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Effect of Culture Media and Temperature on Growth and Sporulation *Colletotrichum gloeosporioides* cause of Anthracnose Disease of Mango

Sanjeev Leharwan*, V.K. Malik, Rajender Singh and Munish Leharwan

[1]*Department of Plant Pathology, CCS Haryana Agricultural University, Hisar-125004, Haryana, India*

**Corresponding author*

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A B S T R A C T

Mango anthracnose disease is one of the major fungal diseases of mango. In the present investigation, the rate of growth of Anthracnose (*C. gloeosporides*) has been compared in various temperature and culture solid media types viz., simple PDA (Potato Dextrose Agar), Oatmeal agar, Nutrient agar, Mango leaf extract agar, Czapek Dox agar. Among all the solid media tested, maximum mycelial growth with excellent sporulation rating was obtained in Potato dextrose agar medium (49.69 mm), which was significantly higher than the other. It was followed by Czapek's Dox agar (47.83 mm) with good sporulation and oat meal agar (47.29 mm) which were found at par with each. Temperature plays an important role in infection and disease development. Maximum mean colony diameter of fungus was recorded at temperatures of 25°C (48.25 mm) and 30°C (46.17 mm) which was significantly superior over all other temperatures. Lowest mean colony diameter was obtained at temperatures of 15°C (14.54 mm) and 20°C (31.65 mm). Among the four temperature level tested, the optimum temperature for conidial germination of *C. gloeosporioides* was found to be 25°C followed by 30°C whereas, least growth and germination was observed at 15°C. This is important for further study of disease management.

Introduction

Mango (*Mangifera indica* L.) is one of the most ancient fruits of India and deserves to be national fruit. It is the favourite fruit of almost every Indian and has been acclaimed as the 'King of fruits'. Mango is grown in more than 80 countries but it is greatly valued in India, where it occupies an area of 2162.8 thousand hectares with a total production of 19686.9 thousand metric tonnes with productivity of 8.7 metric tonnes contributing 40 per cent of the area devoted to total fruit crops (NHB, 2017). The fruit is very popular with the masses due to its wide range of adaptability, high nutritive value, richness in variety, delicious taste and excellent flavor. The fruit is rich with important minerals like potassium, magnesium, sodium, phosphorus, and sulfur. The plant and fruit is commonly infected by a number of fungal, bacterial and other pathogens. Among the fungal diseases anthracnose caused by *C. gloeosporioides* (Penz. & Sacc.) has become one of the

important diseases in fruit production in India. The pathogen produces symptoms viz. blossom blight, twig blight, wither tip, leaf blight and in some severe cases, tree dieback (Ploetz *et al*., 1996). The disease is particularly severe in young leaves and if wet weather prevails during flowering it causes flower set reduction along with yield losses in mango orchards (Kumar and Rani, 2010). In India, fruit losses in the field due to this disease have been estimated to the tune of 2-3 per cent (Prakash, 1998) whereas post- harvest losses, during transit, marketing and storage varied between 47.9- 51.7 per-cent (Prabakar *et al*., 2005). 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 and Erpelding, 2010). The fungus produces good aerial mycelium on different synthetic and semi-synthetic media as reported by Mishra and Tripathi (2015) where they obtained maximum growth and sporulation of *C. gloeosporioides* in Richard's synthetic medium followed by Potato dextrose and Czapek's dox agar media and poor growth without sporulation was recorded in Oatmeal. Different weather parameters play an important role in initiation and progression of the disease. Prakash and Srivastava (1987) observed that pathogen's growth is optimum at 25°C and ceases beyond 35°C. Davis *et al.*, (1987) also reported that temperature range between 20- 30°C was optimum for the growth and sporulation of *C. gloeosporioides* on mango. Considering the intensity of the disease, the present experiment was undertaken to find out the effect of media and temperature on the growth and sporulation of *C. gloeosporioides*.

Materials and Methods

The present investigation was under taken at laboratory conditions at Department of Plant Pathology, College of Agriculture, CCS Haryana Agricultural University, Hisar.

Collection and isolation of pathogen

Leaves having typical symptoms of anthracnose of mango were collected from the orchard of CCS Haryana Agricultural University, Hisar. Infected portions of leaves were cut into small pieces along with some healthy portion, they were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds and then rinsed 3-4 times in distilled sterilized water so that all the traces of mercuric chloride were removed. The bits were then aseptically placed on potato dextrose agar slants and incubated at 28 ± 1^0 C for 7 days. The stock culture was maintained on potato dextrose agar medium at 5 ± 1^0 C and subcultured after every 30 days. Pathogenicity of these isolates was also confirmed suggested by Jaysinghe and Fernando (2009).

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, five media Potato dextrose agar, Oatmeal agar, Nutrient agar, Mango leaf extract agar, Czapek Dox agar was used respectively. All these were autoclaved at 121°C under 15 psi for 20 min. Twenty ml of each medium listed above was poured aseptically into 90 mm diameter Petri plates. After solidification, 5 mm discs of the *C. gloeosporioides* were selected from actively growing culture using a cork borer and a single disc was placed at the center of Petri plate. Each set of experiment replicated thrice and they were incubated at 28^0 C. Colony diameter was measured every day until the colonies reached the edges of the dishes. Sporulation of the pathogen was measured by placing 5 discs of each 5 mm diameter in a test

tube containing 10 ml of sterile distilled water and there after macerating to get uniform dispersion of conidia. A drop was transferred on to the counting chamber of haemocytometer and average number of spores per ml was recorded twice a week.

Incubation at different temperature regime

In order to study the effect of different temperature regimes on mycelial growth and conidial germination. Twenty ml of fresh potato dextrose agar medium was poured aseptically into 90 mm diameter Petri plates. After solidification, five mm discs of the *C. gloeosporioides* were collected from actively growing culture using a cork borer and a single disc was placed at the center of Petri dish. Each set of experiment replicated thrice and they were incubated at 15, 20, 25 and 30°C temperatures. Colony diameter was measured every day until the colonies reached the edges of the dishes. For conidial germination the spore suspension with 20-30 spores per low power microscopic field was prepared in sterilized water. A drop of this suspension was put on the glass slide and kept in humid chamber, already prepared in sterilized Petri dishes just by providing moist filter paper. These Petri dishes were kept at different temperature regimes as mentioned above in incubator and observations were taken after 6 h interval up to 24 h.

Statistical analysis

All treatments were designed in Completely Randomized Design (CRD) with three replications. Experimental data was statistically analyzed using O. P. Sheoran software version 1.0 (CCS HAU, Hisar).

Results and Discussion

The effect of various factors such as media and temperature regimes and their combinations with days after inoculation on the growth and sporulation of *C. gloeosporioides* were studied.

Effect of media on growth and sporulation of *C. gloeosporioides*

It is clear from the data presented in Table 1 and Figure 1 that *C. gloeosporioides* produced different types of colonies on different media. The radial growth data presented in table revealed that among all the media tested the Petri plate completely filled with in 192 h in potato dextrose agar and Czapek's Dox agar followed by oat meal agar (87.00 mm) whereas, it was significantly lower in nutrient agar (56.83 mm) and mango leaf extract agar (53.83) after 192 h of inoculation. Potato dextrose agar was the best suited medium for the growth of fungus with the mean radial growth of 49.69 mm which was significantly higher than the other media. The mean radial growth in Czapek's Dox agar (47.83 mm) and oat meal agar (47.29 mm) were at par but significantly higher than the mean radial growth in nutrient agar (32.10 mm) and mango leaf extract agar (31.13 mm).

In the experiment of sporulation of pathogen, the extent of spore suspension was measured with the help of haemocytometer. It is evident from Table 2 that pathogen produces various extent of sporulation in different media. Sporulation was excellent in potato dextrose agar whereas, it was good in both Czapek's Dox agar and oat meal agar, while the sporulation in nutrient agar and mango leaf extract agar was poor.

Effect of different temperature regimes on the mycelial growth and conidial germination of *C. gloeosporioides*

The result of the study indicated that there were significant differences in mycelial growth recorded at different time period and temperature regimes. Radial growth was

recorded daily and data is presented in Table 3 (Fig. 2). It is clear from the data presented in Table 3 that among the four temperature level tested. The highest mean radial growth (48.25 mm) was observed at 25°C which was significantly higher than other temperature levels. This was followed by 30°C (46.17 mm) whereas, it was significantly lower at 20°C (31.65 mm) and 15°C (14.54 mm). The radial growth showed gradual decline on either side of 25°C (Plate 1).

Table.1 Effect of different culture media on the mycelial growth of *Colletotrichum gloeosporioides*

Different Media	Colony diameter (mm)								
	24h	**48h**	**72h**	**96h**	**120h**	**144h**	**168h**	**192h**	**Mean**
PDA	9.83	18.67	26.00	41.33	55.33	72.17	84.17	90.00	49.69
CDA	8.00	16.83	24.33	40.33	55.17	66.33	81.67	90.00	47.83
NA	8.00	14.67	21.83	29.17	36.00	41.00	49.33	56.83	32.10
OMA	10.33	19.33	29.33	42.83	51.83	62.83	74.83	87.00	47.29
MLEA	7.67	14.33	21.17	28.67	35.50	40.67	47.17	53.83	31.13
Mean	8.77	16.77	24.53	36.47	46.77	56.60	67.43	75.53	
C.D. (p=0.05)		Media						0.86	
		Time period						1.09	
		Media X Time period						2.44	

Table.2 Effect of different culture media on the sporulation of *Colletotrichum gloeosporioides*

S.NO	Different media	Sporulation	Rating
1	Potato dextrose agar	Excellent	++++
2	Czapek's Dox agar	Good	+++
3	Nutrient agar	Poor	+
4	Oat meal agar	Good	+++
5	Mango leaf extract agar	Poor	+

Table.3 Effect of different temperature regimes on the mycelial growth of *Colletotrichum gloeosporioides*

Temperature (°C)	Colony diameter (mm)								
	24h	**48h**	**72h**	**96h**	**120h**	**144h**	**168h**	**192h**	**Mean**
15	5.83	8.17	11.83	13.83	16.17	18.17	19.50	22.83	**14.54**
20	7.50	12.83	18.17	26.83	35.33	42.17	50.20	60.17	**31.65**
25	9.83	18.50	27.00	41.33	53.33	67.33	79.50	89.17	**48.25**
30	8.33	17.00	25.83	37.33	52.17	65.83	75.50	87.33	**46.17**
Mean	7.88	14.13	20.70	29.83	39.25	48.38	56.18	64.88	
C.D. (p=0.05)			**Temperature**				**0.61**		
			Time period					**0.87**	
			Temperature x Time period					**1.74**	

Table.4 Effect of different temperature regimes on the conidial germination of *Colletotrichum gloeosporioides*

Temperature (°C)	Germination (%)				
	6 h	**12 h**	**18 h**	**24 h**	**Mean**
15	0 (4.05)	9.58 (17.91)	22.68 (28.39)	41.07 (39.83)	**18.33 (22.55)***
20	17.52 (24.72)	40.23 (39.35)	56.44 (48.68)	75.45 (60.28)	**47.41 (43.26)**
25	24.66 (29.76)	50.66 (45.36)	69.99 (56.79)	90.39 (72.00)	**58.92 (50.98)**
30	20.08 (26.54)	51.28 (45.72)	68.31 (55.74)	87.72 (69.62)	**56.85 (49.40)**
Mean	15.57 (21.27)	37.94 (37.08)	54.35 (47.40)	73.66 (60.43)	
		Temperature			**(1.69)**
C.D. (p=0.05)		**Time period**			**(1.69)**
		Temperature x Time period			**(3.38)**

*Figures in parentheses are angular transformed values

Fig.1 Effect of different culture media on the mycelial growth of *Colletotrichum gloeosporioides*

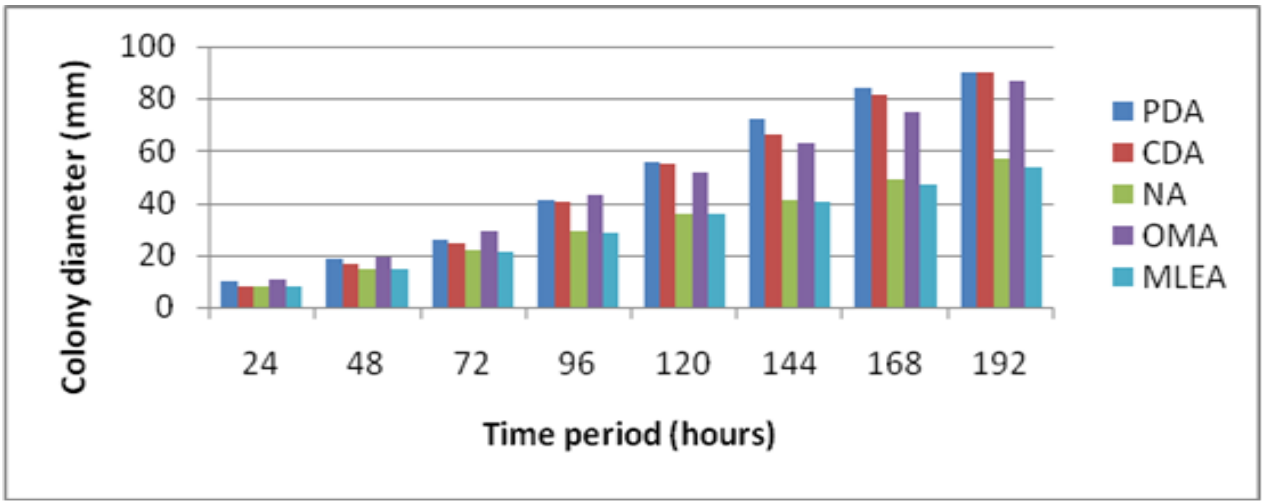


Fig.2 Effect of different temperature regimes on the mycelial growth of *Colletotrichum gloeosporioides*

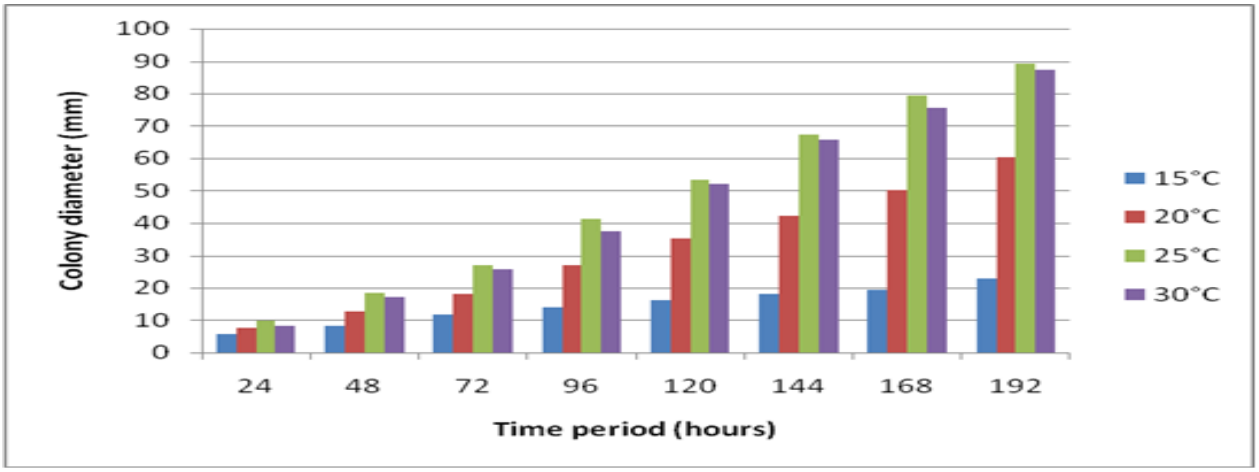
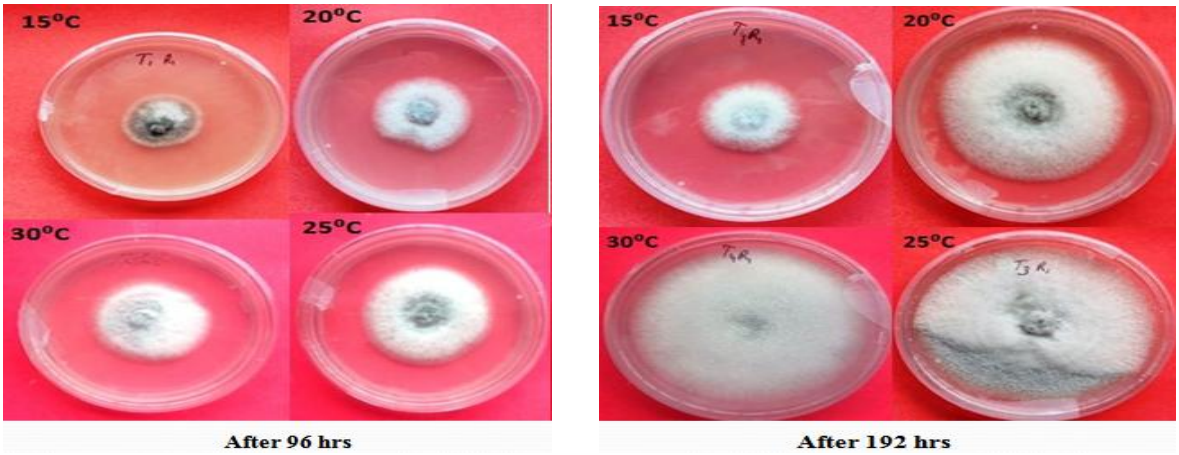


Plate.1 Effect of different temperature regimes on the mycelial growth of *Colletotrichum gloeosporioides*



The data in Table 4 revealed that maximum mean germination of conidia to the extent of 58.92 per cent was supported by 25°C. At the same temperature regime 24.66 and 98.39 per cent conidial germination was observed after 6 h and 24 h of incubation, respectively. The conidial germination showed gradual decline on either side of 25°C after 18 h of incubation registering 22.68, 56.44 and 68.31 per cent germination at 15, 20 and 30°C, respectively as compared to 69.99 at 25°C. After 24 h 41.07, 75.45 and 87.72 per cent germination was recorded at 15, 20 and 30°C, respectively as compared to 90.39 at 25°C. At all the temperature studied, no conidial germination took place at 15°C when observed at 6 h after incubation.

In conclusion, *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 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. Among all the media tested the potato dextrose agar was best suited medium for the growth of fungus and was followed by Czapek's Dox agar. These results are in agreement with that Joshi (2012) and Sarkar and Awasthi (2014).

The extent of sporulation produced on the given media is in confirmation with the finding of Mishra and Tripathi (2015). The optimum range of temperature for this fungus was 25°C to 30°C. However, maximum growth and conidial germination of *C. gloeosporioides* was recorded at 25°C. The result obtained during the investigation was close accordance with Kumar and Rani, 2010 that the temperature of 25°C was found to be good for the mycelial growth and conidial germination. This research is useful for further study of morphology *C. gloeosporioides* fungi.

References

Amusa, N.A., Ashaye, O.A., Oladapo, M.O. and Oni, M.O. (2005). Guava fruit anthracnose and the effects on its nutritional and market values in Ibadan, Nigeria. *World Journal of Agricultural Sciences*, 1: 169-172.

Davis, R.D., Irwin, J.A.G., Cameron, D.F. and Shepherd, R.K. (1987). Epidemiological studies on the anthracnose diseases of Stylosanthes caused by *C. gloeosporioides* in North Queensland and pathogenic specialization within the natural fungal populations. *Australian Journal Agriculture Research*, 38: 1019-1032.

Erpelding, J.E., 2010. Field assessment of anthracnose disease response for the sorghum germplasm collection from the mopti region. *American journal of Agricultural and Biological Sciences*, 5: 363-369.

Green, K.R., (1994). Studies on the epidemiology and control of yam anthracnose. Ph.D. Thesis, University of Reading, Reading, UK

Jayasinghe, C.K. and Fernando, T.H.P.S. (2009). First report of *Colletotrichum acutatum* on *Mangifera indica* in Sri Lanka. *Ceylon Journal of Science*, 38: 31-34.

Joshi, P.V. (2012). Symptomatology, Pathogenicity, Host Range and Cultural Study of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Causing Tip Blight of Jackfruit (*Artocarpus heterophyllus* L.). *Peroidic Research*, 4: 20.

Kumar, R. and Rani, U.S. (2010). Epidemiological and nutritional factors on growth of *Colletorichum gloeosporiodes. Annals of Plant Protection Science*, 18: 159-163.

Kumara, K.L.W. and Rawal, R.D. (2008). Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *C. gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L.). *Tropical Agricultural Research and Extension*, 11: 7-12.

Masyahit, M., Kamaruzaman, S., Yahya, A. and Satar, M.G.M. (2009). The First Report of the Occurrence of Anthracnose Disease Caused by *Colletotrichum gloeosporioides* (Penz.) Penz. Sacc. on Dragon Fruit (Hylocereus spp.) in Peninsular Malaysia. *American Journal of Applied Sciences*, 6: 902-912.

Mishra, N.K. and Tripathi, B.P. (2015). Effect of culture media on growth, colony character and sporulation of three foliar pathogens of guava. The Bioscan, 10(4): 1701-1705.

National Horticulture Board. 2017. Horticultural Statistics at a Glance 2017. 481 pp.

Owolade, O.F., Dixon, A.G.O., Akande, S.R. and Olakojo, S.A. (2009). A combining ability analysis of cassava *Manihot esculenta* crantz genotypes to anthracnose disease. *American Journal of Applied Science*, 6: 172-178.

Ploetz. R.C., Benscher, D., Vazquez, A., Colls, A., Nagel, J. and Schaffer, B. (1996). A re-evaluation of mango decline in Florida. *Plant Disease*, 80: 664-668.

Prabakar, K., Raguchander, T., Parthiba, V. K., Muthulakshmi, P. and Prakasam, V. (2005). Post harvest fungal spoilage in mango at different levels marketing. *Madras Agricultural Journal*, 92 (13):42-48.

Prakash O. 1998. Diseases of mango. In: Mango Cultivation. Prakash O (Ed.). Lucknow: International Book Distributing Co. pp. 301-338.

Prakash, O.M. and Srivastava, K.C. (1987). Mango diseases and their management.

A world review, today and tomorrow's printers and publishers. New Delhi, pp. 175.

Sarkar, S. and Awasthi, D.P. (2014). Effect of different culture medium on linear growth and mycelial dry weight of *Colletotrichum gloesporioides* causal organism of mango anthracnose. *Journal of Plant Protection and Environment*, 11(1): 119-121.

Shin, H.J., Xu, T., Zhang, C.L. and Chen, Z.J. (2000). The comparative study of capsicum anthracnose pathogens from Korea with that of China. *Journal of Zhejiang University*, 26: 629-634.

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