The Pharma Innovation Journal 2022; 11(6): 1127-1131

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(6): 1127-1131
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www.thepharmajournal.com
Received: 16-04-2022
Accepted: 23-05-2022

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Cultural and physiological characterization of *Colletotrichum gloeosporioides* causing anthracnose on *Punica granatum*

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Abstract
Coletotrichum gloeosporioides causing anthracnose is one of the major fungal pathogens that infect during pre and post harvest stages of several host plants. Pomegranate (*Punica granatum* L.) is an arid fruit crop having economical importance with higher export potential. Among all the pathogens of fruit rots, anthracnose caused by *C. gloeosporioides* is becoming major problem in pomegranate. *C. gloeosporioides* was characterized based on its mycelial growth, colony appearance, shape and margins of the colony and sporulation on different solid and liquid media. Among nine different solid media tested, oat meal agar was found to be the best culture media for the growth and development of *C. gloeosporioides* followed by malt extract agar and among different liquid media PDB was found to be best for the growth of pathogens. Highest sporulation was recorded in Richard's agar and Malt extract agar. The effect of temperature and hydrogen ion concentration on growth of pathogen were also studied in which temperature of 30 ^{0}C and pH 6 followed by 7 pH recorded the highest mycelia weight.

Keywords: Pomegranate, anthracnose, *Colletotrichum gloeosporioides* cultural characteristics, morphology

Introduction
Pomegranate (*Punica granatum* L.) is one of the ancient fruits of arid regions of the world belonging to the Lythraceae family. The pomegranate, a small tree has lance shaped and narrow leaves. Pomegranate is known to have table and therapeutic values. Refreshing juice of pomegranate make it more likable fruit. Pomegranates are also used in cooking, meal garnishes, juice blends and alcoholic beverages (Bhowmik *et al.*, 2013) [3].

Pomegranate is native from Iran to Himalayas in northern India. India is world's leading producer of the pomegranate fruit followed by Iran, China, Turkey and United States. Maharashtra is the largest producer of pomegranate followed by Karnataka, Madhya Pradesh, Gujarat, Himachal Pradesh and Rajasthan. Cultivation of pomegranate in India covers an area of 2, 83,000 ha with 29, 15,000 MT production during 2019-20 (Anon., 2020) [2]. Karnataka accounts 25,970 ha area, 2, 68,230 MT production and 10.33 MT/ha productivity during 2018-19. It has spread across different districts of Karnataka *viz.*, Chitradurga, Bellary, Tumkur, Vijayapur and Bagalkot (Anon., 2018) [1].

Pomegranate is affected by several pathogens in field as well as after harvest. This results in huge losses during harvest and storage. Fruit rot is suspected to be caused by, *Colletotrichum sp., Alternaria sp., Cercospora sp., Phytophthora sp., Penicillium spp., Aspergillus spp., Coniella granati and Rhizopus spp.* (Munhuweyia *et al.,* 2016) [9]. Among these fungal pathogens, *Colletotrichm gloeosporioides* (Penz.) Penz. & Sacc. causing anthracnose is the important fruit rot (pre and post harvest) pathogen in pomegranate.

Anthracnose infected fruits show deep sunken lesions on fruit peel around the calyx region and other parts of the fruit surface and necrotic spots on leaves. In severe cases, pathogen is also known to infect the mesocarp, whitish membrane and arils. For the understanding of nature and adaptation of the fungi in different environmental conditions there is a need to study the cultural, morphological and physiological characters of the pathogen.

Materials and Methods

The study was carried out in the Department of Plant Pathology, College of Horticulture, Bagalkot, Karnataka. The pomegranate fruits showing the typical symptoms of anthracnose were selected from the local markets of Bagalkote, Karnataka for the isolation of pathogen. The standard tissue isolation technique was followed for the isolation of the pathogens. Pure culture was done by following single spore isolation technique and in Petri plates on PDA medium and PDA slants in refrigerator and sub cultured once in a month for further studies during the investigation. Isolated pathogen was identified as *Colletotrichum gloeosporioides* through the cultural, morphological and molecular studies.

Cultural characterization of the pathogen

Growth characters on solid media

To study the growth characters of *Colletotrichum gloeosporioides* nine media were selected. Among nine media five were non-synthetic/semi-synthetic *viz*., potato dextrose agar, oat meal agar, V-8 juice agar and malt extract agar and four were synthetic media namely Richard's Agar, Czapek (dox) agar, Sabouraud's dextrose, Luria agar and Mathur's agar media. Media were sterilized for 15 min at 121.6 ^{0}C and 15 lbs pressure for 15 min. To study the growth of the pathogens 18-20 ml of molten media poured into 90 mm petri plates and kept it for solidification. The 2 mm size bit of actively growing cultures was inoculated with the help of Cork borer in the petri plates with the different media and incubated at 28±2 °C. Each treatment was replicated thrice. The fungal growth, colony appearance, shape and margins of the colony and sporulation were recorded 10 days after inoculation. Sporulation scoring was done based on the following grades.

Sl. N0.	Score	Grade	Conidia/microscopic field (400x)
1	++++	Excellent	>75
2	+++	Good	50-75
3	++	Moderate	25-50
4	+	Poor	1-25
5	-	No sporulation	-

Growth characters on liquid media

The growth characters of *Colletotrichum gloeosporioides* was also studied on nine different liquid media *viz*., Potato Dextrose Broth, Oat Meal Broth, V-8 Juice Broth and Malt Extract Broth, Richard's Broth, Czapek (dox) Broth, Sabouraud's dextrose Broth, Luria Broth and Mathur's Broth media. All the required quantity of media were prepared and sterilized at 15 lbs pressure and 121.6 ^{0}C temperature for 15 min. 50 ml of media prepared into 100 ml flask. The 2 mm size bit of actively growing cultures was inoculated with the help of Cork borer in the flasks containing different media and incubated at 28±2 ^{0}C. Each treatment was replicated thrice. The fungal mycelial weight was recorded 10 days after inoculation.

Effect of temperature on growth of the *Colletotrichum gloeosporioides*

Effect of seven different temperatures *viz.*, 15, 20, 25, 30, 35, 40 and 45 ^{0}C was studied on growth of the pathogen. Fifty ml of PDB prepared in 100 ml conical flasks and sterilized at 15 lbs pressure with a temperature of 121.6 ^{0}C for 15 min. The flasks were inoculated with 2 mm mycelial bits of one week old culture to the conical flasks aseptically. The inoculated conical flasks were incubated at different selected temperatures. Each treatment was replicated thrice.

Effect of hydrogen ion concentration (pH) on growth of the *Colletotrichum gloeosporioides*

Eight different pH levels *viz.*, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 were selected to study their effect on growth of the pathogen. The pH was adjusted by using 0.1 N NaOH or 0.1 N HCl. Twenty five ml of PDB was prepared in 100 ml conical flasks and sterilized at 15 lbs pressure with a temperature of 121.6 ^{0}C for 15 min. The flasks were inoculated with 5 mm mycelial bits of one week old culture to the conical flasks aseptically. The inoculated conical flasks were incubated at different selected temperatures. Each treatment was replicated thrice.

Results and Discussion

Effect of different media on growth of *Colletotrichum gloeosporioides*

Among nine different solid media tested, oat meal agar (90.00 mm) was found to be the best culture media for the growth and development of *C. gloeosporioides* followed by malt extract agar (85.67mm) (Table1). The result obtained is in accordance with Mello *et al.* (2004) [8] who recorded oat meal agar as the best growth media for *Colletotrichum gloeosporioides* isolated from green pepper. Highest sporulation was recorded in Richard's agar and malt extract agar which is in contradictory to the results of Prashanth (2007) [13] and Majumdar and Manda (2019) [7] who recorded maximum sporulation on oat meal agar. In the present study least growth sporulation was recorded on PDA and V8- juice agar with 49.00 mm and 49.67 mm mean mycelia diameter respectively but PDA showed highest mycelia growth in studies carried out by Prashanth (2007) [13]; Majumdar and Manda (2019) [7] and Dev *et al.* (2017) [5].

PDA consists of dextrose and potato infusion. Dextrose act as growth stimulant and potato infusion is a nutrient base for the growth and development of most fungi (Castagnoli, 1938). However, oat meal agar is being rich in carbon, nitrogen, protein and other important nutrients may be the reason for the highest growth and sporulation of fungi as compared to PDA (Murray *et al.*, 1999) [10].

The growth pattern of pathogen varied with different media as shown in Fig1. On oat meal agar, *Colletotrichum gloeosporioides* showed flat, whitish grey coloured colony growth giving grey/black pigmentation with irregular margin. Fluffy, dark grey coloured colony having regular margin with light grey pigmentation was observed on malt extract agar (Table1)

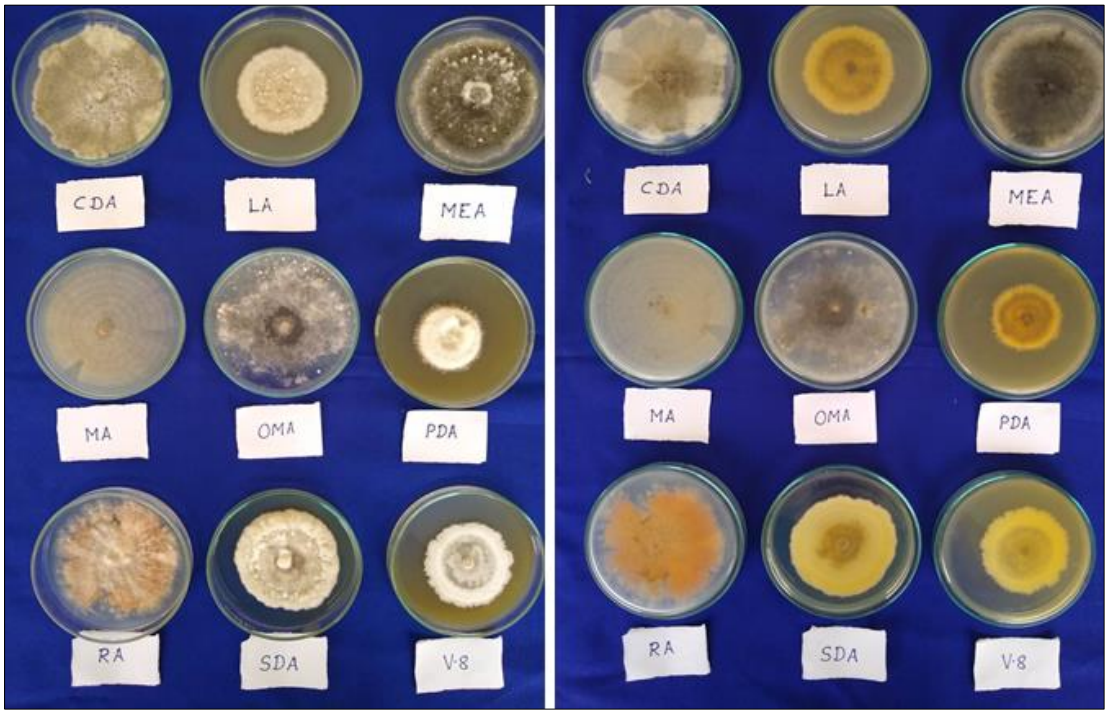
Among different liquid media PDB was found to be best for the growth of pathogen, *Colletotrichum gloeosporioides* with 325.07 mg mean dry mycelia weight (Table2 and Fig2) In the present study, among different media tested PDA showed least growth and PDB showed highest growth *C. gloeosporioides*. This difference between growth of fungi on solid and liquid media may be due to the good aeration and water availability in the liquid media compared to solid media.

Table 1: Comparative growth of *Colletotrichum gloeosporioides* on different solid media

Sl. No.	Solid media	Mean mycelial diameter (mm)	Colony characters			Sporulation
			Colony growth and margin	Colony colour	Pigmentation	
1	Czapek's dox agar	73.00	Fluffy and irregular	Whitish grey	White and grey	++
2	Luria agar	60.33	Fluffy and irregular	White to grey	White to brown	++
3	Malt extract agar	85.67	Fluffy and regular	Dark grey	Grey	++++
4	Mathur's agar	71.67	Flat and regular	Light grey	White	+++
5	Oat meal agar	90.00	Flat and irregular	White to grey	White	+++
6	Potato dextrose agar	49.00	Fluffy and regular	White	Dark grey	+
7	Richard's agar	70.33	Fluffy and irregular	Orange to grey	Orange	++++
8	Sabouraud's dextrose agar	63.00	Fluffy and irregular	White to grey	White to brown	++
9	V8- juice agar	49.67	Flat and irregular	White	White	+
	S.Em ±	0.50				
	CD (1%)	2.02				

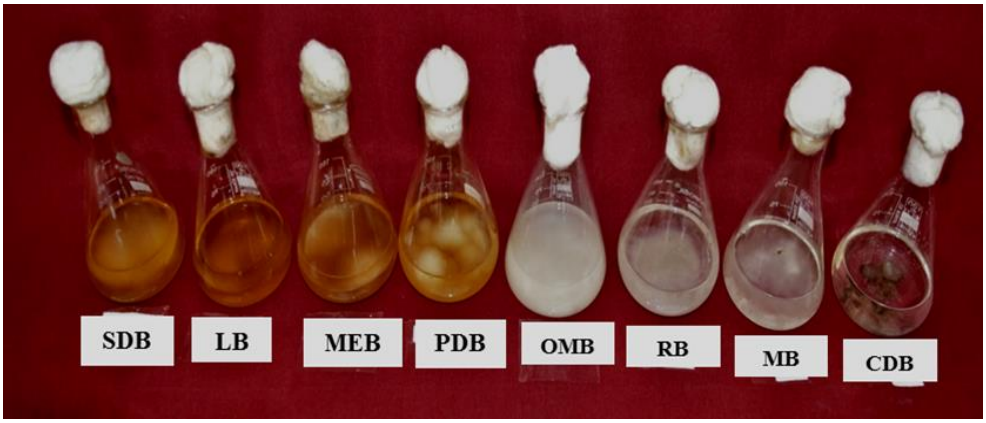
Table 2: Comparative growth of *Colletotrichum gloeosporioides* on different liquid media

Sl. No.	Liquid Medium	Mean mycelial dry weight (mg)
		Colletotrichum gloeosporioides
1	Czapek's dox	124.29
2	Luria	197.88
3	Mathur's	126.75
4	Malt extract	195.21
5	Oat meal	177.33
6	Potato dextrose	325.07
7	Richard's	54.91
8	Sabouraud's dextrose	230.83
S.Em ±		0.54
CD (1%)		2.24



CDA- Czapek's dox agar LA- Luria agar MEA- Malt extract agar MA- Mathur's agar OMA- Oat meal agar PDA- Potato dextrose agar RA- Richard's agar SDA- Sabouraud's dextrose agar V8- V8- juice

Fig 1: Comparative growth of *Alternaria alternata* on different solid media



CDB- Czapek's dox broth LB- Luria broth MEB- Malt extract broth MB- Mathur's broth OMB- Oat meal broth PDB- Potato dextrose broth RB- Richard's broth SDB- Sabouraud's dextrose broth

Fig 2: Comparative growth of *Colletotrichum gloeosporioides* on different liquid media

Effect of different temperature on growth of *Colletotrichum gloeosporioides*

For *Colletotrichum gloeosporioides*, 30 ^{0}C was recorded highest value of dry mycelia weight of 312.09 mg followed by 35^{0}C (298.04 mg). Least growth was recorded at 45, 40 and 15^{0}C with mean dry mycelia weight of 57.63, 121.71 and 112.89 mg respectively (Table3 and Fig3a). Earlier studies carried out by Dev and Somashekhar (2018) [5]; Pandey *et al.* (2012) [11]; Kumara and Rawal (2008) [6] and Prajapati *et al.* (2020) [12] reported similar results which are supporting to the present study. Pomegranate crop can withstand a temperature range of 15 ^{0}C-40 ^{0}C for growth and development. However, the temperature range from 30-35 ^{0}C is suitable for development of pomegranate fruit which correlates with the optimum temperature range for the growth of *Colletotrichum gloeosporioides*.

Table 3: Effect of different temperatures on growth of *Colletotrichum gloeosporioides*

Sl. No.	Temperature (^{0}C)	Mean mycelial dry weight (mg)
		Colletotrichum gloeosporioides
1	15	112.89
2	20	152.59
3	25	288.27
4	30	312.09
5	35	298.04
6	40	121.71
7	45	57.63
S.Em ±		0.56
CD (1%)		2.37

Effect of different temperature on growth of *Colletotrichum gloeosporioides*

Significant difference was noticed among different pH levels. Among the different ph levels, pH 6 recorded highest value of dry mycelial weight of 298.76 mg followed by pH 7 (255.74 mg). Least growth was recorded at pH levels of 3 and 6 with mean dry mycelia weight of 62.97 and 72.08 mg respectively. These results showed that the optimum pH for the growth of *Colletotrichum gloeosporioides* was ranged from 6 to 7 (Table4 and Fig3b). The least growth was noticed at pH 3 (62.97) and pH10 (72.08 mg). These observations show quite good congruence with results of Kumara and Rawal (2008) [6]; Dev and Somashekhar (2018) [5] and Sharma and Kulshreshta (2015). This shows that acidic or slightly acidic conditions are ideal for the growth of isolated pathogens from fruit rot of pomegranate. The pH of pomegranate fruit juice ranges from 4.5 to 6.0 which is comparable with the ideal pH for fungal growth. However, all the stages of pomegranate fruit development are susceptible for anthracnose infection and in this study pH levels from 4-8 were supportive for the growth of *Colletotrichum gloeosporioides*.

Table 4: Effect of different pH on growth of *Colletotrichum gloeosporioides*

Sl. No.	pH	Mean mycelial dry weight (mg)
		Colletotrichum gloeosporioides
1	3	62.97
2	4	127.56
3	5	192.04
4	6	298.76
5	7	255.74
6	8	151.96
7	9	102.18
8	10	72.08
S.Em ±		0.49
CD (1%)		2.01

Fig 3: Effect of different a) temperatures and b) pH on growth of *Colletotrichum gloeosporioides*

Acknowledgement

I am thankful to the Department of Plant Pathology, College of Horticulture, Bagalkot for providing laboratory facilities.

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