DISTRIBUTION OF WHEAT HEAD BLIGHT, IDENTIFICATION AND CHARACTERIZATION OF ASSOCIATED <u>FUSARIUM</u> SPECIES IN SOUTHWESTERN ETHIOPIA

M.SC. THESIS

BY

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Distribution of Wheat Head Blight, Identification and Characterization of Associated *Fusarium* Species in Southwestern Ethiopia

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A Thesis

Submitted to the Department of Horticulture and Plant Sciences, School of Graduate Studies, College of Agriculture and Veterinary Medicine, Jimma University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Agriculture (Plant Pathology)

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DEDICATION

This thesis is dedicated to my beloved parents, my father Kebede Earecho Kitte and my mother Kadeno Kansite Halidaba, whose effortful sacrifice, support and endless encouragement through my life helped me to accomplish things in success!

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in Plant Pathology at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and is deposited at the University Library to make it available to borrowers under the rules of the Library. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of an academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author **Minyahil Kebede Earecho** was born in January 1989 in Derashe district of Southern Nations and Nationalities People's Region (SNNPR) in Ethiopia from his mother **Kadeno Kansite Halidaba** and his father **Kebede Earecho Kitte**. He attended his elementary educations at Holitte elementary school, Gidole elementary and junior secondary school and Ediget elementary and junior secondary School. He finished his first level secondary and preparatory education at Gidole senior secondary and preparatory school, Derashe, SNNPR, Ethiopia. In 2008, he joined ArbaMinch University, College of Agricultural Sciences, Department of Plant Science and graduated with B.Sc. degree in Plant Science in July 2010.

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ABBREVIATIONS AND ACRONYMS

AGP	Agricultural Growth Program
ANOVA	Analysis of Variance
AsARC	Assosa Agricultural Research Centre
AUDPC	Area Under Disease Progress Curve
BC	Before Christ
CSA	Central Statistical Agency
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization
FDK	*Fusarium* damaged kernel
FHB	*Fusarium* Head Blight
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
m.a.s.l.	Meters above sea level
PDA	Potato Dextrose Agar
PSA	Potato Sucrose Agar
SNA	Spezieller Nahrstoffarmer Agar
SNNP	Southern Nation Nationalities and People
SWE	Southwestern Ethiopia
WA	Water Agar

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ABSTRACT

Ethiopia is the second largest wheat (Triticum spp.) producer following Egypt in Africa. However, the crop is challenged by biotic, abiotic, technical and socio-economic constraints. Fusarium head blight (FHB) is one of the biotic factors caused by Fusarium species, which substantially reduces wheat grain yield and quality of the grain worldwide. This investigation was undertaken in order to assess occurrence and importance of FHB of wheat fields in southwestern Ethiopia (SWE), identify, characterize and test the pathogenicity of Fusarium spp. associated with FHB of wheat. Potential wheat growing zones, districts and peasant associations were selected by following purposive multi-stage sampling procedure. While, wheat fields were randomly assessed during early milk to hard dough stages and the disease severity was determined by the modified Horsfall-Barrett scale. Blighted heads were sampled and associated Fusarium spp. were isolated, identified and tested for pathogenicity on Danda'a variety. Results of this study revealed that FHB was 93.9 % prevalent in wheat fields with varying levels of incidence that ranged from 11.3 to 84.6 % in Buno-Bedele, 0 to 100 % in Jimma and 0 to 53.2 % in West-Wollega zone. FHB was high in Buno-Bedele with mean incidence of 38.7 % followed by Jimma (26 %), as opposed to 13.8 % in West-Wollega. Similarly, FHB was severe in Buno-Bedele having mean field severity, infected head severity and FHB index of 28.2 %, 33.2 % and 13.9 %, respectively. On the other hand, West-Wollega and Jimma zones had lower mean field severity of 12.8 % and 14.7 %, respectively. In addition, a total of 269 single conidial isolates of Fusarium was recovered from blighted head samples collected in 52 wheat fields. These isolates were identified into nine Fusarium species. Among them, F. graminearum (29.0 %) and F. culmorum (26.4 %) were the dominant species followed by F. avenaceum (10.4 %), F. poae (7.4 %), F. ussurianum (6.7 %), F. semitectum (6.3 %), F. lateritium (6.0 %), F. sambucinum (6.0 %) and F. heterosporum (1.9 %) in SWE. Pathogenicity tests revealed that isolates of Fusarium spp. caused symptoms of FHB in spikes of a susceptible Danda'a variety. Based on the spikelet infection severity and AUDPC results, F. avenaceum, F. poae, F. lateritium, F. culmorum, F. sambucinum, F. heterosporum and F. graminearum were more aggressive ones that produced higher AUDPC of 1067.2, 1066.3, 856.2, 801.3, 792.4, 670.9 and 546.8, respectively and higher spikelet infection severity of 57.8 % to 100 %. In conclusion, almost all wheat fields and all the wheat varieties grown in the study area were infected by FHB and also seven out of the nine identified Fusarium spp. were more aggressive, while the rest two showed less aggressiveness on Danda'a variety which was grown in most of the farmers field (50 %) in southwestern Ethiopia. These indicated that FHB of wheat will have a chance of becoming a potential disease in southwestern Ethiopia. Therefore, it needs surveillance, further phylogeny study of the causal agents and development of feasible management tactics in order to reduce the probable risk of FHB in southwestern Ethiopia and in Ethiopia at large.

Keywords: *Fusarium* head blight, FHB, Incidence, Pathogenicity, Prevalence, Severity

1. INTRODUCTION

Wheat (*Triticum* spp.), the driving forces of modern civilization (Gustafson *et al.*, 2009), is one of the most ancient (dating back to 9, 600 B.C.) domesticated food crops that serve as the basic stable food of the major civilizations of Europe, West Asia and North Africa (Curtis, 2002; Lim, 2013). Wheat has higher nutritive value than other cereals by containing 12 % water, 60 – 80 % carbohydrates (mainly as starch), 8 – 15 % proteins (17 – 28 % in elite wild genotypes), 1.5 – 2 % fats, 1.5 – 2 % minerals (Fe, Zn, Cu, Mg), vitamins (A, B complex, E) and 2.2 % crude fibers (Charmet *et al.*, 2005; Ortiz-Monasterio *et al.*, 2007; Shewry *et al.*, 2009; Šramková *et al.*, 2009).

Wheat is the second most cultivated cereal crops in the globe next to rice (*Oryza sativa* L.) with a production of 881.16 million tons produced on 244.46 million hectares of land with an average yield of 3.65 tons per hectare. The largest share (53.95 % of tons and 50.72 % of hectares) was by the top four producers namely China, India, Russian Federation and the United States of America (FAO, 2018). Globally, wheat is grown in 125 countries from which Ethiopia ranked 24th in the area allotted for wheat and 28th in tons of wheat production. Whereas, in Africa, Ethiopia is the 2nd largest wheat producer next to Egypt (FAO, 2018).

Wheat is the fourth most widely grown crop (with coverage of 1.70 million hectares) in Ethiopia next to teff (*Eragrostis tef* (Zucc.) Trotter), maize or corn (*Zea mays* subsp. *mays* L.) and sorghum (*Sorghum bicolor* L.) and ranks third in terms of the gross production (4.54 million tons) after maize and teff (CSA, 2017). The wheat crop is grown in Oromia, Amhara, Southern Nation Nationalities and People (SNNP), Tigray and Benishangul Gumuz regional states with an area contribution of 52.97 %, 32.68 %, 7.50 %, 6.35 % and 0.12 %, respectively (CSA, 2017). Moreover, the main wheat belts of Oromia lay in Arsi, Shewa and Bale areas that constituted for 86.02 % of hectares and 90 % of tons to the region's total wheat production (CSA, 2017). Also, the crop is grown as one of the main stable food crops in Jimma, Illubabor and West Wollega zones of Oromia regional state (Dechassa, 2000). Currently, these areas

contributed 4.89 % (43,937.38) of hectares and 3.52 % (93848.88) of tons to the total wheat production of Oromia regional state (CSA, 2017).

Over the past two decades, several government programs and initiatives worked to drive agricultural growth and food security in Ethiopia, resulting in a significant increase in wheat production from 1.10 million tons in 1995/96 (Bergh *et al.*, 2012) to 4.54 million tons in 2016/17 (CSA, 2017). Wheat obtained a great attention because it is one of the poverty alleviation crops of the country (Demeke, 1999). In line with the government's plan and need, there are a lot of works made by the regional, national and international organizations giving due attention to variety development, demonstration, dissemination of wheat technologies and more recently encouraging cluster farming to aid wheat mechanization in Ethiopia (Gebre-ab, 2006).

However, there are many production constraints hanging the wheat productivity in Ethiopia to 2.68 t ha^{-1} (CSA, 2017) below the global yield of 3.65 t ha^{-1} (FAO, 2018). In general, this low yield of wheat is a result of various biotic factors like diseases, insects and weeds; abiotic factors including lack of moisture due to uneven distribution of the rainfall, field size, flooding, hail, soil fertility and acidity problems and continuous farming; technical and socio-economic factors like less adoption of new technology, limited access to credit; and climatic factor like temperature rise beyond optimum for wheat (Barron *et al.*, 2003; Liu *et al.*, 2008; Hailu *et al.*, 2011; Mann and Warner, 2015). Among the pests, fungal diseases like *Puccinia* spp., *Septoria* spp. and *Fusarium* spp. are the main constraints to wheat production in East Africa including Ethiopia. Nowadays, *Fusarium* head blight (FHB) also called scabs or tombstone of wheat obtain the biggest concern (Tesfaye and Pim, 2016).

Fusarium head blight of wheat is one of the most destructive fungal disease of wheat worldwide, especially in humid and semi-humid wheat-growing regions (Goswami and Kistler, 2004; Martinez-espinoza *et al.*, 2014; Lenc, 2015). FHB disease is caused by several species in the genus *Fusarium*, which also infects a number of cereal crops including wheat, barley, oats, rye, corn, canary seed and forage grasses, but wheat, barley and maize are the most affected crops (Clear and Patrick, 2003; Kosová *et al.*, 2009). Infection of wheat kernels by FHB

pathogens contributed to losses in grain yield and quality that includes poor seed germination (or blighted seedlings), shriveled kernels, reduced number of kernels per spike, low protein content in kernels and low baking quality of wheat grains (Gärtner *et al.*, 2008). Besides, FHB also cause health problems to both human and animals because of grains' and straws' contamination with mycotoxins (Grabowski *et al.*, 2012; Darwish *et al.*, 2014). These all bring a less competitiveness of wheat grains in the world market and resulted in lower price or rejection (Goswami and Kistler, 2004).

During the past three decades, FHB has emerged as a major threat to wheat at global levels with an increasing trend of epidemics (McMullen *et al.*, 2012). Several outbreaks with severe on wheat have been experienced since 1990 in different parts of the world. In USA, economic losses of $ 2.7 billion were estimated on wheat due to FHB epidemics during 1998 to 2000s in North Dakota and Minnesota (Nganje *et al.*, 2004a). Similarly, $ 7.7 billion losses were occurred during 1993 to 2001 in wheat and barley of the nine states in the United States (Nganje *et al.*, 2004b). Recently, $ 293 million losses were occurred because of FHB in barley during 2015 and 2016 (Wilson *et al.*, 2017). In China, nine severe and 17 medium FHB epidemics were exhibited during 1950 to 2003 along the Yangtze river (Zhang *et al.*, 2013).

In sub-Saharan Africa (SSA), there is lack of information regarding the FHB epidemics and economic losses on wheat because of the underdeveloped research on the disease (Dweba *et al.*, 2017). Particularly in Ethiopia, there is little information on FHB of wheat that reported the disease as one of the major wheat diseases at high altitude areas (Bekele, 1985) and the 1988 cropping season was one of the scabby season with an incidence of 85 % and severity of 5 – 80 % (Bekele, 1990). Besides, the disease was reported to cause yield losses of 60% and above under experimental conditions in 1989 cropping season of Ethiopia (Snijders, 1989). Moreover, the *Fusarium* spp. associated with FHB of wheat across Arsi, Bale, Gojam, Gonder, Shoa and Wollo areas were identified into 17 *Fusarium* spp. during 1987 and 13 *Fusarium* spp. during 1989 (Bekele, 1990), but their pathogenicity was not verified. Almost two decades later, a new novel specie named as *F. aethiopicum* was phylogenetically identified among the 31 isolates of *F. graminearum* species complex collected from Gugsa-Womberma and Bure in Amhara

region and Arsi-Robe in Oromia region (O'Donnell *et al.*, 2008) this might indicate the existence of species diversity in Ethiopia. Meanwhile, less concern was given to FHB of wheat in Ethiopia even though it is a potent disease and also there is a diversity of *Fusarium* pathogens in the country. In addition, all the past works on FHB in Ethiopia did not enclose southwestern Ethiopia where wheat is grown as one of the stable food crops. Therefore, this study was initiated to provide information on FHB of wheat across southwestern Ethiopia with the following general and specific objectives:

General Objectives

- To assess the distribution and importance of FHB of wheat and also identify and characterize associated *Fusarium* spp. in Southwestern Ethiopia (SWE).

Specific Objectives:

- To assess the distribution and relative importance of FHB in wheat producing agroecologies of SWE
- To identify and characterize *Fusarium* spp. associated with wheat head blight in SWE

2. LITERATURE REVIEW

2.1. Wheat

2.1.1. Origin and diversity

Regarding the origin of wheat, Nikolai Vavilov; a Russian botanist, concluded that South East Asia region is the ancestral homeland of wheat, whereas Robert Braidwood; an archaeologist from the University of Chicago, stated that Near East of the Mediterranean must have been the birthplace of wheat (Kiple, 2001). These resulted in a consensus view for the determination of wheat origin within a geographic range known as the *'Fertile Crescent'*, whose core is within Central Asia and extends to Northern Africa through the Mediterranean (Kimber and Sears, 1987; Hancock, 2012). From there it apparently spread to the Middle East, North Africa, Asia and ultimately Europe (Harlan, 1981).

Archeological pieces of evidence revealed that the domestication of wheat reaches far back into history, stretching from 12,000 BC to 6,500 BC (Kimber and Sears, 1987; Snape and Pánková, 2007; Peng *et al.*, 2011). The cultivation of the wild wheat (most likely Einkorn or wild emmer) was began about 10,000 years ago, as part of the 'Neolithic Revolution' when hunter-gatherers shift to crop domestication or settled agriculture (Lev-Yadun *et al.*, 2000; Kiple, 2001; Katz and Weaver, 2003). This indicated that wheat was one of the first domesticated food crops and it has been the basic staple food of the major civilizations of Europe, West Asia and North Africa (Curtis *et al.*, 2002). Also, the domestication of wheat was achieved through continuous cultivation and selection of mutant wheat forms; which have limited ability to propagate in the wild (Smith, 1995). Hybridization of the diploid and tetraploid was occurred about 9,000 years ago giving a birth to hexaploid bread wheat (Feuillet *et al.*, 2008). Nowadays, wheat is globally produced in 125 countries and traded amongst all nations (FAO, 2018).

The genome of modern wheat cultivars contains three ploidy levels including **diploid**: *2n = 2x = 14* chromosomes having genome A^m or A (*T. monococcum*, *T. urartu*), **tetraploid**: *2n = 4x = 28* chromosomes having genome AB (*T. turgidum*) or AG (*T. timopheevii*) and **hexaploid**:

2n = 6x = 42 chromosomes having genome ABD (*T. aestivum*) or AGAm (*T. zhukovskyi*) (Hancock, 2012).

2.1.2. Taxonomy and Botany

The wheat belongs to phylum: *Angiospermatophyta*, class: *Monocotyledonopsida*, order: *Poales* (*Glumiflorae*), family: *Poaceae* (*Gramineae*), subfamily: *Pooideae*, tribe: *Triticeae*, subtribe: *Triticinae*, genus: *Triticum* L. This tribe contains more than 15 genera and 300 species including wheat, barley, rye, oat, etc. (Kimber and Sears, 1987; Snape and Pánková, 2007). The tribe *Triticeae* is characterized by many different ploidy levels, with both annual (cultivated wheat) and perennial forms. There is no universal agreement on taxonomy or nomenclature for the tribe (Snape and Pánková, 2007). However, the modern wheat cultivars primarily belong to hexaploid bread wheat, *Triticum aestivum* and tetraploid hard or durum-type wheat, *T. turgidum* whereas, other species are relict. Wheat is almost fully self-pollinated (Nevo *et al.*, 2002).

Wheat is a mid-tall annual or winter annual grass (CFIA, 2014), made up of repeating segments which contain a nodes, a hollow internodes, a leaves (that emerge at opposite sides) and a tiller bud found in the axil of the leaf (Kirby, 2002). The leaf sheath wraps around the stem providing support to the shoot. The stem terminates in the spikes of the wheat plant (Setter and Carlton, 2000). Each spike is made up of spikelets, which also contain the florets (composed of the carpel, three stamen and anthers) enclosed by a lemma and a palea. The spikelets are arranged on opposite sides of the central rachis (Setter and Carlton, 2000). The grain is in the shape of an oval and may vary in its length of brush hairs, either long or short (AGDHOGTR, 2017).

2.1.3. Importance

Ethiopia is the second largest wheat producer in Africa next to Egypt (FAO, 2018). The wheat crop was grown in 1.70 million hectares of land and its production reached 4.54 million tons (CSA, 2017). The wheat crop is one of the main crops that plays a great role in the diet and the economy of Ethiopia (Minot *et al.*, 2015; AUCGT, 2017). In 2013, wheat provides 284 kcal/capita/day for more than 90 million people in Ethiopia (FAO, 2017). Since 1984 up to date, the government policy of Ethiopia gives great weight to achieving self-sufficiency in food production through cultivation of poverty alleviation crops like wheat, maize and tef (Demeke,

1999). Besides, several government organizations, national and international initiatives have been cooperated to maximize wheat production through wheat technology innovations, demonstrations and disseminations all across the country. As a result, the current wheat production was showed 3.44 million tons of wheat increments than the 1995/96 wheat harvest (Bergh *et al.*, 2012; CSA, 2017). It is a sluggish increment when compared to wheat consumption in the country; therefore, the government of Ethiopia is enforced to import wheat in order to satisfy the high demand for wheat consumption in the country. These are why the wheat import has grown significantly over the past decade with an import ranged between 25 % to 35 % (Rashid and Lemma, 2014; Minot *et al.*, 2015; AUCGT, 2017).

2.2. Production Constraints

Wheat production in Ethiopia is constrained by several factors including biotic, abiotic, technical, socio-economic, and climatic (Barron *et al.*, 2003; Liu *et al.*, 2008; Hailu *et al.*, 2011; Mann and Warner, 2015). Among the biotic factors, fungal diseases caused by *Puccinia* spp., *Septoria* spp. and *Fusarium* spp. are the chief constraining factors that threaten the wheat production (Hailu *et al.*, 2011; Tesfaye and Pim, 2016). In this study, focus was given to FHB that becomes one of the most important fungal diseases in reducing grain yield, quality and feeding value of the grain due to toxin contaminations in worldwide.

2.3. *Fusarium* Head Blight in Wheat

Fusarium head blight is a dangerous necrotrophic disease of wheat and barley that significantly affect yield and grain quality, particularly in the humid and semi-humid areas (McMullen *et al.*, 2012; Zhang *et al.*, 2012; Kiersten *et al.*, 2015). The disease is also known by different names in different countries: *Fusarium* damaged kernels or tombstone kernels in Canada, scab, head blight, scabby kernels or tombstones in USA and wheat scab in England (Smith, 1884; Saharan *et al.*, 2004). It is also best known as a disease of flowering (anthers are the primary infection site where spores may land and then grow into the kernels, glumes, or other head parts) meaning wheat crops are susceptible in a period up through the soft dough stage of kernel development (Fernandes *et al.*, 2004).

2.3.1. Origin and distribution of *Fusarium* head blight disease

FHB disease of wheat was first reported in 1884 by Smith from England (Smith, 1884). Later on, it spread to other parts of the world and become highly destructive to wheat and barley crops grown in the humid and semi-humid areas including North central U.S.A, Canada, Asia, Eastern and Western Europe, Australia, China, Russia, Brazil, Romania, India, France, and South America (Dickson, 1942; Scott, 1986; Ban *et al.*, 2006; Muthomi *et al.*, 2008; McMullen *et al.*, 2012; He *et al.*, 2013a). In 1980, it was firstly reported from South Africa by Scott on irrigated wheat in the western Transvaal (Scott and de-Jager, 1988). In Ethiopia, *Fusarium* species (causal agent of FHB) were reported to cause considerable damage in some areas of Ethiopia during 1982 (Tesemma and Mohammed, 1982) and three years later (during 1985), FHB was reported as one of the main wheat diseases at higher altitudes areas of Ethiopia (Bekele, 1985).

2.3.2. *Fusarium* head blight epidemics and its economic importance

Fusarium head blight has emerged as one of the main threat to global wheat production in the past three decades with an increasing trend of epidemics (Leonard and Bushnell, 2003; Lenc, 2015). Its outbreaks in United States (U.S.) caused losses of 288,000 metric tons (MT) in 1917, 2.18 million MT in 1919, 2.72 million MT in 1982, 4.78 million MT in 1993 , and 1.3 million MT in between 1998 to 2000 (Nganje *et al.*, 2002). In monetary values, from 1993 to 2001, U.S. lost a total of $ 7.7 billion in wheat and barley production (Nganje *et al.*, 2004b) and also from 1998s to early 2000s, U.S. encountered a total losses of $ 2.7 billion in wheat and barley production due to FHB (Nganje *et al.*, 2004a).

In addition, the other major wheat producing countries including China, Russia, India and France faced FHB epidemics with seasonal and regional variability. In China, 9 severe and 17 medium epidemics were occurred that covered an area of 4 million hectares of wheat from 1950 to 2003 along the mid-lower reaches of the Yangtze river (Parry *et al.*, 1995; McMullen *et al.*, 1997; Zhang *et al.*, 2013).

A few available information indicated that FHB was one among the important wheat diseases at high altitude areas of Ethiopia (Bekele, 1985) that can cause grain yield losses of 60% and above under experimental conditions (Snijders, 1989).

Factors contributing to FHB Epidemics

Different factors have contributed to the increase in FHB epidemics. For example, in 1990s, the increased adoption of conservation (reduced or zero) tillage practices with an expansion of maize production, and climate variability has favored the development of FHB epidemics. Besides, more frequent wet seasons and the use of highly susceptible cultivars lead to a serious economic losses and accumulation of mycotoxins in grains beyond acceptable levels (McMullen *et al.*, 2012).

The climate variability is predicted to raise temperatures or increase in rainfall which may result in favorable conditions for the development of wheat scabs. Numerous successive years of favorable climate can potentially cause a build-up of inoculum, leading to epidemics that increases risk of FHB (Madgwick *et al.*, 2011; West *et al.*, 2012). Moreover, the occurrence of rain and high humidity approximately one week before anthesis is believed to stimulates sporulation of FHB pathogens that can promote risk of FHB (De Wolf *et al.*, 2003) and encourages vegetative spread of mycelium to more florets (Parry *et al.*, 1995; De Wolf *et al.*, 2003). According to the finding of field-based studies conducted in China, the warm and moist conditions during anthesis of wheat are the key factors for FHB epidemics development (Xu, 2003).

2.3.3. Pathogen biology and taxonomy

The FHB disease is caused by members of the *Fusarium* species complex; which comprises more than 16 species (O'Donnell *et al.*, 2000; O'Donnell *et al.*, 2004; Starkey *et al.*, 2007; O'Donnell *et al.*, 2008; Yli-Mattila *et al.*, 2009; Sarver *et al.*, 2011) is one of the most devastating fungal diseases of small grain cereals causing more than 50 % of yield losses (Parry *et al.*, 1995). In addition, they are capable of producing mycotoxin in small grain cereals (van der Lee *et al.*, 2015). The genus *Fusarium* belongs to the *Ascomycota* phylum, *Ascomycetes* class, *Hypocreales* order (Leslie, 1995), while the teleomorphs of *Fusarium* species are mostly classified in the genus *Gibberella*, and for a smaller number of species, *Hemanectria* and *Albonectria* genera (Moretti, 2009). More recently, a comprehensive work reported about 116 species under the genus *Fusarium* (Refai *et al.*, 2015). The predominant species, *F.*

graminearum (teleomorph *Gibberella zeae*), is currently ranked fourth among plant fungal pathogens based on its scientific and economic importance (Dean *et al.*, 2012).

2.3.4. Host range

Fusarium head blight pathogens may infect a number of cereal crops including wheat, barley, oats, rye, corn, rice, canary seed and forage grasses. However, the most affected crops are wheat, barley and maize (Clear and Patrick, 2003; Kosová *et al.*, 2009).

2.3.5. Pathogenesis of pathogens causing FHB

Fusarium spp. have both sexual and asexual life cycles (Figure 1 and 2) (Schmale and Bergstrom, 2003; Ma *et al.*, 2013). During the asexual life cycle, the mycelial structures produce three types of mitotic spores including microconidia from conidiophores, macroconidia from sporodochium and chlamydospores formed on and within hyphae and macroconidia. In addition, the sexual structure called Perithecia produces ascospores which are an important source of inoculum for FHB infection in wheat (Trail and Common, 2000; Ma *et al.*, 2013).

Fusarium propagules from the source of inoculum; where it grown saprophyticaly on crop stubble, disseminated to wheat head passively through rain splash and wind. Rain splash disseminated propagules to short distances (Jenkinson and Parry, 1994; Paul *et al.*, 2004), whereas wind may have disperse ascospores over kilometers or even greater distance in the planetary boundary layer of the atmosphere (Maldonado-Ramirez *et al.*, 2005) and may contaminated the cereal spikes on distant by precipitation or gravitational settling (Keller *et al.*, 2014). Also, propagules can actively dispersed by forcible discharges of ascospores in to air (Trail and Common, 2000). Besides, contaminated arthropod vectors, systemic fungal growth through plants (Parry *et al.*, 1995) and introduction of infected seed into previously non-infested areas (Clear and Patrick, 2000).

When *Fusarium* propagules land on wheat spikes, they adhered to wheat spikes through sticky cell walls of *Fusarium* ascospores which help them to avoid the displacement by wind, rain or other forces (Bushnell *et al.*, 2003). Conidia germination is influenced by temperature, conidia density (Colhoun *et al.*, 1968) and water potential (Sung and Cook, 1981). Relative humidity

above 95 % for 40 to 60 hours accompanied by 25 to 30 °C is the ideal conditions for conidia germination or FHB development (Nazari *et al.*, 2014). If this condition existed, ascospores (conidia) germinated in surface moisture on the spikelet and invade the florets either passively through stomata (Pritsch *et al.*, 2000; Zhensheng and Buchenauer, 2000; Bushnell, 2001) or actively by direct penetration probably with the aid of vast hydrolyzing enzymes (secreted by *Fusarium* conidia) that can degrade the cuticle of wheat florets (Bushnell *et al.*, 2003). Besides, the anthers also provide the initial path for FHB infection in wheat, which might result from the enrichment of wheat anthers with the growth stimulants choline and betaine (Strange *et al.*, 1974; Brown *et al.*, 2010).

Following penetration, *Fusarium* hyphae can spread within the cell apoplast, which leads to significant cytological alterations and finally cause cell death, which is achieved by the secretion of fungal proteases, lipases and carbohydrate-degrading enzymes that can detoxify plant toxins and defense proteins (e.g. amino acids, sugars, fatty acids and ions) to facilitate cell colonization (Divon and Fluhr, 2006; Brown *et al.*, 2010). Symptom development first appear water soaked, then lose their chlorophyll and become straw-colored (Gilbert and Tekauz, 2000). The first symptom of FHB disease tends to occur around the middle of the head, the region where flowering begins (Kirby, 2002; Bushnell *et al.*, 2003). Besides, sporulation occurs within 48 to 76 hours after inoculation (Pritsch *et al.*, 2000) which can be useful for further new secondary infections of wheat plants in the same field mainly by wind dispersed (Keller *et al.*, 2003). Moreover, perithecia, ascospores and vegetative conidia (formed on infected wheat heads and crop stubble) helpful to propagate the disease, while chlamydospores and mycelia allow survival in the soil between crops (Parry *et al.*, 1995).
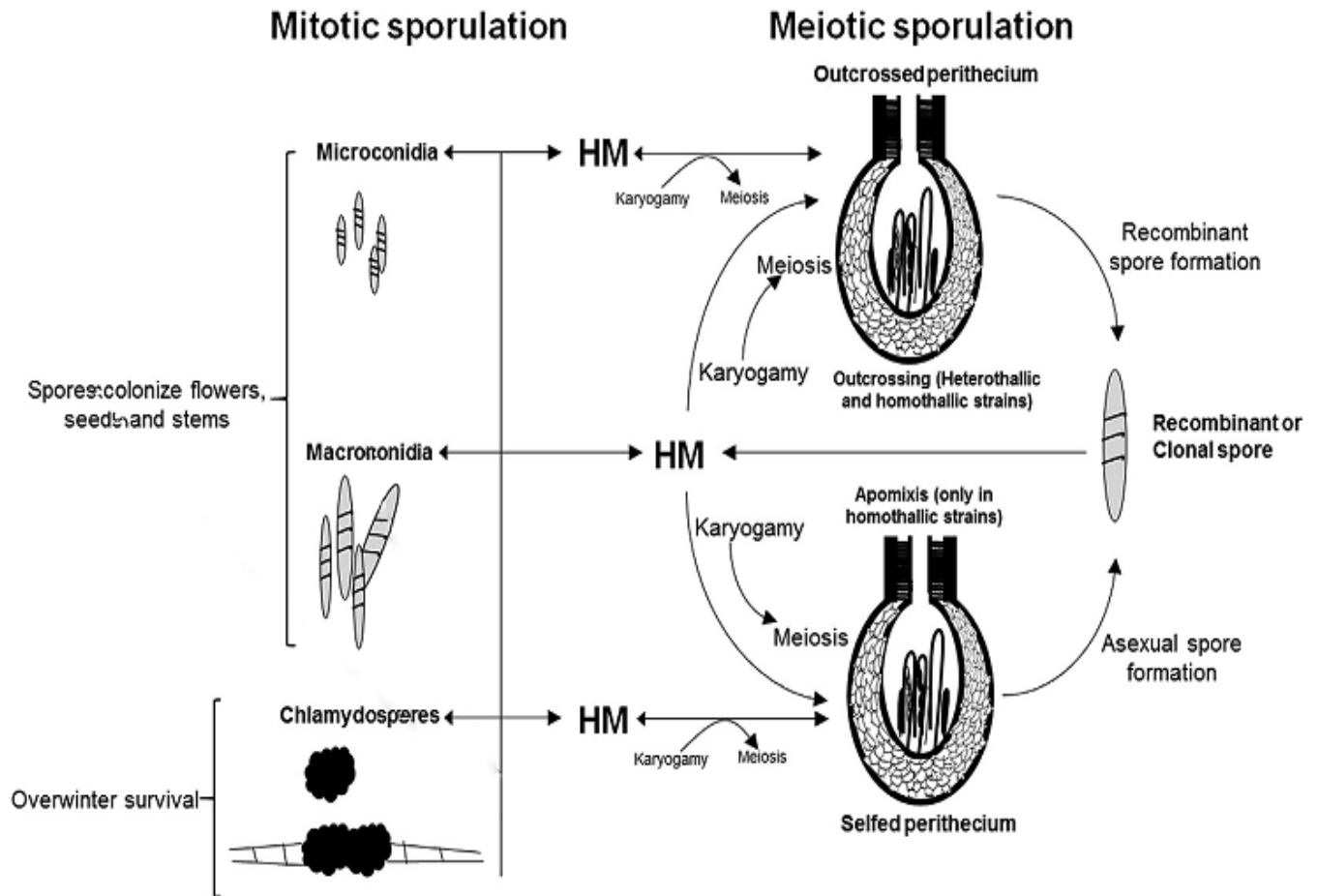
Figure 1. Generalized life cycle of *Fusarium* spp. following plasmogamy, outcrossed and selfed perithecium respectively produced recombinant and conidial meiotic spores. These form haploid mycelium (HM) which in turn form three types of mitotic spores. While conidia (micro- or macroconidia) can colonize the host, chlamydospores, in addition to direct colonization of the crop, can overwinter and develop into perithecium to restart the cycle when conditions are favorable.
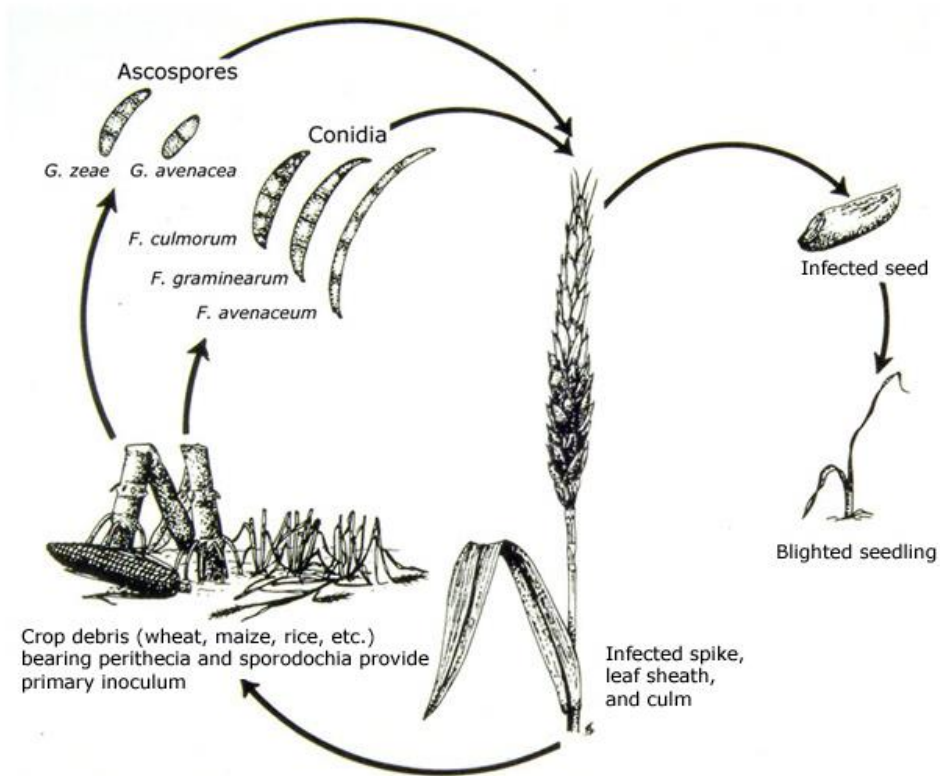
Figure 2. Disease cycle of *Fusarium* spp.

2.3.6. Symptoms of FHB in wheat

Symptoms first begin as water-soaked brownish spots at the base of the glumes and ultimately glumes become bleached in color. The primary symptom of the FHB disease is bleaching of some florets in the head or spike before maturity. Sever infections can cause premature blight or bleaching of the entire spike (Figure 3 and 5). Other symptoms include tan to brown discoloration at the base of the head, a pink or orange colored mold at the base of the florets (around the edges of glumes on infected florets) under moist conditions, and the rachis and peduncle become darken; turning a chocolate-brown color. In general, visible expression of FHB is observed in spikes during the soft to hard dough growth staged as premature ripening accompanied by dark brown discoloration of peduncle and shriveled kernels (*Fusarium* damaged kernels; FDKs) with a chalky (tombstone) appearance. FDKs are shriveled, white, and chalky in appearance (Figure 3) and are lighter in weight than normal healthy kernels. However, the stage of wheat crop affects the degree to which the kernels are infected, early

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infections result in small kernels almost entirely colonized by the fungus but lately infected kernels may resemble healthy kernels. Perithecia (dark fruiting bodies) are produced within the mycelium later in the infection process (Calpas *et al.*, 2003; Burrows *et al.*, 2008; Kiersten *et al.*, 2015; Singh, 2017).

| A | B |
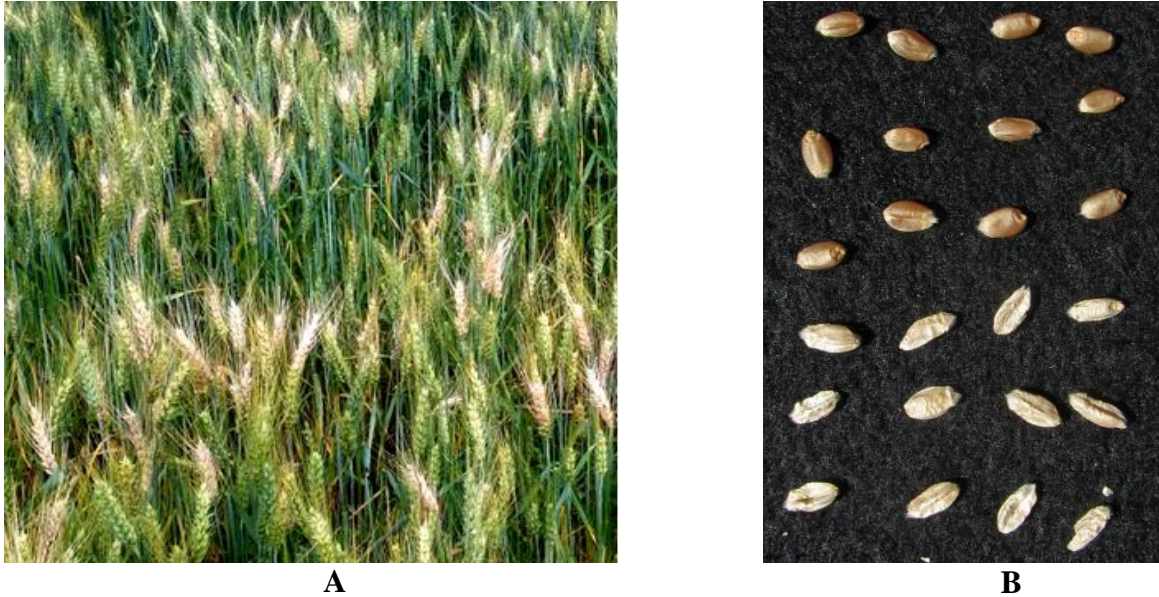
Figure 3. A) Blighted wheat heads due to *Fusarium* infection, (photo taken in **Begi** district of West Wollega zone in Oromia regional state; B) FHB damaged wheat kernels

2.3.7. Management Strategies for *Fusarium* Head Blight of Wheat

2.3.7.1. Cultural options

Utmost cultural management of FHB is given emphasizes on avoiding or reducing the exposure of spikes to spores during flowering and early grain filling. These includes avoiding continuous or short rotations with cereal crops, planting of seeds with no detectable level of *Fusarium* spp., tillage operations that bury infected crop residues, burning of infected crop residues (Salas and Dill-Macky, 2005; Dill-Macky, 2008), mechanical chopping of infected residues to boost rate of decomposition and direct application of soil amendments (including green manures, bentonite clay, urea, and spent lime) on residues can reduce the inoculum potential of residues (Dill-Macky, 2008) all these were advised for FHB management in individual cereal fields and over broader regions of cereal production.

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2.3.7.2. Biological agents

The use of biological control agents including bacteria, yeast and fungi were reported as a potential impact on FHB of wheat disease reduction. *Trichoderma gamsii* (6085) was recommended as a good candidate for controlling *F. graminearum* and *F. culmorum* (Matarese *et al.*, 2012). Bacterial antagonist AS43.3; *Bacillus subtilislamyloliquefaciens* (B-30210), was reported as a potential bio-agent that reduces FHB disease severity by 90 % and incidence by 78 % on durum wheat under greenhouse condition. Whereas, the yeast antagonists OH 71.4; *Cryptococcus* sp. (= *Torula aurea*) and OH 182.9; *C. nodaensis*,were also the most effective in reducing severity of FHB in durum wheat by 57 % and 59 % in field trials, respectively (Schisler *et al.*, 2002). In addition, *Pseudomonas flourescens* strains MKB 158 and MKB 249 and *P. frederiksbergensis* strain 202 were significantly reduced severities of FHB caused by *F. culmorum* both in wheat and barley under greenhouse condition. In the field trail, the two strains of *P. fluorescens* (MKB 158 and MKB 249) were significantly reduced the DON levels by 74 - 78 % in wheat and barley (Khan and Doohan, 2009). From greenhouse trial, *Brevibacillus* sp. strain BRC263 and *Streptomyces* sp. strain BRC87B were recommended for potential control of FHB of wheat (Palazzini *et al.*, 2007).

2.3.7.3. Host resistance to FHB

There are at least five types of resistance to FHB such as type I (resistance to the initial infection), type II (resistance to spread of infection within the head), type III (resistance to the infection of the kernel), type IV (tolerance of infection without substantial effect on yield and quality losses in wheat) and type V (the ability of the host plant to degrade the mycotoxins responsible for virulence) (Gilbert and Tekauz, 2000). At variety levels, QTL for FHB confer a response on two or more types of resistance, that implies incorporation of more types of resistance made the cultivar more stable and durable. The good example is that type I and type II resistance incorporated wheat cultivars, which is more preferable (Jayatilake *et al.*, 2011; Xue *et al.*, 2011; Cainong *et al.*, 2015). Type I resistance is the first line of defense against *Fusarium* pathogens (Schroeder & Christensen, 1963). Morphological and physiological characteristics of wheat plant have been contribute to type I resistance (Bushnell *et al.*, 2003). For instance, traits such as plant height, flowering timing and duration, awn absence/presence,

kernel density, extent of flower opening and row type (i.e. low spikelet density) influence resistance to *Fusarium* infection (Yoshida *et al.*, 2005). Besides, waxy surfaces on head tissue could reduce water availability to *Fusarium* conidia and thus contribute to type I resistance (Yoshida *et al.*, 2005).

Through research works undertaken so far in Ethiopia, bread wheat variety Enkoy has showed good resistance level to FHB disease (Bekele, 1990; Hulluka *et al.*, 1991) although it did not give complete protection and the inherited mechanism has not been known.

So far, there is no wheat cultivar that has been identified and released with complete resistance or immunity to the FHB pathogen. This is due to the reason that resistance to FHB is controlled by many genes with quantitative inheritance; which has slow genetic gain per unit time. However, a promising resistance to FHB was observed in various transgenic research endeavors, implying that alien genes are capable of boosting the genetics of wheat and providing an options in tackling the disease (Xue *et al.*, 2011; Han *et al.*, 2012). Furthermore, the induced resistance due to the phytohormones like methyl-jasmonate, ethylene and salicylic acid existing within the wheat genome under pathogen attack can also have the potential to improve FHB resistance (Makandar *et al.*, 2012).

2.3.7.4.   Fungicides to manage FHB

Fungicides have been widely employed in controlling FHB, but in general they provide a limited control i.e. the best fungicides are not fully effective in controlling FHB. In other words, the effectiveness of fungicides depends on cultivar resistance, climate, economic returns or yield gain, fungicide type, dose rate, application timing, spray quality for adequate coverage of the spike tissues and frequency of application (Picinini and Fernandes, 2002; Mesterhazy *et al.*, 2003; D'Angelo, 2013; Andriolli *et al.*, 2016).

A number of fungicides were suggested for partial control of FHB including carbendazim, hexaconazole, mancozeb, benomyl, prochloraz, propiconazole, tebuconazole and triadimenol are useful for FHB control. But, some countries like South Africa, have no chemicals registered for FHB on wheat and barley. From recent reports, there is a high possibility of fungicide

resistance development due to over-use of the same types of fungicides. For instance, *F. graminearum* isolate was developed resistance to the fungicide tebuconazole in the USA (Spolti *et al.*, 2014) and benzimidazole based fungicide in China (Chen *et al.*, 2009).

2.3.7.5.   Integrated disease management

Fusarium head blight fungal pathogens are one of the main human and animal health impacting pathogens because of their mycotoxins that contaminate small cereals (wheat, barley, oat, ray, etc) and maize, which are the most constituents of world's food (Munkvold, 2003; Wild and Gong, 2009). Enormous efforts are in progress to tackle the problem of FHB in wheat (Paul *et al.*, 2005; Paul *et al.*, 2010). The most effective management approach to mitigate FHB disease-toxin complex in wheat is integrating multiple management strategies (McMullen *et al.*, 2008) including pre- and post-harvest management practices (Jouany, 2007).

Pre-harvest strategies include the use of resistant cultivars, fungicide application, crop rotation, and tillage (Jouany, 2007; Dill-Macky, 2008; Ransom and McMullen, 2008). Whereas, post-harvest practices that can reduce mycotoxin levels include grain blending to dilute contaminated grain using clean grain (Delwiche *et al.*, 2005), screening or sorting based on size, weight, density and kernel morphology and color to separate diseased grain from healthy grain (Delwiche *et al.*, 2005), grain harvesting strategies like removal of light kernels during harvest (Jouany, 2007), reducing *Fusarium* damaged kernels during harvest (Paul *et al.*, 2005; Paul *et al.*, 2006; Beyer *et al.*, 2007b), reducing the speed of combine harvester help to separate and remove infected, lightweight kernels by increasing air blast time (Inch and Gilbert, 2003; Jouany, 2007) and chemical and physical procedures to remove mycotoxins, contaminated grain and infected particles (Young *et al.*, 1986; Trenholm *et al.*, 1991; Jouany, 2007).

3. MATERIALS AND METHODS

3.1. Description of Study Areas

The field survey was conducted in major wheat growing districts particularly Dedo and Seka-Chekorssa in Jimma zone, Bedele and Gechi in Buno-Bedele zone and Begi in West-Wollega zone of Oromia regional state in southwestern Ethiopia (Figure 4 and Table 1).

Table 1. Coordinates, elevations, annual rainfall and mean temperatures of the study area by districts, 2017

| Zone | District | Coordinate | | Altitude | Rain fall | Temperature (°C) | |
		N	E	(m.a.s.l)	(mm)	Min	Max
Jimma	Dedo[a]	07° 25'	37° 00'	880 – 2800	1830.36	12.3	25.5
	Seka-Chekorssa[c]	07° 35'	36° 33'	1560 – 3000	1825.16	10.0	23.0
Buno-Bedele	Bedele[a]	08° 27′	36° 21′	2012 – 2162	2051.1	13.0	26.4
	Gechi[c]	08° 20′	36° 40′	1400 – 2380	1639.0	18.0	25.0
West-Wollega	Begi[b]	9° 15′	34° 45′	1465 – 2100	1024.4	15.2	27.4

[a] National Meteorology agency of Ethiopia, Jimma Meteorological Center, 2017

[b] National Meteorology agency of Ethiopia, Assosa Meteorological centres, 2017. Coordinate and altitude ranges were obtained from districts agricultural and natural resource development office

[c] Obtained from district's agricultural and natural resource development offices of the respective districts

3.2. Sampling Methods

Purposive multistage sampling strategy was employed to select main wheat growing zones, districts within zones and peasant associations within districts across SWE. Random sampling was applied to select wheat fields in each peasant associations. In particular, potential wheat growing districts and peasant associations were selected by consulting zonal agricultural bureaus and district's agricultural and natural resource offices, respectively. Based on the accessibility of wheat fields and the growth stage of wheat crops, this investigation was enclosed a total of 52 wheat fields in five districts within the three zones of Oromia regional state across southwestern Ethiopia.
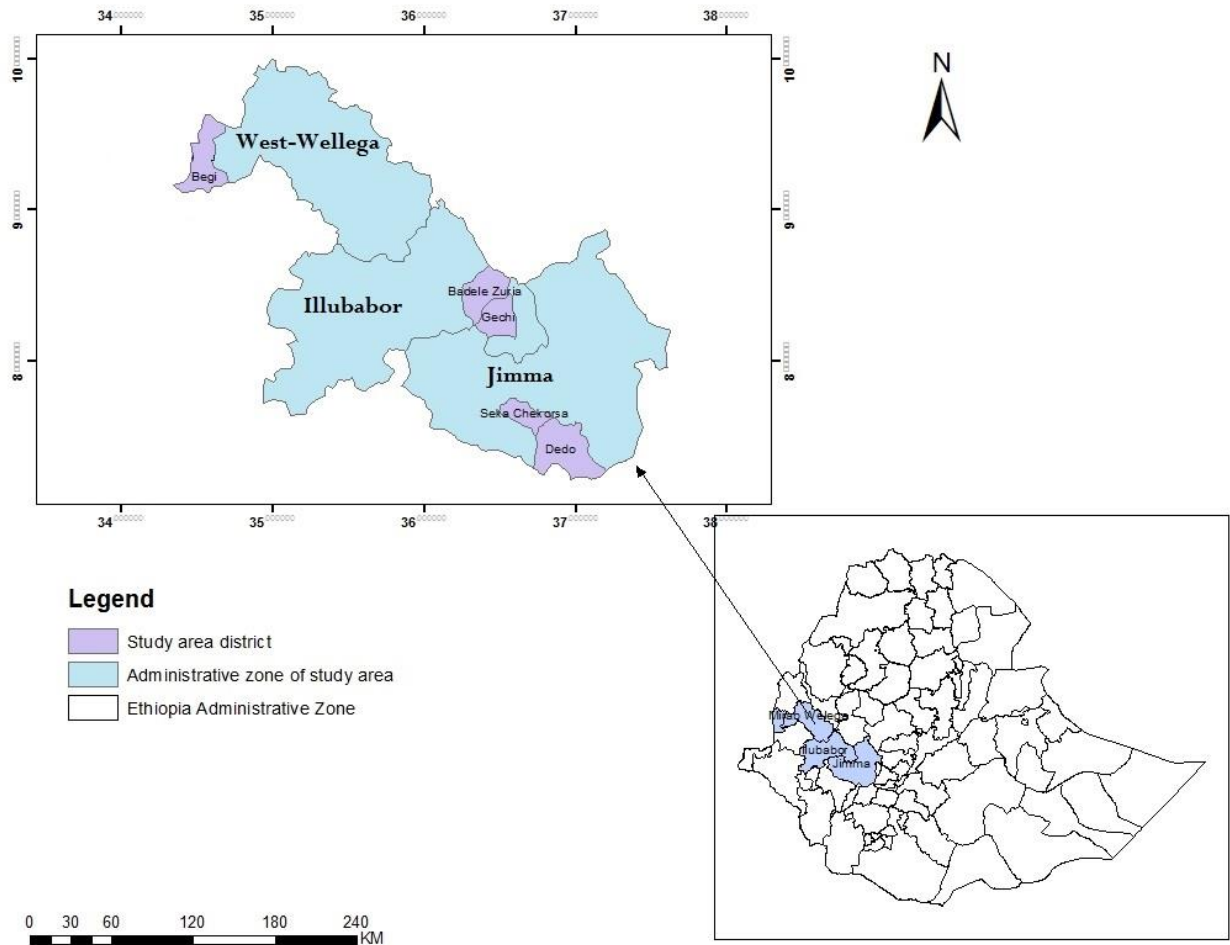
Figure 4. Surveyed zones and districts for FHB of wheat in southwestern Ethiopia, 2017 cropping season.

3.3. Assessment of FHB Disease

A field survey of FHB disease of wheat was carried out once across the selected districts during the main growing season of 2017, particularly from early November to late December when wheat crops reached Zadok's growth stages of 73 (early milk) to 85 (hard-dough) (Zadoks *et al.*, 1974) during which FHB disease symptoms are clearly observed in wheat fields. In the selected field, FHB disease assessment was made along the diagonal of the field by placing 30 cm x 30 cm quadrat randomly in four spots per field (at least 10 m far apart). In each field, all wheat heads or spikes within the quadrat were counted and visually examined in careful manner

for the presence and absence of FHB symptoms. Spikes were considered as diseased when a single spikelet was showed characteristic symptoms of FHB symptom as shown in Figure 5.
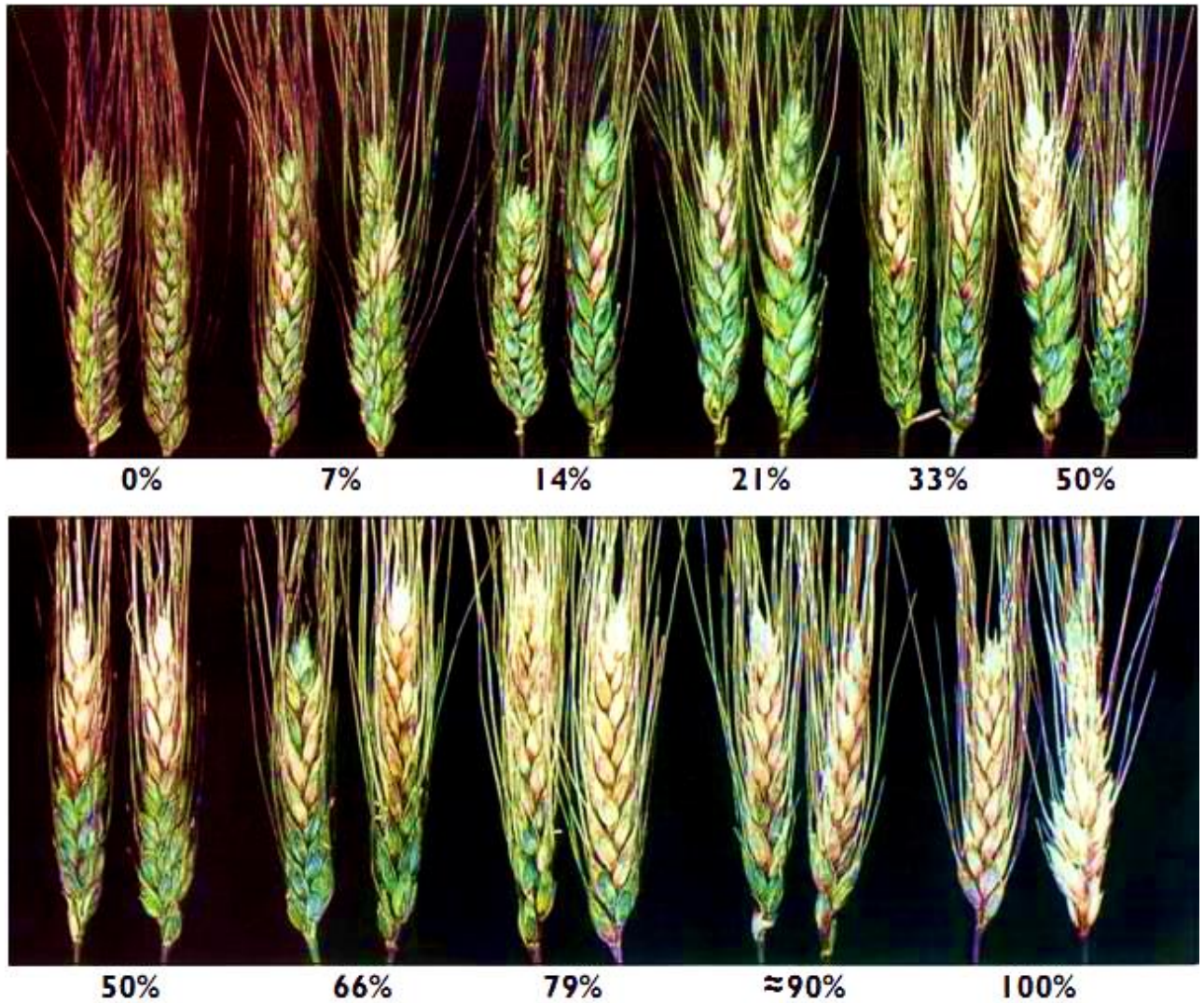
Figure 5. A visual FHB severity rating scale in wheat expressed in percentages

3.3.1. Field survey data

Incidence of FHB was recorded as a percentage of infected wheat spikes (Wegulo *et al.*, 2008), while severity of FHB on wheat spike was recorded following the modified Horsfall-Barrett's scale shown in Figure 5 (Stack and McMullen, 2011) on 10 random spikes within the quadrat of 30 x 30 cm^2. The severity was then partitioned in to FHB field severity which was determined by averaging the scores of all assessed heads per field (including the zero's), whereas FHB

infected head severity which was estimated by averaging only the scores of infected heads per field (Stack and McMullen, 2011). Besides, FHB disease index; which is an estimate of overall disease intensity, was the product of the percent FHB incidence and percent field severity, divided by 100 (Wegulo *et al.*, 2008).

$$\text{FHB incidence}(\%) = \frac{\text{Number of infected wheat spikes within the quadrate}}{\text{Total number of wheat spikes within the quadrate}} \times 100$$

$$\text{FHB field severity}(\%) = \frac{\text{Sum of all scores including zero's within the quadrate}}{\text{Total number assessed spikes within the quadrate}}$$

$$\text{FHB infected head severity}(\%) = \frac{\text{Sum of scores only from infected spikes within the quadrate}}{\text{Total number of infected spikes within the quadrate}}$$

$$\text{FHB index }(\%) = \frac{\text{FHB incidence}(\%) \text{ x FHB field severity}(\%)}{100}$$

To determine the prevalence of FHB of wheat across southwestern Ethiopia, the number of wheat fields with FHB infection and the total number of fields assessed per districts were recorded.

3.3.2. Collection of scabbed wheat spikes

Four to six wheat spikes having typical FHB symptom were collected from each assessed wheat fields and placed inside the paper bags. On and inside (on a piece of paper) the paper bag information including sample code, date of sample collection, GPS readings (Altitude, Latitude and Longitude) and collectors' name were written and placed in ice box for safe handling. Then the samples were ventilated for removal of excess water and air-dried samples were transported to JUCAVM Plant Pathology Laboratory for isolation and identification of associated *Fusarium* species.

3.4. Laboratory Works

3.4.1. Growth media for isolation and identification of *Fusarium* species

A selective malachite-green agar (MGA) and the general potato dextrose agar (PDA) media were used for isolation of *Fusarium* species from samples of blighted wheat spikes collected across Jimma, Buno-Bedele and West-Wollega zones of Oromia region. MGA medium is prepared by autoclaving the mixture of 15 g Peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 2.5 mg Malachite green oxalate and agar 20 g in 1 L distilled H_2O (Bragulat *et al.*, 2004). PDA medium was prepared from 20 g dextrose, 20 g agar and the broth from 250 g white potatoes made up to 1 L and autoclaved. The broth was prepared from unpeeled white potatoes which are washed, diced, boiled until soft and then filtered through a single layer of cheesecloth. A weak nutrient agar; Spezieller nahrstoffarmer agar (SNA), was prepared by autoclaving, the mixtures of 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose and 20 g agar in 1 L of distilled H_2O. To enhance conidia formation, two sterile filter paper pieces, approximately 1 cm^2, were aseptically placed on gelled SNA surface near agar block containing single germinated conidia. Water agar (3%WA) consists of 30 g agar in 1 L of distilled H_2O is used to germinate conidia and aid easy picking of a single germinated conidium for single conidial purification. For examination of colony characteristics (i.e. pigmentation and mycelial growth) potato sucrose agar (PSA) and PDA were used. PSA was prepared by autoclaving 500 ml potato broth (prepared from 250 g white potatoes peeled diced, boiled in 500 ml of distilled H_2O and filtered through 2 layers of cheesecloth), 20 g sucrose and 20 g agar made up to 1 L distilled H_2O. All media used in this study were prepared according to *Fusarium* identification manuals (Nelson *et al.*, 1983; Leslie *et al.*, 2006). The autoclaving temperature was 121 °C for 15 minutes. Besides, all the media were amended by 250 mg of Chloramphenicol per litre of media when the temperature cooled to 50 °C to inhibit bacterial contaminants.

3.4.2. Isolation of *Fusarium* species

Eight seeds were separated from each blighted spike and surface sterilized in 4% (v/v) sodium hypochlorite solution for a minute followed by thrice rinsing in distilled water. The seeds were kept under laminar flow in order to drain out excess moisture from seeds. After draining, four seeds were placed on PDA and another four seeds on MGA. All plates were labeled and then incubated at 25 °C. After 4 to 5 days of incubation, all *Fusarium* resembling colonies were

separately cut out together with agar piece by sterilized needle and placed upside down on the SNA dish close to the sterile filter papers. The needle used was dipped into ethanol and burned off between each colony transfer. The Petri dishes were then labeled, sealed with parafilm and incubated at 25 °C for 7 to 17 days until sporulation.

3.4.3. Single conidium isolate development

For single conidial isolation, a small fungal plug was taken from sporulated SNA cultures and transferred to 3 % WA and a drop of autoclaved distilled water was added onto the fungal plug and the conidia were dislodged by glass roads. The dislodged conidia were spread over the WA by glass road spreader and the plates were incubated at 25 °C for 24 hours. Then after, a hyphal tip derived from a single conidium was cut and transferred to SNA with sterile filter paper pieces (Leslie *et al.*, 2006). The Petri dishes were then labeled, sealed with parafilm and incubated at 25 °C for 7 to 17 days until sporulation. These isolates were used for examination of microscopic and macroscopic features.

3.4.4. Identification and morphological characterization of *Fusarium* species

Isolates of *Fusarium* recovered from blighted wheat spikes across southwestern Ethiopia was identified in to species level based on cultural and morphological characteristics as described by Nelson *et al.* (1983), Leslie *et al.* (2006) and Refai *et al.* (2015). The morphological characteristics include colony features (like mycelial color, pigmentation and growth), macro- and microscopic features (including number of septa per conidia, size, shape, curvature, ends, apical cells, basal cells and type of conidiogenous cell arrangements) and Chlamydospores formation.

Fusarium morphology data

Primary characters; a) Macroconidia characteristics like phialides (mono- or poly-phialides), shape, size, number of septa, shape of the apical and basal cells was noted; b) Microconidia characteristics including presence or absence of microconidia, if present their shape, size and the manner in which they are formed (phialides) were noted; and c) Chlamydospores presence or absence, if present their form (chain or single). Secondary characters; a) Colony morphology features that includes color on PDA (Nelson *et al.*, 1983; Summerell *et al.*, 2003), pigmentation

and hyphal colony growth on PDA and PSA (Nelson *et al.*, 1983; Leslie *et al.*, 2006; Refai *et al.*, 2015).

3.5. Pathogenicity Test

3.5.1. Design, kernel disinfection and pot preparation

Pathogenicity test was conducted from February, 2018 to June, 2018 on Danda'a; a susceptible bread wheat variety, under the Lath-house condition of JUCAVM. The design used to grow the test crop was RCBD with three replications, nine *Fusarium* spp. isolates as treatments and sterile distilled water injection was used as a control treatment. Plastic pots with size of 15 cm x 11 cm x 15 cm were used as an experimental unit. Prior to sowing, the kernels of the test crop were washed under a running tap water for 5 minutes, and then disinfected by 75 % ethanol for 30 seconds followed by 0.5 % NaOCl (sodium hypochlorite) for 1 minute. Finally, the kernels were rinsed twice in sterile distilled water and allowed to dry under a laminar flow hood (Gargouri-Kammoun *et al.*, 2009). After the kernels were dried well, four kernels were sown at a depth of 2 cm in each pot filled with an autoclaved potting mix (containing sand/peat/compost: 1:3:1 v/v). The pots were fertilized with 5 g urea (46% N) before emergence, 5 g NSP at tillering and 5 g urea at booting and also watered twice daily.

3.5.2. Preparation of inocula

The nine identified *Fusarium* spp. were recovered on SNA with sterile filter paper and incubated for 7 – 17 days at 25 °C until sporulation. Then, 10 ml of sterilized distilled water was poured onto each sporulated plate and the conidia were dislodged by using the glass road cell spreader. The suspension was filtered through two layers of sterilized cheesecloth (Gargouri-Kammoun *et al.*, 2009) and the final concentration was adjusted to 5 x 10^5 conidia ml^{-1} with the help of hemocytometer. About 200µl of the already determined inoculum was kept in a 5 ml Falcon tube at 4 °C pending for inoculation (Malbrán *et al.*, 2012; He *et al.*, 2013a).

3.5.3. Inoculation

A single centrally positioned floret of two spikes per seedling pot were injected (Dill-Macky, 2003) at Zadok's growth stage 65 (Zadoks *et al.*, 1974) with the already prepared 10µl inoculum

of each *Fusarium* species. Control (check) spikes were inoculated in the same way by 10 µl of sterile distilled water. Simultaneously, the spikes were tagged and covered with polythene bags for 48 hours to maintain high humidity that can facilitate infection process (Chehri *et al.*, 2011; Christ *et al.*, 2011; He *et al.*, 2013b).

3.5.4. Inspection of disease development

Blighted spikelets per spike due to the infection of inoculated *Fusarium* spp. were carefully inspected at 7, 14, 21 and 28 days after inoculation (Šíp *et al.*, 2008, Malbrán *et al.*, 2012, He *et al.*, 2013b). Finally, each inoculated spike was harvested and re-isolation was performed to confirm the identity of the test pathogen.

3.5.5. Pathogenicity test data

The spikelet infection severity caused by each *Fusarium* spp. was recorded as a percentage of diseased spikelets over the total number of spikelets per spike (Stack and McMullen, 2011) at 7, 14, 21 and 28 days after inoculation. Then, the area under disease progress curve (AUDPC) for isolates of *Fusarium* spp. incorporated in pathogenicity test were determined as follows (Madden *et al.*, 2007):

$$\text{AUDPC} = \sum_{i=1}^{n} \left\{ \left(\frac{y_i + y_{i+1}}{2} \right) (t_i - t_{i-1}) \right\}$$

Where: AUDPC is the area under disease progress curve, n is total number of observation days at the ith observation, y_i is spikelet infection severity at the ith observation, t is time at the ith observation.

3.6. Data Analysis

3.6.1. Filed survey

The prevalence of FHB at district level was determined from the number of wheat fields infected by FHB dividing by the total wheat fields assessed in the district multiplied by 100.

The overall prevalence of FHB across SWE was determined from the sum of all infected wheat fields divided by the total wheat fields assessed and multiplied by 100 as described below:

$$\% \text{ Prevalence} = \frac{\text{Number of fields infected by FHB of wheat}}{\text{Total number of fields assessed}} \times 100$$

Fusarium head blight incidence, field severity, infected head severity and FHB index data were analyzed using three stage nested design procedure of SAS 9.3 statistical software (SAS, 2010). Means were separated using LSD test at significance levels of 0.05. The associations (correlation) of disease incidence, field severity, infected head severity and FHB index with altitude and previous crop were computed using CORR procedure of SAS 9.3 statistical software (SAS, 2010). The relationship of FHB incidence and severity with the independent variables was determined by performing linear regression and multiple regression analysis in SAS 9.3 (SAS, 2010). The three-stage nested model used in analyzing the survey data is described as follows:

$$y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \gamma_{k(ij)} + \varepsilon_{l(ijk)}$$

Where: y_{ijk} is the FHB disease intensity where peasant association k is nested within district J nested within zone i, μ is the overall mean, τ_i is the effect of the ith zone, $\beta_{j(i)}$ is the effect of the jth district within the ith zone, and $\gamma_{k(ij)}$ is the effect of the kth peasant association within the jth district and i^{th} zone, and $\varepsilon_{l(ijk)}$ is the error term.

3.6.2. Pathogenicity test

Analysis of variance for spikelet infection severity and AUDPC data was performed using the general linear model procedure of SAS version 9.3 statistical software (SAS, 2010). The treatment means were separated by LSD test at a probability level of 0.05. Spikelet infection rate of each inoculated species were determined by Minitab 17 software. The RCBD model

used for analyzing pathogenicity data (AUDPC and spikelet infection severity) is described as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where: Y_{ij} is the response (AUDPC or spikelet infection severity) for treatment i observed in block j, μ is the overall mean, α_i is the effect of the ith treatment, β_j is the effect of the jth block, ε_{ij} is the error term for the ith treatment in the jth block.

Finally, aggressiveness of *Fusarium* spp. used in pathogenicity test on Danda'a wheat variety was determined from spikelet infection severity and AUDPC (Mardi *et al.*, 2004; Contreras-Medina *et al.*, 2009).

4. RESULTS AND DISCUSSION

4.1. Occurrence and Extent of FHB on Wheat in Southwestern Ethiopia

4.1.1. Occurrence of FHB on wheat

Fusarium head blight disease of wheat was found wide spread across all districts inspected with an overall prevalence of 93.88 % in southwestern Ethiopia. At district level, the disease was 100 % prevalent in Seka-Chekorssa district of Jimma zone, 100 % in Bedele and Gechi districts of Buno-Bedele zone, 91.70 % in Dedo district of Jimma zone and 80 % in Begi district of West-Wollega zone (Table 2).

Table 2. Altitude ranges, occurrence and incidence of wheat scab by districts in SWE, 2017

Districts	Altitude range of assessed fields	Prevalence (%)	FHB incidence (%)	
			Range	**Mean***
Dedo	2328 – 2613	91.70	0 – 100	26.59[b]
Seka-Chekorssa	2051 – 2462	100	7.6 – 44.2	25.21[b]
Subtotal Jimma	**2051 – 2613**	**95.83**	**0 – 100**	**26.00**
Bedele	1949 – 2009	100	18 – 69.8	38.60[a]
Gechi	2140 – 2269	100	11.3 – 84.6	38.74[a]
Subtotal Buno-Bedele	**1949 - 2269**	**100**	**11.3 – 84.6**	**38.69**
Begi	1711 – 1951	80	0 – 53.2	13.82[c]
Overall	**1711 – 2613**	**93.88**	**0 – 100**	**28.47**
LSD				**10.31**

* Mean values in a column with different letters are significant at $p \leq 0.05$; LSD = least significant difference; FHB = *Fusarium* head blight

In the same way, 100 % prevalence of FHB on wheat was reported during 2014 main cropping season in Ari district of South Omo zone in SNNPR of Ethiopia (Mitiku and Eshete, 2016). Furthermore, in the neighboring country Kenya, prevalence of FHB on wheat fields was reported in an increasing trends from 97 % in 2006 across five agro-ecological zones of Nakuru (Muthomi *et al.*, 2007) to 100 % both in 2008 across 12 agro-ecological zones of Narok, Imenti-North and Nyandarua-North (Muthomi *et al.*, 2012) and in 2013 across three agro-ecological

zones of Narok County (Njeru *et al.*, 2016), respectively. All these shows, FHB of wheat becomes more prevalent in wheat grown areas through time both in Ethiopia and Kenya.

4.1.2. Incidence and severity of FHB on wheat

Results of field severity and infected head severity of FHB was significantly differed at $p < 0.01$ among zones, districts within zones and peasant association within districts and zones. In the same way, incidence and FHB index was varying significantly at $p < 0.01$ among zones and peasant association within districts and zones (Appendix Table 3).

4.1.2.1. Incidence of FHB

Results indicated that FHB was observed with varied incidences ranging from 0 – 100 % in Jimma zone, 11.3 – 84.6 % in Buno-Bedele zone and 0 – 53.2 % in West-Wollega zone during 2017 main cropping season (Table 2). Average incidence of FHB in wheat fields was 38.69 % in Buno-Bedele, 26.00 % in Jimma and 13.82 % in West-Wollega zones of Oromia region (Figure 7). Moreover, higher incidences were recorded in Gechi (38.7 %) and Bedele (38.6 %) districts of Buno-Bedele zone, followed by Dedo (26.6 %) and Seka-Chekorssa (25.2 %) districts of Jimma zone. In contrast, the lowest incidence was recorded from Begi (13.8 %) district of West-Wollega zone (Table 2). At peasant associations level, this investigation revealed that incidence was significantly varied at $p < 0.01$ among peasant associations with higher and statistically the same average incidences of 60.32 %, 48.60 %, 46.65 % and 40.77 % in wheat fields of Gito, Sidisa, Seko and Harotore, respectively. Whereas, lower incidences of 0 to 16.77 % were recorded from Atrosofa, Lalo-Nora, Chona, Aladu-Wabara and Sito peasant associations in descending manner (Table 4, Appendix Table 3).

According to a survey conducted in 1988 cropping season of Ethiopia, FHB incidence of 0 to 35 % was reported at farmer's fields in Holetta and Kulumsa areas, 0 to 56 % at experiment stations, 0 to 57 % at seed production fields and 0 to 84 % at state farms (Bekele and Karr, 1997). Almost after 25 years, during 2014 main cropping season FHB disease of wheat was reported with incidence of 10 to 47 % at farmer's fields in Ari district of South Omo zone, SNNPR, Ethiopia (Mitiku and Eshete, 2016). These indicated that FHB incidence has shown an increasing trend from 1988 to 2017 in Ethiopia. Similarly, in the neighboring country,

Kenya, FHB incidence of 67.7 to 87.5 % at Imenti-North and 74.5 and 84.9 % at Nyandarua-North was reported during 2008 (Muthomi *et al.*, 2012). Then after, during 2013, FHB incidence reaching up to 100 % was reported at Narok County. Kenya (Njeru *et al.*, 2016).
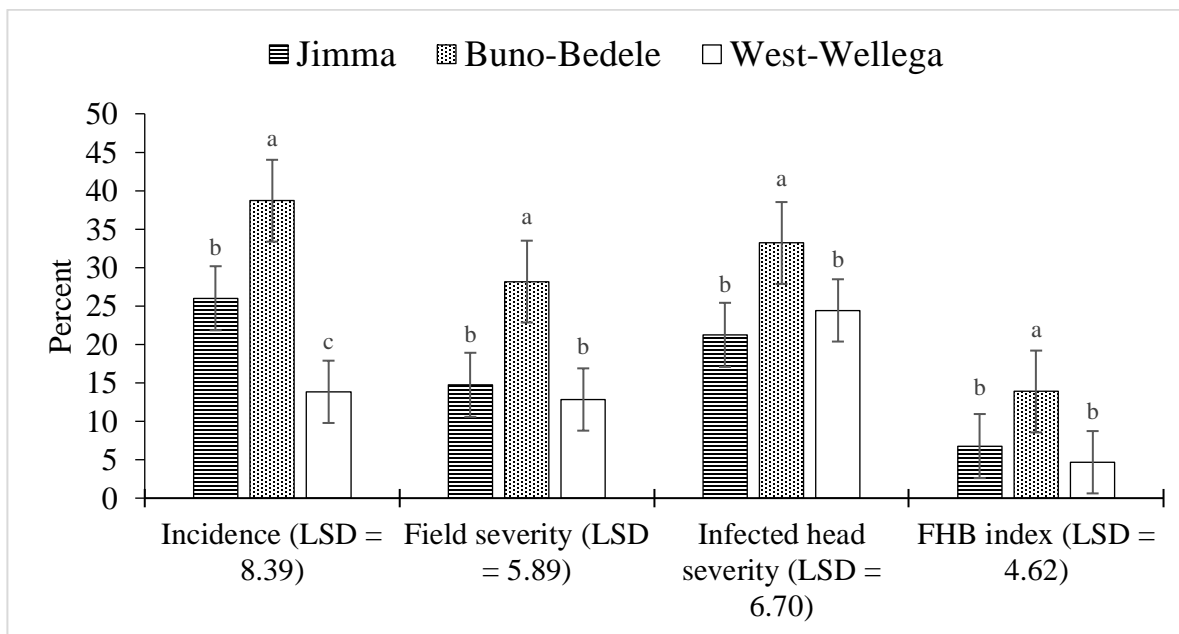
Figure 6. Mean incidence, field severity (average of all assessed heads including the zero scores) and infected head severity (average of all infected heads) of FHB during 2017 in three zones across southwestern Ethiopia. Bars with different letter for respective disease parameters are significantly different at p < 0.0001. LSD is least significant difference; FHB is *Fusarium* head blight.

4.1.2.2. Field severity, infected head severity and FHB index

As illustrated in figure 6, wheat crops showed the highest FHB infections in Buno-Bedele zone having 28.17 % field severity, 33.19 % infected head severity and 13.87 % FHB index. This indicated that FHB of wheat was severe in Buno-Bedele zone than the other zones. On the other hand, statistically, the same field severity (14.74 % and 12.83 %), infected head severity (21.24 % and 24.42 %) and FHB index (6.77 % and 4.67 %) were recorded in Jimma and West-Wollega zones, respectively (Figure 6).

At district levels, the severity of FHB on wheat fields was high in Bedele and Gechi districts of Buno-Bedele zone. The average field severity was 30.10 % in Bedele and 26.94 % in Gechi districts of Buno-Bedele zone. In addition, higher severity of infected heads was observed on wheat crops grown in Bedele and Gechi districts (Table 3). On the other hand, lower and statistically similar field severities of FHB were recorded in wheat fields across Seka-Chekorssa (18 %) and Dedo (12.29 %) districts of Jimma zone and Begi (12.83 %) district of West-Wollega zone (Table 3). In addition, FHB was a problem to wheat during 2017 main cropping season in parts of SWE, where the FHB index ranged from 4.67 % to 14.69 % in infected districts. Higher FHB index of 14.69 % in Gechi and 12.59 % in Bedele districts of Buno-Bedele zone (Table 3).

Table 3. Ranges and means of FHB severity and index by districts in SWE, during 2017

Districts	Field severity (%)		Infected head severity (%)		FHB index (%)	
	Range	**Mean***	**Range**	**Mean***	**Range**	**Mean***
Dedo	0.0 – 59.7	12.29[b]	0.0 – 59.7	16.71[c]	0 – 59.7	7.45[ab]
Seka-Chekorssa	3.8 – 36.8	18.00[b]	10.7 – 44.6	27.28[b]	0.5 – 13.3	5.85[c]
Bedele	17.4 – 45.4	30.10[a]	22.4 – 50.4	37.25[a]	3.9 – 22.5	12.59[ab]
Gechi	3.9 – 48.3	26.94[a]	9.6 – 49.0	30.60[ab]	0.4 – 38.2	14.69[a]
Begi	0.0 – 41.6	12.83[b]	0.0 – 53.7	24.42[bc]	0 – 21.3	4.67[c]
Overall		**19.41**		**26.32**		**9.04**
LSD		**7.24**		**8.23**		**5.68**

* Mean values in a column with different letters are significant at $p \leq 0.05$; LSD = least significant difference; FHB = *Fusarium* head blight; SWE = southwestern Ethiopia

The results of this study revealed that field severity, infected head severity and FHB index was significantly varied ($p < 0.01$) among peasant associations (Appendix Table 3) with higher field severity and infected head severity ranged from 35.2 to 48.04 % and 40.69 to 50.83 % at Gito, Mergamute, Seko and Sidisa, respectively. Statistically, comparable infected head severity was recorded from Harotore (35.48 %) peasant association of Bedele district (Table 4). Besides, the highest FHB index was recorded from Gito (30.02 %) and Sidisa (20.36 %). These indicated that Gito and Sidisa peasant associations were contributed more share to FHB infection of wheat in Gechi and Bedele districts (Table 4), respectively.

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Table 4. Mean percent of FHB incidence, field severity, infected head severity and FHB index on wheat by districts and peasant associations in SWE, 2017 main cropping season

District	Peasant association	Incidence	Field severity	Infected head severity	FHB index*
Bedele	Digaja	30.33[b-f]	21.69[c-f]	30.45[b-f]	6.05[ef]
	Harotore	40.77[a-d]	28.90[b-d]	35.48[a-d]	12.81[b-e]
	Mergamute	38.63[b-d]	45.40[a]	50.83[a]	17.23[b-d]
	Sidisa	48.60[ab]	35.23[a-c]	42.60[ab]	20.36[ab]
	Bido-Jiren	39.38[b-d]	21.08[d-f]	25.93[c-g]	13.54[b-e]
Gechi	Chona	15.80[e-g]	8.13[fg]	13.25[fg]	1.53[f]
	Gito	60.32[a]	48.04[a]	48.49[a]	30.02[a]
	Seko	46.65[a-c]	37.53[ab]	40.69[a-c]	18.78[bc]
	Ilala	24.42[def]	11.28[fg]	18.16[fg]	4.09[ef]
Dedo	Gerima-Guda	34.91[b-e]	17.13[def]	20.46[d-g]	13.14[b-e]
	Gerima-Lamessa	23.41[def]	8.38[fg]	12.79[gh]	3.70[ef]
	Sito	0.00[g]	0.00[g]	0.00[h]	0.00[f]
	Ilketogobe	31.35[b-f]	25.33[b-e]	34.92[a-e]	8.62[c-f]
Seka-Chekorssa	Satema-Goru	27.52[c-f]	15.31[d-f]	21.70[d-g]	6.15[ef]
	Atrosofa	16.77[e-g]	13.35[e-g]	25.22[c-g]	2.79[ef]
Begi	Lalo-Nora	16.24[e-g]	13.99[e-g]	28.93[b-g]	5.32[ef]
	Aladu-Wabara	10.80[fg]	11.38[e-g]	18.77[e-g]	3.87[ef]
LSD		**20.78**	**14.02**	**16.49**	**11.13**

Mean values in a column with different letters are significant at p ≤ 0.05; LSD = least significant difference; * FHB index = *Fusarium* head blight index; CV = coefficient of variation

In North America, when FHB severity is above 10 % it was suggested to use fungicide application (De Wolf *et al.*, 2003). Also, in Brazil a 7 % severity (equivalent to one infected spikelet per spike) in a group of spikes at dough stage resulted in significant reduction in kernel weight per spike, 1000 seed weight and kernel infection (Casa *et al.*, 2004). In the current study, the average field severity of 12.83 to 30.10 % at district levels (Table 3) and 11.28 to 48.04 % in most of the peasant associations (Table 4) indicated that FHB disease of wheat is high in all the assessed zones that needs an intervention to minimize its probable risk on wheat production in southwestern Ethiopia.

4.2. Effect of Cultural Practices on FHB of Wheat in Southwestern Ethiopia

Results of the general linear model (GLM) and LSD tests showed that FHB incidence, was highly affected by the previous crop sown in the field, tillage frequency before sowing the wheat

crop and the wheat varieties cultivated in the study area. In the same way, FHB field severities and infected head severities were also significantly influenced by the previous crop sown in the field, tillage frequency before sowing the wheat crop, the grown wheat varieties and altitudes (agroecologies). In addition, FHB field severity and infected head severity was affected by weed infestation levels and sowing pattern, respectively (Appendix Table 4; Table 5).

The wheat seeds grown in SWE was obtained from different sources including non-governmental organizations, governmental organizations and farmers their own preserved seeds. The highest share of seed provider to farming communities were agricultural offices (36.54 %) of the study area, followed by AGP-II project, local co-operative association (for the farming community of Dedo district), AsARC (for the farming community of Begi district), and farmers their own preserved seed with a magnitude of 13.46 %. Besides, the Cascape project implemented by Jimma University was attributed for 9.62 % of seed source supply particularly to the wheat farming communities of Jimma and Buno-Bedele zones (Table 5).

This study recognized that there is a trial of seed production endeavors by clustering the wheat farmers, but no success was recorded in the study area. As a result, all of the seed provider, except farmer saved seeds, were obtained wheat seeds from other areas like Arsi where seed production was experienced and then distributed to the wheat farming communities of the study area.

As indicated in Table 5, the seed sources such as AGP-II, Cascape project, local co-operative association and agricultural offices were found to contribute more for the occurrence and severity of FHB disease of wheat across the three zones in SWE. The higher incidences were obtained from wheat fields sown by seed provided by AGP-II (47.73 %), Cacape project (40.88 %) and local co-operative association (36.21 %). Whereas, higher FHB field severity and infected head severity was obtained from wheat fields sown by seeds provided by AGP-II (36.42 %) and Cacape project (27.88 %). Also, wheat seeds provided by agricultural offices was attributed for higher infected head severity in the study area (Table 5). These can suggest a need of using *Fusarium* free wheat seeds as a seed source for wheat to prevent the entry of *Fusarium* inoculum in to wheat field. Indeed, it is obvious that seed borne pathogens can cause

enormous crop losses through blighted seedling and kernel infection (Kubiak and Korbas, 1999; Gärtner *et al.*, 2008).

This study revealed that the preceding crops such as finger millet, wheat, maize, tef, and soybean were attributed more to the occurrence and severity of FHB disease of wheat in Jimma, Buno-Bedele and West-Wollega zones of Oromia region, Ethiopia. The higher incidences were recorded from wheat fields previously planted by finger millet (53.2 %), wheat (36.2 %) and maize (35.7 %), followed by tef (29.1 %), potato (24.01 %) and soybean (14.2 %). There is no statistical difference between the prior crops including tef, potato and soybean regarding FHB incidences. However, higher field and infected head severities were recorded on wheat fields previously cultivated by finger millet, wheat and maize with a magnitude of 40.15 % and 48.67 %, 36.8 % and 44.6 %, and 26.25 % and 34.1 %, respectively (Table 5).

Obviously, wheat and maize are the main hosts for *Fusarium* spp. that cause FHB in wheat (Kuhnem *et al.*, 2015). Also, finger millet was assumed as a host for FHB associated *Fusarium* spp. such as *F. graminearum*, *F. culmorum*, *F. moniliforme*, *F. sporotrichoides*, *F. oxysporum* and *F. solani* were reported as seed borne pathogens of finger millet in India (Penugonda *et al.*, 2010; Sobha-Rani and Dorcas, 2016). In addition, the soilborne *Fusarium* spp. (*F. poae,* and other 21 *Fusarium* spp.) were also caused a pathogenic effect on finger millet seedlings in Nigeria (Akanmu *et al.*, 2013). On the bases of these, the pathogenic *F. graminearum, F. culmorum* and *F. poae* which were isolated from all the three zones enclosed by this study may use finger millet as a host and/or as a saprophytic survival. It might be because of this that higher FHB infection was recorded from wheat fields previously planted by finger millet.

Wheat growing farmers across southwestern Ethiopia practiced the traditional ox traction system tillage for land preparation. Particularly, two to five times ploughing of land before sowing the wheat crop was practiced in the study area. Many of the assessed farms (34.61 %) were ploughed five times, while 28.85 % and 26.92 % were tilled four times and three times, respectively (Table 5).

Table 5. The effect of altitude and agronomic practices on mean FHB disease intensity during 2017 in wheat fields across SWE

Agronomic practices	Class	Proportion of fields (%)	DI [a] (%)	FS [b] (%)	IHS [c] (%)	Agronomic practices	Class	Proportion of fields (%)	DI (%)	FS (%)	IHS (%)
	Barley	5.77	5.92cd	2.10d	7.64e	Altitude ranges	1711 – 2269	73.08	29.59	22.81a	32.15a
	Faba bean	5.77	11.04cd	6.19cd	12.31de	of assessed	2328-2613	26.92	25.53	11.84b	17.64b
	Finger millet	7.69	53.16a	40.15a	48.67a	fields	**LSD**		**NS**	**8.92**	**9.69**
	Field pea	3.85	12.61cd	9.20cd	16.73cde		July	48.08	30.97	17.7	24.57
	Maize	19.23	36.85ab	26.87ab	34.34a-c	Planting date	August	51.92	22.92	19.45	29.15
Previous crop	Potato	3.85	24.01bc	5.08cd	11.94de		**LSD**		**NS**	**NS**	**NS**
	Sorghum	5.77	0.00d	0.00d	0.00e		Low	57.69	24.33	18.99	28.51a
	Soybean	3.85	14.33b-d	12.80b-d	42.67ab	Weed	Medium	30.77	34.05	21.15	27.94ab
	Tef	28.85	28.88bc	19.43bc	28.04bcd	infestation [j]	High	11.54	19.71	9.94	16.40b
	Wheat	15.38	36.19ab	36.80a	44.61ab		**LSD**		**NS**	**NS**	**11.61**
	LSD		**23.88**	**16.4**	**18.76**		AGP-II [d]	13.46	47.73a	36.42a	40.81a
	2 times	9.62	4.99c	4.71b	13.97b		AgrOff [e]	36.54	25.35bc	20.65b	31.10ab
Tillage	3 times	26.92	22.43b	18.53a	29.80a		AsARC [f]	13.46	2.83d	2.69d	14.91cd
frequency	4 times	28.85	31.02ab	21.19a	26.20a	Source of Seed	Cascape-JU [g]	9.62	40.88ab	27.88ab	33.12ab
before of sowing	5 times	34.61	36.16a	21.59a	26.17a		Farm saved	13.46	14.24cd	4.30cd	9.29d
	LSD		**9.39**	**6.44**	**7.37**		LC [h]	13.46	36.21ab	18.87bc	25.08bc
	Row	73.08	28.47	21.17a	31.39a		**LSD**		**20.16**	**14.72**	**14.72**
Sowing	Broadcast	26.92	22.79	12.14b	19.58b	Fertilizer	Unfertilized	34.62	22.02	16.06	26.13
pattern	**LSD**		**NS**	**8.42**	**9.1**	application	25 – 50	25	32.55	19.13	25.03
						(Kg ha⁻¹)	100	40.38	27.32	20.47	28.82
							LSD		**NS**	**NS**	**NS**

Mean values with the same letter within a column did not significantly differ at $p < 0.05$; LSD = least significant difference; FHB = *Fusarium* head blight; [a] DI is disease incidence; [b] FS is field severity; [c] IHS is infected head severity; [d] The second agricultural growth program of Ethiopia; [e] Agricultural offices of the respective districts; [f] Assosa agricultural research Centre; [g] Cascape projected implemented by Jimma University; [h] local cooperative in Dedo district; [i] areas with 1500 – 2300 is cool sub-humid (*Woina-dega*) and 2300 – 3200 cool and humid (*Dega*) (MOA, 1998); [j] Weed infestation was recorded as low (for low weed infestation); medium (moderate weed infestation) and high (no weeded fields).

Usually, in the highlands of Ethiopia land preparation was traditionally by paired oxen and four times ploughing before wheat seeding was a common practice among the farmers in Ethiopia (Taa *et al.*, 2000). In this study, higher FHB incidences and severities were recorded from wheat fields tilled three to five times before seeding the crop (Table 5). This might be because of the reason that land tillage practiced in Ethiopia does not totally bury the left-over crop residues into soil, therefore, the farmers either left the remnants as it is or collected them together in small humps within the field. These left-over remnants may help for the saprophytic survival of *Fusarium* propagules that can act as a source of primary inoculum for the succeeding cropping season. Even in case of tractor ploughing, the more intensive tillage did not decrease the FHB incidence as compared to conventional one and sometimes tended to increase it. Possibly due to the returning effect of buried crop residues to the soil surface (Lenc, 2015). To overcome this, inverted tillage (moldboard) was recommended to totally buried the *Fusarium*-infected crop residues deep into soil in developed countries that used tractors for land preparation (Dill-Macky and Jones, 2000; Pereyra *et al.*, 2004; Lenc, 2015; Hofgaard *et al.*, 2016).

Several studies in other countries indicated that agronomic practices in the field such as preceding crops, fertilization, use of pesticides, crop variety, tillage and cultivation have impact on the diversity and spread of *Fusarium* pathogens that caused FHB on wheat (Dill-Macky, 2008; Fernandez *et al.*, 2008; Katz, 2008; Wegulo *et al.*, 2015). Accordingly, maize as preceding crop, cereal rich rotation and zero (minimal) soil tillage favored the spread of *Fusarium* infection on cereals (Fernandez *et al.*, 2008; Wegulo *et al.*, 2015).These clearly implies the fact that preceding crops play a great role in promoting FHB severity on wheat crops grown in the succeeding cropping season either by being a suitable host which increasing the inoculum levels within the field or by producing bulky crop debris suitable for the saprophytic survival (Dill-Macky and Jones, 2000; Beyer *et al.*, 2007a). Remarkably, all the *Fusarium* spp. that cause FHB disease are capable of surviving as saprophytes (Parry *et al.*, 1995) on a range of crop residues including corn, small grain cereals and numerous other grass species and become a primary source of inoculum for FHB disease of Wheat (Keller *et al.*, 2003; Dill-Macky, 2008; Pereyra and Dill-Macky, 2008).

According to the study conducted in Uruguay, 12 *Fusarium* spp. (namely *F. graminearum, F. culmorum, F. tricinctum, F. avenaceum, F. sambucinum, F. semitectum, F. poae, F. acuminatum, F. verticillioides, F. equiseti, F. oxysporum* and *F. solani*) were recovered from residues of wheat, maize, barley, grass weed species and sunflower (Pereyra and Dill-Macky, 2008). In that study, higher colonization of *Fusarium* spp. were observed on residues of wheat and barley than maize, but maize residues can be a sources of primary inoculum for three years (Pereyra and Dill-Macky, 2008).

Study on the effect of crop rotation on FHB development reported that FHB incidence was higher (23.8 %) in soft winter wheat planted following corn-soybean rotation, whereas lower incidence of 0.9 – 6.0 % was recorded in soft winter wheat planted next to corn-pea rotation (Del Ponte *et al.*, 2003). It was reported that field pea stubble harbor *F. graminearum* that would likely become a potential inoculum source reservoir for a wheat crop growing in the following year (Chongo *et al.*, 2001) Greater disease intensity was also reported in wheat fields directly sown into corn residues, when compared to those planted into soybean residue (Dill-Macky and Jones, 2000). Besides, the random distribution of FHB across the rotation reported by Del Ponte *et al.* (2003), indicates an existence of other primary sources of inocula such as aerial spores originated from residues of maize for more than two years within and/or outside of a wheat field (Phalip *et al.*, 2006; Beyer *et al.*, 2007a; Pereyra and Dill-Macky, 2008), windblown infected residues of wheat from one cereal field to the next and transportation of infected crop residues and seeds (Government-of-Alberta, 2018).

For instance, the viable spores of *F. graminearum* at lower atmosphere up to 182.88 m above ground (Maldonado-Ramírez, 2002; Schmale *et al.*, 2002; Del Ponte *et al.*, 2003), *Fusarium* spp. from the nearby surrounding host crops (Phalip *et al.*, 2006) and windblown ascospores from distant; over kilometers (Maldonado-Ramirez *et al.*, 2005) may act as a primary sources of inoculum for wheat infection during flowering through precipitation or gravitational settling (Keller *et al.*, 2014). As a result, wheat plants in fields without cereal residue may also develop FHB disease. In addition, monoculture, reduced tillage and reduced rotations had greatly

increased inoculum levels of *Fusarium* in soil (Dill-Macky and Jones, 2000; Fernandez *et al.*, 2008; Lenc, 2015).

Table 6. Multiple regression for wheat scab incidence and severity on independent variables in southwestern Ethiopia, 2017 main cropping season

Independent variables	FHB incidence			FHB Field severity		
	Coefficients	Type-III SS	P-values	Coefficients	Type-III SS	P-value
Intercept	-13.26	-	-	-23.46	-	-
Altitude (m)	11.90	448.14	0.317	14.86	698.17	0.042
Wheat variety	0.49	6.87	0.901	-1.24	44.69	0.657
Source of seed	-1.78	99.59	0.635	-0.25	1.98	0.925
Frequency of tillage	9.30	2878.02	0.014	6.13	1248.37	0.023
Sowing date	-2.28	42.07	0.758	4.65	175.24	0.381
Sowing pattern	-4.51	104.97	0.626	-2.23	25.68	0.736
Pervious crop	2.14	364.40	0.366	0.63	31.53	0.709
Weed infestation	-6.69	750.18	0.197	-6.62	735.28	0.077
Fertilizer application	0.06	304.08	0.409	0.03	88.50	0.533
R^2		28.3 %			31.3 %	
Adj. R^2		11.8 %			15.4 %	
Pr > F		0.118			0.069	
Intercept	-5.60			-4.08		
Rainfall [a]	0.044	3619.35	0.006	0.03	1773.28	0.008
R^2		15.3 %			14.0 %	
Adj. R^2		13.4 %			12.2 %	

[a] The total rainfall from August to November 2017 during the period when wheat was flowering to hard dough stages; FHB = *Fusarium* head blight; SS = sum of squares

The linear regression analysis revealed that 28.3 % of the variability of FHB incidence and 31.3 % of the variability of field severity were explained by the nine explanatory variables (Table 6). The information brought by the total explanatory variables was not significantly better than what a basic mean would bring for both incidence and field severity. However, frequency of tillage before wheat seeding bring significant information to explain the variability in FHB incidence and field severity. Also, altitude bring significant information to explain the variability of FHB field severity in southwestern Ethiopia. In addition to these, 15.3 % of variability in incidence and 14.0 % of variability in field severity was explained significantly

(p < 0.05) by the total rainfall occurred during wheat flowering to hard dough stages (Table 6). Therefore, frequency of tillage before wheat seeding, altitude and the total rainfall occurred during wheat flowering to hard dough stages were the most influential variable in explaining the variability of FHB in southwestern Ethiopia (Table 6).

4.3. Field Reactions of Wheat Varieties to FHB

Across southwestern Ethiopia, a total of four released wheat varieties (namely Danda'a, Digalu, Kakaba and Kubsa) and one man made variety *Triticale* (which is called as a local cultivar by farming communities of the area) were grown either by row planting (73.08 %) or broadcasting (26.92 %) (Table 5) with field proportion of 50.00 %, 25.00 %, 6.25 %, 6.25 % and 12.50 %, respectively (Table 7).

Table 7. Field reactions of wheat and *Triticale* cultivars to FHB as measured by mean FHB incidence, field severity and infected head severity, 2017

Varieties	Synonym	Year of release	Proportion of fields (%)	Incidence (%)	Field severity (%)	Infected head Severity (%)	FHB index (%)
Danda'a	Danphe#1	2010	50.00	30.46[ab]	21.67[b]	30.59[a]	8.75[ab]
Digalu	HAR-3116	2005	25.00	32.3[ab]	21.84[b]	28.94[a]	10.49[ab]
Kakaba	Picaflor#1	2010	6.25	15.19[c]	18.04[bc]	36.22[a]	5.04[bc]
Kubsa	HAR-1685	1995	6.25	43.12[a]	32.66[a]	38.95[a]	13.99[a]
Triticale	-	-	12.50	14.24[c]	4.30[c]	9.29[b]	0.77[c]
LSD				**12.99**	**8.87**	**10.47**	**7.03**

Mean values with the same letter within a column did not significantly differ at p < 0.05 according to LSD test; LSD = least significant difference; FHB = *Fusarium* head blight disease of wheat

This study found that wheat variety Kubsa was highly infected by FHB having a mean incidence, infected head severity, field severity and FHB index of 43.12 %, 38.95 %, 32.66 % and 13.99 %, respectively. Following Kubsa, the variety Kakaba had showed higher infected head severity (36.22 %), but it had moderate field severity (18.04 %) (Table 7).

Digalu and Danda'a varieties had FHB infections of 32.30 % and 30.46 % incidence, 21.84 % and 21.67 % field severities, 28.94 % and 30.59 % infected head severities, and 10.49 % and 8.75 % FHB index, respectively (Table 7). Similarly, higher severity of 40.11 % was previously

reported on Danda'a variety in South Omo zone, SNNP, Ethiopia (Mitiku and Eshete, 2016). This investigation found that all the released bread wheat varieties grown in southwestern Ethiopia have sustained FHB index ranged from 5.04 % to 13.99 % (Table 7), while the higher and statistically the same FHB index of 13.99 %, 10.49 % and 8.75 % were recorded on Kubsa, Digalu and Danda'a varieties, respectively (Table 7). These indicated that the two most popularly grown wheat varieties (Danda'a and Digalu) were vulnerable to FHB disease of wheat like that of Kubsa variety. This suggests that FHB could pose a major threat to wheat production in the three zones in case an epidemic broke out under favorable weather conditions. This therefore calls the need of searching resistant wheat varieties from all available wheat varieties of Ethiopia in order to intervene the probable risk of FHB in the country at large.

In contrast, lower mean incidence (14.24 %), field severity (4.30 %), infected head severity (9.29 %) and FHB index (0.77 %) were recorded on *Triticale* cultivar across southwestern Ethiopia (Table 7). The *Triticale* cultivars grown in southwestern Ethiopia have a longer plant height that can reached up to 150 cm. According to the study conducted during 2013 and 2014 in Ottawa, Ontario, Canada, taller Eastern spring wheat varieties were showed strong negative relationship with FHB index, meaning that the spikes of the taller plants were less prone to FHB propagules. Also, the microclimate of taller plants are less favorable to FHB disease due to lower relative humidity (Moidu *et al.*, 2015). It might be because of these that the *Triticale* is less vulnerable to FHB infection in southwestern Ethiopia. It needs further investigation to confirm whether plant height or other trait attributed for less FHB damage on *Triticale*.

This study concurred with previous studies conducted in Ethiopia, which reported low levels of FHB infection on local (landrace) cultivars grown by farmers particularly in Arsi, Bale, Gojam, Gonder, and Shoa areas of Ethiopia (Bekele, 1990) and in South Omo zone of Ethiopia (Mitiku and Eshete, 2016). This may need further study to identify the trait that contribute for low infection of FHB in local (landrace) cultivars and then will be used in wheat breeding programs for the development of resistance in wheat varieties.

4.4. Relationship Between FHB Severities, Previous Crop and Altitude

Pearson correlation analysis indicated that there is highly significant (P < 0.01) and strong direct relationship between disease incidence and field severity (r = 0.86), incidence and infected head severity (r = 0.69), incidence and FHB index (r = 0.95), field severity and infected head severity (r = 0.89), field severity and FHB index (r = 0.91) and infected head severity and FHB index (r = 0.77) (Table 8). In the same way, strong and direct correlations between FHB severity and incidence was reported from Kenya having correlation coefficient r = 0.647 (P < 0.001) (Muthomi *et al.*, 2008) and from Passo Fundo, Brazil having r = 0.84 (P < 0.01) (Del Ponte *et al.*, 2005).

Table 8. Pearson correlation analysis to assess the relationship between incidence, field and infected field severity, FHB index and other factors during 2017 cropping season of Ethiopia

	Previous crop	FHB 1ncidence	Field severity	Infected Head severity	FHB index
Altitude (m)	0.35[*]	0.09	-0.16	-0.36[*]	-0.05
Previous crop	1	0.02	-0.05	-0.21	-0.09
FHB incidence		1	0.87[**]	0.69[**]	0.95[**]
Field severity			1	0.89[**]	0.91[**]
Infected head severity				1	0.77[**]
FHB index					1

* Significant at P < 0.05; ** significant at P < 0.01 (Pr. < 0.0001)
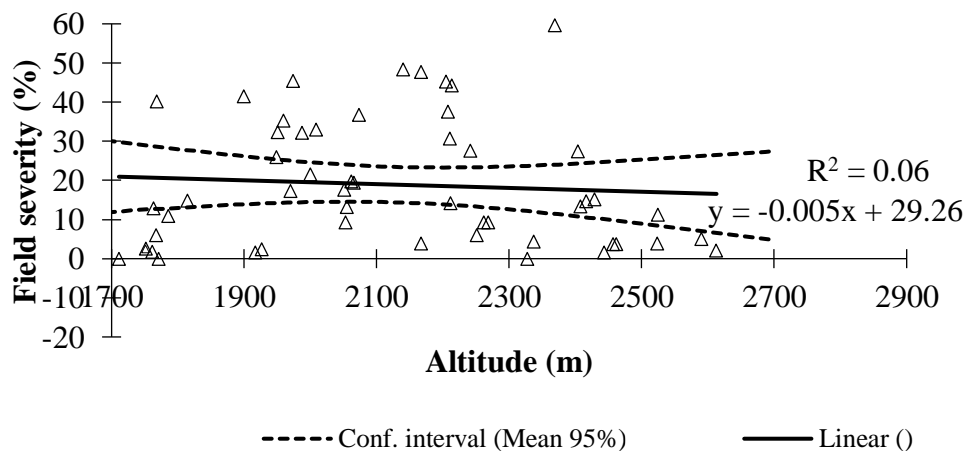
Figure 7. Relationship between field severity of FHB and altitude in SWE

Moreover, significant (P < 0.05) and moderately indirect relationships were observed among altitude and infected head severity (r = -0.36) (Table 8). These results revealed that field severity, infected head severity and FHB index becomes less in higher elevations (*Dega*) areas than mid-altitude (*Woina-dega*) areas (Table 5 & 8) and also 6 % of the variability in field severity was explained by altitude (Figure 7).

Infection of *Fusarium* species was largely dependent on the prevailing weather conditions which also determine disease severity. The risk of infection is associated with warm and humid conditions during flowering (Xu, 2003; Popovski and Celar, 2013). The temperature and rainfall of the surveyed areas between August and November 2017, the period when wheat was flowering to soft dough stages across the three zones in southwestern Ethiopia were shown in Appendix Table 1. During these periods, all the districts enclosed by this study had temperature ranged from 11.8 to 25.9 °C which accompanied by higher rainfall ranged from 40.68 to 323.58 mm. This favors the infection of wheat spikes by FHB pathogens in the study area. Because FHB infection was favored by the frequent rainfall, high humidity and heavy dew during flowering to soft dough stage (Osborne and Stein, 2007; Popovski and Celar, 2013; Nazari *et al.*, 2018; Schöneberg *et al.*, 2018)

4.5. Determination of *Fusarium* spp. Associated with Wheat Head Blight or Scab

A total of 269 single conidial purified strains were recovered from blighted wheat spikes collected during 2017 main cropping season in Jimma, Buno-Bedele and West-Wollega zones of Oromia, southwest Ethiopia. Based on their cultural and microscopic characteristics as indicated in '*Fusarium* species: An illustrated manual for identification', 'The *Fusarium* laboratory manual' and 'Monograph on the Genus *Fusarium*' (Nelson *et al.*, 1983; Leslie *et al.*, 2006; Refai *et al.*, 2015), all isolates were grouped into nine *Fusarium* species. In order of total isolation frequency, the identified *Fusarium* species are *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum*, *F. ussurianum*, *F. semitectum*, *F. lateritium*, *F. sambucinum* and *F. heterosporum* (Table 9; Figure 8). The cultural characteristics of each identified *Fusarium* spp. are provided in Appendix Table 6 & 7.

This investigation revealed that *Fusarium* spp. were isolated with varied frequencies from blighted spikes of wheat across SWE. This may be due factors such as field location, climatic conditions, soil management, crop rotation and cultivation methods (Scala *et al.*, 2016). *F. graminearum* and *F. culmorum* were the most frequent species comprised 29.0 % and 26.4 % of the total number of *Fusarium* isolates, respectively. Whereas, *F. avenaceum*, *F. poae*, *F. ussurianum*, *F. semitectum*, *F. sambucinum* *F. lateritium* and *F. heterosporum* made up of 10.4 %, 7.4 %, 6.7 %, 6.3 %, 6.0 %, 6.0 % and 1.9 %, respectively (Table 9).

Table 9. Isolation frequency (%) of identified *Fusarium* spp. from wheat blighted heads in SWE, 2017 main cropping season

Fusarium species	N	PDA	N	MGA	Total N	Total isolate (%)
F. graminearum Schwabe	46	28.6	32	29.6	78	29.0
F. culmorum (W.G. Smith) Saccardo	32	19.9	39	36.1	71	26.4
F. avenaceum (Fries) Saccardo	21	13.0	7	6.5	28	10.4
F. poae (Peck) Wollenweber	12	7.5	8	7.4	20	7.4
F. ussurianum T. Aoki, Gagkaeva, Yli-Mattila, Kistler & O'Donnell	12	7.5	6	5.6	18	6.7
F. semitectum Berkeley & Ravenel	12	7.5	5	4.6	17	6.3
F. sambucinum Fückel *sensu stricto*	10	6.2	6	5.6	16	6.0
F. lateritium Nees	13	8.1	3	2.8	16	6.0
F. heterosporum Nees *ex* Fries	3	1.9	2	1.9	5	1.9
Total	**161**		**108**		**269**	

Key: N = number of isolates; PDA = Potato Dextrose Agar; MGA = Malchet-Green Agar

The two dominate species namely *F. graminearum* and *F. culmorum* were mostly isolated from blighted wheat spikes sampled from Buno-Bedele zone having an isolation frequency of 44.9 % and 59.2 %, respectively (Table 10). Furthermore, when we noticed species distribution at district levels, *F. graminearum* was most frequently isolated from samples collected across Begi (24.4 %), Bedele (23.1 %) and Gechi (21.8 %), whereas *F. culmorum* was frequently isolated from samples of Gechi (39.4 %), Bedele (19.7 %) and Seka-Chekorssa (19.7 %) (Table 11).

Table 10. Distribution (%) of *Fusarium* spp. isolates in three wheat producing zones in SWE, 2017

Fusarium spp.	N	Jimma	Buno-Bedele	West-Wollega
F. graminearum	78	16 (20.5)	35 (44.9)	27 (34.6)
F. culmorum	71	18 (25.4)	42 (59.2)	11 (15.5)
F. lateritium	16	6 (37.5)	6 (37.5)	4 (25.0)
F. avenaceum	28	15 (53.6)	7 (25.0)	6 (21.4)
F. poae	20	5 (25.0)	6 (30.0)	9(45.0)
F. sambucinum	16	4 (25.0)	8 (50.0)	4 (25.0)
F. ussurianum	18	9 (50.0)	9 (50.0)	-
F. semitectum	17	9 (52.9)	5 (29.4)	3 (17.7)
F. heterosporum	5	2 (40.0)	2 (40.0)	1 (20.0)-

Values in parenthesis is percent frequency; N = isolation number; ⁻ shows the specie is not isolated from samples of the area

Based on this provisional identification, two *Fusarium* spp. namely *F. culmorum*, and *F. ussurianum* were recorded which were not reported by the previous study conducted in Ethiopia, though this needs further confirmation. On the other hand, *F. avenaceum*, *F. graminearum*, *F. poae*, *F. lateritium*, *F. sambucinum*, *F. semitectum* and *F. heterosporum* were recovered from stored wheat grains and blighted wheat heads sampled from wheat fields in Arsi, Bale, Gojam, Gonder, Shoa and Wollo areas (Bekele, 1990).

The occurrence and distribution of *Fusarium* species can vary with the changing climate, crop rotation, cultivar resistance and interactions among different species (Xu *et al.*, 2005). For instance, in some parts of Europe, the predominant species were varied among *F. graminearum,*

F. poae, F. avenaceum and *F. culmorum* (Xu *et al.*, 2005), however, *F. graminearum* was also reported in displacing the *F. culmorum* (Waalwijk *et al.*, 2003). In Belgium, a four-year investigation revealed that the most frequent causal agent of FHB in wheat was *F. graminearum* mainly in areas where corn was cultivated and *F. culmorum*, mainly in areas where small grains were grown (Isebaert *et al.*, 2009). This clearly revealed the effect of cultural practices on *Fusarium* species abundance.

Table 11. Distribution of *Fusarium* spp. by districts in SWE, 2017

Fusarium species	Dedo	Seka-Chekorssa	Bedele	Gechi	Begi
F. graminearum	8 (10.3)	8 (10.3)	18 (23.1)	17 (21.8)	19 (24.4)
F. culmorum	4 (5.6)	14 (19.7)	14 (19.7)	28 (39.4)	8 (11.3)
F. poae	3 (15.0)	2 (10.0)	3 (15.00)	3 (15.0)	5 (25.0)
F. avenaceum	5 (19.2)	10 (38.5)	2 (7.7)	5 (19.2)	4 (15.4)
F. ussurianum	4 (22.2)	5 (27.8)	6 (33.3)	3 (16.7)	-
F. semitectum	4 (23.5)	5 (29.4)	-	5 (29.4)	2 (11.8)
F. lateritium	1 (6.3)	5 (31.3)	3 (18.8)	3 (18.8)	3 (18.8)
F. sambucinum	4 (25.0)	-	3 (18.8)	5 (31.3)	1 (6.3)
F. heterosporum	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)-

Values in parenthesis is percent frequency; ‾ shows the specie is not isolated from samples of the area

In addition to blighted wheat heads, *F. culmorum, F. graminearum* and *F. avenaceum* were isolated from crown rot of bread wheat and durum wheat in Turkey (Gebremariam *et al.*, 2018). Also, in Nebraska, *F. graminearum, F. avenaceum* and *F. culmorum* were recovered from root rot of corn, soybean and wheat (Parikh *et al.*, 2018). In particular, *F. culmorum* was responsible in causing higher seedling blight, whereas *F. graminearum* were severely caused crown rot resulted in greatest yield reduction in wheat (Dyer *et al.*, 2009; Kazan and Gardiner, 2018).

F. graminearum

F. culmorum

F. avenaceum

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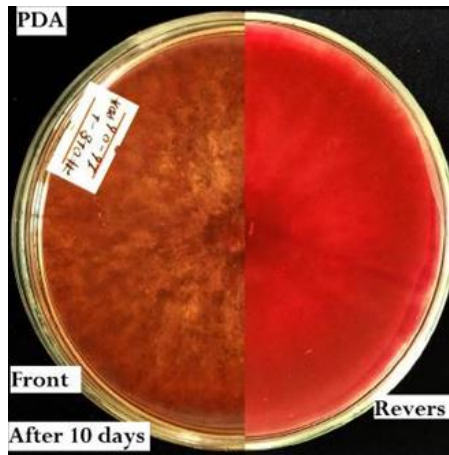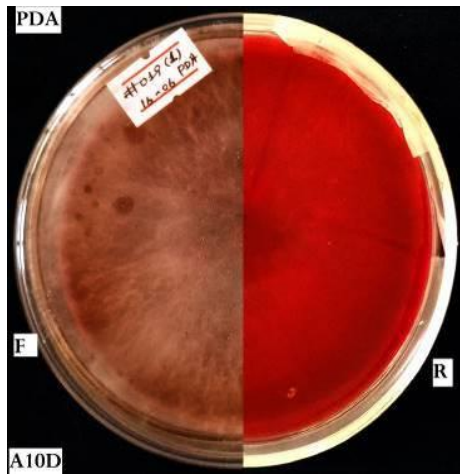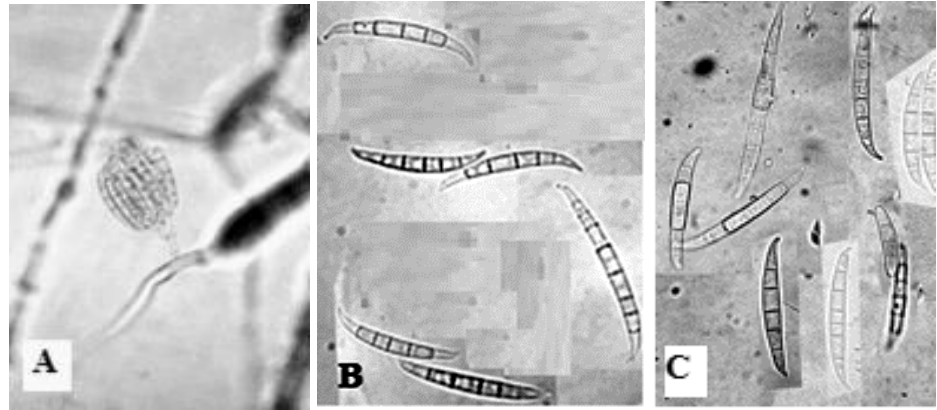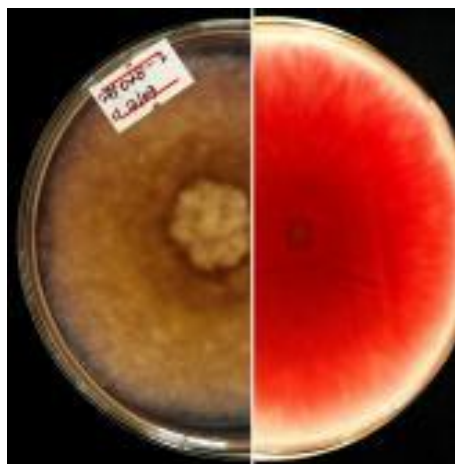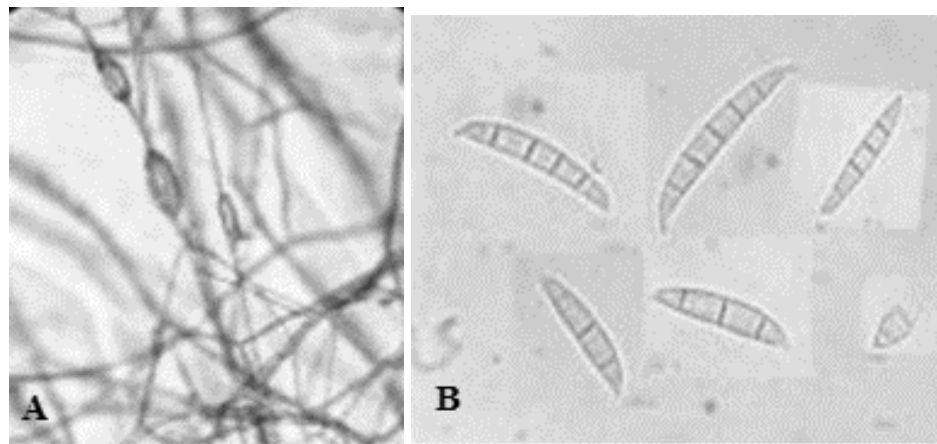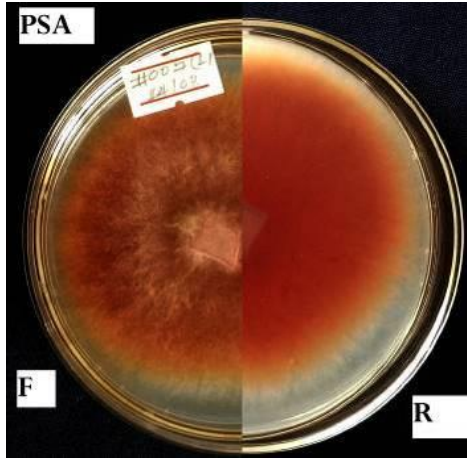
F. lateritium
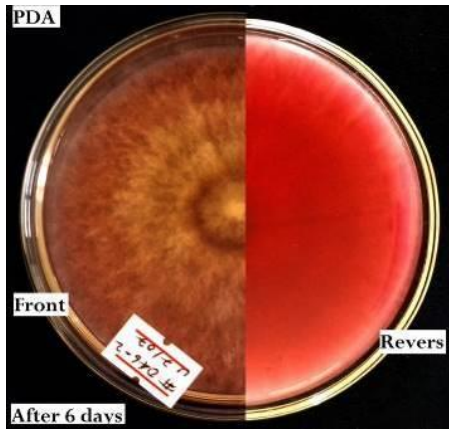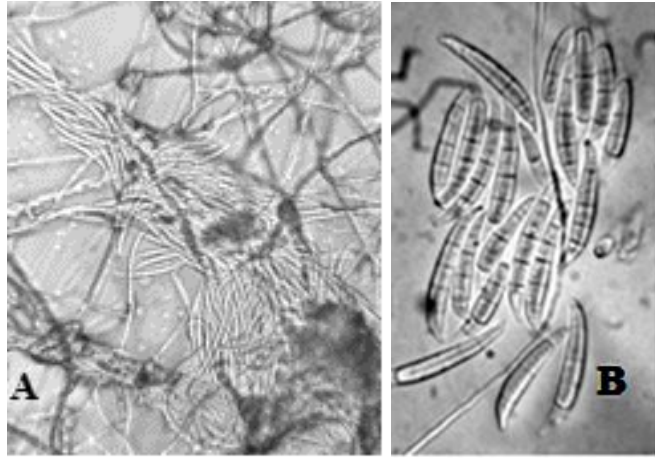
F. poae
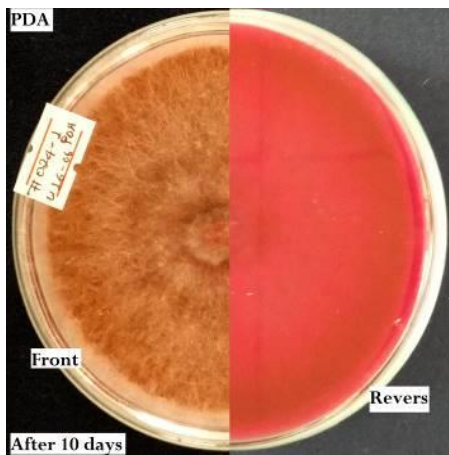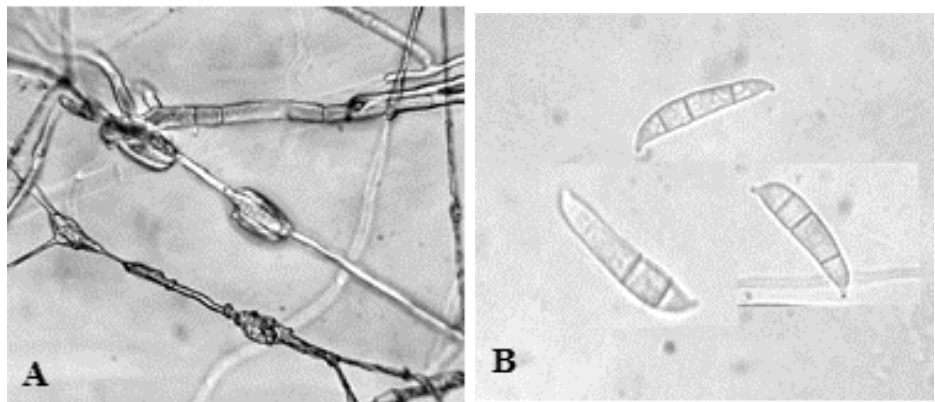
F. semitectum
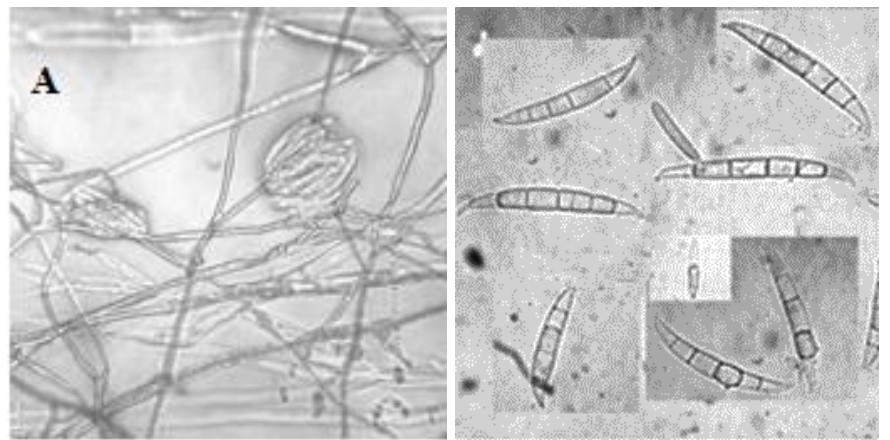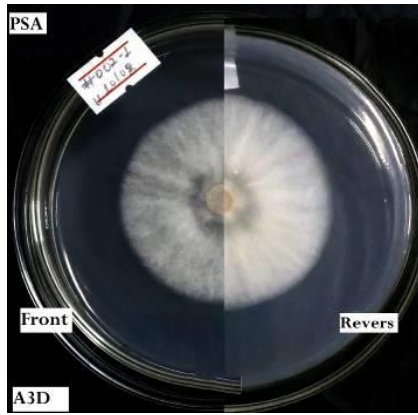
F. ussurianum
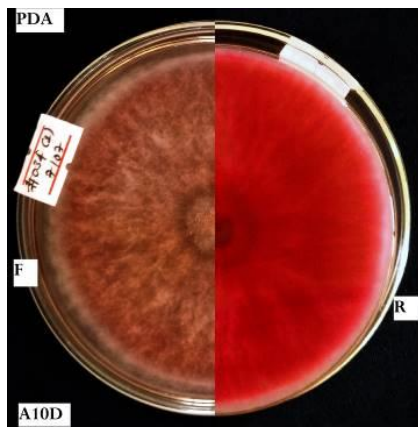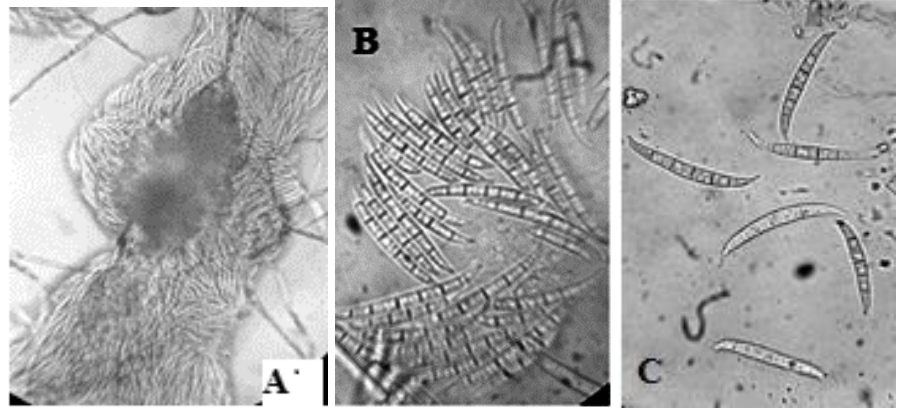
F. sambucinum
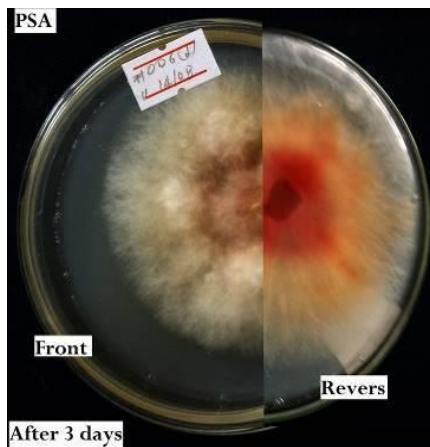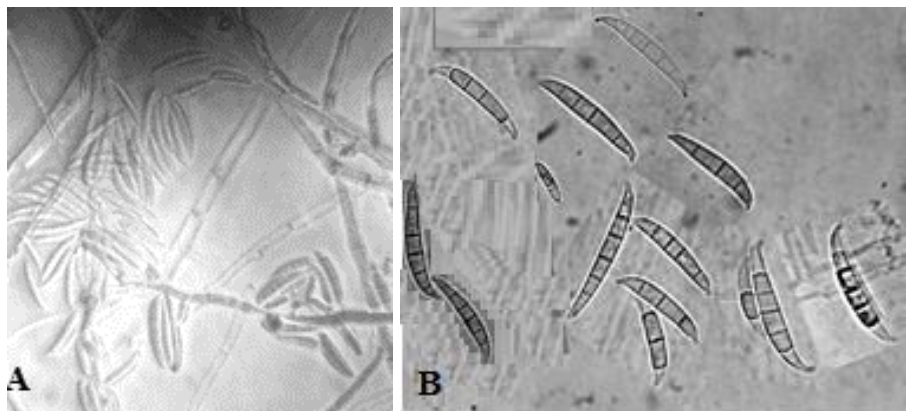
F. heterosporum
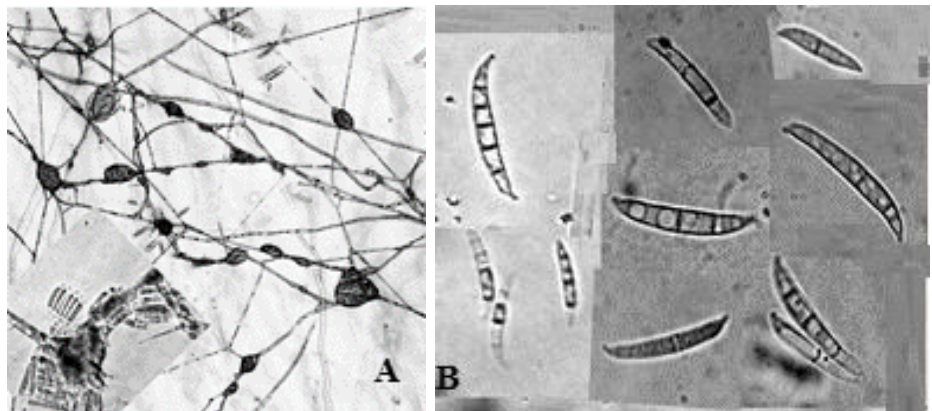
Figure 8. *Fusarium* spp. isolated from blighted wheat spikes sampled across Southwestern Ethiopia, during 2017 main cropping season. Figure 8-A are conidiophores and Figure 8. B – C are conidia of the respective *Fusarium* specie

F. sambucinum and *F. avenaceum* were also reported among the dominant *Fusarium* species that caused dry rot of potatoes in South Africa (Scala *et al.*, 2016). Isolates of *F. sambucinum* (recovered from potato tubers)*, F. avenaceum* (recovered from potato tubers, alfalfa and clover), *F. graminearum* (recovered from cereals) were pathogenic in causing dry rot of potato, while isolates of *F. poae* were weak to none pathogenic to potato (Peters *et al.*, 2008).

4.6. Pathogenicity Test

The pathogenicity of all *Fusarium* spp. identified in this study was assessed using point (single spikelet) injection method (Dill-Macky, 2003). The results indicated that all the tested *Fusarium* spp. caused FHB symptoms on spikes of Danda'a variety. However, no FHB symptoms were observed on spikes inoculated with sterile distilled water (control). Re-isolation from the inoculated kernels agrees with descriptions of the inoculated species, which confirms their pathogenicity under Lath-house condition.

Table 12. Blighted spikelet severity and AUDPC of *Fusarium* spp. under lath-house, 2018

Fusarium spp.	Spikelet infection severity				AUDPC	r	R^2 (%)
	7 DAI	14 DAI	21 DAI	28 DAI			
F. avenaceum	2.6^{cd}	30.5^{ab}	70.7^a	100^a	1067.2^a	0.51**	64.62
F. poae	9.6^a	35.9^a	74.4^a	74.5^{ab}	1066.3^a	0.52**	85.58
F. sambucinum	6.1^{abc}	16.3^{abcd}	52.3^{abc}	83.1^a	792.4^{ab}	0.11	4.50
F. lateritium	5.5^{bc}	21.9^{abc}	54.9^{ab}	85.6^a	856.2^{ab}	0.43**	70.45
F. culmorum	6.0^{abc}	20.3^{abcd}	46.7^{bc}	88.9^a	801.3^{ab}	0.52*	44.51
F. heterosporum	6.4^{ab}	22.9^{abc}	40.9^{bc}	57.8^{ab}	670.9^b	0.26*	45.29
F. graminearum	4.9^{bc}	13.2^{bcd}	29.1^{cd}	66.8^{ab}	546.8^b	0.21**	43.19
F. ussurianum	0.0^d	3.0^{cd}	7.1^{de}	29.8^{cd}	175.2^c	0.42**	60.36
F. semitectum	0.0^d	0.0^d	0.0^e	33.2^{bc}	116.2^c	0.12	5.58
Sterilized distilled water	0.00^d	0.0^d	0.0^e	0.0^c	0.0^c	-	-
LSD	**3.8**	**20.4**	**23.7**	**45.8**	**358.7**		

Mean values in a column with different letters are significant at p ≤ 0.05; AUDPC = area under disease progress curve; DAI = days after inoculation; LSD = least significant difference; r = rate of spikelet bleaching

Fusarium isolates showed significantly varied spikelet infection and AUDPC on Danda'a wheat variety. *F. avenaceum was* the most aggressive species that caused the highest spikelet infection

severity of 100 % at 28 days after inoculation and AUDPC of 1067.2 on spikes of Danda'a variety (Table 12). This specie was isolated from samples collected from the five assessed districts in southwestern Ethiopia (Table 11). It was mainly isolated from samples of Jimma zone (Table 10) particularly in Seka-Chekorssa that attributed 38.5 % isolation frequency (Table 11). Besides, *F. avenaceum* was the third most frequently isolated spp. next to *F. graminearum* and *F. culmorum* (Table 9). Also, *F. culmorum, F. lateritium, F. sambucinum, F. poae, F. graminearum* and *F. heterosporum* were produced statistically comparable spikelet infection severity with that of *F. avenaceum* (Table 12). Likewise, *F. avenaceum, F. poae, F. sambucinum, F. lateritium and F. culmorum* were generated statistically similar AUDPC as compared to that of *F. avenaceum*. However, *F. ussurianum* and *F. semitectum* produced lower AUDPC of 175.2 and 116.2, respectively (Table 12).

All of the nine *Fusarium* spp. showed different rate of FHB development on Danda'a wheat variety (Table 12). Seven of the tested species were able to cause FHB symptom at 7 days after inoculation (DAI). This finding almost agrees with the comparative aggressiveness study conducted in Canada that reported *F. graminearum, F. avenaceum, F. culmorum* and *F. poae* were produced visible infections at 21 and 28 days after inoculation on wheat spikes (Xue *et al.*, 2004). On the other hand, delayed symptom development was observed by *F. ussurianum* and *F. semitectum* until seven and 21 days after inoculation (Table 12).

Based on spikelet infection severity and AUDPC results, *F. avenaceum, F. poae, F. sambucinum, F. lateritium, F. culmorum, F. heterosporum* and *F. graminearum* were more aggressive on Danda'a wheat variety. All of them had caused spikelet infection severity and AUDPC beyond or equal to 57.8 % and 546.8, respectively (Table 12). Whereas, *F. semitectum* and *F. ussurianum* showed less aggressiveness on Danda'a variety with spikelet infection severity of 33.19 % and 29.78 % and AUDPC of 116.2 and 175.2, respectively (Table 12). This finding concurred with the aggressiveness study that reported *F. graminearum* and *F. culmorum* as an aggressive species causing more than 35 % of infected spikelets of wheat in Canada (Xue *et al.*, 2004).

5. SUMMARY AND CONCLUSION

5.1. Summary

Wheat is the main cereal crop that provides 284 kcal/capita/day for more than 90 million people in Ethiopia. However, its cultivation was affected by biotic, abiotic, technical and socio-economic factors. Fungal disease such as *Puccinia* spp., *Septoria* spp. and *Fusarium* spp. are among the main biotic threats to wheat production. Globally, *Fusarium* head blight (FHB) caused by *Fusarium* spp. emerged as one of the destructive fungal diseases of wheat in humid and semi-humid regions. Also, the pathogens of FHB infect several cereals and caused reduction in grain yield, quality and feeding values of grains.

Fusarium head blight of wheat was 93.9 % prevalent across Jimma, Buno-Bedele and West-Wollega zones of Oromia in southwestern Ethiopia. The incidence of FHB varied from 0 – 100 % (26 %), 11.3 – 84.6 % (38.69 %) and 0 – 53.2 % (13.8 %) respectively in Jimma, Buno-Bedele and West-Wollega zones (the value in the parenthesis are average incidence of the respective zone). Severity of FHB was high in Buno-Bedele with field severity, infected head severity and FHB index of 28.17 %, 33.19 % and 13.87 %, respectively. Whereas, lower field severity of 14.7 % and 12.8 % were recorded on wheat fields of Jimma and West-Wollega zones, respectively. In southwestern Ethiopia, the simple linear regression analysis identified the most influential variables in explaining the variability of FHB incidence and severity were frequency of tillage before wheat seeding, altitude and the total rainfall occurred during wheat flowering to hard dough stages.

This study found that varieties under production in SWE had variable field resistance to FHB. Kubsa was highly susceptible, sustaining an average incidence of 43.12 % and average field severity of 32.66 %. Digalu, Danda'a and Kakaba varieties showed moderate field resistance having the mean field severity values of 21.84 %, 21.67 % and 18.04 %, respectively. Most interestingly, the *Triticale* cultivar had better field resistance to FHB. It had the lowest average incidence of 14.24 % and field severity of 4.30 % as compared to the rest aforementioned varieties. This may perhaps necessitate further study for clear identification of traits attributed

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for low FHB infection on *Triticale* cultivars and these traits might be helpful in wheat breeding programs for FHB resistance development.

In addition, the preceding cereals like finger millet, wheat and maize has attributed more to the occurrence and severity of FHB disease in wheat fields. Higher FHB incidence and field severity was observed in wheat fields previously planted to finger millet (53.16 % and 40.15 %), wheat (36.19 % and 36.8 %) and maize (35.67 % and 26.25 %).

A total of 269 single conidial purified isolates were obtained from blighted wheat spikes sampled from 52 wheat fields across Jimma, Buno-Bedele and West-Wollega zones of Oromia in southwestern Ethiopia. Based on their colony and microscopical characteristics, all isolates were grouped in to nine *Fusarium* species. Out of these, two *Fusarium* spp. (namely *F. culmorum* and *F. ussurianum*) were not reported in the previous study conducted in Ethiopia, though this needs further confirmation. Among the nine *Fusarium* spp., *F. graminearum* (29.0 %) and *F. culmorum* (26.4 %) are the most frequently recovered species followed by *F. avenaceum* (10.4 %), while the rest are isolated between 1.9 to 7.4 % frequencies.

The results of pathogenicity test indicated that all the tested *Fusarium* spp. cause FHB symptoms in spikes of Danda'a variety with varied infection rates. Among the nine *Fusarium* spp., *F. avenaceum, F. poae, F. culmorum, F. lateritium, F. sambucinum, F. heterosporum* and *F. graminearum* were more aggressive ones, while *F. ussurianum* and *F. semitectum* were less aggressive as compared to earlier ones.

5.2. Conclusions

This investigation provided information on the extent of FHB disease of wheat and *Fusarium* pathogens that caused blighted spikes of wheat in southwestern Ethiopia as well as their pathogenicity status in causing FHB on Danda'a wheat variety. This study concluded that FHB of wheat is becoming important fungal disease of wheat in southwestern Ethiopia, particularly in Buno-Bedele zone of Oromia region. As well, FHB disease is severe on high yielding improved bread wheat varieties introduced to the localities as compared to *Triticale* variety (which showed low FHB infections). Among the nine identified *Fusarium* spp., *F.*

graminearum and *F. culmorum* were most frequently recovered from blighted wheat spikes in southwestern Ethiopia. Besides, all the identified *Fusarium* species were pathogenic to Danda'a wheat variety (which was grown in 50 % of wheat fields in southwestern Ethiopia). Based on their spikelet infection severity and AUDPC results, *F. avenaceum, F. poae, F. culmorum, F. lateritium, F. sambucinum, F. heterosporum* and *F. graminearum* were more aggressive to Danda'a variety.

This study suggests the need of finding short term solutions such as fungicides efficacy evaluation and evaluation of all the available bread wheat varieties for their resistance to FHB pathogens (*F. graminearum, F. culmorum, F. avenaceum, F. poae, F. heterosporum*, *F. lateritium*, *F. culmorum* and *F. sambucinum*). Additionally, FHB surveillances will be demanded to know FHB pathogen diversity (if possible phylogenetically) and the associated mycotoxins in Ethiopia. As a long-term solution, due emphasize will be necessitated for the development of resistance in wheat varieties and for integrated FHB disease management to minimize the probable risk of FHB in the country.

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7. APPENDICES

Appendix Table 1. Monthly and annual temperature and rainfall of the study area [a] in 2017 main cropping season

Districts	Mean temperature ranges (°C) [b]				Total rain fall (mm)				Relative humidity (%)			
	Aug	Sept	Oct	Nov	Aug	Sept	Oct	Nov	Aug	Sept	Oct	Nov
Dedo	12.7 – 25.5	12.3 – 25.8	12.3 – 25.4	12.2 –25.3	170.38	170.08	220.28	120.38	-	-	-	-
Seka-Chekorssa	-	-	-	-	255.78	323.58	166.38	40.68	79.8	79.7	72.7	
Bedele [c]	13.1 – 23.5	13.1 – 24.9	13.0 – 25.4	11.8 –25.9	312.8	311.0	263.3	117.7	84.1	83.8	75.7	76.7
Begi	15.2 – 24.3	15.0 – 24.4	15.3 – 25.7	14.0 – 25.9	144.9	203.6	95.2	41.3	79.9	79.9	76.0	72.1

[a] The main wheat growing season of the study area is from July to end of December

[b] Temperature range is minimum – maximum

[c] Used to represent both Bedele and Gechi districts in this study

[-] Indicates no available meteorological data

Source: Jimma and Assosa Meteorological Centers

Appendix Table 2. Altitude, date of wheat sowing, growth stages during survey and size of wheat fields by districts, 2017

Districts	Altitude (m)[b]	Sowing dates	Growth stage [a]	Farm size (ha)
Dedo	1711 - 2613	July 14 - Aug 1, 2017	Early milk - Soft dough	0.01 – 3.00
Seka Chekorssa	2051 - 2462	July 22 - Aug 26, 2017	Early dough - Hard dough	0.13 – 0.50
Gechi	2140 - 2269	July 22 - Aug 8, 2017	Early milk - Hard dough	0.25 – 3.00
Bedele	1949 - 2009	July 8 - Aug 9, 2017	Early dough - Hard dough	0.13 – 0.38
Begi	1711 - 1951	July 8 - Aug 31, 2017	Early milk - Hard dough	0.01 – 0.50

[a] During survey periods; [b] Altitude ranges across the study area, where the survey was conducted

Appendix B: Analysis of Variance (ANOVA) Tables

Appendix Table 3. Nested ANOVA table for mean squares incidence and severity of FHB

Source	Degree of freedom	FHB incidence	Field Severity	Infected head severity	FHB index
Model	47	2017.25^{**}	1059.23^{**}	1088.22^{**}	580.06^{**}
Zones	2	8645.28^{**}	5460.81^{**}	3857.87^{**}	1703.43^{**}
Districts (Zones)	2	55.41^{ns}	662.33^{**}	2012.06^{**}	37.28^{ns}
PA(Zones*Districts)	12	1482.66^{**}	1120.84^{**}	1042.87^{**}	519.16^{**}
Error	175	166.9	85.1	165.7	44.05
Mean		**28.47**	**19.41**	**26.32**	**9.04**
CV (%)		**45.37**	**47.53**	**48.91**	**76.42**

^{ns} not significant at $p<0.05$; ** significant at $p < 0.01$; FHB = *Fusarium* head blight; ANOVA = Analysis of variance

Appendix Table 4. ANOVA table of mean squares for the effect of altitude and agronomic practices on FHB of wheat intensity

Source	Degree of freedom	Disease incidence	Field severity	Infected head severity
Model	26	2606.11^{**}	1481.81^{**}	1626.07^{**}
Previous crop	9	1447.90^{**}	467.08^{**}	879.92^{**}
Weed infestation	2	841.36^{ns}	619.15	407.71^{**}
Frequency of tillage	3	4948.67^{**}	1837.89^{**}	1541.52^{**}
Altitude	1	195.58^{ns}	586.39^{*}	331.60^{*}
Wheat variety	4	3350.82^{**}	2103.39^{**}	1881.19^{**}
Sowing pattern	1	134.01^{ns}	380.11^{ns}	1097.86^{*}
Sowing date	1	237.26^{ns}	40.88^{ns}	29.72^{ns}
Seed source	4	710.91^{ns}	158.43^{ns}	183.51^{ns}
Fertilizer application	2	94.41^{ns}	83.72^{ns}	85.38^{ns}
Error	170	392.42	185.07	242.08
CV (%)		**69.54**	**70.20**	**49.86**

^{ns} not significant at $p < 0.05$; * significant at $p < 0.05$; ** significant at $p < 0.01$; ANOVA = Analysis of variance

Appendix Table 5. ANOVA table of blighted spikelet severity and AUDPC mean squares for *Fusarium* spp.

Sources	DF	Spikelet infection severity				AUDPC
		7 DAI	14 DAI	21 DAI	28 DAI	
Model	11	26.9**	401.3*	1912.0**	2493.4**	374732.7**
Replication	2	0.7	119.7	179.8	97.2	20372.4
Treatments	9	32.8**	463.9*	2297.0**	3025.9**	450134.2**
Error	18	5.0	141.1	191.6	711.6	43719.5
CV (%)		54.4	72.5	36.8	43.1	34.3

* significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; AUDPC = Area under disease progress curve; ANOVA = Analysis of variance; DAI = days after inoculation

Appendix C: Culture Characteristics of Isolated *Fusarium* spp.

Appendix Table 6. *Fusarium* spp. cultural characteristics on PDA

Fusarium spp.	Colony growth	Colony color above	Reveres pigmentation	Arial mycelium
F. graminearum	Rapid	White, White-pink, pale-goldenrod, corn-silk	Red, violet red, deep red	Dense
F. culmorum	Rapid	White, white-pink, light-goldenrod	Violet, red, deep-red	Dense
F. lateritium	Relatively slow	White, pale pink	Deep pink, reddish-orange	Sparse
F. avenaceum	Rapid	White, light goldenrod, greyish-rose	Red, deep-pink	Dense
F. poae	Rapid	White	Red, Hot-pink	Dense
F. semitectum	Rapid	White, corn-silk, snow	Red, violet, pink	Dense
F. ussurianum	Relatively slow	White, grayish-brown, reddish-white	Red, brownish-red, violet, white	Dense
F. sambucinum	Rapid	White, tan, wheat	Red, light-pink, light-goldenrod	Dense
F. heterosporum	Rapid	White, pinkish-white	Light-goldenrod, Tan orange	Dense

PDA = Potato dextrose agar

Appendix Table 7. Diameter of colony growth (mm) after three days for *Fusarium* spp. incubated at 25 °C in dark condition

Fusarium spp.	PDA (9 cm Petri plates)	PSA (9 cm Petri plates)	PDA (race tubes)
F. graminearum	50.0 – 90.0	66.5 – 79.5	48 – 62
F. culmorum	45.7 – 90.0	77.5 – 84.5	59 – 80
F. lateritium	22.0 – 60.0	58.0 – 79.0	48 – 58
F. avenaceum	69.5 – 88.5	70.0 – 75.0	52 – 60
F. poae	77.0 – 90.0	70.0 – 81.0	53 – 70
F. semitectum	42.0 – 90.0	47.0 – 73.0	40 – 51
F. ussurianum	20.0 – 70.0	18.5 – 76.0	18 – 45
F. sambucinum	39.5 – 85.0	74.5 – 76.0	56 – 60
F. heterosporum	47.5 – 54.5	54.5 – 58.5	44 – 60

PDA = Potato dextrose agar; PSA = Potato sucrose agar

Appendix D: Check List Used

Data sheet for Prevalence and Intensity of FHB of Wheat in Southwestern Ethiopia (2017)

Part-I: General information about farm

Field code: _____, Date (dd/mm/yy) _____; Farmer's name: _____ Region

_____ Zone _____ District; _____, PA(Kebele) _____, Location (site of

field) _____

Part-II: Field and crop mgt information

1. Field information:

1.1 Size of the wheat field (in *ha*) _____

1.2 GPS data: Latitude (N)

1.3 Longitude (E)

1.4 Altitude (m) _____

1.5 Variety_____ seed sources _____

1.6 Growth stage: Milky (ZGS 73-77) ☐ dough (ZGS 83-85) ☐

　　Hard dough (ZGS 87) ☐

2. Crop management:

2.1 Frequency of tillage/ plough before sowing _____

2.2 Sowing date (dd/mm/yy) _____. Normal　Early ☐　Late ☐　☐

2.3 Panting/sowing pattern: Row　　Broadcast ☐

2.4 Field history (previous crop) _____

2.5 Field sanitation: Weedy or poor　Good ☐　In-between ☐　☐

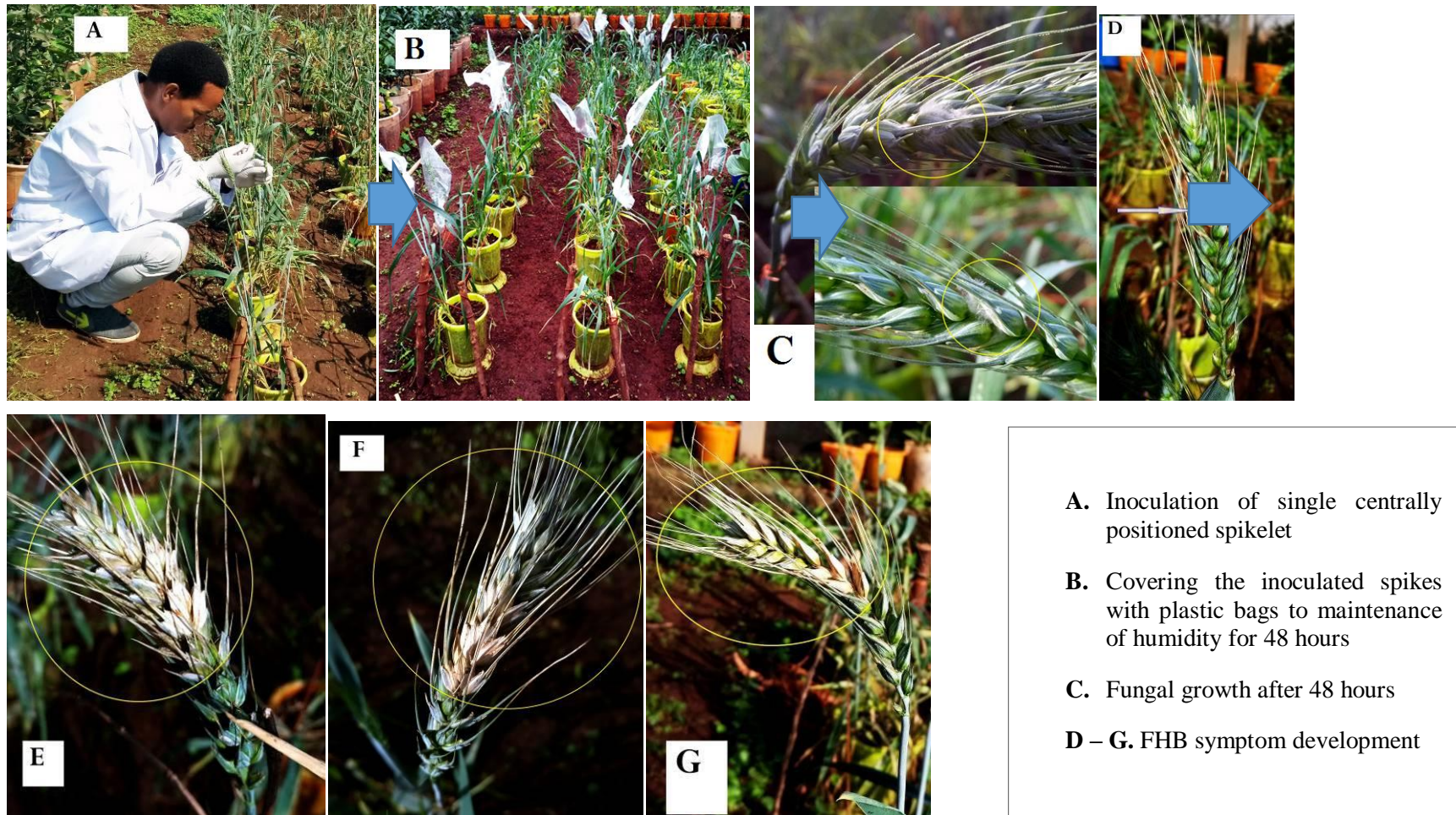2.6 Fertilizer applied DAP(Kg/ha) ____ Urea (Kg/ha) ____ Compost(t/ha) ____

2.7 Crop stand/performance: good　fair ☐　bad ☐　☐

3. Incidence and severity of FHB of wheat

# Q per field	DI	Severity of FHB on wheat spikes per quadrat									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Q1											
Q2											
Q3											
Q4											

Key: Q is quadrat; DI is Disease incidence; #Q is number of quadrats per field; S1 - S10 is # of spikes assessed for severity.

Appendix E: List of Figures in the Appendix

Appendix Figure 1. Procedures followed during pathogenicity test

A. Inoculation of single centrally positioned spikelet

B. Covering the inoculated spikes with plastic bags to maintenance of humidity for 48 hours

C. Fungal growth after 48 hours

D – G. FHB symptom development