

## EFFECTIVENESS OF CULTURAL PARAMETERS ON THE GROWTH AND SPORULATION OF *COLLETOTRICHUM GLOEOSPORIOIDES* CAUSING ANTHRACNOSE DISEASE OF MANGO (*MANGIFERA INDICA* L.)

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### ABSTRACT

*Colletotrichum gloeosporioides* causing anthracnose which is a serious post harvest disease in mango accounting for 15-20% loss. The variation in nutritional and physiological characteristics among five isolates of *C. gloeosporioides* collected from different agro-climatic regions of India was investigated. All the isolates showed differential response in requirements of media, temperature and media pH for growth and sporulation. Malt Extract Agar (MEA) medium was best suited for growth in terms of radial mycelial diameter for all the isolates. Among the studied isolates, Cg 72 (from Maharashtra) showed more virulence and maximum sporulation ( $137.5 \times 10^3 \text{ mL}^{-1}$ ) at 28°C and media pH 6. Maximum growth and virulence at 28°C was observed with Cg 62 isolate. Media of pH 6 was found to be most suitable for the growth of respective isolates (s), but Cg 62 which was collected from Bihar found most virulent in this experiment.

**Keywords:** *Colletotrichum gloeosporioides*, *Mangifera Indica* L., Radial Growth, Sporulation, Culture Media, Temperature, Media pH

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### 1. INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular seasonal fruit found mainly in the tropical and subtropical countries (Shad *et al.*, 2002). It is widely grown in different countries of the world and is attacked by a number of diseases of which anthracnose is one of the most common especially in India and loss due to this disease is substantial (Slade *et al.*, 1987). Anthracnose caused by *C. gloeosporioides* is reported on a wide variety of crop, including almond, avocado, apple, arabica coffee, guava, mango, dragon fruit, cassava, sorghum and strawberry (Amusa *et al.*, 2005; Masyahit *et al.*, 2009; Owolade *et al.*, 2009; Erpelding, 2010).

More recent reports on *C. gloeosporioides* revealed that ambient temperature, pH, free water or relative humidity above 95% were required for conidial germination and aspersorium formation (Shih *et al.*, 2000; Yakoby *et al.*, 2000). The infection was favoured at temperatures ranging from 20 to 30°C. Temperature and moisture requirements for infection are used to build forecasting systems for mango anthracnose a vital component for the disease management (Prakash and Srivastava, 1987). The fungus produces good aerial mycelium in Richard's and Brown's agar and profusely sporulates on oat meal and corn meal agar along with abundant development of acervuli in rings and few setae (Prakash and Srivastava, 1987).

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The optimum pH was between 5.8 and 6.5 and temperature was optimum at 25°C for growth but it ceased beyond 35°C. The fungus grew vigorously on starch and peptone. Glutamic acid and alanine supported best growth and sporulation (Prakash and Srivastava, 1987). Keeping in view of the above facts the present investigation was performed to assess the effect of media, temperature and media pH on the growth and sporulation of isolates of *C. gloeosporioides* collected from Andhra Pradesh, Madhya Pradesh, Bihar, Maharashtra and Uttar Pradesh.

## 2. MATERIALS AND METHODS

### 2.1. Collection and Isolation of Pathogens

The isolates of *C. gloeosporioides* were isolated from the samples collected from Chittoor (Andhra Pradesh), Rewa (Madhya Pradesh), Lucknow (Uttar Pradesh), Muzaffarpur (Bihar) and Dapoli (Maharashtra). A small section of anthracnose infected leaf was surface sterilized with 0.1% HgCl<sub>2</sub> and washed thoroughly with sterile distilled water. It was then inoculated on Potato Dextrose Agar (PDA) medium (Potato 200 g, Glucose 20 g, Agar 15 g, distilled water 1000 mL) and incubated at 28±2°C for 6 days.

A total of 5 *C. gloeosporioides* isolates (Cg 1, Cg 19, Cg 30, Cg 62 and Cg 72) were used in this study. These isolates were isolated, purified and maintained on Potato Dextrose Agar (PDA) slants under controlled temperature. Pathogenicity of these isolates was also confirmed suggested by Bhuvanaeswari and Rao (2001). All the collected isolates of *C. gloeosporioides* were submitted to National Agriculture Important Microbial Culture Collection (NAIMCC), Mau, Uttar Pradesh, India and accessions number were allotted (Table 1).

### 2.2. Preparation of Different Media and Inoculation

The fungal pathogen was inoculated on various types of media to identify the best suited media for its growth and sporulation. In this experiment, fourteen media (Himedia, Mumbai), *viz.*, Potato Dextrose Agar (PDA),

Corn Meal Agar (CMA), Cooke rose Bengal agar base (CRBA), Czapek Dox Agar (CDA), Pseudomonas Agar (PA), Limabean Agar (LA), Conn's Agar (CA), Yeast Dextrose Agar (YDA), Corn Meal Peptone Yeast Agar (CMPYA), Modified Czapek Dox Agar (MCDA), V-8 Juice Agar (VJA), Potato carrot agar (PCA), Malt extract agar base Mycological peptone (MEA) and Oat Meal Agar (OMA) were used 39, 17, 36.54, 49, 37.3, 23, 38, 35, 64, 45.36, 44.3, 24, 50 and 72.5 gram per liter respectively. All these were autoclaved at 121°C under 15 psi for 20 min. Five mm diameter identical fresh culture discs of different isolates (Cg 1, Cg 19, Cg 30, Cg 62 and Cg 72) were grown for 7 days old culture at 27±2°C dissolve in 1 ml distilled water and count.

### 2.3. Preparation of Media at Different level of pH and Inoculation

To assess the optimum media pH for the growth of *C. gloeosporioides* the fresh culture on Malt Extract Agar (MEA) medium of different pH level (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) was used in experiment. The media pH of the medium was adjusted with 0.1N NaOH or 0.1N HCl (Naik *et al.*, 1988). The medium was buffered with disodium hydrogen phosphate citric acid buffer according to the protocol (Vogel, 1951). For inoculation same method was adopted as mentioned earlier. The diameter of colony was observed at 3rd, 5th and 7th days after inoculation while the level of sporulation was recorded at 7th Day After Inoculation (DAI) by modifying the methods (Tastwal and Enagi, 2009).

### 2.4. Incubation at Different Temperature Regime

To study the growth and sporulation of *C. gloeosporioides* at different temperature regime fresh culture was prepared in the solid media *viz.* Malt Extract Agar (MEA). For inoculation same method was adopted as mentioned earlier and culture of different isolates were placed at different temperature regimes to study the best suited temperature level *viz.* (12, 16, 20, 24, 28 and 32°C). Data were recorded with the method (Kumara and Rawal, 2008).

**Table 1.** Morphological characteristic of different isolates from different geographical location of *Colletotrichum gloeosporioides* penz

Culture Code	Culture accession no.	Geographical location	Symptom of anthracnose	Mango substrate	Percent infection	Sporulation	Mean spore size (Length × breadth) (µm)	Colour of mycelial mat
Cg 1	NAIMCC-F-02694	Chittoor (Andhra Pradesh)	Partial	Leaf	50	**	13.49×4.75	White black
Cg 19	NAIMCC-F-02707	Rewa (Madhya Pradesh)	Typical	Leaf	100	***	14.20×5.25	Yellow white
Cg 30	NAIMCC-F-02719	Lucknow (Uttar Pradesh)	Typical	Fruit	70	Nil	16.15×7.28	Grayish white
Cg 62	NAIMCC-F-02730	Muzaffarpur (Bihar)	Typical	Leaf	100	***	15.78×4.38	White
Cg 72	NAIMCC-F-02735	Dapoli (Maharashtra)	Partial	Leaf	75	***	16.87×4.57	Cottony white

(\*; = Less sporulation, \*\*; = Moderate sporulation, \*\*\*; = Profuse sporulation)

## 2.5. Statistical Analysis

All treatments were designed in three factors factorial Completely Randomized Design (CRD) with five replications. Experimental data was statistically analyzed using O. P. Sheoran software version 1.0 (CCS HAU, Hisar). The Dendrogram was generated by Un-weighted Pair-Group Arithmetic Mean (UPGMA) using FREE TREE software version 0.9.1.50.

## 3. RESULTS

The effect of various factors such as media, media pH, temperature regimes and their combinations with days after inoculation on the growth (Table 2) and sporulation of *C. gloeosporioides* were studied for 5 isolates representing different agro climatic zones.

### 3.1. Effect of Media on Growth and Sporulation of *C. Gloeosporioides* Isolates

Malt Extract Agar (MEA) showed higher mean value (54.98 mm) of mycelia growth as compared to other evaluated media followed by Modified Czapek Dox agar medium MCDA (53.01 mm). Cooke rose Bengal agar base (CRBA) media exhibited the least mycelial diameter (18.29 mm). Pseudomonas Agar (PA) and Limabean Agar (LA) medium also recorded lower growth next to CRBA medium but both of them were found to be similar in response (Fig. 1A).

Maximum (46.12 mm) growth was noticed with Cg 72 isolate in over all media while the least growth was recorded in Cg 1 (36.34 mm) (Fig. 1B). Cg 1 showed comparatively slow growth in all the media evaluated for the radial growth. As far as the effects of interaction between media and isolates are concerned, maximum (60.88 mm) growth was recorded with Cg 72 isolate in the MEA media followed by MCDA (60.22 mm).

A significant interaction effect of media and incubation period revealed highest growth in MEA medium followed by MCDA both at 5 and 7th day after inoculation CDA and OMA medium were also at par with MEA medium at 7th days after inoculation. The period of incubation clearly indicated that radial growth of the mycelia increased with increase in incubation period (days after inoculation) (Fig. 1C). The data of interaction between isolates and incubation period clearly showed that mycelial growth in all the isolates increased with advancement of incubation period. CRBA medium did not follow significantly for growth enhancement throughout the incubation period. With respect to the isolate effect, maximum radial growth was

recorded at 7th day in Cg 72 (71.27 mm) while minimum was found in Cg 1 isolate at 3rd day after inoculation. It is also evident that mycelial growth of *C. gloeosporioides* was significantly influenced by interaction of media  $\times$  isolates  $\times$  incubation period. Higher radial growth (87.67 mm) was observed in Cg 19 isolate in Oat Meal Agar (OMA) medium at 7th day of incubation followed by the same isolates in Malt extract agar (86.33 mm), Cg 72 (85.33 mm) and Cg 19 (85.17 mm) in CDA, Cg 72 in MEA (84.0 mm) and MCDA (83.67 mm) at 7th day of incubation.

In the experiment of sporulation in different isolates was also significantly varied by media (Table 3). Highest spore count ( $137.5 \times 10^3 \text{ mL}^{-1}$  of culture suspension) was recorded in Cg 72 isolate on MEA medium followed by Cg 19 on CDA medium ( $102.5 \times 10^3 \text{ mL}^{-1}$  of culture suspension).

### 3.2. Effect of Media pH on Growth and Sporulation of *C. Gloeosporioides* Isolates

The media pH is another important factor like temperature and media that influences growth and sporulation significantly. The effect of different media pH on the growth was observed considering test isolates at different incubation period.

It is evident from the data, that mycelial growth in different isolates significantly differed by pH range of the media. The media pH of 6.0 exhibited higher mycelial growth (54.43 mm) over other level of pH studied (Fig. 2A). Among the isolates, Cg 62 resulted in highest growth (43.86 mm) at this pH followed by Cg 1 (Fig. 2B).

The effect of media pH  $\times$  incubation period interaction with respect to radial mycelial growth was significant. The radial growth pattern was single sigmoid with respect to media pH with peak inferring highest radial growth at 7th day of incubation in media pH 6. Cg 62 and Cg 1 isolate showed the highest radial growth (67.60 mm) at the 7th day of incubation, while minimum was found with Cg 19 isolate at 3rd day of incubation. It is interesting to note that radial growth of all the isolates was at par with each other on 5th day of incubation. However, radial growth of all the isolates increased with the advancement of incubation period (Fig. 2C).

Three factors interactions among media pH, isolates and incubation period revealed that mycelial growth of all the test isolates increased with incubation period in corresponding order. Higher radial growth (83.20 mm) was recorded with Cg 1 isolate followed by Cg 62 (82.40 mm) and Cg 30 (81.40 mm) grown in the media 6.0 pH at 7th day of incubation.

**Table 2.** ANOVA for different isolates of *C. gloeosporioides* under different media, media pH and temperature regimes

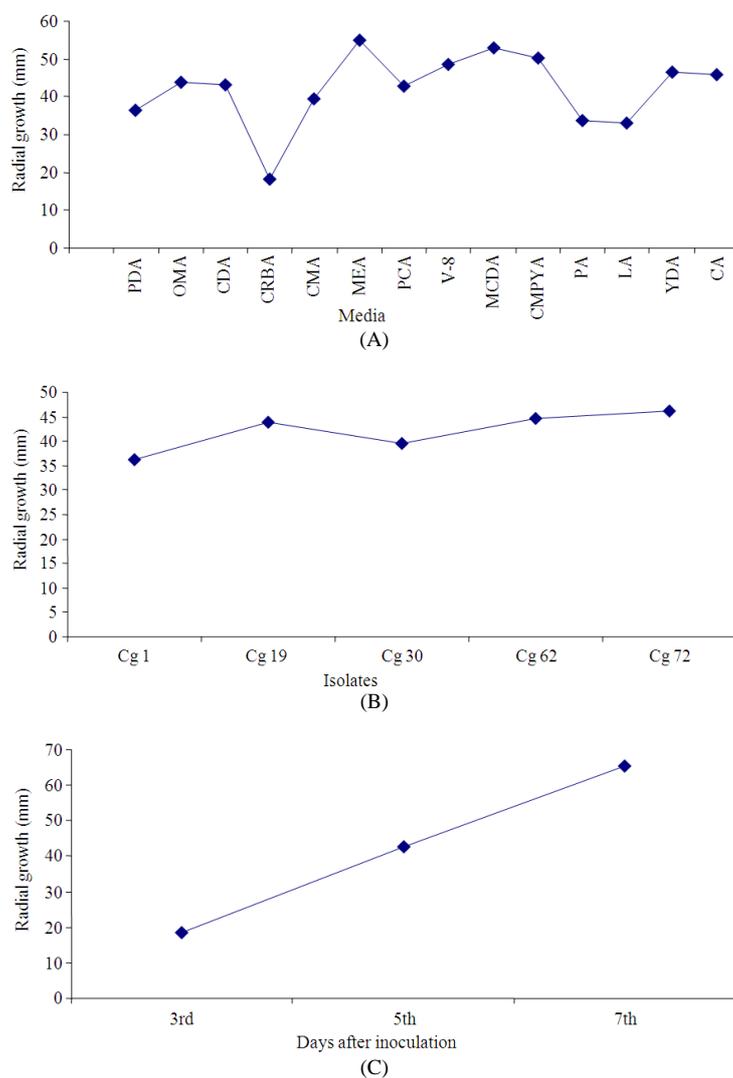
Source of variance	Degree of freedom	Sum of squares	Mean of sum of squares	F-calculated	P value	Critical Deference (C.D.)
Media	13	53381.45	4106.27	393.35	0.00001	1.339
Isolates	4	8248.73	2062.18	197.54	0.00001	0.800
Day after inoculation	2	231091.70	115545.80	11068.40	0.00001	0.620
Media × isolates	52	6161.30	118.49	11.35	0.00001	2.994
Media × Day after inoculation	26	30117.31	1158.36	110.96	0.00001	2.319
Isolates × Day after inoculation	8	2282.16	285.27	27.33	0.00001	1.386
Media × isolates × Day after inoculation	104	5671.09	54.53	5.22	0.00001	5.186
Media pH	5	26177.96	5235.59	2008.54	0.00001	0.519
Isolates	4	1685.27	421.32	161.63	0.00001	0.473
Days after inoculation	2	178894.50	89447.26	34314.81	0.00001	0.367
Media pH × isolates	20	740.12	37.01	14.20	0.00001	1.159
Media pH × Days after inoculation	10	5125.64	512.56	196.64	0.00001	0.898
Isolates × Days after inoculation	8	338.65	42.33	16.24	0.00001	0.820
Media pH × isolates × Days after inoculation	40	1641.72	41.04	15.75	0.00001	2.008
Temperature regime	5	33325.10	6665.02	4697.35	0.00001	0.383
Isolates	4	659.96	164.99	116.28	0.00001	0.349
Days after inoculation	2	176546.00	88272.98	62212.75	0.00001	0.271
Temperature regime × isolates	20	102.86	5.14	3.62	0.00001	0.855
Temperature regime × Days after inoculation	10	6349.33	634.93	447.49	0.00001	0.663
Isolates × Days after inoculation	8	32.38	4.05	2.85	0.00437	0.605
Temperature regime × isolates × days after inoculation	40	118.47	2.96	2.09	0.00023	1.482

**Table 3.** Sporulation of different *Colletotrichum gloeosporioides* isolates under different media pH

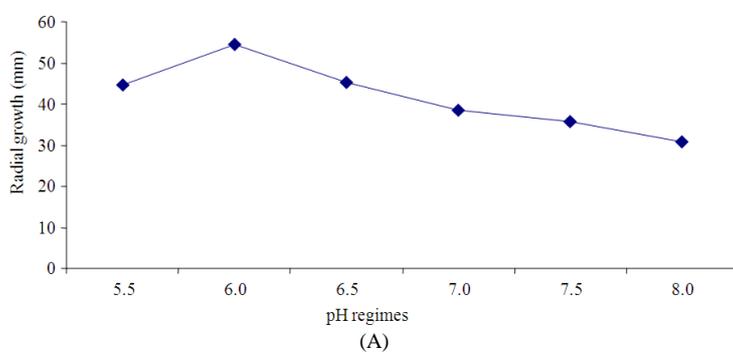
pH ranges	Cg 1	Cg 19	Cg 30	Cg 62	Cg 72	Mean
5.5	74.0000	151.00	200.3300	80.00	135.0000	128.06
6	51.0000	65.00	101.0000	63.00	94.0000	74.80
6.5	12.0000	26.00	40.0000	34.00	21.0000	26.60
7	11.0000	11.00	9.0000	15.00	21.0000	13.40
7.5	7.0000	7.00	10.0000	11.00	15.0000	10.00
8	7.0000	4.33	9.0000	3.66	1.6600	5.13
Mean	27.0000	44.05	61.5500	34.44	47.9400	
<b>Factors</b>	C.D.		SE(d)		SE(m)	
pH ranges	0.6973		0.3485		0.2465	
Isolates	0.6366		0.3182		0.2250	
pH ranges x isolates	1.5590		0.7794		0.5511	

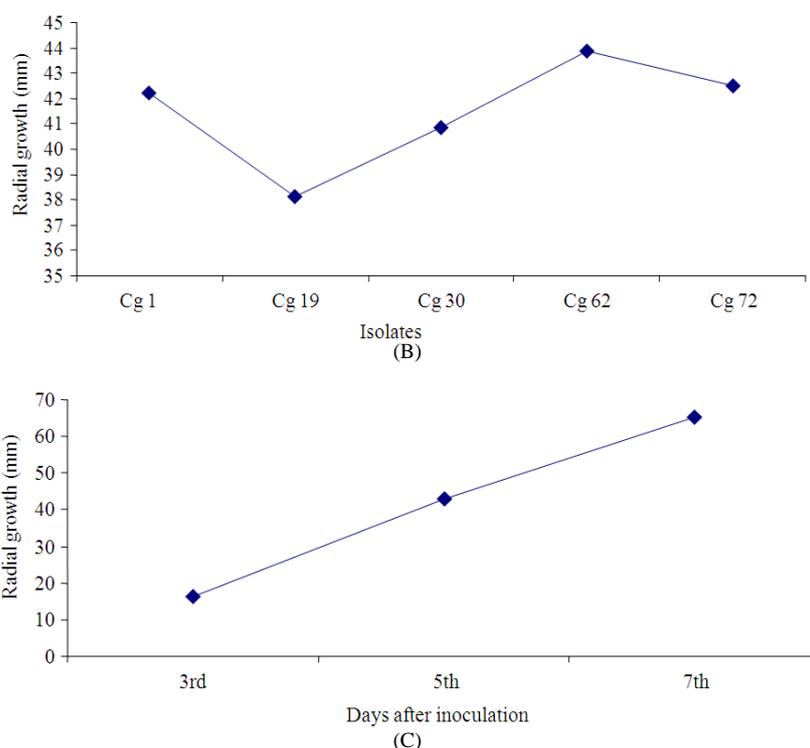
**Table 4.** Sporulation of different *Colletotrichum gloeosporioides* isolates under different temperature regimes

Temperature regimes (°C)	Cg 1	Cg 19	Cg 30	Cg 62	Cg 72	Mean
12	2.00	4.0000	3.33	2.0000	7.3300	3.73
16	6.33	8.0000	9.00	3.0000	12.0000	7.66
20	20.00	16.0000	15.00	15.0000	12.0000	15.60
24	51.00	43.0000	34.66	30.0000	42.0000	40.13
28	103.00	99.0000	111.00	126.0000	114.0000	110.60
32	76.33	59.0000	66.66	60.0000	62.6600	64.93
Mean	43.11	38.1600	39.94	39.3300	41.6600	
<b>Factors</b>	C.D.		SE(d)		SE(m)	
Temperature regimes	0.7466		0.3732		0.2639	
Isolates	0.6816		0.3407		0.2409	
Temperature regimes x isolates	1.6690		0.8344		0.5900	



**Fig. 1.** (A) Radial growth of *Colletotrichum gloeosporioides* on the different media (B) Radial growth of different *Colletotrichum gloeosporioides* isolates under different media (c)Radial growth of *Colletotrichum gloeosporioides* at different incubation period under different media

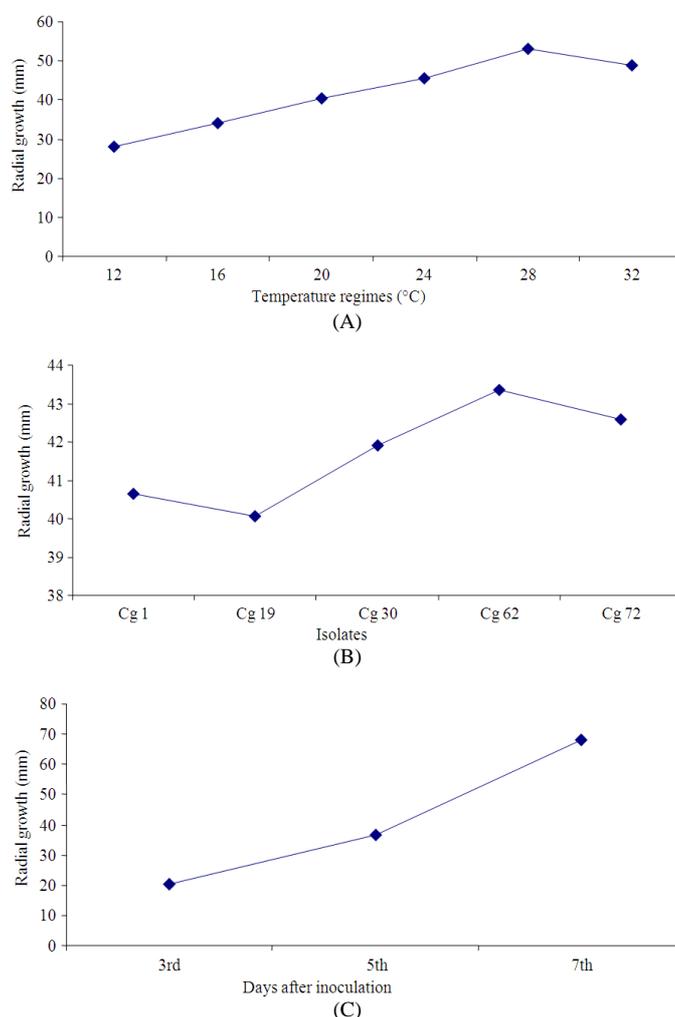




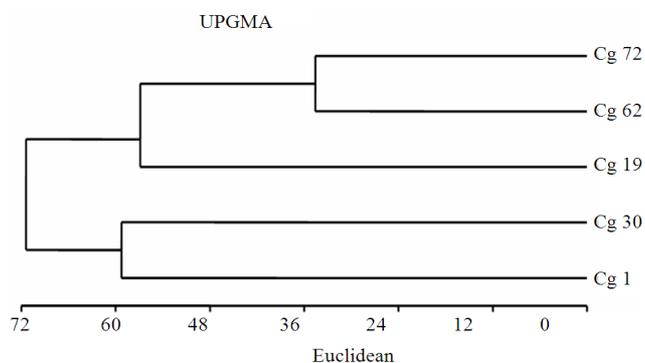
**Fig. 2.** (A) Radial growth of *Colletotrichum gloeosporioides* on the different media pH (B) Radial growth of different *Colletotrichum gloeosporioides* isolates under different media pH (C) Radial growth of *Colletotrichum gloeosporioides* at different incubation period under different media pH

**Table 5.** Sporulation of different *Colletotrichum gloeosporioides* isolates under different media

Media	Cg 1	Cg 19	Cg 30	Cg 62	Cg 72	Mean
PDA	6.0000	5.00	1.3300	7.0000	8.0000	5.46
OMA	3.3300	37.00	81.0000	56.0000	73.3300	50.13
CDA	31.6600	103.00	14.0000	33.6600	22.0000	40.86
CRBA	2.0000	67.00	56.0000	2.3300	41.0000	33.66
CMA	23.0000	5.00	20.0000	21.0000	43.0000	22.40
MEA	20.3300	12.00	3.0000	18.0000	137.6600	38.20
PCA	67.3300	30.33	27.0000	56.0000	90.3300	54.20
V-8	4.000	6.00	12.0000	18.0000	3.0000	8.60
MCDA	3.0000	19.00	4.0000	2.3300	90.3300	23.73
CMPYA	34.0000	51.33	3.0000	12.0000	10.0000	22.06
PA	4.0000	2.33	3.6600	4.0000	5.3300	3.86
LA	3.0000	3.00	2.3300	3.0000	5.0000	3.26
YDA	3.0000	8.00	5.3300	8.0000	2.0000	5.26
CA	21.0000	13.33	2.3300	3.3300	3.0000	8.60
Mean	16.1100	25.88	16.7800	17.4700	38.1400	
Factors	C.D.		SE(d)		SE(m)	
Media	0.7401		0.3723		0.2632	
Isolates	0.6413		0.3116		0.2217	
Media x isolates	1.6426		0.8208		0.5814	



**Fig. 3.** (A) Radial growth of *Colletotrichum gloeosporioides* on different temperature regimes (B) Radial growth of different *Colletotrichum gloeosporioides* isolates under different temperature regimes (C) Radial growth of *Colletotrichum gloeosporioides* at different incubation period under different temperature regimes



**Fig. 4.** Dendrogram generated on the basis of different media, media pH and temperature regimes data by Un-weighted Pair-Group Arithmetic Mean (UPGMA) method from the cultural variability of five *Colletotrichum gloeosporioides* isolates

Sporulation was highest at media pH 5.5 in all the isolates followed by media pH 6. However, as media pH increased the sporulation was increased consistently in all the test isolates (**Table 4**).

### 3.3. Effect of Temperature Regimes on Growth and Sporulation of *C. Gloeosporioides* Isolates

It was observed that growth of mycelia in different isolates significantly varied with temperature regimes and incubation period. Higher (53.17 mm) radial growth of mycelia was observed at 28°C while lesser growth (28.03 mm) was at 12°C (**Fig. 3A**). Among the isolate Cg 62 showed highest growth (43.36 mm) than other isolates, whereas least growth (40.07 mm) was observed in Cg 19 (**Fig. 3B**). A significant effect of temperature x isolates interaction on the radial growth of mycelia was noticed where in maximum growth was recorded with Cg 62 isolate followed by isolate Cg 72 at 28°C.

The radial growth of mycelia increased with advancement of incubation period of the culture (**Fig. 3C**). The interaction between temperature and incubation period of culture also showed significant effect on mycelial growth. Statistically significant average growth (82.24 mm) was observed at 28°C after 7th days of incubation.

It is evident from the data that interaction between isolates and incubation period exhibited significant difference in mycelial growth of all the isolates. Maximum radial growth was recorded at 7th days of incubation with Cg 62 isolate (69.80 mm) while minimum was with Cg 19 isolate (18.57 mm) at 3rd day of incubation. Radial growth of mycelia was significantly influenced by interaction of temperature x isolates x incubation period. Higher radial growth was observed with Cg 62 isolate (84.60 mm) followed by Cg 72 isolate (84.20 mm) at 7th day of incubation in 28°C.

Temperature showed marked effect on the sporulation in the culture of test isolates. The best sporulation was recorded ( $125 \times 10^3 \text{ mL}^{-1}$  of culture suspension) in the culture of Cg 62 isolate at 28°C. Although, it was observed that sporulation in all the culture of test isolates increased in temperature regime upto 28°C, but rate of sporulation decreased when temperature regime of the culture increased from 28° to 32°C during experimentation (**Table 5**).

The dendrogram profile showed that the isolates were grouped into two major cluster, sharing 72% maximum similarity. The entire two cluster consisted test isolates collected from different state showed higher degree of cultural variability. A very close association was found between isolates Cg 1 and Cg 30 collected from Chittoor (Andhra Pradesh) and Lucknow (Uttar Pradesh)

respectively in the first cluster. Second cluster contains Cg 19, Cg 62 and Cg 72 collecting from Rewa (Madhya Pradesh), Muzaffarpur (Bihar) and Dapoli (Maharashtra) respectively showing more than 56% similarity with each other (**Fig. 4**).

## 4. DISCUSSION

*Colletotrichum gloeosporioides*, a filamentous fungus, causing anthracnose disease in fruit crops, is reported to exhibit different requirements of nutrients and optimum conditions either for growth or sporulation (Shin *et al.*, 2000). There is, therefore, a need to study these parameters for mango anthracnose pathogen in order to establish the survivability of *C. gloeosporioides* in soil (Green, 1994). The present study has focused on resolving these issues pertaining to optimum conditions for growth and sporulation of *C. gloeosporioides*.

Growth of mycelium and sporulation are influenced by the medium, pH and temperature (Kumara and Rawal, 2008). These factors independently and or in combination have positive and negative effects in most of the fungi have been reported by several workers. Media containing carbohydrates, lipids, proteins and elements are basic requirements and needed by the microorganisms as these nutrients provide energy for biosynthesis and cell maintenance (Hilton, 1999). Production of biomass in fungi and growth-associated products requires nutrient-balanced media (Hilton, 1999). Some dimorphic fungi require optimal nutrition to produce high biomass, but for sporulation require nutritionally poor media which trigger differentiation of conidia from vegetative growth (Vega *et al.*, 2003).

The effects of medium composition, concentration and temperature on SCC and microcycle conidiation by *C. gloeosporioides* were studied on solid media. Among the evaluated media, MEA was found to be most suitable over other media which showed higher growth of the respective isolates, corroborating with the results (Sudhakar, 2000; Rani and Murthy, 2004). In the other study, it have been noticed that sporulation of *C. gloeosporioides* isolated from capsicum was maximum in oat medium at 25°C (Mello *et al.*, 2004). Earlier, reports showed that PDA and CWA was ideal medium for growth of *C. gloeosporioides*, as the coconut watery endosperm contains considerable amounts of lipids (1.26%), proteins (2.1%), carbohydrates and minerals suitable for maximum growth (Santoso *et al.*, 1996). In the line of above reports also suggested CWA suitable medium for the growth of *C. gloeosporioides* (Marikar, 2009).

In a study on growth of *C. gloeosporioides* under *in vitro* conditions, maximum growth was obtained after 10th day of incubation on potato dextrose broth, with optimum temperature in the range of 25-35°C (Hegde *et al.*, 1990). Temperature affects almost every function of the fungi including sporulation (Lilly and Barnett, 1951). A temperature range of 15-35°C was also suggested to be most suitable for maximum sporulation (Sattar and Malik, 1939). Meanwhile, 15-20°C favoured the conidia formation by *C. lindemuthianum* in culture (Mathur *et al.*, 1950). Further they reported that the sporulation was less at 25°C and ceased at 30°C. Ideally, 20 and 25°C was reported as the most favorable temperature for colony growth and sporulation in many fungi. Recently, maximum growth of *C. gloeosporioides* isolates (from Dapoli, Hessarghatta and Tumkur) at 28°C was identified while, 30°C supported growth of Hassan and Raichur isolates (Sangeetha and Rawal, 2009). Earlier, recorded maximum growth of different isolates of *C. gloeosporioides* at a temperature range of 25-30°C in the mango but sporulation was at an optimum range of 25-28°C (Sangeetha, 2003). In a study *C. gloeosporioides* produced maximum radial mycelial growth at 25°C after 6 days (Prabakar *et al.*, 2003). But in the present study, maximum growth was achieved on 7th day after inoculation.

In the present study, maximum sporulation was observed at 28°C in all five *C. gloeosporioides* isolates which was in conformity with the reports (Banik *et al.*, 1998). The sporulation of fungi of the genus *Colletotrichum* is favored by temperatures in the range of 20-24°C, while temperatures above 30°C may have an inhibitory effect, in total agreement with the results obtained in the present study (Slade *et al.*, 1987). The isolates of *Colletotrichum* respond differently in their growth and sporulation when exposed to different temperature conditions (Jayasinghe and Fernando, 1998).

The pH of the medium determines the rate and amount of growth including many other life processes of fungi (Lilly and Barnett, 1951). A medium with a specific pH which favours the growth but be unfavorable for sporulation or other processes. A medium having pH values between 5 and 6 at the time of inoculation was suitable for sporulation in most fungi which are also in accordance with the present study (Lilly and Barnett, 1951). According to them, fungi generally tolerate more acid than alkali. Similar observations were also recorded by some other workers with various species of *Colletotrichum* (Naik *et al.*, 1988). Similarly, the optimal growth pH of 6, are in agreement with temperature and pH optima reported for this species (Wastie, 1972). In papaya fruit crop *C. gloeosporioides* grew well in a medium of pH 5 (Kumara and Rawal, 2008).

## 5. CONCLUSION

Isolates of *C. gloeosporioides* have shown differential response for the parameters *viz.* media, media pH and temperature regimes in respect of growth and sporulation. The optimum temperatures for maximum growth of *C. gloeosporioides* were 28°C followed by 32°C with 6.0 media pH. Thus, the *C. gloeosporioides* pathogen can grow maximum under the temperature ranging 28 to 32°C with media pH of 5.5 to 6.0. Thus it may be concluded that the temperature and media pH are the critical factors for the growth of pathogen, which might be the main reason for the expression of mango anthracnose symptoms under field conditions in the Northern parts of India.

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