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Arvind Kumar Kurre
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Meghchand Dewangan
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Devendra Nishad
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Shweta mishra
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Arvind Kumar Kurre
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Variability among *Corynespora cassiicola* isolates causing target leaf spot disease collected from soybean growing area of Chhattisgarh

Arvind Kumar Kurre, Meghchand Dewangan, Devendra Nishad and Shweta mishra

Abstract
Target leaf spot disease of soybean caused by *Corynespora cassiicola.* The disease affects leaves, stems, pods and seeds. Leaf lesions are rounded to irregular and reddish brown they vary from speck to big mature spot. Lesions are frequently surrounded by a dull green or yellowish green halo. Eleven isolates were isolated from different soybean growing areas and used for variability studies. Maximum radial growth was observed in two isolates CC-8 and CC-1 (90 mm) and minimum radial growth was observed in two isolate CC-2 and CC-9 (82 mm). There were great variability was observed in colony pigmentation especially on top of the culture *viz.* black, pale brown, brown, dark brown, pale green and gray. There were two patterns were observed on colour of bottom of the colony (dark brown and black), texture (thin and thick) and shape of the colony *viz.* round and polygon.There were enormous variations were observed in conidial morphology of all the isolates. Variations were observed in shape (oval, obclavate, cylindrical or Y, curved or straight), size (10.3–168.8µm long; and 1.3–12.4µm wide) and the number of pseudosepta (0–16).

Keywords: *Corynespora cassiicola*, target leaf, soybean growing

Introduction
Soybean (*Glycine max.* L. Merril) belonging to family Leguminaceae is designated as miracle bean established its potential as an industrially vital and viable oilseed crop in many areas of India. The target leaf spot disease of soybean causes by (*Corynespora cassiicola*) was first reported in 1945 (Olive *et al.*, 1945) [3]. Now it has been found in most of soybean growing states. In Chhattisgarh it has been reported during 2002 from Raipur (Patel, 2005) [4]. The growth of *C. cassiicola* in Potato dextrose agar medium was slender, sub-hyaline to pale brown, hyphae mostly submerged in the sub stratum. Conidiophores arising singly from the mycelium, 3-7 septate, unbranched, erect, straight to slightly curved, pale-brown in colour. Conidia formed singly or in chains, cylindrical to obclavate, straight to slightly curved, sub-hyaline to pale olivaceous brown, smooth walled, 0 - 16 pseudosepta, hilum at the base. Chlamydospores formed in older cultures, which were hyaline, terminal or intercalary and oval in shape. The disease affects leaves, stems, pods and seeds. Leaf lesions are rounded to irregular and reddish brown; they vary from specks to big mature spots. Lesions are frequently surrounded by a dull green or yellowish green halo. Severely affected leaves drop prematurely (Sinclair, 1982) [7]. The fungus over winters on soybean debris and seed. It can survive in a fallow field for two years. The yield losses to an extent of 18-32 percent have been recorded in susceptible soybean lines grown in Mississippi during years when rainfall was above normal in August and September. Qive *et al.* (2011) observed that colony morphology of the fungus showed variation among the isolates. The differences were found either in mycelium colour (white to grey or green), texture (thin to thick, observed from the top), or in colony colour (white to pale brown, red brown, dark brown, dark grey or black, observed from the bottom of the Petri dishes) as well as in the shape of the cultures (round to slightly polygonal). The mean colony diameter was ranged from 36.7 mm to 62.4 mm. A high degree of variability in conidial morphology was observed among isolates. Fernando *et al.* (2009) [1] studied on diversity of *C. cassiicola* cause a devastating disease on *Hevea brasiliensis* and reported thae variation in colony and reproductive morphology, conidia and toxin production, pathogenicity and sensitivity to fungicides among the isolates. Shimomoto *et al.* (2010) [5] analyzed pathogenic variations of 64 Japanese isolates of *C. cassiicola* on perilla, cucumber, tomato, aubergine and sweet pepper, and their multigene phylogeny.

Silva *et al*. (2003) [6] analyzed genetic variation of 42 isolates of *C. cassiicola*, a destructive fungal pathogen of many economically important crop plants including rubber, using RAPD–PCR analysis. Nghia *et al*. (2008) [2] the morphology of the isolates was characteristic of that described for *C. cassiicola*. Variations in colony and conidial morphology.

Material and Method

Experimental site

All the laboratory experiment were carried out at the Department of Plant Pathology, IGAU, Raipur.

General procedure followed

In general, in each Petri dish about 15-20 ml of potato dextrose agar medium was poured, supplemented with streptomycin in order to check the unwanted bacterial contamination. Wherever growth studies were conducted five mm disc of pure culture of *Myrothecium roridum* Tode ex. Fries. by the help of cork borer, was used for inoculation of medium in Petri dish. The inoculated plates were incubated in the 25 °C for three days. Observation for the growth and sporulation were recorded at 10 to 15 days after inoculation.

Media used

Following medium was used during laboratory studies on *Myrothecium roridum* Tode ex Fries.

Media	Composition	Quantities
Potato Dextrose Agar	Potato (peeled and sliced)	200 g
	Dextrose	20 g
	Agar-Agar	20 g
	Distilled water	1000 ml

Collection of diseased sample

The naturally infected leaf of soybean crop with the target leaf spot symptoms were collected from different soybean growing area in Chhattisgarh. Collected samples were brought to the laboratory for critical examination of the symptoms for the identification studied under compound microscope & isolation of the pathogen.

Isolation, Purification & Identification of test fungus

The infected leaves of soybean were cut into small pieces, surface sterilized with 0.1% mercuric chloride (HgCl2) solution followed by three washing with sterile distilled water and placing in moist chamber than after 1 to 2 days fungal mycelium growth were seen than finally small bits of fungus kept on the previously poured and solidified potato dextrose agar medium in Petri plates for isolation of the pathogen. The plates were incubated at 25 °C in an incubator. The plates were observed after mycelial growth from the inoculated mycelium bits. Mycelial were then sub-cultured, purified by hyphal tip method and maintained culture on PDA slant & Petri plate kept on incubator at 25 °C. All the growth characters were recorded and compared with the standard reports publish for confirmation.

Cultural and morphological study of *Corynespora cassiicola* isolates

The eleven isolates of *C. cassiicola* were grown in PDA medium for the cultural and morphological study. The cultural and morphological characters (colony radial growth, mean colony diameter, colony colour and mycelium growth pattern) were recorded after 10 days of inoculation. Slides were prepared from 15 days old culture and the shape and size of conidia and numbers of pseudosepta were recorded.

Table 1: Designation of *Corynespora cassiicola* Isolate and their collection place

S.N.	Isolate	collection place	Block
1	CC-01	Raipur	Raipur
2	CC-02	Salhebharri	Khairagarh
3	CC-03	Bhorampur	Khairagarh
4	CC-04	Chhuhikhadan	Chhuhikhadan
5	CC-05	Lohara	Lohara
6	CC-06	Saja	Saja
7	CC-07	Gandai	Gandai
8	CC-08	Uriyakala	Gandai
9	CC-09	Dharampura	Kawardha
10	CC-10	Bemetra	Bemetra
11	CC-11	Mungeli	Mungeli

Result and Discussion

Variability among *Corynespora cassiicola* isolates collected from soybean growing area of Chhattisgarh Radial growth

The radial growth observed for 11 isolates presented in Table 2 and Figure 1 were different in most of the isolates with highest radial growth of CC -8 (50 mm) and lowest radial growth was observed in two isolates CC-2 and CC-9 (30mm) at 5DAI. Maximum radial growth was observed at 10 DAI in two isolate CC - 8 and CC-1 (72 mm) and minimum radial growth was observed in isolate CC-9 (58mm).

Maximum radial growth was observed in two isolate CC - 8 and CC-1 (90 mm) and minimum radial growth was observed in two isolate CC - 2 and CC-9 (82 mm). But the maximum mean mycelia growth was observed in isolate CC-8 (70.66 mm) followed by CC-1 (70.00 mm) and CC-7 (67.66 mm) and minimum mean growth was observed in isolate CC-9 (56.66 mm) followed by CC-2 (57.00 mm) and CC-11 (60.33 mm). It was also revealed from the data that the growth of pathogen was rapid upto 5DAI after that it was gradually become slow from 5DAI to 15DAI.

Table 2: Variability on mycelial growth of *C. cassiicola* Isolates in *in vitro* condition.

S.N.	Isolate No.	Location	Radial growth of mycelium (mm)*		
			5DAI	10DAI	15DAI
1	CC-1	Raipur	48	72	90
2	CC-2	Salhebharri	30	59	82
3	CC-3	Bhorampur	40	67	86
4	CC-4	Chhuhikhadan	41	64	86
5	CC-5	Lohara	34	61	88
6	CC-6	Saja	40	66	87
7	CC-7	Gandai	46	68	89
8	CC-8	Uriyakala	50	72	90
9	CC-9	Dharampura	30	58	82
10	CC-10	Bemetra	36	63	88
11	CC-11	Mungeli	33	61	87

Colony morphology

Pigmentation: Isolates of *Corynespora cassiicola* depicted great variability in pigment production on PDA medium (Table 3 and Plate 1). Isolate CC-01 produced brown, isolate CC-02 produced pale green, two isolates CC-03 and CC-11 produced pale brown, isolate CC-07 produced grey, isolate CC-09 produced dark brown and five isolates CC-04, CC-5, CC-06, CC-08 and CC-10 are produced black pigment at the top portion of Petri plate and three isolates (CC-01, CC-07, and CC-11) produced dark brown pigment and eight isolates

(CC-02, CC-03, CC-04, CC-5, CC-06, CC-08, CC-09 and CC-10) produced black pigment at the bottom portion of Petri plate on PDA after 15 days of inoculation at 25 ± 2ºC.

Mycelial growth patterns: Mycelial growth patterns were observed on PDA, where CC-01, CC-04, CC-05, CC-07 and CC-08 grew with circular margin or round colony growth pattern and CC-02, CC-03, CC-06, CC-09, CC-10 and CC-11 isolates were growing with irregular margin or polygon colony growth patterns.

Colony texture: Colony textures were observed on PDA medium at 15 DAI, where CC-01, CC-02, CC-04, CC-05, CC-06, CC-07, CC-08, CC-10 and CC-11 isolates produced thick texture while two isolates (CC-03 and CC-09) showed thin colony texture.

Conidial morphology

A high degree of variability in conidial morphology was observed among the isolates of pathogen (Plate 4.5). Differences were observed in shape (oval, obclavate, cylindrical or Y; curved or straight; Fig.3), size (10.3–168.8µm long; and 1.3–12.4µm wide) and the number of pseudosepta (0–16) (Table 4).

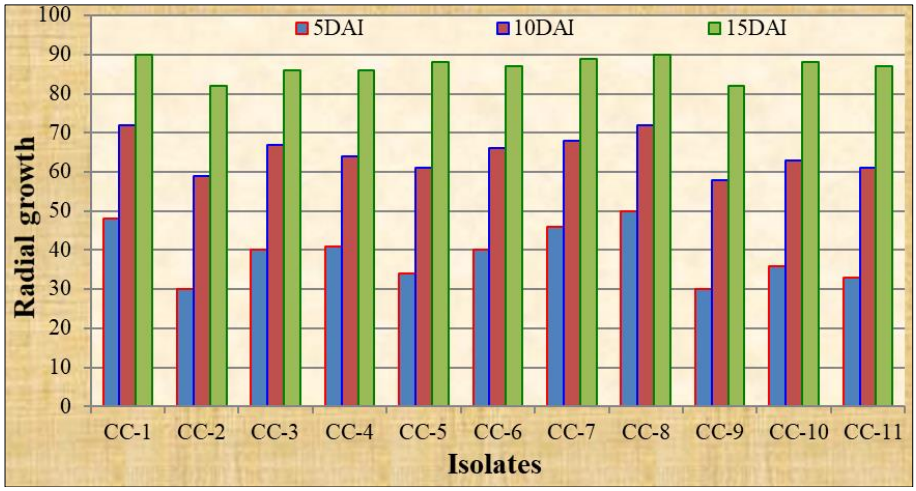

Fig 1: Variability on mycelial growth of *C. cassiicola* Isolates

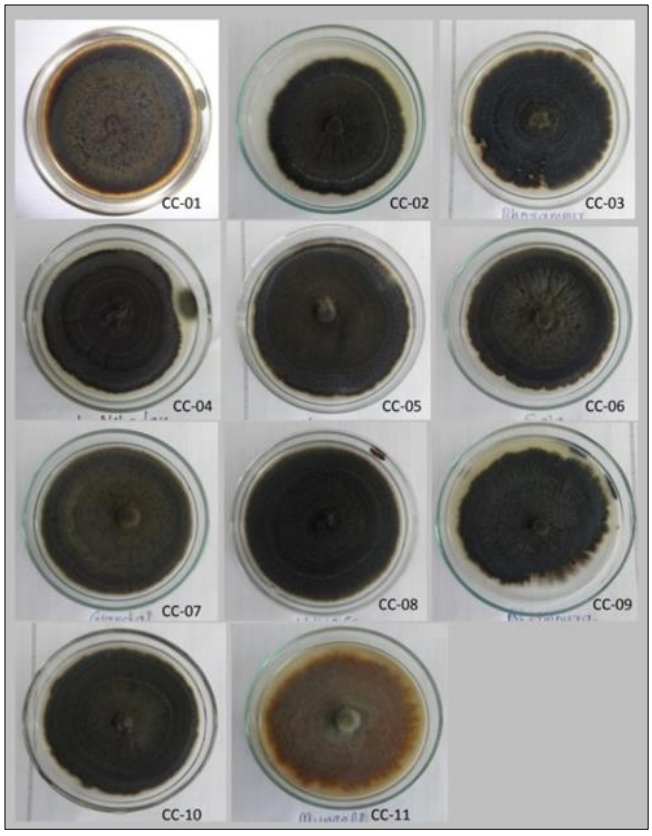

Plate 1: Variability on mycelial growth of *C. cassiicola* isolates

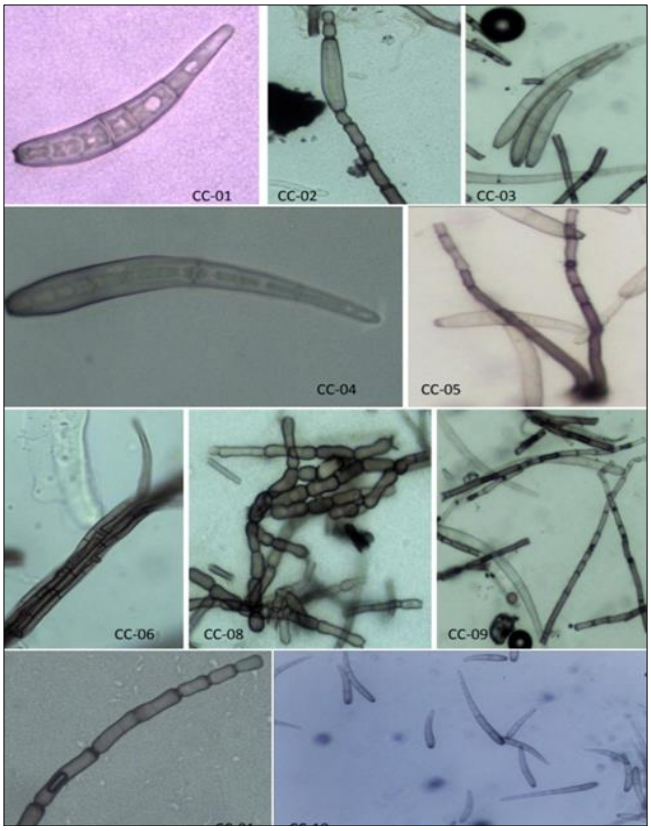

Plate 2: Variability in conidium size and number of pseudosepta of 11 isolates of *C. cassiicola*.

Table 3: Visual characteristics of 11 isolates of *C. cassiicola* cultures after 15 days of incubation

S. N.	Isolate No.	Color		Texture	Shape	Size (mm)
		Top	Bottom			
1	CC-01	Brown	Dark brown	Thick	Round	90
2	CC-02	Pale green	Black	Thick	Polygon	82
3	CC-03	Pale brown	Black	Thin	Polygon	86
4	CC-04	Black	Black	Thick	Round	86
5	CC-05	Black	Black	Thick	Round	88
6	CC-06	Black	Black	Thick	Polygon	87
7	CC-07	Grey	Dark brown	Thick	Round	89
8	CC-08	Black	Black	Thick	Round	90
9	CC-09	Dark brown	Black	Thin	Polygon	82
10	CC-10	Black	Black	Thick	Polygon	88
11	CC-11	Pale brown	Dark brown	Thick	Polygon	87

Table 4: Conidium size and number of pseudosepta of 11 isolates of *C. cassiicola*

S. N.	Isolate No.	Length (µm)			Width (µm)			No. of pseudosepta		
		Min.	Max	Mean	Min.	Max	Mean	Min.	Max	Mean
1	CC-01	16.2	128.0	68.5	3.8	12.4	5.2	1	14	5.1
2	CC-02	13.2	133.4	67.5	2.2	8.9	4.6	0	6	2.4
3	CC-03	11.2	106.6	64.4	6.3	8.8	7.1	0	9	3.8
4	CC-04	13.0	149.5	57.8	2.7	8.3	3.8	0	4	2.8
5	CC-05	22.4	128.7	78.4	5.4	10.1	7.8	0	11	3.2
6	CC-06	11.6	120.4	42.8	2.5	8.6	5.1	1	3	1.6
7	CC-07	15.1	118.4	82.2	4.0	7.0	5.7	1	4	3.3
8	CC-08	14.6	142.5	96.5	2.7	8.5	5.5	0	16	6.1
9	CC-09	10.3	85.2	44.5	1.3	9.3	7.3	0	4	1.1
10	CC-10	13.9	168.8	72.8	3.8	9.2	7.0	1	4	1.9
11	CC-11	16.7	58.4	58.8	4.7	6.5	5.1	0	5	2.3

Conidial length

The variability in conidial length within the isolates was observed and the ratios of these lengths differed among isolates. The maximum length of conidia was observed in isolate CC-08 (96.5 µm) followed by isolate CC-07 (82.2 µm) and isolate CC-10 (72.8 µm) isolate. The minimum length of conidia was observed in CC-01 (42.8 µm) followed by isolate CC-09 (44.5µm) and isolate CC-04 (57.8 µm).

Conidial Width

variability in conidial width was observed among isolates. The maximum width of conidia was observed in isolate CC-05 (7.8µm) followed by isolate CC-09 (7.3 µm) and isolate CC-03 (7.1 µm). The minimum width of conidia was observed in isolate CC-04 (3.8µm).

Number of pseudosepta

The variability in pseudosepta within isolates was observed and the ratios of these pseudosepta differed among isolates. The maximum pseudosepta was found in isolate CC-08 (6.1) followed by isolate CC-01 (5.1) and isolate CC-03 (3.8). The minimum pseudosepta was observed in isolate CC-09 (1.1) followed by isolate CC-06 (1.6) and CC-10 isolate (1.9).

Earlier works have described the variability in colour, texture of the fungal colonies, size and shape of the conidia not only among the isolates obtained from different hosts and geographical regions but also within a single isolate. Nghia *et al*. (2008) [2], observed the differences in morphology of the *C. cassiicola* isolates for mycelium colour, texture, or colony colour as well as the shape of the cultures, and in conidia contour, shape, size and the number of pseudosepta. The similar results are also coincide with the findings of Qive *et al*. (2011). They observed variation in colony morphology of the fungal isolates. They found variation in mycelium colour (white to grey or green), texture (thin to thick), or in colony colour (white to pale brown, red brown, dark brown, dark grey or black as well as in the shape of the cultures (round to slightly polygonal). They also observed variation in colony diameter and conidial morphology among isolates. Differences were observed in shape, size and the number of pseudosepta.

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