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Influence of Incubation Temperature on Variation in Physiology of Growth and Sporulation of *Colletotrichum Gloeosporioides* Isolates Infecting Tropical Fruits

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Abstract

There was profound effect of temperature on isolate variability of *C. gloeosporioides* isolates infecting tropical fruits. Maximum average growth was attended by Cg 91 (9.34 cm day^{-1}) infecting jamun and the lowest average growth rate was attended by Cg 61 (5.02) infecting mango. Isolates Cg 51, Cg 54, and Cg 63 also showed considerable retarded growth. In general, isolates from sweet orange and pomegranate showed moderate to poor growth at temperature range of 18 – 36 ^{0} C. It was observed that the optimum temperature for the growth of *C. gloeosporioides* isolates was 27 – 30^{0}C. The effect of temperature on isolate variability was highly significant. Maximum sporulation irrespective of isolates was attended at 24^{0}C. and was followed by at 27 and 30^0 C. The difference in temperature by 3^{0}C. from 24 to 27 reduced the average sporulation ability considerably. However, with further increase in temperature from 27 to 30^{0}C the amount of sporulation ability was almost constant but declined sharply at 33^{0}C. Few isolates were found to be distinct for the temperature requirements. Isolates, Cg 42 and Cg 62 sporulated at 30^{0}C and isolates Cg 63 Cg 83 and Cg 84 sporulated at 33^{0}C These isolates failed to sporulate at lower temperature levels.

Keywords: Temperature, Colletitrichum gloeosporioides, Isolates, Growth, Sporulation.

1. Introduction

Colletotrichum gloeosporioides is one of the most important fungal plant pathogens worldwide, in tropical and subtropical regions. It causes anthracnose, die back, whither tip, shot hole, leaf blight and post harvest rots in many economically important fruit crops. As a post harvest pathogen, the degree of infection and subsequent invasion is largely influenced by the temperature during ripening, transit, storage and in the market. Pandey, et al. [1] have 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. With reference to climate change, the effect of temperature on behavior of the pathogen having wide host range and ability to develop in an epidemic form needs to be critically studied. As *C. gloeosporioides* is a predominant post harvest pathogen, the ripening temperature is the most crucial factor that determines the degree of severity of infection. Present investigation was therefore undertaken in order to identify most favorable temperature for growth and sporulation so that ripening temperature can be adjusted which will not be favorable for the pathogen development.

2. Materials and Methods

Thirty isolates of *C. gloeosporioides* were obtained from nine different fruit types from four different agro ecological zones of Maharashtra (India). The pathogenicity of each isolate was proved on respective fruit host by following the mycelial bit inoculation method [2]. Pathogenic isolates were accessed with Cg as prefix representing the scientific name of the pathogen followed by two digits. The first digit represents the host type and second digit represents the isolate number from the respective host.

Laboratory experiment was conducted in factorial RBD with 30 treatments (*C.gloeosporioides* isolates), two replications and seven temperature levels. Potato dextrose agar was used as a basal medium. A mycelial bit of 5 mm size of each isolate was placed aseptically at the centre of the solidified Medium in a Petri plate of 10 cm diameter. Such inoculated plates were incubated for 7 days in BOD incubators already set at specific temperature ranging from 18-36^{0}C. After incubation period, the linear growth was measured in mm and the growth rate per day was calculated.

For quantification of sporulation per sq. cm, 3 bits of 5mm (15 sq. mm) diameter were taken from each Petri plate at equal distance from centre to periphery. These bits were suspended in 10 ml sterile water in a test tube. Such test tubes were vigorously shaken using vortex mixture for 5 minutes each to release conidia. The quantification of conidia was done by using haemocytometer and light microscope. The treatment wise sporulation was recorded and amount of sporulation per sq. cm was calculated. The data obtained on growth and sporulation was subjected to Factorial completely Randomized Design (FCRD) and means were compared at 1% significance level.

3.Results and Discussion

3.1. Effect of Different Temperature Levels on Variation in Growthof*C. Gloeosporioides* Isolates

The incubation temperature was found to be most effective parameter for differentiating *C. gloeosporioides* isolates. The differences in the isolates, temperature regimes and their interaction were significant. *C. gloeosporioides* isolates expressed considerable variation in their growth rate irrespective of incubation temperature level. Maximum average growth was attended by Cg 91 (9.34 cm day^{-1}) and was statistically equal with Cg 32, Cg 64 and Cg 81. It was followed by Cg 65 and it was on par with Cg 33, Cg 41 and Cg 43. The lowest average growth rate was attended by Cg 61 (5.02 cm day^{-1}). Isolates Cg 51, Cg 54, and Cg 63 also showed considerable retarded growth which was < 6 cm day $^{-1}$. In general, isolates from sweet orange and pomegranate showed moderate to poor growth at temperature range of 18 – 36 ^{0} C. Six isolates obtained from pomegranate expressed maximum growth at 27^{0}C However, Jayalakshmi [3] reported 30^{0}C as an optimum temperature for *C. gloeosporioides* causing anthracnose of pomegranate.

Effect of temperature on growth of *C. gloeosporioides* irrespective of isolates revealed that the maximum growth of 11.19 cm day^{-1} was recorded at 27 ^{0}C. and it was on par with the growth at 30^{0}C (10.91 cm day^{-1}). This indicated that the optimum temperature range for *C. gloeosporioides* isolates was 27 – 30^{0}C. Extremely low growth of *C. gloeosporioides* isolates in general (3. 19) was attended at 36 ^{0}C.and moderate growth at 24^0 C. Kanapathipillai [4] also reported that *C. gloeosporioides* grew well between temperatures of 25-30°C but at 15 and 35°C growth was reduced. Further, it was stated that optimum temperature range of for growth of *C. gloeosporioides* isolates obtained from eighteen hosts from Malaysia was 28-30^0. The present findings are in agreement with Tasiwal and Benagi [5], Wasanthakumar and Rawal [6] . On similar line variation in the growth rate due to temperature was also reported by Agostini, et al. [7] While studying *C. gloeosporioides* isolates infecting *Citrus* spp. and found that, FGG isolates grew two to three times faster than SGO and KLA isolates. Growth of SGO was faster than KLA

Table-1. Effect of different temperature regimes on variation in the average growth rate (mm day^{-1}) of *C. gloeosporioides* isolates

Hosts	Isolates	18^0 C*	21^0C*	24^0 C*	27^0 C*	30^0 C*	33^0 C*	36^0 C*	Mean
Areca nut	Cg 11	2.50	4.93	9.79	12.57	9.00	7.00	2.85	6.94
Cashew nut	Cg 21	4.28	7.07	8.71	11.93	12.85	6.78	2.85	7.78
Custard apple	Cg 31	4.29	6.64	7.64	11.57	12.85	6.92	2.36	7.46
	Cg 32	2.57	7.64	9.36	12.85	12.85	11.71	6.00	8.99
	Cg 33	5.07	6.78	8.43	12.85	12.85	10.49	3.71	8.60
Mandarine	Cg 41	5.36	7.00	8.57	12.36	10.86	11.42	3.79	8.48
	Cg 42	4.43	7.64	8.78	10.14	7.28	4.14	1.42	6.26
	Cg 43	5.71	7.71	9.43	12.57	12.85	8.71	3.79	8.68
Sweet orange	Cg 51	2.36	5.71	8.07	8.93	6.14	4.64	3.86	5.67
	Cg 52	7.16	8.21	8.93	11.93	11.78	6.00	1.85	7.98
	Cg 53	6.41	7.28	8.43	11.43	8.29	8.43	4.07	7.76
	Cg 54	2.00	4.07	4.50	7.57	11.28	7.93	3.71	5.86
	Cg 55	3.71	7.28	8.14	11.57	12.14	9.00	3.00	7.83
	Cg 56	5.75	6.64	8.49	10.43	12.85	6.92	5.00	8.01
Mango	Cg 61	2.99	4.14	4.71	5.36	9.26	4.71	4.00	5.02
	Cg 62	3.14	6.64	7.28	10.14	8.21	6.71	1.92	6.29
	Cg 63	3.14	5.50	6.43	6.43	7.64	7.07	3.00	5.60
	Cg 64	7.57	8.43	9.00	12.85	12.57	9.93	3.43	9.11
	Cg 65	6.57	8.29	9.36	12.85	12.71	9.00	2.85	8.80
	Cg 66	5.21	8.36	9.07	10.21	12.85	6.28	3.79	7.97
	Cg 67	4.57	6.71	9.50	12.85	10.93	8.79	1.42	7.82
	Cg 68	4.79	6.93	8.71	12.85	11.57	10.50	3.42	8.39
Guava	Cg 71	2.86	6.21	7.85	12.85	12.14	10.57	3.64	8.02
Pomegranate	Cg 81	6.07	7.93	9.21	12.71	12.57	11.28	2.85	8.94
	Cg 82	5.86	6.43	9.28	12.00	10.36	6.78	1.14	7.40
	Cg 83	4.50	7.71	9.50	10.57	7.50	8.93	1.21	7.13
	Cg 84	6.33	7.64	9.71	11.57	12.00	6.21	2.85	8.04
	Cg 85	5.43	6.43	8.14	11.44	10.36	6.93	1.57	7.18
	Cg 86	4.71	7.51	8.00	9.64	10.14	7.86	3.71	7.37
Jamun	Cg 91	6.41	8.07	9.36	12.85	12.57	9.35	6.78	9.34
	Mean	4.72	6.92	8.41	11.19	10.91	8.03	3.19	7.62
S.E. ± (Isolates)			0.14	C.D. 1% (Isolates)					0.52
S.E. ± (‘Temperature range)			0.07	C.D. 1% (Temperature range)					0.25
S.E.± (Isolates x Temp. range)			0.38	C.D. 1% (Isolates x Temp. range)					1.37

* Means of two replications

isolates up to 27^{0}C but was slower than KLA between 27-31^{0}C. The response of individual isolates within isolate groups to temperature was nearly identical. Linear increment in growth of all three groups was observed between 23-27^{0}C peak at 27^{0}C gradual decline between 27-30^{0}C and fast decline between 30-33^{0}C . No growth occurred in any case at 36^{0}C. Denner, et al. [8] also concluded maximum growth of *C. gloeosporioides* infecting avocado grew fast at 28^{0}C.

When the interaction effect was analyzed, it was revealed that in all 14 treatment combinations recorded highest growth rate of 12.85 cm day^{-1} and were on par with other 18 treatment combinations wherein the average growth rate was 12.85 –11.48 cm day-1. Thus 32 treatment interactions were recorded as fast growing. These interactions include the temperature range of 24, 27 and 30^0 C (Table 1).

3.2. Effect of Different Temperature Levels on Variation in Sporulation of *C. Gloeosporioides* Isolates

Perusal of Table 2 revealed that isolates showed considerable variation in the sporulation ability at different temperature regimes, the average maximum sporulation irrespective temperature leveles was recorded by Cg 71 (10.64 X 10^4 conidia sq. cm^{-1}). It was followed by Cg 32, Cg 41 and Cg 33, in the descending order with significant statistical difference. The maximum sporulation irrespective of isolates was attended at 24^{0}C (8.06 x 10^4 conidia sq. cm^{-1}) and was followed by 27 and 30^{0}C respectively and at these temperature levels the sporulation was statistically un differentiable. It was revealed that the difference in temperature by 3^{0}C from 24 to 27 reduced the average sporulation ability by almost 50 %. However, with further increase in temperature from 27 to 30^0 C the sporulation ability was almost constant but declined sharply at 33^{0}C. Six out of eight isolates from mango attended maximum sporulation at 24^0 C. with good sporulation ability up to 30^{0}C. Pandey, et al. [1] also reported that *C. gloeosporioides* isolate of mango Cg 72 (from Maharashtra) showed more virulence and maximum sporulation (137.5 x 10^{3}ml^{-1}) at 28°C. Few isolates were found to be distinct for the temperature requirements. Isolates, Cg 42 and Cg 62, which sporulated at 30^{0}C and isolates Cg 63, Cg 83 and Cg 84 sporulated at 33^{0}C. Isolates Cg 71 and Cg 81 recorded sporulation at all temperature regimes from 18 –36^{0}C. The sporulation in Cg 56, Cg 82 cg 83 and Cg 84 was observed at extreme levels of temperature than the optimum (24 - 27^{0}C). The sporulation at 36^{0}C was very low and 15 isolates failed to sporulate at this level while 14 isolates failed to sporulate at the 18^0 C. Isolates Cg 51 and Cg 52 had a very narrow range of 27 – 33^{0}C for sporulation and followed by isolates Cg 11, Cg 66 Cg 67 and Cg 68 (24 – 33^{0}C).

Table-2. Effect of different temperature regimes on variation in the sporulation of *C. gloeosporioides* isolates (Conidia 1 X 10^4)

Hosts	**Isolates**	**18^0 C***	**21^{0}C***	**24^0 C***	**27^0 C***	**30^0 C***	**33^0 C***	**36^0 C***	**Mean**
Areca nut	Cg 11	0.00	0.00	9.07	2.88	0.00	1.43	0	1.91
Cashew nut	Cg 21	0.00	4.61	20.51	7.79	5.72	2.85	2.23	6.24
Custard apple	Cg 31	0.00	1.75	3.82	0.00	0.00	2.36	2.22	1.45
	Cg 32	2.56	6.84	15.70	8.59	3.97	6.00	6.36	7.14
	Cg 33	2.38	2.23	27.03	2.06	6.04	3.71	3.5	6.70
Mandarine	Cg 41	3.97	6.20	18.60	6.36	8.58	3.79	0	6.78
	Cg 42	0.00	0.00	0.00	0.00	3.04	1.42	0	0.64
	Cg 43	2.86	6.02	6.68	7.63	3.02	3.79	0	3.85
Sweet orange	Cg 51	0.00	0.00	0.00	2.70	3.00	3.86	0	0.94
	Cg 52	0.00	0.00	0.00	4.45	2.53	1.85	0	0.90
	Cg 53	1.90	6.35	9.01	8.00	4.93	4.07	0	4.89
	Cg 54	0.00	1.27	0.00	2.22	2.14	3.71	0	1.03
	Cg 55	0.00	7.95	14.31	7.63	5.70	3.00	5.56	6.30
	Cg 56	13.35	0.00	0.00	0.00	0.00	5.00	1.43	2.83
Mango	Cg 61	0.00	0.00	6.68	0.00	18.29	4.00	6.84	5.11
	Cg 62	0.00	0.00	0.00	0.00	6.20	1.92	0	1.16
	Cg 63	0.00	0.00	0.00	0.00	0.00	3.00	0	0.43
	Cg 64	5.56	3.50	19.24	17.96	4.13	3.43	3.2	8.14
	Cg 65	5.25	7.15	13.35	8.90	8.90	2.85	2.69	7.01
	Cg 66	0.00	0.00	16.06	3.34	16.53	3.79	0	5.67
	Cg 67	0.00	2.72	3.97	3.50	0.00	1.42	0	1.66
	Cg 68	0.00	0.26	7.95	3.66	4.45	3.42	0	2.82
Guava	Cg 71	7.54	5.26	19.24	26.07	5.88	3.64	6.83	10.64
Pomegranate	Cg 81	7.63	3.34	9.08	6.37	7.79	2.85	4.61	5.95
	Cg 82	0.16	0.00	0.00	0.00	2.38	1.14	4.34	1.14
	Cg 83	3.18	0.00	0.00	0.00	0.00	1.21	0	0.63
	Cg 84	0.00	0.00	0.00	0.00	0.00	2.85	0	0.41
	Cg 85	3.66	3.34	5.09	3.32	0.00	1.57	1.11	2.58
	Cg 86	2.70	4.13	4.76	0.00	2.22	3.71	0.95	2.64
Jamun	Cg 91	5.27	4.61	11.76	2.70	6.52	6.78	2.38	5.72
	Mean	2.26	2.58	8.06	4.54	4.53	3.15	1.81	3.78
S.E. ± (Isolates)			0.35	C.D. 1% (Isolates)					1.28
S.E. ± (Temperature range)			0.17	C.D. 1% (Temperature range)					0.62
S.E.± (Isolates x Temp. range)			0.93	C.D. 1% (Isolates x Temp. range)					3.38

* Means of two replications

These findings are in agreement with those reported by Smith [9] who has recorded that the sporulation of fungi of the genus *Colletotrichum* is favored by temperatures in the range of 20-24^{0}C, while temperatures above 30^{0}C may have an inhibitory effect. Sangeetha and Rawal [10] stated that out of 8 *C. gloeosporioides* isolates infecting mango, Dapoli, Hessarghatta and Raichur isolates expressed good sporulationj at 28°C whereas at 25°C good sporulation was recorded in Lucknow and Tiruvur isolates.

When the combined effect of temperature is compared on the basis of growth and sporulation, it can be concluded that the optimum range of temperature for the growth of *C. gloeosporioides* is 27 – 30^0 C while the fungus sporulated profusely at a temperature of 24 to 27^0 C. Quimio [11] reported that the optimum temperature for growth, sporulation and spore germination of *C. gloeosporioides* was 30ºC. The present work is partially in line with the findings in relation to growth but differs for sporulation.

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