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Effect of Different Solid Media on the Growth and Sporulation of *Colletotrichum gloeosporioides* Penz. and Sacc. causing Fruit Rot of Aonla

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A B S T R A C T

Colletotrichum gloeosporioides Penz. and Sacc**.** is associated with aonla fruit rot. Laboratory studies were conducted to study the effect of different solid media on mycelia growth and sporulation of *Colletotrichum gloeosporioides* Penz. and Sacc. Among all the solid media tested, maximum mycelial growth with excellent sporulation rating was obtained on Richard's agar medium (90 mm) within six days and was significantly superior to all the other media tested. This was followed by Potato dextrose agar (88.83 mm) and Corn meal agar (88.40mm) under *in vitro* conditions. The growth characteristics of the fungus such as colour of the colony and sporulation were also different in different culture media. Maximum sporulation of the test fungus was found on Richard's agar media whereas Minimum growth was observed on Host extract dextrose agar medium (68.17 mm).Thus the present work will be useful for further investigation on the physiology of the fungus and management of the disease.

Introduction

Aonla (*Emblica officinalis.*Gaertn.) is one of the major fruit crop in the State of Maharashtra. The anola is affected by number of fungal pathogens such as *Colletotricum gloesporioides.* Penz. and Sacc. (fruit rot) *Ravenelia emblicae* Styd. (rust), *Fusarium* spp. (wilt), *Penicillium citrinum* Thom. (fruit rot or blue mould), *Phomopsis phyllanthi* Punith (soft rot), *Phoma putaminum* Speg. (dry fruit rot), *Aspergillus terreus* (fruit rot) *etc*. Among them, the fruit rot caused by *Colletotricum gloesporioides.* Penz. and Sacc.

is a major disease of aonla fruit and responsible for causing 2- 29 per cent yield loss (Sohi, 1975).

Keeping in view economic importance of aonla and losses incurred due to fruit rot disease, present investigations on the various aspects *viz.*, survey, symptomatology, pathogenicity test, morphological and cultural characteristics, efficacy of different fungicides, bio-agents, plant extracts were undertaken during the season of *Kharif* 2018-2019 at Department of Plant Pathology, College of Agriculture, Badnapur, V.N.M.K.V. Parbhani. The results obtained on the above aspects during the present investigations are being interpreted and presented in the following paragraphs.

Radziah (1985) and Amarjit Singh *et al.*, (2006) revealed that maximum growth and sporulation of *C. gloeosporioides* was obtained on Potato Dextrose Agar (PDA).

Ekbote (1997) observed maximum radial growth of *C. gloeosporioides* on Richards' agar, Potato Dextrose Agar, and Brown's agar.

Vinod Tasiwal and Benagi (2009) opined that best solid medium for growth and sporulation of *C.gloeosporioides* was on V- 8 juice agar and Richards' agar respectively.

Materials and Methods

The present investigation was under taken at laboratory conditions at Department of Plant Pathology, College of Agriculture, Badnapur, VNMKV, Parbhani during the season of *Kharif* 2018. Aonla fruits showing the typical symptoms of fruit rot were collected from the Horticultural Farm, College of Agriculture, Badnapur during the season of *Kharif* 2018 in the months of June, July, August, September, October, November and December. Samples were brought into the department of Plant Pathology, College of Agriculture, Badnapur for isolation and further studies. Infected portions of fruits were cut into small pieces along with some healthy portion, they were surface sterilized with 0.1% mercuric chloride (HgCl2) solution for 30 seconds and then rinsed 3-4 times in distilled sterilized water so that all the traces of mercuric chloride were removed. The bits were then aseptically placed on potato dextrose agar plate and incubated at 28 ± 1^{0} C for 7 days.

The stock culture was maintained on potato dextrose agar medium at 5 ± 1^{0} C and subcultured after every 30 days. Pathogenicity of these isolates was also confirmed suggested by Observations recorded during present investigations were matched with opinion of earlier reporters *viz.,* Gautam (2014) and Shivakumar *et al.,* (2015).

Preparation of different media and inoculation

The following ten culture media available at department of Plant Pathology, Badnapur were used for *in-vitro* experiments conducted during the present investigation. The growth characters of *Colletotricum gloesporioides* was studied on ten solid media. *viz.,* Richards' agar medium, Potato Dextrose Agar, Sabouraud's agar, Czapek- Dox agar, Brown's agar, Fries's agar, Oat meal agar, Corn meal agar, Host extract agar, and Host extract dextrose agar medium. All the media were sterilized at 1.1 kg/cm^2 pressure (121 ºC) for 15 min. To carry out the study 20 ml of each of the medium was poured in 90 mm petri plates.

Such petri plates were inoculated with 5 mm disc cut from the periphery of actively growing culture and incubated at 28 ± 1 ºC temperature. Each treatment was repeated thrice. The colony diameter was recorded daily.

Effect of different culture media on growth and sporulation of *C. gloeosporioides*

Cultural characteristics *viz*., mycelial growth, colony diameter, colony colour and sporulation, *etc.* were recorded. Observations were taken when the fungus covered complete petri plate in any one of the media. The data on radial growth was analyzed statistically.

Experimental details

Design: Completely Randomized Design (CRD)

Replications: Three

Treatment: Ten (culture media)

Sr.No	Name of the medium
1	Richard' agar
2	Potato dextrose agar
3	Corn meal agar
4	Sabouraud's agar
5	Czapek- Dox agar
6	Oat meal agar
7	Brown's agar
8	Fries's agar
9	Host extract agar
10	Host extract dextrose agar

The composition of the media used was given under Appendix I.

The all media were prepared and sterilized by autoclaving at 15 pounds pressure for 20 min which corresponds to a temperature of 121.6^0 C. Fifteen ml of each medium listed above was poured into 90 mm dia. petri plates.

After solidification, five mm disc of *Colletotrichum gloeosporioides* Penz. and Sacc. From an actively growing culture were cut using a cork borer and a single disc was placed upside down at the center of the petri dish. Each set of experiment was replicated thrice and the plates were incubated at room temperature. The measurement of the colony dia. was taken when the maximum growth was attained in any one of the media tested. Cultural characters such as colony dia., colony colour, type of margin and sporulation were also recorded.

The number of conidia were observed microscopically and graded as below.

Results and Discussion

Effect of different solid media on the growth and sporulation of *C. gloeosporioides*

The diversity in cultural characters of *C. gloeosporioides* was studied on nine different solid media under laboratory conditions. The data are presented in table 1, plate 1 and figure 1.

Maximum radial growth was obtained on Richard' agar medium (90 mm) within six days and was significantly superior to all the other media tested. this was followed by Potato dextrose agar (88.83 mm), Corn meal agar (88.40), Sabouraud's agar (87.83 mm), Czapek- Dox agar (86.67 mm), Oat meal agar (80 mm), Brown's agar (76.48), Fries's agar (76.33 mm), and Host extract agar (72.16 mm) Minimum growth was observed on Host extract dextrose agar medium (68.17 mm).

Sporulation was obtained in all the nine media tested. Excellent sporulation of the fungus was recorded on Sabouraud's agar, Potato dextrose agar and Richards' agar media.

Sporulation was fair on Czapek- Dox agar and Host extract agar with respect to mycelia colour, it varied from dull white to gray. The growth varied from slightly raised to slightly fluffy with smooth and entire margins. The

growth of the fungus on PDA was circular, evenly felty, grayish white with entire margin showing diurnal zonations.

Mycelial growth on Sabouraud's agar was like a felted mat with circular entire margins having salmon pink conidial pustules at the centers of the colonies. On the Richards' agar, the fungus produced dull white, slightly fluffy, circular growth having smooth and entire margins. Mycelial growth on Host extract dextrose agar, Fries's agar and Brown's agar were grayish white colour having smooth, circular, entire margins with good sporulation. On Czapek- Dox agar and host extract agar the fungus produced dull white to grayish coloured mycelia growth with slightly raised smooth, circular, entire margin having fair sporulation. On oat meal agar mycelia growth is good with irregular margin, with whitish mycelial growth and on Corn meal agar Moderate growth with smooth margin, Light yellowish to whitish mycelium growth is observed (Table 1, plate I and Fig. 1).

observations of present study are in confirm with earlier records of *viz.,* Radziah (1985) and Amarjit Singh *et al.,* (2006), Hegde (1986), Ekbote (1994) Mesta (1996), Ruchi Garg *et al.,* (2007), Vinod Tasiwal and Benagi (2009).

Table.1 The number of conidia were observed microscopically and graded as below.

Sr.No	Score	Grade	No. of conidia / microscopic field at 100X
1	++++	Excellent	>150
2	+++	Good	101-150
3	++	Fair	51-100
4	+	Poor	1-50
5	-	No Sporulation	-

Fig.1 *In-vitro,* effect of different fungicides on radial mycelium growth of *C. gloeosporioides*

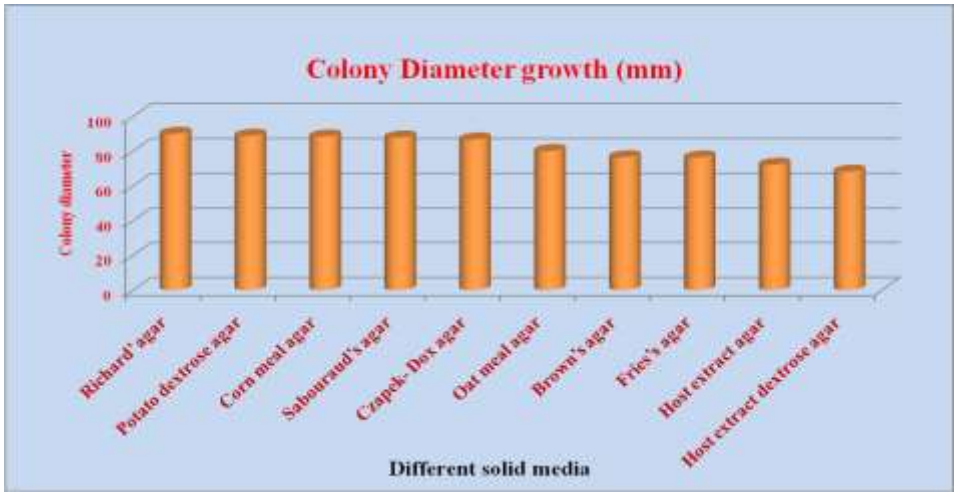
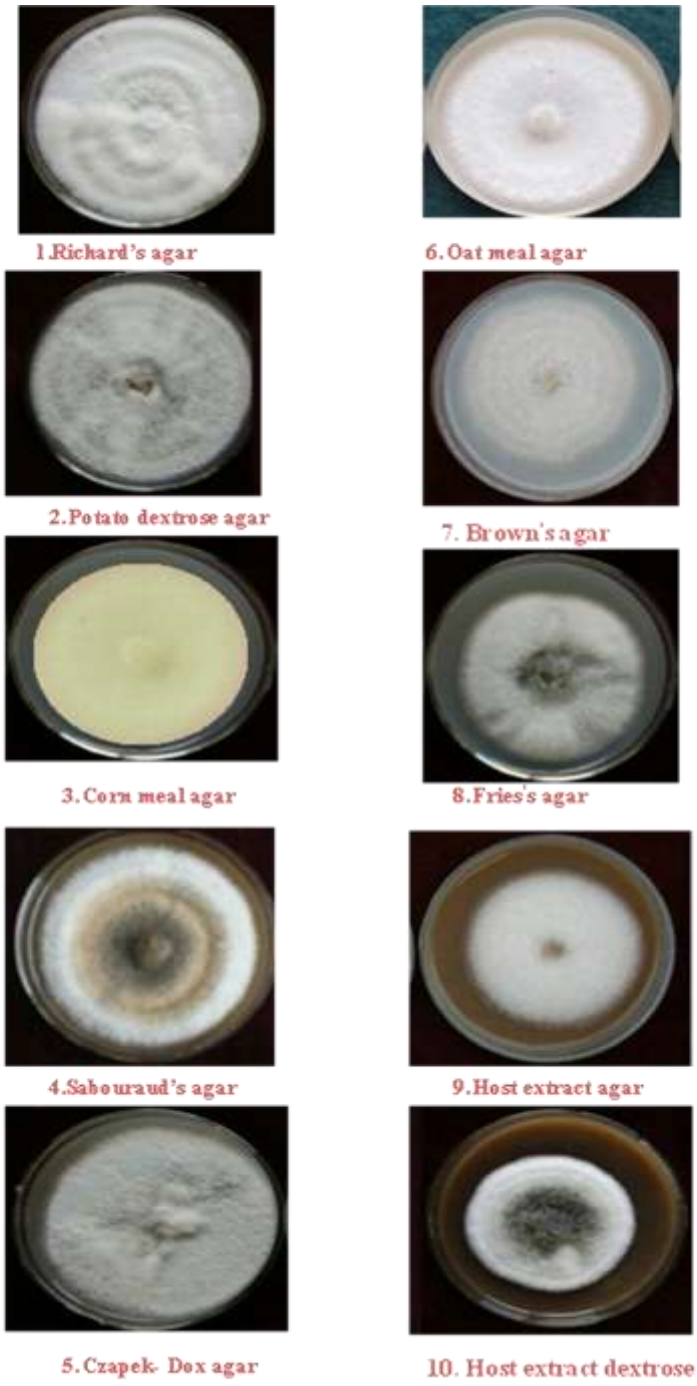


Table.2 Growth and characteristics of *C. gloeosprioides* on different solid media

Sr. No.	Media	Colony* Diameter growth (mm)	Colony growth characters	Sporulation
1.	Richard' agar	90.00 (71.56)	Good growth evenly fluffy with dull white, diurnal zonation, circular, slightly raised colony appeared light salmon pink in colour.	++++
2.	Potato dextrose agar	88.83 (70.47)	Good growth, evenly felty with grayish white, diurnal zonations, entire margin, reverse of colony appeared in the form of distinct olivaceous grey zonation altered with rosy buff zonations	++++
3.	Corn meal agar	88.40 (70.00)	Moderate growth with smooth margin, Light yellowish to whitish mycelium growth.	++++
4.	Sabouraud's agar	87.83 (69.58)	Aerial mycelium even, felted mat with salmon pink conidial pustules evident at the center with white circular and entire margin, diurnal zonation, reverse of the colony light grey in colour.	++++
5.	Czapek- Dox agar	86.67 (68.58)	Evenly felty with grayish white, circular, slightly, raised, entire margin, reverse of colony appeared dark grey colour	+++
6.	Oat meal agar	80.00 (63.43)	Good growth with irregular margin, with whitish mycelium growth.	+++
7.	Brown`s agar	76.48 (60.96)	Greyish white, loosely textured colonies, appeared, circular, entire margins, diurnal zonation, reverse of the colony light grey in colour.	+++
8.	Fries`s agar	76.33 (60.68)	Felyu, dark grey centre with white smooth entire margin, slightly raised, circular, reverse of colony appeared smoky grey in colour.	++
9.	Host extract agar	72.16 (58.15)	Evenly felty with grayish white, circular, entire margin, slightly raised, reverse of colony uncolour.	++
10.	Host extract dextrose agar	68.17 (55.65)	Evenly felty, dark grey centre with white smooth, entire margin, slightly raised, circular, diurnal zonations, reverse of the colony light grey in colour.	++
	SE ±	**0.61**	–	–
	CD (P=0.01)	**1.68**		

*Avg. of three replications, Figures in parenthesis are Arc sine transformation values. (Dia. = Diameter) - : No; +: Poor (1-50 conidia/microscopic field 100x); ++++ EXCELLENT SPORULATION, +++ GOOO SPORULATION, ++ FAIR SPORULATION, + POOR SPORULATION, - NO SPORULATION

Plate - I



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