Research Article

Cultural and Morphological Variability of *Colletotrichum sp* 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* species 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 twenty five isolates of *Colletotrichum* species developed from fruits was established following koch's postulates. Culture colonies varied in their cultural behaviour from *Colletotrichum* species. Potato Dextrose Agar (PDA) supported the maximum growth (9.0 cm). All the isolates of *C. capsici* and *C.gloeosporides* produced black pointed setae, hyaline falcate and cylindrical conidia with single oil globule at the centre. Number of setae per acervulus (12-32) and number of septa per seta (2-4) varied among the isolates. Majority of the isolates produced profuse sporulation. To know the virulence isolates fruits were inoculated and results suggested that isolate Cc3 caused the maximum fruit rot intensity (82.67 per cent) while Cc25 showed least intensity (32.67 per cent).

Keywords: Chilli anthracnose, *Colletotrichum* species, Variability

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Introduction

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is one of the important spice cum vegetable crop in India. 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 [29]. In India chilli is being grown in area of 789000 ha with a production 1389000 tonnes and yield of 1760 kg/ha. [2]. The important chilli growing states are Andhra Pradesh, Karnataka and Tamil Nadu. Inspite of chilli is infected by various biotic and abiotic factors. In biotic several pathogens are causing severe diseases and yield loss. The Chilli anthracnose pathogen *C. capsici* infects diverse host with a high degree of pathogenic variability [24]. The genus *Colletotrichum* causes anthracnose on wide range of fruits, vegetables, cereals, grasses and ornamental plants [3, 6]. *Colletotrichum* is considered as the eighth most importanat plant pathogeneic fungal genus in the world [4]. Among the pathogens *Colletotrichum* species causing anthracnose of chilli has been shown to be caused by at least four species of *Colletotrichum: C. capsici* and *C. gloeosporioides* in India [24], Indonesia [28], Korea [10], Thailand [16]; *C. acutatum* in Australia [26] and Indonesia [15]; and *C. coccodes* in New Zealand [9].

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 [22]. The anthracnose is one of serious diseases on chili to cause the yield loss and to reduce the quantity of marketable fruits. Disease incidence is recorded from 20 to 80% on fruits of *Capsicum annum* and 5 to 20% on fruits of *C. frutescens* infected in the field conditions. It has been reported that a part of post harvest losses of fruit quality deterioration of chilli is due to anthracnose ranges from 21 47% [21]. 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 [22], 25 per cent loss of capsaicin content [19]. Therefore, the objective of this study was to characterize the *Colletotrichum* species associated with chilli anthracnose in Tamil Nadu.

Materials and Methods

Disease Survey and occurence

A roving survey was conducted to assess chilli fruit rot incidence in different chilli growing areas of Tamil Nadu. In each field, five plots each with 5x5 m area were selected. Among the five plots one plot was fixed at the centre of the field and the remaining four plots were fixed at random in different places in the field avoiding border rows. Infected chilli plants were collected in a polythene bag along with soil and labeled properly. They were brought to the laboratory and stored in a refrigerator for further studies. The fruit rot incidence was assessed by counting the number of affected plants out of total number of plants in each plot ($25m^2$). In each area three fields were assessed and the mean disease incidence was calculated. Per cent disease incidence was calculated and furnished in the table by using the formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Anthracnose and fruit rot of chilli incidence was recorded by scoring five plants in each microplot using 0-9 scale [12] as given here under.

Score description	
Grade	**Per cent fruit infection**
0	No symptom
1	1-10%
3	11-25%
5	26-50%
7	51-75%
9	> 75%

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 ± 2^0C) 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 twenty five isolates collected from different parts of Tamil Nadu.

Cultural and morphological characterization of different isolates of Colletotrichum species

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. Twenty days after of incubation at room temperature (28 ± 2^oC), 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 40X X 10X) by using ocular and stage micrometers. The mean values and the range were determined.

Cultural characters of isolates

PDA media were used for the growth of different isolates of the pathogens. Fine sliced pieces of potato tuber, 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 ± 2^0C). 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

Pathogenicity testing

In this study, among 25 isolates were checked for the virulence ability by pathogenicity test. A susceptible K1 (Kovilpatty1) cultivar of *C.capsici* and *C.gloeosporioides* under pot culture experiment at TNAU in the Department of Plant Plathology. Thre replication was maintained. Isolates were cultured on PDA at 27^{o}C under continuous fluorescent light. Prior to inoculation chilli fruits were pin pricked gently with a sterilized needle. Conidia from 15 day old cultures were harvested by adding 5-10mL Of sterilized distilled water to the surface of the cultures, brushing with a soft bristle brush, and filtering through a double layer of cheesecloth. Spore concentration was determined using haemocytometer and adjusted to 10^6 conidia / ml 1 with sterile water. Fruits used as control were inoculated with 20 ul of sterilele distilled water and was used in uninoculated control. Disease reaction of the host was evaluated by measuring the length, width and area of the typical anthracnose lesion which developed on the fruits. Symptoms were evaluated 7-15 days after inoculation (DAI). 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 [13]. The per cent disease index (PDI) was calculated using [13] the infection index,

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

Result and Discussion

Survey and occurrence of disease incidence

Disease survey was conducted in major Chill growing districts. Occurrence of *Alternaria* leaf spot was moderate in most places of the survey. Fruit rot of chilli caused by *Colletotrichum* was observed during the survey. The maximum incidence of fruit rot (60.10%) was recorded in Perumal patty in Theni district. The lowest incidence was recorded Vathrappu in Virudhunagar district. Fruit rot infected chilli plants were collected during the survey and brought to the laboratory and used for isolation of *Colletotrichum* species. Totally Twentyfive isolates of chilli *Colletotrichum* species were isolated from the chilli plants collected during the disease survey. The most important chiilli growing states in India are Andhra Pradesh (49%), Karnataka (15%) Maharasthra (6%) and Tamil Nadu (3%) which constitutes nearly 75 per cent of the total area under chilli [7].

Isolation and 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* species and further confirmed on the basis of colony characters i.e white mycelium become greyish white 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 37.6 - 53.8µ with 2 – 4 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 [25][8][14].

Cultural and morphological characters of Colletotrichum speies

The growth of *C. capsici* and *C. gleosporides* isolates in PDA media tested, the highest mean colony diameter was recorded. Similar observations were made by [18] who found that PDA supported maximum growth and sporulation of *C. capsici*. Similarly [8] reported that *C. capsici* made good growth on PDA followed by Czapek's Dox agar and Richard's agar.[5] found that the maximum growth of *C. curcumae* on Czapek's Dox agar [1] reported that on PDA pathogen *C. capsici* produced white coloured mycelial growth with margins are black and wavy.[17] 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, [20] found that the pathogen produced white to greyish black on different media tested.[23] 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 of isolates

Although the majority of the isolates showed moderate sporulation, Cc3 which was found to be highly virulent ranked first in number of spores produced. Those virulent strains exhibited very rapid growth and high sporulation in the culture. Among the Twenty five (Cc1-5, 13, 15, 20, 23) isolates produced short hyaline conidiophores bearing hyaline falcate conidia singly. The conidia measure 18-23µ x 3.43–3.97µ with a centrally placed oil globule. (Cc 6-12, 14, 16-19, 21, 22, 24) isolates produced short hyaline conidiophores bearing hyaline Cylindrical conidia singly. The setae were black and needle like and number varied from 12-32 with 2-4 septation. These characters agreed with original descriptions given by [8]. [17] Also reported the dimensions of conidia which possessed large oil globule in the centre with the size of 23.3x4.1 µm. Similarly, [12] reported that, pathogen produced white to grey colonies with conidia size of about 19.70-33.60 x 2.23-4.86 µm.

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 Cc3 was significantly found to be most virulent which recorded the highest fruit rot infection (82.67) per cent.

Similarly [11] also found variations in the virulence of the isolates of *C. dematium* in chilli. [27] 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. [12] Also found that the isolates of *C. capsici* produced characteristic symptoms on inoculated chilli fruits after seven days of inoculation. [23] 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.

Conclusion

The pathogen *Colletotrichum* species was found to be associated with fruit rot of chilli in all the disease fruits collected from the chilli growing areas of Tamil Nadu and varied in Morphological characters. All the twenty five isolates of *Colletotrichum* species produced black setae and produce conidia. Among the twenty five isolates Potato Dextrose Agar the maximum growth (9 cm) of the isolates of the *Colletotrichum* species.

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