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Isolation, Identification and Characterization of Colletotrichum kahawae from Infected Green Coffee Berry in Arsi, Southeastern Ethiopia

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Abstract
Colletotrichum kahawae is a causal pathogen of coffee berry disease (CBD). It was reported in Ethiopia for the first time in 1971. Then spread to all major coffee-producing regions within very short period. It was prevalent in most coffee growing areas of Ethiopia and has been characterized in Morpho-cultural attributes. However, characterization of *C. kahawae* in Arsi has been lacked. Therefore, present study was conducted to characterize *C. kahawae* isolates for their Morpho-cultural attributes. Those attributes selected for isolates characterization were conducted following procedures and methods collected from recently done researches with few modification. Accordingly, five representative *C. kahawae* isolates and one *C. gloeosprioides* were isolated and identified from infected green coffee berry sampled from study areas. *Colletotrichum kahawae* isolates were characterized for colony color, radial growth rate and texture in case of morphological character, while conidial shape, size and sporulation capacity in case of cultural characteristic. There were significant variation among isolates related their Morpho-cultural features. Four types of colony color were identified as light gray, dark gray, gray and dim gray. Conidia production capacity varied from 7.5×10^5 – 1.44×10^6, while conidial size varied among and within isolates ranging from 10.5 to 15.5 µm and 2.78 to 3.83 µm for length and width, respectively. More than 50% of conidial shape frequency of each isolate was under conidial shape of type 1 except isolate Shk9. Except conidial size, other Morpho-cultural attributes has been used for identification of *C. kahawae*. Therefore, except conidial size one can be use remaining Morpho-cultural attributes of *C. kahawae* as diagnosis or identification tools. It means not saying traditional characterization is enough for diagnosis. Since it has limit diagnosis and identification among and within pathogen species, further study should be undertaken via molecular tools.
Keywords: *C. kahawae*, conidial, colony, Morpho-cultural features.

1. Introduction

Colletotrichum kahawae is a causal pathogen of coffee berry disease. It was reported in Ethiopia for the first time in 1971 by Mulinge (1972). Then the disease spread to all major coffee-producing regions within very short period except to the lower altitudes, i.e. it has spread and found in all coffee-producing areas in which it has been favored by environmental conditions.

From the range of *Colletotrichum* spp. that are isolated from coffee plants, four groups were initially described based on their morphological traits, namely *C. coffeanum* mycelial, *C. coffeanum acervuli*, *C. coffeanum* Pink and the CBD strain. The three former groups were latter recognized as *C. gloeosporioides* Penz and *C. acutatum* Simmonds, and proved to be non-pathogenic in green coffee berries (Gibbs, 1969; Hindorf, 1970). Only the fourth group was able to infect both wounded and unwounded green berries and was formerly referred to as *C. coffeanum* (Hindorf, 1970).

Colletotrichum coffeanum was described in 1901 based on *Colletotrichum* isolated from coffee in Brazil (Freeman, 1998) where CBD does not exist, and was probably synonymous with *C. gloeosporioides*, which occurs as a saprophyte or weak pathogen of ripe berries and damaged coffee tissue worldwide (Freeman, 1998). Several authors attempted to emend this anomaly but it was not until 1993 that Waller and Bridge described *C. kahawae* as the causal agent of CBD and as a distinct species based on morphological, cultural and biochemical characters (Waller, 1993) and more recently on multi-locus datasets (Prihastuti *et al*., 2009).

Traditional approaches to identification of species belonging to the genus *Colletotrichum* as well as other filamentous fungi have always relied on morphological characteristics (colony color, size and shape of conidia, presence or absence of setae and teleomorph, pathogenicity and cultural criteria (Sutton, 1992; Agrios, 2005). According to Kilambo *et al*. (2013), identification of *C. kahawae* has been based on morphological and cultural characteristics, such as conidial morphology and pigmentation.

It was prevalent in most coffee growing areas of Ethiopia and also characterized based on Morpho-cultural features. However, characterization of *C. kahawae* in Arsi has been lacked. Therefore, present study was conducted to characterize *C. kahawae* isolates for their Morpho-cultural attributes.

2. Materials and Methods

2.1. Description of the study area

Study was conducted under laboratory condition in 2017. It was done in Plant Pathology Laboratory of School of Plant Sciences , Haramaya University. Haramaya University was established in 1954 at Haramaya and to be found in Oromia Regional State, Eastern Ethiopia.

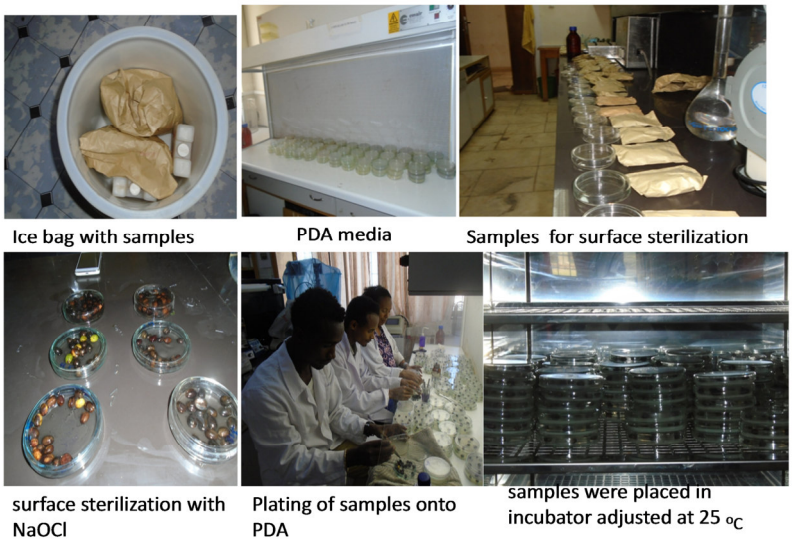
2.2. Sample collection and techniques used

Forty-one samples were collected from 41 plots (farms) overall assessed farms. From each plot (farm), 40 diseased coffee berries totally, 1640 infected green coffee berries with CBD active lesions were collected from overall assessed sites. These sampled berries were collected and placed into sterilized paper bags and sandwiched between newspapers and kept in a cool box for the pathogen to be viable for successful subsequent isolation. Samples were transported to Plant Pathology Laboratory of School of Plant Sciences, Haramaya University. Samples were maintained at 4^{o}C for further analysis.

2.3. Isolation and identification of *C. kahawae*

Colletotrichum kahawae was isolated from the diseased coffee berries with active CBD lesions using the method described by Kilimbo *et al*. (2013), Emana (2014), Abdi and Abu (2015) and Fredrick *et al.* (2017). The infected coffee berries with active lesions (sunken and dark lesions) were selected for fungal isolation. The diseased berries were surface-sterilized with 5% sodium hypochlorite (NaOCl) solution for 3 minutes and then rinsed with sterilized distilled water twice for one minute. Sterilized berries were placed on sterilized tissue paper for drying. Totally 30 coffee berries were used and arranged in three replications, i.e. 10 coffee berries per sample were plated on PDA incubated at 25°C for 5 - 7 days.

For the purpose of fungal identification, advanced mycelia was transferred aseptically to freshly prepared potato dextrose agar PDA. The advanced mycelium was taken from the margin of ten-day-old culture by using sterile scalpel. These all activities were done under air-flow laminar hood to reduce contamination arising from airborne micro-organisms (laboratory weeds). Preliminary confirmatory tests of colony texture of *C. kahawae* isolates on PDA was made based on mycological color chart developed by Rayner (1970). Eventually identification of the pathogen was done under compound microscope.



Identification

Figure 1. Isolation and identification of *C. kahawae* from infected coffee berry.

2.4. Morphological characterization of *C. kahawae*

2.4.1. Colony (mycelial) radial growth

Cultures of 5 *C. kahawae* isolates were inoculated on PDA. Hyphal tip of each isolate was placed at the center of 15 ml PDA dispensed in a 90 mm diameter sterilized Petri dish with three replications (Emana, 2014). Mycelial (colony) radial growth (mm per 24hr) of each isolate was measured manually with ruler. Colony diameter was measured from two perpendicular planes on the reverse side of the Petri-dishes.

2.4.2. Colony color of *C. kahawae* isolates

Colony (mycelia) color on front side and types of pigments from the reverse side of each *C. kahawae* isolate were determined using PDA and MEA using RGB color chart (Rayner, 1970; Anonymous, 2005; Kilimbo *et al.*, 2013; Emana, 2014).

2.4.3. Colony texture (aerial mycelial growth) of C. kahawae isolates

Following procedure employed by Arega (2006) and Emana (2014), vigor of aerial mycelial growth was determined as dense, irregular (scarce) or very scarce type by observing on top side of colony on 10-day-old culture grown on potato dextrose agar (PDA) and malt extract agar (MEA).

2.5. Cultural characterization of *C. kahawae*

2.5.1. Conidial size of *C. kahawae* isolates

Colletotrichum kahawae isolates were incubated on PDA medium at 25^{o}C for 7 days, replicated three times per isolate (Arega, 2006). All types of shapes and most frequent sizes were included at random to minimize further measurement biasedness. Conidial size (length and width) was calculated from 100 conidia per isolate. More conidia were measured for those isolates which had more variable shapes of conidia. Length and width of conidia were measured with ocular micrometer (µm), which was fitted into 10x eyepiece and adjusted at 40x objective of the compound microscope.

2.5.2. Sporulation capacity of *C. kahawae* isolates

Ten-day-old cultures of each *C. kahawae* isolate incubated on PDA was washed by flooding with 10 ml sterile distilled water, rubbed with sterile scalpel and transferred to 50 mL sterile beaker and thoroughly stirred for 10-15 minutes with magnetic stirrer to extract the spores from the interwoven mycelia and then filtered into another sterile beaker through double layer cheese cloth. The number of conidia per milliliter was counted using haemocytometer under compound microscope. The results were determined for each isolate as the average number of conidia per milliliter after taking nine haemocytometer counts (Arega, 2006).

2.5.3. Conidial shape of *C. kahawae* isolates

Frequency of conidial shapes was computed from 14-day-old cultures of *C. kahawae* isolates incubated on PDA (Arega, 2006). The most frequent conidia shapes, i.e. five types of conidia shapes that were described by Hindorf (1973) and Tefestewold (1995), were used. Conidial shape of representative *C. kahawae* isolates were described using ocular compound microscope and the most frequent five conidial shapes which were standardized and used by Hindorf (1973) and Tefestewold (1995) for *Colletotrichum* spp. characterization. The frequency of each shape was computed from 100 conidia per isolate.

2.6. Experimental design, treatments and data analysis:

Completely randomized design (CRD) with three replications was used. Fungal growth medium (PDA and MEA) as constant variable and isolates as treatment were used. Data was analyzed using GenStat software version 16 (Duncan's Multiple Range Test).

3. Results and Discussion

3.1. Isolation and identification of *C. kahawae* and related species

Five representative isolates of *C. kahawae* and one *C. gloeosporioides* were obtained (Table 1). Those isolates identified as *C. kahawae* were recognized by producing dark grey cottony colony, oval conidial morphology and slow growth rate. Five representative *C. kahawae* isolates were isolated from infected green coffee berries sampled from Chole (one isolate), Gololcha (two isolates) and Shanan Kolu (two isolates), while one *C. gloeosporioides* isolate was obtained from infected green berries samples from Gololcha.

Table 1. *Colletotrichum* species detected from infected coffee berries

Isolates code	Garden coffee locality	Pathogenicity	Species
Cho41	Chole	+	*C. Kahawae*
Go33	Gololcha	+	*C. Kahawae*
Go34	Gololcha	+	*C. Kahawae*
Go38	Gololcha	−	*C. gloeosporioides*
Shk9	Shanan Kolu	+	*C. Kahawae*
Shk10	Shanan Kolu	+	*C. Kahawae*

Note : *+ and − stand for pathogenic and non pathogenic of Colletotrichum spp. isolates to coffee berry*

3.2. Frequency of coffee berry contaminated and fungal occurrence

A total of 41 coffee samples were collected from 41 plots (farms) of Arsi garden coffee. From the collected samples, four types of fungal species (*C. Kahawae, F. lateritium*, *Aternaria* spp. and *Phoma* spp.) were isolated and identified (Table 2). Coffee berries were invaded more by *C. kahawae* (87.23%), followed by *F. lateritium* (9.13%), *Phoma* spp. (2.13%) and *Aternaria* spp. (1.5%). The coffee berry samples collected from Shanan Kolu was highly contaminated as compared to Chole and Gololcha districts.

Abdi and Abu (2015) have isolated three kinds of fungal species namely *C. kahawae*, *F. lateritium* and *Phoma* spp. However, present study was demonstrate *Aternaria* spp. as an additional fugal species. The coffee berry samples collected from Abaya was highly contaminated as compared to Bule Hora and Kercha districts (Abdi and Abu, 2015). Obviously secondary micro-organisms or saprophytic microbes do develop on the infected plant tissues to accomplish and extend their life span.

Table 2. Frequency of fungal species from infected coffee berries

Districts	Fungal species	% of berry contaminated by CBD and other fungal spp.
Chole	*C. kahawae*	87.2
	F. lateritium	7.4
	Aternaria spp.	1.9
	Phoma spp.	3.5
Gololcha	*C. kahawae*	91.2
	F. lateritium	6.3
	Aternaria spp.	1.3
	Phoma spp.	1.2
Shanan Kolu	*C. kahawae*	83.3
	F. lateritium	13.7
	Aternaria spp.	1.3
	Phoma spp.	1.7

3.3. Morphological characterization of *C. kahawae*

3.3.1. Mycelial radial growth rate

There was significant ($p \leq 0.05$) difference among isolates in their radial colony growth rate on PDA medium (Table 3). Mean radial colony growth rate of *C. kahawae* isolates ranged between 3.721 and 7.751 mm in 24hrs on PDA medium. High (7.751 mm in 24hrs) and low (3.72 mm in 24hrs) radial growth rate of mycelium was induced by isolates sampled from Gololcha (Go33) and Shanan Kolu (Shk9), respectively.

The result of the present study is agreement with the previous works related with cultural features of the pathogen, *C. kahawae* isolates. Hararghe *C. kahawae* isolates showed different mycelial radial growth rate among themselves that ranged between 17.35 to 59.59 mm in 24hrs (Emana, 2014). Abdi and Abu (2015) presented a similar report on *C. kahawae* isolates from Borena and Guji Zones. Nguyen *et al*. (2010) and Kilimbo *et al*. (2013) also conducted a similar study in Vietnam and Tanzania, respectively. Arga (2006) also characterized *C. kahawae* isolates from the Ethiopian forest coffee for their mycelial radial growth. Arega (2006) also reported on substrate growth preference habit of *C. kahawae* isolates. Accordingly, the researcher demonstrated that *C. kahawae* isolates showed higher mycelia radial growth rate on MEA than on PDA medium due to presence of peptone in MEA. According to him, this indicated the ability of *C. kahawae* isolate to colonize coffee berry easily by decomposing peptone found in the cell wall of the coffee berry by releasing peptidase enzyme.

On the contrary, Tefestewold and Mengistu (1989) earlier reported 6.75 and 6.5 mm in 24hrs growth rates on PDA and MEA media, respectively. Similarly, the recent report showed that 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 (Emana, 2014). The variation among radial growth rates of mycelia of *C. kahawae* isolates indicated their preference for substrate utilization and temperature under which they were cultivated. This may be due to existence of essential growth media, optimum temperature and relative humidity are required for pathogen growth and development.

Table 3. Mean radial growth rate of *C. kahawae* isolates

Isolates	Means	f	P
Go33	7.751a	39.93	0.001
Go34	6.229b		
Cho41	6.062bc		
Shk10	5.476c		
Shk9	3.721d		
LSD (0.05)	0.3199		
CV (%)	7		

Note: *the same letters indicates none significance among isolates.*

3.3.2. Colony texture (mycelia aerial growth)

Colony textures of *C. kahawae* isolates were classified into dense (regular) and scarce (irregular) colony types. About 80 and 20% of the isolates continually indicated dense and irregular (scarce) types of aerial mycelia growth on PDA medium, respectively, while 60 and 40% of the isolates constantly indicated dense and irregular (scarce) types of aerial mycelial growth on MEA medium, respectively (Table 4). More percentage of these *C. kahawae* isolates indicated good (regular) aerial mycelial growth on PDA medium than on MEA medium. Based on this result it can be concluded that *C. kahawae* isolates indicated consistent aerial mycelial growth on PDA medium than on MEA medium in dense or irregular (scarce) types. Similar results were obtained by Arega (2006) for *C. kahawae* isolates collected from Ethiopian forest coffee and by Berhanu (2014) for *C. kahawae* isolate sampled from Hararghe.

According to Berhanu (2014), among Hararghe *C. kahawae* isolates tested for their aerial mycelia growth (vigor), 65 and 90% showed consistently dense aerial mycelia growth on both potato dextrose agar (PDA) and malt extract (MEA) media, respectively; whereas 30 and 5% isolates revealed irregular (scarce) and 5% revealed very scarce aerial mycelia growth on both potato dextrose agar and malt extract agar media, respectively. Similarly, Tefestewold (1995) and Zeru *et al*. (2009) also reported differences in aerial mycelial growth among *C. kahawae* isolates from Kaffa and Illubabor on PDA medium. This variation scientifically showed the existence of genetic variability within the same fungal species and existence of variability on the utilization of different substrates.

Table 4. Colony texture of *C. kahawae* isolates on PDA and MEA

Isolates code	Aerial mycelial growth (vigor)	
	PDA	MEA
Cho41	Dense	Dense
Go33	Dense	Dense
Go34	Dense	Scarce
Shk9	Scarce	Dense
Shk10	Dense	Scarce

3.3.3. Colony color of C. kahawae isolates

There was a variation among *C. kahawae* isolates collected on their colony color. Up on the result of this study, colony color of *C. kahawae* isolates was grouped into 4 classes (light gray, dark gray, gray and dim gray) mycelium (Table 5; figure 2). These groups were made based on the observation from the front side of the culture plate on both PDA and MEA media, while in previous study Arega (2006) was made three groups of colony color (light-gray, dark-gray and gray). Emana (2014) reported that *C. kahawae* isolates from Hararghe were categorized into four types of colony color kindly light gray, dark gray, gray and white mycelia. Previously, Abdi and Abu (2015) reported on mycelia color of *C. kahawae* from Borena and Guji Zones and the report revealed that the young colony produced grey, becoming grey to dark, olivaceous grey, and dark greenish in reverse side of plates.

These all mycelia of *C. kahawae* had cottony appearance, including the present result. Sixty percent (three isolates), 20% (one isolate) and 20% (one isolate) of isolates had dark gray, gray and light gray cottony mycelia on PDA medium, respectively, while on MEA medium they produced 60% (three isolates), 20% (one isolate) and 20% (one isolate) of dark gray cottony, dark cottony and dim gray cottony colonies, respectively. However, the non-pathogenic isolate, *C. gloeosporioides* produced whitish cottony and pale whitish to pinkish cottony mycelium on both PDA and MEA, respectively.

Also, the reverse side of culture plate of these *C. kahawae* isolates showed different pigments. Several researchers reported diverse colony color in both obverse and reverse side of mycelia of *C. kahawae* isolates obtained from different ecologies (Arega, 2006; Emana, 2014; Abdi and Abu, 2015). Likewise the obverse side, the reverse side of the culture plate produced three types of pigments, viz. dark olive green, dark brown and dim

gray. On the PDA medium, 40, 40 and 20% of reverse side of the mycelium of the isolates were dark olive green, dark brown and dim gray, respectively, while on MEA medium, 80 and 20% of reverse side of the mycelium of the isolates were dark olive green and dim gray, respectively. The observed differences among isolates may be related to genetic variability, ecology and utilization of different substrates.

Table 5. Colony colors of *C. kahawae* isolates and *C. gloeosporioides*

Isolates	Colony color on Media			
	PDA		MEA	
	Observed side	Reverse side	Observed side	Reverse side
Cho41	Dark gray cottony	Dark olive green	Dark gray cottony	Dark olive green
Go33	Dark gray cottony	Dark olive green	Gray cottony	Dark olive green
Go34	Dark gray cottony	Dark brown	Dark gray cottony	Dim gray
Go38	Whitish cottony	Pale white	Pale white cottony	Yellowish
Shk9	Light grey cottony	Dim gray	Dim gray cottony	Dark olive green
Shk10	Gray cottony	Dark brown	Dark gray cottony	Dark olive green

Five Arsi *C. kahawae* isolates (ShK9, ShK10, Go33, Go34 and Cho41) and one *C. gloeosporioides* (Cho38)

Colony color of *Colletotrichum* spp. isolates through the reverse side

Figure 2. Colony color of *Colletotrichum* species via front and reverse side.

3.4. Cultural characterization of *C. kahawae* isolates

3.4.1. Sporulation capacity of C. kahawae isolates

Conidial production on 10-day-old mono-conidial cultures showed significant ($p < 0.05$) differences among isolates. Conidial production could range between $7.5x10^5$ and $1.44x10^6$ conidia mL^{-1} by isolate Shk9 and Go33, respectively. The highest (1.44×10^6 conidia mL^{-1}) number of conidia was produced by isolate Go33, followed by Go34 (1.31×10^6 conidia mL^{-1}), Shk10 (1.02×10^6 conidia mL^{-1}), Cho41 (7.8×10^5 conidia mL^{-1}) and Shk9 (7.5×10^5 conidia mL^{-1}) (Table 6)

In a previous study, Tefestewold (1995) observed 1.2-5.2 x 10^5 conidia mL^{-1} and 6.84- 17.20 x 10^6 conidia mL^{-1} production from six isolates of *C. kahawae* on PDA medium and GCA (green coffee seed extract agar). Arega (2006) also has reported the existence of considerable variation in conidia production among *C. kahawae* isolates, which ranged between 6.84 $x10^6$ to 1.720 x 10^7 conidia mL^{-1}. According to this researcher, conidia production was varied between 2.593 x 10^5 (by isolate Y70 from Yayu) and 2.532 $x10^6$ conidia mL^{-1} (by isolate S60 from Sheko). *Colletotrichum kahawae* from Hararghe Zones showed significant variation on their capacity to produce conidial quantity (Emana, 2014). In the same study the mean conidia production capacity of *C. kahawae* isolates ranged between 3.953 $x10^5$ conidia mL^{-1} produced by isolate Bo3 from Boke and 2.6085 x 10^6

conidia per milliliter produced by isolate B2 from Bedeno.

As concluded from previous studies, including the present one, *C. kahawae* isolates sampled from high elevation with low temperature produced large number of conidia as compared with isolates collected from midland to highland. In another way, the finding indicates that *C. kahawae* isolates prefer low temperature and high moisture status rather than high elevation context.

3.4.2. Conidial size of C. kahawae

Conidial size was varied among and within isolates. All isolates had variable mean conidia length and width ranged between 10.5 - 15.5 and 2.78 - 3.83 µm, respectively (Table 6). The average conidial length and width of isolates were 13.224 and 3.526 µm, respectively. The longest and the shortest conidial length was measured on isolate Shk10 and Go34, respectively, while the widest and narrowest conidial width was recorded with isolate Go33 and Shk10, respectively.

Tefestewold (1995) reported that *C. kahawae* isolates had variable mean conidial length that ranged between 13.5 and 19.3 µm and mean conidia width between 2.9 and 5.2 µm. Arega (2006) was also reported that *C. kahawae* isolates had variable mean conidial length and width that ranged between 12.7-15.5 and 3.6-4.8 µm, respectively. The average conidial length and width of isolates were 14.10 and 4.21 µm, respectively (Arega, 2006). The average size of conidia was 14.10 x 4.21 µm, while conidial width and length ranged between 3.6 – 4.8 µm and 12.7 – 15.5 µm, respectively (Kilimbo *et al*., 2013). Kilimbo *et al*. (2013) also reported variability of *C. kahawae* isolates related to their conidial size sampled from different countries of African continent.

In this present study, all isolates indicated variable conidial length and width, even within one isolate, and the observations fit with the findings of previous authors. Talhinhas *et al*. (2005) indicated variability in conidial size within and among strains when studying the diversity of *Colletotrichum* species in olive anthracnose and concluded that it is difficult to distinguish fungal strains using spore size. Similar to his observation, the result of present study ensures presence of high variability on spore/conidial size among and within *C. kahawae* isolates.

Table 6. Conidial size and sporulation capacity of *C. kahawae* isolates

Isolates	Conidial size (L ×W, µm)	Spore production capacity (×10,000 conidia mL^{-1})
Cho41	L 12.7 × W 3.67b	78c
Go33	L 15.5 × W 3.83a	102b
Go34	L 10.5 × W 3.57c	144a
Shk9	L 12.8 × W 3.78b	75c
Shk10	L 14. 7 × W 2.78c	131a
f	31.14	32.57
p	0.001	0.001
LSD (0.05)	4.683	15.78
CV (%)	5.5	8.7

Note: *the same letters indicates none significance among isolates.*

3.4.3. Conidial shape of C. kahawae isolates

The conidial shapes of isolates were variable. About 45-72% of the conidia of the isolates had conidial shape of type 1. The result also indicated that more than 53% of conidia of each isolate fell under conidial shape of type 1 except isolate Shk9. However, isolate Shk9 produced almost type 1 and 2 conidia shapes. Isolate Shk41, Go34 and Shk10 produced all types of conidial shapes but predominantly shape type 1 (Table 7). Hindorf (1970) also reported earlier conidial shape variability of *C. kahawae* isolates. The five types of conidial shapes described by Hindorf (1973) and Tefestewold (1995) were also frequently observed or encountered in different proportions in each isolate in the results of the current experiment.

The result of the present study has conformed previous works. *C. kahawae* isolates from Ethiopian forest coffee showed variable conidial shapes (Arega, 2006). In the same study, isolates showed about 49-88% conidia had conidial shape type 1. More than 55% of conidial shape frequency of each isolate lied under conidial shape type1 except isolates B53, Y75 and G80 (Arega, 2006). It was found that some isolates produced almost type 1 and 2 conidial shapes in equal proportion, while few isolates produced all types of conidial shapes but predominantly type 1 (Arega, 2006).

Emana (2014) also reported conidial shape variability among and within *C. kahawae* isolates collected from Hararghe Zones. In the same study, more than 50% of conidial shape frequency of each isolate were categorized under conidial shape type 1. According to this investigator, isolates, like Ge3, produced almost type 1 and 2 conidia shapes in equal proportions. Some isolates produced all types of conidial shapes except type 5, while few of them produced all types of conidial shapes but most of them produced dominantly type 1 (Emana, 2014).

Table 7. Frequencies of different kinds of conidia shapes produced by *C. kahawae* isolates

Isolates code	% of conidia per shape type				
	1	2	3	4	5
Cho41	72	13	10	5	0
Go33	70	14	9	7	0
Go34	81	9	5	3	2
Shk9	45	39	9	7	0
Shk10	53	28	12	3	4

Note: *1 = cylindrical and round at both ends, 2 = cylindrical acute at one and round at the other end, 3 = clavate-round at both ends starts attenuating from ¼ of its length, 4 = reniform or kidney-shaped, 5 = oblong-elliptical, types.*

4. Conclusion

Colletotrichum species sampled from Arsi Zone coffee growing areas were divided into six representative isolates (Five *C. kahawae* and one *C. gloeosporioides*) depends on their colony morphology and growth rate. *Colletotrichum kahawae* isolates were characterized for their colony color, radial growth rate and texture in case of morphological characteristics, while conidial shape, size and sporulation capacity in case of cultural characteristic. Accordingly, *C. kahawae* isolates were significantly varied from each other with their Morpho-cultural attributes.

However, conidial size was varied among and within isolates. Thus, conidial size was doesn't used as identification and diagnostic tools. Except conidial size, other Morpho-cultural attributes used as identification and diagnostic tools for *C. kahawae*. Therefore, except conidial size one can be use remaining Morpho-cultural attributes of *C. kahawae* as diagnosis and identification tools. It means, not saying traditional characterization is enough for diagnosis. Since it limits diagnosis and identification among and within pathogen species particularly on genetic variability, molecular analysis should be conducted.

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