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Morphological and cultural studies among the isolates of *Colletotrichum capsici* causing chilli fruit rot

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Abstract
Ten isolates of *Colletotrichum capsici* collected from different regions of Vidarbha, Marathwada (Maharashtra), Bidar district (Karnataka) and Guntur district (Andhra Pradesh) showing varied type of pathogenic ability against chilli variety Jayanti and designated as strongly, moderately and weakly pathogenic on the basis of per cent disease intensity. Morphological characters of ten different isolates with respect to radial mycelial growth, conidial characters, setae and acervuli were studied on PDA to know the variability among the isolates. Isolate Cc_1, Cc_6 and Cc_7 exhibited similar colony colour, conidia and acervuli characters, Cc_2 and Cc_4 forming ash colonies with concentric rings, while Cc_3, Cc_8 and Cc_9 showed white to ash colonies and Cc_5 and Cc_{10} form light black colonies. Conidia of all the isolates were sickle shaped having oil globule at the centre but setae were longer than conidial mass in all the isolates. Among the ten isolates of *C. capsici*, maximum radial mycelial growth of 82.42 and 81.94 mm was recorded in Cc_5 and Cc_7 respectively, whereas minimum growth (74.28 mm) was recorded in Cc_3 at 7 DAI. Micrometrical observations with respect to conidia, acervuli and setae revealed the differences. The dimension of conidia i.e. length and breadth among ten isolates ranges from 18.64 – 30.31 x 2.75 – 8.20 µm, whereas acervuli length was 295.96 µm and breadth 292.16 µm in Cc_{10}. Maximum conidia length i.e. 30.31 µm and breadth 8.20 µm was observed in Cc_4 and maximum length of setae (212.98 µm) in Cc_7.

Keywords: *Colletotrichum capsici*, morphology, chilli isolates

Introduction
Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is one of the important spice cum vegetable crop in India. The important chilli growing states are Andhra Pradesh, Karnataka and Tamil Nadu. Inspite of chilli is infected by various biotic and abiotic factors. In biotic several pathogens are causing severe diseases and yield loss. The Chilli anthracnose pathogen *C. capsici* infects diverse host with a high degree of pathogenic variability (Akhtar and Singh, 2007) [1]. The genus *Colletotrichum* causes anthracnose on wide range of fruits, vegetables, cereals, grasses and ornamental plants (Dean *et al.* 2012) [2]. The symptom appears on fruits initially small circular spots appeared on the skin of the fruit. The spots were sunken and light grey coloured with black margin, fruiting bodies *viz*., acervuli were produced on the infected area. The seed borne nature of *C. capsici* may be transmitted from mother plant, which were present throughout the storage period, which cause severe seed rot, seedling decay, twig blight, fruit rot and affect the seed germination of chilli and *C. capsici* able to survive up to the next crop season in the infected seeds (Patil and Moniz, 1973) [8]. The anthracnose is one of serious diseases on chili to cause the yield loss and to reduce the quantity of marketable fruits. Therefore, the objective of this study was to characterize the *Colletotrichum* species associated with the fruit rot of chilli.

Materials and Methods
Collection of disease samples
The disease samples of fruit rot of chilli (plant parts) were collected from different geographical areas of Vidarbha and Marathwada region of Maharashtra (M.S.). Some isolates of *Colletotrichum* causing diseases in chilli were collected from Bidar district of Karnataka and Guntur district of Andhra Pradesh.

Isolation and maintenance of cultures
The samples showing characteristic symptoms of fruit rot, dieback and anthracnose were collected from different localities and cut along with healthy tissues.

The infected bits were washed with sterilized water and surface sterilized in 0.1 per cent mercuric chloride solution for one minute in the Petriplates and subsequently three changes of water was given to remove the traces of mercuric chloride. The bits were dried around the flame of spirit lamp, then transferred to solidified sterile potato dextrose agar (PDA) in Petriplates and were incubated at room temperature (27 ± 2 ºC) for seven days. All the operations were carried aseptically. The fungus growth of *Colletotrichum capsici* was then transferred on PDA slants. The cultures thus obtained were further purified by single spore isolation technique.

Single spore isolation

A spore suspension was prepared in test tubes by transferring a loopful of spores from the culture tube (10 ml distilled sterilized water) and the dilution was adjusted to give 15-20 spores. Loopful of suspension was transferred to 20 ml, 1 per cent melted agar in test tubes and shaken vigorously then poured in sterilized Petriplates and these plates were kept in inverted position after solidification. The position of each spore was marked with India ink by inverting plates. Such spores were then transferred to PDA slants to obtain a single spore culture of each isolate. The single spore isolates, thus obtained were further used in various experiments. The cultures were abbreviated as Cc. (*Colletotrichum capsici*) and reviewed by periodic transfer and maintained on PDA.

Sr. No.	Isolates	Abbreviations	Host	Location
1	*Colletotrichum capsici*	Cc_1	Chilli	Chinchola (Maharashtra)
2	*Colletotrichum capsici*	Cc_2	Chilli	Parbhani (Maharashtra)
3	*Colletotrichum capsici*	Cc_3	Chilli	Bidar (Karnataka)
4	*Colletotrichum capsici*	Cc_4	Chilli	Divatana (Maharashtra)
5	*Colletotrichum capsici*	Cc_5	Chilli	Neemkhed (Maharashtra)
6	*Colletotrichum capsici*	Cc_6	Chilli	Guntur (Andhra Pradesh)
7	*Colletotrichum capsici*	Cc_7	Chilli	Karla (Maharashtra)
8	*Colletotrichum capsici*	Cc_8	Chilli	Washim (Maharashtra)
9	*Colletotrichum capsici*	Cc_9	Chilli	Akola (Maharashtra)
10	*Colletotrichum capsici*	Cc_{10}	Chilli	Jalna (Maharashtra)

Pathogenicity test

Monosporous cultures of isolates were obtained from the fungal cultures. The epidermal layer of fruits, leaves of the susceptible variety of chilli were injured by carborandom powder before inoculation. The spore suspension of each isolate of *Colletotrichum capsici* was used for inoculating the plants in pots by using sterilized cotton swab. The seedlings were kept for predisposition for 24 Hour prior to inoculation by irrigating and covered the moist hesian cloth. Inoculation by cotton swab was made in the evening hour and covered with hesian cloth to provide 100 per cent humidity for spore germination and infection up to 48 Hour. Similarly the fruits were also inoculated by smearing the inoculums on the upper surface and incubated in the humid chamber. After inoculating the plants with *Colletotrichum capsici*, the host plants were examined periodically for development of symptoms. Re-isolations were made and the fungus obtained was compared with the original one.

Morphological variation

Autoclaved PDA was poured in the plates and on solidified media the isolates were inoculated separately. Fungal disc (6 mm) of seven days old cultures were transferred on solidified PDA in plates i.e. one disc per plate. Inoculated plates were incubated at room temperature (27 ± 2 ºC) under 12 hr light and 12 hr darkness. After seven days of incubation variation among the colony characters and growth pattern of *Colletotrichum capsici* were observed.

Measurement of radial mycelial growth

Solidified PDA plates inoculated with isolates of *Colletotrichum capsici* were incubated at room temperature (27 ± 2ºC) and examined for radial mycelial growth on third, fifth and seventh day respectively. The colony diameter was measured in three marked directions at right angles to each other, passing through the center of colony and the averages were worked out.

Micrometry of conidia, setae and acervuli

The dimensions of conidia, setae and acervuli were recorded by using ocular micrometer. Value of one part of ocular micrometer was calibrated under high magnification (15x,40x) by using stage micrometer. The observations were based on hundred conidia, setae, acervuli and the mean size were worked out.

Results and Discussion

Collection, isolation, purification and identification of pathogen

Fruit rot infected plant parts were collected from different geographical areas of Vidarbha and Marathwada region of Maharashtra and some samples were collected from Bidar (Karnataka) and Guntur (Andhra Pradesh). The usual tissue isolation technique was followed to isolate the pathogen from infected plant parts showing fruit rot, anthracnose symptoms. Potato dextrose agar was used as basal medium for isolation of the fungus. The pure culture was obtained using single spore method. The culture thus obtained was identified as *Colletotrichum capsici* on the basis of pathogenic ability and morphological characters as per the CMI publications. Purified cultures of the fungus were maintained on PDA slants for further studies and abbreviated as Cc.

Pathogenicity and symptoms

Pathogenic ability of ten isolates of *Colletotrichum capsici* was tested on a susceptible chilli variety (Jayanti). Observations were recorded on per cent disease intensity after 12 DAI and the results are presented in Table 1. Symptoms under field condition on chilli causing fruit rot appeared in the form of black circular spots on skin spreading along the fruit length and turning to elliptic, heavily infected fruits turned straw coloured and numerous dots like acervuli was observed. The characteristic symptoms on leaves are in the form of irregular to circular areas with brown margins. On fruits grayish black colour elongated spots forming acervuli in advanced stage arranged in elliptical manner was examined. The *Colletotrichum capsici* causing chilli fruit rot was isolated and pathogenicity was proved. These results confirm the findings of Sydow (1913) [14] who reported infection of *Colletotrichum capsici* for the first time in chilli, Verma (1973) [15] isolated *Colletotrichum dematium*, *C. gloeosporioides*, *C. graminicola* and *C. atramentrarium* from the chilli plants infected with dieback and fruit rot. Gotmare (1981) who observed the symptoms of *Colletotrichum capsici*

on chilli as dark brown stripes on twigs and petioles. Similarly, Ramakrishnan (1954) [10] reported the leaf spot disease of chilli and turmeric (*Curcuma longa*) caused by *C. capsici* and Roy *et al*. (1997) [11] confirms the pathogenicity of *C. capsici* on bell pepper by tooth prick inoculation. Similar reports were made earlier by Lubna Massodi *et al.* (2013) [6], Gupta *et al*. (2017) [4] and Sunil Kumar (2017) [13]. Karthik Pandi *et al.* (2018) [5] isolated the pathogen from the symptomatic chilli fruits showing small black circular spots on the skin of the fruits that in the direction of the long axis. The spots were sunken and light grey coloured with black margin. The spots enlarged into larger lesions and on the surface of the lesions acervuli, the fruiting body of the fungus appeared as minute black dots. All the isolates of pathogen was identified as *Colletotrichum* species.

Table 1: Pathogenic ability of *Colletotrichum capsici* isolates showing disease reaction against susceptible variety (Jayanti)

Isolate	Location	PDI (%)	Reaction
Cc_1	Chinchola	43.33	MP
Cc_2	Parbhani	19.50	WP
Cc_3	Bidar	17.67	WP
Cc_4	Divatana	24.33	WP
Cc_5	Neemkhed	37.17	MP
Cc_6	Guntur	51.50	SP
Cc_7	Karla	18.67	WP
Cc_8	Washim	33.83	MP
Cc_9	Akola	51.83	SP
Cc_{10}	Jalna	38.83	MP

Morphological characters

Morphological characters of isolates with respect to radial mycelial growth, conidial characters, setae and acervuli of *Colletotrichum capsici* were studied on PDA. The results are presented in Table 2. Among ten isolates of *C. capsici* Cc_1, Cc_6 and Cc_7 exhibited similar colony colour, conidia and acervuli characters. Cc_2 and Cc_4 forming ash colonies with concentric rings, while Cc_3, Cc_8 and Cc_9 forming white to ash colonies and isolate Cc_5 and Cc_{10} developed light black. Isolate Cc_1, Cc_2, Cc_3, Cc_6, Cc_7 and Cc_{10} conidia were pinkish when appeared in mass, whereas rests of the isolates were saffron in mass. Isolate Cc_3 and Cc_6 form ridges and furrows, whereas Cc_1, Cc_2, Cc_4, and Cc_6 possesses concentric rings and isolate Cc_1, Cc_4, Cc_9 and Cc_{10} developed white patches. Conidia of all the isolates were sickle shaped having oil globule at the centre but setae were longer than conidial mass in all the isolates (Plate 2 and 3). Differences in referred characters might be due to environmental diverse condition or the genetic make of the isolates. Mordue (1971) [7] observed variation in *C. dematium*. Variation in colony colour has been observed by Akhtar Jameel and Singh (2007) [1]. These observations of the present findings lead to confirm the variation among the isolates collected from different locations. Rajyasri Ghosh *et al.* (2016) [9] observed that, the conidia of *Colletotrichum* isolates from soybean and chilli is falcate, fusiform with acute apices. The size ranges from 19.65–21.00 µm in length and 3.0–3.5 µm in breadth. In *C. gloeosporiodes* conidia are hyaline, one celled, cylindrical. The size ranges from 8–12 µm in length and 4–6 µm in width. Similar reports were made by Lubna Massodi *et al.* (2013) [6], Gupta *et al*. (2017) [4] and Sunil Kumar (2017) [13]. Karthik Pandi *et al.* (2018) [5] reported variation in their cultural behaviour of twenty five isolates varied from *Colletotrichum* species in culture colonies. Potato Dextrose Agar (PDA) supported the maximum growth (9.0 cm). All the isolates of *C. capsici* and *C. gloeosporides* produced black pointed setae, hyaline falcate and cylindrical conidia with single oil globule at the centre. Number of setae per acervulus (12-32) and number of septa per seta (2-4) varied among the isolates. Majority of the isolates produced profuse sporulation.

Rate of mycelial growth of different isolates of *C. capsici*

The response of different isolates of *C. capsici* was tested on potato dextrose agar and results are presented in Table 2. Among the ten isolates of *C. capsici*, maximum radial growth of 82.42 and 81.94 mm was recorded in Cc_5 and Cc_7, respectively. It was followed by Cc_1 and Cc_8 isolates recorded 79.18 and 79.00 mm radial mycelial growth, whereas minimum radial growth (74.28 mm) was recorded in Cc_3 at 7DAI. Akhtar Jameel and Singh (2007) [1] reported the differences in radial mycelial growth on PDA of *C. capsici*. Similar observations were also mentioned by Lubna Massodi *et al.* (2013) [6].

Micrometrical observations

Micrometrical observations revealed the differences among the isolates of *C. capsici* causing fruit rot of chilli, measurements with respect to conidia, acervuli and setae were recorded and the observations are tabulated in Table 3. The dimensions of conidia i.e. length and breadth among ten isolates ranged between 18.64–30.31 x 2.75–8.20 µm. The dimension of acervuli measured 106–295.96 x 90.94–292.96 µm whereas, setae length was ranged between 55.69-212.96 µm. Maximum conidial length (30.31µm) and breadth (8.20 µm) was observed in Cc_4, maximum length of setae was 212.98 µm in Cc_7, whereas acervulus size (length) was more i.e. 295.96 µm and breadth (292.16 µm) in Cc_{10} isolate. There were only very minor differences in the size of conidia, acervuli and setae of the isolates grown on PDA. The micrometrical data of present study confirms the reports published by Ramakrishnan (1954) [10], Saxena and Singh (1959) [12] in *C. capsici*. The minor differences in the conidial dimension of different *C. capsici* isolates was reported by Akhtar Jameel and Singh (2007) [1]. Similar reports were also observed by Lubna Massodi *et al.* (2013) [6], Gupta *et al*. (2017) [4], Sunil Kumar (2017) [13] and Karthik Pandi *et al.* (2018) [5] and thus the present results are on the similar line of published literature.

Table 2: Morphological characters of different isolates of *Colletotrichum capsici* on PDA

Isolate	RMG (mm)	Colony characters	Conidial characters	Acervuli
Cc_1	79.18	Colonies dark black, concentric rings with uniform growth and white margins in the centre with a white patch, reverse back black in colour	Conidia sickle shaped having oil globules at the centre but setae are longer than conidial mass and pinkish	Acervuli some what big in size and setae longer than conidial mass.
Cc_2	74.66	Colonies ash with concentric rings and white margins all along the border, reverse back brownish in colour.	Conidia sickle shaped with oil globule and conidial mass is pink in colour	Acervuli bigger with scattered mass of conidia and setae longer than conidial mass

Cc_3	74.28	Colonies white to ash with aerial mycelium forming ridges and furrows, white margins all along the border, reverse back light black.	Conidia sickle shaped, hyaline and pinkish when in mass, oil globule at the centre	Acervuli smaller with scattered setae and setae longer than mass of conidia.
Cc_4	78.83	Ash coloured colonies with white patches in the centre forming a concentric ring at the centre.	Conidia bigger with oil globule and sickle shaped, in mass appear saffron colour.	Acervuli with scattered spore mass and setae longer.
Cc_5	82.42	Colony appears as light black with white border, reverse back light grey in colour without concentric rings.	Sickle shaped with oil globule, hyaline and in mass saffron colour.	Acervuli with dense mass of conidia and setae are present.
Cc_6	75.44	Dark black to white colonies with concentric rings and forming ridges and furrows.	Conidia in mass pinkish colour sickle shaped with oil globule.	Acervuli smaller in size with longer setae.
Cc_7	81.94	Colonies dark black and also reverse back dark black in colour with profuse growth of mycelium.	Conidia in mass pinkish colour and hyaline.	Medium size acervuli with conidial mass and setae.
Cc_8	79.00	White to ash colonies in appearance and form a uniform growth, reverse back light black colour.	Conidia in mass saffron and hyaline.	Acervuli with conidia scattered having longer setae.
Cc_9	78.39	Colony light ash with aerial mycelium and forming profuse growth with white patches.	Conidia hyaline sickle shaped with oil globule in the centre.	Acervuli having longer setae and some big in size.
Cc_{10}	74.61	Light black to ash in colony colour with white patches and no concentric rings.	Conidia minute and in mass pinkish colour.	Bigger acervuli with scattered conidial mass.
'F' test	Sig.	RMG- Radial mycelia growth		
SE (m)±	0.56			
CD at 1%	2.26			

Table 3: Dimension of conidia, acervuli and setae of *Colletotrichum capsici* isolates.

Sr. No.	Isolate	Conidia		Acervuli		Setae
		Length# (µm)	Breadth# (µm)	Length# (µm)	Breadth# (µm)	Length# (µm)
1	Cc_1	19.97 - 26.80	3.65 - 6.44	195.07 - 262.31	177.40 - 245.83	116.42 - 160.38
2	Cc_2	18.64 - 27.31	3.83 - 6.44	160.14 - 260.11	130.44 - 221.50	104.04 - 189.69
3	Cc_3	19.17 - 28.50	4.16 - 6.18	106.62 - 217.11	90.94 - 207.54	55.69 - 84.79
4	Cc_4	24.49 - 30.31	4.51 - 8.20	123.70 - 222.60	111.91 - 223.11	85.48 - 163.41
5	Cc_5	20.02 - 26.79	4.10 - 7.25	159.52 - 259.25	164.75 - 262.93	100.97 - 200.94
6	Cc_6	23.14 - 28.01	5.51 - 8.00	128.04 - 213.51	117.02 - 219.48	56.85 - 142.74
7	Cc_7	24.77 - 30.18	3.49 - 5.87	157.41 - 252.45	154.65 - 244.82	125.60 - 212.98
8	Cc_8	19.82 - 25.47	4.78 - 7.69	156.46 - 256.37	153.94 - 230.68	87.38 - 128.84
9	Cc_9	19.96 - 24.89	2.75 - 5.12	184.53 - 266.91	170.83 - 259.22	118.68 - 195.54
10	Cc_{10}	21.90 - 26.95	5.02 - 6.27	217.85 - 295.96	196.41 - 292.16	117.32 - 169.88

= Range values

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