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Physiological Studies on *Colletotrichum gloeosporioides* Penz. Causing Anthracnose of Mango and its Chemical Control

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Abstract

Colletotrichum gloeosporioides Penz. produced excellent mycelial growth with maximum acervuli and spores on PDA with least on water agar. The best temperature was 25 ± 2°C and pH between 7 to 8 under continuous light. Growth and acervuli production of pathogen was excellent when basal medium was supplemented with glucose as carbon source. Te 60 showed the maximum inhibition on mycelial growth and acervuli production at all concentrations.

Introduction

Anthracnose caused by *Colletotrichum gloeosporioides* Penz. is prevalent in all the mango growing regions of the world (Ploetz *et al.*, 1994). It has been noted that disease attacks leaves, petioles, twigs and fruits at pre and post harvest stages (McGuire and Campbell, 1993). It produces oval to irregular brown coloured spots on leaves, sometimes forming concentric rings on fresh leaves. Under damp conditions, the spots grow rapidly, producing elongated necrotic areas on the affected tissues. They turn black or brown on petioles and cause leaf drop after drying. Elongated black necrotic areas develop on twigs that start drying from tip to downward. In severe cases, affected areas show black small bodies named as acervuli. Sunken black spots develop on the fruits that later cause rottening on the fruits (Hafiz, 1986).

Little work has been reported on the physiology of this pathogen from mango in Pakistan. The present study was carried out to investigate the effect of different physiological factors on the mycelial growth and acervuli production of *C. gloeosporioides.*

Materials and Methods

C. gloeosporioides was isolated from diseased specimens and after purification it was tested for pathogenicity (Akhtar *et al.,* 1998). Mycelial growth and acervuli production was studied in basal medium (BM), corn meal dextrose peptone agar (CMDPA), Richards agar (RA), czapeks dox agar (CA), corn meal peptone agar (CMPA), malt extract agar (MEA), malt extract glucose agar (MEGA), water agar (WA), potato starch agar (PSA) and potato dextrose agar (PDA) medium, as described by Saleem and Nasir (1991). The effect of different temperatures, light periods, and pH were also checked by growing the fungus on PDA medium.

Different carbon sources such as Sucrose (4.75 g), D-Glucose, Fructose, Mannitol (5.00 g), Starch, Adenine (4.50 g), Glycine (6.25 g), L-Asparagine (5.50 g), L-Glutamine (4.25 g), Inosit (4.76 g), Nicotinic acid (3.42 g) and L-Tyrosine (3.02 g) were studied for the growth and acervuli production of fungus. The quantity of these sources were adjusted to give an amount of carbon equivalent to 5.0 g of dextrose for 250 ml of basal medium (Saleem and Nasir, 1991).

Potato dextrose agar medium was amended with different concentrations of mold inhibitors; Thiabendazole, Borax and Boric acid (Ilyas *et al.,* 1982). Petri plates poured with potato dextrose agar medium without mold inhibitors serve as control.

All media in the above studies were autoclaved for 2 minutes at 15 lb/inch^2 pressure and 121°C. Petri plate with 20 ml medium, were inoculated in the centre with mm plugs of 7 days old culture obtained with the help sterilized cork borer into four replicates.

Cultural characters of *C. gloeosporioides* were also noted. The acervuli were counted under stereoscope by taking a 2 mm diameter disc of 7 days old culture from the centre each petri plate by the help of cork borer. Data were analyzed statistically by using the CRD two factor design (Steel and Torrie, 1960).

Results and Discussion

Effect of Different Culture Media: Maximum growth of *gloeosporioides* was observed on potato dextrose age (51.32 mm) and malt extract glucose agar (50.67 mm) after 168 hours of incubation followed by Richards agar, malt extract agar, czapeks dox agar, basal medium, corn meal peptone agar and corn meal dextrose agar, while least on potato starch agar (13.25 mm). Maximum number of acervuli of fungus was found on potato dextrose agar (56.75), followed by potato starch agar (54.50), while corn meal dextrose agar and czapeks dox agar were unable to produce any acervulus (Table 2).

Spore production was excellent in the case of potato dextrose agar, potato starch agar, malt extract agar and malt extract glucose agar. Colour of mycelium a substrate also gave varying responses. Colonies on potato dextrose agar, malt extract agar and malt extract glucose agar were greyish-white while on other media except water agar they were white. Conidia were orange coloured masses forming concentric rings on PDA (Table 1). Same results were presented by Jeffries *et al.* (1990) and Fitzell (1979) they reported that colonies on potato dextrose agar are greyish white to dark grey with salmon coloured masses of spores formed usually in concentric rings on such media.

Effect of Temperature: Maximum growth of fungus was

Table 1: Effect of culture media on *Colletotrichum gloeosporiodes*

Cha./Media	Original colour of medium	Colour of mycelium outside	Colour of mycelium inside	Colour of the substrate	Mycelial growth	Acervuli production	Spore production
Basal medium (BM)	Transparent	White	Light grey	Black	Excellent	Good	Scanty
Corn meal dextrose peptone agar (CMDPA)	Creamy	White	White	Creamy	Excellent	Absent	Poor
Richards agar (RA)	Transparent	White	White	Creamy	Excellent	Poor	Poor
Corn meal peptone agar (CDA)	Transparent	White	White	Black	Excellent	Absent	Absent
Corn meal peptone agar (CMPA)	Creamy	White	White	Yellow	Good	Good	Good
Malt extract agar (MEA)	Creamy	Greyish-White	Greyish-White	Creamy	Excellent	Excellent	Excellent
Malt extract glucose agar (MEGA)	Creamy	Greyish-White	Greyish-White	Light brown	Excellent	Excellent	Excellent
Water agar (WA)	Transparent	No growth	No growth	Transparent	No growth	Poor	Scanty
Potato starch agar (PSA)	Creamy	White	White	Creamy	Excellent	Excellent	Excellent
Potato dextrose agar (PDA)	Creamy	Greyish-White	Greyish-White	Orange with concentric rings	Excellent	Excellent	Excellent

Table 2: Studies on the mycelial growth and acervuli production of *Colletotrichum gloeosporioides* pent.

Parameters		Colony	Acervuli
Media			
	BM	44.21e	37.50d
	CMDPA	42.32f	0.009
	RA	46.50c	29.50e
	CDA	44.82e	0.00g
	CMPA	42.82f	40.25d
	MEA	45.46d	52.75b
	MEGA	50.67b	45.25c
	WA	22.00g	4.25f
	PSA	13.25h	54.50a
	PDA	51.32a	56,75a
Temperature (±2°C)			
	5	0.00f	0.00d
	10	3.57e	0.00d
	15	16,0e	6.75c
	20	32.46c	21.25b
	25	52.21a	29.00a
	30	35.00b	29.50a
	35	17.36d	6.00c
Light			
	2 LL	44.82a	31.00a
	16L	80	24.75ab
	12L + 120	39.60b	30.75a
	8L + 160	38.26d	22.50b
	24	37.29e	27.00ab
pH			
	4	39.04c	28.75bc
	5	39.36b	37.00b
	6	39.07c	46.50a
	7	40.43s	26.00cd
	8	40.29a	19.50de
	9	38.00d	14.50ef
	10	29.36e	8.75f
Carbon sources			
	Sucrose	35.93de	45.75a
	Fructose	35.18e	45,75a
	Mannitol	39.43bc	31.5
	Starch	38.75c	26.75c
	Glycine	26.86g	0.00e
	Tyrosine	35.54h	7.50d
	L-Asparagine	39.39bc	2.25e
	Inosit	39.79b	6.25d
	Glutamine	36.32d	0.00e
	Adenine	28.39f	0.00e
	Nictinic acid	0.001	0.00e
	Glucose	44.21a	47.00a

observed between 25±2°C (52.21 mm) while a little growth was observed at 10°C (3.57 mm) but no growth was observed below 7°C. Best acervuli production (29.00) was observed at 25 - 32°C but no acervulus was found below 17°C. Fitzell (1979) also reported that fungus grows

well and sporulates profusely between temperature of 25- 32°C. Similarly Hafiz (1986) noticed that the optimum range of temperature for the growth of fungus was 25- 29°C, while maximum and minimum ranges of temperature for the mycelial growth are, 35-40°C and 10-15°C, respectively.

Table 3: Effect of different mold inhibitors on mycelial growth (mm) and acervuli production of *Colletotrichum gloeosporioides* Penz.

Treatment	Conc. (ppm)	Colony (mm)	Acervuli (No.)
Tecto-60	5	8.5	0.0
	10	2.0	0.0
	20	0.0	0.0
	50	0.0	0.0
	Control	-	-
Borax	5	75.0	81.0
	10	0	65.0
	20	0	59.0
	50	65.0	39.0
	Control	89.5	75.0
Boric acid	0	84.0	65.0
	10	80.5	51.0
	20	79.0	35.0
	50	75.0	5.0
	Control	-	-

Effect of Different Light Intensities: Continuous light was found to be the most suitable for the maximum mycelia' (44.82 mm) growth and best acervuli production (31.0) of the fungus. Continuous darkness resulted in the least mycelial growth (37.29 mm) and 8 h light alternated with 12 h darkness for least acervuli production (22.50).

Effect of pH: The best mycelial growth was found at pH 7 but less was found in case of pH 10 (Table 2). Maximum number of acervuli were found in the case of pH 6 (46.50) but poor at pH 10 (8.75). It is also clear from Table 2 that fungus prefers acidic range over alkaline range for both mycelial growth and acervuli production. These results are in conformity with the results of Hafiz (1986) who concluded that the growth was same at pH 4 and pH 9 but it fells considerably at pH 3.5 on the acidic side and at pH 8.2 on the alkaline side. These results are supported by Verma (1969), Singh (1971), Ahmed (1973) and Nusrullah (1983).

Effect of Different Carbon Sources: Maximum growth (44.2 mm) was observed when glucose was added as a carbon source (Table 2). The growth of fungus was not found on nicotinic acid even after 168 hours. From Table 2 it is evident that treatment means differed statistically from media added with glucose and also among themselves. But the response of starch, L-Asparagine, inosit, mannitol, fructose and sucrose was almost similar.

The fungus produced maximum number of acervuli (47 on glucose medium while minimum number were observed in case of L-Asparagine (4.50). Glycine, glutamine, and nicotinic acid were unable to produce any acer (Table 2). Glucose, sucrose, fructose , mannitol ands exerted almost similar response but showed stati difference from other carbon sources. Tandon and Chandra (1962), also reported that *C. gloeosporiodies* show poor response to sorbitol (the reduction product of glucose when supplemented as carbon source in artificial material.

Effect of Different Mold Inhibitors: Thiabendazole (Te 60) completely inhibited the growth of fungus ahove ppm and was found effective even at 5 ppm on the my growth and the acervuli production of the fungus. Boron 50 ppm checked the acervuli production. Spalding and Reeder (1972), Jacobs *et al.* (1973) and Feng *et al*. (1991) also reported that anthracnose on mango can be count dipping the fruits in thiabendazole (1000 ppm). Boric was found inefficient against mycelial growth and formation of the fungus (Table 3).

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