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Influence of temperature and relative humidity on anthracnose pathogen growth and diseases development in mango under *in vitro*

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Abstract: The effect of temperature and relative humidity on mycelial growth and conidial germination and the disease development of mango anthracnose caused by *Colletotrichum gloeosporioides* were studied under *in vitro* conditions. The fungus was found to grow between 13 and 40°C. The maximum radial mycelial growth was noted at 25°C followed by 30°C and the minimum growth at 13°C and 40°C. The conidial germination was more at the room temperature (76.67 per cent) followed by 25°C and it was less at 13°C (5.00 per cent). The optimum temperature for the disease development in the inoculated fruits was 25°C followed by 30°C and it was least at 13 and 40°C. At 100 per cent relative humidity, the radial mycelial growth and conidial germination was higher and the incubation time for the disease development on the inoculated fruits was less (3 to 6 days).

Key words : Temperature, RH, Mango anthracnose, Disease development.

Introduction

Mango is a crop of great antiquity and is under cultivation for more than 4000 years in our country. In Tamil Nadu it is a grown in an area of 68,000 hectares with a production of 4.2 lakh tonnes. Tamil Nadu occupies sixth place in terms of area under mango and second in productivity (Anon, 1995). In mango, 36.7 per cent is lost during post harvest period (Madan and Ullasa, 1993). In India the annual estimated post harvest loss was 200 crores in respect of fruits including storage and transit (Dasgupta and Mandal, 1989). Among the several fungi causing damage to mango fruits during post harvest handling, anthracnose caused by *Colletotrichum gloeosporioides* recorded 6.0-15% damage in different parts of India (Tandon, 1967). The influence of environmental factors on the post harvest diseases has not been studied in detail. In the present study, the effect of temperature and relative humidity on the mycelial growth, conidial germination and the anthracnose disease development in mango fruits were studied.

Materials and Methods

Effect of temperature on growth of pathogen

The effect of temperature on mycelial growth, conidial germination and the disease incidence was studied at the Department of Plant Pathology, TNAU, Coimbatore during 1998-99.

Mycelial growth

The oat meal medium in the petridishes were inoculated with the pathogen. The inoculated dishes were incubated at different temperatures *viz.* 13,15,20,25,30,35 and 40°C for 10 days in BOD incubator and suitable control was maintained at 30°. Each treatment was replicated three times. The radial mycelial growth was recorded on 4,6,8 and 10 days after inoculation.

Conidial germination

The cavity slides containing 0.1ml of conidial suspension (10^6 conidia/ml) were placed in petridishes containing moist filter papers at the bottom. The petridishes were incubated at different temperatures as mentioned above. The conidial germination was recorded after 24h of incubation. Three replications were maintained and in each replication 100 conidia were observed for germination.

Disease incidence

The fruits were inoculated by pinprick method mentioned earlier and kept at different temperatures mentioned above. Three replications were maintained for each treatment with five fruits per replication. The disease incidence was recorded 4,6,8 and 10 days after inoculation using the following disease score chart and the PDI was calculated.

Table 1. Effect of temperature on the mycelial growth and conidial germination of *C. gloeosporioides*

Temperature (°C)	Radial mycelial growth (mm)* Days after inoculation				Per cent decrease over control on 10th day	Conidial* germination after 24h (%)	Per cent decrease over control	Remarks
	4	6	8	10				
13	0	0	0	11	87.64	5.00 (12.92)	93.48	Pink coloured acervuli formed on 11th day
15	0	0	12	35	60.67	20.00 (26.15)	73.91	Pink coloured acervuli formed on 10th day
20	20	29	63	77	13.48	56.67 (48.88)	26.09	Pink coloured acervuli formed on 5th day
25	27	44	70	89	0.00	75.00 (60.00)	2.18	Pink coloured acervuli formed on 8th day
30	23	31	65	83	6.74	66.67 (54.84)	13.04	Pink coloured acervuli formed on 8th day
35	19	28	61	75	15.73	48.33 (44.08)	36.96	Pink coloured acervuli formed on 5th day
40	0	0	9	12	86.52	10.00 (17.85)	86.96	Pink coloured acervuli formed on 10th day
Control (30±2°C) (Room temperature)	27	45	70	89	-	76.67 (61.08)	-	Pink coloured acervuli formed on 8th day

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. The values in the parentheses are Arcsine transformed values.

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual ratings} \times 100}{\text{Total number of fruits graded} \times \text{maximum disease grade}}$$

Description	Disease category / Grade
No infection	0
25 per cent fruit surface infected	1
26-50 per cent fruit surface infected	2
51-75 per cent fruit surface infected	3
More than 75 per cent fruit surface infected	4

Table 2. Effect of temperature on the incidence and severity of anthracnose disease in mango fruits

Temperature (°C)	Incubation period (days)	Per cent disease index* Days after inoculation 4	6	8	10	Per cent decrease over control 10th day after inoculation	Remarks
13	10-16	0.00 (2.62)^{d}	0.00 (2.62)^{d}	0.00 (2.62)^{c}	3.33 (10.51)^{c}	96.56	No acervuli formation on fruits
15	6-11	0.00 (2.62)^{d}	5.00 (16.78)^{c}	10.00 (17.85)^{d}	13.33 (21.41)^{d}	86.21	Acervuli formation on fruits on 7th day
20	4-8	11.67 (19.31)^{b}	23.33 (28.88)^{ab}	50.00 (45.00)^{b}	75.00 (60.08)^{d}	22.42	Acervuli formation on fruits on 7th day
25	3-7	23.33 (28.88)^{a}	30.00 (33.16)^{a}	61.67 (51.75)^{a}	95.00 (82.31)^{c}	1.73	Acervuli formation on fruits on 9th day
30	3-7	15.00 (22.60)^{b}	26.67 (30.95)^{ab}	51.67 (45.96)^{b}	85.00 (67.71)^{b}	12.07	Acervuli formation on fruits on 9th day
35	4-6	6.67 (14.97)^{c}	18.33 (25.35)^{b}	40.00 (39.21)^{c}	73.33 (58.91)^{c}	24.14	Acervuli formation on fruits on 6th day
40	8-15	0.00 (2.62)^{d}	0.00 (2.62)^{d}	5.00 (16.78)^{d}	11.67 (19.31)^{d}	87.93	No Acervuli formation on fruits
Control (30 ± 2°C) (Room temperature	3-7	25.00 (30.00)^{a}	30.00 (33.16)^{a}	63.33 (52.73)^{a}	69.67 (79.79)^{a}	-	Acervuli formation on fruits on 11th day

* Mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. The values in the parentheses are Arcsine transformed values.

Effect of relative humidity on growth of pathogen

The effect of relative humidity on the mycelial growth, spore germination and disease incidence was studied.

Mycelial growth

The oat meal agar medium in the petridishes were inoculated with the pathogen as described earlier. The inoculated petridishes were placed in desiccators over different concentrations of sulphuric acid to give appropriate relative humidities *viz.* 36.8, 46.8, 56.8, 66.8, 82.9, 92.9 and 100 per cent (Johnson and Booth, 1983). Each treatment was replicated thrice. The radial growth was recorded on 4,6,8 and 10 days after incubation.

Conidial germination

The cavity slides containing 0.1ml of conidial suspension (10^6 conidia/ml) were placed in petridishes containing moist filter papers at the bottom were incubated for 24h at different relative humidity as mentioned early. Each treatment was replicated thrice and in each replication 100 conidia were counted for the germination.

Disease incidence

The fruits were inoculated by pinprick method and incubated at different relative humidity as mentioned above. Three replications were maintained for each treatment with five fruits per replication. The disease incidence was recorded 4,6,8 and 10 days after inoculation using the

Table 3. Effect of temperature on the mycelial growth and conidial germination of *C. gloeosporioides*

Relative humidity (%)	Radial mycelial growth (mm)* Days after inoculation 4	6	8	10	Per cent decrease (–) over control on 10th day after inoculation	Conidial* germination after 24h (%)	Per cent decrease (–) over control	Remarks
36.8	0[e]	0[d]	0[f]	0[e]	-100.00	0.00 (5.86)[f]	-100.00	-
46.8	0[e]	0[d]	0[f]	0[d]	-100.00	0.00 (5.86)[f]	-100.00	-
56.88	0[e]	0[d]	11[e]	20[d]	-77.27	8.33 (16.78)[e]	88.89	Pink acervuli formed on 9th day
66.8	9[b]	24[c]	33[d]	47[c]	-46.59	41.67 (40.21)[d]	-44.44	Pink acervuli formed on 6th day
82.9	13[b]	33[b]	46[c]	61[b]	-30.68	65.00 (53.76)[c]	-13.33	Pink acervuli formed on 8th day
92.9	23[a]	45[a]	67[ab]	84[a]	-4.55	81.67 (65.65)[b]	+8.89	Pink acervuli formed on 10th day
100.0	28[a]	51[a]	77[a]	89[a]	+1.14	90.00 (71.57)[a]	+20.00	Pink acervuli formed on 5th day
Control (70±10%) (Room temperature)	27[a]	48[a]	72[ab]	88[a]	-	75.00 (60.00)[b]	-	Pink acervuli formed on 12th day

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. The values in the parentheses are Arcsine transformed values.

disease score chart mentioned earlier and the PDI was calculated.

Results and Discussion

Effect of temperature on mycelial growth and conidial germination

The results revealed that the growth was maximum at 25.0°C and temperature below 20°C and above 35.0°C were inhibitory to the growth (Table 1). The growth was reduced when the temperature goes below 25.0°C or above 30.0°C. The formation of acervuli was observed on eighth day at the optimum temperature while it was noticed on fifth day at 20.0 and 35.0°C. The conidia were found to germinate at temperature ranging from 13.0 to 40.0°C. The maximum conidial germination was observed at the room temperature (30±2°C) with 76.67 per cent and it was on par with 25.0°C (Table 1).

Disease incidence

The maximum disease incidence of 96.67 per cent was recorded at 25.0°C and room temperature (30±2°C) after 10 days of inoculation. The optimum temperature for the disease development ranged from 25.0 to 35.0. The aceruvulus formation was quick in the temperature range of 20.0 to 35.0°C (Table 2).

Effect of relative humidity on mycelial growth and conidial germination

The maximum radial mycelial growth of 89 mm was recorded at 100 per cent relative

Table 4. Effect of relative humidity on the incidence and severity of anthracnose disease in mango fruits

Temperature (°C)	Incubation period (days)	Per cent disease index* Days after inoculation 4	6	8	10	Remarks
36.8	-	0.00 (2.62)^{d}	0.00 (2.62)^{c}	0.00 (2.62)^{d}	0.00 (2.62)^{f}	No acervuli formation on fruits
46.8	-	0.00 (2.62)^{d}	0.00 (2.62)^{c}	0.00 (17.85)^{d}	0.00 (2.62)^{f}	Acervuli formation on fruits on 7th day
56.8	8-16	0.00 (2.62)^{d}	0.00 (2.62)^{c}	5.00 (12.92)^{c}	8.33 (16.78)^{e}	Acervuli formation on fruits on 7th day
66.8	4-10	3.33 (10.48)^{c}	13.33 (21.41)^{b}	20.00 (26.15)^{b}	35.00 (36.27)^{d}	Acervuli formation on fruits on 9th day
82.9	4-10	8.33 (16.78)^{d}	16.67 (24.10)^{b}	43.33 (41.17)^{b}	63.33 (52.73)^{c}	Acervuli formation on fruits on 9th day
92.9	3-7	11.67 (19.31)^{b}	20.00 (26.15)^{b}	56.67 (48.88)^{a}	85.00 (67.71)^{ab}	Acervuli formation on fruits on 6th day
100.00	3-6	28.33 (32.16)^{a}	36.67 37.12)^{a}	65.00 (54.32)^{a}	98.33 (82.58)^{a}	No acervuli formation on fruits
Control (70 ± 10%) (Room temperature	3-5	25.00 (30.00)^{a}	30.00 (33.21)^{a}	61.67 (51.76)^{a}	90.00 (71.57)^{a}	Acervuli formation on fruits on 11th day

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. The values in the parentheses are Arcsine transformed values.

humidity, while it was 20mm at 56.8 per cent relative humidity after 10 days of inoculation. The radial mycelial growth was on par at the relative humidity level 100, 92.9 per cent and control (room humidity 70±10 per cent), 10 days after inoculation. The acervuli formation was quickened at relative humidity levels 66.8 and 82.9 per cent while it was slow in the relative humidity levels above 82.9 per cent (Table 3). High humidity of 92.9 per cent and above were found to induce maximum germination. The conidial germination was drastically reduced at relative humidity below 82.9 per cent. The maximum conidial germination of 90.00 per cent was recorded at 100 per cent relative humidity followed by 81.67 per cent of conidial germination in 92.9 per cent relative humidity (Table 3).

Disease development

Maximum disease incidence of 98.33 per cent was observed at the relative humidity level 100 per cent while only 8.33 per cent was observed at 56.8 per cent relative humidity. When the relative humidity was reduced from 82.9 per cent to 66.8 per cent a considerable reduction of 40 to 50 per cent disease incidence was observed (Table 4).

Amongst various factors, temperature plays a key role in influencing the growth and conidial germination. Temperature management is important

in reducing physiological deterioration and preventing moisture loss and shriveling as well as reducing the disease. The metabolic activity of both host and pathogen depends on the action of enzymes that are extremely sensitive to temperature. The relative physiological life of hosts can usually be predicted by respiration. The other host processes linked to respiration are tissue softening, loss of chlorophyll, development of anthocyanin pigments and many others which are temperature sensitive. It was noted that between 0°C and 30°C, for each 10°C increase in temperature there was two to three fold increase in metabolic activity (Eckert and Sommer, 1967).

The fungus showed variation in its conidial germination, growth and disease development at different temperatures, maximum being at 25°C and 30°C, while the least growth was observed at 13°C and 40°C (Table 9,10). These results were in accordance for the growth of *C. gloeosporioides* was 25°C and beyond 35°C the growth ceased. (Quimio, 1975) reported that growth, sporulation, conidial germination and disease development on fruits occurred at wide range of temperature (15 to 30°C) and the optimum temperature lied between 25 to 30°C. Similar results were also reported by Sattar and Malik (1939); Wastie (1972); Fitzell and Peak (1984); Dodd *et al.* (1991) and Makowski (1993).

Relative humidity is one of the major factor determining the growth, conidial germination and disease development in fruits. *C.gloeosporioides* showed variation in its growth, conidal germination and disease development at different relative humidity levels, being maximum at 100 per cent and least at 56.80 per cent (11,12). Baker *et al.* (1940) reported that a relative humidity of 95 per cent for 12h was considered essential for infection and development of *C.gloeosporioides* on the mango fruit. Fitzell and Peak (1984) reported that conidia were formed on the acervuli of the diseased leaves at the relative humidity of 95-97 per cent. Dodd *et al.* (1991) reported that the conidial germination occurred between 95-100 per cent relative humidity. Similar observations were reported by Estrada (1990). Palaniswami (1978) reported the sane trend in the case of *Botryodiplodia theobromae*, the casual agent of stem end rot of mango and banana.

From the present study, it is evident that the fruits stored below 20°C showed less decay when compared to those stored above 20°C. So, for long term storage and fruits may be stored at a temperature below 20°C

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