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Influence of altitudes on the cultural and morphological variation of *Colletotrichum kahawae* isolates, the causal agent of coffee berry disease in the West Region of Cameroon

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Coffee berry disease (CBD) caused by *Colletotrichum kahawae*, is the main disease that affects arabica coffee production in Africa. Isolates of *C. kahawae* were collected from different altitude in the West Region of Cameroon and characterised for cultural and morphological variations. The results showed a significant variation among *C. kahawae* isolates related to their morpho-cultural features with respect to altitude. All isolates had cottony mycelium appearance. Four types of colony colours were identified: Grey, whitish, greyish and pinkish. Mycelia growth rate of isolates varied significantly from 4.32 to 7.11 mm/day. Dominant shape of conidia was cylindrical acute and round ends of CBDBHa1, CBDBHa2 and CBDBHa3 isolates from the high altitude, followed by cylindrical round at both ends of isolates CBDBMa1 and CBDBMa2 from medium altitude. Fusiform and reniform conidia shapes were observed with isolates CBDNBa1 and CBDNBa2 from low altitude. Conidia size and sporulation capacity of *C. kahawae* isolates increased with altitude. Conidia size ranged from 3.82 to 13.46 µm in length and from 1.13 to 1.69 µm in width; while sporulation capacity varied from 169.06 ×10^4 to 438.36 ×10^4 conidia/ml. This study highlights some variations in cultural and morphological characteristics of *C. kahawae* with respect to altitude.

Key words: Altitude, coffee berry disease, *Colletotrichum kahawae*, morpho-cultural characteristics, *Coffea arabica*.

INTRODUCTION

Coffee berry disease (CBD) caused by *Colletotrichum kahawae*, is the most devastating threat to *Coffea arabica* L. production in Africa. CBD broke out in Kenya in 1922 (Hindorf and Omondi, 2011) and later spread to all major

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coffee-producing areas like Angola (1930), Cameroon (1955 to 1957), Uganda (1959), Tanzania (1964) and Ethiopia (1971) (Hindorf and Omondi, 2011; Zenebe et al., 2021). The impact of this disease varies with location and period of year depending on the management practices undertaken, the rainfall pattern and the altitude (Belachew and Teferi, 2015; Garedew et al., 2017). *C. kahawae* attacks coffee berries at all stages of development from flowering to ripening, but the considerable crop loss occurs following the infection of green immature berries (Batista et al., 2017). Yield loss due to CBD is estimated to range from 70 to 80%, but it may reach up to 100% in high rainfall and high altitude areas under conditions where no control measures are undertaken (Silva et al., 2006; Garedew et al., 2017). The disease symptoms appear as small soaked lesions, which rapidly become dark and sunken. Under humid conditions and low temperatures, pink masses become visible on the surface of the lesions (Silva et al., 2006). Berries often drop from the branch at an early stage of the disease. *C. kahawae* conidia dispersal on a tree is by rain splash while the spread from tree to tree and from farm to farm is by coffee pickers, birds or other vectors, but never by wind (Silva et al., 2006; Hindorf and Omondi, 2011).

Coffee berry disease can be controlled by the use of resistant coffee varieties (Gichimu and Omondi, 2010), spraying chemical fungicides (Alwora and Gichuru, 2014), through improved cultural practices (Mouen et al., 2007) and utilisation of natural products, especially plant extracts (Amsalu et al., 2011; Serawit and Tesfaye, 2014; Yemo et al., 2017). However, these methods have faced the problem of variation which exists within *C. kahawae* isolates (Alemu et al., 2020). According to Van der Vossen et al. (2015) a good understanding of the biology of *C. kahawae* could lead to the development of coffee varieties with sufficient disease resistance and disease management strategies. Researched have been conducted across the globe indicating the variation among *C. kahawae* isolates through morphocultural characteristics (Bersisa et al., 2018; Alemu et al., 2020; Amare et al., 2021; Zenebe et al., 2021). Derso and Waller (2003) reported the presence of several morphological and cultural characteristics within *C. kahawae* isolates in Ethiopia and indicated that, it would be useful to look at a profile of several isolates from widely differing coffee types existing in the country. Similarly, Amare et al. (2021) indicated that, apart from coffee tree, variation in CBD severity could be associated with differences in virulence between *C. kahawae* isolates occurring in different coffee growing regions. The variability within *C. kahawae* isolates from different coffee producing areas of Cameroon so far is not known. Thus, the objective of the present study was to determine the influence of altitude on the morphological and cultural variation of *C. kahawae* isolates which affects coffee berries in the West Region of Cameroon.

MATERIALS AND METHODS

Description of the study area and collection of samples

The experiment was carried out in the West Region of Cameroon from June to September 2019 and 2020. This region is one of the main arabica coffee producing areas in Cameroon and covers an area of 13872 km² (Kuit et al., 2008). It is characterized by a mountainous landscape with an altitude varying between 700 and 2700 m. Rainfall varies between 1000 and 2000 mm per year; the climate is the tropical Sudanese type with two seasons of unequal length: a rainy season (mid-March to mid-October) and a dry season (mid-October to mid-March). The average temperature is around 20°C, with a maximum and minimum of 25 and 15°C, respectively (Ngouanet, 2010; Djoukeng et al., 2016). In this region, coffee berries with the symptoms of CBD were randomly collected during the fruiting stage from the Bamboutos and Noun divisions at different altitudes (Table 1). These coffee berries were placed in the appropriate labelled plastic bags and transported to the phytopathology laboratory of the Institute of Agronomic Research and Development (IRAD), Foumbot for isolation of the different *C. kahawae* isolates. A hygro-thermometer was used to capture climatic data such as temperature and moisture during the month of September. This was done daily at 7:00 a.m., 12:00 a.m. and 5:00 p.m. The daily values were averaged monthly to give mean monthly temperature/moisture (Table 1).

Isolation and identification of *C. kahawae* from the infected coffee berries

The infected coffee berries with typical CBD symptoms were first washed with tap water and then fragments of epicarp of about 2 mm² excised using a sterilised scalpel at the level of the CBD lesions. These fragments were surface disinfected in 5% sodium hypochlorite solution for 2 min followed by three rinses with distilled water and then placed on hydrophilic paper to remove excess water (Yemo et al., 2017). The fragments were aseptically plated in Petri dishes containing 20 ml of Potato Dextrose Agar (PDA) medium amended with chloramphenicol (1 g/l) to prevent bacterial growth, and then incubated at 24 ± 2°C. From the daily observations, the mycelia growths visible around the fragments were sub-cultured on a new PDA medium, until pure cultures were obtained. Identification of *C. kahawae* under light microscope was based on cultural and morphological characters with the help of the mycology identification guides (Barnett and Hunter, 1972; Gunnell and Gubler, 1992; Prihastuti et al., 2009). Pure cultures were sealed in Petri dishes with parafilm paper and stored in a refrigerator at 4°C for further studies.

Cultural characteristics of *C. kahawae* isolates

Cultural characteristics of the isolates were evaluated based on their colony colour, appearance and radial growth. Mycelia discs (5 mm in diameter) of 10 day old pure culture of each *C. kahawae* isolate were placed aseptically at the center of Petri dishes containing 20 ml of PDA and incubated at 24 ± 2°C. The colony colour and appearance on the upper side of the Petri-dish for each isolate were assessed after 10 days of incubation. Hence, colony radial growth was also measured manually with a ruler from two perpendicular axes drawn on the reverse side of the Petri dishes

Table 1. Climatic data of the collection sites for the different *Colletotrichum kahawae* isolates.

Division	Altitude (m.a.s.1)	Average temperature (°C)	Average moisture (%)	Isolate code
Bamboutos	High (> 1800)	19.6	80.12	CBDBHa1
	High (> 1800)			CBDBHa2
	High (> 1800)			CBDBHa3
	Medium (1401 - 1800)	19.3	68.33	CBDBMa1
	Medium (1401 - 1800)			CBDBMa2
Noun	Low (≤ 1400)	22.4	66.58	CBDNBa1
	Low (≤ 1400)			CBDNBa2

Source: Authors

while growth rate was determined according to the following formula (Sofi et al., 2013):

$$\text{Growth rate (mm/day)} = \frac{C1 - C0}{2}$$

where C1 = growth observed on a particular day (mm) and C0 = growth observed on the previous day. The experiment was repeated three times.

Conidia morphological characteristics of *C. kahawae* isolates

For each isolate of *C. kahawae*, the conidia suspension was prepared by carefully brushing a 10-day-old pure culture in 10 ml of sterilised distilled water with a fine brush. After shaking with a magnetic stirrer to release the conidia, the suspension was filtered through muslin and a drop of Tween 80 was added to the suspension to homogenise it. A drop of suspension from each isolate was mounted between slide and coverslip and observed under an ordinary microscope at 40X magnification. Measurements of the size (length and width) of 50 conidia taken at random per isolate were made using an ocular micrometer installed in the microscope objective (Keuete et al., 2016; Bersisa et al., 2018). The shapes of these conidia were also recorded. In addition, the sporulation rate (number of conidia/ml) was counted using a haematocymeter. The experiment was repeated three times.

Statistical analysis

Data collected on radial growth, growth rate, conidia size, and sporulation rate were subjected to analysis of variance (ANOVA) using SPSS software, version 22.0. The means were separated with Duncan's Multiple Range Test (DMRT) at 5% level of significance. Cluster analysis was used to determine the relationships between isolates of *C. kahawae* based on cultural and morphological characteristics using XLSTAT software, version 16.0.

RESULTS

Cultural characteristics of *C. kahawae* isolates

The cultural characteristics of *C. kahawae* isolated from infected coffee berries, 10-day-old on PDA medium varied from one isolate to another with respect to altitude. On the basis of colony appearance, all *C. kahawae* isolates had cottony mycelium. With regard to the colony colour, the *C. kahawae* isolates were grouped into four different classes: light grey, greyish, grey and pinkish (Figure 1 and Table 2). These groups were made based on the observation from the front side of the culture plate on PDA. Isolates CBDNBa1 from the low altitude of the Noun division and isolates CBDBMa2 and CBDBHa2 from the medium and high altitudes, respectively of the Bamboutos division produced light grey (45.89%) cottony mycelia. Isolates CBDNBa2 from the low altitude of the Noun division and CBDBHa1 from the high altitude of the Bamboutos division produced greyish (39.52%) cottony mycelia. Colonies produced by isolates CBDBMa1 and CBDBHa3, respectively from the medium and high altitudes of the Bamboutos division had grey (8.51%) and pinkish (6.08%) cottony mycelium, respectively.

Radial growth of *C. kahawae* isolates after 10 day of incubation on PDA culture medium ranged between 70.58 and 84.05 mm (Table 2). The isolates CBDBHa1 and CBDBHa3 from the high altitudes of the Bamboutos division and isolate CBDNBa2 from the low altitude of the Noun division showed the highest radial growth of 84.05, 83.67 and 84.05 mm, respectively; followed by isolates CBDBHa2 from the high altitude (80.48 mm) and CBDBMa2 from the medium altitude (80.57 mm) of the Bamboutos division which had intermediate radial growth. Isolates CBDBMa1 from the medium altitude of the Bamboutos and CBDNBa1 from the low altitude of the Noun division had the lowest radial growth of 76.68 and 70.58 mm, respectively. In addition, radial mycelial growth rate of the different isolates varied between 4.32 and 7.11 mm/day (Table 2). Isolate CBDBHa1 from the high altitudes of the Bamboutos division showed the fastest growth rate of 7.11 mm/day; while, the slowest mycelial growth rate of 4.32 mm/day was recorded with isolate CBDNBa1 from the low altitude of the Noun division which was not significantly different ($P \leq 0.05$)

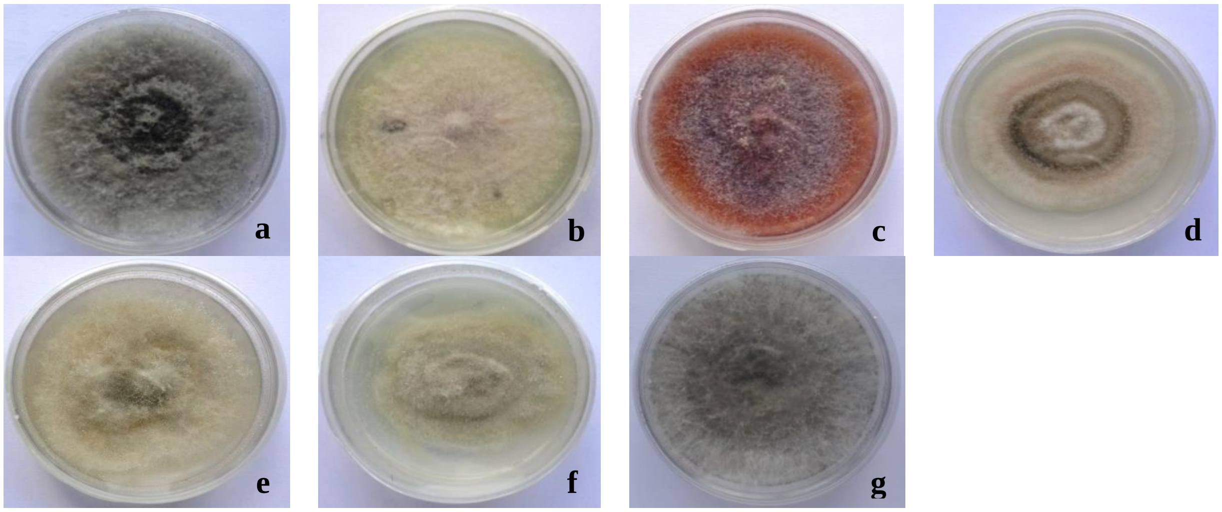

Figure 1. Pure culture different isolates of *Colletotrichum kahawae* on PDA medium. (a) CBDBHa1; (b) CBDBHa2; (c) CBDBHa3; (d) CBDBMa1; (e) CBDBMa2; (f) CBDNBa1; and (g) CBDNBa2.
Source: Authors

Table 2. Comparisons among *Colletotrichum kahawae* isolates on colony texture and colour, radial growth and growth rate.

Isolates code	Colony texture	List colour range	Radial growth day 10 (mm)	Growth rate day 10 (mm/day)
CBDBHa1	Cottony	Greyish	84.05 ± 0.24[a]	7.11 ± 0.30[a]
CBDBHa2	Cottony	Light grey	80.48 ± 0.71[b]	5.84 ± 0.23[b]
CBDBHa3	Cottony	Pinkish	83.67 ± 0.30[a]	5.40 ± 0.35[b]
CBDBMa1	Cottony	Grey	76.68 ± 1.24[c]	5.02 ± 0.28[bc]
CBDBMa2	Cottony	Light grey	80.57 ± 0.51[b]	4.92 ± 0.28[bc]
CBDNBa1	Cottony	Light grey	70.58 ± 1.44[d]	4.32 ± 0.31[c]
CBDNBa2	Cottony	Greyish	84.05 ± 0.22[a]	5.53 ± 0.41[b]

a,b,c: Means in a column followed by the same letter are not significantly different by to Duncan's test at 5% probability level.
Source: Authors

from those of CBDBMa1 (5.02 mm/day) and CBDBMa2 (4.92 mm/day) isolates from the medium altitude of Bamboutos division. The isolates CBDBHa2 and CBDBHa3 from the high altitude of the Bamboutos division and isolate CBDNBa2 from the low altitude of the Noun division showed intermediate growth rate of mycelium with the respective values of 5.84, 5.40 and 5.53 mm/day.

Conidia morphological characteristics of *C. kahawae* isolates

The results of the morphological characters showed considerable variations in conidia shape among isolates of *C. kahawae* with respect to altitude (Figure 2 and Table 3). Isolates CBDBHa1, CBDBHa2 and CBDBHa3 from the high altitude of the Bamboutos division produced cylindrical conidia shape with acute and round ends. While isolates CBDBMa1 and CBDBMa2 from the medium altitude of the Bamboutos division produced predominantly conidia shape with cylindrical and round at both ends. On the other hand, CBDNBa1 and CBDNBa2 isolates from the low altitude of the Noun division, respectively produced fusiform and reniform conidia shapes.

Conidia size of different *C. kahawae* isolates varied significantly ($P \leq 0.05$) with altitude. All *C. kahawae* isolates had variable conidia length and width ranging from 3.82 to 13.46 µm, and 1.13 to 1.69 µm, respectively (Table 3). Isolates CBDBHa1, CBDBHa2 and CBDBHa3 from the high altitude of the Bamboutos division had the

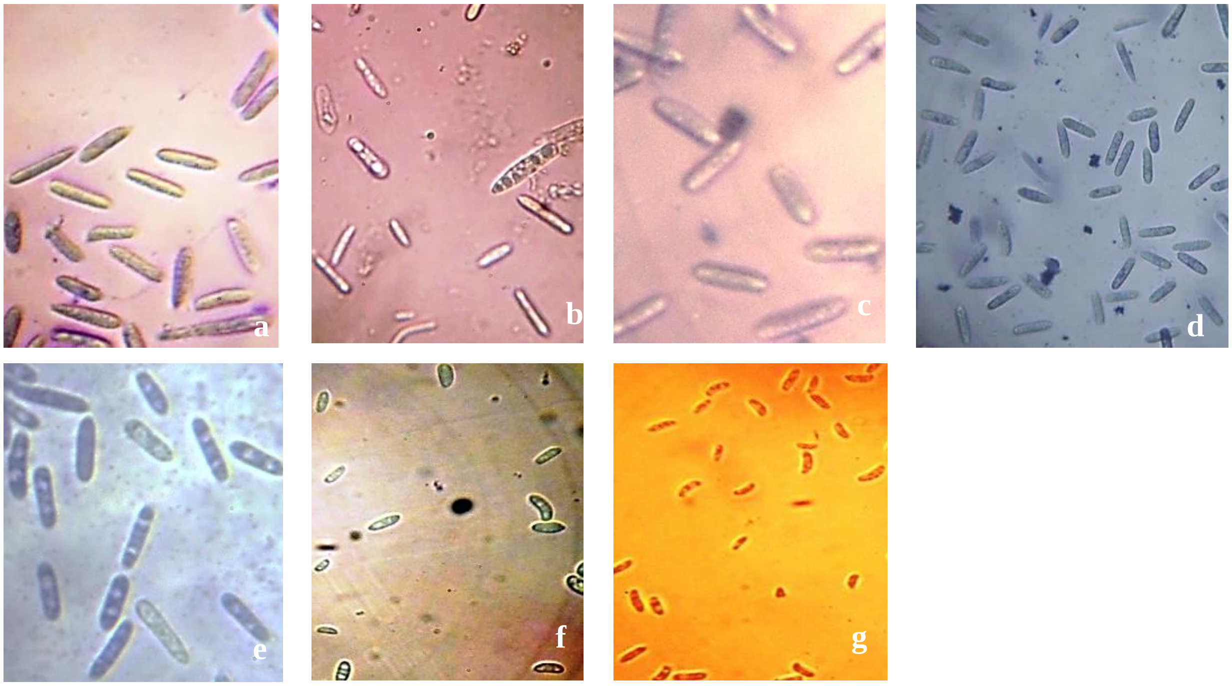

Figure 2. Conidia shape of *Colletotrichum kahawae* isolates. (a) CBDBHa1; (b) CBDBHa2; (c) CBDBHa3; (d) CBDBMa1; (e) CBDBMa2; (f) CBDNBa1; and (g) CBDNBa2.
Source: Authors

Table 3. Variation among isolates of *Colletotrichum kahawae* in sporulation rate, conidia size and shape.

Isolates code	Conidia shape	Conidia size (µm)		Sporulation rate
		Length	Width	($\times 10^4$ conidia/ml)
CBDBHa1	Cylindrical acute and round ends	13.15 ± 0.12^a	1.44 ± 0.04^b	434.20 ± 0.58^a
CBDBHa2	Cylindrical acute and round ends	13.46 ± 0.13^a	1.69 ± 0.03^a	435.69 ± 0.65^a
CBDBHa3	Cylindrical acute and round ends	13.35 ± 0.11^a	1.63 ± 0.04^a	332.02 ± 0.44^b
CBDBMa1	Cylindrical round at both ends	7.91 ± 0.13^b	1.16 ± 0.03^d	301.43 ± 2.34^c
CBDBMa2	Cylindrical round at both ends	8.08 ± 0.12^b	1.19 ± 0.02^d	298.16 ± 2.03^c
CBDNBa1	Fusiform	3.82 ± 0.10^{cd}	1.13 ± 0.02^d	169.77 ± 1.20^d
CBDNBa2	Reniform	4.44 ± 0.08^c	1.29 ± 0.04^c	167.70 ± 0.53^d

^{a,b,c}Means in a column followed by the same letter are not significantly different by to Duncan's test at 5% probability level.
Source: Authors

longest conidia lengths of 13.15, 13.46 and 13.35 µm, respectively and the widest conidia widths of 1.44, 1.69 and 1.63 µm, respectively. While the shortest conidia lengths of 3.82 and 4.44 µm, and the narrowest conidia widths of 1.13 and 1.29 µm, were recorded by isolates CBDNBa1 and CBDNBa2 from the low altitude of the Noun division, respectively. Generally, conidia size of *C. kahawae* isolates increased with increasing altitude. Isolates CBDBMa1 and CBDBMa2 from the medium altitude of Bamboutos division produced conidia which measured 7.91 and 8.08 µm in length and 1.16 and 1.19 µm in width, respectively.

Sporulation rate that was taken from 10 day old cultures showed highly significant differences ($P \leq 0.05$) among isolates with respect to altitude. The sporulation rate increased with altitude and varied from 167.70×10^4 to 435.69×10^4 conidia/ml (Table 3). Isolates CBDBHa1 and CBDBHa2 from the high altitude of the Bamboutos division produced the highest conidia number of 434.20×10^4 and 435.69×10^4 conidia/ml, respectively, followed by isolate CBDBHa3 from the same division at the same altitude (332×10^4 conidia/ml). Whereas, isolates

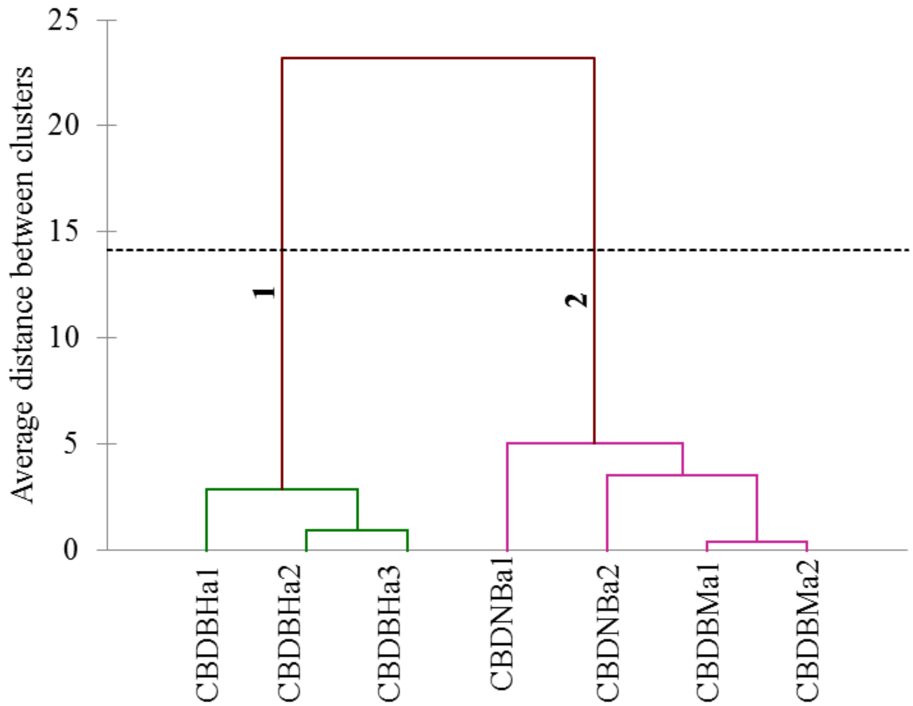

Figure 3. Dendrogram showing relationships between *Colletotrichum kahawae* isolates associated with diseased coffee berries based on cultural and morphological characteristics.
Source: Authors

CBDNBa1 and CBDNBa2 from the low altitude of the Noun division produced the lowest amount of conidia (169.77×10^4 and 167.70×10^4 conidia/ml, respectively) compared to the other isolates. Isolates CBDBMa1 and CBDBMa2 from medium altitude of the Bamboutos division produced the intermediate numbers of conidia (301.43×10^4 and 298.16×10^4 conidia/ml, respectively).

Dendrogram of hierarchical cluster analysis

Dendrogram resulting from the hierarchical cluster analysis of cultural and morphological characteristics of *C. kahawae* isolated from coffee berries collected from different altitudes showed two distinct groups (Figure 3). The first group included three isolates CBDBHa1, CBDBHa2 and CBDBHa3 from the high altitude of the Bamboutos division was characterised by a faster growth, bigger conidia size and higher amount of conidia. The second group composed of two isolates (CBDBMa1 and CBDBMa2) from the medium altitude of the Bamboutos division showed medium growth, medium conidia size and intermediate sporulation rate and the two others (CBDNBa1 and CBDNBa2) from the low altitude of the Noun division with medium growth, smaller conidia size and low number of conidia.

DISCUSSION

The results of this study indicate a significant variation in cultural and morphological characteristics among *C. kahawae* isolates with respect to the altitude where they were collected. This variation in cultural and morphological characteristics is probably due to the fact that isolates of *C. kahawae* were isolated from coffee berries from areas at different altitudes, where a particular rainfall and temperature are observed. These results are in line with those of N'Guettia et al. (2013) who showed that temperature and rainfall could influence the morpho-cultural characteristics of isolates of *C. gloeosporioides*, the causal agent of anthracnose in mango fruit. Based on the cultural characteristics observed in upper side of the plate, all mycelia of *C. kahawae* had cottony appearance divided into four different colours; ranging from grey, light grey, greyish and pinkish. Diverse colony colours on both sides of a culture plate of *C. kahawae* isolates have been reported by many authors. According to Alemu et al. (2020), most of the *Colletotrichum* isolates displayed light grey, grey and dark grey mycelia colours, which is a typical colony characteristic of the *C. kahawae* with easily distinguishable features from the other *Colletotrichum* species. Kilimbo et al. (2013) reported that all mycelia of *C. kahawae* had dark grey cottony colonies, which is a distinctive characteristic observed on *C. kahawae* isolates grown on malt extract agar (MEA) medium, and this distinguished it from other *Colletotrichum* spp. isolated from diseased coffee berries. Similarly, Bersisa et al. (2018) revealed that, isolates of *C. kahawae* had dark grey, grey and light grey cottony mycelia on PDA medium. In addition, the result reported by Emana (2014) showed light grey, dark grey and whitish on both PDA and MEA media.

Mycelia radial growth rate varied significantly among isolates of *C. kahawae* with respect to altitude. Isolate CBDBHa1 from the high altitude of the Bamboutos division showed the fastest growth rate of 7.11 mm/day, while the slowest mycelial growth rate of 4.32 mm/day was recorded by isolate CBDNBa1 from the low altitude of the Noun division. These results are in agreement with the findings of other researchers. Bersisa et al. (2018) and Alemu et al. (2020) recorded 3.72 to 7.75 mm/day and 3.97 to 6.7 mm/day growth rate of *C. kahawae* isolates, respectively in Ethiopia. Kilimbo et al. (2013) and Emana (2014) also conducted a similar study in Tanzania and Ethiopia, respectively and observed a growth rate of *C. kahawae* ranging from 5.0 to 5.5 mm/day on PDA. However, recent reports recorded a relatively lower growth rate of *C. kahawae* isolates ranging from 2.2 to 4.30 mm/day on PDA (Amare et al., 2021; Zenebe et al., 2021). Differences in growth rate observed among the isolates of *C. kahawae* in the present study are probably due to prevailing environmental conditions in the respective altitude of origin of isolates. According to Hudec and Muchová (2010), isolates of *Fusarium* species originating from mountain regions grew significantly faster at low temperature than those from lowland regions. *C. kahawae* isolates associated with coffee differs in mycelial growth, colony characters and growth rates from other *Colletotrichum* isolates which is an important feature for grouping them into separate classes depending on visual observation from the culture plates (Manuel et al., 2010). However, Serra et al. (2006) related that radial growth rate of mycelium is not a stable criterion of differentiation of *Colletotrichum* species, but it plays a significant role in variability within the species.

The morphological characteristics of *C. kahawae* were observed based on their conidia shape and size, they varied significantly among the isolates as also noted in other studies (Kilimbo et al., 2013; Emana, 2014; Bersisa et al., 2018; Alemu et al., 2020; Amare et al., 2021; Zenebe et al., 2021). Isolates CBDBHa1, CBDBHa2 and CBDBHa3 from the high altitude of the Bamboutos division and CBDBMa1 and CBDBMa2 isolated from the medium altitude of the same division produced cylindrical shaped conidia with acute at one end and round at the other end to sometime round at both ends. While isolates CBDNBa1 and CBDNBa2 from the low altitude of the Noun division were dominated by fusiform and reniform conidia shapes. The result of the present study conformed with previous works shown as follows: isolates of *C. kahawae* from Ethiopia showed variable conidia shapes (Emana, 2014; Bersisa et al., 2018). These authors reported that, more than 50% of conidia shape frequencies of each isolate were categorized under conidia shape type cylindrical and round at both ends. Alemu et al. (2020) also reported conidia shape variability among and within *C. kahawae* isolates collected from the same country. In the same study, almost all isolates showed cylindrical with one or both ends obtuse, which is a typical characteristic of conidia of *C. kahawae* and *C. gloeosporioides* to distinguish them from *C. acutatum* (Alemu et al., 2020). Similarly, fusiform conidia shape observed in the present study have also been observed by N'Guettia et al. (2013) and Keuete et al. (2016) in *Colletotrichum* spp. causal agent of anthracnose of several fruits.

In term of conidia size, the result revealed a significant variation among the isolates of *C. kahawae* with altitude. The conidia size of different *C. kahawae* isolates in the present study increased with increasing altitude. Conidia sizes from the high altitudes were significantly bigger than those from the medium and low altitudes. This suggests that altitudes could have a significant influence on the conidia size of *C. kahawae* which may be due to different climatic conditions existing at these altitudes. The results are in agreement with the works of Tsopmbeng et al. (2014) in Cameroon that showed an increase in the sporangia size of *Phytophthora colocasiae* with altitude. Conidia length and width ranged from 3.82 to 13.46 µm and from 1.13 to 1.69 µm, respectively. These values are close to those described by Bersisa et al. (2018) in Ethiopia who observed that conidia size of *C. kahawae* ranged from 10.5 to 15.5 µm in length and from 2.12 to 3.83 µm in width. Similarly, Emana (2014) also reported that *C. kahawae* isolates had variable mean conidial length and width that ranged from 10.51 to 15.78 µm and 2.12 to 4.24 µm, respectively. The average conidia size of *C. kahawae* isolates was 9.87 × 38 µm, while conidia length and width ranged from 9.03 to 10.49 µm and 5.0 to 6.04 µm, respectively (Amare et al., 2021). Alemu et al. (2020) also reported variability of *C. kahawae* isolates related to their conidia size in Ethiopia; according to them, conidia length and width ranged between 12.3 to 17.7 µm and 3.6 to 5.1 µm, respectively.

Another morphological parameter considered in this study was sporulation capacity. It revealed a considerable variation among isolates of *C. kahawae* based on altitude. Sporulation capacity increased with increasing altitude and varied from 167.70 × 10^4 to 435.69 × 10^4 conidia/ml. These values fall within the interval described by Amare et al. (2021) for conidia production that ranged from 182.25 to 432.92 × 10^4 conidia/ml. Similarly, Zenebe et al. (2021) also reported the variation in the number of conidia production ranged from 186.10 to 572.30 × 10^4 conidia/ml. In addition, Emana (2014) and Bersisa et al. (2018) also recorded relatively lower conidia production on PDA ranging between 39.53 to 260.85 × 10^4 conidia/ml and 75 to 144 × 10^4 conidia/ml, respectively. The high amount of conidia production was a characteristic of the virulent pathogen of the *C. kahawae* isolates that produce enough inoculum sources for disease development (Amare et al., 2021). The results also revealed that, isolates of *C. kahawae* sampled from high altitude produced highly significant amount of conidia as compared to isolates collected from medium to low altitudes. Increase sporulation rate with altitude could be related to the fact that *C. kahawae* prefer low temperatures and high humidity prevailing in high altitude areas. According to Bersisa et al. (2018), *C. kahawae* isolates sampled from high altitudes characterised by low temperatures produced large number of conidia as compared with those collected from low and medium altitudes.

Conclusion

The present study highlighted the importance of altitude on cultural and morphological variations of *C. kahawae* isolates. Conidia size and sporulation capacity of *C. kahawae* isolates increased with altitude. Conidia sampled from high altitude were bigger than those from medium and low altitudes. Likewise, isolates of *C. kahawae* isolates collected from high altitude produced the highest amount of conidia than those from the medium and low altitudes. The variability in morphocultural characteristics of *C. kahawae* isolated at different altitude implies that further studies need to be carried out using various advanced supplementary techniques to reveal the diversity of the pathogen in the study area so as to develop efficient disease management strategy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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