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Cultural and Physiological Requirements of *Colletotrichum gloeosporioides* Causing Anthracnose of Mango

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A B S T R A C T

Mango is regarded as 'king of fruits' and is grown mainly in tropical and subtropical regions of the world. Anthracnose is one of the devastating disease, known to cause yield loss from 10 to 80 per cent in orchard and also being post-harvest disease, it cause an economical loss to the tune of 15-20 per cent. The disease is caused by the fungal pathogen *Colletotrichum gloeosprorioides.* Information on the cultural and physiological requirements of the pathogen will aid in formulating the effective management stratergy. Among the ten solid media tested, maximum radial growth of the pathogen was observed in potato dextrose agar (88.23 mm) followed by Sabouraud's agar (78.33 mm) and the least radial growth was recorded in host leaf extract agar (57.09 mm). Sporulation of the fungus was found to be abundant on Richards's agar, least in host leaf extract agar and no sporulation in V-8 juice agar. Under physiological requirements of the pathogen temperature of 25 ^{o}C accompanied with relative humidity of 95 per cent were found optimum for the maximum growth of the pathogen.

Introduction

Mango (*Mangifera indica* L.) is referred as the "king of fruits" preferred by the people for its delicious taste, excellent flavour, high nutritive, medicinal value and great religious-historical significance. It belongs to the family Anacardiaceae (Lakshmi *et al.*, 2011).

The crop is being grown in 87 countries of the world. In India, it occupied an area of 2.2 million hectares and production is around 21.02 million tonnes. Productivity (per hectare) is 8.17 tonnes. It ranks first in production by contributing 52 per cent of the total world production (Anon, 2018).

Although, the productivity of mango is found to be declined due to various biotic and abiotic stresses. Diseases caused by different pathogenic organisms were thought to be the major biotic stresses. Among the different diseases infecting the crop, anthracnose caused by *Colletotrichum gloeosporioides* is the most devastating and found to reduce yield and quality of mango fruits. The disease is known to reduce the yield levels from 10 to 80 per cent under field conditions in

developing countries (Poonpolgul and Kumphai, 2007 and Kumar *et al.*, 2010). It is also a post-harvest disease leading to an economical loss to the tune of 15 to 20 per cent (Ploetz and Prakash, 1997).

Colletotrichum gloeosporioides is species of the genus *Colletotrichum* and reported to occur on various crop species in India, Butler (1918) reported *Colletotrichum gloeosporioides* for the first time in India as a causal organism of coffee leaf spot and in 1924 McRae reported it as a causal organism of anthracnose of mango.

Every organism needs food for its growth, reproduction and survival. Fungi are not exemption to it. Fungi usually obtain their nourishment from the substrate, on which they grow and multiply. In order to culture the fungi artificially in the laboratory, it is essential to provide all the basic nutritional elements in easily accessible form in the medium. All the media are not equally good for all the fungi nor there any universal artificial media on which all the fungi can grow.

Further, temperature and relative humidity are the essential factor for the growth of any fungi. Hence, generating the information on these factorS help us to design the management practiceS. Keeping the fact in view, present investigations were aimed at finding out the nutritional requirements and ideal environmental conditions for the growth of *Colletotrichum gloeosporioides*, the causal agent of anthracnose of mango.

Materials and Methods

Collection and isolation of the pathogen

The mango leaves infected with anthracnose disease were collected from mango orchard at UAS, Raichur and were used for isolation of the pathogen. Small bits measuring about 5 mm size were cut off from the leaves showing lesions in such a way that, it contained both infected and healthy parts. Then these bits were surface sterilized in 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 seconds followed by three washings in sterilized distilled water. The bits were dried by transferring into sterilized discs of blotting paper and then subsequently transferred to potato dextrose agar (PDA) medium under aseptic conditions. The inoculated plates were incubated at 27 ± 1 ^{o}C for seven days for the growth of the pathogen. After the inoculation period is over, observations ere made for the growth of the pathogen.

Cultural studies

Growth and sporulation of the fungus *Colletotrichum gloeosporioides* were studied on different synthetic and non-synthetic media. The media used are listed below.

Synthetic media	Non synthetic media
Asthana and Hawker's agar	Carrot dextrose agar
Czapek's Dox agar	Host leaf extract agar
Richard's agar	Oat meal agar
Sabouraud's agar	Potato dextrose agar
V-8 juice agar	
Malt extract agar	

Twenty ml of each media was poured aseptically into sterilized Petri-plates having size of 90 mm diameter. Five mm disc from an actively growing zone of eight days old

culture of the fungus was placed upside down at the center of solidified medium and the inoculated plates were incubated at 27 ± 1 ºC. Each treatment was replicated for three times.

The radial growth of the fungus in each plate was calculated by measuring linear growth of the colony in three directions and then averaged. The growth was measured, when maximum growth was attained in any one of the media tested. The various cultural characteristics like mycelial growth, type of margin, colour and sporulation on different media were recorded and sporulation was graded as follows. The data on radial growth was analyzed statistically.

Sl. No.	Score	Grade	Description
1	++++	Excellent sporulation	>30 spores/ microscopic field (10X)
2	+++	Good sporulation	21-30 spores/ microscopic field (10X)
3	++	Moderate sporulation	11-20 spores/ microscopic field (10X)
4	+	Poor sporulation	1-10 spores/ microscopic field (10X)
5	-	No sporulation	<1 spores/ microscopic field (10X)

Physiological studies

Effect of different levels of temperature on growth and sporulation of *C. gloeosporioides*

The growth of *C. gloeosporioides* was tested at different temperature levels *viz*., 15, 20, 25, 30 and 35 ºC. Potato dextrose agar medium was poured into 90 mm (diameter) Petri plates. After solidification 5 mm disc from an actively growing 7 days old culture was cut and inoculated at the center of the solidified medium and then incubated separately for 15 days in the incubators adjusted to required temperature levels. Each treatment was replicated thrice. After incubation period radial growth and sporulation of the fungus in each of the incubated plate were recorded as described earlier.

Effect of different levels of relative humidity (RH) on growth and sporulation of *C. gloeosporioides*

Study was conducted to assess the effect of different levels of relative humidity on growth of the pathogen. Five mm disc of seven days old culture of *C. gloeosporioides* was placed at the center of Petri plates containing PDA media under aseptic condition and Petri plates were exposed to 75, 80, 85, 90 and 95 per cent relative humidity levels maintained in the desiccators. Different levels of relative humidity were adjusted by using different quantity of concentrated solution of H_2SO_4. The desiccators were kept at 27 ± 1 ºC with four replications. Observations of colony diameter and sporulation were recorded at 13 days after incubation.

Per cent RH at 27 ºC	H_2SO_4 % Concentration (ml used in 100 ml distilled water)
75	30.00
80	27.00
85	23.00
90	18.00
95	11.00

Results and Discussion

Isolation and identification

The pathogen was isolated from an infected leaves and pure culture of the fungus was maintained on potato dextrose agar medium (Plate 1). The cultural characteristics of the test fungus were studied on different media.

Cultural studies

Table 1 represents the fungus produced varied coloured colonies ranging from white to light grey (Asthana and Hawker's 'A' agar), dirty brown (host leaf extract), white (Czapek's agar, oat meal agar, potato dextrose agar, Richard's Agar, Sabouraud's Agar and carrot meal agar), light brown to white (V-8 juice agar) and light greyish to white (malt extract agar) on the different media tested in the experiment. In respect of colony margin the fungus showed smooth regular margin in majority of the media tested where as it produced smooth irregular margin on Asthana and Hawker's 'A' agar, and carrot meal agar. On host leaf extract agar and Richard's agar fungus produced rough regular margin and on oat meal agar it showed rough irregular margin.

On majority of media fungus produced dense fluffy growth of mycelium where as it showed sparse growth of mycelium on Asthana and Hawker's 'A' agar, host leaf extract agar and malt extract agar. Similar results were obtained by Sangeetha (2003) revealed that, on Richard's Agar, *C. gloeosporioides* produced circular, pure white, raised growth with light pinkish background but on Sabouraud's Agar, circular white cottony, zonate with raised center was noticed and Venkataravanappa (2002) who reported that mycelium was initially white later turn to ash colour on PDA and dark brown on host leaf extract agar. The observations recorded on radial growth and sporulation of the fungus on different solid media were presented in Table 2 and Plate 2, effect of different media on growth of the pathogen was significant and the highest radial growth was observed on potato dextrose agar with 88.23 mm of radial growth.

The next suitable media for significant growth of the pathogen were Sabouraud's agar (78.33 mm), Asthana and Hawker's 'A' agar (76.43 mm) in which growth of the pathogen was on par. The media *viz*., Czapek's agar (72.00 mm) and Richard's agar (70.01 mm) supported the medium growth of the pathogen and significantly least radial growth of 57.09 mm was observed in host leaf extract agar.

Excellent sporulation of the fungus was observed on potato dextrose agar, Czapek's agar and Sabouraud's agar with more than 30 spores per microscopic field (10X) followed by malt extract agar, host leaf extract and Richard's agar with 21-30 spores/ microscopic field (10X) and moderate sporulation was recorded in Asthana and Hawker's 'A' agar, oat meal and carrot meal agar with 11 - 20 spores/ microscopic field (10X) and no sporulation was observed in V-8 juice agar.

In the present study, good growth of *C. gloeosporioides* on PDA medium was attributed to the inherent complex nature of the ingredients supporting the good fungal growth and some additional nutrients as reported by Shivakumar (2015). He has observed the maximum radial growth of *C. gloeosporioides* potato dextrose agar (88.33 mm) followed by Richards's agar (79.50mm) and the least radial growth was recorded on host leaf extract agar (59.08 mm). Similar observations were made by Ekbote *et al.*, (1997), Sudhakar (2000), Venkataravanappa (2002) and Prashanth (2007).

Table.1 Cultural characteristics of *C. gloeosporioides* on different solid media

Sl. No	Media	Colony colour	Type of margin	Mycelial growth
1	Asthana and Hawker's 'A' agar	White to light grey	Smooth irregular	Sparse
2	Czapeck's agar	White	Smooth regular	Dense fluffy
3	Host leaf extract agar	Dirty brown	Rough regular	Sparse
4	Malt extract agar	Light greyish to white	Smooth regular	Sparse
5	Oat meal agar	White	Rough irregular	Dense fluffy
6	Potato dextrose agar	White	Smooth regular	Dense fluffy
7	Richards' agar	White	Rough regular	Dense fluffy
8	Sabouraud's agar	White	Smooth regular	Dense fluffy
9	V-8 juice agar	Light brown to white	Smooth regular	Dense fluffy
10	Carrot dextrose agar	White	Smooth irregular	Dense fluffy

Table.2 Efficacy of different solid media in promoting the growth and sporulation of *C. gloeosporioides*

Sl. No	Different media	Colony diameter (mm)	Sporulation
1	Asthana and Hawker's 'A' agar	76.43	++
2	Czapek's agar	72.00	++++
3	Host leaf extract agar	57.09	+++
4	Malt extract agar	60.16	+++
5	Oat meal agar	67.45	++
6	Potato dextrose agar	88.23	++++
7	Richards's agar	70.01	+++
8	Sabouraud's agar	78.33	++++
9	V-8 juice agar	57.74	-
10	Carrot meal agar	69.82	++
	S. Em ±	**0.64**	
	CD at 1 %	**2.58**	

Sporulation
++++ = Excellent, >30 spores/ microscopic field (10X)
+++ = Good, 21-30 spores/ microscopic field (10X)
++ = Moderate, 11-20 spores/ microscopic field (10X)
+ = Poor, 1-10 spores/ microscopic field (10X)
- = No sporulation, <1 spores/ microscopic field (10X)

Table.3 Effect of different levels of temperature on mycelial growth sporulation of *C. gloeosporioides*

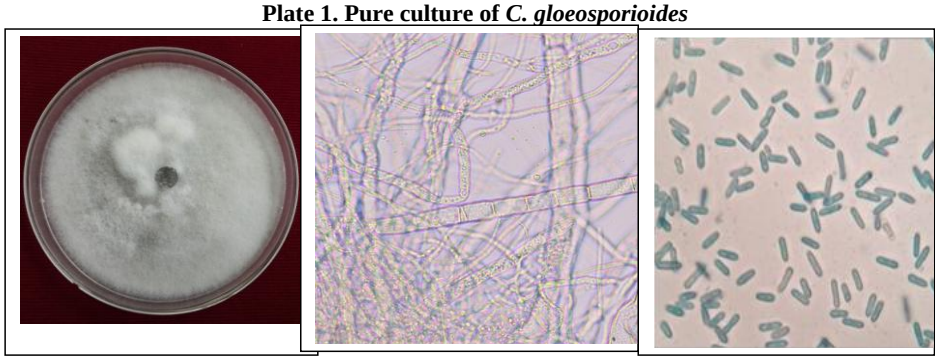
Sl.No.	Temperature (^{o}C)	Mean colony diameter (mm)	Sporulation
1	15	21.74	++
2	20	61.72	++++
3	25	88.63	++++
4	30	86.65	++
5	35	53.92	++
	S. Em ±	**0.85**	
	CD at 1 %	**3.82**	

Table.4 Effect of different relative humidity on mycelial growth and sporulation of *C. gloeosporioides*

Sl. No	Relative humidity (%)	Mean colony diameter (mm)	Sporulation
1	75	63.33	++
2	80	75.00	+++
3	85	79.33	+++
4	90	83.66	++++
5	95	88.33	++++
	S. Em ±	**0.49**	
	CD at 1 %	**2.19**	

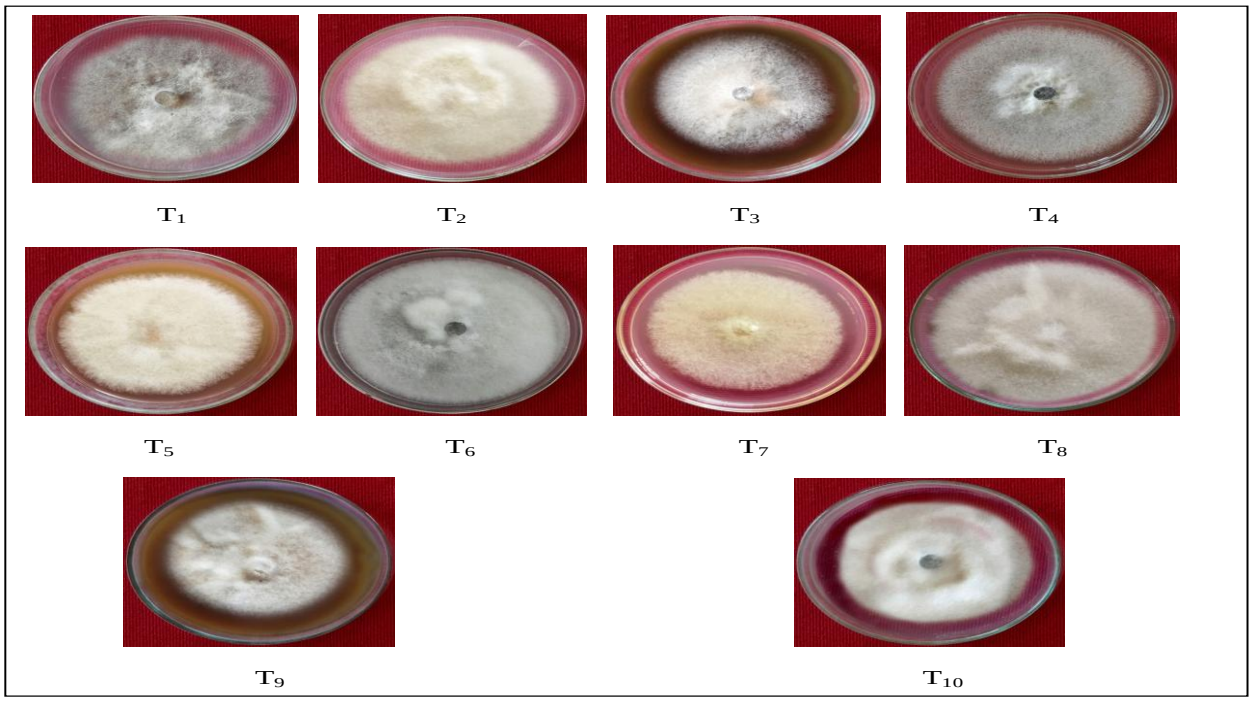
Plate.1 Pure culture of *C. gloeosporioides*

Plate 1. Pure culture of *C. gloeosporioides*



A. Mycelium in Petri | B. Hyaline | C. Conidia

Plate.2 Growth of *C. gloeosporioides* on different solid media



T_1 : Asthana and Hawker's 'A' agar
T_2 : Czapek's agar
T_3 : Host leaf extract agar
T_4 : Malt extract agar
T_5 : Oat meal agar
T_6 : Potato dextrose agar
T_7 : Richards's agar
T_8 : Sabouraud's agar
T_9 : V-8 juice agar
T_{10} : Carrot meal agar

Plate.3 Effect of different levels of temperature on mycelial growth of *C. gloeosporioides*

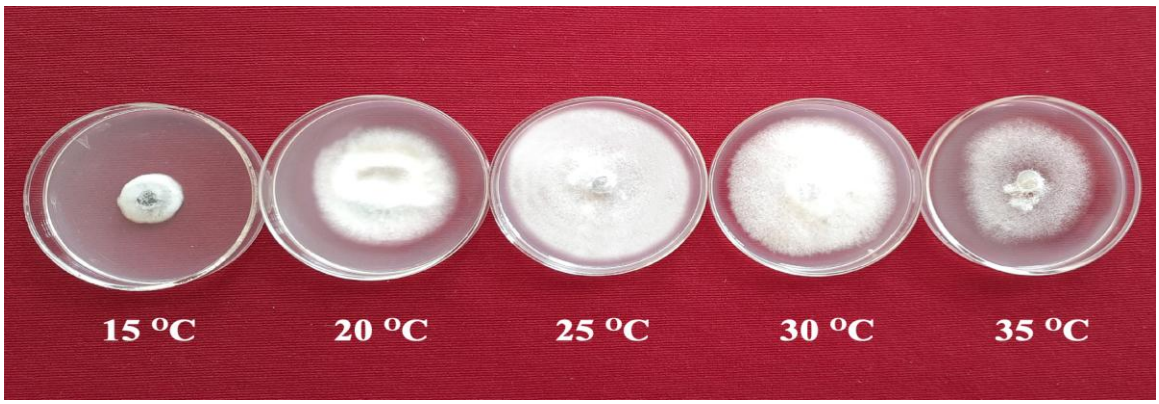
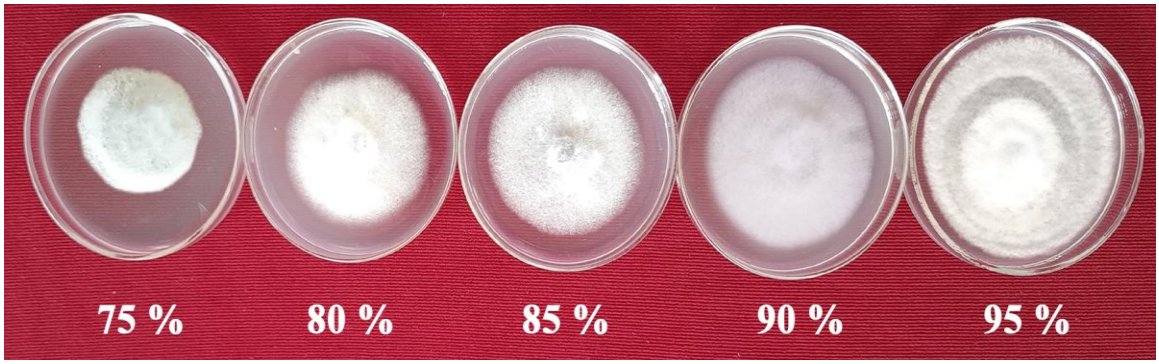


Plate.4 Effect of different relative humidity on mycelial growth of *C. gloeosporioides*



Physiological studies

Effect of different levels of temperature on growth and sporulation of *C. gloeosporioides*

Results of the study revealed that (Table 3 and Plate 3), there was a significant difference in mycelial growth each at five different range of temperatures (15, 20, 25, 30 and 35 ^{o}C). Significantly, maximum mycelial growth of 88.63 mm and excellent sporulation was observed at 25 ^{o}C followed by on par growth (86.65 mm) at 30 ^{o}C and significantly least mycelial growth of 21.74 mm was recorded at 15 ^{o}C. Excellent sporulation was observed at 25 and 20 ^{o}C and moderate sporulation was noticed at 15, 30 and 35 ^{o}C.

According to Kumar and Rani, 2010 and Sanjeev *et al.*, 2019 the optimum temperature for growth and conidial germination of *C. gloeosporioides* was 25 °C followed by 30 °C whereas, least growth and germination was observed at 15 °C which were in accordance with the results of the present study. The present investigations also braced by the findings of several workers that, temperature ranging from 25 to 28 °C to be the optimum for the growth and conidial germination of *C. gloeosporioides* (Prakash and Srivastava, 1987; Venkataravanappa, 2002; Sangeetha and Rawal, 2008 and Pandey *et al.*, 2012).

Effect of different levels of relative humidity on mycelial growth and sporulation of *C. gloeosporioides*

The fungus was subjected to five different relative humidity levels to find out the optimum relative humidity for the maximum growth. Among the five levels of relative humidity ranged between 75 to 95 per cent. Relative humidity of 95 per cent was found to be optimum, which promoted significantly maximum radial growth (88.33 mm) of the pathogen, followed by the next best growth (83.66 mm) at 90 per cent relative humidity There is a significant difference between each relative humidity levels in supporting the growth of the pathogen. Relative humidity of 75 per cent was found to be most unfavorable for the growth, which recorded least radial growth of 63.33 mm and excellent sporulation was recorded at relative humidity of 90 and 95 per cent (Table 4 and Plate 4).

The optimum range of relative humidity for the growth of *C. gloeosporioides* was found to be 90 to 95 per cent (Gulab, 2011). However, observations by different workers revealed the different levels of relative humidity as optimum for the growth of *C. truncatum*. It was 85 to 90 per cent (Laxman, 2006) and 90 95 per cent (Rajashree, 2019) Varaprasad (2000) observed the maximum growth of the fungus at relative humidity of 95 per cent.

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