Plant Archives Vol. 19 No. 1, 2019 pp. 425-430 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PATHOGENICITY VARIATION, MORPHOLOGICAL AND CULTURAL CHARACTERISTIC OF *COLLETOTRICHUM GLOEOSPORIOIDES* ISOLATES

R. Udhayakumar*, S. Usharani and A. Muthukumar

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar-608 002 (T.N.), India.

Abstract

The anthracnose caused by *Colletotrichum gloeosporioides* is one of the most common and serious diseases of mango. In the present study, the pathogenicity variation of *C. gloeosporioides* was assessed by spray inoculation method, detached leaf technique and pin prick method. The data revealed that the level of pathogenicity varied between the isolates. Among the twenty isolates of *C. gloeosporioides*, the isolate I_8 recorded the maximum disease incidence of 30.33 per cent in spray inoculation method, 38.33 per cent in detached leaf technique and 62.00 per cent in pin prick method. In the morphology and culture character assessment, the isolate of *C. gloeosporioides* showed variability with respect to colony characters. Most of the isolates produced grayish white on the PDA medium in Petri dishes. The pigmentations like whitish, grayish brown, greenish grey, pinkish and pinkish brown were predominant in isolates. Among the isolates of *C. gloeosporioides*, the maximum mycelial growth (90.00 mm) was recorded by the isolates I_5, I_8, I_{18} and I_{20} followed by I_3 and I_{14} recorded 88 mm each at seven days after inoculation. All the isolates of *C. gloeosporioides* varied in their ability to produce conidia on PDA. The maximum conidial population was recorded in I_8 (17.47×10^6 conidia ml^{-1}) also the acervuli production was excellent.

Key words : *Colletotrichum gloeosporioides,* pathogenicity variation and culture character.

Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruit crops in the world. It belongs to the family Anacardiaceae and it is called as "King of fruits" (Hayes, 1953). India has the richest collection of mango cultivars and is regarded as the "National fruit" of the country. Mango cultivation is an important agribusiness in India. Eventhough, it has the largest area, the productivity is very low due to a number of diseases. Mango trees are affected by several fungal, bacterial and viral diseases, of which mango anthracnose caused by *Colletotrichum gloeosporioides* Penz. is a highly destructive pathogen that causes considerable damage, inflicting severe qualitative and quantitative losses (Sangeetha and Rawal, 2010). Anthracnose is presently recognized as the most important post-harvest disease of mango worldwide (Gadgile *et al*., 2009; Gupta *et al.,* 2010). It is the major limiting factor in fruit production in all the countries where mangoes are grown, especially where high humidity prevails during the cropping season. The post-harvest phase is the most damaging and economically significant phase of the disease worldwide.

Colletotrichum species are highly variable as manifested by colony morphology, conidial shape, presence and shape of setae and appressoria, pigmentation, fungicide sensitivity, pathogenicity and other traits (Katan, 2000; Martinez *et al*., 2008). Traditional differentiation between *Colletotrichum* species, based on host range or origin, may not be a reliable criterion for this fungus as, *C. gloeosporioides* infects a broad range of host plants (Freeman *et al*., 1998). Differences between isolates are also evident with respect to their relative pathogenicity or virulence (Jeffries *et al*., 1990). With this background, for the better understanding about pathogen, the study was formulated to assess the pathogenicity variation and morphological and cultural characteristic of *C. gloeosporioides* isolates.

***Author of correspondence:** E-mail: udhayaplantpathology@yahoo.co.in

Materials and Methods

Isolation of *C. gloeosporioides*

The pathogen causing anthracnose disease in mango was isolated from diseased leaf and fruit samples. The infected tissue bits were separated with a sterile blade and surface sterilized with 1 per cent sodium hypochlorite solution for 1 min. and subsequently washed three times with sterile distilled water. Then they were transferred into a sterile Petri dish containing Potato Dextrose Agar (PDA) medium (Ainsworth, 1961) amended with streptomycin. The plates were then incubated at room temperature (28±2°C) for four days. The emerging colonies were sub cultured on to PDA slants. Single hyphal tip method was followed for making pure culture and maintained on PDA slants (Aneja, 2003). *C. gloeosporioides* was isolated from all the twenty locations and designated as I_1 to I_{20} (table 1). The identity of all isolates was confirmed by microscopic observations based on morphological characteristics as per the key suggested by Barnett *et al.* (1972).

Table 2 : Pathogenicity variation of *C. gloeosporioides* isolates from various varieties by artificial inoculation on var. Neelam.

Isolate number	Per cent disease index (PDI)		
	Spray inoculation method (Seedlings)	Detached leaf technique (Leaf)	Pin prick method (fruit)
I_1	27.67[b]	36.67[b]	54.00[b]
I_2	10.67[i]	13.67[k]	19.67[i]
I_3	12.33[i]	16.00[j]	22.33[h]
I_4	26.00[c]	35.00[b]	49.67[c]
I_5	19.67[f]	32.67[c]	43.67[d]
I_6	15.00[g]	29.33[e]	33.33[f]
I_7	12.00[i]	14.33[k]	21.67[h]
I_8	30.33[a]	38.33[a]	62.00[a]
I_9	18.67[f]	25.33[f]	35.67[e]
I_{10}	17.00g	24.00[g]	33.00[f]
I_{11}	09.33[j]	13.67[k]	15.67[i]
I_{12}	25.67[c]	36.33[b]	48.33[c]
I_{13}	09.00[j]	11.67[l]	15.00[i]
I_{14}	15.00[g]	22.67[g]	30.33[f]
I_{15}	24.00[d]	34.00[c]	45.67[cb]
I_{16}	14.33[h]	20.33[h]	29.67[g]
I_{17}	21.33[e]	31.33[d]	44.00[d]
I_{18}	19.33[f]	27.67[e]	40.00[d]
I_{19}	14.00[h]	19.00[i]	28.33[g]
I_{20}	23.67[d]	32.00[d]	45.67[c]

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

Preparation of inoculum

Conidial suspension was prepared from 7 days old culture of *C. gloeosporioides* grown on PDA medium. Concentration of conidia in the suspension was adjusted to 1×10^5 conidia ml^{-1} using haemocytometer (Martinez *et al*., 2008).

Pathogenicity test

Pathogenicity of all the twenty isolates of *C. gloeosporioides* was tested by spray inoculation method in mango seedlings of var. Neelam. Also, detached leaf technique and pin prick method were followed to test the pathogenicity in leaves and fruits, respectively. The experiment was conducted in a completely randomized block design with three replications. The highly virulent isolate of *C. gloeosporioides* was used for subsequent studies.

Spray inoculation method

For artificial inoculation of the pathogen, leaves were slightly injured by the pin prick and then inoculated by spraying conidial suspension (1×10^5 conidia ml^{-1}) of each isolate. A hand atomizer was used for spraying the inoculum suspension of each isolate and atomizer was pre sterilized with 90% ethanol before the spraying the inoculum. The inoculated seedlings were covered with transparent polyethylene bags of 100 gauge for 48 h. The inoculated plants were sprayed with sterile distilled water to ensure high humidity to favour conditions for conidial germination and infection (Fitzell, 1979). The fungus was reisolated from the lesions developed and its identity was confirmed.

Detached leaf technique

The young detached leaves of mango were inoculated with conidial suspension of *C. gloeosporioides* isolates containing 1×10^5 conidia ml^{-1} and incubated in transparent polyethylene bags. The bags were placed at room temperature (28 ± 2°C) for 4 days and disease severity was assessed and recorded. For comparison, control treatment was sprayed with sterile water. Four polythene bags (5 leaves per bag) were maintained for each treatment (Martinez *et al*., 2008).

Pin prick method

The conidial suspension of *C. gloeosporioides* isolate containing 1×10^5 conidia ml^{-1} were inoculated into fruits showing uniform size, free from bruise or blemish at full-three quarter stage of maturity by pin prick method (Swinburne, 1976). Inoculation was done at two equidistant points and placed in perforated polythene bags to maintain high humidity and incubated at room

temperature (28 ± 2°C). The symptom expression was recorded at 6 days after inoculation. The fungus was reisolated from the lesions of infected fruits and its identity was confirmed.

Morphological and cultural characteristics of the isolates

Mycelial growth

Fifteen ml of the sterile PDA medium was poured in to sterile Petri plates and allowed to solidify. A 9 mm culture disc of *C. gloeosporioides* was placed at the center of the Petri plate and incubated at room temperature (28±2°C) for 7 days. The radial growth of the isolates was measured at the end of the incubation period. In addition, the mycelial colour and the acervuli production (Akhtar *et al*., 1999) of the isolates were recorded. For each isolate three Petri plates were maintained.

Sporulation

Conidial suspension was prepared from 7 days old cultures of *C. gloeosporioides* by flooding the plates with 10 ml of sterile distilled water, which was then shaken to dislodge the conidia. The number of conidia of each isolate was estimated in a haemocytometer (Martinez *et al*., 2008).

Assessment of loss due to anthracnose in mango

Anthracnose incidence was recorded using the following scales

a. Scale for assessing anthracnose in leaves (Suharban *et al*., 1985)

Grade	Description
0	No spots
1	1-6 spots
2	7-10 spots
3	11-16 spots
4	17-26 spots
5	>26 spots

b. Scale for assessing the anthracnose in inflorescence (Jamadar and Desai, 1997)

Grade	Description
0	No infection observed
1	1-10 per cent
3	10.1-16.0 per cent
6	16.1-26 per cent
7	26.1-60 per cent
9	More than 60 per cent

c. Scale for assessing anthracnose in fruits (Prabakar *et al*., 2008)

Grade	Description
0	No infection
1	25% fruit surface infected
2	26 – 50 % fruit surface infected
3	51 – 75 % fruit surface infected
4	> 75% fruit surface infected

Disease assessment

Disease incidence was estimated by using the different scale and Per cent Disease Index was calculated using the following formula.

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Number of leaf/fruit examined} \times \text{Maximum grade}} \times 100$$

Results and Discussion

Virulence of different isolates of *C. gloeosporioides*

The data presented in table 2 revealed that the level of pathogenicity varied between the isolates. Among the twenty isolates of *C. gloeosporioides*, the isolate I_8 recorded the maximum disease incidence of 30.33 per cent in spray inoculation method, 38.33 per cent in detached leaf technique and 62.00 per cent in pin prick method. This was followed by the isolate I_1 and I_4 in the decreasing order of merit. The Isolate I_{13} was the least virulent as it recorded the least incidence in spray inoculation method, detached leaf technique and pinprick method (9.00, 11.67 and 15.00 per cent, respectively). The Isolate I_8 was found to be more virulent when compared to other isolates. The variation in anthracnose incidence could be well attributed to the difference in virulence of the *C. gloeosporioides* isolates prevalent in the respective areas. The differences in virulence obtained in this study agree with the earlier reports of many workers (Takustu and Rego, 1976; Katan, 2000; Martinez, 2008). Alahakoon *et al* (1994) indicated that the virulence of isolates of *C. gloeosporioides* seems to depend on inoculum density; however, in this work the concentration of inoculum was kept constant, so the differences in virulence found in this investigation are probably due to the existence of more than one race or special form of *Colletotrichum* (Fitzell, 1979; Prusky, 1994; Ploetz and Prakash, 2000). Such behavior could be due to the frequent application of fungicides with a single mode of action (Verdecia, 1999), which could encourage the emergence of more aggressive races that are more difficult to control.

Table 1 : Isolation of *Colletotrichum gloeosporioides* in major mango growing tracts of Tamil Nadu.

Variety	Isolate number	Place of collection	District
Neelam	I_1	Panruti	Cuddalore
Local variety	I_2	Srimushnam	Cuddalore
Banganapalli	I_3	Virudhachalam	Cuddalore
Neelam	I_4	Papparapatti	Dharmapuri
Alphonso	I_5	Palakodu	Dharmapuri
Local variety	I_6	Pennagaram	Dharmapuri
Bangalora	I_7	Koombur	Dindigul
Neelam	I_8	Vedasandur	Dindigul
Local variety	I_9	Sanarpatti	Dindigul
Neelam	I_{10}	Karimangalam	Krishnagri
Banganapalli	I_{11}	Oothangarai	Krishnagri
Neelam	I_{12}	Sirkhzli	Nagapattinum
Alphonso	I_{13}	Vedharanyam	Nagapattinum
Bangalora	I_{14}	Edappadi	Salem
Neelam	I_{15}	Mechari	Salem
Banganapalli	I_{16}	Oomalur	Salem
Bangalora	I_{17}	Periyakulam	Theni
Alphonso	I_{18}	Bodi	Theni
Local variety	I_{19}	Polur	Thiruvanaamalai
Neelam	I_{20}	Keelpennathur	Thiruvanaamalai

Cultural characters of *C. gloeosporioides* isolates

Colour of mycelium

The isolates of *C. gloeosporioides* showed variation with respect to colony colour. The colour of the isolates varied from normal white to light grey, grayish brown, grayish white, greenish grey, pinkish and pinkish brown (table 3). *Colletotrichum* species are highly variable, as manifested by colony morphology, conidial shape, presence and shape of setae, appressoria, pigmentation, fungicide sensitivity, pathogenicity and other traits (Katan, 2000; Martinez *et al*., 2008). The results obtained from the colony characters of *C. gloeosporioides* are in agreement with the reports given by (Jayasinghe *et al*., 1997; Jayasinghe and Fernando, 2009).

Mycelial growth

Among the isolates of *C. gloeosporioides*, the maximum mycelial growth (90.00 mm) was recorded by the isolates I_5, I_8, I_{18} and I_{20} followed by I_3 and I_{14} recorded 88 mm each at seven days after inoculation. The minimum mycelial growth (25.33 mm) was recorded in I_9 (table 3). Physiological studies not only form the basis for culturing pathogens but also prerequisite for effective planning and preparation for a successful crop protection. It also provides the vital information that has direct bearing

Table 3 : Morphological and cultural characteristic of *C. gloeosporioides* isolates.

Variety	Isolate number	Colour of mycelium	Mycelial growth (mm)	Sporulation×10^6 conidia ml^{-1}	Acervuli production
Neelam	I_1	Grayish white	76.67^c	08.64^f	+++
Local variety	I_2	Whitish	72.33^c	06.32^h	++
Banganapalli	I_3	Grayish brown	88.00^b	02.00^j	+++
Neelam	I_4	Grayish white	66.33^d	11.75^d	+
Alphonso	I_5	Whitish	90.00^a	05.22^h	++
Local variety	I_6	Grayish white	58.33^e	15.63^b	+++
Bangalora	I_7	Pinkish brown	78.00^c	03.13^j	+++
Neelam	I_8	Grayish white	90.00^a	17.47^a	+++
Local variety	I_9	Pinkish	25.33^h	02.87^j	+
Neelam	I_{10}	Grayish white	84.67^b	05.22^h	+++
Banganapalli	I_{11}	Grayish brown	50.33^e	07.23^g	+
Neelam	I_{12}	Pinkish brown	81.00^b	17.00^a	+++
Alphonso	I_{13}	Greenish grey	37.67^g	06.32^h	+++
Bangalora	I_{14}	Grayish white	88.00^b	08.33^f	++
Neelam	I_{15}	Pinkish	52.67^e	09.94^e	++
Banganapalli	I_{16}	Grayish white	66.00^d	10.45^e	+++
Bangalora	I_{17}	Pinkish brown	30.67^g	09.67^f	++
Alphonso	I_{18}	Whitish	90.00^a	15.33^b	+
Local variety	I_{19}	Grayish white	45.33^f	03.92^i	+
Neelam	I_{20}	Greenish grey	90.00^a	14.67^c	+++

+ - Poor, ++ - Good, +++ - Excellent; Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

on vigour disease incidence, pathogenesis and cultural characteristics of pathogens. Similar such variations in the mycelial growth among the isolates of *C. gloeosporioides* were observed by earlier workers (Martinez *et al*., 2008; Diedhiou *et al*., 2007). The results of the present experiments revealed that the isolates of *C. gloeosporioides* with faster mycelial growth were more pathogenic and produced higher disease incidence. The present results corroborate with the findings of Sangeetha and Rawal (2010), who reported that the isolates of *C. gloeosporioides* with faster mycelial growth as more pathogenic.

Sporulation and acervuli production

All the isolates of *C. gloeosporioides* varied in their ability to produce conidia on PDA. The maximum conidial population was recorded in I_8 (17.47×10^6 conidia ml^{-1}) also the acervuli production was excellent. This was followed by I_{12} and I_{18} recorded 17.00×10^6 and 15.33×10^6 conidia ml^{-1}, respectively. The minimum conidial population (02.87×10^6) and poor acervuli production was recorded in I_9 (table 3). Similar such variations with regard to the sporulation and acervuli production of *C. gloeosporioides* were observed by earlier workers (Martinez *et al*., 2008; Gunawardhana *et al*., 2009).

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