Pharmaceutical Science and Technology
2020; 4(2): 31-39
http://www.sciencepublishinggroup.com/j/pst
doi: 10.11648/j.pst.20200402.12
ISSN: 2640-4532 (Print); ISSN: 2640-4540 (Online)

Morphological Characterization of *Corticium koleroga*, Cause of Thread Blight on Arabica Coffee

Nagassa Dechassa[1, *], Alemayehu Chala[2], Kifle Belachew[1], Elfinesh Shikur[2]

[1]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, Jimma, Ethiopia

[2]School of Plant and Horticultural Sciences, Hawassa University, Hawassa, Ethiopia

Email address:

nagookooti@gmail.com (N. Dechassa)

[*]Corresponding author

To cite this article:

Nagassa Dechassa, Alemayehu Chala, Kifle Belachew, Elfinesh Shikur. Morphological Characterization of *Corticium koleroga*, Cause of Thread Blight on Arabica Coffee. *Pharmaceutical Science and Technology.* Vol. 4, No. 2, 2020, pp. 31-39. doi: 10.11648/j.pst.20200402.12

Received: November 3, 2020; **Accepted**: November 18, 2020; **Published**: December 22, 2020

Abstract: Coffee thread blight caused by *Corticium koleroga* is one of the fungal pathogens that cause severe damage to *Coffea arabica* in southwest Ethiopia. However, there are very few research findings on the features of the pathogen in Ethiopia. Therefore, the current work was designed with the objectives to characterize pathogen isolates and determine the pathogenicity of the *Corticium koleroga* isolates. For this purpose, diseased samples were collected from 11 districts of southwest Ethiopia during the 2017 cropping season. *C. koleroga* isolates were characterized using macroscopic and microscopic features. Eleven isolates of *C. koleroga* collected from southwest Ethiopia varied in their colony colour ranging from white to floral white, with circular to irregular form and filiform to entire in margin on PDA plates. Growth rate of the isolates was between 6 and 9 mm/day in diameter. Morphological variations in basidiospores length and basidial shape were also evident among the isolates. Average basidiospore size ranged from 10 to 13.75 x 3.75 to 5µ. All isolates were pathogenic to *C. arabica* (74110 susceptible variety), with significantly different ($P<0.01$) lesion size. The most aggressive isolate was Yayu isolate, followed by isolates from Andaracha and Mettu with average lesion size of 95.55, 94.49 and 93.29%, respectively. The current study revealed the identity of *Corticium koleroga* in southwest Ethiopia. Future research should be directed towards molecular characterization of the pathogen.

Keywords: Basidiospore, Isolate, Koleroga, Mycelium, Southwest Ethiopia

1. Introduction

Arabica coffee (*Coffea arabica*) is one of the highly preferred beverages and the most important trade commodity in the world next to oil [1]. Ethiopia was ranked as the first largest *C. arabica* producer in Africa and fourth in the world after Brazil, Colombia and Honduras by producing about 423300.0 Kg (7.4% of world production) in 2017/18 cropping season [2].

Even though *C. arabica* plays a key role in improving livelihood of many Ethiopians, numerous production constraints have been affecting its production and productivity. Abiotic and biotic factors are constraints of coffee production, among which are diseases, attacking coffee parts and reducing the yield, quality and marketability. According to Cavalcante and Sales [3], coffee thread blight (CTB), caused by *Corticium koleroga*, is an important disease of coffee in India, Trinidad and Tobago. In Ethiopia, the disease was first recorded at Gera and Mettu in 1978 [4].

The typical symptom of thread blight disease on coffee at the field is thread-like white to ashen strand on the middle stem of the coffee tree at first and then the grey strands of nodes, internodes of the twigs. The blackening of leaf petiole later spread to leaf blade mostly on the lower surfaces of leaves. The fine strands initiated dark-ashen necrosis and as the whole leaf became involved, the leaf separated at the petiole but usually remained hanging from mycelial strand that grew over the petiole from the branch. The sunken black with ashen myceilial strands was seen as necrotic symptoms of the disease on the berries.

CTB has been known on Ethiopian coffee for many years and has been considered as minor disease, but currently it has been observed as an important disease in wide coffee

growing regions of Ethiopia [5, 6]. This is due to the fact that the disease is linked to several factors related to the environment, pathogen, host and human activities to expand coffee production from place to place. Over exposure of coffee farms to sun light, over production with deficient plant nutrition, difficult adaptation of certain genotypes may also increase the intensity of the disease. Southwestern part of Ethiopia is the major coffee producing area where the damage by CTB is frequently reported with increasing disease incidence and severity from year to year [5, 6].

Nagassa [6] conducted a study at 12 districts of south west Ethiopia indicated that CTB was prevalent and seriously devastated all above ground parts of coffee tree. The disease incidence and severity ranged between 0-46% and 0 to 44%, respectively. The highest mean CTB disease incidence (46%) and severity (44%) were recorded in Masha district followed by Mettu, Andaracha, Alle, Gera and Gomma in that order [6]. Climate condition in Ethiopia favors the proliferation of certain diseases and results in their spreading to neighboring regions where they did not exist before [5, 6].

Human activities to expand coffee production from location to location play a great role in transmission of the disease from place to place [6]. The outbreak of the disease was reported at southwest, West and South Ethiopia on coffee plantations, resulting in considerable damages [5, 6]. Yet the disease is recurring every year and spreading to the neighboring zones of coffee producing areas of the country. Even though, CTB occurred for the past many years in Ethiopia, the identities of the causative pathogen have not yet been characterized.

Therefore, this study was carried out with the following specific objectives to:

1) Verify thread blight causing pathogen in southwestern parts of Ethiopia; and

2) Determine the pathogenicity of the *Corticium koleroga* isolates.

2. Materials and Methods

2.1. Isolation and Macroscopic and Microscopic Identification

2.1.1. Isolation of the Pathogen

Coffee twigs with clear symptoms of thread blight disease were collected from different coffee growing districts of Ilubabor and Jimma zones in Oromia, and Benchimaji and Sheka zones in South Nation, Nationality and Peoples Regional State (SNNPRS). Mettu, Alle, Didu and Yayu from Ilubabor; Gera, Gomma and Shebesombo from Jimma; Shako and Debubbench from Benchimaji; and Andaracha and Masha districts from Sheka zones (Table 1 and Figure 1) were districts from which diseased coffee twigs were collected for the study.

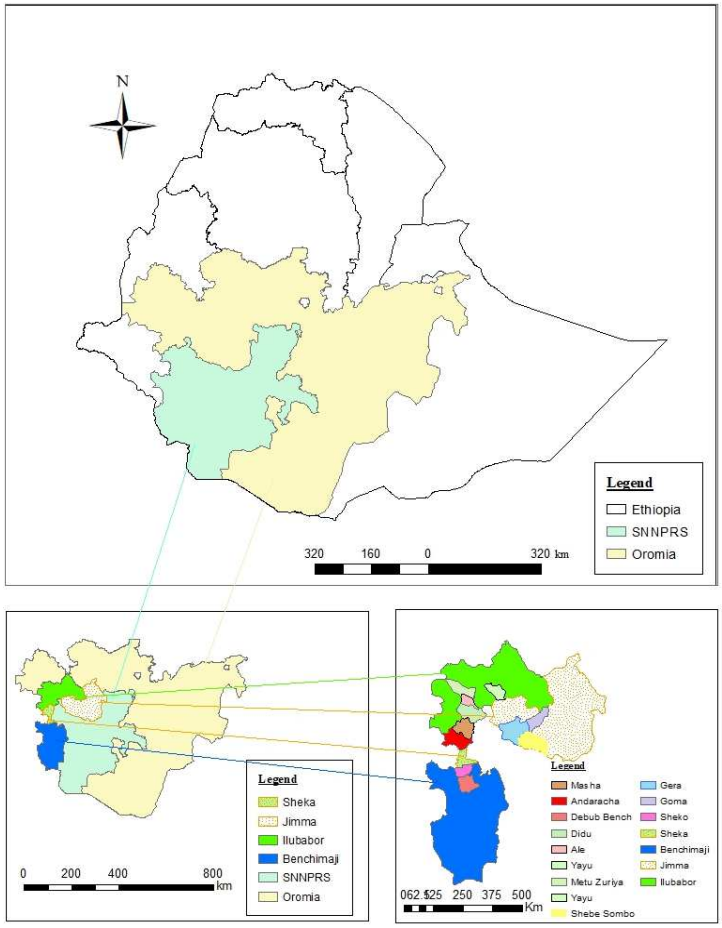


Figure 1. *Map of Ethiopia showing regions, zones and districts of Southwest Ethiopia from which CTB diseased samples were collected.*

Table 1. *Description of coffee thread blight samples and sample collection from 11 districts of southwestern Ethiopia in 2017 cropping season.*

Region	Zone	Districts	Kebele (PA)	Altitude (m.a.s.l.)
Oromia	Jimma	Shebesombo	Angecha	1795
Oromia	Jimma	Gera	Sadiloya	1951
Oromia	Jimma	Gomma	Gembe	1650
Oromia	Ilubabor	Alle	Segibaki	1838
Oromia	Ilubabor	Mettu	Geyi	1555
Oromia	Ilubabor	Didu	Gordomo	1890
Oromia	Ilubabor	Yayu	Dorani	1944
SNNPRS	Benchimaji	Shako	Berhanekontir	1085
SNNPRS	Benchimaji	Debub bench	Abiy-3	1550
SNNPRS	Sheka	Andaracha	Duyina	1816
SNNPRS	Sheka	Masha	Yepho	1760

SNNPRS: Southern Nations, Nationalities and Peoples' Region.

Coffee twigs exhibiting typical symptoms of thread blight were collected from 11 coffee farms.

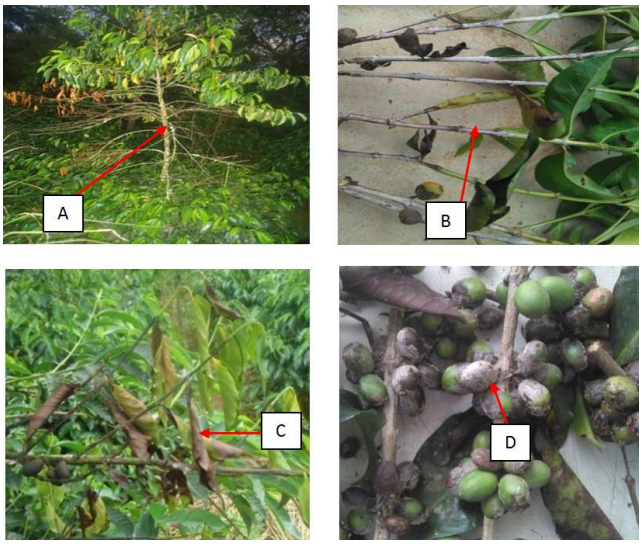


Figure 2. *Thread blight on Coffea arabica was seen as thread-like white to ashen strand on A) Middle stem, B) Branch, C) Leaf and D) Berries observed at Mettu, in 2017 cropping season.*

Pathogen isolation was done following standard procedures in Plant Pathology Laboratory at Jimma Agricultural Research Centre. For isolation of the pathogen, the diseased coffee twigs were cut with a sharp sterilized blade into small bits along with some healthy portions. The sections were then surface-sterilized by dipping into 5% sodium hypochlorite (NaOCl) solution for one minute and rinsed thrice with sterile distilled water (SDW) and finally dried on sterile tissue paper. Then the sections were transferred to potato dextrose agar (PDA) medium in sterilized Petri-plates and incubated at 25°C for five days. The isolates were then purified using hyphal tip isolation technique [7].

2.1.2. Macroscopic Identification

Growth rate of *C. koleroga* isolates

Mycelial discs (5 mm in diameter) of five day old culture of *C. koleroga* isolates were transferred to the centre of PDA plates and incubated at 25°C at dark condition. Three replications were maintained for each isolate in a completely randomized design (CRD). The colony diameter was recorded at 48 hours interval after incubation for ten consecutive days. Growth per day was calculated using the following formula:

$$\frac{\text{Growth observed on a particular day (mm)} - \text{Growth on previous observation (mm)}}{2} \quad (1)$$

The other colony characters, such as form, margin and color were recorded on underside of plate 10 days after incubation.

2.1.3. Microscopic Identification

Ten days old cultures of all the isolates were characterized by using morphological features. Temporary mounts were studied under compound microscope (Germany) for hyphal, basidiospore and basidial features. For each isolate, length and width of 30 randomly taken basidiospores and hyphal width of 30 hyphae per isolate were measured through ocular micrometer under compound microscope at 40x objective lens. Comparisons of morphological characteristics were made using appropriate information sources such as plant pathology guidebooks, global plant protection information system, and the American Phytopathological Society guidelines.

2.1.4. Pathogenicity Test

Pathogenicity tests of the 11 *Corticium koleroga* isolates were conducted on the susceptible *C. arabica* (74110 variety) using the detached twig and leaf methods in air-conditioned growth room. High relative humidity (80-90%) was maintained by humidifier to favour infection at temperature of 23-26°C at 12 hours dark and 12 hours light conditions. The test was carried out on 20 healthy detached coffee twigs with no leaves measuring 20 cm long, about 0.5 cm thick and 20 leaves from the middle branches. Twigs and leaves were surface-sterilized with NaOCl solution and rinsed twice in Sterile Distilled Water (SDW) before inoculation.

Inoculum preparation

Spore suspension was prepared from a seven day old culture of *C. koleroga* isolates by adding 20 mL of SDW to the Petridish with good colony growth, which was then gently swirled to dislodge the spores. The prepared suspensions were transferred to SDW in a sterile beaker and stirred up with magnetic stirrer and filtered through double layer cloth. The suspensions were diluted and spore concentration was adjusted to $2x10^6$ basidiospores per milliliters using haemocytometer (Germany).

Inoculation with suspension

Sterilized detached coffee leaves and twigs were brushed with *C. koleroga* inoculum suspension using sterile inoculating brush. The inoculated coffee leaves and twigs were kept in plastic box with dimension WxLxD, 15 cm x 20 cm x 15 cm containing moist soft paper and covered with transparent plastic sheet on compartment in air-conditioned growth room. The inside of each box was misted with SDW using hand sprayer once in an interval of three days. The treatments were arranged in CRD with three replications.

Data collection

Data on incubation period were recorded. Data on lesion sizes were recorded at 20 days after inoculation. Percentage of infected twigs length was obtained from the lesion length divided by total twig length and multiplied by one hundred [8]. Area of leaf lesions were evaluated using 0-5, score scales on the typical thread blight lesions developed on the leaves, at 20 days after inoculation (Table 2).

Table 2. *Thread blight lesion size scoring scale (0-5 grade) devised by [9] with the slight modification.*

Rating	Area covered with lesions (%)	Symptoms
0	0	No infection
1	0.01–1.0	Very few lesions on leaves
2	1.1-10.0	Few lesions on leaves up to 10% necrotic area covered.
3	10.1–25.0	Lesions covering up to 25% of leaves area covered
4	25.1–50.0	More than 30% of leaves area covered under necrotic lesions.
5	>50	More than 50% of leaves area covered under necrotic lesions.

Percent disease severity index (PSI) was calculated as per [10].

$$\text{PSI} = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves observed} * \text{maximum rating}} * 100 \quad (2)$$

Re-isolation of pathogen

Then 20 days after inoculation, inoculated twigs and leaves showing active thread blight symptoms were re-isolated to PDA Petri-plates and incubated at 25°C for seven days. The obtained cultures were checked for cultural and morphological characters to confirm the fulfillment for Koch's postulates.

2.2. Data Analyses

Data of colony growth rate, hyphal diameter, spore dimension (width and length), and lesion size were analyzed using analysis of variance (ANOVA) with least significant difference at 5% probability level by using SAS Software Version 9.3 [11].

3. Results

3.1. Morphological Characterization of the Pathogen

3.1.1. Macroscopic Identification

Growth rate of mycelia

The study revealed considerable colony growth rate variations among *C. koleroga* isolates collected from different coffee producing regions of Ethiopia. Isolates differed highly and significantly ($p \leq 0.01$) in growth rate in diameter ranging between 6 and 9 mm day^{-1}, with a mean of 8.0 mm day^{-1} in diameter (Table 3). Isolates from midland areas had the fastest (8.6 mm day^{-1} on average) growth rate, while isolates from highland areas grew slowly (mean growth rate of 7.1 mm day^{-1} in diameter). Isolates of *C. koleroga* collected from Andaracha, Shebesombo, Mettu and Shako grew the fastest (8.5 to 9.0 mm day^{-1}), followed by isolates from Yayu, Didu and Masha, which grew at 8.1 to 8.4 mm day^{-1}. Isolates from Agaro, Debubbench Gera and Alle had slow (6.4 to 7.5 mm day^{-1}) growth rate in diameter. In the present study, *C. koleroga* isolates showed periodic changes in their growth rates. All the isolates showed an increasing trend in growth rate from 2 days onward up to 8 days but declined afterwards.

Colony color, elevation and margin

Colony color, form, elevation and margin did not differ very much among the tested isolates of *C. koleroga* on PDA medium (Table 3; Figure 3). The *C. koleroga* isolates produced mycelia with white to floral white front and back side color, filiform to entire margins and irregular to circular in form and produced in abundance. Front side colony color of almost all isolates in the culture were white except isolates form Gera, which seems floral white and the back side colony color of Alle, Andaracha, Gera, Masha, Mettu, Shako and Yayu isolates were floral white. On the other hand, Agaro, Didu, Debubbench and Shebesombo isolates seemed to have white back side colony color. Colony elevations of all isolates were flat. The colony margins of all the isolates were entire, except that of Shako and Masha isolates that had filiform colony margin.

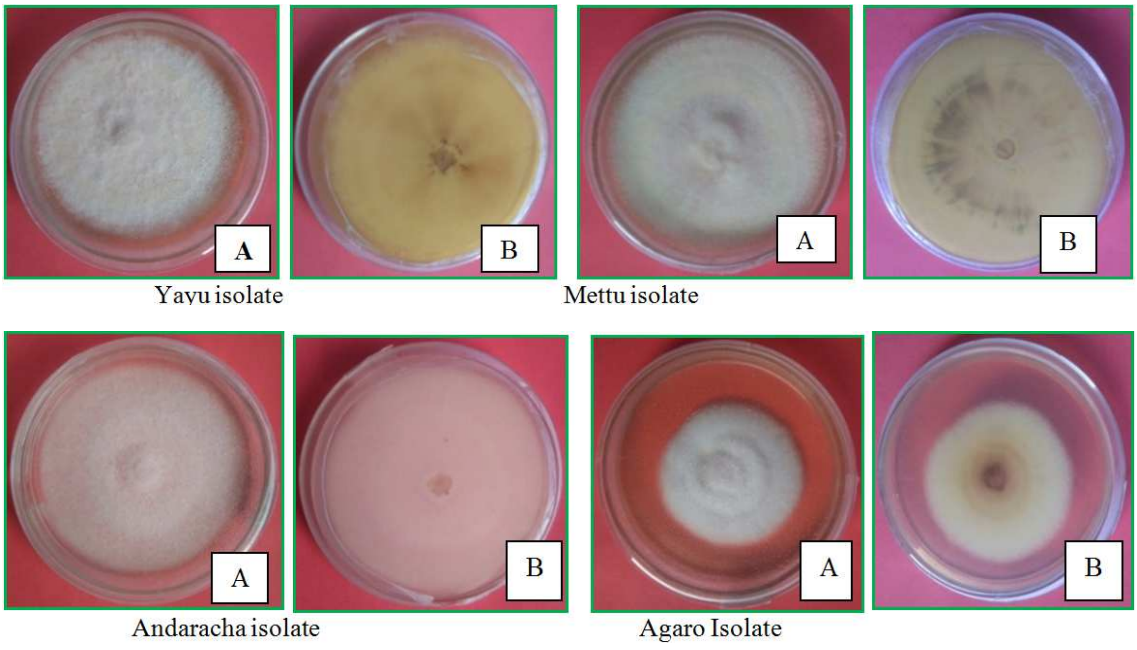
Table 3. *Colony growth rate, hyphal width, spore width and length of C. koleroga isolates of southwestern Ethiopia in 2017.*

Isolates	GR (mm)	HT (mμ) HD		SW (mμ) SW		SL (mμ) SL	
		Range	Average	Range	Average	Range	Average
Andarach	9.00[a]	3.75-5	4.84	3.75-5	4.84	11.25-13.75	12.71[ab]
Shebesombo	8.97[a]	3.75-5	4.84	3.75-5	4.84	11.25-13.75	12.65[ab]
Mettu	8.80[a]	3.75-5	4.79	3.75-5	4.88	12.50-13.75	12.58[ab]
Shako	8.50[b]	3.75-5	4.88	3.75-5	4.79	12.50-13.75	12.83[a]
Yayu	8.40[bc]	3.75-5	4.84	3.75-5	4.75	10.00-12.50	11.75[cd]
Didu	8.13[bc]	3.75-5	4.96	3.75-5	4.96	10.00-12.50	11.67[d]
Masha	8.10[d]	3.75-5	4.84	3.75-5	4.84	12.50-13.75	12.62[ab]
Agaro	7.50[e]	3.75-5	4.84	3.75-5	4.92	10.00-12.50	12.55[b]
Debubbench	7.43[e]	3.75-5	4.92	3.75-5	4.96	10.00-12.50	11.75[cd]
Gera	6.73[f]	3.75-5	4.79	3.75-5	4.79	10.00-12.75	12.67[ab]
Alle	6.46[f]	3.75-5	4.84	3.75-5	4.88	10.00-12.25	12.00[c]
CV (%)	2.04		1.74		2.20		1.23
LSD (0.05)	0.28		0.12		0.18		0.26

GR=Colony growth rate in diameter per day, HT=Hyphal thickness, SW=Spore Width, SL=Spore Length, Means in a column followed by the same letter are not significantly different at $p<0.05$.

Table 4. *Cultural characteristics of Corticium koleroga isolates on PDA at 10 days incubation.*

Isolates	Colony color			Colony	
	Front	Back	Form	Elevation	Margin
Mettu	White	Flora white	Circular	Flat	Entire
Alle	White	Floral white	Circular	Flat	Entire
Yayu	White	Floral white	Circular	Flat	Entire
Gera	Floral white	Floral white	Circular	Flat	Entire
Agaro	White	Floral white	Circular	Flat	Entire
Andaracha	White	Floral white	Circular	Flat	Entire
Masha	White	Floral white	Irregular	Flat	Filiform
Shako	White	Floral white	Irregular	Flat	Filiform
Debubbench	White	White	Circular	Flat	Entire
Shebesombo	White	White	Circular	Flat	Entire
Didu	White	White	Circular	Flat	Entire



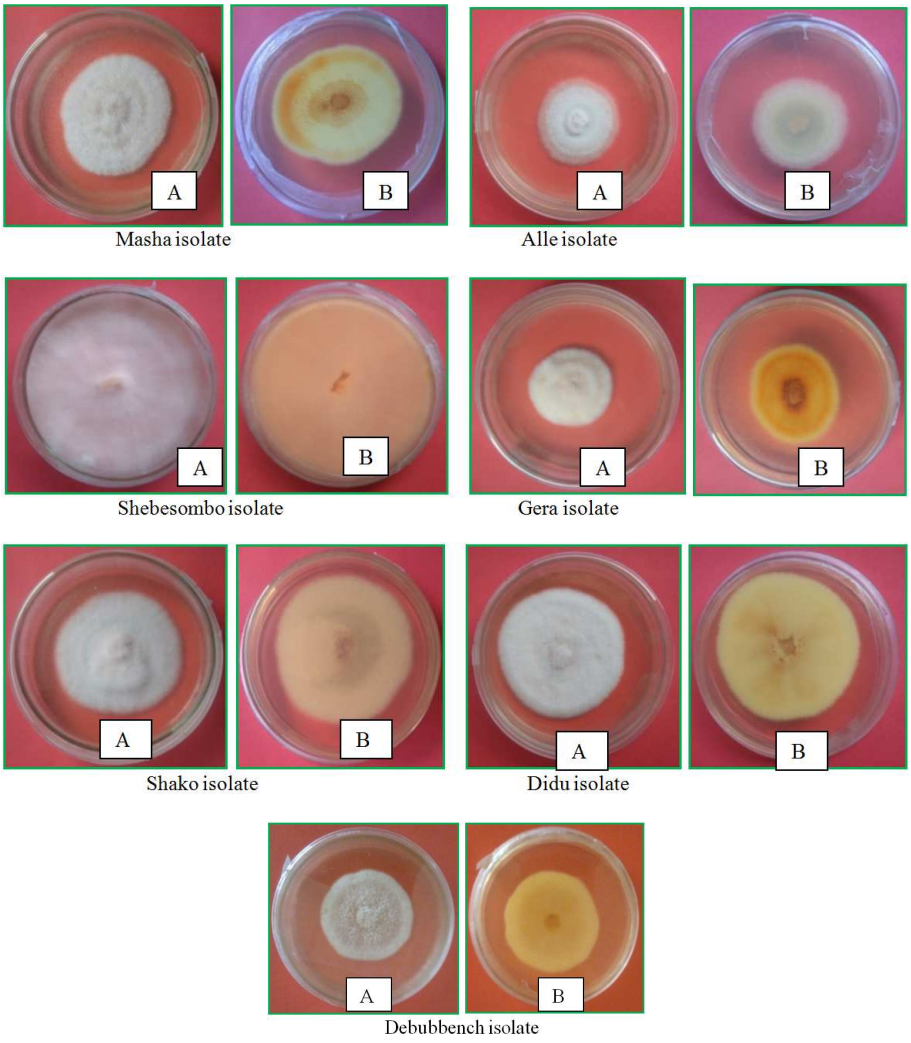


Figure 3. *Colony morphology of C. koleroga isolates on PDA at 10 days incubation: A) Front side and B) Back side reverse.*

3.1.2. Microscopic Identification

Hyphae

Pure culture of *C. koleroga* showing long, hyaline, wide angled branching mycelia (Figure 4B) and more or less uniform hyphal thickness measuring 3.75 to 5.00 mμ (Figure 4C; Table 3) were observed under microscope. Burt [12] also characterized mycelium of this pathogen in similar way.

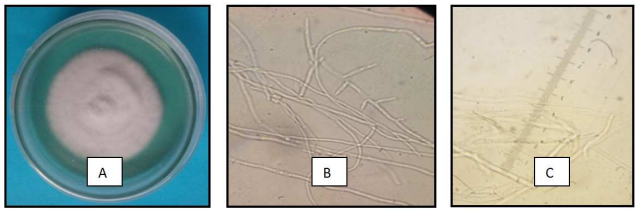


Figure 4. *A) Colony morphology of a 7-day-old culture of isolate from Agaro, B) Hyphae of C. koleroga under microscope, C) Hyphal diameter under 40x objective compound microscope of isolate from Agaro.*

The texture of the hyphae of *C. koleroga* seemed too filamentous because mostly the mycelia of all the isolates were found in compacted form in groups. Once the pathogen started to produce basidiospores, the basidiospores were found scattered over the surface of the hyphae, which is due probably to the presence of gelatinous materials over the surface of hyphae.

Basidia

The result of this study indicated that the basidia of *Corticium koleroga* are ellipsoid to oblong in shape, hyaline in color, not septate, thicker than width of supporting hyphae on which 4-6 basidiospores are directly fixed. It produces the primary basidial cell (probasidium), which is preceded by the final stage of the basidium (metabasidium) and that is collapsed after spore formation (Figure 5). This result is in harmony with previous reports [13-15].

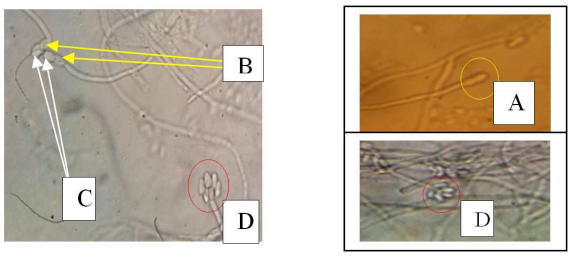


Figure 5. *Fruiting body of the fungus C. koleroga:- A) Basidiole B) Probasidia, C) Metabasidia D) Collapsed basidia and basidiospores under compound microscope of 40x objective lens of isolate from Agaro.*

Basidiospores

Basidiospores produced by isolates of *C. koleroga* collected from different parts of southwestern Ethiopia are fusiform in shape (Figure 6). Statistically there were no significant differences in width of basidiospores among the isolates but there were statistically significant difference ($p \leq 0.05$) among the isolates in spore length. The length and width of basidiospores varied from 10 to 13.75 mμ x 3.75 to 5 mμ, respectively. Basidiospores of *C. koleroga* appeared as smooth, hyaline, narrow and fusiform in shape measuring 10 to 13.75 mμ x 3.75 to 5 mμ in size (Tables 3 and 5).

Table 5. *Morphological characteristics of Corticium koleroga isolates collected from some districts of southwestern Ethiopia in 2017 cropping season.*

Isolates	Basidia				Basidiospore		
	Probasidia	Meta-Basidia	Colour	Shape	Colour	Shape	Septa
Mettu	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Alle	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Yayu	+	+	Hyaline	Ellipsoid	Hyaline	Fusiform	-
Gera	+	+	Hyaline	Ellipsoid	Hyaline	Fusiform	-
Agaro	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Andaracha	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Masha	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Shako	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Debubbench	+	+	Hyaline	Ellipsoid	Hyaline	Fusiform	-
Shebesombo	+	+	Hyaline	Oblong	Hyaline	Fusiform	-
Didu	+	+	Hyaline	Ellipsoid	Hyaline	Fusiform	-

+=Peresent -=Absent.

The basidiospores were found to adhere or cluster frequently in groups of four to six, which would indicate that most probably basidia have six basidiospores (Figure 6A), which agreed with the work of [16, 17]. The mycelia and basidiospores of *C. koleroga* appeared to be attached together into a layer, so that not a basidiospores or mycelium can be removed from the mass without difficulty (Figure 6C) as described by Rogers [14].

The basidiospores were scattered over the surface of hyphae and seated on it and separated from each other with difficulty. Basidiospores were attached and scattered over the mycelia at irregular intervals on the threads without any visible pedicel (Figure 6C). Basidiospores of *C. koleroga* isolates were attached to their mycelium by gelatinous matrix.

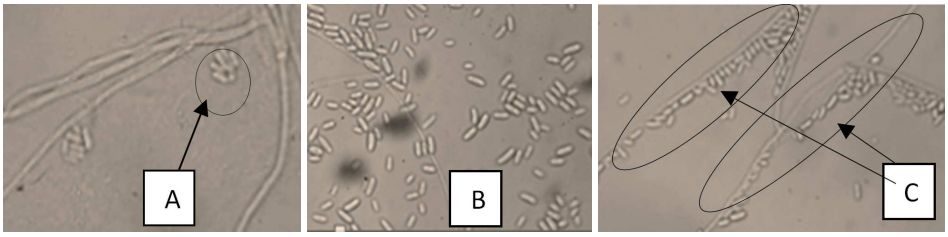


Figure 6. *Basidiospores of C. koleroga under compound microscope at 40x magnification. A) A group of six basidiospores, B) Scattered basidiospores, C) Agglutinated mycilia with basidiospores.*

3.2. Pathogenicity Tests

The pathogenicity tests with eleven isolates were done on detached leaves and twigs of *Coffea arabica* susceptible variety 74110. Lesions of thread blight became visible on detached twigs and leaf petioles at the 6th day after inoculation with the *C. koleroga* isolates from Yayu and Andaracha. These lesions were black to ashen strands on twigs, leaves' petioles and leaves. The initial infection sizes of the disease on twigs and leaves were initially small ≤3 mm in lengthand ≤13% The necrotic symptoms described earlier were the same as that observed on leaves and twigs of *C. arabica* plants by natural infection in the field, whereas no symptoms developed on the control leaves and twigs (Figures 7, 8). Re-isolation of the fungus from symptomatic twig and leaf tissues (inoculated with the isolates) was done on PDA medium. The morphology and cultural characteristics of pure cultures of the re-isolated fungus were the same as that of the original culture of the isolates, fulfilling Koch's postulates.

Longer incubation period of 6 to 10 days was required for symptom development on the detached twigs probably due to non-succulent woody tissue of the coffee twigs and low germination of the basidiospores, whereas relatively shorter incubation period of 5 to 8 days was required for symptoms development on detached leaves because of the availability of the stomata on the leaves in nature. The result of the test revealed that both twigs and leaves of coffee can be infected by the *C. koleroga* isolates. There was highly significant difference ($p \leq 0.01$) in thread blight lesion size among *C. koleroga* isolates in detached twig and leaf inoculation tests.

All isolates of *C. koleroga* tested in the current experiment were pathogenic and destructive on both *Coffea arabica* twigs and leaves. Average lesion size on the twigs varied from 52% by Shebesombo isolate to 94% by Yayu isolate, when evaluated at 20 days after inoculation (Table 6). On the detached leaves, lesion size ranged between 70% in

Debubbench isolate and 99% in Yayu isolate at 20 days after inoculation. These current results suggest a slight difference in CTB intensity on the leaves and twigs with the former being more susceptible in the conditions of the trial.

Figure 7. *Pathogenicity symptom on A) Water sprayed 20 days after sprayed B) Andaracha isolate inoculated on leaf 10 days after inoculation (DAI) C) Andaracha isolate inoculated on leaf 20 DAI.*

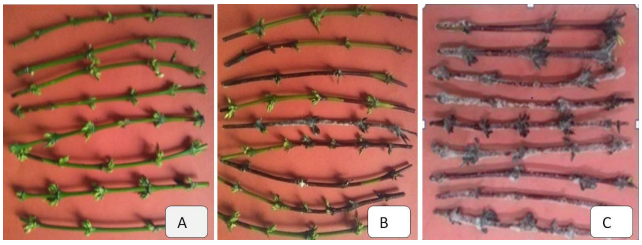


Figure 8. *Pathogenicity symptoms on A) Water sprayed 20 days after sprayed on twigs B) Yayu isolate inoculated on twigs 10 DAI C) Yayu isolate inoculated on twigs 20 DAI.*

These tests also revealed that Yayu and Andaracha isolates of *Corticium koleroga*, were more aggressive on both twigs and leaves than other *C. koleroga* isolates collected from different locations. Both areas represent midlands.

Table 6. *Severity of thread blight on coffee plant parts inoculated with C. koleroga isolates collected from 11 districts of southwestern Ethiopia in 2017.*

Isolates	Lesion size on twig (%)*	Lesion size on leaf (%)*
Yayu	94.43^{a}	96.67^{a}
Mettu	91.33^{a}	95.25^{ab}
Andarach	90.48^{ab}	98.50^{a}
Masha	80.45^{abc}	84.97^{abc}
Shako	77.89^{a-c}	78.63^{abc}
Shebesombo	74.12^{b-d}	74.18^{bc}
Gera	71.08^{cd}	74.53^{bc}
Debubbench	70.78^{cd}	69.50^{c}
Didu	63.78^{c-e}	71.83^{c}
Alle	60.55^{de}	71.00^{c}
Agaro	51.65^{de}	71.07^{c}
Control	0.00^{e}	0.00^{d}
LSD (0.05)	15.33	9.19
CV (%)	8.17	9.89

*=Average of three replications.
Means with the same letter (s) are statistically not significantly different at 0.05 probability level.

4. Discussion

Thread blight of coffee was prevalent in major coffee producing West, South [5] Southwest [6] Ethiopia. It attacks all coffee plant parts except roots and reducing the yield, quality and marketability. The basic keys for identification of *C. koleroga* were growth rate, colony on PDA, hyphae, basidial and basidiospore features [18]. On the basis of macroscopic and microscopic characters, the thread blight-causing fungus was identified as *Corticium koleroga.*

The optimum temperature required for the growth and development of *C. koleroga* is 25°C (Talbot, 1954). In his description of *C. koleroga*, Cooke [19] did not clearly see the perfect state and gave the name *Pellicularia* to the imperfect state, that is, to the vegetative hyphae, which was later rejected by Venkataran [15]. However, Rogers [14] previously reported that *C. koleroga* was characterized by basidia, which are not septate, do not possess stout, swollen sterigmata and that produce basidiospores and germinate directly to form a mycelium. The same author had given a clear account on basidia of *C. koleroga,* terming them apobasidia with the definition of an apobasidium as a basidium whose basidiospores are not apiculate. The same author grouped *C. koleroga* under a group of Homobasidiomycetes other than a Heterobasidiomycete.

Burt [13] concluded that the cell walls of the hyphae of the *C. koleroga* are gelatinous in nature. Texture is a character of *Corticium koleroga*, which can be modified with chemicals, age, thickness and moistness of the pathogen [18]. Currently, the characteristics of the thread blight-causing pathogen isolated from the C*offea arabica* in Ethiopia is quite similar with the original pathogen re-described by Hoehnel [17] from coffee twigs.

5. Conclusions

Thread blight caused by *C. koleroga* is becoming an important disease of coffee in southwest Ethiopia. Based on its signs and symptoms on coffee tree, cultural and morphological characters and pathogenicity test the identity of the pathogen was confirmed to be *Corticium koleroga. C. koleroga* isolates had white to floral cultural colony colour, with filiform to entire margins, growth rate ranging 6 to 9 mm/day, hyphal width ranged 3.75 mμ to 5 mμ and basidiospore size ranged from 10 to 13.75 mμ x 3.75 to 5 mμ. All *C. koleroga* isolates tested for pathogenicity were pathogenic to coffee twigs and leaves with different aggressiveness. Since the present status of CTB is remarkably on increasing trend, it is recommended to:- Study safe, effective and environment-friendly disease management options, such as cultural practices, biological control, fungicides and integrated disease management.

Acknowledgements

The authors would like to thank the Ethiopian Institute of Agricultural Research for the financial support. Special thanks also go to Jimma Agricultural Research Centre (JARC) and Mettu Agricultural Research Sub-Centre (MARsC) for facilitating logistical support.

The authors also acknowledge the colleagues at Jimma Agricultural Research Centre and Mettu Agricultural Research Sub-centre for their helpful suggestions and

technical support during the study.

References

[1] Pendergrast, M., 2009. Coffee second only to oil? Tea & Coffee Trade Journal. April: 38-41.

[2] FAS (Foreign Agricultural Service/USDA). (2018) Coffee: World Markets and Trade.

[3] Cavalcante M, Sales F. (2001) Ocorrência da queima-do-fio (*Pellicularia koleroga*) Emcafezaisem Rio Branco. Empresa Brasileira de Pesquisa Agropecuaria-Embrapa Acre, Rio Branco.

[4] Eshetu D, (1997) Coffee diseases and their significance in Ethiopia. *Agricultural Science,* 17: 723-726.

[5] Kifle B, Demelash T, Legesse H. (2015) Coffee Thread Blight (*Corticium koleroga*): a Coming Threat for Ethiopian Coffee Production. *Journal of Plant Pathology Microbiology,* 6: 303-308.

[6] Nagassa Dechassa, Alemayehu Chala, Kifle Belachew, Elfinesh Shikur. An Investigation on Coffee Thread Blight Caused by *Corticium koleroga* (Cke) Hoehnel and Its Associated Factors in Southwest Ethiopia. *Journal of Drug Design and Medicinal Chemistry*. Vol. 6, No. 3, 2020, pp. 22-29.

[7] Zhu G, Yu Z, Gui Y, Liu Z. (2008) A novel technique for isolating orchid mycorrhizal fungi. *Fungal Divers*, *33* (12), p. 123.

[8] Than P, Jeewon R, Hyde D, Pongsupasamit S, Mongkolporn O, Taylor J. (2008). Characterization and Pathogenicity of *Colletotrichum* Species Associated with Anthracnose on Chilli (*Capsicum* spp.) in Thailand. *Plant Pathology* 57: 562-572.

[9] Verma S. (1991) Factor affecting the development of pink canker of apple. Plant Disease Research 6: 40-45.

[10] Wheeler B. (1969) An Introduction to Plant Disease. John Wiley Sons Ltd., London, pp. 301. 244.

[11] SAS (Statistical Analysis System). (2012) Version 9.3, SAS Institute, Cary, NC, USA.

[12] Burt A. (1918) *Corticium* Causing *Pellicularia* disease of the coffee, Hypochnose of Pomaceous fruits and Rhizoctonia disease. *Annual Molecular Botanical Garden*, 5: 119-132.

[13] Burt A. (1926) Thelephoraceae of North American *Corticium*. *Annual Molecular Botanical Garden,* 13: 173-354.

[14] Rogers D. (1947) A new gymnocarpous heterobasidiomycete with gasteromycetous basidia. *Mycologia,* 39: 556-564.

[15] Venkatarayan S. (1949) The validity of the name *Pellicularia koleroga* Cooke. *Indian Phytopathology* 2: 186-189.

[16] Wakefield G. (1913) Differential characters in some resupinate Hymenomycetes. *Plant Diseases of International Importance*, 4: 113-120.

[17] Hoehnel V. (1910) Fragmente zur Mykologie. *Mitteilung*, 10: 468-526.

[18] Talbot P. (1954) Micro Morphology of the Lower Hymenomycetes. *Bothnlia* 6: 249-299.

[19] Cooke C, (1876) Some Indian fungi *Pellicularia* and affinities of *Pellicularia. Grevillea*, 4: 134-135.