ASSESSMENT AND CHARACTERIZATION OF COFFEE BERRY DISEASE (*Colletotrichum kahawae*) AND RESISTANCE EVALUATION OF GURAGE COFFEE ACCESSIONS

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ASSESSMENT AND CHARACTERIZATION OF COFFEE BERRY DISEASE (*Colletotrichum kahawae*) AND RESISTANCE EVALUATION OF GURAGE COFFEE ACCESSIONS

A Thesis

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We, the undersigned, member of the Board of Examiners of the final open defense by **Dereje Amare** have read and evaluated his/her thesis entitled "**Assessment and Characterization of Coffee Berry Disease (*Colleticotrichum Kahawa*) and Resistance Evaluation of Gurage Coffee Accessions**" and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in **Plant Pathology**

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DEDICATION

I dedicate this work to my mother Bogalech G/Wold and my son Nathan.

STATEMENT OF THE AUTHOR

I declare and affirm that this thesis is my original work. I have followed all ethical and technical principles of a scholar in the preparation, data collection, data analysis and compilation of this thesis. It is submitted in partial fulfillment of the requirement for MS c degree in Plant pathology in Jimma University and is deposited in the college library to be made available to borrowers under the rule of the library. I declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree.

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ACRONYMS

ABT	Attached Berry Test
CBD	Coffee Berry Disease
CLR	Coffee Leaf Rust
CRD	Completely Random Design
CSA	Central Statistical Agency
CWD	Coffee Wilt Disease
DBT	Detached Berry Test
DI	Disease Incidence
GARSC	Gera Agricultural Research Sub Center
GDP	Gross Domestic Product
GZADD	Gurage Zone of Agricultural Development Department
ICO	International Coffee Organization
JARC	Jimma Agricultural Research Center
LSD	Least significant difference
MEA	Malt Extract Agar
MoARD	Ministry of Agriculture and Rural Development
PDA	Potato Dextrose Agar
PSI	Percent Severity Index
RCBD	Randomized Complete Block Design
RGB	Red Green Blue
SNNPR	Southern Nations Nationalities and People Region
USDA	United State Department of Agriculture

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Assessment and Characterization of Coffee Berry Disease (*Colletotrichum kahawae*) and Resistance Evaluation of Gurage Coffee Accessions

ABSTRACT

Arabica Coffee is an important crop in the national economy of Ethiopia. Coffee berry disease (CBD), coffee wilt disease (CLD) and coffee leaf rust (CLR) are the most important coffee diseases in the country. Development of coffee cultivars for different localities having a character of diseases resistant, high yielding and quality coffee is important. Previous research works have little effort to provide varieties that are suitable for Gurage zone southern parts of Ethiopia in addition to the little information available regarding the extent of CBD and related factors in this area. Hence, this study was carried out to assess the magnitude and extent of CBD, characterize the virulence of C. kahawae isolates and evaluate Gurage coffee accessions against CBD. The survey was conducted in three districts of Gurage zone including Cheha, Ezha and Enemorina Ener during July to August 2017. Attach Berry Test and Detach Berry Test evaluations of Gurage coffee accessions were conducted under field and laboratory condition in Randomized Complete Block Design and Completely Randomized Design, respectively. The survey result revealed that CBD was prevalent in all the assessed areas that range from 86.66 to 100% and 38.89 to 59.44% for Disease Incidence and Percent Severity Index, respectively. Out of 33 sample 13 representative C. kahawae isolates from the survey areas and one Gera isolate were isolated from infected green coffee berry which showed significant variations in their Morphological characteristics and pathogenicity. Mean radial colony growth rate of isolate showed significant variation ($p<0.001$) with the range of 2.67 to 4.08 mm/24hrs on PDA in EZA and CA1 isolates, respectively. Conidial size also showed significant difference ($p<0.001$) in the range of 5 to 6.04 and 9.24 to 10.0 µm in width and length, respectively. Similarly, conidia production varied from 182.25 to 432.92×10^4conidia/ml of isolate EK1 and EZD, respectively. All isolates were found to be pathogenic to Arabica coffee with highly significant variation ($P < 0.01$) and infection percentage in the ranges of 45.83 to 68.06%. Gurage coffee accessions Gu-18, Gu-1 and Gu-4 had lower CBD infection level in both field and laboratory experiment, which was 5.4, 8.29 and 11.37 and 32.5, 45.0 and 25.8 %, respectively. Aggressive isolate EZD should be used for screening of coffee variety for CBD resistance evaluations. Future research should focus on evaluating the promising Gurage coffee accessions in seedling inoculation test and in multi-location field trials for several years.

Key words; Incidence, Severity, Virulence, pathogenecity

1. INTRODUCTION

Coffee belongs to the genus *Coffea*, in the family *Rubiaceae.* The genus *Coffea* comprises 124 species; however *C. arabica* L. and *C. canephora* P. are the two commercially important species (Davis *et al.*, 2012). *Coffea arabica* is grown in about 80 countries in the world spanning over 10.2 million ha of land in the tropical and subtropical regions, especially in Africa, Asia and Latin America, which accounts for 70 percent of the world coffee market and the remaining *Coffea canophora* Pierre ex Froehner (Davis *et al*., 2012; Bunn, 2015). It is autogamous, and the only tetraploid taxon (allotetraploid, 2n = 4x = 44) in the Genus *Coffea* (Lashermes *et al*., 1999; Davis *et al*., 2006).

Coffee is the most important cash crop worldwide; more than 125 million people in the coffee growing areas derive their income directly or indirectly from Coffee products (Lashermes *et al.*, 2012). A number of coffee-producing countries in sub-Saharan Africa, including Ethiopia, Uganda, Kenya, Rwanda and Burundi, depend on the export of this commodity for their foreign exchange earnings (Phiri *et al*., 2010). Ethiopia is the center of origin and genetic diversity of Arabica coffee. In Ethiopia coffee farming provides a livelihood income for around 15-16 million peoples, based on four million small-holder farms,10 percent of agricultural production, and about 34 percent of total export earnings over the past decade (Tefera, 2015; Tadesse *et al*., 2015; ICO, 2018). In 2015/16, Ethiopia exported around 180,000 metric tons of coffee with value of 800 million USD, making it first producer in Africa and fifth coffee exporter in the world with market share of 7-10% worldwide (ICO, 2018).

In 2017/18, the country's coffee production was 449,229.81tons in 725,961.24 ha of land with an average yield of 0.619 tons/ha; it shares 5.09% of the area under all crops in the country produced (CSA, 2018). Ninety five percent of Ethiopia's coffee is cultivated by small farm holder and the production is almost exclusively situated in the regions of Oromia and the Southern Nations Nationalities and People Regions (SNNPR). It is produced under plantation coffee (5-8%), forest (8-10%), semi-forest (30-35%) and garden (50-55%) coffee production systems (Workafes and Kassu, 2000; Alemayehu *et al*., 2008; MoARD, 2008; Taye *et al*., 2011; Birhanu, 2017). Gurage zone is one of the coffee growing areas of Ethiopia. This zone encompasses areas suitable for coffee production about 3,934.27 ha of land with a production of

836.68 tons; 0.213 tons per ha in 2016/17 (GZADD, 2017; CSA, 2017). It shares 0.56 percent of the national coffee production and 1.81 percent of SNNP Region coffee production area (CSA, 2017). The national average coffee yield per ha is currently low (0.619 ton/ha). This is partly due to continued reliance on low yielding varieties, poor agronomic practices and widespread and prevalence of pests and diseases (Girma *et al*., 2009). The major important coffee diseases in Ethiopia are coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR) caused by *Colletotrichum kahawae, Gibberella xylarioides* and *Hemileia vastatrix*, respectively (Hindorf, 1998; Girma *et al*., 2009; Abdi and Abu, 2015).

Many research findings have been reported on the status of major coffee diseases in Ethiopia. A survey conducted in 1997 and 1998 in six major coffee growing zones of Oromia region resulted in an average of 31% and 32% CBD severity for the respective years (Melaku and Samuel, 2000). Tesfaye and Sinedu (2000) survey indicated that 40% and 22.8% mean incidence and severity of CBD in 31 districts of SNNPR, respectively. Moreover, mean percent severity of 17.9, 4.0, 5.4 and 2% was reported for Bonga, Yayu, Harena and Sheko forest coffee production system, respectively (Arega *et al*., 2009). However, the overall national average yield loss due to CBD is estimated to be between 20 and 30% (Eshetu, 1997; Eshetu *et al.*, 2000). Kumlachew *et al*. (2016) reflected that the higher disease incidence of 70.7, 65.3 and 59.3% were recorded in Hararghe, Gedeo and Jimma, with correspondingly higher severity of 42.7, 46.7 and 32.0%, respectively and with the national average incidence and severity of 52.5 and 29.9%, respectively.

The genetic diversity of wild Arabica populations far exceeds that of cultivated varieties used in crop production and accessions held in germplasm collections (Labouisse *et al*., 2008). The wild populations also have high functional diversity in terms of disease (Girma, 2005), pest and drought tolerance (Taye, 2006). Development of new coffee cultivars for different localities having a character of diseases resistant, high yielding and possess unique quality profile plays pivotal role for improving coffee productivity and foreign exchange earnings of the country (Girma *et al*., 2009). 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 especially in Ethiopia. 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, 2001).The comprehensive testing of selecting materials for resistance to CBD in the mother trees and their progenies in the laboratory and in different locations, where the epidemic is not only severe but also regularly present, is very vital (Eshetu, 2000).

The mission of the Ethiopian coffee research program was to explore improved coffee cultivars with wider adaptation, by giving more emphasis to the southwestern part of the country. Having this circumstance location specific coffee technology generation and promotion under diverse coffee growing agro ecologies is very important. To date, 37 released pure line CBD resistant cultivars and five hybrid varieties are in production in coffee growing areas of the country, which 10 of them were restricted to mid altitude (<1700 m a s l) where CBD pressure is low (Behailu *et al.*, 2008; Chala *et al.*, 2012; Demelash and Kifle, 2018) and some resistant cultivars showed susceptible under laboratory (Demelash, 2014). However, this research direction has little effort to provide varieties that are suitable for Gurage zone southern parts of Ethiopia. Currently, Jimma Agricultural Research Center has collected 21 coffee landraces from major coffee producing districts of Gurage zones to evaluate their yield, diseases resistance and quality. These accessions are under field evaluation at Gera Agricultural Research Sub Center, Cheha and Enemorina Ener districts of Gurage zone. Nevertheless, there has been little information and work related to CBD status in Gurage zone. Therefore, this research work was executed with the following objective:-

General objective: To assess, characterize *C. kahawae* of Gurage zone and evaluate the reaction of Gurage coffee accessions to coffee berry disease.

Specific objectives

- To assess the coffee berry disease intensity in the major coffee producing districts of Gurage zone
- To characterize *C. kahawae* isolates collected from major coffee producing districts of Gurage zone
- To evaluate pathogenecity of *C. kahawae* isolates of Gurage zone in susceptible cultivars, and
- To evaluate Gurage coffee accessions for resistance to Coffee berry disease

2. LITERATURE REVIEW

2.1. Importance and production of coffee in Ethiopia

At origin, production of coffee provides a livelihood for up to 25 million farmers and their families. Additional economic benefits are accrued by actors along the global value chain, be they traders, roasters, retailers and their workforce or other stakeholders (ICO, 2019). The value of coffee exports amounted to USD 20 billion in 2017/18 (ICO, 2019). In 2016/17, coffee production was 159.1 million bags (i.e. 9.5 millions of tonnes, each bag contain 60 kilograms of green coffee), from which 98.8 were Arabica and 60.4 Robusta. It has a worth an estimated retail value of USD 70 billion and is one of the most traded commodities second in value only to oil and a huge contributor of foreign exchange earnings for developing countries (ICO, 2016). More than 125 million people in the coffee growing areas worldwide derive their income directly or indirectly from its products in cultivation, processing, trading, transportation and marketing (Lashermes *et al.*, 2011).

In Ethiopia, coffee production has passed several stages of production systems since its domestication which can be broadly grouped as forest coffee, semi forest coffee, garden coffee and plantation (Workafes and Kassu, 2000). The coffee crop follows a single annual cycle of growth and fruiting in the country (Wrigley, 1988). However, the growth may vary seasonally according to changes in rainfall and temperature. Apart from being the birth place of *C. arabica*, Ethiopia is the first coffee-producing African country and stands 5^{th} in the world. It contributes 7-10% of world's production and 34% of the total production of coffee in Sub-Saharan Africa, (ICO, 2016; ICO, 2018). Coffee is mainly grown by smallholder farmers on less than 1 ha of land, and earning less than a dollar per day (McCarthy, 2007).

Coffee cultivation plays a vital role both in the cultural and socio-economic life of the nation. Ninety five percent of the total coffee output is produced by over four million small-scale producers (Arslan and Reicher, 2011), and 15-16 million of the Ethiopian population directly or indirectly benefit from coffee value chain. Fifty percent of coffee produced is locally consumed, reflecting the commodity's cultural importance in the country. It remains as a backbone of Ethiopian economy contributing 41% of foreign exchange, sustaining more than 1 million farming household, absorbing 25% employment opportunity and 10% of government revenue

(Petit, 2007; MoARD, 2008). In 2017/18, the country coffee production was 449,229.81tons in 725,961.24 ha of land with average yield of 0.619 tons/ha; it shares 5.09% of the area under all crops in the country produced (CSA, 2018).

2.2 Coffee diseases in Ethiopia

In Ethiopia Arabica coffee is attacked by numerous diseases that reduce its production and productivity significantly. Major coffee diseases are; Coffee berry disease, Coffee wilt disease and Coffee leaf rust which are caused by *Colletotrichum kahawae, Gibberella xylarioides* and *Hemileia vastatrix*, respectively. The minor coffee diseases are; bean discoloration, leaf blight, root-rot, brown eye spot, damping off diseases of seedlings, Fruit-rot and thread-blight which are caused by *Pseudomonas syringe, Ascochyta tarda, Armillaria mellea, Cercospora coffeicola, Rhizoctonia* spp. and *Pythium* spp., *Fusarium* spp. and *Corticium kolleorega*, respectively (Merdassa, 1985; Eshetu *et al*., 2000).

2.3. Coffee Berry Diseases

2.3.1 Occurrence and distribution of *C. kahawae* species

Coffee Berry Disease was first reported in Kenya in 1922 (Mc Donald, 1926). It has since then been reported from most Arabica coffee producing countries in Africa such as Angola in 1930, Congo in 1937, Cameroon in 1957, Uganda in 1959, Tanzania in 1964, Ethiopia in 1971 (Van der Graaff, 1981; Van der Vossen, 1985) and Malawi in 1985 (Lutzeyer *et al.*, 1993). The disease is not known outside Africa (Stephen and Rebecca, 1991).

Since its first report in 1971 (Mulinge, 1973), CBD has spreads to all coffee producing areas of Ethiopia. It is understood that environmental conditions have favored its distribution until it has become a major disease of coffee in Ethiopia, which attacks mainly the green berries. The pathogen is also capable of infecting leaves, stem, bark, flowers and twigs of the coffee plant (Stephen and Rebecca, 1991). Direct loss occurs as a result of flower and young fruit infection. Flowers are susceptible at all developmental stages from the pale green unopened spike. Immature fruit or berries are most susceptible during the expansion phase which occurs from four to fourteen weeks after flowering (Mulinge1970).

Eshetu (2000) reported that 20-30% yield loss due to CBD, however, it may reach up to 100% in some years and in some parts of the country where the rain fall is high at higher altitudes (Van der Graff, 1984). The variation in yield loss estimation in the country might be due to difference in the disease epidemic from region to region and from time to time. Arega *et al*. (2009) reported that the mean incidence ranged between 6.0% and 40.0% and severity ranged between 17.9 and 2.0% at Sheko and Bonga, respectively. Hika *et al.* (2018) indicated that mean CBD incidence ranged between 6.67 - 60%, 23.33 - 65%, and 20 - 80% with mean severity of 42.73%, 54.18% and 55.14% in Chole, Gololcha and Shanan Kolu, respectively.

2.3.2 Morphological and cultural characteristics of the pathogen

According to McDonald (1926) who described the cultural characteristics of *C. kahawae* for the first time; he described that slow growing, cottony, dark greenish grey colonies of the pathogen distinct from other forms of *Colletotrichum* species associated with brown blight on ripening berries, dieback of branches and leaf symptoms.

Gibbs (1969), differentiated *Colletotrichum* species into four strains based on colony characteristics of a single conidial cultures of isolates from host tissue grown on 2% Malt Extract Agar (MEA) as : (a) *C. coffeanum* (*Var. virulans*) with slow growth, profuse grayish-black mycelia and conidia borne directly on hyphae; (b) *C. coffeanum* pink type (CCP) with slow growth, profuse pink areal mycelia, conidia borne directly on hyphae; (c) *C. coffeanum* mycelia type (CCM) with fast growing profuse pace aerial mycelia and conidia borne directly on hyphae and (d) *C. coffeanum acutatum* (CCA) with moderately fast growth, sparse pale aerial mycelia, conidia produced in acervuli. Hindorf (1970) made a detailed morphological study of the isolates of *Colletotrichum* species from coffee from various ecosystems in Kenya and classified pathogenic forms of *C. kahawae* and saprophytic strains of *C. gloeosporioides, C. acutatum,* and *Glomerella cingulata*.

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 and Mengistu 1989). The absence of perithecia, slow growth rate, and pathogenic ability characterize *C. kahawae. C. gloeosporioides* produced fertile perithecia (perfect stage) and was unable to cause CBD. Based on the color of the colony of *C. kahawae* isolates show two groups on PDA,

those dark bluish gray colored (isolates from Sidamo and partly Harerge) and light bluish gray colored (isolates from Kaffa and Illubabor) (Tefestewold, 1995).

The different strains of C*olletotrichum spp.* are distinguished on the basis of conidia shapes and colony characteristics on agar. CBD strains posses a green to dark mycelium after 2-3 days on all media while saprophytic forms exhibit white mycelium (Stephen and Rebecca, 1991). Arega *er al.* (2009) grouped *C. kahawae* into 3 colony color kindly light-gray mycelium, dark-gray mycelium and gray mycelium. Kilimbo *et al.* (2013) reported that 25 *C. kahawae* isolates produced dark grey cottony colonies, while Birhanu (2014) placed *C. kahawae* isolates from Hararghe into four groups based on their colony color on PDA and MEA culture plate; light gray, dark gray, gray and white mycelia. Whereas *C. kahawae* isolates from Borena and Guji showed pale yellowish to pinkish with dense whitish-grey aerial mycelium and a few bright orange conidial masses on the tips of the active growing hyphae on MEA medium (Abdi and Abu, 2015).

Different studies conducted on colony radial growth rate of *C. kahawae* isolates difference were observed in radial colony (mycelia) growth rate. Hindorf (1970) report indicated that the average mycelial radial growth of *C. kahawae* isolates was 1.9 ± 0.5 mm in 24hrs. The mean radial colony (mycelial) growth rate of *C. kahawae* isolates ranged between 0.6 to 5.5 mm in 24hrs, and 1.2 to 6.1 mm in 24hrs on PDA and MEA, respectively (Arega2006). Kilimbo *et al*. (2013) reported the growth rate of *C. kahawae* isolates to be ranged between 5.0 to 5.5 mm per 24hrs at 25°C in MEA media. Birhanu(2014) indicated that the mean radial colony (mycelial) growth rate of *C. kahawae* isolates varied on MEA and PDA, i.e. 4.05 and 5.35 mm in 24hrs, respectively.

In different research works, variability was observed in aerial mycelial growth of *C. kahawae* isolates. The report Arega (2006) categorized isolates into 3 classes based on their aerial mycelial growth (vigor) as kindly dense (47.1%), irregular (scarce) (11.9%) and very scarce (5.9%) colony types on both PDA and MEA media, respectively. The rest 35.2% of isolates made inconsistent aerial mycelia growth on PDA and MEA, dense or irregular (scarce) types.

Conidia production of *C. kahawae* isolates variability was observed in different scholar's research. Tefestewold (1995) recorded that (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. The conidia production of *C. kahawae* isolates of Afro montane varied from 25.93 to 253.22 x 10^4 conidia/ml (Arega, 2006). Kilimbo *et al.* (2008) from Tanzania reported conidia production of *C. kahawae* strains ranging from 13 to 850 conidia/ml.

2.3.3 Epidemiology of CBD

Precipitation, humidity and temperature are the most important factors determining CBD outbreaks, playing a key role on the germination, production and dispersal of spores by the fungus (Omondi *et al.*, 2000). In regions with two rainy seasons, two flowering events are created, which dramatically increases the emergence of epidemics and prolongs the disease active period (Katoh *et al.*, 2009).

The epidemic of CBD is initiated each year by conidia from the bark and, if present, from sporulating mummified berries formed during the previous season (Gassert, 1976). The conidia are water borne and splash distributed. It needs high relative humidity and/or the presence of water for germination. A conidium germinates, forms a germ tube and an appressorium after which the cuticle is penetrated. The whole process generally requires about five hours (Nutman and Robert, 1960). Medium and long distance dispersal or spread is aided by vectors such as man (coffee pickers), birds, wind and possibly insects. Conidia produced in acervuli on developing bark of young twigs and on diseased berries provide the initial inoculum for a CBD epidemic (Wrigley, 1988). Splashes of rain disperse the conidia to new infection sites. In the initial stages of the disease, infected bark may be the only source of inoculum, but as the disease progresses, conidia from berries become more abundant and play a major role in the spread of the disease (Gibbs, 1969; Griffiths *et al.*, 1971).

Mulinge (1970) reported green berries are susceptible at four to fourteen weeks after flowering. Ripening berries are also susceptible while mature berries (16 to 25 week of fruit development) are resistant due to endocarp formation (Silver *et al*., 2006). Nutman and Roberts (I960) reported that the minimum and maximum temperatures for spore germination were 15°C and 30°C, respectively, with an optimum of 22°C. It is generally observed that the incidence of CBD is high on coffee grown in the cooler high altitude zones than in the lower altitude areas.

Mwang'ombe *et al.* (1991) investigated that the effect of temperature on appressoria and lesion formation and found that the optimum temperature for appressoria formation was between 15-

25^{o}C which coincided with the range of temperatures in the cool high altitude coffee growing zones and in the low altitude zone during the cold and wet months. Rainfall in the afternoon provides the conditions for the release of spores, their distribution and the droplets of water on the berries are unlikely to dry out for at least five hours, which enables the spores to germinate and infect the green berries (Wrigley, 1988). At optimum temperature, Firman and Waller (1977) reported that spore germination occur within 4 hours. Typical black sunken anthracnose lesions of CBD initially appear about 1 week after inoculation and most by 2 weeks for berries in the ages between 5 – 14 weeks after flowering (Bock, 1956).

2.3.4 Infection process and disease symptoms of *C. kahawae* species

The infection process is favored by availability of water and suitable temperature. In the infection process, *C. kahawae* uses a hemibiotrophic strategy, which includes a post penetrative asymptomatic biotrophy phase, followed by a destructive necrotrophy phase that culminates in the appearance of disease symptoms and the reproduction of the fungus (Loureiro *et al*., 2012). The infection starts with the germination of the conidia (asexual spore) and differentiation of melanized apressoria on the plant´s surface, a structure used by the fungus to penetrate the cuticle by mechanical pressure(Fig 1), secretion of cutin degrading enzymes, or a combination of both processes (Chen *et al*., 2004; Silva *et al*., 2006). Following penetration, the fungus starts to colonize the host tissues: -in which an infection vesicle is formed from which several other hyphae emerge and grow. This phase involves the transition of hyphae growth in living cells (biotrophy - which may last 24 to 48 hrs after inoculation) to dead cells (necrotrophy). During the symptomless biotrophic phase, the pathogen invades host cells without killing them and feeds on living cells. Subsequently, the pathogen switches to anecrotrophic mode of nutrition, feeding on dead host tissues (Várzea *et al*., 2002, Silva *et al*., 2006).

New conidia are formed and emerge from the cuticle, setting free a new generation of *C. kahawae* spores (Fig. 1) (Silva *et al*., 2006; Loureiro *et al*., 2012). *C. kahawae* infect all stages of the coffee tree from flowers, green berries, ripe fruits and occasionally leaves (Mulinge, 1970). Maximum crop losses occur from infection of green berries with the formation of dark sunken lesions which are sporulates causing their premature dropping and mummification. Infection of the flowers may be economically important, as they are very susceptible at all stages, but it is difficult to measure since they drop shortly after the infection (Omondi *et al*., 2000).

The first symptoms of CBD infection on green immature berries were dark-brown slightly sunken spots. Under suitable environmental conditions, the spots enlarge to cover the whole berry and masses of conidia maybe visible. The lesions may reach the beans that become black and shriveled. If infections were occurred early and climatic conditions favor disease development, berry development is arrested, resulting in dry, black, mummified berries of no commercial value. When the berry ripens and anthracnose fully develops, the bean can become infected and seed borne.

Generally, *C. kahawae* affects any part of the green berries in the expansion phase and may take two forms:

Active lesions (anthracnoses) may start as a dark brown, slightly sunken spot (necrosis) (Nylander *et al*., 2004). Under appropriate conditions the spot eventually enlarges and covers the whole berry, which is reduced to a black and deformed fruit with no commercial value, preventing further processing of the beans. Before the fruit decays completely, if the environment is sufficiently wet, small pinkish masses of spores develop on the surface of the decayed pulp (Nguyen *et al.*, 2009). Active anthracnose lesions commence as small, water-soaked spots that rapidly become dark and sunken (Omondi, 1998). These expand, causing a rot of the whole berry and, under humid conditions, pink spore masses become visible on the lesion's surface. Finally the berries become brown or black and if desiccation occurs, they are mummified.

In *scab lesions*, however, the lesion develops slowly due to a plant resistance response and is characterized by a buff color and the presence of small dark spots. The growth of the lesion is limited, as the fungus dies out, not affecting the normal bean development (Polashock *et al.*, 2009). In scab lesions the pathogen grows sparsely and sporulates poorly or not at all. It can also be observed when infections occur during the less susceptible stages of berry development and on resistant coffee varieties as a defense reaction (Mc Donald, 1932; Masaba and Van der Vossen, 1982; Masaba, 1991). Anthracnose symptoms in other parts of the plant such as leaves and stems are seldom and economically unimportant (Crouch and Beirn, 2009).

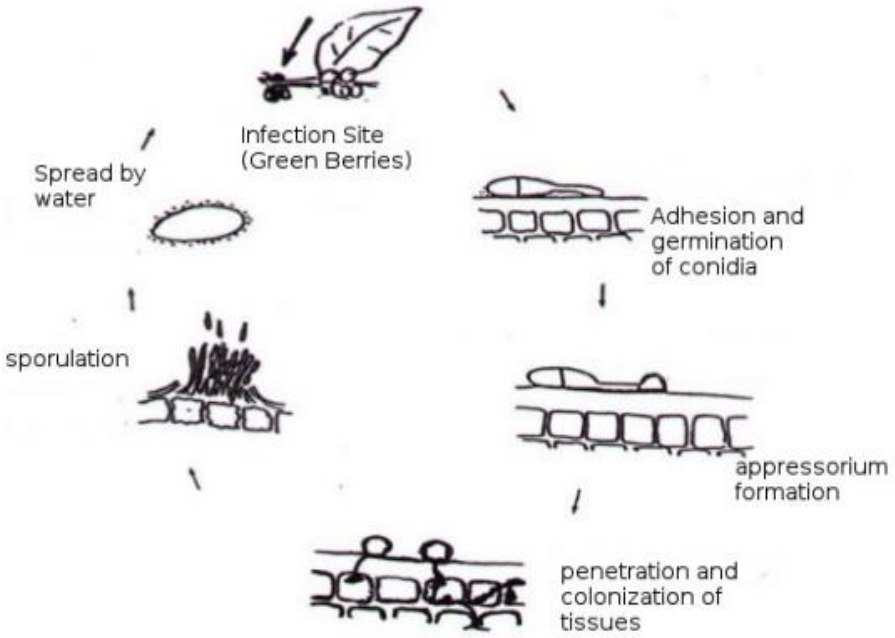

Figure 1 Schematic representation of the infection process of *Colletotrichum kahawae.*
Source; Jeffries & Koomen 1992

2.3.5 Variation in virulence among *C. kahawae* strains

Aggressiveness can be considered as a quantitative measure of the level of disease reached over time. Most of the aggressive pathogen reach at the specific disease level faster than the less aggressive and can be measured by spore production, infection size, latent periods and its severity on the host (Luzolo *et al*., 2010; Pires *et al*., 2016). Factors such as geographical origins are known to determine aggressiveness of *C. kahawae* populations (Manga *et al*., 1998). Moreover, differential interactions between host and *C. kahawae* populations were seldom found in Ethiopia (Eshetu and Waller, 2003). Rodriques *et al.* (1991) reported variation in both aggressiveness and the rate of sporulation and growth of the *C. kahawae*. The variation in both sporulation and rate of growth of the pathogen virulence tests with isolates of *C. kahawae* also confirmed that the CBD pathogen exhibited variation for aggressiveness but no races were detected (Fredrick *et al*., 2015). Susceptible genotypes are clear indicators of aggressiveness difference among pathogen isolates and consequently difference in aggressiveness should always be performed on susceptible genotypes (Castiblanco *et al*., 2018).

2.3.6 Management of CBD

2.3.6.1 Host-plant resistance

Use of resistant varieties is the most important and environmentally friendly method of disease management. Resistance variety is the best option to protect the crop from damage due to biotic factors. It is only the use of variety with durable resistance that provides a permanent solution and guarantees stable cash income to the growers. The report of Vander (2009) from Kenya revealed that none of the *C. arabica* L. genotype showed 100% resistance to all the *C. kahawae* isolates from CIFC's collection although some varieties, like Rume Sudan, Ruiru 11 and derivatives from Timor Hybrid (HDT), showed high resistance levels. In the Ethiopian context, some CBD-resistant national coffee varieties have been generated per coffee-growing region. So far Jimma Agricultural Research Center release 37 pure line CBD resistant cultivars and five hybrids are in production in coffee growing areas of the country of which 10 of them were restricted to mid altitude (<1700 m a s l) where CBD pressure is low (Behailu *et al.*, 2008; Chala *et al*., 2012; Demelash and Kifle, 2018).

The accumulation of salicylic acid and jasmonic acid after pathogen infection induces the expression of defense genes in plants. Thus, artificial induction of plant defense system through signaling molecules could be one of potential methods to reduce the severity of coffee berry disease caused by *Colletotrichum kahawae*. Application of different concentrations of SA, JA, mono potassium phosphate and di potassium phosphate significantly reduced the intensity of coffee berry disease on artificially inoculated coffee hypocotyls by triggering host resistance (Kumlahew *et al*., 2018)

2.3.6.2 Cultural and biological controls

In these methods very few efforts have been made in Ethiopia. There are some reports on case that promote good aeration and rapid drying of the canopy, such as adequate pruning and wide spacing in order to reduce disease incidence. Bedimo *et al*. 2007 showed maintenance pruning, mummified berry removal and inter planting with shade plants helped to significantly reduce CBD intensity on coffee trees. Pruning during the vegetative growth period can leads to the effective means of removing diseased branches, berries, susceptible and old trees. This leads reduce the initial inoculum sources through maintenance pruning and mummified berry removal. Eshetu (1988) reported, in his visit to the former Yeju (North Wollo), there was no CBD

incidence observed and this was attributed to the use of irrigation by coffee farmers in the area, which shifted the susceptible stage of berry development. Shade reduces sunlight and plays an important role in modification of the micro environmental conditions (reduce temperature fluctuations, air movements and could limit the rain intensity) and can work as a barrier and limits the dispersal and development of the pathogen (Jha *et al*., 2014). Bedimo *et al*. (2008) reported 30 and 50% of CBD incidence with and without shade in the coffee plantation respectively, which indicates the significance of the shade.

The groups of soil microorganisms with antagonistic properties towards plant pathogens are diverse, including plant associated prokaryotes and eukaryotes. *Bacillus* and *Pseudomonas* spp. are the most widely used biocontrol agents which have receiving much attention and tested on a wide variety of plant species for their ability to control diseases (Mulatu, 2012). Omondi (1998) observed that *Fusarium stilboides,* and to some extent *Cladosporium tenuissima, Penicillium glaborum, Epicoccum purpurescence, Nigrospora sphaerica* and *Alternaria alternata* have showed promising inhibitory effects with C. *kahawae* based on detached berries. Kebede *et al*. (2018) collected 348 specimens (from leafs, twigs and berries) from Oromia and SNNPs regions to test as the bio-agents for the management of CBD pathogen under laboratory conditions. After stepwise testing of these isolates, they reported 33 bio agents (10 anti-biotic bacteria, 10 antibiotic yeast, 10 antibiotic filamentous fungi and 3 lytic bacteria) as effective bio agents of CBD control under laboratory conditions.

2.3.6.3 Chemical control

Chemical control, particularly the use of cupric fungicide was the first attempt to manage disease outbreaks in the 60's and remains the most common until today (Nylander *et al*., 2004). However, it has revealed somewhat inefficient as are washed away in the rainy seasons, when the fungus strikes most, or because the fungus rapidly develops resistance in the field (Ronquist and Huelsenbeck, 2003; Rydholm *et al.*, 2007). Moreover, smallholders, who produce the majority of the Arabica coffee in most countries of East Africa, are usually unable to carry out the recommended complete spray program, due to the costs associated with the use of fungicides and intensive spray programmers ended up applying one or two fungicide sprays per year (Cisar and TeBeest, 1999). Such occasional sprays were found to induce higher levels of CBD than what would occur in their total absence (Griffiths, 1972). In addition, the CBD fungus was

reported to have developed resistance to some of the recommended systemic fungicides only after 2-3 years of continuous spraying (Cook, 1975; Okioga, 1976).

In Ethiopia fungicide spray against CBD starts six weeks after the main flowering, usually experienced to vary from mid-January to March depending on the rainfall pattern that influences blossoming and the consequent berry development. Six rounds of fungicide applications at 4 weeks (28 days) interval in a crop season have been recommended in the country (Eshetu *et al*., 1995). Spraying of fungicides should be carried out at the right time and correct rate; untimely application and use of incorrect rate of fungicides disregard efficacy of the product. Excessive application may lead to risk of toxicity to the plant or its environment. On the other hand, low levels of treatment introduce the possibility of the pathogen developing tolerance (Eshetu *et al*., 1995). Fungicide spray can limit the development of CBD but inadequate fungicide sprays or improper timing of sprays also result in higher levels of disease pressure than where the sprays have not been applied. This is due to the direct substantial quantitative and qualitative effect of fungicides on the non-target micro flora some of which are antagonistic to the CBD pathogen (Margaret, 2011). The high cost of fungicides (45% of production cost including the labors), appearance of resistant pathogen biotype and other social and health related problems of the conventional agriculture on the environment is the immense topics when thinking to increase the interest of sustainable agriculture and biodiversity conservation (Swami and Alane, 2013).

3. MATERIALS AND METHODS

3.1. Description of the study area

3.1.1 Survey area

CBD incidence and Severity assessment were conducted in the major coffee producing districts of Gurage Zone (Cheha, Enemorina Ener and Ezha) of the SNNPR State (Fig. 2). The area is located between 7.8^{o}- 8.5^{o} latitude and 37.5^{o}-38.7^{o} longitude. The zone has three different agro-climatic zones: 28.3% high land 64.9% midland and 6.8% low land with an altitude range of 1000-3500 m a s l; with an average rainfall of 1350-1800ml; and mean minimum and maximum temperature of 11^{o}C and 30^{o}C, respectively. The soil type was red sandy loam soils (GZADD, 2017).

3.1.2. Attach berry test study sites

The attached berry test was conducted in Gera Agricultural Research Sub-center (GARSC) at field condition. The sub center is found in Oromia Regional State in Jimma zone, Ethiopia, located around 7^{o}7'N latitude and 36^{o}0'E longitude and at an altitude of 1900 m a s l. It represents the high agro ecological zones which receive an annual rainfall of 1877.8mm, with mean minimum and maximum temperatures of 10.4^{o}C and 24.0^{o}C, respectively (Anteneh and Taye, 2015).

3.1.3 Laboratory and Growth room study sites

The laboratory and growth room studies were conducted at Jimma Agricultural Research Center (JARC). The center is found at Oromia Regional State in Jimma zone, Ethiopia, located around 07°46''N latitude and 36°47'E longitude coordinate and at an elevation of 1753 m a s l. It represents the medium agro ecological zones which receive annual rainfall of 1572mm, with mean minimum and maximum temperatures of 11.6^{o}C and 26.3^{o}C, respectively. The major soil type of the center is chromic nitosol and cambiosl of upland and fluvisol of bottom land (JARC, 2004).

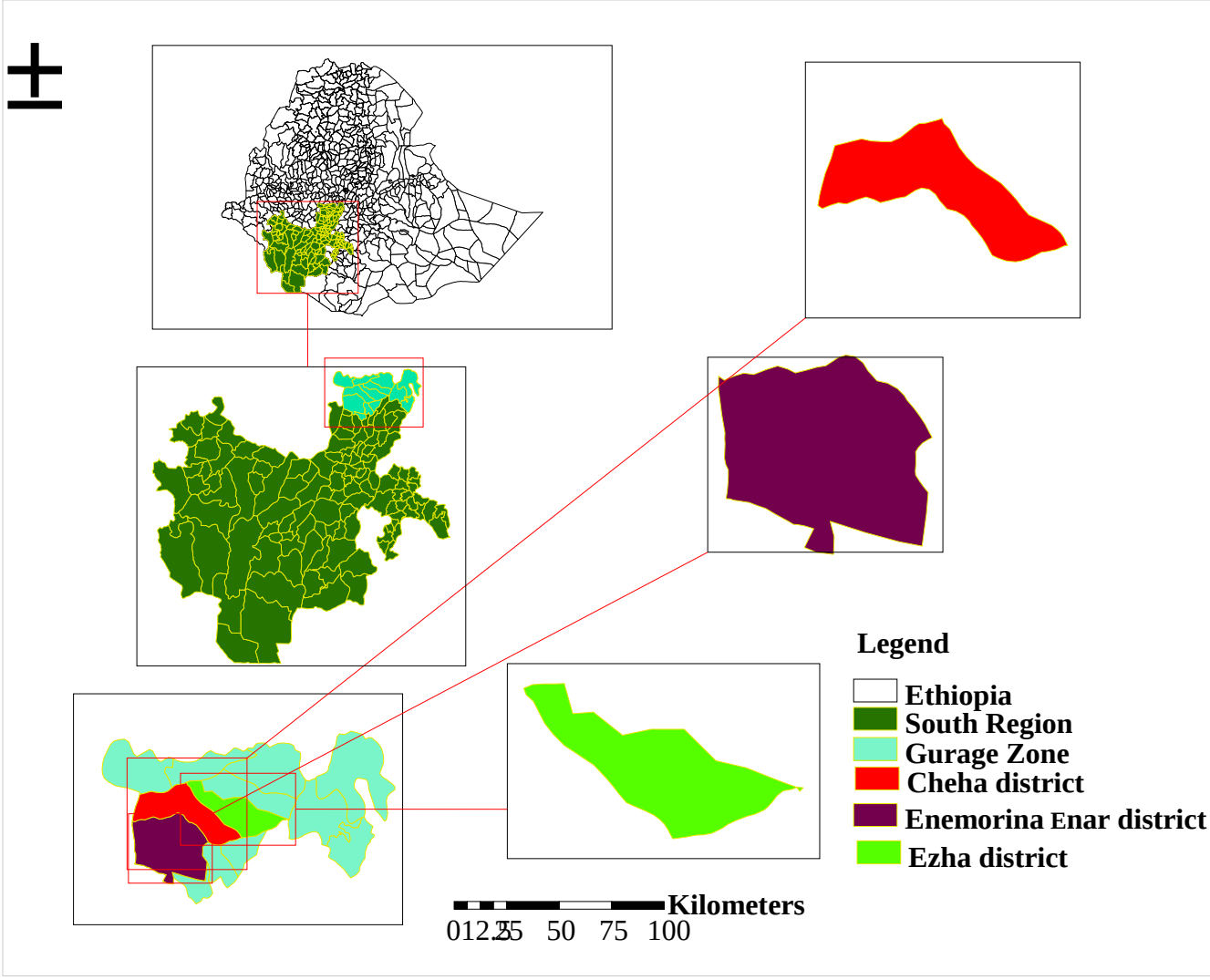

Figure 2 Maps of Survey Area of Gurage zone.

3.2. Survey and disease assessment

Sampling design:

Assessment of CBD was done in major coffee producing districts of Gurage Zone. Three districts Cheha, Enemorina Enar and Ezha were purposively selected based on their coffee production potentials. From each district, three peasant associations were purposively selected based on production potential. Three coffee fields were randomly selected at intervals of 2-3 km along the main and accessible rural roads for Cheha, Enemorina Enar districts and five coffee fields for Ezha a total of 33 coffee fields were randomly selected for assessment for the disease survey.

Field sampling:

CBD assessment was done in the field diagonally following the procedures described by Tesfaye and Ibrahim (2000). Three types of assessments were conducted following the method of Van der Graaff (1981) described as follows:

Assessments:

The assessment of CBD was done in July to August 2017.

(a) Prevalence: each selected farms was visually assessed for presence and absence of CBD. So as to calculate disease distribution as

$$\text{Prevalence} = \frac{\text{number of infected farms}}{\text{Total number of assessed farms}} \times 100$$

(b)Visual assessment (Incidence): Ten trees per farmers' field were randomly taken and diagnosed for the presence or absence of CBD.

$$\text{Disease incidnce (DI)} = \frac{\text{Number of infected trees}}{\text{Total number of trees assessed in the farm}} \text{x}100$$

(c) Severity assessment: Ten coffee trees per farmers' field were randomly selected and each coffee tree was divided into 3 strata of branches (top, middle and bottom). From each stratum one middle branch was taken for data collection. The number of CBD infected and healthy berries were counted and then DS was computed using the formula:

$$\text{Diseases severity (DS)} = \frac{\text{Number of dameged berries}}{\text{Total number of berries counted}} \text{x}100$$

It was rated using standard disease scales of 0-6; where, 0: 0%, 1: ≤2%, 2: 2-5%, 3: 6-10%, 4: 11-50%, 5: 51-99%, and 6: ≥99% of diseased berries (adopted from Abdi and Abu, 2015). The scores were changed into Percentage Severity Index (PSI) for the analysis using the formula of Wheeler.

$$\text{PSI} = \frac{\text{Sum of numerical rating}}{\text{Total number of rated plant X max. score of the scale}} \text{x}100$$

All the farms surveyed were garden type of coffee productions system and intercropped with Enset. Agronomic practices used in the study area were hoeing the farms and adding animal manure for weed management and nutrients for intercropping crop, which was an Enset

important crop in study area. Altitude and the cultivars types were recorded from each farm to determine the relationship with intensity of the disease and pathogen characteristics.

3.3. Isolation and characterization of *C. kahawae* isolates from Gurage Zone

3.3.1. Sample collection

Green diseased coffee berries with active CBD lesions were collected from the Gurage zone. The peasant and farmer's field numbers were mentioned in section 3.2. In each farmer's field, 10 coffee trees were selected in a 6-8 m distance interval depending on size of the field (in a diagonal pattern). A total of 33 farms were surveyed and 33 samples with 20 green coffee berries with active CBD lesions from each farm were collected. Sample berries were picked using sterilized forceps, collected in a sterilized plastic bag, labeled with location (district, farmer field and altitude) and collection date and brought to JARC plant pathology laboratory and kept at 4^{o}C until isolation.

3.3.2. Isolation and identification

Colletotrichum spp. was isolated following the methods described by Tefestewold (1995). The collected berries were cut into pieces with margin of diseased and healthy tissues using sterilized surgical blade. Then samples were surface-disinfected with 5% sodium hypo-chlorite solution for 2 minutes and then rinsed 5 times in sterilized water for 2 minutes. The sterilized samples were dried in laminar flow hood and then, five fragments (cut pieces) of each sample was taken and placed onto Petri dishes containing PDA supplemented with 0.04% streptomycin and incubated at 25^{o}C for 3 to 5 days. The growing edges of any fungal hyphae (mycelial tip) developing from the tissues were sub-cultured aseptically to PDA and inoculated for 7–10 days at 25^{o}C. After morphological and microscopic identification (conidial morphology, conidial lengths, colony growth rate, colony shape and colony colors) from 33 samples 13 representative pure cultures mono conidial *C. kahawae* isolates were preserved in 50% PDA slant method at 4^{o}C for later use. *Phoma* spp., *Aspergillus* spp., *Penicilium* spp. and *Fusarium* spp. were grown on culture of some fragment samples and removed it and sterilize the petri dish to avoid contamination.

3.3.3 Cultural and morphological characterization

Pure culture of 14 representatives *C. kahawae* isolates were isolated from infected green coffee berries from the surveyed areas. From Cheha districts (4 isolates), Ezha districts (5 isolates), Enemorina Ener districts(4 isolates) and 1 Gera isolate were examined cultural and morphological characteristics of *C. kahawae* isolates were studied following the methods and procedures used by Hindorf (1973), Tefestewold and Mengistu (1989), and Tefestewold (1995).

3.3.3.1. Cultural appearances

Ranges of cultural variation in *C. kahawae* population of 13 representative isolates of major coffee growing area of Gurage zone in addition to one isolate from Gera were examined by culturing on PDA containing 0.04% streptomycin and incubated at 25^{o}C in three replications in CRD design for all characters. An isolate was examined for a colony (mycelial) radial growth, colony color, colony shape and aerial mycelial growth characters.

Collected berry samples of representative *C. kahawae* isolates were cultured on PDA. Mycelial tip (newly growing edge) of each isolate was placed at the center of PDA in 9cm Petri-dish. Then, mycelial (colony) radial growth (mm) of each isolate was measured from the reverse side of the Petri-dishes daily with ruler for 10 days starting from the 3rdday of incubation.

The colony (mycelia) color on the upper side and types of pigments on the reverse side of the Petri-dish for each isolate was determined on PDA every 3 days using RGB color chart (Rayner, 1970). Hence, cultures were monitored for 12 days.

Vigor of aerial mycelium growth types of each isolate was observed on upper side of a plate after 10 days of being cultured on PDA. Then, it was examined and recorded as dense (regular), irregular (scarce) or very scarce culture types.

The colony morphology form of each isolates was observed on reverse side of the plate on 8 days of being cultured on PDA. Then, it examined and recorded as round, irregular, filamentous, rhizoid or curled types of culture forms.

3.3.3.2 Morphological characteristics

The isolates of *C. kahawae* collected from major coffee growing area of Gurage zone were cultured on PDA and incubated at 25^{o}C and replicated three times per isolate in CRD design. Then, the conidial size and sporulation capacity character of the isolate was measured.

Isolates was cultured on PDA medium in three replications for 10 days and then conidial size (length and width) were measured on 30 conidia per replication per isolate. Length and width of conidia were measured with ocular micrometer (µm), at 400X magnification of compound microscope.

Sporulation capacity of each isolates was determined from 10 days old culture of the isolates 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 15 minutes with magnetic stirrer to extract the spores from the interwoven mycelia. Finally, the mycelia were filtered into another sterilized beaker through double layer cheese clothes. The number of conidia per ml were counted using haemocytometer. Nine haemocytometer conidia counts were taken for each isolate.

3.4. Pathogenicity test of *C. kahawae* isolates

The 14 representative *C. kahawae* isolates collected from Gurage zone plus Gera isolate were evaluated for their pathogenic ability (virulence) on a detached green berry of susceptible variety (370). The interaction of the variety and the isolates were evaluated following the methods of van der Vossen *et al*. (1976), Bayetta (2001).

3.4.1. Green berry collection

Fifteen weeks old from date of flowering of the expanding coffee berries (Pinard *et al*., 2012) from the susceptible variety (370) were collected. The berries were picked randomly from bottom, middle and top of the coffee tree in order to have a representative sample. Berries were surfaced sterilized with 5% sodium hypochlorite solution for 2 minutes and rinsed three times with sterile distilled water for 2 minutes each and dried using sterile cotton cloth. The wounded stalk end of the berries was removed with a sterile scalpel to avoid contamination with

saprophytic fungi. Eighteen berries per isolates were placed in 3 rows in plastic box on sterilized tissue paper for inoculation, in CRD design in three replications per isolates.

3.4.2. Inoculum preparation and Inoculation

Ten days old mycelia colonies culture of each isolate was washed by flooding with 10 ml sterilized distilled water, rubbed with sterilized scalpel and then transferred to 50 ml sterilized beaker to harvest conidia. The suspension of each isolate was stirred with magnetic stirrer for 15 minutes and filtered through double layers of cheese clothes. After repeating the procedure the spore concentration of each suspension was adjusted to 2 x 10^6conidia/ml and 20µl (Kilambo, *et al*., 2008; Kumau, 2015) of conidia suspension was deposited on the berries using a pipette while shaking time to time when drawing the inoculums. As a control (check) 20µl distilled sterilized water was poured on the berries. Boxes were sealed to provide saturated humid conditions necessary for disease development. Regular opening after every three days was done for 10 minute to allow for aeration of the berries.

The data on infection collected every three days starting from 3rd days post inoculation when CBD symptoms were visible. After 14 days, data on disease intensity (PSI), expressed as pathogenicity level of each isolates were recorded using a scales of 0 to 6 (modified Van der Vossen *et al*., 1976 adopted from Abdi and Abdu, 2015) (Table 1).After scoring each coffee berry individually, average infection percentage (AIP) on each isolates across the replicates was calculated as follows:

$$AIP = \sum [Ir1 + Ir2 + Ir3 + \cdots Irn]/N$$

Where, **I** is the sum of disease score; **n** is the number of replication; **Irn** is the sum of disease score in replication *n;* **N**is the total number of berries scored in the replications (Kamau, 2015).

Table 1 Assessment key for evaluation of coffee berry disease severity in *Coffea arabica*

Disease index	Descriptions
0	Healthy green berries without symptoms
1	Black sunken lesions cover < 2% of the green berries surface
2	Black sunken lesions cover 2-5% of the berries surface; approximately3mm in diameter
3	Black sunken lesions cover 6-10% of the berries surface shows black lesions approximately 5 mm in diameter
4	Black sunken lesions cover 11-50% the berries surface; approximately 7mm in diameter
5	Black sunken lesions cover 51-99% of the berries surface; approximately15 mm in diameter
6	>99% or the whole surface of berries covered with black sunken lesions; mummified berries

Source: Modified Van der Vossen *et al*. 1976

3.5. Reaction of Gurage coffee accessions for CBD

3.5.1. Attach berry test (ABT)

Attached berry test was conducted on 17 Gurage coffee accessions previously established in GARSC in August 2017, those score 0-20% CBD visual assessment data in three consecutive years. The established experiment contains 21 Gurage coffee accessions and one field resistant check (74110) were planted with two replications in RCBD design (Appendix Table 1). The ABT was conducted following the methods described by Van der Graaff (1981). Three trees per accessions were selected and divide the selected trees in three strata of branches (top, middle, and bottom) take one middle braches from each stratum for inoculation. Then, record a number of berries in selected branch after removing diseased, wounded, matured and pinhead berries.

3.5.1.1 Inoculum preparation

In order to obtain the inoculums source, CBD infected green berries with active black lesions were collected from the GARS center. Then, diseased berries were wetted slightly with distilled sterilized water and incubated in a closed plastic box for 24-48 hours which was sufficient time

to produce a reasonable sporulation. Then, a conidia suspension was prepared by rinsing the berries in sterilized distilled water and the conidia density was determined by haemocytometer after repeated purifying of suspension through double layers of cheese clothes.

3.5.1.2. Inoculation of attached berries

The berries in each selected stratum branches were inoculated (≈0.025 ml per berry) with a suspension of 2x 10^6 conidia/ml using hand sprayer in late afternoon to avoid excessive heat (Arega 2006; Kamau, 2015). Then, each branch was kept moist and warm over night for 12 hrs covered in a plastic 'sleeve'. The plastic sleeve was insulated with paper bag to avoid high temperature. After 21 days of inoculation the numbers of healthy and diseased berries were recorded.

3.5.2 Detach berry test (DBT)

Seventeen of the best performing Gurage coffee accessions (score 0-20 % CBD in visual assessment) plus 3 cheks i.e., 741(resistant), 74110 (field resistant and lab moderately resistant) and 370 (susceptible) varieties were used (Appendix Table 1) for this test. A spore suspension was prepared from a 10-day-old culture of *C. kahawae* isolate of EZD, which was more aggressiveness isolate in pathogenecity test experiment. The experiment was laid out in CRD design in three replications containing 20 berries per replication. Similar methods of inoculum preparation, inoculation and data collection procedures were used as described under section 3.4.1 and 3.4.2.

3.6. Statistical analysis

The survey data were analyses by General linear mixed effect model. All laboratory and fieled data were summarized, tested for normal distribution using the normality test and subjected to analyses of variance (ANOVA) using SAS program version 9.3 software (SAS, 2011). Before analysis of variance, the field evaluation data (ABT) was transformed with Arcsine transformation. Fisher's least significant different (LSD) mean separation tests were performed for comparison of genotypes and isolate characters means that showed significantly different. The relationships among disease variables, altitudes of surveyed farms and pathogen characteristics were determined by Pearson correlation analysis using the SAS software (Proc procedure).

4. RESULTS AND DISCUSSION

4.1 Status of CBD in major coffee producing districts of Gurage zone

The mixed models results showed that CBD was prevalent (100%) in all the assessed coffee growing areas (Fig. 3). There was no significant difference (p <0.05) among districts and peasant associations in coffee berry incidence and percent severity index (Fig. 3, Table 2 and Appendix Table 2 and 3). The highest disease incidence was recorded in Bortena peasant associations (100%), where as, the lowest disease incidence was recorded in Sisenaematye peasant associations (Fig. 3).The overall disease incidence ranged from 86.66 to 100%. On the other hand, there was no statistically significant difference (p<0.05) among districts and peasant associations in mean PSI. The highest and lowest PSI was recorded in Kochere (59.44%) and Sisenaematye (38.89%) peasant associations, respectively (Fig. 4, Table 3 and Appendix Table 3). The overall PSI was range between 38.89 to 59.44 % (Fig. 4).

Table 2 Parameter estimates of the generalized linear mixed effect models (GLMM) on the coffee berry incidence.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-1.010	.738		-1.369	.182	-2.522	.502
	District	.052	.055	.461	.944	.353	-.061	.165
	Peasant assotion	-.011	.019	-.325	-.610	.547	-.050	.027
	Altitude	.001	.000	.532	2.575	.016	.000	.002
	Age	-.002	.002	-.220	-1.356	.186	-.006	.001

a. Dependent Variable: DI

b. Predictor: (Constant), Age, District, Altitude, Peasant assotion

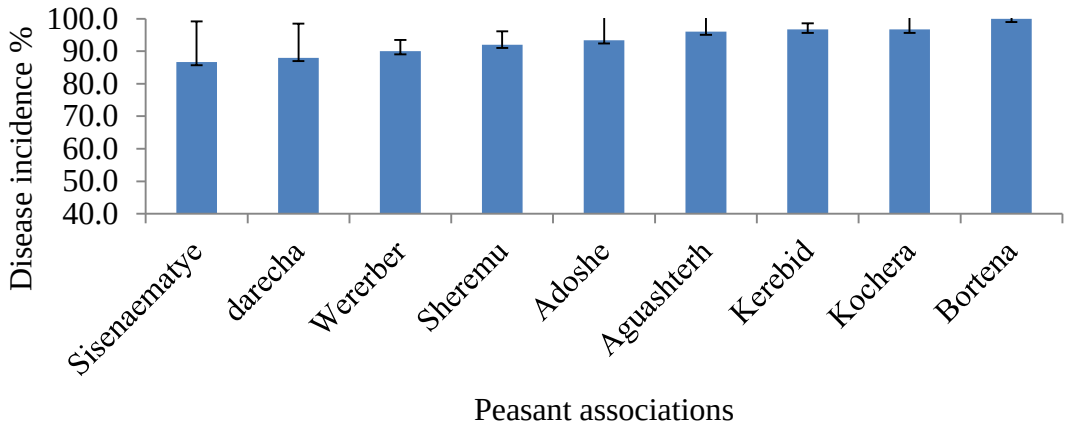

Figure 3 Coffee Berry Diseases recorded at different peasant associations in the three districts of Gurage zone in 2017.

Table 3 Parameter estimates of the generalized linear mixed effect models (GLMM) on the percent severity index

Coefficientsa

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-2.046	.781		-2.618	.014	-3.646	-.445
	District	.064	.058	.497	1.096	.282	-.056	.184
	Peasant assotion	-.017	.020	-.424	-.857	.399	-.058	.024
	Altitude	.001	.000	.600	3.131	.004	.000	.002
	Age	.001	.002	.122	.810	.425	-.002	.005

a. Dependent Variable: PSI
b. Predictor: (Constant), Age, District, Altitude, Peasant assotion

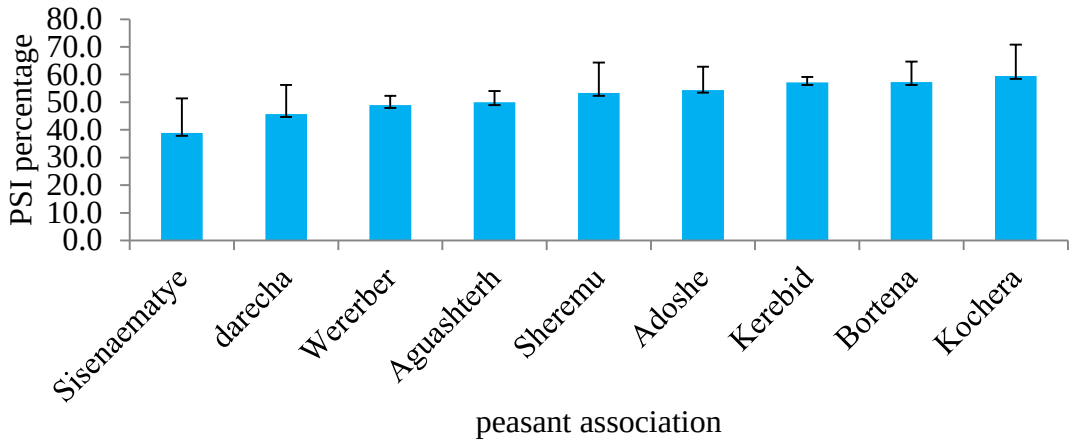

Figure 4 Coffee Berry diseases Percent severity index of nine peasant associations in the three districts of Gurage zone in 2017.

The overall mean DI and PSI recorded across the assessed districts were 93.26% and 51.68% respectively. In this study, the DI and PSI were much higher as compare to the national average disease incidence and severity records (Eshetu 2000; Kumlachew *et al*., 2016) and some areas research reports (Arega *et al*., 2009; Birhanu 2014; Hika *et al*.,2018). This is due to that almost all farmer's in surveyed area grows local coffee landraces which are known for low yield and genetic susceptibility to CBD. The presence of susceptible coffee landraces is one of important parts for the development of CBD epidemic which influence coffee production and reduce the income generation to farmers (Castiblanco *et al*., 2018). Moreover, climate change might could also be contribute to conducive environment for disease development (by increasing rainfall amount, erratic rain fall and increase the leaf wetness hours), which might have increase the period of berry susceptibility in relation to the period of high disease pressure and also affect the potential of some of the previously released good yielding and resistant varieties (Kifle and Demelash, 2015; Kumlachew *et al*., 2016). Leaf wetness duration, relative humidity, soil water potential can affect pathogens directly by altering spore germination and hyphal growth rates or by affecting the rate of inoculum production (Huber & Gillespie, 1992).

There was highly significant ($p < 0.01$) and positive correlation between altitude and incidence ($r = 0.46$) and severity ($r = 0.73$) of CBD indicating strong relationship between altitude and intensity of the disease (Table 4). Increasing in altitude resulted in an increase in disease intensity. All assessed areas in this study occurred in higher altitudes (>1855 m.a.s.l). The

highest disease incidence and severity index was recorded in altitude range of 2006-2025 m.a.s.l with magnitude of 100.0 % and 63.3%, respectively. 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). Kagezi *et al*. (2018) pronounced the impact of CBD is high in the high altitude areas where favorable environmental conditions are found, especially temperature and moisture conditions (i.e. relatively low temperature and high moisture together with susceptible host) exist.

Table 4 Pearson correlation analysis of major factors and CBD intensity (incidence and severity) of coffee berry disease in Gurage zone

	Age	**DI**	**Alt**	**PSI**
Age	1.00	-0.07ns	0.27ns	0.31*
DI		1.00	0.46***	0.73***
Alt			1.00	0.60***
PSI				1.00

Notes: Where DI= disease incidence, Alt= altitude, PSI= percent severity index, *, *** significant level at $p<0.05$ and $p<0.001$, respectively ns = non-significant

The information regarding the existence of the high CBD intensity in Gurage zone was as much important and gives clue for the CBD importance particularly in the study area. Though, Tesfaye and Sinedu, (2000) also reported that 75% disease incidence and 28 - 43% severity in study area, which indicating that the disease can lead to a complete yield loss whenever susceptible landraces are cultivated without adequate control measures (Kifle and Demelash, 2015). So, could be used as valuable input for designing short and/or long term management strategies such as selection of resistant lines that can adapt to various ecological conditions.

4.2 Cultural and Morphological Characterization of *C. kahawae* isolate

4.2.1 Cultural characterization of *C. kahawae* isolate

There was highly significant ($p< 0.001$) difference among isolates in their radial colony growth rate (Table 3 and Appendix Table 4). Mean radial colony (mycelial) growth rate was ranged from 2.67 ± 0.26 to 4.08 ± 0.26 mm/24 hrs in isolates of CA1 (Cheha districts) and EZA (Ezha

districts), respectively (Table 3). Over all mean of radial growth rate of 3.11±0.26 mm/24 hrs was recorded and this results indicated a faster mean growth rate as compared to Hindorf (1970; 1973), i.e. 1.9 ± 0.5 mm/24hrs for the average mycelia growth rate of CBD isolates at 22^{o}C incubation on 2% Oxoid MEA. Waller *et al*. (1993) described the colony growth rate of *C. kahawae* 2-4 mm/24hrs at 25^{o}C 2% MEA. And also Arega *et al*. (2009) reported the mean radial colony (mycelial) growth rate of *C. kahawae* isolates ranged from 0.6 to 5.5 mm/24 hrs in PDA medium. Kilambo *et al*. (2013) recorded growth rates ranging from 5.0 to 5.5 mm/24 hrs of 25 *C. kahawae* isolates. As *C. kahawae* species are slow growing nature in mycelial growth rate in culture medium, this may use as distinguishing criterion of CBD pathogen from other *Colletotrichum* species (like *C. gloesporioides, C. acutatum)* and could serves as indicator of variability within the species (Amsalu *et al*., 2016).

Based on visual observation of the upper side of culture plates of colony appearance (aerial mycelial growth), dense, irregular (scarce) and very scarce types of colony (texture) were identified. Half of the isolates showed dense types of aerial mycelia growth and the rest scare types on PDA media (Table 3). Seventeen *C. kahawae* isolates showed 47.1%, 11.8 and 5.8% dense, irregular and very scarce aerial mycelia growth on both PDA and MEA media, respectively, whereas the rest 35.3% isolates showed inconsistent aerial mycelia growth (Arega *et al*., 2009).

Different colony colors were observed on both sides of the culture plates. Five groups of mycelial color were observed in upper side of plate's *viz.*; Gray white (35.71%), Dark gray white (28.57%), Ghost white (14.28%), Cottony white(14.28%)and floral white (7.14%)(Table 3 and Figure 6). The reverse side of the culture plates also showed; light golden rod (28.57%), pale golden rod (21.42%), lemon chiffon (21.42%), Navajo white (7.14%), antique white (7.14%) and corn silk (7.14%) colony pigmentation (Table 3 and Figure 7). Diverse colony colors have been previously reported on both sides of a culture plates. Light gray, dark gray, gray and white mycelia types of colony color were also observed from Hararghe *C. kahawae* isolates (Birhanu, 2014). Abdi and Abu (2015) also indicated pale yellowish to pinkish with dense whitish-grey aerial mycelium and a few bright orange conidial masses on the tips of the active growing hyphae on MEA media. Diverse colony colors of the pathogens were observed due to by using different growth media and the characteristics' the pathogens. In general, the mycelial colony

color of the isolates are whitish at the 3-5 incubation days; light gray in 6-7 and then 8-10 incubation days changed to dark gray; a distinctive characteristic colony color of *C. kahawae* isolates.

The colony form of the Gurage *C. kahawae* isolates showed two types of colony morphology form, which was most of the isolates was irregular and some isolates showed curled colony shape. These colony forms were the characteristics the filamentous fungi that the *colletotrichum* spp. belongs.

Table 5 Cultural characteristics of *C. kahawae* isolates of Gurage districts.

			Color		**colony growth**
Isolate code	**Form**	**Texture**	**Upper**	**Reverse**	**(mm/day)**
EK1	Irregular	Dense	Gray white	Light gold rod	3.05 ±0.26^{c}
CW	Irregular	Dense	Ghost white	Light rod	3.04 ±0.26 ^{c}
EZS1	Curled	Dense	Cottony white	Lemon chiffon	2.99 ±0.26 ^{c}
EKO	Irregular	Scarce	Gray white	Lemon chiffon	3.06 ±0.26 ^{c}
EZS3	Irregular	Dense	Dark gray white	Light gold rod	3.12±0.26^{bc}
EZA	Irregular	Dense	Gray white	Pale gold rod	**2.67 ±0.26 ^{d}**
EZD	Curled	Scarce	Gray white	Pale gold rod	3.36 ±0.26 ^{b}
CA1	Irregular	Scarce	Dark gray white	Light gold rod	**4.08 ±0.26 ^{a}**
CS	Curled	Dense	Floral white	Light gold rod	3.01 ±0.26 ^{c}
CA2	Irregular	Scarce	Gray white	Pale gold rod	3.05 ±0.26 ^{c}
EK2	Irregular	Dense	Dark gray white	Antique white	2.88 ±0.26^{cd}
GC	Irregular	Scarce	Dark gray white	Navajo white	3.33 ±0.26 ^{b}
EB	Irregular	Scarce	Ghost white	Corn silk	2.91 ±0.26^{cd}
EZS2	Irregular	Scarce	Cottony white	Lemon chiffon	2.97 ±0.26 ^{c}
Mean					**3.11**
LSD					**0.26**
CV (%)					**4.99**

Means followed with the same letter are not significantly different LSD (0.05).

Notes: EK1; EK2; EKO and EB (Enemorina Ener district isolates), CW; CA1; CA2 and CS (Cheha district isolates), EZS1; EZS2; EZS3; EZA and EZD (Ezha District isolates) and GC (Gera isolate)

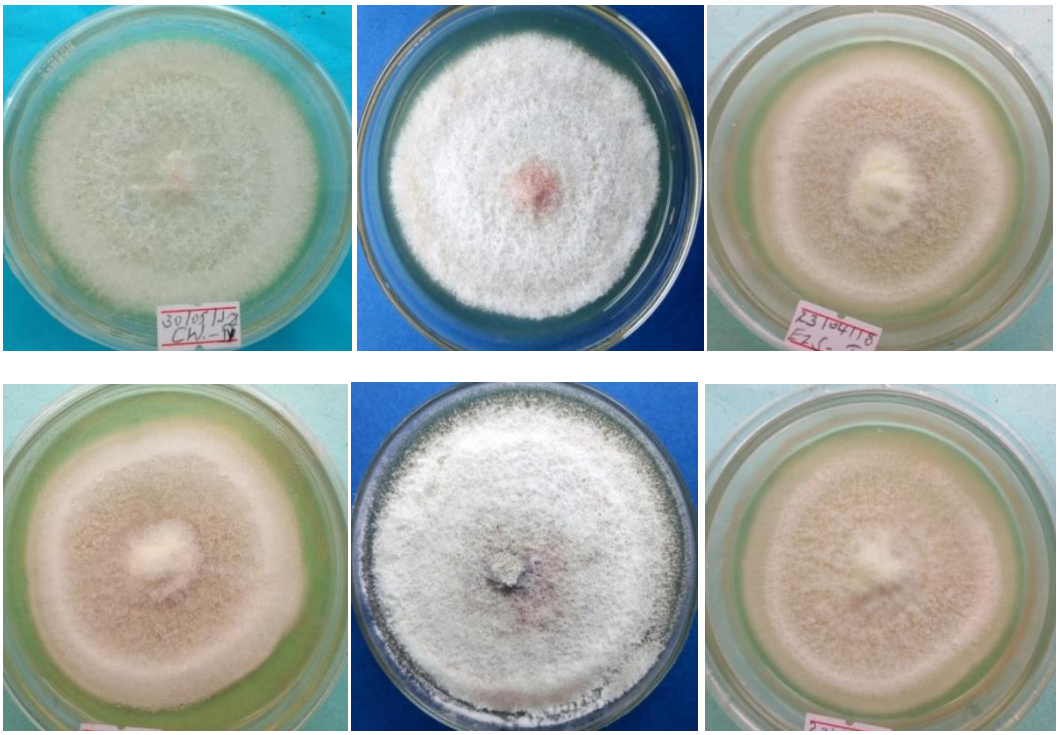

Figure 5 Cultural morphology of 10 days old culture of *C. kahawae* isolates in upper side of the plate.

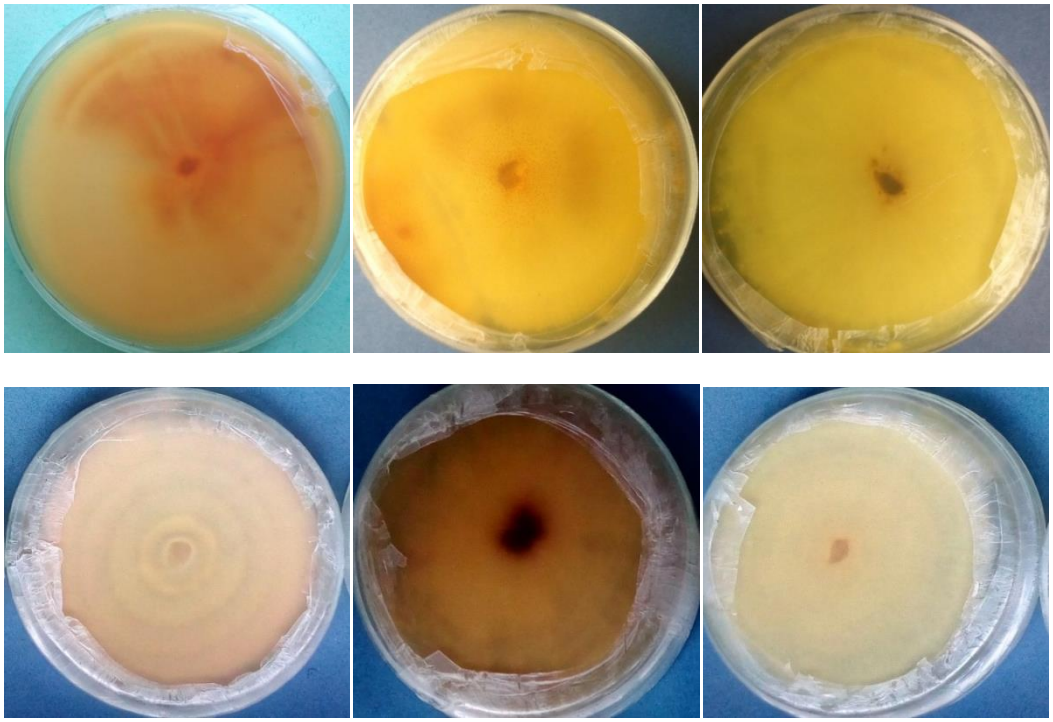

Figure 6 Cultural morphology of 10 days old culture of *C. kahawae* isolates in reverse side of the plate.

4.2.2 Morphological Characteristics *C. kahawae* isolate

There was highly significant ($p< 0.001$) difference among isolates in their conidia size (Table 4 and Appendix Table 5 and 6). All *C. kahawae* isolates had variable conidia length and width, which ranged between 9.03 to 10.49 ±0.54 µm and 5.0 to 6.04 ±0.22 µm, respectively. The average conidial length and width of isolates were 9.87 ±0.54 µm and 5.38 ±0.22 µm recorded, respectively (Table 4). Isolate EZA had the largest mean conidial length (10.49±0.54µm) and the smallest mean conidial length was recorded from isolate EZD (9.24±0.54).While the widest conidial width was recorded on isolate EZS2 (6.04±0.22), and the narrowest mean conidial width was from isolates EB (5.00±0.22) (Table 4).

Table 6 Mean conidia size and conidia production of *C. kahawae* isolate of Gurage zone

Isolate code	Conidia size (µm)		Conidia production
	Width	**Length**	**(x10,000/ml)**
EZS2	**6.04 ± 0.22^{a}**	9.77± 0.54^{bcd}	277.33 ± 27.04^{g}
CA2	5.64 ± 0.22^{b}	9.86 ± 0.54^{bc}	209.79 ± 27.04^{h}
GC	5.55 ± 0.22^{bc}	9.44 ± 0.54^{cde}	395.11 ± 27.04^{bc}
CA1	5.47 ± 0.22^{bcd}	10.22 ± 0.54^{ab}	380.45 ± 27.04^{cd}
EZS3	5.44 ± 0.22^{bcd}	**9.03 ± 0.54^{e}**	392.17 ± 27.04^{bc}
EZS1	5.36 ± 0.22^{cde}	10.14 ± 0.54^{ab}	256.00 ± 27.04^{g}
EK2	5.34 ± 0.22^{cde}	9.72 ± 0.54^{bcd}	355.75 ± 27.04^{de}
EK1	5.33 ± 0.22^{cde}	10.22 ± 0.54^{ab}	**182.25 ± 27.04^{i}**
EKO	5.31 ± 0.22^{de}	10.06 ± 0.54^{ab}	305.97 ± 27.04^{f}
EZD	5.29 ± 0.22^{de}	9.24 ± 0.54^{de}	**432.92 ± 27.04^{a}**
EZA	5.26 ± 0.22^{de}	**10.49 ± 0.54^{a}**	250.83 ± 27.04^{g}
CS	5.25 ± 0.22^{de}	10.08 ± 0.54^{ab}	344.06 ± 27.04^{e}
CW	5.14 ± 0.22^{ef}	9.83 ± 0.54^{bc}	416.33 ± 27.04^{ab}
EB	**5.00 ± 0.22 ^{f}**	10.06 ± 0.54^{ab}	340.25 ± 27.04^{e}
Mean	**5.38**	**9.87**	**324.23**
LSD	**0.22**	**0.54**	**27.04**
CV (%)	**2.49**	**3.25**	**5.84**

Notes: EK1; EK2; EKO and EB (Enemorina Ener district isolates), CW; CA1; CA2 and CS (Cheha district isolates), EZS1; EZS2; EZS3; EZA and EZD (Ezha District isolates) and GC (Gera isolate). Means followed with the same letter are not significantly different at LSD (0.05)

In this study, all isolates showed variable conidial length and width similar previous observations by different authors. Kilambo *et al*. (2013) recorded the conidia length ranged from 8 to 18 mm and width ranged from 2 to 6 mm and showed an overlap of conidia size between isolates thus, making it difficult to distinguish the strains of *C. kahawae* by conidia size. Talhinhas *et al.* (2005) indicated variability in conidia size within and between strains when studying the diversity of *Colletotrichum* species in olive anthracnose and concluded that it is difficult to distinguish fungal strains using spore size.

Sporulation capacity of Gurage *C. kahawae* isolates has been evaluated on 10 days old cultures that revealed highly significant ($P< 0.001$) differences among isolates (Table 4 and Appendix Table 7). Conidia production ranged between 182.25 to 432.92x 10^4 conidia/ml of isolate EK1 and EZD, respectively. Hence, isolate EZD produced highly significant amount of conidia (432.92 ± 27.04x 10^4) followed by isolates CW, GC and EZS3 but was not statistically different. While, isolate EK1 (182.25 ± 27.04x 10^4) was produced the smallest amount of conidia which was highly significant difference among all isolates (Table 4 and Appendix Table 7). The high conidia production was the characteristics of the virulent pathogen of the *C. kahawae* isolates that produce enough inoculum sources for disease development. Tefestewold (1995) observed (1.2-5.2) x 10^5 conidia /ml and (6.84- 17.20) x 10^6 conidia /ml production from six isolates of *C. kahawae* on PDA medium and GCA (green coffee seed extract agar).

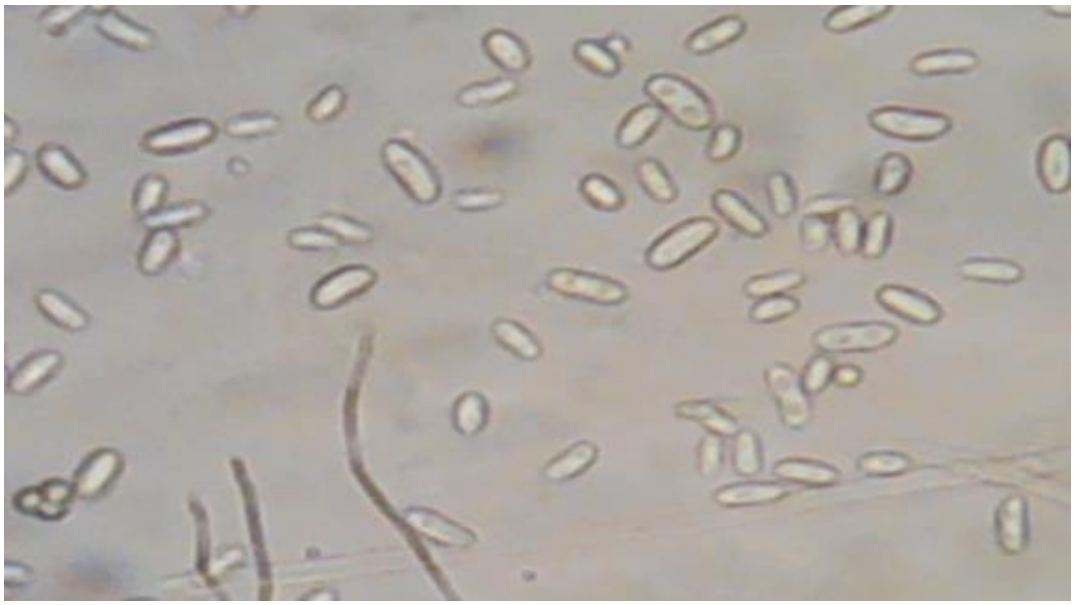

Figure 7 Conidial morphology of *C. kahawae* isolate of Gurage zone.

4.3 Virulence determination of *C. kahawae* isolate of Gurage zone

The result revealed that all isolates were pathogenic to variety 370 and showed distinct and highly significant (p<0.001) variations in the level of aggressiveness (Fig. 10 and Appendix Table 8).The highest level of berry infection was recorded in isolate EZD with 68.06% infection from Ezha districts but statistically not significantly different from GC, CW, EB and CS isolates. The lowest berry infection level were recorded on isolate EZS2 (45.83%) but statistically not significantly different from EK2, CA2, EKO, EK1, CA1, EZA and EZS1 isolate (Fig. 10). In general, the high virulence ability was attributed to high sporulation capacities which usually lead to a high percentage of germinated conidia and appresorial formation in the host tissues, resulting high infection levels. The isolate EZD produce highest amount conidia production and more virulent isolates as compare to the other isolates. In the present study, the symptoms produced by the pathogen on artificial inoculation on the detached berries showed similar symptoms of those observed under natural infection at field. It is true that susceptible genotypes allow the expression of clear differences in aggressiveness when exposed to different pathogen isolates (Castiblanco *et al.*, 2018).

C. kahawae strain can exhibited high virulence because of high sporulation capacity and germination of conidia in the host tissues (Varzea*et al*., 1999, 2002).Variation of *C. kahawae* population may occur due to both aggressiveness and some cultural characters, such as rates of sporulation and growth rate (Rodrigues *et al.*, 1991). Highly aggressive isolates were produced an intermediate amount of conidia. The level of inoculum concentration is also known lead to highest level of infection (Pinard *et al*., 2012). Kamau, (2015) found considerable pathogenic variability among C. *kahawae* isolates that are collected from Kenya on detached green berries. The aggressiveness of the pathogen can be considered as quantitative measure of the level of the disease reached over time. This indicates that the most aggressive pathogen reached at a specific disease level faster than the less aggressive one. The situation can be measured via latent period, spore production, infection, lesion size and disease severity (Pires *et al*., 2016).

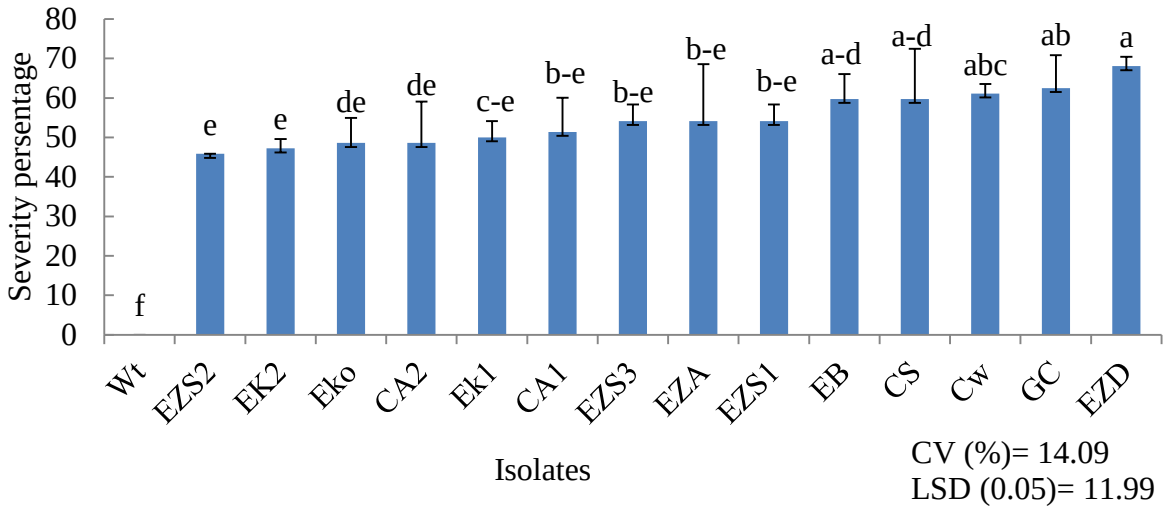

Figure 8 Virulence of *C. kahawae* isolates from Gurage zone on green expanding coffee berries. Means followed with the same letter are not significantly different at LSD (0.05). Distil sterilized water was applied us control (check)

4.3.1 Correlation between virulence and morpho-cultural characteristics

Pearson correlation analysis revealed that highly significant (P<0.001) and strong positive correlation of virulence with the conidia production of isolates (r=0.63) (Table 5). Isolates those produced high conidia productions, infect coffee berries severely than those isolates which produced lower amount of conidia. The isolate EZD produce high amount of conidia which infected coffee berry more severely than the remaining isolates. A related finding was also reported earlier by Varzea *et al.* (2002). Kilimbo *et al.* (2008) also indicated that the conidia productions were weak positively correlated to virulence of isolates (r=0.15).

Pearson correlation result revealed that virulence of the isolates was positive but non-significant correlation with conidia growth rate of the isolates (r=0.14) (Table 5). Positive correlation was found between enlargement of lesion size and sporulation capacity, lesion size and percent berry infection, as well as sporulation capacity and percent berry infection (kilimbo *et al.*, 2008). But this result was not similar to Kilimbo *et al.* (2008) results, in which the lesion size was positively correlated to percent berry infection.

Table 7 Pearson Correlation Coefficients of morpho-cultural characteristics and virulence of the 14 *C. kahawae* isolate of Gurage zone

	Growth rate	Conidia	Width	Length	Virulence
Growth rate	1.00	0.42ns	0.17ns	-0.15ns	0.14ns
Conidia		1.00	-0.24ns	-0.59*	0.63**
Width			1.00	-0.23ns	-0.49*
Length				1.00	-0.34ns
Virulence					1.00

*and **= Significant at 0.05 and highly significant at 0.01 level respectively, ns = non-significant

In this study the conidia size of the isolates were highly variable within and among the *C. kahawae* isolates. In fact, since there was high variation between the same isolate of the pathogen on its conidial size, there was insignificant relationship between pathogenicity (virulence) and conidial size (Arega 2006).The variability in fungal pathogenicity and the close relationship between sporulation and virulence could provide a useful information base for screening coffee germplasm collections for resistance to the pathogen and subsequent breeding programs for durable resistance through the selection of highly sporulated and virulent fungal isolates (Kilambo *et al*., 2008). Hence, the virulent isolate EZD found in this study should be used as screening isolate in coffee CBD resistance evaluation.

4.4 Reaction of Gurage coffee accessions for CBD resistance

4.4.1 Attach berry test

The analysis of variance (ANOVA) for attached berry test showed significant difference among the accessions at $p<0.005$ level (Fig. 11 and Appendix Table 9). The highest CBD severity infection was recorded on accessions Gu-15(30.07%) which showed no significant difference compare to Gu-19, Gu-10, Gu-6 and Gu-13 accessions, but, it has highly significant difference compare to the rest Gurage coffee accessions. The lowest CBD severity infection was recorded on accession Gu-18 (13.21%) which did not statistically different from Gu-7, Gu-12, Gu-1, Gu-8, Gu-20, Gu-9, Gu-4 and check (74110), but it has significant difference compare to the other accessions (Fig.11). The result of this study clearly indicates that certain Gurage coffee accessions that have been better or comparable CBD resistant to the reference

accessions/varieties. Similarly, Tefestewold (1995); Bayetta (2001) and Arega (2006) also reported significant differences in seedling percent infection in reaction to CBD for different coffee accessions.

The variation among coffee accessions for the pathogen response can be associated with the genetic makeup of the accessions. The resistance nature of CBD in coffee has horizontal nature (Van Graff 1981; Bayetta, 2001). The host resistance appears to be largely based on the rapid formation of a cork barrier in the pericarp of the developing fruit distal (point of attachment) from the initial infection site that effectively prevents the pathogen from further invading of healthy tissue which totally absent or incomplete in susceptible host plants (Silva *et al*., 2006; Bayetta, 2001). CBD resistance of Lyamungu coffee hybrids is partly being contributed by wax surface on green coffee berries (Kilimbo *et al*., 2013). Chen *et al*. (2004) showed the green coffee berries possess inherent antifungal compounds that counteract the infection of coffee by *C. kahawae* strains. In the resistant coffee genotypes, restrict the fungal growth associated with a series of hypersensitive reaction (HR) responses. HR indicates the rapid and efficient plant resistance mechanism that leads to a localized plant cell death in response to invasion by a pathogen and is characterized by a rapid loss of membrane integrity in the infected host cells (Hoglund *et al*., 2005; Singh and Upadhyay, 2013).

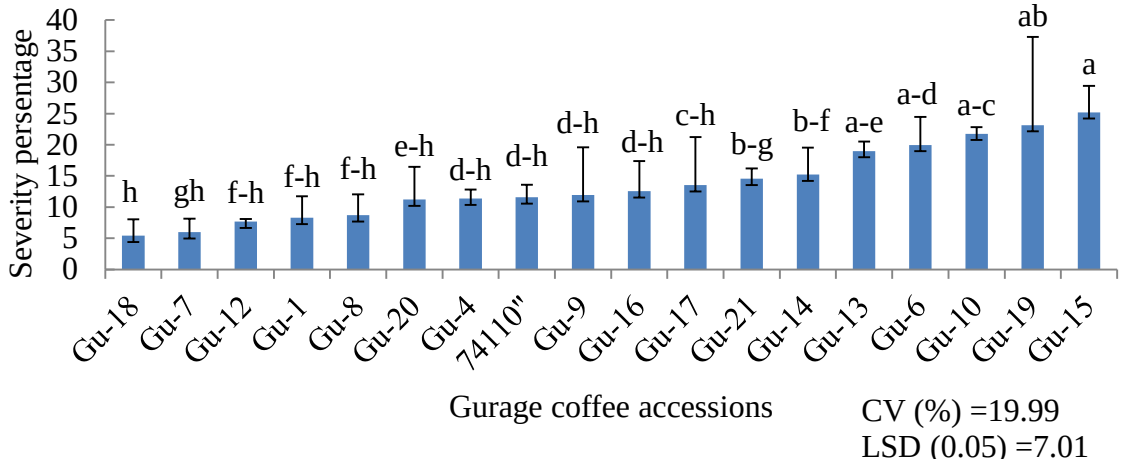

Figure 9 percent CBD infection of Gurage coffee accessions in attach berry test.

Means followed with the same letters are not significantly different at LSD (0.05)

4.4.2 Detach berry test

The study revealed that there was highly significant difference (p<0.001) among Gurage coffee accessions for reaction of CBD resistant (Fig.12 and Appendix Table 10). Coffee accessions Gu-4 and Gu-18 showed lower CBD severity infection (25.83 and 32.50%) and statistically highly significant difference as compare to resistant check (741) and other Gurage coffee accessions. Gu-1, Gu-8 and Gu-12 coffee accessions showed statistically significant difference compare to the two resistant Gurage coffee accessions and not significant difference to the resistant check (741), which showed moderately resistant reaction. The highest CBD severity infections was recorded on coffee accession Gu-7 (88.33%), which was moderately resistant in the field experiment (ABT) and now susceptible in this experiment. The coffee cultivar 74110 was one of the released field resistant cultivars and had moderately susceptible reaction in laboratory (Tefestewold, 1995; Bayetta *et al*., 2000; Kumlachew *et al.*, 2018). Those four Gurage coffee accessions (Gu-7, Gu-19, Gu-16 and Gu-13) showed higher infection percentage more than the well-known susceptible variety (370). The susceptible coffee accessions had their berry surfaces entirely covered by black sunken lesions. On the other hand, those resistance Gurage coffee accessions formed restricted black scab lesions that hindered further penetration of the pathogen into the intercellular parts of the berry (Kumau *et al.*, 2015) (Fig. 13).

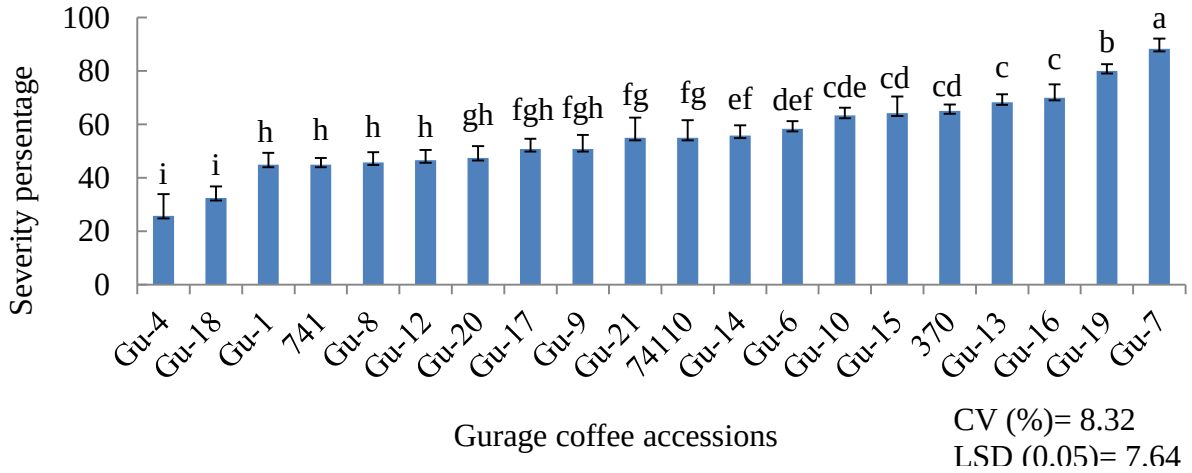

Figure 10 Response of Gurage coffee accessions to *C. kahawae* infection in detached berry test. Means followed with the same letters are not significantly different at LSD (0.05).

Gichuru (1997) findings indicated that scab lesions were the common macroscopic expression of resistance of coffee to CBD. This resistance to CBD is preformed and induced, and it operates at

distinct stages of pathogenesis. The berry resistance could be separated into two types; one against the pathogen penetration and the other against its growth in berries through scab lesion formation (Pinard *et al*., 2012). In Gurage coffee accessions, the resistant accessions should restricted growth of the pathogen (scab formation). Presence of scab CBD lesions formed in resistant or moderately resistant coffee genotypes suggests the mechanism by which further invasion of the CBD pathogen is blocked (Masaba and van der Vossen, 1982; Chen *et al*., 2004). On the other hand, scab formation on the green coffee berry surface is due to cork barrier formation and limits fungal hyphal growth inside the plant tissue (Masaba and van der Vossen 1982). Chen *et al.* (2006) study revealed that epicatechin and catechin present in green coffee berry pericarp of CBD resistant genotypes may prevent CBD by inhibiting appressorial penetration. In the resistant variety the restricted hyphal growth was associated with the hypersensitive-like host cell death (HR), and early accumulation of phenolic compounds both in cell walls and in the cytoplasmic contents. These responses were also observed in the susceptible variety, but in a significantly lower percentage of infection sites and did not prevent the fungal growth, as indicated by the appearance of typical anthracnose symptoms and the presence of acervuli (Silva *et al*., 2006; Loureiro *et al*., 2012). Variations in Gurage coffee accessions could be an opportunity for the next better resistant varietal development through breeding. As resistance in perennial crops like coffee is observed and screened during the late stage of development (Van der Vossen *etal.,* 2015), multi-location repeated evaluation of these accessions over time could be the future line of work for pathologists and breeders.

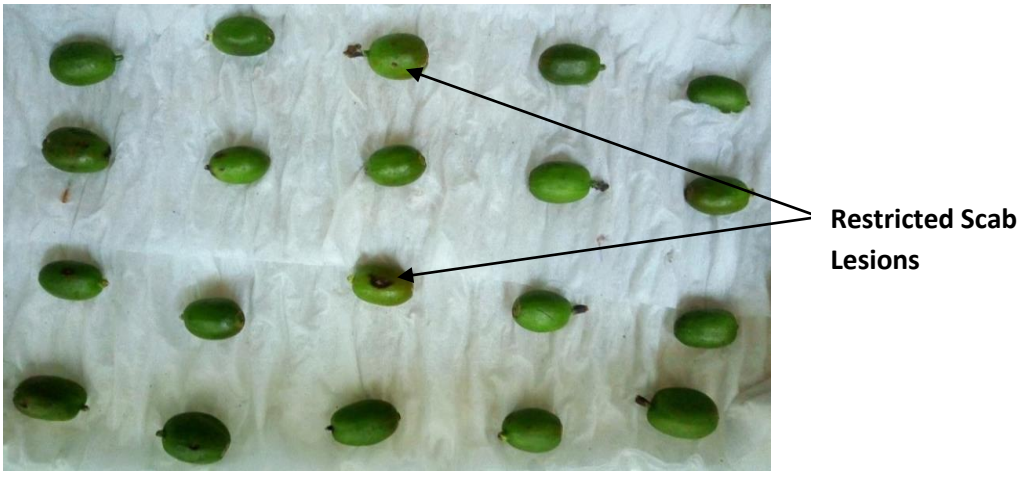

Figure 11 Presence of restricted scab lesions on resistant coffee accessions inoculated with conidia of *C. kahawae* after 21 days.

5. SUMMARY AND CONCLUSION

Coffea arabica L. was grown more than 80 countries in the world and Ethiopia is center of origin and diversity for Arabica coffee. Coffee is an important crop in the national economy of Ethiopia. Coffee production and productivity in Ethiopia was low due to various challenges, among these challenges fungal disease which are coffee berry disease, coffee wilt disease and coffee leaf rust. Coffee berry disease was most important and prevalent in all mid and high altitude areas of the country.

The present study was conducted on assessment of coffee berry disease, characterization of *C. kahawae* isolates in selected coffee growing districts of Cheha, Ezha and Enemorina Ener of Gurage zone and evaluation of Gurage coffee accessions against CBD. The study result showed that the intensity of CBD was prevalent in all assessed areas. However, DI and PSI were varied from districts to districts and from peasant associations to peasant associations in the same districts. This was due the variation in genetic makeup of locale Gurage coffee accessions, the pathogen virulence difference and the environmental condition of the area. The overall mean of DI and PSI ranges from 86.66 to 100% and 38.89 to 59.44%, respectively. Furthermore, the extent of CBD intensity was increased with altitude that revealed positive and strong relationship with DI (r=0.46) and PSI (r=0.73).

Thirteen representatives' *C. kahawae* isolates from three districts of Gurage zone and one isolates from Gera were studied based on their cultural, morphological and pathogenic characters. All the isolates studied for aerial mycelial growth on PDA medium and showed 50% dense and 50% scarce types of mycelia growth. The isolates were grouped into 5 groups based on colony (mycelia) color on the upper side of culture plates viz., Gray white, Dark gray white, Ghost white, Cotton white and floral white. Mean radial colony (mycelial) growth rate of *C. kahawae* isolates were significantly different among them and its growth ranged between 2.67 to 4.08 ±0.26 mm/24hrs on PDA.

The conidial size of the isolates have highly significant difference ($p<0.001$) among the isolates, the mean conidial width and length ranges between 5.00 to 6.04±0.22 µm and 9.24 to 10.48 ±0.54 µm respectively. Similarly isolates were showed highly significant ($p<0.001$) differences in their conidia production capacity. The highest conidia number was produced by isolate EZD

(432.92×10^4ml) isolates from Darech peasant associations and the lowest conidia production was produced by isolate EK1 (182.25×10^4ml) from kerebid peasant associations.

All *C. kahawae* isolates collected from Gurage zone were pathogenic to Arabica coffee variety 370. However, isolates showed highly significant variation (P<0.001) among them on their level of aggressiveness. Among these isolate EDZ was highly aggressive and EZS2 isolates was showed less aggressiveness in detach berry test. Conidia production were positive and significantly correlated to the aggressiveness of the isolates (r= 0.63) and also conidia growth rate was positive and but non-significant correlation to the virulence of the isolates (r=0.14). The conidial size was negatively correlated to the virulence of the isolates.

The resistance evaluation test under field (ABT) and laboratory (DBT) showed significant variations among of Gurage coffee accessions. The mean percent of berry infection ranged from 13.21% (Gu-18) to 30.07% (Gu-15) in field ABT and 25.83% (Gu-4) to 88.33% (Gu-7) in the laboratory DBT. Gurage coffee Gu-18, Gu-1 and Gu-4 were showed low CBD infection percentage under both ABT and DBT experiments. Results of these experiments confirmed that the variations were mainly due to the existence of difference in genetic makeup of the selected lines in reaction to CBD.

Generally, the present study was done in one production season and three districts of Gurage zone, but it will be good when the research was repeated in one more production season and include some other coffee producing districts of Gurage. More virulent *C. kahawae* isolate of EZD were used for future artificial inoculation pathogen for evaluation of coffee accessions. Those coffee accessions that showed low level of infection in CBD resistance evaluation test under field and laboratory conditions are an opportunity for further breading research work and could be the best alternatives to CBD management particularly for the study area. However, these accessions should be evaluated in seedling hypocotyl test and also evaluated under field in multi location and over years

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7. APPENDIXES

Appendix Table 1 List of Gurage coffee accessions with mean CBD score under visual assessment at field conditions of three year data

Sr No.	Gurage coffee accession	Mean CBD%
1	74110	0.24
2	Gu-1	0.74
3	Gu-4	1.43
4	Gu-21	2.01
5	Gu-13	2.59
6	Gu-12	2.93
7	Gu-17	4.74
8	Gu-18	6.49
9	Gu-7	8.37
10	Gu-10	9.11
11	Gu-8	10.85
12	Gu-15	12.02
13	Gu-19	13.00
14	Gu-9	13.91
15	Gu-14	14.13
16	Gu-20	16.05
17	Gu-6	17.33
18	Gu-16	21.17
19	Gu-2	27.78
20	Gu-11	30.38
21	Gu-5	38.38
22	Gu-3	57.33

Appendix Table 2 ANOVA table of coffee berry incidence in nine peasant associations of three Gurage district

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	.072	4	.018	3.199	.028[b]
	Residual	.158	28	.006		
	Total	.230	32			

a. Predictors: (Constant), Age, District, Altitude, Peasant assotion
b. Dependent Variable: PSI

Appendix Table 3 ANOVA table of coffee berry incidence in nine peasant associations of three Gurage district

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	.122	4	.031	4.846	.004[b]
	Residual	.177	28	.006		
	Total	.299	32			

a. Dependent Variable: PSI
b. Predictors: (Constant), Age, District, Altitude, Peasant assotion

Appendix Table 4 ANOVA table of mean radial colony (mycelial) growth rate of *C. kahawae* isolates of Gurage zone

Source	DF	SS	MS	F value	P value
Isolate	13	4.191	0.322	13.35	<0.0001
Error	28	0.676	0.024		
Total	41	4.867			

Appendix Table 5 ANOVA table of mean conidia length of *C. kahawae* isolates of Gurage zone

Source	DF	SS	MS	F value	P value
Isolate	13	6.42	0.48	4.8	<0.001
Error	28	2.88	0.103		
Total	41	9.29			

Appendix Table 6 ANOVA table of mean conidia width of *C. kahawae* isolates of Gurage zone

Source	DF	SS	MS	F value	P value
Isolate	13	2.39	0.18	10.18	<0.001
Error	28	0.51	0.018		
Total	41	2.89			

Appendix Table 7 ANOVA table of mean conidia production of *C. kahawae* isolates of Gurage zone

Source	DF	SS	MS	F value	P value
Isolate	13	322275.77	24790.44	69.03	<0.0001
Error	42	15083.33	359.13		
Total	55	337359.09			

Appendix Table 8 ANOVA table of mean virulence of *C. kahawae* isolates of Gurage zone

Source	DF	SS	MS	F value	P value
Isolate	14	10086.54	720.46	13.94	<0.0001
Error	30	1550.68	51.68		
Total	44	11637.23			

Appendix Table 9 ANOVA table of Evaluating Gurage coffee accessions by attach berry test in the field

Source	DF	SS	MS	F value	P value
Rep	2	6.82	3.41	0.19	0.826
Accession	17	1241.08	73.00	4.09	0.0002
Error	34	606.93	17.85		
Total	53	1854.84			

Appendix Table 10ANOVA table of Evaluating Gurage coffee accessions by detach berry test in the laboratory

Source	DF	SS	MS	F value	P value
Accession	19	12590	662.63	30.88	<0.0001
Error	40	858.33	21.46		
Total	59	13448.33			

Appendix Figure1 Photo of farmer with his coffee farm and survey data taking.


Appendix Figure 2 Brach tagging and inoculation of *C. kahawae* isolate in ABT experiment at Gera.


Appendix Figure 3 Cultural characterization of *C. kahawae* in JARC laboratory.


Appendix Figure 4 Data taking in JARC growth room.

Assessment of CBD Berry Count and Visual Assessment

Districts _____

Peasant association _____ Farmers name _____

Altitude _____ Cultivar used _____

Latitude _____ Type of Shade _____

Age of plant _____

Tree No.	Tree Branch			Total	Mean	CBD		Remark
	Top	Middle	Bottom			Present	Absent	