

**MANAGEMENT OF *FUSARIUM* HEAD BLIGHT OF WHEAT THROUGH  
HOST RESISTANCE AND MICROBIAL AGENTS**

**BY  
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**DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION  
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DECLARATION

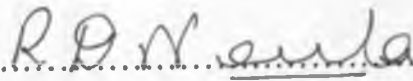
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## **DEDICATION**

Dedication to my Dad Musyimi Mutua and Mum Christine Kasiki Musyimi for their dedicated efforts to educate me, brothers, sisters, friends and relatives for their social and moral support during the study period.

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## LIST OF ACRONYMS AND SYNONYMS

<b>3-ADON</b>	3-Acetyl deoxynivalenol
<b>AEZ</b>	Agro Ecological Zone
<b>ANOVA</b>	Analysis of Variance
<b>ATA</b>	Alimentary Toxic Aleucia
<b>AUDPC</b>	Area Under Disease Progress Curve
<b>CFU</b>	Colony Forming Units
<b>Cv</b>	Coefficient of variation
<b>DON</b>	Deoxynivalenol
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay
<b>EU</b>	European Union
<b>FDA</b>	Food and Drug Administration
<b>FDK</b>	<i>Fusarium</i> Damaged Kernels
<b>FHB</b>	<i>Fusarium</i> head blight
<b>IFS</b>	International Foundation for Science
<b>K.A.R.I</b>	Kenya Agricultural Research Institution
<b>LH 3</b>	Lower Highland 3
<b>LH 4</b>	Lower Highland 4
<b>LH 5</b>	Lower Highland 5
<b>LSD</b>	Least Significant Differences
<b>NIV</b>	Nivalenol

<b>PBS</b>	Phosphate Buffer Solution
<b>PCNB</b>	PentaChloroNitroBenzene
<b>PDA</b>	Potato Dextrose Agar
<b>RCB</b>	Randomized Complete Block Design
<b>SNA</b>	Synthetic Nutrient Agar
<b>SPSS</b>	Statistical package of social studies
<b>UH 2</b>	Upper Highland 2
<b>UH 3</b>	Upper Highland 3
<b>UH 4</b>	Upper Highland 4
<b>ZON</b>	Zearalenone

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## ABSTRACT

*Fusarium* head blight (FHB) is a world wide disease of wheat and other small grain cereals caused by several *Fusarium* species that reduce yield quantity and quality. A study covering 120 farms was done in July-November, 2008 in Nyandarua North, Imenti North and Narok districts of Kenya to determine level of fungal genera inoculum and mycotoxin contamination. FHB incidence was determined as the proportion of diseased heads per 10M<sup>2</sup>. Isolation of the fungus was done from stems, heads, grains, heads and soil samples on PDA. In vitro activity of COTAF<sup>R</sup>, Folicur<sup>R</sup>, PEARL<sup>R</sup>, Thiovit<sup>R</sup>; *Epicoccum*, *Alternaria*, *Trichoderma*, *Penicillium* was determined by paired cultures method in completely randomized design. T-2 toxin mycotoxin contamination in grains was determined by direct competitive Enzyme- Linked Immunosorbent Assay (ELISA). Effect of antagonistic microorganisms, fungicides, varieties and lines on FHB severity and mycotoxin content was tested by co inoculating *F.graminearum* and *F.poa*e together with each of the micro organisms, fungicides and lines.

*Fusarium* head blight was found to be highly prevalent with an incidence of 87 percent. Maize was the other crop grown mostly besides wheat by up to 70 percent. Dimethoate<sup>R</sup> and Folicur<sup>R</sup> were the mostly chemicals used by up to 43 percent and 75 percent respectively. Fungal genera isolated from wheat, wheat residues and soil were *Fusarium*, *Trichoderma*<sup>spp</sup>, *Alternaria*<sup>spp</sup>, *Epicoccum*<sup>spp</sup> and *Penicillium*<sup>spp</sup>. High levels of *Alternaria* and *Epicoccum* showed low levels of *Fusarium*. The major causal *Fusarium* species isolated from wheat, wheat residues and soil were *F. stilboides*, *F. graminearum*, *F. poae*, *F. heterosporum*, *F. fusarioides*, *F. tricinctum* and *F. verticilloides*. Low levels of *Fusarium graminearum* indicated low levels of *Fusarium poae*.

Mycotoxin T-2 toxin detected in the grain samples of up to 23 ppb from areas that had higher isolation rates of *F.poae*.

In vitro activity of fungicides had 100 percent pathogen growth inhibition. Antagonists in vitro reduced pathogen growth up to 50 percent. In vivo activity of antagonists and fungicides did not completely control *Fusarium* head blight but only reduced to low levels of up to 30 percent. Folicur<sup>R</sup> was the most effective fungicide while *Trichoderma* was the most effective antagonist in reducing disease severity. All wheat lines and varieties were susceptible to *Fusarium* head blight and mycotoxin contamination thus posing a threat to human and animal health. Njoro bw1 and Njoro bw2 were less susceptible to FHB compared to Mbuni, KIBIS and R1104.

Results indicated that *F.poae* was more prevalent in all the samples analyzed. The main causal agents were *F.poae* and *F.graminearum* while the major contamination was T-2. Integration of crop rotation, cultural, microbial agents, fungicides and host resistance could be used to manage the disease. Planting of less susceptible varieties to FHB and also use of Folicur<sup>R</sup> in management of FHB. Field trial of the fungicides and microbial agents in the management of FHB should be undertaken.

## CHAPTER ONE

### GENERAL INTRODUCTION

#### 1.1 Background information

Wheat (*Triticum aestivum* L.) is the second most important cereal grain in Kenya (FAO, 2002). Kenya's current national wheat production approximately 3,000,000 tonnes per annum meets only about 50 percent of the national demand (Njau et al., 2006). More over the high increase in population and changing eating habits is expected to substantially increase wheat demand which is estimated to reach 850,000 tonnes per annum in the year 2020 (FAO, 2003).

*Fusarium* head blight (FHB) or scab or *Fusarium* ear blight (FEB) is a highly destructive disease that affects cereal crops and has been reported in most wheat growing areas of the world (Parry et al., 1995). *Fusarium* head blight is caused by *Fusarium* species especially *Fusarium graminearum*; *Gibberella zaeae*, but also *F. culmorum*, *F. tricinctum* (*F. sprotrichoides*), *F. poae*, *F. longipes* (*F. equiseti*), *F. oxysporum*, *F. phylophilum* (*F. proliferatum*), *F. verticilloides* (*F. moniliforme*), *F. fusarioides* (*F. chlamydosporum*), *Microdochium nivale* (*Monographella nivalis*), *F. avenaceum* (*Gibberella avenacea*) and *F. cerealis* (*F. crookwelense*) may also be involved (Marasas et al 1984).

The disease is recorded most frequently under hot, wet climatic conditions where significant yield losses and mycotoxin accumulation in grain is also reported. It has the potential to completely destroy an otherwise healthy crop only weeks before harvest by infecting developing heads at the time of flowering through the soft dough stage of kernel development (Schisler et al., 2002; Fernandez et al., 2008). The disease is unique in its ability to influence every aspect of

the grain industry. Under disease favourable conditions, infection of the spikes leads to poor grain fill and seed quality resulting in yield losses due to reduced kernel size and weight, reduced seed germination, seedling blight and poor stands (Bai and shaner, 1994; McMullen et al., 1997; Parry et al 1995; Argyris et al., 2003).

*Fusarium* species produce several types of secondary metabolites such as trichothecenes which are contaminants of cereal grains such as wheat, corn, barley and oats (Placinta et al., 1999). These fungal metabolites pose serious threats to human and animal health (Bennett and Klich, 2003). Mycotoxin-contaminated grains may be rejected or devalued in commerce (Bai and shaner, 1994; McMullen et al. 1997). The presence of *Fusarium* damaged kernels (FDK) downgrades the grain at the elevator so producers lose quality premiums (McMullen et al., 1997). Milling, baking and pasta-making properties are altered (Dexter et al., 1996, 1997) because the fungus destroys starch granules, cell walls and endosperm proteins (Bechtel et al., 1985; Nightingale et al., 1999). Reduced seed germination (Gilbert and Tekauz, 1995), and the domestic and export markets suffer from loss because of low customer tolerances for FDK (Charmley et al., 1994).

Mycotoxins cause acute or chronic trichothecene- toxicoses, illnesses characterized by dermatitis, vomiting, food refusal, mucous and skin breeding, hemorrhagic diarrhoea and sterility (Verica, 2007). Clinical toxicity includes food refusal, vomiting, tachycardia, hemorrhagic diarrhea, edema, skin and mucous necrosis, destruction of hematopoietic tissue, leukocyte and thrombocyte, leucopenia and nervous disorders. Deoxynivalenol (DON) could be considered the most important wheat contaminant (Bottalico and Perrone, 2002). Its ingestion by animals results

in different symptoms depending on the sensitivity of the species, such as a decrease in weight gain and nutritional efficiency, anorexic syndromes and altered immune function (Pestka, 2007).

Various cultural practices have been proposed to eliminate sources of primary inoculum (Bai and Shaner, 1994). Seed treatment and foliar application of fungicide at anthesis might provide some protection (Mesterhazy, 2003). Several fungicides are registered for application on wheat. However, the cost of treatment, the difficulty of determining the optimum time of application, and lack of any registered fungicides that are highly effective have limited the use of chemical protection against FHB. Even if a fungicide reduces direct yield loss, it may not reduce mycotoxin contamination to a level tolerable for human consumption (Martin and Johnston, 1982; Steffenson, 2003).

Genetic resistance has the potential to provide economical and effective control of FHB. Considerable progress in the search for host resistance has been made in China, Japan, and some other countries in past two decades (Bai et al., 2003; Ban, 1997; Ban, 2000; Lu et al., 2001 and Mesterhazy, 2003). Recent developments in genomic research and biotechnology hold promise for understanding the genetic mechanisms of FHB resistance and allow more effective utilization of FHB resistance genes to develop new resistant wheat.

## **1.2 Problem statement and justification**

*Fusarium* head blight is a widespread disease of wheat and other small grain cereals (Mesterhazy et al., 1995, Parry et al., 1995, and Miedaner, 1997). The disease has the ability to completely destroy a potentially high-yielding crop within a few weeks of harvest, causing economic losses

due to reduced seed quality (Fernandez et al., 2008). Damage from head scab is multifold: reduced yields, discoloured, shriveled “tombstone” kernels contamination with mycotoxins, and reduction in seed quality (McMullen, 1997). The disease also reduces test weight and lowers market grade. Difficulties in marketing, exporting, processing, and feeding scabby grain are experienced. Fungus of the group *Fusarium* spp. infect spikes at anthesis causing heavy losses, and grain harvested from infected wheat may show reduced grain mass, test weight, baking quality and seed quality (Diehl 1984; Bechtel et al., 1985; Wiersma et al., 1996). Moreover, besides yield losses, the most serious concern associated with FHB infection is the contamination of the harvested crop with a range of mycotoxins.

Head blight epidemics have been attributed in large part to increasingly widespread implementation of reduced tillage for soil conservation. This technique results in deposition of larger amounts of crop residues on the soil surface, creating an ideal growth environment for species of *Fusarium*. Maize has particular relevance because of the extraordinary amounts of susceptible residues left after harvest and the established link with increased wheat disease when wheat is planted in rotation with maize (Dill-Macky and Jones, 2000; Bata and Laszitivity, 1999)

Different fungal genera and species have been isolated from Kenyan wheat grains from previous research work (Muthomi et al., 2008). *Fusarium* head blight infection have been reported in Kenya by up to 98 percent infection rate by findings by Muthomi et al., 2001, 2002, 2004, 2007 and 2008; Ndung’u et al 2006; Riungu et al., 2008). Different species infecting wheat have been isolated from wheat heads and grains (Muthomi et al., 2008; Ndungu et al., 2006; Riungu et al., 2008) found *F. poae*, *F. chlamydosporum*, *F. oxysporum*, *F. graminearum*, *F. equiseti*, *F.*

*verticilloides*, *F. avenaceum*, *F. semitectum*, *F. cerealis*, *F. lateritium*, *F. sporotrichoides*, *F. scirpi*, *F. sambucinum*, *F. subglutans* and *F. solani* contaminating wheat grains in Nyandarua and Nakuru district.

This shows wheat production in Kenya is in great risk of FHB outbreak incase favourable conditions prevail. Different mycotoxins T-2 Deoxynivalenol (DON), Zearalenone, aflatoxin have been reported contaminating wheat in Nyandarua and Narok districts of Kenya (Muthomi et al., 2008, Riungu et al., 2008). Fungicides (Folicur<sup>R</sup> and copper oxychloride<sup>R</sup>) and antagonists have been used in the management of FHB in Kenya (Riungu et al, 2008). Although a wide range of fungicides have not used. Therefore the need to screen for more host resistance lines and varieties and also more fungicides to be evaluated for effectiveness in management of FHB.

Therefore the study was carried out with the main objective of determining causal fungi of FHB and toxins possible management strategies in wheat.

The specific objectives were:

- 1 To determine FHB inoculum levels and mycotoxins in wheat producing districts.
- 2 To determine and evaluate chemical, biological and host resistance strategies for management of FHB.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Wheat production areas in Kenya

Wheat is grown in the Rift valley regions of Uasin Gishu, Narok, Marakwet, Elgeyo, Londiani, Molo, Nakuru, Nyandarua, Koibatek and Timau areas (Onsongo, 2007). These areas combined account for 80 percent of Kenya's domestic wheat production (K.A.R.I, 1989). Kenya produces both soft and hard variety wheat, with the later about 70 percent. Wheat varieties grown in Kenya include the high yielding and drought resistant Njoro bw1 and Njoro bw2. However, Njoro bw1 is grown in dry parts such as lower Narok, Machakos and Laikipia with a harvest of upto 20 bags an acre. Njoro bw2 performs well in acidic soils, like parts of Uasin Gishu and Nakuru districts (Noah et al., 2005). It produces 35bags per acre. Other varieties are *duma*, *chozi* grown in dry areas, which yield about 19 bags an acre. *Kenya heroe* and *Kenya yombi* which can produce up to 32 bags per acre.

#### 2.2 Challenges to Wheat production

Challenges to wheat production are still considerable not only because of increasing demand due to increasing population but also due to increased scarcity of water resources (Rosegrant, 1997 and WMO, 1997), ever more unpredictable climate (Fischers et al., 2002), increased urbanization and loss of good quality land away from agriculture (Hobbs, 2007) and decreased public sector investment in agriculture and rural affairs (Falcon and Naylor, 2005).

However low, erratic and unreliable rainfall, land pressure by increasing population, lack or expensive inputs like fertilizers, seeds, insecticides and fungicides. Lack or expensive mechanization, lack of improved technologies or packages, poor infrastructure like transport and

communication. Lack of market for their produce or low prices or exploitation by middlemen/brokers, poor market information, poor farmer, extension and research linkages. Wheat production faces challenges of pests like army worm, cutworm, aphids, bollworm, stalk borers, loopers and caterpillars, its also affected by diseases like rust (stem, yellow and leaf), blight, wilt and smut, also birds like *quellera quellera* pose major threat to the grain before harvesting.

### 2.3 *Fusarium* head blight

*Fusarium* head blight (FHB) is a devastating and insidious disease of wheat and barley in humid and semi humid areas world wide (Steffenson, 2003). *Fusarium* head blight affects all classes of wheat and other small grains (Fernandez et al., 2008). *Fusarium* species are among the most studied plant-pathogenic fungi, with several species causing diseases on maize, wheat, barley, and other food and feed grains. A range of different *Fusarium* species has been associated with the FHB but *Fusarium graminearum*, *F. culmorum* and *F. avenaceum* appear to predominate depending on climatic conditions (Parry et al., 1995). *Fusarium* is a large and complex species containing strains adapted to different environmental conditions all over the world

*Fusarium graminearum* is the most common causal agent of *Fusarium* head blight in many parts of the world. This destructive disease affects wheat, barley and other small grains in temperate and subtropical areas. The disease has the capacity to destroy a potentially high yielding crop within a few weeks, causing economic losses due to reduced seed quality (Fernandez et al., 2008). The fungus is a facultative parasite and normally exists as a saprophyte but can live as a parasite on plants causing disease. *F.graminearum* is a cosmopolitan. Although the fungus is found mainly on wheat, corn and barley, it can also occur in other annual and perennial hosts

*Fusarium graminearum* survives on a wide range of hosts including not only living plants such as wheat, corn, barley, soyabean, and rice, but also on dead tissue of many plant species (Shaner, 2003, Xu, 1993). Crop residues on the soil surface are the major reservoir of pathogens of FHB (Shaner, 2003). Ascospores, macroconidia, chlamydospores, and hyphal fragments all can serve as inoculum (Bai, 1994), but ascospores released from soil surface debris are the principal inoculum that initiates epidemics (Bai, 1994, Shaner, 2003, Xu et al, 1993). Wheat planted after corn or wheat often has significantly more FHB than when it is planted after other crops (Shaner, 2003). Reduced tillage for soil conservation increases the amount of inoculum that can infect wheat (Bai, 1994).

*Fusarium* fungi are facultative saprophytes, and an important part of their life cycle occurs on crop residues (Parry et al., 1995; Miller, 1994; Shaner, 2003), which serve as the main reservoir for inoculum that lead to spike infection. Possible sources of inoculum reported are crop debris, alternative hosts and *Fusarium* seedling blight and ear rot of cereals. *Fusarium* species occur on all vegetative and reproductive organs of plants, causing wilts, rots or blights. They have been isolated from soils of every continent except Antarctica (Windels, 1992). Other species may predominate in cooler climates or where crops other than wheat and corn are dominant (Bergstro, 1993; Parry, 1995; Sutton, 1992; Wong, 1992). The fungus persists and multiplies on infected crop residues of small grains and corn. The chaff, lightweight kernels, and other infected head debris of wheat and barley, returned to the soil surface during harvest, serve as important sites of over wintering of the fungus (McMullen, 1997). Several species of the soil- and residue-borne

fungus *Fusarium* are capable of inciting FHB. Even if no FHB was present, the crop residue may be colonized.

Most species of *Fusarium* are spread by dispersal of conidiospores that are blown or splashed to new infection courts. Airborne spores released from crop residue are deposited on or inside wheat florets where they germinate and initiate infection. The fungus rapidly infects the extruded anthers and then ramifies throughout the developing caryopsis, floral bracts, and rachis (Bai, 1994; Bushnell, 2003). The fungus may also infect by direct penetration of glumes, palea, or rachilla (Bushnell, 2003). Soon after infection, dark-brown, water-soaked spots appear on the glumes of infected florets. Later, entire florets become blighted. The fungus infects other spikelets internally through vascular bundles of the rachilla and rachis in susceptible wheat (Bushnell, 2003). Blight becomes more severe as the fungus spreads within a spike, and eventually the entire spike will become blighted. Infected florets often fail to produce grain, or the grain they produce is poorly filled.

When temperature and moisture are favorable, infection can occur any time after commencement of flowering in wheat, but anthesis is the growth stage most vulnerable to infection (Anderson, 1948). Because of brief period of vulnerability, the fungus is limited to one infection cycle per season (Bai, 1994). Primary infection can occur on several florets of a spike in field conditions. The dark brown symptoms usually extend into the rachis, even down into the stem tissue as the fungus spreads within a spike. The clogging of vascular tissues in the rachis can cause the head to ripen prematurely, so that even grains not directly infected will be shriveled owing to a shortage of water and nutrients (Bai, 1995, Schroeder and Christensen, 1963). If heads are

extensively invaded at a very early stage, kernels may fail to develop entirely, which significantly reduces grain yield and quality. The abundance of primary inoculum and weather conditions, mainly moisture and temperature, during and after anthesis determine the severity of *Fusarium* head blight.

Decreased yield, as well as diminished quality and value of the grain, results in significant worldwide economic losses. Yield loss in small grain cereals due to FHB epidemics has been estimated to be in the order of 10–70 percent (Martin and Johnston, 1982; Windels, 2000). This yield reduction is associated with a decrease of grain number per ear, 1000-grain weight and grain weight per ear (Chelkowski et al., 2000).

## **2.5 Mycotoxins in wheat**

Mycotoxins are natural, secondary metabolites produced by fungi on agricultural commodities in the field and during storage under a wide range of climatic conditions (Rudolf et al., 2008). Mycotoxin type and concentrations is always determined by the mould, substrate and external factors. External factors affecting mould production can be physical, chemical and biological, but almost always in interaction with each other (Verica et al, 2007). These mycotoxins are hazardous to humans and animals (Desjardins and Honn, 1997).

Different strains from the same species may behave differently in mycotoxin production. There is also a regional difference in mycotoxins produced by *Fusarium* species, as a result of genetic variation and climatic factors (Verica et al., 2007). Different mycotoxins have been discovered, exhibiting great structural diversity, which results in different chemical and physicochemical

properties. Aflatoxins and ochratoxins (produced mainly by *Aspergillus* sp.), fumonisins, trichothecenes and zearalenone (produced by *Fusarium* sp.), patulin (produced by *Penicillium* sp.), and ergot alkaloids (produced in the sclerotia of *Claviceps* sp.) receive the most attention due to their frequent occurrence and their severe effects on animal and human health (Bennett and Klich 2003).

Mycotoxins are potent toxins and have a wide range of actions on animals and humans: cyto-, nephro- and neurotoxic, carcinogenic, mutagenic, immunosuppressive and estrogenic effects. Although mycotoxicoses caused by direct consumption of contaminated food and feedstuffs poses the greatest risk to animals and humans, the entry of mycotoxins or their metabolites into the food chain by “carry over” into milk, animal tissue or eggs, for example, should not be underestimated (Rudolf et al, 2008).

Deoxynivalenol (DON) and nivalenol (NIV) are Type B trichothecenes produced predominantly by *F. culmorum* and *F. graminearum*. Isolates of both these species are either DON or NIV producers. DON producers are referred to as Type 1 chemotype. This chemotype is further divided into 1A and 1B depending on the acetylated DON that is produced as a co-contaminant, 3- or 15-acetyl DON, respectively (Jennings et al. 2004a, 2004b). *F. poae* has also been linked to high levels of NIV (Pettersson et al., 1995). HT-2 toxin (HT2) and T-2 toxin (T2) are type A trichothecenes that are produced predominantly by *F. sporotrichioides* and *F. langsethiae* (Kosiak et al., 2003; Thrane, 2004). Deoxynivalenol (DON) is an inhibitor of protein synthesis with a broad spectrum of toxigenicity against animals. Ingestion of contaminated grain or by-products of exposed animals has severe long-term consequences, including immunosuppression,

neurotoxicity, and nutrient uptake alteration (Gilbert, 2000). In domestic animals, particularly swine, extremely low levels of DON induce protracted feed refusal.

The contamination of feedstuffs and foodstuffs with mycotoxins is of increasing concern as changes in agricultural practice and probably climatic changes seem to have increased the prevalence of mycotoxin contamination. Contamination of feeds with mycotoxins accounts for significant economic losses in animal husbandry, as well as in undesirable trade barriers for raw materials and consumable products (Wu, 2006). National and international institutions and organizations, such as the European Commission (EC), the US Food and Drug Administration (FDA), the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) of the United Nations, have recognized the potential health risks to animals and humans posed by food- and feed-borne mycotoxin intoxication and addressed this problem by adopting regulatory limits for major mycotoxin classes and selected individual mycotoxins.

To protect consumers from mycotoxicosis many countries, including the European Union Member States have established maximum allowed levels for the most prevalent *Fusarium* mycotoxins in cereals and cereal products (Van Egmond, 2004, Anonymous, 2005). The EC has set maximum levels for some mycotoxins, including several aflatoxins, ochratoxin A, patulin, deoxynivalenol and zearalenone, in certain foodstuffs. Maximum levels for fumonisins B<sub>1</sub> and B<sub>2</sub> came into force in October 2007. European Union regulation allows a maximum deoxynivalenol (DON) content in unprocessed bread wheat of 1.25 ppm, in bread and bakeries of 0.5 ppm and in baby food of 0.2 ppm (Anonymous, 2005). Due to DON toxicity and its potential effect on the immune system, the European Community have strictly fixed (EC regulation number 1881,

2006) the maximum authorized DON level in unprocessed durum or common wheat grains at 1750 and 1250  $\mu\text{g kg}^{-1}$ , respectively, and in resulting flours or semolina at 750  $\mu\text{g kg}^{-1}$ . Health Canada has established guidelines for DON in soft wheat of 2 and 1 ppm for non-staple foods and baby foods, respectively. Consideration of a review of the maximum levels for deoxynivalenol, zearalenone and fumonisins B1 and B2 as well as the appropriateness of setting a maximum level for T-2 and HT-2 toxins in cereals and cereal products should have been completed by July 2008 (EC, 2006). Further more the EU continues to tighten its regulations on mycotoxin levels.

The FAO has compiled comprehensive worldwide regulations and directives regarding mycotoxins in food and feed as of December 2003 (FAO, 2004). The Joint Expert Committee on Food Additives (JECFA), codex alimentary, a scientific advisory body of FAO and WHO, provides mechanisms for assessing the toxicity of food additives, veterinary drug residues and contaminants, and has recently evaluated the hazards related to several mycotoxins, including fumonisins B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, ochratoxin A, deoxynivalenol, T-2 toxin, HT-2 toxin, and aflatoxin M<sub>1</sub> (WHO, 2002).

The U.S food and Drug administration (FDA) regulates levels of certain mycotoxin for consumption by human and animal. The FDA has established the following advisory levels for DON in food and feed; 1 part per million (ppm) for finished grain products for human consumption with no standard for raw grain going in to milling process. For cattle over 4 months old 10 ppm providing grain at that level does not exceed 50 percent of diet. However, for poultry is set to be 10 ppm providing grain at that level does not exceed 50 percent of diet while 5ppm is

established for swine providing grain at that level does not exceed 20 percent of diet.5ppm for all other animals providing grain at that level does not exceed 40 percent of diet.

**Table 2.1:** Maximum tolerated levels for the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZON) in food commodities

<b>Mycotoxin</b>	<b>Commodity</b>	<b>Limit ug/kg</b>
<b>Trichothecenes</b>		
<b>Deoxynivalenol (DON)</b>	Unprocessed cereals other than durum wheat, oats and maize	1250
	Unprocessed durum wheat, oats and maize	1750
	Cereal flour including maize flour, semolina, maize grits and maize meal	750
	Bread pastries, biscuits, cereal snacks and cereal breakfast	500
	Pasta (dry)	750
	Processed cereal-based food for infants and young children and baby food (dry matter basis).	200
	<b>Zearalenone (also referred to as ZEA,ZON or F2)</b>	
	Unprocessed cereals other than maize	100
	Unprocessed maize	200
	Cereal flour except maize flour	75
	Maize flour, maize meal, maize grits and refined maize oil	200
	Bread, pastries and biscuits	50
	Maize snacks and maize based breakfast cereals	50
	Other cereal snacks and breakfast cereals	
	Processed maize- based foods for infants and young children	20
	Other processed cereal- based food for infants and young children(dry matter basis)	20

Source: EC, 2006

Country	DON ( $\mu\text{g}/\text{kg}$ )	ZON ( $\mu\text{g}/\text{kg}$ )	Commodity
Austria	500	60	Wheat and rye
	750	60	Durum wheat
Brazil		200	Maize
Canada	2000		Uncleaned soft wheat, (intended for infant food)
	1000		
France		200	Maize, small grain cereals
Hungary		50	All foods
Netherlands	All mycotoxins zero tolerance		Cereal products
Romania		30	All foods
Russia	500		Wheat (other than durum)
	1000		Durum wheat
		1000	All cereals, vegetable oil
Uruguay		200	Maize, barley
USA	1000*		Finished wheat products

\*The former FDA "advisory level" for DON of 2000  $\mu\text{g}/\text{kg}$  for wheat entering the milling process was "updated" in 1993, when about 40 percent of the analyzed wheat samples had higher contamination levels.

## 2.6 Sources of resistance to *Fusarium* head blight

Crop management and agro chemical measures are only partly effective to control FHB disease. Therefore the cultivation of *Fusarium* resistant varieties plays key role in integrated *Fusarium* control and the preventions of mycotoxin contaminations (Buerstmayr et al., 2008). Large genetic variation for FHB resistance is available in the wheat gene pool, but often the best regionally adapted and highly productive cultivars are susceptible to FHB. The difficult task for the wheat breeder is to create regionally adapted cultivars that combine high and stable yield and quality performance with resistance to FHB (Buerstmayr et al., 2008). The most efficient strategy to control FHB in wheat is through the development of resistant varieties (Steiner et al., 2008). Resistant to FHB exhibits quantitative variation and its inheritance involves many loci on

different chromosomes (Buerstmayr et al. 2008). Quantitative trait loci (QTL) resistance against FHB have been detected in several European winter wheat populations (Garveis et al., 2003; Shen et al., 2003; Paillard et al., 2004; Schmolke et al., 2005, 2008; Draeger et al., 2007; Klahr et al., 2007; Semagn et al., 2007; Holzapfel et al., 2008; Srinivasachary et al., 2008).

Main sources of resistance to FHB are considered to be from China (Chen et al., 1997), Brazil (Mesterhazy, 1983; Snidgers, 1990), Japan (Ban, 2000) and CIMMYT, Mexico D.F., Mexico (Gilchrist et al., 2000). The expression of resistance to *Fusarium* head scab in wheat include: type I, resistance to primary infection; type II, resistance to subsequent colonization after infection; type III, resistance expressed in developing kernel; type IV, reduced accumulation of mycotoxins; type V, yield tolerance, other types may also exist (Schroeder and Christensen, 1963; Mesterhazy, 1995, Meidaner, 1997). Evidence of expression of type I, II, III is quite strong. Reduced incidence of infection is an indicator of type I and can easily be overwhelmed by environmental conditions (Schroeder and Christensen, 1963. Type II resistance has been extensively studied in wheat and appears to be more stable and less affected by non genetic factors than type I resistance (Bai and Shaner, 1994). Type III manifests itself by a reduction in occurrence of *Fusarium* damaged kernels (FDK). Each of these resistances is believed to be conditioned by multiple genes showing additive effects (Meidaner, 1997).

Much less is known about resistance type IV, reduced mycotoxin accumulation Yield tolerance is the ability of a plant to produce a crop even in the presence of disease at a level that would debilitate most other lines. Yield tolerance is well documented in some other plant disease systems, but evidence for it in FHB (as type V) is largely circumstantial, although a number of researchers believe it to occur (Mesterhazy 1995). Most wheat cultivars available are susceptible

to head scab, none are immune and a few are moderately resistant (Parry et al., 1995). In addition to these types of physiological resistance there are many other heritable traits which may directly or indirectly affect the plant's risk of exposure to *Fusarium*. Such traits as head type, phenology, trapped anthers, plant stature and rate of filling have been implicated as influencing the level of FHB (Parry et al., 1995 and Meidaner, 1997).

Control of DON contamination in the field could probably be obtained by the selection of resistant cultivars (Gervais et al., 2003; Jiang et al., 2007) and a better knowledge of environmental factors (Edwards, 2004; Koch et al., 2006). However, no accession has yet been identified to be completely immune to FHB (Gocho, 1985; Mesterhazy, 1987; Takeda and Heta, 1989; Snidjers, 1990c; Liu and Wang, 1991). Incremental increases in resistance are being reported in hexaploid wheat and to a lesser extent in barley and durum wheat. The development of molecular markers will improve the efficiency of developing FHB in wheat cultivars (Rudd, 2001).

## **2.7 Use of fungicides and microbial agents in management of *Fusarium* head blight**

Though some success in controlling FHB can be expected by plowing fields to bury crop residues infested with *F. graminearum* after harvest (Bai, 1994), minimal tillage practices render this alternative unacceptable. Some progress has been made in finding and analyzing scab resistance in wheat, though all cultivars in current production are susceptible (Bai, 1994). Foliar fungicides applied at anthesis can be useful in reducing scab (McMullen, 1997). Costs and concerns in the public and private sectors over pesticide residues in the environment and in food products render this disease control alternative less attractive.

Biological control an environmentally acceptable method for substantially decreasing the level of disease incited by *F. graminearum*. Though biological control agents (BCA's) have become a more acceptable control alternative for plant pathogens and BCA products are being marketed to a greater extent than ever before. The life cycle of *F. graminearum* suggests that the pathogen is especially susceptible to control using applied microorganisms at anthesis through the soft dough stage of kernel development, when the majority of wheat head infection is by *F. graminearum* (Andersen, 1948). The microbial agents of the present invention are highly useful in agriculture to protect plants from a variety of plant bacterial, fungal, and viral diseases.

These agents can enhance the growth of treated plants. Significantly, these effects are achieved without being hazardous to animals or humans. Biological and Cultural Tests to date, there have been few attempts to develop strategies and microorganisms for biologically controlling FHB (Stockwell et al., 1997); Perondi et al, 1996). There are many reports about the application of antagonistic fungi in controlling plant disease such as the use *Penicillium* species studied by Soyong et al. 2005, *Trichoderma* species by Soyong et al. 2005, *Cheatomium* species by Soyong et al. 2005 *Bacillus* species by Sadfi et al. 2001, and *Paecilomyces* species by Kiewnick and Sikora 2006. Hydrolytic enzymes of antagonistic microorganisms have been considered to play an important role in the biological control of plant pathogens.

## CHAPTER THREE

### STATUS OF FUNGAL CONTAMINATION WITH SPECIAL REFERENCE OF DIFFERENT SPECIES OF *FUSARIUM* AND ITS MYCOTOXINS

#### 3.1 Abstract

*Fusarium* ear blight (FEB), caused principally by the fungal pathogens *Fusarium graminearum* and *F. culmorum*, is a devastating disease of wheat. A survey covering 120 farms was done in Nyandarua North, Imenti North and Narok districts of Kenya during the 2008 growing season to determine *Fusarium* head blight (FHB) and mycotoxin contamination levels in wheat. FHB incidence was determined as the number of blighted ears per 10m<sup>2</sup>. Wheat production practices were determined using structured questionnaire while rainfall and temperature data due to their influence on FHB inoculum were also collected. Isolation of the fungus was done from wheat stems, heads, straws, grains and soil by plating on potato dextrose agar (PDA). Mycotoxin content analysis in the grains was done by competitive Enzyme Linked Immunosorbent Assay (ELISA). FHB was found to be highly prevalent with an incidence of up to 87 percent. During the survey it was found that similar varieties were grown as those grown during the 2002 cropping season. The newer released varieties were mostly grown compared to older released ones with Njoro bw2 grown by up to 45 percent. Maize was the crop grown mostly besides wheat by up to 70 percent while aphids and stem rust were the most reported insect and disease pests by up to 72 percent and 76 percent respectively. Different chemicals were used in the management of pests with Dimethoate<sup>R</sup> and Folicur<sup>R</sup> most used by 43 percent and 75 percent respectively. Mycotoxin detected in the grain samples was T-2 that varied from 3ppb to 22ppb. Different fungal genera isolated from wheat stems, heads, grains, straws and soil samples were *Fusarium*, *Epicoccum*, *Trichoderma*, *Alternaria* and *Penicilium*. The presence of high levels of

*Alternaria* and *Epicoccum* showed low levels of *Fusarium*. *Fusarium* was most isolated from stems, heads and soil while *Epicoccum* was most isolated from straws and grains. Different *Fusarium* species isolated were *F. poae*, *F. graminearum*, *F. stilboides*, *F. verticilloides*, *F. fusarioides*, *F. tricinctum*, *F. heterosporum*. High prevalence of *F. poae* showed low prevalence of *F. graminearum*. The results indicated that FHB and the associated mycotoxins are prevalent in Nyandarua North, Imenti North and Narok districts of Kenya. The main causal agent was *F. poae* and *F. graminearum* while the major mycotoxin in the grain was T-2 with Imenti North having the highest contamination level.

### 3.2 Introduction

Wheat (*Triticum aestivum* L.) is the second most important cereal grain in Kenya after maize (K.A.R.I., 1989). *Fusarium* head blight of wheat and barley is caused by several fungal species that cause similar symptoms (Nicholson, 2008). *Fusarium* head blight is caused by fungal species in genus *Fusarium*. Several species of the soil- and residue-borne fungus *Fusarium* are capable of inciting FHB. A range of different *Fusarium* species has been associated with the disease but *Fusarium graminearum*; *F. culmorum* and *F. avenaceum* appear to predominate depending on climatic conditions (Parry et al., 1995). *Fusarium* is a large and complex species containing strains adapted to different environmental conditions all over the world. *Fusarium* head blight is one of the most destructive diseases of wheat in areas where the weather is warm and humid after the heading of wheat (Tomohiro and Kazuhiro, 2000). It reduces grain yield and quality due to grain shriveling. FHB also produces mycotoxins harmful to animals and human health, such as deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, zearalenone (ZEA) and their

derivatives, in the grain (Ueno et al., 1973; Tanaka et al., 1988; Snijders, 1990e; Atanassov et al., 1994; Yoshizawa et al., 1995; Ehling et al., 1997).

Different strains from the same species may behave differently in mycotoxin production. There is also a regional difference in mycotoxins produced by *Fusarium* species, as a result of genetic variation and climatic factors (Verica et al., 2007). Earlier studies in Kenya show that different species of fungal genera and *Fusarium* species have been isolated from wheat kernels and heads in samples collected from samples collected Nyandarua and Nakuru districts of Kenya. Mycotoxins aflatoxins, deoxynivalenol, T-2 toxin therefore the risk associated with mycotoxin contamination by Muthomi et al., 2002, 2004, 2007, 2008, Riungu et al., 2008 and by Ndung'u et al., 2006. Determination of *Fusarium* species inoculum levels in wheat production regions is of economic importance in Kenya. Since the type and concentration of mycotoxin contamination is determined by *Fusarium* species involved. Therefore this study was carried out to determine FHB inoculum levels in stems, heads, grains, straws and soil samples collected from different wheat growing regions of Kenya and the associated mycotoxins.

### **3.3 Materials and methods**

#### **3.3.1 Survey and sample collection**

A survey was carried out between July and November 2008 before and after harvesting, in 12 different agro ecological zones of major wheat growing regions of Narok, Imenti North and Nyandarua North districts of Kenya. The agro ecological zones included (upper highland 3 and 4, lower highland 3 and 4 of Nyandarua North, lower highland 4, upper highland 2, 3 and 4 of Narok. More so, upper highland 2 and 3, lower highland 2 and 4 of Imenti North whose

characteristics are shown in table 3.1. In each agro ecological zone 10 farms were randomly sampled such that the fifth farm in a transect was selected. If the farm was found to have no wheat planted, then the next or previous farm was selected making a total of 120 farms. Large and small scale farmers were considered in each district. Before harvesting samples of 0.5kg of fresh stem bases and blighted wheat heads per farm were randomly sampled and collected in khaki paper bags and carried in cool box for pathogen isolation. Immediately after harvesting freshly harvested kernels and straws were mixed up and 1-2kgs sub samples collected in khaki paper bags for mycological and mycotoxin analysis. Soil sampling was done by collecting top soil at 5 different points of the farm, which was then mixed up and 1kg sub samples taken for fungal isolation. All samples were stored at 4°C until analyzed.

**Table 3.1:** Characteristics for different agro ecological zones (AEZ) in wheat growing districts covered during the survey.

AEZ	Description	Annual mean rainfall in mm	Length of growing period in days
Lower Highland 3 (LH3)	Wheat/(Maize)-Barley zone	750-900	190-260
Lower Highland 4 (LH4)	Cattle-Sheep-Barley zone	700-1400	115-130
Lower Highland 2 (LH2)	Wheat/Maize-Pyrethrum Zone	1200-1800	320-340
Upper Highland 2 (UH2)	Pyrethrum-Wheat zone	950-1600	310-330
Upper Highland 3 (UH3)	Upper Wheat-Barley zone	700-1000	280-300
Upper Highland 4 (UH4)	Maize-wheat zone	600-850	230-280

Source: Ministry of agriculture and GTZ, 2007.

In each farm disease incidence was determined and 10M<sup>2</sup> quadrants were marked and counted out number of diseased heads per total heads in the quadrant. Other information collected included; wheat varieties grown, disease and insect pests, control measures undertaken, other crops grown besides wheat (Appendix 1), rainfall and temperature data since they affect *Fusarium* inoculum levels (Appendix 3) in the three wheat growing districts.

### 3.3.2 Isolation and culture of *Fusarium* head blight pathogens

Pathogen isolations were done from wheat stems, heads, harvested straws, grains and soil collected from wheat farms. Isolation was done by plating on low strength potato dextrose agar amended with mineral salts (Muthomi, 2001): (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) and 40ppm of antibiotics penicillin, tetracycline, streptomycin and pentachloronitrobenzene (PCNB) were added after cooling the media to 45<sup>0</sup>c. The infected wheat heads, leaves, stem bases and straws were cut into small pieces 0.5 cm long. Each sample was thoroughly mixed and 50 pieces of the heads, straws and stem bases sub samples were taken randomly for mycological analysis. The fifty pieces were surface sterilized in 3 percent sodium hypochlorite with 3 drops of Tween 20 for three minutes and then rinsed off thrice in sterile distilled water. Ten pieces of wheat stems, heads and straws were plated per petri dish and replicated 5 times. A total of 50 pieces for the stems, heads and straws was plated.

Grains per sample were thoroughly mixed and 100 seeds sub sample randomly selected. Hundred kernels were surface sterilized using 3 percent sodium hypochlorite with 3 drops of Tween 20 for three minutes and then rinsed off thrice in sterile distilled water and plated by replicating 10 times per sample. A total of 100 seeds were plated per sample. Isolation from soil samples was

done by dissolving one gram of the sample in 9mls of sterile distilled water and serially diluted up to a dilution of  $10^{-6}$ . The  $10^{-4}$  and  $10^{-5}$  dilutions were plated in PDA by taking 1ml of each sample and evenly dispersing it at the bottom of sterile petri dishes. Then added 20ml of molten Potato Dextrose Agar amended with 40ppm of antibiotics in each petri dish.

The plates were arranged in a complete random design and incubated for 5-7 days at  $25^{\circ}\text{C}$ . Incubation was done at  $22^{\circ}\text{C}$  for 5 to 14 days under 12 h daylight and 12h darkness cycles. Counts were made of the total number of infected seeds or pieces per plate, number with *Fusarium* and *Fusarium* species and other fungal genera per plate. Different fungal genera and *Fusarium* species colonies per petri dish were counted and the number of colony forming units per gram (CFU/g) calculated by multiplying number of colonies by dilution factor for the soil.

### 3.3.3 Identification of fungi and *Fusarium* species and other fungi

*Fusarium* colonies were sub-cultured on both synthetic nutrient agar (SNA): ( $\text{KH}_2\text{PO}_4$  1.0g,  $\text{KNO}_3$  1.0g,  $\text{MgSO}_4$  0.5g, KCL 0.5g, Glucose 0.2g, Agar 20g) (Nirenberg, 1981) and PDA. The cultures were incubated under near-UV light for 14-21 days to induce sporulation. *Fusarium* colonies were identified based on cultural and morphological characteristics like colony colour, pigment production, presence of aerial mycelium in addition to morphological characteristics like conidia shape, septation and conidiophores (Nelson et al., 1983; Leslie and Summerel, 2006 and Seifert, 1996). Other fungi genera were sub cultured on PDA and identified to genus level based on cultural and morphological characteristics.

### 3.3.4 Mycotoxin analysis

T-2 and Deoxynivalenol (DON in wheat grain were analyzed by direct competitive Enzyme-Linked Immunosorbent Assay (ELISA, (AOAC, 1995; Gathumbi, 2001). Each sample was

homogenized and 100g sub-samples were ground to fine powder. Five grams of the ground sample was extracted with 25ml of methanol: water (70/30v/v) for T-2 toxin and 25mls of distilled water for DON toxin.

#### 3.3.4.1 DON analysis

50 microlitres of the prepared sample was pipeted into each microwell and 50 $\mu$ l of peroxidase deoxynivalenol conjugated. 50  $\mu$ l of the anti-deoxynivalenol antibody was added to the microwells mixed by shaking the plate manually and then incubated at room temperature for 30minutes. After incubation the liquid was drained off and the micro well blotted dry into paper towel. The wells were filled with 250 $\mu$ l of washing buffer (10:1) 10mm of phosphate buffer (pH 7.4) containing 0.05 percent Tween 20. The wells were emptied and the remaining liquid poured off and rinsed twice. 100 $\mu$ l of substrate/chromogen containing tetramethylbenzidine was added then mixed gently by shaking the plate manually and incubating for 15minutes at room temperature. 100 $\mu$ l of stop solution containing 1 N sulfuric acid was added to each well then mixed gently by shaking the plate manually. Absorbance at 450nm was determined using spectrophotometer and read within 10 minutes after adding the stop solution.

#### 3.3.4.2 T-2 analysis

Fifty microlitres of the prepared sample was pipeted into each microtiter and 50 $\mu$ l of peroxidase T-2 toxin conjugated. 50  $\mu$ l of the anti-T-2 toxin antibody was added to the micro titer mixed by shaking the plate manually and then incubated at room temperature for one hour at room temperature. After incubation the liquid was drained off and the micro titer blotted dry into a paper towel. The wells were filled with 250 $\mu$ l of distilled water. The wells were emptied and the remaining liquid poured off and rinsed twice. 50 $\mu$ l of substrate containing urea peroxide and 50  $\mu$ l of chromogen containing tetramethylbenzidine was added then mixed gently by shaking the

plate manually and incubating for 15 minutes at room temperature. 100 µl of stop solution containing 1 N sulfuric acid was added to each well then mixed gently by shaking the plate manually. Absorbance at 450 nm was determined using spectrophotometer and read within 30 minutes after adding the stop solution. A calibration curve for the standards for each toxin dilutions was plotted using log<sub>10</sub> of standards concentration against the percentage inhibition of the standards.

### **3.3.5 Data analysis**

Survey data was analyzed using statistical package for social sciences version 12 for windows (SPSS). All other data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of genstat, VSN International limited, 2008 edition III. Differences among the treatment means were separated using the Fisher's protected LSD test at 5 percent probability level. Where necessary data was transformed using square root using the formula;  $\sqrt{n + 0.5}$  where n was the number of observations and SQT was square root and 0.5 was a constant.

## **3.4 Results**

### **3.4.1 Wheat production practices**

During the survey it was found that farmers grew different wheat varieties in the three districts as shown in table 3.2. It was also found that the newer released varieties were mostly grown compared to the older released ones. Njoro bw2 was the most grown variety by up to 67 percent while Chuzi, Bounty and local varieties were least grown varieties by up to 0.8 percent among the three districts. Duma, Mwamba and Njoro bw2 were the most grown varieties by up to 35 percent, 70 percent and 67 percent in Imenti North, Narok and Nyandarua North districts respectively. It was also found that all varieties grown in Narok were known. Twelve percent and 27 percent of the varieties grown in Nyandarua North and Imenti North districts were unknown.

**Table 3.2: Mean percentage of farmers growing different wheat varieties in three wheat growing districts**

Varieties	Imenti North	Nyandarua North	Narok	Mean
Njoro BW 11	5.0	67.5	62.5	45.0
Heroe	0.0	0.0	10.0	3.3
Mwamba	7.5	7.5	70.0	28.3
Kongoni	0.0	2.5	2.5	1.7
Local varieties	0.0	0.0	2.5	0.8
Njoro BW I	7.5	7.5	0.0	5.0
Mbuni	0.0	5.0	0.0	1.7
Kwale	17.5	0.0	57.5	25.0
Duma	35.0	2.5	5.0	14.2
Mamba	2.5	0.0	2.5	1.7
Chiriku	0.0	5.0	0.0	1.7
Popo	2.5	0.0	5.0	2.5
Bounty	0.0	2.5	0.0	0.8
Chuzi	0.0	0.0	2.5	0.8
Not known	27.5	12.5	0.0	13.3

More so, it was found that wheat was not grown in isolation but among other crops as shown in table 3.3. It was also found that vegetables especially Brassicae family, legumes, gramineaous, root, tuber crops and solanaceous were the other crops grown besides wheat. Maize was found to be the other crop mostly grown besides wheat by up to 75 percent compared to onions which was grown by up to 3 percent. Wheat was grown as sole crop by up to 30 percent. Wheat was not grown as sole crop in Imenti North district. Potatoes and maize were the other crops grown mostly besides wheat by up to 85 percent, 75 percent and 70 percent in Imenti North, Nyandarua North and Narok districts respectively. It was found that food security crops or orphaned crops were mostly grown besides wheat compared to non food security crops. It was also found that vegetables were least grown compared to legumes, cereal and tuber crops.

**Table 3.3: Percentage of wheat farmers growing other crops besides wheat in three wheat growing districts**

	Imenti North	Nyandarua North	Narok	Mean
Potatoes	85.0	57.5	35.0	59.2
Peas	57.5	20.0	17.5	31.7
Maize	67.5	75.0	70.0	70.8
Beans	20.0	47.5	42.5	36.7
None	0.0	15.0	30.0	15.0
Cabbage	22.5	17.5	2.5	14.7
Kales	2.5	15.0	2.5	6.7
Onions	2.5	5.0	2.5	3.3
Carrots	20.0	2.5	0.0	7.5

During the survey it was found that different insect, disease and weed pests were major challenges during wheat production as shown in table 3.4. It was also found that up to 12 percent and 22 percent of the disease and insect pests respectively were unknown. Aphids and stem rust were the most reported insect and disease pests by up to 85 percent and 87 percent respectively. Worms, blight, smut and wilts were least reported. Imenti North and Narok reported highest levels of aphid infestation and stem rust infection respectively. Absence of other pests was reported by up to 72 percent. Quillea and weeds were reported by up to 35 percent and 25 percent respectively.

**Table 3.4: Mean percentage of farmers who reported different pests and diseases in three wheat growing districts**

<b>Insect</b>	<b>Imenti North</b>	<b>Nyandarua North</b>	<b>Narok</b>	<b>Mean</b>
Aphids	85.0	67.5	65.0	72.5
Cut worms	2.5	10.0	7.5	6.7
Army worms	0.0	0.0	30.0	10.0
Not known	15.0	22.5	17.5	18.3
Caterpillars	25.0	7.5	15.0	15.8
Boll worms	0.0	5.0	0.0	1.7
<b>Disease</b>				
Blight	7.5	0.0	5.0	4.2
Stem rust	77.5	65.0	87.5	76.7
Yellow rust	7.5	7.5	30.0	15.0
Not known	7.5	12.5	5.0	8.3
Smut	15.0	5.0	0.0	6.7
Wilt	2.5	0.0	12.5	5.0
<b>Other</b>				
Frost	0.0	0.0	10.0	3.3
Quellea	35.0	0.0	5.0	13.3
Weeds	12.5	25.0	2.5	13.3
None	52.5	72.5	27.5	50.8

During the survey it was also found that farmers used different insecticides, fungicides and herbicides in the management of insect, disease and weed pests respectively as in table 3.5. Eighteen percent and 19 percent of the insecticides and fungicides respectively were unknown. Dimethoate<sup>R</sup> and Folicur<sup>R</sup> were the most used insecticide and fungicide by up to 80 percent. It was also found out that wide ranges of chemicals were used in Narok in the management of pests compared to Nyandarua and Imenti North districts. It was also found that farmers used different herbicides and foliar feeds in their wheat fields. Herbicides or foliar feeds were unused by up to 45 percent. Puma complete<sup>R</sup> was the most used herbicide by up to 47 percent while Round up and glean were the least used.

**Table 3.5: Percentage of farmers using different chemicals\* in the control of different pests and diseases in three wheat growing districts**

<b>Insecticides</b>	<b>Imenti North</b>	<b>Nyandarua North</b>	<b>Narok</b>	<b>Mean</b>
Dimethoate	80.0	17.5	32.5	43.3
Bulldock	2.5	20.0	32.5	18.3
Bactril	5.0	0.0	12.5	5.8
Metasystox	0.0	0.0	2.5	0.8
Neural D	2.5	0.0	7.5	3.3
Rinod	0.0	0.0	2.5	0.8
Stratego	0.0	0.0	7.5	2.5
Ateya	0.0	0.0	5.0	1.7
Nduru	0.0	0.0	2.5	0.8
Decis	5.0	0.0	2.5	2.5
Swing	0.0	0.0	7.5	2.5
Thunder	5.0	0.0	2.5	2.5
Ogor	2.5	10.0	7.5	6.7
Cyclone	0.0	0.0	10	3.3
Karate	10.0	0.0	0.0	3.3
Bestox	2.5	17.5	0.0	6.7
Not known	5.0	32.5	17.9	18.5
<b>Fungicides</b>				
Folicur	80.0	70.0	77.5	75.8
Tilt	0.0	10.0	20.0	10.0
Silvacur	0.0	0.0	10.0	3.3
Copper oxychloride	0.0	0.0	2.5	0.8
Impact	0.0	0.0	5.0	1.7
Pearl	0.0	0.0	5.0	1.7
Thiovit	7.5	0.0	0.0	2.5
Not known	20.0	25.0	12.5	19.2
<b>Herbicides/other</b>				
2,4-D	25.0	10.0	20.0	18.3
Puma complete	45.0	47.5	17.5	36.7
None	20.0	40.0	45.0	35.0
Round up	0.0	0.0	2.5	0.8
Glean	0.0	0.0	2.5	0.8
Foliar feed	0.0	2.5	37.5	13.3
Zencur	15.0	0.0	0.0	5.0

\*Chemical names are trade marks

### 3.4.2 Fungal inoculum in wheat stems and immature heads

Different fungal genera isolated and identified from wheat stems among the districts were *Alternaria*, *Epicoccum* and *Fusarium* as in table 3.6. *Fusarium* was the most isolated genera in Imenti north by up to 57 percent while *Epicoccum* was least isolated by up to 16 percent. *Epicoccum* was the most isolated genera by up to 48 percent in Nyandarua North district compared while *Fusarium* was least isolated by up to 31 percent. Samples from agro ecological zone lower highland 4 (LH4) had the highest *Fusarium* and *Epicoccum* infection from Imenti North and Nyandarua North districts respectively. Samples from Imenti North had high *Fusarium* infection while samples from Nyandarua had high *Alternaria* and *Epicoccum* infection. High levels of *Fusarium* showed low levels of *Alternaria* and *Epicoccum* and vice versa among the agro ecological zones and districts. There was no significant difference in fungal genera isolated among the agro ecological zones in Nyandarua North and Imenti North districts.

Different species of *Fusarium* isolated and identified from wheat stems in the wheat growing districts were *F. graminearum*, *F. poae*, *F. heterosporum*, *F. fusarioides*, *F. tricinctum*, *F. stilboides* and *F. verticilloides* as in table 3.6. *Fusarium poae* was the most isolated species by up to 16 percent, the highest being in samples from agro ecological zone lower highland 2 (LH2) while *F. heterosporum* was least isolated by up to 6 percent in Imenti North district. *Fusarium graminearum* was the most species isolated by up to 13 percent, the highest being in samples from agro ecological zone lower highland 3 (LH3) in Nyandarua North district. However, *F. stilboides* and *F. heterosporum* were the least isolated by up to 6 percent in Nyandarua district. There was no significant difference in *Fusarium* species among the agro ecological zones and the

districts. High levels of *F.poae* showed low levels of *F.graminearum* and vice versa among the agro ecological zones and districts.

Disease incidence as the proportion of diseased heads per 10 M<sup>2</sup> was as high as 87 percent among the wheat growing districts. Agro ecological zone LH3 and LH2 had the highest disease incidence of up to 87 percent and 81 percent in Nyandarua North and Imenti North districts respectively. There was no significant difference in disease incidence among the agro ecological zones and districts as shown in table 3.7. Different fungal genera isolated from immature wheat heads were *Alternaria*, *Epicoccum*, *Fusarium* and *Penicillium* as indicated in table 3.7. *Fusarium* was the most genera isolated by up to 58 percent and 71 percent, highest being in agro ecological zone LH2 and LH3 in Imenti North and Nyandarua North districts respectively. *Penicilium* was least isolated by up to 9 percent in Imenti North district. There was no significant difference in the fungal genera isolated among the agro ecological zones.

**Table 3.6:** Percentage isolation of fungi contaminating wheat stems in wheat growing districts

	Different Fungal genera					
	Imenti North			Nyandarua North		
	LH2	LH4	Mean	LH3	LH4	Mean
<i>Alternaria</i>	27.8	26.2	27.0	49.0	19.4	34.2
<i>Epicoccum</i>	15.2	16.3	15.7	27.5	48.9	38.2
<i>Fusarium</i>	57.1	57.6	57.3	23.6	31.8	27.7
	<i>Fusarium</i> species					
	Imenti North			Nyandarua North		
	LH2	LH4	Mean	LH3	LH4	Mean
<i>F.fusariodes</i>	7.3	6.6	6.9	7.0	7.3	7.3
<i>F.graminearum</i>	8.2	10.4	9.3	13.5	13.0	13.2
<i>F.stilboides</i>	6.6	6.6	6.6	6.4	6.7	6.7
<i>F.verticilloides</i>	11.5	11.1	11.3	5.7	9.8	9.4
<i>F.poae</i>	16.9	16.4	16.7	11.7	12.2	12.1
<i>F.heterosporum</i>	6.5	6.4	6.5	6.4	6.7	6.7
<i>F.tricinatum</i>	8.7	8.2	8.5	6.1	7.6	7.4

LH4: Lower highland; LH3: Lower highland 3; LH2: Lower highland 2

Similar spectrum of *Fusarium* species was isolated from immature wheat heads as from wheat stems as indicated in table 3.7. *Fusarium verticilloides* was the most isolated species by up to 12 percent the highest being in samples from agro ecological zone LH4 in Imenti north district. *Fusarium poae* was the species most isolated by up to 13 percent in samples from agro ecological zone LH2. *Fusarium graminearum* was the most isolated species by up to 13 percent in Nyandarua north the highest being in samples from agro ecological LH3. There was no significant difference in the *Fusarium* species isolated from immature wheat heads among the agro ecological zones. High levels of *F. poae* showed low levels of *F. graminearum* and vice versa.

Highest *Fusarium* species inoculum levels were isolated in samples from agro ecological zones with high disease incidence in both districts. *Fusarium* was the most fungal genera isolated in stem and immature head samples from Imenti North district while *Epicoccum* and *Fusarium* were the most fungal genera isolated in stem and head samples from Nyandarua North district respectively. *Fusarium stilboides* and *F. heterosporum* were the least isolated species in stem and head samples collected from Nyandarua North and Imenti North districts.

**Table 3.7:** Percentage isolation of fungi contaminating wheat heads and disease incidence (percent) in wheat growing districts

	Fungal genera					
	Imenti North			Nyandarua North		
	LH2	LH4	Mean	LH3	LH4	Mean
<i>Fusarium</i>	58.0	50.1	54.1	71.6	37.0	54.3
<i>Alternaria</i>	15.5	18.8	17.2	19.9	17.2	18.6
<i>Epicoccum</i>	25.0	21.7	23.4	8.5	45.8	27.2
<i>Penicillium</i>	1.5	9.3	5.4	0.0	0.0	0.0
	<i>Fusarium</i> species					
	Imenti North			Nyandarua North		
	LH2	LH4	Mean	LH3	LH4	Mean
<i>F. fusarioides</i>	7.2	7.7	7.5	8.0	7.3	7.6
<i>F. graminearum</i>	10.8	10.8	10.8	13.5	11.8	12.7
<i>F. stilboides</i>	9.7	7.8	8.5	6.9	7.1	7.0
<i>F. verticilloides</i>	10.9	12.2	11.5	11.6	8.7	10.1
<i>F. poae</i>	13.0	8.6	10.8	13.1	10.9	12.0
<i>F. heterosporum</i>	6.4	7.8	7.1	6.5	6.7	6.6
<i>F. tricinctum</i>	9.2	9.8	9.5	9.2	7.8	8.5
Disease incidence (%)	81.4	67.7	74.5	87.5	82.3	84.9

LH4: Lower highland; LH3: Lower highland 3; LH2: Lower highland 2

### 3.4.3 Fungal inoculum in harvested straws and soils

Similar spectrum of fungal genera was isolated from wheat straws as from wheat stems as in table 3.8. *Epicoccum* was the most isolated genera by up to 28 percent and 33 percent, highest being in samples from agro ecological zones upper highland 4 (UH4) and upper highland 3 (UH3) in Nyandarua North and Narok districts respectively. *Alternaria* was the least isolated genera by up to 20 percent and 25 percent in Nyandarua North and Narok districts respectively. There was no significant difference in the fungal genera isolated among the agro ecological zones and districts.

**Table 3.8:** Percentage isolation of fungi genera infecting wheat straws in different agro ecological zones

AEZ	<i>Alternaria</i>	<i>Epicoccum</i>	<i>Fusarium</i>
<b>Nyandarua North</b>			
LH3	20.2	26.0	25.1
LH4	18.4	26.2	26.0
UH3	19.5	28.0	24.2
UH4	18.2	28.7	24.8
Mean	19.1	27.2	25.0
LSD <sub>(p≤0.05)</sub>	NS	NS	NS
Cv (Percent)	17.8	11.2	6.0
<b>Narok</b>			
LH4	25.9	33.5	23.5
UH2	23.9	32.3	26.4
UH3	23.7	33.7	25.3
UH4	25.4	32.2	25.0
Mean	24.7	32.9	25.0
LSD <sub>(p≤0.05)</sub>	NS	NS	NS
Cv (Percent)	14.4	10.7	12.8

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland; LH3: Lower highland 3; LH2: Lower highland 2; UH3: Upper highland 3; UH4: Upper highland 4; NS: Not significant

*Fusarium poae* and *F.graminearum* were the species of *Fusarium* isolated from wheat straws as shown in table 3.9. *F. poae* was the most isolated species by up to 31 percent and 29 percent, highest being in agro ecological zone LH4 and upper highland 2 (UH2) in Nyandarua North and Narok districts respectively. *Fusarium graminearum* was the least isolated species by up to 27 percent and 16 percent in Nyandarua North and Narok districts respectively. High levels of *F.poae* showed low levels of *F. graminearum*. There was no significant difference in the *Fusarium* species isolated among the agro ecological zones.

**Table 3.9:** Percentage isolation of *Fusarium* species contaminating wheat straws in different AEZ's

AEZ	<i>F. graminearum</i>	<i>F. poae</i>
<b>Nyandarua North</b>		
LH3	24.9	28.7
LH4	24.0	31.9
UH3	27.3	24.9
UH4	24.6	28.4
Mean	25.2	28.4
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	14.4	4.6
<b>Narok</b>		
LH4	13.2	27.4
UH2	15.2	29.4
UH3	16.0	26.9
UH4	13.6	28.4
Mean	14.5	28.0
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	19.1	19.2

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland 4, UH2: Upper highland 2; UH3: Upper highland 3; UH4: Upper highland 4; LH3: Lower highland 3; LH2: Lower highland 2; NS: Not significant

Different fungal genera isolated from soil samples were *Trichoderma*, *Alternaria*, *Fusarium*, *Penicilium* and *Epicoccum* as shown in appendix 6. *Fusarium*, *Trichoderma*, *Alternaria*, *Epicoccum* and *Penicilium* were the fungal genera isolated in samples from Nyandarua North district in decreasing order. *Fusarium* was most isolated in samples from agro ecological zone LH4 by up to  $46.5 \times 10^4$  cfu g<sup>-1</sup> while agro ecological zone UH4 had the least isolation of up to  $2.3 \times 10^4$  cfu g<sup>-1</sup> in Nyandarua North district. *Alternaria* was the most isolated genera in soil samples collected from Narok districts by up to  $78.0 \times 10^4$  cfu g<sup>-1</sup> highest being in samples from agro ecological zone LH4. *Epicoccum* was the most isolated genera in agro ecological zone LH4 by up to  $81.0 \times 10^4$  cfu g<sup>-1</sup> in Narok district. *Trichoderma* was the most fungal genera isolated in samples collected from agro ecological zone UH3 by up to  $85.2 \times 10^4$  cfu g<sup>-1</sup> in Imenti North district.

**Table 3.10** : Different fungal genera spp isolated from soil in different agro ecological zones (AEZ) of wheat growing districts

AEZ	<i>Fusarium</i>	<i>Alternaria</i>	<i>Trichoderma</i>	<i>Epicoccum</i>	<i>Penicillium</i>
<b>Nyandarua North</b>					
LH3	14.9	10.2	8.7	10.8	10.4
LH4	15.6	11.0	7.8	8.9	8.6
UH3	16.5	10.1	7.7	7.2	6.7
UH4	6.8	8.4	4.6	5.8	4.7
Mean	13.45	9.94	7.2	8.2	7.6
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS
Cv ( percent)	21.6	29.9	44.8	24.1	27.2
<b>Imenti North</b>					
LH2	14.1	12.8	12.9	5.6	9.4
LH4	11.5	12.6	13.0	7.7	9.5
UH2	13.8	13.3	12.0	8.5	9.5
UH3	16.3	14.8	12.3	9.8	11.0
Mean	13.9	13.4	12.6	7.9	9.9
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS
Cv ( percent)	22.8	21.9	33.1	36.2	34.9
<b>Narok</b>					
LH4	14.0	17.5	11.8	14.1	13.5
UH2	13.2	15.5	10.4	9.8	11.4
UH3	13.4	13.2	6.5	8.9	8.2
UH4	14.7	16.3	13.7	12.7	13.5
Mean	13.8	15.6	10.6	11.4	11.7
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS
Cv ( percent)	32.1	38.2	51.3	61.7	52.2

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland 4, UH2: Upper highland 2; UH3: Upper highland 3; UH4: Upper highland 4; LH3: Lower highland 3; LH2: Lower highland 2; NS: Not significant; CFU: Colony forming units.

\* Data transformed using formula =Square root (CFU + 0.5)

Similar spectrum of *Fusarium* species was isolated from soil samples as from straws as in appendix 7. *Fusarium poae* was the most isolated species in soil samples collected by up to  $69.7 \times 10^4$  cfu  $g^{-1}$  and  $29.8 \times 10^4$  cfu  $g^{-1}$  from Imenti North and Narok districts respectively. Samples with highest *F.poa*e isolation rates were samples collected from agro ecological zones LH4 and UH3 in Narok and Imenti North district. *F.graminearum* was most isolated species

from soil samples collected from agro ecological zone LH4 by up to  $7.0 \times 10^4$  cfu g<sup>-1</sup> in Nyandarua North district. However, *F.graminearum* was the least isolated species in samples collected in samples collected from There was no significant difference in *Fusarium* species among the agro ecological zones and districts.

**Table 3.11:** Different *Fusarium* species isolated from soil in different agro ecological zones (AEZ) of wheat growing districts

AEZ	<i>F.poae</i>	<i>F.graminearum</i>
<b>Nyandarua North</b>		
LH3	2.7	5.6
LH4	1.1	4.0
UH3	3.9	2.2
UH4	4.2	1.6
Mean	3.0	3.4
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	21.7	60.8
<b>Imenti North</b>		
LH2	11.9	8.2
LH4	9.0	6.3
UH2	10.6	8.8
UH3	13.8	8.2
Mean	11.3	7.9
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	28.6	18.3
<b>Narok</b>		
LH4	11.9	5.7
UH2	12.5	6.1
UH3	11.7	7.2
UH4	13.2	7.3
Mean	12.3	6.6
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	30	50.8

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland 4, UH2: Upper highland 2; UH3: Upper highland 3; UH4: Upper highland 4; LH3: Lower highland 3; LH2: Lower highland 2; NS: Not significant; CFU: Colony forming units.

\* Data transformed using formula =Square root (CFU + 0.5)

### 3.4.3 Fungal contamination and *Fusarium* toxins in harvested grains in three districts of Kenya

Similar fungal genera were isolated from wheat kernels as from soils under wheat crop as shown in table 3.12. *Epicoccum* was the most prevalent genera by up to 35 percent while *Penicilium* was least isolated by up to 10 percent in wheat grains collected from Nyandarua North district. There was significant ( $p \leq 0.05$ ) difference in *Trichoderma*, *Alternaria* and *Epicoccum* genera with no significant in *Fusarium* and *Penicilium* genera among the agro ecological zones in Nyandarua North district. Agro ecological zones UH3, LH3, LH4 and UH4 had the highest level of *Epicoccum* contamination in order of decreasing frequency in Nyandarua district. *Alternaria* was the major contaminant in grains collected from Imenti North district with isolation frequency of up to 28 percent while *Trichoderma* was least isolated from the district by up to 11 percent. The agro ecological zone with high levels of *Epicoccum* and *Alternaria* showed low levels of *Fusarium* as shown in table 3.12. There was significant ( $p \leq 0.05$ ) difference in the fungal genera among the agro ecological zones.

**Table 3.12:** Frequency (percent) of fungi genera spp. contaminating wheat kernels from different agro ecological zones (AEZ) of wheat growing districts

AEZ	<i>Alternaria</i>	<i>Fusarium</i>	<i>Trichoderma</i>	<i>Epicoccum</i>	<i>Penicilium</i>
<b>Nyandarua North</b>					
LH3	13.0	28.5	10.7	37.3	10.4
LH4	15.9	29.9	10.3	33.5	10.4
UH3	13.0	28.5	10.6	37.5	10.5
UH4	15.8	29.4	11.1	33.5	10.2
Mean	14.4	29.1	10.7	35.5	10.4
LSD <sub>(p≤0.05)</sub>	0.1	NS	0.0	0.1	NS
Cv ( percent)	13.4	7.6	3.0	6.3	0.0
<b>Imenti North</b>					
LH2	26.1	24.6	11.0	21.3	17.0
LH4	31.0	19.3	10.1	21.6	18.0
UH2	29.1	20.0	11.7	28.0	11.2
UH3	29.3	21.1	11.7	26.1	11.8
Mean	28.9	21.3	11.1	24.3	14.5
LSD <sub>(p≤0.05)</sub>	0.2	0.1	NS	0.2	0.1
Cv ( percent)	8.1	46.8	4.5	8.3	15.1
<b>Narok</b>					
LH4	21.0	20.6	12.5	31.2	14.7
UH2	14.8	19.4	12.1	41.5	12.2
UH3	14.9	18.0	12.2	43.0	12.0
UH4	31.5	24.7	11.7	19.1	13.0
Mean	20.6	20.7	12.1	33.7	13.0
LSD <sub>(p≤0.05)</sub>	0.1	0.1	NS	0.14	0.1
Cv ( percent)	13.1	47.2	5.7	9.4	12.0

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland 4, UH2: Upper highland 2; UH3: Upper highland 3; UH4: Upper highland 4; LH3: Lower highland 3; LH2: Lower highland 2; NS: Not significant

Similar spectrum of *Fusarium* species was isolated from wheat kernels as from wheat heads and stems as shown in table 3.13. However *F. poae* was most isolated in grains by up to 12 percent and 14 percent from Nyandarua North and Imenti North district respectively. The species most isolated in grains collected from Narok was *F. fusarioides* by up to 12 percent. *Fusarium stilboides* was the species least isolated by up to 6 percent in grains collected from Nyandarua North and Imenti North districts while *F. fusarioides* was least isolated in grains collected from

Narok district. Agro ecological zone UH4 had the highest isolation rate of *F.poa* of up to 17 percent while agro ecological zone UH3 had the least isolation rate of up to 11 percent. There was no significant difference in the species isolated among the agro ecological zones in the districts. It was found that soil and grains had wide range of fungal genera while straws, heads and stems had narrow range of fungal genera. *Fusarium* species were widely isolated from wheat stems, heads and grains with straws and soil having narrow range species range. High levels of *Fusarium* inoculum was isolated in soil, heads, stems, grains and straws in order of decreasing frequency. *Fusarium* was most isolated from head, straw and stem samples while *Epicoccum* was most isolated from soil and grain samples.

**Table 3.13:** Frequency (percent) of *Fusarium* species contaminating wheat grains in different agro ecological zones (AEZ) of three wheat growing districts

AEZ	<i>F.poa</i>	<i>F.gram</i>	<i>F.fusa</i>	<i>F.stil</i>	<i>F.verti</i>	<i>F.hete</i>	<i>F.tri</i>
<b>Nyandarua North</b>							
LH3	12.6	12.1	6.4	6.4	6.4	6.4	6.4
LH4	12.5	12.1	6.5	6.3	6.8	6.4	6.5
UH3	11.5	12.9	6.4	6.4	6.4	6.4	6.4
UH4	12.8	12.2	6.3	6.3	6.5	6.3	6.4
Mean	12.4	12.3	6.4	6.3	6.5	6.4	6.4
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS	NS	NS
Cv ( percent)	13.8	14.7	1.8	0.6	4.8	0.8	2.7
<b>Imenti North</b>							
LH2	17.1	7.3	6.2	6.2	8.3	6.2	6.2
LH4	12.1	7.5	6.2	6.2	6.2	6.6	6.2
UH2	14.9	7.9	6.3	6.3	6.6	6.3	6.3
UH3	15.3	9.0	6.3	6.4	6.7	6.4	6.5
Mean	14.9	7.9	6.3	6.3	6.9	6.4	6.3
LSD <sub>(p≤0.05)</sub>	0.2	NS	NS	NS	0.1	NS	NS
Cv ( percent)	7.6	11.3	0.6	0.0	12.8	0.0	1.6
<b>Narok</b>							
LH4	13.0	7.7	6.5	6.4	8.4	6.4	7.19
UH2	16.2	9.5	6.3	6.3	6.7	6.3	6.33
UH3	1.3	1.4	25.3	0.7	0.7	0.7	0.71
UH4	17.4	6.7	10.4	6.8	9.7	7.2	7.25
Mean	12.0	6.3	12.1	5.1	6.4	5.1	5.4
LSD <sub>(p≤0.05)</sub>	0.1	0.1	NS	NS	0.1	0.0	0.1
Cv ( percent)	11.9	15.0	2.0	3.0	16.6	2.7	5.2

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland 4, UH2: Upper highland 2; UH3: Upper highland 3; UH4: Upper highland 4; LH3: Lower highland 3; LH2: Lower highland 2; NS: Not significant; *F. gram*: *F. graminearum*; *F. stil*: *F. stilboides*; *F. verti*: *F. verticilloides*; *F. hete*: *F. heterosporum* and *F. tri*: *F. tricinctum*.

Contamination with mycotoxin T-2 toxin was detected in all samples analyzed as shown in table 3.14. However, regions with high levels of *F.poa* isolation frequency had high levels of T-2 toxin contamination. The levels of T-2 toxin contamination varied from 9 ppb to 14ppb. Imenti North showed the highest T-2 toxin concentration levels of up to 13 ppb compared to Nyandarua North which showed the lowest concentration levels of up to 12 ppb. Agro ecological zone LH3 had the least level of the T-2 toxin contamination while agro ecological zone UH4 had the

highest level of contamination in Nyandarua North district. Agro ecological zone LH4 had the least T-2 toxin contamination of up to 7ppb while agro ecological UH2 had the highest contamination of up to 16ppb in Imenti North. Agro ecological zone UH2 had the highest level of T-2 toxin of up to 20ppb contamination while agro ecological zone UH3 had the least contamination of up to 8ppb in Narok district. There was no significant difference in T-2 toxin contamination levels among the agro ecological zones.

**Table 3.14:** Contamination levels of T-2 toxin (ppb) in grains collected from farmers in different agro ecological zones (AEZ) of different wheat growing districts of Kenya

	Sample number					Mean
	1	2	3	4	5	
<b>Nyandarua</b>						
LH3	11.0	6.8	3.5	13.2	10.96	9.09
LH4	16.6	16.7	12.5	10.8	11.68	13.65
UH3	9.5	12.2	12.2	16.8	11.10	12.36
UH4	9.8	12.2	23.2	11.7	11.63	13.71
Mean	11.7	12.0	12.9	13.1	11.34	12.20
<b>Imenti North</b>						
LH2	16.2	18.6	11.1	10.8	13.5	14.0
LH4	10.9	13.9	7.5	12.7	12.2	11.4
UH2	16.8	23.0	8.6	12.5	11.0	14.4
UH3	11.6	9.2	12.4	16.8	11.3	12.3
Mean	13.9	16.2	9.9	13.2	12.0	13.0
<b>Narok</b>						
LH4	13.3	10.8	15.8	11.2	18.5	13.9
UH2	10.8	10.9	12.3	20.7	17.3	14.4
UH3	11.3	16.1	11.1	8.4	10.8	11.6
UH4	11.1	11.3	12.5	8.6	10.9	10.9
Mean	11.6	12.3	12.9	12.2	14.4	12.7

LSD: least significant difference; Cv: Coefficient of variation; LH4: Lower highland 4; UH2: Upper highland 2; UH3: Upper highland 3; UH4: Upper highland 4; LH3: Lower highland 3; LH2: Lower highland 2; NS: Not significant

### 3.5 Discussion

Results indicated that disease incidence was prevalent in all the agro ecological zones with high incidence of up to 87.5 percent. This suggests that FHB and the associated mycotoxin contamination could pose a great problem in the three districts surveyed in case of major outbreak. The results were not comparable with the findings by Riungu et al., 2008 who found disease incidence as low as 7 percent. This could have been due to favourable weather conditions for the disease development during the 2008 cropping season. Results showed that different wheat varieties were grown by farmers in the three districts during the survey.

The newer released varieties were found to be mostly grown by farmers compared to the older released varieties. This suggests that the newer varieties could be possessing good qualities like disease tolerance compared to the older ones. However, it could also be due to the fact older released varieties were not available as certified seeds and only recycling was done. This was in line with findings of Muthomi et al., 2007 who found varieties grown in Nyandarua and Nakuru districts as Kwale, Mbuni, Njoro bw2, Njoro bw1, chuzi, popo, mwamba, duma, chiriku, kongoni, Heroe.

Njoro bw2 was the most grown variety by farmers in the three districts this could have been due to its ability to tolerate drought and its high yielding capacity of 35bags per acre (Noah, 2005). Chuzi, Bounty and local varieties were least grown due to their low yielding capacity of 19 bags per acre. Njoro bw2 was mostly grown by farmers due to their ability to resist diseases like head blight. Results showed that Njoro bw2 was the most grown wheat variety by 45 percent of the

farmers. This suggests that it could possess desirable characters like disease tolerance, high yielding and drought tolerance thus making it to be grown most.

Results indicated that different other crops were grown besides wheat this suggests that if they are hosts to the disease they could act as source of inoculum for disease in wheat thus pose a threat to wheat production. Maize was found mostly grown by 70 percent of the farmers besides wheat. This suggests that sources of pathogen primary inoculum would be high. Wheat scab epidemics depend mainly on the amount of primary inoculum rather than secondary inoculum (Dubin et al., 1997). Infections of wheat sown in a field of maize residue may be two to three times more severe (Teich and Nelson, 1984). It also suggests that farmers have shifted to maize production at the expense of wheat production because its considered a food security crop in Kenya.

Develop and maintain crop rotation schedule to avoid planting the same commodity in a field in two consecutive years. Wheat and maize have been found to be particularly susceptible to *Fusarium* species and they should not be used in rotation with each other. Crops such as potato, other vegetables, clover and alfalfa that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculum in the field. Different diseases, insects and weed pests were reported during the survey. Aphids and stem rust were the major pests reported during the season by up to 70 percent of the farmers. Blight was reported by least farmers. This suggests that incase of FHB outbreak farmers would not impose control measures thus leading to economic losses due to quantity and quality reduction.

Different fungicides and insecticides were found to be used in the control of diseases and insect pests in the three districts. More so farmers were found to control weeds using different herbicides in the three regions. Folicur<sup>R</sup> and dimethoate<sup>R</sup> were mostly used in the control of diseases and insects pests respectively. Narok district reported used wide range of chemicals compared to Imenti North and Nyandarua North districts.

All wheat varieties were susceptible to *Fusarium* head blight of wheat and mycotoxin contamination. This suggests that incase of major outbreak it will pose major threat to human and animal health. This was comparable with the findings of other researchers. None of the currently available commercial cultivars are immune to *Fusarium* infection, but differences in partial resistance or tolerance to FHB do occur among different hard red spring wheats. Large genetic variation for FHB resistance is available in the wheat gene pool, but often the best regionally adapted and highly productive cultivars are susceptible to FHB (Buerstmayr et al., 2008). No accession, however, has yet been identified to be completely immune to FHB (Gocho, 1985; Mesterházy, 1987; Takeda and Heta, 1989; Snijders, 1990c; Liu and Wang, 1991).

Most wheat cultivars available are susceptible to *Fusarium* head blight, none are immune and only a few are moderately resistant (Muthomi et al., 2002a; Parry et al., 1995). No source of complete resistance is known, and current sources provide only partial resistance (Rudd et al., 2001). The findings supported the findings that host resistance has long been considered the most practical and effective means of control, but breeding has been hindered by lack of effective resistance genes and by the complexity of the resistance in identified sources according to Rudd et al., 2001. Development of resistant cultivars has been slowed down by poorly adapted and

incomplete resistance sources and confounding environmental effects that make screening of germplasm difficult (Anderson, 2001) thus supporting the findings that there is no varieties resistant to FHB. These fungal metabolites pose serious threats to human and animal health (Bennett and Klich 2003).

Different fungal genera were prevalent in soil, stems, heads, straws and grains. *Fusarium*, *Alternaria*, *Trichoderma*, *Epicoccum* and *Penicillium* were the most prevalent fungal genera isolated from wheat and wheat residues. This was in line with other findings by researchers. *Fusarium*, *Alternaria* and *Epicoccum* isolated from diseased wheat head heads and kernels (Muthomi et al., 2007, 2008, Riungu et al, 2008 and Ndung'u et al., 2006). This was also in line with the findings of Nicholson, 2008 who reported that FHB of wheat and barley as caused by several fungal species. High levels of *Epicoccum* and *Alternaria* showed low levels of *Fusarium*. High levels of *Alternaria* and *Epicoccum* prevalence exhibited low levels of *Fusarium* was comparable to Muthomi et al., 2008; Kosiak et al., 2004; Ndung'u et al, 2006; Riungu et al., 2008 and Sab et al., 2007, who found in their studies that high levels of *Alternaria* and *Epicoccum* in different agro ecological zones had low levels of *Fusarium*.

Isolation of several genera in wheat samples could mean that wheat production is at great risk of wide range of mycotoxin contamination like patulin, mycophenolic, penicilic acid, roquefortins, marcfortine A, andrastin, Gliotoxins and toxins of verruculogen/femitemorgen group (Garon et al., 2006; O'Brien et al., 2006) caused by *Penicillium*. High levels of *Fusarium* in the wheat growing areas could be due to presence of inoculum in the wheat grown land due to ploughing of wheat debris that harbour *Fusarium* which can survive long in the soil due to its saprophytic

nature. According to Sutton et al., 1982 who reported that host debris was principal reservoir of *F. graminearum* though seeds are also sources of inoculum. Also the presence of perithecia can survive for more than 16 months on corn and wheat residue under field conditions.

Different species of *Fusarium* were prevalent in the grains, stems, straws, soil and heads. This was comparable with findings of other researchers (Muthomi et al, 2002, 2007, 2008 Riungu et al 2008, Ndung'u et al., 2006) who in their early studies reported that FHB was prevalent as caused by several *Fusarium* species. More over, as reported by earlier findings that FHB is caused by several *Fusarium* species (Vogelgsang et al., 2008). Also this was comparable with Nicholson, 2008 who reported that *F. graminearum* is the major pathogen to FHB and other species are of significance in many countries. Also in Europe, the predominant FHB pathogens were *F. graminearum* Schwabe, *F. culmorum* (WG Smith) Saccardo, *F. avenaceum* (Fries) Saccardo, and *F. poae* (Peck) Wollenweber. Although *F. graminearum* is globally the most prevalent FHB-causing species, *F. poae* and *F. avenaceum* are repeatedly found in contaminated samples. In cereal surveys from Finland, Germany, Hungary, Ireland, Italy, and the UK, *F. avenaceum* and *F. poae* were among the most frequently isolated FHB pathogens (Birzele et al. 2002; Logrieco et al. 2002b; Jestoi et al. 2004; Xu et al. 2005; Anonymous 2007).

More so, in Switzerland (Therwil, BL) comparing different agricultural production systems, *F. poae* was the most prevalent *Fusarium* species in wheat in 2003 (Gunst et al. 2005). *F. graminearum* was also predominant according to the findings by McMullen et al., 1997 who reported that *F. graminearum* predominated among several species that cause scab. The findings also compare with Ramirez et al., 2006 who isolated *F. graminearum* from wheat in Argentina.

This suggests that mycotoxin contamination caused by *Fusarium* species would be prevalent in those regions.

All grain samples analyzed were contaminated with T-2 toxin. T-2 toxin contamination varied from 6ppb to 23ppb. This could have been due to the presence of *Fusarium poae* species which produce the T-2 toxins. Samples with high levels of *F. poae* had high levels of T-2 toxin. This could be due to the contamination of wheat by FHB species. Results indicated that *F. poae* expressed itself better compared to *F. graminearum*; this could be due to high competitive nature of *F. poae* compared to *F. graminearum*. Mechanisms of action of *F. poae* may be more active than *F. graminearum*. The presence of other crops near the vicinity of wheat would pose major threat of FHB infection to wheat because some crops are alternate hosts to *F. graminearum*.

The presence of *Fusarium* genus in different growth stages of wheat indicates proper management of wheat residues and control of the pathogen at different growth stages is of economic importance. Due to the many pathogens attacking wheat at different stages the synergistic effect in disease severity and mycotoxin production is felt. High contamination levels of the different varieties grown by our farmers with FHB shows no resistance is found in Kenyan wheat varieties. The presence of fungal genera in different wheat growth stages calls for major monitoring and control of FHB till maturity and the post harvest there in. Removal of residues after harvesting is necessary to reduce inoculum level rotation with legumes is advocated for to lower the levels of inoculum. High levels of T-2 toxin to all wheat varieties grown pose a threat to feed and food industry. The integrated approach of managing FHB of wheat is therefore recommended.

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## CHAPTER FOUR

### EVALUATION OF WHEAT LINES AND MICROBIAL ANTAGONISTS IN THE MANAGEMENT OF *FUSARIUM* HEAD BLIGHT OF WHEAT

#### 4.1 Abstract

*Fusarium* head blight (FHB) in wheat is one of the world's most destructive plant diseases, decreasing both grain yield and quality. Laboratory and green house experiments were carried out to determine possible management strategies to *Fusarium* head blight and the associated mycotoxins. In vitro activity of fungicides and antagonists was determined in paired cultures method. Effect of antagonistic microbial agents on FHB severity and mycotoxin content was determined by co-inoculating *F.graminearum* and *F.poa*e together with each of the microbial agents: *Alternaria spp*; *Epicoccum spp* and *Trichoderma spp*. Folicur<sup>R</sup>, COTAF<sup>R</sup>, PEARL<sup>R</sup> and Thiovit<sup>R</sup> fungicides were used as standard checks. Host resistance was determined by re-inoculating different isolates of *F.poa*e and *F.graminearum* to four varieties and fifteen lines in pots replicated four times in randomized complete block design. Ten ears per pot were tagged for disease severity assessment. Results indicated that in vitro activity of fungicides had 100 percent pathogen growth inhibition. Antagonists only suppressed pathogen and did not completely control the pathogen by up to 53 percent. Folicur<sup>R</sup> and *Trichoderma* were the most effective fungicide and antagonist in disease severity reduction respectively. There was significant difference in fungicides and antagonists in disease severity reduction among the varieties and lines. All varieties and lines were susceptible to *Fusarium* head blight with varying levels of susceptibility. Njoro bw2 was least susceptible compared to R1104, Mbuni and KIBIS which were most susceptible. Recommend integrated approach of managing of FHB of wheat.

## 4.2 Introduction

*Fusarium* head blight is one of the world's most destructive plant diseases, decreasing both grain yield and quality (Tan et al., 2008). Fusarial toxins are metabolites by certain species of *Fusarium*. It includes trichothecenes such as T-2, DON, Nivalenol), Zearalenone and fumonisins (Reddy and Raghavender, 2008). They are gaining importance due to their deleterious effects on human and animal health. To reduce the risk of FHB infection and mycotoxin contamination in wheat grains, chemicals are possible remedy.

Management of *Fusarium* head blight and the associated mycotoxins have been based on management strategies such as host resistance, biological agents, tillage, seed treatment, crop rotation and fungicides (Heier et al, 2005; Kriel, 2006; Brown, 2007). However, efficacy of fungicides is limited and their use is prohibited in organic wheat production (Forrer, et al., 2008). Promising options for controlling *Fusarium* head blight include chemical measures and the development of resistant cultivars. Registered fungicides can be somewhat effective in reducing FHB (Jonnes, 2000; Lewis et al., 1993), but residue concerns regarding the use of fungicides late in crop development lessen their attractiveness. Advances in developing resistant cultivars using traditional breeding (Bai et al., 2000) and genetic engineering (Bushnell et al.,1998) are occurring, but all wheat cultivars currently in production remain vulnerable to infection.

Although partially effective in reducing FHB, conventional tillage of fields after harvesting to bury plant residues and reduce available pathogen inoculum (Dill-Macky and Jones, 2000; Yi et al., 2001) is not compatible with the soil conservation practice of minimum tillage. Any competitive advantage conferred by DON would complicate efforts to control *Fusarium* during

its saprophytic growth on crop residues that are left after harvest and constitute the primary inoculum reservoir for outbreaks in subsequent plantings (Matthias et al., 2003).

Biological control of FHB holds considerable promise and includes the treatment of crop residues with antagonists to reduce pathogen inoculum (Bujold et al., 2001) and wheat heads at anthesis to reduce infection (Khan et al., 2001; Perondi et al., 1996; Stockwell et al., 1997). Biological control of FHB is attractive due to its potential for being environmentally benign, compatible with other control measures, and durable. In previous investigations on biologically controlling plant disease, Schisler and coworkers (Schisler et al., 2000) demonstrated that isolates of a fungal pathogen can differ substantially in their amenability to being suppressed with microbial agents. Whether microbial strains also vary in their relative effectiveness depending on the isolate of *G. zeae* used in a bioassay is not known. The importance of the order and the timing of arrival of pathogen and microbial agent inoculum is not known for this microbial system. Biological control shows promise as part of an integrated pest management program for managing FHB (Schisler et al., 2001).

### **4.3 Materials and methods**

#### **4.3.1 Isolation and culture of FHB pathogens and antagonists**

Pathogen isolations were done from wheat stems, heads, harvested straws, grains and soil collected from wheat farms. Isolation was done by plating on low strength potato dextrose agar amended with mineral salts (Muthomi, 2001): (PDA 17g,  $\text{KH}_2\text{PO}_4$  1.0g,  $\text{KNO}_3$  1.0g,  $\text{MgSO}_4$  0.5g, Agar 10g) and 40ppm of antibiotics penicillin, tetracycline, streptomycin and pentachloronitrobenzene (PCNB) were added after cooling the media to  $45^\circ\text{C}$ . The infected wheat heads, leaves, stem bases and straws were cut into small pieces 0.5 cm long. Each sample was

thoroughly mixed and 50 pieces of the heads, straws and stem bases sub samples were taken randomly for mycological analysis. The fifty pieces were surface sterilized in 3 percent sodium hypochlorite with 3 drops of Tween 20 for three minutes and then rinsed off thrice in sterile distilled water. Ten pieces of wheat stems, heads and straws were plated per petri dish and replicated 5 times. A total of 50 pieces for the stems, heads and straws was plated.

Grains per sample were thoroughly mixed and 100 seed sub samples randomly selected. Hundred kernels were surface sterilized using 3 percent sodium hypochlorite with 3 drops of Tween 20 for three minutes and then rinsed off thrice in sterile distilled water and plated by replicating 10 times per sample. A total of 100 seeds were plated per sample. Isolation from soil samples was done by dissolving one gram of the sample in 9mls of sterile distilled water and serially diluted up to a dilution of  $10^{-6}$ . The  $10^{-4}$  and  $10^{-5}$  dilutions were plated in PDA by taking 1ml of each sample and evenly dispersing it at the bottom of sterile petri dishes. Then added 20ml of molten Potato Dextrose Agar amended with 40ppm of antibiotics in each petri dish.

The plates were arranged in a complete random design and incubated for 5-7 days at  $25^{\circ}\text{C}$ . Incubation was done at  $22^{\circ}\text{C}$  for 5 to 14 days under 12 h daylight and 12h darkness cycles. Counts were made of the total number of infected seeds or pieces per plate, number with *Fusarium* and *Fusarium* species and other fungal genera per plate. Different fungal genera and *Fusarium* species colonies per petri dish were counted and the number of colony forming units per gram (CFU/g) was calculated by multiplying number of colonies by dilution factor for the soil.

### 4.3.2 Preparation of inoculum and inoculation

Inoculum of 10 highly pathogenic *F. graminearum* and *F. poae* isolates was multiplied in mung bean broth (Bai and Shaner, 1994). Forty grams of mung bean was cooked in 1000mls of sterile distilled water for 40 minutes which was then cooled and the extract sieved using double layer cheese-cloth to get the mung bean suspension. 100mls of the extract were taken and autoclaved in 250ml at 121°C for 20 minutes. After cooling two agar disc of the pathogen were placed in each conical flask. The suspension was incubated on mechanical shaker (40-50 cycles min<sup>-1</sup>) for 5 days followed by 7 days under stationary conditions.

The fungal growth was macerated in a blender and sieved through double layer cheese-cloth. Spore concentration was then adjusted to  $1 \times 10^6$  spores/ml using haemocytometer. A mixed inoculum was obtained by mixing suspension of different isolates. Inoculum suspension for a mixture of *F.graminearum* and *F.poa*e was diluted to  $1 \times 10^6$  spores/ml and then put in a hand sprayer for inoculation to the varieties in the greenhouse. *Epicoccum*, *Alternaria* and *Trichoderma* sp. were raised on PDA for 14 days at 25°C in cycles of 12 h daylight and 12 h darkness. Antagonists were harvested by flooding the cultures with sterile distilled water and sieved through a double layer of cheese cloth. The fungal inoculum was adjusted to  $1 \times 10^6$  spores per litre using haemocytometer.

### 4.3.3 In vitro Screening of antagonists against FHB pathogens

*Epicoccum* spp, *Trichoderma* spp, *Penicilium* and *Alternaria* spp were screened for antagonism to *F.graminearum* and *F.poa*e isolates in culture. Antagonism was by paired cultures method, where the *F.graminearum* and *F.poa*e agar disc were inoculated at the middle of plate and

antagonist 4 equidistant points 2 cm of plate. Fungicides PEARL<sup>®</sup> (500g/l carbendazim), COTAF<sup>®</sup> (50g/l hexaconazole) and Thiovit<sup>®</sup> (micronised sulphur 80 percent w/w) at the rates of 1ml/ litre, 5mls/litre and 2g/litre, respectively, were used as standard checks. Negative control consisted of *F.graminearum* and *F.poa* each cultured alone. Each treatment was replicated four times and the plates arranged in a completely randomized design on laboratory benches and incubated at 25<sup>0</sup>c for 7 days in cycles of 12h daylight and 12 h darkness (Riungu, 2008). Degree of antagonism was determined by measuring the antagonist colony diameters and percentage inhibition calculated as follows;

$$\text{Inhibition (\%)} = \frac{\text{Colony diameter of Pathogen} - \text{Colony diameter of the Pathogen + Antagonist}}{\text{Colony diameter of Pathogen alone (Control)}}$$

#### 4.3.4 Efficacy of fungal antagonists and fungicides against *Fusarium* Head Blight

*Trichoderma*, *Alternaria*, *Penicillium* and *Epicoccum* species found to be effective antagonists to *F.graminearum* and *F.poa* in vitro were evaluated for potential reduction of FHB and mycotoxin accumulation under green house conditions. Fungicides PEARL<sup>®</sup> (500g/l carbendazim), COTAF<sup>®</sup> (50g/l hexaconazole) and Thiovit<sup>®</sup> (micronised sulphur 80 percent w/w) used in managing other fungal diseases in wheat were also evaluated for potential reduction of FHB and mycotoxin DON under green house conditions. Highly and lowly susceptible wheat varieties namely Mbuni and Njoro BW II were planted and replicated each 4 times in greenhouse following recommended agronomic practices. Inoculation was done at 50 percent flowering (GS 65; Zadoks et al., 1974).

Treatments consisted of spraying the ears with *Trichoderma*, *Alternaria* and *Epicoccum* species together with *F.graminearum* and *F.poa*, *F.graminearum* and *F.poa* together with PEARL<sup>®</sup>,

COTAF<sup>®</sup> and Thiovit<sup>®</sup> fungicides at the rates of 1ml/ litre, 5mls/litre and 2g/litre respectively and *F.graminearum* and *F.poa*e alone. Control plants were sprayed with fungicide alone or sterile distilled water. The antagonists and fungicides were sprayed two days before and after inoculation with *F.graminearum* and *F.poa*e. Inoculation with a mixture of *F.graminearum* and *F.poa*e was repeated 6 days after the first inoculation. Each treatment was replicated four times and arranged in randomized complete block design. The treated heads were covered with polythene bags for 48hrs to maintain relative humidity conducive for infection.

#### **4.3.5 Evaluation of wheat lines for susceptibility to FHB**

Fifteen lines and four wheat seed varieties were obtained from Kenya Agricultural Research Institute (K.A.R.I) Njoro plant breeding research station (Appendix 3). Wheat seeds were planted (20 seeds /pot) in forest soil/farm yard manure medium (2:1 V/V) in pots (22cm diameter) and grown outside the greenhouse until flowering to simulate field conditions. The plants were fertilized at different growth stages using urea (46 percent N) 5g per pot after germination, N-P-K 5g per pot at tillering, urea (46 percent N) 5g per pot at booting. Leaf chewing insects and aphids were controlled using Danadim<sup>®</sup> (dimethoate). The flowering dates were synchronized by early planting of late maturing varieties and late planting of early planting varieties. The wheat ears were spray-inoculated with *F. graminearium* and *F. poae* at 50 percent flowering (GS65, Zadoks et al., 1974) with a hand sprayer, ensuring all the spikelets were exposed to the inoculum.

The ears of control plants were sprayed with sterile distilled water. Each treatment was replicated four times. The ears were covered with polythene bags for 48 hrs to ensure high relative humidity for infection. The plants were allowed to mature in the greenhouse to ensure uniform

environmental conditions and to avoid damage by birds. The experiments were conducted in two greenhouse cropping cycles.

#### 4.3.6 Disease assessment

Ten average-sized ear per pot were tagged and disease severity was assessed based on 1-9 scale every seventh day until yellow ripening. Disease severity was assessed visually as the proportion of spikelets bleached (Miedaner et al., 1996): 1 = no symptoms; 2 = < 5 percent of spikelets bleached; 3 = 5-15 percent of spikelets bleached; 4 = 16-25 percent of spikelets bleached; 5 = 26-45 percent of spikelets bleached; 6 = 46-65 percent of spikelets bleached; 7 = 66-85 percent of spikelets bleached; 8 = 86-95 percent of spikelets bleached; 9 = 96-100 percent of spikelets bleached. Area under the disease progress curve (AUDPC) was calculated from the single ratings recorded over the assessment period (Shaner and Finney, 1977) as follows,

$$\text{AUDPC} = \sum_{i=1}^n [(Y_{i+1} + Y_i)] [t_{i+1} - t_i]$$

Where,  $Y_i$  is the visual score of FHB symptoms at the  $i$ th observation date and  $t_i$  is the time (days) at the  $i$ th observation,  $n$  is the total number of observations. At maturity the ten heads assessed were harvested and threshed separately for weight determination. The harvested kernels (100 kernels per treatment) were plated on culture media for re-isolation of causal pathogen. The experiment was repeated over two greenhouse cycles.

#### 4.3.7 Mycotoxin analysis

T-2 and Deoxynivalenol (DON) in the wheat grain were analyzed by direct competitive Enzyme-Linked Immunosorbent Assay (ELISA), (AOAC, 1995; Gathumbi, 2001). Each sample was homogenized and 100g sub-samples were ground to fine powder. Five grams of the ground

sample was extracted with 25ml of methanol: water (70/30v/v) for T-2 toxin and 25mls of distilled water for DON toxin.

#### 4.3.7.1 DON analysis

Fifty microlitres of the prepared sample was pipeted into each microwell and 50 $\mu$ l of peroxidase deoxynivalenol conjugated. 50  $\mu$ l of the anti-deoxynivalenol antibody was added to the microwells mixed by shaking the plate manually and then incubated at room temperature for 30minutes. After incubation the liquid was drained off and the micro well blotted dry into paper towel. The wells were filled with 250 $\mu$ l of washing buffer (10:1) 10mm of phosphate buffer (pH 7.4) containing 0.05 percent Tween 20. The wells were emptied and the remaining liquid poured off and rinsed twice. 100 $\mu$ l of substrate/chromogen containing tetramethylbenzidine was added then mixed gently by shaking the plate manually and incubating for 15minutes at room temperature. 100 $\mu$ l of stop solution containing 1 N sulfuric acid was added to each well then mixed gently by shaking the plate manually. Absorbance at 450nm was determined using spectrophotometer and read within 10 minutes after adding the stop solution.

#### 4.3.7.2 T-2 analysis

Fifty microlitres of the prepared sample was pipeted into each microtiter and 50 $\mu$ l of peroxidase T-2 toxin conjugated. 50  $\mu$ l of the anti-T-2 toxin antibody was added to the micro titer mixed by shaking the plate manually and then incubated at room temperature for one hour at room temperature. After incubation the liquid was drained off and the micro titer blotted dry into a paper towel. The wells were filled with 250 $\mu$ l of distilled water. The wells were emptied and the remaining liquid poured off and rinsed twice. 50 $\mu$ l of substrate containing urea peroxide and 50  $\mu$ l of chromogen containing tetramethylbenzidine was added then mixed gently by shaking the

plate manually and incubating for 15 minutes at room temperature. 100 µl of stop solution containing 1 N sulfuric acid was added to each well then mixed gently by shaking the plate manually. Absorbance at 450 nm was determined using spectrophotometer and read within 30 minutes after adding the stop solution. A calibration curve for the standards for each toxin dilutions was plotted using log 10 of standards concentration against the percentage inhibition of the standards.

#### 4.3.8 Data analysis

All data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of genstat (VSN International limited, 2008 edition III). Differences among treatment means were separated using the Fisher's protected LSD test at 5 percent probability level. Where necessary data was transformed using square root using the formula;  $= \text{SQT}(n + 0.5)$  where n was the number of observations and SQT was square root and 0.5 was a constant.

### 4.4 Results

#### 4.4.1 In vitro activity of biological and chemical agents against FHB pathogens

All the fungicides in vitro had 100 percent colony reduction for both *F.poa* and *F.graminearum* but the microbial agents differed in their colony reduction in vitro as shown in table 4.1. *Epicoccum* had the highest colony reduction percentage of up to 64 percent while *Penicilium* had the lowest colony reduction for *F.poa*. There was significant ( $p \leq 0.05$ ) difference among the biological agents in the reduction of colony of *F.poa* as indicated in table 4.1. Biological agents differed in colony reduction of *F.graminearum* with *Trichoderma* having the highest colony reduction percentage of up to 53 percent while *Penicilium* had the lowest colony reduction percentage of up to 24 percent. There was significant ( $p \leq 0.05$ ) difference among the biological agents and fungicides in colony reduction of *F.graminearum* and *F.poa* as shown in table 4.1.

**Table 4.1:** Average percentage colony diameter reduction of *F. poae* and *F. graminearum* by different competitive fungi and fungicides.

	<i>F. poae</i>	<i>F. graminearum</i>
<i>Trichoderma</i>	62.6	53.4
<i>Epicoccum</i>	64.9	45.7
<i>Alternaria</i>	60.1	49.3
<i>Penicilium</i>	21.8	24.8
Thiovit <sup>®</sup>	100	100
Folicur <sup>®</sup>	100	100
COTAF <sup>®</sup>	100	100
PEARL <sup>®</sup>	100	100
LSD ( $p \leq 0.05$ )	40	60
Cv ( percent)	2.6	2.1

LSD: least significant difference; Cv: Coefficient of variation

#### 4.4.2 Effectiveness of antagonists and chemical agents in reducing *Fusarium* head blight

Disease severity over time increased with time among all the treatments for the two varieties as in table 4.2. There was significant ( $p \leq 0.05$ ) difference for the 7<sup>th</sup> day in disease after inoculation while thereafter until ripening there was no significant difference among the treatments during the first cycle in Mbuni. Treatment with *Trichoderma* had the highest mean disease severity reduction over time of up to 68 percent in Mbuni inoculated with a mixture of different isolates of *F. poae* and *F. graminearum* as in table 4.2. Treatment with PEARL<sup>R</sup> had the lowest mean disease severity reduction of up to 58 percent in Mbuni.

Treatment with Thiovit<sup>R</sup> had the highest mean disease over time severity reduction of up to 64 percent while treatment with PEARL<sup>R</sup> had the lowest mean disease over time reduction of up to 59 percent during the first cycle among the fungicides. During the second cycle mean disease severity reduction over time in Mbuni was high in treatment with Folicur<sup>R</sup> treatment of up to 75 percent while treatment while *Trichoderma* had the lowest mean disease severity reduction of up

to 64 percent as in table 4.3. There was no significant difference for mean disease over time among all the treatments used. Mean disease severity reduction over time was highest in treatment with Folicur<sup>R</sup> while treatment with Thiovit<sup>R</sup> had the lowest mean disease severity reduction of up to 64 percent among the fungicides. Treatment with *Alternaria* had the highest mean disease severity reduction of up to 67 percent while treatment with *Trichoderma* had the lowest mean disease over reduction during the second cycle in Mbuni.

Mean disease severity for Njoro bw2 increased over time among all the treatments. There was significant ( $p \leq 0.05$ ) difference over time among all the treatments. More over, treatment with *Epicoccum* in Njoro bw2 had the highest mean disease severity reduction of up to 76 percent during cycle one as in table 4.1. However, treatment with Thiovit<sup>R</sup> among the fungicides had the highest mean disease severity reduction of up to 69 percent. More so, treatment with Folicur<sup>R</sup> had the highest mean disease severity reduction of up to 81 percent during cycle two. Treatment with *Trichoderma* had highest reduction of up to 72 percent as shown in table 4.3.

**Table 4. 2:** Disease severity over time on wheat ears inoculated with *F. poae* and *F. graminearum* during cycle one

	Mbuni					Mean
	Days after inoculation					
	7	14	21	28	35	
<i>Alternaria</i>	1.6	2.4	4.0	4.5	4.6	3.4
Fungal mixture	2.7	3.0	3.7	4.2	4.3	3.6
<i>Trichoderma</i>	1.7	2.1	3.0	3.7	3.9	2.9
<i>Epicoccum</i>	1.5	2.4	3.2	3.8	4.1	3.0
COTAF <sup>R</sup>	3.1	3.4	3.5	3.9	4.1	3.6
PEARL <sup>R</sup>	2.7	3.1	3.7	4.2	4.4	3.6
Thiovit <sup>R</sup>	2.3	2.4	3.5	3.8	4.1	3.2
Water	2.6	3.0	3.7	4.3	4.5	3.6
LSD <sub>(p≤0.05)</sub>	0.7	NS	NS	NS	NS	
Cv (Percent)	4.6	3.1	6.8	5.5	4.5	
	Njoro BW II					Mean
	Days after inoculation					
	7	14	21	28	35	
<i>Alternaria</i>	2.0	2.4	2.7	3.5	3.8	2.9
Fungal mixture	2.8	3.2	3.6	3.9	4.2	3.5
<i>Trichoderma</i>	1.9	2.3	2.6	3.2	3.5	2.7
<i>Epicoccum</i>	1.4	1.8	2.3	2.4	2.8	2.2
COTAF <sup>R</sup>	2.4	2.8	3.1	3.5	3.8	3.1
PEARL <sup>R</sup>	2.8	3.2	2.4	3.9	4.3	3.3
Thiovit <sup>R</sup>	1.7	2.3	3.0	3.4	3.5	2.8
Water	1.1	1.2	1.6	1.8	2.0	1.5
LSD <sub>(p≤0.05)</sub>	0.6	0.7	0.8	1.0	1.0	
Cv (Percent)	6.1	6.3	0.9	5.0	4.7	

LSD: least significant difference; Cv: Coefficient of variation; NS: Not significant

**Table 4.3:** Disease severity over time for two wheat varieties inoculated with *F.graminearum* and *F.poa*e during cycle two

	Mbuni					
	Days after inoculation					
	7	14	21	28	35	Mean
<i>Alternaria</i>	2.3	2.7	3.0	3.3	3.5	3.0
Folicur <sup>R</sup>	1.5	2.0	2.4	2.4	2.7	2.2
<i>Trichoderma</i>	2.7	3.0	3.2	3.4	3.6	3.2
<i>Epicoccum</i>	2.3	2.7	3.1	3.3	3.5	3.0
COTAF <sup>R</sup>	2.5	2.9	3.2	3.4	3.6	3.1
PEARL <sup>R</sup>	2.1	2.5	2.8	3.1	3.3	2.7
Thiovit <sup>R</sup>	2.6	2.9	3.2	3.5	3.7	3.2
Water	1.7	2.1	2.4	2.7	3.0	2.4
LSD( $p \leq 0.05$ )	NS	NS	NS	NS	NS	
Cv ( percent)	5.3	4.0	4.1	2.2	1.6	
	Njoro BW II					
	Days after inoculation					
	7	14	21	28	35	Mean
<i>Alternaria</i>	1.9	2.2	2.5	2.9	3.1	2.5
Folicur <sup>R</sup>	1.0	1.4	1.7	2.0	2.2	1.7
<i>Trichoderma</i>	1.8	2.1	2.4	2.9	3.2	2.5
<i>Epicoccum</i>	2.0	2.4	2.7	2.9	3.1	2.6
COTAF <sup>R</sup>	2.2	2.6	2.9	3.3	3.5	2.9
PEARL <sup>R</sup>	2.0	2.4	2.8	3.1	3.4	2.7
Thiovit <sup>R</sup>	2.7	3.1	3.4	3.7	3.9	3.3
Water	2.2	2.5	2.8	3.0	3.2	2.7
LSD( $p \leq 0.05$ )	0.6	0.6	0.7	NS	NS	NS
Cv (Percent)	11.2	8.2	6.4	5.6	4.9	

LSD: least significant difference; Cv: Coefficient of variation; NS: Not significant

Area under disease progress curve (AUDPC) was significant among the treatments for the two varieties as shown in table 4.4. Area under disease progress curve in Mbuni was highest for treatment with PEARL<sup>R</sup> by up to 561 while treatment with *Trichoderma* had the lowest AUDPC of up to 416 during cycle one in Mbuni. Treatment with fungal mixture had the highest AUDPC of up to 543. Treatment with *Trichoderma* had the highest AUDPC of up to 579 while treatment with Folicur<sup>R</sup> had the lowest AUDPC of up to 372 during cycle two. There was significant ( $p \leq 0.05$ ) difference in AUDPC among the treatments in Mbuni.

Treatment with PEARL<sup>R</sup> had the highest AUDPC of up to 545 in Njoro bw2 while treatment with *Epicoccum* had the lowest AUDPC of up to 295 during cycle one. However, among the fungicides treatment with Thiovit<sup>R</sup> had the lowest AUDPC of up to 406 while treatment with PEARL<sup>R</sup> had the highest AUDPC. However, among the antagonists treatment with fungal mixture had the highest AUDPC of up to 543 while treatment with *Epicoccum* had the lowest AUDPC during cycle one. It was observed during the Treatment with Thiovit<sup>R</sup> had the highest AUDPC of up to 611 while treatment with Folicur<sup>R</sup> had the lowest AUDPC of up to 255 during the second cycle. There was significant ( $p \leq 0.05$ ) difference in AUDPC among the treatments.

*Trichoderma* was the most effective antagonist with average disease severity reduction of up to 60 percent while treatment with PEARL<sup>R</sup> had the least effectiveness in average disease severity reduction of up to 68 percent during cycle one in Mbuni. However, among the fungicides Thiovit<sup>R</sup> was the most effective fungicide in disease severity reduction. Treatment with *Trichoderma* had the highest average disease severity reduction compared to fungal mixture which had the lowest disease severity reduction of up to 65% among the antagonists. There was significant ( $p \leq 0.05$ ) difference in average disease severity reduction among the treatments as shown in table 4.4.

During cycle two average disease severity reduction was highest in treatment with Folicur<sup>R</sup> of up to 76% while treatment with *Trichoderma* had the lowest average disease severity reduction as indicated in table 4.5. There was significant ( $p \leq 0.05$ ) difference in average disease severity reduction among the antagonists and fungicides during the second cycle. However, among the

fungicides Folicur<sup>R</sup> had the highest average disease severity reduction while Thiovit<sup>R</sup> had the lowest average disease severity reduction. More over, *Trichoderma* had the highest average disease severity reduction the most effective antagonist while *Alternaria* had the lowest average disease severity reduction in Mbuni.

*Epicoccum* and *Trichoderma* had the highest average disease severity reduction of up to 78 percent and 72 percent in Njoro bw2 as shown in table 4.4 and table 4.5 during the first and second cycle respectively. Fungal mixture and *Epicoccum* had the lowest average disease severity reduction by up to 65 percent and 67 percent during the first and second cycle respectively. However, Thiovit<sup>R</sup> and Folicur<sup>R</sup> had the highest average disease severity reduction while PEARL<sup>R</sup> and COTAF<sup>R</sup> had the lowest average disease severity reduction during first and second cycle respectively. There was significant ( $p \leq 0.05$ ) difference in average disease severity reduction among the treatments.

During the first cycle treatment with *Alternaria* and had the lowest 10 ear grain yield weight reduction up to 6 percent and 12 percent in Mbuni during the first and second cycle respectively. However, treatment with *Epicoccum* and *Trichoderma* had the highest 10 ear grain weight reduction of up to 13 percent and 27 percent respectively during the first and second cycle respectively. Treatment with Thiovit<sup>R</sup> and PEARL<sup>R</sup> had the lowest 10 ear grain weight reduction of up to 6 percent and 9 percent during the first and second cycle respectively. There was significant ( $p \leq 0.05$ ) difference in 10 ear grain weight reduction among the treatments.

Treatment with *Alternaria* and *Epicoccum* antagonists had the lowest 10 ear grain weight reduction of up to 40% in Njoro bw2 during the first and second cycle respectively. Treatment with *Trichoderma* had the highest 10 ear grain weight reduction of up to 61 percent and 55 percent during the first and second cycles respectively as shown in table 4.4 and table 4.5. However, treatment with PEARL<sup>R</sup> and Thiovit<sup>R</sup> fungicides had the lowest 10 ear grain weight reduction of up to 53 percent and 47 percent during the first and second cycles respectively. COTAF<sup>R</sup> and Folicur<sup>R</sup> had the highest 10 ear grain weight reduction by up to 60 percent and 52 percent during the first and second cycles respectively as in table 4.5. There was significant ( $p \leq 0.05$ ) difference in 10 ear grain weight reduction among the treatments in Njoro bw2.

Total grain weight reduction was highest in treatment with PEARL<sup>R</sup> and COTAF<sup>R</sup> while treatment with *Alternaria* had the lowest total weight reduction in Mbuni during the first cycle and second cycle respectively. However in Njoro bw2 treatment with *Alternaria* and Folicur<sup>R</sup> had the lowest total grain weight reduction of up to 26 percent and 19 percent during the first and second cycle respectively. However, *Epicoccum* and *Alternaria* had the highest total grain weight reduction of up to 44 percent during the first and second cycle respectively.

*Alternaria* and *Epicoccum* were the least effective antagonists in reducing prevalence of *Fusarium graminearum* in Mbuni during the first and second cycle respectively as in table 4.4 and table 4.5. However, *Epicoccum* and *Trichoderma* were the most effective antagonists in reducing prevalence of *F. graminearum* by up to 9 percent during the first and second cycle respectively. More over, PEARL<sup>R</sup> and Thiovit<sup>R</sup> were the most effective fungicides in reducing prevalence of *F. graminearum* by up to 8 percent and 2 percent during the first and second cycle

respectively. Thiovit<sup>R</sup> and COTAF<sup>R</sup> were the least effective fungicides in reducing *F.graminearum* prevalence during the first and second cycle respectively. There was significant ( $p < 0.05$ ) difference in *F.graminearum* prevalence reduction among the fungicides and antagonists during the first cycle.

*Epicoccum* and *Trichoderma* were least effective antagonists in reducing prevalence of *F. poae* by up to 52 percent during the first and second cycle respectively in Mbuni. *Alternaria* was the most effective antagonist in reducing *F.poa*e prevalence during the two cycles by up to 25 percent. However, COTAF<sup>R</sup> and Folicur<sup>R</sup> were the least effective fungicides in *F.poa*e prevalence reduction by up to 12 percent during the first and second cycles respectively. PEARL<sup>R</sup> and Thiovit<sup>R</sup> were the most effective fungicides in reducing *F.poa*e prevalence during the first and second cycle respectively. There was no significant difference in *F.graminearum* and *F.poa*e prevalence reduction among the fungicides and antagonists during cycle two in Mbuni.

*Trichoderma* was the least effective antagonist in reducing prevalence of *Fusarium graminearum* in Njoro bw2 as shown in table 4.4 and table 4.5. Fungal mixture and *Alternaria* were the most effective antagonists in reducing prevalence of *F. graminearum* during the first and second cycle respectively. PEARL<sup>R</sup> and Folicur<sup>R</sup> were the most effective fungicides in reducing prevalence of *F.graminearum* during the first and second cycle respectively. COTAF<sup>R</sup> was the least effective fungicide in reducing prevalence of *F.graminearum*. *Epicoccum* and *Trichoderma* were least effective antagonists in reducing prevalence of *F.poa*e during the first and second cycle respectively. *Trichoderma* and *Epicoccum* were the most effective antagonists in reducing

*F.poa*e prevalence during the first and second cycle respectively. PEARL<sup>R</sup> and Thiovit<sup>R</sup> were least effective fungicides by up to 52 percent during the first and second cycle respectively Thiovit<sup>R</sup> and COTAF<sup>R</sup> were the most effective fungicides in reducing *F.poa*e prevalence during the first and second cycle respectively. There was significant ( $p \leq 0.05$ ) difference in *F.graminearum* prevalence among the fungicides and antagonists during cycle one and no significant difference during cycle two.

**Table 4. 4:** Disease severity, grain weight, AUDPC and percentage re-isolation of *F.poa*e and *F.graminearum* during cycle one

	Disease severity	Mbuni			Re-isolation	
		AUDPC	10 ear wgt	Grain weight/pot	<i>F.graminearum</i>	<i>F.poa</i> e
<i>Alternaria</i>	3.0	511.5	4.4	12.9	50.3	25.2
Fungal mixture	3.1	552.8	3.4	9.2	20.7	44.7
<i>Trichoderma</i>	2.6	416.3	3.4	8.2	16.7	47.8
<i>Epicoccum</i>	2.7	437.7	4.1	10.1	9.3	52.1
COTAF <sup>R</sup>	3.2	561.2	2.8	10.5	26.9	49.0
PEARL <sup>R</sup>	3.2	561.9	2.3	6.0	8.3	38.6
Thiovit <sup>R</sup>	2.8	480.4	4.4	10.2	32.0	42.9
Water	3.2	553.5	3.5	8.2	11.7	38.0
Untreated	1.0	105.0	4.7	11.6	0.1	2.9
LSD( $p \leq 0.05$ )	0.4	89.0	NS	NS	1.0	NS
Cv ( percent)	4.5	4.7	4.5	2.7	34.4	4.2

	Disease severity	Njoro bw2			Re-isolation	
		AUDPC	10 ear wgt	Grain weight/pot	<i>F.graminearum</i>	<i>F.poa</i> e
<i>Alternaria</i>	2.6	418.8	4.3	12.7	33.3	27.2
Fungal mixture	3.1	543.2	3.2	9.5	11.0	26.7
<i>Trichoderma</i>	2.4	384.8	2.7	7.4	34.7	20.0
<i>Epicoccum</i>	2.0	295.2	3.0	6.8	14.0	40.0
COTAF <sup>R</sup>	2.8	470.4	2.8	9.5	16.5	43.1
PEARL <sup>R</sup>	3.1	545.1	3.3	11.3	1.5	66.7
Thiovit <sup>R</sup>	2.5	406.5	3.3	10.1	9.8	37.3
Water	1.4	185.7	3.3	8.8	8.7	45.3
Untreated	1.0	105.0	7.1	17.3	8.3	41.3
LSD( $p \leq 0.05$ )	0.3	81.82	2.4	5.1	1.1	NS
Cv ( percent)	3.5	3.4	4.8	5.7	17.6	7.7

LSD: least significant difference; Cv: Coefficient of variation; AUDPC: Area under disease progress curve

**Table 4.5:** Percentage re-isolation of *F.graminearum* and *F.poa*e, disease severity, AUDPC and grain weight during cycle two

	Disease severity	Mbuni			Re-isolation	
		AUDPC	10 ear wgt	Grain weight/pot	<i>F.graminearum</i>	<i>F.poa</i> e
<i>Alternaria</i>	3.0	530.0	2.9	7.5	6.9	18.3
Folicur <sup>R</sup>	2.2	372.0	1.8	5.9	4.9	43.9
<i>Trichoderma</i>	3.2	579.0	2.4	6.9	0.8	51.5
<i>Epicoccum</i>	3.0	533.0	2.4	5.8	11.9	23.2
COTAF <sup>R</sup>	3.1	561.0	2.1	5.6	12.2	31.7
PEARL <sup>R</sup>	2.7	489.0	3.0	7.3	4.1	29.8
Thiovit <sup>R</sup>	3.2	549.0	2.2	6.2	2.2	26.9
Water	2.4	405.0	2.6	4.6	3.1	20.9
untreated	1.0	126.0	3.3	7.7	8.8	44.2
LSD <sub>(p≤0.05)</sub>	0.4	116.7	NS	NS	NS	NS
Cv ( percent)	3.2	2.7	13.2	1.7	29.9	11.0

	Disease severity	Njoro bw2			Re-isolation	
		AUDPC	10 ear wgt	Grain weight/pot	<i>F.graminearum</i>	<i>F.poa</i> e
<i>Alternaria</i>	2.5	436.1	2.0	5.2	1.5	42.6
Folicur <sup>R</sup>	1.7	255.5	3.2	7.5	0.0	45.4
<i>Trichoderma</i>	2.5	427.7	1.7	5.4	3.1	48.4
<i>Epicoccum</i>	2.6	455.7	2.3	6.0	2.1	16.7
COTAF <sup>R</sup>	2.9	514.5	1.6	5.5	12.5	11.0
PEARL <sup>R</sup>	2.7	483.0	1.5	5.6	0.0	32.9
Thiovit <sup>R</sup>	2.4	611.8	2.0	6.1	2.3	52.2
Water	2.7	480.2	1.3	5.8	6.8	35.6
Untreated	1.0	119.0	3.8	9.3	5.5	52.0
LSD <sub>(p≤0.05)</sub>	0.3	107.4	1.9	2.8	NS	NS
Cv ( percent)	6.9	6.6	6.1	4.2	30.2	4.1

LSD: least significant difference; Cv: Coefficient of variation, AUDPC: Area Under Disease Progress Curve; NS: Not significant

#### 4.4.3 Effectiveness of wheat lines in reducing *Fusarium* head blight

Mean disease severity reduction over time was highest in Njoro bw1 of up to 55 percent and least in R1104 of up to 14 percent as shown in table 4.6. The disease severity was significantly ( $p \leq 0.05$ ) different over time among all the lines and varieties during cycle one. Disease severity increased over time in all the lines and varieties. Mean disease severity reduction over time

during cycle two for the lines and varieties inoculated with *F. poae* was highest in R1119 to 53 percent compared to Mbuni had the lowest reduction of up to 18 percent as shown in Table 4.7. Mean disease severity reduction over time for experiment inoculated with *F. graminearum* was highest in Njoro bw2 of 48 percent and least in line R1104 of up to 18 percent. There was a significant ( $p \leq 0.05$ ) difference in *F. poae* and *F. graminearum* mean disease severity reduction over time among all the lines and varieties during cycle two.

**Table 4. 6:** Disease severity on wheat ears over time for different lines and varieties inoculated with a mixture of *F. graminearum* and *F. poae* during cycle one

Variety /line	Days after inoculation					Mean
	7	14	21	28	35	
KIBIS	2.9	5.4	8.8	9.0	9.0	7.0
MBUNI	2.5	4.4	4.5	6.9	7.3	5.1
Njoro bw1	1.1	1.1	1.7	2.4	2.7	1.8
Njoro bw2	1.7	2.5	4.6	5.6	6.4	4.2
R1098	2.7	4.8	6.5	8.3	8.4	6.1
R1107	2.5	4.4	5.1	8.0	8.2	5.7
R1111	3.0	3.2	5.6	6.8	7.2	5.2
R1112	1.2	1.3	1.8	3.8	4.4	2.5
R1114	4.0	6.7	7.8	8.8	8.8	7.2
R1115	1.5	1.7	2.0	3.5	4.1	2.6
R1119	2.7	4.8	5.4	7.4	7.7	5.6
R1121	1.4	1.8	3.7	6.3	6.8	4.0
R1100	2.3	4.1	6.2	7.6	8.0	5.7
R1128	1.9	3.8	8.3	8.8	8.9	6.3
R1130	2.7	4.6	8.2	9.0	9.0	6.7
R1101	2.1	3.4	5.4	8.2	8.4	5.5
R1104	4.1	6.8	8.3	9.0	9.0	7.5
R1105	3.7	6.5	7.0	8.4	8.5	6.8
R1106	4.4	4.8	7.4	7.9	8.1	6.5
LSD <sub>(p≤0.05)</sub>	0.6	0.7	0.8	0.7	0.6	
Cv ( percent)	43.4	34.7	29.7	19.3	16.0	

LSD: least significant difference; Cv: Coefficient of variation

**Table 4.7:** Disease severity on wheat ears inoculated with isolates of *F.poa*e for different lines and varieties over time

Variety /line	Days after inoculation						Mean
	7	14	21	28	35	42	
KIBIS	1.8	2.5	3.2	3.7	4.7	5.5	2.8
MBUNI	2.2	3.2	5.2	6.6	7.4	8.1	5.4
Njoro bw1	1.0	1.0	1.0	2.4	3.1	3.9	1.9
Njoro bw2	1.3	2.1	2.1	2.1	3.8	4.5	2.3
R1098	1.4	2.0	2.8	3.6	4.8	5.8	3.4
R1107	1.0	1.0	1.0	2.0	3.3	4.6	2.2
R1111	1.1	1.1	1.2	2.0	3.3	4.7	2.2
R1112	1.9	2.6	2.6	2.6	4.7	5.4	2.9
R1114	1.2	1.4	1.7	4.0	5.3	6.4	3.3
R1115	1.3	2.0	2.0	2.0	3.8	4.9	2.7
R1119	1.0	1.0	1.0	2.0	2.7	3.6	1.9
R1121	1.6	2.0	2.0	2.5	4.2	5.1	2.9
R1100	1.0	1.1	1.4	2.4	3.6	5.2	2.4
R1128	1.0	1.0	1.0	2.0	3.1	4.4	2.1
R1130	1.0	1.3	1.7	2.0	3.0	4.9	2.3
R1101	1.3	1.8	2.9	4.0	5.0	6.1	3.5
R1104	1.2	1.6	1.7	2.6	2.9	4.7	2.5
R1105	1.2	1.2	2.3	4.9	6.0	7.2	3.8
R1106	1.3	1.8	1.9	2.6	2.8	4.3	2.5
LSD <sub>(p≤0.05)</sub>	0.3	0.4	0.6	0.7	0.8	0.7	
Cv ( percent)	5.9	7.3	12.8	12.5	4.8	3.3	

LSD: least significant difference; Cv: Coefficient of variation

**Table 4. 8:** Average disease severity on wheat ears over time per variety and line inoculated with isolates of *F. graminearum* during cycle two

Variety/line	Days after inoculation						Mean
	7	14	21	28	35	42	
KIBIS	1.5	2.6	3.2	4.2	5.3	6.2	3.9
MBUNI	1.7	3.0	3.9	5.1	5.9	7.0	4.4
Njoro bw1	1.0	1.0	1.0	2.4	3.1	3.9	2.1
Njoro bw2	2.0	2.6	2.6	2.6	6.3	6.9	3.8
R1098	1.8	2.7	4.0	5.2	6.3	7.0	4.5
R1107	1.0	1.0	1.0	2.0	3.6	5.0	2.3
R1111	1.0	1.0	1.0	2.0	3.2	4.7	2.2
R1112	1.6	2.4	2.4	2.4	4.4	5.1	3.0
R1114	1.9	2.7	4.4	4.7	5.6	6.5	4.3
R1115	1.8	2.5	2.5	2.5	4.7	5.5	3.3
R1119	1.0	1.0	1.0	3.2	4.1	4.9	2.5
R1121	1.8	2.5	2.5	2.5	5.2	6.1	3.5
R1100	1.1	1.3	1.4	2.9	5.2	6.4	3.1
R1128	1.0	1.0	1.1	2.3	3.5	4.9	2.3
R1130	1.0	1.3	1.7	2.2	3.4	4.7	2.4
R1101	1.2	1.9	2.6	3.3	4.5	6.1	3.2
R1104	2.0	2.9	5.0	7.4	8.0	8.6	5.3
R1105	1.9	2.7	4.1	5.0	6.0	7.2	4.2
R1106	1.9	2.7	4.7	5.5	6.4	7.6	4.5
LSD <sub>(p≤0.05)</sub>	0.3	0.4	0.7	0.8	0.9	0.8	
Cv ( percent)	5.5	5.7	6.8	3.2	2.4	2.6	

LSD: least significant difference; Cv: Coefficient of variation

Standardized Area under disease severity progress curve (AUDPC) was highest in R1104 by up to 23 and lowest in Njoro bw2 as shown in table 4.9 during cycle one. There was significant ( $p \leq 0.05$ ) difference in AUDPC among the lines and varieties. R1104 had the highest AUDPC during cycle two for treatment with *F. graminearum* as shown in table 4.9 while Njoro bw2 had the lowest AUDPC with significant ( $p \leq 0.05$ ) difference among the lines and varieties. Mbuni had the highest AUDPC after treatment with *F. poae* while R1119 had the least AUDPC during cycle two. There was significant ( $p \leq 0.05$ ) difference among the lines and varieties in AUDPC after inoculation with *F. poae*. All lines and varieties were susceptible to *Fusarium* as in table 4.9. Different levels of *Fusarium* susceptibility were expressed among the lines and varieties.

The most susceptible variety to *Fusarium* was KIBIS while the least susceptible variety was Njoro bw1 during cycle one. R1104 was the most susceptible line to *Fusarium* while Njoro bw1 was the least susceptible variety during cycle two after inoculation with *F.graminearum*. Mbuni was the most susceptible variety to *Fusarium* after inoculation with *F.poa*e while R1119 was the least susceptible line during cycle two. There was significant ( $p \leq 0.05$ ) difference in *F.poa*e and *F.graminearum* susceptibility among the different lines and varieties.

10 ear grain weight and total pot grain weight per lines and varieties were as shown in table 4.9 during the two cycles. Inoculation with a mixture of *F.graminearum* and *F.poa*e to Line R1098 reduced 10 ear grain weight by up to 50 percent and it had the most reduction while line R1104 had the least 10 ear weight reduction of 26 percent. There was significant difference in 10 ear weight reduction among the lines and varieties during cycle one. After treatment with *F.graminearum* Line R10985 had the highest 10 ear grain weight reduction of 9 percent while R1098 had the least reduction of 3 percent. After treatment with *F.poa*e line R1105 had the highest 10 ear grain weight reduction of 8 percent while R1098, R11003 and R1101 had the least reduction of 0.5 percent. There was significant ( $p < 0.05$ ) in 10 ear grain weight reduction difference among the lines and varieties during cycle two.

Grain weight per pot was mostly reduced by 39 percent in Mbuni and least reduced by 32 percent in R1098 during cycle one. After inoculation with *F. graminearum* line R1098 had the highest grain weight reduction per pot of 8.95 percent while line R1106 had the least grain weight reduction of one percent during cycle two. After inoculation with *F. poae* line R1105 had the highest reduction of up to 7 percent of grain weight per pot while Njoro bw1, R1100 and

R1104 had the lowest grain weight reduction of one percent during cycle two. There was significant ( $p \leq 0.05$ ) difference in grain weight per pot reduction among the lines and varieties.

*F. poae* and *F. graminearum* were re-isolated in all the lines and varieties. Njoro bw2 had the highest re-isolation prevalence of *F. poae* of up to 90 percent while line R1105 had the least prevalent of *F. poae* re-isolation of up to 50 percent during cycle one as in table 4.9. *F. poae* was not prevalently re-isolated from lines and varieties inoculated with *F. graminearum* except in line R1104 during cycle two. There was no significant difference in *F. poae* re-isolation among the lines and varieties inoculated with *F. graminearum*. After inoculating varieties and lines with *F. poae* line R1101 had the highest prevalent of *F. poae* re-isolation of 51 percent while Mbuni had the least prevalent. There was no significant difference in *F. poae* re-isolation prevalent among the lines and varieties.

*F. graminearum* was highly re-isolated in Mbuni during cycle one and not re-isolated from Njoro bw1. After inoculating with *F. graminearum* line R1098 had the highest re-isolation prevalent of *F. graminearum* of 55 percent while KIBIS had the least prevalent of 16 percent. After inoculating with *F. poae* some lines had re-isolation prevalence of *F. graminearum* while others did not have *F. graminearum* prevalence. There was significant ( $p \leq 0.05$ ) difference among the lines and varieties for *F. graminearum* re-isolation during the two cycles. All varieties tested exhibited different levels of T-2 toxin contamination. T-2 toxin contamination levels varied from 23ppb to 4 ppb where line R1098 had the highest contamination while R1098 had the least level of contamination.

**Table 4.9:** Disease severity, std AUDPC, grain weight and *F. poae* and *F. graminearum* re-isolation and T-2 levels in ppb during cycle one

	Disease severity	Std AUDPC	10 ear grain wgt	Grain wgt (g)/pot	T-2 toxin	Re-isolation	
						<i>F. poae</i>	<i>F. gra</i>
KIBIS	6.0	17.5	7.6	23.3	15.2	70.7	4.0
Mbuni	4.4	13.1	8.7	26.3	18.8	63.8	20.0
Njoro bw1	1.7	4.7	5.7	20.8	18.1	81.7	0.0
Njoro bw2	3.6	10.1	6.5	25.6	19.7	90.6	1.6
R1098	5.3	15.1	12.2	38.9	18.3	72.9	7.6
R1107	4.9	13.7	11.5	34.9	19.7	67.3	6.7
R1111	4.5	12.1	12.1	36.6	17.0	60.0	4.8
R1112	2.2	6.1	5.6	21.1	24.0	69.7	1.5
R1114	6.2	17.9	10.0	29.1	4.9	56.7	10.7
R1115	2.3	6.1	4.9	21.2	23.6	60.4	4.5
R1119	4.8	13.7	12.4	35.5	21.3	64.2	10.8
R1121	3.5	9.6	6.0	26.3	27.8	68.3	2.1
R1100	4.9	15.1	13.0	39.7	16.6	62.8	11.7
R1128	5.4	19.6	11.8	38.5	16.9	55.2	0.7
R1130	5.8	20.7	10.3	34.2	17.6	66.0	2.0
R1101	4.7	13.3	12.2	32.5	12.5	51.7	19.2
R1104	6.4	23.4	11.3	38.8	22.8	51.7	6.0
R1105	5.9	17.0	15.6	33.0	17.6	50.3	10.7
R1106	5.6	15.2	11.2	42.1	16.3	71.0	6.9
LSD <sub>(p≤0.05)</sub>	0.6	1.5	3.9	9.2		1.5	0.6
Cv ( percent)	1.2	0.9	0.9	0.4		2.2	5.2

LSD: least significant difference; Cv: Coefficient of variation

**Table 4.10:** Disease severity, std AUDPC, grain weight and *F. poae* and *F. graminearum* re-isolation during cycle two

	<i>F. graminearum</i>				Re-isolation
	Disease severity	Std AUDPC	10 ear grain weight	Total grain weight per pot	<i>F. gra</i>
KIBIS	3.4	12.0	2.8	9.3	16.9
MBUNI	3.9	13.8	2.9	9.9	17.4
Njoro bw1	1.9	6.7	3.4	11.4	23.2
Njoro bw2	3.4	12.0	1.1	3.8	18.7
R1098	4.0	14.0	3.9	13.6	23.3
R1107	2.1	7.1	2.0	5.6	54.1
R1111	2.0	7.0	2.4	6.1	35.3
R1112	2.8	9.6	0.4	1.6	55.6
R1114	3.8	13.4	4.7	10.7	23.5
R1115	2.9	10.2	2.9	7.8	28.1
R1119	2.3	8.1	2.6	5.4	21.7
R1121	3.1	10.9	2.5	6.3	46.5
R1100	2.8	9.7	3.3	8.3	40.9
R1128	2.1	7.5	3.3	8.2	17.8
R1130	2.2	7.7	6.4	17.8	24.8
R1101	2.9	10.3	2.5	6.1	29.2
R1104	5.0	17.3	3.2	14.7	40.7
R1105	4.0	13.9	3.0	7.2	40.2
R1106	4.0	15.0	3.6	12.5	28.2
LSD <sub>(p≤0.05)</sub>	0.4	2.2	NS	6.0	1.4
Cv ( percent)	3.3	2.9	3.1	2.9	17.0

LSD: least significant difference; Cv: Coefficient of variation; AUDPC: Area Under Disease Progress Curve; NS: Not significant

**Table 4.11:** Disease severity, std AUDPC, grain weight and *F. poae* and *F. graminearum* re-isolation during cycle two

Variety	Disease severity	<i>F. poae</i>			Re-isolation	
		Std AUDPC	10 ear grain wgt	Grain wgt/pot	<i>F. poae</i>	<i>F. gra</i>
KIBIS	3.3	11.4	1.6	10.0	19.6	0.0
MBUNI	4.8	16.8	2.0	5.2	6.0	17.0
Njoro bw1	1.9	6.8	2.2	6.8	21.9	9.2
Njoro bw2	2.4	8.4	0.3	1.7	35.3	0.0
R1098	3.1	10.7	2.2	6.5	34.4	0.0
R1107	2.0	6.9	2.3	5.0	28.6	0.0
R1111	2.1	7.2	3.3	8.6	29.7	14.8
R1112	3.0	10.4	1.2	3.5	37.7	0.0
R1114	3.0	10.5	1.9	4.4	41.6	0.0
R1115	2.5	8.6	2.3	6.6	32.3	0.0
R1119	1.8	6.1	2.8	7.3	41.9	5.7
R1121	2.6	9.2	3.0	5.8	38.4	0.0
R1100	2.2	7.8	7.7	16.8	34.8	0.0
R1128	1.9	6.8	4.1	11.8	43.9	0.0
R1130	2.1	7.4	4.2	13.9	25.0	4.5
R1101	3.2	11.1	1.9	5.1	51.9	0.0
R1104	2.2	7.9	4.4	15.6	49.5	0.0
R1105	3.4	11.9	1.0	5.3	30.7	0.0
R1106	2.3	7.9	4.4	12.1	31.0	0.0
LSD(p≤0.05)	0.4	1.9	NS	7.0	NS	0.3
Cv ( percent)	6.4	4.7	4.1	6.7	3.7	25.8

LSD: least significant difference; Cv: Coefficient of variation; AUDPC: Area Under Disease Progress Curve; NS: Not significant

#### 4.5 Discussion

All fungicides and microbial agents suppressed *Fusarium* head blight and did not completely control the disease. In vitro activity of fungicides completely reduced FHB. All fungicides and antagonists differed significantly in their effectiveness in reducing FHB. Folicur<sup>R</sup> was the most effective fungicide in reducing the pathogen. All wheat varieties were susceptible to FHB infection. This was in line with findings of other researchers. (Bennett and Klich 2003). None of

the currently available commercial cultivars are immune to *Fusarium* infection, but differences in partial resistance or tolerance to FHB do occur among different hard red spring wheats.

Large genetic variation for FHB resistance is available in the wheat gene pool, but often the best regionally adapted and highly productive cultivars are susceptible to FHB (Buerstmayr et al., 2008). No accession has yet been identified to be completely immune to FHB (Gocho, 1985; Mesterházy, 1987; Takeda and Heta, 1989; Snijders, 1990c; Liu and Wang, 1991). Most wheat cultivars available are susceptible to *Fusarium* head blight, none are immune and only a few are moderately resistant (Muthomi et al., 2002a; Parry et al., 1995). No source of complete resistance is known, and current sources provide only partial resistance (Rudd et al., 2001). The findings supported the findings that host resistance has long been considered the most practical and effective means of control, but breeding has been hindered by lack of effective resistance genes and by the complexity of the resistance in identified sources (Rudd et al., 2001). Development of resistant cultivars has been slowed down by poorly adapted and incomplete resistance sources and confounding environmental effects that make screening of germplasm difficult (Anderson, 2001) thus supporting there is no varieties resistant to FHB.

The level of susceptibility varied among the lines and varieties. There was significance difference in 10 ear grain and grain per pot reduction. All the fungicides in vitro inhibited the growth of *F. poae* and *F. graminearum* completely, therefore confirming earlier findings by Riungu et al., 2008 who found that Copper oxychloride<sup>R</sup> and Folicur<sup>R</sup> completely reduced pathogen diameter in vitro. Among the antagonists in vitro *Trichoderma* had the highest colony reduction this could have been due to chitinase genes which has several important consequences

for the application of *Trichoderma* as a microbial agent against *Fusarium* diseases (Mathias et al., 2003). However, *Penicillium* had the least colony reduction. Among the microbial agents *Alternaria* was found to have the highest grain yield per pot while *Trichoderma* had the least grain yield per pot. This was in contrast with Riungu et al., 2008 who reported that *Trichoderma* reduced disease severity and increased grain yield. The study proved that microbial agents did not completely reduce FHB thus contrasting report by Kolombet et al., 2005.

All fungicides did not completely control the pathogen but only suppressed it thus confirming the earlier findings Chala et al., 2003; Mullenborn et al., 2007 who found that fungicides did not completely control FHB pathogens. Efficacy of fungicides is highly variable and often unsatisfactory Caron, 1990. Some of the variability in this study is related to the timing of the fungicide application and coverage. Other sources of variability are timing and the severity of infection, virulence of isolates and level of resistance in the cultivars planted. All the fungicides were significantly different in reducing FHB and this compared with Mesterhazy et al., 2003.

*Fusarium graminearum* and *F. poae* were the major isolations in the two green house experiments. However, *F. graminearum* was less frequently re-isolated than *F. poae*. This could be due the inability of *F. graminearum* to compete with other *Fusarium* species especially *F. poae*. The results were comparable to Muthomi et al., 2008 who found same species from wheat ears and kernels. Among the biological agents treatment with *Alternaria* gave the highest *F. graminearum* re-isolation while treatment with *Epicoccum* gave the least *F. graminearum* re-isolation.

All wheat lines and varieties were found to be susceptible to FHB. The results were consistent with the findings of other researchers (Muthomi et al 2002, 2007; Ndung'u et al 2007) who found all varieties grown in Kenya susceptible to FHB. Njoro bw1 was found to be the most susceptible variety to FHB while line R1104 was the most resistant variety. This is consistent with Muthomi et al., 2007 who found Njoro bw1 tolerant. Line R1104 had the highest grain weight with Njoro BW I giving the lowest grain weight per pot. This is attributed to the fact that some wheat varieties or lines are susceptible to FHB but can still produce disease and give good yields. However, others may be less susceptible and cannot produce disease thus lowering the yield. The differences in susceptibility to FHB could be due to different genetic resistance factors.

Five mechanisms of resistance to FHB in wheat have been proposed (Schroeder and Mesterházy 1963; Mesterházy, 1995). The terms type I, resistance to initial infection, and type II, resistance to spread of infection, are commonly used by researchers, but types III, IV, and V have been used consistently to describe other mechanisms of resistance (Shaner, 2002), following Shaner (2002); the terms type I, resistance to initial infection, and type II, resistance to spread of infection, are retained, while other mechanisms such as resistance to kernel infection and resistance to toxin accumulation are described but not designated as types. The most common sources of genetic resistance are derived from the Chinese wheat variety Sumai 3 (Shaner 1994; Bai and Shaner 1994; Wan et al. 1997).

Resistant wheat germplasm could be divided into three gene pools: winter wheats from Europe, spring wheats from China and Japan, and spring wheats from Brazil and Italy.

1990d; Liu and Wang, 1991). In Japan, FHB is caused mainly by *F. graminearum* Schwabe (Koizumi et al., 1991a, 1991b). Repeated screening of the genetic resources led to the identification of several cultivars of spring wheat, such as Shinchunaga and Nobeokabouzu-komugi from the Japanese gene pool. Sumai 3, Ning 7840 and Cltr 11028 were identified as resistant cultivars to FHB caused by *F. graminearum* from the Chinese gene pool (Nishikado, 1958c; Gocho, 1985; Ban and Gocho, 1988; Ban, 1991). Among them, one Chinese wheat cultivar, Sumai 3, is considered to be the most useful genetic source for resistance to FHB.

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## CHAPTER FIVE

### GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 General discussion

The results of this study indicate wheat production practices may influence the level of *Fusarium* inoculum in the wheat production regions. This could be due to the fact that some other crops grown near wheat could serve as alternative host to FHB pathogens. This could translate to high economic losses attributed to yield loss and mycotoxin contamination. Since maize was the other crop mostly grown besides wheat it could have contributed to levels of *Fusarium* head blight because its an alternative host to FHB thus serving as source of inoculum. Also the presence of maize among wheat fields could accelerate the effects due to mycotoxin production since its a host to *Aspergillus* which could lead to Aflatoxin contamination. Aphids and stem rust were the most reported insect pests were the most reported diseases. Folicur<sup>R</sup> was the most reported fungicide while dimethoate<sup>R</sup> was the most reported insecticides. This could have been due to the effectiveness of the active ingredient in the chemicals compared to other chemical active ingredient like copper oxychloride which has been used by few farmers.

The presence of different fungal genera isolated from wheat stems, heads, grains, straws and soil could lead to production of more toxins resulting in additive and synergistic effects (Muller and Schwadorf, 1993). The presence of *Alternaria* and *Epicoccum* affects the level of *Fusarium* accordingly and that high level causes low levels of *Fusarium* inoculum. This could have been due to antagonistic effects of *Alternaria* and *Epicoccum* to *Fusarium* genera. The results were comparable with the findings of other researchers (Muthomi et al., 2008) who found that high

levels of *Alternaria* and *Epicoccum* indicated low levels of *Fusarium*. The level of *Fusarium* inoculum varied with different agro ecological zones and districts. *Fusarium graminearum* and *F.poa* competes when both are in presence. The high levels of *F.poa* showed low levels of *F.graminearum* and vice versa. The fungicides and biological agents have significant effects on disease severity, AUDPC, grain weight and more so on mycotoxin production. In vitro activity of fungicides completely reduced the pathogen while under green house conditions fungicides only suppressed disease and complete control was not achieved.

Among the antagonists in vitro *Trichoderma* had the highest colony reduction and *Penicilium* had the least colony reduction. Among the biological agents *Alternaria* was found to have the highest grain yield per pot while *Trichoderma* was shown to have the least grain yield per pot. This was in contrast with Riungu et al., 2008 who reported that *Trichoderma* reduced disease severity and increased grain yield. Biological control holds considerable promise and includes treatment with antagonists to reduce pathogen inoculum and wheat heads at anthesis to reduce infection (Bujold et al., 2001). Biological control is attractive due to its potential for being environmentally benign, compatible with other control measures and durable. The above information justifies the need for use of integrated efforts for management of FHB. Host resistance was demonstrated by varieties which showed low susceptibility to FHB under the two green house experiments conducted. However some varieties exhibited the low susceptibility but gave low yields while others showed high susceptibility and gave high yields.

All fungicides and microbial agents suppressed *Fusarium* head blight and did not completely control the disease. All fungicides and antagonists differed significantly in their effectiveness in

reducing FHB. Folicur<sup>R</sup> was the most effective fungicide in reducing the pathogen. All wheat varieties were susceptible to FHB infection. The level of susceptibility varied among the lines and varieties. There was significance difference in 10 ear grain and grain per pot reduction. All the fungicides in vitro inhibited the growth of *F.poa* and *F.graminearum* completely, therefore confirming earlier findings by Riungu et al., 2008 who found that Copper oxychloride<sup>R</sup> and Folicur<sup>R</sup> completely reduced pathogen diameter in vitro. All fungicides did not completely control the pathogen but only suppressed it thus confirming the earlier findings Chala et al., 2003; Mullenborn et al., 2007 who found that fungicides did not completely control FHB pathogens. Efficacy of fungicides is highly variable and often unsatisfactory Caron, 1990. Some of the variability in this study is related to the timing of the fungicide application and coverage. Other sources of variability are timing and the severity of infection, virulence of isolates and level of resistance in the cultivars planted.

*Fusarium* head blight resistance is by a polygenic system of quantitative trait but the gene effects of dominance influence its resistance. Growing of wheat cultivars resistant to *Fusarium* species is of economic, environment-friendly and effective method of disease control. The best source of resistance to FHB is a complex quantitative trait derived from the wheat cv. Sumai 3. In wheat monogenic gene to gene resistance to *F.graminearum* has not been identified (Bai and Shaner, 2003). Resistance to FHB in some cultivated wheat cultivars is derived from the Chinese cv. Sumai 3 and its derivatives. Sumai 3-derived resistance to FHB is a complex, quantitative trait which confers type II resistance that limits fungal spread from the site of infection (Bai and Shaner 2004). The best control methods combine the planting of cultivars that are partially

resistant to FHB with fungicide application and rotation with non host crops (Bai and Shaner 2004; McMullen et al.1997a and b; Wilcoxson et al., 1993).

Resistance quantitative trait loci (QTL) against FHB have been detected in several European winter wheat populations (Gervais et al. 2003; Shen et al. 2003; Paillard et al. 2004; Schmolke et al. 2005, 2008; Draeger et al. 2007; Klahr et al. 2007; Semagn et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008). Except for a QTL identified near or at the *Rht-D1* locus (Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2008). Effects of QTL found in adapted germplasm are generally smaller compared to those found in Sumai 3 and its derivatives (Anderson et al. 2001; Buerstmayr et al. 2002). Sumai 3 has been used in many breeding programmes worldwide (Bai and Shaner 2004).

Since in vitro pathogen control by fungicides was most complete control then it may be the possibility that poor timely of the application of the fungicides in the green house since previous studies show that foliar fungicides are effective against fungicide provided spray schedule is timed correctly (Wilcoxson, 1996). The level of susceptibility to FHB in different lines and varieties may be due to the presence of some resistance genes in the lines or varieties. High levels of T-2 toxin may be due to the occurrence high infection rates of *F.poa*e and other *Fusarium* species.

## 5.2 General conclusion and recommendations

Since most farmers had maize as the other crop most grown besides wheat its recommended that wheat should not be rotated with maize since it acts as alternate host to FHB pathogen and it may

serve as source of primary inoculum. Njoro bw2 and Njoro bw1 were found to be more tolerant to the disease therefore its recommended farmers should plant Njoro bw1 since its more tolerant to the pathogen and yields more compared to Njoro bw1. This could be due to presence of some FHB tolerant genes which could be exploited to breed varieties tolerant to FHB and mycotoxin contamination. Since Folicur<sup>R</sup> reduced disease severity use of Folicur<sup>R</sup> is recommended since it suppresses FHB pathogen. Wheat production faces challenges due to presence of diseases, insect, weeds and birds, therefore i recommend to use chemical, host resistance and cultural practices to manage the challenges. Use of non host plants during rotation like beans, potatoes and peas would reduce the levels of inoculum.

High levels of *Fusarium* inoculum would pose great threat to animal and human life due to the associated mycotoxins. Antagonists reduced disease severity and there no complete control of the disease. Its recommended to use integrated approach in management of FHB pathogen since fungicides or antagonist were found not to completely control the disease but only suppresses the disease. All lines were found to be susceptible to FHB and their levels of susceptibility varied between the lines and varieties. Therefore i recommend farmers to plant the more tolerant varieties to the FHB. The presence of T-2 toxin poses major threat to animal and human life very low of the toxin concentration would be very toxic to human beings.

### **Recommendations**

- 1 Its recommended to monitor FHB right from the field since it affects all stages of growth.

- 2 High levels of inoculum have been found in wheat stems, heads and grains and in soil therefore control measures should be put at the right time to manage the disease and the associated mycotoxins.
- 3 Other fungal genera infecting wheat should be managed to avoid the accelerated effects of mycotoxins to human and animal health.
- 4 Integrated management efforts should be practiced to eliminate effects due FHB and the associated mycotoxins.
- 5 Breeders should incorporate genes from varieties found tolerable to FHB to breed for more resistance varieties.
- 6 *Fusarium* species to be tested for antagonistic effects on FHB.
- 7 Since FHB and mycotoxins are reported in our Kenyan wheat its advisable for control of the disease.
- 8 Recommends efficacy of the antagonists and fungicides to be tested under field conditions.

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## APPENDICES

### Appendix 1: Questionnaire; survey on wheat in Kenya

Name of the district.....

Agro ecological zone.....

Name of the farmer.....

1. Sources of seed?

A.....

B.....

C.....

D.....

E.....

2. The varieties of wheat grown?

A.....

B.....

C.....

D.....

E.....

3. The acreage on wheat planted?

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4. What are the average yields?

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5. Which are the other crops grown besides wheat?

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7. The measures to control the pests and diseases?

(a)Pesticides (specify)

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(b)Fungicides

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(c) Others (specify)

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8. What is grown before and after wheat?

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**Appendix 2: Characteristics of the lines**

Line	Parentage
R1098	BABAX/LR42// BABAX*2/3/TUKURU
R1107	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3KAUZ*2/TRAP//KAUZ
R1111	ND643/2*WBLL1
R1112	ND643//2*PRL/2*PASTOR(KSSR I)
R1114	BABAX/LR42// BABAX*2/3/BRAMBLING
R1115	BABAX/LR42// BABAX*2/3/TUKURU(KSSR II)
R1119	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1
R1121	PYN/BAU/3/MON//MU//ALD/PVN/4/VEE#6/SARA//DUCULA(KSSR III)
R1100	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3KAUZ*2/TRAP//KAUZ
R1128	EMB16/CBRD//CBRD
R1130	EMBI16/CBRD//CBRD (KSSR IV)
R1101	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KUKUNA
R1104	WAXWING*2/VIVITSI
R1105	WBLL1*2/BRABLING
R1106	THELIN/2*WAXWING

**Appendix 3: Rainfall and temperature data for wheat growing regions for the year 2006-2008**

District	Year	Month	Mean temperature in 0c		Total monthly rainfall in mm
			Minimum	Maximum	
Nyandarua North	2006	January	6.7	22.9	12.7
		February	7.0	24.5	28.7
		March	8.4	23.4	120.6
		April	8.9	21.5	121.0
		May	7.5	22.4	29.4
		June	6.8	21.7	108.8
		July	8.4	20.1	110.3
		August	7.8	20.1	158.0
		September	5.7	21.4	46.8
		October	6.9	22.2	75.1
		November	10.8	19.4	138.3
		December	10.5	19.8	155.2
	2007	January	8.5	20.8	37.5
		February	8.1	22.0	61.5
		March	7.0	23.1	26.4
		April	8.3	22.1	70.2
		May	7.6	22.4	112.9
		June	9.6	20.2	232.2
		July	8.1	19.1	265.6
		August	7.8	19.6	234.3
		September	7.8	20.5	299.1
		October	7.0	20.7	28.0
		November	8.8	20.4	41.5
		December	7.8	21	4.9
	2008	January	7.3	21.8	33.6
		February	6.6	22.9	18.6
		March	8.0	23.0	104.7
		April	7.7	22.0	24.7
		May	6.0	22.4	62.6
		June	7.1	21.2	33.7
		July	8.3	19.8	198.6
		August	7.4	20.2	120.1
		September	7.3	22.0	103.9
		October	9.0	20.9	201.4
		November	9.4	20.0	86.7
		December	7.8	21.4	8.1
2009	January	7.3	22.7	4.2	
	February	7.1	23.8	3.5	
	March	7.8	25.1	0.0	
	April	7.9	24.2	37.0	
	May	8.9	22.5	144.6	
	June	5.7	22.7	28.6	

District	Year	Month	Mean temperature in 0c		Total monthly rainfall in mm
			Minimum	Maximum	
Imenti North	2006	January	11.2	24.8	35.2
		February	12.0	26.4	59.8
		March	14.1	26.1	33.8
		April	14.4	24.1	292.9
		May	13.9	23.4	125.9
		June	12.3	22.7	4.5
		July	DNA	DNA	0.7
		August	12.8	22.5	37.2
		September	13.2	24.1	87.8
		October	14.4	24.3	298.5
		November	14.1	23.0	477.1
		December	13.4	23.3	273.3
	2007	January	12.0	23.6	109.0
		February	12.7	26.7	10.6
		March	13.0	26.2	111.0
		April	14.7	24.0	190.8
		May	14.1	23.5	167.3
		June	12.9	22.4	0.0
		July	12.8	21.6	16.3
		August	13.1	22.1	18.3
		September	12.9	24.6	11.6
		October	14.5	24.6	304.0
		November	13.6	23.7	205.8
		December	11.9	23.9	110.7
	2008	January	11.6	24.5	95.1
		February	11.0	25.2	0.0
		March	13.5	25.8	62.5
		April	13.8	23.8	201.2
		May	14.1	23.1	8.7
		June	12.6	21.6	2.1
		July	12.8	21.0	11.1
		August	13.1	22.8	3.4
		September	12.9	25.6	7.5
		October	14.9	23.7	289.9
		November	13.5	23.8	117.7
		December	11.4	24.6	40.0
2009	January	11.5	24.9	153.5	
	February	12.1	25.1	23.1	
	March	13.3	27.0	46.8	
	April	14.4	25.0	138.0	
	May	14.5	23.4	109.9	
	June	13.3	21.9	19.8	

District	Year	Month	Mean temperature in 0c		Total monthly rainfall in mm
			Minimum	Maximum	
Narok	2006	January	8.6	27.7	101.3
		February	9.1	28.2	61.0
		March	11.7	25.5	123.4
		April	13.3	23.1	299.0
		May	12.3	22.9	59.1
		June	9.8	22.8	1.6
		July	10.2	21.7	24.6
		August	9.0	23.6	32.0
		September	10.8	22.9	21.7
		October	11.6	DNA	1.0
		November	12.5	DNA	271.0
		December	12.5	23.1	156.2
	2007	January	11.4	24.9	105.9
		February	10.8	25.4	143.2
		March	10.1	26.2	59.5
		April	11.8	25.0	89.9
		May	12.6	23.4	97.2
		June	11.6	21.8	34.6
		July	9.5	21.5	10.8
		August	10.6	22.4	22.4
		September	10.0	24.3	69.4
		October	9.3	25.5	9.0
		November	8.6	26.1	30.3
		December	8.9	26.2	39.7
	2008	January	8.9	27.2	7.6
		February	9.9	26.0	100
		March	10.7	25.6	201.3
		April	11.8	23.4	88.9
		May	11.3	23.2	7.8
		June	10.2	22.5	1.4
		July	9.9	22.2	9.2
		August	10.3	23.4	23.1
		September	10.4	25.6	35.1
		October	11.5	25.4	55.5
		November	10.5	25.6	86.3
		December	8.7	27.5	1.6
2009	January	9.8	27.7	52.9	
	February	10.0	27.4	24.4	
	March	10.9	29.1	23.1	
	April	12.8	25.6	105.4	
	May	13.1	23.9	107.7	
	June	10.3	23.8	36.2	

Appendix 4: Different fungal genera spp (CFU10<sup>4</sup> g<sup>-1</sup>)\* isolated from soil in different agro ecological zones (AEZ) of wheat growing districts

AEZ	<i>Fusarium</i>	<i>Alternaria</i>	<i>Trichoderma</i>	<i>Epicoccum</i>	<i>Penicillium</i>
<b>Nyandarua North</b>					
LH3	45.3	9.8	19.4	13.7	7.6
LH4	46.5	16.8	14.7	12.6	6.3
UH3	19.3	7.8	6.8	7.6	7.1
UH4	2.3	2.1	0.7	1.4	0.8
Mean	28.3	9.1	10.4	8.8	5.5
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS
Cv ( percent)	103.8	77.2	146.9	92.6	84.3
<b>Imenti North</b>					
LH2	15.1	10.0	7.1	1.0	8.0
LH4	7.1	8.1	7.6	4.3	5.3
UH2	12.5	10.2	8.8	2.8	7.7
UH3	73.4	38.1	85.2	33.0	24.0
Mean	27.0	16.6	27.2	10.3	11.2
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS
Cv ( percent)	172.7	104.9	213	207	120
<b>Narok</b>					
LH4	33.5	78.0	35.0	81.0	31.8
UH2	27.6	35.6	6.0	8.5	11.7
UH3	27.6	12.7	4.8	9.0	9.8
UH4	28.5	22.9	26.3	24.9	34.7
Mean	29.3	37.3	18.0	30.8	22.0
LSD <sub>(p≤0.05)</sub>	NS	NS	NS	NS	NS
Cv ( percent)	157	109.5	154.2	177	170.1

\* Data raised to 10<sup>4</sup>

**Appendix 5:** Different *Fusarium* species (CFU10<sup>4</sup> g<sup>-1</sup>)\* isolated from soil in different agro ecological zones (AEZ) of wheat growing districts

	<i>F. poae</i>	<i>F. graminearum</i>
<b>Nyandarua North</b>		
LH3	4.0**	4.8
LH4	0.0	7.0
UH3	1.8	8.0**
UH4	1.1	0.0
Mean	8.0**	3.2
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	69.6	157.5
<b>Imenti North</b>		
LH2	11.1	4.0
LH4	3.7	1.4
UH2	9.8	4.2
UH3	69.7	3.7
Mean	23.5	3.3
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	197.6	52.1
<b>Narok</b>		
LH4	29.8	2.8
UH2	26.2	1.9
UH3	18.4	9.2
UH4	25.9	2.5
Mean	25.1	4.1
LSD <sub>(p≤0.05)</sub>	NS	NS
Cv ( percent)	153.2	179.2

\*\*Data raised to 10<sup>3</sup>

\* Data raised to 10<sup>4</sup>