

Original Research Article

Cultural and Morphological Variability in *Colletotrichum capsici* Causing Anthracnose Disease

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ABSTRACT

Keywords

Colletotrichum capsici,
Pathogenicity,
Cultural and
Morphological
variability.

Chilli is an important cash crop and India is the largest grower in the world. *Colletotrichum capsici* is a fungal plant pathogen that infects chilli fruit and causes anthracnose disease under tropical and subtropical condition. The *Colletotrichum capsici* infect chilli in high humidity during mature or premature condition. On the basis of cultural and morphological identification, 12 isolates of *Colletotrichum capsici* were collected from different locations. These isolates were grown on different media and were characterized for colony morphology. Thus, the present study was undertaken to know the pathogenicity and variability, so as to devise better practices of the diseases to study cultural characteristic. In this study, twelve isolates of *Colletotrichum capsici* were cultivated in Petri plates from fruits of chilli, were established following Koch's Postulates. These isolates were grown in PDA and OMA medium. They varied in their cultural behavior ranging from cottony to fluffy, mostly suppressed with regular to irregular margin and the colour of colony ranged between white and gray. Growth rate of isolates was between 4mm–7.72mm/day. The isolates Cc2 and Cc11 were most virulent and more pathogenic on chilli. The cultural and morphological variability and relationship between 12 isolates were investigated using ANOVA. *Colletotrichum capsici* was highly variable based on colony morphology as manifested by colony colour. The mean colony diameter of PDA was 7.4mm and OMA 2.38mm. Morphological characteristics of them were examined by observation of culture for colony diameters, colony morphology, pigmentation, radial growth, colony colour, colony reverse, mycelial growth pattern and sporulation.

Introduction

Colletotrichum capsici is a fungal pathogen causing anthracnose disease in chilli fruits in tropical and subtropical conditions, resulting in qualitative and quantitative losses

(Cannon *et al.*, 2011, Noireung *et al.*, 2012). *Colletotrichum capsici* infecting different hosts have a high degree of pathogenic variability that has to be assessed for

effective development of resistance. The *Colletotrichum capsici* is distributed throughout the tropics and very commonly occurs in chilli growing areas. *Colletotrichum capsici* appeared to be the most severe being able to infect a range of *Capsicum* species and has resistant genotypes (Taylor, 2007). The use of differential hosts was a viable option for the evolution of pathogenic variability and morphological diversity. The symptoms of anthracnose invasion are sunken necrotic lesions on fruits (Waller *et al.*, 2002; Agrios, 2005). The anthracnose lesions on chilli fruit reduced their marketable value (Manandhar *et al.*, 1995). 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% (Rajapakse *et al.*, 2007). *Colletotrichum capsici* infecting diverse hosts and has a high degree of pathogenic variability (Sharma *et al.*, 1999). The use of differential hosts is a viable option for the evaluation of pathogenic variability. Disease symptoms developed only on fully ripened pods or wound chilli fruits. The symptoms typically occur on fruits at maturation under wet autumn conditions which appear as dark sunken lesions with abundant production of orange masses of conidia (Williams *et al.*, 1990).

Colletotrichum capsici is cosmopolitan with either multiple species occurring on a single host or a single species occurring on multiple hosts (Sanders and Korsten, 2003). Fungus-host relationships are broad, imprecise and often overlapping (Freeman *et al.*, 2000). *Colletotrichum capsici* is infect many hosts and may adapt to new

environments (Sanders and Korsten, 2003; Photita *et al.*, 2004) leading to serious cross infection problems in plant production. Different hosts and stages of maturity are important to test the expression of resistance to *Colletotrichum capsici*. The interaction between fruit maturity stage and infection by colonization may depend on *Colletotrichum capsici* (AVRDC, 2002). Thus, the objective of our study was to investigate the variability in *Colletotrichum capsici* populations infecting chillies in North-western region of India by using cultural and morphological appearance.

Materials and Methods

Isolation and identification of *Colletotrichum capsici*

Anthracnose infected chilli pods were collected from different fields situated in Varanasi, Jaunpur, Azamgarh, Ambedkarnager, Faizabad, Barabanki, Sultanpur, Nagpur, Bhuwaneshwar, Mirzapur, Gazipur and Bihar in India. The diseased part of the fruits were cut at advanced margin of lesions into small pieces (5mm diameter). The pieces were surface sterilized in aqueous solution of mercuric chloride (0.1:100w/v), streptocyclin and transferred in PDA after washout.

The PDA plates seized with paraffin film and transferred in incubator at room temperature ($27\pm 2^{\circ}\text{C}$) for 7–10 days in dark condition. After 2 days, margin of mycelial growth was transferred to another Petri plate in aseptic condition. After 3–4 days the culture was revised for the purification of *Colletotrichum capsici* in aseptic condition, for culture growth. *Colletotrichum capsici* identification was based on morphological characters such as size and shape of conidia and appressoria, existence of setae or presence of a teleomorph, and cultural

characters such as colony colour, growth rate and texture (Smith and Black, 1990). These criteria alone are not always adequate to differentiate species because of variations in morphology and phenotype among species under different environmental conditions. The morphology of *Colletotrichum capsici* was observed under a compound microscope with slide (Than *et al.*, 2008).

Now, single spore isolation was performed to obtain pure culture (Ratanacherdchai *et al.*, 2007). The *Colletotrichum capsici* isolates were identified by using key given by Gunnel and Gubler (1992). The *Colletotrichum capsici* produce grey white scattered falcate conidia with black acervuli, and non-uniform shape of mycelium.

Colletotrichum capsici formed smooth circular margin in the colony. The grey whitish mycelium of *Colletotrichum capsici* gradually developed from the second day in culture of isolates. The spore of *Colletotrichum capsici* was 13.21–16.21 μm long and 1.79–3.28 μm wide (Yun *et al.*, 2009).

Purification and maintenance of culture

In purification, isolates were obtained from the lesion without visible sporulation, using the procedure described by Photita *et al.*, (2004). Single spore isolation from the infected fruits with sporulation was also carried out using procedure described by Choi *et al.* (1999).

Spore masses were picked up with a sterilized wire loop and streaked on to the surface of water agar (WA) plates which were then incubated over night. A single germination spore was picked up with a sterilized needle and transferred onto Potato Dextrose Agar (PDA). Pure culture was

stored on 4°C on slants (Yang *et al.*, 2009).

Pathogenicity tests

Pathogenicity tests were done separately for each isolate on host plant using plug inoculation methods following a modified protocol by Sanders and Kirsten (2003). Prior to inoculation, healthy chilli fruits (Hisarshakti) were taken from the fields. All fruits were swabbed with 70% (v/v) ethanol to reduce surface contamination and left for air dry in laboratory for 3–4 min. Fruits were then wounded by gentle pricking with a sterilized needle. Inoculation was proposed by culturing each isolate on PDA at room temperature ($27\pm 2^\circ\text{C}$) for 10 days. Plugs (5 mm diameter) were cut from activity speculating areas near to colony periphery by using a sterilized cork borer and placed. The inoculated fruits were kept in moist chamber at room temperature ($27\pm 2^\circ\text{C}$) for 15 days. The experiments were done with three replications. Data collected as lesion diameter of infected fruits and the coefficient of variation was computed (Ratanacherdchai *et al.*, 2007).

Cultural and morphological study of *Colletotrichum capsici*

The isolate of *Colletotrichum capsici* were grown in different type of media viz. Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), for morphological variation of culture (Bailey *et al.*, 1995). The isolates inoculate in PDA and OMA media. Growth of culture and sporulations are start after 2-3 days (Talhinhas *et al.*, 2005) which given below.

Cultural and morphological study of *Colletotrichum capsici* on PDA

The isolate of *Colletotrichum capsici* was grown in PDA medium for the cultural and

morphological study. The colony growth starts in 1–2 days at 27 ± 2 °C under darkness on PDA. The cultural and morphological character (colony radial growth, colony colour, colony reverse, pigmentation, zonation and nature of growing margin) were recorded with the help of image analyzer after 10 days of inoculation in each replicate (Talhinhas *et al.*, 2005). Slides were prepared from 10 days old culture and number of spores, presence of conidial masses and seate were measured with hemocytometer.

Cultural and morphological study of *Colletotrichum capsici* on OMA

The isolate of *Colletotrichum capsici* was grown in OMA medium for the cultural and morphological study. The above procedure revise and study cultural and morphological character (colony radial growth, colony colour, colony reverse, pigmentation, zonation and nature of growing margin) were recorded after 10 days of inoculation with the help of image analyzer in each replicate (Talhinhas *et al.*, 2005). Slides were prepared from 10 days old culture and number of spores, presence of conidial masses, seate was measured with hemocytometer.

Radical growth of the fungus was measured after 10 days. Colony characteristics of all isolates were taken in three block design with factorial experiment and were statistically analyzed (Fig. 1).

Photograph of cultural characters and mycelium were taken to image analyzer. The length and width ratio was determined and the shape and size were recorded. This was also done with conidia obtained from culture grown on Potato Dextrose Agar (PDA), Oat Meal Agar (OMA). Data were analysed using analysis of variance and

significant difference tests. The colony radius and characteristics (radical growth, colony reverse, pigmentation, margin, zonation, aspect, nature of growing margin-presence of conidial masses, and sporulation) of the isolates were assessed. Colony radius was compared on PDA and Oat Meal Agar medium (Bailey *et al.*, 1995).

Results and Discussion

Morphological characteristics of *Colletotrichum capsici*

Measurement of the mycelial growth was taken for 12 different isolate of *Colletotrichum capsici* from purified culture when inoculated on PDA and OMA plates (Bailey *et al.*, 1995).

Radial growth

Isolates Cc2 and Cc11 are fast growing (70.66 and 77.16mm in PDA and 85.66 and 86.67 in OMA) where as isolates Cc5 and Cc12 are slow growing (43.83 and 40cm in PDA while 76.66 and 82.14 in OMA). The radial growth of *Colletotrichum capsici* (Cc2 and Cc11) is large but radial growth of *Colletotrichum capsici* (Cc5 and Cc12) is very less, after 10 days of inoculation (DAI) as shown in Figure 2 (Talhinhas *et al.*, 2005).

Mean colony diameter

The mean colony diameter of *Colletotrichum capsici* was found to be 7.5 recorded on PDA while mean colony diameter of *Colletotrichum capsici* was 2.34 recorded on the OMA media after 10 DAI. The diameter of isolates Cc2 and Cc11 were 70.66 and 77.16 on PDA while 85.66 and 86.67 on OMA media (Bailey *et al.*, 1995).

Colony Colour

The colony colour of *Colletotrichum capsici* was determined on PDA and OMA. The colony colour of *Colletotrichum capsici* is gray to dark gray (Cc11,Cc7,Cc8,Cc9), Cc12 shows dark gray to graphite gray in PDA and also white colour in OMA media after 10 DAI but in the OMA media isolate Cc1 shows white colour (Maziah *et al.*, 1995) instead as well shown in the Figure 1.

Colony reverse

The isolates were observed for colony reverse in terms of concentric ring and zones formed by acervuli. Most of them were without concentric ring and acervuli. The isolates Cc1, Cc2, Cc3, Cc4, Cc6, and Cc10 showed zonation on PDA but isolates Cc3, Cc5, Cc6 showed concentric ring on the PDA after 10 DAI. The isolates Cc2, Cc3, Cc5, Cc6, Cc7 and Cc8 showed zonation concentric on OMA but isolate Cc1, Cc4, Cc9, Cc10, Cc11 and Cc12 were found without zonation concentric (Talhinhas *et al.*, 2005).

Pigmentation

Pigmentation of all isolates were checked on PDA most of the isolates were found to be producing yellow pigment (Cc1, Cc2, Cc4 and Cc5) and few light orange pigment (Cc3, Cc7), whereas on OMA few isolates produced light orange pigment (Cc10, Cc12) whereas others were non- pigment producers (Maziah *et al.*, 1995) even after 10 days of inoculation (DAI).

Sporulation

All the isolates of *Colletotrichum capsici* showed sporulation on PDA media after 10 DAI and produced spores in the range of $2.6 \times 10^4 - 3.0 \times 10^4$ /ml and $3.19 \times 10^4 - 3.57$

$\times 10^4$ on OMA. The number of spores increases after 10 DAI to about $3.19 \times 10^4 - 3.57 \times 10^4$ /ml on PDA and $3.39 \times 10^4 - 483 \times 10^4$ /ml on OMA. Number of spores was found to be more in Cc2 and Cc11 (Rajapakse *et al.*, 2007).

Mycelium Growth Pattern

The margin of colony of *Colletotrichum capsici* was irregular with fluffy mycelium surface in all the isolates on PDA and OMA. Mycelial growth of Cc3, Cc7 was to be found regular in isolate Cc3 and Cc7 on OMA media (Maziah *et al.*, 1995).

Thus *Colletotrichum capsici* is show different types variability (colony morphology, conidial shape, presence of setae, radial growth, colony colour, colony reverse, mycelial growth pattern, sporulation and pigmentation) in the PDA and OMA media (Maziah *et al.*, 1995). This type of variation in culture surprise of any researchers and also desire for further research. In culture, most of isolates produce cottony, fluffy or suppressed colonies. However, no significant difference were noticed in shape and size of conidia (Sharma *et al.*, 1999)

Figure.1 Cultural / Morphological variation among isolates of *Colletotrichum capsici* on PDA medium at 27±2°C, after 10 days of inoculation

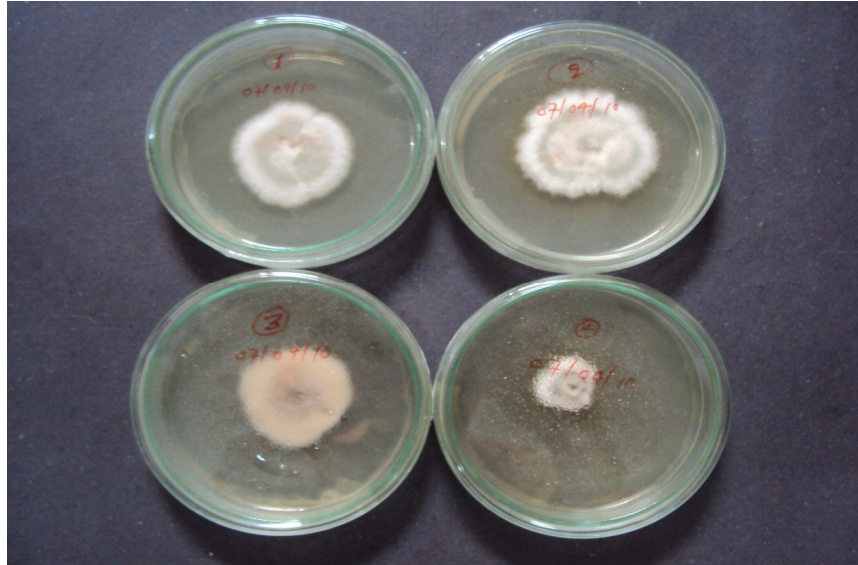
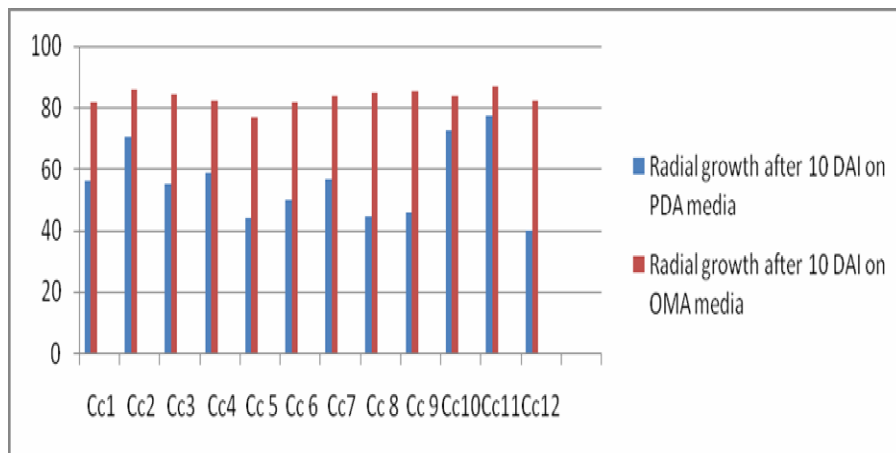


Figure.2 Radial growth of 12 isolates of *Colletotrichum capsici* on PDA and OMA media



Acknowledgement

This study is financially supported by Banaras Hindu University (BHU) through M.Sc. thesis programs (ID. No. /Enrolment No. 08209/303279). Special thanks are conveyed Dr. Lognathan (Senior Scientists), Division of Crop Improvement and Pathology, Indian Institute of Vegetable Research (IIVR) Sahansahpur Jakhni, Varanasi, for their constant encouragement and providing the resources to carry out the research work.

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