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# PATHOGENIC AND MORPHOLOGICAL VARIABILITY IN *COLLETOTRICHUM CAPSICI* ISOLATES CAUSING ANTHRACNOSE OF CHILLI (*CAPSICUM ANNUM* L.)

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## ABSTRACT

Pathogenic and morphological variability among 4 isolates of anthracnose of chilli (*Colletotrichum capsici*) from four states-Rajasthan, Gujarat, Karnataka and Madhya Pradesh were studied. Pathogenic variability was studied in micro plots grown 5 different cultivars. Among the isolates, the maximum colony diameter after 7 days was (88 mm) in UDR Cc-01, it produced maximum number of spore (21.0 x $10^4$ conidia/$mm^2$ medium). The maximum size of conidia was the isolate GUJ Cc-03 which measured 22.6 (20.3–24.8) x 3.7 (3.3–4.1) $\mu$m, maximum size of setae was the isolate BAN Cc-02 which measured 110.3 (98.1-123.5) × 4.8 (4.2-5.3) and maximum size of accervulus was the isolate UDR Cc-01 which measured 200.8 (178.7-224.8) $\mu$m *in vitro* study. In field, the evaluation was done to find out the resistant chilli cultivar and pathogenic potential of different isolates. Isolate UDR Cc-01 caused shortest latent period (48 hrs) in Pusa jwala and longest latent period of 80 hrs in Sadabahar. UDR Cc-01 was the most virulent, as it caused susceptible (S) reaction (disease score 5.5 and PDI 61.2) on Pusa jwala cultivar and moderate susceptible (MS) reaction with score 4.5 and PDI 50.2 in California wonder and disease score 3.7 with 41.3 PDI in Yellow type.

## INTRODUCTION

Chilli (*Capsicum annum* L.) is an important spice cum vegetable crop. Among various diseases, anthracnose caused by *Colletotrichum capsici* is a major problem in India (Chauhan *et al.,* 2014). *Colletotrichum* spp. are among the most important plant pathogens worldwide, causing the economically important disease anthracnose in a wide range of hosts, including cereals, legumes, vegetables and tree fruits (Bailey and Jeger, 1992). Anthracnose disease of chilli caused by *C. capsici* Butl. and Bis. has been a serious problem for chilli cultivation in India. The fungus is distributed throughout the tropics and very commonly occurs in chilli growing areas of India resulting in disease incidence levels ranging between 66% and 84%, and incurring yield loss up to 12-50% (Thind and Jhooty, 1985; Bagri *et al.*, 2004; Sharma *et al.*, 2005). Little information is available on pathogenic variability among the isolates of *C. capsici*. Tamil *et al. (*2006) studied the morphology and physiological variability in nine isolates of *C. capsici*. He found some isolates were distinct from other isolates because of its light colour, average conidial length and saltent type with sector formation in mycelial growth. Similarly, Wijesekara *et al.* (2006) studied the morphological and cultural characteristics of 15 *Colletotrichum* isolates belonging to *C. capsici, C. dematium, C. falcatum [Glomerella tucumanensis], C. gloeosporioides [G. cingulata]* and *C. lindemuthianum*. The data showed variations in morphotaxonomy and dimensions of the conidia, appresoria and setae formation. Ekbote *et al. (*2002) screened fifty-one chilli cultivars for resistance to fruit rot disease caused by *C. capsici*. None was tolerant, one was resistant, three were moderately resistant, five were moderately susceptible, seven were susceptible and nine were highly susceptible to the disease. Montri *et al.* (2009) showed virulent pathotype differences within *C. capsici* isolates based on percent lesion size, appearance of necrotic or water-soaked tissue and presence of acervuli on *Capsicum* species. Therefore, information on the distribution of race or pathotype in chilli growing areas and an accurate method for identification and characterization of *C. capsici* is necessary for effective disease management and development of host resistance in breeding programs (Freeman *et al.,* 1998; Kim *et al.,* 2010). Keeping these views in mind, the experiments were designed to see pathogenic and morphological variability in *Colletotrichum capsici* isolates causing anthracnose of chilli (*Capsicum annum* L.)

## MATERIALS AND METHODS

Cultures of *C. capsici* were isolated from the disease samples of chilli showing variable symptoms collected from different locations and designated as UDR Cc-01: Udaipur (Rajasthan), BAN Cc-02: Bangalore (Karnataka), GUJ Cc-03: Gandhinagar (Gujarat) and MP Cc-04: Indore (Madhya Pradesh). The samples were scaled for Lesion size (length and width) by measuring 15 randomly selected lesions and mean disease score for disease severity was recorded on a 0-9 (0 = No infection, 1 = 1–2%, 3 = 3–5%, 5 = 6–10%, 7 = 11–25%

and 9 = >25% of fruit area infected) as described by Montri *et al*. (2009).

The cultural and morphological studies carried in laboratory by growing the isolates of *C. capsici* petridishes on potato dextrose agar medium keeping four replications of each isolates. The plates were incubated at 28 ± 1ºC for 7 days and observations for colony diameter in mm, spore production and size of conidia, setae and accervulus by each isolate was recorded.

Pathogenic variability in *C. capsici* isolates was studied by inoculating these on different chilli cultivars in micro plots. Five chilli cultivars *viz.,* Pusa jwala, California wonder, Yellow type, Mathania and Sadabahar were used for the experiment. Surface sterilized seeds of each cultivar were separately sown in nursery bed and twenty-five-day-old seedlings of each cultivar were transplanted in micro plots. The size of micro plots was kept 2.75 × 3 m in randomly block design in three replications. There were five plants in each replication at distance of R x R 45 cm and P x P 30 cm. Ninety day-old plants were inoculated with ($1x10^6$ spore/ml) containing tween 20 (polyoxyethylene sorbitan monolaurate) @ 0.1 ml per 250 mL. with each isolate by spraying the spore suspension thoroughly over the plant canopy but control plots of each cultivar for each isolate was having without inoculation served as a control. Polyethylene sheets were used to separate plants when inoculating with different isolates to avoid inoculums drift. Since the weather was mostly dry, water spraying was given thrice a day to provide proper humidity.

Observations for latent period time in hours for development of first chlorotic or necrotic lesion started on second day of inoculation were recorded. The per cent disease index (PDI) was calculated by using following formula described given by Chester (1959).

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all individual disease rating} \times 100}{\text{Total number of plant assessed} \times \text{Maximum disease rating}}$$

Disease reaction was scored on a 0–9 point scale by Montri *et al*. (2009): 0 (highly resistant), no infection; 0-1.0 (resistant), 1–2% of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site; 1.1-3.0 (moderately resistant), >2–5% of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5% of the fruit; 3.1-5.0 (moderately susceptible), >5–15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface; 5.1- 7.0 (susceptible), >15–25% of the fruit area shows necrotic lesion with acervuli; and 7.1-9.0 (highly susceptible), >25% of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervul. The infected plants were then carefully uprooted, placed in polypropylene bags and autoclaved to destroy the pathogen.

Data analysis- The date of latent period and disease severity score were subjected to analysis and least significant difference (critical deviation) determined on 5 per cent (P= 0.05) possibility for host lines, isolates and host lines × isolate

**Table 1: Details of isolates of *Colletotrichum capsici* collected from different states in India**

| S. No | Isolate designation | Place of sample collection /Chilli cultivar | Per cent disease severity* | Lesion symptom | Lesion size** Length (mm) Mean | Length (mm) Range | Width (mm) Mean | Width (mm) Range | Diameter (mm) 7th day*** | Sporulation ($x10^4$ conidia / $mm^2$)**** | Colony characters Growth characters | Colony colour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | UDR Cc-01 | Khamnor (Udaipur), Pusa jwala | 35.1 | Sunken necrotic dry straw tissue, with black concentric ring of acervuli | 28 | 4-42 | 4.5 | 1-6 | 88 | 21 | Wooly with entire margin, center white, raised and fluffy with zonation/ rings | Dirty white later turning in gray, center white |
| 2 | BAN Cc-02 | Bangalore (Karnataka), Banglore local | 34 | Sunken necrotic tissue with circular to elongated, yellowish to straw colourspot, with black concentric ring of acervuli | 23 | 3-35 | 4.8 | 1-7 | 82 | 16.5 | Wooly with entire margin, raised at center, initially submerged later on arial with distinct rings (zonation) | Dirty white turning in dark gray with darkest margin |
| 3 | GUJ Cc-03 | Gandhinagar (Gujarat), Teja | 32.5 | Necrotic dry straw tissue, with black concentric ring of acervuli | 19 | 4-27 | 5.6 | 2-8 | 74 | 13.5 | Wooly with entire margin, raised, fluffy growth in the center with zonation | Light to dark gray |
| 4 | MP Cc-04 | Indore (Madhya Pradesh), MP desi | 25.7 | Reddish yellow lesion, irregular spot | 17 | 2-26 | 4.7 | 1-6 | 80 | 9.8 | Aerial, fluffy with entire margin, centre of colony raised, no zonation ring | Dirty white turning to gray, and white in the center |
| | | | | | | | SEm± | | 1.05 | 0.42 | | |
| | | | | | | | CD ( $P$ = 0.05) | | 3.25 | 1.3 | | |

*Percent disease severity based on 50 Chilli fruits randomly selected from each cultivar of different places, **Mean of 15 replications; *** Mean of four replications **** Mean of 15 replication

interaction.

## RESULTS AND DISCUSSION

The isolates showed considerable variations in anthracnose symptoms, the lesion size ranged from 2 to 42 mm in length and 1 to 9 mm in width across the isolates. The maximum mean lesion size (28x4.5mm) with the range of 4-42mm x 1-6 mm was recorded for the isolate UDR Cc-01 (Table 1).

The four isolates of *C. Capsici* isolated from different places cultivars showed considerable variations in colour of the colony, colony diameter and rate of sporulation on PDA media. The margin/shape and colour of the culture of different isolates varied from wooly with entire margin, raised and fluffy with zonation/ rings that varied in colour from dirty white turning into gray, with white in the center (UDR Cc-01), In BAN Cc-02 wooly with entire margin, raised at centre, initially submerged later on arial with distinct rings (zonation) and in colour dirty white turning in to dark gray with dark margin, while in GUJ Cc-03 wooly with entire margin, form growth zonation, raised and fluffy growth in the centre with light to dark gray colour and the growth was aerial, fluffy with entire margine, centre of colony raised, no zonation rings with dirty white turning to gray and white in the centre (MP Cc-04). Maximum mean colony diameter (88 mm) and maximum number of spores 21.0 x $10^4$ conidia / $mm^2$ medium were counted in the isolate of UDR Cc-01 followed by diameter 82 mm and sporulation 16.5 x $10^4$ conidia / $mm^2$ for BAN Cc-02 after 7 days of inoculation. Isolates from MP Cc-04 showed 80 mm colony diameter and least sporulation 9.8 x $10^4$ conidia / $mm^2$. The least mean colony diameter 74 mm and sporulation 13.5 x $10^4$ conidia / $mm^2$ of isolate GUJ Cc-03. (Table 1). In these isolate of *C. capsici* showed significant variation in conidia, setae and acervuli morphology. Results show that the mean length and width of conidia in different isolates of *C. capsici* ranged from 20.3 (18.0-21.9) to 22.6 (20.3-24.8) x 3.6 (3.2-4.0) to 3.7 (3.3-4.1) $\mu$m, the mean length and width of setae in different isolates ranged from 80.5 (71.6-90.1) to 110.3 (98.1-123.5) x 4.2 (3.7-4.7) to 4.8 (4.2-5.3) $\mu$m and diameter of acervuli in different isolates ranged 135.0 (120.2-151.2) to 200.8 (178.7-224.8) $\mu$m. The maximum length and width of conidia was of the isolate GUJ Cc-03, 22.6 (20.3-24.8) x 3.7 (3.3-4.1) $\mu$m, followed by 22.4 (20.1-25.0) x 3.7 (3.3-4.0) $\mu$m of BAN Cc-02 it was. In MP Cc-04 it was 21.5 (19.7-24.0) x 3.7 (3.0-4.1) and in UDR Cc-01 the mean size of conidia was 20.3 (18.0-21.9) x 3.6 (3.2-4.0) $\mu$m. The maximum length and base width of setae among the isolate was of BAN Cc-02 which measured 110.3 (98.1-123.5) x 4.8 (4.2-5.3) $\mu$m. Setae of MP Cc-04 were next, measuring 98.8 (87.9-110.6) x 4.4 (3.9-4.9) $\mu$m. The smallest size of setae of isolate UDR Cc-01 that measured 80.5 (71.6-90.1) x 4.3 (3.8-4.8) $\mu$m, in the reaming isolate GUJ Cc -03 was 95.8 (85.3-107.3) x 4.2 (3.7-4.7) $\mu$m. The maximum diameter of accervulus was measured 200.8 (178.7-224.8) $\mu$m for the isolate UDR Cc-01, while minimum diameter of accervulus was of MP Cc-04 it was 135.00 (120.2-151.2) $\mu$m. In the remaining two isolates the diameters of acervuli were intermediate of these two isolate. In BAN Cc-02 the mean base diameter of accervulus was 175.5 (156.2-196.5) $\mu$m and in GUJ Cc-03 was 145.5 (129.5-163.0) $\mu$m. (Table 2). Despite these variations, the size of conidia agreed well with the standard description of conidia (16 to 30 x 2.5 to 4.0 $\mu$m) of *C. capsici* (Mordue, 1971) and also about same in Soyabean anthracnose (Nagaraj and Jagirdar, 2014).The micrometrical data of present study confirm the reports published by Saxena and Singh (1959), Guldekar *et al*. (2009) and Jameel *et al*. (2008) and thus the present results are on the similar line of published literature.

The value for latent period, disease score and PDI were statistically ($P$ = 0.05) significant for isolates, cultivars and isolates × cultivars. Among the isolate UDR Cc-01 caused shortest latent period (48 hrs) in Pusa jwala followed by in California wonder (56 hrs) and longest latent period 80 hrs in Sadabahar. The latent periods were 65 and 72 hrs in Yellow type and Mathania, respectively. In BAN Cc-02 showed shortest latent period (60 hrs) in Pusa jwala followed by 66, 74 and 80 hrs in California wonder, Yellow type and Mathania, respectively. It showed longest latent period (84 hrs) in Sadabahar. GUJ Cc-03 showed shortest latent period (70 hrs) in Pusa jwala followed by 74 hrs in California wonder, 79 hrs in Yellow type and 86 hrs in Mathania. It showed longest latent period (90 hrs) in Sadabahar. The shortest latent period of isolate MP Cc-04 was 82 hrs in Pusa jwala and longest in Sadabahar (96 hrs). It showed latent period was 85 hrs in California wonder, 90 hrs in Yellow type and 92 hrs in Mathania.

Among the cultivars, Pusa jwala exhibited the maximum mean disease score (4.4) and PDI (48.6) across the four isolates, followed by California wonder with disease score 3.7 and 40.8 PDI. Yellow type chilli showed disease score 3.1 with PDI 34.4 and disease score 2.0 and PDI 21.7 by Mathania. The minimum disease score 1.4 and PDI 15.7 was in Sadabahar (Table 7 and 8). Of the four isolates UDR Cc-01 of *C. capsici* was the most virulent, as it caused maximum mean

**Table 2: Variations in conidia, setae and acervuli morphology of different isolates of *Colletotrichum capsici*.**

| S. No. | Isolates | Size of Conidia ($\mu$m)* Length Mean | Range | Width Mean | Range | Size of Setae ($\mu$m)* Length Mean | Range | Base Mean | Range | Accervulus Diameter ($\mu$m)* Width Mean | Range |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | UDR Cc-01 | 20.3 ± 0.9 | 18.0-21.9 | 3.6 ± 0.2 | 3.2-4.0 | 80.5 ± 3.7 | 71.6-90.1 | 4.3 ± 0.2 | 3.8-4.8 | 200.8 ± 9.3 | 178.7-224.8 |
| 2. | BAN Cc-02 | 22.4 ± 1.2 | 20.1-25.0 | 3.7 ± 0.2 | 3.3-4.0 | 110.3 ± 5.9 | 98.1-123.5 | 4.8 ± 0.3 | 4.2-5.3 | 175.5 ± 9.4 | 156.2-196.5 |
| 3. | GUJ Cc-03 | 22.6 ± 1.2 | 20.3-24.8 | 3.7 ± 0.2 | 3.3-4.1 | 95.8 ± 5.0 | 85.3-107.3 | 4.2 ± 0.2 | 3.7-4.7 | 145.5 ± 7.6 | 129.5-163.0 |
| 4. | MP Cc-04 | 21.5 ± 1.1 | 19.7-24.0 | 3.7 ± 0.2 | 3.0-4.1 | 98.8 ± 5.2 | 87.9-110.6 | 4.4 ± 0.2 | 3.9-4.9 | 135.0 ± 7.3 | 120.2-151.2 |
| | SEm ± | 0.06 | | 0.01 | | 0.29 | | 0.01 | | 0.53 | |
| | CD ($P$ = 0.05) | 0.18 | | 0.04 | | 0.81 | | 0.03 | | 1.48 | |
| | CD ($P$ = 0.01) | 0.23 | | 0.05 | | 1.07 | | 0.04 | | 1.95 | |

* Mean no. of 50 conidia, setae and accervulus and SD of mean value

**Table 3: Latent period (hours), Disease score, reaction and Disease severity of four isolates of *Colletotrichum capsici* on five chilli cultivars in field condition**

| S. No. | Isolate/Chilli Cultivar | Latent period in hours* | | | | | Disease severity score 0-9 scale* and Disease reaction | | | | | Disease severity (PDI)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pusa jwala | California wonder | Yellow type | Mathania | Sadabahar | Mean | Pusa jwala | California wonder | Yellow type | Mathania | Sadabahar | Mean | Pusa jwala | California wonder | Yellow type | Mathania | Sadabahar | Mean |
| 1. | UDR Cc-01 | 48 | 56 | 65 | 72 | 80 | 64.2 | 5.5(S) | 4.5(MS) | 3.7(MS) | 2.9(MR) | 2.3(MR) | 3.8 | 61.2(38.6) | 50.2(33.9) | 41.3(30.0) | 32.7(26.1) | 25.7(22.8) | 42.2(30.3) |
| 2. | BAN Cc-02 | 60 | 66 | 74 | 80 | 84 | 72.8 | 4.5(MS) | 3.8(MS) | 3.4(MS) | 2.3(MR) | 1.6(MR) | 3.1 | 49.9(33.7) | 41.7(30.2) | 37.5(28.3) | 25.3(22.6) | 17.4(18.7) | 34.4(26.7) |
| 3. | GUJ Cc-03 | 70 | 74 | 79 | 86 | 90 | 79.8 | 4.1(MS) | 3.5(MS) | 2.9(MR) | 1.6(MR) | 1.0(R) | 2.6 | 45.7(31.9) | 38.7(28.8) | 32.5(26.1) | 17.4(18.5) | 11.5(14.8) | 29.1(24.0) |
| 4. | MP Cc-04 | 82 | 85 | 90 | 92 | 96 | 89 | 3.4(MS) | 2.9(MR) | 2.4(MR) | 1.0(R) | 0.7(R) | 2.1 | 37.7(28.4) | 32.5(26.1) | 26.3(23.1) | 11.4(14.8) | 8.2(12.5) | 23.2(21.0) |
| | Mean | \65 | 70.3 | 77 | 82.5 | 87.5 | | 4.4 | 3.7 | 3.1 | 2.0 | 1.4 | | 48.6(33.2) | 40.8(29.7) | 34.4(26.9) | 21.7(20.5) | 15.7(17.2) | |
| | | SEm± | CD ( $P$ = 0.05) | | | | SEm± | CD ( $P$ = 0.05) | | | | | | SEm± | | CD ( $P$ = 0.05) | | | |
| | Isolate | 0.75 | 2.13 | 2.86 | | | 0.03 | 0.08 | 0.11 | | | | | 0.19 | | 0.53 | 0.71 | | |
| | Cultivar | 0.83 | 2.39 | 3.20 | | | 0.03 | 0.09 | 0.12 | | | | | 0.21 | | 0.60 | 0.80 | | |
| | Isolate × cultivar | 1.67 | 4.78 | 6.40 | | | 0.06 | 0.18 | 0.24 | | | | | 0.42 | | 1.19 | 1.60 | | |

*Mean of three replications; S: Susceptible, MS: Moderate susceptible, MR: Moderate resistant, R: Resistant; **Mean of three replications; Figures in parentheses are arcsine √ per cent angular transformed values

disease score (3.8) and PDI (42.2) and less virulent was MP Cc-04, it caused minimum mean disease score (2.1) and PDI (23.2). BAN Cc-02 caused mean disease score 3.1 with PDI 34.4 and GUJ Cc-03 caused mean disease score 2.6 with PDI 29.1.

UDR Cc-01 was the most virulent, as it caused susceptible (S) reaction with disease score 5.5 and PDI 61.2 on Pusa jwala. This isolate exhibited moderate susceptible (MS) reaction to California wonder and Yellow type with disease score 4.5 and 3.7 with PDI 50.2 and 41.3, respectively. It caused moderate resistance (MR) reaction (score 2.9) with PDI 32.7 in Mathania and disease score 2.3 with 25.7 PDI in Sadabahar. This was followed by BAN Cc-02 that caused moderate susceptible (MS) reaction (score 4.5) with PDI 49.9 in Pusa jwala, score 3.8 and PDI 41.7 in California wonder, score 3.4 and PDI 37.5 in Yellow type. This isolate caused moderate resistance (MR) reaction (score 2.3 and 1.6) with PDI 25.3 and 17.4 in Mathania and Sadabahar, respectively. Isolate GUJ Cc-03 caused moderate susceptible (MS) reaction with score 4.1 and 3.5 and PDI 45.7 and 38.7 in Pusa jwala and California wonder, respectively. This isolate caused moderately resistance (MR) reaction (score 2.9) with PDI 32.5 in Yellow type, score 1.6 with PDI 17.4 in Mathania and it gave resistance (R) reaction (score 1.0) with PDI 11.5 in Sadabahar. Isolate MP Cc-04 gave moderate susceptible (MS) reaction with score 3.4 and PDI 37.7 in Pusa jwala. This isolate caused moderate resistance (MR) reaction (score 2.9) with PDI 32.5 in California wonder and score 2.4 with PDI 26.3 in Yellow type. It caused resistance (R) reaction (score 1.0) with PDI 11.4 in Mathania and score 0.7 with PDI 8.2 in Sadabahar. (Table 3).

Similar studies on pathogenic variability has also been reported in nine isolates of *C. capsici* on five chilli cultivars based on cultural characters and pathogenicity (Khirbhat *et al.,* 2004 and Vanan *et al.,* 2005). Kaur *et al*. (2005) evaluation 71 chilli germplasm line against 37*C. capsici* isolates and found that most of the lines were susceptible to almost all the isolates. Similarly, Singh and Vishunavat (2007) evaluated 32 chilli cultivars against anthracnose, No cultivars were found to be immune for anthracnose disease, five cultivars showed resistant reaction and nine cultivars were susceptible to anthracnose.

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