Cultural and Morphological Variability among the Isolates of Colletotrichum spp. Causes Fruit Rot of Papaya

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A B S T R A C T

Papaya is prone to many diseases incited by fungi, bacteria, nematodes and viruses leading to enormous loss in yield. Among all, papaya anthracnose incited by Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. appear to be more severe causing substantial losses to papaya fruits during transit and storage. Papaya anthracnose is the most important disease throughout the year in India and it became a major limiting factor in papaya cultivation. Total nineteen isolates of Colletotrichum spp. studied for their cultural characters at 2nd, 4th, 6th and 8th day after incubation. Significantly highest mycelial growth of Colletotrichum spp. after 4 days was recorded in isolate Cd-8. After 8th day of incubation, significantly highest mycelial growth was recorded in isolates Cd-13 and Cd-15 (90.00 mm). Significantly highest length and breadth of conidia were recorded in an isolate Cd-13 (26.97 & 5.57 µm) and Cd-8 (25.40 & 5.33 µm) as compare to other isolates. Significantly maximum number of setae per acervulus was recorded in isolate Cd-13 (32.00) over all other isolates.

Keywords
Colletotrichum, Fruit rot, Papaya, Cultural characters

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Introduction

The papaya is the fruit of the plant Carica papaya L., the sole species in the genus Carica of the family Caricaceae. It is native of tropical America. It was first cultivated in Mexico (Anon, 2012a). The popularity of papaya fruit has made it ubiquitous in tropical and subtropical regions of the world. It has made its way from kitchen gardens to the commercial orchards in many tropical and subtropical countries because of its highest production of fruits (75 to 100 tons ha⁻¹) and stood next to banana in income (Thamaraikannan and Sengottuvel, 2012).

Pramod et al., (2007) recorded 5.42 to 10.37 per cent disease incidence due to Colletotrichum gloeosporioides in papaya at Combatore markets. Rahman et al., (2008) reported 90 to 98 Per cent incidence and 25 to 38 per cent losses of papaya yield due to
anthracnose disease caused by *C. gloeosporioides* in Malaysia.

**Materials and Methods**

**Collection, isolation and purification**

Fresh naturally infected diseased papaya fruits showing typical characteristic symptoms of Colletotrichum fruit rot were collected from the Sardar Patel vegetable market, Anand and brought to the laboratory in paper bags for isolation of the pathogen. Small pieces of diseased tissues along with adjoining healthy tissues were cut and surface sterilized by dipping in 0.1 per cent NaOCl solution for one minute followed by three successive washings with distilled sterile water and these pieces were placed on Potato Dextrose Agar (PDA) medium (20 ml) poured in Petri plates under aseptic condition. The inoculated plates were incubated for growth of the pathogen at 25 ± 10°C in BOD incubator for seven days.

**Cultural variability**

To study the cultural variability of the isolates of *Colletotrichum* sp. was grown on PDA medium sterilized in autoclave for 20 minutes at 15 lbs p.s.i. The 5 mm disc of pure culture of each isolate was inoculated separately at the center of the pre poured Petri plates from seven days old actively growing culture. All inoculated plates were incubated at 25±1 °C temperature in BOD incubator. Three replications were kept for each isolate. The mycelial growth rate of each isolate was measured after 8 days of incubation. The observations were recorded on cultural characteristics like colony colour and growth pattern after eight days of incubation.

**Morphological variability**

The morphological characters like size (length and width) shape of the conidia, size and total number of acervuli, size and number of setae per acervuli. The observations were recorded in three repetitions within each isolate. The study was carried out using ocular and stage micrometer after mounting them on the slides containing sterile distilled water at magnification of 40X.

**Results and Discussion**

**Cultural variability**

Total nineteen isolates of *Colletotrichum* *spp.* studied for their cultural characters at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> day of incubation. The colony colour and growth pattern of isolates were recorded after eight days of incubation (Table 1 & 2).

**After 2<sup>nd</sup> day**

After 2<sup>nd</sup> day of incubation, significantly highest mycelial growth of *Colletotrichum* *spp.* was found in isolate Cd-13 (21.53 mm). The next best isolate was Cd-12 (20.60 mm) and it was at par with isolate Cd-8 (19.87 mm). The isolate Cd-19 gave 19.53 mm mycelial growth after 2 days of incubation which was at par with isolate Cd-1, Cd-15 and Cd-3 with 19.50, 18.86 and 18.77 mm mycelial growth respectively. The isolate Cd-9 gave 18.50 mm mycelial growth which was at par with isolate Cd-11 (18.00 mm) and Cd-10 (17.87). The next isolate with respect to mycelial growth was Cd-5 (17.60 mm) which was at par with isolate Cd-11 (18.00 mm) and Cd-10 (17.87).

**After 4<sup>th</sup> day**

Significantly highest mycelial growth of *Colletotrichum* *spp.* after 4 days was recorded in isolate Cd-8 and it was at par with isolate Cd-19, Cd-13 and Cd-1 producing 47.33, 46.43, 46.30 and 45.70 mm mycelial growth, respectively. The next best treatment in order
of merit was Cd-10 (44.03 mm) and it was at par with isolate Cd-9 (43.93 mm), Cd-3 (42.80 mm), Cd-15 (42.33 mm), Cd-12 (41.33 mm) and Cd-11 (41.30 mm). The isolate Cd-18, Cd-4, Cd-17, and Cd-5 produced 40.01, 39.37, 38.13 and 37.90 mm mycelial growth, respectively. Significantly lowest mycelium growth of *Colletotrichum* spp. was recorded in isolate Cd-16 (28.33 mm).

**After 6th day**

After 6th day of incubation, the highest mycelium growth was recorded in isolate Cd-13 (68.03 mm) which was at par with isolate Cd-1 and Cd-15 with 67.23 and 66.10 mm mycelium growth, respectively. The next best isolates were Cd-19 (64.03 mm), and it was at par with isolate Cd-8, Cd-3 and Cd-4 with 63.77, 63.20 and 61.80 mm, respectively. The isolate Cd-10 produced 61.24 mm mycelial growth which was at par with isolate Cd-18, Cd-5 and Cd-9 with 61.10, 60.00 and 59.20 mm growth, respectively. Significantly lowest mycelia growth was recorded in isolate Cd-16 (41.03 mm).

**After 8th day**

Significantly highest mycelial growth (90.00 mm) were recorded in isolates Cd-13 and Cd-15 which was at par with Cd-1, Cd-8 and Cd-19 with 89.67, 89.00 and 85.77 mm mycelium growth, respectively. The next best isolate in order of merit was Cd-3 (85.33 mm) which was at par with Cd-4 (83.33 mm) and Cd-18 (83.10 mm). The isolate Cd-5 and Cd-17 produced 80.67 mm and 80.43 mm mycelium growth, respectively. Significantly lowest mycelium growth was recorded in isolate Cd-16 (56.00 mm).

**Morphological variability**

Observations on size of conidia and number of setae per acervulus produced by each isolate were recorded by microscopic observations (Table 3).

**Length of conidia (µm)**

Significantly highest length of conidia were recorded in isolate Cd-13 (26.97 µm) and Cd-8 (25.40 µm) as compare to other isolates. The next best isolate in order of merit was Cd-3 (22.67 µm) and was at par with isolate Cd-16 (22.00 µm) and Cd-1 (21.30 µm). The isolate Cd-18, Cd-4, Cd-17, Cd-10 and Cd-12 recorded 18.80, 17.90, 17.73, 17.50 and 17.33 µm length of conidia, respectively. Significantly lowest length of conidia was recorded in isolate Cd-5 (12.23 µm).

**Width of conidia (µm)**

Significantly highest width of conidia was recorded in isolate Cd-13 (5.57 µm) and was at par with isolate Cd-8 (5.33 µm). The next best isolate in order of merit was Cd-3 with 5.10 µm width and it was at par with Cd-16, Cd-1 and Cd-18 with 5.00, 4.97 and 4.90 µm width, respectively. The isolate Cd-12 has 4.63 µm width, which was at par with isolate Cd-17 (4.57 µm) and Cd-4 (4.40 µm). Isolate Cd-19 recorded 3.90 µm width and it was at par with isolate Cd-14 (3.80 µm), Cd-7 (3.57 µm), Cd-5 (3.57 µm) and Cd-6 (3.53 µm).

**No. of setae/ acervulus**

Significantly highest number of setae per acervulus was recorded in isolate Cd-13 (32.00) over all other isolates. The next best isolate in order of merit was Cd-8 (28.80) which was at par with Cd-15 (27.33). The isolate Cd-3 produced 26.07 setae per acervulus. The next best isolate for acervulus production was Cd-19 (23.03) which was at par with isolate Cd-18, Cd-7, Cd-12, Cd-1 and Cd-17 with 22.53, 22.43, 22.33, 22.30 and 21.67, respectively.
Table.1 Cultural growth variability among the isolates of *Colletotrichum* spp.

| Isolate | Colony diameter (mm) |
|---------|----------------------|
|         | 2\textsuperscript{nd} day | 4\textsuperscript{th} day | 6\textsuperscript{th} day | 8\textsuperscript{th} day |
| Cd-1    | 19.50                | 45.70                | 67.23                | 89.67                |
| Cd-2    | 14.30                | 35.23                | 55.50                | 67.00                |
| Cd-3    | 18.77                | 42.80                | 63.20                | 85.33                |
| Cd-4    | 17.53                | 39.37                | 61.80                | 83.33                |
| Cd-5    | 17.60                | 37.90                | 60.00                | 80.67                |
| Cd-6    | 14.30                | 32.63                | 53.74                | 67.18                |
| Cd-7    | 15.40                | 34.08                | 52.60                | 67.93                |
| Cd-8    | 19.87                | 47.33                | 63.77                | 89.00                |
| Cd-9    | 18.50                | 43.93                | 59.20                | 75.83                |
| Cd-10   | 17.87                | 44.03                | 61.24                | 76.66                |
| Cd-11   | 18.00                | 41.30                | 52.57                | 68.80                |
| Cd-12   | 20.60                | 41.33                | 54.73                | 69.60                |
| Cd-13   | 21.53                | 46.30                | 66.10                | 90.00                |
| Cd-14   | 14.400               | 33.50                | 52.10                | 66.96                |
| Cd-15   | 18.86                | 42.33                | 68.03                | 90.00                |
| Cd-16   | 11.83                | 28.33                | 41.03                | 56.00                |
| Cd-17   | 16.17                | 38.13                | 55.00                | 80.43                |
| Cd-18   | 17.47                | 40.01                | 61.10                | 83.10                |
| Cd-19   | 19.53                | 46.43                | 64.03                | 85.77                |
| S.Em. ± | 0.28                 | 1.03                 | 0.79                 | 1.51                 |
| C.D. at 5 % | 0.80          | 2.95               | 2.26               | 4.33                |
| C.V. %  | 2.75                 | 4.46                 | 2.34                 | 3.37                |
Table 2: Cultural characters of *Colletotrichum* spp. isolates at 25±2 °C on potato dextrose agar after 8 days of incubation

| Isolates   | Colony colour                  | Growth pattern                      |
|------------|--------------------------------|------------------------------------|
| Cd-1       | Dark gray                      | Circular, flat and suppressed       |
| Cd-2       | Whitish pink to gray           | Fluffy, radial growth               |
| Cd-3       | Light grayish                  | Circular, flat and suppressed       |
| Cd-4       | Dull ash to gray colour        | Circular to oval and fluffy         |
| Cd-5       | Dirty white at centre and white at margin | Olive, fluffy and circular          |
| Cd-6       | Dark whitish                   | Suppressed and circular             |
| Cd-7       | Whitish to light green         | Scattered and circular              |
| Cd-8       | White grayish                  | Scattered and circular              |
| Cd-9       | Whitish with gray dots         | Flattened and circular              |
| Cd-10      | Whitish gray to dark gray at centre | Suppressed and radial               |
| Cd-11      | Black colour at centre         | Flattened and circular              |
| Cd-12      | Dirty white with greenish centre | Raised circular fluffy growth       |
| Cd-13      | Dark gray                      | Flattened and circular              |
| Cd-14      | Dirty white                    | Suppressed and circular             |
| Cd-15      | Milky white with gray centre   | Radial, suppressed and flat         |
| Cd-16      | Pinkish white                  | Circular and fluffy growth          |
| Cd-17      | Milky white                    | Flattened and circular              |
| Cd-18      | Whitish pink                   | Radial and flat                     |
| Cd-19      | White with gray centre         | Circular and flat                   |
Table 3 Morphological characters (length and width, µm) of conidia and No. of setae/Acervulus of Colletotrichum spp. isolates

| Isolates | Size of conidia (µm) | No. of setae/Acervulus |
|----------|----------------------|------------------------|
|          | Length | Width |                      |
| Cd-1     | 21.30  | 4.97  | 22.30                 |
| Cd-2     | 13.20  | 3.13  | 18.20                 |
| Cd-3     | 22.27  | 5.10  | 26.07                 |
| Cd-4     | 17.90  | 4.40  | 19.23                 |
| Cd-5     | 12.23  | 3.57  | 16.40                 |
| Cd-6     | 15.63  | 3.53  | 17.13                 |
| Cd-7     | 12.50  | 3.57  | 22.43                 |
| Cd-8     | 25.40  | 5.33  | 28.80                 |
| Cd-9     | 14.73  | 3.63  | 15.70                 |
| Cd-10    | 17.50  | 3.47  | 19.23                 |
| Cd-11    | 13.77  | 2.13  | 16.77                 |
| Cd-12    | 17.33  | 4.63  | 22.33                 |
| Cd-13    | 26.97  | 5.57  | 32.00                 |
| Cd-14    | 13.63  | 3.80  | 14.00                 |
| Cd-15    | 13.10  | 3.43  | 27.33                 |
| Cd-16    | 22.00  | 5.00  | 18.33                 |
| Cd-17    | 17.73  | 4.57  | 21.67                 |
| Cd-18    | 18.80  | 4.90  | 22.53                 |
| Cd-19    | 13.90  | 3.90  | 23.03                 |
| S.Em ±   | 0.47   | 0.14  | 0.54                  |
| C.D. at 5 % | 1.35 | 0.41  | 1.54                  |
| C.V. %   | 4.71   | 5.71  | 4.39                  |
| Cd-1 | Cd-2 |
|------|------|
| ![Cd-1 Image](image1) | ![Cd-2 Image](image2) |

| Cd-3 | Cd-4 |
|------|------|
| ![Cd-3 Image](image3) | ![Cd-4 Image](image4) |

| Cd-5 | Cd-6 |
|------|------|
| ![Cd-5 Image](image5) | ![Cd-6 Image](image6) |

| Cd-7 | Cd-8 |
|------|------|
| ![Cd-7 Image](image7) | ![Cd-8 Image](image8) |
Cultural characters of Colletotrichum spp. isolates of 8th days old culture.

Results in agreement to the present finding were reported by Peres et al., (2002). They studied various cultural and morphological characteristics of Colletotrichum spp. isolates in Brazil. They categorized the Colletotrichum spp. isolated from papaya fruits based on conidial size, conidial shape and colony colour. Damm et al., (2009) categorized Colletotrichum demaitum as ahyaline, aerial, medium close to stem stained pale honey and margins of filter paper gray colour mycelium. After 7 days, colony size and colour of the conidial masses and zonation was recorded and conidial size and shape of 20 arbitrary conidia were measured under the microscope. Papaya isolate showed cylindrical shape of conidia with 17.7 and 6.4 μm mean length and width, respectively (Phoulivong et al., 2010).

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