AJAB

Asian J Agri &Biol. 2017;5(2):77-82.

Original Research Article

Physiological Studies on Colletotrichum Gloeosporiodes Associated With Wither Tip Disease of Citrus and Its Chemical Control

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Received:
August 31, 2016

Accepted:
May 24, 2017

Published:
June 20, 2017

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Abstract

Citrus is the second largest fruit produced in the world and Pakistan is among the 12 large producers of the citrus fruit. It is grown in tropical and subtropical climate all over the world. Besides its high economical & nutritional values citrus is attacked by different pathogen such as fungi, bacteria, viruses and nematodes. Among all the pathogens *Colletotrichum gloeosporiodes* causing citrus wither tip disease is one of the major constrain in citrus production. Therefore current study was conducted to investigate the different physiological characters on the mycelial growth of *C. gloeosporiodes* and its chemical control. Among different fungal nutrient media Potato Dextrose Agar (PDA) gave maximum mycelial growth (7.9 cm) followed by Citrus leaf extract Agar (CLEA) (4.7 cm) and corn meal Agar (CMA) (3.3 cm).Temperature of 30°C favored maximum colony growth (8 cm) followed by 25°C (7.4 cm), 35°C (4.2 cm), 20°C (3.5 cm) and 15°C (2.3cm). A pH level of 6 favored maximum colony growth (7.9 cm) followed by 5 (7.5 cm), 4.5 (5.3cm), 4 (4.8 cm), 7(3.3cm) and 3(2.4 cm).Among the application of different fungicides such as Topsin-M, Copper oxychloride and Aliette at different concentration (300, 600 and 900 ppm). Topsin-M gave maximum result to inhibit the Maximum mycelial growth inhibition of *C. gloeosporioides* (1.1 cm) was produced by Topsin-M 9 days after incubation at 30 °C as compared to control (7.6 cm).

Keywords: Physiological studies, *Colletotrichum gloeosporioides*, chemical control

Introduction

Citrus belongs to *Rutaceae* family, grown in tropical and subtropical domains of the world. China, India and Himalayan region are the major citrus fruit producing regions (NIHORT, 2003). According to (FAO, 2015) Pakistan stands 30th position in citrus production among citrus growing countries and annual fruit production is 2007 thousand tones. As compare to all provinces in Punjab above 95% of citrus fruits are produced and 70 percent production is Kinnow (Niaz*etal*, 2004). Numerous factors are responsible for pre and post-harvest losses in citrus production of which disease play very vital role. Citrus fruits production is drastically reduced by wither tip disease in tropical and subtropical domains (Rohlf,2000).*Colletotrichum gloeosporioides* remains a universal pathogen that cause diseases on many fruits such as mango, papaya, and apple and especially wither tip disease caused on citrus due to this pathogen (Simmonds,1965).Physiological studies on *C. gloeosporioides* was carried out by many researchers

as (Sudhakar, 2000) reported that maximum mycelial growth of *C. gloeosporioides* was observed in five culture medias (Potato dextrose agar, Richard's agar, Brown's agar, Sabouraud's agar, and Oat meal agar). *In vitro* studies revealed that maximum mycelia growth of *C. gloeosporioides* reached after 09 days incubation on PDA, at 25-35 °C (Annonymous, 2001). According to the (Narendra Kumar, 2006) maximum fungal mycelial growth was recorded at pH 6.5 and 6.0 while minimum growth was noticed at pH 4.0.*In vitro* condition the optimum pH for the growth of pathogenic fungai ranges between 5.5 and 7.0.Inhibitory effect of various chemicals viz., benomyl, prochloraz, chlorothalonil, captan and thiram was studied for the control of *C. gloeosporioides* causing wither tip disease of citrus (Eikelenboom, 1964). The present study aimed to investigate the pathogen under different media, temperature, and pH levels requirements for mycelial growth and its chemical control.

Materials and Methods

Collection of diseased sample from Citrus orchard

Samples of the infected plants parts viz. (twigs and leaves) were collected from citrus orchard located in University of Agriculture Faisalabad. The samples were brought in mycology lab wrapping in the new polythene bags and stored in refrigerator at 4 C° for further use.

Physiological (Nutrient media, pH and temperature) studies of the *C. gloeosporiodes* associated with wither tip disease in citrus

Effect of selective Nutrient Medias on *C. gloeosporiodes*

The cultural characters of *C. gloeosporiodes* were examined on PDA, Corn Meal Agar (CMA) and Host leaf extract Agar which was prepared according to the prescribed recipies. After that 5 cm discs from *C. gloeosporioides* cultures were cut by using a sterilized transfer needle and a single disc was placed at the centre of glass cavity blocks. Each set of experiment was replicated thrice and the plates were incubated at 30±1°C for a week. The cultural characters such as colony diameter, colour, type of margin, aerial growth were recorded.

Effect of pH levels on physiology of *C. gloeosporioides*

PH effect on the growth of *C. gloeosporioides* was studied on PDA media. The pH of the medium was adjusted to various levels namely 3, 4, 4.5, 5, 6 and 7 and pH level in each treatment was maintained by electronic pH meter. Medium with known pH level was poured into conical flasks and sterilized. Flasks containing sterilized culture medium were poured into petriplates allow them to solidify and inoculated with fresh culture of *C. gloeosporioides* and incubated for 9 days .Each treatments was replicated thrice. Optimum pH was determined by measuring fungal mycelial growth (cm).

Effect of various temperature levels on *C. gloeosporioides*

Medium was added to 100 ml flasks and sterilized 121^0C and 15psi pressure for 20 minutes. After sterilization media poured into the petriplates was inoculated with fresh culture of *C. gloeosporioides* and kept at temperature levels viz., 15, 20, 25, 30 and 35°C for fungal colony development and three replications were maintained for each treatment. Most suitable temperature for *C .gloeosporioides* was determined by measuring mycelial growth (cm).

In vitro* testing of selected fungicides against *C. gloeosporiodes

Efficacy of selected fungicides (Copper oxychloride, Topsin-M and Aliette) was tested by Poisoned food technique (Hawamdeh and Ahmad, 2001) against *C. gloeosporioides* at various concentrations of 300, 600 and 900 ppm respectively with three replications for each treatment. The actively growing periphery of *C.gloeosporioides* was shifted aseptically to the centre of each petri dish having poisoned medium and incubated at 30 C°. The fungal mycelial growth was noted 3, 6 and 9 days after incubation and percentage mycelial growth inhibition (*I*) was determined by using the following formula:

$$\text{Inhibition of the mycelial radial growth (\%)} = \frac{C - T}{C} \times 10$$

(Perrucci*et al.*, 2003)

C: colony diameter of Control, T: colony diameter of treatment.

Data was analyzed by ANOVA test and differences within treatments were determined by LSD test at P= 0.05 (steel *et al*., 1997).

Results and discussion

Physiological study of *C. gloeosporioides*

Nutrient media effect on mycelial growth of *C. gloeosporiodes*

The nutrient media plays influential role on the growth of *C. gloeosporiodes* as illustrated in Table. 1. According to result revealed that pathogen grew well in a different culture media but the highest colony diameter (7.9) was observed after 9 days on PDA medium followed by host leaf extract medium (4.7 cm) and the minimum colony diameter (3.3 cm) was observed on corn meal agar.

Effect of pH levels on radial growth of *C. gloeosporiodes*

C. gloeosporioides growth was observed on various pH levels on various time intervals as shown in table 2. After 9 days of incubation result indicated that at pH level 6.0 the colony growth (7.9 cm) was maximum followed by 5.0, 4.5, 4.0 and 7. The growth was observed minimum at pH 3. Fungal growth increases at 6.0 by increasing pH up to a certain level after this mycelial growth was decreased at 7.0 pH. According to result at pH levels 5.0 and 6.0 were best among all for radial growth of *C. gloeosporioides* but the pH levels 3.0, 4.0 and 7.0 were not favorable for colony growth.

Effect of various temperature levels on *C. gloeosporiodes*

Radial growth of *C. gloeosporioides* has shown on various temperature levels along with duration in Table 3. Result revealed that maximum radial growth(8.0 cm) was observed at 30°C followed by 20, 25, 35°C while minimum growth was recorded at 15°C and 35°C. The temperature ranged 25 to 30°C was significant for *C. gloeosporiodes*. Statistically significant relationship between radial colony growth and temperature levels was observed.

In vitro* evaluation of fungicides against *C. gloeosporioides

The results after 3, 6 and 9 days shown in table 4, 5 and 6 revealed that selected fungicides showed different efficacy levels in inhibiting the radial growth of *C. gloeosporioides* respectively. Statistical data indicated that Topsin-M was found most effective for controlling the growth of pathogen that showed colony diameter 1.6, 1.3 and 1.1 cm at 300, 600 and 900 ppm after 9 days incubation followed by Copper oxychloride and Aliette respectively.

Table 1: Effect of Nutrient media on fungal colony growth of *C. gloeosporioides*

Media	Duration (days) Colony growth (cm)			Means (cm)
	3 days	6 days	After 9 days	
PDA	2.6 e	3.9 c	7.9 a	4.8 a
CLEA	1.5 g	2.4 f	4.7 b	2.8 b
CMA	0.9 h	1.5 g	3.3 d	1.9 c
Means	1.6 c	2.6 b	5.3 a	

Various characters are likely to be followed by the level of statistical significance of 5% LSD
Alpha = 0.05LSD value for (M) = 0.0841 (D) = 0.0732 and (MxD) = 0.1301

Table 2: Effect of pH Level on mycelial growth of *C. gloeosporioides*

pH	Duration (days)			Means (cm)
	After 3 days Colonygrowth (cm)	After 6 days Colonygrowth (cm)	After 9 days Colonygrowth (cm)	
3	0.7 n	1.2 m	2.4 j	1.4 f
4	1.6 l	2.5 ij	4.8 d	2.9 d
4.5	1.8 k	2.7 h	5.3 c	3.2 c
5	2.5 ij	3.7 f	7.5 b	4.5 b
6	2.6 hi	4.0 e	7.9 a	4.8 a
7	1.1 m	1.6 l	3.3 g	2.0 e
Means	1.7 c	2.6 b	5.2 a	

Various characters are likely to be followed by the level of statistical significance of 5% LSD
Alpha = 0.05LSD value (pH) = 0.0636LSD value (Duration) = 0.0450LSD value (PxD) = 0.1102

Table 3: Temperature effect on mycelia colony growth of *C. gloeosporioides*

Temperature	Duration (Hours) Colony growth (cm)			Means (cm)
	3 days	6 days	9 days	
15°C	0.6 m	1.1 l	2.3hi	1.3e
20°C	1.2 l	1.7 j	3.5f	2.1d
25°C	2.4 h	3.7e	7.4b	4.5b
30°C	2.7 g	3.9d	8.0a	4.8a
35°C	1.4 k	2.2i	4.2c	2.6c
Means	1.6c	2.5b	5.0a	

Various characters are likely to be followed by the level of statistical significance of 5% LSD
Alpha = 0.05 LSD value (Temperature) = 0.0908 (Duration) = 0.0702 and (DxT) = 0.1564

Table 4:*In vitro* evaluation ofselected fungicides 3 days after treatment against *C. gloeosporioides*

Fungicides	Concentrations (ppm) Colony growth (cm)			Means (cm)
	300 ppm	600 ppm	900 ppm	
Copper oxychloride	0.80	0.60	0.40	0.60 c
Topsin-M	0.60 e	0.40	0.30	0.43 d
Aliette	1.20	1.10	0.90	1.06 b
Control	2.50	2.40	2.60	2.50 a
Means	1.27 a	1.12 b	1.05 b	

Various characters are likely to be followed by the level of statistical significance of 5% LSD
Alpha = 0.05 LSD value (F) = 0.0963 LSD value (Conc) = 0.0834 LSD value (FxC) = 0.1667

Table: 5 ***In vitro*** **evaluation of selected fungicides 6 days after treatment against** ***C. gloeosporioides***

Fungicides	Concentrations (ppm) Colony growth (cm)			Means (cm)
	300 ppm	600 ppm	900 ppm	
Topsin-M	1.10 h	0.90 j	0.70 k	0.90 d
Copper oxychloride	1.50 f	1.30 g	1.0 i	1.26 c
Aliette	2.50 c	2.20 d	1.70 e	2.13 b
Control	5.2 a	5.0 b	5.2 a	5.13 a
Means	2.57a	2.35 b	2.15 c	

Various characters are likely to be followed by the level of statistical significance of 5% LSD
Alpha = 0.05 LSD value (F) = 0.0282 LSD value (Conc) = 0.0244 LSD value (FxC) = 0.0489

Table: 6 ***In vitro*** **evaluation of selected fungicides 9 days after treatment against** ***C. gloeosporioides***

Fungicides	Concentrations (ppm) Colony growth (cm)			Means (cm)
	300 ppm	600 ppm	900 ppm	
Topsin-M	1.6 fg	1.3 h	1.1 i	1.33 d
Copper oxychloride	2.1 e	1.7 f	1.5 g	1.76 c
Aliette	3.7 b	3.3 c	2.8 d	3.26 b
Control	7.6 a	7.5 a	7.6 a	7.56 a
Means	3.75 a	3.45 b	3.25 c	

Various characters are likely to be followed by the level of statistical significance of 5% LSD
Alpha = 0.05 LSD value (Fungicides) = 0.0717 LSD value (Concentration) = 0.0621 LSD value (FxC) = 0.1242

Discussion

Citrus is considered as the "common man's fruit" crops. The fruits are immensely important as they are delicious, have high food value, rich in vitamin A and C and also fats and occupy a very prominent place in the diet of human being. Citrus suffers from several diseases and wither tip is one of them cause severe loses. According to the (Deshmukh*et al., 2012*) in an experiment on evaluation of 15 solid media, maximum mycellial growth of *C. gloeosporioides* was noticed on Potato Dextrose Agar media (88.74 mm) followed by Richard's Agar (85.43 mm), Oat Meal Agar (81.51 mm), Malt Extract Agar media (80.25 mm) andCorn Meal Agar (79.40 mm) but no growth was observed on Sach's agar media (0.00 mm). Maximum mycelial growth of *C. gloeosporioides* in Potato dextrose agar was also recorded by (Pandey *et al*., 2012a). For the confirmation of maximum growth at different pH level (Sangeetha, 2003) observed that maximum radial growth of *C. gloeosporioides* at 6.0 pH. Chandra *et al* (2004) observed that the optimal colony growth of *C. gloeosporioides* ranges from 5.2 to 6.4 pH. Among all the physiological factors temperature plays a significant role for increasing the growth of pathogens. It can affect almost all stages of fungi including its growth, spore germination and reproduction. Favourable temperature for the growth of *C. gloeosporioides* is 30ºC observed by (Sangeetha and Rawal, 2008). After the evaluation of five fungicides against *C. gloeosporioides* topsin-M is one the most effective at 0.15 per cent concentration for 100% fungal growth inhibition, followed by Mancozeb (78.51%) at 0.35% (Biradar, 2002).

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