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Growth of *Cercospora capsici* on different solid and liquid media

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It was found in this investigation that in *Cercospora capsici,* Heald and Wolf, the highest mean diameter of colony growth on PDA was 47.33 mm and it was 46.77 mm in Carrrot dextrose agar. It exhibited moderate growth on Carrot leaf decoction agar (37.83 mm), host leaf decoction agar and Asthane Hawker's agar and very least growth on V-8 juice agar. On liquid media maximum dry mycelia weight was obtained (74.33 mg) at 19 days of incubation period on PDB. The maximum dry mycelial weight in host leaf decoction broth (75.20 mg) followed by PDB (73.10 mg) and Carrot leaf decoction broth. The moderate dry mycelial weight observed on Potato sucrose broth, Malt extract broth, Oat meal broth and Richard's broth and least growth in V-8 juice broth. Scanty sporulation was observed on Carrot dextrose broth, Carrot leaf decoction broth, Czapek (Dox) broth, Malt extract broth and Oat meal broth. Non synthetic media support the maximum mycelia growth of the *C. capsici* than the synthetic media.

Key words: *Cercospora capsici*, solid media, liquid media, growth phase

INTRODUCTION

Chilli (*Capsicum annuum* L.) are grown in almost all states under rainfed and irrigated condition for the domestic market and export purpose. The leaf spots of chilli caused by *Cercospora capsici* Heald and Wolf are gaining importance by epidemic of disease in chilli cultivation area. The pathogen has been first isolated and named from bell pepper by Heald and Wolf (1911) and later studied by several researchers (Chupp, 1953; Vasudeva, 1963; Meon, 1990; Lim and Kim, 2003 and Bhat et al., 2008). However, *C. capsici* and *C. unamunoi* are considered to be synonyms to *C. capsicicola* (Bhartiya *et al.*, 2000). Uptil now, there is no much research information available on cultural growth characters *C. capsici* infecting chilli. An attempt is made to study the growth characters and sporulation of *C. capsici* on different culture media.

MATERIALS AND METHODS

Isolation of the pathogen

The causal organism, *C. capsici* was isolated from chilli leaves showing the typical leaf spot symptom of the disease. The infected leaves were cut into small leaf bits and surface sterilized with one per cent sodium hypochlorite solution for two to three minutes and three times repeatedly washed in sterilized distilled water. Then the infected leaf bits were transferred on to Petri dishes (1-2 leaf bits per Petri dish) containing PDA with the help of a sterile forceps and incubated at 25±1°C for 15 days. Further purification and subcultured on PDA was done. Pathogenicity test was carried out. The identification of fungus was done by sequenced the PCR product of ITS rDNA region and sequence were deposited in gene bank with an accession number HM235771.

Growth studies on different solid media

The variation in cultural characters of *C. capsici* was studied on the following synthetic and non-synthetic solid media. The composition and preparation of the media were obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by Hawksworth *et al.*, (1983). Synthetic media like Asthana and Hawker's agar, Czapeck's (Dox) agar, Richard's agar, Sabouraud's agar and V-8 Juice agar media. Non synthetic media like Carrot dextrose agar (CDA), Carrot leaf decoction agar, Host chilli leaf extract

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agar, Malt extract agar (MEA), Oat meal agar (OMA), Potato dextrose agar (PDA), Potato sucrose agar and PDA + $CaCo_3$ media. Twenty ml of each medium listed above was poured into 90 mm diameter Petri plates. After solidification, 5 mm discs of the 15 days old culture of *C. capsici* were cut using a cork borer and a single disc placed at the center of Petri dish. Each set of experiment replicated thrice and the plates were incubated at 25 ± 1° C for three weeks. The cultural characters such as colony diameter, colony colour, type of margin, mycelial width and sporulation were recorded.

Growth studies in liquid media

Growth phase

Twenty ml of potato dextrose broth (PDB) was poured into each of the 100 ml conical flasks. These flasks were sterilized and inoculated with 5 mm mycelial discs cut from the periphery of the 15 day old culture and incubated at 25±1°C. A set of three flasks were harvested every 48 h starting from 5th day of inoculation. Cultures were filtered through Whatman No. 42 filter papers, which were previously dried to a constant weight in hot air oven at 60°C. The mycelial mat on the filter paper was thoroughly washed with distilled water to leach out any salts associated with the mycelium. Subsequently, the filter papers along with the mycelial mat were dried to a constant weight, cooled in desiccators and weighed on an electronic balance.

Selection of basal medium

The composition and preparation of different liquid media used were same as that of the solid media without agar-agar. Twenty ml of each medium was poured in each of the 100 ml flasks. These flasks were sterilized and inoculated with 5 mm mycelial discs cut from the periphery of the 15 day old culture and incubated at 25±1°C and replicated thrice. After 19 days the mycelia was harvested and record dry mycelia weight as described earlier.

RESULTS AND DISCUSSION

Growth on different solid media

The non synthetic media supported the maximum mycelia growth of the fungus than the synthetic media (Table 1). Highest mean colony diameter 47.33 mm growth on PDA followed by CDA (46.77 mm) and PDA + $CaCO_3$ media (45.33 mm). Moderate growth on carrot leaf decoction agar (37.83 mm), host leaf decoction agar (36.88 mm) and Asthane Hawker's agar (35.83 mm) were on par with each other, very least growth was observed on V-8 juice agar (26.91 mm). Fungus did not sporulate in any media even after three weeks of incubation. Maximum growth of *C. cruenta* and *C. beticola* on Czapek (dox) agar followed by Carrot leaf decoction agar (Verma and Agnihotri, 1972). PDA supported maximum growth of *C. moricola* (Kanti, 1975) and *C. sorghi* (Dinesha, 1984). Kwon and Oh (1981) obtained abundant sporulation of *C. canescens* on mungbean leaf decoction-Oat meal agar. Khandar *et al.* (1985) reported that good radial growth of *C. cenescens* on PDA, Oatmeal and Anderson's agar. *C. kikuchii* grew most rapidly on malt extract agar and PDA without sporulation (Chen *et al.*, 1979 and Prashanth, 2004). The maximum numbers of conidia were formed on V-8 juice agar and carrot leaf decoction agar. Queiroz and Menezes (1993) reported high sporulation of *C. nicotianae* on V-8 juice - $CaCO_3$ agar, followed by coconut milk agar and tomato juice - $CaCO_3$ agar and low sporulation on dry tobacco leaves - $CaCO_3$ agar, dry tobacco leaves agar, PDA and PDA-panvit media. *C. abelmoschi* sporulated on carrot leaf extract agar and host (okra) leaf extract agar and Maximum mycelial growth on PDA, host (okra) leaf extract agar and V-8 juice agar. Tobacco

Table 1 : Radial growth and sporulation of *C. capsici* on different solid media

Medium	Radial growth (mm)
Asthane Hawker's agar	35.83
Carrot dextrose agar	46.77
Carrot leaf decoction agar	37.83
Czapek (Dox) agar	41.16
Host leaf decoction agar	36.88
Malt extract agar	41.96
Oat meal agar	40.08
Potato dextrose agar	47.33
Potato dextrose + $CaCo_3$	45.33
Potato sucrose agar	41.94
Richard's agar	40.05
Sabourauds's agar	39.97
V-8 juice agar	26.91
S.Em±	0.66
C.D. at 1%	2.64

extract dextrose agar supported maximum radial growth of *C. nicotianae* followed by Czapek (Dox) agar and carrot leaf decoction agar without sporulated (Pairashi and Jahagirdar, 2007).

Growth on liquid media

Growth phase

Growth phase of *C. capsici* had a significant difference in growth between incubation periods (Table 2). The mycelia growth gradually increased and was highest (74.33) mg on 19 days of incubation and later on the growth decreased significantly. As a result 19 days incubation period was optimum for the growth. PDB supported maximum growth of *C. moricola* at 20 days after inoculation (Kanti, 1975), *C. solani-melongenae* on 22 days (Lakshminarayana, 1981), *C. sorghi* on 16 days (Dinesha, 1984), *C. kikuchii* on 22 days (Prashanth, 2004). *C. nicotianae* on 19 days (Pairashi and Jahagirdar, 2007).

Table.2: Mycelial growth of *C.capsici* on different days of incubation

Days after inoculation	Mean dry mycelia weight (mg)
5	6.13
7	11.66
9	28.50
11	39.66
13	54.00
15	67.63
17	72.00
19	74.33
21	71.16
23	67.00
25	63.00
27	60.05
29	58.16
S.Em±	0.59
C.D. at 1%	2.34

Table. 3: Mycelial growth and sporulation of *C.capsici* on different liquid media

Liquid medium	Mean dry mycelial weight (mg)	Sporulation
Carrot dextroer's broth	71.20	+
Carrot leaf decoction broth	71.80	+
Czapek (Dox) broth	48.80	+
Host leaf decoction broth	75.20	-
Malt extract broth	65.80	+
Oat meal broth	52.70	+
Potato dextrose broth	73.10	-
Potato sucrose broth	68.50	+
Richard's broth	52.50	-
V-8 Juice broth	36.70	-
S.Em±	1.15	
C.D. at 1%	4.71	

+ : Sporulation -: No sporulation

Selection of basal medium

The dry mycelial weight (Table 3) of the fungus maximum obtained in host leaf decoction broth (75.20 mg) followed by PDB (73.10 mg), Carrot leaf decoction broth (71.80 mg) and Carrot dextrose broth (71.50 mg) which were at par with each other. The optimum dry mycelial weight was observed on Potato sucrose broth (6.85 g), Malt extract broth (65.80 mg), oat meal broth (52.70 mg) and Richard's broth (52.50 mg) which were at par with each other and least growth in V-8 juice broth (3.67 mg). Very scanty sporulation was observed on Carrot dextrose broth, Carrot leaf decoction broth Czapek (Dox) broth, Malt extract broth and Oat meal broth. Dange and Patel (1968) obtained maximum growth of *C. beticola* in Czapek (Dox) broth and moderate in Richard's broth, Carrot leaf decoction and poor in Asthana and Hawker's broth. Growth of *C. canescens* and *C. dolichi* was best in Richard's broth and was least growth in *Dolichos* leaf extract (Raghunathan, 1969). Maximum dry mycelial weight of *C. kikuchii* obtained in V-8 juice broth and PDB (Chen *et al.*, 1979; Prashanth, 2004). Pairashi and Jahagirdar (2007) reported that the maximum growth of *C. nicotiane* on Tobacco leaf extract broth followed by Czapek (Dox) broth and Carrot leaf decoction broth.

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