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STUDIES ON THE CULTURAL AND PATHOGENIC VARIABILITY AMONG ISOLATES OF *COLLETOTRICHUM CAPSICI* (SYD.) BUTLER AND BISBY CAUSING ANTHRACNOSE FRUIT ROT INCIDENCE OF CHILLI

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Abstract

In the present study, a roving survey was conducted during *kharif*, 2016-17 in Krishnagiri and Cuddalore districts of Tamil Nadu to assess the occurrence of fruit rot incidence of chilli caused by *Colletotrichum capsici* and the results revealed endemic nature of the disease. Maximum mean severity of the disease was recorded at Kaveripattinam in Krishnagiri district (36.15%) followed by Vadakumangudi in Cuddalore district (34.12%). In general, the crop grown in red soil was found severely affected by anthracnose/fruit rot of chilli. Among the fifteen isolates of *C. capsici,* the isolate Cc2 was found to be more virulent. The fungus produced circular, scattered falcate conidia with black acervuli and creamy white mycelial growth on PDA medium and recorded the maximum mycelial growth (90.00) and sporulation at seventh day after inoculation.

Key words: Roving survey, Anthracnose, *Colletotrichum capsici,* cultural and pathogenic variability

Introduction

Chilli (*Capsicum annuum* L.) one of the most important spice crops in the world belonging to the family Solanaceae, is a well-known commercial crop used both as condiment or culinary supplement and vegetable. It is an important constituent of many foods, adding flavour, colour and pungency. It is grown in tropical and sub-tropical regions of the world for its pungent fruits which are used as both green and ripe (www.seasonal outlook of chilli.com 2008). Chillies are the good sources of Vitamin 'A', Vitamin 'B' and Vitamin 'C' and minerals like calcium, phosphorus, ferrous, sodium and copper in trace amounts. Chillies produce alkaloids, capsaicinoids, carotenoids and red pigments (Capsanthin, Capsorubin and Capxanthin) which make chilli hot and pungent (Panda *et al*., 2010) and such properties increase the demand for chillies all over the world.

India is the single largest producer contributing for about 38% followed by China 7%, Pakistan and Bangladesh contributing about 5% each. The area cultivated with Chilli worldwide is about 3.8 million hectares (9.4 million acres) producing 33 million tons of fresh Chilli (FAO, 2015). The important chilli growing states in India are Andhra Pradesh, Telangana, Maharashtra, Karnataka, Orissa, and Tamil Nadu contributing more than 70 per cent acreage in India. Tamil Nadu ranks 8th in area (41,400 ha) and 12th in production (119 mt) in India (www.horticultural statistics.com, 2016).

The chilli crop is subjected to various diseases caused by fungi, bacteria, viruses, nematodes and physiological disorders at different stages of development. Among the major diseases of chilli, anthracnose/fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is one of the most destructive diseases of chilli in India. Due to this disease more than 50% crop losses have been reported from different parts of India (Ramchandran *et al*., 2007). Hence, the present study was conducted to assess the occurrence of anthracnose/fruit rot in

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Krishnagiri and Cuddalore districts of Tamil Nadu during *kharif* season and assess the cultural and pathogenic variability among the isolates of *Colletotrichum capsici*.

Materials and Methods

Survey for assessment of loss due to anthracnose or fruit rot disease incidence

A field survey was conducted in 15 locations representing Krishnagiri and Cuddalore districts of Tamil Nadu to assess the extent of loss due to fruit rot disease incidence.

Disease assessment

The infected fruits were assessed fruits were based on 3 03 to 3 43 grade scale proposed by Bansal and Grover (1969) and the per cent disease index was worked out.

Grade	Percent disease infection
0	No disease
1	1 – 5 % disease
2	6 – 25 % disease
3	26 – 50 % disease
4	51 – 100 % disease

$$\text{Percent Desease Index} = \frac{\textit{Sum of individual rating}}{\textit{Number of fruits assessed}} \times \frac{100}{\textit{Maximum disease grade}}$$

Isolation of the Pathogen

The fruit of the infected plants showing typical symptoms of the disease was taken and washed with water; cut into small bits, surface sterilized with 0.1% Sodium hypochlorite solution for one minute and then washed in three changes of sterile distilled water. Then they were placed on the PDA medium in sterile Petri plate and incubated at 28±2°C and the isolate was purified by single hyphal tip method. The culture was maintained on PDA slants and used for further studies. The native isolates of *C. capsici* isolated from the different locations in Krishnagiri and Cuddalore districts were designated as Cc1 to Cc15. The mycelia growth of different isolates was assesses at seven days after inoculation. Also the cultural variability viz., mycelial growth, colony colour, colony character and sporulation were assessed following standard procedures and recorded.

Pathogenicity test

The pathogenicity of the isolates was tested on K1 variety of chilli grown in pots using plug inoculation method following modified protocol by Sanders and Korsten (2003). The conidial suspension of the isolate was prepared in sterile water from 12 days old culture grown on PDA slants. The conidial suspension was diluted with sterile distilled water in such a way that it contains approximately 2000 to 3000 conidia/ml. The chilli plants of age 60 to 75 days after transplanting (DAT) were used for inoculation. The plants were kept in humid atmosphere for 24 h by covering them with polythene sheets.

The ripened red chilli fruits were injured with the help of surgical needle (Thind and Jhooty, 1990). 100 ml of the conidial suspension with 10^{-6} cfu/ml of the pathogen was sprayed with hand sprayer on the fruits in the morning hours. Control plants were sprayed with the same volume of sterile distilled water. All the plants were immediately covered with polythene bags sprinkled with sterile water on the inner side to maintain high humidity and kept undisturbed as such for 24 h. Seven days after inoculation the fruits were observed for the disease development. The pathogen was isolated from the infected area of' the inoculated fruits and compared with the original isolate to satisfy Koch's postulates.

Results and Discussion

Survey on the incidence of fruit rot of chilli in Krishnagiri and Cuddalore districts of Tamil Nadu

An extensive survey conducted in major chilli growing areas of Krishnagiri and Cuddalore districts of Tamil Nadu during *kharif* season 2016-17 revealed the endemic nature of the disease (Table 1). Among the 15 different locations of Krishnagiri and Cuddalore districts surveyed for anthracnose/fruit rot incidence, Kaveripattinam registered the maximum incidence of 36.15 per cent followed by Mathur (35.02%), Vadakkumangudi (34.12%), Pochampalli (33.84%), Krishnagiri (31.00%), Pudhuchathiram (30.53%), Rayakottai (28.32%), Pattampakkam (27.93%), Hosur (27.32%), Soolagiri (27.04%), Jeyakondapattinam (26.73%), Santhur (25.15%), B. mutlur (21.53%) and sivapuri (20.12%) in the decreasing order of merit. The minimum fruit rot incidence was recorded in Periyapattu (15.32 %). The native isolates of *C. capsici* were isolated from the respective locations and designated as Cc1 to Cc15. The difference in the per cent disease index in different locations could be due to the presence of different strains of the pathogen differing in their virulence.

Similar to the present work, Krishnakumar (2002) reported 31.3 % fruit rot incidence of chilli due to *C. capsici* with an yield loss of 435kg/ha in Cuddalore district of Tamil Nadu and also reported that maximum percent fruit rot incidence was recorded at Vallampadugai village

Table 1: Survey on the incidence of fruit rot of chilli in Krishnagiri and Cuddalore districts of Tamil Nadu.

S.No.	Isolates	Name of the village	Soil type	Crop stage	Variety	Per cent Disease incidence (%)
Krishnagiri district						
1	Cc1	Hosur	Red soil	Fruiting	CO 1	27.32
2	Cc2	Kaveripattinam	Red soil	Fruiting	Jaya 64	36.15
3	Cc3	Krishnagiri	Red alluvial soil	vegetative	HPH- 1048	31.00
4	Cc4	Mathur	Red soil	vegetative	CO 1	35.02
5	Cc5	Pochampalli	Sandy loam soil	Fruiting	Ananya	33.84
6	Cc6	Rayakottai	Sandy soil	Fruiting	CO 2	28.32
7	Cc7	Santhur	Red soil	Fruiting	CO 4	25.15
8	Cc8	Soolagiri	Clay loam	Fruiting	Local	27.04
Cuddalore district						
9	Cc9	B. Mutlur	Sandy loam	vegetative	Co 1	21.53
10	Cc10	Jeyakondapattinam	Sandy loam	Fruiting	K 1	26.73
11	Cc11	Pattampakkam	Clay loam	vegetative	K 1	27.93
12	Cc12	Periyapattu	Sandy loam	Fruiting	K 2	15.32
13	Cc13	Pudhuchathiram	Sandy loam	vegetative	K 2	30.53
14	Cc14	Sivapuri	Clay loam	vegetative	K 1	20.12
15	Cc15	Vadakkumangudi	Clay loam	Fruiting	Sathur	34.12
	S.EdCD (p=0.05)	—	—	—	—	0.210.85

Data in parentheses indicate angular transformed values.

of Cuddalore district. Similar results have been reported by Pakdeevaraporn *et al.,* (2005) who recorded 50 Percent loss in chilli yield severely infected with *C. capsici*. Ekbote, (2005) reported that fruit rot caused by *C. capsici* was most prevalent disease of chilli in Haveri district of Karnataka and caused 25-48 percent losses in yield. Anamika *et al*., (2014) also reported 55.53-71.10 per cent fruit rot incidence on chilli in Madhya Pradesh. These earlier reports corroborates with the present findings.

Cultural characteristics of various isolates of *C. capsici*

The results revealed that all the fifteen isolates of *C. capsici* exhibited a great variability with respect to mycelial growth, colony colour, colony character and sporulation (Table 2). Among the fifteen isolates, the maximum mycelial growth was recorded by Cc2 (90.00 mm), Cc4 (90.00 mm), Cc5 (90.00 mm), and Cc15 (90.00 mm) at seventh day of inoculation. The minimum mycelial growth was recorded by Cc3. A similar such variation in the mycelial growth among the isolates of *C. capsici* was observed earlier by Krishnakumar, (2002). Massodi *et al*., (2013) also observed morphological variation in *C. capsici* isolates in respect to their colour, conidia size and shape, acervuli production and setae size.

Cultural characters, colony colour and sporulation also varied between the tests isolates. The isolates of Cc2, Cc3, Cc6, Cc7, Cc9, Cc10 and Cc15 produced creamy white, scattered falcate conidia with black acervuli, and non-uniform shape of mycelium. Some isolates such as Cc1, Cc4, Cc5 and Cc8 exhibited light brown mycelial colour, cottony growth and smooth circular margin. The isolates of Cc11, Cc13 and Cc14 showed whitish grey colour and the isolate Cc12 produced grey colour mycelium.

Thind and Jhooty (1990) successfully used morphological and pathological characteristics to categorize 150 isolates of *C. capsici* and *C. gloeosporioides* causing chilli anthracnose. Sharma *et al*., (2005), also reported variation in cultural and morphological traits of 37 isolates of *C. capsici*, and categorised them into five groups as Cc-I, Cc-II, Cc-III, Cc-IV and Cc- V. Akhtar and Singh, (2007) observed differences in colony characters such as shape, zonation, pigmentation and margin of the isolates of *C. capsici* when raised on different culture media. These earlier reports corroborate with the present observations. The sporulation induced by all the test isolates varied from fair (+) to excellent (++++). The isolates *viz.*, Cc2, Cc4, Cc5, Cc15, Cc11, Cc8 and Cc10 produced excellent (++++) sporulation. These results are in agreement with Mesta (1996) and Hegde (1998) with regard to sporulation.

Radial growth of *C. capsici* isolates on different nutrient media

There was considerable variation in the mycelial

Table 3: Radial growth of *C. capsici* isolates on different nutrient media.

S.No	Isolates	Radial growth (mm)			
		PDA	CDA	PCA	Mean
1.	Cc1	86.87	77.66	68.66	77.73
2.	Cc 2	90.00	89.07	87.55	88.87
3.	Cc 3	83.24	79.00	68.00	76.75
4.	Cc 4	90.00	89.50	87.00	88.83
5.	Cc 5	90.00	89.04	87.33	88.79
6.	Cc 6	88.18	82.33	75.37	81.96
7.	Cc 7	81.43	73.00	69.00	74.17
8.	Cc 8	78.64	77.66	67.66	74.65
9.	Cc 9	89.81	84.00	71.44	81.75
10.	Cc 10	79.14	73.57	66.00	72.90
11.	Cc 11	75.42	72.81	70.33	72.85
12.	Cc 12	82.67	76.34	71.05	76.69
13.	Cc 13	77.27	73.40	67.18	72.62
14.	Cc 14	72.34	68.66	64.73	68.58
15.	Cc 15	90.00	89.00	87.00	88.67
	S.EdCD (p=0.05)	0.320.61	0.390.81	0.290.59	0.721.79

PDA - Potato Dextrose Agar Medium; CDA – Czapek'sDox Agar Medium, PCA – Potato Carrot Agar Medium

growth within isolates (*C. capsici*) on different culture media (Table 3). Isolate Cc2 showed maximum growth (90.00 mm, 89.07 and 87.55 mm) on PDA, CDA and PCA media, respectively. While, least growth of 72.34, 68.66 and 64.73 mm was exhibited by the isolate Cc14 on PDA, CDA and PCA media, respectively. The results of the present study on the effect of various culture media on morphological and cultural characteristics and sporulation are in consonance with those reported earlier by several workers (Jash *et al*., 2003; Yadav and Khan, 2008; Hubballi *et al*., 2010). Similar to the present study, Chaudhary and Reeti Singh (2016) reported that, out of seven synthetic and non synthetic media tested, significantly maximum colony diameter and sporulation were recorded in potato sucrose agar medium followed by oat meal agar. Jeyalakshmi and Seetharaman (1999) also reported highest growth of *C. capsici*on potato dextrose agar followed by Czapeck'sdox agar and Richard's agar.

Table 2: Cultural characteristics of various isolates of *C.capsici.*

S.No.	Isolates	Mycelial colour	Mycelial pattern	Mycelial growth (mm) (7 DAI)	Sporulation
1.	Cc1	Light brown	Cottony growth	76.51	++
2.	Cc 2	Creamy white	Fluffy growth	90.00	++++
3.	Cc 3	Creamy white	Fluffy growth	60.13	++
4.	Cc 4	Light brown	Cottony growth	90.00	++++
5.	Cc 5	Light brown	Cottony growth	90.00	++++
6.	Cc 6	Creamy white	Fluffy growth	69.87	++
7.	Cc 7	Creamy white	Fluffy growth	70.58	+++
8.	Cc 8	Light brown	Cottony growth	83.61	++++
9.	Cc 9	Creamy white	Fluffy growth	62.97	++
10.	Cc 10	Creamy white	Fluffy growth	80.39	++++
11.	Cc 11	Whitish Grey	Slightly fluffy	84.82	++++
12.	Cc 12	Grey	Slightly fluffy	73.64	+++
13.	Cc 13	Whitish Grey	Slightly fluffy	79.46	+++
14.	Cc 14	Whitish Grey	Slightly fluffy	68.17	++
15.	Cc 15	Creamy white	Fluffy growth	90.00	++++

Sporulation: Excellent: ++++, Good: +++, Fair: ++, Poor: +

Table 4: Effect of *C. capsici* isolates on the incidence of anthracnose/fruit rot of chilli (var. K 1).

Sl.No.	Isolates	Anthracnose/Fruit rot incidence (%)
1.	Cc1	43.71 (41.58)
2.	Cc 2	68.21 (55.67)
3.	Cc 3	47.83 (43.75)
4.	Cc 4	58.36 (49.81)
5.	Cc 5	56.33 (48.63)
6.	Cc 6	43.25 (41.12)
7.	Cc 7	31.26 (33.99)
8.	Cc 8	33.23 (35.20)
9.	Cc 9	36.42 (37.12)
10.	Cc 10	28.92 (32.53)
11.	Cc 11	37.21 (37.58)
12.	Cc 12	39.85 (39.14)
13.	Cc 13	40.36 (39.44)
14.	Cc 14	35.62 (36.42)
15.	Cc 15	52.12 (46.21)
	S.EdCD (p=0.05)	0.521.45

Data in parentheses indicate angular transformed values.

Pathogenicity of *C. capsici* isolates on chilli var. K1

Among the isolates of *C. capsici* the isolate Cc2 from Kaveripattinam area belonging to Krishnagiri district was found to be highly virulent in causing maximum anthracnose/fruit rot compared to other strains investigated in the present study (Table 4). As observed in the present study Sanders and Korsten (2004) also confirmed pathogenicity of *C. capsici* isolates by inoculating conidial suspension on detached wounded chilli fruits. Generally, penetration of the host plants through wounds has been shown to be significant component of the infection process by

a number of *Colletotrichum* species (Ramachandran *et al*., 2008). Accordingly in the present study also the isolate Cc2 of *C. capsici* proved highly virulent in causing anthracnose/fruit rot, when inoculated on to injured fruit surface.

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