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Pathological and Cultural Variability in *Colletotrichum gloeosporioides* (Penz. & Sacc.) Inciting Anthracnose of Mango

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A B S T R A C T

Mango *(Mangifera indica* L.) is considered as one of the most popular fruits among millions of people in the tropical and sub-tropical area and increasingly in the developed countries. Anthracnose, caused by the fungus *Colletotrichum gloeosporioides*, is the most important pre- and post-harvest disease of mango. Twelve isolates of *C. gloeosporiodes* were obtained from Rewa (4 isolates), Sidhi (3 isolates), Satna (4 isolates) and Jabalpur (1 isolate) districts of Madhya Pradesh and screened in the greenhouse for virulence using spray inoculation method on a year old mango grafts and observed considerable variation in per cent disease index (PDI) and grouped in to different virulence categories. Results indicated that among the twelve isolates Cg_{11} exhibited maximum per cent disease index of 41.15 and Cg_2 recorded lowest per cent disease index of 21.31. Among the different media tested, Potato dextrose agar medium supported significantly the maximum growth of all the twelve isolates of *C. gloeosporioides*. Further, the strains were found to vary morphologically between the isolates under the study.

Introduction

Mango (*Mangifera indica* L.) is known as the national fruit of India and occupies nearly half of the total area under fruits in the country. India is native to mango and is also the largest producer of mangoes with 44.14 per cent of the total world production (Kusuma and Basavaraja, 2014). However, huge losses of the crop are incurred mostly by fungal diseases of which anthracnose caused by the fungus *Colletotrichum gloeosporioides* (Penz. & Sacc.) develops under humid and warm conditions and is the most important pre- and post-harvest pathogen. The genus *Colletotrichum* is a facultative parasite, recently designated as the world's eighth most important plant pathogen (Dean *et al.*, 2012). In India, losses in the field due to this disease have been estimated to be 2- 39 per cent. During storage, important losses, as high as 47.9 percent in July and 51.7 percent in August, were reported by Prabakar *et al.*, 2005. It causes damage to foliage and under crowded and moist conditions causes serious problems in nurseries and young orchards (Kumar and Rani, 2010). It can also cause flower set reduction resulting in yield losses in mango. Anthracnose symptoms occur on leaves, twigs, petioles, flower clusters (panicles), and fruits. On leaves, lesions start as small, angular, brown to blackspots that

can enlarge to form extensive dead areas. The lesions may drop out of leaves during dry weather. The first symptoms on panicles are small black or dark-brown spots, which can enlarge, coalesce, and kill the flowers before fruits are produced, greatly reducing yield. Petioles, twigs, and stems are also susceptible and develop the typical black, expanding lesions found on fruits, leaves and flowers. Ripe fruits affected by anthracnose develop sunken, prominent, dark brown to black decay spots before or after picking. Fruits may drop from trees prematurely. The fruit spots can and usually do coalesce and can eventually penetrate deep into the fruit, resulting in extensive fruit rotting. Most green fruit infections remain latent and largely invisible until ripening.

Thus fruits that appear healthy at harvest can develop significant anthracnose symptoms rapidly upon ripening. Fruits infected at mature stage carry the fungus into storage and cause considerable loss during storage, transit and marketing (Haggag, 2010). The degree of disease severity on host plants depends on the fungal pathotype. Therefore, information on variability among different isolates of *Colletotrichum* is an important aspect of research for developing eco-friendly management of anthracnose of mango.

Materials and Methods

Collection, establishment and pathogenicity of isolates of *C. gloeosporioides*

During 2014-15, an extensive survey was conducted in mango growing areas of Vindhya Region of Madhya Pradesh. Most of the mango orchards suffered severely by anthracnose disease The mango leaves or twigs showing typical anthracnose symptoms were collected in polythene bags from Rewa, Sidhi, Satna and Jabalpur districts of Madhya Pradesh and details of samples and place of collection were presented in Table 1. The pathogen was isolated from the mango leaves or twigs showing typical anthracnose symptoms by tissue segment method on potato dextrose agar medium (PDA). Small bits measuring about 3 mm size were cut off from the leaves showing lesions in such a way that it contained both infected and healthy portions and these bits were surface sterilized in 0.1 per cent mercuric chloride ($HgCl_2$) for 30 seconds followed by three washings in sterile distilled water. The bits were further transferred to sterile discs of blotting paper. The dried bits were subsequently transferred to potato dextrose agar (PDA) medium under aseptic conditions. The petriplates were incubated at 28 ± 2^0C for seven days for the growth of the fungus. The developed fungal colonies were purified by single spore isolation method. The pathogen was identified as *Colletotrichum gloeosporioides* (Penz. & Sacc.), based on its mycelial and conidial characteristics according to (Darshana *et al.*, 2014 and Sampath *et al.*, 2008) and maintained on PDA slants under controlled temperature.

The variation in pathogenicity of different isolates of *C. gloeosporioides* was tested by spray inoculation method. For this purpose one year old Langra grafts were chosen and wounds were made by pin prick method. The spore suspension was made through sterile distilled water. Spore suspension containing a load of 2×10^4 conidia ml^{-1} was inoculated on the wounded areas. Alcohol washed hand atomizer was used separately for spraying inoculums suspension of each isolate. After inoculation, the seedlings were covered with polythene bags for two days to ensure high humidity by spraying sterile distilled water to provide congenial conditions for conidial germination and infection. Three replications were maintained for each isolate. The disease severity was calculated rating scale at 8-10 days after inoculation by adopting 0-5 disease rating scale as detailed below.

Per cent disease index (PDI) was calculated by using following formula given by (Prabakar *et al.*, 2005).

Percent Disease Index (PDI) = (Sum of all numerical ratings X 100)/(Number of samples score X Maximun rating score)

The isolates were classified as highly virulent (HV), moderately virulent (MV) and less virulent (LV) based on PDI values. If PDI is > 40 %, the isolates were categorized under HV and PDI value ranging between 30-40 %, the isolates were grouped under MV. The isolates having less than 30 % PDI, were grouped under LV category.

Colony characteristics and radial growth

Different solid media mentioned below were used for assessing the growth of isolates of *C. gloeosporioides.* The mycelial radial growth (in mm) as well colony colour of mycelia on different media was recorded. The composition and preparations of the following media were obtained from "Ainsworth and Bisby's Dictionary of the fungi" by Ainsworth, 1961 and Plant Pathological methods, fungi and bacteria by Tuite, 1969. Each culture medium was prepared in one liter of water and autoclaved at 120°C at 15 psi for 20 min. These were cooled to 45°C and then poured in 90 mm Petridishes for solidification. Potato Dextrose Agar (PDA) Medium (Potato 200 g, Dextrose 20 g and Agar agar 20 g), Richards agar (Sucrose 50 g, Potassium nitrate 10g, Magnesium sulphate 2.5 g, Ferric chloride 10 ml and Agar agar 20 g), Czapek dox Agar Medium (Sodium nitrate 2 g, Potassium nitrate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 3 g, Sucrose 30 g and Agar agar 20 g) and Corn meal agar (Corn meal in infusion form 50 g, Agar agar 15 g)) were used to study the variability in cultural characteristics of *Colletotrichum gloeosporioides* isolates. For measuring the radial growth rate, all the isolates of *C. gloeosporioides* were inoculated in four replications at the centre of 90 mm PDA plates. Inoculum was in the form of 5 mm mycelial discs taken from margin of colonies grown on PDA plates. The plates were incubated at 25°C and the radial growth was measured (in mm) 6 days post inoculation. Four replications were maintained for each media. Colony radial growth (in mm) and colony colour were recorded six day after inoculation. The data collected were subjected to Completely Randomized Design for their significance (Gomez and Gomez, 1984).

Results and Discussion

Isolation and pathogenicity of *C. gloeosporioides*

A field survey was conducted in different locations of Madhya Pradesh which included different blocks in different districts. In total, 4 districts of Madhya Pradesh including Rewa, Sidhi, Satna and Jabalpur were surveyed for collection of anthracnose infected leaf samples. In these 4 districts, a total number of 9 blocks including 12 different places were surveyed. After subjecting the anthracnose infected leaf samples, in total 12 isolates of *C. gloeosporioides* were isolated and coded as Cg_1 to Cg_{12}. The detail of locations of leaf sample collection and coding of isolates has been given in table 2. The *C. gloeosporioides* isolates produced colonies which were white to grayish white on PDA and produced septate mycelium. Conidia were hyaline, unicellular and cylindrical or elliptical to dumbbell with rounded ends containing one or two oil globules were present towards ends of conidium. The conidia appeared in pinkish slimy drops on the culture plate. The morphology of the pathogen was in accordance with the descriptions given by Darshana *et al.*, (2014).

Langra mango grafts were used to prove the pathogenicity of different isolates of *C. gloeosporioides* using spray inoculation method. It was observed that within ten days of spraying of spore solution, lesions were developed on the inoculated leaves. Numerous brown to black irregular spots appeared on the surface of the leaves. In due course of time, the spots rapidly increased in size and coalesced to form elongated brown necrotic areas. To confirm the pathogen, *C. gloeosporioides* was re-isolated from the infected leaves and it was verified that to be the same as the original culture and hence Koch's postulates were proved for all the twelve isolates of *C. gloeosporioides*. Further, to identify the pathogenic variability among the isolates, per cent disease index (PDI) was calculated and based on PDI, the isolates were classified as less virulent, moderately virulent and highly virulent.

It was evident from the results (Table 3) that variation was observed among the isolates of *C. gloeosporioides* with regard to disease severity resulting in different degrees of per cent disease index (PDI). Based on PDI, two isolates namely Cg_9 and Cg_{11}were grouped under HV category where respectively 40.51 % and 41.15 % PDI was recorded. However 4 and 6 isolates were grouped under LV and MV category respectively. The less virulent isolates produced few numbers of circular brown necrotic spots. However, moderately virulent isolates produced numerous small necrotic spots. The highly virulent isolates produced large circular to irregular confluent necrotic areas.

Colony characteristics and cultural variability of the isolates

The growth characters of *C. gloeosporioides* isolates were studied on four different solid media. The colony radial growth and colony colour were considered as growth characters. The results showed that all the four media tested supported the mycelial growth of all the isolates of *C. gloeosporioides*. Further, it was observed that PDA medium maximum supported the growth of all the isolates of *C. gloeosporioides* followed by Richards agar medium. However, Czapek dox agar medium least supported the growth of isolates of *C. gloeosporioides*. Among different isolates of *C. gloeosporioides*, Cg_9 isolate was fastest growing on all the four tested media.

However, maximum radial growth of 34.33 mm of Cg_9 was recorded on PDA and recorded 34.33 mm radial growth after six days of incubation. Isolate Cg_2 was recorded as slowest growing isolate among all the twelve isolates and minimum radial growth of 13.67 mm of Cg_2 was recorded on Czapek dox agar medium. The detailed data for radial growth of all the twelve isolates on four different media has been presented in table 4.

Table 1: Rating scale for calculating disease severity

Leaf area affected	Score
No infection	0
Up to 5 per cent	1
6 – 10 per cent	2
11 – 20 per cent	3
21 – 50 per cent	4
More than 50 per cent	5

Table.2 Details of anthracnose infected samples collected for the isolation of *C. gloeosporioides* isolates from different districts of Madhya Pradesh

S.No.	Place of collection	Block	District	Code of isolate
1	Kuthulia	Rewa	Rewa	Cg_1
2	Govindgarh	Rewa	Rewa	Cg_2
3	Rewa	Rewa	Rewa	Cg_3
4	Amarpatan	Amarpatan	Satna	Cg_4
5	Bela	Amarpatan	Satna	Cg_5
6	Gurh	Raipur Kurchuliyan	Rewa	Cg_6
7	Gopalpur	Rampur Naikin	Sidhi	Cg_7
8	Kasauli	Sihawal	Sidhi	Cg_8
9	Rampur	Kushmi	Sidhi	Cg_9
10	Jabalpur	Jabalpur	Jabalpur	Cg_{10}
11	Satna	Satna	Satna	Cg_{11}
12	Maihar	Maihar	Satna	Cg_{12}

Table.3 Pathogenic variability among the isolates of *C. gloeosporioides* on Langra mango grafts

S. No.	Isolate	PDI (%)	Category of isolate
1.	Cg_1	22.22 (28.01)	LV
2.	Cg_2	13.33(21.31)	LV
3.	Cg_3	32.22 (34.57)	MV
4.	Cg_4	37.78 (37.88)	MV
5.	Cg_5	17.78 (24.91)	LV
6.	Cg_6	31.11 (33.84)	MV
7.	Cg_7	21.11 (27.28)	LV
8.	Cg_8	33.33 (35.23)	MV
9.	Cg_9	42.22 (40.51)	HV
10.	Cg_{10}	32.22 (34.53)	MV
11.	Cg_{11}	43.33(41.15)	HV
12.	Cg_{12}	31.11 (33.86)	MV
	S.Em±	1.47	-
	C.D. at 5%	4.35	-

Table.4 Effect of different solid media on the growth of *C. gloeosporioides* isolates

Code of isolate	Colony radial growth (in mm)			
	Potato dextrose agar	Richards Agar	Corn Meal agar	Czapek dox agar
Cg_1	21.67	19.67	17.33	16.33
Cg_2	18.00	16.00	14.00	13.67
Cg_3	26.33	23.67	18.33	17.00
Cg_4	30.67	25.67	21.33	22.33
Cg_5	19.33	18.33	15.67	15.33
Cg_6	25.33	22.67	18.33	17.33
Cg_7	20.67	19.33	16.67	15.67
Cg_8	28.00	24.33	19.67	18.33
Cg_9	33.67	28.67	27.00	27.67
Cg_{10}	26.67	23.33	18.67	16.67
Cg_{11}	34.33	29.67	27.33	28.67
Cg_{12}	24.67	22.33	18.67	16.67
S.Em±	0.59	0.4	0.56	0.46
C.D. at 5%	1.72	1.17	1.65	1.36

Table.5 Cultural characteristics (Colony colour) of *C. gloeosporioides* isolates on Different solid media

Code of isolate	Colony colour			
	Potato dextrose agar	Richards Agar	Corn Meal agar	Czapek dox agar
Cg_1	Blackish white	Blackish white	Blackish white	Blackish white
Cg_2	Blackish white	Blackish white	Blackish white	Greyish white
Cg_3	Blackish white	Blackish white	Blackish white	Blackish white
Cg_4	Pink	Pink	Pink	Pinkish white
Cg_5	Orange	Orange	Orange	Orange
Cg_6	Blackish white	Blackish white	Blackish white	Blackish white
Cg_7	Orange	Orange	Orange	Orange
Cg_8	Blackish white	Blackish white	Greyish white	Greyish white
Cg_9	Pink	Pink	Pink	Pinkish white
Cg_{10}	Blackish white	Blackish white	Blackish white	Blackish white
Cg_{11}	Pink	Pink	Pinkish white	Pinkish white
Cg_{12}	Greyish white	Greyish white	Greyish white	Greyish white

The isolates of *C. gloeosporioides* also exhibited variations in respect of colony colour (Table 5). The isolates Cg_1, Cg_6 and Cg_{10} produced blackish white coloured colonies on all the four media. However, isolate Cg_2 and Cg_8 produced blackish white coloured colonies on PDA and Greyish white coloured colonies on Czapek dox agar medium. Isolate Cg_4 and Cg_9 produced pink colour colonies on all the media except Czapek dox agar medium where they produced pinkish white colour colonies. However, isolate Cg_5 and Cg_7 produced orange colour colonies on all the media. There was absence of zonation or concentric rings in all the isolates on all the four tested media.

Mango is one of the most important fruit crop grown in tropical and subtropical regions of India having a great socio-economic significance. It is also known as "King of Fruits". In the present study, the symptoms produced by the pathogen on artificial inoculation were similar to the symptoms observed under natural infection. The symptoms observed under artificial conditions agreed with same type of natural symptom. The symptoms appeared as numerous brown to black irregular spots on the surface of the leaves which rapidly increased in size and coalesced to form elongated brown necrotic areas after some time. Similar symptom of anthracnose was also noticed on banana fruits with sunken lesions and covered with salmon colored acervuli (Sutton and Waterston, 1970).

The results of the present study show that, from large samples of *C. gloeosporioides* isolated from mango and different geographical locations, there is considerable variation among the isolates and the results are in agreement with Pandey *et al.*, 2011). Suvarna *et al.*, 2014 used the same method to study pathogenic variability among the seven isolates of *C. gloeosporioides* on one year old Beneshan grafts.

Every living being requires food for its growth and reproduction; fungi are not an exception to it. Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the medium which are required for their growth and other life processes. Neither all media are equally good for all fungi nor can be a universal substrate or artificial medium on which all fungi can grow well. So, different media were tried for the growth of *C. gloeosporioides*. In the present study, PDA followed by Richards Agar medium supported maximum the growth of *C. gloeosporioides*. This was in conformity with the findings of Singh *et al.*, 2006, who observed the maximum growth of *C. gloeosporioides* of Guava on PDA medium.

The present investigation revealed that the colony colour and growth of *C. gloeosporioides* varied on different media. This might be due to the variation in the nutritional requirement of the fungus. There was variation in colony colour and mycelia growth of different isolates even in the same media. Similar observations were made by Denobys and Baudry, 1995. It can be argued that variation in the isolates may be inherent since isolates were collected from different locality; hence the morphological and physiological characters are influenced by environmental conditions through natural chance mutations which may be responsible for such variability.

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