

INFLUENCE OF CROPPING SYSTEMS AND CROP RESIDUES ON THE OCCURRENCE  
OF *FUSARIUM* HEAD BLIGHT OF WHEAT (*Triticum aestivum* L.) AND ASSOCIATED  
MYCOTOXINS IN NAROK COUNTY, KENYA

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**DECLARATION**

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## **DEDICATION**

I dedicate this thesis to my siblings who have been really supportive of me and my parents who have committed to do whatever it takes to ensure that my siblings and I attain quality education.

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## LIST OF ABBREVIATIONS

AEZ – Agro-ecological Zones

AOAC – Association of Official Agricultural Chemists

CABI – Commonwealth Agricultural Bureau International

CAST – Council for Agricultural Science and Technology

CIMMYT - International Maize and Wheat Improvement Center

CV – Coefficient of Variation

DAP – Diammonium Phosphate

DON – Deoxynivalenol

ELISA – Enzyme Linked Immuno-Sorbent Assay

EU – European Union

FAO – Food and Agricultural Organization

FDA – Food and Drug Agency

FHB - *Fusarium* head blight

GASGA - Group for Assistance on Systems Relating to Grain after Harvest

GS – Growth Stage

HGCA – Houston Gun Collectors Association

IARC – International Agency for Research on Cancer

IFST – Institute of Food Science and Technology I

KARI – Kenya Agricultural Research Institute

KEBS – Kenya Bureau of Standards

LSD – Least Significant Difference

NDP – National Development Plan

OARDC – Ohio Agricultural Research and Development Centre

PCNB – Pentachloronitrobenzene

PDA – Potato Dextrose Agar

SCF – Scientific Committee on Food

SNA – Synthetic Nutrient Agar

WHO – World Health Organization

## ABSTRACT

Wheat (*Triticum aestivum* L.) is an important cereal grain cultivated worldwide. *Fusarium* head blight (FHB) and the associated mycotoxins pose a threat to food security and safety in Kenya, as they cause quantitative and qualitative yield losses, deterioration of grain nutritional value and human and animal health risks. This study assessed the effect of cropping systems and the role of soil and crop residues on the occurrence of FHB and associated mycotoxins in Narok County of Kenya. A field survey covering 51 wheat farms was carried out between June and October 2013. A semi structured questionnaire was used to obtain information on wheat production practices. Top soil, crop residues and wheat spikes were sampled at hard dough stage while kernels were sampled at harvest. The incidence of FHB was determined as the number of blighted heads over the total number of heads within 1m<sup>2</sup> randomly selected quadrants in each farm, while severity was assessed based on proportion of bleached spikelets. *Fusarium* head blight-causing pathogens were isolated from the soil, crop residues, spikelets and wheat kernels by plating on low strength Potato Dextrose Agar (PDA) and the isolated *Fusarium* spp. were identified using cultural and morphological characteristics. Two *Fusarium* mycotoxins, deoxynivalenol (DON) and T-2 toxin in the wheat kernels were analyzed by competitive Enzyme Linked Immuno-Sorbent Assay (ELISA).

Most (>50%) of the wheat farmers were small scale producers who used farm saved seeds, rotated wheat with maize, grew the two crops side by side, grew wheat in consecutive years, left wheat residues as standing hay for livestock and used simple land preparation methods that did not bury previous crop residues. Prevalence of FHB at hard dough stage was 100% while the mean incidence and severity of the disease ranged from 1 to 100% and 1 to 95%, respectively. *Fusarium* spp. were most prevalent in crop residues; *Aspergillus* spp. and *Penicillium* spp. in soil; and *Epicoccum* spp. and *Alternaria* spp. in wheat ears and kernels. The isolation frequency of the fungal pathogens in crop residues, soil, wheat ears and kernels at harvest significantly ( $p \leq 0.05$ ) varied among the agro-ecological zones. The most frequently isolated *Fusarium* spp. were *F. chlamydosporum* (2.6%) and *F. graminearum* (2.0%) in crop residues, *F. oxysporum* (3.4%) and *F. proliferatum* (2.5%) in soil; *F. avenaceum* (3.0%) and *F. poae* (1.9%) in wheat ears; and *F. tricinctum* (4.4%) and *F. poae* (2.9%) in wheat kernels. The incidence of the *Fusarium* spp. in crop residues, soil and wheat ears varied significantly ( $p \leq 0.05$ ) among the agro-ecological

zones but there was no significant ( $p \geq 0.05$ ) difference in the incidence of *Fusarium* spp. in wheat kernels at harvest. All the wheat kernels were contaminated with T-2 toxin while 94% were contaminated with deoxynivalenol. The concentration of T2-toxin in the kernels ranged from 8.8 to 37  $\mu\text{g}/\text{kg}$  (mean = 25.1  $\mu\text{g}/\text{kg}$ ) while that of DON ranged from below limit of detection (18.5  $\mu\text{g}/\text{kg}$ ) to 114  $\mu\text{g}/\text{kg}$  (mean = 9.1  $\mu\text{g}/\text{kg}$ ). The results of this study implied that wheat production practices affect the incidence and severity of FHB and that the disease is caused by a complex of *Fusarium* spp. The *Fusarium* spp. survive in soil and crop residues between cropping seasons thereby providing primary inocula for FHB in a subsequent wheat crop. Although all the wheat kernels were contaminated with T-2 toxin and 94% were contaminated with deoxynivalenol, the toxin levels were within the limits set by the European Commission (EU) and the United States Food and Drug Administration (FDA). This implied that the wheat harvested during the 2013 cropping season in Narok County was safe for human consumption and therefore posed no health risk to humans and livestock. The low incidence of *Fusarium* spp. in spikelets and kernels at harvest could explain the low incidence and severity of FHB in the field and the low levels of DON and T-2 toxin in harvested wheat kernels. It is recommended that inclusion of maize as a rotation crop in wheat production and leaving crop residues on the soil surface after harvesting should be avoided. Additionally, farmers should be encouraged to apply recommended fungicides in the management of FHB; while continuous monitoring and surveillance of DON and T-2 toxin levels in wheat should be promoted. Further studies on the long-term effect of various wheat production practices on FHB are also recommended.

**Key words:** Cropping systems, *Fusarium* head blight, mycotoxin, wheat.



## CHAPTER ONE: INTRODUCTION

### 1.1 Introduction

Wheat is a major cereal grain crop cultivated worldwide in winter and spring. It belongs to the genus *Triticum*, which has thousands of species (Kent and Evers, 1994), with *T. aestivum* sub. *vulgare* and the hard wheat *T. durum* being the most important commercially (Macrae *et al.*, 1993). Wheat forms a predominant basis of human and livestock nutrition worldwide (KARI, 1989). Alternative uses for wheat includes production of biodegradable plastic and paint stripping with wheat starch, wheat-based raw materials for cosmetics, ethanol production and production of meat substitutes (Doty, 2012). In Kenya, wheat is mainly used for domestic and commercial baking (Noah and Waithaka, 2005). Trade in the world for wheat is greater than for all other commercial crops combined (Curtis, 2014). Currently, wheat is the second most important cereal grain in Kenya after maize (KARI, 1989; Gamba *et al.*, 2003), with an annual production of only 350,000 metric tons against a consumption of 900,000 metric tons per year (Odhiambo, 2012).

On a dry matter basis, the grain contains 2 to 3% germ, 13 to 17% bran and 80 to 85% mealy endosperm (Belderok *et al.*, 2014). The bran is rich in B vitamins and minerals. Proteins and carbohydrates each constitute 16% of the total dry weight of bran. The mineral content of bran is 7.2%. Apart from carbohydrates, the mealy endosperm contains fats (1.5%) and proteins (13%): albumins, globulins and the major proteins of the gluten complex namely glutenins and gliadins; proteins that will form the gluten at dough making. The contents of minerals (ash) and of dietary fibers are low; 0.5% and 1.5%, respectively (Belderok *et al.*, 2014). The mineral level is also high (4.5%). Wheat germ is available as a separate entity because it is an important source of vitamin E. The wheat germ has only one half the glutamine and proline of flour, but the levels of alanine, arginine, asparagine, glycine, lysine and threonine are double (Cornell, 2003). The vitamin and mineral content of foods from wheat depends on the amount of bran, germ and endosperm present. Whole grain wheat contains useful amounts of vitamin B complex and vitamin E (Sramkova *et al.*, 2009). The major minerals potassium, iron, magnesium and zinc as well as the trace elements like selenium are also present. While wheat products are an important source of dietary fiber, wheat germ and the outer layer are rich in minerals and vitamins.

Refining and removing these components results in loss of some minerals and vitamins plus some fiber.

Wheat infection by various fungi including; *Alternaria*, *Fusarium*, *Helminthosporium*, *Epicoccum*, *Cladosporium*, *Chaetomium*, *Curvularia*, *Myrothecium*, *Rhizopus* and *Stemphylium* species cause reduction in yields and quality (Zillinsky, 1983; Government of Alberta, 2014). The fungi infect seeds when moisture exceeds 20% and relative humidity exceeds 90% (Tomohiro and Kazuhiro, 2000). *Fusarium* spp. infection of wheat results in many economically important plant diseases such as head blights, ear rots and stem diseases, resulting in severe reduction in crop yield by up to 40% (Niaz and Dawar, 2009). The common *Fusarium* spp. involved in wheat fusariosis are *F. graminearum* (*Gibberella zeae*; the teleomorph stage), *F. culmorum*, *F. crookwellense*, *F. avenaceum* (*G. avenacea*), *F. sporotrichioides*, *F. poae*, and *F. nivale* (Parry *et al.*, 1995). *Fusarium graminearum* and *F. culmorum* are the most aggressive, causing severe blighting of wheat ears (Stack and McMullen, 1985). The infected grains have low market value because their products have undesirable color and odor (Weidenborner, 2001).

Infection of wheat by *Fusarium* spp. can occur during all growth stages but the most susceptible and economically important developmental stage is at flowering (Parry *et al.*, 1995). Rainfall during this stage often results in economically important *Fusarium* head blight (FHB) infections. *Fusarium* head blight of wheat is caused by a complex of soil borne and residue borne *Fusarium* spp. but *F. graminearum*, *F. culmorum*, *F. poae* and *F. avenaceum* predominate depending on climatic conditions (Sutton, 1982; Parry *et al.*, 1995; Miedaner, 1997; Brennan *et al.*, 2003). Diversity of *Fusarium* spp. may be higher than described in earlier studies as *F. graminearum* has been divided into various species (O'Donnell *et al.*, 2004; Starkey *et al.*, 2007; Qu *et al.*, 2008). *Fusarium* Head Blight is one of the most destructive in areas with warm and humid weather especially after heading of wheat (Tomohiro and Kazuhiro, 2000). It reduces grain yield due to sterility of florets and grain shriveling and quality due to production of mycotoxins in kernels harvested from infected ears which adversely affect human and livestock productivity and health (Government of Alberta, 2014). The major mycotoxins associated with *Fusarium* spp. include deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin, zearalenone and their derivatives (Weidenborner, 2001).

## 1.2 Problem statement

Wheat forms a predominant basis for human and livestock nutrition in Kenya. However, wheat production output is lower than the consumption. Low wheat output among small scale farmers is mainly due to low soil fertility and fungal diseases. One of the economically important fungal diseases of wheat is FHB, a disease caused by a complex of residue and soil borne *Fusarium* spp. (Parry *et al.*, 1997; Wagacha *et al.*, 2010). The disease leads to reduction in wheat yields and quality and contaminates grains with mycotoxins (Muthomi *et al.*, 2007; Wagacha *et al.*, 2010; Muthomi *et al.*, 2012). These lead to reduced marketability of the wheat grains (Hershman, 2014). *Fusarium* pathogens pose great threat to food security and safety and contribute to unsustainable socio-economic development.

High incidence of *Fusarium* spp. has been reported to be associated with major FHB associated mycotoxins (Muthomi *et al.*, 2007; Wagacha *et al.*, 2010; Muthomi *et al.*, 2012; Antonissen *et al.*, 2014). Mycotoxins have been associated with a number of human diseases, some chronic and others acute (Wayne, 2007). Acute mycotoxicoses are caused by intake of high doses of mycotoxins and they are characterized by well described clinical signs and symptoms (Antonissen *et al.*, 2014). Muthomi *et al.* (2008), reported low but significant levels of deoxynivalenol (DON), zearalenone, T-2 toxin and aflatoxin B1 in Kenyan wheat. However, higher than the recommended levels of DON were reported by Wagacha *et al.* (2010). Deoxynivalenol is the most economically important *Fusarium* toxin (Wegulo, 2012). Ingestion of DON has high emetic effects and disrupts protein function by inhibiting protein synthesis and results in disorders in lipid metabolism, renal filtration and disturb renal cell DNA methylation and rhabdomyolysis (Sobrova *et al.*, 2010; Kouadio *et al.*, 2013). T-2 toxin inhibits protein synthesis, and cause secondary disruption of DNA and RNA synthesis (EFSA, 2013). It affects the actively dividing cells of lining of gastrointestinal tract, skin, lymphoid, and erythroid cells. T-2 toxin can also decrease levels of antibody, immunoglobulins and certain other humoral factors. Observable effects include weight loss or poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage and decreased productivity (WHO, 2001). Wheat and cereal residues act as sources of primary inocula for FHB and as a pathway for introduction of mycotoxins to the animal feed chain (FSA, 2007; Wagacha *et al.*, 2010; Keller, 2011). *Fusarium* spp. inocula survive in cereal residues and soil until the next season (Martin and Johnson, 1982;

Sutton, 1982; Dill-Macky and Jones, 2000). The reservation of FHB inocula is compounded by wheat-maize rotation. Remains of the infected wheat residues can be easily blown away by wind and/or splashed by rain water (Government of Alberta, 2014).

### **1.3 Justification of the study**

Previous studies in Kenya have focused on the occurrence of *Fusarium* spp. in wheat and associated mycotoxins, quantification of *Fusarium* mycotoxins and exploring disease management options in an integrated approach (Muthomi *et al.*, 2002, 2007a, 2008; Wagacha *et al.*, 2010; Muthomi *et al.*, 2012). However, these studies have not investigated the role of cropping systems, soil and crop residues on accumulation of FHB inoculum. Intercropping cereals is a common practice in many parts of the tropics (Adamu and Sharaiha, 2010). Wheat intercropped with another cereal crop results in reduced yields of one or both of the crops involved as well as increased disease incidence and severity (Ladha *et al.*, 2000). Crop rotation and mixed cropping of wheat with other cereals promotes accumulation of FHB inocula (Silvestro *et al.*, 2013). The cereals act as alternative hosts for *Fusarium* species. All *Fusarium* spp. that infect cereals are capable of surviving saprophytically on crop residues (Parry *et al.*, 1995; Keller, 2011). This then implies that infected crop residues left in the field play a role in survival and spread of *Fusarium* species. They increase the population of *Fusarium* in soils (Bateman *et al.*, 1998; Keller, 2011; Silvestro *et al.*, 2013) and pose health risks of exposing livestock to *Fusarium* related mycotoxins when fed on hay, fodder or where the infected straw act as bedding material (Wagacha *et al.*, 2010). When wheat is continually grown in the same field, wheat residues also acts as primary source of inocula for FHB epidemics. Therefore, there is need to study the role of cropping systems, soil and crop residues on accumulation of *Fusarium* head blight inocula.

#### **1.4 Objectives of the study**

The main objective of the study was to determine the influence of cropping systems and crop residues on occurrence and diversity of *Fusarium* spp. and mycotoxins associated with *Fusarium* head blight of wheat in Narok County, Kenya.

##### **The specific objectives were:**

- i. To evaluate the effect of cropping systems on accumulation of FHB inocula in soils and crop residues.
- ii. To determine the incidence and severity of *Fusarium* head blight of wheat in Narok County, Kenya.
- iii. To determine contamination levels of wheat kernels with deoxynivalenol and T-2 toxin at harvest.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Wheat production in Kenya

Wheat is the second most important cereal grain in Kenya after maize (Gamba *et al.*, 2003). It has an annual production of only 350,000 metric tons against a consumption of 900,000 metric tons per year (Odhiambo, 2012). The difference is often met by imports (Kamau, 2013). The high increase in population and consumption of wheat products is expected to have a bigger impact on wheat production and importation in future with the demand growing higher by 2020.

Wheat varieties grown in Kenya include Kenya Pasa, Kenya Chirika, Kenya Mbuni, Kenya Kwale, Kenya Popo, Kenya Fahari, Kenya Kongoni, Kenya Nyumbu, KS Mwamba, Njoro BW 1, Njoro BW 2, Farasi, Kenya mbega, Kenya Duma among others (Kenya Seed Company, 2014). Most of the varieties are products of research by Kenya Agricultural Research Institute (KARI) (Noah and Waithaka, 2005). Successful wheat production greatly depends on farmers' knowledge about suitable varieties for the different areas. Moreover, varieties with acceptable baking quality, high yields and are resistant to stem rust are recommended (Gamba *et al.*, 2003). Wheat in Kenya is grown on large farms; with the largest wheat growing areas extending from Trans Nzoia to Nzoia, through Elgeyo, to Uasin Gishu, and Marakwet Districts (Noah and Waithaka, 2005). The most important wheat growing areas are Eldoret, Nakuru, Molo and Londiani, where soils are deep and fertile and the yields are high compared to other parts of Kenya. These areas have altitudes ranging between 1200 and 1500m above sea level and annual rainfall varying between 800 and 2000mm, with up to 2500mm on higher grounds per annum (Noah and Waithaka, 2005).

Wheat in Kenya is grown solely or intercropped with other cereals or legumes (Wagacha *et al.*, 2010). Intercropping cereals with other cereals is a common practice in many parts of the tropics (Adamu and Sharaiha, 2010). For example, wheat and maize or wheat and barley is common cereal-cereal intercropping (Wagacha *et al.*, 2010). Cereal – cereal intercropping results in reduced yields of one or both of the crops involved (Ladha *et al.*, 2000), as well as increased disease incidence and severity. Intercropping of wheat can also be between wheat and a legume. The effects of intercropping wheat with a legume are observed in the significantly increased above and below ground biomass of wheat including grain yield and narrowed root/shoot ratio (Gill *et al.*, 2009).

## 2.2 *Fusarium* head blight of wheat

### 2.2.1 Causes of *Fusarium* head blight of wheat

*Fusarium* head blight is the infection of wheat ears caused by a complex of soil borne and residue borne *Fusarium* spp. (Akinsanmi *et al.*, 2004). *Fusarium* head blight is one of the most economically important and destructive disease of wheat and other small grain cereals such as barley and maize in humid and semi-humid wheat growing areas (Cook, 1981; McMullen *et al.*, 1997; Tizaki and Sabbagh, 2012; Landschoot *et al.*, 2013), making it an increasing threat to wheat supply. *Fusarium* head blight is a pre-harvest disease but *Fusarium* spp. can grow post harvest if wet grains are not dried effectively and quickly (Parry *et al.*, 1995). The disease is associated with various *Fusarium* species that includes; *F. graminearum*, *F. poae*, *F. avenaceum*, *F. culmorum*; however, *F. graminearum* predominate in hotter regions while *F. culmorum* and *F. poae* are the predominant species in cooler regions (Parry *et al.*, 1995; Holt, 2014). However, most species produce inocula, grow best and are most pathogenic to wheat heads at warm temperatures and under humid climate (Doohan *et al.*, 2003; Wegulo, 2012). *Fusarium* head blight may be epidemic over large areas in some seasons but commonly varies in severity from field to field or among local areas. The incidence and severity of FHB is influenced by temperature, with higher temperatures (>25°C) causing more disease (Mentewab *et al.*, 2000; Holt, 2014).

*Fusarium* head blight is an increasing threat to world's grain supply and causes various kinds of damage to wheat (Bechtell *et al.*, 1995; Holt, 2014). It reduces the yields due to reduced seed weight of infected seeds, grain quality and contaminates the grains with mycotoxins. Infection of the heads by *Fusarium* spp. may cause reduced kernel set as well as kernel weight, and this is what results in yield reduction. Invasion of the kernels by *Fusarium* also results in destruction of starch granules, storage proteins and cell walls, resulting in poor quality products (Snijders, 1990). The embryo is usually not infected, except in heavily invaded kernels. Slightly infected kernels that have uninfected embryo exhibit reduced germination and vigor (Snijders, 1990).

The prevalence of infection varies from trace to virtually 100% of the heads in the field, and losses may vary correspondingly (Muthomi *et al.*, 2007; Muthomi *et al.*, 2012). The extent of yield reduction depends on the *Fusarium* species involved (Henriksen *et al.*, 2005; Wagacha *et al.*, 2011). When wheat heads are infected, they produce shriveled grains called *Fusarium*

damaged kernels (FDK), which are shrunken and chalky white (Government of Alberta, 2014). Many of the grains may be so shriveled as to be blown out of the back of the combined harvester during harvesting. This reduces yields; however, it also reduces infection levels; since the infected grains are separated from healthy ones by blowing of the wind during harvesting. However, grains infected after flowering may be heavy enough to be harvested together with healthy grains (Government of Alberta, 2014).

### **2.2.2 Sources of *Fusarium* head blight inocula**

*Fusarium* head blight inocula could be from within field or environment (Keller, 2011). Favorable environmental conditions such as frequent rainfall and high relative humidity enhance inoculum production on crop residue and result in disease development (Paulitz, 1996; Fernando *et al.*, 2000). Extended periods of greater than 90% relative humidity and 15-30°C temperature facilitate infection. Continued wet conditions after flowering may increase the potential for the spores to be blown by wind or rain-splashed to nearby ears (Keller, 2011). The primary sources of wheat FHB are wheat, maize and barley residues (Khonga and Sulton, 1988; Dill-Macky and Jones, 2000). The primary cause of FHB, *F. graminearum*, survives as a saprophyte on host residues and overwintered fruiting bodies contributes inoculum for infection of wheat and barley plants in the same field in which the residue is present (Keller, 2011) and more distant fields.

Infected wheat residues grazed to livestock could also act as a pathway for introduction of mycotoxins to the animal feed chains (FSA, 2007; Wagacha *et al.*, 2010). The amount of crop residues at planting is determined by the previous crop, whether cereal or legume, and the type of tillage done (Dill-Macky and Jones, 2000). *Fusarium* head blight incidence and severity is high when soil coverage with cereal residues is high and no or minimum tillage is done. However, tillage alone has little effects on FHB inocula when wheat is rotated with legumes and other short term crops. Spores of *F. graminearum* can be trapped from air during epidemics of FHB (Paulitz, 1996). Inoculum can also come from alternate hosts and cereal plants with *Fusarium* seedling blight/foot rot when planted side by side (Parry *et al.*, 1995; Fernando *et al.*, 1997). The risk of FHB increases with greater concentration of inoculum from within field sources when environmental conditions are moderately favorable for disease (Nita *et al.*, 2006). *Fusarium* head



blight is therefore a result of the complex interaction between weather conditions and agricultural practices including crop rotation, tillage, fungicide application and host resistance (Landschoot *et al.*, 2013).

Symptoms of FHB typically appear near the middle of the wheat or barley spike and progress through the rest of the spike (Bushnell, 2003; Government of Alberta, 2014; Schmale and Munkvold, 2014). In the field, the disease is characterized by premature bleaching of the infected spikelets, and formation of orange spore bearing structures called sporodochia at the base of the glumes. The diseased spikelet may contain visibly affected kernels. Whitish, occasionally pinkish, fluffy fungal growth may occur in the field during wet weather (Stack, 2000; Government of Alberta, 2014). The infection process of *F. graminearum* has several phases; a hyphal mat is formed at the point of infection after spore germination, growth of mycelia to the crown and growth through the crown tissues (Stephens, 2009). As FHB infection progresses, the developing kernels may appear smaller than normal, shriveled and white/pink in color and may contain mycotoxins produced by the fungus (Keller, 2011).

*Fusarium* head blight is also a concern in wheat production because infected wheat kernels may contain mycotoxins. Disease incidence and severity, as well as DON concentrations are said to be positively related to inoculum concentration (Stein *et al.*, 2009). Mycotoxins pose serious health risks to humans and animals (Keller, 2011).

## **2.3 Major *Fusarium* species associated with FHB**

### **2.3.1 *Fusarium graminearum* (teleomorph: *Gibberella zeae*)**

*Fusarium graminearum* is an ascomycete that produces sexual spores called ascospores and asexual spores called macroconidia (Markell *et al.*, 2003; Shaner, 2003). Spore containing structures called perithecia are formed in the residue remaining in the soil surface. In late spring and early summer, the perithecia release ascospores that can be deposited on wheat spikes (Fernando *et al.*, 2000; McMullen *et al.*, 1997; Stack, 1997). Pink/orange masses called sporodochia produce macroconidia. Both ascospores and macroconidia contribute to FHB. However, ascospores play the predominant role in infection of wheat and barley florets from spike emergence through anthesis and grain fill, since they are airborne (Fernando *et al.*, 1997,

2000). *Fusarium graminearum* is predominant in hotter regions (Parry *et al.*, 1995). Mainly, grains infected with *F. graminearum* are of real consequence, but the crown and root tissues can also be infected. *Fusarium graminearum* produces a variety of potent mycotoxins including deoxynivalenol (DON), zearalenone (ZEA) and fusarin C (Bhat and Miller, 1989; Keller, 2011).

### **2.3.2 *Fusarium poae***

*Fusarium poae* is a widespread fungus. However, it is most common in temperate regions where it is usually isolated from seed and grain heads and woody headings (Leslie and Summerell, 2006). In PDA, *F. poae* produces a dense mycelium, white to pink in color that may turn reddish brown as the culture ages. The underside of the colony may vary from white to yellow to deep carmine in color. *Fusarium poae* develops clusters of short branched and unbranched monophialides. Microconidia are numerous, globose or oval to piriform, septate. Macroconidia are rare, typically sickle shaped, septate and have a foot shaped basal cell (Nelson *et al.*, 1983; Desjardins, 2006; Leslie and Summerell, 2006). *Fusarium poae* produces asexual spores that are dispersed by wind and by rain splash to wheat heads (Jenkison and Parry, 1994; Fernando *et al.*, 1997). Compared to *F. graminearum* and *F. avenaceum*, *F. poae* is a relatively weak pathogen, but it produces a large number of mycotoxins (Stenglein, 2009). High incidence of *F. poae* is associated with nivalenol (NIV) contamination (Pettersson *et al.*, 1995; Yli-Matilla *et al.*, 2008a). The highest incidence of *F. poae* corresponds to its density in warm climates. In colder and moister places, *F. poae* frequency is high but its density is lower compared to warmer areas suggesting that it is more adaptable to agro-environmental conditions during grain formation (Rohacik and Hudek, 2005). Mycotoxins associated with *F. poae* are nivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol and beauvericin (Vogelgsang *et al.*, 2006).

### **2.3.3 *Fusarium chlamydosporum***

*Fusarium chlamydosporum* is commonly found in arid and semi-arid regions in the soil and as a saprophyte on a variety of substrates (Leslie and Summerell, 2006). *Fusarium chlamydosporum* has a rapid growth on PDA at 25°C. It has woolly colonies, which are initially white and later turns pink to red brown at the center. It is characterized by production of chlamydospores and

abundant spindle shaped septate microconidia. The chlamydoconidia are brown and the microconidia are clustered and borne on phialides with more than one opening (Wollenweber and Reinking, 1935). *Fusarium chlamydosporum* is an important cause of FHB in Kenya and contaminates wheats with moniliformin (Solfrizzo and Visconti, 1996; Wagacha *et al.*, 2010). It was isolated in the highest incidence from wheat kernels sampled in Nakuru County (Wagacha *et al.*, 2010).

#### **2.3.4 *Fusarium avenaceum* (teleomorph; *Gibberella avenacea*)**

*Fusarium avenaceum* is a soil saprophyte that is often involved in FHB of wheat (Leslie and Summerell, 2006; Vogelgsang *et al.*, 2008). The pathogenicity of *F. avenaceum* is generally lower than that of *F. culmorum* and *F. graminearum*. However, it has been reported as the most frequently isolated *Fusarium* spp. (Kosiak *et al.*, 1997; Langseth *et al.*, 1997; Parry *et al.*, 1995; Muthomi *et al.*, 2012). *Fusarium avenaceum* contaminates grains with enniatins (Jestoi *et al.*, 2004; Logrieco *et al.*, 2002). It is more common in temperate regions but its increased prevalence has also been reported in warmer regions. Like *F. poae*, the pathogenicity of *F. avenaceum* is lower than that of *F. graminearum* and *F. culmorum*. Infection of *F. avenaceum* on wheat spikes results in alteration of cell wall components (Khang *et al.*, 2005).

#### **2.3.5 *Fusarium culmorum***

*Fusarium culmorum* is a soil inhabiting fungus that is a competitive saprophyte and a facultative parasite (Leslie and Summerell, 2006). It is an important pathogen of wheat. Its populations in wheat field soils have been shown to fluctuate throughout the season, increasing greatly in dry conditions that favor its pathogenic activity on stem bases (Goswami and Kistler, 2004; Bateman and Murray, 2001; Bateman *et al.*, 1998; Vigier *et al.*, 1997). The significance of *F. culmorum* in wheat production is attributed to both FHB and mycotoxin production in wheat harvested from infected ears (Wagacha and Muthomi, 2007). *Fusarium culmorum* is the predominant cause of FHB in cooler regions (Parry *et al.*, 1995). Major mycotoxins produced by *F. culmorum* are deoxynivalenol, nivalenol and zearalenone (Leslie and Summerell 2006). Temperatures above 25°C and moist periods longer than 24h favor infection and mycotoxin production by *F.*

*culmorum* (Mentewab *et al.*, 2000; Campell and Lipps, 1998). It produces short, stout, thick walled macroconidia that have curved ventral and dorsal surfaces (Nelson *et al.*, 1983). Chlamydospores may occur singly, in chains or in clumps.

On potato dextrose agar, growth is rapid with dense aerial mycelia; often white but generally yellow to tan. Orange to red-brown sporodochia appears as culture ages. It does not produce ascospores (teleomorph). It produces conidia (asexual spores) which are the main mode of dispersal. The conidia are dispersed by wind or water splashed on wheat heads (Fernado *et al.*, 1997; Jenkinson and Parry, 1994). The conidia infects wheat ears mainly during anthesis (Bai and Shaner, 1996) and the success of infection mainly depends on weather conditions such as temperature (Brennan *et al.*, 2005; Cowger, 2005; Stein *et al.*, 2005; Mentewab *et al.*, 2000), humidity (Cowger, 2005; Nita *et al.*, 2005), cultivar resistance level (Cowger, 2005; Nita *et al.*, 2005; Bai and Shaner, 1996; Llorens *et al.*, 2004) and nitrogen fertilization (Doohan *et al.*, 2003) among others.

#### **2.4 Other fungal diseases of wheat caused by *Fusarium* spp.**

*Fusarium* crown and root rot disease of wheat are perennial problems in cereal agro-systems and cause significant losses in different regions worldwide (Burgess *et al.*, 2001; Paulitz, *et al.*, 2002). Common root rot is caused by *Cochliobolus sativus* and several *Fusarium* spp. (Government of Alberta, 2014). It has several phases: seedling blight, root rot and a possible leaf spot phase later in the season. In mature plants, rot primarily caused by *C. sativus* is the major cause of crop loss. The infection lowers yields since infected plants tend to produce fewer tillers and less seeds per head, some of which ripen pre-maturely (Government of Alberta, 2014).

#### **2.5 Mycotoxins associated with *Fusarium* head blight of wheat**

Mycotoxins are low molecular weight toxic compounds (Negedu *et al.*, 2011) produced by a diverse group of fungi that differ in their morphology, biochemistry and ecological niches (CAST, 2003; O'challagan, 2006). It is a characteristic of the biosynthesis of secondary metabolites, that the amount produced is influenced not only by the environmental and

nutritional parameters at the time of production, but also frequently by the previous growth history and development of the fungi. An infection of wheat with FHB contaminates the wheat with mycotoxins, particularly deoxynivalenol (DON), sometimes called vomitoxin, nivalenol, T-2 toxin, HT-2 toxin, zearalenone (ZEA) and their derivatives (Keller, 2011) and the less frequent diacetoxyscirpenol (DAS), fusarenon X. Because fungi that produce them are widespread and can grow and produce their toxins on a wide variety of food commodities, mycotoxins cause great loss to grain trade and marketing of foods and feeds.

### 2.5.1 Deoxynivalenol

Deoxynivalenol, also called vomitoxin, is a toxic fungus metabolite belonging to the trichothecenes type “B” group. Its chemical name is 12, 13-epoxy 3 $\alpha$ , 7 $\alpha$ , 15-trihydroxytrichothene-9-en-8-on (Pronk *et al.*, 2002). Deoxynivalenol is a polar organic compound, whose molecule contains three hydroxyl groups, which are associated with its toxicity (Nagy *et al.*, 2005). Deoxynivalenol is the most economically important *Fusarium* mycotoxin and has been shown to be a virulence factor in FHB (Keller, 2011). It is called vomitoxin due to its high emetic effect after consumption due to its deleterious effects on the digestive system of monogastric animals (Schmale and Munkvold, 2014). It is transported to the brain where it runs dopaminergic receptors (Sobrova *et al.*, 2010). Ingestion of DON also disrupts protein function by inhibiting protein synthase by binding to the ribosome and activating critical cellular kinases involved in signal transduction related to proliferation, differentiation and apoptosis of cells (Sobrova, 2010). Observable symptoms of DON consumption in humans are nausea, fever, headaches and vomiting (Schmale and Munkvold, 2014).

Deoxynivalenol and its acetylated derivatives are by far the most important trichothecenes. They have been found to have worldwide distribution and various international workshops have focused in them (Larsen *et al.*, 2004). They can contaminate fruits, seeds and by-products; moreover, wheat flour is an important substrate of DON contamination (Birck *et al.*, 2006). The acetylated derivatives are detected at low levels where there are high levels of DON. *Fusarium graminearum* and *F. culmorum* are consistent producers of DON in cereals. Deoxynivalenol and its derivatives are also produced by many other species of *Fusarium* that cause FHB. Within

these two species, there are those strains that produce DON and its derivatives and those that produce nivalenol and fusarenon X as their major metabolites. Intermediates have also been found (Nielsen and Thrane, 2001). Production of DON by *F. pseudograminearum* has also been reported in warm climates (Hocking *et al.*, 2006).

Exposure of humans to DON can be directly through consumption of contaminated cereal grains or indirectly through animal products such as eggs, milk, kidneys and liver (Sobrova *et al.*, 2010). The risk of occurrence of DON in food is due to its ability to withstand high temperatures (Hughes *et al.*, 1999). Deoxynivalenol is very stable even at temperature of 170°C to 350°C; and its concentration cannot be lowered by high temperatures. However, cooked noodles and pasta concentration is reduced by leaching into cooking water (Manthey *et al.*, 2004; Sugita-Konishi *et al.*, 2006; Visconti *et al.*, 2004), because it is water soluble. Deoxynivalenol concentration can be lowered by processing (Cetin and Bullerman, 2006). In the long run, DON does not constitute a threat to public health except in a few cases. The most common effects of acute exposure to DON are decreased weight gain, anorexia, decreased nutritional efficiency and altered immune function and these could be lethal (Pestka, 2007). Deoxynivalenol intoxication in humans can also decrease hematopoiesis by damage to the bone marrow and subsequently result in loss in weight gain (Flannery *et al.*, 2011). It also causes damage to the nervous system, gastrointestinal and cardiovascular systems. In animals, there is subsequent transfer of DON to animal products. In milk, the products of DON metabolism (de-epoxy-DON) have been detected (Keese *et al.*, 2008). Compared to T-2 toxin, DON is less toxic (Sokolovic *et al.*, 2008). However, extremely high doses; not likely to be found in food, can cause shock-like death. Deoxynivalenol can work in synergy with aflatoxin B1 to show great mutagenic effects, than aflatoxin B1 alone (Lei *et al.*, 2013).

### **2.5.2 T-2 toxin and HT-2 toxin**

T-2 toxin and HT-2 toxin often occur together in infected cereals (Scientific Committee on Food, SCF, 2013). The structure of T-2 toxin and HT-2 toxin differ only in the functional group at the C- 4 position. T-2 toxin is the most toxic trichothecenes produced by *Fusarium* spp. (Henriksen, 2000) while HT-2 toxin is less toxic. These toxins often occur in infected cereals

and are mainly produced by *F. poae*, *F. sambunicum* and *F. sporotrichioides* (Eriksen and Alexander, 1998; SCF, 2013) but the most important producer is *F. sporotrichioides*. The fungi producing the two mycotoxins are soil fungi and are important plant pathogens which grow on the crop in the field. After ingestion, T-2 toxin and HT-2 –toxin are very stable during storage/milling and the processing/cooking of food and do not degrade at high temperature (Eriksen and Alexander, 1998). T-2 toxin is rapidly metabolized by mammalian gut microflora into HT-2 toxin derivative, and is absorbed into the blood after ingestion (SCF, 2013). Metabolism of T-2 toxin continues in the liver and is distributed in the organism with little or no accumulation in any specific organ or tissue.

Contaminations of T-2 toxin in animals and humans have the effects of inhibiting DNA and RNA synthesis (WHO, 1990), inhibiting the activity of peptidyl transferase and consequently protein synthesis due to the affinity of T-2 for the 60s ribosomal subunit (European Food Safety Authority, EFSA, 2013). Also, T-2 toxin affects the permeability of cell membranes and thus causing changes in the phospholipid turnover (Grandoni *et al.*, 1992) and hemolysis of red blood cells (Rizzo *et al.*, 1992). T-2 toxin ingestion also inhibits the mitochondrial electron transport system and causing apoptosis (Ueno *et al.*, 1995; Yang *et al.*, 2000; Shifrin and Anderson, 1999). Out of all the known trichothecenes, T-2 toxin is the most cytotoxic (Garg, 2000).

### **2.5.3 Zearalenone**

Zearalenone is a potent estrogenic secondary metabolite of some *Fusarium* spp. (OARDC, 2012; Schmale and Munkvold, 2014). Zearalenone and its derivatives are mainly produced by *F. culmorum* and *F. graminearum*, which occur frequently in cereals all-over the world. Other producers of zearalenone and its derivatives are *F. equiseti* and *F. crookwellense*. Zearalenone is not produced in significant amounts prior to harvest, but is readily produced in wheat and small grains during storage. Zearalenone production is triggered by alternating low and moderate temperatures in storage. Zearalenone is not destroyed by heat, long storage, roasting or addition of mold retardants (OARDC, 2012).

Zearalenone is potent at levels as low as 0.5 parts per million (Gillespie, 2010). It is primarily associated with infertility, abortion or other breeding problems, in swine and dairy cattle (OARDC, 2012). In addition to oestrogen function, zearalenone also causes oxidative stress (Hou *et al.*, 2013). Conception rates may also be reduced in dairy heifers when fed on greater than 12.5 parts per million of zearalenone. When contaminated rations are consumed by animals, they develop a condition known as hyper-esterogen (OARDC, 2012).

#### **2.5.4 Nivalenol**

Nivalenol is a toxic trichothecene metabolite produced by strains of *F. culmorum*, *F. crookwellense*, *F. poae* and *F. equiseti* (Nielson and Thrane, 2001). Nivalenol and fusarenon X have a high degree of similarity with DON and are found in the same commodities. Nivalenol is toxic in much lower concentrations than DON (Khatibi *et al.*, 2014).

#### **2.5.5 Fumonisin**

Fumonisin are a group of mycotoxins that are primarily produced by *F. verticillioides* and *F. proliferatum* in maize and to a lesser extent in other cereal crops (Schmale III and Munkvold, 2014). Fungal contamination and fumonisin production in cereals is increased if kernels are physically damaged, especially by insect infestation. There are at least 28 forms of fumonisins designated as A-series, B-series, C-series and P-series but fumonisin B1, B2 and B3 are the most common. Fumonisin B1 is the most economically important form and is toxic to and cause apoptosis of the liver and the kidneys. A study done in Hungary detected fumonisins in wheat (Olga, 2009). Depending on the type of animal, age, sex, nutritional and health conditions, fumonisins cause different clinical symptoms, ranging from carcinogenic to neurotoxic (IARC, 1993). They are inhibitors of sphinganine N-acyltransferase, which is an important enzyme in lipid metabolism thereby disrupting the pathway (Nelson *et al.*, 1993).



### **2.5.6 Other mycotoxins contaminating wheat**

Other fungi infecting wheat also produce an array of toxins. These toxins can be produced while wheat is growing in the field or during harvest as is the case for *Fusarium* toxins, or after harvest and during storage. *Alternaria* contaminates grains with alternariol and tenuazonic acid before or during harvest. The storage fungi (e.g. *Penicillium* and *Aspergillus*) are capable of growing at lower water contents than the field fungi and they tend to contaminate the grains in silos and other storage places (Negedu *et al.*, 2011).

Aflatoxin, a toxin produced by *Aspergillus* spp., is commonly detected in wheat and is the most harmful to human and animal health. *Aspergillus* spp. are principally found in the soil and decaying matter. There are four main classes of aflatoxins; B1, B2, G1 and G2. When aflatoxin B1 and B2 are metabolized in animals, they produce hydroxylated metabolites aflatoxin M1 and M2, respectively. Aflatoxin is produced by *Aspergillus flavus*, *A. parasiticus*, *A. nominus* and *A. clavatus* with *Aspergillus flavus* being the most common producer (Cary *et al.*, 2005). Exposure to aflatoxin, the most prevalent of all the classes of mycotoxins is widespread. A study done in Gambia, Guinea Conakry, Nigeria and Senegal on human exposure showed that 98% of the subjects tested positive for aflatoxin markers (Wild, 1996; Glaston *et al.*, 2000; Moss, 2008). Aflatoxin expresses its effects on humans and animals as a mixture of aflatoxin or in synergy with other substances. Naturally occurring mixtures of aflatoxin have been classified as class one human carcinogens (IARC, 1993) and it has been associated with male infertility. High levels of aflatoxin B1, B2 and G1 are associated with hepatocellular carcinoma (Li *et al.*, 2000). In synergy with other agents such as Hepatitis B virus, aflatoxin cause liver cancer (Turner *et al.*, 2000). Aflatoxin B1 is the most toxic aflatoxin type and is almost always present in foods and feeds (Haladi and Alves, 2012).

## **2.6 Factors affecting occurrence and production of mycotoxins in wheat**

### **2.6.1 Environmental factors**

Environmental factors such as temperature, humidity and rainfall have a great influence on incidence and severity of FHB as well as mycotoxin production in wheat (Vigier *et al.*, 1997; Di Menna *et al.*, 1991; Jimenez *et al.*, 1996). Frequent rainfall and high relative humidity enhance

inoculum production on residue, resulting in disease development (Fernando *et al.*, 2000; Paulitz, 1996). Extended periods of greater than 90% relative humidity and 15-30°C temperature facilitate infection. Continued wet conditions after flowering may increase the potential for the spores to be blown by wind or rain-splashed to nearby spikes (Keller, 2011). Wet weather at flowering promotes *Fusarium* development (HGCA, 2012). Rainfall during the period from onset of ripening to harvest also affects occurrence and production of mycotoxins (Russell *et al.*, 2010). The region where wheat is grown also affects mycotoxin occurrence. High humidity in coastal areas tends to increase the levels of particular *Fusarium* mycotoxin (HGCA, 2012). Temperature stress of the growing crop is also important. Temperature and moisture content in storage grains determines whether the fungi will grow and/or produce mycotoxins (GASGA, 1997).

### **2.6.2 Agronomic factors**

Agronomic factors affecting occurrence of mycotoxins include soil, cultivation, nitrogen fertilization, fungicides, crop rotation and host genotype (Bottalico and Perrone, 2002). Previous crop residues on the soil surface are a major source of inoculum. This is especially a risk factor if the residues are of the same crop or of an alternative host for the *Fusarium* pathogen, like maize or barley. The spores of the mycotoxin producing fungi overwinter in the residues (Keller, 2011). Direct drilling of residues into the soil encourages development of these fungi. Some mycotoxins are also influenced by nitrogen levels of the soil, grain and/or straw. For instance, high nitrogen application at anthesis increases DON levels in the wheat (Van der Burgt and Timmermans, 2009). Occurrence and production of mycotoxins can be influenced by the cropping system. Intercropping, mixed cropping or crop rotation with other cereals raises the chances of mycotoxin occurrence in wheat. Crop rotation of wheat with maize is the greatest risk factor for the occurrence of FHB. This is probably because maize produces more residues which act as site for inocula to overwinter and build-up (FSA, 2007).

Other agronomic factors affecting occurrence of mycotoxins include variety, weeds and insects, use of fungicides as well as timing of harvest (FSA, 2007). Different varieties vary in their resistance to FHB and therefore to the risk of mycotoxin contamination. Grass weeds can act as

alternative hosts for FHB and consequently act as source of secondary infection to wheat with FHB. Application of ear spray at a robust rate can reduce the amount of FHB and subsequent mycotoxin production. Delay of harvest due to wet weather once a crop has ripened may result to production of more mycotoxins (FSA, 2007).

### **2.6.3 Biotic factors**

*Fusarium* infected seeds may be partially responsible for mycotoxin production in wheat (Wong *et al.*, 1995). The occurrence and amount of mycotoxin also depends on the species of the fungus present and the crop variety. Some wheat varieties are more resistant to *Fusarium* infection than others (HGCA, 2012). Insect infestation of stored grains damage the grain tegument and produce carbonic acid and water, contributing to the increased humidity which then increase grain respiration and consequently temperature. This favors fungal multiplication and mycotoxin production. Also, insects due to their movement help to disseminate the spores of the fungus all over the grain bulk (Santos and Montovani, 1997).

## **2.7 Implications of mycotoxins on human and animal health**

*Fusarium* species is associated with a number of human and animal toxicoses. However, only rarely has a direct connection been established between mycotoxins and mycotoxicosis (Pronk *et al.*, 2002; Wayne, 2007; Eeckhout *et al.*, 2013). The toxic effects of mycotoxins are diverse and depend on the type of mycotoxin, extent of exposure i.e. duration and dose, age, nutritional status and health of the affected individual and as a result of combined effects (antagonistic, additive and/or synergistic) of two or more mycotoxins as well as toxicological effects at low levels (Pronk *et al.*, 2002; Bhatnagar *et al.*, 2002).

### **2.7.1 Mycotoxins and human health**

Mycotoxins have been associated with a number of human diseases, some chronic and others acute. Human mycotoxicoses mainly occurs due to ingestion of mycotoxin contaminated food

(Bhat *et al.*, 1989). Humans can also be exposed to mycotoxins by inhalation of mycotoxin-containing spores and dust, and direct skin contact with infected wheat kernels (Trenholm *et al.*, 1989). Mycotoxins can also be carried over to food for human consumption via animal products such as meat, milk and eggs (Olsen *et al.*, 1993; Pettersson *et al.*, 1989; Veldman *et al.*, 1992). Given the current trade patterns, mycotoxicoses may result from contaminated locally grown or imported wheat (Peraica *et al.*, 1999). The impact of regular intake of low levels of mycotoxins on human health is likely to be significant with a number of possible consequences that include impaired growth and development, immune dysfunction, increased susceptibility to infectious disease and the disease consequences of alteration in DNA metabolism (Wayne, 2007).

Implications of mycotoxins in humans manifest in alimentary toxic aleukia, nausea, vomiting, abdominal pain, diarrhea, dizziness and headache; throat irritation, blood in stool, fullness, fatigue and fever (Li *et al.*, 1999). Mycotoxins also reduce vaccinal immune response which increase susceptibility to infections and reduce vaccine efficacy. Chronic ingestion of small amounts of trichothecenes may result in an important secondary effect: the predisposition to infectious disease as a result of suppression of the immune system (Kuiper-Goodman, 1985; Miller and Atkinson, 1987). Aflatoxins have been associated with diabetes, liver cancer, chronic gastritis and certain respiratory diseases (Bhat *et al.*, 1989).

### **2.7.2 Mycotoxins and animal health**

Animals demonstrate variable susceptibility to mycotoxins. This depends on genetic, physiological and environmental factors. All ages are affected by mycotoxins but young animals are more susceptible (IFST, 2009). Animal mycotoxicoses associated with trichothecene producing *Fusarium* includes haemorrhagic syndrome, feed refusal, emetic syndrome, ill thrift, gastrointestinal lesions, economic impact of reduced animal productivity, increased incidence of disease due to immune suppression, interferences with reproductive capacity, scabby grain intoxication or red-mould disease as well as death due to mycotoxin poisoning (D'Mello *et al.*, 1999; Frank *et al.*, 2007; WHO, 2001).

In comparison to other animals, poultry species tend to be resistant to effects of fumonisin, DON, and ZEA. However, the presence of these mycotoxins in poultry feeds is an indication that molds

have occurred and this can generate other mycotoxins as well as reduce the nutritive value and palatability of feeds. Therefore, the presence of fumonisins, DON or ZEA in poultry feeds is of concern (Frank *et al.*, 2007).

Deoxynivalenol has been associated with reduced milk production in dairy cattle, vomiting by swine consuming contaminated feeds or feed refusal, inhibition of reproductive performance and immune function in several animal species (Frank *et al.*, 2007). Zearalenone mimics the effects of estrogen. At high doses, zearalenone interferes with conception, ovulation, implantation, fetal development and viability of newborn animals. At lower doses, zearalenone increases the size or early maturity of mammary glands and reproductive organs (Frank *et al.*, 2007).

T-2 toxin and its chemical relatives cause irritation, hemorrhage and necrosis throughout the digestive tract, depress the regenerative process in the bone marrow and spleen, impair immune system function and cause changes in reproductive organs. Clinical signs in affected animals include weight loss, poor feed utilization, lack of appetite, vomiting, bloody diarrhea, abortion and in severe cases death. Fumonisins impair immune system function, damage liver and kidney, decrease weight gains and increase mortality rate as well as cause leukoencephalomalacia in horses and respiratory difficulties in swine (Frank *et al.*, 2007).

## **2.8 Mycotoxin detection and quantification**

There are several methods that have been used to detect and quantify mycotoxins (Pronk *et al.*, 2002). These are; Thin-layer chromatography (TLC), High-performance liquid chromatography (HPLC) with post or pre-column derivatization and either UV detection, fluorescence detection (FLD) or mass spectrometric (MS) detection, supercritical fluid chromatography (SCF), capillary gas chromatography (GS) with either electron-capture detection (ECD), flame ionization detection (FID) or MS detection (Schothorst and Jekel, 2001). For screening purposes, Enzyme Linked Immuno-Sorbent Assay (ELISA) can be used (Krska *et al.*, 2001). Samples for mycotoxin detection with ELISA do not require clean up after extraction and therefore the method can be applied directly to the crude extract and the results are quickly available. Enzyme Linked Immuno-sorbent Assay methods are very sensitive and allow simultaneous analysis of

many samples. No short operator training is required and it is easy to use to give quantitative results with little investment. However, the method has high uncertainty of the results.

## 2.9 Regulation of mycotoxin contamination in wheat

There are legal limits for *Fusarium* mycotoxins in wheat intended for human consumption and guidance limits for grain for feed (HGCA, 2012). The limits are set to avoid highly contaminated wheat from entering the food chain as well as to encourage all measures to minimize *Fusarium* mycotoxins contamination to be taken in the field and storage stages of the production chain (FSA, 2007). The limits and regulations are influenced by various factors, both scientific and socioeconomic. Crop assurance schemes have been designed to help farmers comply with food law (HGCA, 2012). Standards have been set by various organizations including; Kenya Bureau of Standards (KEBS), European Commission (EC), Food and Drug Administration (FDA), on the maximum of each mycotoxin acceptable in food and feed. The limits differ with the kind of food and the mycotoxin type. *Fusarium* toxins limits in cereals for human consumption are expressed in µg/kg (ppb). Mycotoxin limits in cereals for animal consumption are expressed in mg/kg (ppm). There are no standards for certain mycotoxins in foods and feeds.

**Table 1:** Maximum limits of various mycotoxins (µg/kg) in cereals based on EU, FDA, and KEBS standards

Mycotoxin	Foodstuff/feedstuff	EU	FDA	KEBS
Deoxynivalenol	Unprocessed cereals and their products	1750		
	Processed cereals	1000	1000	
	Animal feeds		5000	
Zearalenone	Unprocessed cereals(except maize)	100		
	Cereals for direct human consumption	75		
T-2 toxin and HT-2 toxin	Cereals	100	500	
Fumonisin	Unprocessed maize	4000		1000
	Maize based food for human consumption	1000		
Aflatoxin	All cereals and products	4	20	10
Ochratoxin	Unprocessed cereals	5		
	Products for unprocessed cereals	3		

\*parts per billion (µg/kg)

EU –European Union; FDA – Food and Drug Authority; KEBS – Kenya Bureau of Standards

Source: [services.leatherheadfood.com/eman/FactSheet.aspx?ID=79](http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=79)

Alakonyai *et al.*, (2009); KEBS, (2007)

## 2.10 Strategies for mycotoxin control and prevention

The ideal control measure for mycotoxins is their elimination from the food chains (Negedu *et al.*, 2011). However, this is practically impossible because most fungal producers of the mycotoxins are naturally found in the soil and air, making it difficult to prevent their contact with agricultural products (Negedu *et al.*, 2011). Control of factors which enhance molds and their toxin production can help reduce mycotoxin contamination of agricultural commodities. Prevention and reduction of mycotoxin production can be achieved by avoiding maize as the previous crop and minimizing previous crop residues on the soil surface, choosing more resistant varieties, weed and insect control, timely harvest and drying, use of fungicides and use optimum nutrient inputs (FSA, 2007). The most important of these factors are high moisture content (20 to 25%), high relative humidity (70% and above), and warm temperature (20 to 30°C) (Ma *et al.*, 2002; Langseth *et al.*, 1995). Insects and mites damage pre-dispose crops to mold invasion and mycotoxin production. These can be controlled by biological antagonism (Galvano *et al.*, 2001), chemical inhibition (Fanelli *et al.*, 2003; Hope *et al.*, 2003) and plant breeding to achieve more resistant cultivars (Ma *et al.*, 2002). The use of multi-resistant cultivars significantly reduces use of fungicides in low input cropping systems (Champeil *et al.*, 2004).

Fungal growth and mycotoxin production in stored grains and seeds can be controlled by properly drying and maintenance of the dry state. Drying should take place as soon after harvest and as rapidly as feasible (GASGA, 1997). Maintenance of foods below a water activity of 0.7 is an effective technique used throughout the world for controlling fungal spoilage and mycotoxin production in foods. It is also important to avoid grain damage before and during drying and storage as damaged grains are more susceptible to fungal infection and therefore mycotoxin contamination. Keeping numbers of insects in stored grains at a minimum reduces grain damage and accumulation of moisture from insect activity and thus control fungal growth and mycotoxin (GASGA, 1997). Proper storage conditions of moisture and temperature, proper ventilation and humidity reduce mycotoxin production in stored grains.

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Study area

A field survey was conducted in Narok County, one of Kenya's leading wheat growing regions. The County has altitude ranging from 1400 to greater than 2400 m above sea level and a temperature range between 8°C and 28°C. The County receives two rainy seasons with annual rainfall range from 500 mm to 1000 mm, with up to 1800 mm in higher altitudes (Gamba *et al.*, 2003; Ministry of Agriculture, 1987). Narok County has sandy loam soils developed from igneous rocks that are shallow to deep and excessively drained. The agro-ecological zones where wheat is grown are lower highland 3 (LH3), lower highland 2 (LH2), upper highland 3 (UH3) and upper highland 2 (UH2) (Table 2) (Ministry of Agriculture, 1987).

**Table 2:** Characteristics of wheat growing agro-ecological zones of Narok County

AEZ	Description	Annual average rainfall (mm)	Altitude (m)
LH3	Wheat/Maize-Barley zone	900- 1100, 850 - 1050	1850 - 2150
LH2	Maize/Wheat-Pyrethrum zone	1100 -1300, 100 - 1200	1980 - 2280
UH3	Wheat-Barley zone	1000 - 1200	2150 - 2370
UH2	Wheat-Pyrethrum zone	1100 -1800	2280 - 2970

Source: Ministry of Agriculture and GTZ (2007); Ministry of Agriculture, 1987

### 3.2 Determination of wheat production practices in Narok County

#### 3.2.1 Survey and sampling design

A field survey covering 51 wheat farms was carried out between June and October 2013, at mid anthesis (GS 65-69) and at harvest (GS 92), in three agro-ecological zones (AEZ); 15 wheat farms in lower highland 3 (LH3), 18 in lower highland 2 (LH2), and 18 in upper highland 3 (UH3). This was done by selecting in a transect the fourth wheat growing farm. A semi structured questionnaire (Appendix 1) was used to obtain information on wheat production practices. Information which was gathered included cultivars of wheat cultivated, sources of planting seeds, field preparation practices, pre-season practices, rotation programs and insect pests and diseases management among others.



### **3.2.2 Sample collection**

At mid anthesis (GS 65-69), approximately 100g of top soil, 250g of crop residues and wheat ears were sampled in each farm. Soil was collected randomly from the top 5cm from five different points in the farm then mixed to make a composite sample from which 100g sub-sample was taken for fungal isolation. Six to ten wheat ears were cut randomly from different points in the farm. At harvest (GS 92), freshly harvested kernels were sampled in a Kraft bag for mycological and mycotoxin analysis, from the same farms where the first sampling was done. Wheat kernels at harvest were sampled by fetching approximately half a kilo of composite sample from the total harvest of the whole farm. The samples were stored at 4°C until analyzed.

### **3.3 Assessment of FHB incidence and severity**

Incidence of *Fusarium* head blight was determined at mid anthesis (GS 65-69), as the number of blighted heads over the total number of heads within five, 1m<sup>2</sup> randomly selected quadrants in each farm, while severity was measured on a scale of 0 – 9 as the average proportion of the bleached spikelets (1 = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 25-44%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%; Miedaner *et al.* 1996).

### **3.4 Determination of the incidence of *Fusarium* spp. in soil, crop residues, wheat ears and wheat kernels**

#### **3.4.1 Preparation of culture media**

Fungal pathogens were isolated on low strength potato dextrose agar (PDA) amended with minerals and antibiotics (Muthomi, 2002): (PDA 17g, KH<sub>2</sub>PO<sub>4</sub> 1.0g, KNO<sub>3</sub> 1.0g, MgSO<sub>4</sub> 0.5g, Agar 10g). Fifty milligrams of penicillin, tetracycline, streptomycin and pentachloronitrobenzene (PCNB) each were added after the media was cooled to 45°C. Isolated *Fusarium* colonies were subcultured on PDA and synthetic nutrient agar (SNA) (Nirenberg, 1981): (KH<sub>2</sub>PO<sub>4</sub> 1.0g, KNO<sub>3</sub> 1.0g, MgSO<sub>4</sub> 0.5g, KCl 0.5g, Glucose 0.2g, Agar 20g).

### 3.4.2 Isolation of fungi from soil, wheat ears, crop residues and wheat kernels

The soil samples were prepared for isolation by sieving through a number 22 standard testing sieve (0.833 mm opening) to get a fine powder. Isolation from soil samples was done by dissolving 1g of the sample in 9 ml of sterile distilled water to form a stock solution ( $10^{-1}$  dilution), which was serially diluted to  $10^{-3}$ . Two hundred microliter aliquots of the  $10^{-3}$  dilution were plated on low strength PDA modified with minerals and antibiotics and each sample replicated three times. The wheat spikelets and crop residues were cut into small pieces (0.5cm long) while for the kernels, sub samples were taken randomly. Spikelets, crop residues and wheat kernels were surface sterilized in 3% sodium hypochlorite for three minutes and rinsed three times in sterile distilled water. Five pieces of crop residues, spikelets and kernels were plated on each petri plate on low strength PDA and replicated three times. The plates were incubated for 5-7 days at 25°C under 12h day light and 12h darkness cycles. A count of the total number of infected spikelets, residues and kernels per plate was made and different fungal genera and *Fusarium* spp. colonies per Petri dish were made for soil samples. The number of colony forming units per gram (CFU/g) was calculated as follows:

$$\text{Number of fungi/g soil} = \frac{\text{no. of colonies}}{\text{amount plated} \times \text{dilution}}$$

The relative isolation frequency of each genus was calculated as follows (Gonzalez *et al.*, 1999):

$$\text{Frequency (\%)} = \frac{\text{number of isolates of a genus}}{\text{total number of genera}} \times 100$$

### 3.4.3 Identification of *Fusarium* species

*Fusarium* colonies were sub-cultured on PDA and SNA. *Fusarium* cultures on SNA were incubated at 25°C at near UV-light to facilitate sporulation while those on PDA were incubated at 25°C for 14-21 days. Slide cultures of *Fusarium* spp. were made by placing SNA agar squares on a microscope slide raised with a V-shaped glass rod in a sterile glass petri plate covered with a sterile filter paper at the bottom. Some of the *Fusarium* mycelia or spores were transferred from the isolate to the four edges of the agar block using an inoculating needle. A clean cover slip was placed on the surface of the agar block, the filter paper wetted with sterile distilled water

and the plate partially sealed with parafilm™ and incubated at 25°C for 14-21 days. Slides for light microscopy were prepared by removing the agar block and then adding a drop of lactophenol cotton blue on the slide and cover slip added to cover the growth. The slide preparations were used for identification and taking photos of morphological characteristics of common *Fusarium* spp. isolated at ×1000 using light microscope (LEICA DM 500, Leica Microsystems, Wetzlar, Germany). The microscope was fitted with a camera (LEICA ICC 50, Leica Microsystems, Wetzlar, Germany). Wet mounts for identification of other fungal genera were prepared by transferring some spores onto a microscope slide with a drop of lactophenol cotton blue and carefully placing a coverslip.

Fungal genera were identified based on the cultural and morphological characteristics such as mycelia color, colony pigmentation, spore shape, septation and sporophores. *Fusarium* spp. was identified to species level using manuals by Nelson *et al.*, (1983) and Leslie and Summerell, (2006). *Fusarium* cultures on PDA were used for cultural characterization based on growth, presence of aerial mycelium, color of aerial mycelium and reverse colony color while cultures on SNA were used for microscopic identification based on spore and conidiophore morphology. Features for microscopic identification were macroconidia morphology: size, shape of basal and apical cells; microconidia: present or absent, whether produced on chains or false heads, shape: type of conidiophores, types of phialides and chlamydospores: present or absent, arrangement.

### **3.5 Determination of mycotoxin levels in wheat kernels at harvest**

#### **3.5.1 Sample preparation and mycotoxin extraction**

One hundred grams of wheat kernels sampled at harvest (GS 92) were ground to fine powder. Five grams of the ground sample were extracted with 25ml of distilled water for deoxynivalenol and 25ml of methanol/ distilled water (70:30v/v) for T-2 toxin. The extracts were stirred on a magnetic stirrer for 3 and 10 minutes for DON and T-2 toxin, respectively. The extracts were then centrifuged at 350 revolutions per minute for 10 minutes. Samples for T-2 toxin were diluted with an equal volume of sample dilution buffer provided in the Ridascreen® T-2 toxin kit.

### **3.5.2 Mycotoxin detection and quantification**

Mycotoxin content in the wheat kernels were determined by direct competitive Enzyme Linked Immuno-Sorbent Assay (ELISA) (AOAC, 1995; Gareis *et al.*, 1989; Gathumbi, 2001). The 96 well microlitre polystyrene (Maxisorp<sup>®</sup>, Nunc, Denmark) provided in the Ridascreen<sup>®</sup> DON (Art. No. : R5906) and Ridascreen<sup>®</sup> T-2 toxin (Art. No. : R3801) kits plates were filled with 50µl of standard or prepared samples, using a new pipette tip for each standard or sample. Fifty microlitres of enzyme conjugate for each toxin and 50µl of anti-deoxynivalenol antiserum DON143/16 for DON and T-2 toxin antibody were added to each well. The liquids were mixed by shaking gently and incubated at room temperature for 30 minutes for DON and 1 hour for T-2 toxin. The liquids were damped out of the wells into a sink and the wells washed using washing buffer. One hundred microlitres of DON substrate/chromogen and 50µl of both T-2 toxin substrate and chromogen were added to each well and incubated in the dark for 15 min for DON and 30 min for T-2 toxin. 100µl of stop solution was added, shaken gently and absorbance determined using the spectrophotometer ELISA reader (RIDA<sup>®</sup>SOFT Win) at 450nm wavelength. A calibration curve for the standards for each toxin was plotted using log<sub>10</sub> of standards concentration against the percentage inhibition of the standards and used to determine the concentrations of the toxins in the samples in parts per billion.

### **3.6 Data Analyses**

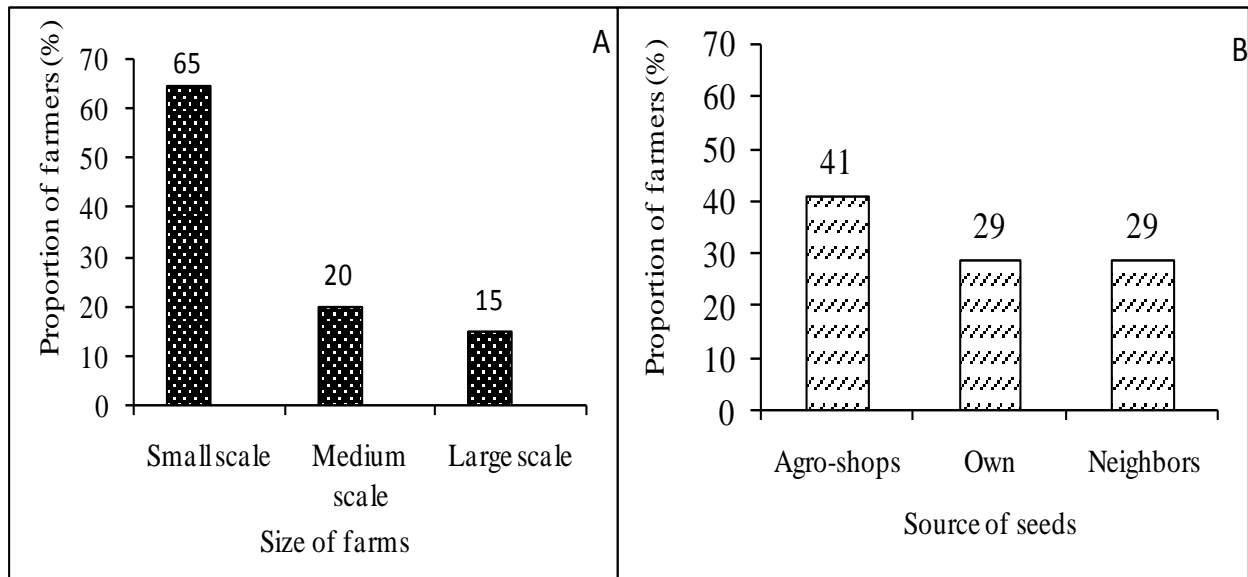
Survey data was reported as percentage. Data obtained from isolations was subjected to Analysis of Variance (ANOVA) using the PROC ANOVA procedure of GENSTAT version 15 and differences among the treatment means were compared using Fisher's Protected LSD at 5% probability level. Data that was not normally distributed was transformed to arcsine before analysis.

## CHAPTER FOUR: RESULTS

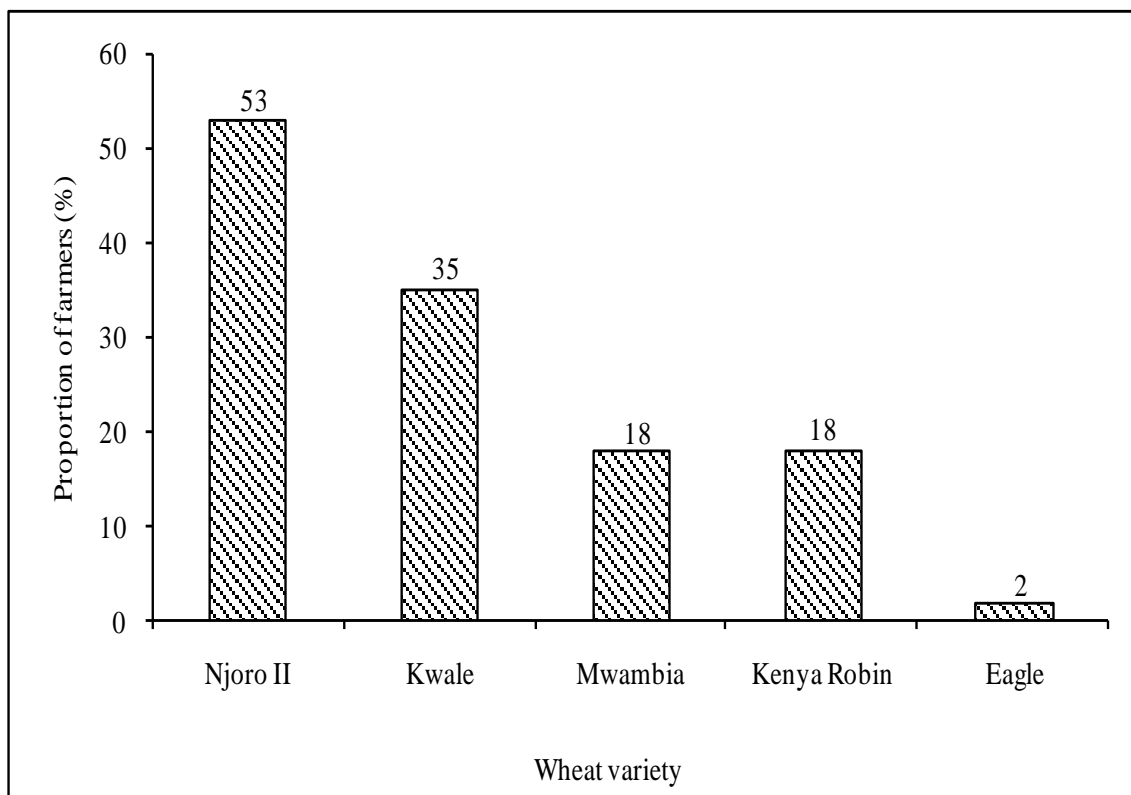
### 4.1 Wheat production practices in Narok County, Kenya

#### 4.1.1 Agronomic practices

Small scale farms were classified as farms which had less than 8 hectares, medium scale farms ranged between 8 and 20 hectares while farms greater than 20 hectares are large scale. Majority (65%) of wheat farmers in Narok County were small scale while the rest were medium scale and large scale (Figure 1A). Most wheat farmers did not have a specific place where they sourced their seeds for planting. The farmers mainly used their own seeds and from neighbors and then mixed them before planting. However, there were those who obtained planting seeds from agro-shops and other sources (Figure 1B). Five varieties of wheat - Kwale, Njoro II, Mwambia, Kenya robin II, and Eagle - were grown by wheat farmers in Narok County (Figure 2). The most popular variety across all the agro-ecological zones was Kwale, which was grown by 53% of farmers while the least popular was eagle grown by only 2% of the farmers, in LH2.



**Figure 1:** Percentage of farmers growing wheat in different farm sizes (A) and sources of wheat seeds (B) in Narok County

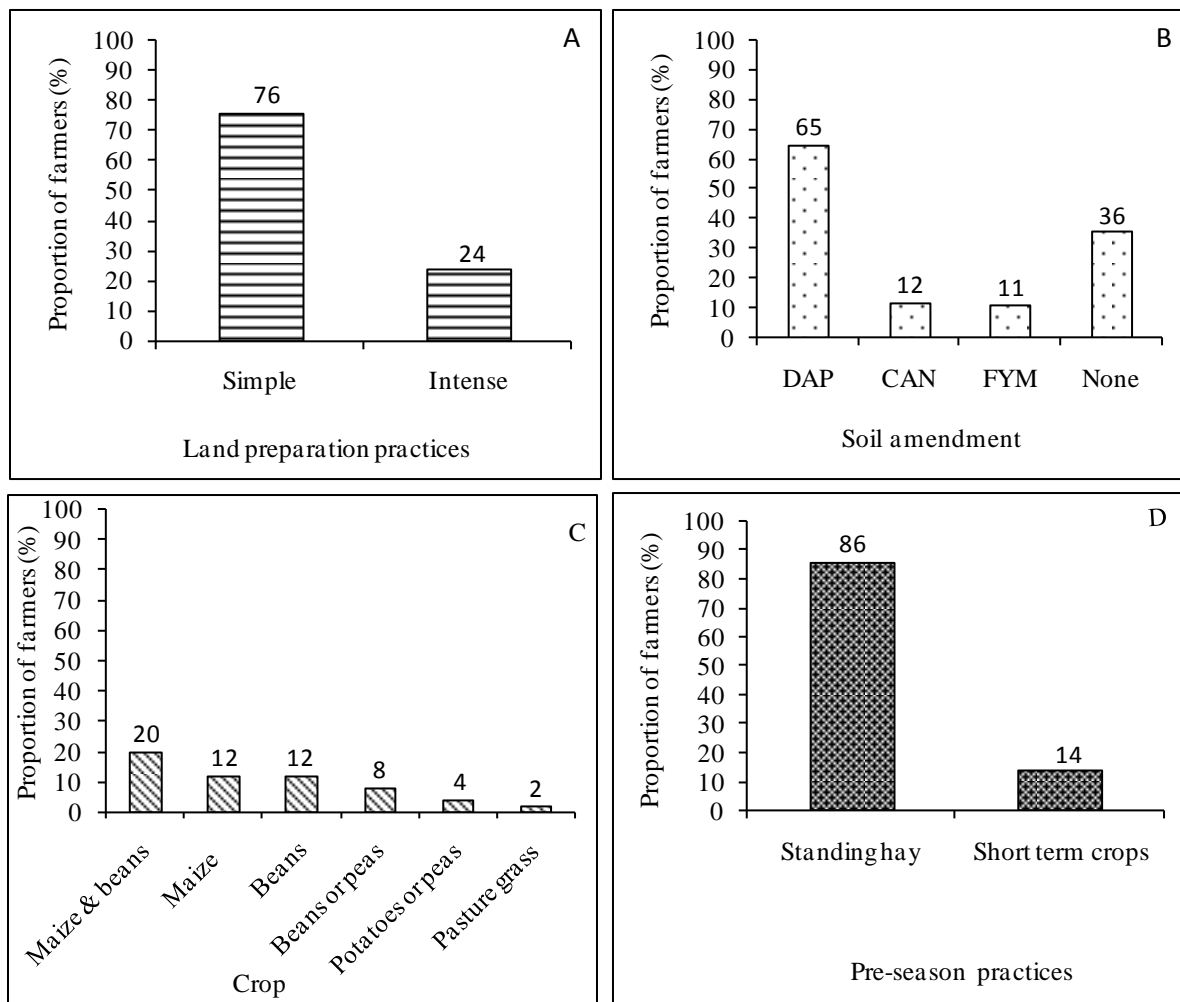


**Figure 2:** Percentage of farmers growing different wheat varieties during the 2013 cropping season in Narok County

At the start of the season, wheat farmers in Narok County used diverse field preparation practices. Tillage methods included ploughing, harrowing and chisel ploughing (Figure 3A). Combinations and intensity of these cultivation methods resulted in the tillage being simple or intense. Majority of the farmers practiced simple land preparation practices - ploughing and harrowing - while the rest practiced chisel ploughing and/or harrowing. However, all farmers used the tractor plough for land preparation. Prior to planting, after ploughing, some farmers applied the herbicide Round-up<sup>®</sup>. Herbicide Round-up<sup>®</sup> is a broad spectrum systemic herbicide that is used to kill broad-leaf weeds and grasses. At planting, majority of wheat farmers (65%) applied diammonium phosphate (DAP) while the others did not apply any soil amendment (Figure 3B). A small number of farmers who did not apply DAP while planting wheat applied

farmyard manure when planting other crops like maize and potatoes. Additionally, 12% of wheat farmers who used DAP at planting also applied CAN foliar fertilizer on wheat before heading stage.

Intercropping of wheat with other crops was not common in Narok County. However, 2% of the farmers intercropped wheat with the pasture grass, *Boma rhodes*. Wheat farmers also cultivated other crops in the farms between wheat cropping seasons or in rotation programs. Other crops commonly grown by the wheat farmers were maize, beans, peas and potatoes (Figure 3C). The proportion of wheat farmers that practiced crop rotation was 57% while the rest of the farmers cultivated wheat in consecutive seasons. When wheat was rotated with maize, majority (98%) of the farmers left maize stovers as standing fodder for livestock; with a few (2%) burning the residues before land preparation. Majority ( $\geq 50\%$ ) of the farmers grew wheat and maize in neighboring fields. Most farmers (86%) in Narok County grew wheat for one season annually. After harvesting, wheat residues were left as standing hay for livestock consumption until the next wheat cropping season (Figure 3D). Livestock were left to graze freely in the farms and neighboring farms, where wheat or maize were mainly grown. The rest (14%) of the wheat farmers planted short term crops like beans, peas and tomatoes between wheat cropping seasons.



**Figure 3:** (A) Proportion of farmers practicing various methods of land preparation, (B) Applying various soil amendments, (C) Other food crops grown in fields where wheat was cultivated and (D) Handling of wheat residues after harvesting and pre-season practices

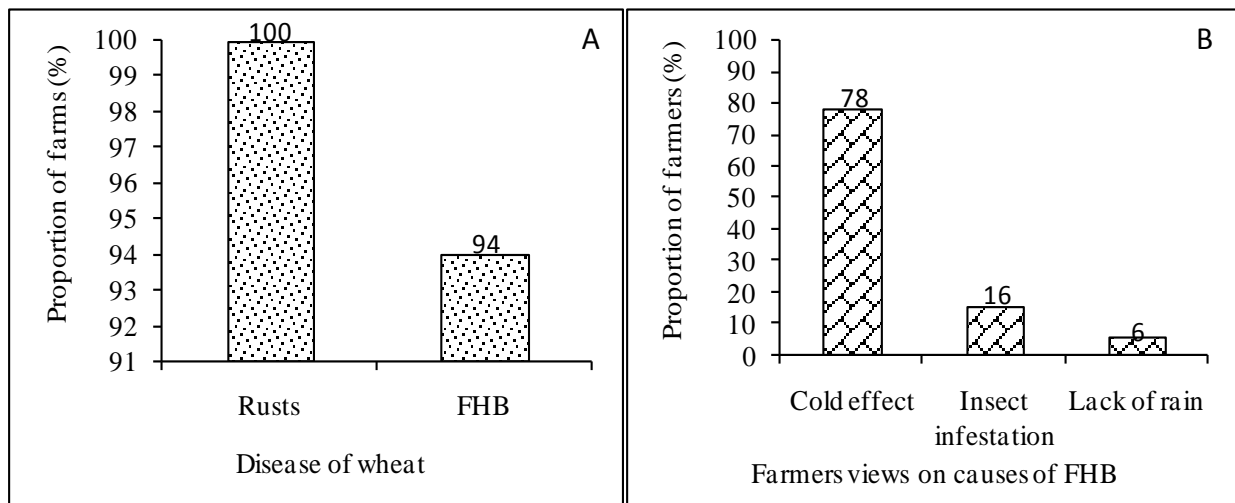
DAP – Diammonium phosphate; CAN – Calcium Ammonium Nitrate; FYM – Farm Yard Manure

#### 4.1.2 Diseases associated with wheat in Narok County

The most common diseases affecting wheat in Narok County were stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*) and *Fusarium* head blight (Figure 4A). Rusts were more prevalent with the farmers having greater knowledge on stem rust than leaf rust. Although many farmers (94%) could describe FHB or identify it in the fields, majority (63%) of the farmers



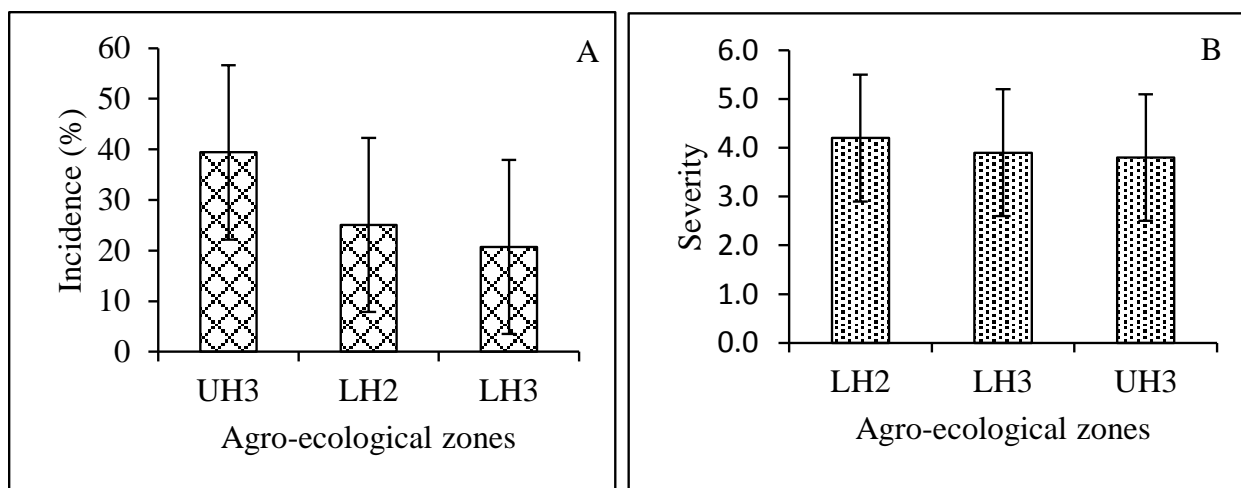
thought that FHB was “caused” by cold while others (37%) considered it to be another symptom of rust. After showing photos of wheat ears manifesting symptoms of FHB to the farmers, 94% of the farmers said it was familiar and common. Even after showing the photos of FHB diseased wheat ears, majority (78%) of the farmers referred to the disease as “cold effect”, 16% considered it to be insect infestation while 6% thought it was early maturity due to lack of enough rains (Figure 4B). The farmers applied the same fungicides such as Folicur<sup>®</sup> in managing rust and FHB while those that considered it to be caused by insect infestation applied common insecticides such as Bulldock<sup>®</sup>. The most common insect pest of wheat was aphids.



**Figure 4:** Farmers views on (A) Diseases associated with wheat, (B) Causes of FHB

#### 4.2 Prevalence, incidence and severity of FHB of wheat in Narok County

The prevalence of FHB in Narok County was 100%. The disease incidence and severity varied among farms and among agro-ecological zones (Figure 5). In majority of the wheat farms there was inter-farm variability in FHB incidence and severity. Incidence of FHB varied between 1% and 100% while severity varied from 1 to 95%. There were no significant ( $p \geq 0.05$ ) differences in FHB severity among the three AEZs but incidence significantly ( $p \leq 0.05$ ) varied among the AEZs. Infected wheat ears were characterized by bleaching (Figure 6).



**Figure 5:** Incidence and severity of FHB of wheat in different agro-ecological zones in Narok County, Kenya

<sup>a</sup>Severity score based on scale by Miedaner *et al.* (1996): 1 = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 25-44%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%  
 Bars accompanied by Least Significant Difference (LSD) values ( $p \leq 0.05$ ).



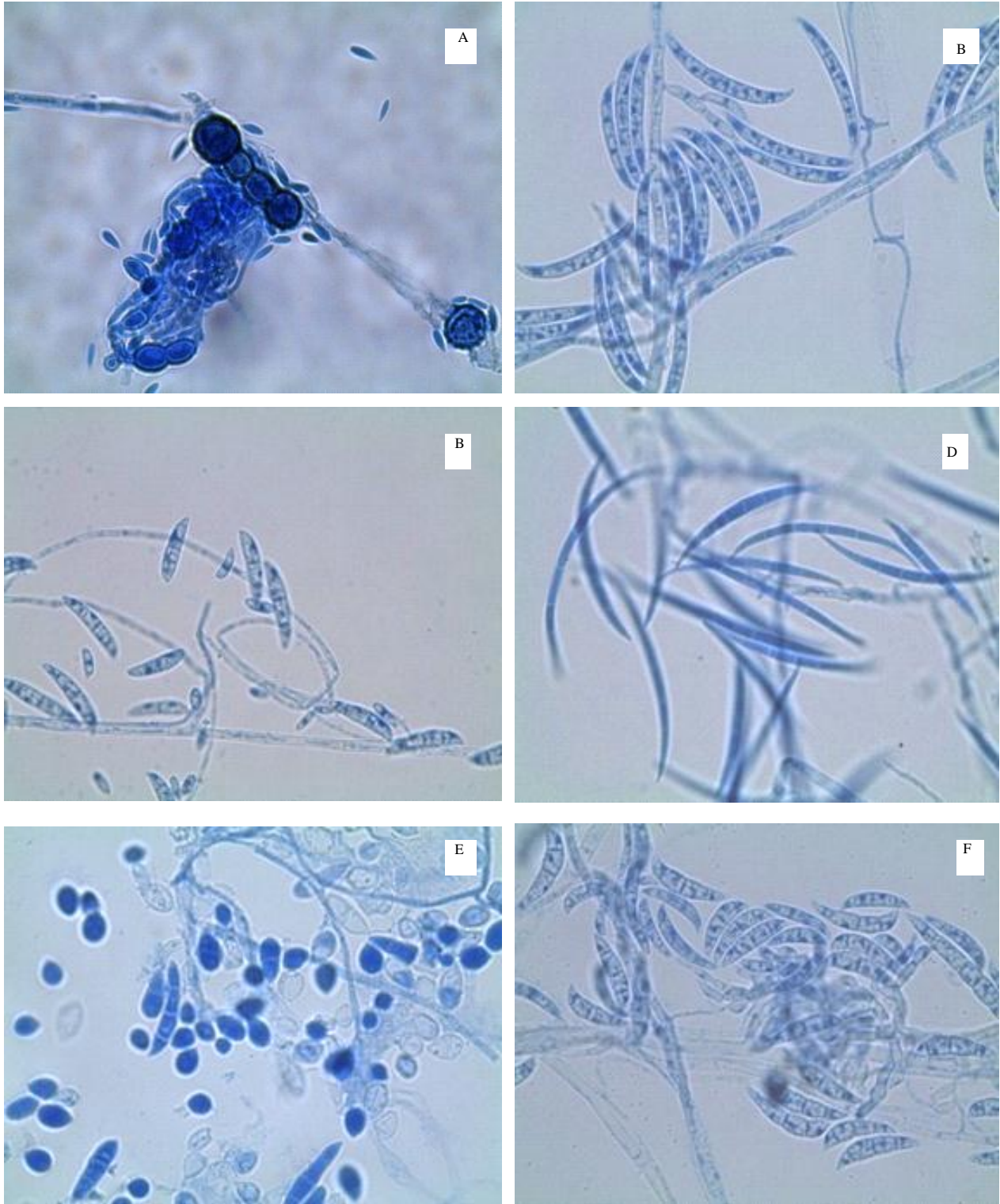
**Figure 6:** Wheat ears showing bleaching symptoms caused by FHB infection in Narok County, Kenya

#### 4.3 Morphological characteristics of major *Fusarium* spp. in crop residues, soil, wheat ears and kernels sampled in wheat fields in Narok County

*Fusarium chlamydosporum*, the predominant species from crop residues produced comma shaped, non-septate microconidia and chlamydospores that occurred singly, in chains and in clumps on the aerial mycelia (Figure 7A). *Fusarium graminearum* isolates produced thick walled macroconidia that were moderately curved and had a well developed foot shaped basal

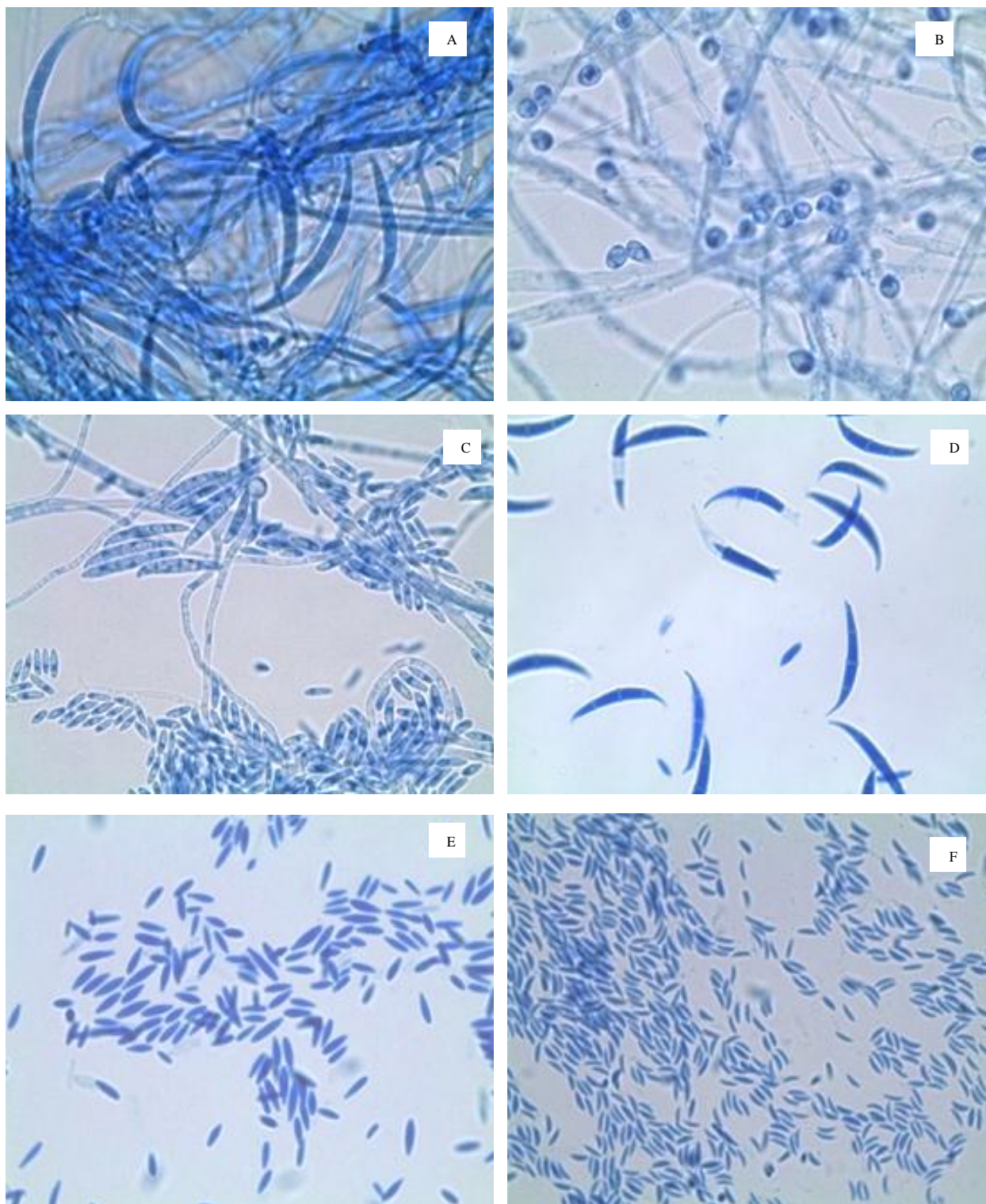
cell (Figure 7B). The predominant species isolated from soil, *F. oxysporum*, produced non-septate kidney shaped microconidia, slightly curved 3-septate macroconidia and smooth walled, singly held chlamydospores (Figure 7C). *Fusarium avenaceum* and *F. tricinctum* were the predominant species in wheat ears and kernels, respectively. *Fusarium avenaceum* produced long and slender macroconidia with some isolates producing 1- to -2 septate fusoid microconidia (Figure 7D). *Fusarium tricinctum* produced napiform and oval shaped, non – 1 septate microconidia with some having a papilla and relatively slender, falcate to almost lunate and up to 3 - septa macroconidia (Figure 7E). *Fusarium sambucinum* produced falcate, comparatively short and uniform sized macroconidia that had pointed apical cell and foot shaped basal cell (Figure 7F). *Fusarium equiseti* produced long slender macroconidia with pronounced dorsal ventral curvature and abundant chlamydospores that occurred in chains and clumps (Figure 8A).

*Fusarium poae* produced abundant globose, non - septate microconidia that had a papilla and found in clusters (Figure 8B). *Fusarium proliferatum* produced both micro- and macroconidia (Figure 8C). *Fusarium proliferatum* macroconidia were relatively slender, relatively straight, thin walled and had curved apical cell, poorly developed basal cell and 3 – 5 septa while the microconidia were club to pyriform shaped, non-septate and with a flattened base. *Fusarium scirpi* produced microconidia, macroconidia and chlamydospores (Figure 8D). The macroconidia were relatively slender but widest at the middle, with strong pronounced dorsal ventral curvature, tapering elongated apical cell and well developed foot cell, whereas the microconidia were ellipsoidal shaped, non-septate and few. *Fusarium verticillioides* produced oval to club shaped, non-septate microconidia that were in long chains and aggregates (Figure 8E). *Fusarium nivale* produced abundant comma shaped macroconidia (Figure 8F). *Fusarium solani* produced oval shaped, non - 1 septate microconidia and less frequently, wide, straight and stout macroconidia (Figure 9A). *Fusarium semitectum* produced abundant pyriform to obovate microconidia that were non-1 septate but occasionally 2-3 septate (Figure 9B). *Fusarium subglutinans* produced oval, non - septate microconidia on false heads on the aerial mycelia (Figure 9C).



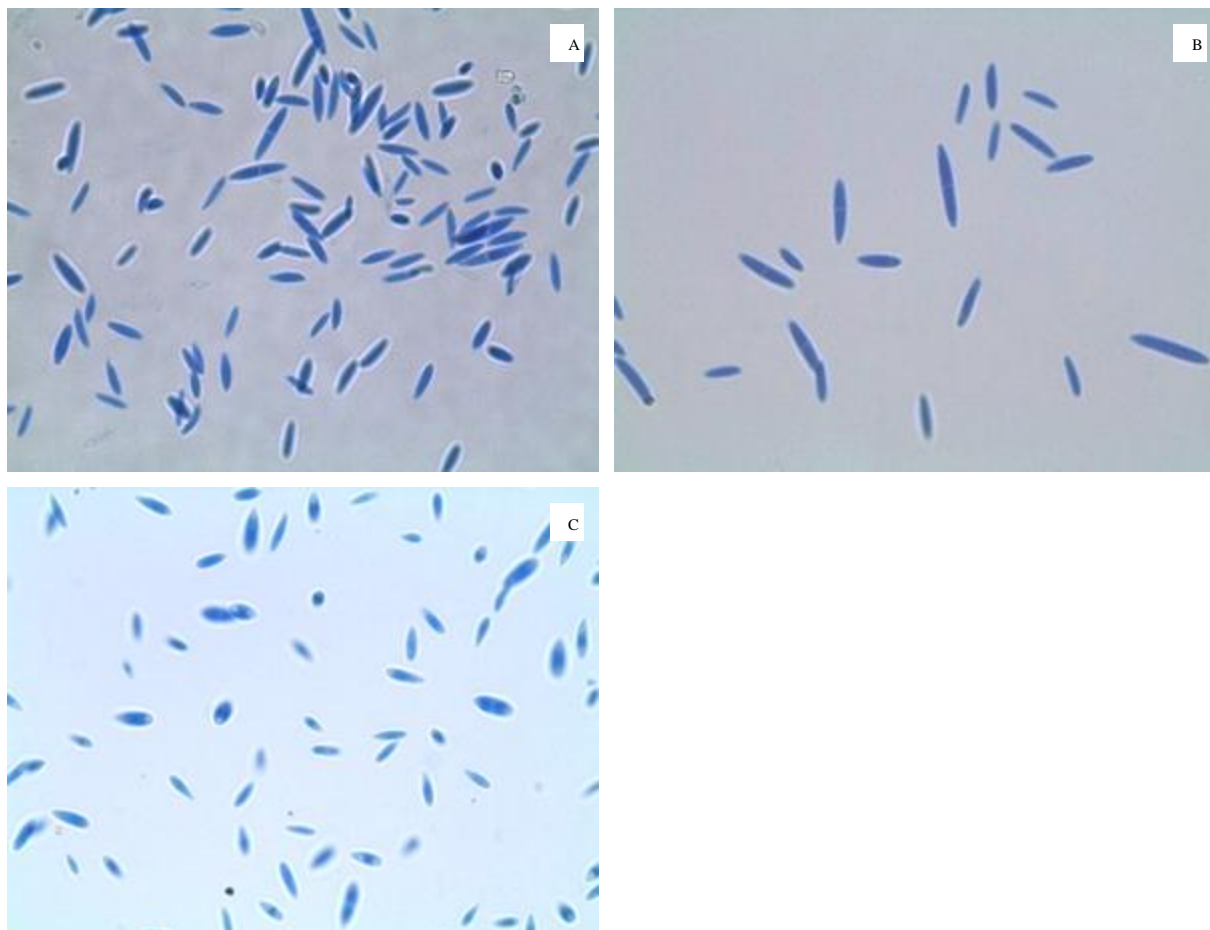
**Figure 7:** Morphological characteristics of *Fusarium* spp. isolated from crop residues, soil, wheat ears and kernels from wheat farms in Narok County

A: *F. chlamydosporum*; B: *F. graminearum*; C: *F. oxysporum*; D: *F. avenaceum*; E: *F. tricinctum*; F: *F. sambucinum*



**Figure 8:** Morphological characteristics of *Fusarium* spp. isolated from crop residues, soil, wheat ears and kernels from wheat farms in Narok County

A: *F. equiseti*; B: *F. poae*; C: *F. proliferatum*; D: *F. scirpi*; E: *F. verticillioides*; F: *F. nivale*



**Figure 9:** Morphological characteristics of *Fusarium* spp. isolated from crop residues, soil, wheat ears and kernels from wheat farms in Narok County

A: *F. solani*; B: *F. semitectum*; C: *F. subglutinans*

#### 4.4 Incidence of *Fusarium* spp. in crop residues, soil, wheat ears and kernels sampled in wheat fields in different agro-ecological zones of Narok County

##### 4.4.1 Incidence of *Fusarium* spp. in crop residues

Fungal pathogens isolated from crop residues were *Fusarium* spp., *Pythium* spp., *Trichoderma* spp., *Chaetomium* spp., *Rhizoctonia* spp., *Aspergillus* spp., *Helminthosporium* spp., *Macrophomina* spp. and *Epicoccum* spp. (Table 3). Other fungi isolated in low frequencies included *Alternaria* spp., *Colletotrichum* spp., *Curvularia* spp., *Cladosporium* spp. and

*Penicillium* spp. The incidence of fungal pathogens in crop residues varied significantly ( $p \leq 0.05$ ) with an overall infection rate of 117%, where 54% were *Fusarium* spp. Seventeen percent of the residues had multiple fungal infections. The isolation frequency of the fungal pathogens significantly ( $p \leq 0.05$ ) varied among the agro-ecological zones. *Fusarium* spp. and *Pythium* spp. were isolated in the highest frequency in the three agro-ecological zones with a mean incidence of 5.1 and 2.2%, respectively.

The major *Fusarium* spp. isolated from crop residues in decreasing order were: *F. chlamydosporum*, *F. graminearum*, *F. equiseti*, *F. sambucinum*, *F. scirpi* and *F. avenaceum* (Figure 10). The incidence of *Fusarium* spp. in crop residues varied significantly ( $p \leq 0.05$ ) among the AEZs. *Fusarium chlamydosporum* was the most prevalent in LH3, *F. graminearum* in LH2 while *F. equiseti* in UH3 (Table 4).

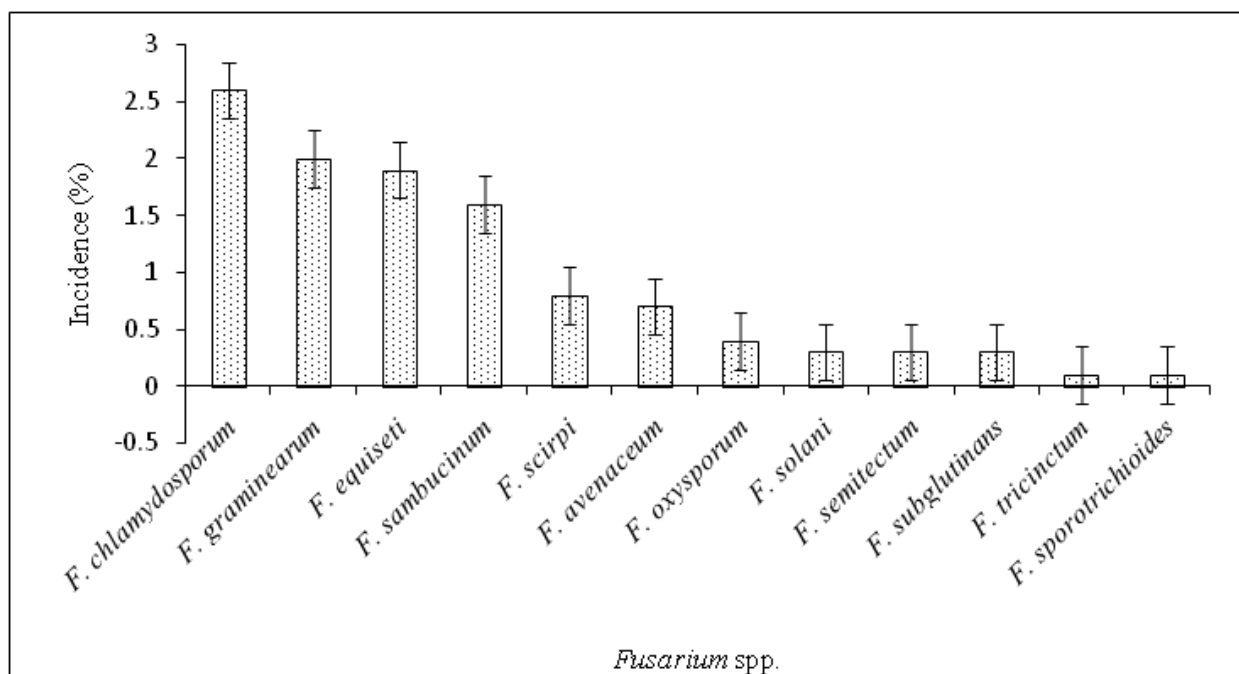
**Table 3:** Isolation frequency (%) of fungal pathogens from crop residues sampled in wheat fields in different agro-ecological zones of Narok County

Fungal spp.	LH3	LH2	UH3	Mean
<i>Fusarium</i>	4.9 a	5.0 a	5.5 a	5.1 a
<i>Pythium</i>	2.3 b	2.6 b	1.7 b	2.2 b
<i>Trichoderma</i>	0.4 d	1.5 c	1.7 b	1.2 c
<i>Chaetomium</i>	0.2 de	1.1 cd	1.0 c	0.8 d
<i>Rhizoctonia</i>	0.9 c	0.7 de	0.7 cd	0.8 d
<i>Aspergillus</i>	0.0 e	0.3 e	0.0 e	0.1 e
<i>Helminthosporium</i>	0.0 e	0.0 e	0.3 de	0.1 e
<i>Macrophomina</i>	0.0 e	0.2 e	0.0 e	0.1 e
<i>Epicoccum</i>	0.1 de	0.1 e	0.0 e	0.1 e
Others <sup>a</sup>	0.3 de	0.7 de	0.7 cd	0.5 d
Mean	0.8	1.1	1.0	1.0
LSD ( $p \leq 0.05$ )	0.4	0.8	0.5	0.4
CV (%)	30.1	42.6	28.1	40.1

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3.

<sup>a</sup> – fungi isolated in low frequency and unidentified fungal species.



**Figure 10:** Mean isolation frequency (%) of *Fusarium* spp. from crop residues sampled from wheat fields in Narok County

Bars accompanied by standard error of the means

**Table 4:** Isolation frequency (%) of *Fusarium* spp. in crop residues sampled from wheat fields in different agro-ecological zones in Narok County

<i>Fusarium</i> spp.	LH3	LH2	UH3	Mean
<i>F. chlamydosporum</i>	4.3 a	2.2 ab	1.2 c	2.6 a
<i>F. graminearum</i>	1.8 b	2.9 a	1.3 c	2.0 ab
<i>F. equiseti</i>	0.1 e	2.0 ab	3.7 a	1.9 ab
<i>F. sambucinum</i>	0.7 de	1.5 cd	2.5 b	1.6 bc
<i>F. scirpi</i>	1.5 bc	0.1 e	0.7 cd	0.8 cd
<i>F. avenaceum</i>	0.4 e	0.7 de	1.1 b	0.7 e
<i>F. oxysporum</i>	0.4 e	0.0 e	0.9 cd	0.4 e
<i>F. solani</i>	0.3 e	0.6 cd	0.1 e	0.3 e
<i>F. semitectum</i>	0.0 e	0.4 e	0.5 cd	0.3 e
<i>F. subglutinans</i>	0.7 de	0.0 e	0.1 e	0.3 e
<i>F. tricinctum</i>	0.1 e	0.2 e	0.0 e	0.1 e
<i>F. sporotrichioides</i>	0.2 e	0.0 e	0.0 e	0.1 e
Mean	0.9	0.9	1.0	0.9
LSD ( P ≤ 0.05)	1.0	1.0	1.0	0.8
CV (%)	68.4	68.4	55.6	96.1

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3.



#### 4.4.2 Incidence of *Fusarium* spp. in soil

The major fungal genera in decreasing order were: *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp. and *Trichoderma* spp. (Table 5). *Penicillium* spp. and *Aspergillus* spp. were the most prevalent with a mean population of  $1.4 \times 10^6$  and  $4.4 \times 10^5$  CFU/g soil, respectively. The mean fungal population in the soil was  $1.8 \times 10^5$  CFU/g while the mean population of *Fusarium* spp. in was  $1.7 \times 10^5$  CFU/g. The incidence of the fungal pathogens in soil significantly ( $p \leq 0.05$ ) varied among the AEZs. *Penicillium* spp. was the most prevalent in LH3, *Aspergillus* spp. in LH2 and *Fusarium* spp. in UH3.

The *Fusarium* spp. isolated in decreasing incidence were: *F. oxysporum*, *F. proliferatum*, *F. sambucinum*, *F. equiseti*, *F. chlamydosporum*, *F. graminearum*, *F. scirpi*, *F. solani*, *F. verticillioides* and *F. nivale* (Figure 11). The incidence of *Fusarium* spp. in soil varied significantly ( $p \leq 0.05$ ) among the AEZs (Table 6). *Fusarium oxysporum* was the most prevalent in LH3 and UH3 while *F. proliferatum* was the most prevalent in LH2.

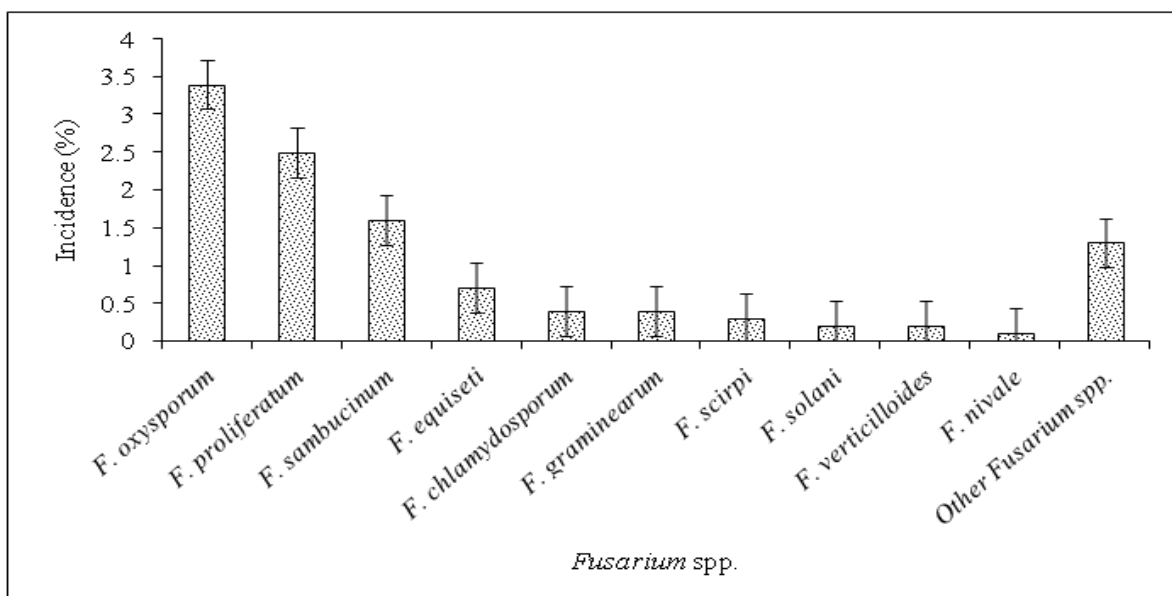
**Table 5:** Isolation frequency (%) of fungal pathogens isolated from soil sampled from wheat fields in different agro-ecological zones of Narok County

Fungal spp.	LH3	LH2	UH3	Mean
<i>Penicillium</i>	8.4 a	6.6a	3.7 a	6.2 a
<i>Aspergillus</i>	2.4 b	1.5b	1.7 b	1.8 b
<i>Fusarium</i>	1.0 c	0.7c	1.2 c	1.0 c
<i>Trichoderma</i>	0.5 d	0.6cd	0.7 de	0.7 cd
<i>Macrophomina</i>	0.1 e	0.2de	0.5 def	0.2 d
<i>Rhizoctonia</i>	0.2 de	0.2de	0.3 def	0.3 d
<i>Chaetomium</i>	0.2 de	0.2de	0.2 def	0.2 d
<i>Pythium</i>	0.1 e	0.0e	0.1 f	0.1 d
<i>Curvularia</i>	0.0 e	0.0e	0.2 ef	0.1 d
<i>Colletotrichum</i>	0.1 e	0.0e	0.1 f	0.1 d
Others <sup>a</sup>	0.3 de	0.6c	0.8 cd	0.6 cd
Mean	1.2	1.0	0.9	1.0
LSD ( $p \leq 0.05$ )	0.4	0.4	0.5	0.6
CV (%)	18.4	25.2	33.0	65.9

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3.

<sup>a</sup> – unidentified species.



**Figure 11:** Mean isolation frequency (%) of *Fusarium* spp. from soil sampled from wheat fields in Narok County

Bars accompanied by standard error of the means

**Table 6:** Isolation frequency (%) of *Fusarium* spp. in soil sampled from wheat fields in different agro-ecological zones in Narok County

<i>Fusarium</i> spp.	LH3	LH2	UH3	Mean
<i>F. oxysporum</i>	3.0 a	2.2 ab	5.1 a	3.4 a
<i>F. proliferatum</i>	2.2 ab	2.9 a	2.5 b	2.5 b
<i>F. sambucinum</i>	1.8 abcd	1.5 b	1.5 bc	1.6 c
<i>F. equiseti</i>	0.2 de	0.4 c	1.6 bc	0.7 de
<i>F. chlamydosporum</i>	0.3 cde	0.0 c	0.9 bc	0.4 d
<i>F. graminearum</i>	0.3 cde	0.2 c	0.5 c	0.4 d
<i>F. scirpi</i>	0.2 de	0.3 c	0.3 c	0.3 d
<i>F. solani</i>	0.4 bcde	0.1 c	0.0 c	0.2 d
<i>F. verticilloides</i>	0.3 cde	0.1 c	0.2 c	0.2 d
<i>F. nivale</i>	0.0 e	0.2 c	0.0 c	0.1 d
Other <i>Fusarium</i> spp.	2.1 abc	0.5 c	1.2 bc	1.3 cd
Mean	1.0	0.8	1.3	1.0
LSD ( $p \leq 0.05$ )	1.8	1.0	1.6	0.9
CV (%)	109.0	74.7	76.7	91.6

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3.

#### 4.4.3 Incidence of *Fusarium* spp. in wheat ears at hard dough stage

The major fungal pathogens infecting wheat ears were *Epicoccum* spp., *Alternaria* spp., *Chaetomium* spp. *Fusarium* spp. and *Aspergillus* spp. (Table 7). *Penicillium* spp., *Cladosporium* spp. and *Helminthosporium* spp. were isolated in low frequency (<0.1%). The mean infection rate of wheat ears was 131%, with 31% multiple infection rate of the plated wheat spikelets. *Epicoccum* spp. and *Alternaria* spp. were the most prevalent with mean infection rate of 48% and 22%, respectively. *Fusarium* spp. had a mean infection frequency of 7%. The incidence of the fungal pathogens in wheat ears significantly ( $p \leq 0.05$ ) varied among the agro-ecological zones. *Epicoccum* spp. was the most prevalent in all the agro-ecological zones.

The *Fusarium* spp. isolated from wheat ears in order of decreasing incidence were: *F. avenaceum*, *F. poae*, *F. graminearum*, *F. equiseti*, *F. chlamydosporum*, *F. sambucinum*, *F. tricinum* and *F. scirpi* (Figure 12). The incidence of the *Fusarium* spp. varied significantly ( $p \leq 0.05$ ) among the agro-ecological zones with no variability in LH3 (Table 8). *Fusarium avenaceum* was the most prevalent in all the three agro-ecological zones. *Fusarium scirpi*, *F. tricinum* and *F. subglutinans* were isolated in low frequency in the three agro-ecological zones.

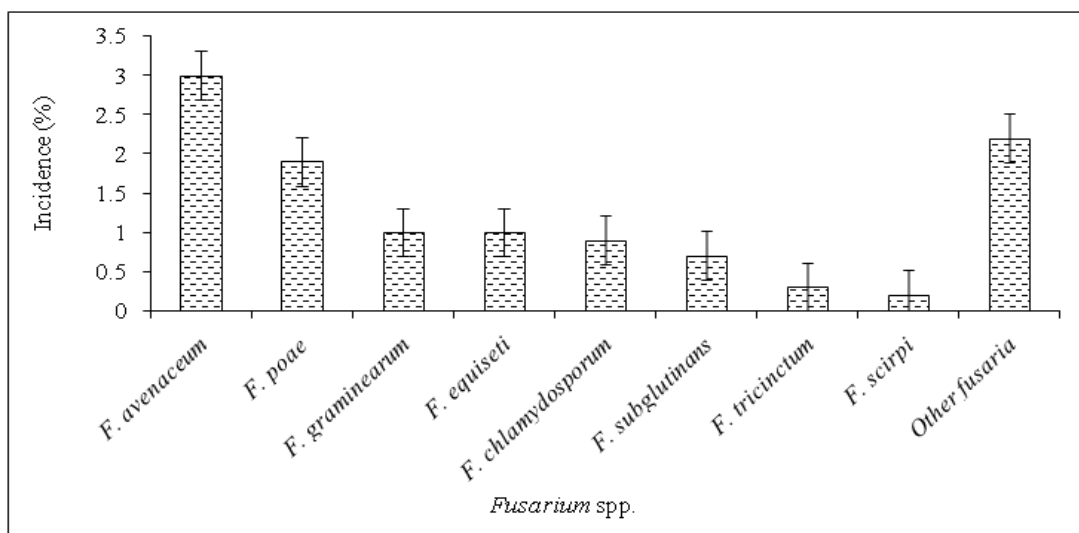
**Table 7:** Isolation frequency (%) of fungal pathogens infecting wheat ears sampled at hard dough stage in different agro-ecological zones of Narok County

Fungal spp.	LH3	LH2	UH3	Mean
<i>Epicoccum</i>	5.2 a	3.6 a	3.3 a	4.0 a
<i>Alternaria</i>	3.2 b	1.7 c	0.7 cd	1.9 b
<i>Chaetomium</i>	0.2 abc	2.7 b	2.6 b	1.8 b
<i>Fusarium</i>	0.4 c	0.8 d	1.0 c	0.7 c
<i>Aspergillus</i>	0.0 e	1.0 d	1.0 c	0.7 c
<i>Macrophomina</i>	0.0 e	0.8 d	0.3 cde	0.4 cde
<i>Ulocladium</i>	0.1 e	0.4 def	0.3 cde	0.3 cde
<i>Stemphylium</i>	0.0 e	0.5 def	0.2 cde	0.2 cde
<i>Rhizoctonia</i>	0.0 e	0.0 e	0.5 de	0.2 cde
<i>Pythium</i>	0.0 e	0.1 e	0.2 de	0.1 f
<i>Trichoderma</i>	0.1 e	0.0 e	0.2 de	0.1 f
Others <sup>a</sup>	0.4 c	0.7 de	0.7 cd	0.6 cde
Mean	0.6	0.8	0.7	0.7
LSD ( $p \leq 0.05$ )	0.3	0.6	0.5	0.5
CV%	24.6	46.0	38.4	78.2

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3

<sup>a</sup> - Fungal pathogens isolated in low frequency, unidentified fungi



**Figure 12:** Mean isolation frequency (%) of *Fusarium* spp. from wheat ears sampled at harvest in Narok County

Bars accompanied by standard error of the means

**Table 8:** Isolation frequency (%) of *Fusarium* spp. from wheat ears at hard dough stage in different agro-ecological zones in Narok County

<i>Fusarium</i> spp.	LH3	LH2	UH3	Mean
<i>F. avenaceum</i>	1.5 a	3.5 a	4.0 ab	3.0 a
<i>F. poae</i>	0.0 a	2.0 ab	3.5 ab	1.9 abc
<i>F. graminearum</i>	0.5 a	2.0 ab	0.5 c	1.0 bcd
<i>F. equiseti</i>	1.5 a	0.0 c	1.5 bc	1.0 bcd
<i>F. chlamydosporum</i>	1.0 a	1.5 ab	0.0 c	0.9 bcd
<i>F. subglutinans</i>	0.0 a	2.0 ab	0.0 c	0.7 bcd
<i>F. tricinctum</i>	1.0 a	0.0 c	0.0 c	0.3 cd
<i>F. scirpi</i>	0.5 a	0.0 c	0.0 c	0.2 d
Other Fusaria <sup>a</sup>	0.0 a	1.0 ab	5.6 a	2.2 ab
Mean	0.7	1.3	1.7	1.2
LSD ( $p \leq 0.05$ )	2.2	2.7	2.7	1.6
CV (%)	188.7	116.6	91.7	139.7

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3

<sup>a</sup> - unidentified *Fusarium* spp.

#### 4.4.4 Incidence of *Fusarium* spp. in wheat kernels at harvest

The major fungal species isolated from wheat kernels sampled at harvest were *Epicoccum* spp., *Alternaria* spp., *Stemphylium* spp., *Aspergillus* spp., *Penicillium* spp. and *Chaetomium* spp.

(Table 9). The mean infection rate of wheat kernels with fungal pathogens was 131% where 41% of the infections were *Epicoccum* spp., 37% *Alternaria* spp. and 4% *Fusarium* spp. There were 31% multiple infections of wheat kernels with various fungal pathogens but mostly with *Epicoccum* spp. and *Alternaria* spp. The incidence of the fungal pathogens was significantly ( $p \leq 0.05$ ) different among the three agro-ecological zones. *Epicoccum* spp. and *Alternaria* spp. were the most prevalent fungi and were isolated in the highest frequency among all the three agro-ecological zones. *Pythium* spp. and *Rhizoctonia* spp. were isolated at low incidence of less than 0.1%.

*Fusarium* species isolated from wheat kernels at harvest in decreasing order were *F. tricinctum*, *F. poae*, *F. equiseti*, *F. nivale*, *F. sambucinum*, and *F. graminearum* (Figure 13). There was no significant ( $p \geq 0.05$ ) difference in the incidence of *Fusarium* spp. in wheat kernels. However, there was variation in the incidence of various *Fusarium* spp. among the three AEZs. *Fusarium* spp. isolated in the highest incidence in LH3, LH2 and UH3 were *F. nivale*, *F. poae* and *F. equiseti* and *F. tricinctum*, respectively (Table 10).

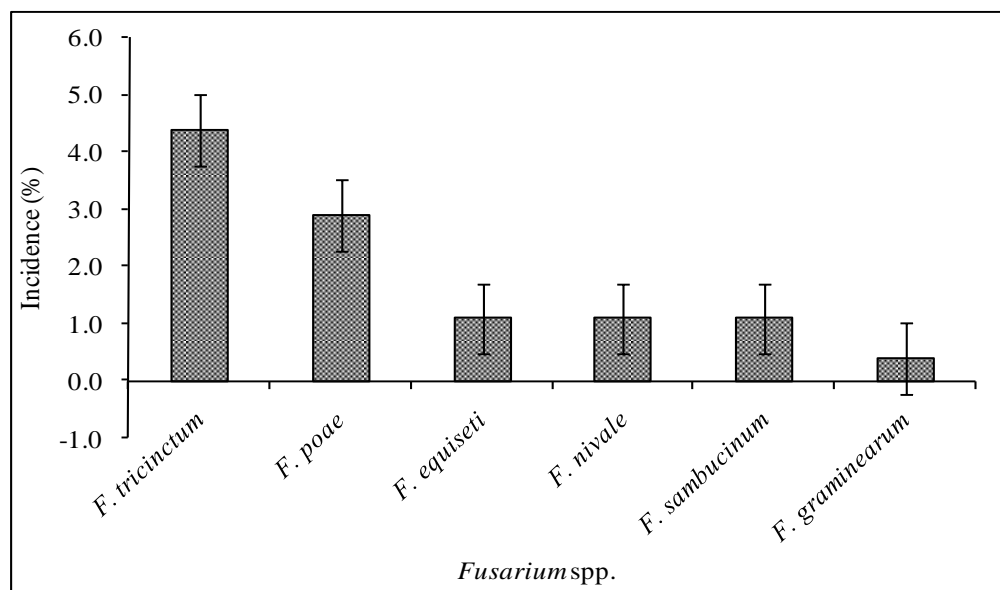
**Table 9:** Isolation frequency (%) of fungal pathogens isolated from wheat kernels sampled at harvest from different AEZs of Narok County

Fungal spp.	LH3	LH2	UH3	Mean
<i>Epicoccum</i>	2.6 a	3.2 a	4.6 a	3.5 a
<i>Alternaria</i>	2.7 a	3.7 a	3.0 b	3.2 a
<i>Stemphylium</i>	1.2 b	1.2 b	1.0 cd	1.2 b
<i>Aspergillus</i>	0.5 cd	1.0 bc	1.3 c	1.0 b
<i>Chaetomium</i>	0.5 cd	0.5 cde	0.8 de	0.6 c
<i>Penicillium</i>	0.6 c	0.6 cd	0.4 fg	0.5 c
<i>Fusarium</i>	0.2 cde	0.3 de	0.5 ef	0.3 cd
<i>Macrophomina</i>	0.2 cde	0.3 de	0.4 fg	0.3 cd
<i>Ulocladium</i>	0.2 cde	0.1 de	0.2 fg	0.1 d
<i>Helminthosporium</i>	0.0 e	0.0 e	0.3 fg	0.1 d
<i>Trichoderma</i>	0.3 cde	0.0 e	0.0 g	0.1 d
Others <sup>a</sup>	0.3 cde	0.1 de	0.1 g	0.2 d
Mean	0.7	0.8	0.9	0.8
LSD ( $p \leq 0.05$ )	0.5	0.6	0.4	0.3
CV (%)	44.6	41.0	25.5	47.0

Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3.

Others<sup>a</sup> – unidentified fungal pathogens



**Figure 13:** Mean incidence (%) of *Fusarium* spp. isolated from wheat kernels sampled at harvest in Narok County

Bars accompanied by standard error of the means

**Table 10:** Incidence (%) of *Fusarium* spp. in wheat kernels sampled at harvest from different agro-ecological zones in Narok County, Kenya

<i>Fusarium</i> spp.	LH3	LH2	UH3	Mean
<i>F. tricinctum</i>	0.0a	2.2 a	11.1 a	4.4 a
<i>F. poae</i>	2.2a	3.3 a	3.3 b	2.9 a
<i>F. equiseti</i>	0.0a	3.3 a	0.0 b	1.1 a
<i>F. nivale</i>	3.3a	0.0 a	0.0 b	1.1 a
<i>F. sambucinum</i>	0.0a	2.2 a	1.1 ab	1.1 a
<i>F. graminearum</i>	1.1a	0.0 a	0.0 b	0.4 a
Mean	1.1	1.8	2.6	1.8
LSD ( $p \leq 0.05$ )	4.6	4.0	8.6	3.6
CV (%)	235.3	120.9	187.0	206.1

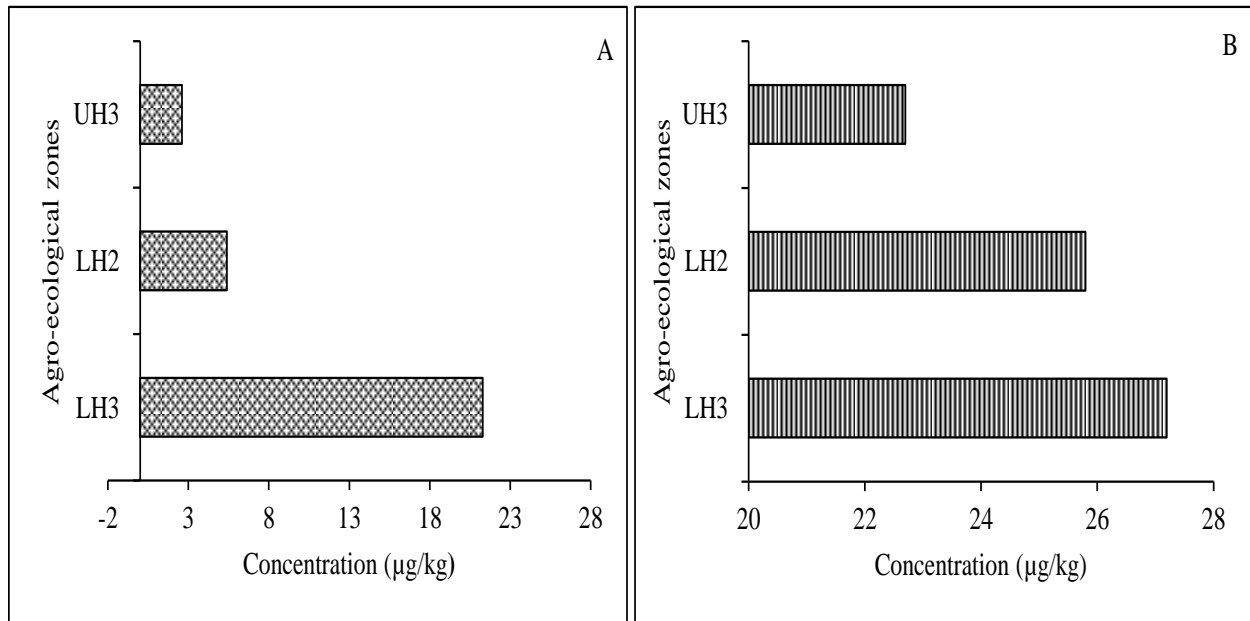
Means followed by different letters within columns are significantly different ( $p \leq 0.05$ ).

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3

#### 4.5 Mycotoxin contamination of wheat kernels at harvest

All the wheat sampled from Narok County was contaminated with T-2 toxin while 94% were contaminated with DON. The concentrations ranged between 8.8 to 37 and below detectable limit (18.5) to 144  $\mu\text{g}/\text{kg}$ , for T-2 toxin and DON, respectively (Figure 14). There was high

variation in mycotoxin concentrations among wheat samples from different AEZs (Table 11). Although the mycotoxin levels were relatively low, the incidence for both DON and T-2 toxin were highest in LH3 and lowest in UH3. Both DON and T-2 toxin levels of all the wheat samples met the FDA and EU standards of 1000 and 1750 for DON and 500 and 100  $\mu\text{g}/\text{kg}$  for T-2 toxin, respectively.



**Figure 14:** Levels ( $\mu\text{g}/\text{kg}$ ) of deoxynivalenol (A) and T-2 toxin (B) in wheat kernels from different agro-ecological zones of Narok County

LH3 – lower highland 3; LH2 – lower highland 2; UH3 – upper highland 3

**Table 11:** Concentration ( $\mu\text{g}/\text{kg}$ ) of deoxynivalenol and T-2 toxin in wheat kernels sampled at harvest from different agro-ecological zones in Narok County

Sample ID	Agro-ecological zones	Deoxynivalenol	T-2 toxin
001	LH3	144.2	25.6
002	LH3	80.1	25.7
003	LH3	20.9	30.7
004	LH3	3.0	27.7
005	LH3	7.4	29.0
006	LH3	9.3	26.0
007	LH3	4.5	26.1
008	LH3	3.3	20.6
009	LH3	8.3	26.0
010	LH3	5.2	31.9
011	LH3	7.4	29.0
012	LH3	7.1	37.0
013	LH3	8.0	32.2
014	LH3	5.5	27.4
015	LH3	5.7	13.6
016	LH2	7.8	22.4
017	LH2	6.5	23.5
018	LH2	5.2	34.3
019	LH2	8.3	30.2
020	LH2	10.5	26.4
021	LH2	5.3	24.3
022	LH2	1.9	22.6
023	LH2	3.1	27.4
024	LH2	7.4	20.4
025	LH2	3.3	18.3
026	LH2	4.8	26.8
027	LH2	6.0	29.3
028	LH2	2.7	25.3
029	LH2	3.3	30.5
030	LH2	5.9	26.4
031	LH2	5.9	22.2
032	LH2	3.5	22.2
033	LH2	6.5	31.3
034	UH3	2.8	27.7
035	UH3	5.0	32.0
036	UH3	3.6	34.7
037	UH3	7.4	31.9
038	UH3	0.0	26.9
039	UH3	4.8	19.4
040	UH3	2.6	8.8
041	UH3	0.0	25.7
042	UH3	1.6	28.0
043	UH3	1.5	35.0
044	UH3	0.0	15.4
045	UH3	4.5	12.0
046	UH3	2.4	11.6
047	UH3	2.5	13.4
048	UH3	2.7	14.2
049	UH3	2.3	12.5
050	UH3	2.2	23.9
051	UH3	1.0	35.6
Mean		9.1	25.2



## CHAPTER FIVE: DISCUSSION

### 5.1 Wheat production practices in Narok County, Kenya

Most of the wheat farmers in Narok County were small scale producers who used farm saved seeds. Most of the small scale farmers did not use chemicals to control pests and diseases of wheat possibly because of their high costs. Some of the small scale farmers lacked knowledge on key wheat production practices like crop rotation, land preparation methods, common diseases of wheat and their management among others. These farming practices are known to promote FHB incidence and severity (FAO, 2001). Infected seeds for planting could result in high incidence of the disease as the seeds are a primary source of inocula besides reducing germination rate and seedling vigor (Government of Saskatchewan, 2007; Wegulo *et al.*, 2008; Government of Alberta, 2014). Infected seeds for planting could be the reason for the high FHB prevalence in Narok County where most farmers did not plant certified seeds.

Five varieties of wheat - Kwale, Njoro II, Mwamba, Kenya Robin and Eagle - grown by farmers in Narok County were infected with FHB. Kwale was the most widely grown wheat variety in Narok County. Although the variety is high yielding, it is moderately susceptible to FHB (Muthomi *et al.*, 2007). The popularity of the variety Kwale in Narok and Kajiado districts was confirmed in a previous survey (Ndiema *et al.*, 2007). Wheat varieties differ in their susceptibility to FHB. Therefore, the variety of wheat grown could determine the levels of FHB and mycotoxin contamination (Dill-Macky and Jones, 2000; Nopsa and Fredy, 2010). Environment greatly influences the susceptibility of a variety to *Fusarium* damage of kernels, DON contamination and yield reduction (Nopsa and Fredy, 2010).

High susceptibility of a variety to FHB under field conditions translates to high mycotoxins contamination as well as high yield losses due to shrunken, light weight kernels that are usually lost during seed cleansing (Government of Saskatchewan, 2007; Cirlini *et al.*, 2014). However, some varieties may be susceptible but often escape the disease because they flower before *Fusarium* spores are produced (Government of Saskatchewan, 2007). The reduction in the weight of kernels depends on the *Fusarium* spp. causing the head blight (Henriksen *et al.*, 2005; Wagacha *et al.*, 2011). Also, some varieties are less susceptible to FHB but more to mycotoxin contamination (Nopsa and Fredy, 2010). Most wheat varieties in the world have a limited range

of resistance to FHB (FSA, 2007). Previous studies have demonstrated that all wheat varieties grown in Kenya are susceptible to FHB (Muthomi *et al.*, 2002; Ndung'u 2005; Muthomi *et al.*, 2007). This implies that FHB epidemic break out could pose a serious threat to wheat production in the country.

Most farmers left wheat residues as standing hay for livestock consumption until the next wheat cropping season. Livestock spread infected wheat residues all over the fields and from one field to others as they grazed freely. Wheat residues act as sources of primary inocula for FHB and as a pathway for introduction of mycotoxins to the animal feed chain (FSA, 2007; Keller, 2011). The remains of the infected wheat residues can also be easily blown away by wind and/or splashed by rain water (Government of Alberta, 2014). All *Fusarium* spp. that cause FHB are capable of surviving as saprophytes on crop residues (Parry *et al.*, 1994). The findings of this study concur with the findings by Dill-Macky and Jones (2000) who reported a positive relationship between previous crop residues, tillage and the level of FHB. The researchers showed that FHB incidence and severity was highest when soil coverage with crop residue was high; when no tillage treatment was done and disease incidence was significantly influenced by previous crop residues and tillage. However, tillage has little effect on FHB inocula following crop rotation or mixed cropping with legumes (Dill-Macky and Jones, 2000) and other short term crops. The researchers also found out that differences in the quantity and type of crop residues may affect disease development. High proportion of farmers who rotated wheat with maize and those who did not practice crop rotation could have contributed to the high prevalence of FHB in Narok County. Steinkellner and Langer, (2003) reported a high diversity of *Fusarium* species in conservation tillage plots than in plough based tillage plots.

Over 50% of the farmers grew wheat and maize side by side, 32% rotated wheat with maize and 43% grew wheat in consecutive years. Infected maize and wheat just a few meters away from the wheat farms could act as alternative hosts and FHB inocula overwinter in their residues and consequently become sources of inocula in the next season. Side by side growing of wheat and maize also contributes to spread of FHB inocula (Wagacha *et al.*, 2010). Fernando *et al.* (1997) reported that FHB declined by 50% at distances 1 to 10 m from the inocula sources and by 90% at distances of 10 to 25 m. Del Ponte *et al.* (2003) observed that within field sources of *G. zeae* ascospores were not strong contributors to FHB compared to airborne spores coming from

outside of those fields. This therefore implies that *Fusarium* spp. inocula can be obtained from more distant sources. Side by side growing of wheat in the fields could also result in secondary infection from infected wheat heads in the neighboring wheat fields. Wheat infected with *Fusarium* spp. at heading stage may serve as source of inocula for wheat in the neighboring fields. This can explain the findings by Del Ponte *et al.* (2003) that under favorable conditions, severe FHB symptoms can develop in wheat fields where there is no significant inocula coming from within field. However, Snijders (1990) suggested that secondary infections do not play an important role in the development of FHB symptoms but still lead to significant toxin contamination.

Rotation of wheat with maize and intensive cereal production compounds reservation of FHB inocula in the soil because every time the *Fusarium* spp. inocula overwinter in the wheat and other cereal residues (Martin and Johnson, 1982; Sutton, 1982; Seaman, 1982) and act as sources of inocula for FHB. Maize and wheat are the most susceptible cereal host crops to *Fusarium* infections and associated mycotoxins. The growing of wheat after maize is the greatest agronomic risk factor for the occurrence of FHB (FSA, 2007). Seaman (1982) and Dill-Macky and Jones (2000) observed that when wheat and maize were grown in rotation, they left a lot of residues on which FHB inocula could develop. *Fusarium* infection of wheat sown in a field with maize residue may be two to three times more severe (Teich and Nelson, 1984). Guo *et al.*, (2010) and Landschoot *et al.* (2013) showed that conventional tillage made significant reduction in *Fusarium* spp. inocula levels. When wheat stubbles are left on soil after harvest, it partly decomposes but about 30% of the dry matter is left even after two years (Parry *et al.*, 1995; Pereyra *et al.*, 2004; Guo *et al.*, 2010). In the case of minimum or no tillage, large amounts of these stubbles remain at the soil surface making it favorable for *Fusarium* spp. to act as FHB inocula when conditions of moisture and humidity become favorable. Tillage method and wheat variety grown has a more significant influence on FHB incidence in the subsequent season compared to wheat – maize rotation (Landschoot *et al.*, 2013). However, repeat wheat – maize rotation results in high FHB incidence when coupled with favorable weather conditions. This could have been the reason for high FHB prevalence in Narok where 76% of farmers practiced simple land preparation- ploughing and harrowing. However, only *Fusarium* spores produced at wheat flowering stage become primary inocula (Wilcoxson *et al.*, 1992; Inch *et al.*, 2005).

Stem rust and FHB were the common diseases of wheat. Rusts are more serious if plants are attacked at milk stage or earlier (CABI, 2005). Stem rust is caused by *Puccinia graminis* leading to badly shriveled kernels, many of them being so light and chaffy that they are blown out with chaff during threshing (CABI, 2005; Government of Alberta, 2014). The remaining grains may be shrunken to one-half or two-thirds normal size. However, stem rust does not overwinter and therefore does not usually cause significant yield losses. A recent study showed that worldwide, significant losses by rusts are rare, though the disease can occur at significant levels in particular fields or throughout a particular growing season (Marsalis and Goldberg, 2013). This makes rust a less important disease of wheat compared to FHB, which many farmers confused with rust. Many farmers did not recognize FHB and thought it was caused by lack of rains, insect infestation while others thought it was as a result of cold weather. The reason why most farmers could not recognize the disease was because of its symptom manifestation, the bleaching which could be caused by many other aspects such as drought (Snijders, 1990; Stack, 2000; Government of Alberta, 2014). Earlier studies reported high prevalence of FHB in Nakuru and Narok, respectively (Muthomi *et al.*, 2007; Muthomi *et al.*, 2012).

## **5.2 Prevalence, incidence and severity of *Fusarium* head blight of wheat in Narok County**

*Fusarium* head blight was present in all the 51 farms in all agro-ecological zones in Narok County. The incidence was highest in UH3 and lowest in LH3. The high incidence agrees with findings by Muthomi *et al.* (2008; 2012), who reported high incidence of up to 97% and 88% in the 2006 cropping season in Nakuru and in the 2008 cropping seasons in Narok, Imenti North and Nyandarua, respectively. The different environmental conditions in the different agro-ecological zones could have caused the variations in the disease incidence among the three agro-ecological zones (Doohan *et al.*, 2003; Brennan *et al.*, 2005; Chen *et al.*, 2006; Klahr *et al.*, 2007a; Muthomi *et al.*, 2008). The high incidence in UH3 could be attributed to more rainfall (1000-1200mm) and high humidity in UH3 which could favor colonization of wheat heads by *Fusarium* spp. (Chen *et al.*, 2006; Klahr *et al.*, 2007a; Muthomi *et al.*, 2008), especially if these conditions prevail at flowering.

The differences in the disease prevalence between the findings of this study and previous studies mentioned above could be attributed to unfavorable weather conditions of moisture and temperature during the 2013 cropping season (Appendix II) as well as farm practices by the farmers in Narok County and those by farmers in other study areas (FAO, 2001; Ndiema *et al.*, 2007). *Fusarium* head blight infection occurs at a temperature range of 15 - 30°C and relative humidity greater than 90% (Holt, 2014). These parameters were relatively low during the 2013 cropping season and therefore the low FHB incidence and severity. Farmers in Narok County rotated wheat with maize, grew wheat in consecutive years, left wheat residues as standing hay for livestock, planted uncertified seeds and used simple land preparation methods that did not bury previous crop residues. These are known to cause increase in the level of primary FHB inocula that infect cereal residues and consequently disease incidence and severity (FAO, 2001; Holt, 2014).

### **5.3 Incidence of *Fusarium* spp. in crop residues, soil, wheat ears and kernels sampled in wheat fields in different agro-ecological zones of Narok County**

The common fungi isolated were: *Fusarium* spp., *Pythium* spp. and *Trichoderma* spp. from crop residues; *Epicoccum* spp., *Alternaria* spp. and *Chaetomium* spp. from wheat ears and kernels; *Aspergillus* spp. and *Penicillium* spp. from soil. A previous study in Kenya (Muthomi *et al.*, 2012) reported *Penicillium* spp. as the the predominant fungal genus in soil. *Apergillus* spp. and *Penicillium* spp. are mycotoxin producers. Their high incidence therefore implies that wheat and other crops grown in these fields could be contaminated with associated mycotoxins and this may pose a health risk to humans and livestock. *Aspergillus* spp. mainly *A. flavus* and *A. parasiticus* produce aflatoxin commonly in most kinds of foods in tropical countries (Hocking *et al.* 2006). Food borne *Penicillium* spp. naturally produces ochratoxin A, Citrinin, Patulin and chaetoglobosins (Hocking *et al.* 2006). *Fusarium* spp. were diverse in the four sample types. High diversity of *Fusarium* spp. was also reported by Wagacha *et al.* (2010), Muthomi *et al.*, (2012) in Nakuru County, Kenya. Isolation of diverse *Fusarium* spp. is an indicator that FHB is caused by a complex of *Fusarium* spp. (Muthomi and Mutitu 2003; Muthomi *et al.*, 2007;

Wagacha *et al.*, 2010; Muthomi *et al.*, 2012). The spectrum of *Fusarium* spp. involved varies with the region and weather conditions during the vegetation period.

The high incidence of *Epicoccum* spp. and *Alternaria* spp. in this study concur with the findings of Kosiak *et al.*, (2004), Ndung'u , (2006), Sab *et al.*, (2007), Muthomi *et al.*, (2008) and Wagacha *et al.*, (2010). As in previous studies, high incidence of *Epicoccum* spp. from wheat ears and kernels was the cause of low incidence of *Fusarium* spp. A study by Ogorek and Plaskowka (2011) showed that *Epicoccum nigrum* has antagonistic properties against *F. avenaceum*, *F. graminearum* and *F. oxysporum*. This could therefore explain the inverse relationship in the incidence of *Fusarium* spp. and *Epicoccum* spp. Both *Epicoccum* spp. and *Alternaria* spp. are saprophytes that cause grey or black discoloration of the wheat heads resulting in sooty moulds, black point or smudge (Zillinsky, 1983). Some *Alternaria* spp. are known to produce the mycotoxins alternariol, alteneune, tenuazonic acid and altertoxin I-III (Weidenborner 2001; Sab *et al.*, 2007) while *Alternaria alternata* causes huge losses in yields (Williamson, 1997).

Infection rate with *Fusarium* spp. were highest for crop residues with 54% incidence. Crop residues act as substrate for *Fusarium* spp. ascospores to overwinter and become sources of FHB inocula when conditions of moisture and temperature become favorable by releasing the ascospores on wheat heads (McMullen *et al.*, 1997; Dill-Macky and Jones, 2000). Extended periods of greater than 90% relative humidity with temperature between 15°C and 30°C facilitate infection of wheat ears by spores of *Fusarium* spp. (De Wolf *et al.*, 2003). The prevailing weather conditions, especially humidity, during the survey period in Narok were not conducive for enhanced infection of wheat by *Fusarium* spp. Cross infection of the nearby wheat ears occur by wind blowing or water splashed when these conditions continue after flowering (Paul *et al.*, 2004; Cowger and Sutton, 2005; Maldonado-Ramirez *et al.*, 2005).

A complex of 12 *Fusarium* spp. were isolated from crop residues with *F. chlamyosporum*, *F. graminearum*, *F. equiseti* and *F. sambucinum* being the most common species. These findings concur with the findings by Muthomi *et al.*, (2007) and Wagacha *et al.*, (2010) who reported that *F. chlamyosporum* and *F. equiseti* were isolated in higher frequencies than *F. graminearum* from kernels at harvest. This could be attributed to the fact that *F. graminearum* does not

produce chlamydospores. *Fusarium chlamydosporum* and *F. equiseti* produce chlamydospores that may overwinter in crop residues and when conditions of moisture and temperature become favorable, these *Fusarium* spp. could act as FHB inocula and subsequently contaminate the wheat with associated mycotoxins (Dill-Macky and Jones, 2000; Guo *et al.*, 2010).

Some strains of *F. chlamydosporum* produce moniliformin and other secondary metabolites such as acuminatopyrone, chlamydosporol and steroids (Rabie *et al.*, 1978; Solfrizzo and Visconti 1996). *Fusarium graminearum* causes blighting of infected wheat ears and is highly virulent and the main cause of FHB of wheat, as well as the most important producer of DON and zearalenone especially in warm areas (Brennan *et al.*, 2005; Leslie and Summerell, 2006). However, despite its high virulence, it was isolated in lower frequency than *F. chlamydosporum*. Other mycotoxins produced by *F. graminearum* are nivalenol, zearalenone, fusarin, culmorins, steroids and fusarochromanone (Leslie and Summerell, 2006). *Fusarium equiseti* produces nivalenol, T-2, diacetoxyscirpinenol, beauvericin and butenolide while *F. sambucinum* produces enniatins, beauvericin, fusarin C, fusaric acid and steroids (Leslie and Summerell, 2006).

*Fusarium* spp. were also isolated from most of the soil samples but in lower incidence than from crop residues. *Fusarium oxysporum* and *F. proliferatum* were found to be the predominant species in soil. The *Fusarium* spp. isolated in this study and the dominance of *F. oxysporum* and *F. proliferatum* were also reported by other researchers (Nwanma and Nelson, 1993; Lattifah *et al.*, 2007; Lukall, 2012; Silvestro *et al.*, 2013). However, the dominance of *F. oxysporum* and *F. proliferatum* contrasts the findings by Muthomi *et al.*, (2012) and Jeschke *et al.*, (1990) who reported *F. poae* and *F. graminearum* as the dominant species in soil. *Fusarium proliferatum* has a wide range of hosts and produces high levels of fumonisins in maize (Leslie and Summerell, 2006), a crop which in this study was used as a rotation crop with wheat. *Fusarium proliferatum* also produces beauvericin, fusaproliferin, fusaric acid, and fusarins. *Fusarium oxysporum* is a cosmopolitan soil saprophyte (Leslie and Summerell, 2006) that causes many plant diseases, including vascular wilts, damping off, crown rots and root rots (Jarvis and Shoemaker, 1978; Summerell and Rugg, 1992).

In soil, *Fusarium* spp. persist as mycelia, chlamydospores and conidia (McMullan and Stack, 1983). Keller (2011) used experimental fields that had no or little residues of maize and small

grains from previous year crop but still recovered *F. graminearum*. This implies that FHB inocula can survive in the soil. In addition to *F. oxysporum* and *F. proliferatum*, other *Fusarium* spp. isolated from the soil samples included *F. graminearum*. There were significant differences in the incidence of *Fusarium* spp. in soil among the three agro-ecological zones. The differences could be attributed to soil types (Larkin *et al.*, 1993) and cropping systems. The presence of *Fusarium* spp. in agricultural soils could impact on crop health and consequently on the amount and quality of human food and animal feeds (Keller, 2011). This shows that soil could be a potential source of FHB inocula as well as inocula for other crop diseases.

The main *Fusarium* spp. isolated from wheat ears at hard dough stage were *F. avenaceum*, *F. poae*, and *F. graminearum*. Previous studies in Kenya reported similar findings on dominant *Fusarium* spp. but the dominance differs (Muthomi *et al.*, 2007, Muthomi *et al.*, 2008, Muthomi *et al.*, 2012, Wagacha *et al.*, 2010). *Fusarium avenaceum* produces enniatins, moniliformin, cyclic peptides, and fusarins (Desjardins and Proctor, 2001; Jestoi *et al.*, 2004). *Fusarium avenaceum* contaminates grains with enniatins more than any other *Fusarium* spp. *Fusarium poae* is a cosmopolitan species but more common in temperate regions. This could explain its highest incidence in UH3 compared to other AEZs. Although a weaker pathogen of wheat associated with FHB, *F. poae* is associated with contamination of infected wheat with trichothecenes nivalenol and T-2 toxin (Leslie and Summerell, 2006). Co-occurrence of these major FHB causal pathogens has the implication of co-occurrence of the major mycotoxins. Other species may be isolated from wheat ears but they do not cause bleaching of wheat ears. However, they may cause damage to the spikelets and result in low disease severity and latent infection of seeds (Parry *et al.*, 1995).

*Fusarium tricinctum* and *F. poae* were the main *Fusarium* spp. isolated from wheat kernels at harvest. Isolation of *F. poae* in high incidence from kernels concurs with the findings of Muthomi *et al.* (2007), and Muthomi *et al.* (2012) while the high incidence of *F. tricinctum* concurs with the findings by Bottalico and Perrone (2002), Wagacha *et al.*, (2010) and Castariates *et al.* (2011). The high incidence of *F. poae* and *F. tricinctum* in wheat kernels at harvest makes them important components of the FHB causing *Fusarium* spp. complex and consequently major contaminants of harvested wheat with mycotoxins. *Fusarium tricinctum* is a temperate species that grows in highlands where temperatures are low and humidity is high, and



this can explain its high incidence in UH3 in Narok County. *Fusarium tricinctum* is generally regarded as a plant pathogen and contaminates cereals with fusarin, enniatins and moniliformin (Leslie and Summerell, 2006).

#### **5.4 Mycotoxin levels in wheat kernels at harvest**

There were low concentrations of DON and T-2 toxin from wheat kernels sampled at harvest. The detection of these toxins concurs with the findings by Muthomi *et al.* (2008), Wagacha *et al.* (2010) and Muthomi *et al.* (2012). However, the findings contrasted the findings by Wagacha *et al.* (2010) who reported high DON concentrations of up to 1310 µg/kg. Deoxynivalenol is a potent mycotoxin and a virulent factor for *F. graminearum* (Hallem Adem *et al.*, 2011). Deoxynivalenol is mainly produced by *F. graminearum* and *F. culmorum* and is the most economically important *Fusarium* mycotoxin (Keller, 2011). Deoxynivalenol has high emetic effect after consumption; it is transported to the brain where it runs dopaminergic receptors (Sobrova *et al.*, 2010). Deoxynivalenol ingestion also disrupts protein function by inhibiting protein synthesis by binding to the ribosome and activating critical cellular kinases involved in signal transduction related to proliferation, differentiation and apoptosis of cells (Sobrova, 2010). Oral repetitive administration of low doses DON results in disorders in lipid metabolism, renal filtration disturb and renal cell DNA methylation and rhabdomyolysis (Kouadio *et al.*, 2013). Although DON is less toxic compared to other trichothecenes, it is the most commonly detected *Fusarium* toxin and most economically important mycotoxin of small grains (Wegulo, 2012). Late infection of wheat with FHB results in less damage to kernels but high DON concentrations. The high levels of DON in LH3 could be due to the high incidence of *F. graminearum* in the zone. Deoxynivalenol levels in kernels at harvest are correlated with the disease incidence (Cowger and Arellano, 2013) and therefore the low mean DON levels were due to low FHB mean incidence.

The 100% prevalence of T-2 toxin in this study concurs with the findings by Muthomi *et al.*, (2012). T-2 toxin is one of the major toxins produced by *F. poae* and *F. sporotrichioides* and is known to pose serious threats to human and animal health (Bennet and Klich, 2003; EFSA, 2013). T-2 toxins are not destroyed by milling but it becomes unevenly distributed between

fractions of the cereal (EFSA, 2013). T-2 toxin inhibits protein synthesis, and cause secondary disruption of DNA and RNA synthesis. It affects the actively dividing cells of lining of gastrointestinal tract, skin, lymphoid, and erythroid cells. T-2 toxin can also decrease levels of antibody, immunoglobulins and certain other humoral factors. Observable effects include weight loss or poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage and decreased production (WHO, 2001). Production of T-2 toxin is greatest with increased humidity and a temperature of 6 – 24°C (Anonymous, 2014). The levels of T-2 toxin contrast the incidence of their major producers, *F. poae*, in kernels at harvest. The levels of T-2 toxin were highest in LH3 and lowest in UH3 while the incidence of *F. poae* was highest in UH3 and lowest in LH3. *Fusarium poae* is one of the main producers of T-2 toxin.

The detected DON and T-2 toxin levels could be due to the moderate temperature, rainfall and relative humidity in the Narok's 2013 cropping season (Appendix II). Temperature, rainfall and relative humidity were on the lower range for production of DON and T-2 toxin. Environmental factors influencing the development of FHB; temperature, moisture, and relative humidity influence accumulation of the toxins (Wegulo, 2012). Wheat production practices might also have influenced the levels of the toxins. A recent study (Landschoot *et al.*, 2013) showed that DON accumulation in wheat can be influenced by tillage method, susceptibility of the wheat variety to FHB and repeated wheat – maize rotation. However, the researchers reported that the quantitative effects of these factors depend on FHB pressure. The low levels of DON and T-2 toxin could therefore be related to the low FHB incidence and severity.

## CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

Farming practices have been shown to cause increase in the level of primary FHB inocula and consequently the disease incidence and severity (Ndiema *et al.*, 2007). Wheat farmers in Narok County practiced: rotation of wheat with maize, growing wheat side by side with wheat, growing wheat in consecutive years, leaving wheat residues as standing hay for livestock, planting uncertified seeds and using simple land preparation methods that did not bury previous crop residues. These practices are known to contribute to the build up of FHB primary inocula and consequently to high FHB prevalence, incidence and severity in the wheat fields. There was 100% prevalence of FHB of wheat in Narok County. However, FHB incidence and severity were relatively low. This study therefore provided baseline information on the incidence and severity of FHB in Narok County.

Diverse *Fusarium* spp. were isolated from crop residues, soil, wheat ears and kernels. This showed that FHB is caused by a complex of *Fusarium* spp. However, certain *Fusarium* spp. dominated in soil, crop residues and wheat ears and kernels. *Fusarium chlamydosporum* was dominant in crop residues, *F. oxysporum* in soil, *F. avenaceum* in wheat ears and *F. tricinctum* in kernels at harvest.

The high infection rate of crop residues with *Fusarium* spp. showed that crop residues play an important role in the survival of FHB inocula between cropping seasons and consequently to the disease. *Fusarium* spp. overwinter as saprophytes on crop residues and act as source of FHB primary inocula in subsequent seasons. Isolation of *Fusarium* spp. from soil indicated that *Fusarium* spp. survive in the soil which makes soil an important source of FHB inocula when moisture and temperature become conducive for germination and sporulation of *Fusarium* spp. (Bateman *et al.*, 2001). In soil, *Fusarium* spp. persists as mycelia, chlamydospores and conidia Keller (2011).

Although all the wheat kernels were contaminated with T-2 toxin and 94% were contaminated with deoxynivalenol, the toxin levels were within the limits set by the European Commission (EU) and the United States Food and Drug Administration (FDA). This implied that the wheat

harvested during 2013 cropping season in Narok County was safe for human consumption and therefore posed no health risk to humans and livestock.

## 6.2 Recommendations

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

- i. Inclusion of maize as a rotation crop in wheat production should be avoided. Besides acting as an alternative host to *Fusarium* spp., maize stovers are difficult to decompose remaining in soil for up to two years thereby playing a critical role as source of primary inocula for FHB.
- ii. It is important to avoid leaving crop residues on the soil surface after harvesting. The level of cereal residues associated pathogens and their development can be reduced by burying crop residues through intense tillage. This greatly reduces the amount of cereal residues on the soil surface on which *Fusarium* spp. could overwinter and become source of FHB inocula in the following cropping season.
- iii. Farmers should be encouraged to apply recommended fungicides to their wheat crop especially at early flowering to protect the opening florets from *Fusarium* spores dispersed by wind or splashed by rain water. However, unnecessary fungicide applications are both economically and environmentally unfriendly as they are expensive and could also end up polluting the environment.
- iv. Monitoring and surveillance programs should include mycotoxins such as DON and T-2 in addition to aflatoxin, as their levels of occurrence are dependent on seasonal variations.
- v. Further research on various wheat production practices and the extent of their effects on accumulation of FHB inocula should be conducted to come up with a combination of practices which would reduce accumulation of FHB in the soil.
- vi. Since all wheat varieties grown by farmers in Narok County were susceptible to FHB, breeding of varieties which are resistant to the disease should continuously be undertaken.

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**APPENDICES**

Appendix I: *Fusarium* head blight survey questionnaire

**Section I: Background information**

Farmer ID.: ----- Name of farmer: ----- Date: -----/-----/2013  
Age: ----- Sex: (M) (F) Village: ----- Agro-Ecological Zone: -----  
Latitude: ----- Longitude: ----- Elevation (m): -----  
Head of household (M/F): ----- Highest level of education: -----

**Section II: Information on production practices**

- I. How many years have you practiced wheat production? -----
- II. Area under wheat production (acres): -----
- III. Varieties of wheat grown: -----
- IV. Sources of seeds: a) Own ----- b) Neighbor ----- c) Market----- d) Agro-shop-----
- V. Other crops grown on the farm -----  
-----  
-----
- VI. What method(s) of field preparation do you practice? -----  
-----  
-----
- VII. Pre-season practices -----  
-----  
-----
- VIII. Do you mix wheat crop with other crops? *(Yes) (No)*
- IX. If yes, with what crops? -----  
-----
- X. Do you practice crop rotation in wheat production? *(Yes) (No)*
- XI. If yes, with what crops? -----  
-----
- XII. Do you use any soil amendments in wheat production? *(Yes) (No)*
- XIII. If yes, which ones? -----
- XIV. What are the most common diseases of wheat in your wheat field? -----  
-----

XV. What methods of pest and disease control do you employ? -----  
-----  
-----  
-----

XVI. Do you know this disease? (Show farmer a photo of wheat ears showing symptoms of FHB) (*Yes*) (*No*)

XVII. What method(s) do you use to control it? -----  
-----  
-----

XVIII. How do you handle the crop residues after harvest? -----  
-----  
-----  
-----

XIX. Yield per harvest (kg)? -----

XX. FHB Incidence -----

XXI. FHB Severity -----

**Other observations** -----  
-----  
-----

**Severity score:** 1 = no symptoms, 2= <5%, 3 = 5-15%, 4 = 16-25%, 5 = 25-44%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%

**Incidence:** No. of infected plants infected per 1m<sup>2</sup>/ total heads

**Vote of Thanks**

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**Thank the farmer and give advice on how to manage the disease**

**Appendix II:** Monthly precipitation (mm), temperature (°C), and relative humidity (%) data recorded at the Narok Meteorological Weather Station for the year 2013.

Month	Total Precipitation	Minimum temperature	Maximum temperature	Relative humidity 06Z	Relative humidity 12Z
January	63.4	10.5	25.7	74.0	46.0
February	73.6	9.3	26.6	70.0	37.0
March	103.4	11.7	26.6	74.0	41.0
April	240.7	13.7	23.8	87.0	63.0
May	57.0	12.2	22.4	86.0	60.0
June	2.7	9.7	22.0	76.0	53.0
July	16.4	8.7	23.3	81.0	47.0
August	14.9	9.4	22.7	81.0	49.0
September	75.1	10.5	25.4	73.0	43.0
October	Nil	10.2	25.9	65.0	34.0
November	12.7	12.9	26.0	72.0	39.0
December	80.5	11.7	25.0	78.0	52.0

Relative humidity 06Z – relative humidity taken at 9.00am; relative humidity 12Z - relative humidity taken at 3.00pm

Source: Ministry of Environment, Water and Natural Resources, State Department of Environment, Meteorological Service, Kenya (2014)