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Morphological and Cultural Variability of C*olletotrichum* Spp. Causing Anthracnose of Chilli (*Capsicum annuum* L.) in Andhra Pradesh

Vyshnavi Deshmukh, T Vijaya Lakshmi, B Tanuja Priya, A Rajani and B K M Lakshmi

College of Horticulture, Dr YSR Horticultural University, V RGudem, A. P.

ABSTRACT

Anthracnose disease, caused by *Colletotrichum* spp. is one of the major economic constraints to chilli production in tropical and subtropical regions. The different isolates of *Colletotrichum* species were collected from different chilli growing areas of Andhra Pradesh during the survey conducted for recording anthracnose disease. The isolates were evaluated for their morpho-cultural characteristics and pathogenic variability on chilli fruits. The pathogenic behaviour of the 15 isolates of *Colletotrichum* spp. developed from fruits was established following Koch's postulates. All the isolates of *C. truncatum* and *C. gloeosporioides* produced black pointed setae, hyaline falcate and cylindrical conidia with a single oil globule at the centre. Colony growth rate (6.0 to 8.8 mm day^{-1}), shape of the conidia, length and width of the conidia, colony colour, colony texture varied among the isolates. The majority of the isolates produced profuse sporulation. To know the virulence of the isolates fruits were inoculated with all the isolates and the results suggested that isolate CC14 caused the maximum anthracnose intensity (53.0 per cent) while CC2 showed the least intensity (16.5 per cent).

Keywords: *Anthracnose,Chilli, Colletotrichum truncatum and C. gloeosporioides and variability.*

Chilli (*Capsicum annuum* L.) is an important vegetable as well as a spice crop cultivated worldwide for its pungent fruits, which are used as both green and ripe, having nutritional and medicinal value. Chilli is a good source of vitamins like A, B and C and minerals like calcium, phosphorus, iron, sodium and copper in trace amounts. India is the largest producer, consumer and exporter of chilli in the world and is cultivated in an area of 7.43 lakh hectares with a production of 19.14 lakh tonnes in 2020-21 (www.indiastat.com). Andhra Pradesh ranks first in area and production of chilli all over India. Though Andhra Pradesh ranks first in chilli cultivation, the intensity of anthracnose has been found to be increasing and has become a constraint for profitable production.

Anthracnose of chilli, caused by *Colletotrichum truncatum*, is one of the major and devastating diseases of chilli and causes severe losses (10-60%) both in yield and quality of the chilli depending upon the varieties (Bansal and Grover, 1969). About 50% of the crop losses have been reported from different parts of India.

The chilli anthracnose pathogen, *C. capsici* infects diverse host's with a high degree of pathogenic variability (Akhtar and Singh, 2007). *Colletotrichum* is considered as the eighth most important plant pathogenic fungal genus in the world (Cannon *et al.*, 2012). Among the pathogens, *Colletotrichum* species cause anthracnose of chilli and has been shown to be caused by at least four species *viz.*, *C. capsici* and *C. gloeosporioides* in India (Akhtar and Singh,

2007), Indonesia (Masoodi *et al.,* 2012), Korea (Simmonds, J.H, 1965), Thailand (Mayee and Datar, 1986), *C. acutatum* in Australia (Raj *et al.,* 2014) and Indonesia (Prasad *et al.,* 2000) and *C. coccodes* in New Zealand (Pakdeevaraporn *et al.,* 2005).

The virulence degree of disease symptoms on host plants depends on the fungal pathotype. Sharma et al. (2005) reported the existence of 15 pathotypes of *C. capsici* based on disease symptom development on inoculated fruit of *C. annuum* genotypes. Therefore, information on the variability and distribution of race or pathotype of *C. truncatum* in chilli growing areas of Andhra Pradesh needs to be generated and hence the present study was taken up with the objective of morphological and cultural variability of different isolates of *Colletotrichum* spp. collected from chilli growing areas of Andhra Pradesh

MATERIAL AND METHODS

Survey and collection of anthracnose infected samples

A random survey was conducted in four major chilli growing districts *viz*., Guntur, Krishna, Prakasam and Kurnool districts of Andhra Pradesh for recording disease incidence and collection of diseased fruit samples. A total of 15 villages covering 15 mandals in four districts were surveyed. For recording anthracnose incidence, total number of fruits and the number of affected fruits on l0 randomly selected plants in each field were counted and the PDI was calculated. Chilli fruit samples showing characteristic symptoms of anthracnose were collected from fields surveyed i.e., one sample from each village. Collected samples were kept in perforated polythene covers and kept for isolation.

Isolation of the pathogen

Experiments were conducted during the crop season 2021-22 for cultural and morphological characterization. Laboratory experiments were carried out in the Department of Plant Pathology, DrYSRHU, Horticultural Research Station, Lam, Guntur district, Andhra Pradesh.

The diseased parts were cut into small bits with the help of a sterilized blade. Then surface sterilized with 1% sodium hypochlorite solution under aseptic conditions in laminar air flow chamber and washed thoroughly for three times with sterilized water to remove traces of chemical. Excess moisture was removed by placing these in the fold of sterilized blotting paper. The pieces are then transferred into Petri plates with the help of sterilized needles. The Petri plates were previously sterilized and poured with PDA media amended antibiotic streptocyclin to prevent bacterial contamination. The Petri plates were kept at 25±2 °C for 7-10 days in an incubator. Single spore isolation technique was followed for obtaining pure culture.

Pathogenicity test of isolated *Colletotrichum* spp., to prove Koch postulates

Red ripe chilli fruits were collected from the field and sterilized using 1% sodium hypochlorite solution followed by drying using sterilized filter paper. After drying mild pinprick was made on the fruit with the sterilized needle and then conidia suspension 2µl of concentration 1×10^6 per ml was placed over the wound. Spore concentration was adjusted by haemocytometer. The inoculated fruits were observed for disease development and the pathogen was reisolated and observed under microscope to prove Koch is postulates.

Cultural, morphological and pathogenic variability of the *Colletotrichum* spp.,

The experiment was conducted to study the variation in the morphological and cultural characters of isolates of *Colletotrichum* spp., collected from

different chilli growing areas of Andhra Pradesh. All 15 isolates of *Colletotrichum* spp., isolated from different locations aseptically inoculated on PDA media separately, and the plates were incubated at 25±2^{0}C.

Cultural characteristics

The cultural characters of isolates of *Colletotrichum* spp. were recorded from a culture grown on PDA. PDA of 20 ml was poured into each of the previously sterilized Petri plates. Five mm discs were cut using sterilized cork borer, from the margin of seven days old colony of the fungal culture grown in Petri plates. One disc was placed in the centre of each plate and incubated at 25±2 ^{o}C for seven days. The observations regarding colony colour, colony texture and type of mycelial growth of the pathogen were taken.

Morphological characteristics

The slides of isolated fungus were prepared in lactophenol from seven-day old culture. For morphological studies, observations for colony growth rate (mm day^{-1}), shape of conidia, length and width of 100 conidia, (µm) (using fluorescent microscope), presence or absence of setae, margins (smooth or wavy). Colony growth rate was measured at different intervals by using the formula:

Average Linear Growth Rate (ALGR) (mm/day)

$$= \frac{C_8 - C_0}{8}$$

C_8 – diameter of the colony on 8th day after inoculation

C_{0} - diameter of the colony on the day of inoculation

Pathogenic variability

After proving the Koch postulates, each isolate was inoculated on detached red ripe chilli fruits of Gemini 116 variety by pinprick method as followed for the pathogenicity tests. The inoculated fruits were observed for development of the disease and the diameter of the lesion was recorded at 3, 7, 10 days after inoculation. The per cent fruit area is recorded by using the 0-5 disease rating scale. (Jeyalakshmi and Seetharaman, 1998) grades were given based on the fruit area infected i.e., 0-no disease, 1-up to 5% infection, 2- >5-10% infection, 3->10-25%, 4->25-50%, 5 - > 50%. Then per cent fruit area infected was calculated by the formula:

Per cent fruit area infected =

$$\frac{\text{Sum of numerical ratings} \times 100}{\text{Total no of fruits observed x maximum grade}}$$

This was done for all the isolates. The isolate which showed significantly highest per cent fruit area infected was identified as more virulent and was used for screening of genotypes.

Variability

After taking observations on the morphological and cultural characters of each isolate, variability among different isolates was studied.

Statistical analysis

In the present study, CRD was used for analysing the data. The value of F at 5% level of probability was calculated using Fischer and Yates (1968) for the appropriate degrees of freedom (d.f). The analysis of variance was used to study the differences among treatments in the experiments. The treatment difference was obtained and compared with critical difference if it was less than C.D then there was no significant difference in treatment effect.

RESULTS AND DISCUSSION

The experimental findings of research work on the morphological and cultural variability of C*olletotrichum* spp. which causes anthracnose of chilli in Andhra Pradesh are presented hereunder.

Survey and collection of anthracnose infected chilli fruit samples

A field survey was conducted in four important chilli growing districts *viz*., Guntur, Krishna, Prakasam and Kurnool districts of Andhra Pradesh to record the recording incidence of anthracnose in chill. The survey was carried out during the fruit development and ripening stages of the chilli fruits. During survey all the required data is collected from the farmers of the respective fields.

Five mandals in Guntur district, four mandals in Prakasam district and three mandals each in Krishna and Kurnool districts were surveyed, one village from each mandal was selected. From each village, three fields were surveyed and, in each field, 10 plants were selected at random, examined for disease, and the per cent disease incidence was calculated and presented in the Table 1.

Table 1. Survey for anthracnose disease in chilli

District	Mandal	Village	Latitude And Longitude	PDI (Range)
Guntur	1.Chebrolu	Narakodur	LAT 16°13’22.8”N LONG 80°30'54.4"E	26.28 - 28.55
	2. Amaravati	Mandepudi	LAT 16°28'01.1"N LONG 80°19'60.0"E	36.35 - 39.86
	3.Pedakurapadu	Lagadapadu	LAT 16°28'59.6"N LONG 80°13'26.0"E	27.35 - 35.41
	4. Tadikonda	Jonnalagadda	LAT 16°22'30.1"N LONG 80°27'06.3"E	29.34 - 30.23
	5. Sattenapalli	Sattenapalli	LAT 16°24'12.2"N LONG 80°08'32.5"E	36.73 - 42.39
Prakasham	1.Tripuranthakam	Mittapalem	LAT 16°33'52.8"N LONG 80°05'21.4"E	29.02 - 32.12
	2. Pullalacheruvu	Pullalacheruvu	LAT 16°09'33.8"N LONG 79°25'37.0"E	34.46 - 35.16
	3.Yerragondapalem	Yerragondapalem	LAT 16°01'58.0"N LONG 79°18'40.5"E	30.75 - 32.62
	4. Parchuru	Parchuru	LAT 15°57'56.5"N LONG 80°16'37.4"E	20.86 - 25.83
Krishna	1.Chandarlapadu	Konayapalem	LAT 16°44'32.6"N LONG 80°13'30.6"E	22.57 - 30.15
	2.Kanchikacherla	Keesara	LAT 16°42'22.4"N LONG 80°21'51.8"E	25.35 - 25.66
	3.Nandigama	Nandigama	LAT 16°46'53.3"N LONG 80°17'23.1"E	24.99 - 25.81
Kurnool	1.Nandyala	Nandyala	LAT 15°27'20.7"N LONG 78°28'51.3"E	30.04 - 35.44
	2.Kurnool	Kurnool	LAT 15°48'26.1"N LONG 78°00'30.4"E	23.60 - 25.35
	3.Nandikotkur	Nandikotkur	LAT 15°51'48.6"N LONG 78°16'01.0"E	33.70 - 40.91

In Guntur district, the incidence ranged from 42.39 to 39.86%. In Prakasam, it ranged from 20.86 to 35.16%. In Krishna district incidence ranged from 22.57 to 30.15% and 23.60 to 40.91% was recorded in Kurnool. Among all the surveyed villages, the highest incidence (42.39%) was recorded in Sattenapalli village of Sattenapalli mandal in Guntur district followed by 40.91% in Nandikotkur village of Kurnool district. The lowest incidence (20.86%) was recorded in Parchuru village of Parchuru mandal in Prakasam district. Similar results of disease incidence are recorded with Ekbote (2002) who surveyed the prevalent diseases of chilli (*Capsicum annuum*) in 6 taluks in the Haveri district of Karnataka. Fruit rot caused by *C. capsici* was found to vary around 42.00% and similar results were reported by Chigoziri and Ekefan (2013) from Gboko and Ohimini, South Nigeria. Anamika *et al*. (2012) surveyed to assess the incidence of anthracnose of chilli in five locations in Rewa Province. The percentage incidence of anthracnose was observed to be in the range of 55.53 and 71.10%. It was revealed that the predominance presence of the anthracnose disease is varied by environmental factors

and inoculum presence in a particular place. The disease incidence varied from field to field may be due to the variety of the soil type and other favourable environmental characters like relative humidity, temperature, and rainfall.

Isolation, morphological identification, and variability among *Colletotrichum* spp.,

Isolation and purification

The fruits showing typical anthracnose symptoms were collected from 15 different chilli growing areas of Andhra Pradesh. The pathogen was successfully isolated from the infected fruits using the tissue segment method and standard procedures were followed in the isolation of the pathogen. Isolates were named as isolate-1 (CC-1), isolate-2 (CC-2), isolate-3 (CC-3), isolate-4 (CC-4), isolate-5 (CC-5), isolate-6 (CC-6), isolate-7 (CC-7), isolate-8 (CC-8), isolate-9 (CC-9), isolate-10 (CC-10), isolate-11 (CC-11), isolate-12 (CC-12), isolate-13 (CC-13), isolate-14 (CC-14), isolate-15 (CC-15).

Single spore isolation technique is used for obtaining pure culture on PDA. Pure cultures obtained by this method are further used for the identification and characterization of the pathogen. All the 15 isolates were maintained by this technique. The isolated pathogen was further maintained by subculturing by hyphal tip method. Pathogen cultures are maintained in both slants and Petri plates for further studies. Continuous subculturing of the pathogen is avoided as it decreases the virulence of the pathogen.

Pathogenicity test

All 15 isolates cultured from collected diseased fruits are subjected to pathogenicity test to prove Koch's postulates. Chilli variety Gemini 116 is used for the test. A set of 5 fruits are used for the pathogenicity. Proper standard procedure is followed for the pathogenicity test. Most of the isolates showed symptoms starting from 3rd day. After 10th day the pathogen is reisolated and cultured. All the isolates produced the same cultures and hence proved the Koch postulates. Various workers have reported the pathogenic nature of *C. capsici* in chilli fruit rot disease (Parey *et al.*, 2013, Sangdee *et al.*, 2011, Ratanacherdchai *et al.*, 2010 and Than *et al.*, 2008).

Identification of the pathogen causing anthracnose in chilli

Macroscopic and microscopic studies using many different characters are taken into consideration for the identification of the pathogen. The pathogen involved in anthracnose disease of chilli was identified as Colletotrichum spp., based on reports of Kulshrestha *et al*. (1976), Ahmed (1986) and Desai and Prasad (1955). Typical colony characters like fairly white to light mouse grey coloured colony, circular, fluffy mycelium with black coloured acervuli which were scattered all over the colony which can be seen with the naked eyes and later the microscopic observations *viz.* presence of acervuli and conidial shape of *Colletotrichum* spp.,

Identification of virulent isolate

All the isolates were inoculated on chilli fruits separately and observed for disease development. Observations on the development of the lesion were recorded as per 0-5 scale on three, seven and 10 days after inoculation and the per cent fruit area was calculated and presented in the Table.2

The results showed that per cent fruit of infected ranged from 4.50 to 36.50 at three DAI, 16 to 52 at seven DAI and 16 to 56 at 10 DAI. There were significant differences were observed. Among the isolates tested, isolate 14 is significantly more virulent than other isolates and less virulence was recorded by isolate 2. These results confirm the findings of Sydow (1913) who reported infection of

Table 2. Per cent fruit area infected by the isolates of *Colletotrichum* spp.,

Isolate No.	Name of the isolate	Per cent area infected on the fruit		
		3 DAI	7 DAI	10 DAI
Isolate-1	CC-1	9.50 (17.95)	27.00 (31.29)	31.50 (34.13)
Isolate-2	CC-2	4.50 (12.24)	15.50 (23.18)	16.50 (23.96)
Isolate-3	CC-3	21.00 27.26)	37.00 (37.45)	43.00 (40.96)
Isolate-4	CC-4	12.50 (20.70)	20.00 (26.55)	23.00 (28.65)
Isolate-5	CC-5	13.00 (21.13)	33.50 (35.35)	35.00 (36.26)
Isolate-6	CC-6	17.00 (24.34)	38.50 (38.34)	41.00 (39.80)
Isolate-7	CC-7	11.00 (19.36)	21.00 (27.26)	27.50 (31.62)
Isolate-8	CC-8	8.50 (16.94)	17.50 (24.72)	20.00 (26.55)
Isolate-9	CC-9	10.50 (18.90)	30.50 (33.51)	33.50 (35.35)
Isolate-10	CC-10	19.00 (25.83)	39.00 (38.63)	38.50 (38.34)
Isolate-11	CC-11	16.50 (23.96)	36.50 (37.15)	39.00 (38.63)
Isolate-12	CC-12	14.00 (21.96)	36.00 (36.86)	36.00 (36.86)
Isolate-13	CC-13	14.00 (21.96)	31.50 (34.13)	39.50 (38.92)
Isolate-14	CC-14	36.50 (37.15)	52.00 (46.13)	53.00 (46.70)
Isolate-15	CC-15	14.50 (22.37)	32.50 (34.74)	36.00 (36.86)
C.D (P=0.05)		1.975	1.95	2.607
SE(m)		0.65	0.64	0.86
CV (%)		4.15	2.68	3.417

C. capsici for the first time in chilli. Differences in the aggressiveness of *C. capsici* isolates have been reported previously by Taylor *et al.* (2007). These results corraborate with the findings of Christopher *et al.* (2013) and Sharma *et al.* (2005).

Morphological variability of the *Colletotrichum* spp.

Morphological variability of the isolates of *Colletotrichum* spp. was assessed by comparing the characters *viz.* colony growth rate (mm day^{-1}), shape conidia, length, width of conidia, (µm) (using fluorescent microscope, presence, or absence of setae), margins (smooth or wavy) airpresented in the Table 3.

m The results revealed that the growth rate ranged from 5.75 to 8.88 mm/day. Among the isolates, significant differences were observed and the highest growth rate was observed in isolate CC-14 (8.88 mm/day) and followed by CC-3 (8 mm/day). The shape of the conidia is falcate for all the isolate except for isolates CC-3 and CC-15 which are cylindrical. Conidial length ranged from 23.20 to 18.59 µm of falcate conidia and 12.01 to 10.72 µm of cylindrical conidia. The conidial width ranged from 3.13 to 3.67 µm for falcate conidia whereas the width ranged from 4.02 to 4.26 µm for cylindrical conidia. Setae are present in all the isolate except in CC-3. Margins of isolates CC-1, CC-2, CC-4, CC-6 and CC-9 showed regular margins and remaining isolates CC-3, CC-5, CC-7, CC-8, CC-10, CC-11, CC-12, CC-13, CC-14, CC-15 showed irregular margins. The conidial size and shape of a pathogen were prominently used to determine the variants within a given population (Shenoy *et al.* 2007). In *C. gloeosporioides* conidia are hyaline, one celled and

Table 4. Cultural characters of the isolates of *Colletotrichum* spp.

Isolate Name	Colony Colour		Texture	Mycelial growth pattern
	Upper surface	Bottom side		
CC-1	Blackish Gray	Dark Brown	Not cottony	Concentric
CC-2	Blackish Gray	Black	Cottony	Concentric
CC-3	Olive Gray	Brown	Wooly	Concentric
CC-4	Black	Brown	Not cottony	Concentric
CC-5	Blackish Gray	Black	Not cottony	Concentric
CC-6	Blackish Gray	Light brown	Not cottony	Concentric
CC-7	Blackish Gray	Black	Not cottony	Not Concentric
CC-8	Light Gray	Black	Cottony	Concentric
CC-9	Light Gray	Black	Cottony	Concentric
CC-10	Light Gray	Light Gray	Cottony	Concentric
CC-11	Black	Light Brown	Cottony	Concentric
CC-12	Light Gray	Light Brown	Cottony	Concentric
CC-13	Black	Light Brown	Not Cottony	Not Concentric
CC-14	Light Gray	Black	Cottony	Concentric
CC-15	Light orange	Light orange	Cottony	Concentric

cylindrical. The size ranges from 8–12 µm in length and 4–6 µm in width. Similar reports were made by Lubna *et al*. (2013) and Gupta *et al*. (2017) and thus the present results are on the similar line of published literature. Results were in congruence with those of Shenoy *et al*. (2007) who reported that the average conidial size of *C. capsici* isolated from Maskalipalayam, Coimbatore in India, varied from 16- 25 µm in length and 3.0-4.0 µm in width. Butler (1973) reported *C. capsici* conidium size of 17-28 × 3-4 mm. These observations confirmed the measurement of *C. capsici.*

Cultural variability of the *Colletotrichum* spp.

The cultural variability is assessed by observing colony colour, colony texture and mycelial growth following standard procedures and presented in Table 4.

The upper surface of the isolates varied from blackish gray, light gray, black, olive gray and light orange. Among the isolates, CC-1, CC-2, CC-5, CC-6, CC-7 shown blackish gray, CC-8, CC-9, CC-10, CC-12, CC-14 shown light gray, CC-4, CC-11, CC-13 showed black, CC-3 shown olive gray and CC-15 shown light orange colour colony. The bottom side of the colony colour varied from black to dark brown, brown, light brown and light orange. These observations were like those obtained by Selvakumar (2007) and Srideepthi (2017) reported that the upper surface of each isolate varied from whitish to gray, brown, black or pale colors while the reverse surface on the PDA plate appeared black, dark gray or brown. The texture of isolate CC-3 was wooly, eight isolates showed cottony texture and the remaining six isolates showed non cottony textured mycelium. The mycelial growth pattern was concentric in all the isolates except in CC-7 and CC-13. These results observed in the present study confirm with the findings of several earlier workers. Akhtar and Singh (2007) reported the differences in radial mycelial growth on PDA of *C. capsici* and also variation in colony colour has been observed. Similar

observations were also mentioned by Lubna *et al.* (2013). Sangdee *et al.* (2011) evaluated ten isolates of *C. capsici* causing chilli anthracnose for their morphological, cultural, and pathogenic variability on chilli fruits. The cultural variability of *C. capsici* isolates on basal culture medium Potato Dextrose Agar was reported earlier by Hartman and Wang, 1990. Karthik pandi *et al.* (2018) reported variation in their cultural behaviour of twenty-five isolates varied from *Colletotrichum* species in culture colonies.

CONCLUSION

During the survey for recording chilli anthracnose in Andhra Pradesh, the high incidence (42.39%) was recorded in the Guntur district followed by 40.91% in the Kurnool district. Less incidence (20.86%) was recorded in the Prakasam district. The pathogen was isolated from the disease samples of 15 different locations in Andhra Pradesh and pathogenicity was established to prove Koch's postulates. Based on the morphological and cultural characters of the isolate the pathogen was identified as *C. truncatum* and *C. gloeosporioides*. The studies indicated that the isolates of *Colletotrichum* spp. collected from different locations of Andhra Pradesh varied among themselves on pathogenicity, morphological and cultural characters.

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