

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.908.385>

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

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ABSTRACT

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

Article Info

Accepted:
26 July 2020
Available Online:
10 August 2020

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 Coimbatore 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 40_x.

Results and Discussion

Cultural variability

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

After 2nd day

After 2nd 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 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 (18.00mm) 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-4 (17.53 mm) and Cd-18 (17.47 mm). Significantly lowest mycelial growth was recorded in isolate Cd-16 (11.83 mm)

After 4th 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 (42.80mm), Cd-15 (42.33mm), Cd-12 (41.33) and Cd-11 (41.30). 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.*

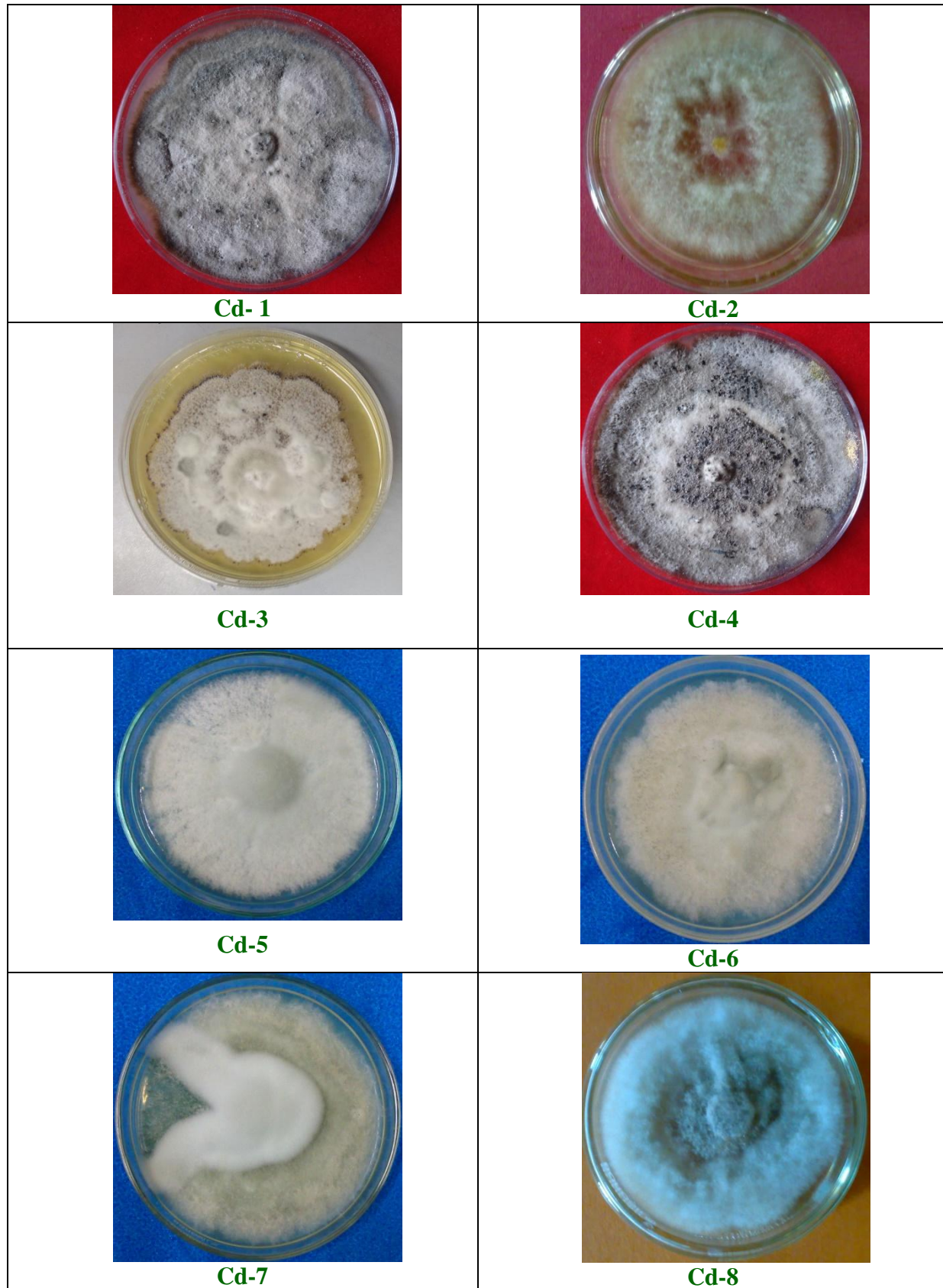
Isolate	Colony diameter (mm)			
	2 nd day	4 th day	6 th day	8 th day
Cd-1	19.50	45.70	67.23	89.67
Cd -2	14.30	35.23	55.50	67.00
Cd -3	18.77	42.80	63.20	85.33
Cd -4	17.53	39.37	61.80	83.33
Cd -5	17.60	37.90	60.00	80.67
Cd -6	14.30	32.63	53.74	67.18
Cd -7	15.40	34.08	52.60	67.93
Cd -8	19.87	47.33	63.77	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

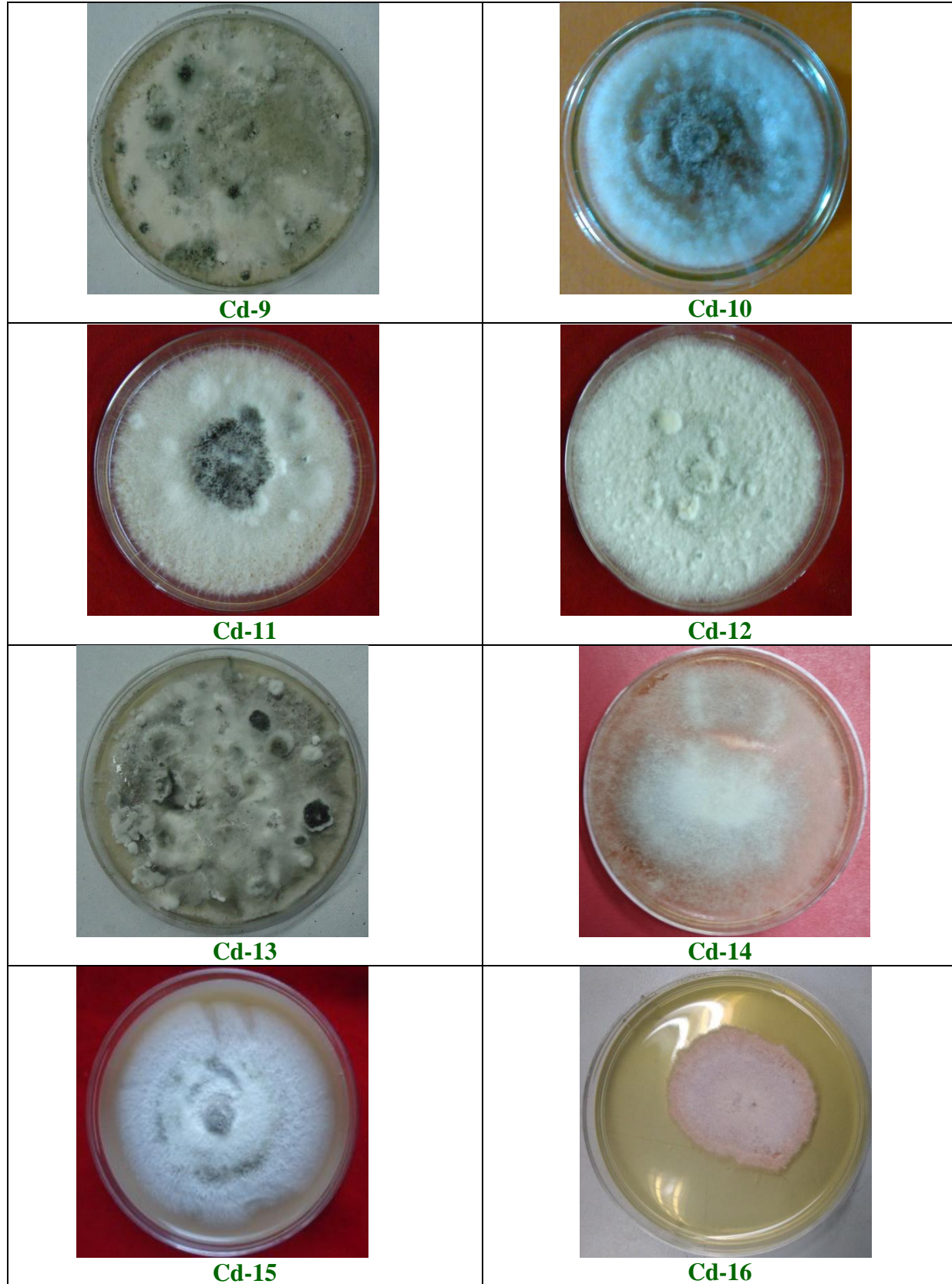
Table.2 Cultural characters of *Colletotrichum spp.* isolates at 25±2 °C on potato dextrose agar after 8 days of incubation

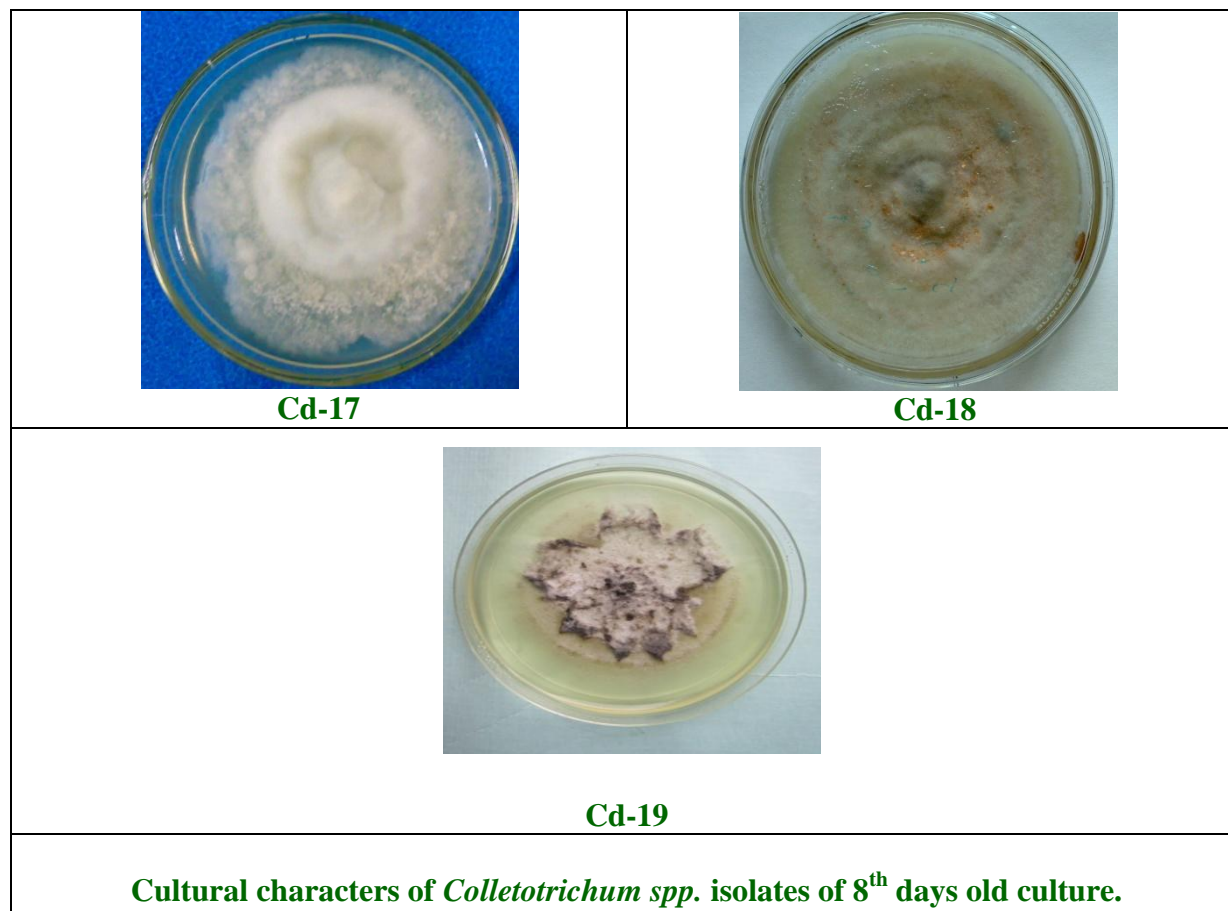
Isolates	Colony colour	Growth pattern
Cd-1	Dark gray	Circular, flat and suppressed
Cd -2	Whitish pink to gray	Fluffy, radial growth
Cd -3	Light grayish	Circular, flat and suppressed
Cd -4	Dull ash to gray colour	Circular to oval and fluffy
Cd -5	Dirty white at centre and white at margin	Olive, fluffy 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 \pm	0.47	0.14	0.54
C.D. at 5 %	1.35	0.41	1.54
C.V. %	4.71	5.71	4.39







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 dematium* 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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How to cite this article:

Prajapati, H. N. and Patil, R. K. 2020. Cultural and Morphological Variability among the Isolates of *Colletotrichum* spp. Causes Fruit Rot of Papaya. *Int.J.Curr.Microbiol.App.Sci*. 9(08): 3344-3353. doi: <https://doi.org/10.20546/ijemas.2020.908.385>