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Studies on the cultural and nutritional characteristics of *Colletotrichum gloeosporioides*, the causal organism of papaya anthracnose*

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Abstract: Papaya is one of the important fruit crop of India. Cultural studies of *Colletotrichum gloeosporioides* causal agent of anthracnose of papaya on different solid media showed that the V-8 juice agar and Richard's agar were good for growth and sporulation. The fungus in liquid medium there was a increase in growth upto ten days ofincubation and there was decrease in growth for eleventh day onwords. Temperature requirement of the fungus was found the 300C where good growth was observed. The fungus exposed to alternate cycles of light and darkness produced maximum growth and sporulation when compared to continuous light and continuous darkness.

Key words: *Colletotrichum gloeosporioides*, solid media liquid media, light, temperature

Introduction

Anthracnose of papaya caused by *C. gloeosporioides* is prevalent where papaya is grown and it is major post harvest disease during transit, storage and market. The fungus derive food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon which all the fungi can grow and reproduce. Therefore studies were conducted in different suitable media to identify surface medium for the growth and sporulation of *C. gloeosporioides.*

Material and methods

Selection of basal medium for growth and sporulation of the fungus was done by using potato dextrose agar, malt extract agar, oat meal agar, host leaf extract agar, corn meal agar, potato carrot agar, papaya fruit agar, V-8 juice agar, Richard's agar, Czapek's agar, Sabouraud's agar, Elliott's agar, Tochinal's agar, Sach's agar and Brown's agar. Twenty ml of each media was poured aseptically into Petriplates of 90 mm diameter. Five mm discs from an actively growing zone of ten days old culture was placed upside down at the centre of the solidified medium and were incubated at 27 ± 1ºC. Each treatment was replicated thrice.

The radial measurements of the colony of the fungus were taken when the maximum growth was attained in any one of the media tested. The various cultural characters like, rate of growth, type of margin, colour and sporulation on different media were recorded. The measurements of colony diameter on different media were measured.

Thirty ml of potato dextrose broth was added into each of the 100 ml conical flasks and sterilized at 1.1 kg/cm2 pressure for 15 minutes. These flasks were allowed to cool and then inoculated with 5 mm discs of inoculum as described earlier and incubated at 27 ± 1ºC. Each treatment was replicated for three times. Culture was filtered through whatman No. 42 filter paper discs of 12.5 cm diameter, which were dried to a constant weight at 60ºC in an electrical oven. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove salts if any associated with it. One set of mycelium from the flasks was harvested on second day after inoculation. Subsequent harvesting was done at an interval of two days up to twenty days. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60ºC, cooled in a desicator and weighted immediately on an analytical electrical balance.

The composition and preparation of different fifteen liquid media used, were the same as that of solid media except that, the agar was not added. All the liquid media were sterilized and the flasks were inoculated under aseptic condition and were incubated at 27 ± 1ºC for ten days. The mycelial growth was harvested, dried and weighed. The data were analysed statistically. The best synthetic media was found and used as a basal media for further studies.

Richard's liquid medium was used in this experiment with its nitrogen, carbon and sulphur source kept unchanged as they were found to be the best. The pH of the medium was also adjusted to 6.5, since the maximum growth of the fungus was obtained at this pH. The different temperatures maintained for the growth of *C. gloeosporioides* were 10, 15, 20, 25, 30 and 35ºC. Mycelial discs measuring 5 mm taken from Petriplates. For each temperature level four replications were maintained. The fungus was inoculated under aseptic condition and incubated for ten days. The data were analysed statistically.

Richard's broth was used in this experiment. Conical flasks of 100 ml capacity and each contain 30 ml of liquid medium were inoculated and exposed to different lengths of light hour's *viz.,* alternate cycles of twelve hours and twelve hours darkness, continuous light and continuous darkness in an environmental chamber. Petriplates were inoculated with 5 mm discs taken from the periphery of ten days old pure culture. Each treatment was replicated seven times and incubated at 27 ± for ten days. Dry mycelial weight was obtained as described earlier

Results and discussion

Among the fifteen solid media evaluated, maximum radial growth of *C. gloeosporioides* was observed on V-8 juice agar

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(87.67 mm) followed by oat meal agar (83.63 mm), Richard's agar (82.65 mm), corn meal agar (80.00 mm), malt extract agar (79.20 mm), potato carrot agar (79.00 mm), papaya fruit agar (78.17 mm), potato dextrose agar (75.50 mm), Tochinal's agar (75.50 mm), Czapek's agar (74.83 mm) and host leaf extract agar (74.27 mm) and potato dextrose agar and Tochinal's agar were found on par with each other. Elliott's agar (48.45 mm) and Brown's agar (34.83 mm) were found to be next best solid media for radial growth of the fungus . No growth was noticed in case of Sach's agar media. The results are in confirmation with that of Durairaj (1956) in case of *C. capsici*, Ekbote *et al*. (1997); Sudhakar (2000) and Rani and Murthy (2004) in case *C. gloeosporioides*. Sporulation of fungus was found to be abundant on Richard's agar, V-8 juice agar, oat meal agar and malt extract agar. They were in agreement with observation of Ekbote *et al.* (1997); Akthar (2000); Sudhakar (2000) and Rani and Murthy (2004) in case of *C. gloeosporioides* (Table 1).

Fungi possess an ability to utilize a wide range of nutrients as a source of energy. Among the liquid media used for growth of *C. gloeosporioides*, Richard's broth (581.33 mg) and malt extract broth (575.00mg) were supported maximum growth both were on par with each other followed by potato dextrose broth (449.17 mg), Brown broth (425.00 mg), Tochinal's broth (420.33) and oat meal broth (339.00 mg). Czapek's broth (256.13 mg), Sabouraud's broth (253.67 mg), papaya fruit broth (249.03 mg), V-8 juice broth (217.67 mg), Sach's broth (145.23 mg), corn broth (136.33 mg) and host leaf extract broth (93.33 mg) were found to be next best media. Least mycelial growth was observed in potato carrot broth (43.10 mg). Hiremath *et al*. (1993) and Ekbote *et al*. (1997) reported that, maximum dry mycelial

Table 1: Colony growth, mycelial weight and cultural characters of *Colletotrichum gloeosporioides* on different solid and liquid media

Sl. No.	Solid media	Mean colony diameter (mm)	Mean dry mycelial weight* (mg)	Growth characters	Sporulation
1.	Brown's agar	34.83	425.00	Good growth, whitish raised mycelia with irregular margin	++
2.	Corn meal agar	80.00	136.33	Good growth, whitish mycelia with smooth margin	++
3.	Czapek's agar	74.83	256.13	Good growth, margin smooth, whitish mycelium	+++
4.	Elliott's agar	48.45	80.33	Poor growth, dirty brown growth	-
5.	Host leaf extract agar	74.27	93.33	Moderately growth, white irregular margin and lightbrown colour mycelia	+
6.	Malt extract agar	79.20	575.00	Moderately growth, white mycelial growth	++
7.	Oat meal agar	83.63	339.00	Good growth, white colony with regular margin	+++
8.	Papaya fruit agar	78.17	249.03	Good growth, white mycelia with regular margin	+++
9.	Potato carrot agar	79.00	43.10	Good growth, white mycelia with regula margin	+++
10.	Potato dextrose agar	75.50	449.17	Moderately growth, white mycelia with regular smooth margin	++
11.	Richard's agar	82.65	581.33	Moderate growth, margin are irregular , dirty white growth mycelium	++
12.	Sabouraud's agar	68.67	253.67	Poor growth	-
13.	Sach's agar	0.00	145.23	Moderate growth, white mycelia with regular margin	++
14.	Tochinal's agar	75.50	420.33	No growth was observed	-
15.	V-8 juice agar	87.67	217.67	Poor growth, dirty white mycelia growth	+
	Mean	68.15	284.31		
	SEm ±	0.857	2.32		
	CD at 1%	3.33	9.02		

*Mean colony diameter after 10 days of incubation.

Table 2. Effect of incubation on dry mycelial weight of *Colletotrichum gloeosporioides* on potato dextrose broth

Sl. No.	Days after seeding	Mean dry mycelial weight (mg)
1	2	97.83
2	4	153.26
3	6	259.03
4	8	327.33
5	10	486.07
6	12	479.19
7	14	454.30
8	16	328.45
9	18	302.11
10	20	288.40
Mean		317.60
SEm ±		1.05
CD at 1%		4.24

Table 3. Effect of different temperature levels on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation

Sl. No.	Temperature (°C)	Mean dry mycelial weight (mg)
1	10	179.75
2	15	248.00
3	20	427.50
4	25	505.50
5	30	558.50
6	35	300.50
Mean		369.88
SEm ±		0.70
CD at 1%		2.86

growth of *C. gloeosporioides* was recorded in Richard's broth (Table 1).

Fungus reached maximum dry mycelial weight of 486.07 mg when it was incubated for 10 days, thereafter it shows decreased dry mycelial weight. This indicated that there may be occurance of autolysis. Lilly and Barnett (1951) also reputed antolysis after maximum growth where cellular enzymes begin to digest the various cell constituents. Similar finding were also observed by Ekbote (1994), Hiremath *et al*. (1993) in case of *C. gloeosporioides*.

Among the external abiotic factors which influence the growth of fungi, temperature plays an important role. Temperature affects almost every function of fungi, including growth, spore germination and reproduction. The fungus under study grew best at temperature of 30ºC (505.50 mg dry mycelial weight), whereas optimum range is between 20 to 30ºC. Similarly, Naik (1985) reported, 20-30ºC as an optimum temperature range for a *C. gloeosporioides* and also Hegde and Hegde (1986) achieved maximum growth of *Colletotrichum gloeosporioides* at 30ºC and temperature range for the good growth was 20 to 35ºC. Sattar and Malik (1939) have reported the optimum temperature for the sporulation of *C. gloeosporioides* to be 30ºC. Quimio (1973) reported that the optimum temperature for growth, sporulation and spore germination was 30ºC (Table 3).

Light has a profuse effect on growth of fungi. The preliminary studies carried out in present investigation with *C. gloeosporioides* indicated a maximum radial growth (89.29 mm colony diameter and 593.71 mg dry mycelial weight) was observed when exposed to alternate cycles of light and darkness. This was followed by continuous light (72.14 mm diameter and 468.29 mg dry mycelial weight) and continuous darkness resulted in 68.86 mm colony diameter and 359.86 mg dry mycelial weight of fungi. Kamanna (1996); Sudhakar (2000); Ashoka (2005) and Narendra Kumar (2006) observed that exposure of *C. gloeosporioides* to alternate cycles of 12 hours light and 12 hours darkness resulted in maximum growth. Similarly Chowdhuary (1936) found that continuous light or darkness was found to inhibit sporulation of *Colletotrichum graminicola* (Ces) G. Wilson, but cultures exposed to alternate light and darkness were found to sporulate earlier and more conspicuous. Mishra and Siradhana (1980) showed that disease was more when the pathogen was exposed to diurnal light compared to continuous light or darkness (Table 4).

Table 4. Effect of light intensity levels on dry mycelial weight and radial growth of *Colletotrichum gloeosporioides* after ten days of incubation

Sl. No.	Treatments	Mean colony dry mycelial weight (mg)	Mean colony diameter (mm)
1	Continuous light	468.29	72.14
2	Continuous dark	359.86	68.86
3	Alternate cycles of 12 hr light and 12 hr darkness	593.71	89.29
	Mean	473.95	76.76
	SEm ±	1.34	0.37
	CD at 1 %	5.47	1.50

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