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Cultural and morphological variability amongst *Colletotrichum capsici* isolates collected from Marathwada region of Maharashtra state

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Abstract
An extensive roving survey carried out during both the year 2017-18 and 2018-19 in the eight districts of Marathwada region for the incidence and intensity of anthracnose of chilli. The disease was found to be predominant in all the eight districts of Marathwada region. Based on symptomatology, cultural and morphological characteristic, pathogenicity test and microscopic observations, the test pathogen was identified as *Colletotrichum capsici* (Syd.) Butler and Bisby. All the eight representative isolate of *C. capsici* collected during survey exhibited a wide range of variability in respect of cultural and morphological characters. However, the isolates Cc7 followed by Cc1, Cc2 and Cc3 exhibited maximum mycelial growth of 84.33, 80.90, 75.13 and 68.26 mm, respectively. Maximum conidial size of 25.52 × 2.14 µm was produced by the isolate Cc1 followed by the isolates Cc6 (23.52 × 1.83 µm) and Cc2 (23.12 × 2.85 µm). Maximum growth of *C. capsici* was recorded on Richard's agar (88.94 mm) followed by Czapek's dox agar (88.53 mm), Oat meal agar (87.83 mm), Sabouroud agar (87.31 mm), Beijerinka agar (86.63 mm), and Potato Dextrose Agar (84.37 mm).

Keywords: *Colletotrichum capsici*, cultural and morphological variability

Introduction
Chilli (*Capsicum annuum* L) also called as red pepper is an important spice-cum-vegetable crop of the world. India is the largest producer of dry chilli in the world. However, it suffers from biotic diseases caused by fungi, bacteria, viruses and nematodes resulting in huge loss of the crop. Among the fungal diseases infecting chilli crop, anthracnose or ripe fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has become a serious problem limiting the profitable cultivation and seed production throughout the major chilli growing regions of India (Gopinath *et al.,* 2006; Ramachandran *et al.,* 2008) [5, 7]. Therefore, present investigations on anthracnose (*C. capsici*) disease of chilli were undertaken to find out the cultural and morphological variability amongst different isolates of Marathwada region of Maharashtra state.

Material and methods
A roving survey to record anthracnose incidence was conducted during the year 2017-18 and 2018-19, covering 184 and 189 chilli crop fields of 08 districts, distributed under three agro-climatic zones of Marathwada region of the Maharashtra state. During survey different isolates of *Colletotrichum capsici* were collected and categorized. From this samples eight samples are selected for further studies as below.

Description of eight isolates collected from different districts of Marathwada region

Sr. No.	Agro climatic zones	Location	Isolate name
1	Aurangabad	Vaijapur	Cc1
2	Beed	Georai	Cc2
3	Hingoli	Sengao	Cc3
4	Jalna	Ambad	Cc4
5	Latur	Renapur	Cc5
6	Nanded	Loha	Cc6
7	Osmanabad	Bhoom	Cc7
8	Parbhani	Purna	Cc8

Cultural characteristics of *C. capsici* isolates

The experiment was conducted to study the variation in the morphological and cultural characters of isolates of *C. capsici* collected from different locations. Eight isolates of *C. capsici* isolated from eight districts were aseptically inoculated on PDA medium separately and the plates were incubated at 27 ± 2 ^{0}C for ten days.

Observations on shape of colony, colony colour, colony diameter, colony elevation were recorded after ten days of incubation and on sporulation were recorded at 15 days of incubation.

Morphological variability

For studying morphological characteristics, the temporary mounts on glass slide in a drop of Lactophenol cotton blue stain of the pure culture (10-12 days old) of *C. capsici* test isolates were prepared separately, covered with cover slip and observed under research microscope. Observations on conidial shape and size, number of setae per acervulus, length of setae, colour and size of acervuli were recorded. Measurements of conidia, setae and acervuli were recorded at 400X magnification under research microscope by using ocular micrometer calibrated with stage micrometer and mean dimensions were recorded.

Observations on colony diameter, and colony characteristics were recorded after a week of incubation and that of sporulation, after 10 - 12 days of incubation. The sporulation was graded as per the scale (Dasgupta, 1981) given below.

Scale used for recording sporulation data

Sr. No.	Grade	Description	Av. No of conidia / microscopic field
1	--	No sporulation	--
2	+	Poor	1-10
3	+ +	Fair /moderate	11-30
4	+ + +	Good	31-50
5	+ + + +	Excellent	>51

Result and Discussion

Cultural variability

Cultural characteristics *viz*., growth rate and amount, colony colour, colony elevation, shape, colony margin, colony texture, zonation etc. of the test isolates were studied using PDA as basal culture medium.

Results (Table 1) revealed variability in cultural characteristics of the eight test isolates of *C. capsici*. Among the test isolates, colony diameter / growth was ranged from 53.80 to 84.33 mm. However, it was highest with the isolate Cc-7 (84.33 mm), followed by isolates *viz*, Cc-1 (80.90 mm), Cc-2 (75.13 mm) and Cc-3 (68.26 mm). Growth rate was fast in Cc-1, Cc-4, Cc-7, where it was medium in Cc-2, Cc-3, Cc-5, Cc-7 and slow in Cc-6. Colour of the colonies varied from white (Cc-1, Cc-2, Cc-6, Cc-8), light grey (Cc-3, Cc-5) and dark grey (Cc-4, Cc-7).

These results observed in the present study are in confirmity with the findings of several earlier workers. Sangdee *et al*. (2011) [9] evaluated ten isolates of *C. capsici* causing chilli anthracnose for their morphological, cultural and pathogenic variability on chilli fruits. Cultural variability of *C. capsici* isolates on basal culture medium Potato Dextrose Agar was reported earlier by several workers (Singh, 1985; Hartman and Wang, 1990; Wijesekara and Agarwal, 2006 and Ghugul, 2007) [10, 6, 11, 4].

Morphological variability of *C. capsici* isolates

The data presented in Table 2 indicated that all the eight representatives isolate of *C. capsici* exhibited a wide range of variability in respect of conidial dimensions and length of setae. Conidial size of the test isolates was ranged from 17.29 × 3.73 µm (Cc_8 isolate) to 27.32 × 2.89 µm (Cc_1 isolate). However, the largest sized conidia (27.32 × 2.89 µm) was produced by the isolate Cc_1. The second and third larger conidia were produced by the isolates Cc_2 (25.52 × 3.54 µm) and Cc_6 (25.12 × 2.57 µm). This was followed by the isolates *viz.*, Cc_4 (22.72 × 2.49 µm), Cc_5 (22.52 × 2.83 µm), Cc_3 (21.32 × 2.95 µm) and Cc_7 (20.02 × 3.31 µm). The isolate Cc_8 (17.29 × 3.73 µm) produced comparatively small sized conidia. Conidia of all the isolates were falcate, blunt to narrow at ends with oil globuse at centre. There was no variation observed among the isolates with respect to colour of conidia as the colour of conidia of all the isolates was hyaline.

Length of setae of the test isolates was ranged from 99.26 µm (Cc_7 isolate) to 116.23 µm (Cc_2 isolate). However, maximum length (116.23 µm) was recorded in Cc_2 isolate. This was followed by the isolates *viz.*, Cc_3 (112.34 µm), Cc_8 (110.77 µm), Cc_6 (108.92 µm), Cc_1 (108.1 µm), Cc_5 (103.87 µm) and Cc_4 (101.78 µm). Comparatively short length of setae (99.26 µm) was recorded in the isolate Cc_7. There was no distinct variation observed among the isolates with respect to shape and colour of the setae as all the test isolates produced dark brown setae which were smooth, septate, rigid, hardly swollen at the base and slightly tapered towards the paler acute apex.

The results obtained in present study on morphological variability of *C. capsici* isolates tested are similar to those reported earlier by several workers. Butler (1973) reported *C. capsici* conidium size of 17-28 × 3-4 µm. Wilson and Vijayan (1980) reported conidium size of 20.8-27.2 × 3.2-4 µm. Rangaswami (1993) reported *C. capsici* conidium size of 11-24 x 4-5.5 µm and contained centrally placed oil globules. Chidananda Swamy and Kulkarni (2003) reported that setae of *C. capsici* were brown, septate, tapering and measuring 97.32 to 134.73 µm on host and 53.41 to 151.40 µm in culture medium. Conidiophores were single celled hyaline smooth walled, falcate or sickle shaped with blunt ends and measured15.42 × 2.93 µm. Sangdee *et al*. (2011) [9] reported conidium size of 23.5-35.0 × 2.5-3.75 µm. These observations confirmed with the measurement of *C. capsici.*

Table 1: Cultural variability among *C. capsici* isolates of chilli

Sr. No.	Isolates	Mean colony diameter (mm)*	Shape of colony	Colour of colony	Topography	Sporulation
1.	Cc_1	80.90	Circular	White	Flat	++++
2.	Cc_2	75.13	Irregular	White	Flat	++
3.	Cc_3	68.26	Circular	Light gray	Raised	+++
4.	Cc_4	53.80	Irregular	Dark gray	Flat	++++
5.	Cc_5	65.14	Circular	Light gray with Conc. rings	Raised	+++
6.	Cc_6	60.14	Irregular	White	Flat	+
7.	Cc_7	84.33	Circular	Dark gray	Raised	++
8.	Cc_8	59.10	Circular	White	Flat	++++
	S.E. ±	0.7				
	CD (P= 0.01%)	2.1				

+ = Poor ++ = Fair +++ = Good ++++ = Excellent

Table 2: Morphological variability among the isolates of *C. capsici*

Isolate No.	Conidia (µm)						Setae (µm)		
	Max. (µm)		Min. (µm)		Mean (µm)		Length		
	Length	Breadth	Length	Breadth	Length	Breadth	Max. (µm)	Min. (µm)	Mean (µm)
Cc_1	29.12	3.64	25.52	2.14	27.32	2.89	126.2	90	108.1
Cc_2	27.92	4.24	23.12	2.85	25.52	3.54	131.26	101.02	116.23
Cc_3	23.12	3.76	19.52	2.14	21.32	2.95	137.26	87.43	112.34
Cc_4	24.72	3.24	20.72	1.75	22.72	2.49	119.12	84.45	101.78
Cc_5	25.52	3.60	19.52	2.06	22.52	2.83	119.42	88.33	103.87
Cc_6	26.72	3.31	23.52	1.83	25.12	2.57	131.62	86.23	108.92
Cc_7	21.03	4.04	19.01	2.58	20.02	3.31	118.51	80.02	99.26
Cc_8	19.17	4.52	15.41	2.94	17.29	3.73	127.49	94.05	110.77
S.E. ±	--	--	--	--	0.99	0.68	--	--	0.58
CD (P= 0.01%)	--	--	--	--	2.99	1.86	--	--	2.24

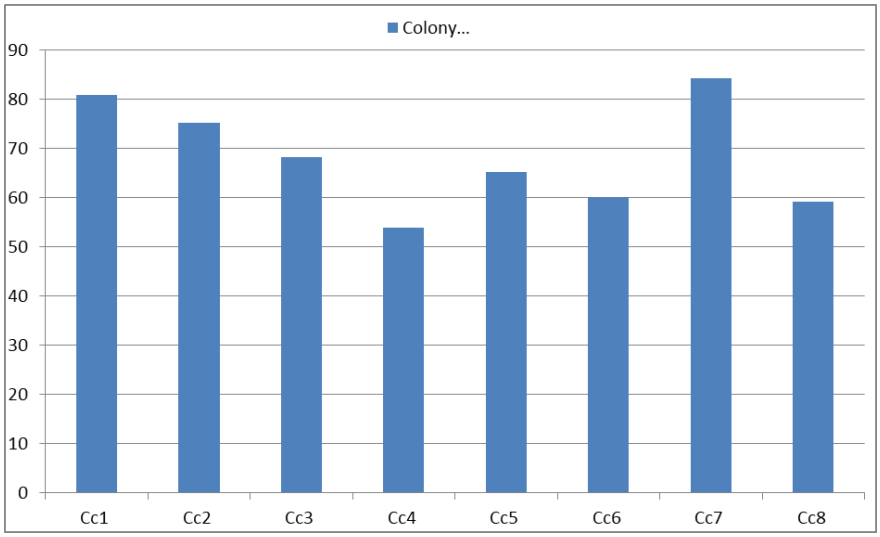

Fig 1: Cultural variability among the isolates of *C. capsici*

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