International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 2227-2230

Received: 12-05-2018
Accepted: 17-06-2018

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Cultural and morphological variability in *Colletotrichum gloeosporioides* inciting mango anthracnose

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Abstract
Anthracnose disease of mango causes by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. Is one of the most devastating pathogen in mango, which behaves differently to varied environmental conditions in their symptom developement, severity and adaptibility. So, an invitro study was conducted to know cultural and morphological variability of ten different isolates of pathogen in major mango growing districts of Karnataka. Our study was found that, among the ten isolates, numerically maximum radial growth observed in Cg-8 (88.83 mm) isolate followed by Cg-1 (88.67 mm) and least was observed in Cg-7 isolate (87.17 mm). Mycelial growth pattern and pigmentation varied from circular to suppressed and white, pinkish to black respectively. Spore size also varied among the isolates, large sized spores (8.12-10.41 x 2.12-3.06 µm) were observed in Cg-1isolate, while, small sized spores (5.08-7.02 x 1.97-2.52 µm) were in Cg-10 isolate. Sporulation was also varied among the isolates, excellent sporulation (++++) was found in Cg-5 and Cg-7 isolates, whereas, poor (+) was found in Cg-10 isolate.

Keywords: Mango, *Colletotrichum gloeosporioides*, mycelial growth, sporulation

Introduction
Mango (*Mangifera indica* L.) is one of the most important and popular fruit crop in tropical and sub-tropical regions (Shad *et al.* 2002) [20], belonging to the family Anacardiaceae, which is grown in more than 110 countries of the world. Although mango is considered to be a hardy plant, it is susceptible to various diseases, insect pests and physiological disorders. Fungi are the major group of pathogens responsible for nursery, field and fruit rot diseases.
Among the various fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. Is a major limiting factor in fruit production in all mango growing areas of the world with high rainfall (Ullasa 1998) [24]. The disease was first identified in India by McRae in 1924 [16]. The pathogen causes black spot, leaf blight, blossom blight, fruit rot and in severe cases die-back (Sangeetha 2003) [19] (Akem 2006) [2]. In general, such symptoms are similar in all mango host from different localities, but the isolates of the pathogen may vary in its degree of aggressiveness as well as in its cultural and morphological characters. Species concepts based on morphological criteria are generally broad for the genus *Colletotrichum*, and wide variations for cultural and morphological characters, pathogenicity and host range have been reported among isolates of *C. gloeosporioides* (Alahakoon and Brown 1994) [4].
Cultural and morphological character like colony diameter, type growth, pigmentation, number of spores and spore size is account for pathogenicity and adaptability of the pathogen to varied environmental conditions. The cultural and morphological variability of *C. gloeosporioides* of different regions in Karnataka state is lacking. So, by considering the fact, different isolates from all around the Karnataka were collected and studied.

Material and Methods
Collection and isolation of pathogen
Mango leaves infected with anthracnose were collected from different districts of Karnataka (India) *viz*., Bengaluru Rural, Chitradurga, Chikkaballapur, Dharwad, Haveri, Kolar, Raichur, Ramanagara, Shivamogga and Tumakuru during survey and used for isolation of the fungus *in-vitro*.

Disease samples collected from different locations of Karnataka were designated as follows

Location (Districts)	Name of isolate
Kolar	Cg-1
Chikkaballapur	Cg-2
Dharwad	Cg-3
Tumakuru	Cg-4
Haveri	Cg-5
Shivamogga	Cg-6
Chitradurga	Cg-7
Raichur	Cg-8
Bangaluru Rural	Cg-9
Ramanagara	Cg-10

The isolation of the fungus was made by following standard tissue isolation technique as described below.

The infected portions along with some healthy part were cut and surface sterilized using 1 per cent sodium hypochlorite solution for 60 seconds. These bits were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants and incubated at room temperature (27±1 °C) and observed periodically for fungal growth and sporulation. The pathogen was identified as *Colletotrichum gloeosporioides* based on its mycelial and conidial characteristics as per standard mycological keys (Barnett *et al.* 1972) [7] and maintained on PDA Petri plates at 27±1 °C for further studies.

Cultural and morphological studies

Twenty ml of PDA medium was poured aseptically into 90 mm diameter Petri plates. After solidification, five mm discs of the *C. gloeosporioides* was selected from actively growing culture using a cork borer and a single disc placed at the centre of Petri dish. Each set of experiment replicated three times and they were incubated at 27±1 °C for 12 days. Growth of culture and sporulation are start 2-3 days after inoculation (Talhinhas *et al*. 2005) [23].

The cultural and morphological characters like, colony characters such as diameter, pigmentation (white, light brown, brown or pink), type of mycelial growth (circular or irregular and raised or flattened), spore size, no of spores were studied to differentiate different isolates.

Assessment of sporulation of the fungus

Conidial suspensions prepared by harvesting the conidia from 12 day old culture Petri plates using sterile distilled water and collected into the test tube. From this spore suspension, a drop was transferred on to the counting stage of the haemocytometer and average number of spores per ml was determined. The isolates were categorized as poor, average, good and excellent on the basis of conidial population as given below.

S. No	Average No. of conidia ml^{-1}	Score	Grade
1.	$< 2.0 \times 10^4$	+	Poor
2.	2.0×10^4- 4.0×10^4	++	Average
3.	4.0×10^4 - 8.0×10^4	+++	Good
4.	$> 8.0 \times 10^4$	++++	Excellent

Results

The response of different isolates of *C. gloeosporioides* was tested on potato dextrose agar (PDA) and pertaining results are presented in Table 1, Figure 1 and Plate 1.

All the ten isolates of *C. gloeosporioides* were collected from different parts of Karnataka, has shown considerable difference in relation to radial growth, colony character, pigmentation and sporulation on potato dextrose agar (PDA) after 12th day of incubation at 27±1 ºC.

Radial Growth

There is not much variation among the isolates with regarding to mean radial growth (Table 1). Among the ten isolates, numerically maximum radial growth in Cg-8 (88.83 mm) isolate followed by Cg-1 (88.67 mm), Cg-4 (88.67 mm), Cg-5 (88.63 mm), Cg-9 (88.50 mm), Cg-10 (88.17 mm), Cg-2 (88.00 mm), Cg-3 (87.83 mm), Cg-6 (87.83 mm). Whereas, least radial growth (87.17 mm) was observed in Cg-7 isolate. All the isolates are on par with each other and there was no significance difference in radial growth of *C. gloeosporioides* among isolates.

Mycelial growth pattern and pigmentation

The four isolates of *C. gloeosporioides viz.*, Cg-2, Cg-4, Cg-5 and Cg-9 were shown white raised colonies. While, the other three isolates (Cg-10, Cg-7 and Cg-8) produced light pinkish colonies. The isolates Cg-1 and Cg-6 were produced brown suppressed and brown fluffy raised colonies respectively. Whereas, Cg-3 produced black suppressed colony.

Sporulation

Observations in spore size also varied among the isolates. Large sized spores (8.12-10.41x2.12-3.06 µm) were observed in Cg-1 isolate, whereas, small sized spores (5.80-7.02x1.97-2.52 µm) were recorded in Cg-10 isolate. Maximum sporulation with excellent grade was recorded in Cg-5 and Cg-7 isolates. Good sporulation was observed in Cg-1, Cg-2, Cg-3, Cg-4 and Cg-9 isolates, while, moderate sporulation was noticed in Cg-6 and Cg-8 isolates. The poor sporulation was observed in Cg-10 isolate.

Discussion

Ten isolates were collected from different geographical locations of Karnataka were grown in the universal media (PDA). Maximum radial growth was recorded in isolate Cg-8 (88.83 mm) whereas, least radial growth (87.17 mm) was recorded in Cg-7 isolate. The ten isolates of *C. gloeosporioides* also showed diverse response in attaining maximum growth. The similar findings were also reported by Ekabote *et al.* (1997) [11] and Jayalakshmi (2010) [13] but differ with the findings Akthar (2000) [3], Sudhakar (2000) [22], Prashanth (2007) [17], Rani and Murthy (2004) [18] and Ashutosh *et al.* (2012) [6].

Isolates varied significantly in cultural land conidial characteristic, produced white to black colony with regular to irregular and fluffy raise to fluffy flat growth. All the isolates produced initially white colony later become dull brown to black with regular margins. The conidia appear as whitish or pinkish to brown in mass. These results are in agreement with that of Aruna (2008) [5], Smith and Black (1990) [21] in strawberry, Agostini *et al.* (1992) [1] in citrus, Bernstein *et al*. (1995) [8] in Peach, Apple and Pecan, Litz (1997) [15] and Prashanth (2007) [17] in Pomegranate.

The size of the spores also varied among the isolates. Large sized spores (8.12-10.41 x 2.12-3.06) were observed in Cg-1isolate, while, small sized spores (5.08-7.02 x 1.97-2.52) were noticed in Cg-10 isolate. There was a considerable variation in the extent to sporulation among the isolates on PDA medium. The excellent sporulation was recorded in Cg-5 and Cg-7 isolates after 12 days of incubation followed by good in Cg-1, Cg-2, Cg-3, Cg-4 and

Table 1: Cultural and morphological characteristics of isolates of *Colletotrichum gloeosporioides* on potato dextrose agar

S. No	Isolates	Radial growth (mm)	Mycelial colony	Pigmentation	Spore Size (µm)	Sporulation
1	Cg-1	88.67	Medium white suppressed	Brown	8.12-10.41 × 2.12-3.06	+++
2	Cg-2	88.00	White raised circular	Whitish	7.21-7.65 × 2.24-2.70	+++
3	Cg-3	87.83	Brown suppressed circular	Black	6.73-8.38 × 2.29-2.63	+++
4	Cg-4	88.67	White raised irregular	White	6.08-7.50 × 2.10-3.08	+++
5	Cg-5	88.63	Brown fluffy raised	White	6.50-8.16 × 1.57-2.23	++++
6	Cg-6	87.83	Brown fluffy raised	Brown	7.87-8.02 ×2.38-2.64	++
7	Cg-7	87.17	Medium white raised	Light pinkish	8.12-8.66 × 1.78-2.12	++++
8	Cg-8	88.83	Medium white suppressed	Light pinkish	7.10-8.20 × 2.01-2.23	++
9	Cg-9	88.50	White fluffy circular	White	6.62-7.61 × 2.12-2.64	+++
10	Cg-10	88.17	White raised circular	Pinkish	5.80-7.02 × 1.97-2.52	+
S.Em ±		0.56				
C.D. at 1%		NS				

NS: Non-Significant

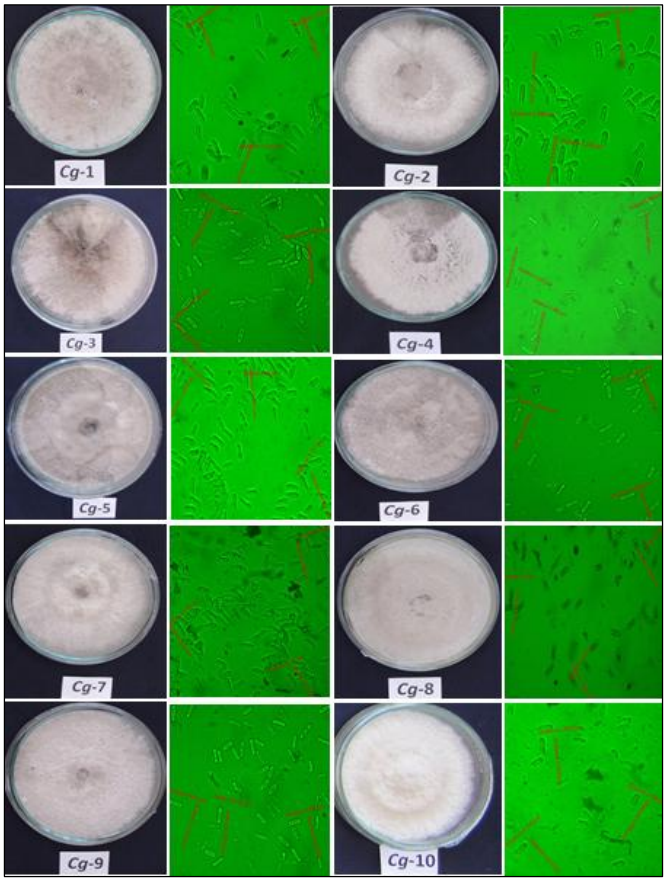

Plate 1: Cultural and morphological characteristics of isolates of *Colletotrichum gloeosporioides*

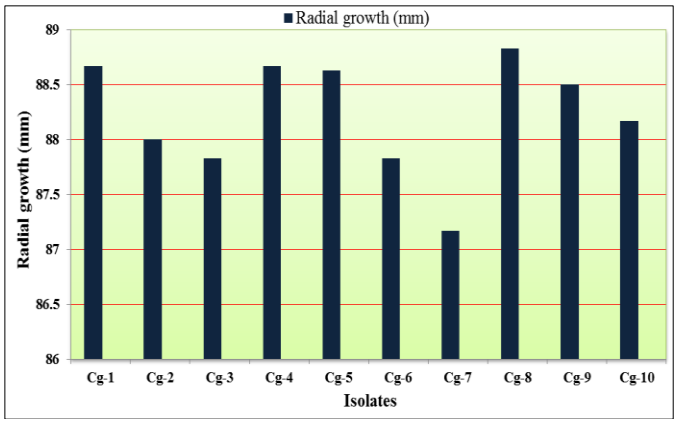

Fig 1: Cultural Characteristics of ten isolated of *Colletotrichum gloeosporioides* on potato dextrose agar

Cg-9. While, moderate sporulation were observed in Cg-6 and Cg-8 isolates. The poor sporulation was noticed in Cg-10 isolate (Table. 1). The present study is in conformity with Holliday (1980) [12], Jefferies *et al*. (1990) [14], Ekabote (1994) [10], Chowdappa and Mohan Kumar (2012) [9].

Conclusion

Thus *Colletotrichum gloeosporioides* is show different types variability like (colony morphology, radial growth, colony colour, mycelial growth pattern, sporulation and pigmentation) in the PDA. In culture, most of isolates produce cottony, fluffy or suppressed colonies. However, no significant difference was noticed in shape and size of conidia among the different isolates. Thus, the results of the present investigation revealed that existence of cultural and morphological variability among the *C. gloeosporioides* isolates.

References

1. Agostini JP, Timmer LW, Mitchell DJ. Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides* from citrus. Phytopathology. 1992; 76:1176-1178.
2. Akem CN. Mango anthracnose disease: present and future research priorities. Plant Pathology Journal. 2006; 5(3):266-273.
3. Akthar KP. Fresh potato extract the best source for the growth of *Colletotrichum gloeosporioides* causing anthracnose of mango and *Fusarium subglutinans* isolated from malformed inflorescence of mango. Pakisthan Journal Phytopathology. 2000; 12:134-136.
4. Alahakoon PW, Brown AE. Host range of *Colletotrichum gloeosporioides* on tropical fruits in Sri Lanka. International Journal of Pest Management. 1994; 40:23-26.
5. Aruna. Studies on cultural, morphological, pathogenic variability and biological control of *Colletotrichum gloeosporioides* Penz. Causing mango anthracnose (Coastal and Telangana regions of Andhra Pradesh). M.Sc. (Agri.) Thesis, Acharya N.G. Ranga Agricultural University, Hyderabad, India, 2008.
6. Ashutosh P, Lallan PY, Muthukumar M, Ugam KC, Brajesh KP. Effectiveness of cultural parameters on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose disease of mango (*Mangifera indica* L.). Journal of Biological Science. 2012; 12(4):123-133.
7. Barnett HL, Barry Hunter B. Illustrated Genera of Imperfect Fungi. Burgoes Publishing Company, Minnesota, 1972.

8. Bernstein B, Zehr EI, Dean RA, Shabi E. Characteristics of *Colletotrichum* from peach, apple, pecan and other hosts. Plant Disease. 1995; 79:478-482.
9. Chowdappa P, Mohankumar SP. Existence of two genetically distinct populations of *Colletotrichum gloeosporioides* Penz. In mango from India. Pest Management in Horticulture Ecosystem. 2012; 18:161-170.
10. Ekabote SD. Studies on anthracnose of mango (*Mangifera indica* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, India, 1994.
11. Ekabote SD, Padagunnur GM, Patil MS, Chattannavar SN. Studies on the cultural and nutritional aspects of *Colletotrichum gloeosporioides*, the causal organism of mango anthracnose. Journal of Mycology and Plant Pathology. 1997; 27:229-230.
12. Holliday P. Fungus disease of tropical crops. Cambridge University Press, Cambridge, 1980, 256.
13. Jayalakshmi K. Studies on anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, India, 2010.
14. Jefferies P, Dodd JC, Jeger MJ, Plumbley RA. The biology and control of *Colletotrichum* species on tropical fruit crops. Plant Pathology. 1990; 3:343-366.
15. Litz RE. The mango botany, production and uses. Commonwealth Agricultural Buareaux, International Library of Congress Cataloging in Publication Data. 1997, 257-280.
16. McRAE W. Economic Botany part-III. Mycology. Annual Report, Board of Science and Advisory. India, 1924, 31-35.
17. Prashanth. Investigations on anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc. Of Pomegranate (*Punica granatum* L.). M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, India, 2007.
18. Rani SG, Murthy MK. Cultural and nutritional characterstics of *Colletotrichum gloeosporioides*, the causal organism in cashew anthracnose. Journal of Mycology and Plant Pathology. 2004; 34:317-318
19. Sangeetha CG. Studies on anthracnose of mango caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India, 2003.
20. Shad MA, Ansari TM, Pervez H, Rubab Mahmood T. Changes in sugar, amino acid and mineral content of leaves of two mango varieties affected by quick decline disease. Journal of Biological Science. 2002; 2:694-696.
21. Smith BJ, Black LL. Morphological, cultural and pathogenic variation among *Colletotrichum* species isolated from strawberry. Plant Disease. 1990; 74:69-76.
22. Sudhakar. Biology and management of *Stylosanthes* anthracnose caused by *Colletotrichum gloeosporioides* (Penz.). Penz. And Sacc. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, India, 2000.
23. Talhinhas PS, Sreenivasaprasad J, Neves-Martins, Oliveira H. Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. Microbiology. 2005; 71:2987-2998.
24. Ullasa BA. Influence of time of application of some fungicides on their efficacy against mango anthracnose caused by *C. gloeosporioides*. Plant Disease Research. 1998; 4:196-197.