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Distribution of Coffee Berry Disease (*Colletotrichum kahawae*) in Seyo Nole District, Oromia Region of Ethiopia, and *In-vitro* evaluation of plant extracts and Fungicides against the pathogen

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Abstract

Coffee Berry Disease (CBD), caused by *Colletotrichum kahawae* is a significant challenge to Arabica coffee cultivation in Ethiopia. This study aimed to assess the current status of CBD in the Seyo-Nole district of the West Wellega Zone and evaluate the effectiveness of various plant extracts and fungicides against the pathogen. Ninety coffee farms were surveyed to determine the disease's prevalence and severity. Diseased berries were collected from each farm for pathogen isolation, characterization, and in-vitro antifungal management evaluation. The evaluation of botanicals and fungicides was conducted using food poison techniques, measuring the radial growth of the fungus to determine antifungal activity. Seven aqueous and five ethanol extracts of medicinal plants were tested at 25% (w/v) concentration, along with two fungicides at 2000 ppm. The survey results showed significant variation in CBD distribution and status among and within Peasant Associations, with disease incidence ranging from 25% to 45.3% and disease severity from 23.5% to 45.23%. The in-vitro antifungal assay results indicated that both fungicides and medicinal plant extracts significantly inhibited ($p<0.01$) the pathogen. Propiconazole and Difenoconazole achieved the highest inhibition (100%). Among the aqueous plant extracts, Papaya Leaf Extract showed the highest inhibition (97.71%), followed by Eucalyptus Leaf Extract (96.50%), with the lowest inhibition recorded for Orange Leaf Extract (77.10%). The ethanol extracts exhibited superior results, with Papaya Leaf Ethanol Extract achieving 99.28% inhibition, followed by Eucalyptus Leaf Ethanol Extract (98.34%), and the lowest inhibition by Acacia Leaf

Ethanol Extract (87.29%). The use of Propiconazole, Difenoconazole, and plant extracts from *Carica papaya*, *Eucalyptus saligna*, and *Datura stramonium* for the management of Coffee Berry Disease. The plant extracts exhibiting significant inhibition should undergo further testing and formulation.

Key words : Arabica Coffee, Coffee Berry Disease, in-vitro, inhibition, medicinal plant, plant extracts.

Ethiopia is the origin of highland coffee (*Coffea arabica* L.), which is one of the country's most valuable cash crops. Coffee is the most important agricultural export crop, accounting for 60% of foreign exchange revenues[19]. The coffee industry generates roughly 40% of the country's GDP and employs nearly 5 million farmers, with a quarter of the nation's population dependent on coffee for their livelihood[11]. In Ethiopia, 764,863.16 hectares were dedicated to coffee cultivation in 2018/19, producing 494,574.36 tons with an average productivity of 0.64 tons per hectare, accounting for 30% of total production[8]. Of the 25 top coffee-producing districts, 18 are located in the Oromia region, while the rest are in the Southern Nations, Nationalities, and Peoples' Region[13]. Seyo Nole district is a leading coffee producer in the Oromia region's West Wollega Zone, covering a substantial area of land. According to data from the Seyo Nole Agriculture and Natural Resource Office, the total area covered by coffee in the district is 158,120 hectares (Seyo Nole Agricultural and Natural Resource Office, unpublished, 2022).

Despite its significant importance, coffee cultivation faces numerous challenges, including Coffee Berry Disease (CBD) caused by the fungus *Colletotrichum kahawae*. Due to CBD and other factors, the average green coffee bean yield per hectare per year is 0.64 tons, much lower than the global average of 0.8 tons per hectare and the Brazilian average of 1.3 tons per hectare. Alemu *et al.,* [4] reported that CBD incidence ranges between 10-80% in Borena, 40-100% in Gedeo and West Hararghe, 10-90% in Illubabor, and 30-90% in Jimma and Sidama, with 30-80% in West Wollega. A survey indicated that West Hararghe (71%) and Gedeo (65%) had higher CBD rates, whereas Jimma had the lowest at 59% [4]. Nationally, CBD severity on local landraces averages 24-30% [9]. The disease has since spread to all coffee-producing regions, thriving under favorable environmental conditions. The severe economic threat posed by CBD to Ethiopia is profound, as coffee exports account for 25% of the country's foreign currency earnings[12]. CBD occurrence and intensity vary by location and season due to host sensitivity, pathogen aggressiveness, and environmental conditions[3]. During the 2014 cropping season, the national average incidence and severity of CBD were 52.5% and 29.9%, respectively, indicating a rising trend[3]. Consequently, C. kahawae was classified as a quarantine pathogen and a biological weapon[5,15].

The use of chemical fungicides is the most common practice for managing CBD, but this can lead to fungal resistance[21]. Continuous

and inappropriate use of chemical fungicides is not a sustainable solution due to increased investment costs, high levels of toxic residues, and concerns for human health and environmental impact[7]. Fungicides like Propiconazole 25% EC and Difenoconazole 25% EC have been effective against C. kahawae, but products like Dyrene and Octave were banned due to their side effects[9]. Moreover, fungicides can negatively affect beneficial microorganisms that antagonize the CBD pathogen[16]. The high cost of pesticides, the emergence of fungicide-resistant pathogen biotypes, and the social and health-related problems of conventional agriculture have increased interest in sustainable agriculture and biodiversity conservation. Additionally, millions of coffee farmers face challenges with low coffee prices and a growing interest in organically grown coffee worldwide[16].

Plant extracts with antifungal properties are gaining attention due to their specificity, biodegradability, and low toxicity[20]. For instance, Allium sativum extracts have been evaluated against *C. camelliae*, showing complete inhibition of spore germination[20]. In Ethiopia, there is limited knowledge on the antifungal action of medicinal herbs against fungal phytopathogens. However, extracts from plants like *Allium sativum* have shown potential for inhibiting plant pathogenic fungi[4], suggesting they could be a better biological alternative to chemical fungicides. Therefore, this study aims to assess the effectiveness of plant extracts and fungicides against *Colletotrichum kahawae* in vitro and the current status of Coffee Berry Disease in the Seyo Nole district of West Wollega Zone and develop a management strategy to increase coffee yield.

Description of the Study Area :

The study was conducted in the Seyo-Nole district, located 475 km west of Addis Ababa, Ethiopia. The geographical coordinates of Seyo-Nole are 9.5380° N latitude and 36.3030° W longitude, with an elevation of 1845 meters above sea level (Table-1). The area receives an annual rainfall ranging from 1500 to 1600 mm and experiences temperatures ranging from 13.7°C to 32.9°C. The predominant soil type in the study site is sandy loam

Table-1. Description of Coffee Berry Disease Survey Peasant Associations (PAs) at Seyo Nole District of West Wollega Zone in 2022.

No	Kebeles	Latitude(0N)	Longitude(0E)	Altitude (m.a.s.l)
1	Ganka Incini	8.421	35.241	1724
2	Gudatu Debesso	8.532	35.485	1795
3	Alito Gatiro	8.454	35.247	1624
4	Haro Anani	8.575	35.489	1843
5	Oda Lalisa	8.654	35.242	1920
6	UlmayaAba Galmo	8.756	35.488	1864
7	Kontomi Guto	8.451	35.453	1521
8	Qore Sibo	8.352	35.654	1679
9	Guto Didibe	8.751	35.432	1565

Source**:** Seyo Nole Agricultural and Natural Resource office Report unpublished, 2022.

Survey procedure :

The survey involved interviews with coffee farm owners and community members in all sampled locations. The research team consulted with experts and development agents (DAs) from the Agricultural Development Office of the District to understand the objectives of the survey and identify potential coffee production areas. Nine Peasant Associations (PAs) were selected, representing three agro-ecological zones: lowland, mid-altitude, and highland. From each PA, coffee farms were randomly selected as sample plots, at intervals of 2-5 kilometres, following the methodology outlined by Morgan *et al.,*[17].

In each selected farm, observations were made to collect critical disease data and assess the farming system through a transect walk. Additionally, farm owners were interviewed using structured questionnaires to gather primary information about their coffee farms. The questions covered aspects such as coffee varieties, the age of the farm, the number of trees, major problems in coffee production, and disease management practices employed. Overall, 90 field observations and interviews were conducted to achieve the study's objectives.

Data collection

Coffee Berry Disease (CBD) Assessment :

In each selected Peasant Association (PA) of the Seyo Nole district, CBD assessments were conducted at intervals of 2-5 kilometres across the farmers' coffee fields. The assessment considered the presence and absence of coffee farms within each PA. For each selected farm, two types of variables were measured: disease incidence and disease severity. The following procedures were used for the assessments :

Disease Incidence :

In each farmer's field, five plots of 10m x 10m were randomly selected in a zigzag pattern. The presence or absence of the disease on each tree within these plots was visually diagnosed. Disease incidence was then calculated as the number of diseased trees divided by the total number of observed trees and expressed as a percentage. The percentage of disease incidence was computed using the following formula: This methodology follows the approach outlined by Morgan *et al.,*[17].

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected coffee trees}}{\text{Total number of assessed trees in the farm field}} \text{ X } 100$$

Disease severity (Berry counting) :

From each plot assessed for disease incidence, five trees were randomly selected. Each selected tree was divided into three strata of branches: top, middle, and bottom. From each stratum, two branches were chosen to evaluate disease severity. Both CBD-damaged and healthy berries on these branches were counted. The percentage of diseased berries relative to the total number of berries counted was calculated to determine disease severity, which was then expressed as a percentage. The percentage of disease severity was computed using the following formula:

$$\text{Disease severity} = \frac{\text{Damaged counted berry}}{\text{Total counted berry}} \text{X} 100$$

This method ensures a comprehensive

assessment of disease severity by considering different parts of the tree and various branches within each stratum.

On-Farm CBD Severity scoring :

On-farm CBD severity scoring was carried out using similar procedures on the same plants selected for DI assessment. Three branches from three strata (top, middle, and bottom) were selected from each tree. CBD severity was estimated using a 0-6 disease score scale (Table-2), via critical observation of the lesion size and its extent (spread) on the diseased berry parts using a field guide[14]. Accordingly, the percent severity index (PSI) was computed as follows :

$$PSI) = \frac{\text{sum of numerical rating}}{\text{Total number of rated plant} \times \text{max,score of the scale}} X100$$

Table-2. Assessment Key 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; approximately 3mm 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; approximately 15 mm in diameter
6	>99% or the whole surface of berries covered with black sunken lesions; mummified berries

Foot note: *adopted from Mohammed and Jambo, 2015 with slight modification*
This method ensures a comprehensive assessment of disease severity by considering different parts of the tree and various branches within each stratum, providing an accurate representation of the disease's impact on the coffee plants.

Pathogen isolation and characterization :

Colletotrichum kahawae was isolated from green infected coffee berries. Pericarp fragments from infected green berries were thoroughly washed in tap water, cut into small pieces of about 2 mm^2, surface disinfected with 70% alcohol, rinsed in sterilized distilled water, and plated on Petri dishes containing 20 ml Potato Dextrose Agar (PDA) medium. The plates were incubated at 25°C for 3-5 days. Hyphal tips of the growing fungus were then transferred to freshly prepared PDA medium. This process was repeated several times to obtain pure cultures. The fungus was identified under a compound microscope based on cultural characteristics, following identification guides and species descriptions by Waller *et al.,*[23]. Macroscopic characterization of C. kahawae isolates was conducted according to procedures by Zeru and Biratu[6,24]. Cultural variations of the isolates were examined by comparing cultures grown on PDA plates in three replications. The mycelial tips of old cultures were aseptically transferred to the center of PDA plates and incubated at 25°C in three replications. Cultural features such as colony colour, texture, and mode of growth were examined 7 days after incubation. Additionally, the radial growth of each isolate was measured using a ruler from two directions

on the reverse side of the Petri dishes at 3-day intervals. Mean diameter of the fungal colony growth and the rate of radial growth were calculated.

Preparation of plant extracts :

Fresh leaves of *Carica papaya*, *Cymbopogon citratus*, *Datura stramonium*, *Ocimum gratissimum*, *Eucalyptus saligna*, *Acacia nilotica*, and *Croton macrostachyus* were collected from the Ambo area. The collected samples of each plant species were washed under tap water, air-dried at room temperature, and ground into powder using an electrical grinder. The powder from all samples was carefully stored at 4°C.

For aqueous extracts, 100 g of the powdered samples were dissolved in 500 ml of distilled water and mixed thoroughly. The mixture was filtered using cheesecloth, followed by Whatman filter paper after 48 hours of incubation at room temperature. The stock extracts were transferred into labelled sterile bottles and stored at 4°C [1]. A 70% ethanol extract was prepared according to the method by Alad and Irobi.[2] with slight modifications. 100 g of each dried powder were dissolved in 500 ml of 70% ethanol for 48 hours. The extract was filtered through cheesecloth, followed by Whatman No. 1 filter paper. The organic solvent was evaporated under an oven at 30-40°C at room temperature. The remaining extract was diluted by adding an appropriate quantity of sterilized distilled water to make a final dilution of 25% extract. The stock extracts were transferred into labelled sterile screw-capped bottles and stored at 4°C for future use.

In Vitro Assay :
Experimental design :

The in vitro antimicrobial assay of plant extracts and standard chemicals was conducted in the plant pathology lab of Ambo University, Mamo Mezemir Campus. The experiment was laid out in a Completely Randomized Design (CRD) with 15 treatments and three replications. Seven individual botanicals (*Carica papaya, Cymbopogon citratus, Datura stramonium, Ocimum gratissimum, Eucalyptus saligna, Acacia nilotica, and Croton macrostachyus*) were evaluated using both aqueous and ethanol extractions. Two chemical fungicides, Score (Difenoconazole 25% EC) and Tilt (Propiconazole 25% EC), were also evaluated along with a control.

Poisoned food technique :

The poisoned food technique[18] was used to screen the antimicrobial potential of plant extracts at concentrations of 15%, 20%, and 25% for both aqueous and ethanol extracts. Similarly, the two fungicides were evaluated at concentrations of 1000 ppm, 1500 ppm, and 2000 ppm. Preliminary evaluations indicated that the most effective concentrations were 25% for plant extracts and 2000 ppm for fungicides.

The required amount of each plant extract and fungicide was mixed thoroughly with autoclaved and cooled (40°C) Potato Dextrose Agar (PDA) medium in conical flasks to obtain the desired concentrations. Plain PDA medium without treatment served as the control. The PDA medium amended with treatments was poured aseptically into Petri plates (90 mm dia.). After the medium

solidified, all plates were inoculated aseptically with 5 mm culture discs of the test pathogens obtained from a week-old actively growing pure culture. The disc was placed in an inverted position at the center of the Petri plates, and the plates were incubated at 27±1°C. Each treatment was replicated thrice. The plates were wrapped with parafilm to minimize contamination and incubated at 25±1°C in an incubator. When the medium in the untreated control plates was fully covered with mycelial growth of the test pathogens, radial mycelial growth was measured in all treatment plates. Observations on the colony diameter of each fungus were recorded at 24-hour intervals until the control plates were completely filled with mycelial growth. Percent inhibition of mycelial growth in treated plates was calculated using Vincent's[22] formula:

Percent of inhibition (I) %=(GC-GT/GC) *100,
Where: C = Growth (mm) of test fungus in untreated control plates, T = Growth (mm) of test fungus in treated plates.

Statistical analysis :
Efficacy of plant extracts and Fungicides :

The efficacy data from the in vitro assays of plant extracts and fungicides against C. kahawae were subjected to analysis of variance (ANOVA). This statistical method was used to determine if there were significant differences among the treatments. The ANOVA was conducted at two levels of significance: $p<0.01$ (highly significant) and $p<0.05$ (significant).

Statistical procedures :

The use of these statistical methods ensured a thorough analysis of the data, providing clear insights into the distribution of coffee berry disease, the characteristics of C. kahawae isolates, and the efficacy of various plant extracts and fungicides in controlling the pathogen. Where ANOVA detected significant differences among treatments, the treatment means were separated using the Least Significant Difference (LSD) test. This post-hoc analysis helped to identify which specific treatments differed significantly from each other. The LSD test was chosen for its simplicity and effectiveness in comparing means when ANOVA indicates a significant F-value.

Incidence of Coffee Berry Disease :

The survey results indicated a significant variation in the distribution and status of Coffee Berry Disease (CBD) among and within Peasant Associations (PAs) in the Seyo Nole district. Disease incidence ranged from 23.5% to 45.3% across the assessed PAs. Specifically, the mean incidence was lowest at Alito Gatiro (23.5%) and highest at Haro Anani (45.3%), with an overall average mean incidence of 34% (Fig. 1). The highest disease incidence was recorded at Haro Anani (45.30%), followed by Oda Lalisa (43.10%) and Kontomi Guto (38.40%). Conversely, the lowest incidences were noted at Gudatu Debesso (24.25%) and Alito Gatiro (23.50%). These findings underscore the significant impact of CBD on coffee trees throughout the surveyed fields. The high incidence of CBD in certain areas, such as Haro Anani, may be attributed to the particularly high rainfall and relatively high altitude, which create favourable conditions for the development of the disease. Garedew *et al.,*[10] explained that high rainfall, high humidity or wetness, and relatively low temperatures persisting for long

periods are conducive to CBD development, particularly at higher altitudes. This correlation was observed in the current study, where areas with higher rainfall and altitude experienced higher incidences of CBD.

There was remarkable variation in CBD distribution among and within the kebeles of the Seyo Nole district. The present findings align with earlier reports by Kumlachew *et al*. (2016), who found that CBD incidence ranged between 10% and 80% in Borena, 40% and 100% in Gedeo and West Hararghe, 10% and 90% in Illubabor, 30% and 90% in Jimma and Sidama, and 30% and 80% in West Wollega. Similarly, higher CBD incidences were reported in West Hararghe (71%) and Gedeo (65%), with Jimma recording an incidence of 59%. The increasing trend of CBD incidence in Ethiopia has significant implications for both domestic and foreign coffee markets. Alemu *et al.,*[3] reported that the current status of CBD in Ethiopia is rising, posing a threat to coffee production and exports. This increasing trend necessitates the implementation of effective disease management strategies to mitigate the negative impact of CBD on coffee production.

In summary, the findings highlight the importance of understanding the environmental and management factors contributing to CBD incidence. Targeted interventions, such as improved disease management practices and the introduction of resistant coffee varieties, are crucial for reducing the impact of CBD in high-incidence areas. Further research and continuous monitoring are essential to develop sustainable solutions to combat this significant threat to Ethiopia's coffee industry.

Severity of Coffee Berry Disease :

The survey results revealed a wide range of disease severity, varying from 23.5% to 45.23% across different Peasant Associations (PAs) in the Seyo Nole district. The mean

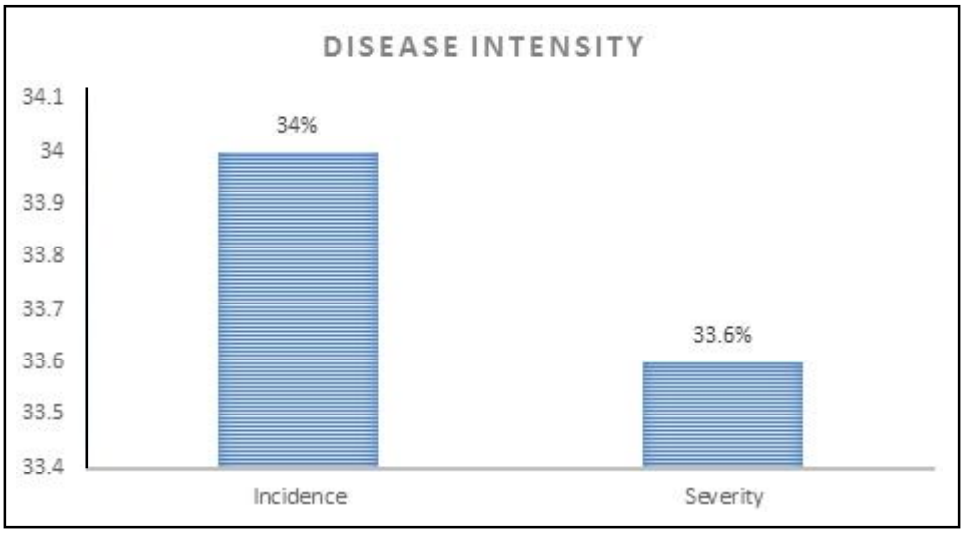

Figure 1. Mean disease intensity in Seyo Nole district

severity was lowest at Alito Gatiro (23.5%) and highest at Haro Anani (45.23%), with an average of 33.6% 9 (Table-3). Notably, Haro Anani exhibited the highest disease severity, followed by Oda Lalisa and Kontomi Guto. Conversely, Gudatu Debesso and Alito Gatiro recorded the lowest disease severity levels. Several factors may contribute to the observed disease intensity, including climatic changes, the cultivation of homogeneous coffee cultivars, and crop management practices. The spread and magnitude of the epidemics could be attributed to these factors, along with the predominant self-fertility of *C. arabica* and uniform cultural practices over an extended period.

The significant economic costs associated with disease control using chemical interventions underscore the importance of addressing CBD effectively. Therefore, determining the current incidence and severity of CBD in different Peasant Associations (PAs) is crucial for implementing appropriate disease management strategies. This assessment provides valuable insights into the distribution and severity of CBD in the Seyo Nole district, laying the foundation for targeted management approaches to mitigate the impact of the disease on coffee production.

Growth characterization of C. kahawae Isolates :

The growth characteristics of *C. kahawae* isolates were assessed based on visual inspection of culture plates. Across the nine culture plates (petri dishes) containing the isolates, varying forms of colony texture were observed. Approximately 44.4% of the isolates exhibited a scarce colony texture, while 33.3% displayed a dense texture, and 22.3% showed

Table-3. Coffee Berry Disease Incidence and Severity in Seyo Nole District, West Wollega Zone, Oromia Region

No	Name of sample site	Incidence mean %	Severity mean %
1	Ganka Incini	27.50d	27.60d
2	Gudatu Debesso	24.20d	24.30d
3	Alito Gatiro	25.00d	23.50d
4	Haro Anani	45.30a	45.23a
5	Oda Lalisa	43.10a	40.00a
6	Ulmaya Aba Galmo	36.40b	36.46b
7	Komtomi Guto	38.40b	38.42b
8	Qore Sibo	34.30c	34.34c
9	Guto Didibe	33.02c	33.02c
CV%		12.3	18.5
LSD		0.23	0.12

Significant difference among treatments at ($p<0.05$), treatment means were separated using the Least Significance Difference (LSD). Means with the same letters are not differing significantly

a very scarce texture. Moreover, the colony colour of the isolates differed between the front and back sides of the culture plates. On the front side, the colony colours included 44.4% dark grey, 11.11% light grey, 33.33% white Gray, and 11.11% flowery white. On the back side, the majority (44.4%) exhibited light grey and light golden rod colony colours, followed by 33.33% with light grey colony colour (Table-4). During the initial 4-6 days of incubation, most C. kahawae isolates displayed a whitish mycelial colour, which gradually transitioned to light grey after further incubation. This observation is consistent with previous reports of cultural variations among *C. kahawae* isolates from different regions. Understanding the growth characteristics of *C. kahawae* isolates provides valuable insights into the variability of the pathogen, which can aid in the development of targeted management strategies for controlling coffee berry disease.

Table-4. Macroscopic characterization of *Colletotrichum kahawae* isolates

Isolate code	Colony aspect	Colony color		colony growth (radius) mm/day
		Front	Back	
KG	Scarce	Floral white	light gray	3.25b
GD	Dense	dark gray	dark gray	2.75bc
QS	Dense	dark gray	light Golden	2.1bc
Gde	Scarce	light gray	light gray	2.5bc
AG	very scarce	white gray	light gray	4.3a
GI	very scarce	white gray	light gray	4.1a
HA	Dense	dark gray	dark olive green	2.5bc
OL	very scarce	white gray	light golden rod	3.5b
UAG	Dense	dark gray	light golden rod	3.25b
CV%				3 .13
LSD				1.05

Foot Note: KG- Kontomi Guto Gde-Guto Didibe HA-Haro Anani, GD-Gudatu Dabbesso AG-Alito Gatiro OL-Oda Lalisa QS- Qore Sibo GI-Ganka Incini UAG-Ulmaya Aba Galmo

Colletotrichum species are often distinguished using cultural features as a taxonomic criterion. Differences in mycelial growth, colony characteristics, and growth rates among Colletotrichum isolates associated with coffee have allowed them to be categorized into various classes through visual inspection of cultures. However, distinguishing between Colletotrichum species culturally can be challenging and requires experience and careful observation, as some species found in coffee, such as C. gloeosporioides, C. capsici, and C. kahawae, are closely related, making their cultural identification difficult. Kebati *et al.,*[15] also contributed to the understanding of Colletotrichum species. Their work likely provided valuable insights into the characteristics and behaviour of these fungi.

Table-5. Effect of medicinal plant leaf extract and method of extraction (aqueous, ethanol and fungicides) on radial growth of *Colletotrichum kahawae* in vitro test

Method of extraction	Medicinal plants	Radial growth(mm)	Inhibition%
Aqueous extraction	PLE	2.03^{gh}	97.713^{ab}
	OrLE	20.42^{b}	77.10^{g}
	DLE	7.85^{f}	91.24^{c}
	CLE	17.35^{cd}	80.70^{ef}
	ALE	18.18^{bc}	79.80^{f}
	OLE	15.50^{d}	82.75^{e}
	ELE	3.13^{g}	96.50^{b}
Ethanol extraction	PLE	0.63^{h}	99.28^{a}
	OrLE	7.10^{f}	92.10^{c}
	ALE	11.25^{e}	87.49^{d}
	OLE	8.68^{f}	90.32^{c}
	ELE	1.48^{gh}	98.34^{ab}
Chemical	Propiconazole	0^{h}	100.00^{a}
	Difenoconazole	0^{h}	100.00^{a}
	Control	90.00^{a}	0^{h}
CV%		7.74	1.39
LSD		4.5	3.5

Foot Note: PLE -Papaya Leaf Extract, OrLE- Orange Leaf Extract, DLE- Datura Leaf Extract, CLE - Croton Leaf Extract, ALE- Acacia Leaf Extract, OLE- Osmium Leaf Extract, ELE- Eucalyptus Leaf Extract.

Evaluation of Fungicides and Plant Extracts against Colletotrichum kahawae :

The efficacy of various fungicides and plant extracts against Colletotrichum kahawae, the causative agent of coffee berry disease, was assessed through in vitro studies. Seven aqueous and five ethanol extracts of medicinal plants, along with two fungicides, were tested at concentrations of 25% (w/v) and 2000 ppm, respectively. The radial growth of the fungus was measured to determine the antifungal activity. Significant ($p<0.01$) interactions were observed between the type of fungicides, medicinal plants used, and extraction methods, indicating varied inhibitory effects on Colletotrichum kahawae. Overall, both fungicides and plant extracts significantly inhibited the mycelial growth of Colletotrichum kahawae compared to the untreated control. The efficacy of aqueous plant extracts ranged from 77.10% to 97.71%, with Papaya Leaf Extract (PLE) demonstrating the highest inhibition at 97.71%, followed by Eucalyptus Leaf Extract (ELE) at 96.50%. Ethanol extracts showed inhibition ranging from 87.48% to 99.28%, with Papaya Leaf Ethanol Extract (PLEE) exhibiting the highest inhibition at

99.28%, followed by Eucalyptus Leaf Ethanol Extract (ELEE) at 98.34%. (Table-5). Fungicides Propiconazole and Definoconazole displayed the highest inhibition at 100%, followed by Papaya Leaf Ethanol Extract (PLEE) at 99.28% and Eucalyptus Leaf Ethanol Extract (ELEE) at 98.34%. Notably, Papaya Leaf Extract (PLE) and Eucalyptus Leaf Extract (ELE) showed significantly higher inhibitory effects on Colletotrichum kahawae compared to other extracts.

Furthermore, the growth of mycelia was significantly reduced in plates treated with fungicides and plant extracts compared to the control, indicating their potential for disease management. This study highlights the potential of certain plant extracts, particularly from Carica papaya and Eucalyptus saligna, as effective antifungal agents against *Colletotrichum kahawae*. These findings contribute to the development of eco-friendly strategies for controlling major fungal diseases, suggesting that plant extracts may contain active compounds capable of delaying disease development. Further research is warranted to explore the mechanisms underlying the antifungal activity of these plant extracts and their application in disease management practices.

Coffee Berry Disease (CBD), caused by Colletotrichum kahawae, poses a significant threat to coffee production in the Seyo Nole district, an important coffee-producing area in West Wollega. The survey revealed that CBD affects approximately 30% to 37% of coffee berries in the region. Results from the in vitro antifungal experiments demonstrate the efficacy of both fungicides and medicinal herbs in suppressing the growth of Colletotrichum kahawae. Notably, Propiconazole and Definoconazole achieved complete inhibition (100%) of the pathogen. Among the aqueous plant extracts, Papaya Leaf Extract (PLE) and Eucalyptus Leaf Extract (ELE) showed the highest level of inhibition. Additionally, Ethanol Extract of Papaya Leaf (EEPL) exhibited the highest success rate, with a 99.28% inhibition rate. Farmers in the Seyo Nole district should implement recommended CBD management strategies to combat the high incidence and severity of the disease. This proactive approach is essential to prevent further disease buildup and minimize crop losses. Coffee growers can effectively manage CBD by using fungicides such as Score (Definoconazole 25% EC) and Tilt (Propiconazole 25% EC). These fungicides have demonstrated significant efficacy in inhibiting the growth of Colletotrichum kahawae and can serve as integral components of CBD management programs. Natural plant extracts showing antifungal potential, as observed in this study, should be further evaluated under field conditions. Field trials will provide valuable insights into the practical efficacy of these extracts as CBD management tools and inform recommendations for their use in commercial coffee production. By implementing these recommendations, coffee growers in the Seyo Nole district can effectively manage CBD and safeguard their crops against this devastating disease, ensuring the continued productivity and sustainability of the region's coffee industry.

Data Availability

Data is available in the manuscript.

Conflict of interest

The authors do not have any possible conflicts of interest.

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