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Virulence Variation of *Colletotrichum gloeosporioides* (Penz.) Penz. and Evaluation of Varietal Susceptibility against Mango Anthracnose

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ABSTRACT

Anthracnose caused by *Colletotrichum gloeosporioides* is a major disease in many parts of the world where the climate is suitable for mango production to be the most important field and post harvest phase. The aim of the present work was to analyze virulence nature of *C. gloeosporioides* in the differentiation of isolates obtained from mango fruits and their potential to cause disease in different varieties. Survey was conducted in ten different districts of Tamil Nadu at post harvest phase. From the survey twenty six isolates of *C. gloeosporioides* were isolated and identified using morphological characters. Among the five artificial inoculation methods tested, pinprick plus spore suspension spray was the best suitable method. By this method out of twenty six isolates used, MCG 16 was identified as virulent isolate based on lesion diameter, per cent disease incidence and virulence index produced on inoculated fruits. MCG 16 was further used to test the varietal susceptibility of twelve different mango varieties and found that *Neelum* was the highly susceptible variety, in which the progression the lesion diameter was increased with increasing the incubation period.

Key words *mango, Colletotrichum gloeosporioides, virulence, varietal susceptibility*

Mango (*Mangifera indica* L.) is one of the high potential fruits in India, suitable for different agro-ecological zones of tropics and warmer sub-tropics. Like other horticultural crops mango is attacked by several pathogens which impair the quality and quantity of the fruit. The expected production of the king of fruits is likely to be 60 per cent down compared to previous years (Anon, 2013). However, huge losses of the crop are incurred mostly by fungal diseases. Anthracnose disease caused by *C. gloeosporioides* (Penz.) Penz. (Teliomorph: *Glomerella cingulata* (stoneman) Spauld. and H. Schrenk) is a major constraint to the production, persistence and utilisation of mango worldwide. Diseases caused by *Colletotrichum* are particularly troublesome on perennial crops and frequently cause significant economic losses (Dodd, *et al.*, 1992; Waller, 1992). On mango, anthracnose symptoms occur on leaves, twigs, petioles, flower clusters (panicles) and fruits. The post harvest phase is the most damaging and economically significant phase of the disease worldwide. Post harvest loss of horticulture produce varies between 5-39 per cent of total production. Ripe fruits affected by anthracnose develop sunken, prominent, dark brown to black decay spots before or after picking (Meer, *et al.*, 2013).

Accurate taxonomic identification is necessary for plant breeding purposes and disease management (Freeman, *et al.*, 1998). There is large variation among and within *Colletotrichum* species in pathogenicity, culture appearance and uncertain relationships with host plants (Sutton, 1992). Traditional approaches for identification of species belonging to the genus *Colletotrichum* as well as other filamentous fungi have always relied on morphological characteristics such as colony colour, size and shape of conidia, presence or absence of setae and teleomorph (Liyange, *et al.*, 1992), growth rate (Adaskaveg and Hartin, 1997) and cross infectivity (Wasantha Kumara and Rawal, 2004).

Establishment of disease by artificial inoculation is essential for studying various aspects of plant pathology. The wound/drop method has been shown to be useful to select resistant varieties of chili from susceptible varieties (Lin, *et al.,* 2002). Giri, *et al.*, 2013 demonstrated that spore suspension drop along with agarose inoculation method was most ideal as this fixed the inoculum on the target site. Each and every isolates of different pathogens found to differ in the level of expression of symptoms. The symptom expression varies between the isolates, in relation to the resistant and susceptible cultivars. Kolte and Sapkal, 1994 found that the virulence pattern of different isolates of *A. alternata* vary depending on the nitrogen level in the mycelium.

The study of resistance to anthracnose through screening in different genotypes was conducted by several workers in different crops *viz*., chilli pepper (Prasath, *et al*., 2007), cassava (Kunkeaw, *et al*., 2010), grapevine (Shelke, *et al*., 1997) and mango (Pandey, *et al*., 2011). The same species isolated from different hosts, has different cross infection ability and this should be considered when establishing new species. There have been several studies concerning cross infection of *Colletotrichum* species especially with *C. acutatum* and *C. gloeosporioides* species complexes (Abang, 2003; Sanders and Korsten, 2003; Kim, *et al*., 2009). Infection of fruits may be dependent on

Table 1. Collection and isolation of *C. gloeosporioides* isolates

Isolate	Variety	Place
MCG 1	Neelum	Rajapalayam
MCG 2	Mallika	Krishnagiri
MCG 3	Himayuddin	Dharmapuri
MCG 4	Raspuri	Krishnagiri
MCG 5	Mulgoa	Palani
MCG 6	Nadusala	Coimbatore
MCG 7	Himayuddin	Periyakulam
MCG 8	Banganapalli	Tenkasi
MCG 9	Mulgoa	Paiyur
MCG 10	Alphonso	Tirunelveli
MCG 11	Alphonso	Paiyur
MCG 12	Kalapad	Nagercoil
MCG 13	Sindhooram	Trichy
MCG 14	Mulgoa	Erode
MCG 15	Alphonso	Thiruvallur
MCG 16	Neelum	Theni
MCG 17	Neelum	Thanjavur
MCG 18	Banganapalli	Kanyakumari
MCG 19	Sindhooram	Namakkal
MCG 20	Neelum	Salem
MCG 21	Bangalora	Dharmapuri
MCG 22	KaruNeelum	Paiyur
MCG 23	Bangalora	Nagapattinam
MCG 24	Neelum	Madurai
MCG 25	Neelum	Kalakad
MCG 26	Mulgoa	Kayathar

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environmental factors such as variety of fruits, humidity, temperature and concentration of inoculum (Freeman, *et al*. 1998), rather than which *Colletotrichum* species colonizes it.

MATERIALS AND METHODS

Collection and establishment of isolates of *C. gloeosporioides:*

The anthracnose diseased samples were collected and isolation was carried out, following a standard tissue isolation method described by Rangaswami, 1958. The pathogen was identified up to species level based on their cultural and morphological characters according to Ekbote, *et al*., 1997. Pathogenicity was tested on mango fruits following the method described by Phoulivong, *et al*., 2012.

Methods of inoculation:

Artificial inoculation methods *in vitro* are commonly used to test the pathogenicity of a fungal species, as it is easy to control environmental conditions. Fully matured green unripe mango fruits and the fast growing isolate of *C. gloeosporioides* were used for this study. Common inoculation methods were practiced as reported by Manjunath, 2009. Three replications were maintained in each method. Eight days after inoculation, the lesion diameter was measured.

Table 2. Virulence variability in *C. gloeosporioides* isolates

Isolates	*lesion diameter (mm)	PDI	Virulence index
MCG 1	6.30^{def}	10.08^{gj} (18.47)	0.96^{ghi}
MCG 2	20.30^{b}	16.54^{bcd} (23.95)	1.57^{bcd}
MCG 3	17.70^{b}	16.08^{bcd} (23.59)	1.53^{bcd}
MCG 4	1.50^{g}	4.66^{l} (12.44)	0.44^{k}
MCG 5	8.40^{cd}	10.87^{fghij} (19.21)	1.03^{fghi}
MCG 6	4.40^{f}	5.67^{kl} (13.74)	0.54^{jk}
MCG 7	9.50^{cd}	13.56^{defg} (21.56)	1.29^{defg}
MCG 8	6.30^{def}	9.74^{hij} (18.14)	0.93^{hi}
MCG 9	38.00^{a}	19.15^{abc} (25.90)	1.82^{abc}
MCG 10	8.50^{cd}	11.55^{efghi} (19.82)	1.10^{efgh}
MCG 11	38.00^{a}	19.90^{ab} (26.44)	1.90^{ab}
MCG 12	4.80^{f}	7.93^{jk} (16.32)	0.75^{ij}
MCG 13	21.50^{b}	17.55^{bcd} (24.72)	1.67^{bcd}
MCG 14	10.10^{c}	14.70^{cdef} (22.49)	1.40^{cdef}
MCG 15	5.10^{ef}	9.11^{ij} (17.53)	0.87^{hi}
MCG 16	40.50^{a}	22.59^{a} (28.33)	2.15^{a}
MCG 17	0.00^{h}	0.00^{m} (0.16)	0.00^{l}
MCG 18	22.80^{b}	17.68^{bcd} (24.81)	1.68^{bcd}
MCG 19	8.90^{cd}	11.67^{efghi} (19.93)	1.11^{efgh}
MCG 20	10.30^{c}	15.33^{cde} (23.00)	1.46^{bcde}
MCG 21	7.60^{cd}	10.54^{ghij} 18.90)	1.00^{ghi}
MCG 22	17.70^{b}	15.45^{bcde} (23.10)	1.47^{bcde}
MCG 23	0.00^{h}	0.00^{m} (0.16)	0.00^{l}
MCG 24	0.00^{h}	0.00^{m} (0.16)	0.00^{l}
MCG 25	8.90^{cd}	13.48^{defgh} (21.49)	1.28^{defg}
MCG 26	21.50^{b}	17.76^{bcd} (24.87)	1.69^{bcd}
Control	0.00^{h}	0.00^{m} (0.12)	0.00^{l}

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5 % level by DMRT; Values in parentheses are arcsine transformed values.

Virulence of isolates:

Various isolates of *C. gloeosporioides* were inoculated on ripened fruits of mango by following pin prick plus spore suspension spray method as explained above. Three replications were maintained for each isolates with five fruits for each replication .The symptoms were observed

on eleven days after inoculation. Lesion diameter and PDI was calculated.

The Per cent Disease Index (PDI) was calculated by using the formula (Prabakar, *et al.,* 2005).

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of observations}} \times \frac{100}{\text{Maximum rating observed}}$$

The numerical values of per cent 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 index (PDI) X Latent period^{-1}

Varietal susceptibility studies:

The most virulent isolate of *C. gloeosporioides* was used for varietal screening according to the method suggested by Hong and Hwang, 1998. Inoculated fruits were placed in moist chamber at room temperature and data was collected as lesion size (mm) of each fruit tested.

RESULTS AND DISCUSSION

Anthracnose is the most common post harvest diseases in and around mango growing countries of the world. By analysing the morphological characters, pathogenicity, virulence potential and cross infectivity, twenty six isolates of *C. gloeosporioides*, were identified as pathogen of mango anthracnose.

Isolation and identification of pathogen from anthracnose infected mango fruits:

Twenty six isolates of *C. gloeosporioides* were isolated from the infected mango fruits of different varieties which showed anthracnose symptoms (Table 1; Plate 1). Symptoms appeared as brown to black lesions which occur larger than 2 cm on mango fruits. Identification of isolates was done based on the morphological descriptions of *Colletotrichum* species outlined by Mordue, 1971 and Sutton, 1992. Distinctness in spore morphology and colony characteristics among the isolates, resulted in morphological groups being identified and that was correlated with *C. gloeosporioides,* regardless of the host species from which they were obtained. In all the twenty six isolates, the conidia were hyaline with oil globules and the size ranges from 8.29 to 11.52 µm x 2.60 to 6.30 µm. The basic growth pattern and colony type for all isolates remained constant when grown out on PDA. But within the described groups, there was some variability in the colony growth, colour and pigmentation of mycelium, size of the conidia and rate of sporulation. This result confirms the observation of Photita, *et al.,* 2005 and Meer, *et al*., 2013.

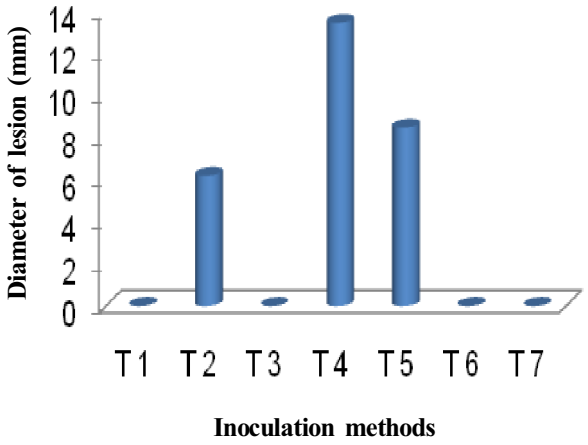

Fig. 1. Different method of artificial inoculation

T 1	Mycelial disc alone
T 2	Mycelia disc + Pin prick
T 3	Spore suspension spray
T 4	Spore suspension spray + Pin prick
T 5	Spore injection
T 6	Control - Pin prick alone
T 7	Healthy control

Pathogenicity tests indicated that all isolates caused similar anthracnose symptoms and reisolation from lesions always yielded an isolate with characteristics similar to the one used to inoculate the fruit. The fruits maintained as control did not show any symptoms (Plate 2). This supports the reports of several workers implicating *C. gloeosporioides* as the causal agent of anthracnose of mango (Sangeetha and Rawal, 2009; Jayasinghe and Fernando, 2009).

Method of inoculation:

Several studies have been conducted for comparing different inoculation methods against different pathogens for screening different varieties. For a successful screening, an adequate amount of inoculum is necessary. The conventional method like spraying has the disadvantage of causing considerable variation in spore distribution (Tuite, 1969). Accuracy and precision were improved by applying a drop of inoculum with a modified hypodermic needle (Lapwood and Mckee, 1966) or capillary pipette (Toussoun, *et al.,* 1960). The data obtained from the present experiments, although preliminary, gives a clear indication that there were indeed differences between the methods used. The results of different methods of artificial inoculation indicated that the lesion diameter was significantly varied with that of different methods of inoculation. Among all the methods tested, the lesion diameter was more in spore suspension spray plus pin prick method (13.50 mm) employed, followed by spore injection

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Table 3. Varietal susceptibility of mango against *C. gloeosporioides*

S. No.	Varieties	Diameter of lesion (mm) / Days after inoculation			
		3 DAI	5 DAI	7 DAI	11 DAI
1.	Banganapalli	2.23^{b}	7.12^{bcd}	14.18^{de}	21.79^{cd}
2.	Karuneelum	1.52^{c}	6.00^{cd}	9.45^{ef}	17.09^{d}
3.	Mulgoa	1.27^{c}	5.54^{d}	8.40^{f}	16.94^{d}
4.	Mallika	1.37^{c}	5.49^{d}	7.35^{f}	16.72^{d}
5.	Nadusala	2.49^{ab}	8.59^{bc}	19.95^{bc}	27.45^{c}
6.	Neelum	2.69^{ab}	12.98^{a}	29.93^{a}	38.08^{a}
7.	Banglora	2.26^{b}	7.48^{bcd}	16.38^{bcd}	24.18^{cd}
8.	Raspuri	2.92^{ab}	7.67^{bcd}	15.75^{bcd}	25.04^{cd}
9.	Kalapad	3.03^{a}	14.33^{a}	26.27^{a}	35.43^{ab}
10.	Senthuram	2.21^{b}	7.27^{bcd}	14.70^{cde}	22.55^{cd}
11.	Kudhathat	1.22^{c}	6.15^{cd}	7.88^{f}	17.01^{d}
12.	Imampasand	2.29^{b}	9.02^{b}	21.00^{b}	28.40^{bc}

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

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and mycelia disc plus pin prick method which accounts 8.50 and 6.20 mm, respectively (fig. 1). Because this wound drop method is more favourable since wounding allows the pathogenic isolate internal access to the fruit and enhances infection. This study is consistent with inoculation studies by Baayen and Schrama, 1990 in carnation, Buckley, *et al.,* 2009 in corn and Priyanka, *et al.,* 2013 in Indian mustard.

Variation in virulence of isolates:

Pathogenicity was the parameter that best separated the *C. gloeosporioides* isolates according to their virulent nature. Montri, *et al.,* 2009 showed virulent pathotype differences within *C. capsici* isolates based on percent lesion

Plate 1. Symptoms and culture of *C. gloeosporioides* causing mango anthracnose

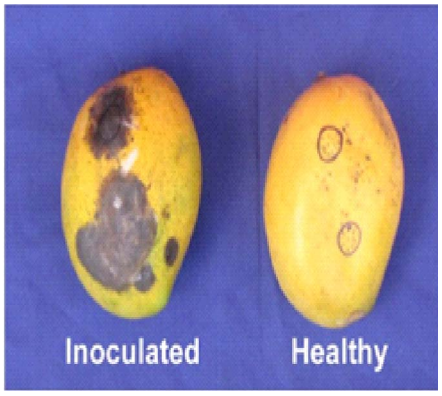

Plate 2. Pathogenecity test

size, appearance of necrotic or water-soaked tissue and presence of acervuli on *Capsicum* species.

In the present study, purified isolates were artificially inoculated on the mango fruits with spore suspension spray plus pin prick method and virulence were measured by lesion diameter on eleven days after inoculation revealed differences between isolates (Table 2). Among the sixteen isolates, MCG 16 produced highest mean lesion diameter (40.50 mm), followed by MCG 9 and MCG 11 (38.00 mm). Out of twenty six isolates, three isolates (MCG 17, MCG 23 and MCG 24) were not produced any lesions on fruits. The development of anthracnose symptoms on different fruits was statistically compared based on the percentage of lesion area, per cent disease index and virulence index. Of the twenty six isolates of *C. gloeosporioides*, maximum per cent disease index was recorded in MCG 16 (22.59 %) followed by MCG 11 and MCG 9 (19.90 and 19.15 %, respectively). Similarly, the isolate MCG 16 which is isolated from neelum variety and collected from Theni district produced highest virulence index of 22.15 than other isolates (Plate 3).

Based on the parameters of lesion diameter, per cent disease index and virulence index, out of twenty six isolates tested, three isolates *viz*., MCG 17, MCG 23 and MCG 24 considered as avirulent isolates. The isolate MCG 16 produced highest lesion diameter with highest virulence index, in the light of this, MCG 16 being the most virulent among the twenty six isolates. This was supported by Pandey, *et al.,* 2011 where he reported *C. gloeosporioides* isolates produced highest lesion growth over other isolates on fruit surface on mango hybrids. It can be argued that variation in the isolates may be inherent since isolates were collected from different sites; hence, the physiological characters are influenced by environmental conditions through natural chance mutations which may be responsible for such variability.

Plate 3. Virulence variability in *C. gloeosporioiddes* isolates

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Varietal susceptibility studies :

Fungus-host relationships are broad, imprecise and often overlapping. *Colletotrichum* species are cosmopolitan with either multiple species occurring on a single host or a single species occurring on multiple hosts and may adapt to new environments (Sanders and Korsten, 2003; Photita, *et al.,* 2004), leading to serious cross infection problems in plant production. The virulent isolate MCG 16 was used to study the varietal susceptibility of twelve different mango varieties of *viz*., *Banganapalli, Banglora, Imampasand, Kalapad, Karuneelum, Kudhathat, Mulgoa, Mallika (Neelum* X *Dashehari), Nadusala, Neelum, Raspuri* and *Senthuram* by artificial inoculation (Table 3). Typical anthracnose lesions were produced by MCG 16 on all the varieties tested. The development of anthracnose symptoms on different varieties were statistically compared based on lesion diameter at 3, 5, 7 and 11 days after inoculation on the fruit (Plate 4).

The fruits were started to produce anthracnose symptoms as lesions on three days after inoculation. The results revealed that, the range of lesion diameter on 3 DAI was from 1.22 to 3.03 mm. The size of the lesions was gradually increased as days progress. Eleven days after the inoculation, the lesion size was significantly higher in

1. Banganapalli **2. Karuneelum**
3. Mallika **4. Mulgoa**
5. Nadusala **6. Senthuram**
7. Kudhathat **8. Imampasand**
9. Banglora **10. Rasuri**
11. Kalapad **12. Neelum**

Plate 4. Varietal screening of *C. gloeosporioides* on different varieties of mango fruits

Neelum (38.08 mm) followed by *Kalapad* (35.43 mm), *Imampasand* (28.40 mm), *Nadusala* (27.45 mm). The least lesion size was recorded in *Mallika* (16.72 mm) on 11 days after inoculation which is *on par* with *Mulgoa* (16.94 mm), *Kudhathat* (17.01 mm) and *Karuneelum* (17.09 mm). Among the twelve different mango varieties tested, the lesion diameter was constantly superior in neelum variety compared to others over the incubation period and considered as highly susceptible to the virulent isolate MCG 16. This also reflected the ability of the virulent pathogen to cause larger spots on the highly susceptible fruits. The ability of *C. gloeosporioides* to infect a wide range of fruit crops was also reported by Quimio and Quimio, 1975 and Shabi, *et al.,* 1997. The study of pathogenic variability of *Colletotrichum* species is therefore important and understanding of the host range of a particular pathogen may help in efficient disease control and management (Weckert, *et al.,* 2007).

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