.

Another reason for the very low fumonisin B1 levels in finger millet could be the competing mycoflora that could be different from sorghum. The competing mycoflora may inhibit the synthesis of fumonisins by *Fusarium* species or they may degrade the mycotoxin as soon as it is produced (Velluti *et al.*, 2000). The results demonstrate that fumonisin-producing isolates of *Fusarium* were well adapted to grow on sorghum than finger millet. The finger millet could be having nutritional components in them that could act as inhibitors of fumonisin biosynthesis (Harris and Burns, 1973). Lastly, the fumonisin B1 producers may prefer sorghum as their major host as opposed to finger millet since sorghum could be having chemicals in their tissues that facilitate faster attack and penetration as compared to finger millet for the corresponding *Fusarium* species.

The fumonisin B1 levels detected in sorghum were higher than the recommended, in the US, Food and Drug Administration (FDA) recommended that the fumonisin levels should not be higher than 4 μg/g in human foods (FDA, 2000). In Switzerland tolerance levels for fumonisin B1 of 1 μg/g in food products intended for human consumption have been proposed (Marasas, 2001). In Kenya there are no recommended tolerance levels to fumonisins in sorghum which can be detrimental to both man and livestock. However Marasas (1995) recommended that fumonisin B1 levels of 10 μg/g to 100 μg/g of a sample are considered dangerous for pigs and horses respectively.

Sorghum samples from Nyamira district had the highest fumonisin levels with mean of 76.63 μg/g followed by Kakamega 41.86 μg/g, Teso 53.11 μg/g and Busia 17.59 μg/g. The differences could be due to the different varieties sampled from the regions.

Geographical differences may also influence *Fusarium* species associated with sorghum. According to Nelson *et al.*, (1983), the areas with low moisture content have low fumonisin production. Nyamira receives rainfall of about 1200-2200 mm annually thus high moisture content compared to Busia which receives about 900-1500 mm annually. The high moisture content in Nyamira may support *Fusarium* growth and subsequently more production as compared to Busia. The levels of fumonisins detected in sorghum were higher than those in Brazil (Josefa *et al.,* 2004) where *F. verticillioides* in sorghum produced between 0.12 µg/g and 5.38 µg/g. These low levels were because only one *Fusarium* isolate was used rather than all the isolates in the grain that could yield more fumonisins.

The sorghum varieties Ochuti and Wagiita had the highest levels of fumonisins of 129.92 µg/g and 128.68 µg/g respectively. KARI Mtama-1 and Esila had no fumonisin levels detected meaning that levels were below 1.75 µg/g while Ikhumba had 1.75 µg/g. The differences in fumonisin levels could be attributed to the colour/tannin amounts present in the sorghum varieties. Ochuti and Wagiita are brown in colour thus have tannins thus accumulation fumonisins could be due to soft seed coat that allows the penetration of the *Fusarium* species. KARI Mtama-1 and Ikhumba are white and could be having hard seed coats that prevents penetration and accumulation of fumonisins. Esila is light brown and had no fumonisin detected and this could be because it contains chemicals like polyphenols that prevents growth of *Fusarium* species and production of fumonisins.

From Nyamira district, Ochuti and Wagiita had the highest levels of fumonisin B1 of 129.92 µg/g and 128.68 µg/g respectively while Migogo had the lowest levels of 38.11 µg/g. Wagiita is preferred by most farmers in the district and this can cause mycotoxicoses, therefore farmers are supposed to be educated on the dangers of fumonisins and advised to adopt Migogo that has lower fumonisin B1 levels. Samples from Kakamega had IS8193 with the highest fumonisin B1 levels of 66.48 µg/g while Ikhumba had the lowest levels of 1.75 µg/g. Farmers are advised to adopt Ikhumba which is a white variety that had the lowest minimum fumonisin B1 detection levels.

From Busia district, AF28 with 25.89 µg/g had the highest levels of fumonisin B1 while Esila had no fumonisin B1 detected. Farmers are advised to adopt Esila as it had 0% incidences of *Fusarium* species and no fumonisin B1 detected therefore safe for both man and livestock consumption. Samples from Teso had Wagiita with the highest fumonisin B1 with over 200 µg/g while KARI Mtama-1 had no fumonisin B1 detected. KARI Mtam-1 had incidences of 41.53% on the grains but no fumonisins were detected. This means that the *Fusarium* species growing on the grains were saprophytes and not fumonisin producing species therefore farmers are advised to grow and consume KARI Mtama-1.

The fumonisin B1 levels in finger millet varieties were not significantly different and were within the detection limits of 1.75 µg/g, while others like Mokomoni, Nafusi and U15 had their fumonisin levels below the detection limits. The very low levels of fumonisin B1 in finger millet may be attributed to the kinds of *Fusarium* species isolated from the grains. Isolates from the finger millet grains were predominantly *F. compactum* and *F. equiseti* which may produce enniatins and equisetin mycotoxins respectively but not fumonisin B1. Other isolated species include *F. oxysporum, F. graminearum*, *F. heterosporum* and *F. solani* which are non-fumonisin producers. However *F. graminearum* produces zearalenone and DON which are dangerous to man and domesticated animals. The colour of the finger millet grains and their geographical region could not significantly influence the fumonisin B1 levels in the grains as opposed to the sorghum grains.

The aflatoxin B1 analysis revealed that sorghum varieties from Busia (AF28, Seredo and Esila) and Teso districts (KARI Mtama-1, Nakhadabo, IS8193 and Wagiita) had very low levels of below 2 ppb which is the detection limit. This implies that these varieties do not pose aflatoxin B1 related problems. AFB1 and FB1 cause cancer in man but the rate at which cancer is produced by AFB1 is higher that in FB1 (Josefa *et al.*, 2004). In a mycological study of sorghum grain storage silos in Botswana, up to 25% of the total fungal counts were *Aspergillus flavus* and *A. parasiticus* (Mpuchane *et al.*, 1997). In this study, the aflatoxin B1 concentrations ranged 5-25 μg/kg. In another study in Botswana by Siame *et al.*, (1998), *A. flavus* was recovered in sorghum grains but there was absence of aflatoxin B1 and he suggested that there are seasonal or annual variations in aflatoxin B1 contamination in sorghum storage. Therefore, there is need for continued surveillance of grains in the storage silos to determine the levels of aflatoxins (Siame *et al.*, 1998). The absence of aflatoxin B1 and presence of *A. flavus* could be because of the presence of some *Fusarium* species that prevents aflatoxin B1 production. Marina et *al.*, (1998) found that *F. verticillioides* and *F. proliferatum* are generally competitive and dominant against *Aspergillus flavus* and *Penicillium* species. This can lead to significantly reduced aflatoxin contamination in infected grains (Zummo and Scott, 1992).

No aflatoxin analysis was done for finger millet grains since none of the *Aspergillus* species was isolated from the grains

CHATER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The study established that there was significant difference in the incidences of *Fusarium* species at $p<0.05$ between sorghum grains (29.66%) and finger millet grains (4.87%). This is because sorghum grains have nutritional requirements that necessitate the growth of *Fusarium* species than finger millet grains. This implies that consumption of sorghum grains could cause mycotoxin related problems as compared to finger millet grains
- Sorghum grains had fumonisin producing *Fusarium* species isolated while finger millet grains had non-fumonisin producing *Fusarium* species. This implies that there is a high risk of consuming sorghum grains and their products as they pose a danger to fumonisin related problems
- There was significant difference in the incidences of *Fusarium* species isolated between the districts from sorghum at $p<0.05$. Nyamira was the highest at 65.47% and Kakamega district lowest at 11.12%. this could be due to the highest number of farmers growing sorghum in the area that increases the inoculum
- Wagiita variety had the highest incidences of *Fusarium* species isolated from both Nyamira and Teso districts. The variety could be having nutritional components that provides good environment for the growth of the fungi. Esila variety had no *Fusarium* species isolated therefore this variety should be bred in large amounts and be distributed to farmers as it does not pose any fumonisin problems.

- There was significant difference in the fumonisin B1 levels at $p<0.05$ between the sorghum grains at 48.81 μg/g and finger millet grains at 1.13 μg/g. This was due to the fumonisin producing *Fusarium* species isolated in sorghum grains while only non-fumonisin producing *Fusarium* species were isolated from finger millet grains. This implies that the consumption of sorghum grains especially Wagiita and Ochuti that had very high levels of FB1 is dangerous to both man and domesticated animals. The levels obtained were higher than the accepted limits of 4 μg/g. The significantly high levels could cause cancer and other health related problems.
- The study established no significant difference in the fumonisin levels between the sampled districts. Nyamira had 76.63 μg/g while Busia had 17.59 μg/g. The higher levels in Nyamira could be attributed to the varieties sampled and the high amounts of rainfall experienced in the area
- There was no fumonisin B1 detected in KARI Mtama-1and Esila while very low levels in Ikhumba (1.75 μg/g) which was the detectable limit. This implies that these varieties are safe for consumption without any fear of fumonisin contamination to man and domesticated animals
- Finger millet varieties had very low levels of fumonisins which was below the detectable limits (1.75 μg/g) while some had no fumonisins detected. The grains should be encouraged for consumption as they pose no fumonisin related problems especially for weaning children
- The aflatoxin B1 levels detected in the sampled varieties were below the limits of 2 ppb despite the isolation of *A. flavus* from the grains. This is due to the season of

sampling and also the presence of *F. verticillioides* that inhibits aflatoxin B1 production. This implies that sorghum varieties sampled are safe from aflatoxin related problems

6.2 Recommendations

- Breeders should come up with sorghum varieties that are resistant to *Fusarium* growth and FB1 biosynthesis
- Farmers should be sensitized on the dangers of FB1 in sorghum so as to adopt varieties with low fumonisin levels
- Farmers should be advised to grow KARI Mtama-1, Esila and Ikhumba varieties of sorghum as they have minimal levels of FB1. there is also need to carry out more screening for these varieties for other mycotoxin accumulation and fungal infection
- There need to carry out more research on white varieties of sorghum as they had no fumonisins detected in this study (KARI Mtama-1 and Ikhumba)
- Fumonisin tolerance limits for sorghum and its products need to be introduced in Kenya both for human and livestock
- Continued surveillance of fumonisin B1 and aflatoxin B1 levels in Western Kenya in sorghum and finger millet as it may vary from year to year
- There is need to carry out research on other mycotoxins present in sorghum and finger millet like citrinin, zearalenone, moniliformin, patulin, ochratoxins, aflatoxins and trichothecene

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APPENDICES

Appendix 1.0: Media for isolation and identification of *Fusarium* species

Appendix 1.1: Peptone PCNB Agar medium/ Snyder and Nash (1962)

15g Peptone

1.0g KH_2PO_4

0.5g $MgPO_4.7H_2O$

1.0g PCNB

20g Agar

1000ml Water

300ppm Streptomycin sulphate after autoclaving

Appendix 1.2: Spezieller Nähstofarmer Agar

KH_2PO_4	1.0g
KNO_3	1.0g
$MgSO_4.7H_2O$	0.5g
KCL	0.5g
Glucose	0.2g
Sucrose	0.2g
Agar	20g

Appendix 2.0: Analysis of Variance

Appendix 2.1: One-way ANOVA: incidence versus district

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
District    3    179.08     59.69    10.27    0.000
Error     252   1464.25      5.81
Total     255   1643.33


Level       N      Mean     StDev
Busia      60     2.720     3.007
Kakamega  100     1.677     1.869
Nyamira    16     4.058     2.288
Teso       80     3.460     2.532

Pooled StDev =    2.411
```

Appendix 2.2: One-way ANOVA: incidence versus variety

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
Variety    12    322.13     26.84     5.26    0.000
Error     307   1566.33      5.10
Total     319   1888.46


Level       N      Mean     StDev
AF28       40     2.825     2.777
Esila      20     0.710     0.000
Essuti     20     1.799     1.651
Gopari     20     2.701     1.892
Ikhumba    20     1.143     1.086
IS8193     40     3.183     2.744
KARI Mta   20     3.222     2.127
Livoywa    20     0.833     0.548
Migogo     20     3.065     2.248
Nakhadab   20     3.152     2.240
Ochuti     20     3.511     2.373
Seredo     20     3.737     3.196
Wagiita    40     3.839     2.511

Pooled StDev =    2.259
```

Appendix 2.3: one-way ANOVA: Fusarium in sorghum versus *Fusarium* Species

```
Analysis of Variance for C6
Source     DF        SS        MS        F        P
Fus. Sp.    3     31.18     10.39     2.70    0.052
Error      76    292.48      3.85
Total      79    323.66


Level        N      Mean     StDev
F. compa    20     2.632     1.862
F. grami    20     2.754     1.950
F. Oxysp    20     3.672     1.810
F.nygama    20     4.122     2.203

Pooled StDev =    1.962
```

Appendix 2.4: One-way ANOVA: incidence versus Sorghum variety in Nyamira district

```
Analysis of Variance for C6
Source     DF        SS        MS        F        P
s.var.      3     16.44      5.48     1.36    0.263
Error      76    307.22      4.04
Total      79    323.66


Level        N      Mean     StDev
Gopari      20     2.701     1.892
Migogo      20     3.065     2.248
Ochuti      20     3.511     2.373
Wagiita     20     3.903     1.381

Pooled StDev =    2.011
```

Appendix 2.5: One-way ANOVA: Percentage incidence versus sorghum varieties in Busia district

```
Analysis of Variance for C10
Source     DF        SS        MS        F        P
S. var.     2    121.25     60.62     8.38    0.001
Error      57    412.16      7.23
Total      59    533.41


Level        N      Mean     StDev
AF28        20     3.714     3.388
Esila       20     0.710     0.000
Seredo      20     3.737     3.196

Pooled StDev =    2.689
```

Appendix 2.6: One-way ANOVA: incidence versus Fusarium species in sorghum in Busia district

```
Analysis of Variance for C10
Source     DF        SS        MS        F        P
F. var      3    234.11     78.04    14.60    0.000
Error      56    299.30      5.34
Total      59    533.41


Level       N      Mean     StDev
F. compa   15     6.127     4.049
F. grami   15     1.777     1.390
F. Oxysp   15     1.287     1.229
F.nygama   15     1.690     1.242

Pooled StDev =    2.312
```

Appendix 2.7: One-way ANOVA: percentage incidence in Teso versus sorghum variety

```
Analysis of Variance for Teso
Source     DF        SS        MS        F        P
s. var      3      6.09      2.03     0.31    0.819
Error      76    500.36      6.58
Total      79    506.45


Level       N      Mean     StDev
IS8193     20     3.693     2.402
KARI Mta   20     3.222     2.127
Nakhadab   20     3.152     2.240
Wagiita    20     3.774     3.320

Pooled StDev =    2.566
```

Appendix 2.8: One-way ANOVA: incidence of *Fusarium* versus *Fusarium* species in Teso district

```
Analysis of Variance for Teso
Source     DF        SS        MS        F        P
Fus. sp.    3    279.85     93.28    31.29    0.000
Error      76    226.60      2.98
Total      79    506.45


Level       N      Mean     StDev
F. compa   20     6.652     1.542
F. grami   20     2.668     1.949
F. Oxysp   20     2.651     1.826
F.nygama   20     1.872     1.555

Pooled StDev =    1.727
```

Appendix 2.9: One-way ANOVA: Incidence of *Fusarium* species Kakamega versus sorghum variety

```
Analysis of Variance for inc. kak
Source     DF        SS        MS        F        P
s.variet    4     41.44     10.36     3.23    0.016
Error      95    304.42      3.20
Total      99    345.86


Level       N      Mean     StDev
AF28       20     1.937     1.637
Essuti     20     1.800     1.651
Ikhumba    20     1.143     1.086
IS8193     20     2.673     3.023
Livoywa    20     0.832     0.548

Pooled StDev =    1.790
```

Appendix 2.10: One-way ANOVA: Incidence of *Fusarium* Kakamega versus *Fusarium* species

```
Analysis of Variance for inc. Kakamega
Source     DF        SS        MS        F        P
f.sp.       3     58.55     19.52     6.52    0.000
Error      96    287.31      2.99
Total      99    345.86


Level       N      Mean     StDev
F. compa   25     2.910     2.853
F. grami   25     0.808     0.490
F. Oxysp   25     1.501     1.333
F.nygama   25     1.489     1.348

Pooled StDev =    1.730
```

Appendix 2.11: One-way ANOVA: percentage incidence versus District of finger millet

```
Analysis of Variance for percentage incidence
Source     DF        SS        MS        F        P
District    3      0.17      0.06     0.05    0.984
Error     276    294.50      1.07
Total     279    294.67


Level       N      Mean     StDev
Busia      60     1.200     0.988
Kakamega   60     1.140     1.222
Nyamira    80     1.141     1.003
Teso       80     1.139     0.937

Pooled StDev =    1.033
```

Appendix 2.12: One-way ANOVA: percentage incidence versus millet variety in all districts

```
Analysis of Variance for percentage incidence
Source     DF        SS        MS        F        P
millet v   10      8.07      0.81     0.76    0.670
Error     269    286.60      1.07
Total     279    294.67


Level       N      Mean     StDev
Aran       20     0.955     0.754
Enyaikur   20     1.388     1.234
Enyankun   20     0.710     0.000
Gulu E     20     1.078     0.898
Ikhulule   40     1.136     1.080
KNE 688    20     1.200     1.005
Mokomoni   20     1.388     1.234
Nafusi     20     1.273     1.558
Namadimw   20     1.200     1.005
P224       60     1.118     0.921
U15        20     1.323     1.088

Pooled StDev =    1.032
```

Appendix 2.13: One-way ANOVA: incidence versus millet variety in Nyamira district

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
mil. var    3     6.236     2.079     2.16    0.100
Error      76    73.214     0.963
Total      79    79.450


Level       N      Mean     StDev
Enyaikur   20    1.3880    1.2345
Enyankun   20    0.7100    0.0000
Mokomoni   20    1.3880    1.2345
P224       20    1.0775    0.8976

Pooled StDev =   0.9815
```

Appendix 2.14: One-way ANOVA: incidence versus *Fusarium* species in Nyamira district

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
F. speci    3      0.92      0.31     0.30    0.827
Error      76     78.53      1.03
Total      79     79.45


Level       N      Mean     StDev
F. grami   20     1.209     1.242
F. oxysp   20     1.200     1.005
F.compac   20     0.955     0.754
F.nygama   20     1.200     1.005

Pooled StDev =    1.016
```

Appendix 2.15: One-way ANOVA: incidence in Kakamega versus millet variety

```
Analysis of Variance for inc. in
Source     DF        SS        MS        F        P
mill. va    2      1.10      0.55     0.36    0.700
Error      57     87.00      1.53
Total      59     88.10


Level       N      Mean     StDev
Ikhulule   20     1.194     1.258
Nafusi     20     1.273     1.558
P224       20     0.955     0.754

Pooled StDev =    1.235
```

One-way ANOVA: incidence in Kakamega versus *Fusarium* species in finger millet

```
Analysis of Variance for inc. in
Source     DF        SS        MS        F        P
f.sp.       3      3.93      1.31     0.87    0.461
Error      56     84.17      1.50
Total      59     88.10


Level       N      Mean     StDev
F. grami   15     1.191     1.344
F. oxysp   15     1.297     1.717
F.compac   15     1.363     1.121
F.nygama   15     0.710     0.000

Pooled StDev =    1.226
```

Appendix 2.16: One-way ANOVA: incidence versus finger millet variety in Teso district

```
Analysis of Variance for inc. in
Source     DF        SS        MS        F        P
f.millet    3     1.501     0.500     0.56    0.643
Error      76    67.828     0.892
Total      79    69.329


Level       N      Mean     StDev
Aran       20    0.9550    0.7541
Ikhulule   20    1.0775    0.8976
KNE 688    20    1.2000    1.0055
P224       20    1.3225    1.0884

Pooled StDev =   0.9447
```

Appendix 2.17: One-way ANOVA: incidence versus *Fusarium* species in Teso district

```
Analysis of Variance for inc. in
Source     DF        SS        MS        F        P
f.specie    3     3.902     1.301     1.51    0.219
Error      76    65.427     0.861
Total      79    69.329


Level       N      Mean     StDev
F. grami   20    1.4450    1.1519
F. oxysp   20    1.0775    0.8976
F.compac   20    1.2000    1.0055
F.nygama   20    0.8325    0.5478

Pooled StDev =   0.9278
```

Appendix 2.18: One-way ANOVA: incidence versus finger millet variety in Busia district

```
Analysis of Variance for inc. in
Source     DF        SS        MS        F        P
f.mill.     2      0.60      0.30     0.30    0.742
Error      57     57.02      1.00
Total      59     57.62


Level       N      Mean     StDev
Gulu E     20     1.078     0.898
Namadimw   20     1.200     1.005
U15        20     1.323     1.088

Pooled StDev =    1.000
```

Appendix 2.19: One-way ANOVA: incidence versus *Fusarium* species in Busia district

```
Analysis of Variance for inc. in
Source     DF        SS        MS        F        P
fus.spec    3     3.201     1.067     1.10    0.358
Error      56    54.423     0.972
Total      59    57.624


Level       N      Mean     StDev
F. grami   15    1.2000    1.0144
F. oxysp   15    1.5267    1.1955
F.compac   15    0.8733    0.6326
F.nygama   15    1.2000    1.0144

Pooled StDev =   0.9858
```

Appendix 2.20: One-way ANOVA: incidence versus plant

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
Plant       1    368.10    368.10   100.83    0.000
Error     598   2183.13      3.65
Total     599   2551.23


Level       N      Mean     StDev
1         320     2.723     2.433
2         280     1.153     1.028

Pooled StDev =    1.911
```

Appendix 2.21: One-way ANOVA: incidence *Fusarium* species versus district

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
District    3     66.05     22.02     5.28    0.001
Error     596   2485.18      4.17
Total     599   2551.23


Level       N      Mean     StDev
Busia     120     1.960     2.356
Kakamega  160     1.476     1.672
Nyamira   160     2.218     1.924
Teso      160     2.300     2.231

Pooled StDev =    2.042
```

Appendix 2.22: One-way ANOVA: incidence in sorghum roots versus sorghum varieties

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
s. varie    4     23.40      5.85     0.68    0.609
Error      95    819.77      8.63
Total      99    843.17


Level       N      Mean     StDev
Esila      20     3.348     2.863
IS8193     20     2.137     2.341
Mtama-1    20     2.883     2.945
Seredo     20     2.717     3.320
Wagiita    20     3.495     3.126

Pooled StDev =    2.938
```

Appendix 2.23: One-way ANOVA: incidence in sorghum roots versus *Fusarium* species

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
Species     3    457.63    152.54    37.98    0.000
Error      96    385.54      4.02
Total      99    843.17


Level       N      Mean     StDev
F. compa   25     6.505     2.441
F. grami   25     2.337     2.250
F. Oxysp   25     1.940     2.075
F.nygama   25     0.882     0.858

Pooled StDev =    2.004

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0103
```

Appendix 2.24: One-way ANOVA: incidence in sorghum roots versus incidence on stem

```
Analysis of Variance for incidence
Source     DF        SS        MS        F        P
inc. on     1      2.23      2.23     0.29    0.590
Error     198   1517.19      7.66
Total     199   1519.42


Level       N      Mean     StDev
1         100     2.705     2.609
2         100     2.916     2.918

Pooled StDev =    2.768
```

Appendix 2.24: One-way ANOVA: incidence on stem versus sorghum variety

```
Analysis of Variance for inc. on
Source     DF        SS        MS        F        P
s.var.st    4     33.65      8.41     1.25    0.296
Error      95    640.37      6.74
Total      99    674.02


Level       N      Mean     StDev
Esila      20     2.387     2.744
IS8193     20     1.783     1.906
Mtama-1    20     2.840     2.759
Seredo     20     3.031     2.780
Wagiita    20     3.483     2.683

Pooled StDev =    2.596

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.00657
```

Appendix 2.25: One-way ANOVA: Fumonisin versus crop

```
Analysis of Variance for Fumonisin
Source     DF        SS        MS        F        P
Crop        1     16979     16979     9.68    0.004
Error      28     49099      1754
Total      29     66079


Level       N      Mean     StDev
Finger m   14      1.12      0.87
Sorghum    16     48.81     57.21

Pooled StDev =    41.88
```

Appendix 2.26: One-way ANOVA: Fumonisin versus district

```
Analysis of Variance for Fumonisin
Source     DF        SS        MS        F        P
District    3      2964       988     0.41    0.749
Error      26     63114      2427
Total      29     66079


Level       N      Mean     StDev
Busia       6      9.38     12.51
Kakamega    8     26.60     41.31
Nyamira     8     38.75     48.10
Teso        8     27.21     69.88

Pooled StDev =    49.27
```

Appendix 2.27: One-way ANOVA: fumonisin in sorghum versus districts

```
Analysis of Variance for F. in sorghum
Source     DF        SS        MS        F        P
District    3      6337      2112     0.59    0.631
Error      12     42753      3563
Total      15     49089


Level       N      Mean     StDev
Busia       3     17.59     13.72
Kakamega    5     41.86     47.01
Nyamira     4     76.63     39.65
Teso        4     53.11     98.01

Pooled StDev =    59.69
```

Appendix 2.28: One-way ANOVA: Fumonisin in sorghum versus Sorghum variety

```
Analysis of Variance for F. in sorghum
Source     DF        SS        MS        F        P
Sor. Var.  12     32442      2703     0.49    0.840
Error       3     16648      5549
Total      15     49089


Level       N      Mean     StDev
AF28        2     25.89      0.59
Esila       1      1.75      0.00
Essuti      1     31.41      0.00
Gopari      1     81.15      0.00
Ikhumba     1      1.75      0.00
IS8193      2     66.48     80.45
Livoywa     1     26.44      0.00
Migogo      1     38.11      0.00
Mtama-1     1      0.00      0.00
Nakhadab    1      2.83      0.00
Ochuti      1    129.92      0.00
Seredo      1     25.54      0.00
Wagiita     2    128.68    100.87

Pooled StDev =    74.49
```

Appendix 2.29: One-way ANOVA: Fumonisin in finger millet versus districts

```
Analysis of Variance for F. in f.millet
Source     DF        SS        MS        F        P
Distr.      3     0.401     0.134     0.14    0.933
Error      10     9.443     0.944
Total      13     9.844


Level       N      Mean     StDev
Busia       3    1.1667    1.0104
Kakamega    3    1.1667    1.0104
Nyamira     4    0.8750    1.0104
Teso        4    1.3125    0.8750

Pooled StDev =   0.9717
```

Appendix 2.30: One-way ANOVA: Fumonisin versus finger millet variety

```
Analysis of Variance for F. finger millet
Source      DF        SS        MS        F        P
f.mill var  10      6.27      0.63     0.53    0.806
Error        3      3.57      1.19
Total       13      9.84


Level       N      Mean     StDev
Aran        1     1.750     0.000
Enyaikur    1     1.750     0.000
Enyankun    1     1.750     0.000
GuluE       1     1.750     0.000
Ikhulule    2     0.875     1.237
KNE688      1     1.750     0.000
Mokomoni    1     0.000     0.000
Nafusi      1     0.000     0.000
Namadimw    1     1.750     0.000
P224        3     1.167     1.010
U15         1     0.000     0.000

Pooled StDev =    1.091
```