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Cultural, morphological and pathogenic variability of *Colletotrichum capsici* (Syd.) Butler and Bisby causing anthracnose of chilli (*Capsicum annuum* L.)

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ABSTRACT: *Anthracnose disease is one of the major economic constraints to chilli production in tropical and subtropical regions. The different isolates of Colletotrichum capsici causing chilli anthracnose were collected from different places of Tamil Nadu. The isolates were evaluated for their morphological and cultural characteristics, pathogenic variability on chilli fruits. Pathogenic behaviour of the ten isolates of* C. capsici *developed from fruits was established following koch's postulates. Culture colonies varied in their cultural behaviour from cottony to fluffy with irregular to margins. Potato Dextrose Agar (PDA) supported the maximum growth (8.15 cm) of all the isolates of* C. capsici. *All the isolates of* C. capsici *produced black pointed setae, hyaline falcate conidia with single oil globule at the centre. The length (18-23 ì) and width (3.43– 3.97 µ) of the conidia, number of setae per acervulus (12-32) and number of septa per seta (2-6) varied among the isolates. Majority of the isolates produced profuse sporulation. To know the virulence isolates fruits were inoculated and results suggested that isolate* CC_1 *caused the maximum fruit rot intensity (72.27 per cent) while* CC_9 *showed least intensity (2.93 per cent). The isolate* CC_1 *produced acervuli in the form of concentric rings on the infected fruits.*

Keywords*: Chilli Anthracnose, Colletotrichum capsici, Variability*

INTRODUCTION

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is of the important spice cum vegetable crop in India. It is grown throughout the year and used as green and dried stages their pungency and colour, it is an integral part if Indian diet and used at tine in every Indian home. Indian chilli is being exported to over 90 countries and has become a good foreign exchange earner. India ranks second next to China in the vegetable production in the world (Vitkar *et al.* 2007). In India chilli is being grown in area of 7.69 lakh hectares with a production of 12.39 lakh tonnes and a productivity of 1.61 t/ha (Basavaraj 2008). The important chilli growing states are Andhra Pradesh, Karnataka and Tamil Nadu. The pathogen *C. capsici* inciting anthracnose disease in chilli (*Capsicum annuum* L.) has been found to be crop wherever it is grown. The symptom appears on fruits initially small circular spots appeared on the skin of the fruit. The spots were sunken and light grey coloured with black margin, fruiting bodies *viz*., acervuli were produced on the infected area. The seed borne nature of *C. capsici* may be transmitted from mother plant, which were present throughout the storage period, which cause severe seed rot, seedling decay, twig blight, fruit rot and affect the seed germination of chilli and *C. capsici* able to survive up to the next crop season in the infected seeds (Ramesh Das 2007). Anthracnose disease caused by the fungus *C. capsici* is the most destructive disease of chilli, which cause pre and post emergence damping off, leaf spots, premature fruit drop, mummification of unripe green fruits and fruit rot, which contribute 50-100 % loss in India (Amusa et al 2004). Anthracnose caused the healthy green fruits lost 31 per cent and red ripe fruits lost 46 per cent ascorbic acid after 14 days of pathogenesis (Ramesh Das 2007), 25 per cent loss of capsaicin content (Prasad *et al*., 2000). The Chilli anthracnose pathogen *C. capsici* infects diverse host with a high degree of pathogenic variability (Sharma *et al*. 2005). Identification of species by morphological characters like shape, existence of setae, colony colour, size and formation of conidia is extensively dependable since they are grown in different media

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and environment. Thus the objective of the present study was investigated on the variability pathogen infecting chilli in Tamil Nadu.

MATERIALS AND METHODS

Collection of pathogen isolates

Chilli fruit showing the typical symptoms of fruit rot were collected from different places of Tamil Nadu state. The pathogen was isolated in Potato Dextrose Agar (PDA) medium using the collected samples. The infected lesions were cut into small pieces by means of a sterile scalpel and surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec. and washed repeatedly by using sterile distilled water. Then the bits were placed onto sterilized Petri plates containing solidified PDA medium under aseptic conditions in the culture room. The plates were incubated at room temperature (28 ± 2°C) for five days after incubation. The tip of hyphal growth radiating from the infected tissue was transferred onto PDA slants. The fungus was purified again by single hyphal tip method and maintained on PDA slants. The above procedure was adopted in respect of all the ten isolates collected from different parts of Tamil Nadu.

Variability studies of the isolates

Nine mm culture discs from a 15 days old PDA culture of the pathogen were taken by using sterilized cork borer and placed at the centre of sterile Petri plates containing 20 ml of PDA under aseptic conditions. Fifteen days after of incubation at room temperature (28 ± 2°C), the mycelial growth and morphological characters of the isolates were observed. The morphological characters *viz*., mycelial growth, colour, septation of mycelium, size and shape of the conidia were observed. In addition to this number of setae per acervulus, number of septa per setae and sporulation were observed. Measurements of 100 spores were taken under the microspore (Magnification 45_X X 10_X) by using ocular and stage micrometers. The mean values and the range were determined.

Cultural characters of isolates

The following media were used for the growth of different isolates of the pathogens *viz*., Potato dextrose agar, Oat meal agar, Ripe chilli fruit extract agar, Green chilli fruit extract agar, Czapek' Dox medium and Richard's medium. Fine sliced pieces of potato tuber, ripe chilli fruit, and green chilli fruit were boiled for 10 minutes and the extracts were filtered. To the extract, other ingredients of the medium were added and volume was made up to 1000 ml with distilled water and autoclaved at 1.4 Kg cm $^{-2}$ for 15 minutes. Twenty ml of the sterilized warm medium was poured into sterilized Petri plates and allowed to solidify. The isolates were inoculated at the centre of the plate by placing 7 days old nine mm PDA culture disc of the pathogen. The plates were incubated at room temperature (28 ± 2°C). Three replications were maintained. The radial growth of the mycelium was measured at eight day after inoculation. The colony colour and growth pattern on the culture media were also recorded.

Virulence of the isolates

The red ripped fruit were used for inoculation. The fruit surface was sterilized with 0.1 per cent $HgCl_2$; washed with sterile distilled water and the fruits were inoculated with the spore suspension of the isolates (5×10^5 spores/ml) by spore suspension method. Fifteen fruits were inoculated with each isolate with three replications in each. The number of fruits infected and the intensity of fruit rot was calculated. The intensity of the fruit rot was calculated as Per cent Disease Index (PDI) as per the grade chart using the formula proposed by Mc Kinney (1923). And the virulent Melur isolate was used for study. The per cent disease index (PDI) was calculated using Mc Kinney (1923) the infection index,

$$PDI = \frac{Sum\ of\ numerical\ ratings}{Total\ number\ of\ fruits\ observed} \times \frac{100}{Maximum\ category\ value}$$

Data analysis: The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI) Biometrics unit, the Phillippines (Gomez and Gomez 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease indices were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Pathogen identification

The pathogen was isolated from the symptomatic chilli fruits showing small black circular spots on the skin of the fruits that in the direction of the long axis. The spots were sunken and light grey coloured with black margin. The spots enlarged into larger lesions and on the surface of the lesions acervuli, the fruiting body of the fungus appeared as minute black dots.

All the isolates of pathogen was identified as *Colletotrichum capsici* and further confirmed on the basis of colony characters i.e white mycelium become greyish white with age and produces short hyaline conidiophore bearing hyaline falcate conidia singly. The conidia with a centrally placed oil globule. The setae were black and needle like and the length varied from 32.6 - 53.8µ with 2 - 6 septation. The pathogen was purified and cultures of these isolates were maintained on Potato Dextrose Agar (PDA) slants. Similar type of pathogen characters have been observed and reported by (Singh 1995; Jeyalakshmi and Seetharaman 1999; Muthu Kumar and Bhaskran 2007).

Growth variability of isolates

The growth of *C. capsici* isolates in different solid media tested, the highest mean colony diameter was recorded in Potato Dextrose Agar (PDA) with 8.15 cm, followed by oat meal agar (8.14 cm), Czapek's Dox Agar (8.09 cm), Richard's Agar (7.63 cm), Ripe chilli agar (7.57 cm) and green chilli agar (7.34 cm). The isolate CC_1 exhibited the highest mean mycelial growth of 8.58 cm on all the six media tested, followed by CC_2 (8.38 cm), CC_3 (8.17 cm), CC_4 (8.08 cm), CC_5 (7.90 cm), CC_6 (7.72 cm), CC_7 (7.61 cm), CC_8 (7.40 cm), CC_9 (7.23 cm) and least in CC_{10} (7.08 cm). The isolate CC1 produced black colonies in Richard's agar, PDA, oat meal agar, white colonies in Czapek's Dox agar and it produce greyish white colonies in ripe and green chilli agar medium. In the present study, PDA supported the maximum growth of *C. capsici* isolates followed by oat meal agar, Czapek's Dox Agar and Richards agar (Table 1). Similar observations were made by Patil and Moniz (1973) who found that PDA supported maximum growth and sporulation of *C. capsici*. Similarly Jeyalakshmi and Seetharaman (1999) reported that *C. capsici* made good growth on PDA followed by Czapek's Dox agar and Richard's agar. Ghorge and Kodmelwar (1986) found that the maximum growth of *C. curcumae* on Czapek's Dox agar. Akhtar *et al.* (2007) reported that on PDA pathogen C. capsici produced white coloured mycelial growth with margins are black and wavy. Parey *et al.* (2013) found that *C. capsici* produced fairly white to light mouse grey, circular, fluffy mycelium with black coloured acervuli which were scattered all over the colony growth on PDA and supported maximum growth. Also, Raj *et al.* (2014) found that the pathogen produced white to greyish black on different media tested. Sangdee *et al.* (2011) found that isolates of *C. capsici* produced cottony colonies on PDA with a colour of greyish-white to dark grey on the ventral surface whereas the reverse of the colonies was mainly black and pathogen produced conidia varied between 23.5 to 35.0 µm in size.

Sporulation variability of isolates

The sporulation of *C. capsici* isolates in different solid media tested, the isolate CC_1 exhibited profuse sporulation on all the media except Richards agar in which moderate sporulation was observed. The isolate CC_2 shown moderate growth on all media and CC_3 shown profuse sporulation on PDA media and

Table 1
Growth and sporulation of *Colletotrichum capsici* isolates in different media

Isolates	PDA		Oat meal		Czapek's		Richards		Ripe chilli		Green chilli		Mean
	G*	S	G	S	G	S	G	S	G	S	G	S	
CC_1	8.90	+ + +	8.77	+ + + +	8.97	+ + +	8.63	+ +	8.23	+ + +	8.13	+ + +	**8.58**
CC_2	8.77	+ +	8.63	+ +	8.60	+ +	8.43	+ +	7.93	+ +	8.00	+ +	**8.38**
CC_3	8.53	+ +	8.43	+ +	8.37	+ + +	8.17	+ +	7.67	+ +	7.77	+ +	**8.17**
CC_4	8.37	+	8.33	+	8.27	+ +	8.03	+	7.83	+	7.63	+	**8.08**
CC_5	8.27	+	8.17	+	8.10	+	7.77	+	7.73	+ +	7.37	+ +	**7.90**
CC_6	8.07	+	8.10	+	7.87	+	7.47	+	7.63	+	7.17	+	**7.72**
CC_7	7.80	+	7.93	+	8.03	+	7.17	+	7.50	+	7.20	+	**7.61**
CC_8	7.73	+	7.77	+	7.77	+	7.03	+	7.27	+	6.83	+	**7.40**
CC_9	7.57	+ +	7.67	+ +	7.57	+ +	6.87	+ +	7.03	+ +	6.70	+ +	**7.23**
CC_{10}	7.50	-	7.57	-	7.37	+	6.73	-	6.73	+	6.60	+	**7.08**
Mean	**8.15**		**8.14**		**8.09**		**7.63**		**7.57**		**7.34**		

CC – *Colletotrichum capsici* isolates
G – Colony diameter in cm, S – Sporulation
+ Sparse, + + abundant and + + + profuse sporulation: - no sporulation

CD (P=0.05%)
Isolates : 0.06
Media : 0.05
Isolates × Media : 0.15

Czapek's Dox Agar media only, CC_4 shown moderate sporulation in Czapek's Dox media, in rest of the media isolates were shown sparse sporulation. The isolates of CC_6, CC_7 and CC_8 showed sparse sporulation in all the media and the isolate CC_9 shown moderate sporulation on all the media. The isolate CC_{10} shown no sporulation on Richard's agar, PDA, oat meal agar and sporulated in Czapek's Dox agar, Ripe chilli agar, Green chilli agar media. Although the majority of the isolate showed moderate sporulation, CC_1 which was found to be highly virulent ranked first in number of spores produced. This is in conformity with report of Christopher et al (2013) who reported that virulent strains exhibited very rapid growth and high sporulation in the culture. All the ten isolates produced short hyaline conidiophores bearing hyaline falcate conidia singly. The conidia measure 18 - 23 µ x 3.43 – 3.97 µ with a centrally placed oil globule. The setae were black and needle like and number varied from 12-32 with 2-6 septation (Table 2). These characters agreed with original descriptions given by Jeyalakshmi and Seetharaman (1999). Parey *et al.* (2013) also reported the dimensions of conidia which possessed large oil globule in the centre with the size of 23.3x4.1 ìm. Similarly, Masoodi *et al.* (2012) reported that, pathogen produced white to grey colonies with conidia size of about 19.70-33.60 x2.23-4.86 µm.

Table 2
Conidial size of the *Colletotrichum capsici* isolates

Isolates	*Length (µm)**	*Width (µm)**	*Setae per acervuli**
CC_1	23.00	3.93	32.00
CC_2	21.33	3.97	28.00
CC_3	22.67	3.90	26.67
CC_4	22.67	3.90	24.33
CC_5	21.33	3.57	27.33
CC_6	18.00	3.47	19.00
CC_7	18.67	3.43	12.00
CC_8	20.33	3.57	23.00
CC_9	20.33	3.73	17.00
CC_{10}	19.33	3.67	21.67
CD (P=0.05%);	1.7	0.2	2.18

* Mean of three replications
CC – *Colletotrichum capsici* isolates

Virulence of the isolates

The pathogen isolates were inoculated artificially on chilli fruits by spore suspension spray after pinpricking to test the virulence, the isolate CC_1 was significantly found to be most virulent which recorded the highest fruit rot infection of 98.67 per cent with fruit rot intensity of 72.27 PDI. The isolate CC_1 was the most virulent one followed by CC_2, CC_3, CC_6, CC_4, CC_5, CC_7, CC_8, CC_{10} while CC_9 was found to be the least virulent as evidenced by the degree of disease intensity on the host (Table 3). There was variation in pattern of acervuli produced on chilli fruits. The isolate CC_1, CC_2, CC_3, CC_5, CC_6, CC_7 produced acervuli in concentric rings while CC_4, CC_8, CC_9 and CC_{10} produced the same in scattered manner. Similarly Kumar and Mahmood (1986) also found variations in the virulence of the isolates of *C. dematium* in chilli. Thind and Jhooty (1990) reported that the most virulent isolate recorded the maximum fruit rot incidence and produced acervuli in a scattered manner. However, the results of the present study revealed that the most virulent isolate produced acervuli in concentric rings. Masoodi et al (2012) also found that the isolates of *C. capsici* produced characteristic symptoms on inoculated chilli fruits after seven days of inoculation. Sangdee *et al.* (2011) reported the pathogenic variability of isolates, which produced small lesions and tissue collapse, acervulus production and sporulation on chilli fruits after inoculation. Various disease scores based on the acervulus development time on inoculated fruits were observed and categorized into three groups.

Table 3
Virulence of *Colletotrichum capsici* isolates

Isolates	*Percent fruit infected**	*PDI**
CC_1	98.67	72.27
	(82.67)	(58.22)
CC_2	77.33	61.33
	(61.59)	(51.55)
CC_3	69.33	51.20
	(56.39)	(45.69)
CC_4	53.33	35.47
	(46.91)	(36.55)
CC_5	49.33	32.67
	(44.62)	(34.61)
CC_6	61.33	44.27
	(51.56)	(41.71)
CC_7	41.33	28.80
	(40.01)	(32.46)
CC_8	29.33	17.07
	(32.78)	(24.40)
CC_9	5.33	2.93
	(13.17)	(9.84)
CC_{10}	13.33	9.07
	(21.37)	(17.52)
CD (P=0.05%)	**3.36**	**0.76**

* Mean of three replications
Data in parentheses are arc sine transformed values

CONCLUSION

The pathogen *Colletotrichum capsici* was found to be associated with fruit rot of chilli in all the diseased fruits collected from the ten conventional chilli

growing areas of Tamil Nadu and varied in morphological characters. All the isolates of *C. capsici* produced black pointed setae, hyaline falcate conidia with single oil globule at the centre with profused sporulation. Among the solid media, Potato Dextrose Agar supported the maximum growth (8.15 cm) of all the isolates of *C. capsici* followed by oat meal agar, Czapek's dox agar, Richards Agar, riped chilli agar and green chilli agar. The isolate CC_1 exhibited the profuse sporulation on all the media except Richards Agar in which, moderate sporulation was observed.

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