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In-vitro Effect of Different Abiotic Stresses on the Growth and Sporulation of *Colletotrichum gloeosporioides* causing Anthracnose of Mango

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ABSTRACT

Background: Mango productivity was very much affected due to a major fungal pathogen, *Colletotrichum gloeosporioides* causing anthracnose mango rot. The present study was carried out to investigate the influence of abiotic factors for the support of superficial growth of isolated fungus and finding a minimum inhibitory concentration of different fungicides.

Methods: Among four different culture media tested, the highest radial growth and sporulation of the fungus were recorded in Oatmeal agar (OMA) (84 mm) followed by Conn's agar (CA), Czapek Dox agar (CDA) and Potato dextrose agar (PDA). Among the different pH tested, pH 7.0 was found to be the best in supporting the good radial growth (69 mm) followed by pH 6.0 (56 mm), pH 5.5 (49 mm), pH 7.5 (43 mm) and pH 8.0 (37 mm). Among the various temperature tested, 25°C (69.32) was found to be the best followed by 20°C (52.53 mm), 30°C (65.23 mm) and 35°C.

Result: Among the fungicides tested, Zineb 68% + Hexaconazole 4% WP (avtar) was found best as the radial growth was observed to be 45, 41, 36, 32, 25 mm at 5, 10, 25, 50 and 100 ppm, respectively as compared to 80 mm in control. The fungicide Tricyclazole 18% + Mancozeb 62% WP (Merger) was found to be the least effective in checking the radial growth of *C. gloeosporioides* even at 100 ppm concentration.

Key words: Anthracnose, *Colletotrichum,* Fungicides, Mango, pH, Temperature.

INTRODUCTION

Mango (*Mangifera indica* L.) belongs to the family-*Anacardiaceae* is one of the most important fruit crops, growing in almost all parts of the world (Ann *et al.,* 1997). India is one of the major producers of mango in the world. Other important mango-producing countries are China, Mexico, Thailand, Pakistan, the Philippines, Indonesia, Nigeria and Brazil. Although India is the largest producer of mango in terms of productivity its ranks sixth. The low productivity is mainly due to the disease associated with mango. Anthracnose is presently recognized as the most important field and post-harvest disease of mango worldwide (Ekbote, 1994). 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 (FAO, 2000). This disease is so destructive that crop losses can reach upto 60 per cent or higher during heavy rain season, disease control was obtained with fungicides chlorothalonil, thiram and carbendazim (Jeffries *et al.,* 1990). Prochloraz is an imidazole fungicide that has eradicative but not systemic properties and the mode of action of this fungicide was thought to be inhibition of the ergosterol biosynthesis pathway (Kumar and Rani, 2010). Unlike triazole fungicides, prochloraz was found to be very effective in controlling anthracnose disease (McMillan, 1984). Spraying of the chemical fungicides such as Carbendazim, Zinep, Manep and Copper oxychloride which showed that the pathogen inoculum and disease in cidence could be reduced only 23.83 and 50.16 per cent; respectively disease levels during flower setting until harvesting were compared in rainy (off-season fruits) and summer seasons (Noiaium and Soytong, 1999). The introduction of benzimidazole fungicides such as benomyl, carbendazim and thiophanate-methyl in the early 1960s revolutionized disease control as reported (Ploetz and Prakash, 1997). (Prior *et al.,*1992) Reported that spraying of the chemical fungicides such as carbendazim, zineb, maneb and copper oxychloride showed that the pathogen inoculums and disease incidence could reduce only 23.83 and 50.16 per cent respectively. Benzimidazoles like carbendazim, thiophante-methyl and benomyl were most effective

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compared to non-systemic fungicides in controlling mango anthracnose as reported (Saju *et al.,* 2012). The best control was obtained with fungicides chlorothalonil, thiram and carbendazim (Jeffries *et al.,* 1990). Prochloraz is an imidazole fungicide that has eradicative but non-systemic properties and the mode of action of this fungicide was thought to be inhibition of the ergosterol biosynthesis pathway (Kumar and Rani, 2010). This fungicide also has been used in mango anthracnose control in many countries (Sangeetha and Rawal, 2009). Systemic fungicides are important tools because they are capable of moving within a plant system. Therefore, present work was carried out to standardize fungicide concentration to control *C. gloeosporioides* growth and also to study growth performance against different abiotic factors such as different pH, temperature and nutrient formulation with sporulation.

MATERIALS AND METHODS

Field surveys were carried out at the experimental form of School of Agriculture and Horticulture, Kalasalingam Academy of Research and Education, Virudhunagar district, Tamil Nadu. The pathogen *Colletotrichum gloeosporioides* Penz, the causal organism of mango anthracnose was isolated by standard protocol and purification followed sporulation study from infected leaves of mango by isolation method from leaves (Saxena, 2002) on potato dextrose agar medium (PDA).

Standardization of media for the growth and sporulation of *C. gloeosporioides*

Four different fungal media was used to identify the status of *C. gloeosporioides* growth and sporulation. In this study, media such as Oatmeal agar (OMA), Potato dextrose agar (PDA), Conn's agar (CA) and Czapek Dox agar (CDA) were screened to select the medium supporting the highest growth of the fungus. All the media were adjusted to pH-7.0 and sterilized at 15 lbs /inch^2 for 20 min. In sterilized Petri plates, 18-20 ml of sterilized medium was poured. The plate was shaken uniformly and the medium in plates was allowed to solidify. These were inoculated by placing in the culture of the plate 5 mm mycelial disc having conidial growth cut from the margin of the 5-day old culture of the test fungus. Inoculated Petridishes was incubated at 27±2°C for 6 days. The radial growth was measured at the interval of 24 h till the 7th day. The medium, which supported the highest radial growth, was selected for further studies. For the sporulation study, a 5 mm mycelial disc of actively growing culture of *C. gloeosporioides* was dipped in sterilized distilled water about 5-10 times. About 10 µl spore suspension solution was then observed on the slide under the microscopic field. The numbers of spores were counted using standard methods for spore count.

Effect of different temperatures on the growth of *C. gloeosporioides*

Four temperature regimes *viz.* 20, 25, 30 and 35°C were selected to study the best temperature regime supporting the radial growth of the pathogen. These media were adjusted to pH 7.0 and then in sterilized Petri-plates, 18-20 ml of sterilized medium was poured. The plates were shaken uniformly and the medium in plates was allowed to solidify. After solidification of the medium in the plates, these were inoculated by placing in the center of the plate 5 mm mycelium disc having conidial growth cut from the margin of the 7-day old culture of the fungus. Inoculated Petri discs were kept in an incubator at different selected temperatures *viz.* 20, 25, 30 and 35°C for 7 days. The radial growth was measured after 24 hours at regular intervals for 7 days.

Effect of different pH on growth of *C. gloeosporioides*

Effect of different pH values *viz.*, on Prepared different pH (5.5, 6.0, 7.0, 7.5 and 8.0) of PDB media adjusted by adding sodium hydroxide (NaOH) (0.1N) or hydrochloric acid (0.1N HCl) and additionally added 2% solidification agent agar with pH was measured using electrical pH meter before sterilization within autoclave at 121°C. Twenty 20 ml of sterilized media was poured into sterilized Petri plates. The plates were shaken uniformly and the medium in plates was

Table 1: Effect of different media on radial growth of *C. gloeosporioide*s.

Medium	Radial growth of *C. gloeosporioide*s (mm)						
	Days						
	1	2	3	4	5	6	Mean
Potato dextrose agar (PDA)	11.21	24.52	27.42	36.72	47.31	52.01	33.19
Oat meal agar (OMA)	13.22	28.51	49.21	69.5	75.81	84.21	47.58
Czapek dox agar (CDA)	11.71	28.32	43.18	58.22	70.33	82.33	46.54
Conn's agar (CA)	10.32	24.50	39.21	53.96	60.31	70.51	43.14
		Media		Days		Media and Days interaction	
SED		0.0355		0.0434		0.0868	
CD (0.05)		0.0712**		0.0873**		0.1746**	

**Significant at 0.01.

*Mean of five replications.

allowed to solidify. After solidification of the medium in the plates, these were inoculated by placing in the center of the plate 5 mm mycelium disc having conidial growth cut from the margin of the 7 days old culture of Petri dish were kept in an incubator at 37±2°C for 7 days. The radial growth was measured after 24 hours at regular intervals for 7 days.

Evaluation of fungicides for the inhibition of growth of *C. gloeosporioides*

Five fungicides *viz.* Zineb 68% + Hexaconazole 4% WP (Avtar), Blastin (Tricyclazole WP.75), Tricyclazole 18% + Mancozeb 62% WP (Merger), Mancozeb 75% WP (Indofil M-45), Blitox-50 (Copper oxychloride) were evaluated for control of *C. gloeosporioides in vitro* for inhibition of radial growth on the media. The above fungicide were tried at 5 ppm, 10 ppm, 25 ppm, 50 and 100 ppm were prepared then sterilized at 15 lbs/ inch^2 for 20 minutes and poured in sterilized Petri plates. The plates were shaken uniformly and the PDA containing the desired concentration of fungicide in plates was allowed to solidify. The plates were inoculated by placing a 5 mm mycelium disc having fungal growth cut from the margin of the 7 day's old culture of the pathogen. Inoculated Petri dishes were incubated at 27±2°C and observations were recorded by measuring the colony diameter of the pathogen and check. and evaluated against *C. gloeosporioides.*

Statistical analysis

Treatment means were compared using ANOVA and the level of significance considered was p>0.01(Scheinpflug and Kuck, 1987).

Table 2: The effects of different media on the sporulation of *C. gloeosporioides*.

Medium	Sporulation	Rating
PDA	Low	+
OMA	High	++++
CDA	Good	+++
CA	Moderate	+

RESULTS AND DISCUSSION

Symptoms

The characteristic symptoms are numerous oval or irregular vinaceous brown or deep brownish spots of various sizes scattered all over the leaf surface under damp conditions. The fungus grows rapidly forming elongated mars brown or mummy brown necrotic areas measuring 20-25 mm in diameter which when old becomes ruptured and blighted. They do not become much larger as the leaf grows but often become dry and fall out giving the older leaves a 'shot hole' appearance.

Effect of different media on radial growth of *C. gloeosporioides*

Results of the experiment revealed that among the four media Oatmeal agar was best (84 mm) supporting the good radial growth of the pathogen followed by Czapek's dox agar and conn's agar (Table 1). Oatmeal agar was selected for further studies of the pathogen.

Effect of different media on sporulation of *C. gloeosporioides*

It was clear from the results (Table 2) that high sporulation was observed in the case of Oatmeal agar media. Czapek Dox agar and Conn's agar supported moderate sporulation. Low sporulation was observed in the case of PDA (Table 2).

Effect of different temperatures on radial growth of *C. gloeosporioides*

Results of the experiment revealed that among the four temperatures tested, 25°C was found best in supporting the good radial growth (69 mm) of *C. gloeosporioides* followed by 30°C (65 mm), 20°C (52 mm). The temperature regime of 35°C did not support the radial growth of the pathogen as only 12 mm radial growth was observed (Table 3). Temperature 25°C was selected for further studies of the pathogen.

Effect of different pH values on radial growth of *C. gloeosporioides*

Results of the experiment revealed that among five pH values, pH 7.0 was found best in supporting the good radial

Table 3: The effects of different temperature on radial growth of *C. gloeosporioides*.

Temperature (°C)	Radial growth colony diameter (mm)							
	Days							
	1	2	3	4	5	6	7	Mean
20	6.07	12.43	16.21	24.33	36.09	43.23	52.53	27.27
25	11.21	21.33	28.31	37.43	44.09	60.32	69.32	38.86
30	7.33	16.95	23.61	33.72	33.72	57.71	65.23	34.04
35	5.95	12.31	8.43	3.21	0	0	0	4.27
		Temperature			Days		Temperature and days interaction	
SED		0.03149			0.03726		0.08332	
CD (0.01)		0.06281**ns			0.07431**ns		0.16617**ns	

**ns=Not Significant at 0.01.

*Mean of four replications.

Table 4: Different pH effects on the radial growth of *C. gloeosporioides.*

pH value	Mean radial growth (mm)							
	Days							
	1	2	3	4	5	6	7	Mean
5.5	9.41	20.72	23.51	27.60	30.82	36.71	49.42	28.31
6.0	11.21	24.42	27.08	30.21	35.31	40.21	56.82	32.18
7.0	12.71	26.62	28.31	32.31	41.61	51.32	65.51	36.91
7.5	6.31	18.81	21.80	24.53	28.41	32.51	43.31	25.09
8.0	4.53	15.61	17.20	19.95	23.32	32.21	37.61	21.49
	pH			Days		pH and Days interaction		
SED	0.03305			0.03911		0.08745		
CD (0.01)	0.06593**ns			0.07800**ns		0.17442**ns		

**ns=Not significant at 0.01.

*Mean of four replications.

Table 5: Fungicide effects on the radial growth of *C. gloeosporioides.*

Fungicides	Mean radial growth (mm) Concentration (ppm)					
	5	10	25	50	100	Mean
Mancozeb 75% WP (Indofil M-45)	45.21	41.31	40.07	36.31	36.31	39.84
Blastin (Tricyclazole WP.75)	47.30	44.21	41.21	35.82	31.31	39.97
Zineb 68% + Hexaconazole 4% WP (Avtar)	45.52	41.61	36.61	32.30	25.41	36.29
Blitox-50 (Copper oxychloride)	56.07	50.21	44.31	36.71	28.30	43.12
Tricyclazole 18% + Mancozeb 62% WP (Merger)	58.89	53.21	46.51	40.21	32.07	46.18
Control	80.21	80.21	80.61	80.31	80.21	80.31
	Fungicide		ppm		Fungicide and ppm interaction	
SED	0.06012		0.05488		0.13443	
CD (0.01)	0.12026**ns		0.10978**ns		0.26891**ns	

**ns=Not significant at 0.01.

*Mean of four replications.

growth (65 mm) of *C. gloeosporioides* followed by pH 6.0 (56 mm), pH 5.5 (49 mm), pH 7.5 (43 mm) and pH 8.0 (37 mm) (Table 4). pH 7.0 was maintained in all further studies.

Effect of fungicides on the radial growth of *C. gloeosporioides*

All the fungicides tested were found effective in checking the radial growth of *C. gloeosporioides.* At all the concentrations tried, Zineb 68% + Hexaconazole 4% WP (Avtar) was found best as the radial growth was observed to be 45, 41, 36, 32, 25 mm at 5, 10, 25, 50 and 100 ppm respectively as compared to 80 mm in control. The fungicide Tricyclazole 18% + Mancozeb 62% WP (Merger) was least effective in checking the radial growth of *C. gloeosporioides* even at a 100 ppm concentration (Table 5).

In Conclusion, *in vitro* effect of various abiotic factors such as nutrient constitution media, temperature, pH against the growth performance of mango pathogen, *Colletotrichum gloeosporioides* was studied. Spalding and Reeder, 1978 reported good growth of *C. gloeosporioides* on Pomegranate between 15-35°C with an optimum at 28°C. (Rangaswami and Mahadevan, 1999) Reported based on the growth of different isolates of *C. gloeosporioides* at different temperatures reported that isolation from Lucknow, Arambakam and Triuvur grow well at 25°C while isolating from Dapoli, Hessarghatta and Tumkur at 28°C and isolation from Hassan and Raichur at 30°C. (Russell, 1995) Recorded maximum growth of *C. gloeosporioides* at 25°C and 29°C respectively. The present study evaluated five different fungicides in various concentrations 5, 10, 25, 50 and 100 ppm. The fungicide Zineb 68% + Hexaconazole (Avtar) was found to be best than other treatments which were following the results of previous studies. The new fungicides were found to work well against the pathogen in the present study. (Jeffries *et al.,* 1990) Conducted experiments to evaluate the effects of 10 fungicides formations and hyphal growth of *C. gloeosporioides* (*Glomerella cingulata)*, the causal agent of persimmon anthracnose. The results showed that the fungicide has a distinct control effect against anthracnose. The best control way obtained with chlorothalonil, thiram and carbendazim. (McMillan, 1984) Reported that, unlike triazole fungicides, prochloraz was

found to be very effective in controlling anthracnose disease. The fungicide also has been used in mango anthracnose control in many countries (Sangeetha and Rawal, 2009). (Ploetz and Prakash, 1997) Reported that systemic fungicides were important tools because they are capable of moving within a plant system. The introduction of benzimidazole fungicides such as benomyl, carbendazim and thiophanate-methyl in the early 1960s revolutionized disease control. Among the tested fungicides, tricyclazoles were found to be superior for controlling the incidence of pathogen (Hua *et al.,* 2001) reported the effectiveness of different fungicides against *C. gloeospori oides* infecting large cardamom, the *in vitro* tests showed that the pathogen was highly sensitive to copper oxychloride 50 WP (0.3%) followed by mancozeb 75 WP (0.3%) and combined formulation of car-bendazim + mancozeb (12 + 63) WP (0.3%).

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