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Survey and isolation of *Colletotrichum capsici* causing chilli anthracnose disease from Southern districts of Tamil Nadu

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Abstract

Chilli (*Capsicum annum* L.) is a vegetable as well as spice crops, cultivated in tropical and subtropical regions of the world. In chilli, anthracnose otherwise called as fruit rot disease causes enormous loss to chilli growing farmers. To identify the disease incidence spot survey was conducted on the incidence of chilli anthracnose during 2020-2021 at three districts in Southern districts of Tamil Nadu. Maximum disease incidence (55.19%) was observed in Subramaniapuram village of Thoothukudi district and Pudhupatti village (50.37%) of Virudhunagar district. The fungus *Colletotrichum capsica* was isolated and morphological characteristics were studied for each isolates.

Keywords: *Colletotrichum capsici*, chilli, Disease incidence, Fruit rot

1. Introduction

Chilli (*Capsicum annum* L) is a popular commercially cultivated hot tasting berry crop and it is the fourth major crop cultivated globally and belongs to Solanaceous family. Anthracnose disease is one of the major constraints to chilli producers resulting in huge yield loss. In severe cases, pre and post-harvest yield losses accounts up to 50 per cent in India (Sahitya *et al.*, 2014) [12]. The disease has been reported to cause 30-76 per cent yield loss in Tamil Nadu (Datar, 1995) [3]. Anthracnose is characterized by circular or angular, depressed, sunken lesions with concentric rings of acervuli which contains pink to orange conidial masses. Generally, warm and wet climate (rainy weather) with approximate temperature of 27 °C, RH 75 to 80% and soil pH 5 to 6 favors the disease development (Roberts *et al.*, 2001) [11]. Keeping in view on the crop losses caused by this devastating pathogen and to identify anthracnose hot spots in southern districts, the present investigation has been carried out on survey of chilli anthracnose disease incidence in Southern districts of Tamil Nadu and isolation of *Colletotrichum capsici* and morphological characterization of the various isolates have been done.

2. Materials and Methods

2.1. Survey and collection of Diseased Sample

Roving survey was conducted to assess the severity of fruit rot disease in Southern districts of Tamil Nadu state *viz.*, Thoothukudi, Tenkasi and Virudhunagar. Five villages *viz.*, Subramaniapuram (Thoothukudi), Pudupatti (Virudhunagar), Kuralyampatti (Thoothukudi), Vadakarai (Tenkasi) and Aryanaykipuram (Thoothukudi) were surveyed. In each selected villages, disease severity was assessed by grading the fruits using 0-9 scale (TNAU, 1980) at fruiting stage.

Grade	Description
0	No disease
1	Less than 1% infection
3	1 – 10% infection
5	11 – 25% infection
7	26 – 50% infection
9	26 – 50% infection

Per cent Disease Index (PDI) was calculated by using formula given by Mc Kinney's (1923).

$$\text{Percent Disease Index} = \frac{\text{Sum of all individual disease ratings} \times 100}{\text{Total No. of plants observed} \times \text{Maximum disease grade}}$$

2.2. Isolation of *Colletotrichum* spp. and Morphological characterization

Infected fruit samples were collected from five places of Thoothukudi, Tenkasi and Virudhunagar districts. The infected tissue was cut into small pieces and surface sterilized with 0.1 percent mercuric chloride solution for 30 seconds followed by washing with series of sterile distilled water for 2-3 times. The sterilized PDA medium was poured into the sterilized Petri Plates and allowed to solidify. Sterilized infected pieces of chilli fruit rot samples were placed in three places at equal distance and incubated at 28 °C for 10 days. The growing young fungal hyphal tip was transferred to the sterile plates containing PDA medium aseptically for purification by following the hyphal tip method (Dhingra and Sinclair, 2017) [5]. The pure cultures of all the five isolates were maintained in PDA slants. Further subculturing was done from the slants and after ten days of incubation, the

plates were observed for the growth and morphological characters of the isolates viz., colour of mycelium, radial mycelial growth, shape and colour of the conidia.

2.3. Pathogenicity

Chilli fruits were collected from field and the contaminants were removed by using sodium hypochlorite solution 0.1% for 5 minutes then gently washed with distilled water for three times and finally dried using sterilized filter paper. After drying mild pin prick was made on the fruit with the sterilized needle and then 10µl conidia suspension (5×10^6 conidia/ml) was placed over the wound. The inoculated fruits were kept under 48 hrs of dark and light at the temp 25 °C (Suthan Raj, 2013) [10]. The fruits were observed upto 14 days and the diseases intensity was evaluated by using a modified 0–9 point scale based on the disease scoring system given by Dasgupta (1981) [2].

0	No infection(Highly Resistant)
1	Necrotic lesion or large water-soaked lesion(Resistant)
3	Acervuli present (Moderately Resistant)
5	Necrotic lesion, surface covered 25%of fruit surface,possible acervuli) (Susceptible)
7	>25% of the fruit showing necrosis, (Moderately Susceptible)
9	>25% of the fruit showing necrosis, abundant acervuli(Highly Susceptible)

2.4. Statistical Analysis

The experimental results were recorded and analyzed using WASP (Web Agri Stat Package). The data with percentage values were transformed into Arc sine at critical difference at 5% significant levels.

3. Results

3.1. Survey on the occurrence of anthracnose of chilli

The roving survey revealed that the disease was the three districts surveyed in 2020-2021. The overall disease severity ranged from 29.81to 55.19 percent. The highest Percent Disease Index (55.19%) was noticed in Subramaniapuram village of Thoothukudi district followed by Pudupatti village of Virudhunagar district (50.37%). The least PDI of 29.81%was recorded in Aryanaykipuram village of Thoothukudi district. *Colletotrichum capsici* isolated from all the five villages were named as CcI 1, CcI 2, CcI 3, CcI 4 and CcI 5(Table1).

3.2. Symptomatology

Majority of cases, diseases was observed on young fruit as circular, black sunken spot surrounded by grey followed by necrotic spot. In severe case, the pathogen spreads over the twig and tender fruit petiole causing die back symptoms. Matured fruit showed dark brown sunken spot with black colour acervuli at the center of the spot in a concentric pattern and finally fruit decayed (Fig.1).

3.3. Pathogenicity

All the five isolates of *C. capsici* could able to produce the typical symptoms of anthracnose on the fruit when artificially inoculated by pinprick method. The pathogenic isolates were reisolated from the artificially infected chilli fruit and the

Koch's postulate was proved. Among the five isolates, CcI 1 showed highest disease severity (>15-25%) followed by CcI 2 and CcI 3 both exhibited > 5-10% disease severity (Table 2, Fig. 2).

3.4. Morphological Variations of *C. capsici*

Different isolates of *C. capsici* were studied on PDA medium and the cultural and morphological characters were studied. Result revealed that all the isolates of *C. capsici* produced fluffy mycelial growth. Among the isolates, CcI 1, CcI 3 and CcI 4 showed whitish black mycelial growth whereas CcI 2 and CcI5 produced cottony growth. Maximum radial growth of 82.59 mm was observed in CcI 1 and found to be virulent which has taken 12 days to completely cover the plates. All the isolates produced hyaline conidia. The shape of the conidia is falcate (CcI 1 and CcI 3), cylindrical (CcI 2 and CcI 4) and fusiform in CcI 5. Among the isolates, good sporulation was found in all the isolates except CcI 4 which exhibited moderate sporulation (Table 3, Fig. 3). Significant variations were observed with respect to conidial dimensions among the isolates. The length of the conidia ranged from (12.46-18.69µm). Highest length of conidia was observed in CcI 1 (18.69µm) followed by16.25µm in CcI 2and 14.85µm in CcI 3. The minimum conidial length of12.56µm and 12.46µm was recorded in the isolate CcI 4 and CcI 5, respectively. Width of the conidia ranged from (3.13–6.83µm). The highest width of 6.83 µm was observed in CcI1followed by 5.46µm in CcI 2 and 5.24µm in CcI 3.The least conidial width size of 3.23µm and 3.13µm were recorded in isolate CcI 4 and CcI 5, respectively. The isolate CcI 1 and CcI 3 only found to produce acervuli with setae and in other isolates acervuli production was not observed (Table 4, Fig. 4.).

Table 1: Incidence of chilli anthracnose in Southern districts of Tamil Nadu

S.NO	Isolate Name	Latitude And Longitude	Village Name	District	Variety Name	Symptoms On	Percent Disease Index (Pdi)
1.	CcI 1	LAT N9°6' 34.0308" LONG E78°14'15"48.1212"	Subramaniapuram	Thoothukudi	MUNDU	Mature fruits	55.19 ^a (47.870)
2.	CcI 2	LAT N 8°41'59.7012 LONG E 77°56'38.76"	Pudupatti	Virudhunagar	K2	Tender fruits	50.37 ^b (44.908)
3.	CcI 3	LAT N9°6' 54.468" LONG E 78°12'19.872"	Kuralyampatti	Thoothukudi	MUNDU	Mature fruits	45.78 ^c (43.70)
4.	CcI 4	LAT N9.045° LONG E 77.27°	Vadakarai	Tenkasi	K1	Mature fruits	37.04 ^d (37.411)
5.	CcI 5	LAT 9°6' 38.916" LONG E 78°14' 58.5816"	Aryanaykipuram	Thoothukudi	MUNDU	Immature fruits	29.81 ^e (42.076)

*Mean of three replications

*Values in the parentheses are arcsine transformed values

*Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

Table 2: Severity levels of the various isolates of Chilli Anthracnose disease

Score/ Isolates	Diseases Severity Level					Severity
	CcI 1	CcI2	CcI3	CcI4	CcI5	
0	-	-	-	-	-	No infection
1	-	-	-	-	-	Larger necrotic lesion
3	-	-	-	>2-5%	>2-5%	Water soaked lesion on fruit surface
5	-	>5-10%	>5-10%	-	-	Necrotic lesion and acervuli present
7	>15-25	-	-	-	-	Necrotic lesion and acervuli

Table 3: Variation among the isolates of Chilli anthracnose caused by *Colletotrichum capsici*

S. No	Isolate	Colour Of Mycelial Mat	Growth Pattern	Topography	Radial Mycelia Growth (Mm)	Days To Cover The Plate(Days)	Sporulation
1.	CcI 1	Whitish black	Wavy	Mycelial fluffy growth	82.59 ^a	12	+++
2.	CcI2	Cottony white	wavy	Mycelia fluffy growth	78.65 ^b	11	+++
3.	CcI3	Whitish black	circular	Wavy fluffy growth	68.97 ^c	11	+++
4.	CcI4	Whitish brown	circular	Wavy fluffy growth	63.7 ^d	10	++
5.	CcI5	Cottony white	circular	Mycelial fluffy growth	58.41 ^e	9	++

(-) no sporulation, (+) poor sporulation, (++) moderate sporulation, (+++) good sporulation, (++++ excellent sporulation;

*Mean of three replications

*Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

Table 4: Conidial variation among the isolates of *Colletotrichum capsici*

S.NO	Isolate	Colour	Shape Of Conidia	Length Of Conidia(µm)	Width Of Conidia(µm)	Acervuli	Setae
1.	CcI 1	Hyaline	Falcate	18.69 ^a	6.83 ^a	PRESENT	PRESENT
2.	CcI2	Hyaline	cylindrical	16.25 ^b	5.46 ^a	ABSENT	ABSENT
3.	CcI3	Hyaline	Falcate	14.85 ^b	5.24 ^a	PRESENT	PRESENT
4.	CcI4	Hyaline	cylindrical	12.56 ^c	3.23 ^b	ABSENT	ABSENT
5.	CcI5	Hyaline	Fusiform	12.46 ^c	3.13 ^b	ABSENT	ABSENT

*Mean of three replications

*Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

**Fig 1:** Symptom of chilli anthracnose infected fruit

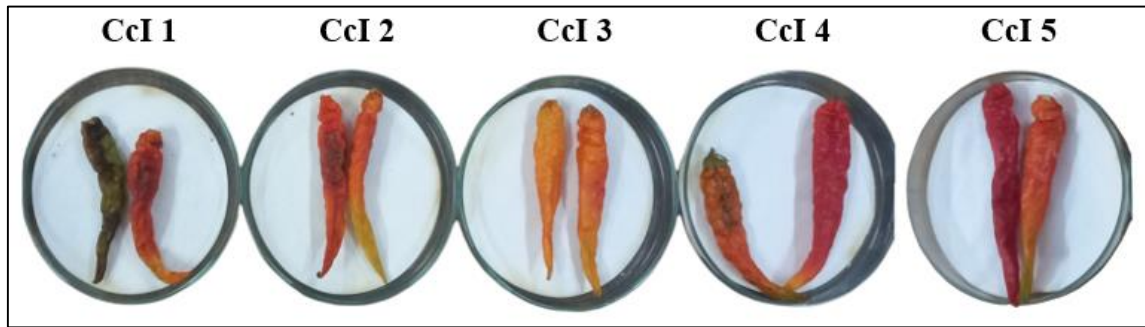


Fig 2: Pathogenicity study for *Collectotrichum capsici* isolates

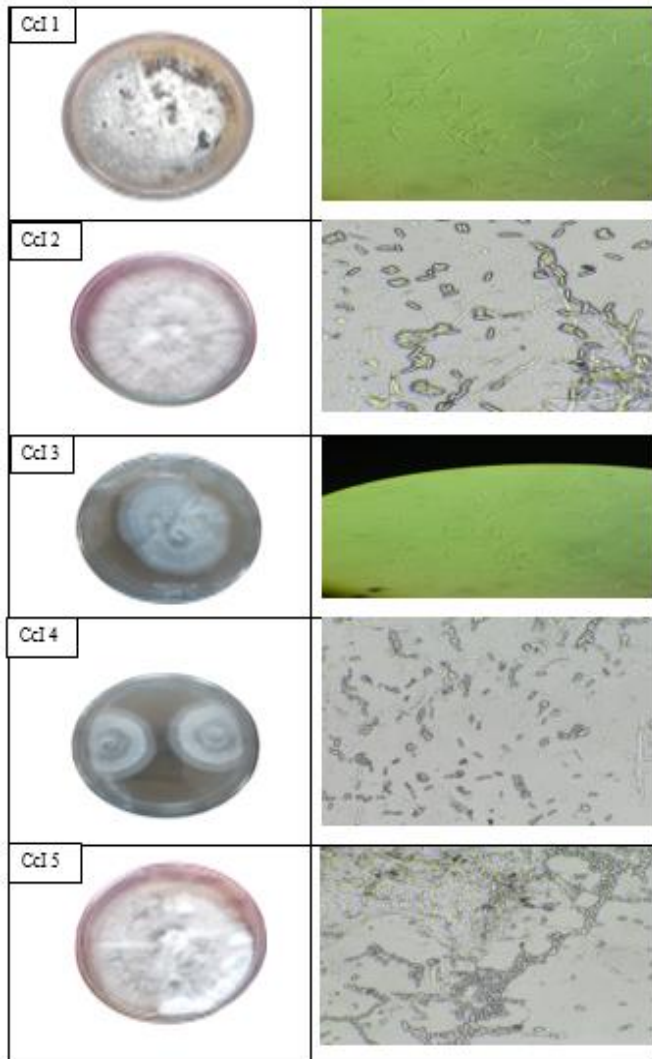


Fig 3: Growth and Morphological characters of *Collectotrichum capsici* isolates

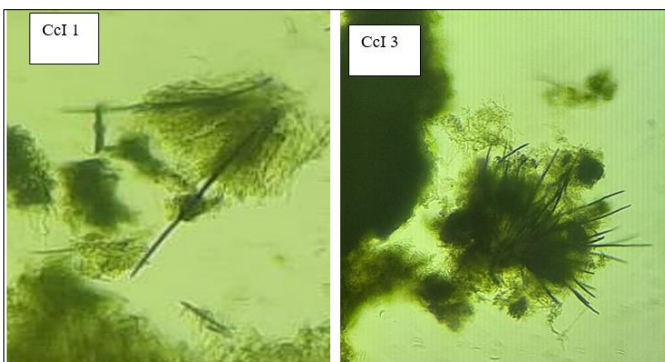


Fig 4: Acervuli of *Collectotrichum capsici*

4. Conclusion

The anthracnose disease incidence and morphological characters of *Collectotrichum capsici* were studied and observed in Southern district of Tamil Nadu. Among the surveyed areas, highest level of disease incidence was noticed in Subramaniapuram village of Thoothukudi district. The fruit rot inciting fungi *Collectotrichum capsici* isolated from different villages showed morphological variations in growth and cultural characters. Among the isolates, CcI1 collected from Subramaniapuram village of Thoothukudi district showed highest conidial length of 18.69 μ m and width of 6.83 μ m compared to other isolates, the least conidial length of 12.46 μ m and width of 3.13 μ m was observed in CcI5 which was collected from Aryanaykipuram village of Thoothukudi District.

The above conidial character of 18-27 μ m \times 1.8-4.1 μ m and 12-21 μ m \times 3.5-6 μ m were already studied and reported by Prajapati *et al.*, (2020) [9] and Ghosh *et al.*, (2016) [6] respectively. The variation in spore size was noticed by Hanumanthappa *et al.*, (2018) [7] and found that conidiophores of *Collectotrichum sp* isolates were hyaline and septate, containing ovoid to cylindrical, falcate conidia that were one celled with one or two oil globules and were 21.5 x 4.1 μ m size. de Silva *et al.*, (2019) [4] reported that variability occurred in conidial character among the different *Collectotrichum sp*. Bailey, 1992 [1] and Saket Kumar *et al.*, (2015) agreed that the radial mycelial growth varied with different isolates(43.83-70.86mm). From this study, it is concluded that maximum occurrences of chilli anthracnose was observed in Subramaniapuram village, Thoothukudi district of Tamil Nadu and they differed both culturally and morphologically

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