



Variability among the *Corynespora cassiicola* Isolates causing Target Leaf Spot in Cotton

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Abstract : Six isolates of *C. cassiicola* obtained from different areas of cotton cultivation in Andhra Pradesh were analysed for morphological variability including pigmentation, growth pattern, surface texture, margin, elevation, zonation, aerial hyphae, growth rate of the mycelium, length and width of the conidia and number of pseudosepta. The isolate from Dachepalli (C3) was statistically superior with 7.96 cm colony diameter after nine days of incubation at 28±1°C whereas C2 from Rajupalem (7.92 cm) was *on par* followed by 7.61 cm in C6 isolate of Nakkabokkalapadu. Average growth rate was found to be higher in C2 (0.040 cm/h) isolate followed by C1 (Reddigudem), C5 (Martur), C6 (0.037 cm/h) isolates. The highest mean conidial length of 77.3 µm was recorded in C3 isolate followed by 51.03µm (C4 of Jagarlamudi) and 46.64 µm (C2). The minimum width of conidia was observed in C4 (5.7µm) supervened by C6 (5.87µm) and C3 (6.9µm). Mean number of highest pseudosepta (4.12±2.04) was found in C4 followed by C3 (3.64±0.99). This cultural and morphological variability indicate its adaptability to wider host range.

Keywords: *Corynespora cassiicola*, cotton, cultural, morphological, variability

Cotton (*Gossypium hirsutum*), commonly referred to as "White Gold" is a major fibre crop across the world. Cotton fibre is used as a raw material in the textile, pulp, and paper industries, while cotton seed oil is utilised in food, cosmetics, chemicals, medicines and other sectors. Cotton seed cake is also fed to cattle (Proto *et al.*, 2000). Cotton is a fiber producing crop of global importance that is cultivated in tropical and subtropical regions of more than 80 nations worldwide. Cotton is farmed all over the world for its natural fibre and oil.

Corynespora cassiicola is becoming a major concern in the production of several high economic value crops. The pathogen causes target leaf spot disease of cotton and reported from central and south India (Salunkhe *et al.*, 2019; Siva Prasad *et al.*, 2022). Variation in length, width and number of pseudosepta in conidia of *Corynespora* was reported by different workers (Kwon *et al.*, 2005; Conner *et al.*, 2013;

Fulmer *et al.*, 2012). In view of the regular occurrence of *Corynespora* target spot of cotton in Andhra Pradesh since 2017, an investigation was carried out to understand the variability of the pathogen.

A roving survey was conducted during *kharif* 2022-2023 to assess the disease severity of *Corynespora* target spot in two major cotton growing districts of Andhra Pradesh *i.e.*, Guntur and Prakasam. In each district two mandals and in each mandal three villages were surveyed. Five plants each at five locations *i.e.*, at four corners of each field and one at the centre were selected to record the severity of *Corynespora* target spot.

The disease severity of cotton *Corynespora* target spot was assessed by using 0-4 scale and per cent disease index (PDI) was calculated.

$$\text{PDI} = \frac{\text{Sum of all the numerical ratings}}{\text{Total number of leaves scored} \times \text{Maximum disease grade}} \times 100$$

Pathogen isolation

Leaf bits of five mm² with healthy and infected leaf portions were cut, surface sterilized using 1 per cent sodium hypo chloride for a minute and rinsed with three changes of sterile distilled water to remove the disinfectant. Leaf bits were blot dried before transferring aseptically on to PDA plates and then incubated at 27±1 °C (Silva *et al.*, 2003) in incubator. Three-day old mycelial bits developed from diseased leaf bits were aseptically transferred to glass slide and observations were made to confirm their identity based on morphological characters (conidia). The obtained pathogen cultures were sub-cultured on PDA after confirmation. The fungus was identified using cultural features given by Conner *et al.*, (2013), Schlub *et al.*, (2007), Mmbaga *et al.*, (2015).

Purification

Different isolates obtained from infected tissue were purified by single spore isolation method. Three millilitre of sterile distilled water was added to 10day old culture tube to get spore suspension which was later diluted serially to get desired concentration and 100 µl was aseptically transferred for single spore isolation (Miyamoto *et al.*, 2009; Silva *et al.*, 2003) through spread plate method using 2 per cent solidified water agar. After incubating at 27±1°C for 4-5 h, a well isolated, germinated spore was located and marked using microscope. Circular disc of the medium corresponding to the marked single spore was picked up using a sterilized cork borer and was aseptically transferred to PDA medium. Pure culture obtained was subcultured on PDA slants and incubated at 27±1°C till the mycelium was fully grown over medium. The culture slants were then preserved in a refrigerator at 4°C using the method for studying variables including Pigmentation (Top and Bottom), Growth pattern, Surface texture, Margin, Elevation, Zonation, Aerial hyphae, Growth rate of the mycelium, length and width of the conidia and number of

pseudosepta.

Growth rates were evaluated by measuring the mycelial growth in petri plates in two equilateral directions at three, six and nine days after incubation. Conidial length and width were measured by using optical microscopy (Lanscope) and photographed.

Six isolates of *C. cassicola* were collected based on the symptoms, as pinhead sized reddish brown spots which turn brown with dark reddish brown margin from cotton crop in Guntur, and Prakasam districts and designated as C 1-6 after confirmation of pathogenicity (Table 1).

Cultural characteristics

Variability was observed to a great extent among the six isolates of *C. cassicola* from cotton in PDA culture media (Plate 1, Table 2). Growth pattern was irregular (C1), circular (C2 and C6), oval (C4) and filamentous (C3 and C5); colony texture was fibrous (C1, C4 and C6), floccose (C3 and C5) and fluffy (C2); margin was fili form (C1, C2 and C3), entire (C4 and C6) and fibrous (C5); elevation was flat (C1 and C3), umbonate (C2), crateriform (C5) and raised (C4 and C6). Pigmentation at the bottom of the plate in different isolates varied from black, light pink (C2) to dark pink (C6), pale black (C4) to black (C1), black with pale pink (C3) or pale yellow colour patches (C5). Zonation was absent (C1, C2, C3 and C6) or present (C4 and C5); aerial hyphae were present (C1, C4 and C6) or absent (C2, C3 and C5). Colour of the mycelium varied from white (C2, C3, C5 and C6) to light grey (C4) or dark brown (C1). Prosper *et al.*, (2018) observed green-grey, dark grey, grey and pink pigment among *Corynespora* isolates of rubber. Presence and absence of zonations was reported Udayasiri *et al.*, (2013) reported that the pigmentation varied among the isolates with brown, black and grey colours.

Colony growth

Colony diameter in the six isolates of *C. cassicola* from cotton was observed by inoculating seven days old culture of each isolate on PDA at 28 ± 1 °C. Three days after incubation, C1 isolate showed the highest colony diameter of 2.87 cm followed by C4 (2.86 cm). Other isolates, C3, C6 and C2 recorded 2.62, 2.36 and 2.35 cm respectively; C5 expressed the lowest growth of 1.88 cm.

Six days after incubation, the isolate C4 recorded the highest growth of 5.07 cm. The isolates C 2, C 3, C 5 and C1 expressed growth to the tune of 4.78, 4.71, 4.55 and 4.52 cm respectively; C6 registered the lowest growth of 3.46 cm.

The isolate C3 showed the highest growth of 7.96 cm followed by 7.92 cm in C 2, at nine days after incubation. Isolates C 6, C1, C 5 and C4 recorded 7.61 cm, 7.54, 7.41 and 7.11 cm respectively. Udayasiri *et al.*, (2013) observed maximum growth after 8 days of incubation.

Among six isolates of *C. cassicola* from cotton, the average growth rate was found to be highest in C4 isolate (0.036 cm/h) followed by 0.035 cm/h in C1 and C3 isolates, 0.034 cm/h in C2. The lowest growth rate (0.303 cm/h) was recorded in C5 and C6 isolates (Table 3).

Significant differences in the growth of different isolates of rubber (Akpaja *et al.*, 2015), soybean isolates (Kurre *et al.*, 2019) and cotton isolate of *C. cassicola* in different media (Siva Prasad *et al.*, 2021) were reported earlier.

Variability in conidia

The heterogeneity in conidial length was seen within isolates, and the ratios of these lengths changed between isolates. The conidial dimensions ranged from 20.2-78.05 x 5.33-8.96 μm which were in accordance with Salunkhe *et al.*, (2019). Similar observations were reported earlier in cotton, blackgram,

soybean etc. (Conner *et al.*, 2013, Peiris *et al.*, 2015, Soni *et al.*, 2016, Kurre *et al.*, 2019).

Conidial length

The maximum length was observed in C3 (64.40 \pm 17.60 μm) followed by C-5 (56.75 \pm 16.7 μm) and C6 (50.9 \pm 20.58 μm). The minimum length was observed in C2 (40.96 \pm 6.91 μm) followed by C4 (38.43 \pm 10.78 μm) (Table 3, 4 and Fig 1,2). Conidia in some of the isolates were in chains of 2-6 (Ahmed *et al.*, 2013).

Conner *et al.*, (2013) reported 50 to 209 μm long and 7 to 15 μm wide conidia in cotton. The maximum frequency of spore length in the range of 25 and 74.9 μm was reported in okra (Ahmed *et al.*, 2013). Variation in conidial length (15-275 μm), width (3.75-12.50 μm) and shape (straight, curved, cylindrical and obclavate) was observed by Peiris *et al.*, (2015) in *C. cassicola* isolates from different host plants.

Conidial width

The maximum width was observed in C5 (8.25 \pm 0.65 μm) followed by C2 (7.58 \pm 1.02 μm). The minimum width was observed in C6 (6.23 \pm 0.70 μm) supervised by C1 (6.33 \pm 1.09 μm) and C4 (6.93 \pm 0.96 μm).

Prosper *et al.*, (2018) reported that the shape of conidia is cylindrical, straight for some and slightly curved for others; with average size of 11.77 to 52.87 μm in length and 1.66 to 5.71 μm in width in *C. cassicola* causing leaf fall disease in rubber.

Number of pseudosepta

Cotton isolates of *C. cassicola* showed variation in the number of pseudosepta present in their conidia. The maximum number of pseudosepta was observed in C4 (4.12 \pm 2.04) succeeded by C3 (3.64 \pm 0.99) and minimum number of pseudosepta was observed in C2 (1.6 \pm 0.64) and C6 (2.36 \pm 1.11) (Table 3,4 and

Table 1. Cotton isolates of *Corynespora cassiicola* analysed for cultural and morphological characters

S. No.	Isolates	Village	Mandal	District
1.	C1	Reddigudem	Rajupalem	Guntur
2.	C2	Rajupalem	Rajupalem	Guntur
3.	C3	Dachepalle	Dachepalle	Guntur
4.	C4	Jagarlamudi	Yeddanapudi	Prakasam
5.	C5	Martur	Martur	Prakasam
6.	C6	Nakkabokkalapadu	Ballikurava	Prakasam

Table 2. Variation in cultural characters among *Corynespora cassiicola* isolates of cotton on PDA

S. No.	Isolates	Growth pattern	Surface texture	Margin	Elevation	Pigmentation bottom of the plate	Zonation hyphae	Aerial	Colour of the mycelium
1	C1	Irregular	Fibrous	Filiform	Flat	Black	0	Yes	Dark brown
2	C 2	Circular	Fluffy	Filiform	Umbonate	Light pink	0	No	white
3	C 3	Filamentous	Floccose	Filiform	Flat	Black with pale pink	0	No	white
4	C 4	Oval	Fibrous	Entire	Raised	Pale black	2	Yes	light grey
5	C 5	Filamentous	Floccose	Fibrous	Crateriform	Black with pale yellow	1	No	white
6	C 6	Circular	Fibrous	Entire	Raised	Pink	0	No	White

Table 3. Variability in conidial dimensions of *Corynespora cassiicola* isolates from cotton

S. No.	Isolates	Conidial Dimensions (μm)				Pseudosepta	l:b ratio
		Length	Population mean	Width	Population Mean		
1.	C 1	33.81e	43.28±11.64	7.16c	6.33±1.09	3.44±1.55	4.72
2.	C 2	46.64c	40.96±6.91	8.63a	7.58±1.02	1.60±0.64	5.40
3.	C 3	77.30a	64.40±17.60	6.90d	7.54±0.78	3.64±0.99	11.20
4.	C 4	51.03b	38.43±10.78	5.71e	6.93±0.96	4.12±2.04	8.98
5.	C 5	35.11d	56.75±16.7	7.82b	8.25±0.65	3.04±0.84	4.48
6.	C 6	21.74f	50.9±20.58	5.87f	6.23±0.70	2.36±1.11	3.70
SEm±	0.23		0.08				
CV(%)	7.00		5.70				
C.D. (p=0.05)	0.81		0.26				

Table 4. Population means of conidial dimensions of *Corynespora cassiicola* infecting cotton

Characters	Population mean (μm)	Upper limit (μm)	Lower limit (μm)	Range (μm)
Conidial length	49.13±2.06(14.04)	78.05	20.2	20.2-78.05
Conidial width	7.15±2.06(0.88)	8.96	5.33	5.33-8.96

Table 5. Variability in the colony diameter of *Corynespora cassiicola* isolates of cotton

S. No.	Isolates	Colony diameter (cm)			Growth rate (cm/h)			Average growth rate (cm/h)
		3 DAI	6 DAI	9 DAI	3 DAI (cm/h)	6 DAI (cm/h)	9 DAI (cm/h)	
1	C 1	2.87 (1.69 ^a)	4.52 (2.21 ^{cd})	7.54 (2.74 ^b)	0.040	0.031	0.035	0.035
2	C 2	2.35 (1.53 ^c)	4.78 (2.18 ^b)	7.92 (2.81 ^a)	0.033	0.033	0.037	0.034
3	C 3	2.62 (1.61 ^b)	4.71 (2.17 ^{bc})	7.96 (2.82 ^a)	0.036	0.033	0.037	0.035
4	C 4	2.85 (1.69 ^a)	5.07 (2.25 ^a)	7.11 (2.67 ^c)	0.040	0.035	0.033	0.036
5	C 5	1.88 (1.37 ^d)	4.55 (2.13 ^{bcd})	7.41 (2.72 ^b)	0.026	0.032	0.034	0.031
6	C 6	2.36 (1.53 ^c)	3.46 (1.86 ^e)	7.61 (2.75 ^b)	0.033	0.024	0.035	0.031
	CD (p=0.05)	0.19	0.24	0.20				
	SEm±	0.064	0.08	0.068				
	CV (%)	2.17	1.53	0.78				

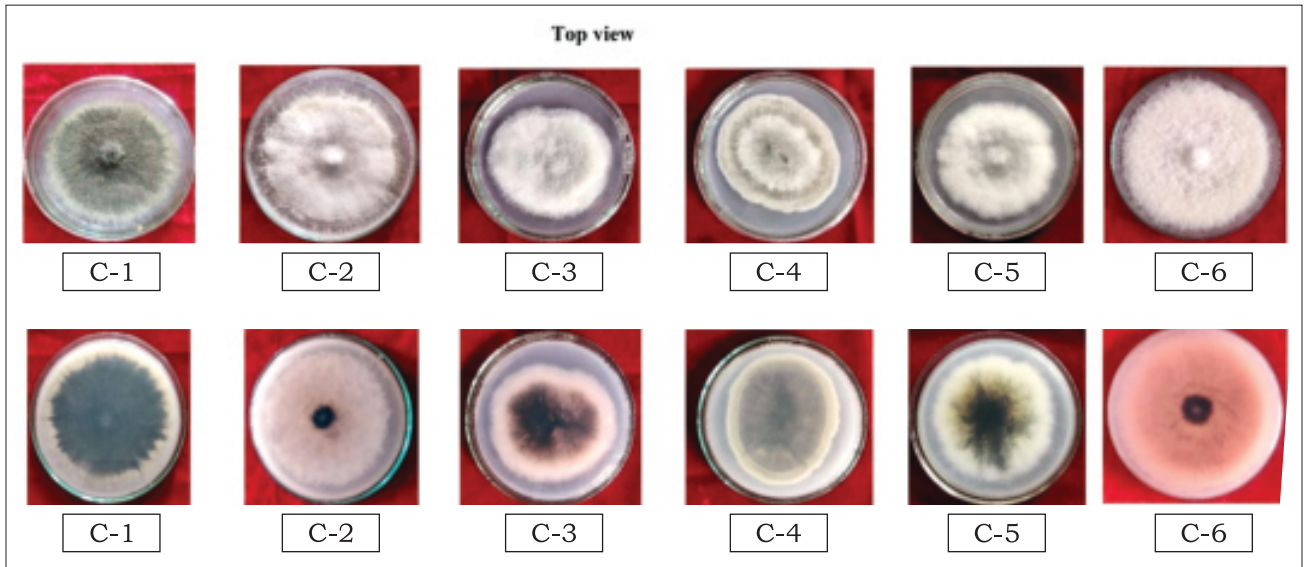


Plate1. Variability in pigmentation of *Corynespora cassiicola* isolates of cotton

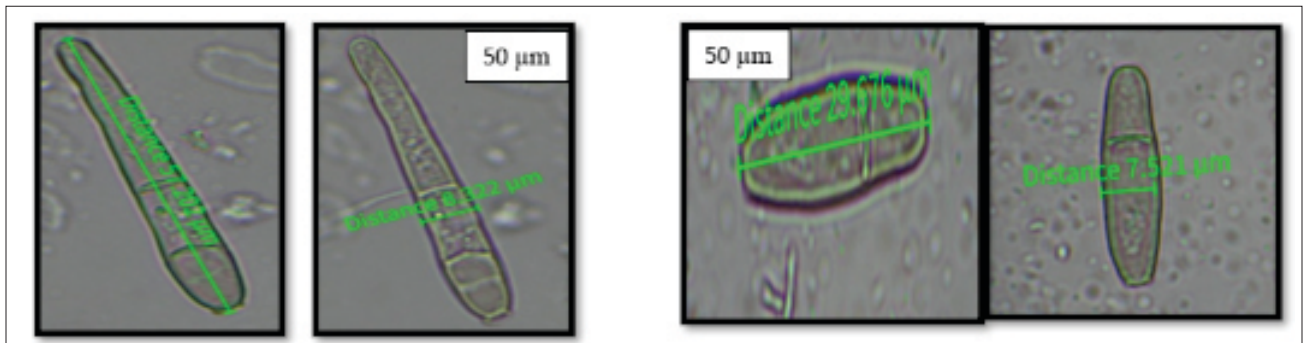


Fig. 1. Conidial measurements of *Corynespora cassiicola* infecting cotton

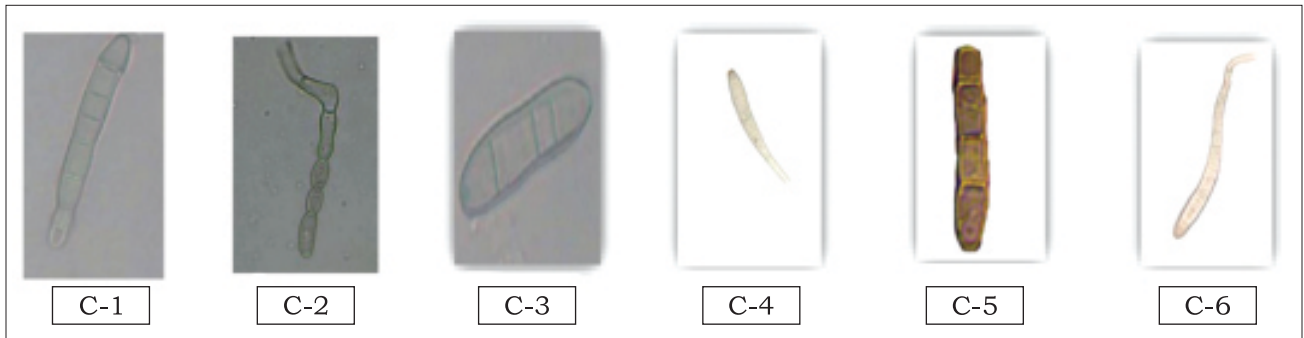


Fig. 2. Variability in conidial morphology of *Corynespora cassiicola* infecting cotton

Fig.1,2). The conidia produced singly or in acropetal chains, were obclavate, cylindrical, straight or curved, 62–127 × 5–8 μm (mean 100 × 6.8 μm) with 5–20 cells separated by hyaline pseudoseptate, and dark brown to pale brown in lettuce (Chairin *et al.*, 2017). Reported that conidia of *C. cassiicola* had 4-20 pseudosepta

and isthmus measured 42.7-197.6 μm length and 9.3-21.5 μm width which were correlated with the above results. Onesirosan *et al.*, (1974) reported that the conidia were extremely variable in size, ranging from 60 to 250 x 5 to 13 μm, with an average of 167 x 8.3 μm.

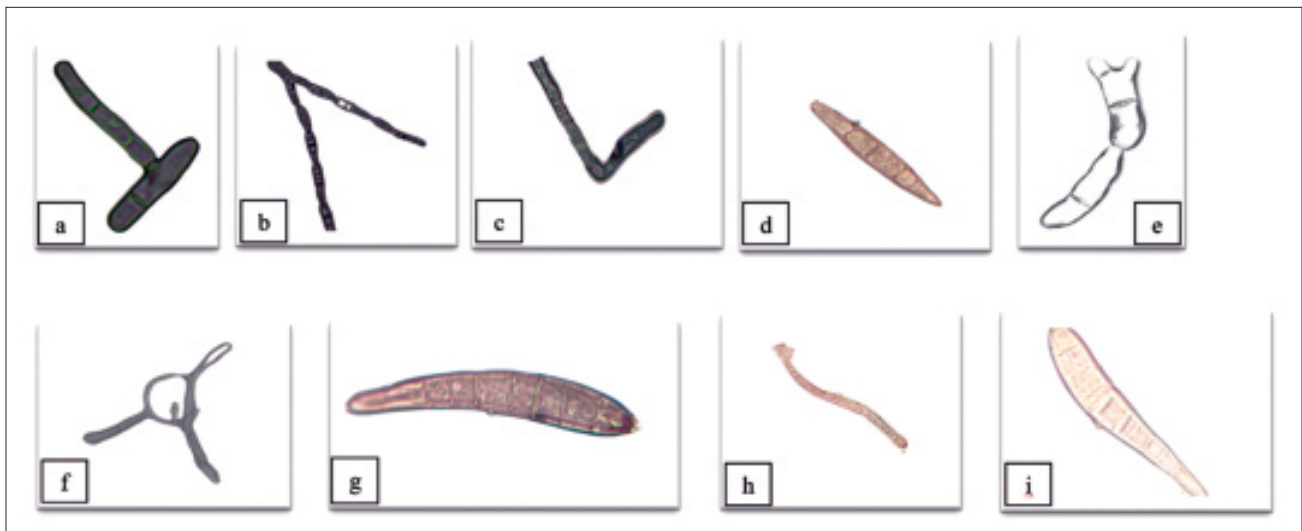


Fig. 3. Variability in shapes of conidia of *Corynespora cassiicola* in cotton
(a) T-Shape (b) V-Shape (c) L-Shape (d) fusiform (e) bifurcate (f) irregular (g) clavate (h) filiform (i) pyriform

Cotton isolate of *C. cassiicola* contained 4 to 15 pseudosepta (Conner *et al.*, 2013). Toulet *et al.*, (2022) reported that the size of the conidia ranged from 6 to 120 μm in length and 2.4 to 12 μm in width, with an average of 19.90–46.80 μm long and 4.09–5.30 μm wide in leguminous crop soybean.

Shapes of conidia

A great variability was observed among the shapes of conidia. The observed shapes are cylindrical, obclavate, oval, curved or straight and some are 'L' and 'I' shape (Fig. 3). *Corynespora* conidia formed as either solitary or catenary, were obclavate to cylindrical in shape. Yan -Xian QI *et al.*, (2011) reported different shapes of conidia (oval, obclavate, cylindrical or Yshape; curved or straight). Kurre *et al.*, (2019) observed a high degree of variability in conidial morphology among the isolates of soybean such as shape (oval, obclavate, cylindrical or Y; curved or straight), size (10.3–168.8 μm long; and 1.3–12.4 μm wide) and the number of pseudosepta (0–16).

CONCLUSION

C. cassiicola isolates infecting cotton expressed variability in pigmentation, growth pattern, surface texture, margin, elevation, zonation, aerial hyphae, growth rate of the mycelium, length and width of the conidia and number of pseudosepta indicating cultural and morphological diversity to adapt to its wider host range.

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