



# CULTURAL, MORPHOLOGICAL AND PATHOGENIC VARIABILITY IN ISOLATES OF *COLLETOTRICHUM CAPSICI* CAUSING ANTHRACNOSE OF CHILLI IN EASTERN U.P.

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## Abstract

Anthrachnose of chilli (*Capsicum* spp.) due to causes *Colletotrichum capsici* major losses throughout India, where chilli plants are grown. Therefore, the present experiment was carried out to understand the behaviour and biology of the pathogen so as to incorporate proper management strategies to reduce the economic loss and get maximum yield. A total of twenty one *Colletotrichum capsici* isolates, associated with necrotic lesions of chilli leaves and fruit were collected from chilli producing areas in Eastern U.P. Isolated pathogen of *Colletotrichum* spp. was readily identified by its falcate shaped conidia and abundant setae in the necrotic lesions. Pathogenic behaviour of 21 isolates derived from chilli fruit was established following Koch's Postulates. Variability in cultural, morphological and pathogenic characteristics was observed in all the isolates of *Colletotrichum capsici* derived from both ripe and unripe chilli fruits.

**Keywords:** Chilli, *Colletotrichum capsici*, morphological, cultural and pathogenic variability.

## Introduction

Chilli is an important cash crop of India and it is the largest producer, consumer and exporter of dry chilli and other products around the world. Chilli plays a very important role in commercial sector. There are many medicinal, nutritional and economically benefits of its production. Chilli is not only important ingredient in foods but it is also used for culinary and remedies applications. Specially, it is used in pharmaceutical industries, preparation of oleoresin, cosmetics, and other industrial resources. This crop suffers heavy losses in yield due to many diseases especially dieback and fruit rot diseases occurs on leaves, stems and fruit of host plants (Sutton, 1992.) Anthracnose caused by *Colletotrichum capsici* (Syd.) is widespread throughout the chilli growing areas of India (Jeyalakshmi, 1996). Anthracnose affects the yield directly and indirectly by infecting stems, leaves and fruits causing flower drop. Anthracnose caused by *Colletotrichum* spp. is a major problem of ripened fruit called as ripe fruit rot occurs worldwide wherever chilli plants are cultivated. *Colletotrichum capsici* is capable of causing disease on virtually all plant parts of the chilli during growth of pathogens. *Colletotrichum capsici* is one of the most important genera of plant pathogenic fungi with many species known to cause disease in plant crops worldwide. Chilli anthracnose usually develops under high humid conditions when rain or irrigation occurs after the fruit formation and ripening of yield losses up to 84% (Thind and Jhooty, 1985). Latent infections during immature green fruits that express at the fruit ripening stage, reduce the quality and quantity of

chilli fruits and causes crop loss up to 50% world-wide (Pakdeevaporn *et al.*, 2005). Small anthracnose lesions on chilli fruits reduce their marketable value due to black spot appear on fruits (Manandhar *et al.*, 1995). Proper identification of these pathogens is important for mitigating the risk of incursion of new pathogens which if happens, may have devastating consequences for the local industries. In addition, exact identification of the species is important for resistance breeding programs and in identifying the host-range of species.

## Materials and Methods

The survey was conducted during *Rabi* season from 2014-15 in five districts of eastern Uttar Pradesh *i.e.* Varanasi, Chandauli, Mirzapur, Bhadohi, and Jaunpur (Table 1). The diseased leaves and fruits of chilli samples showing typical and unpredictable anthracnose or fruit rot symptoms were collected from the farmer fields.

## Identification of isolated pathogen of *Colletotrichum capsici*

A total of twenty one isolates associated with anthracnose symptoms on chilli fruit and leaves were collected during the harvest season of chilli from different districts of Eastern U.P. in India collected and isolated of fungal pathogens. Infected portion of fruits and leaves were cut into small pieces of 5 to 6 mm and 1.5-2.5 mm width were cut at the juncture of diseased and healthy portion with the help of disinfected blade after surface sterilizing. These bits were surface sterilized in 0.2% mercuric chloride (HgCl<sub>2</sub>) solution for about 15 seconds

followed by three times washing with sterilized distilled water in petriplates under aseptic conditions using laminar air flow chamber to remove the traces of mercuric chloride. After blot drying with sterilized filter paper, these bits were placed in sterilized petriplates containing 20 ml of potato dextrose agar medium (PDA) mixed with streptomycin sulphate to avoid bacterial contamination. These plates were incubated at  $27\pm 1^\circ\text{C}$  for five days to obtain good growth of fungus. They were sub cultured in PDA slants and or on 2% water agar and purified by hyphal tip culture method. The culture thus obtained was subjected to purification of *Colletotrichum capsici* from collected samples of chilli showing anthracnose lesions were used the procedure described by Than *et al.* (2008) and Saxena *et al.* (2014).

### Morphological characterisation

**Cultural variability:** Different cultural media was used for studying the growth of *Colletotrichum capsici* isolates. Rate of growth was measured with the help of scale. Morphological characters including colony character, colour, and conidial masses and number of acervuli formation for each isolate were recorded only on PDA, while rate of growth of mycelia was recorded on different culture media (Saxena *et al.*, 2014).

**Composition and preparation of culture media:** The cultural characters of *Colletotrichum* spp. were studied using the following five culture media: PDA (potato dextrose agar), MEA (malt extract agar), CMA (corn meal agar), LnA (Lenoleic agar) and OMA (oat meal agar). The composition of media were obtained from "Ainsworth and Bisby's Dictionary of the fungi" by Ainsworth. The composition and preparation of different media are as given Potato dextrose agar (Potato peeled 200g, Dextrose 20g Agar-agar 20g, Distilled water 1000 ml to make up final volume). Malt extract agar (malt extract 25g, Agar-agar 20g, Distilled water 1000 ml to make up final volume). Corn meal agar (Corn meal 20g, Agar-agar 20g, Distilled water 1000 ml to make up final volume). Yeast extract dextrose agar medium (Yeast extract 7.5g, Dextrose 20g, Agar-agar 15g, Distilled water 1000 ml to make up final volume). Oat Meal Agar Medium (Rolled oat meal 250g, Agar-agar 15g, Distilled water 1000 ml to makeup final volume).

**Growth rate of fungus:** To provide a uniform assessment of pathogen growth rates, 21 different isolates cultured on different growth media. The plates were inoculated with 8 mm mycelium disc from the actively growing mycelial of each isolate and were incubated at  $27\pm 2^\circ\text{C}$ . The radial growth rate of mycelium on different media was calculated after 9 days of incubation using measuring scale. The experiments were performed in three replicates and mean was calculated.

**Type and colony colour:** Cultures grown on PDA at  $27\pm 2^\circ\text{C}$  were used for morphological observation. Colony colour, texture, conidial masses and acervuli formation was observed after nine days incubation in B.O.D.

### Microscopic characterisation of the isolates

For the measurement of spores, spore suspension of the isolates was prepared aseptically using sterile distilled water and a drop was placed on sterile glass slide for observation under the microscope at 10X and 40X power using calibrated stage and ocular micrometer. Identification was made after comparing the microscopic and morphological features of the pathogen *Colletotrichum capsici* with the available standard literature for establishing their identity by (Saxena *et al.*, 2014). The size and shape of conidia, setae and number of acervuli were determined on PDA using a slide culture technique. Isolated culture were mounted in lacto-phenol and cotton blue, the length and width measured for 20 randomly selected of conidia and setae harvested from every culture of each isolate were recorded. Colony diameter of every culture was recorded nine days after incubation and growth rate was calculated as the 9 day (mm/day).

### Pathogenicity testing

**Preparation of inoculums:** Nine days old cultures of *Colletotrichum capsici* grown on PDA in BOD maintained at  $27\pm 2^\circ\text{C}$  were used for making conidial suspension. The plates were flooded with sterile distilled water and gently scrapped by sterile loop to collect the conidia from the culture plates and filtered between two layers of muslin cloth and conidial suspension concentration was adjusted to  $1 \times 10^6$  conidia  $\text{ml}^{-1}$  using Haemocytometer and used as standard inoculums for carrying out different studies.

**Detached fruit method:** Pathogenic variability was studied by method of Rajapakse (2002). The green and red chilli fruit purchased from the local market and used for pathogenic variability in this experiment. The fruits were surface sterilised with 1% sodium hypochlorite solution for 5 minutes and rinsed with sterile distilled water for two to three times. The fruits were blotted dry with a sterile tissue paper and inoculated with the help of sterile needle using either the wound or non-wound method. The conidial suspension (10 $\mu\text{l}$ ) was injected at the wounded and non wounded site. The fruits were then kept in moist chambers maintained at  $27\pm 2^\circ\text{C}$  with 98% relative humidity. Un-inoculated but wounded fruits served as control. The rate of lesion progression on ripe and unripe fruits was measured every day. Rate of lesion progression in ripe unripe fruits and leaves were evaluated with the help of the descriptive rating scale proposed by Montri *et al.* (2009) using 0-9 scale rating for assessment of the disease symptom of anthracnose symptoms after 9 days. Disease severity was scored on six categories were made on the basis of % twig/fruit area infected, as per the following category (0 = healthy, 1 = 1-2%, 3 = 3-5%, 5 = 6-10%, 7 = 11-25%, and 9 = >25% fruit area involved). The pathogen was re-isolated on PDA media after 9 days using direct isolation to morphologically identify and compare with original isolate to proof the Koch's postulates rules.

## Analysis of data

Data were analyzed using analysis of variance value C.D. @ 5% with Duncan's multiple range tests (DMRT) and completely randomized design (CRD) values used with Web Agri Stat Package 2.0. (ICAR, Research Complex for Goa).

## Results and Discussion

Twenty one distinct isolates of *Colletotrichum capsici* were obtained from the farmer field to collect the infected green and red chilli fruits and leaves from the major chilli growing areas of eastern Uttar Pradesh, India (Table 1). On the basis of differences in morphological characteristics such as shape and size of the conidia and setae, and number of acervuli, colony characteristics and pathogenicity test, the fungus was identified as *Colletotrichum capsici*.

Different culture media were used for analysing the growth rate of *Colletotrichum* isolates. Among all the five different culture media, maximum radial growth of all the isolates of test fungus after nine days was observed in potato dextrose agar (PDA) and oat meal agar media *i.e.*, 90 mm, which was significantly superior over all other culture medium and the lowest was lenoleic agar (32.33 mm) on isolate CCa10 followed by corn meal agar (38.33 mm) on isolate CCa12, malt extract agar (41.33 mm) on isolate CCa16 and oat meal agar (44.67 mm) on isolate CCa16 respectively (Table 2). Morphological characters including colony character, colour, and conidial masses and number of acervuli formation for each isolate were recorded only on PDA.

Isolates of *Colletotrichum* spp. differed with respect to their cultural characteristics. The isolates of *Colletotrichum capsici* grown on PDA showed variation in their colony colour (Figure 1). Colony colour varied from whitish to dark grey and brownish colony. Mostly the colonies had cottony or fluffy mycelial growth with regular and irregular margin. The cottony growth was observed in nine isolates *viz.*, CCa3, CCa5, CCa9, CCa10, CCa11, CCa15, CCa19, CCa20 and CCa21, fluffy growth in twelve isolates *viz.*, CCa1, CCa2, CCa4, CCa5, CCa6, CCa7, CCa8, CCa12, CCa13, CCa14, CCa16, CCa17, CCa18 and CCa19 (Table 3). The colony margins varied from regular to irregular. Regular margins were observed in thirteen isolates *viz.*, CCa1, CCa2, CCa3, CCa4, CCa7, CCa8, CCa9, CCa13, CCa14, CCa16, CCa18, CCa19 and CCa20 whereas, rest eight isolates had irregular margins. Similar results have been reported by Masoodi *et al.* (2013) and Saxena *et al.* (2014).

Length and breadth of conidia varied significantly between 18.1-27.1  $\mu\text{m}$  and 1.6-2.3  $\mu\text{m}$  respectively. Maximum conidial length was observed in CCa4 isolate and minimum length was observed in CCa1. Similarly maximum and minimum conidial breadth was observed in CCa16 and CCa2, CCa4 and CCa7 respectively (Table 4). In all the isolates of *Colletotrichum capsici*, falcate shaped conidia were observed (Fig. 2). Length and breadth of setae varied

significantly between 87.2-151.4  $\mu\text{m}$  and 3.3-5.3  $\mu\text{m}$  respectively. Maximum and minimum length was observed in CCa3 and CCa2 respectively. Similarly, maximum and minimum breadth was observed in CCa3 and CCa4 respectively (Table 4). An insight into data further revealed that as irrespective of the isolates, acervuli production ranged from 20-35/5 mm mycelial disc. The least production of 20 of acervuli/5 mm mycelia disc was recorded in isolate CCa2, CCa13 and CCa20 and maximum production was found in CCa3 (Table 4). Similar observations have been reported by Masoodi *et al.* (2013).

Twenty one isolates of *Colletotrichum capsici* from chilli fruits collected from five districts of eastern Uttar Pradesh were inoculated on to the variety of chilli cultivar VNR-305 purchased from the local market and grown in the pots and these fruits were harvested and used for recent experiment. Pathogenic variability was studied by method of Rajapakse (2002) and disease severity of ripe and unripe chilli fruit was scored using 0-9 scale proposed by Montri *et al.*, 2009. The disease intensity was recorded lowest in CCa8, CCa10, CCa12 and CCa20 in ripe chilli and CC10 in unripe chilli and highest diseases intensity was recorded in CCa3, CCa7, CCa13 and CCa19 in ripe chilli fruit. The data of Table 5 revealed that the isolates exhibited at different virulent pattern when inoculated on the ripe and unripe chilli fruits. CCa3, CCa7, CCa13 and CCa19 isolates of *Colletotrichum capsici* were found to be most virulent strains for chilli fruit. So the present finding was similar to that of Saxena *et al.* (2014), but the growth stages of chilli as the subject of intervention is new.

## Conclusion

During the survey of the disease in the districts of eastern Uttar Pradesh, the highest incidence and intensity of Anthracnose of chilli was observed in the locations of Magaraha, IIVR, Chunar and Pachwar. The pathogen *Colletotrichum capsici* was isolated in the pure form and pathogenicity was established following Koch's postulates. The pathogen was identified as per cultural, morphological and pathogenic behaviour as *Colletotrichum capsici*. These studies indicated prevalence of pathogenic variability among all the isolates. Among all the 21 isolates of *C. capsici*, four isolates (CCa3, CCa7, CCa13 and CCa19) were reported the most virulent strains for chilli (*C. annuum*). Comprehending the pathogenic variability of a pathogen in a given area along with its aggressiveness on the crop is of immense importance in order to choose proper management strategies for the disease. More emphasis should be given at different growth stages of the crop in order to reduce the pathogen inoculum in the field, thereby reducing the disease incidence. Still, further studies are required to interpret the complexity of the plant patho-system to efficiently manage the spread of the disease.

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### References

- Ainsworth GC, Bisby GR, Staplers JA, David JC and Kirk PM (2001). Dictionary of the Fungi, 9<sup>th</sup> edn. CABI Publishing, Wallingford, UK.
- Jeyalakshmi C (1996). Studies on fruit rot and die-back disease of chilli (*Capsicum annum* L.) incited by *Colletotrichum capsici* (syd.) Butler and Bisby. M.Sc. (Ag.) Thesis. Tamil Nadu Agricultural University, Madurai. India. 216p.
- Manandhar JB, Hartman GL, Wang TC, (1995). Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Disease* **79**: 380-383.
- Montri P, Taylor PWJ and Mongkolporn O (2009). Pathotypes of *Colletotrichum capsici*, the causal agent of chili anthracnose, in Thailand. *Plant Disease*, **93**: 17-20.
- Masoodi Lubna, Anwar Ali, Ahmed Shahzad and Sofi TA (2013). Cultural, Morphological and Pathogenic Variability in *Colletotrichum capsici* causing Die-back and Fruit Rot of Chilli. *Asian journal of Plant Pathology*, **7(1)**: 29-41.
- Pakdeevaporn P, Wasee S, Taylor PWJ and Mongkolporn O (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. *Plant Breeding*, **124**: 206-208.
- Rajapakse RGAS and Ranasinghe JADAR (2002). Development of variety screening method for anthracnose disease of chilli (*Capsicum annum* L.) under field condition. *Tropical Agricultural Research and Extension*, 5(1 & 2).
- Sutton BC (1992). The genus *Glomerella* and its anamorph *Colletotrichum*. In *Colletotrichum: biology, pathology and control* (eds. JA Bailey and MJ Jeger). *CAB International: Wallingford*, 1-26.
- Saxena A, Raghuwanshi R and Singh HB (2014). Molecular, phenotypic and pathogenic variability in *Colletotrichum* isolates of subtropical region in north-eastern India, causing fruit rot of chillies. *Journal of Applied Microbiology*, **117**: 1422-1434.
- Thind and Jhooty (1985). Relative prevalence of fungal diseases of chilli fruits in Punjab. *Indian Journal of Mycology and Plant Pathology*, **15(3)**: 305-307.
- Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O and Taylor PWJ (2008). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology*, **57**: 562-572.



**Table 1: Collection of pathogen (*Colletotrichum capsici*) from different district of eastern Uttar Pradesh (India)**

| S. No. | Isolates Name | Isolation Source    | Location              | District  | Latitude     | Longitude    | Fungal spp.                   |
|--------|---------------|---------------------|-----------------------|-----------|--------------|--------------|-------------------------------|
| 1      | CCa1          | Ripe chilli fruit   | BHU Horticulture Farm | Varanasi  | 25°15'49.5"N | 82°59'35.0"E | <i>Colletotrichum capsici</i> |
| 2      | CCa2          | Ripe chilli fruit   | Nuwan                 | Varanasi  | 25°14'38.1"N | 82°58'21.2"E | <i>Colletotrichum capsici</i> |
| 3      | CCa3          | Ripe chilli fruit   | Magaraha              | Mirzapur  | 25°09'04.8"N | 82°49'57.3"E | <i>Colletotrichum capsici</i> |
| 4      | CCa4          | Ripe chilli fruit   | Chiraigaon            | Varanasi  | 25°21'41.6"N | 83°03'32.8"E | <i>Colletotrichum capsici</i> |
| 5      | CCa5          | Ripe chilli fruit   | Rajatalab             | Varanasi  | 25°16'04.5"N | 82°50'14.5"E | <i>Colletotrichum capsici</i> |
| 6      | CCa6          | Ripe chilli fruit   | Cholapur              | Varanasi  | 25°28'18.4"N | 83°00'23.0"E | <i>Colletotrichum capsici</i> |
| 7      | CCa7          | Ripe chilli fruit   | IIVR                  | Varanasi  | 25°10'56.0"N | 82°52'36.1"E | <i>Colletotrichum capsici</i> |
| 8      | CCa8          | Ripe chilli fruit   | Narayanpur            | Mirzapur  | 25°12'14.0"N | 83°03'16.5"E | <i>Colletotrichum capsici</i> |
| 9      | CCa9          | Ripe chilli fruit   | Kachhariya            | Varanasi  | 25°14'26.0"N | 82°48'52.0"E | <i>Colletotrichum capsici</i> |
| 10     | CCa10         | Unripe chilli fruit | Arjunpur              | Varanasi  | 25°19'30.4"N | 82°46'07.5"E | <i>Colletotrichum capsici</i> |
| 11     | CCa11         | Ripe chilli fruit   | Marikpur              | Jaunpur   | 25°37'02.4"N | 83°02'08.9"E | <i>Colletotrichum capsici</i> |
| 12     | CCa12         | Ripe chilli fruit   | Karanjkala            | Jaunpur   | 25°48'35.2"N | 82°40'13.9"E | <i>Colletotrichum capsici</i> |
| 13     | CCa13         | Ripe chilli fruit   | Chunar                | Mirzapur  | 25°04'59.9"N | 82°54'02.3"E | <i>Colletotrichum capsici</i> |
| 14     | CCa14         | Ripe chilli fruit   | Bhaupur               | Varanasi  | 25°20'29.8"N | 82°50'24.5"E | <i>Colletotrichum capsici</i> |
| 15     | CCa15         | Ripe chilli fruit   | Uttari                | Chandauli | 25°28'12.2"N | 83°11'18.7"E | <i>Colletotrichum capsici</i> |
| 16     | CCa16         | Unripe chilli fruit | Mughalsarai           | Chandauli | 25°14'24.6"N | 83°06'57.0"E | <i>Colletotrichum capsici</i> |
| 17     | CCa17         | Ripe chilli fruit   | Saraiya               | Varanasi  | 25°26'40.0"N | 82°56'53.8"E | <i>Colletotrichum capsici</i> |
| 18     | CCa18         | Ripe chilli fruit   | Raghupur              | Bhadohi   | 25°21'44.9"N | 82°42'31.2"E | <i>Colletotrichum capsici</i> |
| 19     | CCa19         | Leaves and fruits   | Pachwar               | Varanasi  | 25°21'54.2"N | 82°47'29.3"E | <i>Colletotrichum capsici</i> |
| 20     | CCa20         | Ripe chilli fruit   | Jayapur               | Varanasi  | 25°12'42.1"N | 82°49'11.4"E | <i>Colletotrichum capsici</i> |
| 21     | CCa21         | Unripe chilli fruit | Paho                  | Mirzapur  | 25°10'16.4"N | 82°47'23.8"E | <i>Colletotrichum capsici</i> |

**Table 2: Growth rates of all the isolates of *Colletotrichum capsici* on different culture media after nine days after inoculation (mm)**

| Isolates  | PDA                      | MEA                      | CMA                       | LnA                        | OMA                      |
|-----------|--------------------------|--------------------------|---------------------------|----------------------------|--------------------------|
| CCa1      | 72.33±2.52 <sup>e</sup>  | 54.00±1.73 <sup>e</sup>  | 48.67±2.52 <sup>hi</sup>  | 41.33 ± 1.53 <sup>f</sup>  | 61.33±1.53 <sup>i</sup>  |
| CCa2      | 73.67±2.08 <sup>cd</sup> | 61.33±3.06 <sup>d</sup>  | 54.67±3.51 <sup>fg</sup>  | 44.00 ± 2.65 <sup>f</sup>  | 69.67±2.52 <sup>fg</sup> |
| CCa3      | 90.00±0.00 <sup>a</sup>  | 75.00±3.00 <sup>b</sup>  | 66.67±1.53 <sup>c</sup>   | 62.33 ± 2.52 <sup>b</sup>  | 90.00±0.00 <sup>a</sup>  |
| CCa4      | 81.67±1.15 <sup>b</sup>  | 63.33±2.89 <sup>d</sup>  | 62.33±2.08 <sup>d</sup>   | 54.33 ± 2.52 <sup>cd</sup> | 73.33±3.06 <sup>de</sup> |
| CCa5      | 75.67±2.08 <sup>cd</sup> | 75.00±1.00 <sup>b</sup>  | 57.00±2.65 <sup>de</sup>  | 43.67±1.53 <sup>f</sup>    | 76.67±2.31 <sup>cd</sup> |
| CCa6      | 83.67±1.15 <sup>b</sup>  | 78.67±1.53 <sup>a</sup>  | 51.67±2.52 <sup>gh</sup>  | 76.00 ± 2.65 <sup>a</sup>  | 80.00±3.00 <sup>bc</sup> |
| CCa7      | 90.00±0.00 <sup>a</sup>  | 73.33±2.08 <sup>bc</sup> | 71.67±2.52 <sup>b</sup>   | 57.67±2.52 <sup>b</sup>    | 77.67±2.52 <sup>cd</sup> |
| CCa8      | 62.67±2.08 <sup>g</sup>  | 70.67±3.06 <sup>c</sup>  | 45.33±1.53 <sup>ijk</sup> | 42.33±2.52 <sup>f</sup>    | 81.67±1.53 <sup>b</sup>  |
| CCa9      | 72.00±2.00 <sup>e</sup>  | 50.00±2.00 <sup>fg</sup> | 48.33±2.52 <sup>hi</sup>  | 56.33±1.53 <sup>c</sup>    | 53.33±1.53 <sup>j</sup>  |
| CCa10     | 50.33±2.08 <sup>i</sup>  | 70.33±2.52 <sup>c</sup>  | 41.33±1.53 <sup>lm</sup>  | 32.33±2.52 <sup>g</sup>    | 60.33±2.52 <sup>i</sup>  |
| CCa11     | 72.67±0.58 <sup>de</sup> | 63.33±1.53 <sup>d</sup>  | 51.00±1.73 <sup>gh</sup>  | 49.67±1.53 <sup>c</sup>    | 66.67±1.53 <sup>gh</sup> |
| CCa12     | 56.67±1.53 <sup>h</sup>  | 47.67±3.06 <sup>gh</sup> | 38.33±3.51 <sup>m</sup>   | 40.67±1.15 <sup>f</sup>    | 50.33±2.08 <sup>jk</sup> |
| CCa13     | 73.67±2.52 <sup>cd</sup> | 62.33±1.53 <sup>d</sup>  | 57.67±2.52 <sup>ef</sup>  | 52.00±2.00 <sup>de</sup>   | 65.67±1.53 <sup>h</sup>  |
| CCa14     | 66.33±1.53 <sup>f</sup>  | 53.67±1.53 <sup>ef</sup> | 46.00±2.00 <sup>ijk</sup> | 41.67±3.51 <sup>f</sup>    | 58.33±1.15 <sup>i</sup>  |
| CCa15     | 55.33±2.52 <sup>h</sup>  | 44.33±2.08 <sup>hi</sup> | 42.67±2.52 <sup>kl</sup>  | 56.67±2.52 <sup>c</sup>    | 48.00±2.00 <sup>kl</sup> |
| CCa16     | 49.67±2.52 <sup>i</sup>  | 41.33±2.52 <sup>i</sup>  | 43.67±1.53 <sup>kl</sup>  | 41.33±1.53 <sup>f</sup>    | 44.67±2.52 <sup>i</sup>  |
| CCa17     | 83.00±2.65 <sup>b</sup>  | 72.33±1.53 <sup>bc</sup> | 78.00±2.00 <sup>a</sup>   | 62.33 ± 2.52 <sup>b</sup>  | 74.00±2.65 <sup>de</sup> |
| CCa18     | 76.00±2.00 <sup>c</sup>  | 62.67±2.08 <sup>d</sup>  | 60.67±1.15 <sup>de</sup>  | 57.33 ± 2.08 <sup>c</sup>  | 68.00±2.00 <sup>gh</sup> |
| CCa19     | 88.33±2.08 <sup>a</sup>  | 75.00±2.00 <sup>b</sup>  | 56.33±2.52 <sup>f</sup>   | 65.33 ± 2.52 <sup>b</sup>  | 78.67±2.52 <sup>bc</sup> |
| CCa20     | 56.00±2.65 <sup>h</sup>  | 69.67±2.08 <sup>c</sup>  | 56.33±2.52 <sup>f</sup>   | 75.33 ± 2.08 <sup>a</sup>  | 72.33±2.08 <sup>ef</sup> |
| CCa21     | 64.33±2.08 <sup>fg</sup> | 61.00±2.65 <sup>d</sup>  | 40.67±3.06 <sup>lm</sup>  | 42.00 ± 3.00 <sup>f</sup>  | 53.67±1.53 <sup>j</sup>  |
| SE(m)     | 1.177                    | 3.715                    | 1.367                     | 1.331                      | 3.505                    |
| C.D. (5%) | 3.372                    | 1.297                    | 3.914                     | 3.813                      | 1.224                    |

For numerical characters, values observed by the same letter in a column did not differ significantly C.D. @ (5%) in Duncan's Multiple Range Test (DMRT)

**Table 3: Morphological characteristics of different isolates on potato dextrose agar medium (PDA)**

| S. No. | Isolates | Colony colour          | Colony character          | Conidial masses        | Acervuli formation  |
|--------|----------|------------------------|---------------------------|------------------------|---------------------|
| 1      | CCa1     | White to grey colony   | Cottony, regular margin   | Brown conidial masses  | Abundant at centre  |
| 2      | CCa2     | White to grey colony   | Cottony, regular margin   | Orange conidial masses | Concentric rings    |
| 3      | CCa3     | White to grey colony   | Cottony, regular margin   | Brown conidial masses  | Abundant at centre  |
| 4      | CCa4     | Dull grey colony       | Fluffy, regular margin    | Brown conidial masses  | Sparse acervuli     |
| 5      | CCa5     | White to orange colony | Cottony, irregular margin | Orange conidial masses | Abundant at centre  |
| 6      | CCa6     | White to grey colony   | Fluffy, irregular margin  | Brown conidial masses  | Formed at periphery |
| 7      | CCa7     | White to grey colony   | Cottony, regular margin   | Brown conidial masses  | Abundant at centre  |
| 8      | CCa8     | White to grey colony   | Fluffy, regular margin    | Orange conidial masses | Abundant at centre  |
| 9      | CCa9     | Grey to orange colony  | Cottony, regular margin   | Orange conidial masses | Sparse acervuli     |
| 10     | CCa10    | Brown colony           | Cottony, irregular margin | Brown conidial masses  | Abundant at centre  |
| 11     | CCa11    | Grey to brown colony   | Cottony, irregular margin | Brown conidial masses  | Concentric rings    |
| 12     | CCa12    | Grey to white colony   | Fluffy, irregular margin  | Brown conidial masses  | Sparse acervuli     |
| 13     | CCa13    | Light grey colony      | Fluffy, regular margin    | Brown conidial masses  | Sparse acervuli     |
| 14     | CCa14    | White to orange colony | Fluffy, regular margin    | Orange conidial masses | Abundant at centre  |
| 15     | CCa15    | White to grey colony   | Cottony, irregular margin | Brown conidial masses  | Abundant at centre  |
| 16     | CCa16    | White colony           | Fluffy, regular margin    | Orange conidial masses | Abundant at centre  |
| 17     | CCa17    | White to grey colony   | Fluffy, irregular margin  | Brown conidial masses  | Abundant at centre  |
| 18     | CCa18    | Grey colony            | Fluffy, regular margin    | Brown conidial masses  | Sparse acervuli     |
| 19     | CCa19    | White colony           | Cottony, regular margin   | Brown conidial masses  | Concentric rings    |
| 20     | CCa20    | Whit to grey colony    | Cottony, regular margin   | Orange conidial masses | Abundant at centre  |
| 21     | CCa21    | White to grey colony   | Cottony, irregular margin | Brown conidial masses  | Formed at periphery |

**Table 4: Variability in shape and size of conidia and setae and number of acervuli of *Colletotrichum capsici***

| S. No.       | Isolates | Conidia length              | Conidia breadth (µm)       | Conidia shape | Setae length (µm)             | Setae breadth (µm)          | No. of acervuli/5 mm mycelial disc |
|--------------|----------|-----------------------------|----------------------------|---------------|-------------------------------|-----------------------------|------------------------------------|
| 1            | CCa1     | 18.1± 5.17 <sup>f</sup>     | 2.1 ± 0.69 <sup>bcd</sup>  | Falcate       | 112.0 ± 28.9 <sup>cdefg</sup> | 4.6 ± 0.99 <sup>bcde</sup>  | 25±2.11 <sup>bc</sup>              |
| 2            | CCa2     | 22.2± 6.06 <sup>bc</sup>    | 1.6 ± 0.53 <sup>efg</sup>  | Falcate       | 87.2 ± 16.2 <sup>ghij</sup>   | 3.9± 0.90 <sup>ghij</sup>   | 20±2.45 <sup>defg</sup>            |
| 3            | CCa3     | 23.1 ± 6.15 <sup>b</sup>    | 1.5 ± 0.44 <sup>fg</sup>   | Falcate       | 151.4 ± 36.9 <sup>a</sup>     | 5.3 ± 0.87 <sup>a</sup>     | 35±4.51 <sup>a</sup>               |
| 4            | CCa4     | 27.1± 6.68 <sup>a</sup>     | 1.6 ± 0.44 <sup>fg</sup>   | Falcate       | 107.0 ± 34.6 <sup>defg</sup>  | 3.3 ± 0.66 <sup>j</sup>     | 27±2.89 <sup>c</sup>               |
| 5            | CCa5     | 19.6 ± 3.11 <sup>cdef</sup> | 2.2 ± 0.61 <sup>ab</sup>   | Falcate       | 98.4 ± 27.4 <sup>fgh</sup>    | 4.2 ± 0.85 <sup>defgh</sup> | 25±2.01 <sup>cd</sup>              |
| 6            | CCa6     | 19.8 ± 2.45 <sup>cdef</sup> | 1.8 ± 0.51 <sup>bcd</sup>  | Falcate       | 104.6 ± 26.6 <sup>efgh</sup>  | 4.0 ± 1.06 <sup>fgh</sup>   | 26±2.03 <sup>cd</sup>              |
| 7            | CCa7     | 21.0 ± 3.10 <sup>bcd</sup>  | 1.6 ± 0.42 <sup>fg</sup>   | Falcate       | 97.0 ± 19.8 <sup>fgh</sup>    | 4.0 ± 0.97 <sup>fghi</sup>  | 32±2.11 <sup>a</sup>               |
| 8            | CCa8     | 21.8 ± 3.44 <sup>bcd</sup>  | 1.8 ± 0.64 <sup>bcd</sup>  | Falcate       | 99.2 ± 17.1 <sup>fg</sup>     | 3.9 ± 0.85 <sup>fghi</sup>  | 28±4.03 <sup>ab</sup>              |
| 9            | CCa9     | 19.6 ± 2.63 <sup>cdef</sup> | 2.0 ± 0.78 <sup>bcd</sup>  | Falcate       | 113.2 ± 29.0 <sup>bcd</sup>   | 4.5 ± 0.98 <sup>cdef</sup>  | 26±2.45 <sup>bc</sup>              |
| 10           | CCa10    | 18.9 ± 3.10 <sup>def</sup>  | 1.8 ± 0.55 <sup>bcd</sup>  | Falcate       | 94.4 ± 23.6 <sup>efgh</sup>   | 5.1 ± 1.05 <sup>ab</sup>    | 25±2.65 <sup>de</sup>              |
| 11           | CCa11    | 20.8 ± 3.79 <sup>bcd</sup>  | 1.9 ± 0.62 <sup>bcde</sup> | Falcate       | 102.8 ± 29.9 <sup>efgh</sup>  | 4.3 ± 1.02 <sup>defg</sup>  | 22±2.22 <sup>def</sup>             |
| 12           | CCa12    | 19.5 ± 4.06 <sup>cdef</sup> | 1.7 ± 0.37 <sup>defg</sup> | Falcate       | 99.3 ± 24.1 <sup>efg</sup>    | 3.7 ± 0.85 <sup>hij</sup>   | 25±2.10 <sup>def</sup>             |
| 13           | CCa13    | 20.5 ± 3.32 <sup>bcd</sup>  | 2.0 ± 0.64 <sup>abcd</sup> | Falcate       | 112.6 ± 28.8 <sup>bcd</sup>   | 4.0 ± 0.98 <sup>efgh</sup>  | 20±3.11 <sup>defg</sup>            |
| 14           | CCa14    | 22.4 ± 3.21 <sup>bc</sup>   | 2.1 ± 0.59 <sup>abc</sup>  | Falcate       | 107.3 ± 21.0 <sup>bcd</sup>   | 4.9 ± 1.13 <sup>abc</sup>   | 22±3.23 <sup>def</sup>             |
| 15           | CCa15    | 18.4 ± 4.19 <sup>ef</sup>   | 1.8 ± 0.58 <sup>cdef</sup> | Falcate       | 117.7 ± 31.3 <sup>b</sup>     | 4.0 ± 0.78 <sup>fghi</sup>  | 26±2.00 <sup>cde</sup>             |
| 16           | CCa16    | 20.7 ± 2.54 <sup>bcd</sup>  | 2.3 ± 0.52 <sup>a</sup>    | Falcate       | 123.4 ± 28.4 <sup>bc</sup>    | 4.2 ± 0.80 <sup>cdefg</sup> | 25±2.71 <sup>def</sup>             |
| 17           | CCa17    | 19.4 ± 4.28 <sup>cdef</sup> | 1.9 ± 0.63 <sup>bcd</sup>  | Falcate       | 96.4 ± 12.5 <sup>fghi</sup>   | 4.2 ± 0.99 <sup>efgh</sup>  | 22±2.51 <sup>defg</sup>            |
| 18           | CCa18    | 21.4 ± 5.27 <sup>bcde</sup> | 1.7 ± 0.47 <sup>g</sup>    | Falcate       | 107.4 ± 30.1 <sup>bcd</sup>   | 4.4 ± 0.92 <sup>defg</sup>  | 28±2.09 <sup>ab</sup>              |
| 19           | CCa19    | 18.8 ± 2.44 <sup>def</sup>  | 1.9 ± 0.49 <sup>fg</sup>   | Falcate       | 107.3 ± 32.5 <sup>bcd</sup>   | 4.7 ± 0.97 <sup>bcd</sup>   | 21±2.83 <sup>efg</sup>             |
| 20           | CCa20    | 19.8 ± 2.67 <sup>cdef</sup> | 2.2 ± 0.71 <sup>bcd</sup>  | Falcate       | 97.7 ± 23.1 <sup>efg</sup>    | 4.1 ± 0.80 <sup>efgh</sup>  | 20±1.89 <sup>efg</sup>             |
| 21           | CCa21    | 20.3 ± 3.17 <sup>bcd</sup>  | 2.0 ± 0.48 <sup>bcd</sup>  | Falcate       | 118.6 ± 29.7 <sup>bc</sup>    | 3.6 ± 0.91 <sup>ij</sup>    | 25±2.68 <sup>bc</sup>              |
| <b>SE(m)</b> |          | <b>0.458</b>                | <b>0.985</b>               |               | <b>6.030</b>                  | <b>0.207</b>                | <b>0.625</b>                       |
| <b>C.D.</b>  |          | <b>3.075</b>                | <b>0.353</b>               |               | <b>17.628</b>                 | <b>0.576</b>                | <b>3.115</b>                       |

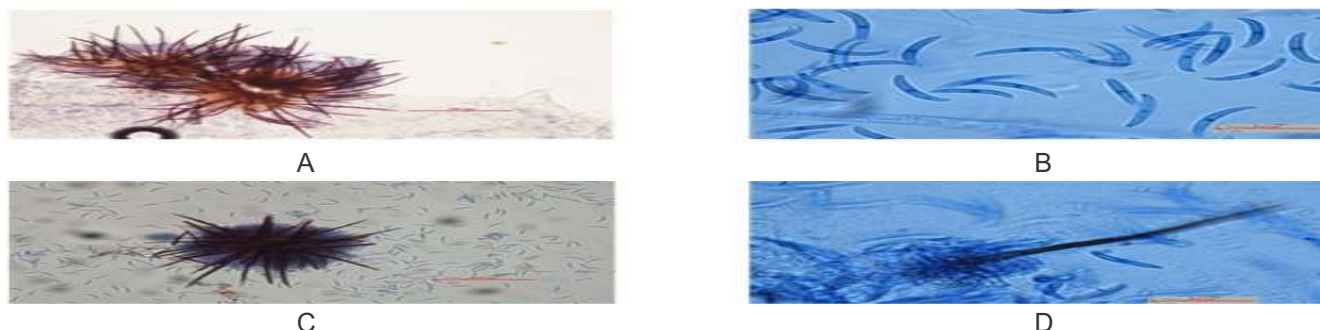
For numerical characters, values observed by the same letter in a column did not differ significantly C.D. @ (5%) in Duncan's Multiple Range Test (DMRT)

**Table 5: Per cent fruit infection and disease severity ratings of different isolates of *Colletotrichum capsici* by detached fruit method**

| S. No. | Isolates | Green Chilli Fruits |                        | Red Chilli Fruits |                        |
|--------|----------|---------------------|------------------------|-------------------|------------------------|
|        |          | % Fruit Infection   | Disease Severity Scale | % Fruit Infection | Disease Severity Scale |
| 1      | CCa1     | 15.07               | 7                      | 23.9              | 7                      |
| 2      | CCa2     | 6.4                 | 5                      | 18.3              | 7                      |
| 3      | CCa3     | 21.53               | 7                      | 38.35             | 9                      |
| 4      | CCa4     | 10.44               | 7                      | 19.83             | 7                      |
| 5      | CCa5     | 7.24                | 5                      | 21.31             | 7                      |
| 6      | CCa6     | 3.9                 | 3                      | 13.46             | 7                      |
| 7      | CCa7     | 19.17               | 7                      | 30.55             | 9                      |
| 8      | CCa8     | 6.9                 | 5                      | 9.61              | 5                      |
| 9      | CCa9     | 5.5                 | 5                      | 20.23             | 7                      |
| 10     | CCa10    | 1.3                 | 0                      | 9.09              | 5                      |
| 11     | CCa11    | 7.98                | 5                      | 12.5              | 7                      |
| 12     | CCa12    | 4.1                 | 3                      | 8.77              | 5                      |
| 13     | CCa13    | 17.14               | 7                      | 26.81             | 9                      |
| 14     | CCa14    | 10.7                | 7                      | 18.53             | 7                      |
| 15     | CCa15    | 7.3                 | 5                      | 13.92             | 7                      |
| 16     | CCa16    | 2.1                 | 1                      | 15.3              | 7                      |
| 17     | CCa17    | 12.8                | 7                      | 19.01             | 7                      |
| 18     | CCa18    | 7.05                | 5                      | 16.15             | 7                      |
| 19     | CCa19    | 16.66               | 7                      | 28.3              | 9                      |
| 20     | CCa20    | 1.1                 | 1                      | 8.47              | 5                      |
| 21     | CCa21    | 6.9                 | 5                      | 11.2              | 7                      |



**Fig.1: Different isolates of *Colletotrichum capsici* grown on potato dextrose agar (PDA)**



**Fig. 2: Microscopic observations of (A) acervuli (B) conidia (C) acervuli with setae and conida (D) setae**