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Interaction of putative virulent isolates among commercial varieties of mango under protected condition

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Abstract
The present study was undertaken to know the interaction of the putative *Colletotrichum gloeosporioides* isolates from different regions of Karnataka. The cultural characteristics among the 10 isolates studied, showed that isolates Cg-1, Cg-2, Cg-4, Cg-5, Cg-6, Cg-7, Cg-8, Cg-10 were circular with smooth margin both on 7th and 12th day of inoculation. Excellent sporulation was observed in Cg-8 and Cg-4 isolate, good sporulation was observed in Cg-3 and Cg-9 isolates whereas, medium sporulation in Cg-1, Cg-2, Cg-5, Cg-6 and Cg-7 isolates and poor sporulation was observed in Cg-10 isolate. Among the varieties tested, none of them showed immune and resistant reaction to the disease under shade house condition. Dasheri (20%) exhibited moderately resistant reaction, Totapuri (28%) and Himayudhin (29.70%) exhibited moderately susceptible reaction whereas, Mallika (35%) and Kesar (47.30%) exhibited susceptible reaction. Alphanso (62.50%), Neelam (56.20%) and Raspuri (75%) exhibited highly susceptible reaction.

Keywords: *C. gloeosporioides*, mango anthracnose, virulence

Introduction
Mango (*Mangifera indica* L.) the most popular fruit crop grown throughout the tropics and subtropics is one of the most favoured fruits in the international market. It is the king of fruits because of its attractive fragrance, flavour, excellent taste, sweetness and beautiful colour. Mango productivity is affected by many problems limiting its production. Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., is the most important disease prevalent in all mango growing areas of the world and is often associated with high rainfall and humidity (Arauz, 2000) [2]. The pathogen affects young leaves, flower panicles and forms latent infections on the fruit (Dodd *et al*., 1992) [7], is also present in quiescent form in immature fruits. The disease is more significant in the postharvest stage. Emergence of new races of the pathogen is one of the main concerns now as it has become very difficult to manage this disease.

Material and methods
Collection, isolation, identification and proving the pathogenicity of the pathogen
The mango leaves infected with anthracnose were initially collected from mango orchards of different agro climatic zones of Karnataka as mentioned in Table 1 and used for isolation of the pathogen using Potato Dextrose Agar (PDA). Colonies which developed from such culture was periodically observed for mycelia growth and sporulation under microscopic. Mycelial and spore character was used as a means of identification of the pathogen. After identification, single spore isolation was done and the pure culture was maintained for further use. Pathogenicity and Koch's postulate were proved on the seedlings.

List of agro climatic zones

Sl. No.	Agro climatic zones	Isolates	Location
1	North eastern transition zone	Cg-1	Bidar
2	North eastern dry zone	Cg-2	Raichur
3	Northern dry zone	Cg-3	Bagalkot
4	Central dry zone	Cg-4	Tumkuru
5	Eastern dry zone	Cg-5	Bengaluru
6	Southern dry zone	Cg-6	Hassan
7	Southern transition zone	Cg-7	Mysuru
8	Northern transition zone	Cg-8	Belagavi
9	Hilly zone	Cg-9	Sirsi
10	Coastal zone	Cg-10	Mangaluru

Cultural variability of *C. gloeosporioides* isolates

All the isolates were grown for 4-19 days at 27 ± 1 ºC and cultural variability among the isolates in terms of colony character, type of margin, topography, colony colour, pigmentation and sporulation were recorded. The sporulation was graded as follows.

Grading for sporulation

Score	Grade	Conidia/microscopic field (400 x)
++++	Excellent	> 75
+++	Good	50-75
++	Moderate	25-50
+	Poor	1-25
-	No sporulation	---

Growth studies of isolates on PDA media

Colony growth of each isolates in terms of diameter was measured. Recording of observations started 48 hours after initiation of the experiment until the colonization of the entire surface of the culture medium. Per day growth rate for each isolates were calculated by using following formula.

$$\text{Per day growth rate} = \frac{\text{Diameter of Petri plate} - \text{Growth of the culture}}{\text{Number of days to cover the entire Petri plate}}$$

Prospecting the isolates for virulence *per-sey*

To know the virulence of the isolates collected from different agro climatic zones, all the isolates of *C. gloeosporioides* was inoculated to one year old seedlings of Alphonso by spray inoculation method containing a load of 2 x 10^4 conidia ml-1. The relative humidity of the plants were maintained for two days by spraying sterile distilled water to provide congenial condition for conidial germination and infection (Shivakumar *et al*., 2015) [17]. Three replications were maintained for each isolate. Based on the lesion development on the leaf area virulence of the isolates were identified. The disease severity was calculated at 8-10 days after inoculation by adopting 0-5 disease rating scale as mentioned below (Prabakar *et al.*, 2005) [11]:

Scale for anthracnose

Grade	Leaf area affected (%)
0	No infection
1	Up to 5
2	6-10
3	11-20
4	21-50
5	>50

Per cent Disease Index (PDI) was calculated by using following formula

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

Based on the PDI, (Prabakar *et al*., 2005) [11] the isolates were classified as highly virulent, moderately virulent and less virulent as given below. Highly virulent isolates were used for further studies.

Grouping for the virulence

Group	PDI (%)
Highly virulent	>40
Moderately virulent	>30-40
Less virulent	<30

Interaction of virulent isolates among commercial varieties of mango under protected condition

Evaluation of promising varieties of mango against *C. gloeosporioides* was carried out to identify the resistant varieties using virulent isolates. Different commercially growing mango seedlings *viz.*, Alphonso, totapuri, dasheri, baneshan, raspuri, mallika, neeelum, kesar, himayudhin varieties were collected from the nursery and maintained in Poly-house at College of Horticulture, Bengaluru whereas, the mature fruits were also collected from field and maintained in laboratory. All the seedlings and fruits were inoculated using pin prick method with a spore suspension of 2 x 10^4 conidia ml-1. The disease incidence was recorded using the 0-5 scale as described by Narasimhudu (2007) [9] and Per cent Disease Index was calculated.

Disease scale /Rating for disease resistance

Disease Resistant	Rating	PDI (%)
Immune	0	No infection
Resistant	1	1-10
Moderately Resistant	2	11-20
Moderately Susceptible	3	21-30
Susceptible	4	31-50
Highly Susceptible	5	>50

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

Results

Cultural variability of *C. gloeosporioides* isolates on potato dextrose agar media

All the 10 isolates were cultured on potato dextrose agar to know the difference in the cultural characters. The colony characters of Cg-1, Cg-2, Cg-4, Cg-5, Cg-6, Cg-7, Cg-8, Cg-10 isolates were circular with smooth at margin both on seventh and 12th day of inoculation, whereas, Cg-3 isolate was circular to oval colony with smooth margin on both seventh and 12th day of inoculation and Cg-9 isolate was circular with smooth wavy at margin (Table 1). The mycelial growth at the centre of the culture were flat, puffy cottony growth with raised centre in Cg-1 and Cg-8 isolate, whereas, aerial puffy cottony growth with raised center in Cg-2 Cg-7 and Cg-10 isolate. Cg-3, Cg-4, Cg-6 isolate showed aerial puffy cottony growth with uniform center, Cg-9 isolate showed flat colony, cottony growth with uniform center whereas, Cg-5 isolate showed spongy cottony growth with raised center at seventh day of inoculation.

On 12th day of inoculation Cg-1, Cg-4, Cg-7, Cg-8 and Cg-9 isolates showed flat colony, puffy cottony growth with uniform center whereas, Cg-3, Cg-6 and Cg-10 isolates showed aerial colony, puffy cottony growth with uniform center. Cg-5 isolate showed flat colony, spongy cottony growth with raised center. Some special appearance was also noticed in Cg-2 isolate as aerial colony, puffy cottony growth with raised center with black appearance. The mycelial colour of the culture were white in Cg-1, Cg-2, Cg-3, Cg-4, Cg-8 and Cg-10 isolates, greyish white in Cg-5 isolate. Cg-6 isolate showed dull white mycelial colour whereas pinkish white and grey to ash colour in Cg-9 isolate and Cg-7 isolate respectively at seventh day of inoculation at 12th day of inoculation showed greyish white mycelial colour in Cg-1,Cg-7 and Cg-8 isolates whereas, white in Cg-2, Cg-3, Cg-5 and Cg-10 isolates. Cg-4 isolate showed orangish white mycelial colour, Cg-6 isolate dull white colour and pinkish white in Cg-9 isolate.

Table 1: Cultural variability of different isolates of *Colletotrichum. gloeosporioides* of mango on potato dextrose agar media

Isolate	Pigmentation in young culture			Pigmentation in matured culture		
	7th day		Remarks	12th day		Remarks
	Centre	Margin		Centre	Margin	
Cg-1 (NETZ)	Dull white	White	No ring structure appearance	White	White	Orange ring structure at margin
Cg-2 (NEDZ)	Dull orange white	Dull orange	No ring structure appearance but black dots at centre	Dull orange white	Dull orange	No ring structure appearance but black dots at centre
Cg-3 (NDZ)	White	White	No ring structure appearance	White	White	No ring structure appearance
Cg-4 (CDZ)	Dull orange	Orange	More circular orange appearance	Orange	Orange	More circular orange appearance
Cg-5 (EDZ)	Prominent dot black	Dull white	Prominent black ring appearance	Dull white	White	Orangish black ring appearance at margin
Cg-6 (SDZ)	Dull white	Dull white	No ring appearance	Dull white	White	No ring appearance
Cg-7 (STZ)	Dull dot black	Dull dot black	Concentric black dot with orange ring appearance at margin	Dot black	Dot black	Concentric black dot with orange ring appearance at margin
Cg-8 (NTZ)	Dull grey	Dull white	Orange dull grey ring structure at margin and centre	Dull grey	White	Orange dull grey ring structure at margin and centre
Cg-9 (HZ)	Dull orange	Orange	More circular orange appearance	Dull orange	Orange	More circular orange appearance
Cg-10 (CZ)	Dull white	White	Circular orange ring appearance	White	White	Circular orange ring appearance

Morphological variability of *C. gloeosporioides* isolates on potato dextrose agar media

The morphological characteristics of the conidia of different isolates of *C. gloeosporioides* were recorded from the PDA culture and infected host tissue. Excellent sporulation was observed in Cg-8 and Cg-4 isolate (Table 2). Good sporulation was observed in Cg-3 and Cg-9 isolates whereas, medium sporulation in Cg-1, Cg-2, Cg-5, Cg-6 and Cg-7 isolates and poor sporulation was observed in Cg-10 isolate. On PDA media, conidia of all the 10 isolates showed hyaline, cylindrical to oval shape with both the apices rounded or one apex rounded and the other end pointed. Oil globules in the conidia varied from 0-2 in all the 10 isolates. On host surface, conidia of all the isolates were hyaline, cylindrical to oval shape with both the apices rounded or one apex rounded and the other end pointed with 0-2 oil globules in a conidia. The mycelial growth of the isolates ranged from 5-9.5 cm on PDA containing Petri plates.

Table 2: Cultural and morphological variability of different isolates of *C. gloeosporioides* of mango on potato dextrose agar media

Isolate	Mycelial growth (cm)		Sporulation	Shape of conidia	Oil globules
	7th day	12th day			
Cg-1 (NETZ)	6.5	9.0	+ +	Cylindrical to oval	0-2
Cg-2 (NEDZ)	7.5	9.5	+ +	Cylindrical to oval	0-2
Cg-3 (NDZ)	7.7	9.5	+ + +	Cylindrical to oval	0-2
Cg-4 (CDZ)	7.0	8.5	+ + + +	Cylindrical to oval	0-2
Cg-5 (EDZ)	5.0	7.5	+ +	Cylindrical to oval	0-2
Cg-6 (SDZ)	6.9	8.0	+ +	Cylindrical to oval	0-2
Cg-7 (STZ)	6.5	9.0	+ +	Cylindrical to oval	0-2
Cg-8 (NTZ)	7.5	9.0	+ + + +	Cylindrical to oval	0-2
Cg-9 (HZ)	7.6	8.5	+ + +	Cylindrical to oval	0-2
Cg-10 (CZ)	6.2	8.5	+	Cylindrical to oval	0-2

Sporulation Conidia /microscopic field (400 X)
++++ (Excellent) >75
+++ (Good) 50 - 75
++ (Medium) 25 - 50
+ (Poor) 1 - 25
- (No sporulation) No conidia

To prospect the isolates for virulence *per-sey*

The isolates were inoculated to leaves and result obtained as the day's increases lesion size and spots also increases. Size of the lesion varied for different isolates inoculated. Cg-8 inoculated plants showed maximum lesion size of 0.20 cm followed by Cg-2 (0.18 cm) (Table 3). Other isolates showed less lesion size Cg-1, Cg-4 and Cg-6 inoculated plants showed 0.12cm, Cg-7 (0.15 cm), Cg-3 (0.13 cm), Cg-9 (0.10 cm), and Cg-10 (0.11 cm). More number of lesions also developed in Cg-8 inoculated plants with 236 spots in less time. The result indicated that maximum PDI was recorded in Cg-8 (NTZ) *i.e.*, 54.4% followed by Cg-2 (49.6%) and Cg-7 (42.4%) and classified as highly virulent. Cg-1(36.8%) and Cg-4 (32.8%) were classified as moderately virulent whereas, Cg-3 (28.8%), Cg-5 (27.2%), Cg-6 (28.8%), Cg-9 (24%) and Cg-10 (16.8) were classified as less virulent (Table 4).

Table 3: Evaluation of virulence of *Colletotrichum gloeosporoides* causing anthracnose of mango using Alphonso seedlings

Sl. No.	Isolates	Plant part used	Date of inoculation	Size of the lesion (cm)	Number of lesions	PDI (%)
1	Cg-1 (NETZ)	Leaves	27/01/2017	0.12	222	36.8
2	Cg-2 (NEDZ)	Leaves	27/01/2017	0.18	224	49.6
3	Cg-3 (NDZ)	Leaves	28/01/2017	0.13	155	28.8
4	Cg-4 (CDZ)	Leaves	28/01/2017	0.12	209	32.8
5	Cg-5 (EDZ)	Leaves	27/01/2017	0.10	140	27.2
6	Cg-6 (SDZ)	Leaves	27/01/2017	0.12	155	28.8
7	Cg-7 (STZ)	Leaves	27/01/2017	0.15	182	42.4
8	Cg-8 (NTZ)	Leaves	27/01/2017	0.20	236	54.4
9	Cg-9 (HZ)	Leaves	27/01/2017	0.10	128	24
10	Cg-10 (CZ)	Leaves	28/01/2017	0.11	80	16.8

Table 4: Classification of different isolates of *C. gloeosporioides* based on PDI

Group	PDI (%)	Isolates
Highly virulent	>40	Cg- 8, Cg-7, Cg-10
Moderately virulent	>30-40	Cg-1, Cg-4
Less virulent	<30	Cg-3, Cg-5, Cg-6,Cg-9, Cg-10

Interaction of putative virulent isolates among commercial varieties of mango under protected condition

In order to identify the resistant sources screening of nine varieties of seedlings in shade house and eight varieties of mature fruits under laboratory condition was carried out. The PDI on varieties were recorded under shade house and lab conditions. The commercial variety were evaluated using 0-5 scale (Narasimhudu, 2007) [9]. Of the nine varieties screened, none of them found to be free from the disease but the varieties differed in severity. Among the varieties tested, none of them showed immune and resistant reaction to the disease under shade house condition (Table 5 and 6). Dasheri (20%) exhibited moderately resistant reaction. Totapuri (28%) and Himayudhin (29.70%) exhibited moderately susceptible reaction whereas Mallika (35%) and Kesar (47.30%) exhibited susceptible reaction. Alphanso (62.50%), Neelam (56.20%) and Raspuri (75%) exhibited highly susceptible reaction among other varieties to this pathogen. Among the eight varieties tested under lab condition, none of them were found to be free from disease in postharvest condition. Mallika (20%) exhibited moderately resistant reaction whereas, Kesar exhibited moderately susceptible reaction. All other varieties such as Alphanso (38%), Dasheri (46%), Neelam (46%), Baneshan (48%), Raspuri (36%) and Totapuri (47%) showed susceptible reaction.

Table 5: Evaluation of varieties of mango seedlings against *Colletotrichum gloeosporioides* of mango based on number of spots

Sl. No.	Varieties	No of spots			PDI	Reaction
		3 rd day	7 th day	14 th day		
1	Dasheri	91.33	111.67	123.67	20.00	MR
2	Himayudhin	86.00	105.00	140.33	29.70	MS
3	Neelum	105.67	118.33	271.67	56.20	HS
4	Mallika	160.00	175.00	181.67	35.00	S
5	Totapuri	140.67	155.67	157.67	28.00	MS
6	Kesar	145.33	163.67	171.00	47.30	S
7	Baneshan	225.33	243.67	246.67	30.00	MS
8	Raspuri	324.00	349.67	350.67	75.00	HS
9	Alphanso	250.00	252.00	254.67	62.50	HS
	Total	152.83	167.47	189.80	-	-
	S.Em ±	6.76	11.93	11.93	-	-
	CD @5%	19.94	35.19	35.20	-	-
	CV	7.66	12.33	10.89	-	-

Table 6: Evaluation of varieties of mango fruits against *Colletotrichum gloeosporioides* of mango based on size of the lesion

Sl. No.	Varieties	Size of the lesion		PDI	Reaction
		5th day	10th day		
1	Alphanso	0.88	1.69	38.00	S
2	Dasheri	0.77	1.91	46.00	S
3	Neelum	0.94	2.03	46.00	S
4	Baneshan	1.28	2.02	48.35	S
5	Mallika	0.35	0.82	20.22	MR
6	Kesar	0.51	0.84	22.50	MS
7	Raspuri	1.85	1.35	36.00	S
8	Totapuri	0.40	2.06	47.53	S
	Total	0.70	1.27	-	-
	S.Em ±	0.042	0.04	-	-
	CD @1%	0.17	0.18	-	-
	CV	10.59	6.16	-	-

Discussion

Mango anthracnose is one of the most important and destructive disease affecting the fruit quality. All isolates showed some variation in the culture. It may be beacouse all isolates were isolated from different geographical conditions. Similar results were also recorded by earlier researchers (Sutton, 1980; Zakaria and Bailey, 2000; Prashanth, 2007; Prasanna kumar, 2001; Pandey *et al*., 2012; Darshan *et al*., 2014; Chowdappa *et al*., 2012) [20, 21, 13, 12, 10, 6, 5] On host surface, conidia of all the isolates are hyaline, cylindrical to oval shape with both the apices rounded or one apex rounded and the other end pointed with 0-2 oil globules in a conidia. The mycelial growth of the isolates ranged from 5.0 -9.5 cm on PDA containing Petri plates. The results are in conformity with Prasanna, 2001; Rajesh, 1999; Serra *et al*., 2006; Adhikary *et al*., 2013; Zakaria and Bailey, 2000 [12, 14, 15, 1, 21].

The results indicated that maximum PDI was recorded in Cg-8 (NTZ) i.e., 54.4% followed by Cg-2 (49.6%) and Cg-7 (42.4%) and classified as highly virulent. Cg-1(36.8%) and Cg-4 (32.8%) were classified as moderately virulent whereas, Cg-3 (28.8%), Cg-5 (27.2%), Cg-6 (28.8%), Cg-9 (24%) and Cg-10 (16.8%) were classified as less virulent. The findings were compared with result obtained from Suvarna *et al.* (2014), whereas, Cg 14 exhibited maximum per cent disease incidence (56.40) and Cg 19 recorded lowest per cent disease incidence (23.43). Archana *et al*. (2014) [3] found MCG16 was identified as virulent isolate based on the lesion development, per cent disease incidence and virulence index produced on inoculated mango fruit; (Shivakumar *et al*., 2015; Sofi *et al*., 2013) [17, 18].

Among the fruits, Mallika showed moderately resistant and Kesar showed moderately susceptible whereas, other cultivar exhibited susceptible reaction. These results are in agreement with the reports of Sharma and Badiyala, 1999 [16]; Archana *et al*., 2014 [3]; Haq *et al*., 2013 [8]; Bhagwat *et al*., 2015 [4].

Conclusion

The different isolates of *Colletotrichum gloeosporioides* isolated from the different regions of Karnataka showed variability. The isolates, Cg-8, Cg7 and Cg 2 were highly virulent and Dasheri exhibited moderately resistant reaction.

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