

Temperature Requirement of Different Isolates of *Colletotrichum gloeosporioides* Isolated from Mango

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Abstract: Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the most important diseases of mango. During survey, the disease samples of fruits affected by anthracnose were collected from Arambakam, Dapoli, Hassan, Hessarghatta, Lucknow, Raichur, Tiruvur and Tumkur and isolations were made. Studies were conducted to find out the temperature requirement of different isolates by incubating them at 15, 20, 25, 28 and 30°C. The study indicated that the temperature of 25°C was found to be good for the growth of Arambakam, Lucknow and Tiruvur isolates. Maximum growth of Dapoli, Hessarghatta and Tumkur isolates were recorded at 28°C whereas 30°C supported good growth of Hassan and Raichur isolates, nine days after inoculation. As regards to sporulation, Dapoli, Hessarghatta and Raichur isolates were good at 28°C whereas, 25°C supported good sporulation of Lucknow and Tiruvur isolates. Hassan and Tumkur isolates showed moderate sporulation at 28°C and 25°C supported moderate sporulation of Arambakam.

Key words: *Colletotrichum*, Anthracnose, Sporulation

INTRODUCTION

Mango (*Mangifera indica* L.) is considered as one of the most popular fruits among millions of people and grown through out the tropical and subtropical regions of the world. It is ranked as one of the best fruits in the international market because of its delicious taste and high caloric value. This fruit has become an essential fruit crop in Asia, Southern and Central America as well as in many parts of Africa. India stands first in global mango production where it is mainly grown in Andhra Pradesh, Uttar Pradesh, Karnataka and Bihar. Because of its diverse production conditions and the vast area grown, mango suffers from a number of diseases, some of them taking heavy toll on the crop and representing limiting factors. India stands first in global mango production (52%). However, the productivity of mango in India is affected by various post harvest diseases which reduce the fruit quality and cause severe losses, because they lead to completely unmarketable fruits. Although blemished fruit can be sold in the local market, this practice results in economic loss due to the considerable differences between the export and local prices. The mango tree and

more especially the fruit is the host of a large number of pathogens among which fungi are the major agents of fruit rot after harvest. Fungal pathogens involved in mango rotting after harvest include *Colletotrichum gloeosporioides* responsible for mango anthracnose, *Alternaria alternata* and *A. tenuissima* that cause alternariose, *Botryodiplodia theobromae* and *Dothiorella* spp. responsible for stem end rot and *Phoma mangiferae* [1-4]. Termination of fungal quiescence appears to be related to the reduction of antifungal compounds [5] and or the production of ethylene by the ripening fruit [6]. As the fruit ripens, a reduction in the concentration of phenolic compounds active against *C. gloeosporioides* and *A. alternata* is observed [7]. The change in nutritional status of the host upon ripening has also been suggested as a factor in quiescence termination, but experimental evidence is contradictory [8].

Among the various diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the most serious disease widely distributed in all mango growing regions of the world [9-13] and is a major constraint on the expansion of export trade of mango [14]. It affects both vegetative and reproductive structures.

Initial infection starts from leaves and spreads to flowers causing blossom blight, which destroys inflorescence (flower panicles) leading to considerable reduction in fruit set and yield loss. The disease incidence from different countries has been reported to be 32 per cent in South Africa [15], 64.6 per cent in Costa Rica during 1990 [16] and could reach almost 100 per cent in fruits produced under wet or very humid conditions [4]. Post harvest decay due to anthracnose was 29.6 per cent in Himachal Pradesh, India during 1990-92 [17]. To know the outbreak of the disease, the favourable environmental condition prevalent in a particular region should be known. Hence the present work was conducted to understand better the interactions between fungi involved in post harvest rotting of mango and the temperature requirement for its growth

MATERIALS AND METHODS

Isolation of the Pathogen: The pathogen *C. gloeosporioides* was isolated from infected mango leaves. Isolation was made by cutting a small section of anthracnose infected portion, which was surface sterilized with 0.1% HgCl₂ solution and rinsing in sterilized distilled water. It was then placed on Potato Dextrose Agar (PDA) medium (Potato-200g, Glucose-20g, Agar agar-20g, Distilled water-1000ml), in sterilized Petri plates and incubated at 28 ± 2°C. The pure culture was maintained in PDA slants.

Eight isolates of *C.gloeosporioides* from mango were isolated, representing a range of geographical origins (Table 1). Actively growing cultures of all isolates were established on Richard's Agar (Magnesium sulphate-0.25g, Potassium di-hydrogen phosphate-5.00g, Potassium nitrate-10.00g, Potato starch-10.00g, Sucrose-50.00g, Distilled water-1000.00ml) from stock collections maintained in cool-stored culture tubes for long-term preservation. The plates were inoculated with a 5 mm

mycelial plug cut with a sterile cork borer from the margin of a 7-day-old colony of all the eight isolates to be tested. Plates were not sealed and were placed in an incubator maintained at 15, 20, 25, 28 and 30°C. Radial growth was measured twice a week in two perpendicular directions until colonies reached the edges of the dishes. Four plates were used for each isolate x temperature combination. Daily growth rates were analyzed in a two-way ANOVA, with temperatures and isolates as main factors.

RESULTS

The results of the study indicated that there were significant differences between isolates, temperatures and their interaction when observed three days after inoculation. Among the temperatures, for all the isolates, the maximum mean colony diameter was at 28°C (26.55mm) followed by 25°C (21.55mm), which was significantly higher than other temperatures. Temperature of 15°C (5.64mm) showed least growth of all the isolates. All the isolates recorded maximum growth between temperature ranges of 25 to 28°C.

Temperature of 28°C was found to be favorable for maximum growth of Arambakam isolate (23.19mm), Dapoli isolate (23.81mm), Hessarghatta isolate (23.88mm), Lucknow isolate (23.19mm) and Tumkur isolate (23.81mm) three days after inoculation (Table 2). Maximum growth of Hassan isolate (24.13mm) and Raichur isolate (30.21mm) was recorded at 30°C three days after inoculation. However, 25°C supported good growth of Tiruvur isolate (25.44mm) followed by 28°C (46.75 mm). The results presented in Table 3 indicate significant differences between isolates, temperature and also their interaction when observed six days after inoculation. Temperature of 28°C supported maximum growth of 61.91mm in general for all the isolates which was significantly superior over all the temperatures. Good growth of 58.34 mm was also recorded at 25°C with least growth at 15°C (19.09 mm). Temperature in the range of 25-28°C supported good growth of all the isolates.

Among the isolates, 25°C favored maximum growth of Arambakam (57.38 mm), Lucknow isolate (57.37mm) and Tiruvur isolate (75.81mm) which was statistically on par with growth at 28°C. However, the growth of Dapoli isolate (60.5 mm), Hessarghatta isolate (56.69mm), Tiruvur isolate (75.81mm) and Tumkur isolate (60.5mm) were maximum at 28°C and significantly superior over temperature of 25°C. Raichur and Hassan isolates showed maximum growth at 30°C with 82.63 mm and 51.75mm

Table 1: List of different isolates of *Colletotrichum gloeosporioides*

Isolate	Location (State)	Organ	Mean Spore size
			(Length x breadth) (µm)
Arambakam	Tamil Nadu	Fruit	14.49 x 4.76
Dapoli	Maharashtra	Fruit	14.23 x 5.23
Hassan	Karnataka	Fruit	16.66 x 7.74
Hessarghatta	Karnataka	Fruit	16.84 x 4.97
Lucknow	Uttar Pradesh	Fruit	16.16 x 7.25
Raichur	Karnataka	Fruit	16.23 x 7.45
Tiruvur	Tamil Nadu	Fruit	15.54 x 3.85
Tumkur	Karnataka	Fruit	16.88 x 4.48

Table 2: Effect of temperature on growth of different isolates of *Colletotrichum gloeosporioides* (3 days after inoculation)

Colony diameter (mm) of different isolates									
Temperature (°C)	Arambakam	Dapoli	Hassan	Hessarghatta	Lucknow	Raichur	Tiruvur	Tumkur	Mean
15	9.81	4.19	4.00	4.25	9.81	4.38	4.50	4.19	5.64
20	5.00	10.69	6.50	6.88	5.00	11.13	22.50	10.69	9.79
25	20.87	17.36	12.63	7.63	20.86	23.25	52.44	17.38	21.55
28	23.19	23.81	19.00	23.88	23.19	28.75	46.75	23.81	26.55
30	6.21	21.75	24.13	5.09	6.21	30.21	23.75	21.75	17.39
Mean	13.02	15.56	13.25	9.54	13.02	19.54	29.99	15.53	16.19
	S.Em.±	CD (1%)							
Isolates (I)	0.623	1.729							
Temperature(T)	0.788	1.864							
I x T	1.762	3.609							

Table 3: Effect of temperature on growth of different isolates of *Colletotrichum gloeosporioides* (6 days after inoculation)

Colony diameter (mm) of different isolates									
Temperature (°C)	Arambakam	Dapoli	Hassan	Hessarghatta	Lucknow	Raichur	Tiruvur	Tumkur	Mean
15	22.94	13.94	12.69	10.44	22.94	17.69	38.17	13.94	19.09
20	13.19	50.94	34.44	34.19	13.19	52.44	49.49	50.94	37.35
25	57.38	56.81	42.81	50.75	57.37	68.94	75.81	56.81	58.34
28	56.24	60.50	49.63	56.69	56.24	82.62	72.88	60.50	61.91
30	10.88	52.93	51.75	27.75	10.88	75.06	30.69	52.93	40.05
Mean	32.12	47.02	38.26	35.96	32.12	59.35	53.41	47.02	43.16
	S.Em.±	CD (1%)							
Isolates (I)	0.824	2.289							
Temperature(T)	1.043	2.466							
I x T	2.331	4.776							

Table 4: Effect of temperature on growth of different isolates of *Colletotrichum gloeosporioides* (9 days after inoculation)

Colony diameter (mm) of different isolates									
Temperature (°C)	Arambakam	Dapoli	Hassan	Hessarghatta	Lucknow	Raichur	Tiruvur	Tumkur	Mean
15	25.44-	34.06-	26.31-	30.06-	25.44-	41.31-	61.44-	34.06-	34.77
20	21.69-	69.94+	55.75-	62.06+	21.69-	76.25+	72.50-	69.94+	56.22
25	81.31++	69.50++	66.86-	67.56++	81.31+++	72.00++	81.06+++	69.50++	73.64
28	75.94+	86.81+++	71.60++	86.25-	75.94++	87.44++	80.88++	86.81++	81.46
30	15.94-	79.81-	86.00-	58.15-	15.94-	89.88-	41.38-	79.81-	58.36
Mean	44.06	68.02	61.31	60.82	44.06	73.38	67.45	68.02	60.89
	S.Em. +	CD (1%)							
Isolates (I)	2.370	6.579							
Temperature(T)	2.997	7.090							
I x T	6.703	13.728							

- Nil sporulation + Poor sporulation ++ Moderate sporulation +++ Good sporulation

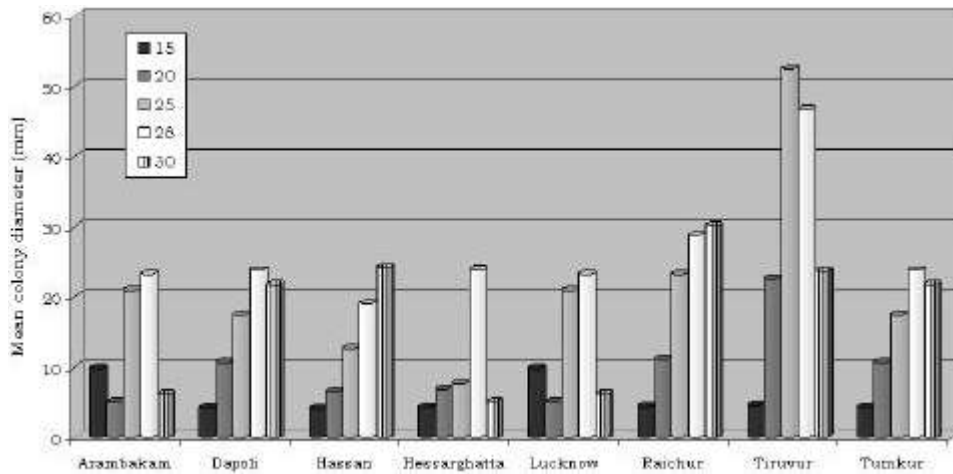


Fig. 1: Temperature requirement of different isolates of *Colletotrichum gloeosporioides* on solid media (3 D.A.I.)

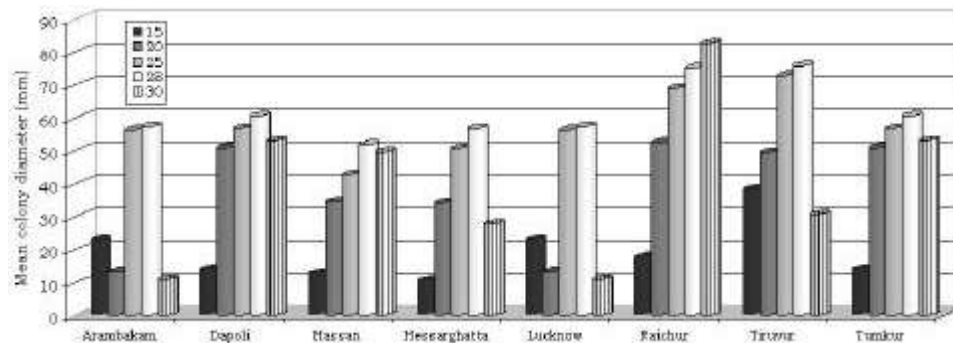


Fig. 2: Temperature requirement of different isolates of *Colletotrichum gloeosporioides* on solid media (6 D.A.I.)

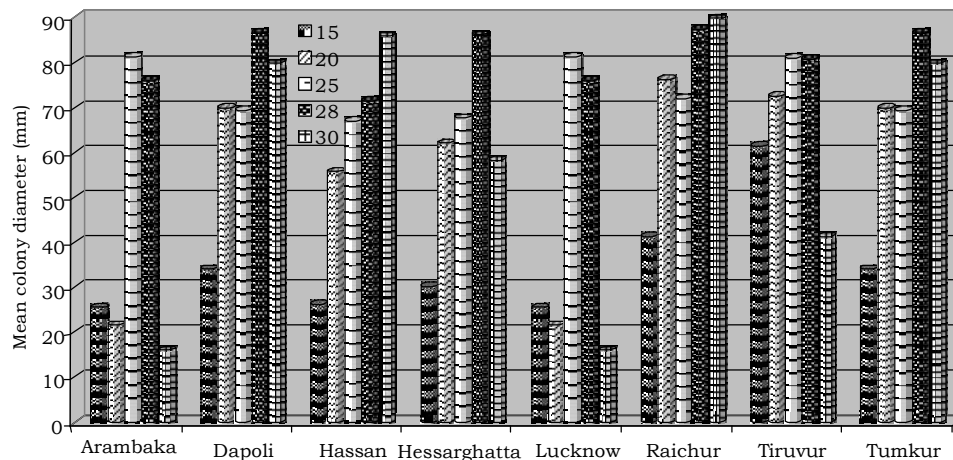


Fig. 3: Temperature requirement of different isolates of *Colletotrichum gloeosporioides* on solid media (9 D.A.I.)

growth respectively followed by 75.06mm and 49.63mm growth at 28°C. Least growth of all the isolates except Arambakam and Lucknow isolate was at 15°C.

The data in Table 4 and Figure 3 revealed significant differences between isolates, temperature and their interaction when recorded nine days after inoculation.

Among the temperatures in general for all the isolates, 28°C supported maximum mean growth of 81.46 mm which was significantly superior to 25°C with mean growth of 73.64 mm. Mean growth at 25°C and 30°C were on par with each other. Among the isolates, temperature of 25°C supported maximum mean growth of Arambakam

(81.31mm), Lucknow (81.31mm) and Tiruvur (81.06mm) isolates which was on par with the mean growth at 28°C. Maximum growth of Dapoli (86.81mm), Hessarghatta (86.25mm) and Tumkur (86.81mm) isolate was recorded at 28°C which was superior to all other temperatures. Hassan and Raichur isolates recorded good growth at 30°C. Based on the growth of different isolates of *C.gloeosporioides* at different temperatures, the isolates can be grouped as:

Group I: Arambakam, Lucknow and Tiruvur (25°C)

Group II: Dapoli, Hessarghatta and Tumkur (28°C)

Group III: Hassan and Raichur (30°C)

The sporulation of Arambakam was moderate sporulation at 25°C and poor at 28°C whereas no sporulation was recorded at 15, 20 and 30°C. Dapoli and Hessarghatta isolate showed good sporulation at 28°C with moderate sporulation at 25°C, poor sporulation at 20°C and no sporulation at 15 and 30°C. Moderate sporulation of Hassan isolate was recorded at 28°C with no sporulation at other temperatures whereas good and moderate sporulation at 25 and 28°C respectively was recorded in both Lucknow and Tiruvur isolates with nil sporulation at 15, 20 and 30°C. Similarly Raichur and Tumkur isolates recorded moderate sporulation at 25 and 28°C with poor sporulation at 20°C and no sporulation at 15°C and 30°C.

DISCUSSION

The studies conducted to find out the role of different temperature on growth and sporulation of *C.gloeosporioides* revealed that temperature range from 25-30°C favored good growth and sporulation of Arambakam, Dapoli, Hassan, Hessarghatta, Lucknow, Raichur, Tiruvur and Tumkur isolates. Temperature of 15°C did not favor the growth of any of the isolates. This difference in the temperature requirement of different isolates could be due to the difference in the climatic condition of the regions where disease exists and the fungus could have adapted to that particular climate. Since Arambakam and Tiruvur are coastal regions where the night temperature is low, it showed maximum growth at 25°C. Hassan and Raichur being hot regions, maximum growth was recorded at 30°C.

Similarly, Quimio and Quimio [18] and Ahmed [19] recorded good growth of *C.gloeosporioides* at a temperature range of 20-30°C whereas Saxena [20] noticed good growth of *C.gloeosporioides* on pomegranate between 15-35°C with optimum at 28°C. Quesada and

Lopez [21] and Banik *et al.* [22] reported good growth of *C.gloeosporioides* at 28°C, whereas Rajak [23] and Ekbote [24] recorded maximum growth of *C.gloeosporioides* at 25°C and 29°C, respectively. Good sporulation of Dapoli, Hessarghatta and Raichur isolates were observed at 28°C, whereas 25°C supported good sporulation of Lucknow and Tiruvur isolates. Hassan and Tumkur isolates showed moderate sporulation at 28°C and 25°C supported moderate sporulation of Arambakam isolate. This results show that *C.gloeosporioides* can sporulate between a temperature ranges of 25-28°C. Similar reports on good sporulation of *C.gloeosporioides* at 28°C was made by [21] Quesada and Lopez, whereas [19] Ahmed reported good sporulation at a temperature range of 15-35°C, optimum being between 20-30°C.

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