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INVESTIGATION INTO THE SIGNIFICANCE OF TEMPERATURE WITH RESPECT TO PATHOGEN GROWTH, *Colletotrichum gloeosporioides* - A DEVASTATING FUNGAL PATHOGEN CAUSING MANGO ANTHRACNOSE

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ABSTRACT

Mango is the most important fruit covering about 35 per cent of area and accounting of 22 per cent total production of total fruits in India, which is highest in the world with India's share of about 49.62 per cent, is severely affected by mango anthracnose caused by *Colletotrichum gloeosporioides* a devastating disease responsible for 30 – 60 per cent post harvest losses. Out of 276 (bacteria - 247 + fungi - 29) leaf endophytes screened against the aggressive pathogen, the potential endophytic bacteria were cloned and identified. Further, the talc based formulations of novel bacteria were developed to control the anthracnose disease and also to minimize the risk in use of fungicides. As a part of common practice, the formulations/fungicides are applied prior to the infection and during the favourable environmental conditions, the fungus which is in dormant state becomes active and cause disease symptoms. As the environmental conditions and geographical factors plays a major role, it is necessary to understand the favourable temperature for the growth of the fungus in order to recommend the scheduled spray time to the farmers. In the present study, an investigation into the significance of temperature with respect to pathogen growth (sporulation) was studied using Analysis of Variance (ANOVA) with Post hoc test using advanced statistical software SAS Ver. 9.2. The research findings concluded that the temperature between 25°C – 3 0°C was most favourable for the growth of the *C. gloeosporioides* and also revealed that there is a significant difference between three temperature conditions of radial growth, growth rate and sporulation ($p < 0.0001$). These findings help in allowing the farmers to take suitable measures in order to control the anthracnose disease and fetch more income.

KEYWORDS: Mango; anthracnose disease; temperature; SAS.

INTRODUCTION

Mango (*Mangifera indica* L.) is native to India and South East Asia. India is the largest producers of mangoes in the world when compared to half of the global production and the largest exporter. Andhra Pradesh is a leading state in production and productivity of mango in India. Devastating disease like anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. reduce quality of fruit produced under wet or very humid conditions. The post harvest phase is the most economically significant throughout the world. Post harvest produce and responsible for 30 to 60 per cent of harvest losses. It is the major pre and postharvest disease of mango (Arauz, 2000), and can result in serious decay of fruit during marketing and after sale. The incidence of this reach almost 100 per cent in fruits produced under wet or very humid conditions. The post harvest phase is the most economically significant throughout the world. Post harvest thermal and chemical treatments reduces anthracnose severity of the fruits but the adverse effect of synthetic chemical residues on human health, environment and the development of resistance in the pathogen to chemicals used for controlling the disease have lead to intensified efforts to develop alternative methods. Biological control using microbial antagonists has emerged as one of the most promising alternatives, used either alone or as integrated control strategy to reduce the use of fungicides. But the information on the growth of the fungal pathogen with respect to the temperatures and geographical regions is scanty which directly linked with the spray schedules. Now, to identify any variations in the growth parameters of the pathogen at different temperatures provides much insight information in controlling the disease is the need of the hour. The meteorological factors which form the basis of such predictions usually are prevailing temperatures and wet periods. Although it is known that temperature affects

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spore germination and infection, which in turn influence disease development, the period required for infection will be largely influenced by prevailing temperatures, provided wetting is unlimited. If the wetting period is short, infection will only be successful if the temperature is optimal. Considering the severity of the disease and the losses associated with it, an investigation was made to study the growth parameters of *C. gloeosporioides* may assist in better understating the relationship of the growth and temperature in specific geographical locations for better timing of fungicide applications.

MATERIAL AND METHODS

Isolation of pathogen

Mango leaves infected with *Colletotrichum gloeosporioides* Penz., were collected during the roving survey was conducted to estimate the disease incidence in major mango growing regions of Andhra Pradesh for the estimation of per cent disease incidence. Isolations were made from the leaves showing typical symptoms of anthracnose disease by tissue segment method (Rangaswami and Mahadevan, 1999) on potato dextrose agar medium (PDA). These bits were transferred to sterile discs of blotting paper. The dried bits were subsequently transferred to sterile PDA medium in Petri plates under aseptic conditions. The Petri plates were incubated at 28 ± 2°C and the fungal growth was observed after seven days.

Purification and identification of pathogen

The fungal colonies developed were purified by single spore isolation method (Rangaswami and Mahadevan, 1999) and the pathogen was identified based on its mycelia and conidial characteristics as per standard mycological keys (Barnett and Hunter, 1972).

Cultural and morphological characterization

Potato dextrose agar medium (PDA) was used to assess the differential growth among the isolates of the fungus. Morphological characteristics such as colour of the colony and size of the conidia (length and width) were recorded for each isolate. Length and width of the conidia were measured by Motic image software programme inbuilt in Motic BA200 binocular microscope.

Measurement of radial growth of the fungus

The variation among the isolates of *C. gloeosporioides* in colony growth, the radial growth of the fungus in each Petri plate (replications) was recorded after seven days of incubation *i.e.,* when the growth of the fungus in some of the Petri plates was maximum. The colony growth was measured along two diameters at right angles and averaged.

Measurement of growth rate of the fungus

The radial growth of the fungus in each Petri plate (replications) for each isolate was measured at every 24 hours interval in order to determine the growth rate.

Assessment of sporulation of the fungus

Sporulation of the individual isolates of fungus on PDA medium was assessed. Five discs of each 5 mm diameter size were cut with a sterile cork borer at a radius of 20 mm from the centre of the growth. The discs were placed in a test tube containing 10 ml of sterile distilled water and macerated with the help of glass rod so as to get uniform dispersion of conidia. From this spore suspension, a drop was transferred on to the counting chamber of the haemocytometer and average number of spores per ml was determined. The conidial population was categorized as poor, average, good and excellent as given below:

Categories	Average no. of conidia ml^{-1}	Rating
Poor	$< 1.5 \times 10^4$	+
Average	$> 1.5 \times 10^4 – 4.0 \times 10^4$	++
Good	$> 4.0 \times 10^4 – 9.0 \times 10^4$	+++
Excellent	$> 9.0 \times 10^4$	++++

RESULTS AND DISCUSSION

Among three different temperatures tested, the temperature 26°C – 30°C was found to be most favorable temperature for the growth of fungal pathogen (radial growth, growth rate and sporulation). The effect of temperature on the growth parameters of fungal pathogen *C. gloeosporioides* collected from all three different regions of Andhra Pradesh showed significant difference with respect to different temperatures ($p<0.0001$) (Table 1 and Fig 1). Among all the three different regions of Andhra Pradesh, the fungal parameters ware found to be more or less similar. Hence, the results conclude that the growth parameters of *C. gloeosporioides* showed that there is no significant difference with respect to different geographical locations ($p>0.05$) (Table 2 and Fig. 2). The fungal growth parameters viz., radial growth, growth rate

Table 1. Effect of temperature at 20°C – 35°C on *C. gloeosporioides*

Growth	N	Temperatures (Mean ± SD)			p-value
		20°C – 25°C	26°C – 30°C	31°C – 35°C	
Radial Growth (mm)	15	64.58 ± 4.73	86.46 ± 2.49	53.85 ± 3.87	< 0.0001*
Growth Rate (mm/day)	15	10.09 ± 1.27	12.83 ± 0.73	8.07 ± 2.10	< 0.0001*
Sporulation (No. of conidia/ml) [10^4]	15	2.58 ± 0.55	5.09 ± 0.64	1.66 ± 0.71	< 0.0001*

* : Very high significant.

Table 2. Comparison of growth parameters of isolates collected from different regions at 20°C to 35°C

Growth	N	Region (Mean ± SD)			p-value
		Chittoor	Kadapa	Kurnool	
Radial Growth (mm)	15	67.54 ± 14.98	67.47 ± 14.98	69.87 ± 13.51	0.875 (NS)
Growth Rate (mm/day)	15	9.85 ± 2.26	9.94 ± 2.57	11.20 ± 2.43	0.242 (NS)
Sporulation (No. of conidia/ml) [10^4]	15	3.12 ± 1.78	2.99 ± 1.60	3.21 ± 1.48	0.932 (NS)

NS : Not Significant

Table 3. Overall comparison of fungal growth parameters at three different temperatures

Region	Growth	Temperature (Mean ± SD)			p-value
		20°C – 25°C	26°C – 30°C	31°C – 35°C	
Chittoor	Radial Growth (mm)	61.82 ± 3.82	87.18 ± 2.09	53.63 ± 1.26	< 0.0001
	Growth Rate (mm/day)	9.78 ± 1.09	12.45 ± 0.24	7.32 ± 0.44	< 0.0001
	Sporulation (No. of conidia/ml) [10^4]	2.60 ± 0.71	5.29 ± 0.92	1.47 ± 0.41	< 0.0001
Kadapa	Radial Growth (mm)	64.20 ± 6.13	85.67 ± 3.42	52.53 ± 5.49	< 0.0001
	Growth Rate (mm/day)	9.57 ± 1.48	12.91 ± 0.79	7.33 ± 0.76	< 0.0001
	Sporulation (No. of conidia/ml) [10^4]	2.30 ± 0.55	4.92 ± 0.25	1.76 ± 1.23	< 0.0001
Kurnool	Radial Growth (mm)	67.72 ± 2.04	86.52 ± 2.04	55.38 ± 3.96	< 0.0001
	Growth Rate (mm/day)	10.93 ± 0.97	13.14 ± 0.92	9.54 ± 3.26	0.047
	Sporulation (No. of conidia/ml) [10^4]	2.84 ± 0.29	5.06 ± 0.67	1.74 ± 0.14	< 0.0001

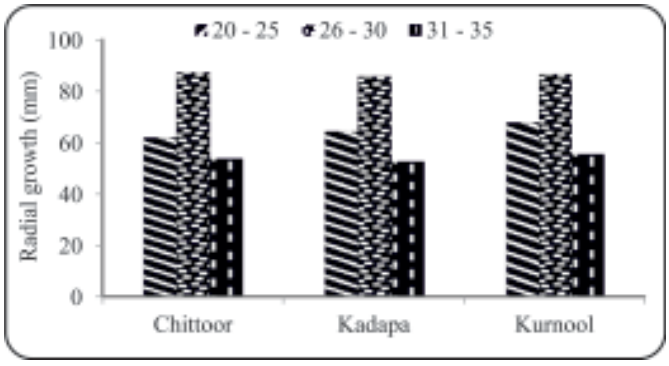

Fig. 1. Comparison of radial growth (mm) at different temperatures (°C) in different regions

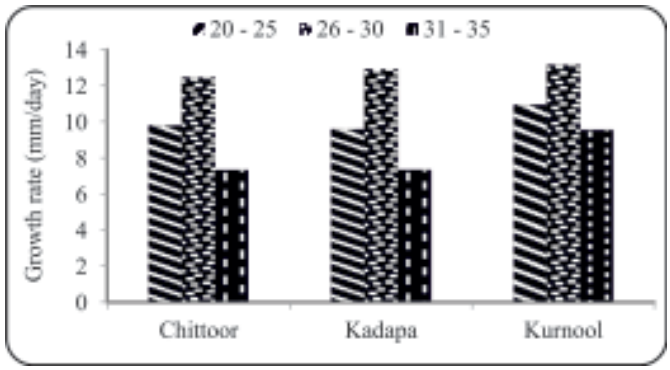

Fig. 2. Comparison of growth rate (mm/day) of fungal pathogen at different temperatures (°C) in different regions

and sporulation were found to be higher at 26°C – 30°C compared with 20°C – 25°C and 31°C – 35°C in all three regions has shown that significance difference ($p < 0.0001$) (Table 3 and Fig. 3).

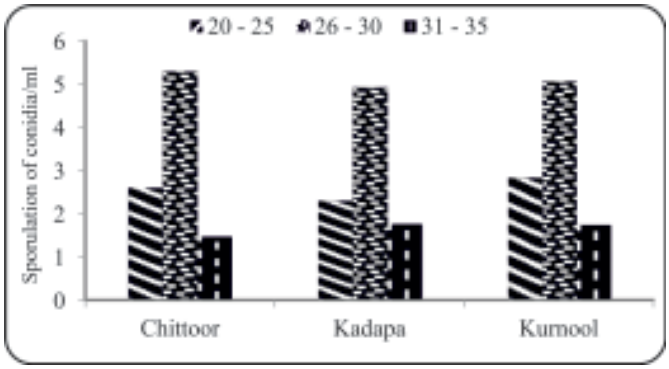

Fig. 3. Comparison of sporulation (No.of conidia/ml) at different temperatures (°C) in different regions

In Chittoor region, the radial growth has higher mean (87.18) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (61.82) and 31°C – 35°C (53.63). The growth rate has higher mean (12.45) at 26°C–30°C when compared with the mean values of 20°C – 25°C (9.78) and 31°C – 35°C (7.32). The sporulation has higher mean (5.29) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (2.60) and 31°C – 35°C (1.47). The results stated that there is a significant effect of temperature on growth parameters of fungal pathogen *C. gloeosporioides* in three different regions of Andhra Pradesh ($p<0.0001$).

In Kadapa region, the radial growth has higher mean (85.67) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (64.20) and 31°C – 35°C (52.53). The growth rate has higher mean (12.91) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (9.57) and 31°C – 35°C (7.33). The sporulation has higher mean (4.92) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (2.30) and 31°C – 35°C (1.76). The results stated that there is a significant effect of temperature on growth parameters of fungal pathogen *C. gloeosporioides* in three different regions of Andhra Pradesh ($p<0.0001$).

In Kurnool region, the radial growth has higher mean (86.52) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (67.72) and 31°C – 35°C (55.38). The growth rate has higher mean (13.14) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (10.93) and 31°C – 35°C (9.54). The sporulation has higher mean (5.06) at 26°C – 30°C when compared with the mean values of 20°C – 25°C (2.84) and 31°C – 35°C (1.74). The results stated that there is a significant effect of temperature on growth parameters of fungal pathogen *C. gloeosporioides* in three different regions of Andhra Pradesh ($p<0.0001$).

The current study findings are in accordance with earlier reports by Banik *et al*., (1988) and Quesada and Lopez (1980) observed that 28°C favoured the good growth of *C. gloeosporioides*, whereas Rajak (1983) and Ekbote (1994) recorded maximum growth of *C. gloeosporioides* at 25°C and 29°C respectively. Quimio and Quimio (1975) and Ahmed (1985) who recorded good growth of *C. gloeosporioides* at a temperature range of 20 – 30°C. The present investigations prove that *C. gloeosporioides* shall sporulate between a temperature range of 25°C – 28°C. Similar reports on good sporulation of *C. gloeosporioides* at a temperature range of 15°C – 35°C, optimum being between 20°C – 30°C recorded by Ahmed (1985) whereas 28°C was best temperature as per findings made by Quesada and Lopez (1980).

CONCLUSION

The research findings concluded that the optimal temperature for germination was between 25°C and 30°C in the all three regions of Andhra Pradesh from where the *C.gloeosporioides* isolates were collected. Poor germination was recorded at both the 20°C – 25°C and 31°C–35°C temperatures and the study revealed in understanding the sensitivity of temperature for the mycelial growth and conidial germination of the fungal pathogen. This information helps the researchers as well as farmers for the prediction of pathogen infection during the season and would encourage in planning spray schedules.

REFERENCES

Ahmed, K.M. 1985. Effect of temperature and light on the growth and sporulation of *C. gloeosporioides* Penz. *Bangladesh Journal of Botany*. 14: 155-159.

Arauz, L.F. 2000. Mango anthracnose: economic impact and current options for integrated management. *Plant Disease*. 84:600–611.

Banik, A.K., Kaiser, S.A.K.M and Dhua, R.S. 1988. Influence of temperature and humidity on the growth and development of *Colletotrichum gloeosporioides* and *Diplodia natalensis* causing post harvest fruit of mango. *Advances in Plant Sciences*. 11(2): 51-57.

Barnett, H.L. and Barry, B. Hunter. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minnesota, U.S.A.

Ekbote, S.D. 1994. Studies on anthracnose of mango (*Mangifera indica* L.) caused by *Colletotrichum gloeosporioides* (Pens.) Penz. and Sacc. *M.Sc. Thesis* submitted to University of Agricultural Sciences, Dharwad, p. 101.

Quesada, G.L and Lopez, H.E. 1980. Sexual form and culture media for *Colletotrichum gloeosporioides* pathogen of mango in Cuba. *Ciencias de la Agricultura*. 7: 11-17.

Quimio, T.H and Quimio, A.J. 1975. Notes on Philippine grape and guava anthracnose. *Plant Disease Reporter*. 59: 221-224.

Rajak, R.C. 1983. Nutritional requirement of two species of *Colletotrichum*. *Acta Botanica Indica.* 11: 155-160.

Rangaswami, G and Mahadevan, A. 1999. Diseases of crop plants in India, 4th edn. Prentice Hall of India Pvt. Ltd., New Delhi, p. 65-66.