Research Paper :

Effect of pH levels, carbon and nitrogen sources on the mycelial growth and bio- mass production of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

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SUMMARY

Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.)Penz. and Sacc. is one of the important diseases in mango. The present studies was concerned with different pH levels, culture media, carbon and nitrogen sources which were tested against *C. gloeosporioides.* The results revealed that among the pH levels tested, 7.0 was the best for the mycelial growth (88.3mm) and mycelial dry weight (730.5 mg) of *C. gloeosporioides.* Among the ten culture media tested, PDA was found to be best in mycelial growth (84.8mm), mycelial dry weight (625.4mg) and excellent in acervuli production of *C. gloeosporioides,* while least on water agar. With regard to different carbon and nitrogen sources tested, the pathogen produced maximum mycelial growth and mycelial dry weight when the basal medium was supplemented with manitol (79.5mm and 590.8mg) as a carbon source and ammonium nitrate (86.6mm and 680.8mg) as a nitrogen source.

Key words :
Colletotrichum gloeosporioides, pH, Culture media, Carbon and nitrogen sources.

The mango (*Mangifera indica* L.) is an important fruit crop in India. It belongs to the family Anacardiaceae and it is popularly known as "king of fruits" or "apple of tropics". In India, it is grown in an area of 1.01 millon ha. with a production of 9.45 million ton (Prabakar *et al.,* 2008). India stands first in global mango production 51% (FAO, 1999). However, the productivity of mango is affected by various fungal diseases. Among them, anthracnose incited by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is more responsible for serious loss in mango production. It destroys the developing and developed fruits both in field and storage conditions. It causes leaf, blossom blight and tree die-back in the orchard and can subsequently give rise to rotted fruits during storage and thus, poses severe problems.

All the fungi have specific requirement for their nutrition. Carbon and nitrogen are the most important and essential elements, besides others, for their infection, growth and reproduction. Hence, the present study was carried out to investigate the effect of different culture media, pH, carbon and nitrogen sources on the *in vitro* growth of *C. gloeosporioides*.

MATERIALS AND METHODS

Isolation of pathogen :

The pathogen was isolated from infected fruits of mango which was collected from local market. The isolation was done as per the method described by Sundravadana *et al.* (2007). After obtaining pure culture, the identification was done based on the conidial characters and acervuli production. Then it was confirmed as *C. gloeosporioides.*

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Effect of different pH levels :

Liquid medium:

Erlenmeyer flasks of 250 ml size, containing 50 ml of Potato dextrose broth and various pH levels were prepared *viz.*, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. The pH of the medium was adjusted with 0.1 N NaOH (or) HCl. The plates were inoculated with 9 mm mycelial discs of the pathogen obtained from 7 days old grown on PDA in Petri plates and incubated for 10 days. After incubation period, the mycelial mat was harvested and kept in a sterilized filter paper. Then the mycelium along with the filter paper was dried in hot air oven at 105^{0}C for 48 h. Then the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Solid medium :

To assess the influence of pH levels on linear growth of *C. gloeosporioides*, on Potato dextrose agar (PDA) medium was prepared at various pH levels *viz.*, 4.0, 5.0,

6.0, 7.0, 8.0, 9.0 and 10.0. The pH of the medium was adjusted with 0.1 N NaOH (or) HCl. The plates were inoculated with 9 mm mycelial discs of the pathogen obtained from 7 days old grown on PDA in Petri plates and incubated at room temperature (28± 2^0C) for 7 days. The mycelial growth was measured at the end of the incubation period. Three replications were maintained for each treatment.

Effect of different culture media :

Liquid medium:

Erlenmeyer flasks of 250 ml size, containing 50 ml of the various liquid media *viz.*, Basal medium (BM), Corn meal dextrose peptone agar (CMPDA), Richards agar (RA), Czapek's dox agar (CDA), 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 were sterilized and inoculated with 9 mm mycelial discs of the pathogen obtained from 7 days old grown on PDA in Petri plates. The flasks were incubated at room temperature (28± 2^0C) for 10 days. After incubation the period, mycelial mat was harvested and kept in a sterilized filter paper. Then the mycelium along with the filter paper was dried in hot air oven at 105^0C for 48 h. and the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Solid medium :

Fifteen ml of the various sterilized agar media *viz.*, Basal medium, Corn meal dextrose peptone agar, Richards agar, Czapek's dox agar, Corn meal peptone agar, Malt extract agar, Malt extract glucose agar, Water agar, Potato starch agar and Potato dextrose agar media were poured into 90 mm sterile Petri plates. The plates were inoculated with 5 mm mycelial discs of the pathogen obtained from 7 days old grown on PDA in Petri plates and incubated at room temperature (28± 2^0C) for 7 days. The mycelial growth of the pathogen was measured at the end of the incubation period. Three replications were maintained for each treatment (Akhtar, 1999).

Carbon sources :

Liquid medium :

The *in vitro* growth of the fungus was tested with ten different carbon sources *viz.,* dextrose, fructose, lactose, maltose, mannitol, starch, glycin, glutamine, glucose and sucrose. Richards medium was taken as the basal medium for the study. In Richards broth, sucrose was replaced with various carbon sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the medium was adjusted to 7.0. After that, the medium was inoculated with 5 mm mycelial disc of pathogen obtained from 7 days old culture grown on PDA. The inoculated media were incubated for 10 days at room temperature (28 ± 2^0C). At the end of the incubation period, the mycelial mats were filtered through previously dried and weighed filter paper and dried in hot air oven at 105^0C for 48 h. and the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Solid medium:

Richards medium was amended with various carbon sources on equivalent weight basis and were dispensed in sterile Petri plate at fifteen ml quantities. After cooling, they were inoculated with 9 mm mycelial disc of the pathogen obtained from 7 days old culture grown on PDA in Petri plates and incubated for 7 days. The linear growth of the pathogen was measured in mm at the end of the incubation period.

Nitrogen sources :

Liquid medium :

The *in vitro* growth of the fungus was tested with seven different nitrogen sources *viz.,* ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, potassium nitrate, sodium nitrate and sodium nitrite were taken as the basal medium for the study. In Richards broth, sodium nitrate was replaced with various nitrogen sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the medium was adjusted to 7.0. After that, the medium was inoculated with 5 mm mycelial disc of pathogen obtained from 7 days old culture grown on PDA and the media were incubated for 10 days at room temp. (28 ± 2^0C). At the end of the incubation period the mycelial mats were filtered through previously dried and weighed filter paper and dried in hot air oven at 105^0C for 48 h. and the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Solid medium :

Richards medium was amended with various nitrogen sources on equivalent weight basis and were dispensed in sterile Petri plate at fifteen ml quantities. After cooling, they were inoculated with 9 mm mycelial disc of the pathogen obtained from 7 days old culture grown on PDA in Petri plates and incubated for 7days. The linear growth of the pathogen was measured in mm at the end of the incubation period.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Effect of pH levels :

Effect of different pH levels were tested (Table 1) the maximum mycelial growth and dry weight (88.3 mm; 730.5 mg) was obtained in neutral pH (pH 7.0) followed by pH 8.0. However, either increase or decrease in this pH level was highly detrimental to the growth of pathogen. In general, this pathogen prefered acidic range over alkaline range for both mycelial growth and mycelial dry weight (Table 1). These results are in conforming with the findings of Singh (1971); Ahmed (1973); Nusrullah (1983) and Akhtar *et al.* (1999).

Effect of Different culture media :

The results of this present study revealed that maximum mycelial growth and dry weight of *C. gloeosporioides* was observed on Potato dextrose agar and broth (84.8 mm; 625.4mg) which was followed by Malt extract glucose agar and broth (78.5 mm; 583.1mg). The least mycelial growth and dry weight was obtained on Water agar (3.2 mm;15.3mg). In addition to this acervuli production was excellent and also produced greyish-white colour mycelium on PDA medium (Table 2). Similar findings were also observed by Jeffries *et al.* (1990), Akhtar *et al.* (1999) and Fitzell (1979).

Table 1 : Effect of various pH levels on the mycelial growth and mycelial dry weight of *C. gloeosporioides*

pH	Mycelial growth (mm)	Mycelial dry weight (mg)
4.0	63.0	580.1
5.0	71.6	630.6
6.0	76.9	666.8
7.0	88.3	730.5
8.0	86.5	678.4
9.0	60.5	565.4
10.0	52.1	420.1
S.E. ±	0.4364	4.714
C.D.(P=0.05)	0.8973	9.6921

Mean of three replications

Carbon sources :

The data presented in the Table 3 revealed that all the nitrogen sources favoured the growth. Among the 10 nitrogen sources tested, mannitol gave maximum mycelial growth (79.5 mm) and mycelial dry weight (590.8 mg) which was followed by fructose (78.1 mm and 580.5 mg) while least mycelial growth (55.4 mm) and mycelial dry weight (386.8 mg) was recorded in lactose as carbon source (Table 3). Similar findings was also observed by Chaturvedi (1965); Reddy (2000); Sangeetha and Rawal (2008). The above results lend support to the present findings.

Nitrogen sources :

Among the seven different nitrogen sources tested, ammonium nitrate gave the maximum mycelial growth (86.6 mm) and mycelial dry weight (680.8 mg) of *C. gloeosporioides* (Table 4). This was followed by potassium nitrate and sodium nitrate. Whereas, the least mycelial growth and bio-mass production was observed with ammonium chloride amended medium (70.4 mm; 478.1 mg). Similar observations were made by Sangeetha and Rawal (2008). Nitrogen is an important component required for protein synthesis and other vital functions by *C. gloeosporioides*. The pathogen, *C. gloeosporioides* of mango utilized potassium nitrate more efficiently and ammonium nitrate less efficiently for the growth and

Table 2 : Effect of different culture media on the mycelial growth and cultural characteristics of *C. gloeosporioides*

Media	Colour of mycelium	Mycelial growth (mm)	Mycelial dry weight (mg)	Acervuli production
BM	Light grey	66.8	464.8	Good
CMDPA	White	55.3	314.1	Excellent
RA	White	68.5	504.0	Excellent
CDA	White	66.4	455.0	Excellent
CMPA	White	63.8	430.6	Good
MEA	Greyish-white	58.4	380.6	Excellent
MEGA	Greyish-white	78.5	583.1	Excellent
WA	No growth	3.2	15.3	Poor
PSA	White	25.8	124.0	Excellent
PDA	Greyish-white	84.8	625.4	Excellent
S.E. ±		0.5164	7.6646	
C.D.(P=0.05)		1.0617	15.7583	

Mean of three replications

Table 3 : Effect of different carbon sources on the mycelial growth and mycelial dry weight of *C. gloeosporioides*

Carbon sources	Mycelial growth (mm)	Mycelial dry wt (mg)
Sucrose	71.5	556.1
Fructose	78.1	580.5
Mannitol	79.5	590.8
Starch	60.1	434.8
Glycin	57.6	386.8
Lactose	55.4	397.6
Maltose	55.8	588.4
Dextose	78.4	440.6
Glucose	60.3	472.8
Glutamine	66.4	500.6
S.E. ±	0.7149	4.6348
C.D.(P=0.05)	1.4699	9.5292

Mean of three replications

Table 4 : Effect of different nitrogen sources on the mycelial growth and mycelial dry weight of *C. gloeosporioides*

Nitrogen sources	Mycelial growth (mm)	Mycelial dry wt (mg)
Ammonium nitrate	86.6	680.8
Pottasium nitrate	83.2	638.4
Sodium nitrate	78.6	573.6
Sodium nitrate	76.0	540.8
Calcium nitrate	74.3	501.0
Ammonium sulphate	75.8	520.8
Ammonium chloride	70.4	478.1
S.E. ±	0.7766	5.4715
C.D.(P=0.05)	1.5968	11.2495

Mean of three replications

sporulation as reported by Naik (1985) and Saxena (2002).This is contradictory to the present findings.

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