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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**Abstract**

*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 (88.40 mm) 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.

**Keywords**

Aonla (*Emblica officinalis*, Gaertn.), *Colletotrichum gloeosporioides* Penz. and Sacc.

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**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), *Penicillum 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 *Colletotrichum 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±1º C for 7 days.

The stock culture was maintained on potato dextrose agar medium at 5±1º 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 gloeosporioides* 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/cm² 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.
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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 number of conidia were observed microscopically and graded as below.

**Results and Discussion**

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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 conidal 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).

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. gloeosporioides* 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*
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