

## Cross-infection Potential of *Colletotrichum gloeosporioides* Penz. Isolates Causing Anthracnose in Subtropical Fruit Crops

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**ABSTRACT.** Anthracnose, caused by *Colletotrichum gloeosporioides* Penz. is considered the most important disease in fruit crops in the humid tropics that contributes significantly to preharvest and postharvest losses in mango, papaya, guava, custard apple, pomegranate and other subtropical fruit crops. The objective of this study was to test the ability of the pathogen *C. gloeosporioides* isolates from seven alternate fruit crops viz., mango, acid lime, custard apple, pomegranate, papaya, cashew and guava to cause disease by cross infection between fruit crops. Information on less infection would facilitate the design of an integrated approach for controlling the preharvest and postharvest losses due to anthracnose under mixed cropping systems especially in mango. Cross inoculation experiments demonstrated variation in the level of host preference and Percent Disease Index (PDI) among *C. gloeosporioides* isolates. The results revealed that among different fruit crops mango, cashew, pomegranate and custard apple were highly susceptible to the anthracnose disease. Isolate of *C. gloeosporioides* obtained from mango developed anthracnose symptoms on seedlings on all alternative fruit crops tested except on papaya, but it developed the symptoms on fruits of papaya. Mango isolate recorded maximum PDI of 86.7 on fruits of custard apple and minimum on acidlime fruits (12.8 PDI). The *C. gloeosporioides* isolates obtained from acid lime, custard apple, pomegranate, cashew and guava could infect the mango leaves and fruits except the papaya isolate which failed to infect the leaves, but produced infection on fruits of mango. Maximum PDI of 19.8 was recorded on mango leaves when inoculated with isolate from cashew, and a minimum PDI of zero and 3.4 was recorded when the leaves were inoculated with papaya and acid lime isolates respectively. Among different isolates of *C. gloeosporioides*, the cashew isolate was more virulent on mango leaves and fruits, followed by the custard apple and guava isolates.

**Key words:** *Colletotrichum gloeosporioides*, Cross inoculation, Fruit crops.

### INTRODUCTION

Mango is one of the world's most important and esteemed fruits of the tropical and subtropical countries and is cultivated extensively as a commercial fruit crop in India, China, Indonesia, Thailand and Mexico. By virtue of its wide range, delicious taste, superb flavor, very high nutritive and medicinal value as well as great religio-historical significance, it is called the "King of the fruits" (Hayes, 1953). In India, it occupies an area of 19.61X10<sup>5</sup> ha (36.7% of the total area devoted for fruit crops) with an annual production of 12537.9 MT,

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(21.3% of total fruit production of India) and a productivity of 6.2 MT per ha. (National Horticulture Board Report, 2008-09).

Various biotic and abiotic stresses cause immense loss to mango crop throughout the world. Among biotic stresses, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. in Penz.(the fungus teleomorph *Glomerella cingulata* (Stoneman) Spauld & Schrenk) is the most important and prevalent disease in all mango growing regions. It contributes significantly to preharvest and postharvest losses in mango and other fruit crops such as cashew, pomegranate, guava, acid lime and papaya. It is also manifested as leaf spot (Bird's eye disease), blossom blight or fruit rot (Prakash *et al.*, 1996). Crop losses caused by *C. gloeosporioides* generally occur as a direct reduction in quantity or quality of the harvested produce. Freeman and Shabi (1996) reported that mango, papaya, custard apple, guava and pomegranate suffered mostly due to latent infection of the pathogen.

In general, mango is cultivated as sole crop in a wide range of soils and climatic conditions. However in early stages of its growth (up to 15 years) it is cultivated along with cashew, guava, custard apple and acid lime as a mixed crop. The problem of anthracnose incidence is compounded by the mixed cropping system of horticulture practiced in Andhra Pradesh. Hence a cross inoculation study was conducted to test the ability and level of host preference of different isolates of *C. gloeosporioides* isolated from tropical fruit crops to cause disease on mango and isolates of mango on other fruit crops, to provide information that would facilitate to develop an integrated system for controlling post harvest losses of produce due to anthracnose disease.

## METHODOLOGY

*C. gloeosporioides* isolations were made from the leaves of mango (*Mangifera indica* L.), acid lime (*Citrus aurantifolia*), custard apple (*Annona squamosa*), pomegranate (*Punica granatum* L.), papaya (*Carica papaya*), cashew (*Anacardium occidentale* L.) and guava (*Psidium guajava* L.) showing typical symptoms of anthracnose disease by the tissue segment method (Rangaswami and Mahadevan, 1999), on potato dextrose agar medium (PDA). The fungus was further purified by single spore isolation method (Dhingra and Sinclair, 1993). The pathogen was identified as *Colletotrichum gloeosporioides* Penz., based on its mycelial, conidial characteristics through standard mycological keys (Barnett *et al.*, 1972). Isolates were stored on PDA for further studies.

### Pathogenicity study

#### The effect on seedlings

Pathogenicity of the different isolates of *Colletotrichum gloeosporioides* was tested by the spray inoculation method (Fitzell, 1979) on leaves of mango, acid lime, custard apple, pomegranate, papaya, cashew and guava seedlings. Each isolate of *C. gloeosporioides* collected from different fruit crops were artificially inoculated on mango leaves and the mango isolate was inoculated on leaves of other fruit crops seedling and subsequent anthracnose disease symptom development on leaves of seedlings were recorded to study the cross infection. For this leaves were slightly injured by the pin prick method and then inoculated by spraying spore suspensions of conidia ( $4 \times 10^4$  conidia ml<sup>-1</sup> water) 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 of the inoculum each time. The inoculated seedlings were covered with transparent polyethylene bags of (100 gauge) 15x 10" for 48 h to ensure high humidity by spraying sterile distilled water to provide favorable conditions for conidial germination and infection.

### The effect on fruits

Cross infection potential of isolates on different fruit crops were tested using matured healthy fruits of mango var. Baneshan, acid lime var. Balaji, custard apple var. Balanagar, pomegranate var. Bhaguva, papaya var. Red lady, cashew var. BPT 9 and guava var. Allahabad Safeda. Surface sterilized fruits were slightly injured with sterile needles aseptically and spray inoculated with *C. gloeosporioides* spore suspension ( $4 \times 10^4$  conidia ml<sup>-1</sup> of water with 0.01% Tween 20). Fruits sprayed with sterile distilled water served as the control. Each set of fruits was incubated separately in moist chambers and covered with perforated polythene bags to maintain a high humidity necessary for infection and these bags were removed 48 hrs after inoculation and maintained at room temperature (28±2°C). Lesion development was measured daily for each fruit. The fungus was re-isolated from the lesions of infected fruits and its identity was confirmed.

Three replications each containing eight seedlings /fruits were inoculated and maintained for each fungal isolate. The experiment was repeated twice. Observations on type of lesion development and number of days taken for lesion development on leaves and fruits were recorded after inoculation. The disease severity on leaves and fruits were assessed using a 0-5 scale.

### Disease severity

Disease severity on plant parts was recorded using a five point rating scale which was recommended by All India Co-ordinate Research Project on Sub-tropical Fruit Crops, Lucknow, India based on the percentage of leaf or fruit area affected by the disease, presented below.

Leaf / fruit area affected	Grade
No infection	0
Up to 5 percent	1
6 – 10 percent	2
11 – 20 percent	3
21 – 50 percent	4
More than 50 percent	5

### Percent disease index (PDI)

Based on the numerical ratings given above a 'Percent disease index' for leaf blight and fruit rot was calculated using the formula (Mayee and Datar, 1986) given below:

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of numerical ratings} \times 100}{\text{No. of units * examined} \times \text{Maximum grade}}$$

\* Unit = Leaves / fruits.

### Virulence index (VI)

The numerical values of Percent disease index and latent period were used to calculate the Virulence index using the following formula (Thakur and Rao, 1997).

Virulence index (VI) = Per cent disease reaction (PDI) X Latent period

### Statistical Analysis

The experiment was carried out using a completely randomized design (CRD) with three replications, analysis was done to compare significant differences among the isolates for pathogenicity, Latent period and Virulence index (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

*C. gloeosporioides* has an extensive host range particularly in sub tropical areas. Cross-inoculation experiments demonstrated variations in pathogenicity on their original host and on mango and mango isolates on other fruit crops. The level of host preference among *C. gloeosporioides* isolates from seven subtropical fruit crops and the susceptibility of the hosts varied significantly.

### Pathogenicity of *C. gloeosporioides* isolates on their original host

*C. gloeosporioides* isolates were established as the causative organisms of anthracnose disease in the fruit crops tested and recorded maximum per cent disease index, virulence index and minimum incubation period on their original host (Table 1). The results revealed that among different fruit crops cashew (64.3, PDI), custard apple (61.8, PDI) and mango (51.4, PDI), were highly susceptible to anthracnose disease than acid lime, papaya and pomegranate. A minimum mean PDI of 21.9% was recorded in acid lime seedlings. Incubation period of *C. gloeosporioides* on an average was higher on leaves (9.7 days) compared to fruits (4.8 days). However, on fruits the maximum PDI and virulence index were recorded maximum on custard apple (98.6% and 20.6%, respectively) followed by cashew (88.4% and 18%, respectively). *C. gloeosporioides* isolates produced larger lesions on their original host when compared with the alternate hosts (Wijeratnam *et al.*, 2008).

In the present study, the isolates of *C. gloeosporioides* from different fruit crops showed that *C. gloeosporioides* is the pathogen responsible for anthracnose in fruit crops. Sanders and Korsten (2003) reported that cross inoculation potential of *C. gloeosporioides*, isolates from avocado and mango showed larger lesions on their original hosts and produced lesions on all other hosts except citrus. Similar cross infectivity studies carried out by *Colletotrichum spp.* isolates from cashew, mango, papaya and passion fruit produced necrotic and depressed lesions on fruits, except on passion fruit, which was susceptible to its isolates only (Lima Filho *et al.*, 2003)

**Table 1. Pathogenicity of *C. gloeosporioides* isolates on their original host**

Isolated from	Percent disease index*			Incubation period (DAI)*			Virulence index*		
	Leaves	Fruits	Mean	Leaves	Fruits	Mean	Leaves	Fruits	Mean
Mango	30.4 (33.4) <sup>b</sup>	72.3 (58.3) <sup>c</sup>	51.4 (45.8) <sup>b</sup>	7.7 <sup>d</sup>	7.7 <sup>a</sup>	7.6	3.8 <sup>b</sup>	9.4 <sup>d</sup>	6.6 <sup>c</sup>
Acid lime	23.4 (28.9) <sup>c</sup>	20.5 (26.9) <sup>e</sup>	21.9 (27.9) <sup>d</sup>	15.3 <sup>a</sup>	7.5 <sup>a</sup>	11.4	1.5 <sup>c</sup>	2.7 <sup>e</sup>	2.1 <sup>d</sup>
Custard apple	25.1 (30.0) <sup>c</sup>	98.6 (84.6) <sup>a</sup>	61.8 (57.3) <sup>a</sup>	7.4 <sup>d</sup>	2.6 <sup>d</sup>	5.0	3.4 <sup>b</sup>	37.9 <sup>a</sup>	20.6 <sup>a</sup>
Pomegranate	29.1 (32.6) <sup>b</sup>	43.5 (41.3) <sup>d</sup>	36.3 (36.9) <sup>c</sup>	10.9 <sup>b</sup>	4.9 <sup>b</sup>	7.9	2.6 <sup>bc</sup>	9.0 <sup>d</sup>	5.8 <sup>c</sup>
Papaya	23.6 (29.1) <sup>c</sup>	43.9 (41.5) <sup>d</sup>	33.8 (35.3) <sup>c</sup>	8.3 <sup>c</sup>	3.9 <sup>bc</sup>	6.1	2.6 <sup>bc</sup>	11.1 <sup>d</sup>	6.8 <sup>c</sup>
Cashew	40.2 (39.4) <sup>a</sup>	88.4 (70.3) <sup>b</sup>	64.3 (54.8) <sup>a</sup>	7.1 <sup>e</sup>	4.5 <sup>b</sup>	5.7	5.7 <sup>a</sup>	18.0 <sup>c</sup>	11.8 <sup>b</sup>
Guava	22.7 (28.5) <sup>c</sup>	76.2 (60.9) <sup>c</sup>	49.5 (44.7) <sup>b</sup>	11.3 <sup>b</sup>	2.9 <sup>d</sup>	7.1	2.0 <sup>c</sup>	27.1 <sup>b</sup>	14.5 <sup>b</sup>
Mean	27.8	63.3		9.7	4.8		3.1	16.5	
SEm ±	0.8	1.5	2.1	0.2	0.3	0.5	0.7	1.2	1.7
C.D (P=0.05)	1.6	3.1	4.3	0.4	0.7	0.9	1.3	2.5	3.6

\* Means of three replications in each treatment. \*\*Figures in parentheses are arc sin transformed values.  
 \*\*\* Means with same letters were not significantly different within each column

**Cross infectivity of mango isolate of *C. gloeosporioides* on alternate fruit crops**

The isolate of *C. gloeosporioides* from mango caused infection on the leaves and fruits of each of these hosts; however, there was a significant variability in the degree of virulence. In fact, it showed typical symptoms of anthracnose on leaves and fruits of all the crops except on leaves of papaya. Table 2 indicates that there was a significant difference in preference of alternate host, maximum PDI of 22.6% was recorded on cashew leaves, whereas minimum PDI (0%) was recorded on papaya followed by acid lime (4%) and pomegranate (8.4%) seedlings.

**Table 2. Cross infection potential of *C. gloeosporioides* mango isolate on alternate fruit crops**

Isolated from	Percent disease index*			Incubation period(DAI)*			Virulence index*		
	Leaves	Fruits	Mean	Leaves	Fruits	Mean	Leaves	Fruits	Mean
Acid lime	4.0(11.4)	12.8(20.9)	8.4 (16.1)	19.9	11.5	15.7	0.1	0.7	0.4
Custard apple	12.6(20.7)	86.7 (68.7)	49.6 (44.7)	12.6	4.5	8.5	0.9	19.1	10.0
Pomegranate	84(16.8)	21.8 (27.7)	15.1 (22.3)	18.0	7.8	12.9	0.4	2.7	1.6
Papaya	00(0.0)	26.8 (31.1)	13.4 (15.5)	0.0	5.9	2.9	0.0	4.4	2.2
Cashew	22.6(28.6)	69.3 (56.3)	46.0 (42.4)	11.5	5.9	8.7	1.9	11.6	6.8
Guava	10.5(18.9)	60.4 (51.0)	35.5 (34.9)	18.4	3.8	11.1	0.5	15.8	8.2
Mango	30.4 (33.4)	72.3 (58.3)	51.3 (45.8)	7.7	7.6	7.6	3.8	9.4	6.6
Mean	15.6	50.0		12.6	6.7		1.1	9.1	
SEm±	0.6	1.2	1.8	0.1	0.3	0.4	0.2	0.5	0.7
C.D (P=0.05)	1.4	2.6	3.7	0.3	0.7	1.0	0.5	1.0	1.4

\* Means of three replications in each treatment. \*\*Figures in parentheses are arc sin transformed values.  
 \*\*\* Means with same letters were not significantly different within each column

The mango isolate was more aggressive on fruits of custard apple (86.7, PDI), cashew (69.3, PDI) and guava (60.4, PDI) with an incubation period of 4.5, 5.9 and 3.8 days respectively than the other fruit crops. In contrast *C. gloeosporioides* collected from mango was less vigorous on acid lime fruits compared to other fruits inoculated and a PDI of 8.4 was recorded with a maximum incubation period of 11.5 days. In case of papaya, inoculation mango with isolate succeeded to produce symptoms on fruits, 26.8 PDI with an incubation period of 5.9 days. The mean maximum Virulence index of 10.1 was recorded on custard apple and minimum virulence of 0.49 was recorded in acid lime followed by pomegranate (1.6) which was similar with each other. Similar results were reported by Quimo and Quimo (1975) on differences in the degree of pathogenicity of mango isolates of *C. gloeosporioides* on mango, citrus and papaya to cause infection on fruits of each of these hosts but there was variability in the degree of pathogenicity.

### **Cross inoculation studies of *C. gloeosporioides* collected from different fruit crops on Mango**

The isolates obtained from cashew, acid lime, custard apple, pomegranate and guava could infect the leaves and fruits of mango, except the papaya isolate which failed to infect the leaves of mango, but produced infection on mango fruits (Table 3). The isolate from cashew was more aggressive on leaves and fruits of mango and it recorded the maximum per cent disease index of 19.8 on leaves and 40% on fruits compared to other isolates with mean maximum VI of 4.1. Papaya, acid lime and pomegranate isolates were least effective on mango and caused a minimum PDI on leaves of mango (0, 3.4 and 4.3%, respectively) and 21.8, 9.3 and 14.3%, respectively on fruits. These isolates exhibited the symptoms of anthracnose on leaves of mango after maximum incubation period of 17.3 days when pomegranate isolate was inoculated, followed by acid lime isolate (16.8 days) which similar to each other.

On fruits of mango, a mean minimum incubation period of 3.7 days was recorded with the papaya isolate on mango and mean maximum incubation period of 12.6 and 12.4 days were recorded with acid lime and pomegranate isolates respectively. Papaya isolate recorded a Virulence index of zero on leaves of mango and 2.8 on mango fruits with a mean virulence index of 1.4. It showed the lower preference of mango by papaya, acid lime and pomegranate isolates. Similar results were reported by Sharma and Verma (2007) that the isolates obtained from mango, citrus, guava had capability to infect the host of each other except the acid lime leaves. However, Xiao *et al.* (2004) studied mango, citrus and strawberry and proved the cross inoculation potential of these isolates among themselves.

Simmonds (1965) demonstrated that *C. gloeosporioides* isolates from fruit crops could readily cross-infect over a wide host range, however, isolates were most aggressive in infecting the host from which they were originally isolated. In the present study the isolates of *C. gloeosporioides* collected from fruit crops such as citrus, pomegranate, cashew and guava caused anthracnose disease on mango seedlings and fruits. But the disease index was less on mango compared to PDI on their original host. *C. gloeosporioides* from papaya failed to develop the anthracnose symptoms on leaves of mango, but it induced the anthracnose disease on mango fruits more than acid lime and pomegranate isolates of *C. gloeosporioides*. However mango isolate was more aggressive on custard apple and cashew than the other tested fruits even on mango, the original host.

**Table 3. Cross infection potential of *C. gloeosporioides* collected from different fruit crops on mango**

Isolates	Percent disease index on mango*			Incubation period(DAI)*			Virulence index*			
	Leaves	Fruits	Mean	Leaves	Fruits	Mean	Leaves	Fruits	Mean	
Mango	30.4 (33.4)	72.3 (58.3)	51.2 (45.9)	7.7	7.6	7.6	3.9	9.4	6.7	
Acid lime	3.4 (10.5)	9.3 (17.6)	6.4 (14.0)	16.8	8.5	12.6	0.2	1.1	0.6	
Custard apple	11.2 (19.5)	19.2 (25.9)	15.2 (22.7)	9.6	7.6	8.66	1.1	2.5	1.8	
Pomegranate	4.3 (11.8)	14.3 (22.1)	9.3 (16.9)	17.3	7.4	12.4	0.2	1.9	1.1	
Papaya	0.0 (0.0)	21.8 (27.8)	10.9 (13.8)	0.0	7.5	3.7	0	2.8	1.4	
Cashew	19.8 (26.4)	40.0 (39.2)	29.9 (32.8)	9.7	6.9	8.3	2.5	5.8	4.1	
Guava	6.5 (14.7)	22.5 (28.3)	14.5 (21.5)	13.1	7.4	10.2	0.5	3.0	1.7	
Mean	10.8 (16.6)	28.4 (31.3)		10.6	7.5		1.2	3.8		
SEM ±		0.7	1.3	---	0.2	0.4	0.6	0.1	0.2	0.3
C.D(0.05)		1.4	2.7	NS	0.5	0.8	1.3	0.3	0.5	0.7

\* Means of three replications in each treatment. \*\*Figures in parentheses are arc sin transformed values

\*\*\* Means with same letters were not significantly different within each column

A single host may be infected by several *Colletotrichum* sp or a single species of *Colletotrichum* may infect more than one host (Alahakoon *et al.*, 1994; Bernstein *et al.*, 1995; Freeman and Shabi 1996 and Xiao *et al.*, 2004). As many hosts susceptible to *C. gloeosporioides* are cultivated, the losses in fields where mango, cashew, custard apple and guava are grown in close proximity could be high. Similar results were reported by Freeman *et al.* (1998) in mango, avocado, papaya and citrus in cross inoculation studies. Based on the present results, it can be concluded that in nature, inoculum may be dispersed from one crop to other crops. Further, such ability of the pathogen may provide an opportunity to the pathogen to survive during adverse periods in crops. In epidemiological studies it is essential to consider the source of inoculum from these sources in addition to infected debris.

The differences in capability of various isolates of *C. gloeosporioides* to invade different kinds of fruits tested can be attributed to variations in the composition of each kind of fruit. The available data of the present experiment suggests that the *C. gloeosporioides* is more pathogenic on fruits than on leaves. The isolates from mango and papaya failed to infect the leaves of alternate hosts but successfully penetrated the intact fruits. This might be due to accessibility of substrates such as pectin and cellulose for induction and secretion of cell wall degrading enzymes or due to the presence of cutinolytic enzymes secreted by the pathogen (Dickman, 1994). Prusky and Plumbley (1992) suggested that the susceptibility of fruits to *Colletotrichum* infection is related to the level of antifungal inhibitors present in these fruits. Vercesi *et al.* (1997) found that fruit exudates may significantly affect pathogen growth at an early stage of infection.

Isolates obtained from mango and cashew were the most pathogenic on leaves of the other fruit crops. This suggested that adaptation of *C. gloeosporioides* on relatively resistant hosts has resulted in the generation of more virulent isolates of the pathogen. Omar (2001) collected five isolates of *C. gloeosporioides* from diseased guava fruits and it successfully invaded mango, pear and apple fruits. However, the five isolates showed different pathogenic potential towards the four tested fruits. Enzyme activity was significantly different among these isolates.

## CONCLUSION

The cross inoculation experiments demonstrated variations in the level of host preference among *C. gloeosporioides* isolates from different fruit crops. Among the different fruit crops, mango, cashew and custard apple were highly susceptible to the anthracnose disease. The isolates of *C. gloeosporioides* obtained from acid lime, custard apple, pomegranate, cashew and guava could infect the mango leaves and fruits except papaya isolate which produced infection only on mango fruits. Among different isolates of *C. gloeosporioides*, the cashew isolate was more virulent on mango leaves and fruits followed by custard apple and guava isolates. This study established the possibility of cross infection between host organisms in the case of seven isolates of *C. gloeosporioides* with respect to examined varieties of hosts. This could result in the development of model orchards of mango mixed cropping systems, with the motto of an integrated, safe and convenient means of reducing post harvest losses in fruit crops.

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**Plate 1. Pathogenicity of mango isolates of *C. gloeosporioides* on other fruit crops**



**Plate 2.** Symptoms developed by cashew and custard apple isolates of *C. gloeosporioides* on mango