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Physiological Characteristics of *Colletotrichum gloeosporioides*: The Causal Agent of Arecanut Leaf Spot

Sangamesh M Kalyani*[1], Lokesh M S[1], Ravikumar B[2], Vinaykumar M Mudenur[2], Y C Vishwanath[4], M P Basavarajappa[1], Noorulla Haveri[1] and Manjunath Hubballi[1]
Department of Plant Pathology, College of Horticulture, Bagalkot, Karnataka-587104, India

ABSTRACT

The experiment aimed to study the physiological characteristics of the *Colletotrichum gloeosporioides* at College of Horticulture Bagalkot, Karnataka, India. During 2024, the pathogen was cultured in 40 ml of potato dextrose broth and its dry mycelial growth was recorded over 20 days at two-day intervals. The highest mycelial growth was recorded on the 12th day after incubation (DAI) with a dry weight of 284.7 mg, followed by the 10th day with 246.3 mg. The study also examined the pH effect on mycelial growth. The pathogen exhibited the highest dry mycelial growth at pH 6, with a weight of 362.3 mg, followed by pH 7, which showed a mycelial weight of 314.7 mg. The least mycelial growth was recorded at pH 10, with a weight of 176 mg. Temperature also played a crucial role in the growth of *C. gloeosporioides*. The optimal temperature for the growth of *C. gloeosporioides* exhibited the highest mycelial growth at 25°C, reaching 290.5 mg. The next most favourable temperature was 30°C, where the growth measured 214.3 mg. There was no growth observed at 40°C and 45°C. Therefore, the temperature range of 25 to 30°C was considered optimal for the growth of pathogen. The maximum growth (88.3 mm) under alternate cycles of 12 hours of darkness and 12 hours of light, with excellent sporulation. It can be concluded that *Colletotrichum gloeosporioides* exhibits its highest growth potential at pH 6, 25°C and under alternate cycles of 12 hours of darkness and 12 hours of light.

Keywords: Growth, Light, pH, Physiology, Temperature

INTRODUCTION

Arecanut (*Areca catechu* L.) is a monocotyledonous palm belonging to the family Arecaceae with a chromosome number of 2n=32. It is widely grown in subtropical and tropical regions of Asia and East Africa. Arecanut is vulnerable due to recent outbreak of arecanut leaf spot which affect the production and productivity. The leaf spot disease was caused by *Colletotrichum gloeosporioides*. Fungal growth is significantly influenced by factors such as light, substrate pH and temperature. These environmental conditions play a crucial role in regulating fungal growth, sporulation and various other biological activities. The production of spores, which is vital for fungal dissemination and subsequent infection, is also strongly affected by environmental factors. These conditions determine whether a species will sporulate and at what rate it will multiply (Eicher and Ludwig, 2002). Understanding the physiological requirements of plant pathogens is essential for developing effective control strategies (Jaruhar and Prasad, 2011). In this context, the present study focuses on examining the effects of physiological factors on the vegetative growth and sporulation of *Colletotrichum gloeosporioides*.

MATERIALS AND METHODS

Studies on the growth phase of pathogen

The 40 ml of potato dextrose broth (PDB) was prepared in 100 ml conical flasks and autoclaved. A 5 mm mycelial bit from a pure 12 day old culture was inoculated into each flask after cooling. The flasks were then incubated at 27±1°C. Starting from the day of inoculation, three flasks were harvested every two days until the 20th day. The mycelial mat was collected by filtering through filter paper (Whatman no.1). which, along with the mycelium, was dried at 60 °C and then weighed.

Corresponding Author's Email - sangameshkalyani9@gmail.com
1Department of Plant Pathology, College of Horticulture, Bagalkot, Karnataka-587104, India
2Department of Plant Pathology, Horticulture Research and Extension Centre, Devihosur (Haveri) - 581 110, Karnataka, India.
3Department of Entomology, Horticulture Research and Extension Centre, Devihosur (Haveri) - 581 110, Karnataka, India.
4Department of Plantation, Spices, Medicinal and Aromatic Crops, KRCCH Arabhavi, Karnataka, India.

Table 1. Growth phase of isolated pathogens on potato dextrose broth *Colletotrichum gloeosporioides*

Sr. No.	Days after incubation	Mycelial dry weight in mg (Mean)
1	02	89.3
2	04	137.9
3	06	206.5
4	08	213.7
5	10	246.3
6	12	284.7
7	14	263.7
8	16	254.1
9	18	238.9
10	20	229.1
	S. Em ±	2.60
	CD (P=0.01)	10.48

Effect of different temperatures on the growth of pathogen

To investigate effect of different level of temperatures on pathogen growth, six different temperatures 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C and 45 °C were used. A total of 40 ml of PDB was prepared in 100 ml flasks, sterilized by autoclaving and inoculated with a 5 mm disc of fungus from a 12-day-old culture. The inoculated conical flasks were incubated at the selected temperatures, with the replicates (3) for each condition. After 12 days of incubation, the dried mycelium weight was measured, and the data were analysed statistically.

Effect of hydrogen ion concentration (pH) on the growth of pathogen

To study the pH effect on the growth of pathogen, seven different pH levels were selected: 4, 5, 6, 7, 8, 9 and 10. The pH of the PDB was adjusted using 0.1N NaOH or 0.1N HCl. Potato dextrose broth served as the basal medium and autoclaved. A 5 mm culture bit of the pathogen was inoculated into 40 ml of PDB, which had been autoclaved. Three replicates were maintained for each pH level. The dry mycelial weight of the pathogen was measured, and the data were analyzed statistically.

Effect of light on the growth and sporulation of pathogen

The effect of light and darkness on growth of the pathogen and sporulation were studied on PDA media by exposing the pure culture to continuous dark, continuous light and alternating with 12 hours of light and 12 hours of darkness. The inoculation of culture to PDA containing Petri dishes were done with seven replications per treatment. The plates were incubated at 27±1 °C. Observation on mycelium diameter and sporulation were recorded.

RESULTS AND DISCUSSION

Studies on the growth phase of pathogen

The growth of *Colletotrichum gloeosporioides* varied significantly depending on the day of mycelial harvest (Table 1). The highest dry mycelial growth was recorded on the 12th day after incubation with a dry weight of 284.7 mg, followed by the 10th day with 246.3 mg. In contrast, the lowest dry mycelial weight of 89.3 mg was observed after just two days of incubation. Afterwards, the growth of mycelium decreased with an increased number of days. Similarly, Tejashwini (2023) got a higher dry mycelial weight of pathogen (337.2 mg) on the 12th day of inoculation on PDB.

Effect of different temperatures on the growth of pathogen

Th data (Table 2) regarding the effect of temperature on growth of pathogens. *Colletotrichum gloeosporioides* exhibited the highest mycelial growth at 25°C, reaching 290.5 mg. The next most favourable temperature was 30°C, where the growth measured 214.3 mg followed by 173.7 mg at 35°C and 169.1 mg at 20°C. There was no growth observed at 40°C and 45°C. Therefore, the temperature range of 25 to 30°C is considered optimal for the growth of *C. gloeosporioides*. These findings are similar with Kommula *et al* (2017), who obtained the maximum mycelial growth of *Colletotrichum capsici* (72.4 mm) at 25°C. On the other hand, Dev and Somasekhara (2018) reported higher mycelial weight of *C. gloeosporioides* at 30°C, followed by 25°C.

Table 2. Effect of temperature on growth of pathogen *Colletotrichum gloeosporioides*

Sr. No.	Temperature(°C)	Mean mycelial dry weight (mg)
1	15	99.0
2	20	169.1
3	25	290.5
4	30	214.3
5	35	173.7
6	40	0.00
7	45	0.00
	S. Em ±	2.93
	CD (P=0.01)	8.88

Table 3. Effect of pH on growth of pathogen *Colletotrichum gloeosporioides*

Sr. No.	pH	Mean mycelial dry weight (mg)
1	04	196.1
2	05	210.3
3	06	362.3
4	07	314.7
5	08	250.7
6	09	212.7
7	10	176.0
	S. Em ±	5.50
	CD (P=0.01)	16.66

Effect of hydrogen ion concentration (pH) on the growth of pathogen

Colletotrichum gloeosporioides exhibited the highest dry mycelial growth at pH 6, with a weight of 362.33 mg, followed by pH 7, which showed a mycelial weight of 314.7 mg (Table 3). The least mycelial growth was recorded at pH 10, with a weight of 176 mg and pH 8 at 250.7 mg. It indicated that acidic media is preferred by the fungus for its growth. Sharma and Kulshreshta (2015), Chaudhari *et al* (2017) and Dev and Somasekhara (2018) also reported the maximum growth of *C. gloeosporioides* at pH 6.0. However, Kumara and Rawal (2008) obtained the maximum weight at pH 5.

Effect of light on the growth and sporulation of pathogen

The effect of light and darkness on the growth of *C. gloeosporioides* was examined. After 10 days of incubation, *C. gloeosporioides* exhibited the maximum growth (88.29 mm) under alternating cycles of 12 hours of light and 12 hours of darkness, with excellent (++++) sporulation. This was followed by growth under continuous light (77.29 mm) and continuous darkness (64.57 mm), which showed moderate (++) and good (+++) sporulation, respectively. This agrees with the findings of Dev and Somasekhara (2018) recorded the maximum radial growth of *C. gloeosporioides* (85.60 mm) for 12 hrs light and 12 hrs dark compared to the continuous light (61.3 mm) and continuous dark (54.3 mm) exposure. Similar findings were also recorded by Kamanna (1996), Alexander *et al* (2004) and Tejaswini (2023) on *C. gloeosporioides*. (Table 4)

CONCLUSION

The study concluded that pH, temperature and light conditions significantly influence the mycelial growth of *Colletotrichum gloeosporioides*. The pathogen exhibited the maximum dry mycelial growth at pH 6 followed by pH 7. Temperature played a crucial role, with optimal growth observed at 25°C, followed by 30°C. No growth was observed at 40°C and 45°C, indicating that the ideal temperature range for the pathogen is between 25°C and 30°C. Light conditions also influenced growth, with the highest mycelial growth and excellent sporulation occurring under alternating 12-hour light and dark cycles. This was followed by continuous light and continuous darkness which exhibited moderate to good sporulation. These findings highlight the optimal environmental factors that regulate the growth and development of *C. gloeosporioides* under *in vitro* condition.

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Table 4. Effect of light on the growth and sporulation of pathogen *Colletotrichum gloeosporioides*

Treatment	Mean diameter(mm)	Sporulation
Continuous light	77.29	+++
Continuous dark	64.57	++
12 hours light and 12 hours dark	88.29	++++
S. Em±	0.09	-
CD (P=0.01)	0.37	-

- No sporulation; + Poor (1-25 spores/microscopic field 400 X); ++ Moderate (25-50); +++ Good (50-75); ++++ Excellent (>75)

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