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J. Mycopathol, Res, 55(3) : 269-274, 2017;
ISSN 0971-3719

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J. Mycopathol. Res. ***55(3)*** *: 269-274, 2017;* (ISSN 0971-3719)

Physiological profiling of *Colletotrichum falcatum*, the causal agent of Sugarcane Red rot disease

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Received : 24.05.2017 Accepted : 19.06.2017 Published : 30.10.2017

Growth of fungi *Colletotrichum falcatum*, a sugarcane red rot pathogen, to different ranges of temperature and pH were studied. Results indicated that nine *C. falcatum* isolates had variations in their tolerance to different abiotic stress conditions, they were exposed. Though more alkaline and acidic liquid media have shown mycelial growth to certain extent, pH value 6 was optimum for pathogen growth. Highest dry mycelial were recorded for isolate cfGAN (662.3mg) on liquid media. Among the temperature range studied, 28-32°C supported significantly the maximum growth of all the isolates. Out of nine isolates, cfKAM reached its best up to 8.5cm mean mycelial diameter on OMA after seven days of incubation at 28°C. This information is useful for prediction of environmental and soil condition for infection period in field. This study reveals that lower temperature and slight acidic condition of soil can promote the disease.

Key words: Sugarcane, red rot, *Colletotrichum falcatum*, pH, temperature, pathogen, disease

INTRODUCTION

Red rot is the most severe disease of standing sugarcane worldwide, caused by the *Colletotrichum falcatum* Went. In India, red rot disease is a major constraint for sugarcane production and resulting in elimination of many popular varieties from cultivation (Chona, 1954). The pathogen undergoes continues genetic changes in relation to the host sugarcane varieties, which subsequently leads to changes in the virulence behaviour of the fungus (Satyavir, 2003). This disease causes 29.07% losses cane yield and 30.8% in sugar recovery (Hussnain Z, 2006).

Age of the sugarcane stalk, time of infestation and susceptibility of the cultivar determines different types of symptoms. The presence of white spots in stalks otherwise rotten internodal tissues with grayish to whitish mycelia appear when the crop is at the peak end of the majestic growth phase during August-September. Tiwari *et al.* (2010) reported that the disease became highly destructive in the north western part of the country due to favourable environment of high humidity and most favourable temperature during crop season in this area. Manjunath (2009) reported optimum temperature between 25-28°C for growth of C1 isolate of *C. gloeosporioides* isolated from noni. Physiological conditions such as low-high temperature, acidic-basic soil and low-high humidity play important role in pathogenicity spread of disease. The conditions necessary for survival and successful infection differ among pathogens. Lilly and Barnett (1951) reported that pH values between 5 and 6 is suitable for most fungal growth, because fungi generally tolerate more acid than alkali. This present study was undertaken to evaluate different abiotic parameters to investigate the growth variability of *C. falcatum* isolates.

MATERIALS AND METHODS

In this study *Colletotrichum falcatum* pathogen viz., cfNAV, cfVES, cfPAR, cfTIM, cfMAR, cfGAN, cfKAM, cfCHA and cfMAD were isolated from the stems of red rot infected sugarcane from South Gujarat (Prittesh *et al*. 2016). All the isolates were morphologically identified and maintained on oat meal agar slant at 4°C.

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Sterile Czapekdox broth was used at five pH level 5.0, 6.0, 7.0, 8.0 and 9.0 to scrutinize the effect on mycelial growth of each isolates. The pH was obtained over the ranges 5–9 by adding the required amount of buffer 0.1 N Citric acid or 0.1 N NaOH. An eight mm mycelial disc was transferred from the margin of the 7-day-old culture to the 250 ml conical flask with 100ml Czapek dox broth. Experiment was conducted in replication of three. After 12 days of incubation at 30±1°C, mycelia were harvested and dried in oven at 50°C till the consistent weight obtained.

The effect of temperature on mycelial growth rate was evaluated on OMA. Mycelial discs of 5 mm in diameter were transferred from the margins of the 7 day old growing cultures of each test isolate to the center of each OMA plate. Inoculated plates were placed in plastic bags and incubated at 20, 24, 28, 32 or 36 ^{0}C in the dark. Three replicates for each isolate were arranged in a random complete block design. Observations on colony radius of all isolates recorded on 3, 5 and 7 day after inoculations (DAI).

In this study, five solid media viz. PDA, OMA, potato malt agar (PMA), Sabouraud's dextrose agar (SDA) and Rose Bengal Agar medium (RBA) were used to evaluate growth rate and colony characters (mycelia colour, texture and topography) for each isolate.All the media were prepared according to label directions (HiMedia, India). Seven day-old pre-cultured 5 mm discs of each iso-late were used as inoculum. Inoculated Petri dishes were incubated at 30±1°C in the dark. There were three replicate plates of each medium per isolate. The diameter of the each of the test isolates was recorded at 3, 5 and 7 DAI in two axes perpendicular to one another. Studies of sporulation on different solid media used, was also undertaken. A five mm disc of the culture was cut from the nearest center portion of the plate and put in 1 ml ster-ilized water and shaken well, so that the spores were dislodged. 50µl of this spore suspension was placed on a haemocytometer and the number of spores in 5 squares at random was counted.

The experiment was performed following Randomized Block Design (RBD) with threereplication of each treatment. The significance of differences between the treatments was evaluated by one way analysis of variance (ANOVA) at the significance level of 95 %. The means of diameter and dry weight of mycelia of all treatments were compared ($P > 0.05$).

RESULTS AND DISCUSSION

In the present investigation, physiological studies indicate that there was variation in their growth requirement of *C. falcatum* pathogens. Influence of different pH values on dry mass of nine sugarcane red rot isolates in Czapek dox broth is shown in Table 1. The results clearly indicated that *C. falcatum* isolates could grow within the pH ranges 5 to 9 at varying degree. The growth of isolates was measured in terms of dry mycelial weight collected from liquid media after 12 days of incubation at 30±1 °C. Among the pH range studied, in general for all the isolates the maximum dry mycelial weight was recorded at pH 6 for cfGAN (662.3), which was significantly higher than other pH and isolate interaction. Abbott (1938) recorded that the pH5.5 was optimum from measurement of the diameters of growth on solid media. Out of nine isolates tested during the experiment, cfNAV, cfTIM, cfMAR, cfGAN and cfCHA could showed maximum growth at pH6, cfVES and cfKAM at pH7 and cfPAR at pH8. On an average, entire pH range studied, the highest total dry mycelial weight was recorded for cfGAN (459.2). More acidic and alkali environment does not support the growth of *C. falcatum* isolates. Dry mycelial weight was enhanced from pH5 to pH7, but it was dropped down gradually towards pH4 and pH9. Wang *et al.* (2013) reported similar results that the dry mass of *E. vermicola* was enhanced at pH 5 and 6 after 7 days in Potato dextrose broth, while the growth of dry mass was inhibited greatly at pH 9 and 10. It might be because of strong acidic or basic condition degrade the DNA of the chromosomes, proteins and suppress the mycelial growth in Czapekdox broth. The pH of the medium can affect the growth of the fungus externally by the degree of dissociation of inorganic ions in the solution and since dissociation plays a part in the movement of ions into the fungus and internally by changes in pH in the mycelium (Agnihotri, 1963).

Radial colony growth of nine *C. falcatum* isolates showed high variability at five different temperatures viz. 20°, 24°, 28°, 32° and 36°C (Table 2). However, growth rate was more homogeneous between isolates at 28 and 32°C. Initially there was no significant difference in growth rate at 3 DAI. Mean colony diameter was increased rapidly after

Table 1 : Effect of pH on *C. falcatum* growth

	Dry mycelial weight of *C. falcatum* isolates in mg									
pH	cfNAV	cfVES	cfPAR	cfTIM	cfMAR	cfGAN	cfKAM	cfCHA	cfMAD	Mean
5	121.6	110.6	117	271	226	328.6	165	248.3	117.6	189.5
6	492.3	253	283	488	597.6	662.3	426.3	475.6	359	448.5
7	396.6	500	408	370	411	479	493.6	307.3	466	425.7
8	364.3	398	481.3	228.6	545.6	443	410.6	244	420.6	392.9
9	305	348.6	258.6	196.3	502	383.3	345	205.6	313.6	317.5
Mean	336	322.06	309.6	310.8	456.4	459.2	368.1	296.2	335.4	

*Data represent mean of three replication value. Statistically analyzed by one way ANOVA

	Isolates(I)	Treatment(T)	Interaction(I x T)
CD (0.05)	4.38	3.27	9.81
SEM (±)	1.55	1.61	3.48

Table 2 : Effect of temperature on mycelial growth of *C. falcatum*. Values are the mean of three replications. Data were analyzed with one way ANOVA. (P< 0.05)

(a) 3 days after inoculation

	Radial mycelial growth mean diameter in centimeter									
Temp (° C)	cfNAV	cfVES	cfPAR	cfTIM	cfMAR	cfGAN	cfKAM	cfCHA	cfMAD	Mean
20	0.93	0.53	1.13	1.5	0.56	0.7	0.8	0.76	0.93	0.87
24	2.4	2.76	2.56	2.7	2.76	2.63	2.73	2.36	3.06	2.66
28	2.46	2.23	2.7	2.46	2.3	2.03	2.83	2.23	2.06	2.36
32	3.23	3.46	3.5	3.96	3.56	3.16	3.26	3.26	3.33	3.41
36	0.33	0.6	0.16	0.46	0.23	0.3	0.16	0.73	0.13	0.34
Mean	1.87	1.91	2.01	2.21	1.88	1.76	1.95	1.86	1.90	

	Isolates (I)	Treatment (T)	Interaction (IxT)
CD (0.05)	0.22	0.16	0.49
SEM (±)	0.07	0.05	0.17

(b) 5 days after inoculation

	Radial mycelial growth mean diameter in centimeter									
Temp (°C)	cfNAV	cfVES	cfPAR	cfTIM	cfMAR	cfGAN	cfKAM	cfCHA	cfMAD	Mean
20	3.26	2.8	2.93	2.96	2.96	3.46	3.26	3.13	3.7	3.16
24	4.03	3.9	5.16	5.7	5.9	5.9	6.16	5.46	6.2	5.37
28	5.33	5.33	5.8	6.33	6.3	5.96	6.06	6.1	6.36	5.95
32	6.06	6.83	6.63	6.96	7.16	7.13	6.63	7.06	6.86	6.81
36	0.73	0.7	0.73	0.76	0.66	0.6	0.5	1.2	0.23	0.67
Mean	3.88	3.91	4.25	4.54	4.59	4.61	4.52	4.59	4.67	

	Isolates (I)	Treatment (T)	Interaction (IxT)
CD (0.05)	0.24	0.18	0.55
SEM (±)	0.08	0.06	0.19

(c) 7 days after inoculation

	Radial mycelial growth mean diameter in centimeter									
Temp (°C)	cfNAV	cfVES	cfPAR	cfTIM	cfMAR	cfGAN	cfKAM	cfCHA	cfMAD	Mean
20	4.53	5.37	4.77	4.63	4.7	5.93	6.1	6.57	6	5.4
24	7	7.37	7.1	8	7.93	7.87	7.97	7.1	8.23	7.61
28	7.73	7.36	7.77	8.03	7.9	7.83	8.5	8.13	7.3	7.83
32	8.03	8.23	8.2	8	8.13	8.17	8.1	7.87	8.33	8.11
36	0.83	1.2	0.8	1	1	0.7	0.6	1.3	0.53	0.88
Mean	5.62	5.90	5.72	5.93	5.93	6.1	6.25	6.19	6.078	

	Isolates (I)	Treatment (T)	Interaction (IxT)
CD (0.05)	0.18	0.13	0.41
SEM (±)	0.06	0.04	0.14

Table 3 : Growth of *C. falcatum* isolates on different media. Linear growth represented as Mean±SD. PDA- Potato Dextrose Agar, OMA- Oat Meal Agar, PMA- Potato Malt Agar, SDA- Sabouraud's Dextrose Agar, RBA- Rose Bengal Agar

		Radial mycelial growth mean diameter(cm)					
Isolates	Culture media	3rd DAI	5th DAI	7th DAI	Mycelial colour	Mycelial texture and topography	Log CFU/ml on 7th day
cfNAV	PDA	1.76±0.05	3.8±0.30	5.86±0.05	Light grey	Thin sparse	5.342
	OMA	2.73±0.20	5.6±0.45	7.7±0.30	Dull white	Thick fleecy	5.146
	PMA	2.73±0.11	6.1±0.10	7.76±0.50	White	Thick fleecy	5.602
	SDA	2±0.30	3.93±0.20	5.73±0.55	Dark grey	Thick fleecy	4.778
	RBA	2.1±0.10	4.2±0.10	6.2±0.10	Light white	Thick fleecy	4.301
cfVES	PDA	2.23±0.11	4.53±0.05	6.1±0.20	Light grey	Thick fleecy	5.502
	OMA	2.33±0.11	5.53±0.11	7.4±0.17	White	Thick fleecy	5.301
	PMA	3.13±0.05	6.93±0.05	8.3±0.17	Dull white	Thick fleecy	5.77
	SDA	2.56±0.05	5.16±0.05	7.03±0.15	Greyish white	Thin sparse	4.903
	RBA	1.96±0.05	5.26±0.11	6.13±0.11	White	Thick fleecy	4.954
cfPAR	PDA	2.63±0.11	5.3±0.05	6.43±0.05	Dull white	Thick fleecy	5.39
	OMA	3.26±0.05	5.96±0.15	8.16±0.11	White	Thick fleecy	5.34
	PMA	3.56±0.05	7.06±0.05	8.43±0.11	Dull white	Thick fleecy	5.777
	SDA	2.76±0.05	6.33±0.05	7.26±0.11	Greyish white	Thin sparse	5.869
	RBA	2.73±0.23	4.86±0.1	6.83±0.05	White	Thick fleecy	5.079
cfTIM	PDA	2.3±0.36	5.26±0.25	7.73±0.05	White	Mild fleecy	5.301
	OMA	3.1±0.05	7.03±0.25	8.23±0.05	Grey	Thin sparse	5.602
	PMA	3.1±0.10	6.1±0.30	7.83±0.05	Whitish grey	Thick fleecy	5.491
	SDA	2.1±0.20	5.33±0.15	7.93±0.20	White	Thin sparse	5.255
	RBA	2.56±0.05	5.2±0.26	7.26±0.11	White	Thin sparse	4.845
cfMAR	PDA	2.73±0.15	6.43±0.15	8±0.10	Dull white	Thin sparse	5.477
	OMA	2.56±0.05	6.7±0.10	8.43±0.05	Grey	Thin sparse	5.505
	PMA	2.56±0.20	6.83±0.15	8.06±0.05	Whitish grey	Thick fleecy	5.079
	SDA	2.73±0.25	6.13±0.05	7.93±0.37	Dull white	Thin sparse	5.301
	RBA	2.66±0.05	5.56±0.20	7.23±0.40	White	Thick fleecy	5.414
cfGAN	PDA	2.6±0.40	5.5±0.60	7.86±0.15	White	Thin sparse	5.447
	OMA	5.16±0.73	6.73±0.05	8.36±0.15	Dull white	Thin sparse	5.079
	PMA	5.16±0.59	6.93±0.05	8.1±0.10	Greyish white	Thick fleecy	5.079
	SDA	2.23±0.20	5.26±0.41	7.63±0.05	Greyish white	Thin sparse	5.176
	RBA	2.4±0.20	5.3±0.60	6.53±0.55	White	Thin sparse	4.778
cfKAM	PDA	2.13±0.15	5.76±0.35	7.76±0.05	White	Thin sparse	5.322
	OMA	3.96±0.35	7.06±0.30	8.43±0.05	Light white	Thick fleecy	5.113
	PMA	3.96±0.11	7.1±0.26	8.33±0.05	Greyish white	Thick fleecy	5.531

(Contd. part Table 3)

	SDA	2±0.10	4.86±0.35	7.36±0.15	Whitish grey	Thin sparse	5.041
	RBA	2.5±0.17	5.2±0.1	7.26±0.05	White	Thick fleecy	5.342
	PDA	1.93±0.30	4.53±0.45	7.03±0.45	White	Thin sparse	5.204
	OMA	3.06±0.05	7.6±0.20	8.33±0.15	Grey	Thick fleecy	5.414
cfCHA	PMA	3.06±0.11	5.86±0.50	7.93±0.25	White	Thick fleecy	5.414
	SDA	2.3±0.36	4.5±0.10	7.56±0.30	Dull white	Thin sparse	5.505
	RBA	2.6±0.10	5.36±0.05	7.36±0.05	White	Thick fleecy	5.041
	PDA	2.26±0.05	5.1±0.30	7.73±0.25	White	Thin sparse	5.176
	OMA	2.9±0.05	5.6±0.10	8.26±0.05	Light grey	Thick fleecy	5.255
cfMAD	PMA	2.9±0.10	6.53±0.55	8.23±0.15	Dull white	Thick fleecy	5.305
	SDA	2.06±0.05	4.3±0.10	7.36±0.30	Dull white	Thin sparse	5.255
	RBA	3±0.20	5.63±0.05	7.5±0.10	Dull white	Thin sparse	5.113

3 days of incubation and almost get doubled between 3 to 5 day. The optimum temperature was in the range of 24 and 32 ^{0}C which support good growth of all the isolates. A temperature of 25°C was reported to be the optimum for the growth of *C. gloeosporioides* on mango, almond and avocado (Moriwaki *et al*. 2003). Surprisingly there was very less growth of each isolate recorded at 36 °C. Isolates cfKAM (6.1cm) and cfCHA (6.57cm) could grow very well even at 20 ºC and reach up to more than 6cm colony diameter after 7 days of incubation. The highest mean colony diameter was recorded 3.96cm for cfTIM at 3 DAI and 7.16cm for cfMAR at 5 DAI. Temperature of 32°C supported maximum mycelial growth of above 8cm in general for each isolates which were significantly superior over all the temperatures studied. The mycelial growth of *C. gloeosporioides* isolated from anthurium was maximum at 25°C compared to incubation of the fungus at 30°C (Nandinidevi, 2008). Good fungal mycelial growth of 6.25cm was recorded for cfKAM and minimum 5.62cm for cfNAV at entire temperature range studied. No significant difference in mycelial growth was recorded at 28 and 32 °C. Thangamani *et al.* (2011) reported that the growth of *C. musae* was maximum at pH range of 6.50-7.00 and temperature range of 25-30°C.

The growth of each isolate on five solid media viz., PDA, OMA, PMA, SDA and RBA was regular and progressive. The data presented in table- 3 reveals that among the five media evaluated, the maximum radial mycelial growth was observed on OMA (8.43±0.05cm) for cfMAR and cfKAM and on PMA medium (8.33±0.05cm) for cfPAR. Oat meal agar and potato malt agar were found par with each other. Among the nine isolates cfNAV reported to have minimum growth even after seven days on PDA and SDA and could reach 5.86±0.05cm and 5.73±0.55cm respectively. Mycelial colour, texture and topography of nine *C. falcatum* isolates on five solid media have been recorded after seven days of incubation (Table 3). Colony colour had a white to grayish range of colour. Among the nine isolates, cfNAV, cfVES and cfPAR have grown with evenly distributed concentric ring of orange colour acervuli around the inoculums of P1 Group. Other isolates observed to have orange to blackish acervuli all over plates with uneven distribution on all the media studied and grouped as P2 (Prittesh *et al*. 2016). Spores counted with the help of haemocytometer after seven days of incubation shown good sporulation of all nine *C. falcatum* isolates. Maximum Log cfu/ml i.e., 5.869 was recorded for cfPAR on SDA and minimum Log cfu/ml 4.301 on RBA for cfNAV.

All the *C. falcatum* isolates were grown best at pH6 and temperature 28 - 30°C. These data are in accordance with Abbott (1938), who has recorded optimum temperature of about 30 – 32.5°C for the growth of *C. falcatum*. *In vitro* growth of pathogens can be correlates with their growth in soil condition. These data suggest that the pathogen can be more virulent and spread very fast in environmental condition with temperature less than 32°C and slightly acidic soil. Furthermore, the high humidity supports the good growth of *C. falcatum*. So, monsoon period (July- August; low temperature and high humidity) is vulnerable time to the attack of *C. falcatum*, resulting in complete devastation of the crop.

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