Journal of Plant Sciences
2024, Vol. 12, No. 3, pp. 70-75
https://doi.org/10.11648/j.jps.20241203.11

Research Article

Intrinsic and Extrinsic Factors Affecting Growth and Sporulation of *Corticium koleroga (Cke) Hoehnel*

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Abstract

A numerous production constraints have been affecting coffee production and productivity. Among the constraints, coffee diseases attacking fruits, leaves, stems and roots that reducing coffee yield and marketability are economically important across the country. Coffee thread blight disease considered as a minor for 40 years in Ethiopia is currently emerging as a significant bottleneck to the sector. Recently, the occurrence and significant damage due to this disease have been frequently reported from different coffee production areas. However, there is the lack of profiled information on intrinsic and extrinsic character of the pathogen. Hence, this study was initiated to determine the effect of temperature and artificial media on the growth and sporulation of C. koleroga. Collected sample from Metu sub-center have been isolated and purified. Then after, purified isolate was characterized on five different artificial media namely PDA, MEA, Sabouraud, yeast and Czapeck and three temperatures (20, 25 and 30 ℃) ranges for growth and sporulation. The result revealed, there was a significant difference ($P<0.001$) among media and temperature ranges in the sporulation and radial growth rate of the pathogen. The fastest radial growth 3.57 and 3.51mm/day was recorded from PDA and Sabouraud media when incubated at 25 ℃, respectively. Whereas, the lowest growth rate was observed on yeast extract agar. On the other hand, the highest spore amount (116 spores/ml) was recorded from Sabouraud medium followed by potato dextrose agar and Yeast extract agar media at 25 ℃. The study confirmed that the three media (Sabouraud, Yeast extract agar and potato dextrose agar) with 25 ℃ temperature are the best combination for proper radial growth and sporulation of Corticium koleroga. In general future studies should focus on alternative intrinsic and extrinsic factors for the growth and sporulation of this pathogen.

Keywords

Corticium koleroga, Media, Phytopathogenic Sporulation

1. Introduction

Coffee is not only one of the highly preferred international beverages but also one of the most important trade commodities in the world next to petroleum [1]. *C. arabica* is the most important agricultural commodity in Ethiopia, contributing around one quarter of its total export earnings [2]. Ethiopia is Africa's largest coffee producer and the world's fifth largest coffee producer and exporter next to Brazil, Vietnam, Colombia and Indonesia, contributing about 7 to 10% of total world coffee production. Coffee farming provides a livelihood income for around 15 million Ethiopians

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Received: 11 January 2024; **Accepted:** 2 February 2024; **Published:** 10 May 2024

(16% of the population), based on four million small-holder farmers [3].

In Ethiopia, *C. arabica* grows under very diverse and wider ranges of altitudes, temperature, rainfall, humidity and soil types [4]. The main coffee growing areas of Ethiopia are found within the Southwest, Southeast and Eastern parts of the country (Oromia and Southern Nations, Nationalities and Peoples' Regions), with modest and minor production in the north (Amhara and Benishangul Gumuz Regions, respectively) under different types of production systems. Coffee grown under these diverse environments showed wide genetic variations within and between populations of different regions for yield, quality, disease resistance and other traits. reported The total area of coffee production in the country is estimated to be about 700,475 hectares with the estimated annual national production of about 6.7ton/hec [2]. Even though *C. arabica* is the key cash crop and top foundation of the Ethiopian economy. Nevertheless, numerous production constraints have been affecting its production and productivity. Abiotic and biotic factors are the major constraints of coffee production in the country among which are fungal diseases, attacking the shoot and root systems of coffee trees and reducing the yield and marketability. Fungal diseases are the most important factors that contribute to the reduction of coffee production. According to Cavalcante and Sales [5] thread blight caused by the phytopathogenic fungi (*Corticium koleroga*) is an important disease of coffee in India, Trindad and Tobego. In Ethiopia, the disease had first been recorded in 1978 at Gera and Metu [6, 7] and was known for more than 40 years and considered as minor coffee disease. Southwest part of Ethiopia is the major coffee producing area where the damage by coffee thread blight is frequently reported with increasing disease intensity from year to year [8]. Climatic conditions in Ethiopia favor the proliferation of certain diseases and results in their spreading to regions where they did not exist [8, 9], a similar situation has applied to thread blight. Coffee thread blight outbreak was first seen at Gumer (Limmu coffee plantation farm) in 2008 with mean disease incidence and severity of 49.2 and 9.8, respectively [8]. Four years later (in 2012), around 34 hectares of coffee farm devastated at Bebeka coffee estate due to thread blight disease was the second outbreak [10]. Yet the disease recurs every year and spreading to the neighboring zones of coffee producing areas of the country. Previously, thread blight disease intensity was assessed mainly by focusing on plantation coffee production system across the limited coffee agro-ecologies. However, the disease has become very important almost in all coffee production systems and different coffee-producing areas of Ethiopia [11]. This fact suggests that continuous change in weather variables, coffee habitat, cultural practices and production systems as a whole brings about a shift in the status of coffee thread blight disease occurrence and intensity across the locations and time. So, the outbreak and increasing rate of thread blight disease is associated with many biophysical factors [11].

In fact, fungi exhibit various responses to light intensity, duration of exposure to light and temperature. Exposure to light is needed by some fungi for sporulation [12], whereas other fungi sporulate better in the dark. Behavior of a fungus depends upon its nutritional response. Phytopathogenic organisms express similarities in broader behavior for their basic nutritional needs, yet they maintain their individuality in choosing specific substances [13]. It is now well established that phytopathogens show greater diversities in utilizing the same elements from different nutrient media (natural, semi-synthetic and synthetic culture media). These culture media always contain essential elements needed for proper growth and sporulation of the plant pathogen.

Pathogen Sporulation

Intrinsic and extrinsic factors play very important roles in maintaining a microbiologically safe food system. Intrinsic factors including those that are internal to the food itself, such as nutrient content, PH level, water activity and other antimicrobial components acting as defense mechanisms against microbes. Extrinsic factors are imposed by the environment in which the food product is present, such as temperature, relative humidity, presence of competitor microorganisms. In general, the fungi are able to grow under laboratory conditions Paterson [14] but the production of efficient and inexpensive commercial preparations requires mastering the competence for an effective and low-cost cultivation of the fungi, as well as for making them produce a great amount of spores.

Nagassa [11], indicated that *Corticium koleroga* sporulates very low on PDA and MEA at 27 ℃. Difficulty in obtaining abundant sporulation in the culture of many species of *Corticium* may be the limiting factor for studies of biology, systematics, and inoculation of the genus. Hence, it is necessary to understand the nutritional and environmental requirements that influence mycelia growth and sporulation. The food environment can support or reduce the ability of microorganisms to persist, establish and growth. Each one presents as a natural characteristic of a food ingredient or adjusted through manufactured processes. In order to improve the sporulation and selectivity towards obtaining a huge amount of spore of *C. koleroga*. Its growth media and temperature range were not well identified previously therefore; the focus of this study was initiated to fill this gap as well.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was carried out during 2021-2022 at Jimma Agricultural Research Centre (JARC) in the Coffee pathology laboratory. JARC is found in the Oromia Regional State in the Jimma zone Southwestern part of Ethiopia. It is located around 07 °46'N latitude and 36 °47'E longitude coordinates and at an elevation of 1753 m.a.s.l. It is 358 kilometers away from Addis Ababa and 12 kilometers from Jimma town in the

West direction. It represents the medium agro-ecological zones that receive an annual rainfall of 1572mm. Its mean minimum and maximum temperatures are 11.6℃ and 26.3℃respectively. The major soil types of the center are chromic nitosols and cambisols of upland and fluvisol of bottomland.

2.2. Pathogen Isolation and Characterization

C. koleroga was isolated from diseased coffee parts and culture maintained on PDA and Malt extract Agar plates. Five-millimeter mycelia discs were cut with a sterile cork borer from the advancing margin of colonies and kept on 5 different artificial growth media on Petri plates and incubated at 20, 25 and 30℃. Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek's and Sabouraud's medium (Table 1). Were used for the study of mycelia and spore formation. Twenty milliliters of the media have been poured after sterilization on 90 millimeter Petri plates. Five-millimeter mycelial blocks from 7-day old culture of *Corticium koleroga* were inoculated on the center of media. Colony diameter was measured in millimeter as a basis of growth. Growth of the cultures was measured after three days and sporulation after fifteen days of incubation under various conditions and hemocytometer was used for measuring the degree of sporulation.

Table 1. *List of Treatments used in the study and their arrangement.*

Artificial Media	Temperature	Trt	Trt. Code
Potato Dextrose Agar (PDA)	20 ℃(T1)	T1	PDAT1
Potato Dextrose Agar (PDA)	25 ℃(T2)	T2	PDA T2
Potato Dextrose Agar (PDA)	30 ℃(T3)	T3	PDAT3
Malt Extract Agar (MEA)	20 ℃(T1)	T4	MEAT1
Malt Extract Agar (MEA)	25 ℃(T2)	T5	MEAT2
Malt Extract Agar (MEA)	30 ℃(T3)	T6	MEAT3
Sabouraud Glucose Agar (SGA)	20 ℃(T1)	T7	SGAT1
Sabouraud Glucose Agar (SGA)	25 ℃(T2)	T8	SGAT2
Sabouraud Glucose Agar (SGA)	30 ℃(T3)	T9	SGAT3
Czapek Concentrate Agar (CCA)	20 ℃(T1)	T10	CCAT1
Czapek Concentrate Agar (CCA)	25 ℃(T2)	T11	CCAT2
Czapek Concentrate Agar (CCA)	30 ℃(T3)	T12	CCAT3
Yeast Extract Agar (YEA)	20 ℃(T1)	T13	YEAT1
Yeast Extract Agar (YEA)	25 ℃(T2)	T14	YEAT2
Yeast Extract Agar (YEA)	30 ℃(T3)	T15	YEAT3

2.3. Data Analysis

All the recorded data were subjected to Mycroft excel and its means arc sin transformed. SAS software (version 9.4) package was used for analysis. Duncan's multiple range test at $P \leq 0.05$ was used for mean separation between treatments.

3. Results and Discussion

Different cultural pigmentation such as margin, shape and elevation were observed among the tested media on the pathogen front and reverse growth (Table 2, Figure 1).

Table 2. Cultural features of C.koleroga pathogen on different media.

Groth media	PH	Margin	Shape	Elevation	pigmentation	
					front	reverse
Czapek	7.3	Filamentous	Filiform	Raised	Alice blue	light goldn yellow
MEA	4.7	Entire	Irregular	Raised	brown	light goldn rod
PDA	3.5	Entire	Irregular	Raised	Ghost white	pale golden rod
Sabouraud	5.2	Entire	Circular	Flat	smoky white	Orange
Yeaex tract agar	7.2	Entire	Irregular	Flat	smoky white	light goldn yellow

Significant differences ($p<0.001$) on both tested growth media and temperature ranges (Table 3). An abundant amount of spores harvested from Sabouraud medium which is (99.4%) greater than Malt extract agar, (89.3%) greater than Czapeck and almost the same amount of spore harvested from both potato dextrose agar and Yeast extract agar media at temperature 25 ℃ (Table 3). This may be due to Peptones as efficient sources of nutrients Sabouraud medium. Peptone is an enzyme (gastric-enzymes) that produces protein derivative (hydrolysate), composed primarily of peptides and amino acids that are used in the cell culture including that of bacteria, yeast, fungus and mammalian cells. It is recommended for the ability of an organism to ferment a specific carbohydrate which helps in the differentiation of genera and species. Yeast extract provides microorganisms and cells with essential nutrients such as vitamins, trace elements, growth factors, amino acids and peptides with balanced composition, which could improve product synthesis. On the other hand, the pathogen preferred 25 ℃ temperature and Potato dextrose agar medium for initial growth. Some fungal pathogens grow at a fast rate but need a long time to sporulate. which means, at the initial stage they need many nutrients to grow and later prefer some stresses to sporulate.

Table 3. Radial growth and conidial sporulation of C. koleroga on different temperature ranges and growth media.

Growth media	Radial growth mm/day			Conidial sporulation/ml		
	25 ℃	20 ℃	30 ℃	25 ℃	20 ℃	30 ℃
Czapek	3.33^{b}	3.22^{b}	3.28^{ab}	58^{b}	32.33^{c}	36^{d}
Malt Extrat Agar	3.32^{b}	3.03^{c}	3.03^{c}	26.33^{c}	9.67^{d}	51.67^{c}
Potato Dextrose Agar	3.57^{a}	3.48^{a}	3.33^{a}	114^{a}	43.33^{b}	74^{b}
Sabouraud	3.51^{a}	3.25^{b}	3.22^{b}	115.67^{a}	52^{a}	84.33^{a}
Yeas extract agar	2.15^{c}	2.14^{d}	2.30^{d}	114^{a}	38^{b}	67.67^{b}
Mean	3.18	3.02	3.03	85.6	35.07	62.73
LSD	0.12	0.17	0.0.9	15.04	5.53	8.07
CV(%)	2.09	2.98	1.56	9.33	8.38	6.83

The pathogen responded different colony color (Figure 1), shape, size and sporulation capacity. Temperature also has a great role on the growth and sporulation of the pathogen. *C. koleroga* can grow and sporulate in all tested media and temperature ranges but the growth and sporulation capacity were different at different temperatures.

Figure 1. *Cultural growth feature of Corticium. koleroga at different growth media of 25 ℃ temperature for and reverse side.*

4. Conclusion

The nutrients and environmental factors can influence the ability of microorganisms to persist, establish and grow since they can present as natural characteristics of a food ingredient or be adjusted through manufactured processes. Intrinsic and extrinsic factors play very important roles in maintaining a microbiologically safe condition for growth and sporulation of this coffee thread blight-causing pathogen. Even though the coffee thread blight pathogen grows on growth media such as potato dextrose agar, Malt extract agar, Czapek, yeast extract agar and Sabouraud, its sporulation and rate of growth have a significant difference among the growth media. Our findings indicated that the three media (Sabouraud, Yeast extract agar and Potato dextrose agar) in combination with 25 ℃ temperature are the best for *Corticium koleroga* isolation, identification and characterization; whereas Sabouraud medium is better for sporulation of coffee thread blight. Generally, this experiment concludes that *Corticium koleroga* rapidly grows on media rich in vitamins and highly sporulates on media containing peptides and amino acids. In addition, studies have to be conducted on other combinations of intrinsic and extrinsic factors using isolates from different locations.

Funding

There is no official or private publication source, although the Ethiopian Institute of Agricultural Research provided the funding for the study's completion.

Acknowledgments

This research was funded by Ethiopian Institute of Agricultural Research (EIAR). The authors are greatly indebted to the staff of the Plant Pathology Sections who were involved in the experiment from start to end.

Conflicts of Interest

The authors declare no conflicts of interest.

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