PREVALENCE AND CONTROL OF SEEDBORNE FUNGAL PATHOGENS OF WHEAT IN FARMERS SAVED SEEDS OF SELECTED LOCATIONS IN NORTHERN TANZANIA

EDITH LAURENCE KADEGE

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ABSTRACT

Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases. Laboratory and screen house experiments were carried out at Selian Agriculture Research Institute Arusha, to investigate the extent of seed-borne fungal pathogen problems from farmers saved seeds in Northern wheat growing Zone. A total of 45 untreated seed samples of wheat were collected from farmers saved seeds in Karatu, Hanang and Siha Districts. Each sample was physically inspected and pure seeds were separated from abnormal seeds and inert matter. Seed samples collected at Siha were of high quality compared to seed samples collected at Hanang and Karatu. Pure seeds ranged from 98.2–99.2%, abnormal seeds 0.49–1.4% and inert mater 0.15–0.80%. *Alternaria alternata, Bipolaris sorokiniana, Dresclera tritici, Fusarium graminearum, Fusarium moniliforme, Aspergillus flavus, Cladosporium sphaerormum, Epicoccum purpurascers, Pyricularia oryzae* and *Penicilium Corylophilum* were isolated and identified using blotter, potato dextrose agar (PDA) and agar plate methods. The most predominant seed-borne fungi were *Cladosporium sphaerormum* (9.8%), *Alternaria alternata* (9.2%) and *Aspergillus flavus* (8.7%). The lowest percent seed infection (10.5%) was recorded in samples collected at Siha District, (17.5%) at Hanang District and (20.4%) at Karatu District. The percentage fungal seed infection was higher in seed samples collected at Karatu with infection rate of 32.8% and lowest for seeds sample collected at Siha with infection of 25.4%. The overall seed infection in the region was 29.1% causing yield losses of 1.2 t/ha on average. Seed treatment with Metalaxy plus, Mancozeb and Baytan was found to increase percent seed germination by 17.2, 14.3, and 12% respectively and yield by 27.8, 20.4 and 17.8% respectively. Farmers saved seeds in

Hanang, Karatu and Siha districts are heavily infected with fungi and it is recommended that they should be treated with appropriate fungicides prior planting.

DECLARATION

I EDITH LAURENCE KADEGE do here by declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

_____ _____

Edith Laurence Kadege **Date**
(MSc candidate)

The above declaration is confirmed by;

_____ _____

Prof. H.F.J Lyimo **Date**
(Supervisor)

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
≤	Less than or equal to
µm	Micrometer
ANOVA	Analysis of variance
CIDA	Canadian International Development Agency
cm	Centimeter
Co Ltd	Company limited
COSTECH	Commission for Science and Technology
CRD	Complete Randomized Design
CV	Coefficient of Variation
DAP	Di-ammonium phosphate
DMRT	Duncan Multiple Range Test
DNA	Dioxyribosnucleic acid
EAC	East African Community
eg	Example
FAO	Food and Agriculture Organization
Fig	Figure
g	Gram
GS	Growth stage
h	Hour
ha	Hectare
i.e	That is
ICRISAT	International Crops Research Institute for the Semi Arid Tropic

ISTA	International Seed Testing Association
kg	Kilogram
m	Metre
min	Minutes
ml	Milimeter
Mt	Mountain
NAFCO	National Agricultural Food Cooperation
NaOCl	Sodium hypochlorite
NUV	Near Ultra Violet
^{O}C	Degree Celsius
P	Probability
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
PF	Percentage frequency
RAP	Random amplified polymorphic
SE	Standard error
SHF	Small holder farmers
spp	Species
t	Ton
USA	United States of America
WP	Wettable powder

CHAPTER ONE

1.0 INTRODUCTION

Wheat (*Triticum aestivum* L.), family Poaceae (Rehman *et al*., 2011) is a worldwide cultivated grass, grown mainly for food and cash earning. It provides about 20% of the world's food calories and it is a staple food for nearly 40% of the world's population. Wheat is extensively grown across the whole of Europe, Central Asia, Middle East, Northern Africa, Northern India, North East China, United States, Mid West Southern central Canada and in several other countries in the Southern hemisphere. Wheat is also grown in many tropical countries such as Kenya, Tanzania, Ethiopia, Paraguay, Bolivia, Peru and Mexico (FAO, 1996). The four largest wheat producing countries in the world are China, India, United States and Russian (Shuaib *et al*., 2007).

In Tanzania, wheat is the fifth most important cereal after maize, sorghum, millet and rice (FAO, 1996). It is grown in the Northern highland regions (Arusha and Kilimanjaro) on a large-scale basis and in the Southern highlands (Mbeya, Iringa, and Rukwa) by small and medium-scale farmers (Kilima, 2006). Annual wheat production in Tanzania is estimated to be 96 000 metric tons (Kilima, 2008). However, assessment made in 2008 and 2009 has shown production decline trend from 262 to 176 tons annually which is a decrease of about 67% (Kilima, 2008 and Kuwite *et al*., 2010).

Current average wheat production in Tanzania is only 1.6 t /ha which is very low when compared to 2.7 t/ha realized in developed countries (Kuwite *et al*., 2010).

Several factors contribute to low productivity, such as insect pests, diseases, soil infertility, poor agricultural practices and inadequate high yielding varieties. With the continuous use of farmer saved seeds and continuous cropping of wheat in the same field, population of soil-borne fungal wheat pathogens are likely to build up causing serious economic yield losses in wheat.

Most farmers in Tanzania plant uncertified seed saved from previous harvests, borrowed from neighbours or purchased from local markets; factors that encourage wide spread and introduction of new diseases (Louwaars and Marrewijk, 1996). This habit has variously been attributed to prohibitively high prices of certified seed; desire to grow new varieties and the fear of losing the traditional varieties that have special attributes (Witcombe *et al*., 1999; Wanyera, 2008)). Parastatal certified wheat seed systems in Tanzania supply only 10% of the total seed planted each year, whereas informal seed systems supply 90% of the seeds used by smallholder producers (Temu and Mtenga, 2008). Many of the diseases that reduce yields in wheat are mainly seed-borne (Abdulsalaam and Shenge, 2007). Crop losses due to seed-bone fungi in Tanzania are said to be in the range of 8 to 43% (Mbwaga and Hayden, 2003). Studies of seed-borne pathogens are necessary to determine seed health status to ensure seeds of high germination percentage are used by farmers to realize high crop yield. A complex of seed-borne fungi including genera of *Tilletia*, *Ustilago*, *Bipolaris*, *Fusarium*, *Alternaria*, *Drechslera*, *Stemphylium*, *Curvularia*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Penicillium* have been convincingly reported as the most frequent seed-borne fungi of wheat throughout the world (Glazek, 1997; Hashmi and Ghaffar, 2006; Rehman *et al*., 2011; Suproniene *et al*., 2011). However little is known on the status of seed-borne pathogens in wheat-growing regions in the Northern parts of

Tanzania. This information is necessary to enable local growers understand the seed-borne fungal pathogens status infecting wheat and type of fungicide(s) to be used as a control measure. No study has been carried out to identify the diverse population of seed-borne fungal pathogens prevalent in Northern wheat growing Zone of Tanzania. The management of these pathogens during the seed-borne phase is considered to be the cheapest disease control strategy (Abdulsalaam and Shenge, 2007). Moreover, effective management of the seed borne diseases can only be implemented effectively if the causal pathogens are correctly identified as fungicides are not cross-effective among fungal species. Many farmers in the Northern wheat growing Zone are using seeds served from previous harvests whose health status has not been tested. The present study therefore, was undertaken with the objective to investigate the internal and extent seed-borne fungal pathogens in farmer saved seeds in selected areas of Northern wheat growing Zone in order to recommend better wheat disease management strategies in these area.

1.1 The Overall Objective

To investigate the extent of seed-borne fungal pathogen problem, via seeds for better disease management strategies of wheat in selected areas of Northern wheat growing Zone.

1.2 The Specific Objectives

i. To determine prevalent seed-borne fungal pathogens present in farmers saved seeds and levels of seed infection in the study areas.

ii. To assess suitable seed dressing fungicides for the control of seed borne fungi in the farmer's saved seeds from the study areas.

iii. To explore the effects of seed-borne fungal pathogens on wheat yield.

1.3 Description of the Study Area

1.3.1 Hanang District

Hanang is one of the five Districts of the Manyara Region in Tanzania. Its geographical coordinates are 4° 31' 0" South, 35° 23' 0" East and its original name (with diacritics) is 'Katesh'. It is bordered to the North by the Mbulu and Babati Districts, to the Southeast by the Dodoma Region and to the Southwest by the Singida Region. The main economic activities in Hanang District are subsistence and commercial farming. More than 80% of Hannang population depends on agriculture as their mainstay. The labour force is mainly engaged in small scale farming where hand hoes are the main farm implements. The maximum rainfall is 627 mm per year, the maximum temperature is between 20.1^{o}C and 25.1^{o}C; the soils are young and volcanic in origin and range from moderate to very fertile soils. They also range from poorly drained (clay) soils to well drained (loamy sand) soils. Though termed moderately fertile soils, they are however generally deficient in Nitrogen and Phosphorus.

1.3.2 Karatu District

Karatu is one of the five Districts in the Arusha Region of Tanzania. It is bordered by the Ngorongoro District to the North, the Shinyanga Region to the West, the Monduli District to the East, and the Manyara Region to the South and Southeast. Average

temperature is 28^{o}C and rainfall 650 mm per year. The area contain a very fragile vegetation cover which could one day turn into desert if the area continue to be over cultivated and overgrazed. The main economic activities include agriculture which employs about 72% of the population and livestock keeping which employs about 11% of the total population. Other economic activities are mining, tourism, small scale industries and business entrepreneurship. These contribute about 17% of employment to the Regional population. Soil fertility range from poorly drained (clay) soils to well drained (loamy sand) soils.

1.3.3 Siha District

Siha District (formerly part of Hai) is in Kilimanjaro Region. Siha District is a new governing District formed in July, 2005. It is located in an area of remarkable beauty on the plateau between Mt. Kilimanjaro and Mt. Meru in Tanzania. Siha is one of the seven Districts of the Kilimanjaro Region of Tanzania. It is bordered to the South and West by the Arusha Region, to the North by Kenya, and to the East by Moshi Rural and Rombo Districts. Average temperatures is ranging from 17°C to 34°C, rainfall varies greatly from place to place. The humid and intensively cultivated highland area receives 1 000–1 500 mm of rainfall annually, with the probability of at least 1 000 mm even in the driest years. Agriculture is the dominant economic activity, employing more than 80% of the population in the District. This is practiced on a smallholder scale, on plots of 0.5 to 5 hectares per family, the main crops grown are: maize, beans, wheat, sunflower, sorghum, cassava, bananas and other fruits especially citrus types and mangoes. People also keep small numbers of livestock's, few traditional (humped zebu) cows, goats, sheep, pigs and freely feeding poultry.

Figure 1: Map of Tanzania showing locations of Karatu, Siha and Hanang Districts

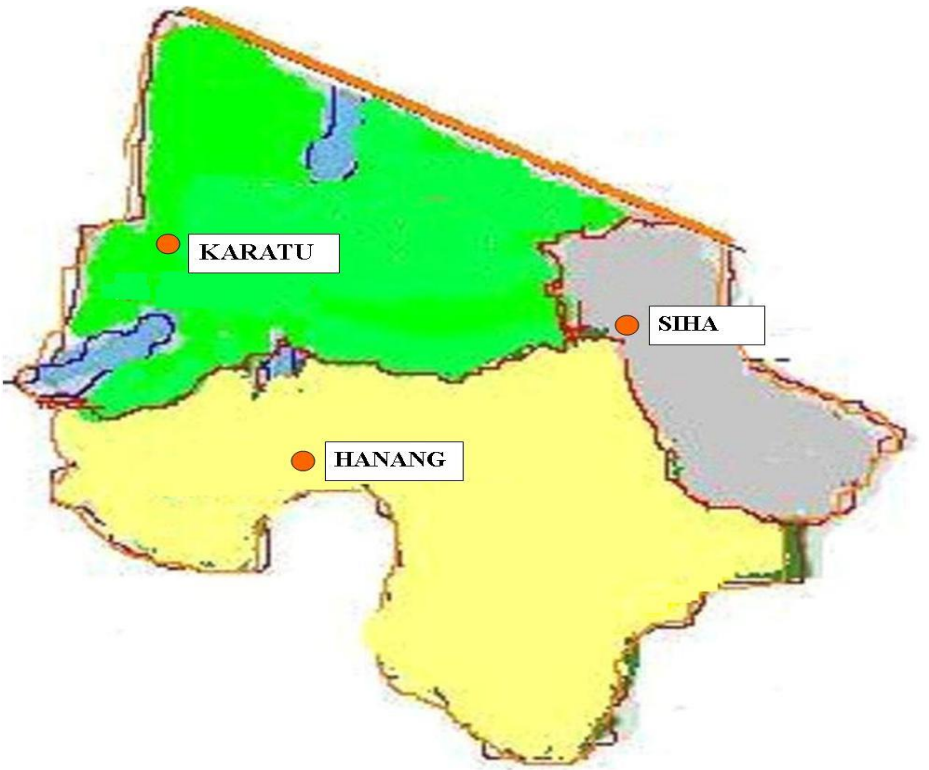

Figure 2: Map showing Karatu, Hanang and Siha Districts

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wheat Production in Tanzania

German missionaries introduced wheat crop in the Southern highlands of Tanzania to small-scale farmers in the 19th Century (Rudgers, 1992). Follow the 1974/75 food shortage; significant large-scale wheat plantations were initiated at Hanang, in the Northern highlands of Tanzania, with assistance of the Canadian International Development Agency (CIDA) to implement the Tanzanian food self-sufficiency policy (Benson *et al*., 2007). A survey of population, wheat consumption, imports and food self-sufficiency in eight Eastern, Central and Southern Africa countries conducted in 1994 revealed that Tanzania was self sufficient in wheat only by 53% (Pingali and Rajaram 1999; Kyaliet *et al*., 2010). Survey conducted in 1996 indicated that the National Agricultural Food Cooperation (NAFCO)'s Hanang Wheat Complex farms produced 40% of the total wheat production (Prestes *et al*., 2007). However, to the present day, annual wheat production in Tanzania has never satisfied its demand (Kamau, 2011) and current production capacity stand at 100 000 mt tones annually (FAO, 2009).

2.2 Important Wheat Seed-bone Fungi

Infected or contaminated seeds serve as major source of inoculum for large number of plant pathogens which may infect the seeds and survive as spore or resting structures on or within the seeds (Saberi *et al*., 2004). Wheat seed harbor several species of fungi, which can reduce seed quality and cause plant disease. Fungi carried on or within seeds reduce seed germination, seedling emergence lead to less vigorous seedling (Anjorin and

Mohammed, 2009). Seed-borne fungal pathogen present externally or internally may cause seed abortion, seed rot and seed necrosis (Khanzada *et al*., 2002). Some plant pathogenic fungi kill seedlings shortly after they emerge, whereas others cause serious disease epidemics after being transmitted from seeds to seedlings. Seed-bone diseases also affect the growth and productivity of wheat (Weber *et al*., 2001). Seed-borne fungi reported in wheat include *Alternaria* spp, *Aspergillus* spp, *Cladosporium* spp, *Claviceps* spp, *Cochliobolus* spp, *Curvularia* spp, *Dilophosphora* spp, *Drechslera* spp, *Didymella* spp, *Fusarium* spp, *Gaeumannomyces* spp, *Gibberellazeae* spp, *Hymenella* spp, *Lasiopiplodia* spp, *Leptosphaeria* spp, *Leptosphaeria* spp, *Monographella* spp, *Mycosphaerella* spp, *Nigrospora* spp, *Pseudoseptoria* spp, *Puccinia* spp, *Pyrenophora* spp, *Sclerophthora* spp, *Setosphaeria* spp, *Tilletia* spp, *Urocystis* spp and *Ustilago* spp. (Weidenboner *et al*., 1996; Klyszejko *et al*., 2005).

Major seed borne fungal diseases of wheat in Eastern and Southern Africa according to Marthur *et al*. (2002) are *Ustilago* spp (Smut), *Tilletia* spp (Common bunt), *Fusarium* spp (Head scab), and *Helminthosporium* spp (Spot blotch). The important seed-borne diseases of wheat in Tanzania are *Helminthosporium* spp (Spot blotch). *Alternaria* spp (black point) and *Fusarium* spp (Scab or head blight) (Kuwite *et al*., 2010). Anjorin and Mohammed, (2009) isolated seed-borne fungi (*Aspergillus* spp, *Alternaria* spp, *Cladosporium* spp, *Penicillium* spp and *Ulocladium* spp) associated with wheat grains under laboratory conditions. Zare *et al*. (2006) reported the association of *F. culmorum* (15.5%), *F. Graminearum* (13.1%), *B. sorokiniana* (24.4%), *Drecshslera tritici-repentis* (4.5%), *A. alternata* (8.5%), *Cladosporium sphaerospermum* (24.2%),

Penicillium spp (4.7%), *A. niger* (5%), *A. flavus* (9%) and *Rhizopus* (12%) with wheat seeds when assayed under blotter method. In the study, the number of colonies of *Aspergillus* and *Rhizopus* were increased by 27 and 64 %, respectively under agar method where as the colonies of *Cladosporium, Penicillium, Fusarium* and *Phoma* were reduced in numbers. Gohari *et al*. (2007) also isolated the fungi *Aspergillus flavus, Alternaria alternata, Cladosporium* spp, *Penicillium* spp and *Ulocladium* spp in wheat seeds. Supronienе *et al*. (2011) and Habib *et al*. (2011) reported the predominance genera of *Alternaria alternata, Ulocladium alternariae, A. flavus, A. niger, Chaetomium globosum, F. proliferatum, Cladosporium cladosporioides, Rhizopus* spp. and *Penicillium* spp associated with wheat seeds when assayed under agar method compared to blotter method. Hajihasani *et al*. (2012) isolated surface-borne saprophytes including *Fusarium graminearum*, *F. culmorum*, *Bipolaris sorokiniana, Alternaria alternata*, *Aspergillus niger, A. flavus*, and *Penicillium* spp from the seeds of three wheat cultivars employing standard blotter method.

It has long been noted that seed-borne fungal pathogens are responsible for reducing seed quality, protein and carbohydrate contents, reduction or elimination of germination capacity as well as seedling damage, which result in the reduction of crop yield (Fakhrunnisa *et al.*, 2006, Mushtaq and Hashmi, 2007, Rehmanet *et a*l., 2011; Supronienе *et al*., 2011). Over the last decades, many studies have been made to test and detect seed-borne diseases of wheat throughout the world. For example, studies of seed microflora in Canada revealed that a total of 35 genera and 59 species of seed-borne fungi exist in seed samples of wheat (Zare *et al*., 2006). Hajihasani *et al*. (2012) reported that 17 genera and 45 species of seed-borne fungi were associated

with wheat seeds in Pakistan. Khanzada *et al*. (2002) isolated six fungal species from wheat seeds using standard blotter method, the most common were *Alternaria alternate, Aspegillus flavus, Fusarium monoliforme, Fusarium graminerium, Cladosporium* spp and *Bipolaris sorokiniana*.

Hashmi and Ghaffar (2006) identified 21 species of fungi from wheat seeds by employing standard blotter method and deep freezing method; predominant species found were *Absidia* spp*, Alternaria spp, Aspergillus* spp*, A. candidus, A. flavus, A. niger, A.sulphureus, Cephalosprium* spp*, Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera halodes, D. hawaiiensis, D. tetramera, Fusarium moniliforme, Fusarium oxysporum, F. pallidoroseum, F. subglutinans, Penicillium* spp*, Rhizoctonia solani and Rhizopus* spp. Of the fungi isolated *Alternaria alternata, Aspergillus flavus, A. niger, Cephalosporium* spp, *Penicillium* spp*, and Rhizopus* spp.

Rehman *et al.* (2010) isolated some species of fungi from freshly harvested wheat seeds using agar plate method, eight genera and 13 species were isolated, along with *Alternaria alternata* other species were *Alternaria tenussima, Fusarium nivale, Fusarium graminearum, Fusarium heterosporum, Fusarium proliferatum, Fusarium sporotrichioides, Fusarium tricinctum, Fusarium semitecum, Aspergillus niger, Mucor* spp, *Rhizopus* spp, *Curvularia lunata*, *Bipolar specifera* and *Stemphylium herbarum.*

Ojuederie *et al*. (2009) isolated surface-borne saprophytes *Alternaria alternata, Aureobasidium pullulans, Cladosporium* spp, *Penicillium* spp, *Rhizopus stolonifer, Stemphylium* spp, *Vlocladium atrum* and several other pathogens like *Ascochyta pisi, Botrytis cinerea, Fusarium oxysporum* and *Phoma medicoginis* from chickpea seed by employing standard blotter method. Carey *et al*. (2005) reported the association of *Aspergillus* (12%), *Cladosporium* (56%), *Fusarium* (75.5%), *Penicillium* (7.3%), *Phoma* (25.5%) and *Rhizopus* (12%) with soybean seeds when assayed under blotter method. David *et al*. (2007) reported the predominance of genera of *Aspergillus, Fusarium* and *Penicillium* with pea seeds when assayed under agar method compared to blotter method. Chauhan *et al*. (2009) isolated *Alternaria alternata* and *Fusarium oxysporum* from two Chickpea genotypes CNJ and E14 under laboratory conditions. Kiran *et al*. (2005) isolated nine fungal species belonging to eight genera namely; *Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme, Helminthosporium sativum, Mucor* spp, *Penicillium notatum* and *Rhizopus nigricans* from seven seed samples of chickpea.

2.3 Detection of Seed-borne Fungal Pathogens

The detection of pathogenic seed-borne fungi and seed diseases is an important aspect of disease management. Determining the presence of seed-borne pathogens allow managers to apply the appropriate controls or modify management practices to avoid the problem in the future (Carey e*t al.*, 2005). Presence of diseased seeds in seed-lots cannot be reliably detected by visual examination (Kolotelo, 2007). Radiographic assays of seeds provide an efficient, non-destructive method to determine internal seed damage (Michelle *et al*., 2008). Internal seed contents can be examined by

cutting the seed open and looking for mycelium or symptoms of disease (Michelle *et al*., 2008). Seed-borne pathogens can also be present on seeds without obvious disease symptoms (Agarwal and Sinclair, 1997).

The presence of pathogenic fungi on seeds is most often determined through laboratory culture and identification where samples of seeds are placed on various media and fungi that grow from the seeds are evaluated (Guizhen and Thomas 2002). Although this technique is widely employed, it is time consuming and may not detect pathogens at low levels. Competitive saprophytic fungi on seeds are an additional problem because they can obscure the presence of a pathogen (Murakishi, 2002 Niaz; Dawar, 2009). However, for some fungal species, such as *F. oxysporum*, evaluation of isolates from seeds on living seedlings is necessary to determine pathogenicity (Littke, 2008). Many detection methods have been developed over the years for various seed-borne pathogens. Standard blotter method has been found to be efficient method of detecting seed-borne fungal pathogens in wheat seed (Walcott *et al*., 1998; Paulsen, 2002). Following ISTA (2005) rules, the method involves planting 400 seeds on some layers of moistened filter paper. Methods used in routine seed health testing include seed health testing procedure which involve techniques such as; direct examination of dry seeds, examination of germinated seeds, examination of organism removed by washing, examination after incubation (both blotter and agar plates), examination of growing plants (for example the seedling symptom test), embryo count methods and molecular and serological techniques (Toussaint *et al*., 2001). Other methods include the use of a selective medium for specific pathogen (Cockerill and Smith, 2002). However, with advances in molecular techniques, emphasis in

fungal identification and taxonomy has changed from morphological approach (for example spore size and shape) to a more functional approach based on aspects of life cycle, mechanism of spore production and release, DNA relationships and physiological attributes (Kolotelo, 2007). DNA analysis techniques such as the polymerase chain reaction (PCR) and Random Amplified Polymorphic (RAP) analysis are the most commonly used tools (Toussaint *et al*., 2001).

2.4 Epidemiology of Seed-borne Diseases

Prevalence of pathogens significance to quarantine suggest the potential of seed transmission as a factor that could lead to epidemic of seed-borne diseases into new areas where they were not previously been reported (Gabrielson, 1996). They could also relate the potential of seed damage or yield losses caused by diseases derived from the seed-borne inoculums of the pathogen. Yield loss caused by disease epidemic is important in determining pathogens with quarantine significance (Savary *et al.,* 1998). In seed health testing detection frequency means that, number of seeds which are infected (based on 400 seeds tested) is equivalent to the inoculum level (Bearchell *et al*., 2008).

Infected seeds with either spores on their coats or mycelium under their coats are likely to be the main source of transport for these pathogens into new areas. In addition, the infected seed left on the ground after harvest also serve as a source of infection of newly planted crop (Makun *et al*., 2007). The environmental factors such as temperature, moisture and relative humidity affect the epidemics of seed-borne diseases, e.g. seeds are more infected by *Alternaria alternata* at 30ºC. Generally, a

high temperature of 15-30ºC is required for the growth and survival of this fungus in the seeds and high relative humidity of more than 65% is required for spore germination (Sangeetah and Siddaramaiah, 2007).

2.5 Infection Cycle of Seed-borne Diseases

Infection cycle of seed-borne fungal diseases is shown on Fig. 1. Seed-borne inoculums re-infect the seed during the development of a disease: The sequence of events involve initial seed borne inoculums transported by various means and once the seeds are sown the fungus infect the developing plant followed by disease establishment and development in the field due to subsequent infection cycles that lead to crop damage and yield losses as shown below.

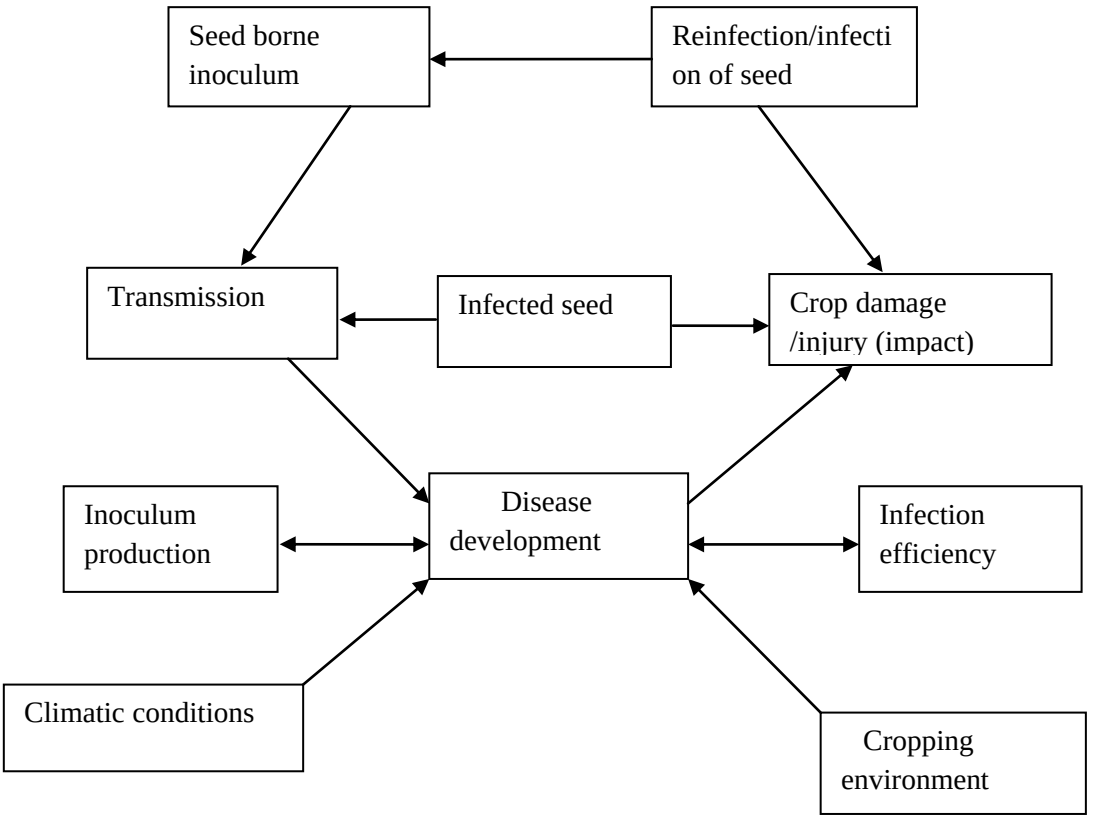

Figure 3: Infection cycles of seed-borne fungal pathogens

Source: Mew and Gonzales (2006)

Various factors affect the seed-borne infection cycle including weather conditions, cropping practices, resistance or susceptibility of the variety, virulence of the pathogen and the amount of inoculums produced for secondary spread and efficiency of the inoculums (Kishore, 2010). It is often assumed that for a pathogen to be a seed-borne, it must be seed-transmitted. Mc Gee (1995) reported that it is only very few seed-borne pathogens have their transmission clearly been established. Once a disease is established in a crop, its intensity will depend on factors that influence the infection cycle. Climatic condition and crop management practices are crucial for disease development. For example, in rice the infection frequency of *Pyricularia oryzae* is very low, yet the disease potential under a conducive environment is very high.

Once seedlings are infected from seed-borne inoculums, even at a low infection rate, millions of conidia are produced for secondary infection (Savary *et al*., 1998). On the other hand, seed-borne *F.moniliforme* often induces bakane with only one cycle of infection. Therefore, the initial inoculums for *F.moniliform* are important in the epidemiology of the disease. Once the seed borne inoculums is minimized the disease is likely to be controlled (Rehman *et al*., 2011). However in an epidemiological research, seed transmission and establishment of disease derived from seed-borne inoculums should be considered (Mc Gee 1995). These data are essential for assessing the importance of seed-borne fungal pathogens.

2.6 Mechanisms of Seed Infection

Seed-borne microorganisms can be considered in four main classes (Gabrielson, 1996). According to these authors, the first class consisted of pathogens in which the

seed is the main source of inoculum. Under these conditions, when the seed infection is controlled, the disease is also controlled. The second class consisted of important pathogens in which the seed-borne phase of the disease is of minor significance as a source of inoculum. Examples are those in which the crop residues in the field are the major source of inoculum. The third and the largest group of seed-borne microorganisms consisted of those that have never been shown to cause disease as a result of their presence on seeds.

The fourth class is a group of microorganisms that can infect the seed either in the field or in storage and reduce yield and seed quality. Examples of common field seed-borne fungi are *Diplodia, Fusarium, Cladosporium, Alternaria, Helminthosporium, Drechslera, tetramera,* and *Curvularia*. The storage fungi are *Aspergillus* and *Penicillium* which can invade most types of seeds under high-moisture storage conditions (Ilyas *et al.,* 1998; Bhutta and Hussain, 1999; Ijaz *et al*., 2012). The process of seed infection is influenced by the conditions under which the crop grows (Glazek, 1997).

Factors that influences the process of infection includes the host and its genotype, the pathogen and its genotype and environmental factors. Agarwal and Sinclair (1997) reported that the establishment of a pathogen in any part of the seed is refereed as seed infection. It can be systemic, through vascular system or plasmodesmata or directly by natural or artificial wounds. The same pathogen can infect the seed using one or more of these mechanisms (Agarwaland Sinclair, 1997). Most of the systemic seed-borne fungi reach and infect the embryo through the flower or from the

peduncule of the fruit, via funiculus, Agarwal and Sinclair, (1997); Bateman and Kwasna (1999); Khanzada *et al*. (2002) indicated that most of the systemic seed-borne fungi reach and infect the embryo through the flower or from the peduncule of the fruit, via funiculus. Monaco (2009) found that during the infection, some pathogens follow the same path as the pollen grains do and spores of some fungus reach the stigma and germinate, producing hypha that reaches the ovary through the style, where they can stay as a dormant mycelium until seed germination. e.g *Ustilago nuda* and *U. triticci* in barley and wheat, and *Alternaria alternata* in sweet pepper. Fungi like *Ustilago nuda* and *U. triticci* penetrate through the wall of the ovary as a result of the germination of the teliospores on the stigma or the wall of the ovary. The pro-mycelium goes through the wall and other tissues until it reaches the embryo (Mew and Gonzales, 2006).

In some other cases, penetration occurs through breakages on the testa, establishing itself in the endopleura or the endosperm (Khanzada *et al*., 2002; Bateman and Kwasna, 1999). The pathogen or its parts can stick or can get mixed with the seeds during any of the processes during seed re-collection; harvesting, extraction, threshing, selection or packing (Khanzada *et al*., 2002). Pathogens that stick to seeds during harvest or postharvest do so by their spores e.g clamydospores, oospores, teliospores, uredospores, Spores of the following fungi can be carried on seed coat surfaces e.g *Alternaria alternata, Drechslera tritici, Bipolaris sorokiniana, Fusarium moniliform, Tilletia caries, T. foetida* and *T.* contraversa, and Urocystis agropyri in wheat (Mew and Gonzales, 2006).

2.7 General Characteristic of Important Seed-bone Fungal Diseases of Wheat

2.7.1 Storage mould caused by *Aspergilus flavus*

Aspegillus flavus has upright, simple conidiospores that rise from prominent foot cells and terminate in a globose or clavate swelling. The swellings bear clusters of phialides that produce globose, one celled conidia (phialospores, 2.5-5 µm in diameter in dry, basipetal chains). In mass, conidia often are green but can be variously colored (Wiese, 1998). Mould on seed will result to seed infection and decreased seed quality. Mould primary invade embryo and cause damage and non-germinable to many kernels (Richardson 1990; Wiese, 1998).

2.7.2 Black Point (Kernel Smudge) caused by *Altenaria alternata*

This disease occurs in most wheat-growing regions of the world. The disease was first reported in 1962 in India (Marthur and Barry, 2005) where it is reported to have caused considerable damage in certain parts of Bihar and Bengal states. A severe epidemic in wheat grown in India was observed in 1983 where it caused 75% reduction in grain weight (Marthur and Barry, 2005) *Alternaria alternata* reduces seedling emergence and yield of the subsequent crop (Aulakh *et al*., 2000; Chauhan *et al*., 2009).

Colonies on potato dextrose agar (PDA) are initially light grey, later turning black to olivaceous black but lighter towards the margin. Conidiophores are single or in small groups arising from the substratum, simple or branched, straight sometimes geniculate, pale, mid-olivaceous or golden brown. They are 40-50x2-6 µm, with several conidial scars. Conidia form long, branched or unbrached chains.

They are obclavate, obpyriform ovoid or ellipsoid, often with a short conical or cylindrical beak; pale to golden brown smooth or verrucose, with longitudinal or oblique septa, 20-65x 10-18 µm with a beak 10-20x2-5 µm long (Haware *et al.*, 2004; Mercado *et al.*, 2006).

2.7.3 Scab (Head Blight) caused by *Fusarium graminearum* and *Fusarium moniliform*

Scab occurs in all regions of the world where humid conditions exist during the flowering and grain filling stages (Leslie *et al*., 2006). The distribution of the causal fungi varies with geographical and climate, especially temperature. The most severe scab develops when abundant fungal sporulation coincides with wheat anthesis such as in fields and regions where wheat is cropped continuously or is cropped rotationally with other suspects such as maize or rice. Cropping systems in which residues of infected crops remain on the soil surface further enhance scab development. Scab results in significant reductions in grain yield and quality. Underestimated losses are associated with *Fusarium*-produced mycotoxins even in lightly-infected grains which can cause mycotoxicoses in animals and humans that consume scabby grain (Marasas, 2008). Grain from blighted fields has reduced value as a seed crop.

A high percentage of seeds may be infected by *Fusarium* spp, and may fail to germinate or may give rise to blighted seedlings, especially if planted into warm dry soils (Marthur and Barr, 2005) and Leslie *et al*. (2006) observed that *F.graminearum* aggressively invaded most portions of the wheat kernel except the embryo. They are 3-7 septate, thick-walled straight to moderately sickle shaped, unequally curved with

the ventral surface almost straight and dorsal surface smoothly arched. The basal cell is constricted as a snout. Macroconidia are borne on both branched and unbranched monophialides. Sporodochia are uncommon, but when they occur they are red brown to orange. Clamydospores (10-12 µm in diameter) often formed within macroconidia are slow to form in culture, superficial, purple to black perithecia (150-350 µm in diameter). Asci contain eight hyaline, 0-3 septate ascospores (17-25x3-5 µm) (Leslie *et al*, 2006).

2.7.4 Spot Blotch caused by *Bipolaris sorokiniana*

Colonies on PDA are olivaceous brown to black, lighter towards the periphery. The margin is mostly smooth but sometimes wavy with dark bands young colonies which are pinkish-white. Abondant conidia in older colonies make the entire colony black to deep olivaceous brown, roughly circular with concentric rings and the margin is smooth to irregular, rarely light pinkish white (Bach *et al*., 2004). Conidiophores on seed are brown short erect, mostly single bearing 1-6 conidial at the end and on the side at short distances in the upper half. Conidia are ellipsoid, dark brown mostly straight or slightly curved, thik-walled but lesser toward the end. They are broadest in the middle and the ends are rounded. The basal cell has a subhyaline terminal portion with a clear scar Conidia are 68-99x 17-24 µm and 6-9 septate (Seog and Byung, 2000), Initial symptoms on the leaves are small, light-brown to almost black spots, which are circular linear or elliptical (Anonymous, 1998).

Stem and node infection results in lodging, normally, individual spikelet's are infected but under favorable conditions the whole ear including the awns is severely

diseased. Lesions on glumes are oblong with a dark brown margin. Under favorable conditions conidia can be seen easily in the spots. Seeds in heavily infected seed lots are shriveled and have elliptical to oblong lesions which are lighter in the center (Shahzad *et al*., 2009).

2.8 Management Strategies of Seed-borne Diseases

2.8.1 Cultural methods

Disease management is a key component to realize high-yield in wheat production. In most years, it is possible to produce high wheat yields without paying attention to disease control (Agarwal and Sinclair, 1997). Most diseases are best managed through the use of multiple tactics, both proactive e.g., crop rotation, delayed and/or staggered planting plates, use of resistant varieties of varying maturities, proper fertility, and application of seed treatment and/or foliar fungicides and reactive e.g., application of foliar fungicides and timely harvest (Carey *et al.,* 2005). Seed-borne and soil borne diseases are controlled primarily by seed treatment and crop rotation. Resistance is generally not available for these diseases (Shahzad *et al*., 2009). Following planting and fertility management pre-seed treatment with suitable fungicide is recommended for successful disease management. The management of wheat pathogens during the seed-borne phase is considered to be the cheapest disease control strategy (Shenge *et al*., 2010).

2.8.2 Chemical Seed Treatments

Seed treatments have the potential to damage seeds; therefore, seed treatments should be used only when the gain in germination and seedling survival is greater than the potential loss (Barnett *et al.*, 1999, Barnett and McGilvray 2002, Barnett and Varela

2003, Allen *et al.,* 2004). Treating seed as an effort to control seed-borne and soil-borne diseases has been employed since the middle of the 17th Century. Use of chemical seed treatment was started in the United Kingdom to control Bunt of wheat (Maude, 1996). Chemical treatments, in particular, can be toxic to seeds and should be used with caution (Mbaka *et al*., 2012). Chemical fungicide treatments such as Captan and Thiram are still the most widely used products thus research on a better ways to apply and to reduce the effective rates of these chemicals must continue (Wang and Davis, 1997).

Thiram (tetramethylthiuram disulfide) is commonly used in nurseries as a bird and animal repellent, as well as a fungicide. In particular, thiram has been effective against *Fusarium* spp in wheat seeds (Barnett and Varela 2003; Littke, 2008). Besides these popular topical fungicides, there are wide range of systemic fungicides e.g. Dithane and Metalaxyl plus Mancozeb, Difenoconazole, Tebuconazole, Thiabendazole, Triadimenol and Triticonazole which help control seed-borne diseases and offer some disease control for the seedling; however, under conditions of high disease pressure, they may often fail (Wang and Davis, 1997 Marcia *et al*., 2011). Some of these chemicals Mancozeb, Captan, Carbamate, Metalaxy plus, Thiabendazole, and Triadimenol (Hendrick, 1996) have the potential to be harmful to the soil and non soil environment as well as carry the potential to be phytotoxic to the seed and the emerging seedling (Smith *et al.,* 2000; Sigler and Turco, 2002; Kinney *et al.,* 2005 and Bending *et al.,* 2007). However, as a result of this, there are more and more seed treatment research underway examining the ability of ecofriendly methods of treating seed to control disease(s) (Buss *et al*., 2001).

2.9 Evaluation of Seed Dressing Fungicides

Several techniques for evaluating fungicides have been described from time to time. Poisoned food technique is the most commonly practiced method for evaluating fungicides under laboratory conditions (Johnson and Sekhar, 2012). Javaid *et al*, (2006) evaluated some fungicides against seed-borne mycoflora of wheat, during their study they found that the effect of fungicides was not significant against the seed-borne mycoflora.

However, different tested fungicides showed variable response against the frequently occurring species of *Drechslera australiensis* and the other rarely occurring fungal species. Dithane and Metalaxyl plus Mancozeb were found to be the most effective fungicides against *Aspergillus terreus, Alternaria alternata* and *Fusarium oxysporum.* All the applied doses of Metalaxyl plus Mancozeb significantly reduced the incidence fungal species and completely arrested the growth of *F. oxysporum.* Malaker and Mian (2009) evaluated effect of seed treatment in controlling black point disease of wheat. Seed treatment with either Metalaxy plus -200 or Homai-80WP had no significant effect on black point incidence but both the fungicides were equally effective in increasing plant population, number of spikes and grain yield. Seedling mortality was found significantly lower in plots sown to seeds treated with either, Metalaxy plus - 200 or Homai-80WP than sowing with untreated seeds. Number of grains per spike was increased significantly by using both the fungicides but the effect was more pronounced when Metalaxy plus - 200 was used. However, no significant increase in spike length, spike weight or 1 000-grain weight was observed when the seeds were treated with any of the two fungicides. The increase in grain

yield due to seed treatment was attributed to increase in number of spikes per square meter and grains per spike. Khanzada *et al*. (2002) during their study on effect of seed dressing fungicide for the control of seed-borne mycoflora of wheat observed that maximum germination of wheat seedling was higher with seed treated with Baytan followed by Metalaxy plus, Benlate, Captain and Dithane M-45 respectively. There was no significance difference in the germination of seeds treated with Derosal and the greatest root length was observed in seed of all varieties treated with Baytan and Vitavax followed by Benlate. The root length was significantly decreased in seeds treated with Rizolex and Derosal, but it was higher than the untreated seeds of all the seeds in all varieties treated with Baytan followed by Metalaxy plus and Benlate. Whereas, shoot length was significantly decreased in seedlings obtained from seed treated with Rizolex. Shoot length of seedlings obtained from Captain, Dithane M-45, Derolex was also greater than the seedlings germinated from untreated seeds.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The present investigations were carried out in the laboratory and screen house during November 2011 to June 2012 at Selian Agricultural Research Institute, Arusha, Tanzania located at latitude 03^{o} 21' 51.5" S and longitude 036^{o} 38' 05.3 " E. The details of materials used and the methodology followed in conducting the experiments are described in this chapter.

3.1 Field Study

A total of 45 untreated seed samples of wheat one kilogram each were collected from farmers saved seeds at Karatu, Hanang and Siha Districts located in Arusha region Northern Tanzania. The three Districts are famous in the production of wheat in the Northern wheat Zone. In each District, 15 farmers were randomly selected, information on varieties grown, post harvest handling practices (storage facilities) were collected. 1kg seed samples collected from each selected farmer were packed in a paper bags labeled and stored at room temperature for further processing and assay of seed-borne pathogens. Detailed information of varieties collected in various Districts is shown in Appendix 1.

3.2 Laboratory Study

3.2.1 Physical inspection of dry seeds

Forty five wheat seeds samples one kilogram each for all varieties in all three sampled locations, were packed into three different bags (for each paper bag labeled) depending on where the sample was collected, (samples from Karatu in bag A,

samples from Hanang in Bag B, and samples from Siha in Bag C respectively) then all three bags were stored at room temperature. Each sample was physically inspected with unaided eye on the basis of which they were separated into pure seeds, seeds of other crops and inert matter. One kilogram (kg) of each sample was poured into a plastic tray. Pure seeds were separated from abnormal seeds and inert matter and each of these components were weighed separately and their various weights recorded. Seeds with physical abnormalities, like shriveling of the seed coat, reduction or increase in seed size, discoloration or spots in the seed coat were classified under abnormal seeds. Inert matter included soil, sand, stones, plant debris, fungal fruiting bodies etc. During analysis samples from one location were analyzed until all samples were finished then continue with sample from another location.

3.2.2 General laboratory procedures

3.2.2.1 Glassware cleaning

Petri dish glassware's were used for all laboratory experimental studies. They were kept for a day in the cleaning solution containing 60 g potassium dichromate, 60 mls of concentrated sulphuric acid, in 1 liter of water. Then they were cleaned by washing with detergent solution followed by rinsing several times in tap water and finally with distilled water.

3.2.2.2 Sterilization

All glassware's used in this study were sterilized in an autoclave at 1.1 kg/cm pressure for 20 min and kept in hot air oven at 60º C for 1 hr. Both solid and liquid media were sterilized at 121 °C and 1.1 kg /cm pressure for 15 minutes.

3.2.3 Identification of fungi

3.2.3.1 Standard blotter method

The standard blotter method developed by Doyer in 1938 and later included in the International Seed Testing Association Rules of 2005 was used in the identification of seed- borne fungal pathogens in this study. Four hundred seeds of each variety were tested in 3 replications. Three pieces of blotting papers of 90 mm size were moistened with distilled water and placed in 90 mm sterilized Petri dishes. After draining excess water, untreated seeds were placed at the rate of 25 seeds per Petri dish at equal distance. The plates were incubated at room temperature (20± 2ºC) under alternate cycles of 12 hours near ultra violet (NUV) light and darkness. After seven days of incubation the seeds were examined under stereoscopic–binocular microscope (Type Leica ATC 2000) for the associated fungi and they were identified based on "habit and colony characters in culture (Burgess *et a*l., 1994, Mathur and Kongsdal, 1994). Incidence of different fungal pathogens was also recorded. Fungal species found growing on the surface of seeds were identified and their percentage frequency (PF) of occurrence was calculated using the following formula:

$$PF = \frac{\text{No. of seeds on which fungus appear}}{\text{Total No. of seeds}} \times 100$$

3.2.3.2 Water agar test

The seeds were externally sterilized by 1% sodium hypochlorite (NaOCl) solution for 1-2 min and then placed at the rate of 10 seeds per petri dish containing 20 ml of 2% water agar (Habib *et al*., 2007). The Petri dishes were incubated for 7 days under

conditions described in section 3.3.3.1. The isolated fungi were identified using light microscope (Type Leica ATC 2000) after slides were stained with Lactophenol cotton blue (Henselova and Hudecova, 2001; Gwary *et al*., 2006).

3.2.3.3 Potato dextrose agar test

Potato dextrose agar (PDA) was prepared and the fungi identified by blotter method and water agar were inoculated onto the sterile PDA and incubated for 7 days under conditions described in section 3.3.3.1. At the end of which the fungi were identified based on their colony colour, spore morphology and type of mycelia growth using the light microscope (Type Leica ATC 2000) (Habib *et al*., 2007, Begum *et al*., 2004; Chuku *et al*., 2007; Sheikh, 2009).

3.3 Screen House Study

3.3.1 Evaluation of the effect of seed dressing fungicides on seed germination, seedling growth, seed-borne diseases and grain yield of wheat seed

Planting was done on 1st February 2012 in 20cm diameter pots, containing sterilized soil and each fungicide treatment was replicated three times. 1.5 g of Di-ammonium phosphate (DAP) was applied in each pot thereafter covered with small amount of soil to avoid burning of seeds. 15 seeds were planted per pot and later thinned to ten seedlings per pot after one week. Seeds were treated with fungicides prior planting as follows: Twenty gram triplicate seed samples containing 500 grains were treated with three different fungicides separately at the rate of 0.25gm per 500 seeds. The slurry treatment was used whereby preparation was done by mixing the required amount of fungicide in one liter of water. The slurry was added into the seeds in polyethylene bags. The seeds were shaken manually for 5 minutes to ensure uniform distribution of

fungicide on seed surfaces. The fungicides were applied as surface seed treatment. The fungicides used with their active ingredients and trade names as seed treatment is shown in (Appendix 2). The doses used for different fungicide is shown in Appendix 3. Pots were watered every other day. Hand weeding was carried out three weeks after planting and at one week interval until harvesting. Complete randomized design (CRD) with three replications was adopted. The untreated seeds were planted in separate pots as control. Disease severity was assessed using 0-9 scale described by Prescott (1975) (Appendix 4) on five plants selected randomly per pot. Similar plants were assessed throughout.

3.3.2 Data collection and statistical analysis

Weekly data collection started 14 days after planting and continued until harvesting, Plants were observed for disease expression using 1-9 scale (Appendix 4). The following growth parameters were also recorded; percentage of germinated seedlings, root length, shoot length, fresh biomass root, fresh biomass shoot, plant height (cm) near soil liner, number of grain per spike, 100 grain weight and grain yield per pot. All data collected were subjected to Analysis of Variance (ANOVA) procedures using Mstat-c version 2.1 statistical program. Statistical model used: $Y_{ij} = \mu + t_i + eij$ where; μ= overall mean; t_i = treatment effect and eij= random error. Mean separation test was done using Duncan's Multiple Range Test at $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Laboratory Study

Due to variation of types of varieties cultivated in the three Districts, two commonly used varieties (Mbayuwayu and local) were selected for laboratory and screen house studies to reveal location differences.

4.1.1 Physical inspection of dry farmers saved wheat seeds

The results of physical inspection of dry wheat seeds is shown on (Table 1). There were significance differences ($P \leq 0.05$) in the number of pure and abnormal seeds for samples collected in different locations. The proportion of inert matter also differs significantly between locations. Varieties and location x varieties interaction on seed quality differ significantly ($P \leq 0.05$) (Appendix 5). Wheat seed samples collected at Siha had highest average pure seeds for both varieties 994 and 992g followed by Hanang 988 and 983g and Karatu 986 and 984g for Mbayuwayu and Local variety respectively. Abnormal seeds and inert matter also differed significantly between varieties and locations (Table 1). Generally wheat seed samples collected at Siha were of high quality compared to seeds collected in other locations.

Table 1: Physical qualities of dry seeds for two varieties collected in Hanang, Karatu and Siha District

Variety	Location	Pure seed (g)	Abnormal seed (g)	Inert matter (g)
Mbayuwayu	Hanang	988.00 [a]	10.40 [a]	1.60 [a]
	Karatu	986.50 [b]	11.75 [a]	1.80 [a]
	Siha	994.00 [a]	4.90 [b]	1.10 [b]
Local	Hanang	983.50 [b]	13.95[a]	1.05 [b]
	Karatu	984.50 [b]	14.45[a]	2.55 [b]
	Siha	992.00 [a]	6.5 [b]	1.5 [b]
Mean		988.08	10.33	1.6
CV %		8.3	27.7	63.7
SE ±		3.354	2.862	1.019

1 000g seeds examined
Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05

4.1.2 Identification of fungi from farmer saved wheat seed samples collected at Siha, Hanang and Karatu District

A total of ten fungal species viz *Alternaria alternata, Bipolaris sorokiniana, Dresclera tritici, Fusarium graminearum, Fusarium moniliforme, Aspergillus flavus, Cladosporium sphaerormum, Epicoccum purpurascers, Penicilium corylophilum* and *Pyricularia* spp were isolated from 45 wheat seed samples collected at Hannang, Karatu and Siha Districts (Table 2, 3 and 4). The frequency of association of wheat seed with fungal microflora was significantly influenced by the location and varieties (P≤ 0.05) (Appendix 6). The highest percentage infection was recorded in Local variety collected on both three locations. Karatu District registered the highest average percentage seed infection (32.8%) followed by Hanang (28.6%) and Siha (25.4%) respectively (Table 2, 3 and 4), Similar result were recorded on Mbayuwayu variety (30%) in Karatu followed by Hanang (26, 5%) and Siha (25%) (Table 2, 3 and 4). *Alternaria alternata* was found to be the most frequently specie associated with the wheat grains on local variety in all Districts exhibiting (20.8, 11.5 and 7%)

incidence for Hanang, Karatu and Siha respectively, followed by *Aspegillus flavus* (25, 11.5 and 5.25%) for Karatu, Siha and Hanang respectively (Table 2, 3 and 4). *Cladosporium sphaerormum* was observed to be the most frequently occurring species on Mbayuwayu variety with (9.8, 8.8 and 6%) for Hanang, Siha and Karatu respectively (Table 2, 3 and 4). Generally, Local variety was observed to have highest percentage seed infection as compared to Mbayuwayu variety (Table 2, 3, 4 and 5). Seed samples collected from Siha were more clean with the lowest percent seed infection as compared to seed samples collected from Hanang and Karatu (Table 2, 3 and 4).

Table 2: Fungi identified from samples collected in Hanang District

Variety	Type of seed-borne fungi isolated	No. of infected Grains[1]	% seed infection	Total fungi infection (%)
Local	*A.alternata*	83	20.75	
	F.graminearum	12	3	
	C.sphaerormum	20	5	
	A.flavus	21	5.25	37.0
	F.moniliform	7	1.75	
	E.purpurascers	2	0.5	
	B.sorokiniana	1	0.25	
	P. corylophilum	2	0.5	
Mbayuwayu	*A.alternata*	29	7.25	
	C.sphaerormum	39	9.75	
	A.flavus	29	7.25	
	E.purpurascers	3	0.75	
	F.moniliforme	3	0.75	26.5
	P. corylophilum	1	0.25	
	Pyricularia	2	0.5	
	P. corylophilum			
Selian	*A.flavus*	46	11.5	
	A.alternata	29	7.25	
	C.sphaerormum	40	10.0	33.5
	B.sorokiniana	14	3.5	
	P. corylophilum	5	1.25	
Riziki	*A.alternata*	34	8.5	
	A.flavus	30	7.5	
	C.sphaerormum	4	1.0	17.5
	E.purpurascers	2	0.5	

[1]Per 400 seeds examined

Table 3: Fungi identified from samples collected in Karatu District

Variety	Type of seed-borne fungi isolated	No. of infected Grains[1]	% seed infection	Total fungi infection (%)
	A.alternata	46	11.5	
Local	*A.flavus*	100	25.0	
	C.sphaerormum	8	2	
	F.gramineum	5	1.25	
	Pyricularia	3	0.75	42.0
	P. corylophilum	6	1.5	
	A.alternata	60	15	
Mbayuwayu	*A.flavus*	21	5.25	
	C.sphaerormum	24	6	
	F.moniliforme	10	2.5	30.0
	E.purpurascers	3	0.75	
	B.sorokiniana	1	0.25	
	D.tritici	1	0.25	
	A.flavus,	45	11.25	
Chiriku	*A. alternate*	36	9.0	23.0
	C.sphaerormum	6	1.5	
	E.purpurascers	5	1.25	
	C.sphaerormum	103	25.75	
Lumbesa	*A.alternata*	23	5.75	34.5
	A.flavus	12	3.0	
	C.sphaerormum	89	22.25	
Tausi	*A.alternata*	16	4.0	
	A.flavus	14	3.5	30.3
	E.purpurascers	2	0.5	
	A.flavus	51	12.63	
Sifa	*C.sphaerormum*	18	4.5	20.4
	E.purpurascers	5	1.25	
	A.alternata	2	0.5	
	F.gramineum			
	C.sphaerormum	144	36.0	
Azimio	*A.flavus*	40	10.0	49.8
	A.alternata	13	4.25	
	D.tritici	2	0.5	

[1]Per 400 seeds examined

Table 4: Fungi identified from samples collected in Siha District

Variety	Type of seed-borne fungi isolated	No. of infected Grains[1]	% seed infection	Total fungi infection (%)
Local	*A.flavus*	46	11.5	
	A.alternata	28	7	
	C.sphaerormum	21	5.25	25.0
	E.purpurascers	3	0.75	
	B.sorokiniana	2	0.5	
	C.sphaerormum	35	8.75	
Mbayuwayu	*A.alternata*	32	8.0	
	A.flavus	12	3	
	E.purpurascers	6	1.5	23.8
	B.sorokiniana	3	0.75	
	D.tritici	7	1.75	
	Pyricularia	3	0.75	
	P. corylophilum	2	0.5	
	A.alternata	57	14.25	
Duma	*A.flavus*	27	6.75	
	C.sphaerormum	20	5.0	32.8
	Pyricularia	4	1	
	D.tritici	7	1.75	
	B.sorokiniana	12	3	
	F.graminearum	4	1	
Kenya mwamba	*C.sphaerormum*	21	5.25	
	A.alternata	11	2.75	10.5
	F.graminearum	6	1.5	
	A.flavus	3	0.75	
	E.purpurascers	1	0.25	
Ngamia	*A.alternata*	83	20.83	
	F.graminearum	12	3	
	C.sphaerormum	21	5.33	35.1
	A.flavus	20	5.08	
	F.moniliform	7	1.75	
	E.purpurascers	2	0.375	
	B.sorokiniana	1	0.25	
	P. corylophilum	2	0.5	

[1]Per 400 seeds examined

Table 5: Mean percent infection for two varieties widely grown in Hanang, Karatu and Siha Districts

Location	Variety	% Seed infection
Hanang	Local	37.6 [a]
	Mbayuwayu	32.6 [b]
Karatu	Local	35.4[a]
	Mbayuwayu	34.8[a b]
Siha	Local	32.4[a]
	Mbayuwayu	30.8[b]
Mean		33.9
CV %		23.88
SE ±		7.696

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05

4.1.3 Culture characteristics of the identified seed-borne fungi

Aspegillus flavus: The growth of the fungus on seeds produced green, yellow fluorence (Plate 1). Conidiophores were observed as simple, upright. Conidia were 2-5 µm in diameter with basipetal chains, conidia were green to yellowish in colour (Plate 2).

Alternaria alternata: Growth of the fungus was brown to black. Conidia were in long chains (Plate 5). Conidiophore were short 40-50 × 3-4 µm; single or branched with corresponding number of primary chains, which were simple straight pale or golden brown (Plate 5). The color of fungal colonies was usually dark brown to dark olive green brown, but quite often lighter and almost white colonies (Plate 6).

Fusarium graminearum: Growth of the fungus was whitish and powdery; microconidia were in chains and false heads, formed abundant, thick, pinkish white mycelia, with grayish margins (Plate 7). Macroconidia are hyaline, inequilaterally

fusoid delicate thin walled with an elongated sharply curved pedicellate basal cell, 3-7 septate (Plate 8). Colony was whitish to grayish in colour (Plate 9).

***Bipolaris sorokiniana*:** Conidiophore of the fungus were brown, short erect bearing 1-5 conidia. Conidia or conidiophores appears as palm tree with mycelium produced on infected seed. Conidia was ellipsoid, dark brown to black, smooth, mostly straight, wall was thick less toward the end, broadest in the middle, ends rounded (Plate 10). Colonies were velvet-like, dark olive; plane, totally covered by short conidiophores, with black conidia in their apex (Plate 11).

Dresclera tritici: Fungus produced dense, white to grey mycelium covering mostly the whole seed. Few light-brown, slender conidia were visible on mycelium. Conidia were smooth-walled, single straight, sylindrical with rounded apex and a characteristic conidia basal cell, 1-9 distoseptate (Plate 12). Colony was observed as dark green or dark grayish in colour, (Plate 13).

Cladosporium sphaerormum: Growth of the fungus was grey to grayish brown, produce septate brown hyphae, Conidiophores and conidia were erect and dark in colour. They occur in branching chains which were closer to its end, appear like ends of brush in shape measuring 3-4 μm. *Cladosporium sphaerospermum* produces elongate and septate shield cells (Plate 14). The colour of colony is olivaceous grey to whitish from the front which is light to heavy (Plate 15).

Epicoccum purpurascers: The fungus produced brown to black sporodochia which were in group. Sporodochia were rough and granular with small brownish black, more or less spherical conidia on short and stout conidiophores, the conidia wall were

verrucose to tubercular or areolate (Plate 16). Colony was observed as powdery which were whitish in colour (Plate 17).

Plate 1: *A. flavus* in seeds on water agar

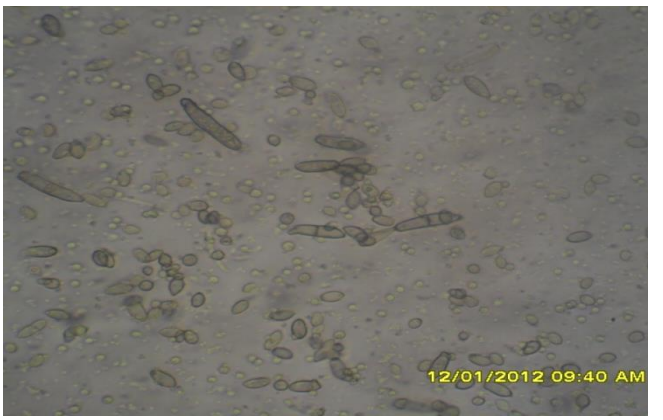

Plate 2: Conidia of *A. flavus* under microscopy (x 40 magnification)

Plate 3: Colony of *P. corylophilum* on PDA

Plate 4: *A. altenata* in seeds on water agar

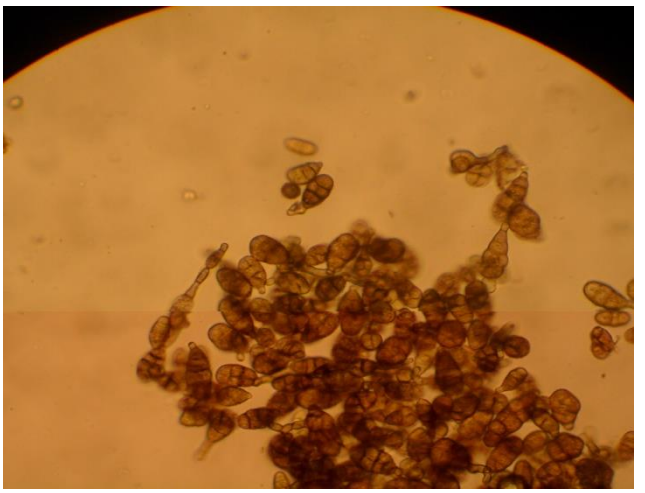

Plate 5: Conidia of *A. altenata* under microscopy (x 40 magnification)

Plate 6: Colony of *A. altenata* on PDA

Plate 7: *F. graminearum in* seeds on water agar

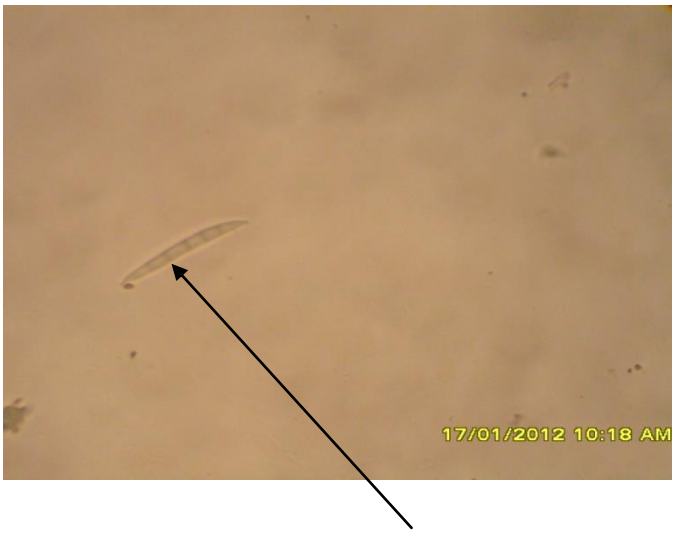

Plate 8: Conidia of *F. graminearum* under microscopy (x 40 magnification)

Plate 9: Colony of *F.graminearum* on PDA

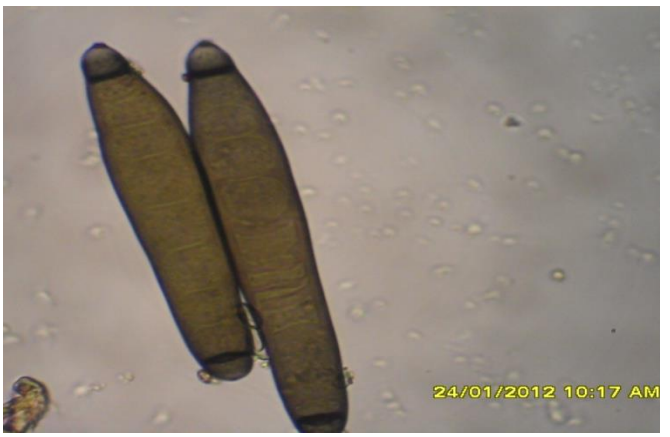

Plate 10: Conidia of *B. sorokiniana* under microscopy (x 40 magnification)

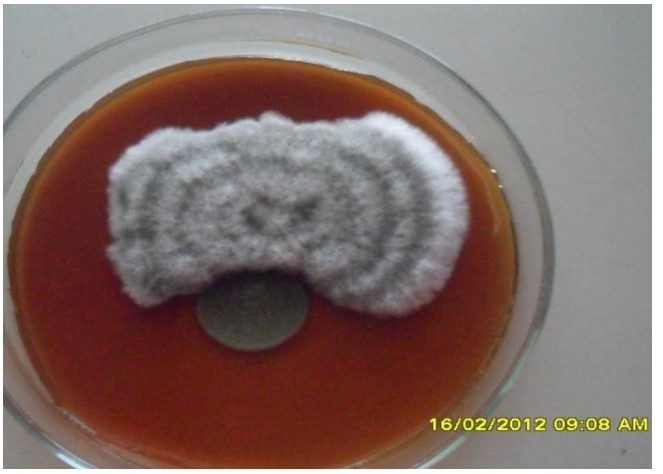

Plate 11: Colony of *B. sorokiniana* on PDA

Plate 12: Conidia of *D. tritici* under microscopy (x 40 magnification)

Plate 13: Colony of *D. tritici* on PDA

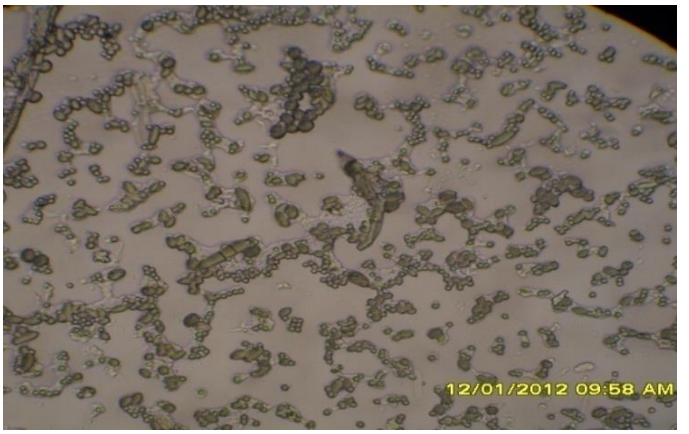

Plate 14: Conidia of *C. sphaerormum* under microscopy (x 40 magnification)

Plate 15: Colony of *C.sphaerormum* on PDA

Plate 16: Conidia of *E.purpurascers* under microscope (x 40 magnification)

Plate 17: Colony of *E.purpurascres* on PDA

4.2 Screen House Study

4.2.1 Evaluation of seed dressing fungicides for seed samples collected in Karatu District

4.2.1.1 Effect of varieties on wheat growth variables for seed samples collected in Karatu District

The effect of varieties on wheat growth variables (percent seed germination, shoot length, root length, fresh shoot biomass and fresh root biomass) is shown on Table 6.

Highly significance differences were observed on fresh root biomass at (P≤ 0.001), while there were no significant differences between varieties on percent seed germination, shoot length, root length and fresh shoot biomass at (P≤ 0.05) (Table 6 and Appendix 7). Shoot length, root length, fresh shoot biomass and fresh root biomass was observed to be higher on Mbayuwayu variety compared to local variety (Table 6).

Table 6: Effect of varieties on wheat growth variables for seed samples collected in Karatu District

Variety	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	90.08 [a]	13.79 [a]	3.21 [a]	1.15 [a]	0.06 [b]
Local	89.50 [a]	13.62[a]	3.11 [a]	1.09[a]	0.5 [a]
Mean	89.79	13.71	3.16	1.12	0.05
CV%	0.6	0.8	2.9	0.7	2.6
SE ±	1.15	0.13	0.13	0.005	0.003

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05

4.2.1.2 Effect of fungicide on wheat growth variables for seed samples collected in Karatu District

Significance difference between fungicides was observed on fresh shoot biomass and fresh root biomass at (P≤ 0.001). No significance differences were observed on percent seed germination, root length and shoot length between fungicides (Table 7 and appendix 7). Percentage seed germination was higher in wheat seed samples treated with Baytan, followed by Metalaxy plus, Mancozeb and control respectively (Table 7). Shoot length was higher with wheat seed sample treated with Metalaxy plus, followed by Mancozeb, Baytan and control respectively. Root length and fresh

shoot biomass were higher on wheat seed sample treated with Metalaxy plus and Baytan. Fresh root biomass was higher on wheat seed samples treated with fungicides compared to control though the differences less significant (Table 7).

Table 7: Effect of fungicide on wheat growth variables for seed samples collected in Karatu District

Fungicide	% Germination	Shoot length (cm	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Metalax plus	91.50 [ab]	15.61[a]	4.24 [a]	4.17 [a]	1.13 [ab]
Mancozeb	89.33 [b]	14.97 [a]	2.87[b]	2.94[b]	1.21 [a]
Baytan	94.67 [a]	10.82 [c]	4.123[a]	4.04[a]	1.23[a]
Control	78.83 [c]	13.42 [b]	1.56[c]	1.46[bc]	1.02[b]
Mean	88.58	13.69	3.19	3.15	1.12
CV %	2.7	2.4	3.9	0.9	10.5
SE ±	1.37	0.83	0.31	0.19	0.005

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05, Control = No fungicide treatment.

4.2.1.3 Combination of variety and fungicides on wheat growth variables for samples collected in Karatu District

Significance differences were observed on varieties x fungicides interaction in all wheat growth variables for all varieties at (P≤ 0.001) (Appendix 7). Highest percentage seed germination was observed on Baytan fungicide treatment for both varieties followed by Metalaxy plus and Mancozeb respectively (Table 8). Baytan, Metalaxy plus and Mancozeb increased seed germination by 18, 16, and 12.7%, respectively on Mbayuwayu variety and 21.6,15.7, and 13.6%, respectively on Local variety compared to control. Fungicide application also exhibited variable effects on other wheat growth variables (Table 8). Baytan and Control were observed to have higher shoot length, but the differences were higher on Local variety compared to Mbayuwayu variety (Table 8). Root length was higher on Local variety treated with

Metalaxy plus followed by control, Baytan and Mancozeb in that order. Root length on Mbayuwayu variety was higher on Metalaxy treatment for both varieties. There were no significance difference between fungicides on fresh shoot biomass and fresh root biomass except for Baytan treatment on Mbayuwayu variety (Table 8).

Table 8: Combination of variety and fungicides on wheat growth variables for seed samples collected in Karatu District

Variety	Fungicide	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	Metalax plus	92.00 [a]	13.70[ab]	3.21[a]	1.15[a]	0.05[a]
	Mancozeb	89.33 [b]	13.67[ab]	3.09[a]	1,09[a]	0.05 [a]
	Baytan	93.67 [a]	14.82 [a]	2.76[b]	0.03[b]	0.039 [b]
	Control	79.00 [c]	14.60 [a]	2.94[b]	1.20[a]	0.03[a]
Local	Metalax plus	91.00 [b]	10.88 [c]	4.04[a]	1.23[a]	0.08[a]
	Mancozeb	89.33 [c]	13.52 [b]	1.46[c]	1.21[a]	0.07 [a]
	Baytan	95.67 [a]	15.21[a]	2. 63[b]	1.43[a]	0.03 [a]
	Control	78.67 [d]	14.43 [ab]	2.91[b]	1.31 [a]	0.04[a]
Mean		88.58	13.69	2.87	3.15	1.12
CV %		2.98	3.85	10.41	1	15.16
SE±		1.52	0.30	0.19	0.18	0.03

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05, Control = No fungicide treatment

4.2.2 Evaluation of seed dressing fungicides for seed samples collected in Hanang District

4.2.2.1 Effect of varieties on wheat growth variables for seed samples collected in Hanang District

The effect of varieties on wheat growth variables (percent seed germination, shoot length, root length, fresh shoot biomass and fresh root biomass) for samples collected in Hanang is shown on (Table 9). Significance difference on varieties was observed only on fresh shoot biomass at (P≤ 0.001), while all other growth variables showed no significance differences (Appendix 8 and Table 9). Percent seed germination was

higher on Local variety compared to Mbayuwayu variety, but all other variable were higher on Mbayuwayu than Local variety (Table 9).

Table 9: Effect of varieties on wheat growth variables for seed samples collected in Hanang District

Variety	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	90.08 [a]	13.79 [a]	3.21[a]	1.15 [a]	0.05[a]
Local	89.50 [a]	13.62[a]	3.1 [a]	1.09[b]	0.05[a]
Mean	89.79	13.70	3.16	1.12	0.05
CV%	0.6	0.8	2.9	0.7	2.6
SE ±	1.15	0.12	0.13	0.005	0.03

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05

4.2.2.2 Effect of fungicide on wheat growth variables for seed samples collected in Hanang District

Fungicide treatments differed significantly on percent seed germination, root length and fresh shoot biomass at (P≤ 0.001) (Appendix 8). Percentage seed germination was higher on wheat seeds treated with Baytan, followed by Metalaxy plus, Mancozeb and control respectively (Table 10). Shoot length was higher with wheat seed sample treated with Metalaxy plus, followed by Mancozeb, control and Baytan respectively. Root length was higher on wheat seed sample treated with Metalaxy plus and Baytan (Table 10). There were differences between fungicides on fresh root biomas though the differences were not significant statistically (Table 10).

Table 10: Effect of fungicide on wheat growth variables for seed samples collected in Hanang District

Variety	% Germination	Shoot length (cm)	Root length(cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Metalax plus	92.50[b]	15.61[a]	4.17[a]	1.29[a]	0.03[a]
Mancozeb	90.17 [c]	14.752[a]	2.93[b]	1.20[a]	0.04[a]
Baytan	94.33 [a]	10.82[c]	4.05 [a]	1.23[a]	0.04[a]
Control	82.17 [d]	13.43[b]	1.49[c]	1.02 [b]	0.04[a]
Mean	89.79	13.66	3.16	1.12	0.05
CV%	2.2	2.5	6.4	0.8	12.3
SE ±	1.63	0.007	0.18	0.007	0.004

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05, Control = No fungicide treatment.

4.2.2.3 Combination of variety and fungicides on wheat growth variables for seed samples collected in Hanang District

Varieties x fungicide interaction were highly significance on root length, shoot length, fresh shoot biomass and fresh root biomass at (P≤ 0.001) (Appenix 8). Shoot length and fresh root biomass did not differ significantly in all fungicide treatments (Appendix 8). Germination percentage was observed to be higher with Baytan treatments followed by Metalaxy plus and Mancozeb respectively (Table 11). Baytan, Metalaxy plus and Mancozeb increased seed germination by 12.9, 12.5 and 10.5%, respectively on Mbayuwayu variety and 16.7, 12.6 and 8.9%, respectively on Local variety as compared to control (Table 11). Baytan and control were observed to have the highest shoot length compared to Metaxy plus and Mancozeb (Table 11). Root length was higher on Local variety treated with Metaxy plus while on Mbayuwau variety treated with Metalaxy plus and Mancozeb although all differences were not significant.

Table 11: Combination of variety and fungicides on wheat growth variables for seed samples collected in Hanang District

Variety	Fungicide	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	Metalaxy plus	93.00[a]	13.86[b]	3.21[a]	1.15 [b]	0.003[b]
	Mancozeb	91.33[a]	13.74[b]	3.11[a]	1.09 [b]	0.005 [b]
	Baytan	93.33[a]	14.76[a]	2.97[a]	1.46[a]	0.05 [a]
	Control	82.67[b]	14.93[a]	2.93[a]	1.20[ab]	0.4 [a]
Local	Metalaxy plus	92.00[b]	10.87 [c]	4.045 [a]	1.23[ab]	0.08[a]
	Mancozeb	89.00[c]	13.64 [b]	1.49[c]	1.02 [b]	0.04 [b]
	Baytan	95.33[a]	15.13 [a]	2. 86[b]	1.43[a]	0.05[b]
	Control	81.67[d]	15.10 [a]	2.88[b]	1.31[a]	0.5[a]
Mean		89.79	14.03	2.94	1.24	0.10
CV %		3.14	2.93	10.02	1.10	15.65
SE ±		0.50	0.23	0.18	0.05	0.17

Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05, Control = No fungicide treatment

4.2.3 Evaluation of seed dressing fungicides for seed samples collected in Siha District

4.2.3.1 Effect of varieties on wheat growth variables for seed samples collected in Siha District

The effect of varieties on wheat growth variables (percent seed germination, shoot length, root length, fresh shoot biomass and fresh root biomass) for samples collected in Siha is shown on Table 12. Varieties differed significantly on fresh root biomass and fresh shoot biomass at (P≤ 0.001), while other growth variables did not differ significantly between varieties (Appendix 9). Germination percentage did not differed significantly between varieties. Wheat growth variables were higher on Mbayuwayu variety (Table 12).

Table 12: Effect of varieties on wheat growth variables for seed samples collected in Siha District

Variety	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	89.50 [a]	13.84 [a]	3.19 [a]	1.15 [a]	0.5[a]
Local	89.50 [a]	13.60 [a]	3.08 [a]	1.09 [b]	0.05 [b]
Mean	89.5	13.72	3.14	1.15	0.05
CV%	0.7	0.8	3.2	0.5	6.5
SE ±	0.73	0.13	0.15	0.004	0.003

Means followed by the same letter within columns do not differ significantly according to DMRT at $P \leq 0.05$

4.2.3.2 Effect of fungicide on wheat growth variables for seed samples collected in Siha District

There was no significance differences observed between fungicides on shoot length, but all other growth variable showed significance differences at ($P \leq 0.001$) (Appendix 9 and Table 13). Germination percentage was highest in wheat seed samples treated with Baytan, followed by Metalaxy plus, Mancozeb and control respectively (Table 13). Baytan was observed to have lower shoot length compared to control; shoot length was higher on wheat seed sample treated with Metaxy plus (Table 13). Root length was higher with wheat seed samples treated with Metalaxy plus and Baytan than wheat seed samples treated with Mancozeb and control. Fresh shoot biomass and fresh root biomass differences were significant (Table 13).

Table 13: Effect of fungicide on wheat growth variables for seed samples collected in Siha District

Variety	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Metalax plus	92.17 [b]	15.70 [a]	4.15 [a]	1.02 [b]	0.03b
Mancozeb	90.33 [c]	14.89 [a]	2.92 [b]	1.20 [a]	0.04 [ab]
Baytan	94.33 [a]	10.78 [c]	4.00 [a]	1.22 [a]	0.08 [a]
Control	81.17 [d]	13.51 [b]	1.48 [c]	1.02 [b]	0.03 [b]
Mean	89.5	13.72	3.14	1.12	0.05
CV%	2.0	2.3	10.4	0.9	13.3
SE ±	1.04	0.19	0.19	0.005	0.004

Means followed by the same letter within columns do not differ significantly according to DMRT at $P \leq 0.05$, Control = No fungicide treatment.

4.2.3.3 Combination of variety and fungicides on wheat growth variables for seed samples collected in Siha District

Varieties x fungicide interaction highly differed significantly on shoot length, fresh shoot biomass at ($P \leq 0.001$ and fresh root biomas at ($P \leq 0.05$) (Appendix 9). Germination percentage and root length did not differ significantly between varieties in all fungicide treatments (Appendix 9). Baytan treatment was observed to have the highest seed germination percentage followed by Metalaxy plus and Mancozeb respectively (Table 14). Baytan, Metalaxy plus and Mancozeb increased seed germination by 14.3, 12.2 and 11.8%, respectively on Mbayuwayu variety and 19.2, 16.3 and 11.5%, respectively on Local variety compared to control (Table 14). Shoot length was higher with wheat seed samples treated with Baytan followed by Mancozeb, Metalaxy plus and control respectively. Root length was higher on control on both Mbayuwayu and Local variety. There were significant differences between fungicide treatment on fresh shoot biomass and fresh root biomass (Table 14).

Table 14: Combination of variety and fungicides on wheat growth variables for seed samples collected in Siha District

Variety	Fungicide	% Germination	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)
Mbayuwayu	Metalaxy plus	91.67 [b]	13.60 [b]	3.078[b]	1.15 [ab]	0.05 [a]
	Mancozeb	91.33 [b]	13.69 [b]	3.22[b]	1.09 [b]	0.05 [a]
	Baytan	93.33 [a]	14.89 [a]	2.92[b]	1.42 [a]	0.002 [b]
	Control	81.67 [c]	10.78 [c]	4.00 [a]	1.20 [a]	0.04 [a]
Local	Metalaxy plus	93.00 [b]	13.21 [b]	2.96 [c]	1.22 [ab]	0.08 [a]
	Mancozeb	89.00 [c]	13.51 [b]	3.09 [a]	1.02[b]	0.03[a]
	Baytan	95.33 [a]	15.10 [a]	2. 87 [b]	1.4 [a]	0.002 [b]
	Control	80.67 [d]	11.06 [c]	3.76 [a]	1.31[a]	0.04[a]
Mean		89.5	13.23	3.05	1.23	0.04
CV %		2.01	2.34	10.29	0.99	14.03
SE±		1.04	0.18	0.19	0.02	0.03

Means followed by the same letter within columns do not differ significantly according to DMRT at $P \leq 0.05$, Control = No fungicide treatment.

4.2.4 Effect of fungicide seed treatment of farmers saved seeds on the development of wheat diseases

4.2.4.1 Effect of Metalaxy plus, Mancozeb and Baytan on *Alternaria alternata* the cause of black point of wheat

Symptoms of black point were first visible on leaves seven weeks after planting. Symptoms appeared as small discolored lesions with irregular and dark brown to grey surrounded by a bright yellow margin. Black point disease assessment for the first 28 days after planting showed that there were no significant differences on disease reaction between varieties, fungicides and interaction of varieties x fungicides (Fig 4). The second and third assessments conducted 42 and 56 days after planting indicated significant variations in disease severity among the varieties. Fungicides shown to differ significantly in the controll of black point for seed samples collected at Hannang while for seeds collected at Siha and Karatu there were no significance

differences. Significance difference were observed on interaction of varieties x fungicide in the control black point for seed samples collected at Siha at ($P \leq 0.05$), while there were no significance difference for seed samples collected at Karatu and Hanang (Appendix 10, 11 and 12). Seed treatment with Metalaxy plus was more effective in reducing severity of black point in wheat caused by *Alternaria alternata* followed by Mancozeb and Baytan respectively (Fig 4). Seed treatment with any of the fungicide significantly reduced the disease compared to untreated control (Table 15, 16 and 17).

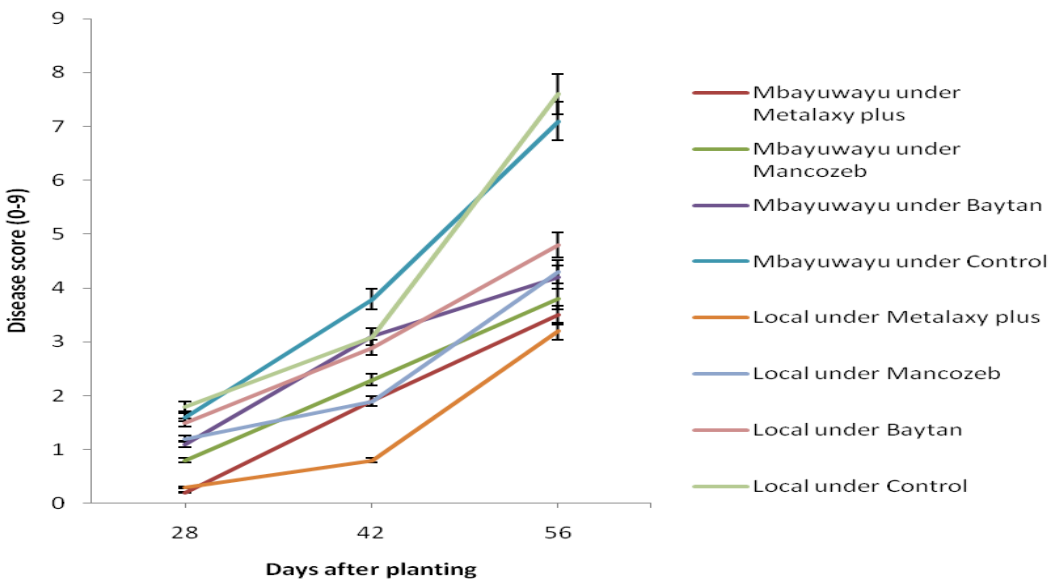

Figure 4: Effect of Metalaxy, Mancozeb and Baytan seed dressing fungicides on development of black point

4.2.4.2 Effect of Metalaxy plus, Mancozeb and Baytan on *Bipolaris sorokiniana* the cause of spot blotch of wheat

Six weeks after planting symptoms of spot blotch on leaves were observed as small light brown to black spots (Plate 18), some plants were lodged (post-emergence

damping off) due to stem infection and lesion were also seen on awn glume and spikelets. Initially, spot blotch disease symptoms were visible as traces (only in one pot with few plants) but with time some other pots showed symptoms of the disease. Spot blotch exhibited variable effects on varieties used, however, the effect was statistically insignificant in all three locations, There were no significant difference on fungicide application in the control of spot blotch for seed samples collected at Karatu, Hanang and Siha (Appendix 10, 11 and 12). There were significance differences on varieties x fungicides interaction on disease severity for seed samples collected at Karatu at ($P \leq 0.05$) (Appendix 10, 11 and 12), while for Hanang and Siha there were no significance difference. Seed treatment with Metalax plus, Mancozeb and Baytan significantly controlled *Bipolaris sorokiniana.* Metalaxy plus was the most effective followed by Mancozeb and Baytan respectively (Table 15, 16 and 17). Untreated seeds were severely infected with spot blotch compared to treated seeds, (Fig 5).

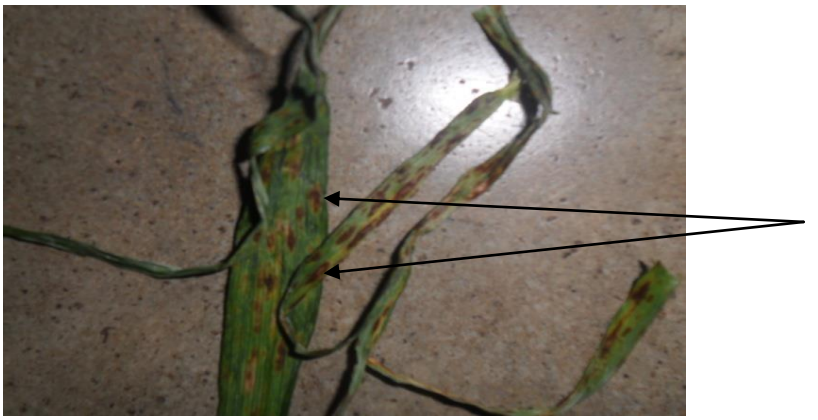

Plate 18: Spot blotch showing small light brown to black spots on wheat leaves

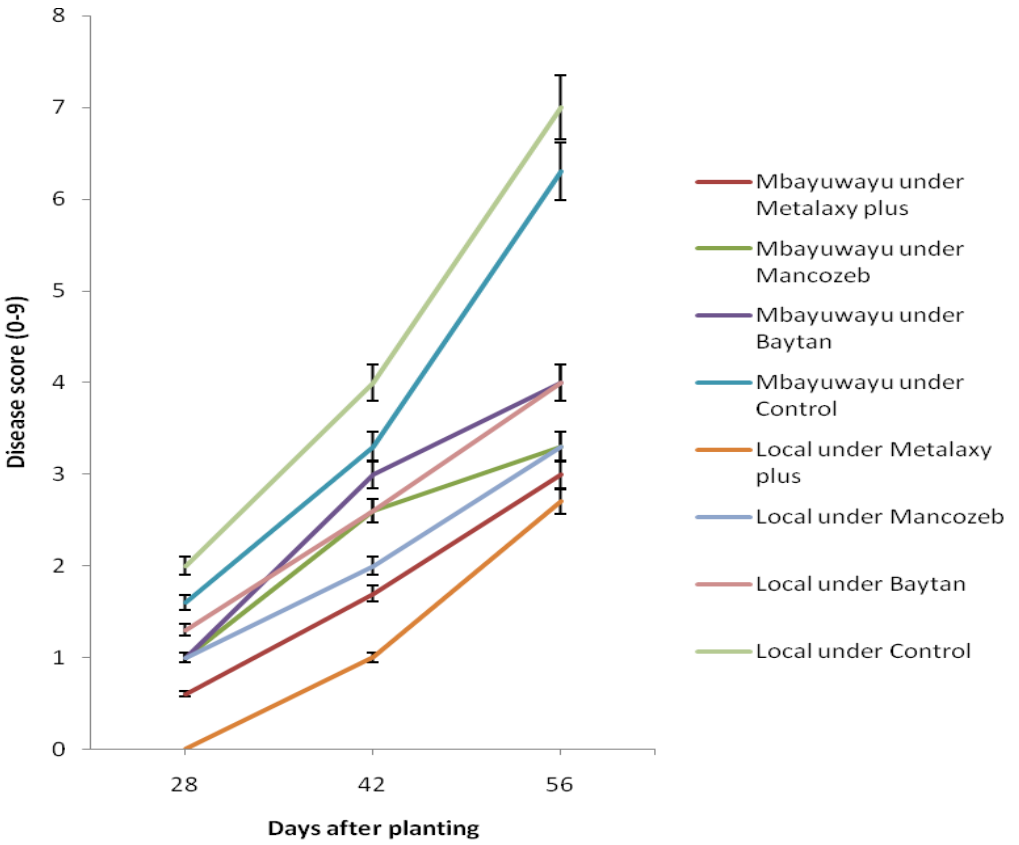

Figure 5: Effect of Metalaxy, Mancozeb and Baytan seed dressing fungicides on development of spot blotch

4.2.4.3 Effect of Metalaxy plus, Mancozeb and Baytan on *Fusarium gramineum* the cause of fusarium head blight of wheat

Symptoms of Fusarium head blight were less severe on spike and seeds, very few spikelets and seeds showed symptoms of Fusarium head blight. Symptoms of Fusarium head blight appeared as bleached on spikelets while some parts of spikelet remained green (Plate 19). On head and stem brown to purplish discoloration were observed with shriveled seeds.

There was no significant difference between varieties on Fusarium head blight for seed samples collected in all three locations (Appendix 10, 11 and 12). Fungicides

were observed to have significance differences on controlling Fusarium head blight for seed samples collected at Karatu, Hanang and Siha at ($P \leq 0.001$). There were significance difference on varieties x fungicides interaction in controlling Fusarium head blight for seed samples collected at Hanang, while for seed samples collected at Karatu and Siha no significance difference were observed (Table 15, 16 and 17). Metalaxy plus was the best fungicide in the controll of Fusarium head blight where disease was observed only in one pot with single spikelet infected while on Mancozeb and Baytan treatments, some few plants were observed to have a disease symptoms. High incidence of Fusarium head blight symptoms were observed on untreated seed pots (Fig 6, Table 15, 16 and 17).

Plate 19: Fusarium head blight on spikelets

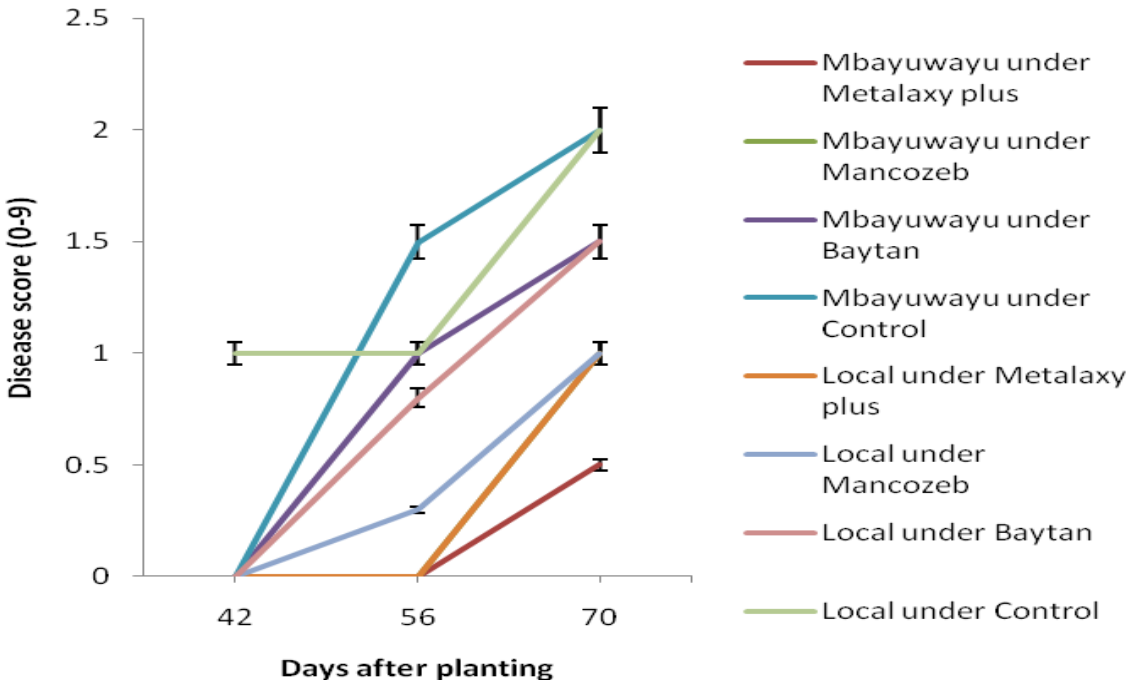

Figure 6: Effect of Metalaxy, Mancozeb and Baytan seed dressing fungicides on development of fusarium head blight

4.2.5 Effect of fungicide seed treatment on yield and yield components

4.2.5.1 Samples collected in Karatu District

There was no significance difference between varieties on plant height, number of grain per spike, 100 grain weight and grain yield at (P≤ 0.001) Appendix 10. Fungicide treatments differed significantly on plant height, number of grain per spike, 100 grain weight and grain yield at (P≤ 0.001) (Appendix 10). Varieties x fungicide interaction showed significance difference on 100 grain weight and grain yield at (P≤ 0.001) (Appendix 10). Plant height, number of grain per spike, 100 grain weight and grain yield per pot were higher on Metalaxy plus treatment compared with Mancozeb and Baytan. Metalaxy plus, Mancozeb and Baytan increased plant height, number of grain per spike, 100 grain weight and grain yield per pot compared to control (Table

15). Metalaxy plus, Mancozeb and Baytan increased grain yield by 44.9, 32.9 and 28.1% respectively on Mbayuwayu variety, 7.8, 4.8 and 3.1% respectively on Local variety compared to control (Table 15).

Table 15: Combination of varieties and fungicides on diseases, yield and yield components for samples collected in Karatu District

Variety	Fungicide	Disease score			Plant height (cm)	Number of grain per spike	100 grain weight	grain yield per pot (g)
		BP	SB	FH				
Mbayuwayu	Metalax plus	2.10^{ab}	1.20^{c}	0.00^{d}	46.77^{a}	13.33 ^{a}	4.92 ^{a}	9.06 ^{a}
	Mancozeb	2.40^{a}	2.20^{b}	0.97^{c}	45.13 ^{a}	11.00 ^{b}	4.69 ^{a}	8.31 ^{a}
	Baytan	2.60^{a}	2.88^{b}	1.33^{b}	40.53 ^{b}	10.33 ^{c}	4.63 ^{a}	8.003 ^{a}
	Control	2.97 ^{a}	4.40 ^{a}	2.43 ^{a}	40.60 ^{b}	6.00 ^{d}	3.03 ^{b}	6.25 ^{b}
Local	Metalax plus	2.53^{b}	1.67^{c}	0.43^{c}	45.30 ^{a}	11.33 ^{a}	3.51 ^{a}	6.35 ^{a}
	Mancozeb	2.87^{b}	2.30^{b}	0.97^{bc}	44.50 ^{a}	10.00 ^{b}	3.33 ^{a}	6.17 ^{a}
	Baytan	2.77 ^{b}	2.63^{b}	1.10^{b}	38.00 ^{c}	9.33 ^{b}	3.22^{a}	6.11 ^{a}
	Control	3.63^{a}	4.30^{a}	2.53^{a}	41.90 ^{b}	4.67 ^{c}	2.73 ^{b}	5.89 ^{b}
Mean		2.61	2.69	1.22	42.84	9.50	3.76	7.02
CV %		16.06	16.6	28.2	3.13	6.08	2.92	1.36
SE±		0.24	0.26	0.36	0.77	0.33	0.06	0.07

BP=Black point; SP=Spot blotch and FH= Fusarium head blight

Means followed by the same letter within columns do not differ significantly according to DMRT at $P \leq 0.05$

Control = No fungicide treatment.

4.2.5.2 Samples collected in Hanang District

Significance differences were observed only on 100 grain weight and grain yield per pot at ($P \leq 0.001$). Also significance differences were observed on number of grain per spike, 100 grain weight and grain yield at ($P \leq 0.001$) (Appendix 11). Plant height, number of grain per spike, 100 grain weight and grain yield per pot were higher on Metalaxy plus treatment compared to Mancozeb and Baytan (Table 16). Metalaxy plus, Mancozeb and Baytan increased plant height, number of grain per spike, 100 grain weight and grain yield per pot compared to control (Table 16). Grain yield was increased by 45.1% in Metalaxy plus treatment, 33.5% in Mancozeb and 30.9% in

Baytan on Mbayuwayu variety, while on Local variety grain yield increased by 13.9% in Metalaxy plus, 9.6% in Mancozeb and 9.2% in Baytan treatments compared to control (Table16).

Table 16: Combination of varieties and fungicides on disease, yield and yield components for samples collected in Hanang District

Variety	Fungicide	Disease score			Plant height (cm)	Number of grain per spike	100 grain weight	grain yield per pot (g)
		BP	SB	FH				
Mbayuwayu	Metalaxy plus	0.767 [b]	0.97 [c]	0.10 [c]	42.17 [c]	12.33 [a]	4.92[a]	9.056[a]
	Mancozeb	1.63 [a]	1.53 [b]	1.00[b]	44.83 [b]	11.00 [a]	5.36 [a]	8.33 [a]
	Baytan	1.83 [a]	2.20 [a]	0.87[bc]	46.2 [a]	10.33[b]	4.29 [a]	8.17 [a]
	Control	3.000[a]	2.43 [a]	2.30 [a]	43.0 [b]	6.70 [c]	3.04 [b]	6.24 [b]
Local	Metalaxy plus	0.83 [c]	1.30 [b]	0.40[b]	44.7 [a]	12.33 [a]	3.51 [a]	6.53 [a]
	Mancozeb	1.53 [b]	1.77 [b]	0.60 [b]	41.3 [b]	11.00 [a]	3.33 [a]	6.28 [a]
	Baytan	2.07 [a]	2.30 [a]	1.20 [ab]	41.2 [b]	9.33 [b]	3.22 [a]	6.16 [a]
	Control	3.17 [a]	2.97 [a]	2.60 [a]	43.4 [a]	5.33 [c]	2.79[b]	5.73 [b]
Mean		1.85	1.93	1.13		43.35	3.81	7.06
CV %		19.60	20.34	16.64	6.12	33.73	2.84	2.91
SE ±		0.24	0.29	0.20	1.54	1.61	0.06	0.12

BP=Black point; SP=Spot blotch and FH= Fusarium head blight
Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05, Control = No fungicide treatment.

4.2.5.3 Samples collected in Siha District

There were significance differences on 100 grain weight and grain yield per pot at (P≤ 0.001). Also significance difference were observed on number of grain per spike, 100 grain weight and grain yield at P≤ 0.001 (Appendix 12). Metalaxy plus was the most effective in increasing plant height, number of grain per spike, 100 grain weight and grain yield per pot compared to Mancozeb, Baytan treatment and control (Table 17). Grain yield per pot increased by 43.9% in Metalaxy plus treatment, 32.4% in

Mancozeb and 27.3% in Baytan on Mbayuwayu variety while on Local variety grain yield increased by 10.9, 9.5 and 7.3% in Metalaxy plus, Mancozeb and Baytan respectively compared to control (Table 17).

Table 17: Combination of varieties and fungicides on diseases, yield and yield components for samples collected in Siha District

Variety	Fungicide	Disease score			Plant height (cm)	Number of grain per spike	100 grain weight	grain yield per pot (g)
		BP	SB	FH				
Mbayu wayu	Metalaxy plus	1.9 [b]	0.97 [b]	0.10 [b]	47.33 [a]	13.67 [a]	4.93 [a]	9.11 [a]
	Mancozeb	2.6 [b]	0.73 [b]	0.87 [b]	44.40 [b]	11.67 [ab]	4.70 [a]	8.38 [a]
	Baytan	1.2 [c]	1.10 [a]	0.87 [b]	43.00 [b]	10.33 [b]	4.63 [a]	8.06 [a]
	Control	3.40[a]	1.97 [a]	2.30 [a]	41.10 [c]	5.67 [c]	3.04 [b]	6.33 [b]
Local	Metalaxy plus	1.2 [b]	1.17 [a]	0.40 [c]	44.20 [a]	12.67 [a]	3.52 [a]	6.40 [a]
	Mancozeb	2.2 [a]	0.73 [b]	1.00 [b]	43.10[ab]	11.00 [a]	3.33 [a]	6.32 [a]
	Baytan	2.1 [a]	1.97 [a]	1.30 [a b]	43.03[ab]	10.00 [ab]	3.22 [a]	6.19 [a]
	Control	2.8 [a]	1.50 [a]	2.50 [a]	41.80 [b]	5.33 [b]	2.80 [b]	5.77 [b]
Mean		2.17	1.27	1.17	43.50	10.04	3.77	7.07
CV %		22.6	16.00	20.41	4.96	7.22	12.08	2.77
SE ±		0.41	0.29	0.24	0.88	0.41	0.27	0.11

BP=Black point; SP=Spot blotch and FH= Fusarium head blight
Means followed by the same letter within columns do not differ significantly according to DMRT at P ≤ 0.05, Control = No fungicide treatment

CHAPTER FIVE

5.0 DISSCUSION

5.1 Physical Inspection of Dry Farmers Saved Wheat Seeds

Pure and abnormal seeds differed significantly between varieties and locations. The differences in physical qualities between locations could be attributed by poor seed health status that were used for planting, prevailing local weather conditions, geographical positions, post harvest management practices including storage conditions and seed storage periods. Ingle (1990) in his study of seed-borne fungi of peal millet, transmission and control reported that seed samples collected from farmers field were more discoured attributed by frequent rains during seed development stages, high seed moisture content, prolong storage period which resulted to poor quality seeds with low pure seeds, high abnormal seeds and inert matter. The quality of seed at the time of sowing depends on the quality of the seeds that entered storage and how well it was stored (ICRISAT, 2007; Safránková *et al*., 2010 Manjaro, 2012). Scmidf (2000) and Ferguson *et al.* (2004) found that a seed does not improve in storage as such inferior seeds will end with inferior seeds, no matter how much care is taken. Seed health is an important quality attribute and seeds used for planting should be free from abnormal seeds, inert matter and pests as reported by Bishaw *et al*. (2006) and Osborn *et al*. (2010).

Wheat seed samples collected from Siha were of high quality compared to other locations. This may be due to the fact that many of the seed samples collected in this location were subjected to better post harvest management practices, such as harvesting by combine harvester, dried properly on tapeline the processes that could

have minimized fungi contamination and infection. Seeds in Hanang and Karatu were harvested by hand and spread on the ground before been threshed by hand; all these might have increased chances of fungal contamination and infection. After the crop is harvested, it undergoes several operations that, if improperly done, may result in serious losses caused by insect pest and seed-borne fungal pathogen (Gwinner *et al*., 1996; Nautiyal, 2007). However, it should always be recognized that an intact grain is an essential item for successful storing (Gwinner *et al*., 1996; Malaker *et al.,* 2008). Dirty, cracked or broken grains provide an entry point for infestation by insects, pathogens, and moulds during storage (Gwinner *et al*., 1996; Oyekale *et al*., 2012). Damage to grains may happen due to improper application of post-harvest practices such as harvesting, threshing, drying or transporting. Threshing may inflict a degree of physical damage to the grains (Nautiyal, 2007). Deterioration of the grain quality can be due to improper post harvest management practices and storing conditions, which leads to contamination with fungi or insect infestation as reported by Gwinner *et al*. (1996). The overall assessments have shown that 29.1% of farmers saved seed in the study area were contaminated with variety of fungi which reduced their viability. Seed deterioration in the study area is mainly caused by poor post harvest practices that included poor storage of farmers saved seeds that make them more prone to fungal contamination and infection. The study has also revealed that most of the farmers in the Northern zone store their wheat grains in earthen pitcher which allows contamination and infection by fungi.

5.2 Assessment of Germination for Fungicide Treated Farmers Saved Seeds

Significant increase in germination percent of both varieties when seeds were treated with fungicides compared to untreated controls could reflect response of wheat seeds

to fungicide treatments that reduced or killed seed borne fungi. Most of the seeds used in the trials were heavily infected with seed borne fungi as reflected by low germination percentage in untreated seeds and individual fungi that were isolated and evident in seeds. Similar results were reported by other workers who observed that when seed treated fungicides were used resulted to an appreciable increase in germination over control (Siddiqui and Zaman, 2004; Kandolo, 2008). Seed dressing with systemic fungicides is a conventional method used for the control of many seed borne infection (Malaker and Mian 2009). The beneficial effect of fungicidal application in wheat has been emphasized by several workers, (Siddiqui *et al.*, 1997; Siddiqui *et al*., 1999). Mercury based fungicides are said to have some toxicity in damaged and cracked seeds but healthy seeds without any injury have shown to improve germination. Marthe *et al*. (2009) obtained increased germination of wheat seeds after treatment with mercury fungicides. However, the mercury seed dressing fungicides have been withdrawn because of their toxicity and residual effects on humans (David *et al*., 2007). Siddiqui *et al*. (1999) reported that the viability of wheat seeds treated with Captan and Thiram, Farmerzeb, and Baytan was significantly higher than untreated seeds. It has long been noted that seed-borne fungal pathogens are responsible for reducing seed quality reduction or elimination of germination capacity as well as seedling damage, which result in the reduction of crop yield (Mushtaq and Hashmi, 2007; Fakhrunnisa *et al*., 2006).

5.2.1 Effectiveness of Metalaxy plus, Mancozeb and Baytan seed treatment in the control of *Alternaria alternata*, *Bipolaris sorokiniana* and *Fusarium gramineum* in farmer saved seeds

The disease severity on plants treated with Metalaxy plus, Mancozeb and Baytan seed treatment fungicide differed significantly reflecting the potential of different fungicides to control seed borne diseases in the study area. Very few farmers in the study area treat seeds before planting resulting to wide spread of seed borne diseases causing high crop losses. Metalaxy plus has shown to offer effective control for most of the seed borne fungi in the study area compared with Mancozeb and Baytan. Therefore, these fungicides should be recommended for the area in the control of black point. The fungicide was more effective for seed samples collected from Karatu and less effective for seed samples from Siha and Hanang suggesting prevalence of stains of the pathogen which might have developed resistance to the fungicide at Siha and Hanang. One of the problems with fungicide application is the development of new races that render fungicides ineffective as a result of continuous use of the fungicide or application of low doses of fungicides (Reigner, 2005; McGrath and Davey 2007; Smith 2008). However, more studies are required to reveal strain diversity of this pathogen in the study areas and their sensitivity to different fungicides. Reis, (2009) reported that, infected seeds with *Bipolaris sorokiniana* are one of the most important sources of primary inoculum that survives between wheat cropping seasons and that the use of healthy seeds provide means of reducing primary inoculum, foliage infection and seedling blight. The predominant black point fungus, *B. sorokiniana* is reported to be highly seed-transmitted and more than 80% seed to plant transmission of this pathogen has been reported in wheat (Reis *et al.,* 2009).

Ilyas *et al.* (1998) found that the use of seed dressing fungicides is an option to reduce the primary inoculum of *B. sorokiniana* and other seed and soil-borne fungi. In this study, Metalaxy plus was observed to be the best in reducing spotch blotch; similar results were reported by Zobaer *et al*. (2007) who observed lowest incidence of *Bipolaris sorokiniana* in farmers saved seed treated with metalaxy plus followed by sun drying. Kabir *et al.* (2007) also found that Dithane, Baytan, Triticonazole and Metalaxy plus to be equally effective for control of *Bipolaris sorokiniana*. Seed dressing with Metalaxy plus Mancozeb and Baytan in the control of *Alternaria altenata*, *Bipolaris sorokiniana* and *Fusariam gramineum* also gave similar results where the effectiveness of these fungicides varied across locations suggesting existence of different strains of the pathogens that can resist the fungicides as described earlier. Butt *et al*. (2011) observed that seed treatment with different fungicides (Mancozeb, Thiabendazole, Triadimenol Metalaxy plus) exhibited variable significant effects on the occurrence of *F. moniliforme* and *F, gramineum* Furthermore, Robert (2012) reported that when Topsin and Mancozeb were used as seed dressing fungicides suppressed the growth of *F, gramineum* by 50%. Yagouda, (2010) found that the seed treatment with Iprodione, Baytan and Metalaxy plus each at the rate of 2g/kg of seed were most effective in the control of *Fusarium gramineum* and *Fusarium moniliform* on wheat.

Meisner and Ahmed (1996) demonstrated that Metalaxy plus 200 (Carboxin [37.5 %] and Thiram [37.5 %] as an effective broad spectrum wheat seed treatment fungicide, both for externally and internally seed-borne diseases of wheat suggesting wide spectra of these fungides in controlling diverse strains of the pathogen found in

wheat growing areas. It is an evident from this study that farmers saved seed are heavily infected with fungi requiring fungicides treatment prior planting. Moreover, pre-harvest foliar application of chemicals should also be applied to reduce the internal seed-borne fungi and when combined with seed treatment produce healthy seed.

5.2.2 Effect of fungicide treatments on yield

Seed treatment with Metalaxy plus, Mancozeb and Baytan resulted to increased number of grain per spike, 100 grain weight and total grain yield on both three locations and varieties. The increased yield in treated seeds was probably due to reduced intensity of the diseases observed compared to untreated controls. Carmona, (2008) reported an increase of 100 grain yield by 11% when seed treatment fungicide was used when compared to untreated control. The results of this study have shown that seed samples with less percentage of seed-borne fungi and with highest percentage pure seeds registered the higher yields compared to seed samples which were found to have highest percentage infection and lowest percent pure seeds. Abdulsalaam and Shenge (2007) reported that, among various factors that affect seed health, the most important are the seed borne fungi that not only lower seed germination, but also reduce seed vigour resulting in low yield. Seed treatments together with quality seed help emerging crops by controlling most seed- and soil-borne diseases, allowing the crop to get a good start (quick, uniform plant emergence and better seedling vigour). However, seed treatments could protect young plants against seedling diseases but do not prevent later infection by cereal leaf diseases or

root rots therefore continuous sprays will be necessary to control subsequent infections (Ransom and McMullen, 2008).

Mbayuwayu variety was observed to have higher grain yield as compared with local variety on both locations. The variety could have genes for high yielding compared with local variety despite the fact that the former variety had high incidence of seed borne diseases than the latter variety. Increased grain yield follow application of seed treatment with various seed dressing fungicides have also been reported by other workers (Kamaluddin, 1996; Ilyas *et al.,* 1998). Meisner and Ahmed, (1996) found that seed treatment with Metalaxy plus increased plant stand by 23% and grain yield by 18% under farmer's field condition. Ahmed (1996) reported that wheat seed treatment with systemic fungicides, such as Baytan, Raxil and Metalaxy plus, significantly increased crop stand, grain yield and yield attributes. In the U.S., various studies have demonstrated yield increases in winter wheat due to seed treatment with fungicide. For example, Wegulo *et al*. (2009) showed that up to 42% yield loss was prevented by dressing seed with fungicides to winter wheat. Kelley, (2001) found that over a period of six years, the fungicide Propiconazole Metalaxy plus significantly increased winter wheat yield 77% of the time.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results have revealed the types and geographical distribution of the seed-borne fungi prevalent in Northern wheat growing zone. None of the wheat varieties collected in the Northern wheat growing Zone of Tanzania was found to be free from seed-borne fungal pathogens. Most prevalent seed born fungi were *Alternaria alternata, Aspegillus flavus* and *Cladosporium sphaerormum*. Seed treatment with either Metalaxy plus, Mancozeb and Baytan was found effective in reducing black point, spot blotch and fusarium head blight incidence resulting from seed borne infection which also increased germination percent on Mbayuwayu variety by 15.2, 13.7 and 12.3% in Baytan, Metalaxy plus and Mancozeb treatments respectively and 19.2, 14.9 and 13.3% on local variety for Baytan, Metalaxy plus and Mancozeb treatments respectively compared to control. Seed treatment with Metalaxy plus, Mancozeb and Baytan was also was found to control Fusarium head blight significantly where 90% of treated plants were free from Fusarium head blight diseases widely distributed in Northern wheat zone. Moreover, seed treatment was found to increase grain yield by 44.6, 32.9 and 28.8% on Mbayuwayu variety with application of Metalaxy plus, Mancozeb and Baytan respectively and on local variety by 10.9, 7.9 and 6.7% with Metalaxy plus, Mancozeb and Baytan respectively, as compared to the control. Mbayuwayu (improved variety) was observed to have high grain yield potential as compared with local variety. Seed samples with low infection rates were found to be better on grain yield as compared to seed samples with higher infection rates.

6.2 Recommendations

Seed production in disease-free areas or under effective disease control and field inspection schemes is very important to obtain disease-free seed. Pathogen free seeds sowing could be a better option to control wheat diseases. The results have revealed that farmers saved seeds in Hanang, Karatu and Siha districts are heavily infected with fungi and it is recommended that they should be treated with appropriate fungicides prior planting. Seed health testing and seed treatment before sowing is necessary. Treatments of seed with seed dressing fungicides will improve germination and increase grain yield per unit area as demonstrated in this study. Apparently results have shown that seed treatment with Metalaxy plus, Mancozeb and Baytan should be recommended for the control of Fusarium head blight, Black point and spot blotch. Moreover, the use of improved varieties such as Mbayuwayu is highly recommended to be used by farmers as it has shown to have high yield as compared with local variety. More research should be done in order to determine economical importance of seed-borne diseases of wheat in infested Districts since yield losses caused by these fungal pathogens are not yet known in Northern zone. Seed production in disease free areas or under effective disease control and field inspection scheme is very important to obtain disease free seed. Thus understanding disease epidemiology, its transmission rate and economic threshold, combined with seed health testing in the study areas is very important, as these could help to define the need for seed treatment.

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APPENDICES

Appendix 1: Details of farmers saved wheat seed sample collected in Karatu, Hanang and Siha Districts

Location	Varieties collected	Remarks
Hanang	Mbayuwayu, Local, Riziki, Selian and Azimio	Most of seed were stored in plastic containers some in polyethylene bag and Earthen pitcher.
Karatu	Mbayuwayu, Local, Chiriku, Azimio, Lumbesa, Tausi and Sifa	Most of seed were stored in Earthen pitcher some in plastic containers and Sifa was stored on polyethylene bags.
Siha	Local, Kenya mwamba, Duma, Ngamia and Kaiege	Most of the seed were stored in polyethylene bags and plastic containers.

Appendix 2: The Active Ingredient of Fungicides Used in the Experiments

Name of fungicide	**Chemical composition**
Baytan	B – (4 – chlorophenoxy – a – (1,1 – dimethylethyl – 1 H – 1, 2, 4 - triazole – 1 – ethanol CA.
Mancozeb	Mangnese ethylenebisdithio carbamate plus zinc – 75%
Metalaxy plus	5,6 – dihydro – 2 – methyl – 1 – 1, 4 – oxathin – 3, carboxaitide

Appendix 3: Doses and Concentration of Three Fungicides Used in the Experiments.

Fungicides	Dose (gm)	Concentration (g/100ml)
Metalaxy plus	0.25 **R**	0.050
Mancozeb	0.25 **R**	0.050
Baytan	0.25 **R**	0.050

R: Recommended dose of fungicides; Source *Mycopath* (2006), 4(1): 45-49

Appendix 4: Scale used to assess the intensity of foliar diseases on wheat

Rating	Description with severity levels
O	Free from infection
OE	Free from infection, but probably represents an escape
1	A few isolated lesions on only the lowest leaves
2	Scattered lesions on the second set of leaves with first leaves lightly infected
3	Light infection of lower third of plant; lower most leaves infected at moderate to severe levels
4	Moderate infection of lower leaves with scattered to light infection extending to the leaf immediately below the middle of the plant
5	Severe infection of lower leaves; moderate to light infection extending only to the middle of plant
6	Severe infection of lower third of plant moderate on middle leaves and scattered lesions beyond the middle of the plant
7	Lesions severe on lower and middle leaves with infection extending to the leaf below the flag leaf, or with trace on the flag leaf
8	Lesions severe on lower and middle leaves; moderate to severe infection of upper third of plant; flag leaf infected in amounts more than a trace
9	Severe infection on all leaves; spike also infected to some degree.
N	No scoring possible due to necrosis as a result of other disease factors

1-3 denote resistant; 4-6 moderately resistant; 7-8 susceptible; 9 highly susceptible; N no scoring possible

Appendix 5: Mean squares physical inspection for seed samples collected in Karatu, Hanang and Siha districts of Northern zone

	Mean Squares		
Source of variation	Pure seed	Abnormal seed	Inert matter
Replication	3.70	2.17	0.003
Location	72.58***	65.03***	0.69***
Varieties	24.08***	20.54***	0.12***
Location x varieties	2.08***	0.96*	0.75***
Error	11.25	8.19	1.04

*** p= 0.001

Appendix 6: Mean squares on percent infection for two varieties widely grown in Hanang, Karatu and Siha Districts

	Mean Squares
Source of variation	% Infection
Replication	252.3
Location	103.8
Varieties	1.4
Location x Varieties	118.8*
Error	234.6

* p= 0.05

Appendix 7: Mean squares on wheat growth variable for seed samples collected in Karatu District

	Mean squares				
Source of variation	Germination %	Shoot length	Root length	Fresh shoot biomass	Fresh root biomass
Replication	16.17	0.62	0.21	0.09	0.0003
Varieties	0.17	0.07	0.003	0.07	0.020***
Fungicide	282.28	27.43	26.09	9.42***	0.07***
Varieties x Fungicide	2.50***	0.33***	0.35***	0.63***	0.02***
Error	5.64	0.17	0.29	0.11	0.001

*** p= 0.001

Appendix 8: Mean squares on wheat growth variable for seed samples collected in Hanang District

	Mean squares				
Source of variation	Germination %	Shoot length	Root length	Fresh shoot biomass	Fresh root biomass
Replication	4.01	0.0031	0.21	0.006	0.0003
Varieties	2.04	0.07	0.06	0.21***	0.0002
Fungicide	172.43***	27.44	9.21***	0.73***	0.004
Varieties x Fungicide	5.04	0.41***	0.61***	0.02***	0.0001
Error	7.96	0.16	0.10	0.001	0.005

*** p= 0.001

Appendix 9: Mean squares on wheat growth variable for seed samples collected in Siha District

	Mean squares				
Source of variation	Germination %	Shoot length	Root length	Fresh shoot biomass	Fresh root biomass
Replication	3.50	0.09	0.08	0.0003	0.0007
Varieties	0.00	0.33	0.08	0.02***	0.0001***
Fungicide	201.22***	27.98	9.12***	0.07***	0.0004***
Varieties x Fungicide	7.44	0.27***	0.61	0.02***	0.0001*
Error	3.21	0.10	0.11	0.001	0.0003

* p= 0.05; *** 0.001

Appendix 10: Mean squares on yield and yield components for seed samples collected in Karatu District

	Mean squares						
Source of variation	Disease score BP	Disease score SB	Disease score FH	Plant height	Number of grain per spike	100 grain weight	Grain yield per pot
Replication	0.03	0.06	0.02	0.74	0.38	0.0001	0.007
Varieties	0.20	0.02	0.03	4.17	10.67***	7.55***	18.89***
Fungicide	1.27	9.06	5.33***	58.8***	53.00***	2.14***	2.86***
Varieties x Fungicide	0.72	0.14***	0.11	3.94	0.33	0.45***	1.50***
Error	1.33	0.13	0.25	1.95	0.33	0.01	0.01

*** p= 0.001

Appendix 11: Mean squares on yield and yield components for seed samples collected in Hanang District

	Mean squares						
Source of variation	Disease score BP	Disease score SB	Disease score FH	Plant height	Number of grain per spike	100 grain weight	Grain yield per pot
Replication	0.43	0.02	0.28	1.08	1.007	0.006	0.009
Varieties	0.05	0.54	0.11	12.18	2.04	8.57***	18.99***
Fungicide	5.40	2.81	5.27	0.48***	44.59***	2.53***	3.49***
Varieties x Fungicide	0.03	0.05	0.19***	18.42	0.71	0.83***	1.17***
Error	0.23	0.41	0.03	4.62	0.50	0.21	0.04

*** p= 0.001

Appendix 12: Mean squares on yield and yield components for seed samples collected in Siha District

	Mean squares						
Source of variation	Disease score BP	Disease score SB	Disease score FH	Plant height	Number of grain per spike	100 grain weight	Grain yield per pot
Replication	0.88	0.006	0.09	1.002	1.04	0.001	0.007
Varieties	0.33	0.14	0.43***	5.13	2.04	7.33***	19.39***
Fungicide	0.88	1.23	4.85***	19.27***	64.15***	2.02***	3.19***
Varieties x Fungicide	3.02*	0.45	0.03	4.29	0.15	0.50***	1.23***
Error	0.98	0.76	0.03	7.96	0.42	0.01	0.05

* p= 0.05; *** 0.001