ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

DIVERSITY OF ARABICA COFFEE POPULATIONS IN AFROMONTANE RAINFORESTS OF ETHIOPIA IN RELATION TO *COLLETOTRICHUM KAHAWAE* AND *GIBBERELLA XYLARIOIDES*

In Partial Fulfillment of the Requirements of the Degree of Master's of Science in Biology, Applied Microbiology

By

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July 2006
Addis Ababa

1. INTRODUCTION

Ethiopia has served in the past and continues to serve as the source of germplasm for several economically important cultivated crops around the world. Among these crops, the most important gift of Ethiopia to the world is coffee known as *Coffea arabica* L., which had and still has a tremendous economic, social and spiritual impact on many people of different geographical locations, cultural backgrounds and psychological behaviors (Tefestewold Biratu, 1995).

Coffee is a non-alcoholic and stimulant beverage crop, and belongs to the family Rubiaceae and the genus *Coffea*. Coffee is not only one of the highly preferred international beverages, but also one of the most important trade commodities in the world next to petroleum (Tefestewold Biratu, 1995). Ethiopia is believed to be the country of origin of arabica coffee that makes over 90% of the world's production (Paulos Dubale and Demel Teketay, 2000). Current contributions of coffee is more than 60% of the country's foreign exchange earning, over 5% of the GDP, 12% of the agricultural out put, and 10% of the government revenues (CSA, 2002). It also employs 25% of the domestic labour force (IAR, 1996). About 55% of the production is exported and the rest is consumed locally (Mesfin Ameha, 1991).

Coffee production systems in Ethiopia are grouped into four broad categories namely, forest coffee, semi-forest coffee, garden coffee and coffee plantations (MCTD, 1992). They account 10, 34, 35 and 21% of the total production, respectively. The most important cultivation areas are southwestern and southern Ethiopia.

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 Dubale and Demel Teketay, 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 Biratu, 1995). Many abiotic and biotic factors are the major constraints of coffee production in the country the most important of which are diseases caused by many etioletic agents, mainly the fungi. The crop is prone to a number of diseases that attack fruits, leaves, stems and roots, and reduce the yield and marketability (Eshetu Derso, 1997). The major coffee diseases in Ethiopia are coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR). The most important diseases both in severity and wide distribution are coffee berry disease (CBD) and coffee wilt disease (CWD).

Coffee berry disease (CBD) is a disease caused by fungal pathogen, an anthracnose of green and ripe coffee berries, which is induced by *Colletotrichum kahawae* (*Colletotrichum coffeanum*). *Colletotrichum kahawae*, taxonomically, belongs to order Melanconiales of the fungi imperfecti (Hindorf, 1975; Agrios, 2004). It is one of the three species of *Colletotrichum* that have been isolated from coffee berries, leaves and branches: *C. kahawae* (the only parasitic species to green coffee berries, originally designated as *C. coffeanum*), *C. gloeosporioides* and *C. acutatum* (Hindorf, 1970; Waller *et al*., 1993; Tefestewold Biratu, 1995).

The overall national average loss due to coffee berry disease is estimated to range 25-30%, which amounts to well over 600 million Ethiopian Birr (ETB) or 73.6 million USD (1 USD=8.15 ETB) per annum (Eshetu Derso, 1997; Eshetu Derso *et al.*, 2000). CBD can be controlled by the use of resistant coffee varieties, spraying fungicides or by cultural practices. Development of resistant varieties save the nation's valuable foreign exchange spent on fungicides, fuel and spray machinery and further avoids the hazardous effect of pesticide pollution of the environment. The exhaustive testing of selecting materials for resistance to CBD in the mother trees and their progenies in the laboratory and in locations, where the epidemic is not only severe but also regularly present, is very vital (Eshetu Derso, 2000). Resistance to CBD in coffee arabica most probably is horizontal/quantitative (Robinson,1974; Van der Graaff, 1984) in nature and controlled by 3-5 recessive genes (Mesfin Ameha and Bayetta Belachew, 1984).

Many research works have been conducted concerning with CBD pathogen (*C. kahawae*) and its variability. Based on colony color and ability to form saltation *C. kahawae* isolates from Harerge was subdivided into two i.e, fungi that form 'dark mycelium' and 'grayish mycelium' , which showed light grayish to white color other than the colony color by forming saltation (Tefestewold Biratu and Mengistu Hulluka, 1989). Waller *et al.* (1993) found common morphological, biochemical and pathogenic characteristics of isolates of *C. kahawae* taken from its range of distribution in Africa. They found also the nomenclature of CBD pathogen, *C.*

coffeamum, is confuse with the etiologic agent of coffee leaf spots in Brazil and introduced a new species name *C. kahawae*.

According to Van der Graaff (1981) differential interactions between host and pathogen population seldom found in Ethiopia but positive effects were small and it is improbable that they were caused by gene-for-gene specificity. Tefestewold Biratu (1995) studied variations/similarities within *C. kahawae* isolates collected from Harerge, Illubabor, Kaffa and Sidamo areas and recorded presence of variations in aggressiveness and absence of races within *C. kahawae* population based on pathogenicity test. Eshetu Derso and Waller (2003) found that *C. kahawae* isolates, collected from Yirgachefe, Gore and Gera garden coffee areas, were pathogenic to the hypocotyls of susceptible coffee cultivars and varied in their aggressiveness.

The other important fungal disease of coffee, and probably the second most important disease next to CBD in Ethiopia, is CWD (Van der Graaff, 1978; Merdassa Ejetta, 1985). CWD is a vascular wilt disease syndrome, which is commonly referred to as tracheomycosis, and induced by *Gibberella xylarioides* Heim & Saccas (*Fusarium xylarioides* Steyaert is the conidial stage).

CWD is known to attack all species of *Coffea*, including the wild indigenous lines in Tropical Africa (Wrigley, 1988; Coste, 1992). The disease was reported to be prevalent on *Coffea excelsa* in Central Africa Republic and Cameroon, on Robusta varieties in Zaire and Ivory Coast (Booth, 1971; Coste, 1992) and found on Arabica coffee in Ethiopia, mainly in plantations near Agaro, Jimma and Bonga in early 1970's (Kranz and Mogk, 1973). The disease incidence is high where coffee is grown under advanced cultural practices and minimal in the less managed forest coffee (Van der Graaff, 1983).

It is apparent that tracheomycosis increasingly becoming more and more important, especially in plantations. The actual disease assessment indicated that the incidence varied from 45% at Gera to about 69% at Bebeka (Girma Adugna *et al.*, 2001**)**. 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 (2003) technical report indicated that the national incidence and severity of CWD in Ethiopia were 27.9% and 3%, in monitory terms it causes an estimate loss of more than 3.7 million US dollar annually, respectively. However, the incidence and severity varied from place to place in a range of 0-100% and 0-25%, respectively.

The hitherto research activities on CWD have been concentrated on selection of resistant coffee varieties due of the severity of the disease and difficulties encountered in applying chemical treatments (Coste, 1992). The development and use of resistant germplasms through selection, from an enormously heterogeneous population of arabica coffee, is the only long-term solution to the tracheomycosis problem. Thus, in order to achieve reliable results in wilt disease resistance, field evidence on mature coffee trees should be supported by intensive greenhouse seedling tests. Conversely, coffee cultivars known to have promising performance in the seedling test must be observed for certain periods of time under field conditions essentially in areas where they will be released (Girma Adugna, 1998). According to Girma Adugna *et al.* (2001) significant difference among the cultivars in percent tree death caused by *G. xylarioides* under field conditions was observed. Resistance to *G. xylarioides* in coffee arabica is of a horizontal nature (Pieters and Van der Graaff, 1980; Girma *et al*., 2005).

Cultural comparisons, pathogenicity tests and RAPD-PCR markers corroborated existence of host specialization into at least two pathogenic forms within *Gibberella xylarioides* populations. Thus, two formae speciales, namely *Gibberella xylarioides* f.sp. *abyssiniae* (anamorph: *Fusarium xylarioides* f.sp. *abyssiniae*) for the fungal strains attacking only *Coffea arabica* and *Gibberella xylarioides* f.sp. *canephorae* (anamorph: *F. xylarioides* f.sp. *canephorae*) pathogenic to *C. canephora* and *C. excelsa* are known (Girma Adugna *et al.*, 2005). According to Girma Adugna *et al*. (2005) this subdivision enables to design effective coffee wilt disease management strategies, develop resistant cultivars/lines and formulate further breeding programs towards each population group.

To control coffee berry disease (CBD) and coffee wilt disease (CWD) there is a strong consensus that growing resistant varieties is the most appropriate cost effective means of managing these diseases. There may be a great danger for the coffee based industries and the people depending on them if the production of coffee fails due to unforeseen biological calamities such as outbreaks of diseases or pests. The genetic wealth of arabica coffee found in the Ethiopian afromontane rainforests at present is of great importance for breeding work to develop desirable stature and morphology, to improve the quality and quantity of coffee and to develop resistant varieties to diseases, pests and abiotic stress (Paulos Dubale and Demel Teketay, 2000). Conservation of this important genetic resource, both *in situ* and *ex situ* is a landmark for coffee consumers.

Although certain CBD resistant cultivars have been identified and commercialized, the amount of work is not in proportion to the crop's economic importance and the amount of genetic diversity available for improvement in its center of origin (Bayetta Belachew, 2001). To conserve and use of forest coffee sustainably little is known about the resistance potential diversity across the afromontane rainforest coffee germplasms in relation to major diseases (CBD and CWD).

It has also been recognized that a better knowledge of both, the pathogens and the coffee plant diversity will allow the development of novel and economical approaches to develop durable resistant varieties. According to Eshetu Derso and Waller (2003), although, it is believed that the appearance of physiologic races within *C. kahawae* isolates as individual entities is unlikely, it would be useful to look at a profile of several isolates from widely differing coffee types existing in the country, in a locality over time. Since the inception of CBD resistant selection and breeding program at Jimma Agricultural Research center (JARC), 19 CBD resistant cultivars were released to growers, based on regular field observations on the farm, 6 of them were withdrawn from production from time to time due to their manifestation of either high CBD, rust or wilt diseases and/or low yield (Bayetta Belachew *et al.*, 2000).

Studies on variability within *G. xylarioides* populations were conducted and confirmed the existence of one pathogenic form in relation to *Coffea arabica* in Ethiopia (Girma *et al.*, 2005). Although many works have been undertaken in many parts of coffee producing areas of Ethiopia in relation with incidence, severity, distribution, and resistance to CBD, the variability within *C. kahawae* population, the cause of CBD in forest *Coffea arabica* of Ethiopia is not yet well known.

Therefore, in the present study, the occurrence and importance of major coffee diseases in the indigenous population of coffee, variations of indigenous forest arabica coffee selections in relation to major coffee diseases (CBD and CWD) and variation within *C. kahawae* isolates from different forest coffee areas viz., Harena (Bale), Bonga, Berhan-Kontir (Sheko) and Yayu were studied.

2. OBJECTIVES

1. To evaluate the diversity of indigenous forest *Coffea arabica* populations in afromontane rainforests of Ethiopia in relation to CBD and CWD.
2. To examine the variation of *Colletotrichum kahawae* isolates, the cause of CBD collected from coffee populations in afromontane rainforests of Ethiopia.

3. LITERATURE REVIEW

3.1. Arabica coffee and its 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. Forests in Southwestern of Ethiopia are the primary center of origin and center of genetic diversity of *Coffea arabica* (Sylvian, 1958; Meyer, 1965; Melaku Werede, 1984).

Vast areas of forest coffee were observed in southwestern areas, viz. Yayu and Anfillo (Workafes Woldetsadik and Kassu Kebede, 2000). It is a wild type of coffee grown spontaneously in the humid hot forests of southwestern parts of the country in the administrative zones of WestWellega, Illubabor, and Sheka-Kefa Bench-Maji.

According to Paulos Dubale and Demil Teketay (2000) Wild animals and birds disseminating seeds within the forest community assist spontaneous regeneration. The forest is also covered by heterogeneous species of overhead shade trees. The occurrence of wild coffee types with distinct phenotypic differences in the forests around Sheko, Tepi and Bebeka; Gewata and Geisha in Kefa, Obacherko in Gera, Geba-Doggi valley near Yayu in Illubabor and Eba forest in Anfillo, all in Southwest Ethiopia, and the average yield of forest coffee has been estimated to be in the order of 200-250 kg/ha (Paulos Dubale and Demel Teketay, 2000). 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.

Semi-forest coffee production system is commonly found in Illubabor, Jimma, Keffa-Sheka, Benchi-Maji and west Wellega zones. 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 Woldetsadik and Kassu Kebede, 2000). These types of plantations are known traditionally as semi-forest type and are believed to have evolved from forest coffee production system. The farmers slash the weeds and shrubs in the relatively light forests and fill in the open spaces with local seedlings. 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 coffee represents about 24% of the total land covered by coffee, contributes about 20% of the total coffee production in the country and its average yield has been estimated to be in the order of 400-500 kg/ha (Paulos Dubale and Demil Teketay, 2000).

In the coffee improvement Weredas of 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 Woldetsadik and Kassu Kebede, 2000). Improved management of row planted coffee in Harerge, in South and Southwestern part of the country has been used as intensive coffee production system. Spacing, planting pattern and tree density depends on the type of selections planted. The coffee plantation development enterprise runs highly intensified plantations. These large plantations (state coffee farms) of about 21,000 hectares are distributed into seven different farms in Limu, Tepi and Bebeka.

3.2. Coffee diseases in Ethiopia

Coffea Arabia L. in Ethiopia is attacked by numerous diseases that reduce its production and productivity significantly. Coffee berry disease (CBD) is the top major disease of coffee in Ethiopia, which attack mainly the green berries of coffee. CBD was first observed in Ethiopia in 1971 (Mulinge, 1973). Since then it spreads and found in all coffee producing areas in which it has been favored by favorable environmental conditions.

Following the advent of CBD and modernization of the crop production system leads to the replanting of limited number of CBD resistant cultivars, which brought deforestation and rehabilitation of diverse coffee population. Side by side modern cultural practices are widely employed (Van der Graaff, 1981; 1983). These practices comprise more weeding and digging to remove noxious grasses. This results in more wounding of the trees and better chances for transmission of the pathogen of coffee vascular wilt disease (*Gibberella xylarioides*) (Vander Graaff, 1981; Pieters and Van der Graaff, 1980; Van der Graaff, 1983). Damage caused by *Gibberella stilboides* in Ethiopia seems to be confined to a collar rot of young seedlings, some susceptible collections were observed at Jimma research station, and root rot damage was rare and might be caused by basidiomycetes. (Van der Graaff, 1981). Leaf rust (*Hemileia vastatrix*) was reported by Sylvian (1958).

Coffee leaf rust (*Hemileia vastatrix*), CWD (*Gibberella xylarioides*), bean discoloration (*Pseudomonas syringae*), leaf blight (*Ascochyta tarda*), root-rot (*Armillaria mellea*), brown-eyespot *(Cercospora coffeicola*) and damping off diseases of seedlings (*Rhizoctonia* spp., and *Pythium* spp.), Fruit-rot (*Fusarium* spp.), and thread-blight (*Corticium kolleorega*) were recorded associated with coffee (Merdassa Ejetta, 1985; Eshetu Derso *et al*. 2000)

3.3. Major diseases of coffee in Ethiopia

3.3.1. Coffee berry disease (CBD)

3.3.1.1.Occurrence and distribution of coffee berry disease (CBD)

CBD is a major cause of crop loss of arabica coffee in Africa and a dangerous threat to production elsewhere. The disease is an anthracnose of green and ripe berries induced by *Colletotricum kahawae*. McDonald first detected CBD in 1922 in Kenya causing about 75% crop loss (Gibbs, 1969). Since then the disease was found in many estates of the Rift valley in Kenya. By the 1950s CBD had established in the east, the main coffee growing areas (Rodrigues *et al*., 1992).

Apparently, the free movement of coffee plant materials from CBD infected areas has been the main factor in distribution of this disease throughout all important arabica growing areas in Africa. The disease was reported in Angola around 1930, Zaire in 1937, Cameroon 1955-1957, Uganda in 1959, Tanzania in 1964, Ethiopia 1971(Van der Graaff, 1981) and in Malawi in 1985 (Lutzeyer *et al*., 1993). CBD was also confirmed in Malawi, Zimbabwe and Zambia in 1985 (Masaba and Waller, 1992). It is not known outside of Africa, although a leaf spot and ripe berry anthracnose caused by related *Colletotrichum* species has been reported from Guatemala and Brazil (Griffiths *et al*., 1991).

In Ethiopia CBD first reported in 1971 (Mulinge, 1973; Van der Vossen and Walyaro, 1980). Then spread to all major coffee producing regions within very short period except to the lower altitude. Big plantations, garden and forest coffee, with and without shade all were infested alike (Tefestwold Biratu, 1995). So environmental issues except low altitudes did not make much difference.

Merdassa Ejetta (1985) reported yield losses of 51% at Melko and 81% at Wondo Genet due to CBD. In 1994 crop season prevalence of CBD was conducted in Oromiya Region and Southern Nations Nationalities and Peoples Region (SNNPR) and the result indicated 38.8 and 17.2% of mean percent prevalence of the disease, respectively (IAR, 1997). According to the result CBD pressure was very high at higher altitudes in the southwest region, while severe disease was recorded in valleys of Sidamo zone. According to Tefestewold Biratu (1995) CBD severity varied from year to year and among woredas and regions. In Amhara region where CBD occurs, survey result showed that an average CBD severity for the 1996/97-crop season was 38% (Tesfaye Alemu and Ibrahim Sokar, 2000).

Survey conducted in 1997 and 1998 in six major coffee growing zones (in 32 woredas) of Oromiya region showed an average of 31% and 32% disease severity for the respective years (Melaku Jirata and Samuel Assefa, 2000). CBD incidence and severity assessment in 10 zones and 31 woredas of Southern Nations Nationalities and Peoples Region (SNNPR), conducted in September 1998, resulted with 40% and 22.8% mean incidence and severity of the disease, respectively (Tesfaye Negash and Sinedu Abate, 2000).

Losses due to CBD on individual farms vary considerably and in high rainfall and high altitude areas, losses may reach up to 100% (Van der Graaff, 1981). Resistant varieties play significant role in combating CBD.

3.3.1.2. Biology of *Colletotrichum kahawae* (Synonymous- *C. coffeanum*)

C. kahawae can infect all stages of the crop from flowers to the ripe fruits and occasionally leaves, but maximum crop losses occur following infection of green berries with the formation of dark sunken lesions with sporulation.

The perfect state for some species of *Colletotrichum*, occurring on coffee, has been proved to be the ascomycete *Glomerella cingulata*. These fungi are generally polyphagous. In 1901 Noack detected for the first time *Colletotrichum acutatum* in Brazil causing leaf spots and dieback (branches) of *C. arabica* L. (Hindorf, 1975). But it was not pathogenic to green coffee berries. Rayner (1952) had confirmed distinct forms that referred to *Colletotrichum coffeanum*, in the context of a fungus causing CBD, which was first detected by McDonald (1922).

Gibbs (1969) and Hindorf (1970) tried to identify *Colletotrichum* species from various parts of coffee in Kenya. Gibbs (1969) categorized the isolates (from coffee berries and bark) into four groups of which three were non-pathogenic and the fourth one invariably infected both wounded and unwounded berries and caused CBD. Based on sporulating capacity (conidia production), shape and size of conidia, production of acervuli and pathogenicity on berries, who assigned and described the CBD pathogen isolate (‘var. virulans’) as slow growing, profuse grayish-black aerial mycelium, and conidia borne directly on hyphae.

Hindorf (1970; 1973) classified the isolates using detailed cultural and morphological characteristics and relating his findings to Gibbs (1969). Hindorf (1970) grouped *Colletotrichum* isolates from Kenya coffee into 3, viz. *C. coffeanum* (now it is *C. kahawae*) as the only isolate causing CBD, *C. acutatum* (never causes serious damage to coffee but found on the host part damaged by biotic or abiotic factors) and *C. gloeosporioides* (causes anthracnose of leaves, die-back of branches, and brown blight of ripe berries). It was reported that *Glomerella cingulata* is the perfect form of *C. gloeosporioides* and never be for *C. kahawae* (which perhaps is a clone of *Glomerella cingulata* that fails to produce the perfect state in vitro). The pathogenic fungus produced conidia that were variable in size and shapes, at the tip of solitary hyphae, never in acervuli. According to Sutton (1980) the fungus that cause CBD produces colonies of dense or floccose pale chocolate brown aerial mycelium, sometimes grayish with a lighter center, reverse greenish gray, lacking acervuli and sclerotia, and setae are usually absent.

The germination of the one-celled, cylindrical and hyaline conidia of *C. kahawae* takes place only in the presence of free water (Hindorf, 1975). Optimum temperature appears to be at 22^0C ($18\text{-}23^0$C *in vivo*, $15\text{-}25^0$C *in vitro*). After germination, germ-tubes grow rather slowly, and 4 to 5 hours later, dark brown thick-walled appressoria are formed at their tips. The appressoria stick strongly to the host cuticle and penetrate it by means of infection pegs. Inter-cellular mycelium is formed sparsely. The incubation period lasts from 5 days to 3 weeks, average being around 8 days. Soon after a black necrotic lesion develops, the fungus produces fruiting bodies, the acervuli in which masses of pink conidia are formed.

C. kahawae and *C. gloeosporioides* were the only two species of fungus isolated from coffee tissue samples collected from Habro and Kuni districts in Harerge region (Tefestewold Biratu and Mengistu Hulluka, 1989). The absence of perithecia, its slow growth rate, and its pathogenic ability were the distinct characteristics of *C. kahawae*, while *C. gloeosporioides* produced fertile perithecia (perfect stage) and was unable to cause coffee berry disease.

Tefestewold Biratu (1995) studied variations/similarities among *Colletotrichum* isolates collected from Harerge, Illubabor, Kaffa and Sidamo areas and found 3 spp. viz. *C. kahawae*, C. *gloesporioides* and *C. acutatum*. Variation of a representative range of *Colletotrichum* isolates from diseased coffee berries (collected from Yirgachefe, Gore, Gera and Limu garden coffee areas) was further studied using morphological and pathological criteria and the result showed that both *C. kahawae* and *C. gloeosporioides* occur in diseased berries, probably as sequential colonizers of diseased tissues (Eshetu Derso and Waller, 2003). They indicated that *C. kahawae* isolates were pathogenic to hypocotyls of susceptible coffee cultivars where as *C. gloeosporioides* isolates were not pathogenic.

3.3.1.3. Cultural and morphological variation within *C. kahawae* and other *Colletotrichum* spp. occurring on coffee

According to Gibbs (1969) CBD pathogen 'var. virulans' showed conidia production capacity 5 x 10^4 conidia/cm^2/h on active coffee berry lesion and 10 x 10^4/cm^2/hr on infected ripe berry.

Based on cultural and morphological characteristics, Hindorf (1970) described the 3 *Colletotrichum* spp. occurring on *C. arabica* in Kenya. CBD pathogen strain culture becomes initially white mycelium changed after 4-6 days to gray and eventually to dark olive brown and its conidia shape and size are variable. Average radial growth of colony and conidia size recorded from CBD pathogen (*C. kahawae*) isolates were 1.9 ± 0.5 mm/24h and 13.1 ± 0.6 x 3.87 ± 0.2, respectively (Hindorf, 1970 and 1973; Hindorf and Muthappa, 1974). They indicated that shape of conidia variable, mostly straight, cylindrical, and rounded at both ends. Hindorf (1973) and Tefestewold Biratu (1995) described the most frequent shapes of conidia and assigned as standard shapes of conidia of *Colletotrichum* spp: 1 = cylindrical and round at both ends, 2 = cylindrical acute at one and round at the other end, 3 = clavate-round at both ends starts attenuating from ¼ of its length, 4 = reniform or kidney shaped, 5 = oblong-elliptical, types.

The culture of *C. kahawae* from Kenya grows slowly, changes the color into complete black produces conidia sparsely on solitary hyphae (only in darkness) and is able to infect green coffee cherries very easily. In contrary the fast growing culture, remaining whitish producing a large number of conidia in black acervuli and being not pathogenic to green coffee cherries, these are the typical characteristics of *C. gloeosporioides* (Hindorf and Muthappa, 1974). Two species of *Colletotrichum* were identified from isolates of Harerge, i.e. *C. kahawae* and *C. gloeosporioides*. Based on colony color and ability to form saltation the first group, *C. kahawae* further subdivided in to two: ‘dark mycelium’ which showed little variation in the group, i.e. green and grayish to bluish white which later turned to dark green or olive brown to brown as the mycelia got older where as the second subgroup comprises ‘grayish mycelium’ form, which showed light grayish to white color other than the colony color by forming saltation (Tefestewold Biratu and Mengistu Hulluka, 1989). As they indicated the rate of mycelial growth was distinctly slower in *C. kahawae* (6.5-6.7 mm/24 hr) than in the *C. gloeosporioides* mycelium form (6.7-12.9 mm/24 hr) and sizes and shapes of *C. kahawae* were variable, and the average size of the conidia was 15.3 x 3.5 µm from *C. kahawae* isolates where as 13.6 x 3.4 µm from *C. gloeosporioides*.

Based on the color of the colony of *C. kahawae* isolates detected on PDA two groups could be made, those dark bluish gray colored (isolates from Sidamo and partly Harerge) and light bluish gray colored (isolates from Kaffa and Illubabor) (Tefestewold Biratu, 1995). He also observed (12-52) x 10^4 conidia/ml and (684-1720) x 10^4 conidia/ml production from 6 CBD pathogen isolates on PDA and GCA (green coffee seed extract agar), respectively, and existence of variation in conidia production between batches of cultures. Most precisely taking morphological, biochemical and pathogenical characteristics into consideration can identify the CBD causing fungus *C. kahawae* (Hindorf *et al.*, 1997).

Eshetu Derso and Waller (2003) observed that *C. gloeosporioides* isolate from Limu (non pathogenic to green coffee berries) produced a faster growing grayish aerial mycelium and developed a black color in the substrate where as the pathogenic isolate (*C. kahawae*) from Yirgachefe showed slow growth and grayish aerial mycelium.

3.3.1.4. Coffee berry disease development and pathogen (*C. kahawae)* variability

The conidia of *C. kahawae* are distributed by water splashes. They require the presence of water or 100% relative humidity and optimum temperature of about 22^{o}C for germination (Nutman and Roberts, 1960). Conidia germinate into germ-tubes, which in turn produce appressoria. The appressoria adhere to the plant cuticles and produce infection pegs, which penetrate the cuticle to cause infection. Under optimum conditions, the time between infection and lesion development (incubation period) vary between two to three weeks (Van der Graff, 1981) or two to four weeks (Mulinge, 1970). Generally, high rainfall, high humidity or wetness, and relatively low temperatures that persist for long periods favor CBD development and the disease is invariably severe at higher altitudes where these conditions generally prevail (Cook, 1975).

CBD is critical between expanding and the endosperm stages of berries which occurred from 6th week up to 23rd week after the main flowering of coffee in Ethiopia (Gassert, 1979). The critical stage for CBD occurrence in Kenya is between 8th and 24th week after flowering (Mogk and Hindorf, 1975) and 22nd week in Cameroon (Muller, 1984).

In Ethiopia, CBD development and severity is high above 1750 m.a.s.l., moderate between 1500m and 1750m, whilst below 1500m CBD is not a problem at all. Gera, one of the hot spot areas where screening for resistance was carried out, is located at an altitude of 1900m. It is characterized by annual rainfall of 1800mm, 10^{o}C minimum and 24^{o}C maximum temperature, and 70% relative humidity. In July and August when infection reaches at maximum, the minimum temperature is 11^{o}c and maximum temperature is about 22^{o}c, relative humidity is about 80%. At Jimma where the severity is moderate, the altitude is 1753m and the respective monthly average minimum and maximum temperatures are about 11^{o}c and 26^{o}c with 70% relative humidity and a total annual rainfall of 1590 mm (Bayetta Belachew, 2001).

According to Van der Graaff (1981) differential interactions between host and pathogen population seldom found in Ethiopia but positive effects were small and it is improbable that they were caused by gene-for-gene specificity.

Tefestewold Biratu (1995) found the presence of variations in aggressiveness and absence of races within *C. kahawae* isolates, and existence of significant differences among coffee selections/isolates and their infection effects.

Eshetu Derso and Waller (2003) found that *C. kahawae* isolates (collected from Yirgachefe, Gore and Gera garden coffee areas) were pathogenic to the hypocotyls of susceptible coffee cultivars and varied in their aggressiveness. According to them, although, it is believed that the appearance of physiologic races within the *C. kahawae* isolates is unlikely, it would be useful to look at a profile of several isolates from widely differing coffee populations existing in the country, in a locality over time.

3.3.2. Coffee wilt disease (CWD)

3.3.2.1. Occurrence and distribution of coffee wilt disease (CWD)

CWD is a Vascular wilt disease syndrome, which is commonly referred to as tracheomycosis, induced by *Gibberella xylarioides* Heim & saccas (*Fusarium xylarioides* Steyaert is the conidial stage), is one of the major diseases of coffee and probably the second most important disease next to CBD (Van der Graaff and Pieters, 1978; Merdassa Ejetta, 1985).

The *Fusarium* wilt disease on *Coffea arabica* was first observed in Ethiopia (in Keffa province) (Stewart, 1957). He described the wilting symptom and identified the causal organism to be *Fusarium oxysporium* f.sp. *Coffea*. Stewart and Dagnachew Yirgu (1967) had noted the presence of CWD on arabica coffee. Later on, the fungus inciting tracheomycosis was authentically confirmed to be *G. xylarioides*, of which *F. xylarioides* is the imperfect state. This was based on comparative studies of the isolates collected from dying arabica coffee trees of different origin (including isolates from *Coffea arabica* L., Ethiopia) and different *Coffea* spp. (Kranz and Mogk, 1973).

Vascular wilt caused by *G. xylarioides* is an endemic disease in all coffee growing areas of Ethiopia and no economic importance under traditional low-management growing conditions, but when coffee is grown under modern cultural practices it often reaches epidemic proportions (Pieters and Van der Graaff, 1980). It was regarded as endemic throughout the southwestern coffee growing regions of the country (Van der Graaff, 1981; Merdassa Ejetta, 1985). The disease incidence is high where coffee is grown under advanced cultural practices although it is said to be minimal in less managed forest coffee (Vander Graff, 1983). Merdassa Ejetta (1985) had assessed the incidence of the disease in singletree progenies of different coffee lines for six years (1979-1984) at Gera, and obtained tree loss ranging up to 87%. The actual disease assessment indicated that the incidence varied from 44% at Gera to about 69% at Bebeka and CWD develops to an important disease on arabica coffee. In addition there were certain variations between coffee fields at each locality which probably could be ascribed to genetic makeup and age of coffee cultivars, cultural practices and environmental condition at specific location (Girma Adugna *et al*., 2001**).**

According to Girma Adugna (2004) CWD was widespread in semiforest, garden and plantation coffee production systems of Ethiopia and the incidence was significantly varied from 3.6 to 15.5, 27.2 to 43.5 and 17.3 to 65.2%, respectively, indicating that the disease is more important in plantation followed by garden based production systems.

3.3.2.2. Coffee wilt disease development and pathogen (*Gibberella xylarioides*) variability

The pathogen enters tree roots either through wounds or directly through root hairs and the epidermis of the small roots (Toole, 1941). However, as Van der Graaff and Pieters (1978) suggested that the fungus did not persist in the soil as it seldom forms chlamydospores. Once a wilt pathogen has penetrated a suitable host through wounds, it moves to the vascular tissue. The pathogen then spread throughout the plant by means of mycelia growth or conidia, primarily microcondia, produced in infected xylem vessel elements. As the disease development progresses, the fungus invades tissues adjacent to the xylem tissues such as pith, cambium, phloem, and cortex. At this time, symptom expression is severe, and a portion of the plant or the entire plant may succumb to the disease (Nelson, 1981).

The disease manifests itself after a prolonged incubation period, by expression of disease symptoms including a rapid wilting and shedding of the foliage, and then affected tree dies and finally perithecia of the fungus are formed in the bark of the lower parts of the stem after complete tree death (Kranz and Mogk, 1973).Trees die-back starts unilaterally and later extends to the whole tree and the affected trees die in 2 to 3 months after appearance of the first symptoms (Van der Graaff and Pieters,1978). Inspection of dying trees revealed bluish-black or brown-reddish streaks under the bark and stromata (fruiting bodies) producing perithecia can be observed in the bark of dead trees (Girma Adugna *et al*., 2001).

Girma Adugna *et al*. (2005) found two pathogenic forms within *Gibberella xylarioides* populations and suggested that the information enables to design effective coffee wilt disease management strategies, develop resistant cultivars/lines and formulate further breeding programs towards each population group.

3.4. Resistance in *Coffea arabica*

3.4.1. Resistance to CBD in *Coffea arabica*

Many explorers (Meyer, 1965) and (Sylvian, 1958; Melaku Werede, 1984; Bayetta Belachew, 2001) indicated that the genetic variability within arabica coffee populations, in the Southwestern highlands of Ethiopia, is extremely high. Immediately after the outbreak of CBD in the 1971 and 1972 the genetic potential of the forest, semi forest and garden coffee populations, a survey programme was initiated in those populations where epidemics of the disease had occurred. Genetic variability within arabica coffee populations, in the southwester highlands of Ethiopia, is extremely high. It was observed that within the coffee populations there was a wide range of variation; from complete susceptibility to high levels of resistance. Resistant trees occurred at frequencies of 0.1% to 1% and one tree in every 10,000 possessed both resistance and high yield and quality (Robinson, 1976). Based on this survey a massive selection programme for resistance was launched using these indigenous populations. In the group of mother trees at Gera, certain trees had consistently more disease than others (Van der Graaff, 1981).

A search for resistance was also made among 200 arabica cultivars, which had been introduced from abroad earlier in 1969. However, none of them were found resistant except Rume Sudan, which showed some level of resistance, although not as resistant as the local types (Bayetta Belachew, 2001).

Partial to complete dominance of the susceptible genes to the resistant genes was consistently found and 3 to 5 major genes of an additive nature were suspected to be involved in the control of resistance to CBD in the arabica coffee population, in Ethiopia (Mesfin Ameha and Bayetta

Belachew, 1984).

Resistance in 741 of the cultivars could be considered as horizontal or non-race specific where as 74110 and 744 showed susceptible and mixed (susceptible for some and resistant for others) reactions, respectively (Tefestewold Biratu, 1995). 74110 is one of the released resistant cultivars and still in production (Bayetta Belachew *et al*., 2000). Since the inception of CBD resistant selection and breeding program at Jimma Agricultural Research center, 19 CBD resistant cultivars were released to growers, based on regular field observations on the farm, 6 of the cultivars were withdrawn from production from time to time due to their manifestation of either high CBD, rust or wilt diseases and/or low yield.

CBD resistant cultivars have been identified and commercialized for immediate use, and currently different works have been carried out on the genetic improvement of arabica coffee in many countries. Nevertheless, the amount of work is not in proportion to the crop's economic importance and the amount of genetic diversity available for improvement in its center of origin (Bayetta Belachew, 2001).

Tefestewold Biratu (1997) reported that seeds of CBD resistant coffee selections in Ethiopia and cultivars in Kenya had higher caffeine content than the susceptible ones.

3.4.2. Resistance to CWD in *C. arabica*

Van der Graaff and Pieters (1978) observed a varietal pattern of attack by *G. xylarioides.* The seedling test and conidium germination test on six host genotypes with four *G. xylarioides* isolates support the conclusion that the resistance is of a horizontal nature (Pieters and Van der Graaff, 1980). Coffee cultivars 74165 and 35/85 appeared to be consistently resistant both in the field and in the greenhouse when significant interaction between coffee cultivars and *G. xylarioides* isolates occurred both in percent seedling deaths and incubation periods (Girma Adugna, 1998). According to Girma Adugna et al. (2001) significant difference among the cultivars in percent tree death caused by *G. xylarioides* under field conditions was observed.

There were evidences indicating variations in resistance or tolerance levels in Arabica coffee cultivars under field conditions (Girma Adugna, 2004). According to Girma Adugna et al. (2005)

there were also highly significant differences among cultivars, the isolates and cultivar-isolate interactions in seedling test using *G. xylarioides,* suggesting presence of certain qualitative vertical reaction with quantitative horizontal resistance.

4. MATERIALS AND METHODS

4.1. Descriptions of study sites

The study was conducted both in the field and in the laboratory. Field studies were conducted in afromontane rainforest coffee populations (FCPs), in Harena (P-1), Bonga (P-2), Berhan-Kontir (P-3) and Yayu (P-4) in Southeast and Southwest of Ethiopia (Figure 1). In each forest coffee population two representative sites (A and B) were selected, each with 100 m x 100 m, and from each site 10 indigenous forest coffee trees were selected randomly (Table 1). The trees were tagged for seed collection (used for seedling inoculation test) and further evaluation (for attached berry test in Bonga and Yayu). In each forest coffee area, major coffee diseases assessment and sampling were conducted and the laboratory works were conducted at Jimma Agricultural Research Center.

Table 1. Description of forest coffee tree selection sites

Forest unit	Forest coffee site	Latitude (N)	Longitude (E)	Altitude (m.a.s.l)
Harena (Bale) (P*-1)	Majete (A)	6°30’	39°45’	1420
	Majete (B)	6°29’	39°44’	1490
Bonga (P-2)	Yabito (A)	7°18’	36°03’	1780
	Alemgono (B)	7°30’	36°30’	1750
Berhan-Kontir(Sheko) (P-3)	Shimi (A)	7°04’	35°25’	1180
	Giz-Meret (B)	7°05’	35°30’	1120
Yayu (P-4)	Haromelaka (A)	8°20’	35°50’	1477
	Haromelaka (B)	8°23’	35°47’	1400

*Coffee population

4.1.1. Harena forest coffee

The Harena forest coffee is found in Oromiya region, Bale zone, and Man-Angetu woreda in Harena afromontane rainforest. Harena rainforest is part of Bale Mountains National Park. The Harena forest lies between 1300 and over 3000 m.a.s.l. The forest coffee occurs only in altitudes ranging between 1300 and 1850 m.a.s.l. (Feyera Senbeta, 2006). The soil in the coffee zone is acidic to slightly acidic with a pH between 5.3 and 6.6. The rainfall pattern in the area is bi-modal 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 Woldemariam, 2003). Coffee is one of the crops that plays significant role for the livelihoods of the community living in the surrounding areas.

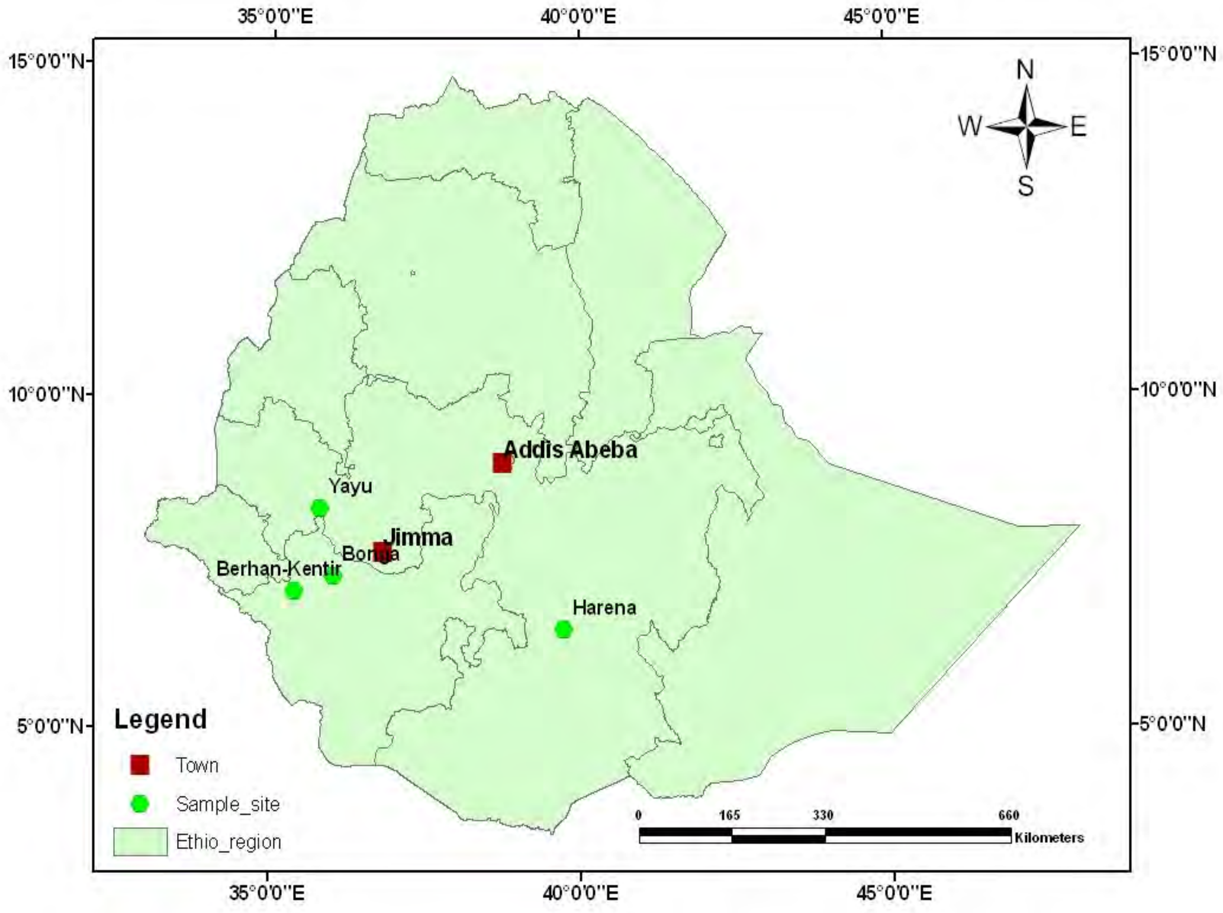


Figure 1. Locations of forest coffee sites

4.1.2. Bonga forest coffee

Bonga forest coffee is found in Bonga rainforest in South Nations and Nationalities Peoples Region (SNNPR), Kefa zone, Gimbo woreda. Meteorological data from Wushwush village at 1725 m.a.s.l (7^o13'N; 36^o17'E) indicated that the annual rainfall is around 1800 mm, the wettest months being May and June; average temperature is around 19^oC (NMSA, 1996; Feyera Senbeta, 2006).

4.1.3. Berhan-Kontir forest coffee

It is found in SNNPR, Bench-Maji zone, Sheko woreda in Berhan-Kontir forest. Berhan-Kontir forest area is located near Giz-Meret village between 35^o15'-35^o30' East and 06^o55'-07^o05' 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 Dubale and Demel Teketay, 2000). A total of 5, 500 ha of this forest is delineated to serve as the forest coffee conservation area. The annual rainfall and mean annual temperature are 2200 mm and 22^oC, respectively (NMSA, 1996).

4.1.4. Yayu forest coffee

It is found in Oromiya region, Illubabor zone, and Yayu woreda in Geba-Dogi rainforest area. Geba-Dogi forest area contains dense forest through which the road from Addis Ababa to Metu passes. The forest is located between 35^o45'-36^o05' East and 08^o15'-08^o37' North, and stretches along the two sides of Geba and Dogi rivers (Paulos Dubale and Demel Teketay, 2000). Big trees, shrubs and herbs including coffee plants growing in the wild and 18,600 ha of the forest is delineated to serve as the forest coffee conservation area. It receives annual rainfall of 1800 mm and mean annual temperature of 21^oC (Tadesse Woldemariam, 2003). The forest covers about 10000 hectares and out of this 5,500 hectares is covered by forest coffee.

4.1.5. Jimma Agricultural Research Center (JARC)

It is found in Oromiya region, Jimma zone. It is located around 7^o46'N latitude and 36^o E longitude coordinate and at an elevation of 1753 m.a.s.l. It is 343 kilometer away from Addis Ababa and 8 kilometer away from Jimma town (in the west direction). It represents the medium agro-ecological coffee growing zones, which receives annual rainfall of 1550 mm. Its mean minimum and maximum temperature is 11.3^{o}C and 26^{o}C, respectively.

4.2. Assessment of major coffee diseases

Assessment of CBD and CWD was conducted in the four forest coffee areas (Harena, Bonga, Birhan-Kontir and Yayu) from July to September 2005.

4.2.1. Assessment of coffee berry disease (CBD)

In each forest coffee area, CBD assessment was taken every 3-5 km interval across the forest coffee by considering the existing field variation especially land gradients, and presence and absence of forest coffee. In each 100 m x 100 m plot , two types of assessments were conducted on the same trees diagonally following procedures used by Tesfaye Alemu and Ibrahim Soka (2000):

(a) Berry counting- 10 trees/plot were randomly selected and each tree divided into 3 strata of branches (top, middle and bottom). From each stratum two branches were selected to calculate disease intensity. Depending on the forest coffee field sizes across each forest coffee area 5-7 plots were assessed. CBD damaged and healthy berries were counted and then percentage of diseased berries over total counted berries calculated.

(b) Visual assessment of 10 trees per plot were randomly taken and diagnosed for presence and absence of the disease on each tree. Thereafter disease incidence was calculated as (number of diseased trees/total observed trees) x 100.

4.2.2. Assessment of coffee wilt disease (CWD)

In each forest coffee, CWD assessment was taken every 2-4 km distance between plots across the forest coffee area. Depending on the forest coffee field sizes across each forest coffee area 5-8 plots were assessed. In each 100 m x 100 m plot, 30-50 trees were diagnosed diagonally and consecutively following Girma *et al.* (2001) and CABI (2003) procedure. Healthy and diseased (dying and dead) trees showing typical characteristic symptoms (internal and external) of the disease and/or sign of the pathogen were visually observed. Internal symptoms (black and/or brown strips) were observed after the diseased trees bark was slightly scratched off by knife to expose the wood. 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.

4.3. Testing of indigenous forest coffee germplasms for resistance to CBD and CWD

4.3.1. Testing of indigenous coffee germplasms for resistance to CBD

Attached berry test (ABT) and seedling inoculation test were conducted in field and in laboratory conditions, respectively to evaluate differences in resistance among forest coffee germplasms.

4.3.1.1. Attached berry test (ABT)

Attached berry test was conducted following methods and procedures used by Van der Graaff (1981). ABT was not conducted on selected indigenous forest coffee trees in Sheko and Harena because there was no CBD occurrence in or near the selected sites, but occurred only in pocket areas very far from the selected coffee trees. The test was conducted at the sites of Bonga and Yayu on selected indigenous forest coffee trees in August 2005. Three branches of the selected coffee trees (1 from top, 1 from middle, 1 from bottom) were marked bearing healthy berries of the same age. In order to obtain the inoculum for further infections, green CBD infected berries with active black lesions were collected from the respective sites. Then diseased berries were wetted slightly with distilled sterilized water and incubated in closed plastic boxes for 24-48 hours to produce a reasonable sporulation. After incubation, a suspension of conidia was prepared by rinsing the berries in sterile water and the conidia density was determined by means of haemocytometer.

The berries in the expanding stage on marked branches were inoculated with a suspension of 2×10^6 conidia/ml using a hand sprayer. Each branch was then being kept moist and warm over night for 12 hours in a plastic ‘sleeve’. The plastic sleeve was covered with paper bag to avoid high temperature due to insulation. Individual branches of a tree were used as replications. Number of healthy and diseased berries was recorded 21 days after inoculation. The disease index (DI) was calculated as the ratio of diseased to total (healthy plus diseased) berries and the fractions were analyzed after angular transformation.

4.3.1.2. Seedling inoculation test

Seeds were collected from 75 different indigenous forest coffee trees, described under 4.1, from all forest coffee areas to check their abilities for susceptibility or resistance to CBD. Seedling tests were conducted following the same methods and procedures described under 4.4.4.2. Isolate G81 from Gera was used as the inoculum source. Resistant (741, 754, 75227) and susceptible (370) cultivars were included as standard checks (Bayetta Belachew *et al.*, 2000; Bayetta Belachew, 2001). The experiment was arranged in randomized complete block design and replicated three times.

4.3.2. Testing of indigenous coffee germplasms for resistance to CWD

4.3.2.1. Coffee seedling raising

Seedlings of 67 forest coffee trees from all forest coffee areas and standard checks like coffee cultivar SN5 as a susceptible, cultivar Catimor-J19 as resistant and 7440 as moderately resistant cultivar (Girma Adugna, 2004) were grown in growth-room. The seed lots of each germplasm were first soaked in distilled sterile water for about 48 hours after removing the parchment. The soaked seeds (25 seeds/pot) of each germplasm were sown into heat sterilized and moistened sandy soil in disinfected plastic pots (each has 5652 cm^3 capacity). Sterile water was regularly, every two days, applied to maintain adequate moisture for seed germination, emergence and growth of the plants throughout the experimental period.

4.3.2.2. Inoculum preparation and inoculation

70 days after sowing, a representative isolate of *Gibberella xylarioides* from Plant Pathology Section of JARC was taken and multiplied for inoculation following the method of Pieters and Vander Graaff (1980) with some modifications of Girma Adugna and Mengistu Hulluka (2000). The stock culture of the representative 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. At the same time fresh coffee branches were collected from healthy trees, cut into small pieces of 15 cm and the bark was slightly scratched off to expose the wood.

The branches were placed in a test tube (3.75 cm^3) having a small roll of well-moistened cotton wool underneath and then sterilized in an autoclave. Each of a batch of 10 twigs was inoculated with 2-3 ml of conidia suspension of the isolate and incubated for 10 days under standard conditions. The conidia used for seedling inoculation were obtained by thoroughly rinsing off the branches with good colony growth with sterile water in a sterile beaker. The suspension of the isolate was stirred up with magnetic stirrer and filtered through double layers of cheese clothes. The spore concentration of the inoculum suspension was adjusted to about 2.3×10^6 conidia/ml.

The coffee seedlings were inoculated with a viable conidial suspension of the isolate by stem nicking procedures (Pieters and Van der Graaff, 1980; Girma Adugna and Mengistu Hulluka, 2000). A sterile scalpel was first immersed into the suspension, then the stem of each seedling was nicked at about 2 cm from the soil level and a drop of nearly 1 milliliter was placed in the notch.

The treated plants were immediately kept in air-conditioned growth room with high relative humidity (>95%) and optimum temperature (23-25^{o}C) for infection. After 10 days, the inoculated seedlings were transferred to a greenhouse (with temperature of 15-30^{o}C and relative humidity of 60-80%) the experiment was laid out in randomized complete block design with three replications. The number of dead and healthy seedlings per pot was recorded at fourteen days interval for 6 months starting a month after inoculation. Percent wilt was calculated from cumulative number of dead over total number of seedlings (dead plus healthy), 210 days after inoculation, and the death rates were analyzed after angular transformation.

4.4. Sample collection, isolation and identification for characterization of *C. kahawae* isolates from forest coffee

4.4.1. Sample collection

To study the population variation of the pathogen involved in causing CBD in the afromontane rainforest coffee areas (Harena, Bonga, Sheko and Yayu) of Ethiopia, diseased coffee berries with active CBD lesions were collected. In each forest coffee area 2-6 CBD infested sites were identified, 4-5 coffee trees were selected and from which samples of 15-20 green coffee berries with active CBD lesions (Tefestewold Biratu, 1995) were collected during August-September 2005. Samples were picked using disinfected forceps, collected in a sterilized plastic bag, kept in icebox and transported to Jimma Agricultural Research Center, Plant Pathology laboratory. Samples were maintained at 4 ^{o}C until they were isolated.

4.4.2. Isolation and Identification

Colletotrichum isolation was carried out following methods and procedures described by Gassert (1979) and Tefestewold Biratu (1995). Five samples from each forest coffee tree were washed in 5% sodium hypo chlorite solution for 2 minutes and rinsed 5 times (2 minutes each) with sterilized distilled water, placed on sterilized moist tissue paper in disinfected plastic boxes with lid to induce conidial production. The boxes were maintained at room temperature (18 – 25 ^{o}C) until conidial mass and/or fungal growths were observed. Conidial producing berries were aseptically transferred to 100 ml beaker containing sterilized distilled water. Conidia were harvested by gentle shaking manually. Serial dilutions (10^{-1}–10^{-5}) were made according to Gibbs (1969) and 0.2 milliliter of each suspension was plated on PDA amended with 100-ppm streptomycin sulfate. The number of dilution/sample (3–5) was based on the washed fungal conidia density that was observed in a drop of the suspension on a glass slide under light microscope. The inoculated media was incubated for 3–5 days at 22–25^{o}C. After identification, monoconidial isolates of *C. kahawae* were subcultured on PDA for 10–14 days in an incubator adjusted to 22–25 ^{o}C. Pure cultures from monoconidial isolates were preserved in sterile distilled water at room temperature (18-25 ^{o}C) for later.

4.4.3. Cultural and morphological characterization of *C. kahawae* isolates from forest coffee areas

Cultural and morphological characteristics of *C. kahawae* isolates were studied following the methods and procedures used by Hindorf (1973), Tefestewold Biratu and Mengistu Hulluka (1989), and Tefestewold Biratu (1995).

4.4.3.1. Cultural appearances

Ranges of cultural variation in *C. kahawae* population were examined using representative isolates of each forest coffee area, including isolates from CBD hot-spot area of Gera by culturing on potato dextrose agar (PDA) and malt extract agar (MEA) adjusted at pH 5.5 ± 0.1 and incubated at 25^{o}C in darkness. For all experiments the ingredients of PDA were potatoes infusion form 200 g/l and dextrose 20 g/l where as the ingredients of MEA were 30 g/l malt extract, 5 g/l peptone and 15 g/l agar.

4.4.3.1.1. Colony (mycelial) radial growth

Cultures of 17 *C. kahawae* isolates (15 from forest coffee and 2 from Gera) were inoculated on PDA and MEA. Hyphal tip of each isolate was placed at the center of 15 ml PDA and MEA dispensed in a 96 mm diameter sterilized petridish with three replications. Mycelial (colony) radial growth (mm) of each isolate was measured with ruler, colony diameter from two perpendicular planes on the reverse side of the Petri-dishes.

4.4.3.1.2. Colony color

Colony (mycelia) color on obverse side and types of pigments from the reverse side of each *C. kahawae* isolate were determined on PDA and MEA using RGB color chart (Anonymous, 2005). Cultures were observed for 3 weeks. Observation on the presence and absence of sector formation, i.e., tendency to form sectors (saltation) was also made.

4.4.3.1.3. Aerial mycelial growth

Vigor of aerial mycelium growth: dense, irregular (scarce) or very scarce type was observed on obverse side from 10 days cultures on PDA and MEA.

4.4.3.2. Morphological characteristics

17 representative isolates of *C. kahawae* were cultured on PDA adjusted at pH 5.5 ± 0.1 and incubated at 25^{o}C in darkness and replicated three times per isolate. Parameters, viz. conidia sizes, sporulation capacity and shapes of conidia frequency were taken from 7, 10 and 14 days old cultures, respectively.

4.4.3.2.1. Conidial Size

C. kahawae isolates were incubated on PDA medium for 7 days, replicated 3 times per isolate. All types of shapes and most frequent sizes were included at random to minimize further measurement biasness. Conidial size (length and width) was computed from 75-150 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.

4.4.3.2.2. Sporulation capacity

10 days old cultures of each *C. kahawae* isolate, incubated on PDA was washed by flooding with 10 ml sterilized distilled water, rubbed with sterilized scalpel and transferred to 50 ml sterilized beaker and thoroughly stirred for 10-15 minutes with magnetic stirrer to extract the spores from the interwoven mycelia and then filtered into another sterilized beaker through double layer cheese clothes. 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 9 haemocytometer counts.

4.4.3.2.3. Shape of conidia

Frequency of conidial shapes was tallied from 14 days cultures of *C. kahawae* isolates incubated on PDA. The most frequent conidia shapes, 5 types of conidia shapes that were described by Hindorf (1973) and Tefestewold Biratu (1995) were used. Conidial shape of representative *C. kahawae* isolates were described using binocular compound microscope and the most frequent 5-conidial shapes which were standardized and used by Hindorf (1973) and Tefestewold Biratu (1995) for *Colletotrichum* spp. characterization. The frequency of each shape was tallied from 150 conidia per isolate.

4.4.4. Pathogenicity tests

4.4.4.1. Pathogenicity test of *Colletotrichum* isolates on detached berries

The detached berry test (DBT) was employed to confirm the infectivity of 18 Colletotrichum isolates, which were collected from Harena (4), Bonga (6), Sheko (2) and Yayu (6), respectively. Fully expanded green coffee berries were collected at Jimma Agricultural Research Center (JARC) and surface sterilized in laundry bleach for 2 min and washed in distilled water. Twenty-five berries were arranged in a plastic box lined with tissue papers for the inoculation of each isolate. A plastic with hollows was used as a substrate to maintain a high level of humidity. Inoculum of each isolate was prepared from 10 days old cultures grown on potato dextrose agar (PDA) by washing with distilled sterilized water. A drop (25 µl) of the conidia suspension ($2x10^6$ conidia/ml) was placed at the center of each berry. Then the boxes were hermetically closed after inoculation to maintain the high relative humidity needed for the infection process and symptom development. After 14 days number of infected, healthy and rotten berries was recorded.

4.4.4.2. Pathogenicity test of *C. kahawae* isolates on coffee seedlings

The pathogenic variability in *C. kahawae* population was studied by inoculating representative isolates on resistant, moderately resistant and susceptible cultivars. Representative isolates from Harena (H40, H41, H43), Bonga (B52, B53, B55), Berhan-Kontir/Sheko (S60, S61), Yayu (Y70, Y73, Y75) and one isolate from Gera (G81) were inoculated on seedlings of resistant cultivars

(754, 741), one moderately resistant in laboratory and resistant in field (74110) and susceptible cultivar (370) to investigate their interaction. The interaction of selected cultivars and CBD isolates was evaluated following the methods and procedures used by Van der Graaff (1981), Tefestewold Biratu (1995) and Bayetta Belachew (2001).

Coffee seedlings were raised in growth-room from freshly harvested seeds of each cultivar. To obtain seedlings, ripe cherries of each cultivar were picked from field and dried under shade after removing the pulp by hand. Then seeds of each coffee cultivar were prepared by removing the parchment and soaked in sterilized distilled water and kept for 48 hours. Thereafter, seeds were sown (40 seeds/box) in heat sterilized and moistened sandy soil in disinfected plastic boxes (each has 2295 cm^3 capacity) arranged on benches and covered with chipwood in growth-room. 5 weeks after sowing, the emerging seedlings were kept in growthroom at 20-25 ^oC with 12 hours photoperiod until unfolding stage of seedlings. Two days before inoculating the hypocotyls at unfolding stage, temperature was adjusted to 20^oC and seedlings were sprayed with sterilized water and covered with plastic sheet for 48 hours to obtain 100% relative humidity. Batches of 3 plastic boxes from each coffee selection containing 25 seedlings/box were inoculated by stem brushing procedure with fine camel hairbrush. Mycelia colonies of each isolate were carefully slashed with a scalpel from the PDA medium while washing with distilled sterilized water to harvest conidia from 10 days old cultures. The suspension of each isolate was stirred with magnetic stirrer for 10-15 minutes and filtered through double layers of cheese clothes. After repeating the procedure again the spore concentration of each suspension was adjusted to 2×10^6 conidia/ml. Randomize complete block design was employed to see the interactions of isolates and coffee cultivars. The second reinoculation was after 48 hours in the same procedure and maintained wet for additional 48 hours by closing with plastic cover. The temperature was adjusted to $20\text{-}25^oC$ for 3 weeks. The reaction of each seedling of coffee cultivars against the isolates was assessed 15 and 21 days after inoculation using the symptom classifications (0-4 scale) according to Van der Graaff (1981). A disease index (DI) for each assessment was expressed as a percentage of the maximum possible infection using the equation used by O'sullivan and Kavanagh (1991), and Tefestewold Biratu (1995):

$$\mathbf{DI = 100\,(w + 2x + 3y + 4z) / 4(v + w + x + y + z)},$$

where v = number of seedlings in class 0, w = number of seedlings in class 1, x = number of seedlings in class 2, y = number of seedlings in class 3, z = number of seedlings in class 4. The fractions were analyzed after angular transformation.

Reisolation from each coffee selection, and isolate interaction was conducted to check the presence of the pathogen.

4.5. Data analysis

All data were analyzed following the respective statistical procedures and treatment means were compared using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984; Townend, 2002). MSTAT-C and SAS microcomputer statistical software packages were employed to perform analysis of variance (ANOVA) and mean comparisons. Excel microcomputer statistical software was also employed to manipulate graphs.

5. RESULTS

5.1. Occurrence of CBD and CWD in the afromontane rainforest coffee areas of Ethiopia

CBD, CWD and other diseases were found associated with forest coffee in Harena (Bale), Bonga, Berhan-Kontir (Sheko) and Yayu afromontane rainforests of Ethiopia. Coffee berry disease (*Colletotrichum kahawae*) and coffee wilt disease (*Giberella xylarioides*) were mainly observed in forest coffee at different intensity. Coffee leaf rust (*Hemileia vastatrix*) and leaf blight (*Ascochyta tarda*) were also observed in the forest coffee.

The frequency and intensity of CBD varied among and within forest coffee areas. Coffee berry disease was observed in Yayu and Bonga forest coffee consistently. In Yayu it was also found at low altitude but the intensity was lower than the higher altitude. In Sheko and Bale, the disease was found in pocket areas (limited areas) of the forest coffee areas. Survey results indicated that the disease incidence ranged 0-50%, 20-60%, 0-20% and 0-50% and severity 0-15%, 12.5-22.5%, 0-6.5% and 0-7.8% were observed in forest coffee areas of Bale, Bonga, Sheko and Yayu, respectively. The mean incidence ranged between 6.0% at Sheko and 40.0% at Bonga where as the intensity of the disease varied between 2.0 and 17.9% at Sheko and Bonga, respectively (Figure 2 and 3).

Coffee wilt disease was found in all assessed forest coffee areas that has been being posing considerable coffee tree losses. Its incidence was found 0-16%, 0-10%, 0-6% and 0-30% in forest coffee areas of Harena, Bonga, Sheko and Yayu, respectively. The mean incidence varied between 2.4% at Sheko and 16.9% at Yayu (Figure 2).The disease has been being highly expanding and damaging the coffee trees particularly in Yayu and Harena. Coffee farmers in Yayu, Bonga and Harena accustomed to work in group and use cutlasses (bushman knives), indiscriminately and without take care of coffee trees not to wound due to lack of awareness, to slash weeds around coffee trees once per year from July to mid September.

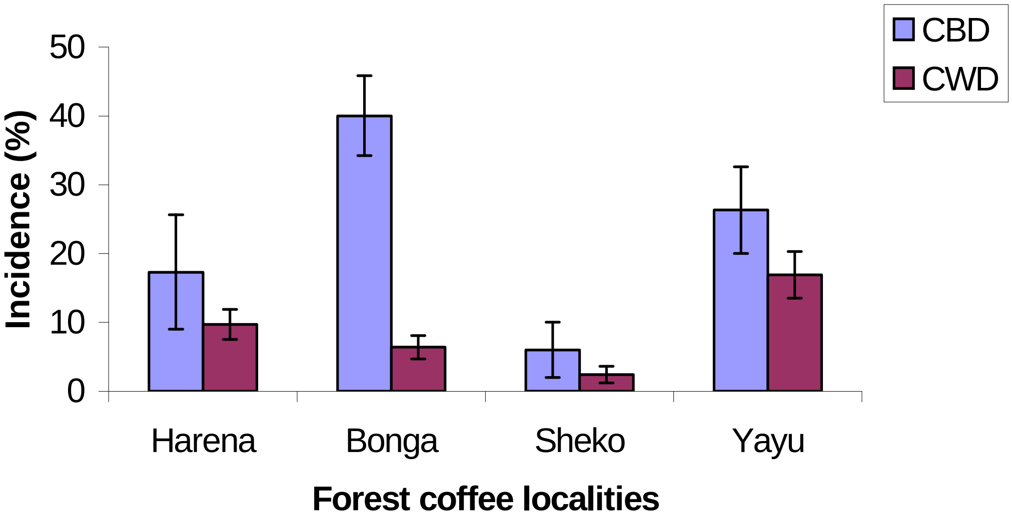


Figure 2. Incidence of CBD and CWD in afromontane rainforest coffee areas of Ethiopia (error bars are standard errors).

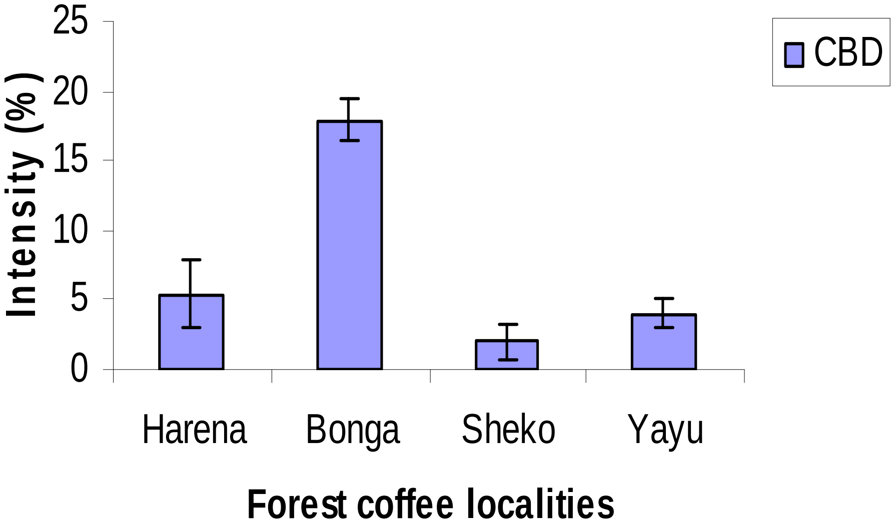


Figure 3. Intensity of CBD in afromontane rainforest coffee areas of Ethiopia (error bars are standard errors).

5.2. Variation of indigenous forest coffee germplasms in resistance to CBD and CWD

5.2.1. Variation of indigenous forest coffee germplasms in resistance to CBD

Resistance/susceptibility of indigenous forest coffee germplasms in reaction to CBD was tested in the laboratory and in the field using seedling inoculation and attached berry tests, respectively.

5.2.1.1. Results of seedling inoculation test

Differences in CBD levels were observed among indigenous forest coffee selections from Yayu, Bonga, Birhane-Kontir and Harena. There existed highly significant ($P < 0.05$) differences among indigenous forest coffee selections in seedling percent infection in reaction to CBD (Appendix 1 and 2). Out of the tested selections from all forest coffee areas 68% revealed significantly ($P = 0.05$) low level of seedling infection rate as compared to the standard susceptible check (Table 2 and 3; Figure 4 and 5).

Selections from Yayu, Bonga, Berhan-Kontir and Harena showed mean seedling infection rate (in reaction to CBD), 69-100%, 57.5-100%, 75.3-100% and 23.3-100%, respectively (Table 2 and 3). 95%, 33%, 40% and 90% of the tested selections from Yayu, Bonga, Berhan-Kontir and Harena revealed significantly ($P = 0.05$) low level of infection rate as compared to the susceptible check (Table 2 and 3). Two selections (BA25 and YB25), one from Bonga and one from Yayu respectively, showed significantly ($P = 0.05$) very low level of infection, i.e. 69.0% and 57.5%, respectively in seedling test (Table 2) as compared to susceptible check. These two lines showed resistance reaction (<30%) performance, 8.3 and 18.5%, respectively with attached berry test in the field (Table 4).

Two selections (HA21 and HB29) from Harena indicated significantly ($P = 0.05$) low level of infection percentage, 23.3 and 27.3%, respectively, which had the same significant level with the standard resistant checks (741 and 754) (Table 3). They showed resistant reaction (<30%). Generally, the seedling test and attached berry test indicated the presence of diversity among the indigenous forest coffee selections in resistance to CBD.

Figure 4. Comparison between standard resistant check, 741 (A) and forest coffee selection (HB29) (B).

Figure 5. Comparison between forest coffee selections that showed low level of infection (YA26, YA29 and YB27, SHB29, and BB21) (C) and standard susceptible check, 370 (D).

Table 2. Percent infection of seedlings from Yayu, Bonga and Sheko indigenous forest arabica coffee selections inoculated with *C. kahawae* isolate

Coffee selection	Mean	Coffee selection	Mean
YA20	92.7 e-g	BB20	98.7 a-c
YA21	92.6 e-g	BB21	76.3 jk
YA22	84.0 hi	BB23	100.0 a
YA23	95.0 d-f	BB26	100.0 a
YA24	92.7 e-g	BB27	99.3 ab
YA25	96.0 c-e	BB29	100.0 a
YA26	74.4 jk	SHA20	99.3 ab
YA27	84.8 hi	SHA21	100.0 a
YA28	90.0 f-h	SHA22	99.3 ab
YA29	76.7 jk	SHA23	99.3 ab
YB20	98.0 b-d	SHA24	100.0 a
YB21	97.3 cd	SHA25	94.0 d-f
YB22	100.0 a	SHA26	100.0 a
YB23	96.7 c-e	SHA27	100.0 a
YB24	95.7 de	SHA28	100.0 a
YB25	69.0 k	SHA29	100.0 a
YB26	94.3 d-f	SHB20	90.0 f-h
YB27	75.7 jk	SHB21	98.0 b-d
YB28	86.8 g-i	SHB22	97.3 c-d
YB29	82.1 ij	SHB23	99.3 ab
BA20	98.7 a-c	SHB24	100.0 a
BA21	89.5 f-h	SHB25	98.7 a-c
BA22	85.3 hi	SHB26	94.7 d-f
BA23	99.3 ab	SHB27	92.3 e-g
BA24	87.4 g-i	SHB28	95.0 d-f
BA25	57.5 l	SHB29	75.3 jk
BA26	99.3 ab	370*	100.0 a
BA27	100.0 a	75227**	47.7 m
BA29	100.0 a		
Mean			91.0
CV (%)			5.0

-* Susceptible check, ** Resistant check.

-YA20-YB29, BA20-BB29, and SHA20-SHB29 are indigenous forest coffee selections from Yayu, Bonga, and Birhane-Kontir (Sheko) forest coffee respectively.

-Means followed with the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT).

Table 3. Percent infection of seedlings from Harena indigenous forest arabica coffee selections inoculated with Colletotrichum kahawae isolate

Coffee selection	Mean
HA20	98.7 ab
HA21	23.3 h
HA22	87.7 g
HA23	97.0 b-d
HA24	96.0 b-e
HA25	95.0 b-g
HA26	97.3 bc
HA27	93.0 c-g
HA28	95.3 b-f
HA29	94.7 b-g
HB20	92.7 c-g
HB21	91.3 d-g
HB22	89.8 e-g
HB23	88.3 fg
HB24	92.1 c-g
HB25	97.7 ab
HB26	90.0 e-g
HB27	86.6 g
HB28	97.0 b-d
HB29	27.3 h
370*	100 a
754**	20.3 h
741**	21.3 h
Mean	81.4
CV (%)	6.9

-*Susceptible check, **Resistant check.

-Means with the same letter are not significantly different according to DMRT.

5.2.1.2. Results of attached berry test (ATB)

The test was conducted at Yayu and Bonga forest coffee sites on selected indigenous forest coffee trees. There existed highly significant ($P < 0.05$) differences among Bonga forest coffee selections and among Yayu forest coffee selections in percent infection of attached berries (Appendix 3 and 4). Out of the tested selected trees, 13 and 16 selections from Yayu and Bonga showed low level of infection (< 30%), 4.7-26.6% and 1.4-25.6%, respectively (Table 4).

Table 4. Percent infection of attached berries at Yayu and Bonga indigenous forest coffee selections inoculated with *C. kahawae* at each site in the field

Bonga		Yayu	
Coffee Selections	Mean	Coffee Selections	Mean
BA20	24.8 bc	YA21	16.0 C-F
BA21	7.9 c-e	YA22	28.9 B-D
BA22	9.0 c-e	YA23	31.3 B-D
BA23	25.5 bc	YA24	17.2 C-F
BA24	14.3 c-e	YA25	18.5 C-F
BA25	8.3 c-e	YA26	41.4 A-C
BA26	14.3 c-e	YA27	32.1 A-D
BA27	23.6 b-d	YA28	8.9 D-F
BA28	81.5 a	YA29	56.7 AB
BA29	9.5 c-e	YA30	22.1 C-F
BA30	8.4 c-e	YA31	35.0 A-D
BA31	4.2 e	YA32	9.9 D-F
BB21	44.4 b	YB20	4.7 F
BB23	10.1 c-e	YB21	10.4 D-F
BB24	25.6 bc	YB24	16.1 C-F
BB25	45.0 b	YB25	8.3 EF
BB26	11.6 c-e	YB26	41.0 A-C
BB27	32.6 bc	YB27	61.4 A
BB28	75.2 a	YB28	26.6 C-E
BB29	43.2 b	YB29	10.0 D-F
BB30	1.4 e		
BB31	5.4 de		
Mean	23.9		24.8
CV (%)	34.2		34.1

-BA20-BB31 and YA21-YB29 are indigenous forest coffee selections from Bonga and Yayu forest coffee, respectively.

-Means followed with the same letter are not significantly different according to DMRT.

5.2.2. Variation of indigenous forest coffee germplasms in resistance to CWD

There existed highly significant ($P < 0.05$) difference among indigenous forest coffee selections both in percent dead (wilt) seedlings and incubation period (Appendix 5 and 6). HA26 showed no seedling death at all. HA23 and HA26 showed the same significant ($P = 0.05$) level of seedling death rate with the standard resistant check (Table 5; Figure 7/I and 7/III). HA21 and HA23 resulted with significantly ($P = 0.05$) low percentage of dead seedlings (< 30%), i.e., 9.3 and 3.7% and with incubation period of 85.3 and 42.7 days, respectively (Table 5 and 6).

They followed by HA29, HA22, HA27, HA28 and BA21 which revealed 15.1, 18.4, 23.3, 23.5 and 26.2% mean wilt (dead) seedlings, which had mean incubation period of 146.7, 142.0, 118.7, 137.3 and 120.7 days, respectively. One selection of Yayu (YA20), five selections of Bonga (BA21, BA22, BA23, BA25, BB21) and seven selections of Harena (HA21, HA22, HA23, HA26, HA27, HA28, HA29) revealed significantly ($P = 0.05$) low level of percent seedling death, i.e., 58.0%, 52.3-61.1% and 0-23.5%, respectively, as compared to the moderately resistant check (7440) which showed 77.2% seedling death (Table 5). 5%, 44%, 5% and 90% of the tested selections of Yayu, Bonga, Berhan-Kontir (Sheko) and Harena (Bale) indicated significantly ($P = 0.05$) low percent wilt (dead) seedlings and the rest showed high seedling death rate as compared to the susceptible check (Table 5; Figure 7/II and 7/IV). Typical partial wilting symptom was `observed at first and progressed until the diseased seedling completely wilted (dead) (Figure 6).

BA24, BA28, SHA28, HA20 and HA24 showed the same significant level of percent wilt (dead) seedlings with the moderately resistant check, 72.2%, 65.2%, 72.7%, 73.1% and 77.4%, respectively (Table 5). The test indicated that there was great diversity among the indigenous forest coffee selections, which selected from the 4 Afromontane rainforest coffee areas, in reaction to CWD.

Table 5. Percent wilt of seedlings from Yayu, Bonga, Sheko and Harena indigenous forest arabica coffee selections inoculated with G. xylarioides isolate

Coffee selection	Mean	Coffee selection	Mean
YA20	58.0 pq	BB28	89.0 g-m
YA21	87.5 g-m	SHA20	95.8 b-h
YA22	87.5 g-m	SHA21	95.9 b-h
YA23	93.1 c-k	SHA22	87.2 g-m
YA24	85.1 j-n	SHA23	94.3 b-j
YA25	92.6 c-k	SHA24	86.5 h-n
YA26	96.7 a-g	SHA25	96.0 b-h
YA27	81.0 k-n	SHA26	96.3 bh
YA28	96.0 b-h	SHA27	82.0 k-n
YA29	81.3 k-n	SHA28	72.7 n-p
YB20	82.1 k-n	SHA29	90.9 f-k
YB21	97.2 a-e	SHB20	98.7 ab
YB22	88.4 g-m	SHB21	95.9 b-h
YB23	85.7 I-n	SHB22	94.7 b-j
YB24	95.5 b-I	SHB23	100.0 a
YB25	97.0 a-f	SHB24	98.7 ab
YB26	96.5 b-g	SHB25	98.7ab
YB27	97.2 a-e	SHB26	98.5 ab
YB28	94.4 b-j	SHB27	96.0 b-h
YB29	90.6 f-l	SHB28	90.9 f-k
BA20	90.0 f-m	SHB29	97.8 ac
BA21	26.2 r	HA20	73.1 n-p
BA22	57.9 q	HA21	9.3 s
BA23	52.3 q	HA22	18.4 r
BA24	72.2 n-p	HA23	3.7 st
BA25	57.1 q	HA24	77.4 l-o
BA26	82.2 k-n	HA25	94.0 b-j
BA27	85.7 I-n	HA26	0.0 t
BA28	65.2 o-q	HA27	23.3 r
BA29	93.1 c-k	HA28	23.5 r
BB20	85.2 I-n	HA29	15.1 r
BB21	61.1 pq	Catimor.J.19*	0.0 t
BB23	83.6 j-n	7440*	77.2 m-o
BB26	92.1 e-k	SN5**	92.4 d-k
BB27	97.3 a-d		
Mean			77.4
CV (%)			8.0

-*Resistant check, ** Susceptible check.

-YA20-YB29, BA20-BB28, SHA20-SHB29 and HA20-HA29 are indigenous forest coffee selections from Yayu, Bonga, Birhane-Kontir (Sheko) and Harena (Bale) forest coffee, respectively.

-Means followed with the same letter are not significantly different according to DMRT.

Table 6. Incubation periods (days) after inoculating seedlings of indigenous forest arabica coffee selections from Yayu, Bonga, Sheko and Harena, inoculated with *G. xylarioides* isolate

Coffee selection	Incubation period	Coffee selection	Incubation period	Coffee selection	Incubation period
YA20	102.0 a-g	BA23	97.3 a-h	SHB20	60.0 g-i
YA21	74.0 d-i	BA24	116.0 a-f	SHB21	74.0 d-i
YA22	69.3 e-i	BA25	83.3 d-i	SHB22	78.7 d-i
YA23	78.7 d-i	BA26	78.7 d-i	SHB23	74.0 d-i
YA24	69.3 e-i	BA27	74.0 d-i	SHB24	64.7 f-i
YA25	74.0 d-i	BA28	102.0 a-g	SHB25	69.3 e-i
YA26	83.3 d-i	BA29	74.0 d-i	SHB26	60.0 g-i
YA27	83.3 d-i	BB20	102.0 a-g	SHB27	69.3 e-i
YA28	46.0 h-i	BB21	69.3 e-i	SHB28	64.7 f-i
YA29	78.7 d-i	BB23	97.3 a-h	SHB29	74.0 d-i
YB20	92.7 b-i	BB26	92.7 b-i	HA20	128.0 a-d
YB21	83.3 d-i	BB27	83.3 d-i	HA21	85.3 c-i
YB22	64.7 f-i	BB28	83.3 d-i	HA22	142.0 a-b
YB23	97.3 a-h	SHA20	55.3 g-i	HA23	42.7 i-j
YB24	83.3 d-i	SHA21	69.3 e-i	HA24	109.3 a-g
YB25	97.3 a-h	SHA22	78.7 d-i	HA25	90.7 b-i
YB26	88.0 d-i	SHA23	74.0 d-i	HA26	0.0 j
YB27	69.3 e-i	SHA24	83.3 d-i	HA27	118.7 a-f
YB28	88.0 c-i	SHA25	64.7 f-i	HA28	137.3 a-c
YB29	88.0 c-i	SHA26	83.3 d-i	HA29	146.7 a
BA20	74.0 d-i	SHA27	83.3 d-i	Catimor.J.19*	0.0 j
BA21	120.7 a-e	SHA28	83.3 d-i	7440*	95.3 a-i
BA22	104.3 a-g	SHA29	64.7 f-i	SN5**	104.7 a-g
Mean					82.5
CV (%)					31.75

-*Resistant check, ** Susceptible check.

-Incubation periods indicate the number of days between inoculation and the first date of symptom appearance.

-0 (zero) values indicate no incubation period, i.e.; there was no infection symptom until termination of the experiment.

-YA20-YB29, BA20-BB28, SHA20-SHB29, and HA20-HA29 are indigenous forest coffee selections from Yayu, Bonga, Sheko and Harena (Bale) forest coffee, respectively.

-Means followed with the same letter are not significantly different according to DMRT.

Figure 6. Partial (Pw) and complete wilting (Cw) symptoms of CWD on inoculated seedlings with *G. xylarioides*.

Figure 7. Comparison among standard resistant check, Cat.J.19 (I), standard susceptible check, SN5 (II), and HA26 (resistant) (III) and HA25 (susceptible)(IV) from forest coffee selections inoculated with *G. xylarioides*

5.3. Characterization of C. kahawae isolates from afromontane rainforest coffee areas of Ethiopia

5.3.1. Isolation and identification of *C. kahawae* and related species from diseased green coffee berries

C. kahawae and related *Colletotrichum* species were isolated from diseased coffee berries, which were collected from afromontane rainforest coffee areas of Ethiopia (Table 7). In this study attention has been given to isolates that were pathogenic to green coffee berries.

Table 7. *C. kahawae* and other *Colletotrichum* isolates from 4 Afromontane rainforest coffee areas, isolated from diseased berries

Isolate Code	Forest coffee locality	Pathogenicity	Species
H40	Harena (Bale)	+	*C. kahawae*
H41	Harena (Bale)	+	*C. kahawae*
H42	Harena (Bale)	-	*C. gloeosporioides*
H43	Harena (Bale)	+	*C. kahawae*
B50	Bonga	+	*C. kahawae*
B51	Bonga	+	*C. kahawae*
B52	Bonga	+	*C. kahawae*
B53	Bonga	+	*C. kahawae*
B54	Bonga	-	*C. gloeosporioides*
B55	Bonga	+	*C. kahawae*
S60	Berhan-Kontir (Sheko)	+	*C. kahawae*
S61	Sheko	+	*C. kahawae*
Y70	Yayu	+	*C. kahawae*
Y71	Yayu	+	*C. kahawae*
Y72	Yayu	-	*C. gloeosporioides*
Y73	Yayu	+	*C. kahawae*
Y74	Yayu	+	*C. kahawae*
Y75	Yayu	+	*C. kahawae*

+ = Pathogenic to green coffee berries
- = Not pathogenic to green coffee berries

5.3.2. Cultural and morphological characteristics of *C. kahawae* isolates from forest coffee

5.3.2.1. Cultural (colony) appearances

The following data have been presented from the result of cultural characterization of *C. kahawae* isolates that had been isolated from CBD infected green berries. Pure culture of 15 *C. kahawae* isolates representing the four afromontane areas and 2 isolates from Gera were examined for colony (mycelia) aerial growth (vigor), pigmentation, sectoring and radial growth of colony (mycelia).

The isolates categorized into 3 classes on the basis of aerial mycelial growth (vigor): dense, irregular (scarce) and very scarce colony types. 47.1, 11.9 and 5.9% of isolates consistently indicated dense, irregular (scarce) and very scarce types of aerial mycelial growth on both PDA and MEA media, respectively (Table 10). The rest 35.2% of isolates made inconsistent aerial mycelia growth on PDA and MEA, dense or irregular (scarce) types.

Based on the observation from the obverse side of the culture plate, 3 groups of mycelial color forms were observed: lightgray mycelium, darkgray mycelium and gray mycelium forms (Table 9; Figure 9/A-C). In the first group, 9 (on PDA) and 8 (on MEA) *C. kahawae* isolates had white mycelial color upto the first 4-6 incubation days and then changed into lightgray mycelia on both PDA and MEA media while they had been being observed from the obverse side of the culture plates (9/A). But isolate B52, Y70 and Y71 revealed darkolivegreen or darkolivebrown as they got old and others remained lightgray. The second group, 3 (on PDA) and 2 (on MEA) isolates, manifested darkgray color for 15 days incubation (9/B). The third group, initially lightgray and changed into gray after 5-7 incubation days and then changed to darkolivegreen after 15 days incubation (9/C-D). On the reverse side of culture plates, colonies of the first group revealed whitishyellow or lightyellow colors in the first 4-6 incubation days and then changed into various combinations of colors (Table 9).

The second group manifested lightgray color in the first 4-6 incubation days and then changed into various colors combination. The third group showed lightorange and/or rosybrown color and then changed into various colors combinations, as they got old.

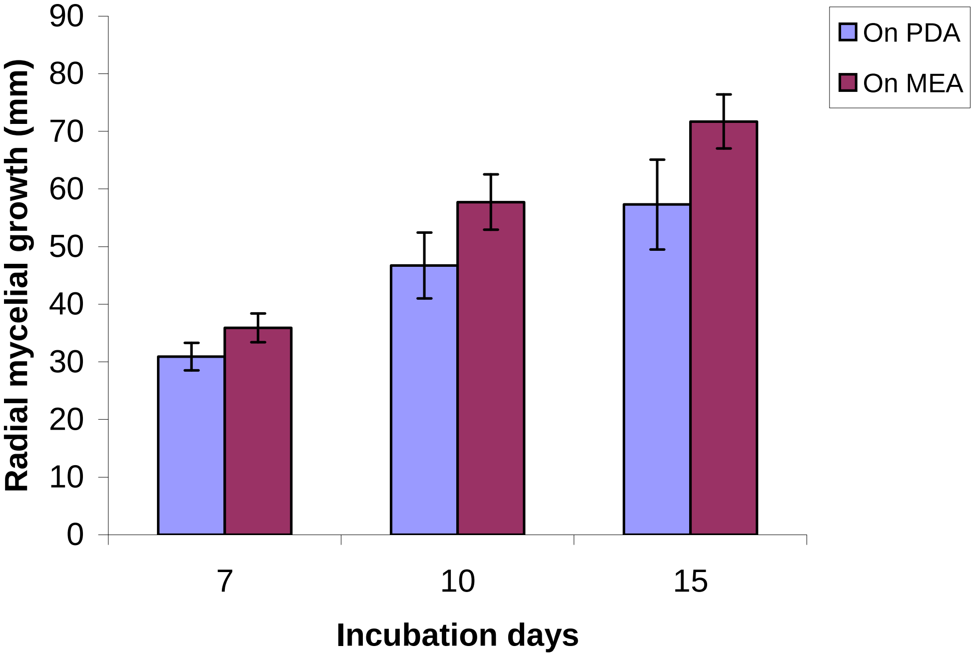
There was highly significant ($P < 0.05$) difference among isolates in their radial colony growth rate both on PDA and MEA (Appendix 7 and 8). Radial colony (mycelial) growth of isolates was faster on MEA than PDA. The mean colony diameter of *C. kahawae* isolates on PDA were 35.9, 57.7 and 71.7 mm after 7, 10, and 15 incubation days, respectively and 30.9, 46.7 and 57.3 mm on MEA, after 7, 10, and 15 incubation days, respectively (Figure 8; Appendix 9). Mean radial colon (mycelial) growth rate of *C. kahawae* isolates ranged between 0.6 and 5.5 mm/24 hr, and 1.2 and 6.1 mm/24 hr on PDA and MEA, respectively (Table 8). Mean radial colon (mycelial) growth rate of *C. kahawae* isolates was varied on PDA and MEA, i.e., 4.4 ± 0.36 and 5.1 ± 0.35 mm/24 hr, respectively.

Table 8. Radial mycelial (colony) growth rate (mm/24 hr) on PDA and MEA incubated at 25 ^{o}C

Isolate[1]	Media	
	PDA	MEA
H40	4.9 a-c	5.2 D
H41	4.6 b-d	5.2 D
H43	4.7 b-d	6.0 AB
B50	4.6 cd	5.9 A-C
B51	4.9 a-c	5.4 CD
B52	4.8 b-d	5.7 A-D
B53	4.5 cd	6.1 AB
B55	4.2 de	5.5 A-D
S60	5.5 a	6.0 A-C
S61	4.6 cd	5.4 B-D
Y70	3.6 e	4.0 E
Y71	4.8 b-d	5.3 D
Y73	5.3 ab	4.4 E
Y74	5.5 a	6.1 A
Y75	0.6 f	1.2 F
G80	3.6 e	4.4 E
G81	4.4 cd	5.5 B-D
Mean	4.4	5.1
CV (%)	8.1	6.8

[1] Three (H40, H41, H43), five (B50, B51, B52, B53, B55), two (S60, S61), five (Y70, Y71, Y73, Y74, Y75 and two (G80, G81) *Colletotrichum kahawae* isolates were collected from Harena, Bonga, Sheko , Yayu and Gera, respectively.

-Means followed with the same letter are not significantly different according to DMRT.



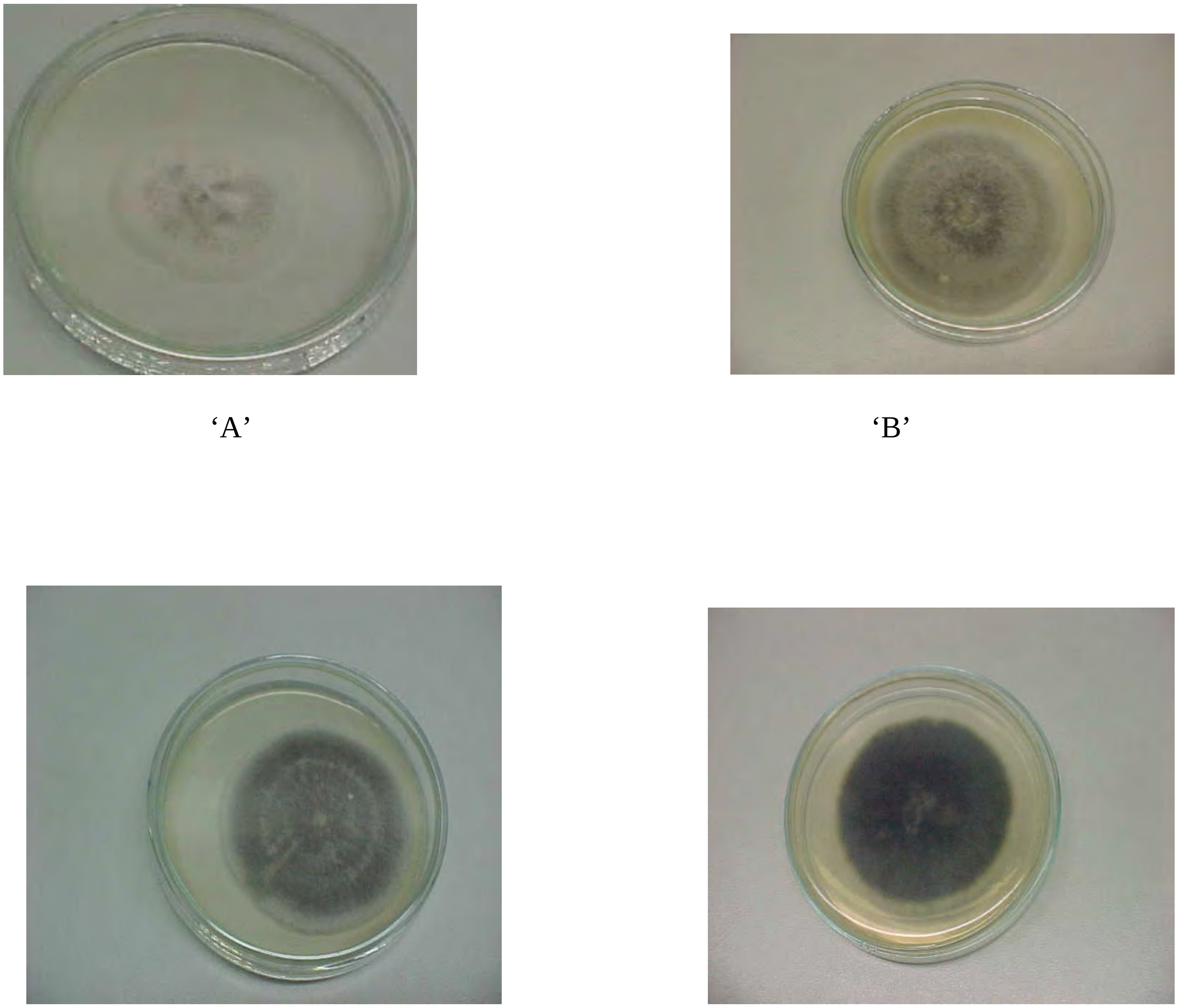
Figgure 8. Radial mycelial growth of C. kahawae isolates on PDA and MEA after 7, 10 and 15 incubation days (error bars are standard deviations).

Table 9. Colony color of isolates on PDA and MEA observed from 10 days cultures

Isolate[1]	Colony color on Media			
	PDA		MEA	
	TOP	REV	TOP	REV
H40	Gray	Darkolivegreen	Dimgray	Darkolivegreen
H41	Gray	Darkolivegreen	Dimgray	Darkolivegreen
H43	Darkgray	Darkolivegreen	Darkgray	Darkolivegreen
B50	Gray	Dimgray	Gray	Dimgray
B51	Darkgray	Darkolivegreen	Gray	Darkolivegreen
B52	Lightgray	Darkolivegreen	Gray	Darkolivegreen
B53	Lightgray	Dimgray	Lightgray	Dimgray
B55	Gray	Rosybrown	Darkgray	Darkolivegreen
S60	Lightgray	Gray	Lightgray	Dimgray
S61	Lightgray	Dimgray	Lightgray	Dimgray
Y70	Lightgray	Darkolivegreen	Lightgray	Darkolivegreen
Y71	Lightgray	Darkolivegreen	Lightgray	Darkolivegreen
Y73	Lightgray	Dimgray	Lightgray	Dimgray
Y74	Darkgray	Gray	Gray	Gray
Y75	Lightgray	Rosybrown	Lightgray	Rosybrown
G80	Lightgray	Rosybrown	Lightgray	Rosybrown
G81	Gray	Darkolivegreen	Gray	Darkolivegreen

[1] Three (H40, H41, H43), five (B50, B51, B52, B53, B55), two (S60, S61), five (Y70, Y71, Y73, Y74, Y75) and two (G80, G81) *Colletotrichum kahawae* isolates were collected from Harena, Bonga, Sheko , Yayu and Gera, respectively.

-**TOP:** Colony color from above; **REV:** Colony color from the reverse side of the petri-plate.



‘A’ ‘B’

‘C’ ‘D’

Figure 9. Aerial mycelial (colony) colors from obverse side: lightgray (A), darkgray (B), gray (C) and darkolivegreen (D).

Table 10. Comparisons of tendency to form sector and aerial mycelial growth of C. *kahawae* isolates on PDA and MEA

Isolate	Sectoring or saltation On PDA	Aerial mycelia growth (vigor)	
		PDA	MEA
H40	Absent	+	+
H41	Present	+	+
H43	Absent	++	+
B50	Absent	++	+
B51	Present	+	+
B52	Absent	+	+
B53	Present	+	+
B55	Absent	++	+
S60	Absent	+	+
S61	Absent	++	++
Y70	Present	+	++
Y71	Present	+	+
Y73	Absent	++	+
Y74	Absent	++	++
Y75	Present	+++	+++
G80	Absent	++	+
G81	Present	+	+

[1] Three (H40, H41, H43), five (B50, B51, B52, B53, B55), two (S60, S61), five (Y70, Y71, Y73, Y74, Y75) and two (G80, G81) *Colletotrichum kahawae* isolates were collected from Harena, Bonga, Sheko , Yayu and Gera, respectively.

-**Aerial mycelia growth (vigor):** += Dense, ++= Irregular (scarce), +++= Very scarce.

5.3.2.2. Investigation on morphological characteristics

5.3.2.2.1. Size of conidia

All *C. kahawae* isolates had variable mean conidia length and width, which ranged between 12.7-15.5 µm, and between 3.6-4.8 µm, respectively. The average conidia length and width of isolates were 14.10 and 4.21 µm, respectively.

5.3.2.2.2. Sporulation capacity

Conidial production that had been taken from 10 days old cultures showed highly significant ($P < 0.05$) differences among isolates (Appendix 10). Conidia production varied between 25.93 x 10^{4} conidia/milliliter (by isolate Y70 from Yayu) and 253.22 x 10^{4} conidia/ml (by isolate S60 from Sheko). Isolate 60 produced significantly ($P = 0.05$) high amount of conidia, followed by isolates from Bonga (B52, B53) and Yayu (Y74) (Table 11). Isolate B52, which was significantly ($P = 0.05$) aggressive on all coffee selections (Table 13), produced intermediate amount of conidia as compared to S60. It was observed that there was variation in conidia production in different batches.

Table 11. Comparison of conidia production of C. kahawae isolates on PDA after 10 days of incubation at 25°C

Isolate	Conidia production (x10,000/ml)
H40	115.22 c
H41	113.33 c
H43	104.67 cd
B50	50.89 g
B51	89.78 de
B52	148.22 b
B53	146.44 b
B55	87.33 de
S60	253.22 a
S61	73.78 ef
Y70	25.93 h
Y71	106.56 cd
Y73	66.25 fg
Y74	136.91 b
Y75	49.78 g
G80	73.44 ef
G81	76.89 ef
Mean	10.1
CV (%)	11.9

-Means followed with the same letter are not significantly different according to DMRT.

5.3.2.2.3. Shape of conidia

Isolates showed a frequency range of 49-88% conidia shape of type 1 (cylindrical and round at both ends) (Table 12). The result indicated that more than 55% of conidial shape frequency of each isolate failed under conidial shape of type 1 except isolates B53, Y75 and G80 (Table 12). Isolate B53 produced almost type 1 and 2 conidia shapes in equal proportion. Isolate Y75 and G80 produced all types of conidia shapes but dominantly type 1.

Table 12. Comparisons of frequencies of different kinds of conidia shapes produced by *C. kahawae* isolates on PDA.

Isolates	PDA				
	1	2	3	4	5**
H40	79	5	14	1	0
H41	88	3	9	1	0
H43	81	6	12	1	0
B50	87	5	8	0	0
B51	82	7	11	0	0
B52	84	4	12	0	0
B53	49	49	1	0	1
B55	81	6	13	0	0
S60	83	2	14	1	0
S61	67	13	18	2	0
Y70	65	14	20	1	0
Y71	59	13	25	3	0
Y73	69	12	17	1	1
Y74	77	7	13	3	0
Y75	49	28	15	4	4
G80*	53	28	12	6	1
G81*	74	6	17	3	0

****Conidial shapes:** 1= cylindrical and round at both ends, 2= cylindrical acute at one and round at the other end, 3= clavate-round at both ends starts attenuating from one fourth of its length, 4= reniform or kidney shaped, 5= Oblong-elliptical.

* Isolates from Gera used as check.

5.3.3. Variation in pathogenicity of *C. kahawae* isolates

C. kahawae isolates (H40, H41, H43, B52, B53, B55, S60, S61, Y70, Y73, Y75 and G81) were inoculated on seedlings of 3 (741, 754 and 74110) widely cultivated cultivars in Ethiopia, which were released as resistant coffee cultivars, and 1 susceptible (370) arabica coffee cultivar. All isolates were pathogenic to all 4 arabica coffee types but varied in aggressiveness (Table 13). Coffee types also showed from highly susceptible to resistant reaction to isolates. Lesions of various sizes and numbers were manifested by all isolates inoculated on 4 coffee types. The incubation periods on 370, 74110, 741 and 754 were 5, 5-7, 6-8 and 6-8 days after inoculation depends on the aggressivity of isolates. Highly aggressive isolates manifested the symptom earlier than the weak ones.

There existed highly significant ($P < 0.05$) differences among coffee cultivars, isolates and cultivar x isolate interaction (Appendix 11). Coffee type 370, which was highly susceptible to all isolates, reacted in classes 1-4 (from single tiny brown dot with 1 or 2 narrow brown lession to black lession girdling the stem and top killed) for isolates H43, B55, S61, Y73, and G81 where as in class 4 for isolates H40, H41, B52, B53, S60 and Y70. Y75 was weakly infecting isolate as compared to other isolates and the only isolate to which 8% of seedlings of 370 showed no symptom (in class 0). 8 days after inoculation, all isolates except Y75 produced symptoms on seedlings of 370 in classes 1-4 where as isolate 75 produced symptoms in classes 0-4.

Coffee type 370 showed significantly ($P = 0.05$) high mean percent CBD infection (96.7%) and followed by 74110 with 78.0% mean CBD infection (Table 14). 8 days after inoculation, all isolate produced symptoms in classes 0-4 on seedlings of 74110. Isolate B52 was significantly ($P = 0.05$) aggresive isolate followed by isolates Y73, H40, B53 and H41 (Table 14). Isolate Y75 produced significantly ($P = 0.05$) low infection percentage. Seedlings of 741 and 754 showed resistant reaction, i.e., resulted with low mean percent of CBD infection, 15.0 and 15.5%, respectively. Cultivar 741 reacted in classes 0-1 (resistant reaction) for isolates S60, S61 and Y70 where as in classes 0-2 for isolate Y75 and in classes 0-4 for other isolates. Cultivar reacted in classes 0-1 (resistant reaction) for isolates B53 and S61, in classes 0-2 for isolates Y70, Y75 and G81 and in classes 0-4 for other isolates.

Sources of variations contributed by coffee cultivars, isolates and their interactions in this experiment were 92.58, 3.75 and 3.67%, respectively. Highly significant ($P < 0.05$) differences among cultivars, among isolates and among their interactions were obtained in the first 15 days after inoculation (Appendix 12). As the incubation time increased from 15 to 20-21 days the reaction progressed at decreasing rate on all cultivars and progress in reaction was negligible beyond 21 days after inoculation. Almost all seedlings of 370 were top killed within the first 15 days after inoculation (Appendix 12).

Table 13. Percent infection of seedlings of *Coffea arabica* cultivars inoculated with 12 *Colletotrichum kahawae* isolates collected from afromontane rain forest coffee areas and one isolate from Gera

	Coffea arabica cultivar				
Isolate[1]	741	754	74110	370	Mean[2]
H40	14.0 gh	20.3 g	88.7 b-d	100 a	55.8 AB
H41	12.7 gh	17.8 gh	85.3 b-e	100 a	54.0 A-C
H43	14.2 gh	15.4 gh	78.3 d-f	98.0 a	51.5 CD
B52	20.2 g	17.9 gh	89.5 bc	100 a	56.9 A
B53	16.5 gh	13.8 gh	86.6 b-e	100 a	54.2 A-C
B55	17.7 gh	18.9 gh	77.8 d-f	98.3 a	53.2 B-D
S60	12.3 gh	13.7 gh	80.3 c-f	100 a	51.6 B-D
S61	10.7 gh	14.3 gh	76.9 ef	97.6 a	49.9 D
Y70	9.0 h	15.0 gh	79.0 d-f	100 a	50.8 CD
Y73	14.6 gh	18.5 gh	92.7 b	98.3 a	56.0 AB
Y75	16.0 gh	10.8 gh	21.0 g	70.3 f	29.5 E
G81	21.8 g	9.3 h	79.3 d-f	98.3 a	52.2 B-D
Mean[2]	15.0 L	15.5 L	78.0 K	96.7 J	

[1] Three (H40, H41, H43), three (B52, B53, B55), two (S60, S61), three (Y70, Y73, Y75) and one (G81)

Colletotrichum kahawae isolates were collected from Harena, Bonga, Sheko , Yayu and Gera,

respectively.

[2] Means followed with the same letter are not significantly different according to DMRT.

-CV = 9.5%.

6. DISCUSSION

Coffee diseases such that CBD and CWD, the two important coffee diseases were found associated with coffee growing in afromontane rainforest of Harena (Bale), Berahane-Kontir (Sheko), Bonga and Yayu. CBD incidence and severity varied from one forest coffee area to other depending on environmental condition and genetic diversity of forest arabica coffee. It is apparent for these surveys that CBD was wide spread in Bonga forest coffee areas and followed by Yayu. The mean percent incidence of CBD was 40, 26.3, 18.6 and 6% for Bonga, Yayu, Harena and Berhan-Kontir (Sheko), respectively.

The estimated mean percent severity was 17.9, 4.0, 5.4 and 2% for Bonga, Yayu, Harena and Sheko, respectively. There was no CBD occurrence at bottom land of Harena (around Majete) and Berhan-Kontir (around Gizmeret) forest coffee areas. It is the first information to report in both areas for the existence of CBD infestations in pocket (limited parts) of Harena (around Mekabaldo) and Sheko (around Wesheka) forest and semi-forest coffee areas. Similarly, Bayetta Belachew (2001) explained high CBD occurrence related with high humidity with high altitude around Gera. High incidence of CBD may be explained by the particularly high rainfall found in relatively high altitudes of Bonga and to some extent in Yayu. As Cook (1975) explained that high rainfall, high humidity or wetness, and relatively low temperatures that persist for long periods favour CBD development and the disease is invariably severe at higher altitudes where these conditions generally prevail.

The incidence and the intensity of the disease varied from one forest coffee area to other. Similarly, the variation in intensity and incidece of CBD in other coffee production systems also repoted by many authors. In 1994 crop season, prevalence of CBD was conducted in Oromiya Region and Southern Nations Nationalities and Peoples Region (SNNPR) and the result indicated 38.8 and 17.2% of mean percent prevalence of the disease, respectively (IAR, 1997). According to the result CBD pressure was very high at higher altitudes in the southwest region, while severe disease was recorded in valleys of Sidamo zone. According to Tefestewold Biratu (1995) CBD severity varied from year to year and among woredas and regions. In Amhara region where CBD occurs, survey result showed that an average CBD severity for the 1996/97-crop season was 38%

(Tesfaye Alemu and Ibrahim Sokar, 2000). Survey conducted in 1997 and 1998 in six major coffee growing zones (in 32 woredas) of Oromiya region showed an average of 31% and 32% disease severity for the respective years (Melaku Jirata and Samuel Assefa, 2000). CBD incidence and severity assessment in 10 zones and 31 woredas of Southern Nations Nationalities and Peoples Region (SNNPR), conducted in September 1998, resulted with 40% and 22.8% mean incidence and severity of the disease, respectively (Tesfaye Negash and Sinedu Abate, 2000).

Coffee wilt disease was prevalent in all assessed forest coffee areas where it has been being posing considerable coffee tree losses. Its mean incidence ranged from 2.4% at Behan-Konitr (Sheko) to 16.9% at Yayu forest coffee area. The difference in genetic diversity of indigenous arabica coffee as well as variation in the intensity of cultural practices (exposure of coffee trees to wounding) from one forest population to other and within the same forest coffee population, could be the cause of variations in the extent of CWD incidence across forest coffee areas. According to Girma Adugna *et al*. (2001) CWD incidence ranged from 45 to 69% at Gera and Bebeka respectively. The variations could be ascribed to susceptibility of coffee cultivars, intensity of cultural practices and environmental conditions.

Even if CWD was reported in earlier times, 49 years ago, on arabica coffee in Ethiopia, this survey result showed very low mean percent incidence across the forest coffee areas as compared to the national CWD survey result of Ethiopia, this is because of the diversity among indigenous forest arabica coffee germplasms and low cultural practices in the forest coffee areas. CWD biological survey result report indicated that the national average incidence and severity of the disease in Ethiopia were about 27.9 and 3%, respectively (CABI, 2003). The report also indicated that the incidence and severity of the disease in Ethiopia varied from place to place, 0-100% and 0-25% respectively.

The result also showed incidence variation from one forest coffee areas to other, even from one particular plot area to another plot area within one forest coffee population. The disease has been being highly expanding by different mechanisms and damaging the coffee trees. According to the observation and information (personal communications with farmers in the localities) in each forest coffee area, coffee farmers in Yayu, Bonga and Harena accustomed to work in group and use cutlasses (bushman knives), indiscriminately and without take care of coffee trees not to wound due to lack of awareness, to slash weeds around coffee trees once per year from July to mid September.

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. According to Booth (1971) the fungus is known to penetrate coffee trees through wounds either above or below the ground. Girma Adugna et al. (2001) also reported that any parts of infected trees, except seeds, and possibly the adjacent asymptomatic coffee trees and soils serve as survival organ and could be potential sources of the pathogen for infection. They also emphasized that the movement and use of infected coffee trees for firewood and for fencing purposes, and the indiscriminate use of farm tools could be mechanisms for disease spread.

In Harena forest coffee, flocks of cattles have grazed and moved along the coffee trees while they have been being damaging mechanically the coffee trees, seedlings and carrying the inoculum on their bodies from area to area. In Berhan-Kontir (Sheko) people intensively use drying and dead coffee trees as firewood and carry them across the forest coffee that could be the means for distribution and spread of the disease in the area. 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.

Considerable variations among indigenous forest coffee selections in reaction to CBD were detected from the results of seedling inoculation and attached berry tests for resistance to CBD. Results of these experiments confirmed that the variations were mainly due to the existence of difference in genetic make up of the selected lines in reaction to CBD. According to Robinson (1976) resistant observed at frequencies of 0.1 to 1% and one tree in every 10,000 possessed both resistance and high yield with good quality. A search for resistance among 200 arabica cultivars, which had been introduced from abroad earlier in 1969, indicated that none of them were found resistant except Rume Sudan, which showed some level of resistance, although not as resistant as the local types (Bayetta Belachew, 2001). Partial to complete dominance of the susceptible genes to the resistant genes was consistently found and 3 to 5 major genes of an additive nature were suspected to be involved in the control of resistance to CBD in the arabica coffee population, in Ethiopia (Mesfin Ameha and Bayetta Belachew, 1984).

Indigenous forest coffee selections that selected from 4 different forest coffee areas revealed significant variations among them in percent CBD infection. Selections from Yayu, Bonga, Berhane-Kontir (Sheko) and Harena (Bale) showed mean seedling infection rate, 69.0-100% 57.5-100%, 75.3-100% and 23.3-100%, respectively. The result indicated that there existed widest range of infection rate among Harena selections followed by Bonga and Yayu selections. But a difference in disease infection level among Sheko selections was very narrow even if statistically significant, 60% of the tested selections depicted the same significant level with that of the standard susceptible chek.

This result indicated that there existed variations among selections that selected from all sites generally and there was also a variation within individual coffee selections of each forest coffee population (locality) in reaction to CBD. Considerable variation also examined among forest coffee selections in percent infection of attached berries in ABT conducted on Yayu and Bonga forest coffee selections in the field. Percent infection of berries varied between 1.4 and 81.5% at Bonga, and between 4.7 and 61.4% at Yayu. 13 and 16 selections from Yayu and Bonga showed low level of infection (< 30%), 4.7-26.6% and 1.4-25.6%, respectively, as compared to others.

In seedling test, considerable variations were also observed among indigenous forest coffee selections in their wilt (dead) seedlings percentage, in reaction to CWD. Similarly, Girma Adugna *et al*. (2001) observed significant difference among the cultivars in percent tree death caused by ***G. xylarioides*** under field conditions. Mean wilt (dead) percent seedlings varied between 0 to 100%. It was also varied among coffee selections of each forest coffee area (population) i.e., coffee selections from Yayu, Bonga, Berhan-Kontir (Sheko) and Harena revealed 58.0-97.2%, 26.2-97.3%, 72.7-100% and 0-94% seedling death, respectively, in reaction to CWD. HA26 showed no seedling death that implies the existence of resistant reaction. There were evidences indicating variations in resistance or tolerance levels in arabica coffee cultivars under field conditions (Girma Adugna, 2004). One selection of Yayu (YA20), five selections of Bonga (BA21, BA22, BA23, BA25, BB21) and seven selections of Harena (HA21, HA22, HA23, HA26, HA27, HA28, HA29) revealed significantly low level of percent seedling death, i.e., 58.0%, 26.2-61.1% and 0-23.5%, as compared to the moderately resistant check (7440) which showed 77.2% seedling death. Van der Graaff and Pieters (1978) also observed a varietals pattern of attack by *G. xylarioides.* Varietals difference in reaction to *G. xylarioides* was also observed in the field (Pieters and Van der Graaff, 1980; Girma *et al*., 2001).

5%, 44%, 5% and 90% of the tested selections of Yayu, Bonga, Berhan-Kontir (Sheko) and Harena (Bale) indicated significantly (P = 0.05) low percent wilt (dead) seedlings and the rest showed high seedling death rate as compared to the susceptible check. 70% of the test Harena (Bale) forest coffee selections indicated significantly low percent wilt (dead) seedlings (< 30%), varied between 0 and 23.5%. 6 selections of Bonga ranged their percent dead seedlings from 26.2% to 72.2%, which were in between percent wilt seedlings of the standard resistant and moderately resistant checks. Out of the tested selections of Berhan-Kontir (Sheko), 95% showed susceptible reaction as compared to susceptible check. This implies selection s from Berhan-Kontir have narrow genetic base in reaction to CWD.

Selections from Yayu, Bonga, and Harena showed wider range of variation where as selections from Birhan-Kontir (Sheko) showed very narrow range of variation, as compared to others, against CWD. This indicated that in the course of resistant coffee variety development it is possible to get wider alternative resistance gene pool from Harena and Bonga followed by Yayu indigenous forest coffee germplasms. But Berhan-Kontir forest coffee has narrow genetic base in reaction to both CWD and CBD.

From these experiments' results it could be concluded that there is great heterogeneity among the indigenous forest coffee selections, selected from the 4 afromontane rainforest coffee areas, in general in resistance to CBD and CWD and great heterogeneity within Harena, Bonga and Yayu coffee populations in particular where as less heterogeneity within Berhan-Kontir forest coffee population in reaction to CBD and CWD.

15 representative *C. kahawae* isolates from forest coffee areas of Ethiopia and 2 isolates from Gera were studied based on their cultural characters, morphological characters and physiological character. As Hindorf *et al.* (1997) stated for correct identification and characterization one still

needs to isolate the fungal population, detecting morphological features *in vitro* such as mycelium colour, growth rate, conidial production and testing the pathogenicity on hypocotyls or berries. Accordingly, 17 isolates (15 from forest coffee areas 2 from Gera) were used for detail quantitative and qualitative analysis of CBD pathogen.

From the diseased berries two species were identified: *C. kahawae* as pathogenic and *C. gloeosporioides* which was most probably as saprophytic or sequential colonizer of dead tissues that is the same result with other authors (Gibbs, 1969; Hindorf, 1970; Tefestewold Biratu and Mengistu Hulluka, 1989; Tefestewold Biratu, 1995; Eshetu Derso and Waller, 2003).

Out of 17 *C. kahawae* isolates tested for their aerial mycelial growth (vigor) 47.1% showed consistently dense aerial mycelial growth on both PDA and MEA media where as 11.8 and 5.8% isolates revealed irregular (scarce) and very scarce aerial mycelial growth on both PDA and MEA media, respectively. The other 35.3% isolates showed inconsistent aerial mycelial growth. Isolates could be grouped into 3 based on their colony color manifestation on the obverse side of PDA and MEA culture plates viz., lightgray, darkgray and gray mycelial forms. Isolates from Sheko (S60, S61), Bonga (B52, B53), Yayu (Y70, Y72, Y73, Y75) and Gera (G80) showed distinct lightgray mycelium form. In this group, *C. kahawae* isolates had white mycelial color upto the first 4-6 incubation days and then after changed into lightgray mycelia on both PDA and MEA media. But isolate B52, Y70 and Y71 revealed darkolivegreen or darkolivebrown as they got old and others remained lightgray. Similar result was reported by Hindorf (1970), it was observed that *C. kahawae* isolates initially white mycelium changes after 4-6 days to gray and eventually to dark olive brown. Similarly, Tefestewold Biratu (1995) also reported light bluish gray colored *C. kahawae* isolates from Kaffa and Illubabor on PDA.

In the second group, isolates H43 (from Harena), B51 (from Bonga), and Y74 (from Yayu) showed darkgray (color intensity between lightgray and gray) colony color in the first 15 days incubation period. In other study similar colony colors of *C. kahawae* isolates were observed. Tefestewold Biratu and Mengistu Hulluka (1989) found *C. kahawae* isolates from Harerge isolates that were grayish mycelium form (light grayish to white). In the third group, isolates from Harena (H40, H41), Bonga (B50, B55) and Gera (G81) revealed initially light gray and changed into gray after 5-7 incubation days and then after 15 days into darkolivegreen. Similarly, Hindorf (1975) reported that the culture of *C. kahawae* forms a gray to blackish colony on malt extract agar.

On the reverse side of culture plates, colonies of the first group revealed whitishyellow or lightyellow colors in the first 4-6 incubation days and then changed into various combinations of colors. The second group manifested lightgray color in the first 4-6 incubation days and then changed into various colors combination. The third group showed lightorange and/or rosybrown color and then changed into various colors combinations.

In agreement to the results of Tefestewold Biratu (1995) the colors of the C. kahawae isolates were consistent within 12-30^{o}C. 41.2% of isolates made sectoring or saltation, which differ in color from the mother isolates. Sectors in all groups were lighter in color and/or some isolate like Y75 showed orange or lightyellow color, which indicated the existence of mutation. As Tefestewold Biratu and Mengistu Hulluka (1989) recorded, saltants/sectors originated in any part of the colony of the mother isolates. These strains indicated unstable characteristics, i.e., some were revertible while others remained stable.

The result revealed that the radial colony (mycelial) growth of isolates was faster on MEA than PDA. The mean colony diameter of *C. kahawae* isolates were 35.9, 57.7 and 71.7 mm on MEA, and 30.9, 46.7 and 57.3 mm on PDA after 7, 10, and 15 days of incubation, respectively. Mean radial colony (mycelial) growth rate of *C. kahawae* isolates ranged between 0.6 and 5.5 mm/24 hr on PDA, and between 1.2 and 6.1 mm/24 hr on MEA.

Mean radial colony (mycelial) growth rate of *C. kahawae* isolates was varied on PDA and MEA, i.e., 4.4 ± 0.36 and 5.1 ± 0.35 mm/24 hr, respectively. It was high as compared to Hindorf (1970; 1973) and Hindorf and Muthappa (1974) result that they reported 1.9 ± 0.5 mm/24hr for average mycelial growth rate of CBD pathogen isolates at 22^{o}C incubation on 2% Oxiod malt extract agar. But the result was similar with that of Tefestewold and Mengistu (1989) report that they recorded 6.5 and 6.7 mm/24hr growth rates on PDA (25 ± 1^{o}C) for dark mycelia and grayish mycelia isolates of CBD pathogen, respectively. Very low but significant growth rate difference was observed among the pathogenic isolates at similar temperatures (Tefestewold Biratu, 1995). He added also that the most favorable media at 25^{o}C was green coffee seed extract agar (GCA), on which *C. kahawae* grew about 8 mm/day followed by PDA (6.7 mm/day), MEA and lima bean agar (LBA) (6mm/day) and V-8 (5.6 mm/day).

In this result distinctly different mycelial growth rate on PDA and MEA was observed, i.e., growth rate on MEA was much faster than on PDA. Faster growth rate on MEA was mainly due to the presence of peptone (5 gram/litre) in MEA substrate composition. Utilization of peptone by the isolates revealed that *C. kahawae* isolates could release peptidase to degrade peptone. Waller et al., (1993) also recorded 4 mm/24 hr for the growth rate of CBD pathogen that was very closer to this result.

The morphological characteristics of the *C. kahawae* isolates were observed on PDA. The sizes and shapes of conidia were variable. The average size of conidia was 14.10 x 4.21µm. Conidia width and length ranged as 3.6 – 4.8 µm and 12.7 – 15.5 µm, respectively. In agreement with those of Hindorf (1973), Hindorf and Muthappa (1974), Tefestewold Biratu and Mengistu Hulluka (1989), and Tefestewold Biratu (1995), the range of conidial sizes were observed from 7 days cultures. Hindorf (1973) recorded 13.1 ± 0.6 x 3.8 ± 0.2 µm and (10.8 – 23.0) x (3.4 – 4.7) µm average and range of conidia sizes, respectively. Hindorf and Muthappa (1974) found 13.1 x 3.8 µm and (11 – 23) x 3.4 – 4.7) µm average and range of conidia sizes from Kenyan *C. kahawae* isolates. Tefestewold Biratu and Mengistu Hulluka (1989) reported 15.3 x 3.5 µm average conidia size of Harerge *C. kahawae* isolates. On PDA *C. kahawae* isolates had variable mean conidia length between 13.5 and 19.3 µm and mean conidia width between 2.9 and 5.2 µm (Tefestewold Biratu, 1995).

The conidia shapes of *C.kahawae* isolates were variable. Conidia shapes variability of *C. kahawae* also reported by Hindorf (1970). From this experiment, as described by Hindorf (1973) and Tefestewold Biratu (1995), the 5 types of conidia shapes were frequently observed in different proportion when detected from each isolate. But conidia shape of type 1 (cylindrical and round at both ends) was dominantly observed from all isolates and accounted a proportion which ranged between 49 and 88%.

Due to interwoven nature of aerial mycelial growth all conidia produced could not be released or washed out easily during harvesting. However, relatively a higher number of conidia were obtained from higher conidia producing isolates by thorough stirring the mycelia suspension with magnetic stirrer for 10-15 minutes. Considerable variation was observed among *C. kahawae* isolates in their conidia production. Conidia production varied between 25.93 x 10^4 (by isolate Y70 from Yayu) and 253.22 x 10^4 conidia/ml (by isolate S60 from Sheko). Isolate S60 produced significantly high amount of conidia, followed by isolates from Bonga (B52, B53) and Yayu (Y74) which had produced 148.22 x 10^4, 146.44 x 10^4 and 136.91 x 10^4 conidia/ml, respectively. Isolate B52, Which showed significantly aggressive reaction on all coffee cultivars, produced intermediate amount of conidia as compared to isolate S60. It was observed that there was variation in conidia production in different batches of cultures. Tefestewold Biratu (1995) observed (12-52) x 10^4 conidia/ml and (684-1720) x 10^4 conidia/ml production from 6 CBD pathogen isolates on PDA and GCA (green coffee seed extract agar). He repoted also the existence of variation, in conidia production, between batches of cultures.

Pathogenicity test of 12 isolates on seedlings of 4 *Coffea arabica* L. cultivars indicated that there was a highly significant difference among cultivars, isolates, and cultivar x isolate interactions. Cultivars 741 and 754 seedlings exhibited resistant reactions to all isolates from afromontane forest coffee areas of Ethiopia and Gera implying horizontal resistant reaction where as cultivar 370 revealed highly susceptible reaction to all isolates. Cultivar 74110 also showed susceptible reaction to all isolates except isolate Y75 (from Yayu) to which low level of mean percent (21.0%) infection was recorded. Inheritance of resistance to coffee berry disease studies by Mesfin Ameha and Bayetta Belachew (1984) suggested that CBD resistance in 741 and 754 cultivars was controlled by recessive three to five major genes of additive nature where as susceptibility was controlled by partial to complete dominant genes. According to Bayetta Belachew *et al.* (2000) 74110 was one of the released resistant cultivars and still in production, on the other hand Tefestewold Biratu (1995) reported that the cultivar was field resistant and had unusually susceptible hypocotyl reaction.

Significant differences of the main effects, i.e., differences between host genotypes and/or differences between isolates in the analysis of variance indicated that resistance and pathogenicity are horizontal in nature (Van der Plank, 1978; Tefestewold Biratu, 1995). This seedling inoculation test result confirmed that high aggressiveness in *C. kahawae* population associated with lower latent period and high mean percent infection of inoculated coffee hypocotyls.

The differential effects in cultivar x isolate interactions analysis revealed that isolates B52 (from Bonga), Y73 (from Yayu), H40 (from Harena), B53 (from Bonga) and H41 (from Harena), followed by B55 (from Bonga), G81 (from Gera) and S60 (from Sheko), induced high percent seedling infection on all arabica coffee types as compared to other isolates. These isolates showed significantly high aggressiveness on 74110 and 370 coffee types where as less aggressiveness on cultivars 741 and 754. Isolate Y75 (from Yayu) was the weakest isolate on cultivar 74110, causing 21.0 % mean seedling infection, however, this cultivar exhibited high susceptible reaction by other isolates with mean percent infection ranging between 76.9 to 92.7%. The isolate also revealed the lowest infection rate (70.3%), less aggressive reaction on seedlings of coffee type 370 as compared to other isolates. Cultivar 741 showed 15.0 % seedling infection.

7. CONCLUSION AND RECOMMENDATIONS

Major Coffee diseases such that CBD and CWD were assessed in afromontane rainforest coffee areas viz., Harena (Bale), Berahane-Kontir (Sheko), Bonga and Yayu. CBD incidence and severity varied from one forest coffee area to other depending on environmental condition and genetic diversity of forest arabica coffee. The mean percent incidence of CBD was 40, 26.3, 18.6 and 6% for Bonga, Yayu, Harena and Berhane-Kontir (Sheko), respectively. The estimated mean percent severity was 17.9, 4.0, 5.4 and 2% for Bonga, Yayu, Harena and Sheko, respectively. It is apparent for this survey that CBD was wide spread in Bonga forest Coffee areas and followed by Yayu.

Coffee wilt disease was prevalent in all assessed forest coffee areas where it has been being posing considerable coffee tree losses. Its mean incidence ranged from 2.4% at Berhan-Konitr (Sheko) forest area to 16.9% at Yayu forest coffee area with certain variations among sampled forest coffee field plots in each forest coffee locality. The difference in genetic diversity of indigenous arabica coffee as well as variation in the intensity of cultural practices (exposure of coffee trees to wounding) from one forest population to other and within the same forest coffee population, could be the cause of variations in the extent of CWD incidence across forest coffee areas. Even if CWD was reported in earlier times, 49 years ago, on arabica coffee in Ethiopia, this survey result showed very low mean percent incidence across the forest coffee areas, this is because of the diversity among indigenous forest arabica coffee germplasms and low cultural practices in the forest coffee areas. This result also showed incidence variation from one forest coffee areas to other, even from one particular plot area to another plot area within one forest coffee population.

Considerable variations among indigenous forest coffee selections were detected from the results of seedling inoculation test and attached berry test in resistance to CBD. Results of these experiments confirmed that the variations were mainly due to the existence of difference in genetic make up of the selected lines in reaction to CBD. Selections from Yayu, Bonga, Berhan-Kontir (Sheko) and Harena (Bale) showed mean disease infection rate in seedling test from 69.0-100%, 57.5-100%, 75.3-100% and 23.3-100%, respectively. In ABT, percent infection of berries varied between 1.4 and 81.5% at Bonga, and between 4.7 and 61.4% at Yayu.

In seedling test, significant variations were observed among indigenous forest coffee selections in resistance to CWD. Mean wilt (dead) percent seedlings varied between 0 to 100%. It was also varied among coffee selections of each forest coffee area (population) i.e., coffee selections from Yayu, Bonga, Berhan-Kontir (Sheko) and Harena revealed 58.0-97.2%, 26.2-97.3%, 72.7-100% and 0-94% seedling death, respectively, in reaction to CWD.

Selections from Yayu, Bonga, and Harena showed wider range of variation where as selections from Birhane-Kontir (Sheko) showed very narrow range of variation, as compared to others, against CWD and CBD. This indicated that in the course of resistant coffee variety development it is possible to get wider alternative resistance gene pool from Harena and Bonga followed by Yayu indigenous forest coffee germplasms. But Berhan-Kontir forest coffee has narrow genetic base in resistance to both CWD and CBD. These observations allow first remarks on possible CBD and CWD tolerant or resistant selections in the indigenous coffee of Ethiopia increasing the value of afromontane rainforest coffee areas. 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 population and evaluation for diseases resistant (with particular focuses for major diseases, CBD and CWD), high yield, better quality and other characteristics to enhance the production and productivity of coffee in Ethiopia and the world.

15 representative *C. kahawae* isolates from forest coffee areas of Ethiopia and 2 isolates from were studied based on their cultural characters, morphological characters and physiological character. Out of 17 *C. kahawae* isolates tested for their aerial mycelial growth 47.1% showed consistently dense aerial mycelial growth on both PDA and MEA media where as 11.8 and 5.8% isolates revealed irregular (scarce) and very scarce aerial mycelial growth on PDA and MEA media, respectively. The other 35.3% isolates showed inconsistent aerial mycelial growth.

Isolates could be grouped into 3 based on their colony color manifestation on the obverse side of PDA and MEA culture Petriplates viz., light gray, darkgray and gray mycelial forms.

Our result revealed that the radial colony growth of isolates was faster on MEA than PDA. The mean colony diameters of *C. kahawae* isolates were 35.9, 57.7 and 71.7 mm on MEA and 30.9, 46.7 and 57.3 mm PDA after 7, 10, and 15 days of incubation, respectively. Mean radial colony growth rate of *C. kahawae* isolates ranged between 0.6 and 5.5 mm/24 hr, and 1.2 and 6.1 mm/24 hr on PDA and MEA, respectively.

The morphological characteristics of the *C.kahawae* isolates were observed on PDA. The sizes and shapes of conidia were variable. Conidia width and length ranged as 3.6 – 4.8 µm and 12.7–15.5 µm, respectively. From this experiment, as described by other authors, the 5 types of conidia shapes were frequently observed in different proportion when detected from each isolate. But conidia shape of type 1 (cylindrical and round at both ends) was dominantly observed from all isolates and accounted a proportion which ranged between 49 and 88%. Due to interwoven nature of aerial mycelial growth all conidia produced could not be released or washed out easily during harvesting. However, relatively a higher number of conidia was obtained from higher conidia producing isolates by thorough stirring the mycelia suspension with magnetic stirrer for 10-15 minutes. Considerable variation was observed among *C. kahawae* isolates in their conidia production. Sporulation capacity of isolates varied between 25.93 x 10^4 (by isolate Y70 from Yayu) and 253.22 x 10^4 conidia/ml (by isolate S60 from Sheko).

No race difference was observed within *C. kahawae* isolates, however, certain cultural, morphological variation as well as significant variation in aggressiveness were detected among them. Pathogenicity test of 12 isolates on seedlings of 4 *Coffea arabica* L. cultivars indicated that there was a highly significant difference among cultivars, isolates, and cultivar x isolate interactions. This seedling inoculation test result confirmed that high aggressiveness in *C. kahawae* population associated with high mean percent infection of inoculated coffee hypocotyls. As many authors reported, the result showed that the resistance manifested by resistant cultivars was horizontal or non-biotype specific. The difference in virulence and aggressiveness implies care should be taken in that to develop resistant varieties aggressive isolates should be used for successful screening of coffee germplasms before the release of the new developed cultiva(s).

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9. APPENDICES

Appendix 1. Analysis of variance table for percent infection of seedlings from Yayu, Bonga and Sheko indigenous forest arabica coffee selections inoculated with *Colletotrichum kahawae* isolate

Sources	df	SS	MS	F- value	Probability
Replication	2	20.0	10.0	1.04	0.3552
Selections	58	26082.5	449.7	46.98	0.0001
Error	116	1110.3	9.6		
Total	176	27212.9			

Appendix 2. Analysis of variance table for percent infection of seedlings from Harena indigenous forest arabica coffee selections inoculated with *Colletotrichum kahawae* isolate

Sources	df	SS	MS	F- value	Probability
Replication	2	94.2	47.1	2.12	0.1318
Selections	23	25712.0	1117.9	50.26	0.0001
Error	46	1023.2	22.2		
Total	71	26829.4			

Appendix 3. Analysis of variance table for percent infection of attached berries of Yayu indigenous forest coffee selections inoculated with *C. kahawae* in the field

Sources	df	SS	MS	F- value	Probability
Replication	2	44.4	22.2	0.24	0.7853
Selections	19	7260.6	382.1	4.19	0.0001
Error	38	3464.1	91.2		
Total	59	10769.1			

Appendix 4. Analysis of variance table for percent infection of attached berries of Bonga indigenous forest coffee selections inoculated with *C. kahawae* in the field

Sources	df	SS	MS	F- value	Probability
Replication	2	236.6	118.3	1.38	0.2622
Selections	21	16692.7	794.9	9.29	0.0001
Error	42	3594.3	85.6		
Total	65	20523.6			

Appendix 5. Analysis of variance table for percent wilt of seedlings from Yayu, Bonga, Sheko and Harena indigenous forest arabica coffee selections inoculated with *G. xylarioides* isolate

Sources	df	SS	MS	F- value	Probability
Replication	2	567.7	283.9	10.72	0.0001
Selections	68	91362.7	1343.6	50.73	0.0001
Error	136	3602.0	26.5		
Total	206	95532.4			

Appendix 6. Analysis of variance table for incubation periods (days) after inoculating seedlings of indigenous forest arabica coffee selections from Yayu, Bonga, Sheko and Harena, inoculated with *G. xylarioides* isolate

Sources	df	SS	MS	F- value	Probability
Replication	2	16533.17	8266.59	12.05	0.0001
Selections	68	128053.08	1883.13	2.75	0.0001
Error	136	93283..50	685.91		
Total	206	237869.75			

Appendix 7. Analysis of variance table for radial growth rate of 17 *C. kahawae* isolates on PDA

Source	df	SS	MS	F- value	Probability
Replication	2	0.5	0.3	1.9	0.161
Isolates	16	59.4	3.7	28.9	0.0001
Error	32	4.1	0.1		
Total	50	64.0			

Appendix 8. Analysis of variance table for radial growth rate of 17 *C. kahawae* isolates on MEA

Source	df	SS	MS	F- value	Probability
Replication	2	0.4	0.2	1.5	0.2391
Isolates	16	68.8	4.3	35.5	0.0001
Error	32	3.9	0.1		
Total	50	73.0			

Appendix 9. Radial mycelia (colony) growth of 17 C. kahawae isolates incubated at 25^{o}C after 7, 10, and 15 incubation days

	Media Type					
Isolate	PDA			MEA		
	7	10	15	7	10	15*
40	34.0	53.3	64.8	36.3	61.0	75.5
41	32.5	51.2	62.0	36.3	59.3	74.7
43	32.8	48.8	55.0	42.2	66.8	82.0
50	32.0	45.5	53.3	41.5	67.8	82.7
51	34.5	55.8	66.8	37.7	60.8	77.5
52	33.3	49.0	59.8	40.0	64.2	79.2
53	32.0	48.5	58.5	42.5	70.0	85.0
55	29.0	45.0	54.2	38.7	64.3	81.0
60	38.3	57.3	76.7	41.8	68.0	87.8
61	32.0	52.3	71.7	38.0	62.0	82.7
70	25.0	39.0	52.2	28.0	41.5	54.5
71	33.7	47.8	61.3	36.8	58.5	80.2
73	37.2	55.2	65.0	30.5	48.8	62.2
74	38.5	53.5	69.7	42.8	72.2	88.2
75	4.3	6.0	7.3	8.0	8.8	10.7
80	25.2	39.3	54.2	30.7	46.5	64.2
81	30.8	45.7	63.0	38.2	60.7	80.7
Mean	30.9	46.7	58.6	35.9	57.7	73.5

*Incubation days

Appendix 10. Analysis of variance table for sporulation capacity of 17 *C. kahawae* isolates on PDA

Source	df	SS	MS	F- value	Probability
Replication	2	382.2	191.1	1.32	0.28.6
Isolates	16	130579.3	8161.2	56.47	0.0001
Error	32	4624.5	144.5		
Total	50	135586.1			

Appendix 11. Analysis of variance table of 12 C. *kahawae* isolates on seedlings of 4 coffee cultivars, 21 days after inoculation

Source	df	SS	MS	F- value	Probability
Replication					
Cultivars	3	101958.7	33986.2	1642.9	0.0001
Isolates	11	4134.6	375.9	18.17	0.0001
Cultivars x Isolates	33	4040.8	122.4	5.9	0.0001
Error	96	1985.9	20.7		
Total	143	112120.0			

Appendix 12. Comparisons of pathogenicity of 12 *C. kahawae* isolates on 4 cffee cultivars 15 days after inoculation

	Coffea arabica cultivar				
ISOLATE[1]	741	754	74110	370	Mean[2]
H40	9.3 kl	20.3 hi	86.3 c-e	100 a	54.0 A
H41	12.5 I-k	15.1 h-k	80.3 d-f	100 a	52.0 AB
H43	13.0 h-k	13.2 h-k	76.3 fg	96.3 b	49.7 B-D
B52	12.6 h-k	13.8 h-k	88.2 cd	100 a	53.7 A
B53	11.4 jk	10.4 jk	84.5 c-e	100 a	51.6 AB
B55	14.7 h-k	13.3 h-k	75.5 fg	97.6 ab	50.3 A-C
S60	11.0 jk	12.0 I-k	77.5 e-g	99.6 ab	50.0 A-C
S61	8.3 kl	9.7 kl	75 fg	97.3 b	47.6 C
Y70	8.1 kl	11.5 I-k	78.2 e-g	100 a	49.5 B-D
Y73	12.9 h-k	14.8 h-k	91.7 c	98.3 ab	54.4 A
Y75	4.0 l	8.0 kl	19.0 h-j	68.8 g	25.0 D
G81	21.7 h	9.0 kl	75 fg	98.3 ab	51.0 A-C
Mean[2]	11.6 o	12.6 o	75.6 n	96.4 m	

[1] Three (H40, H41, H43), 3 (B52, B53, B55), 2 (S60, S61), 3 (Y70, Y73, Y75) and 1 (G81) *Colletotrichum kahawae* isolates were collected from Harena, Bonga, Sheko , Yayu and Gera, respectively.

[2] Means followed with the same letter are not significantly different according to DMRT.

- CV = 9.7%.

Declaration

I, the under signed, declare that this thesis is my original work. It has never been submitted in any institution and that all sources of materials used for thesis have been dully acknowledged.

Name: Arega Zeru

Place: Addis Ababa University

Signature: _________

Date: August 2, 2006

ACKNOWLEDGEMENTS

First of all I would like to thank the Almighty God who blessed each and every minute of my life in doing all my work in his way. I am deeply indebted to my advisors Dr. Fasil Asefa (Department of Biology, Addis Ababa University), and Dr. Girma Adugna (Plant Protection Division in JARC, EIAR) for their continues help during the study period and their critical comments of the manuscript. I would like to extend my thanks to Dr. H. Hindorf (ZEF, University of Bonn) for facilitating my work by providing important reference materials and critical comments of the manuscript. Furthermore, thanks would be directed to Ato Million Abebe, Dr. Bayetta Belachew and Ato Tadesse Eshetu for their cooperation and encouragement.

I am thankful to Ato Demelash Teferi, Ato Sisay Tesfaye, Ato Teshome Tadesse, Ato Biyadige Kassa, Ato Mesfin Seyum and Mamo Abiye for their unreserved assistance during field and laboratory works and data collections. I also thank Ato Kemal Abamegal, Ato Kemordin Abafita, Ato Kedir Abajobir and Wondimu Bekele for their continues follow up and management of seedling raising and facilitating laboratory works. I would like to forward my thanks to Dr. Tadesse Woldemariam for his wise coordination of Conservation of Forest Coffee of Ethiopia (CoCE) project in Ethiopia and facilitating the required finance and materials to my work. I deserve special thanks for Abraham Tesfaye, Tefera Mengistu, Chemeda Abdeta, Chala Jefuka, Woyessa Garedew, Tesfaye Yakob, Esayas Mendesil, Teklemariam Yidgu and other members of the staff of Jimma Agricultural Research Center (JARC) for their encouragement.

I would like to express my respectful thanks to my family, especially my uncle, Kebede Afele, my father, Zeru Melaku, my sincere wife Alem Tesfaye as well as Adanech Beyene, Woinshet Kebede, Bosena Afele, Truwork Tesfaye and all other extended families for their encouragement and support. I am grateful to the Center for Development Research (ZEF) for financing the project. I am also grateful to Ethiopian Institute of Agricultural Research (EIAR) for allowing the study and facilities to work with and Addis Ababa University (AAU) for providing all required facilities.

Many heart-felt thanks go to all who participated directly or indirectly for the successful accomplishment of my thesis work.

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ACRONYMS and ABBREVATIONS

ABT	Attached berry test
ANOVA	Analysis of variance
ASIC	Association International du Café
CABI	CAB International
CBD	Coffee berry disease
CLR	Coffee leaf rust
CSA	Central Statistics Authority
CV	Coefficient of variation
CWD	Coffee wilt disease
DBT	Detached berry test
DMRT	Duncan’s Multiple Range Test
EMSA	Ethiopian Meteorology Service Agency
ETB	Ethiopian Birr
g	gram
GCA	Green coffee seed extract agar
GDP	Gross domestic production
ha	hectare
hr	Hour
IAR	Institute of Agricultural Research
JARC	Jimma Agricultural Research Center
kg	kilogram
l	litre
LBA	Lima bean agar
m	meter
masl	Meter above sea level
MEA	Malt extract agar
ml	Milliliter
MCTD	Ministry of Coffee and Tea Development
MSTAT-C	Microcomputer statistics, software
NFPA	National Forest Priority Area
NMSA	National Meteorology Service Agency of Ethiopia
ppm	Parts per million
PCR	Polymerase Chain Reaction
PDA	Potato dextrose 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
USD	United States Dollar
µm	Micrometer
µl	Microlitre

ABSTRACT

Coffee berry disease (CBD), caused by *Colletotrichum kahawae* and coffee wilt disease (CWD), which is caused by *Gibberella xylarioides,* are the major coffee diseases in Ethiopia. Assessment of CBD and CWD was conducted in Harena, Bonga, Birhan-Kontir and Yayu from July to September 2005. These diseases were prevalent in all surveyed forest coffee areas of Ethiopia. The mean percent incidence and intensity of CBD varied from 6.0-40.0% and 2.0-17.9% across forest coffee areas. The mean incidence of CWD varied from 2.4 to 16.9% across forest coffee areas.

Seedling inoculation tests conducted on indigenous forest coffee selections from 4 different forest coffee areas revealed significant variations ($P < 0.05$) among the selections both in percent seedling CBD infection, inoculated with *C. kahawae* and in percent wilt seedlings, inoculated with *G. xylarioides*. Selections from Yayu, Bonga, Berhan-Kontir and Harena showed mean seedling CBD infection rate from 69.0-100%, 57.5-100%, 75.3-100% and 23.3-100% where as percent wilt seedlings varied from 58.0-97.2%, 26.2-97.3%, 72.7-100% and 0-94%, respectively. This indicated that in the course of resistant coffee variety development it is possible to get wider alternative resistant gene pool from Harena and Bonga, followed by Yayu indigenous forest coffee germplasms. These observations allow first remarks on possible CBD and CWD tolerant or resistant selections in the indigenous forest coffee of Ethiopia, which increase the value of afromontane rainforest coffee. Hence it is important to conserve and use sustainably the indigenous coffee germplasms both *insitu,* and *exsitu*, by conducting intensive selection from more diverse coffee population and evaluation for diseases resistant (priority on CBD and CWD), high yield, better quality and other characteristics.

15 representative *C. kahawae* isolates were obtained from forest coffee areas of Ethiopia and 2 isolates from Gera were studied based on their cultural and morphological characters. Isolates could be grouped into 3 based on their colony color manifestation on the obverse side of potato dextrose agar (PDA) and malt extract agar (MEA). The cultures exhibited lightgray, darkgray and gray mycelia forms. Mean radial colony growth rate of *C. kahawae* isolates ranged between 0.6 and 5.5 millimeter (mm)/24 hour (hr), and between 1.2 and 6.1mm/24 hr on PDA and MEA, respectively. Conidia width and length ranged as 3.6–4.8 µm and 12.7–15.5 µm, respectively. Highly significant ($P < 0.05$) variation was observed among *C. kahawae* isolates in their sporulation capacity, and varied between 25.93×10^4 and 253.22×10^4 conidia/ml.

No race difference was observed within *C. kahawae* isolates; however, certain cultural, morphological variations as well as significant variation in aggressiveness were detected among them. Pathogenicity test of 12 isolates on seedlings of 4 *Coffea arabica* L. cultivars indicated that there was a highly significant difference ($P < 0.05$) among cultivars, isolates, and cultivar x isolate interactions. The resistance manifested by cultivars was horizontal or non-biotype specific. The difference in virulence and aggressiveness implies that care should be taken in while developing resistant varieties. Aggressive isolates should be used for successful screening of resistant coffee germplasms before releasing any newly developed coffee cultivar(s).

Key words/phrases: Afromontane rainforest indigenous coffee, *Coffea arabica,* *Colletotrichum kahawae*, *Gibberella xylarioides*