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Morphological characters of *Colletotrichum gloeosporioides* from various hosts

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Abstract
Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the most damaging disease causing huge losses in pre and post harvest conditions. Studies were conducted to find out morphological characteristics and sporulation under 16/8 hours light and darkness condition *in vitro* on PDA. Isolates were obtained from *Mangifera* indica, *Citrus aurantifolia*, *Catharanthus roseus*, *Psydium guavajava*, *Punica granatum*, *Carica papaya*, and *Glycine max*. Pathogenicity of these isolates was proved on their native hosts. It was revealed that there was variation in growth. Maximum radial mycelial growth was attained by Cg_6 isolate (89mm) at 8th DAI and minimum radial mycelial growth was in Cg_2 isolate. Maximum conidial length was observed in Cg_1 isolate (12.57-19.62µm) while maximum breadth was observed in Cg_6 isolate (3.0-7.5µm). It was clearly observed in all the isolates that there were no visible setae. Variation in colony colour was observed and acervuli were brownish to blackish in colour with raised conidial mass. Sporulation study revealed that Cg_6, Cg_5 and Cg_3 isolates showed good sporulation while Cg_1, Cg_2, Cg_4 and Cg_7 isolates showed poor to medium sporulation under 16/8 h condition of light regime.

Keywords: *Colletotrichum gloeosporioides*, morphological study, sporulation study

1. Introduction
Colletotrichum is an economically important genus of fungus belonging to family-Melanconiaceae, order-Melanconiales, class-Coelonycetes and subdivision- Deuteromycotina. Recently it is classified under phylum- Ascomycota, class- filamentous Ascomycetes (Pyrenomycets), order-Phyllachorales, genus-*Glomerella* (*Colletotrichum*) (Agrios, 2005) [2].
Filamentous fungi of the genus *Colletotrichum* are economically significant plant pathogen worldwide. The telomorph stage of *Colletotrichum* is *Glomerella* and both stages are widely prevalent in hot and humid climate. According to Phoulivong *et al*. (2010) [7] *Colletotrichum* species is caused above ground plant parts of the crops as well as fruit trees affected by *Colletotrichum* anthracnose and in the case of fruit infection, there is reduction in yield quantity or quality. The symptoms include anthracnose, leaf spot, twig blight, fruit spot in various crops which reduces economic value of the produce. It affects the mango severally and causes leaf spot, blossom blight and wither tip. It also affects inflorescence. Sanders and Korsten (2003) [8] observed that *Colletotrichum gloeosporioides* could cause infection on mango, chilli, pepper, and guava. According to Amusa *et al.,* (2005) [3] anthracnose of guava is one that impairs the quality of fruits while anthracnose is the second most important disease of pomegranate next to bacterial blight. Joshi *et al.,* (2014) [4] observed it is more severe in the fruiting stage of Mrigbahar cropping season leading post harvest rotting of fruits. Sadafuli is a horticultural and medicinal plant which when attacked by *C. gloeosporioides* severely; growth of the plant is adversely affected. Considering the importance of this disease to infect wide range of host plants, it was thought worthwhile to undertake study of identification of the disease on some natural hosts by studying morphological characters after occurrence of disease symptoms on their native hosts.

Materials and Methods
The present study was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2017-2018.

Materials required

Collection of samples

The diseased samples of leaves of citrus, sadafuli, soybean and fruits of mango, guava, pomegranate and papaya showing symptoms of *Colletotrichum* infection were collected from the premises of farm of College of Agriculture, Nagpur.

Methods adopted

Isolation and maintenance of culture

The samples showing symptoms of anthracnose were collected from different localities by cutting infected part with healthy tissues. The infected bits were washed with sterilized water and surface sterilized by 0.1% mercuric chloride solution for one minute in the petriplates and subsequently three changes of distilled water were given to remove the traces of mercuric chloride. The bits were dried and then transferred to solidified sterile PDA in petriplates and were incubated at room temperature (27±2 ℃) for seven days. All the operations were carried out aseptically. The fungal growth of *C. gloeosporioides* was then transferred on PDA slant. The culture thus obtained was further purified by hyphal tip method and maintained on PDA slant for further use.

Identification of pathogen

The isolated fungus was identified on the basis of colony character, type of conidia, and acervuli. The pathogen was identified as *C. gloeosporioides*.

Preparation of host for pathogenicity

For proving pathogenicity, seedlings of mango, periwinkle and soybean were grown in earthen pots which were filled with sterilized soil. The host plants were regularly irrigated till they were well established in pots. Whereas healthy fruits of acid lime, guava, pomegranate and papaya were used for disease inoculation.

Inoculation for pathogenicity

In order to prove Koch's postulates, pathogenicity test was carried out. The epidermal layer of leaves of mango, periwinkle and soybean was damaged by smearing carborandom powder before inoculation. The suspension of each isolate of *C. gloeosporioides* was used for inoculating the plant in pots by spraying the seedlings with the help of atomizer. Fully matured unripe fruits of acid lime, guava, pomegranate and papaya were washed thoroughly under running tap water. The fruits were blot dried and surface sterilized with 5% sodium hypo chloride and again washed with sterile distilled water, blotted dry on sterilized filter paper. The fruits were injured (pinprick) with sterilized needle and the spore suspension (4×10^4 spores/ml) of the pathogen was prepared using a seven days old PDA culture sprayed over the fruits. The fruits inoculated with sterile distilled water served as control. The inoculated fruits and seedlings were covered with polythene bags and kept under the moist chamber. After inoculating the plants, they were examined regularly for disease establishment.

The fungus was reisolated from artificially inoculated seedlings and fruits showing typical anthracnose symptoms and the culture obtained was confirmed for its morphology and colony characters.

Morphological variation

Autoclaved PDA was poured in the petriplates and allowed it to solidify. Fungal disc (5mm) of seven days old culture was transferred on solidified PDA for each isolate. Inoculated plates were incubated at room temperature (± 27˚C) under 12 hrs lightness and 12 hrs darkness. On solidified PDA, the culture was examined for radial mycelial growth on 2nd, 4th, 6th and 8th days after inoculation. The colony diameter was measured in two directions at right angle to each other passing through the centre of the colony.

Results and Discussion

Isolation and identification of pathogen

Collection of disease sample and isolation of pathogen *C. gloesporioides* isolates from various hosts like mango, acid lime, periwinkle, guava, pomegranate, papaya and soybean were identified on the basis of morphological characters. The usual tissue isolation technique was followed to isolate the pathogen from infected plant parts showing fruit rot and anthracnose symptoms. The pathogen was identified as *C. gloeosporioides* having pink, white, grayish colony colour, conidia were hyaline oblong cylindrical and which carry oil globule in the centre. Agostini *et al*., (1992) [1] was described that slow growing isolate of acid lime produced deep orange pigment and colony had smaller conidia.

Pathogenicity on native host

For proving pathogenicity, seedlings of mango, periwinkle and soybean grown in sterilized earthen pots while fruits of acid lime, guava, pomegranate and papaya were used for disease inoculation. All of the isolates were identified using morphological characters, colony growth and conidial characters confirmed by Phoulivong *et al*., (2010) [7].

Table 1: Pathogenicity of *C. gloeosporioides* on their native hosts

Botanical name	Part used	Isolates	Initiation of symptoms DAI	Spot size (mm)	Per cent disease intensity	Symptoms	Category
M. indica	Leaves	Cg_1	7	2-3.5	7.40	Brownish to black colour necrotic spots were found on leaves	WP
C. aurantifolia	Fruits	Cg_2	8	1-1.5	2.77	Reddish brown irregular spots. In advance stage it progresses showing dark brown colour	WP
C. roseus	Leaves	Cg_3	5	1-2	4.08	On leaves brownish colour necrotic spots developed and leaves fall down	WP
P. guavajava	Fruits	Cg_4	10	1-3	3.75	Brownish necrotic spots developed	WP
P. granatum	Fruits	Cg_5	7	1-1.5	18.84	Dry, brown to black discolouration.	WP
C. papaya	Fruits	Cg_6	5	2-3	19.44	Large dark brown water soaked lesions on fruits	WP
G. max	Leaves	Cg_7	6	1-1.5	19.4	Brown irregular spots on leaves	WP

Morphlogical characters

Morphological characters of different *C. gloeosporioides* isolates with respect to mycelial growth, conidial characters, setae and acervuli were studied on PDA. *C. gloeosporioides* showed high variability in morphological characters and pathogenicity on PDA. The results are presented in Table 2, figure 1 and 2 and Plate 1. All seven isolates of *C. gloeosporioides* were designated as Cg_1, Cg_2, Cg_3, Cg_4, Cg_5, Cg_6 and Cg_7 which produced white, pink and grayish colour of colony. Conidia of all isolates were oblong to cylindrical having one fat globule at center but setae were not clearly observed. Acervuli had brownish to blackish coloured raised conidial mass. Meshram (2014) [6] were noticed the isolates of *C. gloeosporioides* varied greatly in colony colour and appearance. The colour of *C. gloesporioides* varied from white to grey, dark orange or pink grey while the reverse side of the colony was of white, dark grey orange or a mixture and with regular colony margins. The growth pattern was either circular with the mycelia showing a uniform growth pattern or radial ring like pattern. Radial mycelial growth was measured in the form of diameter on 2nd, 4th, 6th and 8th day after inoculation on PDA and the data presented in Table 3. Among all seven isolates, Cg_6 isolate had maximum mycelial growth 32 mm at 2nd DAI and 89 mm at 8th DAI, followed by Cg_3 isolate 30 mm at 2nd DAI and 79 mm at 8th DAI. Isolate Cg_5 had 88 mm mycelial growth at 8th DAI. Isolate Cg_2 registered minimum mycelial growth all the intervals 27mm at 2nd, 36 mm at 4th, 64 mm at 6th and 72 mm at 8th DAI. The *C. gloeosporioides* complex is well known to exhibit high variability in conidial size and shape and in colony morphology.

Micrometric observations

The micrometric observations of *C. gloeosporioides* isolates, conidial shape, and size were observed on PDA medium and are given in Table 3. The highest conidial length of 12.57µm was noted in Cg_1 isolate followed by Cg_3 isolate 12.5µm. The breadth of conidia was ranging from 2.75-7.5µm. Isolate Cg_6 had maximum breadth ranging 3.0-7.5µm.

Sporulation study

The effects of light regimes i.e. 16 hrs light and 8 hrs darkness on fungal development of different isolates of test fungus were evaluated on PDA media at 3rd, 5th and 7th days interval. The result presented in Table 4. Cg_3, Cg_5 and Cg_6 isolates had good sporulation while Cg_4 and Cg_7 with medium sporlation at 16/8 h light and dark condition. Cg_1 and Cg_2 isolates showed poor sporulation at this condition on PDA media. Thus, the result revealed that 16/8 h light and darkness condition improves sporulation of Cg_3, Cg_5 and Cg_6 isolates of *C. gloeosporioides*. Mello *et al.,* (2004) [5] obtained good growth of *C. gloesporioides* in continous light condition at 25˚ C. In the present investigation, variation among the isolates noticed in respect of sporulation was noticed at 7th DAI.

Table 2: Morphological characters of different *C. gloeosporioides* isolates on PDA

Isolates	Colony	Conidia	Acervuli
Cg_1	Circular dull white grayish colour of colony having aerial mycelial	Cylindrical, hyaline with one fat globule atcentre	Brownish to blackish colour in which conidial mass raised
Cg_2	white grayish colony having saffron coloured raised conidial mass atcentre	Oblong, hyaline with one fat globule in centre	Brownish to blackish colour in which conidial mass raised
Cg_3	Circular white grayish colour colony having regular growth	Oblong and hyaline with fat globule at centre.	Brownish to blackish colour in which conidial mass raised
Cg_4	Colony is white grayish in colour with cottony growth	Cylindrical, hyaline with fat globule at centre	Brownish to blackish colour in which conidial mass raised
Cg_5	Intermixed black and white colour colony, fluffy raised circular mycelial growth with light pinkish pigmentation	cylindrical or slightly dumbbell, hyaline with one to two oil globules	Brownish to blackish colour in which conidial mass raised
Cg_6	White to dull white with smooth margin	Oblong, hyaline with oil globules at centre	circular, pinkish colour, covered with numerous conidia
Cg_7	Dull white to greyish colour of colony	cylindrical, hyaline with oil globules at centre	brownish to blackish colour in which conidial mass raised

Table 3: Radial mycelial growth (mm) and dimension of different *Colletotrichum gloeosporioides* isolates on PDA at various interval.

Isolates	Radial mycelial growth (mm)				Dimension of conidia(µm)	
	2nd	4th	6th	8th	Length	Breadth
Cg_1	28	38	67	79	12.57-19.62	2.75-3.86
Cg_2	27	36	64	72	11.2-17.6	3.2-5.7
Cg_3	30	50	79	87	12.5-16.5	4.8-5.5
Cg_4	27	46	71	81	11.31-13.31	4.29-5.0
Cg_5	29	52	77	88	11.4-20.0	4.25-6.62
Cg_6	32	55	78	89	9.0-20	3.0-7.5
Cg_7	28	44	75	81	8.0-13.1	3.9-6.0

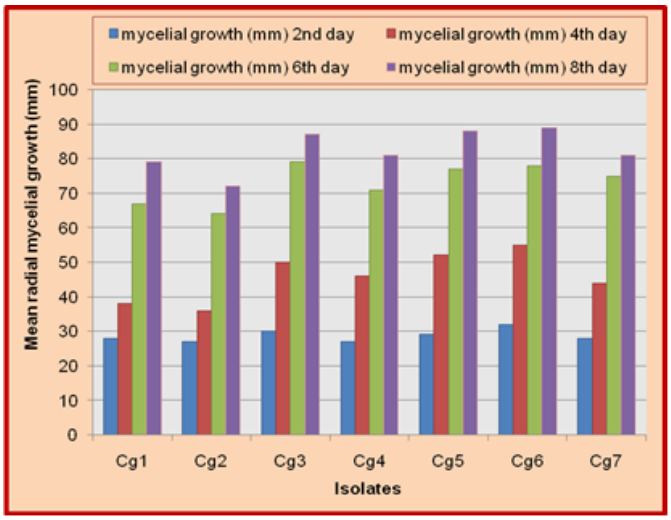

Fig 1: Mean radial mycelial growth of different isolates of *C. gloeosporioides*

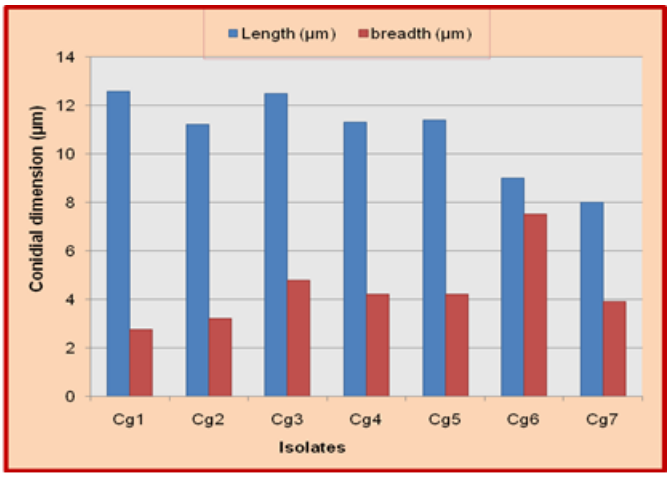

Fig 2: Conidial dimension of isolates of *C. gloeosporioides*

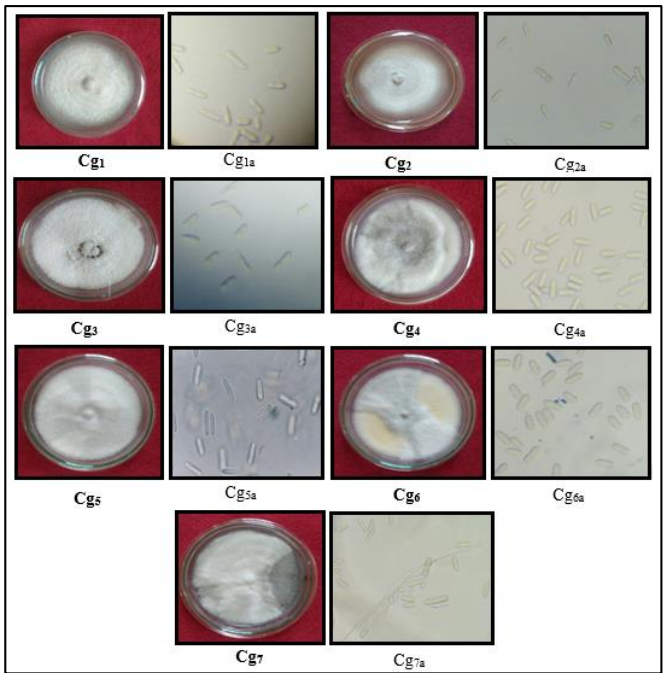

Fig 3: Morphological characters of *C. gloeopsporioides* isolates

Table 4: Effect of continuous 16/8 h light and dark on sporulation of different isolates of *C. gloeosporioides*

Isolates	Sporulation		
	3rd	5th	7th
Cg_1	-	-	+
Cg_2	-	+	+
Cg_3	+	+	+++
Cg_4	-	+	++
Cg_5	+	++	+++
Cg_6	+	+++	+++
Cg_7	+	++	++

+ - poor ++ - medium +++ - good

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