CHARACTERIZATION OF *Gibberella xylarioides* ISOLATES AND TESTING THE REACTIONS OF SOME COFFEE COLLECTIONS IN THE FOREST POPULATIONS IN SOUTHWEST AND SOUTHEAST ETHIOPIA

M.Sc. Thesis

Sihen Getachew Yimer

January, 2011
Jimma University

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Sihen Getachew Yimer

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SCHOOL OF GRADUATE STUDIES

JIMMA UNIVERSITY

As Thesis research advisor, I hereby certify that I have read and evaluated this Thesis prepared under my guidance by Sihen Getachew Yimer, entitled: "**Characterization of *Gibberella xylarioides* isolates and testing the reactions of some coffee collections in the forest populations in southwest and southeast Ethiopia"** and I recommend that it be submitted as fulfilling the Thesis requirement.

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As members of the examining Board of the final MSc. Thesis Open Defense, we certify that we have read and evaluated the thesis prepared by **Sihen Getachew Yimer** and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Plant Pathology.

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DEDICATION

I dedicate this thesis to my Sister, Seblewerk Getachew Yimer.

STATEMENT OF AUTHOR

I declare that this thesis is my original 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 at the Jimma University College of Agriculture and Veterinary Medicine and is deposited at the Jimma University Library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author was borne on July 23, 1986 G.C in Tenta District, North Wello of Amahara Region. She attended elementary and high school education from Semptember, 1992-2001 G.C at Abiote Elementary and Adjibar Senior Secondary School. Between 2001 and 2003 G.C, she attended preparatory education at Dessie Hotie Preparatory and Comprehensive High School. She joined Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), at Department of Crop Production and Protection in October, 2004 and graduated with B.Sc. degree in June 30, 2007. Soon after her graduation, she was employed by the Ministry of Education (MoE) and has been working at Wollega University in Agronomic Irrigation Department as assistance graduate (instructor). After serving for a year, she joined Jimma University School of Graduate Studies to persue her M.Sc. study in Plant Pathology in October 2008.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASIC	Association International du Café
CABI	Center for Agricalture and Bioscience international
CBD	Coffee berry disease
CLR	Coffee leaf rust
CSA	Central Statistics Authority
CV	Coefficient of variation
CWD	Coffee wilt disease
DMRT	Duncan's Multiple Range Test
DF	degree of freedom
ETB	Ethiopian Birr
g	gram
EEA	Ethiopian Economic Association
EIAR	Ethiopian Institute of Agricultural Research
GDP	Gross domestic product
ha	hectare
hr	Hour
IAR	Institute of Agricultural Research
JARC	Jimma Agricultural Research Center
kg	kilogram
l	litre
m	meter
masl	Meter above sea level
ml	Milliliter
MCTD	Ministry of Coffee and Tea Development
MS	mean squares
MSTAT-C	Microcomputer statistics, software
NFPA	National Forest Priority Area
NMSA	National Meteorology Service Agency of Ethiopia
PCR	Polymerase Chain Reaction

PDA	Potato Dextrose Agar
PSA	Potato Sucrose Agar
RAPD	Random amplified polymorphic DNA
RGB	Red Green Blue
SAS	Statistical analysis system, software
SNA	Synthetic low nutrient agar
SNNPR	Southern Nations Nationalities and Peoples Region
SPSS	Statistical program for social sciences
SS	Some of squares
USD	United States Dollar
μm	Micrometer
μl	Microlitre
WARDO	Woreda Agricultural and Rural Development Office

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CHARACTERIZATION OF *Gibberella xylarioides* ISOLATES AND TESTING THE REACTIONS OF SOME COFFEE COLLECTIONS IN THE FOREST POPULATIONS IN SOUTHWEST AND SOUTHEAST ETHIOPIA

ABSTRACT

Coffee wilt disease which is caused by Gibberella xylarioides (Fusarium xylarioides) is one of the highly destructive diseases that affect plantations and smallholder coffees resulting in yield reduction, and also affecting forest coffee. However, the characteristics and variability of the pathogen, and the reactions of forest coffee accessions against the pathogen are not well studied in the forest coffee areas of southwest and southeast Ethiopia. Thus, the objectives of this study were to characterize and determine the variability of Gibberella xylarioides populations and also to test and determine the reactions of some coffee accessions collected in the major forest coffee areas of southwest and southeast Ethiopia. These studies were conducted both in the field, laboratory and greenhouse. Disease assessment and sample collection was conducted in four rainforest coffee areas (three fields/location) of Bonga (Keffa), Sheko (Brehane-Kontir), Yayu (Ilubabor) and Harenna (Bale) in southwest and southeast Ethiopia and the laborartory and greenhouse study works were conducted. The experimental design of this experiment was laidout as complete randomized design (CRD) with three replications in laboratory study while evaluations of coffee accessions and the host-pathogen interaction studies were also conducted in randomized complete block design (RCBD) with 5 isolates x 13 accessions factorial treatment combinations replicated three times. Coffee wilt was prevalent in most assessed forest coffee trees, semi-forest and plantation coffee trees and its incidence significantly increased with coffee tree losses that ranged from 12.8% to 27.6% at Bonga (Kaffa) and at Sheko (Brehan-kontir) in the southwest. The disease was more severe in Harenna (Bale) varying between 26.2 and 28.5% in southeast forest coffee areas. In cultural and morphological characterization of Gibberella xylarioides populations, most of the isolates from the southwest forest coffee areas had similar appearances in colony pigmentations, aerial and radial growths but slightly different from those isolates collected in the southeast forest coffee (Harenna). Morphologically, most of the isolates were similar in features like shape and size of both conidia (macro and micro) and septations. The reactions of 14 accessions collected in some forest coffee areas of Bonga and Yayu (southwest) and Harenna (southeast) were tested at seedling stage by inoculating with Yayu isolate (Y21) in the greenhouse at JARC. The result indicated highly significant differences among accessions ranging between resistant and susceptible host reactions. Accessions of Harenna HA1, HA2 and HA6 had very low (0 – 10 %) seedling deaths being highly resistant while accessions of Bonga and Yayu (BO1, BO2, YA1 and YA2) showed complete seedling death (90%) being highly susceptible to the pathogen isolates in the forest. There were highly significant differences ($p < 0.01$) both in percent seedling deaths and incubation periods in the host-pathogen interactions study where by seedlings of 12 coffee accessions were inoculated with four Gibberella xylarioides isolates representing forest populations of both organisms. Based on the results, the accessions of Harenna (P4 and P6) revealed resistance reaction to most isolates with low mean percent seedling deaths (21.9 and 21.5%); whereas Sheko (P38) and Bonga (P27) accessions were susceptible to most isolates and showed high mean percent seedling death (85.7 and 79.2%), respectively. Harenna isolate was more aggressive than most of the isolates including the one from plantation coffee (Gera). In comparing the combined forest coffee accessions by isolate interactions (differential effects), Harenna (H11) isolates induced a higher rate of percent seedling death on accessions P41, P47 (90%) and P49 (85.97%) than Sheko (SH21) isolate on the same accession of P41 (9.47%), P47 and P49 (0.0%). In conclusion, coffee wilt is an important disease of forest coffee impacting the conservation of wild coffee genetic

resources although there exist variations in host reactions to the pathogen. The fungus populations collected in these forest coffee areas showed basically similar cultural and morphological structures of the species Gibberella xylarioides although clear differences in some features like growth nature, pigmentation and aggressiveness. The pathogen strains in the forest coffee areas are as aggressive as those isolates in plantation coffee.

1. INTRODUCTION

The genus *Coffea* belongs to the family Rubiaceae and includes approximately 100 species (Stoffelen, 1998). The two main species of coffee cultivated on a world scale are Arabica coffee (*Coffea arabica* L.) and Robusta coffee (*Coffea canephora*), which account for about 98 – 99% of the world coffee production. Out of this, 90% of the world coffee market is based on arabica coffee (Cambrony, 1992; Coste, 1992; Wintgens, 2004). Both species can grow best on deep, free- draining, loamy soils, with a good water holding capacity and a slightly acid soil (P^H 5-6) and soil fertility is important for good production (Paulos, 1994).

Coffee is one of the most important commodities in the international agricultural trade, representing a significant source of income to several coffee producing countries including Ethiopia. The country's economy is based on agriculture, and about 90% of the population earns its livelihood from the land, mainly as subsistence farmers (EEA, 2001). Coffee is by far the number one export crop and, with 41%, contributes decisively to the country's foreign currency income, 25% employment opportunity and 10% of the government revenues. The average annual production amounts to more than 200,000 tones and 95% of the produce is from the garden, semi-forest and forest coffee systems by small-scale farmers while nearly 5-6% comes from large-scale plantation coffee (MoARD, 2008).

Arabica coffee (*Coffea arabica*) has its center of origin in the highlands of southwest and southeast Ethiopia where wild coffee populations grow naturally in the undergrowth of the Afromontane rainforests at altitudes between 1,000 and 2,000 m. The wild populations are highly endangered by land-use pressure on the montane rainforests (Demel, 1999, Tadesse *et al*., 2002; Tadesse, W. 2003; Feyera, 2006; Schmitt, 2006). Poverty and conflicting property rights make farmers convert forests into agricultural or pastoral land, thereby threatening the entire biodiversity of the forests. As wild coffee is collected by local people, the biodiversity of the forest habitat is also threatened by management interventions to increase the productivity of the wild coffee stands (Tadesse *et al.* 2002).

Ethiopia is the only country in the world where coffee grows wild as an understorey shrub or small tree in the afro-montane rainforests (Paulos and Demel, 2000). It is believed that forests

harbor a large genetic pool of Arabica coffee that represents a potential source to develop the crop for the benefit of present and future human generations in the world (Sylvian, 1958; Tefestewold, 1995). In this case, the economic value of *Coffee arabica* genetic resources contained in Ethiopian highland rainforests is estimated to amount around USD 1458 million and USD 420 million at 5 and 10% respective discount rates (Hein and Gatzweiler, 2005).

Diseases are the most important factors that contribute to the reduction of coffee production in Africa as well as in the world. In general, *Coffea arabica* is susceptible to most diseases attacking coffee compared to other *Coffea* species (Van der Graaff and Pieters, 1978; Bertrand *et al*., 2001). The major coffee diseases in Ethiopia are coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR). The former two are by far the most important diseases both in severity and prevalence (Arega *et al*. 2008; Girma *et al*., 2009).

Coffee wilt is the major disease caused by *Gibberella xylarioides* Heim & Saccas, a teleomorphic state of *Fusarium xylarioides* Steyaert, is a typical vascular disease commonly called tracheomycosis. Tracheomycosis or vascular wilt of coffee historically was first observed in 1927 on *Coffea excelsa* in Central Africa Republic and the causal pathogen was first identified and reported as *Fusarium xylarioides* in 1946 by Steyaert (Flood, 1997; Girma and Hindorf, 2001; Oduor *et al*., 2003). The disease was observed again in Zaire in the early 1980s (Flood, 1996) and noticed for the first time in Uganda in 1993, and since then it has been causing economic losses to robusta coffee in both countries (Flood and Brayford, 1997; CABI 2003).

In Ethiopia, the occurrence of *G. xylarioides* on *C. arabica* was established in the early 1970s by Kranz and Mogk (1973) after isolating and identifying the fungus from dying coffee trees. Survey works conducted so far in the major coffee growing areas have demonstrated that vascular wilt is becoming the main factor of coffee tree death in the country (Van der Graaff and Pieters 1978, Girma *et al*., 2009). The disease incidence seemed be high where coffee is grown under advanced cultural practices although it was said to be minimal in less managed forest coffee (Van der Graaff 1983). For instance, Merdassa (1986) assessed the incidence of the disease in single tree progenies of different coffee lines for six years (1979 - 1984) at Gera,

and obtained tree loss ranging from 0.3 to 87.0 %. It is apparent that tracheomycosis increasingly becoming more and more important, especially in plantation coffee. The actual disease assessment indicated that the incidence varied from 45% at Gera to about 69% at Bebeka (Girma *et al*., 2001b**)**. In addition, there were certain variations in the incidence of CWD between coffee fields at each locality that may be ascribed to differences in their genetic makeup and age of coffee cultivars, cultural practices and environmental condition at specific location**.** The CABI technical report indicated that the national incidence and severity of CWD in Ethiopia were 27.9% and 3%, respectively, in monitory terms it caused an estimate loss of more than 3.7 million US dollar annually (CABI, 2003).

The occurrence of CWD was reported after assessment in four forest coffee areas in south-west and south-east afromontane rainforests as Arega (2006) has very recently reported as high as 20% mean incidence of CWD in the southeast and southwest forest coffee areas of Bonga (Kafa), Sheko (Berehane kontir) Yayu (Ilubabor) and Harenna (Bale). He also noted increasing occurrence of CWD in some forest areas like in Harenna (Bale) and Bonga (Keffa). However, no isolates of *Gibberella xylarioides* was collected and characterized yet from these areas, although the damage is significantly impacting conservation forest coffee populations.

Nevertheless, one of the reasons speculated earlier for low incidence and severity of the disease in the forest coffee was less aggressiveness of the fungus population under such intact conditions (Girma, 2004). Thus, it is important to characterize and determine the fungus variability in cultural characteristics and pathogenicity in the forest coffee populations, and also examine the reactions of some coffee accessions collected in the major montane forest areas of southwest and southeast Ethiopia against the pathogen.

Objectives of the study

1. To characterize and determine the variability of *Gibberella xylarioides (Fusarium xylarioides)* populations and
2. To test and determine the reactions of some coffee accessions collected in the major forest coffee areas of southwest and southeast Ethiopia.

2. LITERATURE REVIEW

2.1. Coffee Production Systems in Ethiopia

Coffea arabica L. is indigenous to Ethiopia and the principal source of foreign currency. It is mainly produced in the southern, south western and eastern parts of the country. In Ethiopia, coffee production has passed several stages of production systems since its domestication which can be broadly grouped as forest coffee, semi forest coffee, garden coffee and plantation coffee (Workafes and Kassu, 2000).

2.1.1. Forest Coffee

Forests in southwestern Ethiopia are the primary center of origin and center of genetic diversity of *Coffea arabica* (Sylvian, 1958; Meyer, 1965; Melaku, 1984). According to Paulos and Demil (2000) wild animals and birds disseminating seeds within the forest community assist spontaneous regeneration. Wild coffee types with distinct phenotypic differences occurr in the forests around Sheko, Tepi and Bebeka; Gewata and Geisha in Kefa, Obacherko in Gera, and Geba-Doggi valley near Yayu in Illubabor and Eba forest in Anfillo, all in southwest Ethiopia. The average yield of forest coffee has been estimated to be in the order of 200-250 kg/ha (Paulos and Demel, 2000).

In the forest coffee production systems, coffee trees are self-growing and spontaneously regenerating under natural intact forest where different species of trees provide shade to the coffee. The forest is also covered by heterogeneous species of overhead shade trees. The system accommodates extremely diverse biological composition and heterogeneous in genetical and morphological characteristics of coffee which can serve as source of gene pool (Tadesse *et al.*, 2002). This system mostly accounts for 8.3% of total area (Alemayehu *et al*., 2008) and 10% of the total coffee production of the country (Degnet, 2004). Moreover, economic valuation of these forest coffee's genetic resources were estimated to be around 1458 million USD at 5% discount rate and 420 million USD at 10% discount rate, respectively (Hein and Gazwiler, 2005*)*. However, the forest coffee ecosystem is under severe threat of extinction. Currently, the only 2-3% of total area of the country remains covered with high forest and disappearing at an

alarming rate of 100,000-120,000 hectare per annum (Simayehu *et al*., 2008). These rate of destruction are attributed to population pressure (Degnet, 2004, Tadesse *et al*., 2008), continuous degradation, over exploitation of forest products and expansion of commercial plantation such as tea and coffee increasing settlement and land use pressure The management of forest coffee is limited to only a single slashing of the broad-leaved weeds at the beginning of the cropping season followed by harvesting (Tadesse *et al*., 2008).

2.1.2 Semiforest Coffee

The semiforest coffee production is similar to forest coffee except influenced by human intervention for management and use of naturally growing coffee trees in the forest. Farmers sometimes transplant self-regenerated coffee seedlings under the forest canopy to fill spaces between mother trees. Agronomic practices like thinning over story of forest trees and slashing could be applied to improve production and productivity and/or to facilitate harvesting. It is commonly found in Illubabor, Jimma, Keffa-Sheka, Benchi-Maji and west Wellega zones (Tadess *et al.*, 2002).

Forest coffee lands of considerable sizes that are located near the main roads, rural towns or peasant villages are covered with coffee trees standing in scattered manner and are managed with little cultural practices such as weeding and shade regulation (Workafes and Kassu, 2000). These types of plantations are known traditionally as semi-forest type and are believed to have evolved from forest coffee production system. According to MCTD (1992), it was estimated that semi-forest coffee occupies nearly 136,000 hectares (34%) of the total area of coffee land in the country. Currently, semi-forest production system accounts for 30% of total area (Alemayehu *et al*., 2008) and contributes about 35% to the total coffee production.

2.1.3. Garden Coffee

Garden coffee production system consists of small areas of backyard coffee found in patches near homestead and predominantly found in southern and eastern Hararghe regions. However, basically, it could exist in all the coffee producing regions of the country and in majority of the cases coffee trees are not shaded (Masresha, 1996) or light to medium shade levels are often maintained. In Sidamo, Gedeo, West Harerge and West Wellega, the observed coffee

production system is garden type, located near the residence houses and with an area of less than 0.5 hectares (Workafes and Kassu, 2000). Intercropping with different food crops like sorghum (*Sorghum bicolor*), maize (*Zea mays*), Chat (*Catha edulis*) and enset (*Ensete ventricosum*), frequent weeding (2-3) and farmyard manure and crop residue applications are practiced (Workafes and Kasu, 2000). Garden coffee is produced on about 56.7% of total coffee area in the country accounting for about 50% to the total coffee production of the country.

2.1.4. Plantation Coffee

Plantation coffee production system is an intensified coffee production on about 5% of total area (Alemayehu *et al*., 2008) and contributes about 5-6% to the national coffee production. The system includes large coffee plantations owned by the state farms (Coffee Plantation Development Enterprise, CPDE); small plantations owned by small-scale farmers and private investors. Particularly, CPDE practice pruning, mulching use recommended inorganic fertilization supplemented with organic fertilizers, integrated weed and pest management, shade regulation and planting of high yielder and disease resistant varieties for boosting production and productivity. Rejuvenation is also practiced when trees become exhausted (Workafes and Kassu, 2000).

2.2. Diseases of Arabica Coffee in Ethiopia

Many biotic and abiotic factors are the major constraints of coffee production in Ethiopia of which the most important once are diseases caused by fungi. About ten fungal diseases and one bacterial disease have been reported to attack the crop. The major coffee disease includes coffee berry disease (CBD) and coffee wilt diseases (CWD) (Merdassa, 1986). While coffee leaves rust (CLR) can be considered as potentially important disease of coffee in Ethiopia (Eshetu, 1997). CBD is a disease caused by the fungal pathogen *Colletotricum kahawae*, inducing an anthracnose of green and ripe coffee cherries.

Incidence of coffee berry disease (CBD) varies from one area to another and depends on types of plantation. Eshetu *et al.,* (2000) reported that the over all national disease severity due to CBD on landrace was between 24-30%. But on susceptible cultivar under favorable environment, 100% loss could occur. The existence of immense genetic diversity of Arabica

coffee in the country allowed managing the disease with development and use of resistant varieties.

Coffee wilt disease, which is also, called vascular wilt disease or tracheomycosis completely kills coffee plant and known to attack all species of *Coffea* (Wrigly, 1988). The disease has been known to be the main factor of coffee tree death (Van der Graaff and Pieters, 1978) and regarded as an endemic disease throughout the south and southwestern coffee producing regions of Ethiopia (Eshetu *et. al.,* 2000). Currently the disease is prevalent in most coffee growing areas with an estimated national disease incidence of 28.7% accounting to a total yield loss of 16.7% (CABI, 2003). The damage caused by the disease varies from location to location according to coffee production systems, age of coffee tree and environmental conditions. Its incidence was higher in plantation coffee mainly in research centers, small-scale farmers' holdings and in large estate commercial plantations (Girma *et al.,* 2001a; Girma, 2004). Girma *et al*., (2001b) reported that CWD incidence ranged from 45-69% on coffee plantation fields at Gera and Bebeka respectively.

CLR which is caused by *Hemileia vastatrix* first reported in Ethiopia in 1934 and according to Meseret (1991) and Chala, (2009) the importance of CLR was increasing with estimated national percent tree attack of 12.9 and latter raised to 36% after 10 years. Although integrated disease management approach is required to conserve and use forest coffee sustainabley in Ethiopia, there was very little information concerning major coffee disease incidence in afromontane rain forest coffee areas in south west and south east Ethiopia (Arega, *et al*., 2008).

2.3. *Fusarium* Wilt Diseases of Crops

Fungal wilt continues to be important and frequently a devastating plant disease in many areas in the world. The greater number of fungal wilt disease known in higher plant is caused by the species of the genera *Fusarium* and *Verticillium*. *Fusarium* species are ubiquitous fungi found not only in the temperate and tropical areas of the world but also occur in such diverse environments as the arctic and desert (Booth, 1971).

They are saprophytes or pathogen that attack plants and animal including man. It causes cortical rots, head blight, leaf spots, root and fruit rots, canker, dieback and vascular wilt disease (Nelson, 1981). Vascular wilt diseases caused by fungi are usually highly destructive whether they occur in cultivated crops or in indigenous species. *Fusarium* is a filamentous fungus widely distributed on plants and in the soil. It is found in normal mycoflora of commodities, such as rice, bean, soybean, and other crops. While most species are more common at tropical and subtropical areas, some inhabit in soil and cold climates. Some *Fusarium* species have a teleomorphic state (Wintgens, 2004). *Fusarium* is one of the emerging causes of opportunistic mycoses (Geiser *et al.*, 2005).

2.4. Symptomatology of *Fusarium* Wilt Diseases

Fungal wilt diseases are caused by a rather diverse group of microorganism and the diversity of the symptom complex is as great as or greater than the organism involved (Green, 1981). The feature that is unique to vascular wilt *Fusarium* is that the pathogen establishes itself systematically in the water conducting systems of the host directly after penetration and remains in the conductive tissue of the host xylem until the disease syndrome is well advanced (Green, 1981). The disease syndrome is due to therefore either to the actual presence of the pathogen or its propagules in the host vascular tissue or to the “polluting” effects of the factor in the host and or the interaction of the pathogen with the host (Beckman, 1987).

The external vascular wilt disease symptom both on herbaceous and woody angiosperm commonly include chlorosis, one sided and total wilting. Severely infected plants wilt and die while plant affected to a lesser degree may become stunted and unproductive herbaceous plants such as infected tomato grown in the green house. Vein clearing and dropping or epinasty of the petioles is the early symptoms. Foliar chlorosis followed by necrosis and defoliation develop first on the older leaves and spread either upward along the elongated stem or in the rosette type plant (Green, 1981).

The leaves turn yellow, dry up and fall although sometimes this only happens on the canopy (hemiplegia) branches die and finally with in a few month or even a few weeks the entire plant wither and die. These symptoms however can be classified as first degree symptoms. Common to

cases, they can not lead to a precise diagnosis of the disease (Wintgens, 2004). Second degrees are symptom more specific to the disease itself therefore need to be examined. They can be found on the trunks lodged in small cracks of the barks where there is a long black pellet the perthicium of the pathogen develop, one of the ways is checking for the disease to examine the layer under the bark. Wilting coffee plant is due to colonization of the sap vessels by the mycelium and the tyloses that block water and sap circulation.

The infection can set at any time from the cotyledon stage to maturity. The deaths of the tree depend on the age, the vigor of the plant and agro environmental condition and occur within 9-18 months. Both microconidia and macroconidia abound shortly before death and when the tree dies. The infection spread via the microconidia, the macroconidia and ascospores because the perithicia continue to develop for a long time after the death of the tree (Wintgens, 2004).

2.5. Climatic Factors and Wilt Disease Development

Fusarium wilt is reported to be a warm weather disease. Mean temperatures above 23°C favour fungal growth and disease incidence and the wilt phase of this disease is most evident when temperatures are high (Davis et al., 1996). Warm moist conditions may promote infection but the wilt symptoms are most severe in hot dry conditions (Beckman, 1987).

Environment greatly affects disease and disease resistance of the plant. Environmental factor may change the fitness of pathogen relationship (Walker, 1965). Temperature often has a great effect on host genotype. Beckman (1987) reported that the resistance of Cucurbitaceous to *Fusarium oxysporum* is best expressed at 30^{o}C and that many resistant hosts became susceptible at 6^{o}C where as the optimum growth of the fungus occur at 26-28^{o}C. On the other hand resistance to *Fussarium* wilt in cabbage is greatest at low temperature and decrease rapidly as temperature is increased particularly from 20-26^{o}C. *Fusarium* wilt of tomato developed over a broad range of temperature (20-34^{o}C) but occurred most rapidly and severely at 27^{o}C (Beckman, 1987).

Plant receiving little or no light generally becomes susceptible to wilt disease. Light of low intensity or short duration promoted rapid disease development in the susceptible cultivar but

less rapid development in the resistance tomato cultivar. Masert *et al.,* (1981) have noticed that *Fusarium* wilt of muskmelon symptom occur more quickly and are more intense when the relative humidity of their air is between 50 and 60 percent and they rarely occur when it goes beyond 80 or 90% in artificial inoculation test.

2.6. *Fusarium* Wilt Disease Cycle

The Fusaria are often soil born (soil-based) fungi because of their abundance in soil and their frequent association with plant roots, either as parasites or saprophytes. However, may have active or passive means of dispersal in the atmosphere and common colonizer of aerial plant parts, where they may cause disease of economic importance. The wide spread distribution of most *Fusarium* population can probably be attributed to their ability to colonize a wide range of substrate and their efficient mechanism for dispersal in time and/ or space. Moreover, these two key factors contribute to their ability to readily adapt to new ecological niches created by man (Burgess, 1981). In the absence of a suitable substrate these fungi persist as resistant (dormant) hyphae in plant residues colonized parasitically or saprophytically or as discrete propagules such as Chlamydospores and resistant conidia. (Burgess, 1981); Beckman (1987) and Schippers and Van Eck (1981) implicated that it is these structures and their recycling capacity that permit many pathogenic forms of fusaria to persist in most soils long after a suitable crop has been removed. *Fusarium* wilt pathogens can also survive in the absence of susceptible host plant by invasion and colonization of other plant that show no visible symptoms of the disease.

The means by which wilt pathogen penetrate host tissue and enter vascular elements may differ but as summarized by MacHardy and Beckman (1981) and Beckman (1987) are of two distinct types. Some pathogenic forms penetrate root directly, where as other must enter through wounds or wounds greatly enhance vascular colonization. Many forms of specialist *F. oxysporum* are able to penetrate host tissue directly and do not require a wound for penetration (Nelson, 1981). Hepple (1963) has shown that invasion in peas by *F. oxysporum f. sp. pisi* occur through senescent cotyledons and progresses into the vascular stele through cotyledonary trace.

In certain host plant wounding appears to be necessary before infection can take place. In sweet potato the principal mode of infection of sprouts by *F. oxysporum f. sp. batatas* was by the way

of vascular wounds such as freshly cut stem, fresh leaf scars (McClure, 1949). Infection did not occur through uninjured stem and roots root eruption wounds or the callus of a healed basal eruption wound. McClure (1949) further ascertained that tobacco was infected by the same organism when roots were cut or damaged by transplanting, but remained healthy when inoculated without wounding. *F.oxysporum f. sp. perniciosum* was indicated to enter mimosa tree roots either through wound or directly through root hairs and epidermis of the small roots (Toole, 1941). In general once wilt pathogen has penetrated a suitable host it moves to vascular tissue. The pathogen then spread throughout the plant by means of mycelial growth or conidia, primarily microconidia produced in infected xylem vessel elements. As the disease development progresses the fungus invade tissue adjacent to the xylem elements such as pith cambium, phloem, and cortex. At this time symptom expression is severe and the portion of the plant or entire plant succumb to the disease (Nelson, 1981).

2.7. History of the Taxonomic System of the Genus *Fusarium*

Following Link's diagnosis of the genus *Fusarium* in 1809, many researchers were concerned with diagnosis and identification of *Fusarium* species that caused disease on plant host. At one point, following link's treatment more than 1000 *Fusarium* species were recognized which were mostly isolated from diseased plant and because there were no guidelines and regulations applied in naming these isolates, the taxonomy of the genus *Fusarium* was in disarray (Leslie and Summerell, 2006). In 1821 fries validated the genus *Fusarium* according to the term of the International botanical code and included it in his order in the taxonomy of *Fusarium* was the population of "Die Fusarien" by Wollenweber and Reinking (1935).

2.7.1. Wolenweber and Reinking (1935)

In the 1930's Wolenweber and Reink formulated a taxonomic system that grouped *Fusarium* species within section (Wolenweber and Reink 1935). The separation within sections was based on variable cultural characters. The characteristics used to separate sections were: (i) the presence or absence of macroconidia (ii) the shape of the microcondia, (iii) the presence or absence of chlamydospores, the location of chlamydospores (v) the shape of macroconidia and, (vi) the shape of basal or foot cells on the macroconidia. Taxa within the sections were divided in to species, varieties and forms on the basis of (i) the color of the stroma, (ii) the presence and

absence of sclerotia, (iii) the number of septations in the macroconidia (Wolenweber and Reink 1935). Species in each section were grouped based on shared morphological features. The work of Wolenweber and Reink is foundation of most modern taxonomic systems.

Their taxonomic system described 65 *Fusarium* species and 77 sub-specific varieties and forms within 16 sections. The section that recognized were *Macroconia, submicrocera, pseudomicrocea, discolor, Roseum, Elegans, Liseola, sporotrichiella, Gibbosum, Martiella, Ventricosum, Arachnites, Arthrospriella, Eupionnotes, Lateritium and spicarioides (*Wolenweber and Reinking 1935; Joffe 1986; Windels,1992).

2.7.2. Snyder and Hansen (1940-1945)

In the 1940's Snyder and Hansen protested against the species distinctions of the Wollenweber and Reinking (1935) system. They argued that *Fusarium* cultures not initiated from single spores can display variable morphological features. Therefore, according to Snyder and Hansen (1940, 1941, 1945), the large morphological variations emphasized by Wollenweber and Reinking systems were due to Wollenweber and Reinking cultures not irritated from single spores. Hence they regarded the morphological variation of the Wollenweber and Reinking system as having taxonomic value. This prompted them to reduce the number of *Fusarium* species described by Wollenweber and Reinking (1935) to nine species (Nelson *et al.,* 1983).

They did not follow the grouping of species into sections and the nine species they recognized within the genus *Fusarium* corresponding to the Wollenweber and Reinking (1935) sections were *F. oxysporum* (section *Elegans*) *F. solani* (Sections *Martilla* and *Ventricomsum*): *F. moniliforme* (section *Liseola*) *F. roseum* (sections *Roseum, Arthrosporiela, Gibbosum* and *Discolor*): *F. lateritium* (section *Lateritium*); *F. ticinctum* (section *Sprotrichiella*); *F. nivale* (Section *Arachnites). F.rigidiuscula* (section *Spicarioides*) and *F.episphaeria* (sections *Eupionnotes* and *Macroconia*) (Nelson *et al.,* 1983). Since taxa within the Wollenweber and Reinking (1935) sections were polyphyletic the Snyder and Hansen, (1941) systems led to huge losses of information on a number of *Fusarium* species previously described (Booth, 1971; Domsch *et al.,* 1980 Messiaen and Cassini, 1981: Joffe 1986; Windels, 1992; Leslie and Summerell, 2006). For a number of years, different researchers strictly followed the

Wollenweber and Reinking's system or the Snyder and Hansen's system while others combined the two in their own taxonomic systems. Examples of such combined taxonomic system include that of Gordon (1952), Messiaen and Cassini (1981), Booth (1971), Gelach and Nirenberg (1982) and Nelson *et al*., (1983).

2.7.3. Gordon (1952)

In the 1950's Gordon first adopted the system of Snyder and Hansen (1945) but later based his work on that of Wollenweber and Reinking (1935). He combined some isolates. Gordon Classified 26 *Fusarium* species within 14 sections and also included 5 varieties and 69 forms of *F. oxysporum.* He kept the four Wollenweber and Reinking's sections (*Discolor*, *Roseum*, *Arthrosporiella* and *Gibbosum*) instead of replacing them as *F. roseum*, as Snyder and Hansen had done. He also considered the sexual phase of the species in his taxonomic descriptions (Domsch *et al*., 1980: Joffe, 1986: Leslie and Summerell, 2006), an aspect not addressed in the Wollenweber and Reinking and Snyder and Hansen's systems.

2.7.4. Messiaen and Cassini (1968)

Messiaen and Cassini based their taxonomic system on that of Snyder and Hansen (1940, 1941). The only difference in their work was that they adopted the use of botanical varieties instead of cultivars at subspecies level in *F. rosum*. Species *F. sambucium, F. culmorum, F. graminearum* and *F. avenaceum* were all made varieties of *F. roseum* (Messian and Cassini, 1968; Booth, 1971).

2.7.5. Booth (1971)

In this work Booth recognized 51 *Fusarium* species within 12 sections namely *Arachnites* (*Submicrocera*), *Martiella* (*Ventricosum), Episphaeria (Eupionnotes* and *Macroconia*), *Sporotrichiella, Arthrisporiella (Roseum), Coccophilum (Pseudomicrocera* and *Macroconia), Lateritium, Liseola Elegans, Gibbosum* and *Discolor.* He also introduced the use of morphology of the conidiogenous cells as an additional species level diagnostic character. He used this character to distinguish within some of the species in sections *Liseola* and *Sporotrichiella*. Booth's taxonomic system was identical to that of Gordon (1952) (Booth, 1971; Domsch *et al.*, 1980; Joffe 1986; Windels, 1992: Leslie and Summerell, 2006).

2.7.6. Gerlach and Nirenberg (1982)

In 1982, Gerlach and Nirenberg published an atlas that recognized 78 *Fusarium* species and 55 varieties the 16 sections recognized by Wollenweber and Reinking (1935). Their system is considered as an update of Wollenweber and Reinking (1935) but it is also very similar to that of Booth (1971) (Gerlach and Nirenberg, 1982; Joffe, 1986).

2.7.7. Nelson, Toussoun and Marasas (1983)

Shortly, after the publication of Gerlanch and Nirenberg (1982), Nelson *et al., (*1983) published a taxonomic system that combined the best features of the taxonomic systems of Wollenweber and Reinking (1935), Snyder and Hansen (1940), Joffe (1986), Messiaen and Cassini (1968). In their species descriptions they did not recognize the presence of polyblastic conidiogenous cells as a diagnostic characters as done by Booth (1971). They recognized 30 Fusarium species within 12 sections namely sections *Eupiomotes, Arachnites, sprotrichiella Roseum. Arthrosporiella Gibbosum, Discolor, Lateritium, Liseola, Elegans and Martiella-Ventricosum.* There were about 16 additional species that had insufficient descriptions and illustrations that they documented in their publications (Nelson *et al.,* 1983; Leslie and Summere, 2006).

In summary, there are currently more than 100 species recognized within the genus *Fusarium,* However, due to taxonomic revisions of the morphological species concept which was initially employed in *Fusarium* species identification, this number is expected to rise (Leslie and Summerell, 2006). Also, as much as there are a number of taxonomic systems that have been proposed for *Fusarium*, some systems have shown to be more popular than other amongst the Fusarium research communities. This is at least true for those researches that still employ morphological characters as the basis for species identification. The taxonomic system of Nelson *et al.* (1983) (Rheeder *et al.,*1996; Viljioen *et al.,* 1997; Marasas *et al.,*1998) is one example of such a system.

2.8. Morphological and Cultural Criteria Used in the Taxonomic Systems

All the major *Fusarium* taxonomic systems (Wollenweber and Reinking, 1935; Booth, 1971; Gerlach and Nirenberg, 1982; Nelson *et al.,* 1983) are based on morphological and cultural

criteria. In the Nelson and co-workers (1983) taxonomic system, the grouping of species into sections was based on cultural characteristics such as the growth rates colony morphology and pigmentation. The morphology of the macroconidia from sporodochia, macroconidia from aerial mycelium conidiophores and chlaymydospores were also characters used to group species into sections. Cultural characteristics are observed on potato sucrose agar (PSA) and potato dextrose agar (PDA) and the morphology of the macroconidia, microconidia, conidiophores and chlamydospores should be done on cultures grown on synthetic low nutrient agar (SNA) (Booth, 1971, Nirenberg, 1998).

Proper growth conditions are important in *Fusarium* species identification; therefore, cultures are usually grown in an alternating temperature of 25^{o}C day /20^{o}C night and incubated in diffuse daylight or in light from fluorescent tubes (Nelson *et al*., 1983). PSA and PDA are media considered to be rich in nutrients such as carbohydrates and because of their high available carbohydrate content. They promote mycelial growth rather than sporulation in fungal cultures. Furthermore, PSA is mostly used in *Fusarium* cultures identification to induce pigmentation and observe cultural growth rates, which are important secondary characteristics in species identification (Booth 1971; 1975). No complete identification of *Fusarium* species can be made based on cultures grown on potato dextrose agar (PDA) since the high nutrient concentration of the medium induces morphological mutation in *Fusarium* isolates (Booth, 1971, Nirenberg, 1982).

Synthetic nutrient agar and carnatin leaf agar (CLA) on the other hand is a medium that is low in nutrients compared to PSA and promotes sporulation rather than mycelial growth in *Fusarium* cultures under correct growth conditions. Result in consistent morphological characters produced by *Fusarium* species (Gerlach and Nirenberg, 1982 and Burgess *et al.*, 1991). Carnatin leaf agar (CLA) and Synthetic nutrient agar (SNA) gained their popularity in *Fusarium* species identification after Fisher *et al.,* (1982) showed them to be a good media to grow and preserve *Fusarium xylarioides* cultures over long period of time. The ability of *Fusarium* species to sporulate so very well in SNA could be attributed to its low available carbohydrate content (Gerlach and Nirenberg, 1982).

2.8.1 Variations in the Genus *Fusarium*

2.8.1.1 Cultural and Morphological Variations

Cultural and morphological variation among the genus *Fusarium* is now generally accepted as banal truism (Armstrong and Armstrong, 1975). Morphological variants among natural isolates of *Fusarium oxysporum* are known to occur although they may not be as frequent in some species. However, several morphological types have been isolated directly from nature (Armstrong *et al.*, 1940, Oswold, 1949). These authors have also reported that strains isolated directly from nature were more uniform usually having abundant aerial mycelia and few macroconidia. In laboratory culture of *Fusarium* freshly isolated from nature might remain unchanged through several repeated agar sub-cultures, or it might gradually change its morphology or it might suddenly produce sectors of growth radically different in appearance from the parent (Puhalla and Bell, 1981). The range of variation is great, but certain types are predominant. The most common variations are reduction in aerial mycelium or growth rate, increase in microspore production and colony pigmentation and changes in chlamydospores and microcondia production (Puhalla and Bell, 1981).

Ingeneral, studies of morphological variation in *Fusarium* are conflicting and often confusing as stated by Puhalla (1981). The changes are not continuous but occur in discrete steps and the altered morphology may appear as sectors or patches in the parent colony, or it may arise among the spore's progeny of an apparently normal colony (Puhalla, 1981). The spontaneous morphological variants of *Fusarium* are, however, stable and do not revert to the parental types during vegetative sub-culture (Puhalla and Bell, 1981; Puhalla, 1981) rather the variant may give rise to secondary variants (Armstrong *et. al.*, 1940). Nonetheless Puhalla (1981) implicated that the extreme morphological variability in agar culture may not occur among natural isolates of the fungus.

2.8.1.2. Variation in Pathogenicity

Natural isolates of certain pathogenic species of *Fusarium* showed significant difference in pathogenicity. Some of attack only certain plant species: others attack on entirely different groups of plant species whereas still others may overlap on both those host range (Puhalla and

Bell 1981; Puhalla, 1981). Such host specialization groups of the isolate are termed as *formae specials.* The concept of *formae speciales* (specialized form) was used to delineate pathogenic strain that were morphologically and culturally indistinguishable from saprophytic strains of the same species but different in their ability to parasitized specific host (Booth 1975; Windles 1992). Specialization for different hosts was originally predominantly for strain of *F. oxysporum* but rather this term has been applied for *F. solani*, *F. lateritium* and several other *Fusaria* (Booth, 1971, and Armstrong, 1975).

According to Booth (1971) and Armstrong (1975) more than 70 agricultural crops, have been described. Further sub-division of *formae speciales* of *F. oxysporum* capable of causing vascular wilt disease of many agricultural crops has been described. Further sub-division of *forma specialis* into race (physiological race) often have been made based on virulence to particular set of differential host cultivars that vary in disease resistance. Variation in pathogenicity has also been found in laboratory cultures of wilt *fusaria*. This variation usually affected only the virulence of the isolates and not its host ranges (Puhalla and Bell, 1981). Unlike morphological variation such change in pathogenicity may be reversible a variant less aggressive than the parent could produce secondary variants with increased aggressiveness (Puhalla, 1981).

2.9. Coffee Wilt Disease *(Gibberella/Fusarium xylarioides)*

Gibberella xylarioides Heim & Saccas, a teleomorphic state of *Fusarium xylarioides* Steyeart, is a specific coffee pathogen causing an extensive necrosis of the vascular tissue leading to wilting and death of infected plants. The syndrome is a typical vascular disease commonly called tracheomycosis or sometimes known as carbuncullariosis. The fungus was earlier reported to be a well-known pathogen of other *Coffea* species in West and Central Africa. *Coffea excelsa* (Excelsa coffee) plantations were seriously attacked in the Central African Republic and Cameroon; and a number of *C. canephora* (Robusta coffee) varieties were decimated in Ivory Coast and in Zaire by this disease in the 1950s (Booth 1971; Wrigley, 1988 and Coste 1992). The disease was observed again in Zaire in the early 1980s (Flood, 1997) and noticed for the first time in Uganda in 1993, it is now causing economic losses to robusta coffee in both countries (Flood and Brayford, 1997).

Kranz and Mogk (1973) isolated *F. xylarioides* from infected coffee. The pathogen also attacks *Coffea arabica* and is endemic in all coffee growing areas of Ethiopia (Flood, 1997; Girma and Hindorf, 2001; Girma, 2004; Lepoint *et al.*, 2005). During the 1950s and 1960s, it was considered to be the most serious disease of coffee in Africa and destroyed millions of coffee trees (Oduor *et al.*, 2003; Girma, 2004). Coffee wilt disease has markedly increased through out coffee producing areas of the country (Girma, 1997, Girma *et al.*, 2001b; Girma and Hindorf, 2001a, Girma, 2004). There were certain variation between coffee fields at different localities and that probably could be ascribed to genetic makeup and age of coffee cultivar, cultural practice and environmental conditions at specific location (Girma, 1998).

The disease severity varied among coffee types in the field, for instance at Gera in a field consisted of various coffee selections including both wilt susceptible and resistance checks and significant difference was obtained in percent coffee tree death mean percentage tree death (loss) in this field ranging from 95-60 for susceptible standards check (74304) to 12.20 for selection 8150 (Girma, 1997). It is probably a minor disease but attracted attention through causing serious losses in Ivory cost and Zaire (1948-1949). The crop in central West Africa is largely of *Coffea canephora* (Robusta type) which occur wild in Africa equatorial forests. Since the establishment of settled agriculture in Ethiopia 2000 years ago, coffee has been grown in the wild forests of the Southwestern highland massive of Kaffa and Buno regions (Tadesse *et.al.*, 2002), of the several species of coffee, Ethiopia is the center of origin and diversity of *Coffea arabica* L., which accounts for 60% of coffee exports in the world (Tadesse *et.al.*, 2002). Currently, the national average of coffee yield is about 472 kg/ha. That is much below the research results of 1500-2000 kg ha^{-1}. Such a substantial loss is to large extent determined by the wide spread of coffee wilt diseases which are usually caused by such plant pathogenic fungi as *Fusarium* species (Geiser *et al*., 2005).

Systematic elimination of affected plants over vast areas combined with the development of breeding programmes effectively reduced its impact to a minor disease (Kimani *et al*., 2002; Lewis Ivey *et al.*, 2003). However, the incidence has begun to increase dramatically and spread throughout Central and East Africa (Lewis Ivey *et al.*, 2003; Rutherford, 2006). Since 1993, farmers began reporting a wilt disease of coffee in western Uganda near the border with the

Democratic Republic of Congo and later in 1995, in Central Africa Republic (Lewis Ivey *et al*., 2003; Geiser *et al*., 2005; Rutherford, 2006).

This disease is clearly becoming a serious threat to coffee production in Africa and the cause of its reemergence is due to arising of a new, aggressive strain or biotypes of the pathogen. Isolates from other species of coffee (*C. arabica, C. excelsa*) and parts of Africa (Ivory Coast, Ethiopia) gave different band patterns. These results are surprising for a heterothallic fungus which produces its sexual stage in nature and support the hypothesis that a new, more aggressive strain of the pathogen may have arisen within the wider gene pool of the pathogen population in Africa (Lewis Ivey *et al*., 2003; Geiser *et al*., 2005; Lepoint *et al.*, 2005).

About 20 species of *Fusarium* have been recorded from coffee worldwide, but only four species are known to be pathogenic to coffee (Barnett and Hunter, 1972; Flood and Brayford, 1997; Flood, 2003; Gesier *et al.*, 2005). These are *F. solani* causing lethal root disease; *F. stillboides* inciting bark disease; *F. oxysporum* and *F. xylarioides* causing wilt diseases (Waller and Brayford, 1990; Stover, 1992; Waller and Holderness, 1997; Gesier *et al.*, 2005). Formae speciales of *F. solani* and *F. oxysporum* can be recovered from coffee root, husks and soil samples obtained from infected trees with wilt disease (Flood, 1997) and inducing different types of wilting on coffee in different geographical regions. *Fusarial* bark diseases of coffee caused by the same *F.stillboides* is an important factor limiting Arabica coffee production in the low and medium altitude distinct of Kenya (Flood and Brayford, 1997; King' ori, 2001).

2.9.1. Biology and Taxonomy of *Fusarium xylarioides (G. xylarioides)*

The sporodochial macroconidia are 1-3 septated, frequently falcate slightly curved with distinct visible foot and basal cells (Booth, 1971; Girma and Mengistu, 2000). Microconida of the aerial mycelia are usually 0-1 septated, and often variable in shape from slightly curved to allantoidal, and comma or U-shaped. Three asexual spores (macroconidia, microconidia and chlamydospores) and the fourth sexual spore (ascospores) allow the pathogen for the production of highly variable population, in addition to the parasexual cycle (Flood, 2003; Girma, 2004; Rutherford, 2006).

The sporulating stage of each fungus develops within one or two days on the split stem of diseased coffee, provided that stems are kept moist. *Fusarium* produces sickle-shaped conidia on sporodochia. *Fusarium xylarioides* survives for 2 to 11 years or five to ten years in the soil as "saprophyte" because it produces resting spores or chlamydospores. Moreover, the sexual spores (ascospores) produced in the perithecia may be able to act as survival spores (Flood, 2003).

The taxonomy of *Fusarium* is based on the morphological characters including the presence or absence, the shape and the dimensions of microconidia, macroconidia basal cells and chlamydospores, and the growth and color development on different media are used as markers in practice (Gerlach, 1978; Flood and Brayford, 1997; Lewis Ivey *et al*., 2003; Gesier *et al*., 2005). All *Gibberella* species are sexual states or teleomorphs of *Fusarium* species, which are destructive plant pathogens (Samuels *et al*., 2001: Desjardins, 2003). The anamorphic stage (G. *xylarioides*) *F. xylarioides* was first described by Steywart from stem samples of diseased coffee trees obtained from *coffea excelsa*.

The teleomorph form was observed by Saccas in 1949 on dead trees of *C. noearnolandiana* and described and renamed as the *Gibberella xylarioides* (Gerlach and Nirenberg, 1982; Nelson *et al.*, 1983). The fungus was indicated as one of heterothallic ascomycetes having male and female strains, which can be identified based on the colony appearance and conidial morphology (Barnett and Hunter, 1972; Gerlach, 1978; Gerlach and Nirenberg, 1982; Nelson *et al.*, 1983; Girma and Mengsitu, 2000).

2.9.2. Survival and Spread of Coffee Wilt Pathogen

The pathogen survives in the soil in the form of microconidia, macroconidia, chlamydospores and perithecium with ascospores. The pathogen appears to be a soil inhabiting fungus which can penetrate through wounds either above or below ground. Inside the coffee the fungus invades the water conducting system (xylem) and blocks the movement of water upwards from the roots to the rest of the plant. The timeing from first symptoms to death of the tree varies from days in young plants to eight months and in trees more than ten years old (Girma, 2004). Once the

fungus infects the coffee tree, all affected trees eventually die. According to Rutherford (2006) some farmers call the disease" Coffee AIDS".

F.oxysporum f. sp. pemicisum was indicated to enter mimosa tree roots either through wounds directly through root hair and the epidermis of the small roots (Toole, 1941).The coffee wilt disease cycle is not fully understood but the pathogen was supposed to be a soil inhibiting fungus (Booth, 1971, Flood and Brayford, 1997) suggested that the fungus did not persist in the soil as it seldom from chlomydospores. Flood and Brayford (1997) refering observation of chlomydospores with in the host tissue by the earlier workers have argued that the pathogen may be present in the organic debris of the soil.

They have also implicated that the ascospores may act as alternative to chlamydospores although condia could not survive long. Booth (1981) pointed out that in the genera of Hypocreales the chlomydospores or somatic tissue has much greater survival value than the ascospore. According to Wrigley (1988) the lateral and feeder roots of coffee spread on the surface plate parallel to soil surface for a distance of 1.2 to 1.8 meters from the trunk, and *F. xylarioides* is abundantly recovered from root parts of symptomatic and asymptomatic trees (Flood,1997; Flood and Brayford,1997; Girma *et al*., 2001; Girma, 2004). The pathogen spreads 2 meters up to four plants on either sides of the inoculated focus plant through the infection of the roots in greenhouse experiment (Girma, 2004 and Rutherford, 2006).

Closely spaced trees are more liable to wounding and cross inoculation while slashing or hoeing coffee fields. According to Girma (2004) almost all coffee trees have wounds at the crown level or few centimeters above, and on average healthy trees have 1-3 wounds per coffee stem. Weeds are slashed frequently, some times more than ten times a year, depending on the dominating weed flora in plantation coffee. Most of coffee trees are found with wound at least once at all locations, where slashing is employed to control coffee weeds (Girma, 2004). When seedlings with healthy roots are transplanted into either naturally or artificially infested soils, no wilting symptoms appeared. Infection exhibits when the tap roots are injured and transplanted into naturally or artificially infested soils, and also only on those seedlings inoculated by stem wounding through ditching with *F. xylarioides* infested scalps or by injecting the conidial

suspensions with needles (Pieters and van der Graaff, 1980; Girma and Mengistu, 2000; Lewis Ivey *et al.*, 2002; Girma *et al*., 2005b, 2007a),

The stem nicking or root drenching inoculation methods also elaborate the roles of contaminated farm implements in cross inoculating coffee trees as well as disseminating the coffee wilt pathogen in the field (CABI, 2003; Girma *et al*., 2004, CABI, 2005). Replanting susceptible cultivars in the infected field increases the fungus inoculum density (CABI, 2003). Pieters and Van der-Graff (1980) reported that among socioeconomic factors contributing to the spread of CWD, particularly in Ethiopia, is the frequent replacing with several seedlings (3-8) per uprooted wilted trees. The infection of the young replants undoubtedly suggests that the fungus survives in stumps, root debris or in the soil for 2-3 years perithecia of *Fusarium xylarioides* containing a great number of viable ascospores with 95% germination rate in the soil (CABI, 2003, Girma, 2007). So, that these sexual spores are the most important source of inoculum in the CWD epidemics. High infection of susceptible C*offea arabica* seedlings is observed after inoculating with field-collected ascospores suggesting that the perithecial stage is the primary source of inoculum in the field. The major function of the sexual state of the fungus is largely to serve as a survival mechanism, rather than maintaining diversity in the population structure (CABI, 2003, Girma, 2007).

The conidia can be spread by water (rain splash and flooding), wind, and human activity and penetrate the host through wounds caused by weeding and pruning. Wood pieces used for vegetative propagation may be an important source of spread, while coffee seeds (berries) have been shown not to transmit the disease. Wind spread may occur over long distances (Flood, 1997; Flood, 2003; Rutherford, 2006). Human activities, such as pruning, weeding with a hoe and transporting affected trees for use as firewood or fencing can spread the fungus (Flood, 2003; Rutherford, 2006). When a tree is deliberately or accidentally wounded, during pruning, weeding around the trees and even harvesting, the fungus may enter and cause disease (Gesier *et al*., 2005; Rutherford, 2006).

2.9.3. Prevalence of Coffee Wilt Disease

Coffee wilt disease also known as tracheomycosis occurs in almost all surveyed coffee fields under forest, semiforest, garden and plantation coffee production systems in almost all coffee areas of the country. The magnitude and extent of damage varied among and within coffee fields, districts and the production systems depending on various interacting factors such as genetic makeup and age of coffee, cultural practices and prevailing climatic conditions of the areas(Girma and Hindorf, 2001; Girma, 2004).

2.9.3.1. CWD in the Forest and Semi-forest Coffee

The occurrence of CWD was reported after assessment in four forest coffee areas in south-west and south-east afromontane rainforests with incidence ranging between 5% at Sheko and 30% at Yayu. Although it was indicated that the damage was minimal in the dense stands of coffee (Van der Graaff, 1983; Merdassa, 1986), this was the first report that showed presence of CWD on forest coffee trees. Arega (2006) demonstrated increasing occurrence of CWD in some forest areas like in Harenna (Bale) and Bonga (Keffa). The mean incidence in semi-forest coffee ranged from 3.6% at Mettu to 15.5% at Gera situated in south-west coffee-producing areas, and the severity varied between 18.6% and 25.4% in some coffee fields at Yirgacheffe (Girma, 2004). Similar situations were observed in Bale, Jimma, Ilubabor, and West Wellega zones (CABI, 2003).

2.9.3.2. CWD in Garden Coffee

CWD is prevalent in the southern region, specifically in the three major quality-coffee-producing districts, namely, Wonago, Kochore and Yirgacheffe of Sidama and Gedeo zones, with highest incidence in Yirgacheffe followed by Kochore and Wonago. The severity of wilting in the sample fields in Yirgacheffe varied between 27.2% and 43.5% in the garden coffee as compared to that of the semi-forest coffee (Girma, 2004). Although the disease was not evenly distributed in most coffee-growing areas of Southern Nation, Nationalities and Peoples region, the average incidence (35%) and severity (5.0%) was higher than in other regions. It was particularly high in the Sidama and Gedeo zones, with an incidence over 90% and severity of 25%. The incidence of CWD was also above 35% in garden coffee of West

Gojam zone of Amhara regional state, but it was very low in Wolaita (Southern Nation, Nationalities and Peoples) and West Harerghe (Oromiya) (CABI, 2003).

2.9.3.3. CWD in Plantation Coffee

The disease incidence is more severe in plantation coffee such as at research centres, on larger farmer holdings (1 to 5 ha) and in large estate commercial farms. CWD is commonly encountered in the research plots at Gera and Jimma amounting to 42.5% and 48.2%, respectively (Girma 2004). It is serious in the farmers' coffee plantations at the Gera, Chira and Gechi districts, with respective mean incidence ranging from 21.7% to 25.5%, from 32.3% to 77% and from 35% to 60%, respectively. The overall mean coffee tree loss in the farmers' plantation was more than 30%, and in total, about 10 ha of plantation coffee had been abandoned completely. The lowest percentage of the disease was recorded in the farmers' plantation at Tobba (17.3%), whereas the highest was at Bebeka (65.2%). Girma *et al*. (2001) confirmed that the disease was more severe in plantation coffee at Bebeka, Teppi, Gera and Jimma. Van der Graaff (1979) remarked that some spectacular failures of the modern plantations system could be due to *G. xylarioides*, and when comparisons are made across production systems, the disease is more destructive in garden and plantation coffees than in forest and semi-forest coffee systems.

The latter two systems are composed of heterogeneous coffee populations possessing varying levels of resistance and less human interference. However, in the former systems, characterized by relatively homogenous coffee trees and high levels of intervention, the disease spreads from tree to tree, from row to row and from one block to the other developing throughout the field (Girma, 2004). A remarkable increase in CWD severity of (11.5%) was recorded over a 6-month period in nine districts (weredas) of Gedeo and Sidama zones of Ethiopia (CABI, 2003).

2.9.4. Pathogenic Diversity and the Role of Sexual Structures in *G. xylarioides*

The population structure of *Gibberella* (*Fusarium*) *xylarioides* was studied in recent and historical strain collections employing pathogenicity tests and RAPD-PCR analysis. In the pathogenicity test that consisted of 11 isolates and nine cultivars from arabica and Robusta coffees, the Arabica isolates were pathogenic only to seedlings of arabica coffee with

significantly varying degrees of aggressiveness across cultivars, but incompatible with that of Robusta coffee seedlings. In contrast, the Robusta strain was specifically compatible with seedlings of Robusta coffee without showing any infection symptom in all arabica cultivars. There existed also highly significant ($P < 0.001$) differences among arabica cultivars, the isolates and cultivar-isolate interactions both in percent seedling deaths and incubation periods suggesting horizontal resistance in the host, aggressiveness in the pathogen and vertical resistance/virulence combinations, respectively (Girma 2004; Girma *et al*., 2005). Likewise strains collected in the recent CWD outbreaks on Robusta coffee in Democratic Republic of Congo (RDC002), Uganda (CABI,2003) and Tanzania (TZ008, TZ009) were pathogenic to all seedlings of eight Robusta coffee lines but caused no infection on that of Arabica coffee (Girma *et al.*, 2007b). These two findings were consistent with the previous report of Girma and Mengistu (2000) but it was not congruent with the work of Pieters and van der Graaff (1980).

In RAPD-PCR analysis of 22 *G. xylarioides* strains of the recent and historical collections from Arabica, Robusta and Excelsa coffees; the Ethiopian Arabica isolates clustered into a single homogeneous population although distinctly polymorphic to the recent and historical strains from Robusta and Excelsa coffees. The historical arabica strain in 1971 was slightly different from all the recent isolates in 2001 in Ethiopia illustrating little genetic change in the population structure over the last 3 decades (Pieters and van der Graaff, 1980).

The host-pathogen interactions and RAPD-PCR markers corroborated existence of host specialization into at least two pathogenic forms within *G. xylarioides* populations; that lead to designate *formae speciales*, namely; *Gibberela xylarioides* f. sp. *abyssiniae* (anamorph: *Fusarium xylarioides* f. sp. *abyssiniae*) for the fungus strains attacking only *C. arabica* and limited to Ethiopia and *G. xylarioides* f. sp. *canephorae* (anamorph: *F. xylarioides* f. sp. *canephorae*) pathogenic to *C. canephora* and *C. excelsa* (Girma *et al*. 2001). *G. xylarioides* produces perithecia in dark stromatic fruiting bodies in the barks of stems of dead coffee trees in the field after 2 – 3 months (Van der Graaff and Pieters, 1978; Girma *et al.* 2001).

2.9.5. Coffee Wilt Disease (*Gibberella xylarioides*) Management

2.9.5.1. Quarantine Measures

For areas currently free from tracheomycosis strict quarantine measures help to prevent its entry and spread. Movement of coffee materials (seedlings, husks, and other organs) between affected and unaffected areas should be restricted as much as possible (Hakiza, and Mwebesa, 1997). These measures need to be backed up with dissemination of information about the disease to farmers, extension workers, scientists and the general public. Dissemination of information on the symptoms of the disease is essential to allow monitoring and early detection of the disease. For countries bordering affected countries cordon sanitors can be constructed. This involves the destruction of all affected coffee in border areas and encouragement of farmers to grow crops other than coffee (Flood, 1997; Girma, 2004; Rutherford, 2006).

2. 9.5.2. Resistant Variety

Production of resistant cultivars is the best option for controlling CWD in the longer term. This method was very successful in controlling outbreaks of the disease in 1950s and 1960s in West and Central Africa, where affected coffee is uprooted and destroyed and the fields replanted with resistant cultivars of *C. canephora* such as cultivar `robust`, but recently resistance is broken down due to emergence of a new form of the fungus (Flood and Brayford, 1997). In Ethiopia, breeding programmes were initiated for *C. arabica* (Vander-Graff and Pieters, 1978; Pieters and Vander-Graff, 1980) but the disease remains a problem in some areas of the country. Some farmers are conducting their own selection since, even in very badly affected areas, a few trees may survive. Farmers are replanting with seeds from these plants might be resistant.

2.9.5.3. Cultural Practices

Systematic elimination of affected plants over vast areas combined with the development of breeding programmes effectively reduced its impact (Flood, 1997; Hakiza and Mwebesa, 1997). Affected trees and trees adjacent to affected trees should also be uprooted and burnt although they may appear healthy because while symptoms of the disease may not be visible, the fungus may be inside the plant (Rutherford, 2006). When symptoms are recognized quickly and uprooting and burning done efficiently, the farmers may save some of the crops (Flood and

Brayford, 1997; Girma, 1997; Lepoint *et at.,* 2005; Leslie *et al.,* 2005). If the farmers delay, the infected trees act as sources of inoculum to other trees and leads to whole crop losses. Trees cut down as control measure should not be used as fuel, as affected trees drugged through healthy trees in the farm will aggravate the spread of the disease. Diseased trees must be burnt where they are uprooted. To prevent spread from one field to another in large plantation, it is recommended that a 300 m strip of land should be cleared of coffee (by uprooting & burning) ahead of the disease front (Girma, 1997, Hakiza, and Mwebesa, 1997; Flood, 2003; Rutherford, 2006). Any kind of wounding to the tree will allow the fungus to enter. Wounding may occur through weeding and pruning with machete or hoe, or even by livestock feeding on and around the tree (Flood, 2003; Rutherford, 2006). Great care should be taken to minimize damage to the tree and all tools should be sterilized with fire or with disinfectant before moving to another tree.

Mulches and soil amendments including cow dung and urine have been claimed to control the disease, but bring only temporal improvement to infected trees by increasing plant vigor and stimulating new growth of roots, shoots, and leaves (Flood, 1997; Hakiza, and Mwebesa, 1997; Rutherford, 2006). Improvement will also be partially due to the encouragement of organisms such as *Trichoderma* and *Aspergillus* in the soil that compete with the wilt fungus (Thangavelu *et al*., 2003). Mulches and soil amendments are therefore unlikely to control the disease in already infected trees, but may be useful in preparing the land for replanting after affected trees have been uprooted and burnt (Cooney and Lauren, 1998; Rutherford, 2006).

Following destruction of the diseased trees and preparation of the land, replanting should not be carried out for at least two years to allow the inoculum of the fungus in the soil to decrease (Girma and Mengistu, 2000; Girma, 2004; Rutherford, 2006). Replanting should be done with plants raised from disease free cuttings and seeds collected from areas that are free from the disease (Girma and Mengistu, 2000).

2.9.5.4. Chemical Control

The pathogen is thought to live in the soil and inside the plant, making it hard to target the fungus even with systemic fungicides (Tesfaye and Kapoor, 2004). Control of CWD with

systemic fungicides can be an alternative option, but again, its economic viability is likely to be low, and the risk of contaminating the coffee beans with the fungicide should be investigated. There is also the possibility of the pathogen adapting to systemic chemicals. Systemic fungicides are already being used in the control of CLR, mostly by large-scale coffee farmers. Even if systemic fungicides are found to be uneconomical to use for controlling CWD or are found in the coffee bean, they may be the only solution for saving valuable coffee germplasm materials that have useful agronomic traits but are susceptible to CWD. Many CWD-affected coun tries are losing their valuable germplasm. Uganda has lost coffee germplasm due to CWD. In addition, Uganda has lost *Coffea kapagota* at the Entebbe Botanical Gardens due to this disease. An effective systemic fungicide could therefore be used to protect these valuable materials. Preliminary studies with benomyl under greenhouse conditions in Uganda demonstrated that the fungicide may control CWD, but frequent drenching every month was required (Hakiza and Mwebesa, 1997).

2.9.5.5. Biological Control

"Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms accomplishing naturally or through manipulation of the environment, host or antagonists, or by mass introduction of one or more antagonists"(Baker and Cook, 1974).

Biological control is the strategy for reducing disease incidence or severity by direct or indirect manipulation of microorganisms (Papavizas, 1985; Zhang *et al*., 1994; Paullitz and Bekanger, 2000; Tesfaye and Kapoor, 2004 and Negash, 2007). Antagonists that produce antibiotics kill pathogens and eradicate or control them from substrate. Some microorganisms occupy the niches and exclude pathogens from becoming established, thereby protecting plants from infection. Biological control has attracted great interest because of increasing regulation and restriction of fungicides or unnecessary control attempts by other means. It is especially attractive for soil borne diseases because it needs critical evaluation of economics of the country *Trichoderma harzianum* and *T. viride* are the most studied of all the *Trichoderma* species for biological control and the most effective in reducing diseases caused by soil borne plant pathogens (Baker, 1987; Cortes *et al.,* 1998; Rocco and Perez, 2001; Tesfaye Alemu and

Kapoor, 2004). *Trichoderma* has rapid growth and development, and also produces a large number of enzymes, induced by the presence of phytopathogenic fungi. Its high tolerance to extreme environmental conditions and habitat, where fungi are the cause of various diseases, makes it an efficient agent of control; equally, it can survive in media with high levels of pesticides and other chemicals. So the application of *Trichoderma* directly on the soil offers greater protection to the crops (Cooney and Lauren, 1998).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted both in the field, in the laboratory and in the greenhouse. Field studies were conducted in afromontane rainforest coffee populations in Bonga (Kafa), Sheko (Berhane Kontri), Yayu (Ilubabor) and Harenna (Bale) forest coffee areas in southwest and southeast Ethiopia, and laboratory and greenhose studies were conducted at Jimma Agricultural Research Center (JARC). The study areas are described as follows.

3.1.1. Bonga Forest Coffee

Bonga forest coffee is found in Bonga rainforest in Gimbo woreda of Kafa zone in South Nations and Nationalities Peoples Region (SNNPR). Meteorological data from Wushwush village at 1725 m a.s.l ($7^0$13´ N; $36^0$7´E) indicated that the annual rainfall is around 1800 mm, the wettest months being May and June; mean average temperature is around 19^0 C (Feyera, 2006).

3.1.2. Sheko /Berhan-Kontir Forest Coffee

Sheko forest coffee is found in Berhan-Kontir forest in Sheko woreda of Bench-Maji zone of SNNPR. Berhan-Kontir forest area is located near Giz-Meret village between $35^0$15´-$35^0$30´ East and 06^0 55´-07^0 05´ North. It forms a part of the 417,000 ha of Sheko forest demarcated as one of the national forest priority areas (NFPA) in the country (Paulos and Demel, 2000). A total of 5, 500 ha of these forests are delineated to serve as forest coffee conservation area. The annual rainfall and mean annual temperature are 2200 mm and 22^{o}C, respectively (NMSA, 1996).

3.1.3. Yayu Forest Coffee

Yayu forest coffee is found in Geba-Dogi forest area in Yayu woreda of Illubabor zone in Oromiya region. It contains dense forest through which the road from Addis Ababa to Metu passes; being located between $35^0$45´-$36^0$05´ east and $08^0$15´-$08^0$37´ north, and stretches along the two sides of Geba and Dogi rivers (Paulos and Demel, 2000). Big trees, shrubs and herbs including coffee plants growing in the wild and 18,600 ha of the forest are delineated to serve as the forest coffee conservation area. The forest covers about 10,000 hectares and out of these

5,500 hectares is covered by forest coffee. It receives annual rainfall of 1800mm and mean annual temperature of 21^{o}C (Tadesse, 2003).

3.1.4. Harenna Forest Coffee

The Harenna forest coffee is found in Maneangetu woreda of Bale zone in Oromiya region. Harenna rainforest is part of Bale Mountains National Park that lies between 1300 and over 3000 m.a.s.l. Most forest coffee occurs only in altitudes ranging between 1300 and 1850 m.a.s.l. and coffee plays significant role for the livelihoods of the community living in the surrounding areas (Feyera, 2006). The rainfall pattern in the area is bimodal type, i.e., March through April and August through October. Annual rainfall is about 1000 mm and the mean annual temperature is 18 ^{o}C (Tadesse, 2003).

3.1.5. Jimma Agricultural Research Center (JARC)

Jimma Agricultural Research Centre (JARC) is located 363 km South West of Addis Ababa at 7°40'N Latitude and 36°47'E Longitudes. The Center has a temperature of Max: 26.2^{0}C and Min: 11.3^{0}C with 1529.5 mm average rain fall. The Research Centre, which is found under the umbrella of the Ethiopian Institute of Agricultural Research, specializes in agricultural research, which includes serving as National coordinating center for coffee and spices research (EIAR, 2004).

3.1.6. Gera Agricultural Research Sub-center

The study area, Gera district, is located 450 km away from the capital Addis Ababa in South Western of the Regional State of Oromia. Its location lies between 70^{o}27'-70^{o}55" N latitude and 360^{o} 01'- 360 ^{o} 24'E longitude. It is located 95 kms West of Jimma town, and it is one of the 17th districts of the zone with an area of 1443.4 km^{2}. In its area coverage Gera ranks eighth out of the total district in the zone. The maximum average annual rainfall is estimated at 1900 mm. The maximum annual range of temperature, which is recorded in winter season, is 25°C while the minimum annual temperature recorded during summer season is 40^{o}C (WARDO, 2008/9).

3.2. Disease Assessment and Sample Collection

In this study each forest coffee area of Bonga, Sheko/ Berhane kontri, Yayu and Harenna, three representative sample fields, each with 5m x 10m were randomly selected for coffee wilt disease (CWD) assessment and disease sample collections. In each sample field, CWD was assessed by diagnosing infected coffee trees and then counting the number of diseased and healthy trees per plot. Then the numbers of healthy and diseased trees counted, and the incidence of CWD was computed as (number of diseased trees/total number of observed coffee trees) x 100. At the same time, two to three stem pieces measuring 15-20 cm were collected from three to four samples of infected coffee trees with partial wilting symptoms in the same field and transported to the Plant Pathology laboratory at Jimma Agricultural Research Center (JARC) (Girma *et al*., 2001, Girma, 2004; Arega *et al*., 2008). For comparison purpose samples were collected from semi-forest and plantation coffee at Jimma and Gera Research Sub-center, respectively.

3.3. Isolation and Identification of the Fungus

Isolation of the pathogen from the samples collected in the forest coffee areas were made in the laboratory of Plant Pathology at Jimma Agricultural Research Center (JARC). Accordingly samples from the stem were first remove the outer epidermal part (bark) from all specimens and then small section (0.5-1.0 cm) are exercised from the boundary of discolored and healthy wood using sterile scalpels. These sections (4-6 per specimen) were transferred in to plastic Petridish having about 5ml of 10% chlorox ($NaHO_3$) or sedex and then uniformly agitated for 1 min and immediately rinsed with sterile distilled water in three changes (each change for 1min). After disinfection and blotting each section (four per specimen) was plated using sterile forceps on to Petridish (9 cm diameter) containing 15ml of potato sucrose agar (PSA) amended with antibiotic (Streptomycin sulfate). Then petridishes were incubated in a growth chamber subjected to a 10-12 hr dark/ florescent light cycle at temperature of 25 ± 2^{o}c (Booth, 1971, Girma *et al*., 2001, Girma 2004). The fungus developing out of the plated section was purified on standard media employing hyphal tip transfer techniques. The pure culture grown for 7 – 12 days under the same condition was identification of *G. xylarioides* (*F. xylarioides*) isolates was confirmed in comparison to standard refernce cultures preserved at Plant Pathology laboratory of Jimma

Agricultural Research Center (Girma *et al*., 2001; Girma, 2004) and morphological characterstics of the fungus as described by Booth (1971).

3.4. Characterization of *Gibberella xylarioides*

The cultural and morphological features of the pathogen was made by macroscopic (cultural appearances) and microscopic (morphological structures) features of *G. xylarioides* isolates collected in the forest coffee areas representing all the fields compared with the standard isolates from semi-forest and plantation coffee production systems.

3.4.1 Cultural Appearance

The cultures of 30 representative *G. xylarioides* isolates (24 from forest coffee areas, 3 from semiforest and 3 from plantation coffees) with three replications (30x3) were taken from a true-to-type culture by hyphal tip transfer and growing on potato sucrose agar (PSA) under standard condition 10-12 hr dark/ flurescent light cycle at temperature of 25 ± 2^{o}c (Booth, 1971, Grima *et al*., 2001, 2004). Thereafter, the following qualitative and quantitative phenotypic scoring was made at 4, 7, 10, 14 and 21 days of incubation periods (Girma *et al*., 2001; Girma, 2004).

3.4.1.1. Apical Mycelial Growth Type and Colony Density

The varying degrees of colony evaluation (the apical growth nature) from the agar surface were taken as raised, slightly raised and apprased, while colony density was recorded as dense intermediate and scares (Girma *et al*., 2001; 2004).

3.4.1.2. Colony Colour

Colony (mycelia) color on obverse side and types of pigments from the reverse side of the Petridish for each *G. xylarioides* isolate was determined on PSA using RGB color chart (Anonymous, 2006). Cultures were observed at 4, 7, 10, 14 and 21 days (Booth, 1971; Girma, 2004 and Arega, 2006).

3.4.1.3 Colony Radial Growth

Mycelial (colony) radial growth (mm) of each isolate was measured with ruler, colony diameter from two perpendicular planes on the reverse side of the Petridishes.

3. 4. 2. Morphological Characteristics

Cultures that were selected as typical representative isolate of the fungus developed from hyphal tip transfers were used. They were cultured on SNA for 10-14 days under the standard conditions as mentioned above. The colony growth in each Petridish was flooded with 10ml of sterile distilled water and rubbed gently from the agar surface to free the fungus conidia. The spore suspension was poured in to sterile beaker and uniformly stirred and then filtered into another sterile beaker through double layer of cheese clothes and the following parameter was recorded (Girma *et al*., 2001; 2004).

3.4.2.1. Shape and Size of Conidia

Frequency of *G. xylarioides* conidial shapes was tallied and the most frequent conidia shapes were described using binocular compound microscope and the most frequent conidial shapes which were standardized following Booth (1971) and Girma and Mengistu (2000) for *Fusarium* spp. characterization. The frequency of each shape was tallied from 450 - 500 conidia per isolate. Conidial size (length and width) was computed from 450 to 675 conidia per isolate. More conidia were measured for those isolates which had more variable shapes of conidia. Length and width of conidia were measured with ocular micrometer (μm), which was fitted into 10x eyepiece and adjusted at 40x objective of the compound microscope. At the same time, the number of conidia per milliliter was counted using haemocytometer. The results were determined for each isolate as the average number of conidia per milliliter after taking three haemocytometer counts. The presence and absence of fungus fruiting bodies such as chlamydospores and perithecia arising in the culture was also checked and noted.

3.5. Evaluation of the Resistance of Some Coffee Accessions Collected in Forest Areas

Following the methods described below, seedlings of from some forest coffee accessions including Catimor J-21 as resistant and SN-5 as susceptible checks were raised and inoculated with one *G. xylarioides* isolate obtained from Yayu forest coffee to determine the reactions of the accession against the pathogen.

3.5.1. Raising Coffee Seedlings

Coffee seeds were prepared from mother trees representing of some forest coffee areas in southwest (Bonga and Yayu) and southeast (Harenna) of Ethiopia. The seed lots of each accession was first soaked in distilled sterile water for about 48 hours after removing the parchment. The soaked seeds (25-30 seeds/pot) of each accession were sown into heat sterilized and moistened sandy soil in disinfected plastic pots (25cmx18cmx7cm). Sterile water was regularly applied to maintain adequate moisture for seed germination, emergence and growth of the plants throughout the experimental period (Girma, 2004; Girma *et al.,* 2009).

3.5.2. Inoculum Preparation of *Gibberella xylarioides* Isolate

The stock culture of Yayu (Y21) isolate was used to initiate colony growth by sprinkling grains of sand on to Petridishes with SNA followed by further sub-culturing on the same medium for about a week. Then at the same time fresh coffee branches were collected from healthy trees, cut in to small pieces of 15cm length and the bark was slightly scratched off to expose the wood. After this, the branch was placed in test tubes ($3.75cm^3$) having a small roll of well-moistened cotton wool underneath and sterilized in an autoclave. Each of a batch of the twigs was inoculated with 3ml of conidia suspension of the isolate and incubated for 14 days under the standard conditions. The conidia were harvested by thoroughly rising off the branches with sterile water in a sterile beaker. The suspension of the isolate was stirred up with magnetic stirrer and filtered through double layer of cheesecloth. The spore concentration of the isolate was determined using haemocytometer and then adjusted to $2.3x10^6$ conidia/ml (Girma and Mengistu; 2000, Girma, 2004).

3.5.3. Seedling Inoculation Test

Seventy days after sowing coffee seedling (25 per pot) of all accessions (14 accessions and 2 checks) they were inoculated at cotyledon stage with viable conidial suspension of the isolate by stem nicking procedures described by Pieters and Van der Graaff (1980), Girma and Mengistu (2000) Girma *et al.,* (2009). A sterile scalpel was first immersed into the suspension, then the stem of each seedling was nicked out (1.0-1.5cm long) at about 2 cm from the soil level and a drop of nearly one millitter (1 ml) was placed in the notch. In order to create more humid conditions for infection, all treated plants were immediately covered with transparent plastic

sheet and maintained in the air-conditioned (23^{o}C) growth room of JARC. After ten days the inoculated seedlings were transferred in to greenhouse and the treatments were placed on experimental benches in randomized complete block design (RCBD) with three replications.

3.6. Host –pathogen Interaction Study

Interaction between coffee accessions and *G. xylarioides* (*F. xylarioides*) was studied through inoculation test of coffee seedlings as desctribed above (section 3.5.1 – 3.5.3.). The pathogenic variability in the fungus populations was compared among isolates representing the four forest coffee areas and with one isolate from plantation coffee on seedlings 12 of forest coffee accessions originated from Harenna, Bonga, Sheko, and Yayu which were established at JARC in 2005; and standard check (7440 candidate CWD resistant) (Girma and Mengistu 2000; Girma, 2004).

3.7. Experimental Designs

The experiments were conducted both in greenhouse and in the laboratory. The laboratory experiment were laid out in completely randomized design (CRD) with three replications while evaluations of coffee accessions and the host-pathogen interaction study was conducted in randomized complete block design (RCBD) with 5 isolates x 13 accessions factorial treatment combinations replicated three times.

3.8. Re-isolation of the Fungus (*G. xylarioides)* from the Inoculated Seedlings

Each isolate was retrieved by plating from the seedling tissue (section) from the stem and root parts of a symptomatic and asymptomatic sample (3 - 5 seedlings/ pot) of the inoculated coffee seedlings following the method described above (3.3). For confirmation the cultural and morphological features of each re-isolate was studied in comparison with that of the representative parent isolates used for inoculation following the procedures described above (3.4.1 – 3.4.2)

3.9. Data Collection and Analysis

3.9.1. Data Collection

Cultural and morphological characteristics of the pathogen (*G. xylarioides*) was observed and recorded as outlined above. Cultural appearance such as apical mycelial growth, colony density, colony color and colony radial growth rate and morphological characteristics which included, shape of conidia, dimension (size) of microconidia, macroconidia and sporulation capacity were observed and recorded in the laboratory. The number of healthy and wilting seedlings per pot was recorded at 14 days interval for six months starting a month after inoculation (Pieters and Van der Graaff 1978; Girma and Mengistu 2000). The first date on which symptoms appeared was also recorded. Characteristic external wilting symptom development was noted periodically and described. Accordingly, the presence and /or absence of wood discoloration (internal symptom) were examined on five sample seedlings per treatment at the end of the test period. Incubation period and percentage of dead seedling were computed from the cumulative number of wilted seedlings six months after inoculation over the total number of originally inoculated seedlings (Girma, 2004; Girma *et al*., 2009).

3.9.2. Data Analysis

All the collected data were analyzed following the respective statistical procedures such as simple statistic by using SPSS or excel for quantitative and qualitative parameters. Analysis of Variance (ANOVA) was carried out using SAS computer software (9.2 versions) (SAS Institute, 2008). Based on the significant test results, treatment means were separated by Duncan's multiple range test (DMRT).

4. RESULTS

4.1 Distribution of Coffee Wilt Disease in Forest Coffee Areas

Coffee wilt disease was prevalent in forest coffee areas in southwest and southeast Ethiopia (Fig. 1) and its incidence varied from 0 to 13%, 0 to 28%, 0 to 20%, and 26 to 29% in forest coffee areas of Bonga (Kafa), Sheko (Berehane Kontir), Yayu (Illubabor) and Harenna (Bale), respectively (Table 1). The mean incidence was low at Bonga (9.4%) and high at Sheko (22.7%) and Harenna (27.1%) when compared to the overall mean of about 20.1% incidence of CWD recorded in the forest coffee areas of southwest and southeast Ethiopia in 2009 (Table 1). In 2010 survey period, the mean incidences were about 16.2, 17.5, 29.2 and 27.2 % at Bonga, Sheko, Yayu and Harenna forest coffee areas, recepectivly (Fig. 1). Similar trend was observed during the two survey years, although slight change was noted at Bonga and Yayu forest coffee areas.

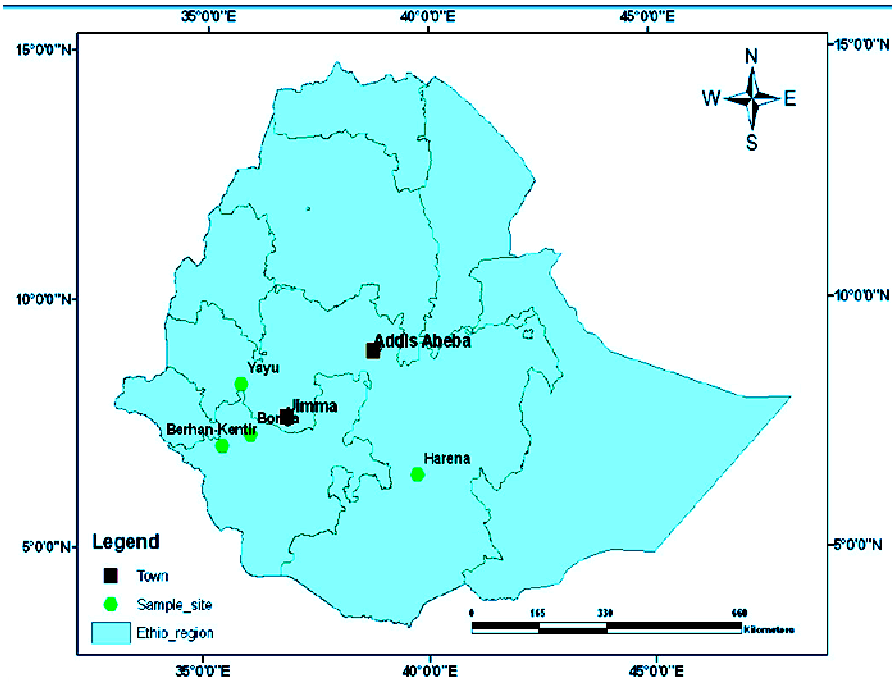

Figure 1. Distribution of coffee wilt disease in forest coffee areas of southwest and southeast Ethiopia.

Table 1. Incidences of coffee wilt disease in the forest coffee areas of southwest and southeast Ethiopia in 2009.

Location *	Range **	Mean & SD
Bonga	0.0 - 12.8	9.4±1.1
Sheko	0.0 - 27.6	22.7±12.3
Yayu	0.0 – 19.6	18.6±9.0
Harrena	26.2 - 28.5	27.1±4.8
Means & SD		20.1±6.6
CV (%)		48.9

*The actual data were transformed into angular value before analysis. **Sample field (n = 3)

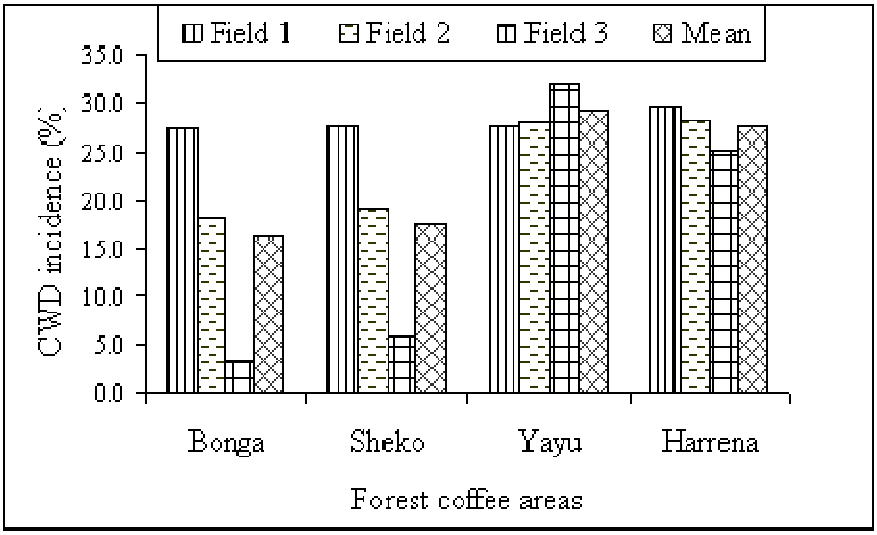

Figure 2. Incidences of coffee wilt disease in the forest coffee areas of southwest and southeast Ethiopia in 2010.

4.2. Isolation and Identification of the Fungus from Collected Samples

A total of 30 (93.75%) *Gibberella xylarioides* isolates were identified from 36 samples of coffee wilt disease (CWD) infected trees randomly collected from three fields in the four forest coffee areas, namely Bonga, Sheko (Berhane kontri) and Yayu in southwest and Harenna in southeast Ethiopia. The remaining 6.25% of the samples produced unidentified organisms like other *Fusarium* spp. Almost all of the six smples collected from Jimma (JARC) and Gera gave *G. xylarioides* (*Fusarium xylarioides*) (Fig. 3). The identification of isolated target fungus was based on the cultural and morphological descriptions of Booth (1971) and in refernce to standard cultures of the fungus preserved at JARC (Girma and Mengistu, 2000; Girma, 2004). Isolation and identification results indicated high proportion of CWD causing pathogen (*G. xylarioides*) obtained from Bonga (95.8%), whereas low proportion was recorded from Sheko (86.1% *G. xylarioides)* forest coffee areas in the southwest Ethiopia (Fig. 3).

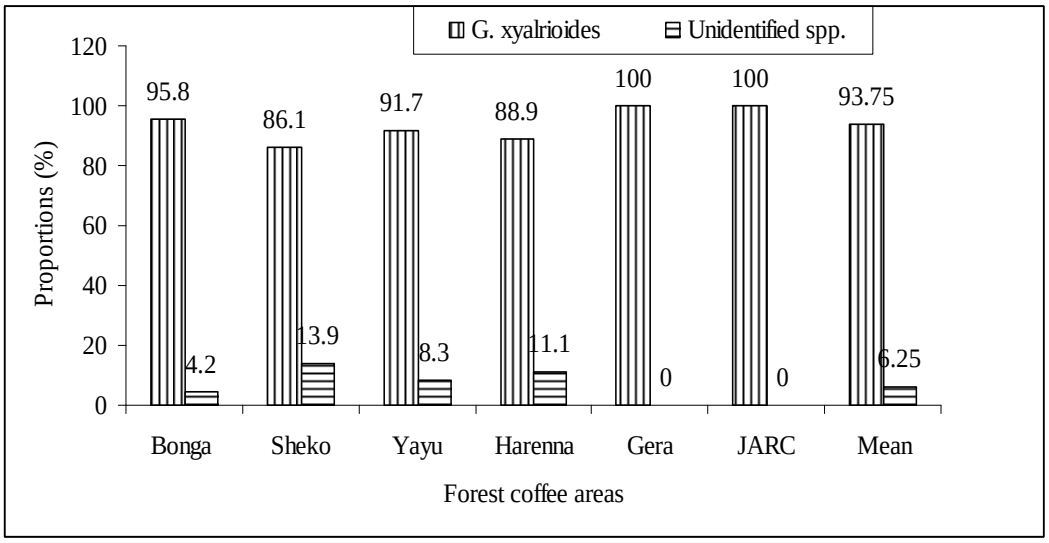

Figure 3. Proportions (%) of *Gibberella xylarioides* isolates and other unidentified spp. from the samples collected in southwest and southeast forest coffee areas of Ethiopia in 2009.

4. 3. Cultural and Morphological Characteristics of *Gibberella xylarioides*

4. 3.1 Cultural Characteristics

4. 3.1.1. Areal Mycelial Growth Type

The cultures of 30 representative *Gibberella xylarioides* isolates from southwest and southeast forest coffee areas including six isolates from Gera and Jimma were categorized into three classes based on colony mycelia growth types namely appressed (flat), slightly raised (intermediate) and raised with slight variation among the areas (Table 3). Out of 30 isolates studied for their aerial mycelial growth (vigor), about 80% was slightly raised and 20% was appresed (flat) from Bonga, Sheko and Yayu; while 66.7% and, 33.3% of the isolates from Harenna were slightly raised and raised type respectively. All the isolates (100%) from Gera and Jimma had slightly raised mycelia growth type (Table 2). Aerial mycelium growth of Harenna (Bale) isolates was different from the fungus isolates collected from Bonga, Sheko and Yayu (southwest) forest coffee areas as 33.3% of the isolates were raised.

4. 3.1.2. Colony Density

Colony density of *Gibberella xylarioides* isolates varied from slightly dense (intermediate) to dense growth type. It was observed that 100% of the isolates from Bonga and Sheko forest areas and from Gera and Jimma was slightly dense while some proportions of those from Yayu and Harenna showed dense mycelial growth (Table 3).

Table 2. Frequency of aerial mycelial growth of *Gibberella xylarioides* isolates from forest coffee populations in southwest and southeast Ethiopia in 2009.

Location	Frequency of Aerial mycelial growth (%)		
	Slightly raised	Raised	Appressed (flat)
Bonga (n= 5)	80	0	20
Sheko (n= 5)	80	0	20
Yayu (n= 8)	80	0	20
Harenna (Bale) (n= 6)	66.7	33.3	0
Gera (n= 3)	100	0	0
Jimma (n= 3)	100	0	0
Mean & SD	84.5 ± 13.1	5.6 ± 13.6	10 ± 10.9

n = number of representative isolates from each locations

Table 3. Frequency of colony density of the *Gibberlla xylarioides* isolates in southwest and southeast of Ethiopia in 2009.

Location	Frequency of colony density	
	Slightly dense	Dense
Bonga (n= 5)	100	0
Sheko (n= 5)	100	0
Yayu (n= 8)	88.9	11.1
Harenna (Bale) (n= 6)	83.3	16.7
Gera (n= 3)	100	0
Jimma (n= 3)	100	0
Mean & SD	95.4 ± 7.4	4.6 ± 7.4

n = number of representative isolates from each locations

4. 3.1.3. Colony Colors/pigmentations

The cultures of *Gibberella xylarioides* isolates showed typical color or pigmentation of the fungus when grown in potato sucrose agar (PSA) under standard conditions of 10 - 12 hr light/dark cycle at 23 ± 2 ^{o}C for 10 to 14 days (Booth 1971, Girma and Mengistu 2000; Girma 2004). When seen from the upper side (obverse side), the mycelial color of the isolates were generally grayish white and on the reverse side, the fungus produced various pigments into the culture media. Grayish white (with or without bluish spots around the center), purplish white, light violet and light bluish colors were commonly observed between 10 and 14 days of incubation (Table 4).

All isolates collected from Harenna (Bale) showed distinctly white (83%) and grayish white (17%) pigments (Table 4) but those *G. xylarioides* isolates collected from Bonga and Sheko isolates revealed light bluish (60%) and grayish white (40%). Pigmentation of Yayu isolates were white violate (50%) in reverse side of the plate (Table 4). Isolates collected from southeast (Harenna forest coffee) were different with grayish to white color as compared to those isolates collected from southwest forest coffee areas of Bonga, Sheko and Yayu as most of them showed varying pigmentations bluish white, violate and white purplish colony.

Table 4. Proportion (%) of colony colors (pigmentations) of *Gibberella xylarioides* isolates between 10 and 14 days of incubation period on potato sucrose agar under standard conditions

Location	Colony colors (pigmentation)									
	Obverse side (upper side)				Reverse side (Lower side)					
	B+W	GW	W	LP	B	B+W	GW	LB	LV	W
Bonga (n= 5)	40	60	-	-	40	-	-	60	-	-
Sheko (n= 5)	40	60	-	-	40	40	-	20	-	-
Yayu (n= 8)	12	50	-	38	-	15	-	15	70	-
Harenna (n= 6)	-	16.8	83.2	-	-	-	16.8	-	-	83.2
Gera (n= 3)	100	-	-	-	-	-	-	100	-	-
Jimma (n= 3)	100	-	-	-	-	-	-	100	-	-

*Colour (pigmentations) notation according to RGB color chart (RGB Hex= as RGB color codes); Where: B= Bluish; W = White; LB = light blue; LV= Light Violate; LP = light purplish; GW = Grayish White; B+W= Bluish white (White with bluish spot on the center of the plate).

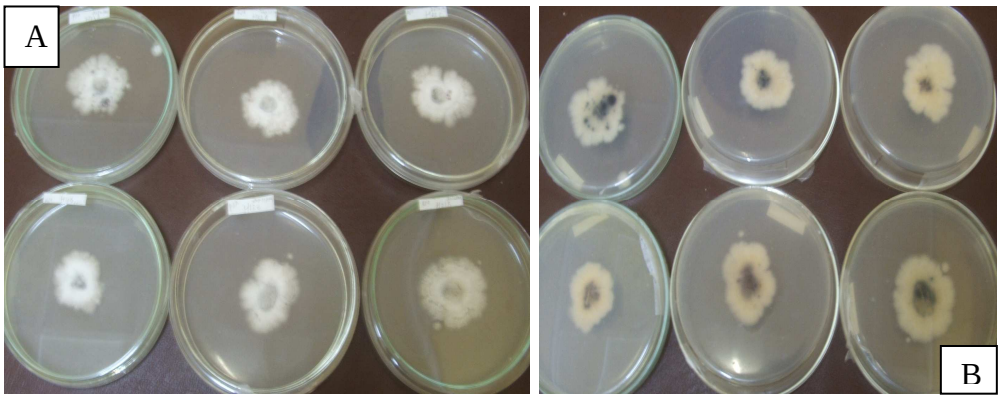

Plate 1. Pigmentation of *Gibberella xylarioides* isolates collected from Harenna forest coffee populations in southeast of Ethiopia, A, obverse side B; reverse side of the plates after 14 days of incubations.

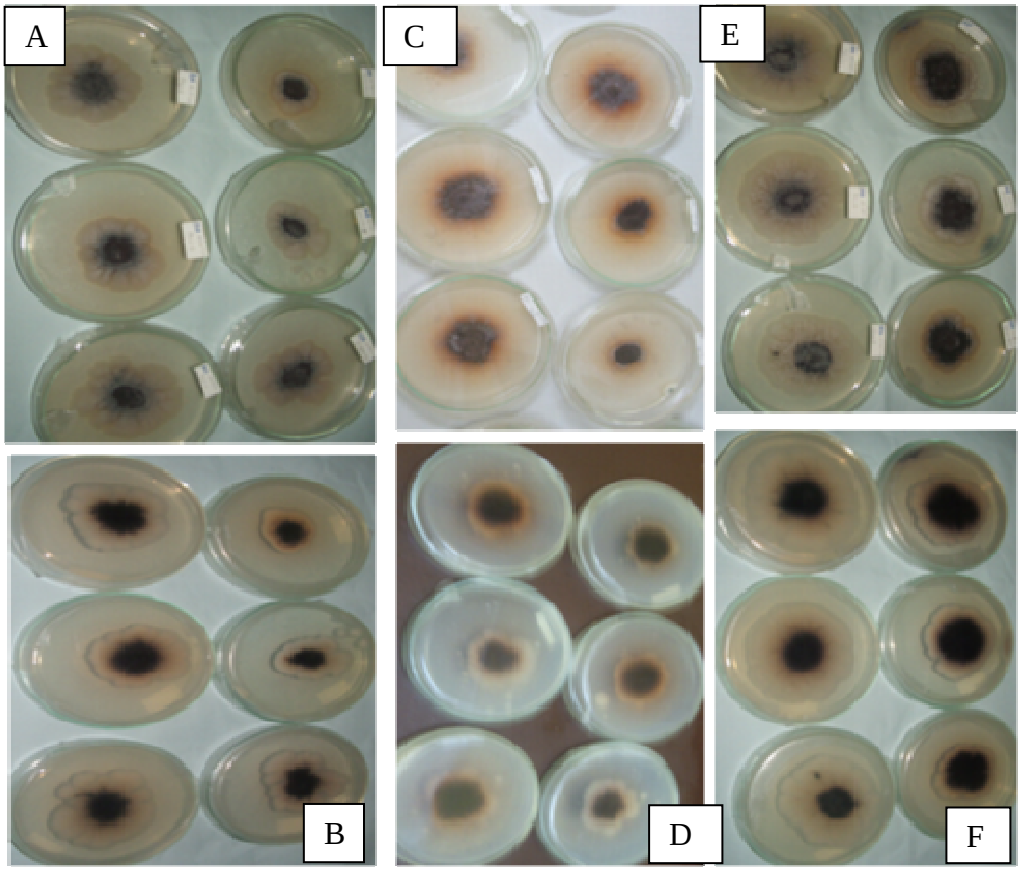

Plate 2**.** Pigimentation of *Gibberella xylarioides* isolates collected from Southwest forest coffee areas of Ethiopia. A, obverse and B, reverse side of isolates Bonga; C, obverse and D, reverse side of Sheko isolates E, obverse and F, reverse side of Yayu isolates

4. 3.1.4. Colony Radial Growth

The radial colony growth of *G. xylarioides* isolates were measured at 4, 7, 10, 14, and 21 days of incubation periods and the mean diameter were 16.3, 23.8, 32.6, 42.9 and 63.4 mm for Bonga isolates and 23.1, 31.4, 42.2, 56.1 and 71.1 mm for Sheko isolates, respectively. Radial growth rate of Bonga was analyzed at 4^{th} day incubation period 4 mm growth rate per day, whereas, at 7, 10, 14 and 21 incubation period the rate increased by 3mm per day. The Sheko isolate at 4^{th} day's incubation period increased by 5.75mm radial growth rate where as it decreased from 10 days incubation period as comperd to Harenna isolate but increased as compared to Yayu and Bonga isolates. Sheko isolates had faster radial growth rate than others, while, Jimma isolates were slower than others. The Harenna isolates had faster growth rate than those isolates from

southwest forest coffee areas including Jimma and Gera isolates during its first 10 days of incubation period and then became slower than the others after wards (Fig. 4).

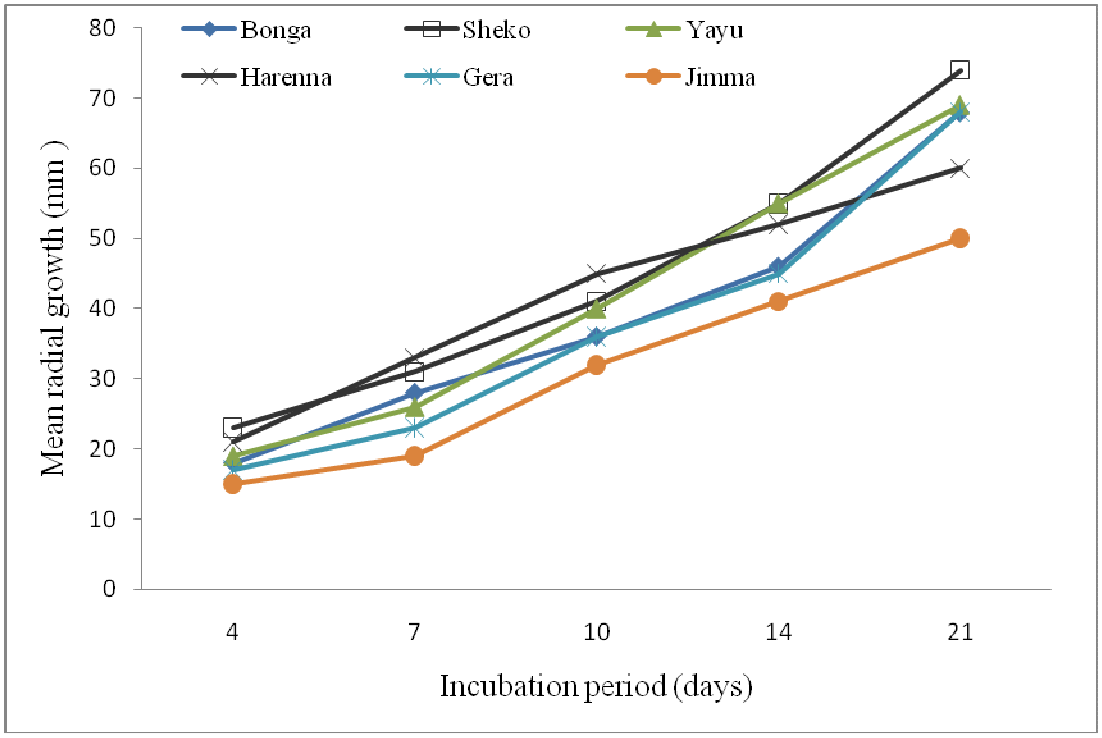

Figure 4. Mean radial colony growth rate of *Gibberella xylarioides* isolates collected from forest coffee areas of southwest and souteast Ethiopia

4. 3.2. Morphological Characteristics

4. 3.2.1. Conidia Shape and Dimension:

The *Fusarium* anamorph state of *Gibberella xylarioides* (*Fusarium xylarioides*) has two types of conidia namely macroconidia and microcondia, which are normally variable in shape and size with 1 – 3 septation. Some of macroconidia are cylindrical, slightly curved and curved with hooked ends. The microconidia have 0 – 1 septate and they are smaller in size and varying in shape; but the allentoid and u-shaped conidia were more frequent in this study. The macroconidia, in general, ranged from 17.6 x 2.8 µm to 25.0 x 2.9 µm while the microcondia varied between 10.9 x 2.9 µm and 11.8 x 2.9 µm on average (Table 5).

Table 5. Conidia shape and size of *Gibberella xylarioides* after 10 – 14 days incubation periods on PSA under standard conditions.

Location	Shape and Size (L x W) (µm)				
	Microconidia		Macroconidia		
	U-shaped	Allentoide	Curved	Cylindrical	Slightly curved
Bonga	11.2 x 2.8	12.1 x 2.9	21.4 x 2.9	24.9 x 2.9	25.7 x 2.9
Sheko	12.1 x 2.9	12.5 x 2.9	20.7 x 2.9	21.4 x 2.7	27.5 x 3.0
Yayu	13.4 x 2.9	13 x 2.9	20.1 x 2.8	25.2 x 2.9	25.4 x 2.9
Harrena	9.6 x 2.9	10.9 x 3.1	22.3 x 2.8	20.8 x 2.8	24.1 x 2.9
Gera	9.6 x 2.9	12.5 x 2.5	18.6 x 2.7	21.9 x 2.7	23 x 2.7
Jimma	9.6 x 2.9	10 x 3.1	22.3 x 2.8	20.8 x 2.8	24.1 x 2.9
Mean	10.9 x 2.9	11.8 x 2.9	17.6 x 2.8	22.5 x 2.8	25.0 x 2.9

4.4. Evaluation of the Reactions of Coffee Accessions Collected in the Forest Areas

In testing the reactions of some forest coffee accessions against an isolate from Yayu showed highly significant ($P<0.01$) differences both in percent seedling death (wilt) and incubation period (appendix Table 5 and 6). Accession HA2, HA6 and HA8 showed no seedling death at all, while accession HA5, BO3, HA7, BO4 and HA1 resulted in significantly ($P<0.01$) low percentage of dead seedlings of 31.7, 30.2, 21.8, 20.6 and 10.9% with mean incubation period of 94.7, 84.7, 84, 82.7 and 80 days, respectively, which was comparable to the standard checks Catimor J-21 (Table 6). Higher pecent seedling death was observed on accessions BO1, BO2, HA3, HA4 and YA1 than the standard susceptible check SN-5 (Plate 3). The result indicated that there were variabilities in resistance to CWD among the forest coffee accessions randomly collected from the three forest coffee areas of southwest (Yayu and Bonga) and southeast (Harenna) Ethiopia.

Table 6. Reactions of some forest coffee accessions inoculated with *G. xylarioides* isolate (Y21) at seedling stage in the greenhouse at Jimma Agricultural Research Center (2009).

Forest coffee accessions	Transformed mean value	Incubation period (mean number of days)
BO1	90 a	118 d
BO2	90 a	118 d
BO3	30.2 c	84.7 a
BO4	20.6 d	82.7 a
YA1	90 a	105.3 e
YA2	90 a	100 e
HA1	10.9 e	80 ba
HA2	0.0 f	0.0 f
HA3	90 a	118 e
HA4	90 a	110 e
HA5	31.7 c	94.7 bc
HA6	0.0 f	0.0 f
HA7	21.8 d	84 d c
HA8	0.0 f	0.0 f
Catimor J-21	32.1 c	94.7 a
SN-5	66.6 b	99.3 dc
Mean	47.1	80.6
CV (%)	7.4	6.7

*Actual mean percentage seedling death values transformed to arcsin square root before analysis (Gomez1984).** $p<0.01$

Means with the same letter (s) are not significantly different. BO1 – BO4, YA1 and YA2, HA1 – HA8 were forest coffee accessions randomly collected from Bonga, Yayu and Harenna (Bale), respectively. Catimor J-21 and SN-5 were CWD resistant and susceptiable checks, respectively (Girma, 2004).

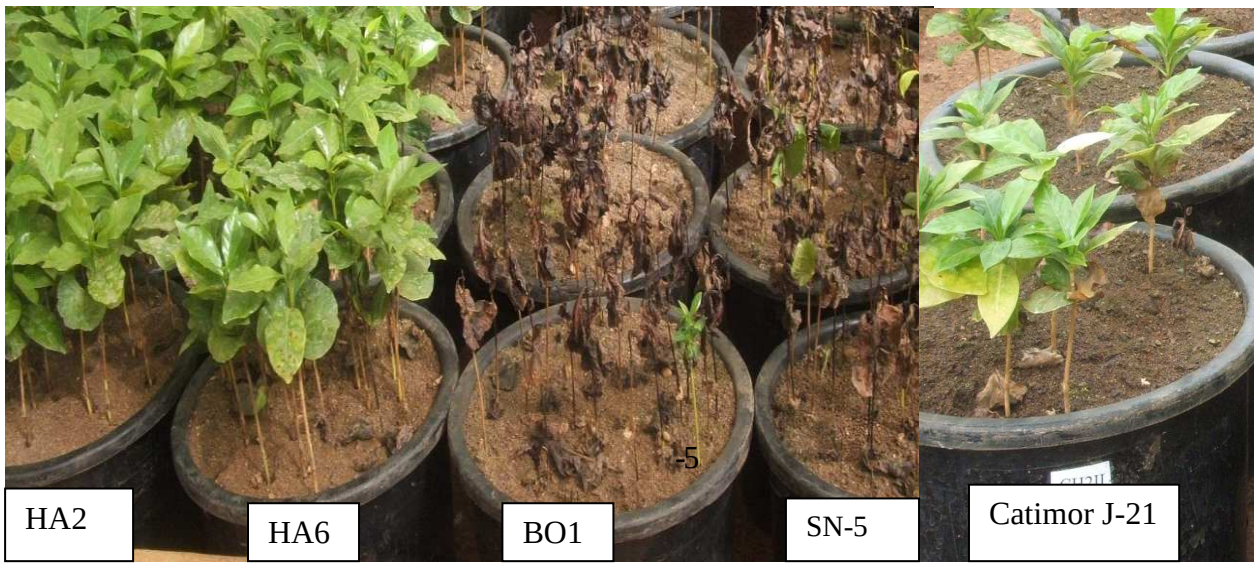

Plate.3. Comparisons of variations among resistant forest coffee accessions (HA2 and HA6) and susceptible accessions (BO1 and YA2) inoculated with *Gibberella xylarioides* in the greenhouse at JARC (2009/10). SN-5 and Catimor J-21 were CWD susceptiable and resistant checks respectively.

4. 5. Interactions of Forest Coffee Accessions by *Gibberella xylarioides* Isolates

4. 5.1 Description of Symptoms on Inoculated Coffee Seedlings

There were typically two types of external symptoms observed on artificially inoculated and infected coffee seedlings in the greenhouse. The first symptom type was chlorosis and defolation where the leaves of infected coffee seedlings showed progressive change in color from green to yellowish (Plate 4 A) and eventually drop from the young seedlings before drying (Plate 4 B).

The second symptom was necrotic type where the leaves showed epinasty, dullness, and loss of turgidity in green leaves. These epinastic leaves later desiccate and wilt completely. This type of symptom did not involve shading of the leaves (defoliation) rather they retained on the seedlings (Plate 5 A and B). Internally the vascular tissues of most of the inoculated coffee seedlings had a characteristic brownish/bluish black streak visible on the wood.

Plate 4. Chlorotic-defoliation external symptom of CWD on inoculated seedlings with *Gibberella xylarioides* in the greenhouse at Jimma Agricultural Reaserch Center (JARC).

Plate 5. Necrotic-non defoliating external symptoms of CWD on inoculated coffee seedlings with *Gibberella xylarioides* isolates in the greenhouse at JARC

The infection symptom types varied with forest coffee accession by isolate combinations. Seedlings of Harrena (P11xB23) and other accessions (P27xB23, P41xB23, P49xB23, P59xB23) inoculated with Bonga isolate and accession-isolate combination of P21xY21, P34xY21 P38xY21 and P41xG11, uniformly manifested chlorotic–defoliation wilting symptom.

Seedlings of coffee accession–isolate combinations of P21xB23, P27xH11, P27xY21, P34xSH21, P38xB23, and 7440xB showed necrotic symptom type of CWD. There were no external symptoms exhibited on most seedlings of all accessions treated by Sheko isolates except on accessions P21, P27, P34, and P38.

In all cases the fungus isolates were retrieved by plating small sections from the stem and root parts of symptomatic and asymptomatic sample of the inoculated coffee seedlings, and the cultural and morphological characteristics of each re-isolate was confirmed in comparison with that of the original parent (preserved) cultures used for inoculation.

4.5.2. Forest coffee accessions by *Gibberella xylarioides* Isolates Interactions

Five *G. xylarioides* isolates namely B23, SH21, Y21 and H11 representing the four forest coffee areas of Bonga, Sheko, Yayu and Harenna, respectively, and one isolate (G11) obtained from infected coffee tree in plantation coffee at Gera were used to inoculate seedlings of 13 forest coffee accessions originated from forest and a moderately resistant released variety 7440. There were highly significant ($P < 0.01$) differences in percent seedling deaths and incubation periods under glasshouse conditions (Appendix 7 and 8). Seedlings of forest coffee accessions P38 (Sheko), P27 (Bonga) P34 (Sheko) showed significantly ($P < 0.05$) higher mean percent deaths of 85.69, 79.15 and 76.53 (Table 7), with incubation periods of 72.1, 65.9 and 71 days, respectively (Table 7; Plate 6). On the contrary, significantly ($p < 0.05$) lower percent seedling deaths of 21.91, 21.49, 33.64 and 35.73 was observed on accessions of P4, P6 and P11 (Harenna), and P17 (Bonga), respectively, with incubation periods of 39.3, 53.4, 66.4 and 63.8 mean number of days, respectively (Table 8). Seedlings of Harenna accessions P4 and P6 exhibited higher resistance reaction than the moderately resistance check 7440 to all the four isolates (B23, SH21, Y21, and G11) implying horizontal resistance reaction, while accessions of Sheko (P38 and P34), Bonga (P27) and Yayu (P47 and P49) showed highly suceptiable reactions to most of isolates (Table 7 and Plate 6).

G. xylarioides isolates H11, B23, G11, and Y21 caused significantly higher mean seedling deaths with 78.65, 63.37, 61.53 and 56. 35 mean percentages with mean incubation days of 88.7, 57.9, 66.9 and 64.9 days, respectively, than isolate SH21 (Sheko isolate) with 20.83%

seedling death (Table 8and Plate 6). Among the isolates, Harenna (H11) isolate was most aggressive to all accessions as compared to the others including an isolate from plantation coffee (G11). Sheko isolate (SH21) was the least one while Y21 isolate was moderately aggressive isolate (Table 7; Plate 7 B, D and E). In accession by-isolate interactions (i.e differential effects), seedlings of Harenna accessions (P4, P6 and P11) were highly resistant (0 – 30%) to all but susceptiable to Harenna isolate (H11) which caused 90% death. All Yayu accessions (P47, P49 and P59) were highly resistant to only Sheko isolate (0.0%) but attacked by the four *Gibberlla xylarioides* isolates (B23, Y21, H11 and G11) (Table 7 and Plate 7). This result indicates host-pathogen specificity with some vertical resistance reactions and virulence in the pathogen populations originated from the four major forest coffee areas.

Table 7. Percent seedling deaths of forest coffee accessions inoculated with five *Gibberella xylariodes* isolates collected in southwest and southeast Ethiopia in the green house at Jimma Agricultural Research Center (2009/2010).

Forest coffee accessions[2]	*Gibberella xylarioides* isolates[1]					
	B23	SH21	Y21	H11	G11	Mean
P4	10.00 jk	0.00[3] k	0.00 k	90.00 a	9.57 jk	21.91 G
P6	13.17 jk	0.00 k	4.10 k	77.73 a-e	12.43 jk	21.49 G
P11	30.43 ij	0.00 k	31.33 ij	78.77 a-e	27.67 ij	33.64 F
P17	44.23 g-i	13.87 jk	16.53 jk	55.87 e-h	48.13 f-i	35.73 EF
P21	45.13 g-i	63.33 b-h	66.33 b-g	56.83 d-h	47.30 f-i	55.79 D
P27	82.13 a-c	64.93 b-h	79.30 a-d	90.00 a	79.37 a-d	79.15 AB
P34	90.00a	42.70 hi	69.93 a-f	90.00 a	90.00 a	76.53 AB
P38	90.00 a	76.53 a-e	86.17 ab	85.77 a-c	90.00 a	85.69 A
P41	90.00 a	9.47 jk	90.00 a	90.00 a	84.27 a-c	72.75 B
P47	90.00 a	0.00 k	90.00 a	90.00 a	80.57 a-c	70.11 BC
P49	86.17ab	0.00 k	90.00 a	85.97 a-c	90.00 a	70.43 BC
P59	85.53 a-c	0.00 k	90.00 a	63.03 c-h	69.10 a-f	61.53 CD
7440	67.00 a-g	0.00 k	18.83 jk	68.47 a-f	71.47 p	45.15 E
Means	63.37 X	20.83 Z	56.35 Y	78.65 W	61.53 XY	

[1]B23, SH21, Y21, H11and G11 were *G. xylarioides* isolates obtained from Bonga, Sheko, Yayu, Harena and Gera, respectively.
[2]P4, P6, & P11 (Harenna accessions), P17, P21, P27 (Bonga accessions), P34, P38 & P41 (Sheko accessions), P47, P49 & P59 (Yayu accessions) and 7440 used as moderately resistant respectively. CV = 25.41%.
[3]0.0 indicate no seedling death was observed during the trial period.
Means followed with the same letter(s) are not significantly ($P < 0.05$) different from each other according to Duncun's Multiple Range Test (DMRT). LSD values for the forest coffee accessions, the isolates and the interactions comparisons were 10.31, 6.39, and 23.05, respectively.

Table 8. Incubation periods (mean number of days) of five *Gibberella xylarioides* isolates *inoculated on seedlings of thirteen forest coffee accessions in green house at Jimma Agricultural Research Center (2009/2010)*.

Forest coffee Accessions[2]	*Gibberella xylarioides* isolates[1]					
	B23	SH21	Y21	H11	G11	Mean
P4	28.33h-j	0.00 j[3]	0.00 j	106.00 a	62.33c-h	39.33 D
P6	56.33 d-i	0.00 j	23.67 ij	97.67 a-c	89.33 a-e	53.40 CD
P11	61.67c-i	0.00j	94.00 a-d	96.67 a-c	80.00 a-g	66.47 A-C
P17	61.00 c-i	28.33 h-j	80.00 a-g	80.67 a-g	69.00 a-g	63.80 A-C
P21	42.67 g-i	64.00 b-h	66.33 b-h	94.33 a-d	47.33f-i	62.93 A-C
P27	57.00 d-i	71.00 a-g	73.33 a-g	66.67 b-g	61.67 c-i	65.93 A-C
P34	61.67c-i	73.33 a-g	66.33 b-h	89.67 a-e	64.00 b-h	71.00 AB
P38	71.00 a-g	71.00 a-g	81.33 a-f	73.33 a-g	64.00 b-h	72.13 A
P41	66.67 f-i	49.67 f-i	61.67 c-i	83.00 a-f	57.00 d-i	63.60 A-C
P47	52.33 e-i	0.00 j	66.33 b-h	96.67 a-c	61.67 c-i	55.40 A-D
P49	57.00 d-i	0.00j	71.00a-g	75.00a-g	71.00 a-g	54.80 B-D
P59	57.00 d-i	0.00j	71.00a-g	92.33 a-d	66.33 b-h	57.33 A-C
7440	80.00 a-g	0.00j	88.67 a-e	101.3 ab	75.67 a-g	69.13 A-C
Means	57.90 Y	27.49 X	64.90 Z	88.72 W	66.87 Z	

[1]B23, SH21, Y21, H11 and G11 were *G. xylarioides* isolates obtained from Bonga, Sheko, Yayu, Harenna, and Gera, respectively.

[2]P4, P6 and & P11 (Harenna accessions), P17, P21 and P27 (Bonga accessions), P34, P38 and P41 (Sheko accessions), P47, P49 and P59 (Yayu accessions). 7440 used as CWD moderately resistant selection.

[3]0.0 = indicates no incubation period (no external symptom was observed until end of the trial).

Means followed with the same letter(s) are not significantly ($P < 0.05$) different from each other according to DMRT. LSD values for the cultivars, the isolates and the interactions comparisons were 17.13, 10.62 and 38.28, respectively. CV = 38.75%.

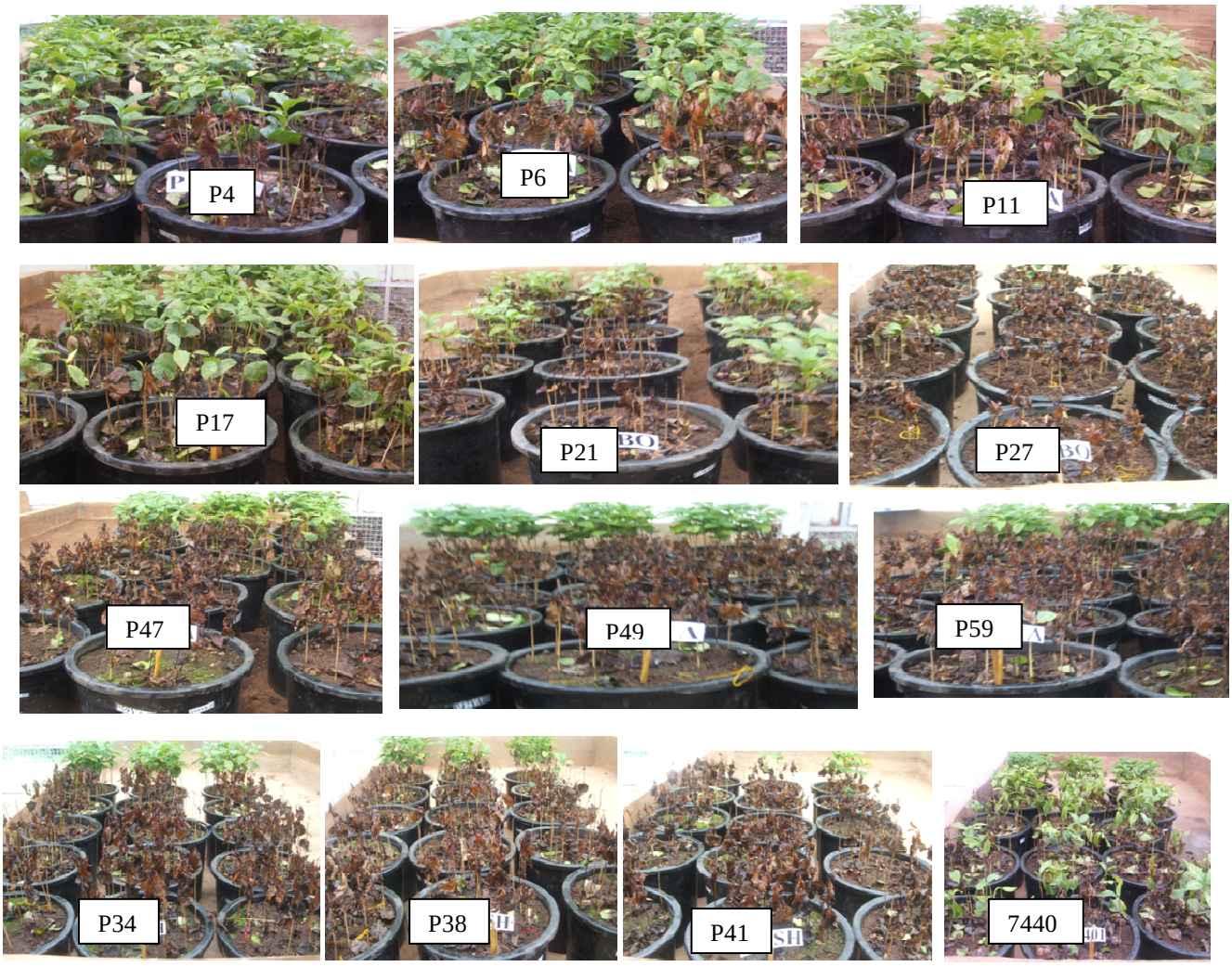

Plate 6. Reaction of forest coffee accessions to *Gibberella xylarioides* collected from southwest and southeast forest coffee areas of Ethiopia. P4, P6, P11 are Harenna accessions; P17, P21, P27 are Bonga accessions; P34, P38, P41 are Sheko accessions and P47, P49, P59 are Yayu accessions.

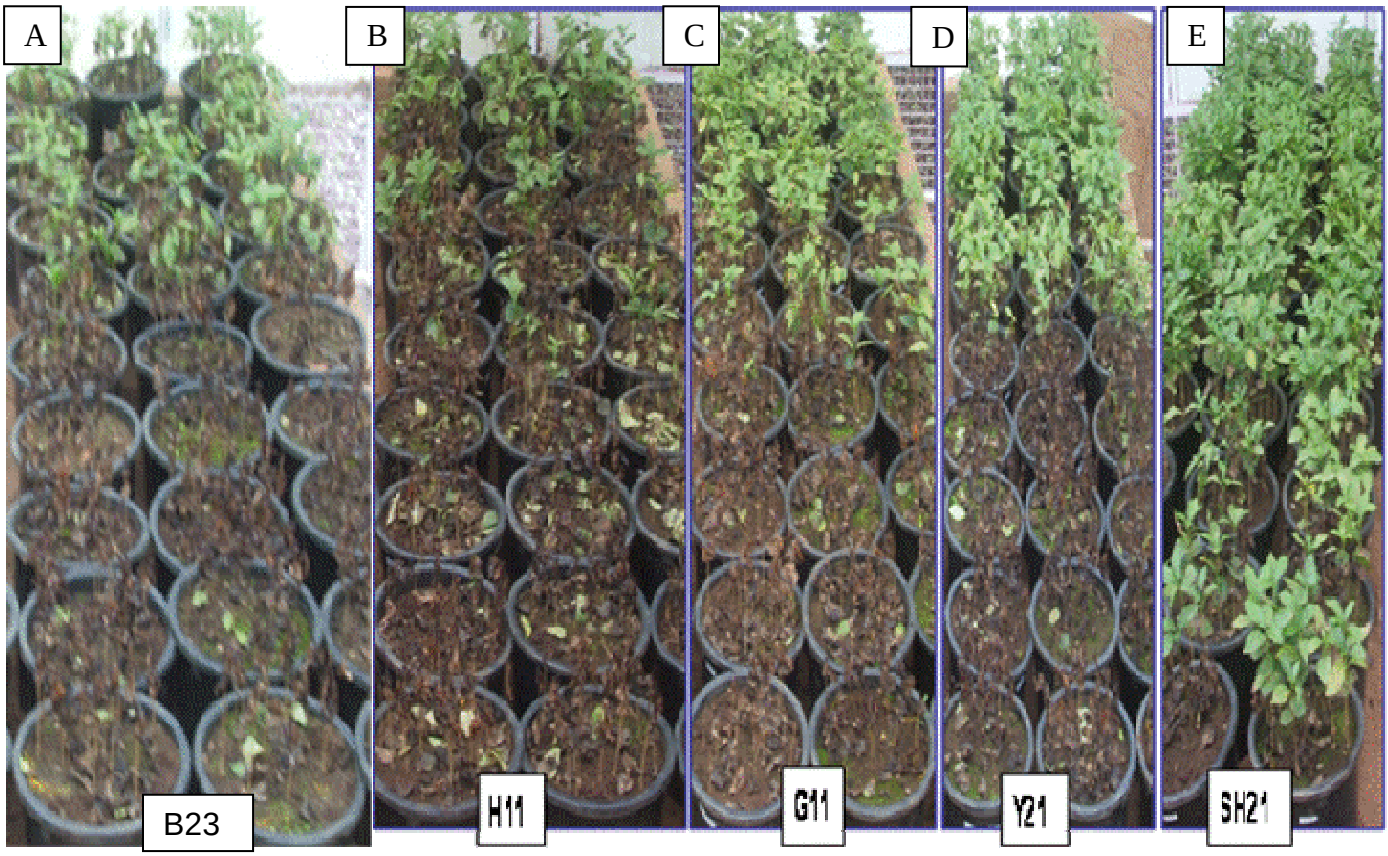

Plate 7. Variation in aggressiveness of *Gibberella xylarioides* isolates on seedlings some forest coffee accessions collected from southwest and southeast Ethiopia. B23 indicates Bonga isolate; H11 indictes Harenna isolate; G11 indicates Gera isolates; Y21 indicates Yayu isolates and SH21 indicates Sheko isolates

5. DISCUSSION

Coffee wilt disease (tracheomycosis) occurs in the fields of all coffee production systems-forest, semi-forest, garden and plantation coffees, with increasing order of importance varying in the extent of damage among and within coffee fields and districts. These variations were depending on different interacting factors, mainly susceptibility of coffee trees, intensity of cultural practices and environmental conditions (Girma and Hindorf, 2001; CABI, 2003; Girma, 2004). In the present study the incidence and severity of the disease was assessed in four forest coffee areas, considering three sample fields per locality accompanied by collecting three infected trees from each field and isolating and identifying the causal pathogens. During the two year survey (2009/10), CWD incidence was higher in the forest coffee areas of Harenna (southeast) followed by Yayu and Sheko (southwest). The mean percent incidence during the first assessment was 9.4% at Bonga (Kafa), 22.7% at Sheko (Behan-Konitr), 18.6% at Yayu (Illubabor) and 27.1% Harenna (Bale), respectively (Table 1). During the second year, the incidence increased to 16.2, 17.5, 29.2, and 27.5 % in the respective forest areas (Fig. 1). Arega (2006) reported that coffee wilt disease incidence ranged from 6.1% at Bonga, 2.4% at Sheko (Behan- Konitr) to 16.9% at Yayu and 10.1% at Harenna in rainforest coffee areas.

Although there was variations in the disease incidence from sample plot to plot and from one forest coffee areas to the other, the result showed that coffee wilt disease is increasing from time to time. This was may be because of the differences in genetic diversity of coffee populations (susceptibility/ resistance), aggressiveness of the pathogen and environmental conditions and some human interference to exploit forest coffee.

According to the observation in each forest coffee field in Yayu, Sheko and Harenna accustomed to work in the forest area and use cutlasses (bushman knives), indiscriminately and without taking care of coffee trees not to wound due to lack of awareness, to slash weeds around coffee trees once per year. The possibility of wounding and contaminating the coffee trees with the pathogen contaminated cutlasses (bushman knives) from diseased coffee trees or soil is high when they slash the dense weed masses cover around coffee trees. The other means of the occurrence of CWD is presence of infected tree for long time which is used for as primary

inoculum for infection of the disease. In Harenna forest coffee, flocks of cattles have grazed and moved along the coffee trees while they are damaging mechanically the coffee trees, seedlings and carrying the inoculum on their bodies from area to area. In Berhan-Kontir (Sheko) people intensively use infected coffee trees as firewood and carries them across the forest coffee that could be the means for distribution and spread of the disease. All the above mechanisms, most probably, aggravated the spread and distribution of the disease in the forest coffee and increasing the damaging effects of the disease in the areas.

There were high proportions (93.75%) of *Gibberella xylarioides* (*Fusarium xylarioides*) isolates identified from most of the samples of infected coffee trees randomly collected in these forest coffee areas. The identification of the pathogen was undertaken based on cultural and morphological characteristics of the fungus species described by Booth (1971) and Girma and Mengistu (2000), in comparison to previous works and reference cultures properly preserved in the Plant Pathology laboratory of Jimma Agricultural Research Center. This is may be the first assessment works supported by isolation and identification of the causal pathogen *Gibberella xylarioides* of wilting trees in the various forest coffee areas. Further more cultural and morphological features of the fungus populations were characterized on recommended media namely (potato sucrose agar (PSA) and synthetic low nutrient agar (SNA) and under standard conditions. Accordingly, 24 isolates from forest coffee areas of Bonga, Sheko, and Yayu in southwest and Harenna (southeast) were studied in comparison with six *Gibberella xylarioides* isolates representing plantation (3 sample) coffee at Gera and semi-forest coffee at Jimma (3 sample).

Based on mycelial growth type, most of *G. xylarioides* isolates were slightly raised and slightly dense (80%) and appressed (20%) while Harenna isolates were slightly different in that about 66.7 percent were slightly raised and 33.3 percent had raised mycelial growth type (Table 3). The average colony radial growth rate on PSA was 2.2 - 4.4 mm per day although there was some variation among the isolates. The result revealed that the radial colony growth rate of isolates varied between southwest and southeast, as the Harenna isolates were slow growing type as opposed to that of Bonga, Sheko and Yayu. There were variations in colony pigmentation (colors) among the location and most of Harenna isolates (southeast) were white to grayish white

but isolates from Bonga, Sheko and Yayu (southwest) showed light bluish to bluish, purplish and violet in appearances.

The morphological characteristics of *G. xylarioides* isolates were observed on SNA (synthetic low-nutrient agar). The sizes and shapes of conidia were variable. The average size of macroconidia ranged from 22.5 to 25.0 μm in length and 2.8- 2.9 μm in width, while microcondial size varied from 10.0 to 13.4 μm in length with similar size in width to the macroconidia. Sporulation capacity of isolates varied among the locations and conidia production ranged between 2.15 x 10^6 (by isolate B23 from Bonga) and 2.18 x 10^6 conidia/ml (by isolate SH21 from Sheko), 2.2 x 10^6 conidia/ml (by isolate Y21 from Yayu, 2.3x10^6conidia/ml (by H11 from Harenna) and 2.14 x 10^6 conidia/ml (by G11 from Gera). Girma (1998), Girma and Mengsitu (2000) reported similar findings on the cultural and morphological characteristics of large number of the fungus isolates collected from Bebeka, Tepi, Gera and Jimma coffee growing areas in the southwest Ethiopia.

Evaluation of reactions of some coffee accessions collected from Bonga and Yayu in the southwest and Harenna in the southeast including a susceptible (SN-5) and a resistance (Catimor J-21) checks was studied by inoculating a representative *G. xylarioides* isolate from Yayu forest (Y21) under greenhouse condition at Jimma (JARC). There were highly significant differences among forest coffee accessions and seedlings of Harenna accession; HA2, HA6 and HA8 had slight seedling deaths of less than 10 percent exhibiting higher resistance even as compared to the standard check (Catimor J-21). On the other hand, accessions from Bonga (BO1 and BO2) and Yayu (YA1 andYA2) showed significantly higher susceptibility with 90% seedling death than the susceptible check (SN-5). There were some accessions like BO4 and HA1 showing moderate tolerance reactions. Arega (2006) also reported existence of variations in the forest coffee populations and about seven accessions from Harenna revealed significantly low level of percent seedling death due to coffee wilt disease in the greenhouse study.

In the host-pathogen interaction study, seedlings of twelve forest coffee accessions (one standard check 7440) were independently inoculated with four *Gibberella xylarioides* isolates each of them representing Bonga (B23), Sheko (SH11) and Yayu (Y21) in the southwest and Harenna (H11) in the southeast forest coffee areas of Ethiopia. These host-pathogen combinations were

compared among each other and against the fungus isolate originated from plantation coffee at Gera (G11). The result showed that there were highly significant ($P < 0.01$) differences among forest coffee accessions and among *G. xylarioides* isolates; and the interactions between the accessions and the isolates both in percent seedling deaths and incubation periods. As already reported by Girma and Mengistu (2001) and Girma *et al.* (2005), this study also indicated the existence of horizontal resistance in the host, and variations in aggressiveness in the fungus populations; while the significant interactions (i.e. the differential effects) between coffee accessions and *G. xylarioides* isolates implicated some vertical resistance in the host populations and virulence in the pathogen strains. In this case, the Harenna accessions namely P4 and P6 revealed horizontally high resistance, P11 and P17 were moderately tolerant with low mean percent seedling deaths of about 20 to 35 percent, respectively (Table 7). The Harenna isolate was more aggressive than the isolates from other forest areas with mean seedling deaths of 79 percent and even highly pathogenic than the isolates from plantation coffee (G11) that caused 61.5 percent (Table 7). Harenna isolate (H11) was more pathogenic than Sheko (SH21) isolates on coffee accessions of P4, P6, P21, P41 and P47 but less pathogenic on accession P17 than Sheko isolates. Bonga isolates (B23) seem to be moderately aggressive on most coffee accessions (P34, P38, P41, P47 and P49), Sheko isolates became less aggressive or weekly pathogenic to P4 (Table 7).

On the contrary, accession P38 (Sheko accession) and P27 (Bonga accession) were susceptible to most isolates as they showed high percentage seedling death (Table 7). Harenna isolate was aggressive than Sheko isolates on most forest coffee accessions while Sheko isolate was the least one. Bonga isolate was moderately aggressive as those isolates from plantation coffee at Gera (G11) (Table 7 and 8). In comparing the combined forest coffee accession - isolate interactions (differential effects) on percent seedling death showed that Harenna (H11) isolates induced a higher rate of percent seedling death on accession P41, P47 (90%) and P49 (85.97%) than Sheko (B21) isolates on the same accession of P41, P47(0.0%) and P49 (0.00%). Yayu (Y21) isolate was moderately aggressive next to Bonga isolate on the accessions of P47, P49 and P59 (90%) seedling death than Sheko isolate on the same accession of P47, Y49 and P59 (Table. 7). Girma *et al*., (2005) reported similar results on coffee cultivars *vs. G. xylarioides* isolate interactions; there was variability in the aggressiveness of the pathogen and vertical resistance of Arabica

coffee cultivars under greenhouse condition. Results of these study confirmed that the variations were mainly due to the existence of difference in the genetic make up of the accession in reaction to coffee wilt disease. In this case the resistance was predominantly horizontal, while the significant cultivar x isolate interaction (differential effect) presented some evidence for vertical resistance (race specificity) in Arabica coffee.

There were typically two types of external symptoms observed on inoculated seedlings with *G.xylarioides* isolates. The first one was chlorotic-defolating type while the second type was necrotic-none defoliating symptoms of coffee wilt disease and both types of CWD symptoms varied with forest coffee accession by isolate combinations. There was also no external symptom exhibited on seedlings of some accessions treated by Sheko isolates except accessions P21xSH21, P27xSH21, P34xSH21, and P38xSH21. However, the pathogen was re-isolated from these apparently healthy looking seedlings in the experiment. In general, such symptoms of CWD are characteristics of almost all wilt diseases on perennial woody plants including coffee (Girma, 2004).

These investigations on coffee wilt disease and its causal pathogen in the forest coffee populations indicated that the disease is increasingly becoming equally important as the case in semiforest, garden and plantation coffee. The fungus populations are found to be very pathogenic even more aggressive than those *G. xylarioides* isolates collected from plantation coffee. In this regard, coffee wilt disease is becoming one of the major biotic factors endangering the conservation of wild forest coffee in the montane rainforests of the country. Although some isolate population like that of Harenna (south east) showed variations in cultural characteristics such as colony pigmentation, growth rate and aerial mycelial growth, from those groups in southwest, they are in the domain of the fungus description given by many authors. The differences among the sample plots along with the variations obtained in seedling inoculation tests for evaluating the reactions of accessions to the fungus pathogen revealed that there are many promising resistant coffee genotypes to be used for variety development.

6. SUMMARY AND CONCLUSION

Coffee is vital to the economy of Ethiopia, providing a major source of foreign exchange earnings and, as a cash crop, supporting the livelihoods of millions involved in cultivation, processing, marketing, and export. However, *Coffea arabica* is susceptible to most diseases mainly coffee berry disease, coffee wilt and leaf rust. Among these, coffee wilt disease caused by *Gibberella xylarioides* (*Fusarium xylarioides*) is one of the highly destructive diseases that affect plantations and smallholder coffee resulting in yield reduction. The disease inflicted losses to coffee production in Africa since 1927 but has been largely contained with host resistance and in some instances wide-scale sanitation practices. It is also known to attack semi forest coffee, garden, plantation, and forest coffees in Ethiopia.

Prevalence of CWD reported as high as 20% incidence in the southeast and southwest forest coffee areas of Bonga (Kaffa), Sheko (Berhane-kontir), Yayu (Ilubabor) and Harenna (Bale) in 2006. This assessment also indicated that the occurrence of CWD has been increasing in some forest areas like in Harenna (Bale) and Bonga (Kaffa). However, no isolates of *Gibberella xylarioides* was collected and characterized yet from these areas, although the damage was significantly influencing forest coffee conservation. Thus, the objectives of this study were to characterize and determine the fungus variability in cultural and morphological characteristics, pathogenicity in the forest coffee populations, and also examine the reactions of some forest coffee accessions collected in the major montane forest areas of southwest and southeast Ethiopia against the pathogen.

The study was conducted both in the field, in the laboratory and in the greenhouse. Field studies were conducted in rainforest coffee populations in Bonga (Kafa), Sheko (Berhane Kontri), Yayu (Ilubabor) and Harenna (Bale) forest coffee areas in southwest and southeast Ethiopia. In this study period (2009 – 2010), the disease severity was assessed and number of CWD infected and healthy coffee trees were recorded. At the same time, samples of infected coffee trees (three samples per location) were collected and brought to the laboratory for further study. In addition, some specimens were included from semi-forest and plantation coffees at Jimma and Gera, respectively.

Coffee wilt disease was prevalent in most assessed forest coffee areas with significantly increasing coffee tree losses that ranged from 12.8% to 27.6% at Bonga (Kaffa) and at Sheko (Brehan-kontir) in southwest. The disease is more severe in Harenna (Bale) varying between 26.2 and 28.5% in southeast forest coffee areas. The survey results in both years showed that CWD is expanding at a rapid rate and affecting the coffee genetic resource conservation in the most forest areas. The causal agent of most of the tree deaths was confirmed to be *Gibberella xylarioides* as on the average 93.7% of the samples from infected coffee trees in all forest areas produced the fungus while isolating and identifying in the laboratory.

In cultural and morphological characterization of *G. xylarioides* populations, most of the isolates from the southwest forest coffee areas had similar appearances in colony pigmentations, aerial and radial growths but slightly different from those isolates collected in the southeast forest coffee. The Harenna isolates are slightly raised and raised type with white and grayish white colony color whereas most of those strains from Bonga, Sheko and Yayu including isolates from Jimma (semiforest) and Gera (plantation) had dominantly the slightly raised and slightly dense colony with pigmentations varying from greyish white, light bluish, purplish white, and light violet between 10 and 14 days after plating. Morphologically, most of the fungus isolates were similar in features like shape and size of both conidia (macro and micro) and sepations.

The reactions of 14 accessions collected in some forest areas of Bonga and Yayu (southwest) and Harenna (southeast) were tested at seedling stage by inoculating with Yayu isolate (Y21) in the greenhouse at JARC. The result indicated highly significant differences among accessions ranging between resistant and susceptible host reactions. Accessions of Harenna HA1, HA2, HA6 and HA8 had very low (0 – 10 %) seedling deaths being highly resistant while accessions of Bonga (BO1 and BO2) and Yayu (YA1 and YA2) showed complete seedling death (90%) being highly susceptible to the pathogen isolates in the forest.

There were highly significant ($p < 0.01$) differences both in percent seedling deaths and incubation periods in the host-pathogen interactions study where by seedlings of 12 coffee accessions were inoculated with five *Gibberella xylarioides* isolates representing forest

populations and including one check from plantation of both organisms. The significant variations among accessions and among isolates (both main effects) indicated horizontal host resistance and horizontal pathogenicity in the pathogen populations (aggressiveness); and significant interactions between the accessions and the isolates (i.e., the differential effects) implicated vertical resistance in the host and virulence in the pathogen strains collected in the forests.

Based on the results, the accessions from Harenna (P4 and P6) revealed resistance reaction to most isolates with low mean percent seedling death (21.9 and 21.5%); whereas Sheko accessions (P38) and Bonga (P27) were susceptible to most isolates and showed high mean percentage seedling death (85.7 and 79.2%), respectively. Harenna isolate was more aggressive than most of the isolates including the one from plantation coffee (Gera). In comparing the combined forest coffee accessions by isolate interactions (differential effects), Harenna (H11) isolates induced a higher rate of percent seedling death on accessions P41, P47 (90%) and Y49 (85.97%) than Sheko (SH21) isolate on the same accession of P41, P47 (0.0%) and P49 (0.00%). Yayu (Y21) isolate was moderately aggressive next to Bonga isolate on the accessions of Y47, P49 and P59 (90%) seedling death than Sheko isolate on the same accession. Besides, these accessions-isolates interactions associated with the manifestation of two characteristic coffee wilt symptoms namely chlorotic-defoliating and necrotic-non defoliating types.

In conclusion, coffee wilt is an important disease of forest coffee impacting the conservation of wild coffee genetic resources although there exist variations in host reactions to the pathogen. The fungus populations collected in these forest coffee areas showed basically similar cultural and morphological structures of the species *Gibberella xylarioides* although clear differences in some features like growth nature, pigmentation and aggressiveness. The pathogen strains in the forests coffee areas equally as aggressive as those isolates in plantation coffee. Generally, accessions from Bonga, and Harenna showed higher range of variation where as accessions from Sheko (Birhane-Kontir) showed very low range of variations, as compared to others against coffee wilt disease. This indicate that in the course of resistant coffee variety development it is possible to get many resistant genetic material from Harenna and Bonga followed by Yayu indigenous forest coffee germplasms. So it is important to conserve the forest indigenous coffee

germplasms both *insitu* and *exsitu* and use sustainably by first applying intensive selection from more diverse coffee populations and evaluation for resistance to CWD and along with applying proper management practices shouled be encaraged for these high yield, better quality and other characteristics to enhance the production and productivity of coffee in Ethiopia and the world.

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8. APPENDICES

Appendix 1. Analysis of variance (ANOVA) table of percent incidences of coffee wilt disease from four forest coffee of southwest and southeast Ethiopia in 2009

Source	DF	SS	M S	F- Value	Pr > F(Probability)
Replication	2	66.054	33.027	0.6	0.555
Sample	2	1330.83	665.414	12.19	0.0003
Location	3	2069.3	689.766	12.63	<.0001
Error	22	1201.26	54.6026		
Total	35	5457.26			

Appendix 2. Analysis of variance (ANOVA) table of percent incidences of coffee wilt disease from four forest coffee of southwest and southeast Ethiopia in 2010.

Source	DF	SS	MS	F -Value	Pr > F (Probability)
Replication	2	99.19	49.595	2.38	0.12
Sample	2	757.89	378.95	18.15	<.0001
Location	4	2084.4	521.09	24.96	<.0001
Error	19	396.69	20.878		
Total	35	4954.7			

Appendex 3. Origins of *G. xylarioides* isolates in the forest coffee areas of southweast and southeast of Ethiopia

Location	Forst coffee site	Plot No	Sample No	Sample plant part	Symptom type of the sample
Bonga	Gimbo-Yabito	1-(Komba)	1	Stem	PW
		1-(Komba)	2	Stem	CW
		1-(Komba)	3	Stem	PW
		2-(Kayakella)	1	Stem	PW
		2-(Kayakella)	2	Stem	PW
		2-(Kayakella)	3	Stem	PW
Sheko	Shimi (west)	1-(Shadiga)	1	Stem	CW
		1-(Shadiga)	2	Stem	PW
		1-(Shadiga)	3	Stem	PW
	Gizemerate(east)	2-(Sanka)	1	Stem	PW
		2-(Sanka)	2	Stem	PW
		2-(Sanka)	3	Stem	PW
Yayu	Haromelka	1-(Haromelka)	1	Stem	CW
		1-(Haromelka)	2	Stem	PW
		1-(Haromelka)	3	Stem	PW
		2-(Haromelka)	1	Stem	CW
		2-(Haromelka)	2	Stem	PW
		2-(Haromelka)	3	Stem	PW
		3-(Haromelka)	1	Stem	PW
		3-(Haromelka)	2	Stem	CW
		3-(Haromelka)	3	Stem	PW
Harenna	Majete	1-(Majete)	1	Stem	PW
		1-(Majete)	2	Stem	PW
		1-(Majete)	3	Stem	CW
		2-(Majete)	1	Stem	PW
		2-(Majete)	2	Stem	PW
		2-(Majete)	3	Stem	PW
Gera	GSARC	GSARC	1	Stem	PW
Jimma	JARC	JARC	1		

*PW= indicates partial wilt symptom type
*CW= indicates complete wilt symptom type

Appendix 4. Analysis of variance (ANOVA) table of radial growth of *Gibberella xylarioides* isolates collected from forest coffee populations

Source	DF	SS	MS	F Value	Pr > F(Probability)
Replication	2	48.9744	24.4872	6.08	0.004
Location	5	1622.15	324.43	80.58	<.0001
Time	4	23556.5	5889.12	1462.67	<.0001
Error	58	233.525	4.02629		
Total	89	26231.1			

Appendix 5. Analysis of variance (ANOVA) table of evaluation of seedling of forest coffee accessions against to *Gibberella xylarioides* isolates collected from forest coffee populations of southwest and southeast Ethiopia

Source	DF	SS	MS	F Value	Pr > F(probability
Replication	2	27.29124	13.64562	0.28	0.7599
Coffee accessions	15	64415.8795	4294.39197	87.19	<.0001
Error	30	1477.53655	49.25122		
Total	47	65920.7073			

Appendix 6. Analysis of variance (ANOVA) table of incubation period of inoculated forest coffee seedlings with *Gibberella xylarioides* isolates collected from forest areas of southwest and southeast Ethiopia

Source	DF	SS	MS	F Value	Pr > F
Replication	2	1887.16667	943.58333	0.8	0.4588
Coffee accessions	15	78951.66667	5263.44444	4.46	0.0002
Error	30	35400.8333	1180.0278		
Total	47	116239.6667			

Appendix 7. Analysis of variance (ANOVA) table of Host –pathogen interactin forest coffee seedlings inoculated with *Gibberella xylarioides* isolates collected from southwest and southeast forest coffee area of Ethiopia

Source	DF	SS	MS	F Value	Prob
Replication	2	85.467	42.734	0.2099	
Coffee accessions	12	89079.876	7423.323	36.4650	0.0000
Gibberella xylarioides isolates	4	71547.100	17886.775	87.8638	0.0000
Coffee accession X *G.xylarioides* isolates	48	61467.101	1280.565	6.2904	0.0000
Error	128	26057.460	203.574		
Total	194	248237.004			

Appendixes 8. Analysis of variance (ANOVA) of incubation period of inoculated forest coffee seedlings with *Gibberella xylarioides* isolates collected from forest area

Source	DF	SS	MS	F Value	Prob
Replication	2	5145.549	2572.774	4.5796	0.0120
Coffee accessions	12	14590.738	1215.895	2.1643	0.0171
Gibberella xylarioides isolates	4	76070.892	19017.723	33.8517	0.0000
Coffee accessions X *G.xylarioides* isolates	48	63317.108	1319.106	2.3480	0.0001
Replication	2	5145.549	2572.774	4.5796	0.0120
Error	128	71909.785	561.795		
Total	194	231034.072			

Appendix plate 1. Comparison of forest coffee seedling before inoculation and after inoculation with *Gibberella xylarioides* isolates collecterd from southwest and southeast forest coffee area of Ethiopia