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Studies on cultural and morphological characteristics of *Colletotrichum gloeosporioides* (Penz.) Sacc. in sweet orange at Marathwada region of Maharashtra

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Abstract
The present investigation was carried out to study on pathological pre harvest fruit drop aspects of sweet orange in Marathwada region of Maharashtra. This experiment was conducted in the department of Plant Pathology, College of Agriculture, Badnapur and Sweet orange research station, (SORS) Badnapur, Dist-Jalna (VNMKV, Parbhani) M.S. in year 2016-17. The experiment was laid out in Completely Randomised Design-CRD, with three replications and ten treatments. Morphogenic characteristics of *C. gloeosporioides* was studied on different media and wide range of variability in respect of conidial and setae dimensions was observed. The conidial size *Colletotrichum gloeosporioides* ranged from18.21 to 26.62 µm. The large sized conidia (26.33-26.62x3.16-4.74 µm) were produced on the PDA which was followed by Richards agar, host leaf extracts, oat Meal Agar, Corn meal Agar, Czapak Dox Agar, Yeast mannitol Agar and Ashby's mannitol Agar. However, small sized conidia were noticed on plain agar and Yeast extract Agar. The maximum average setae length was recorded on potato dextrose agar while maximum setae width was observed on Ashby's mannitol Agar. Potato Dextrose Agar (PDA) was found to be most suitable medium for growth (90.00 mm). The second and third best medium found were Czapak Dox Agar (89.82 mm) and Oat Meal Agar (88.43 mm). Rest of the culture media recorded radial mycelial growth in the range of 49.67 mm (Host leaf extract Agar) to 85.38 mm (Richard's Agar). All the culture media tested, exhibited wide range of colony colour, colony texture, sporulation, conidial dimensions and setae dimensions.

Keywords: Cultural, morphological, characteristics, Colletotrichum gloeosporioides (Penz.) Sacc., sweet orange, Marathwada, Maharashtra

Introduction
Citrus belongs to the family Rutaceae. It is long-lived perennial fruit crop, grown in more than 100 countries across the world (Savita *et al.*, 2012) [14]. Citrus fruits which have been cultivated from times immemorial are native of tropical and sub-tropical areas of Southern Asia. The oranges are believed to have been originated in China, the lime in India, the lemon in Arabia and the Pummelo in Malayan Archipelago (De Condolle, 1886) [5]. The most important commercial citrus cultivars in India are the mandarin (*Citrus reticulata* Blanco) followed by sweet orange (*Citrus sinensis* L. Osbeck) and acid lime (*Citrus aurantifolia* Swingle).

In Maharashtra, the sweet oranges are grown mainly in the Jalna, Aurangabad, Nanded, Parbhani, Beed and Osmanabad districts of Marathwada region of central Maharashtra and Ahmednagar, Pune and Nasik districts of western Maharashtra. Area under sweet orange cultivation in Maharashtra is 35.29 thousand ha, with the production of 461.85 thousand MT of fruits and productivity is 8.8 MT/ha. (Anonymous 2016) [1].

In Marathwada region the area of sweet orange cultivation is increasing at faster rate year after year but production is not increasing as the same proportion due to the numerous production constraints *viz.*, non-availability of disease free planting material, poor management practices, irregular bahar practices. Besides this, crop is attacked by more than 150 diseases and disorder caused by fungi, bacteria, virus and virus like particles right from nursery level to bearing stage resulting in considerable losses.

Among the fungal pathogens *Colletotrichum gloeosporioides* (Penz.)Sacc. induces pre harvest fruit drop or Anthracnose or wither tip or twig blight. *Phytophthora* spp. induces root rot, foot rot, gummosis, leaf fall and fruit rot. Other fungal diseases of sweet orange include pink disease (*Pellicularia salmonicolor*), powdery mildew (*Acrosporiumtingitaninum*), greasy spot (*Mycosphaerella citri*), scab (*Elsinoe fawcettii*), dry root rot (*Fusarium solani*) and pre-harvest stem end rot which is majorly caused by *Colletotrichum gloeosporioides* (Penz.) Sacc.

Fawcett (1912) [6] reported the stem-end rot of citrus fruits in Florida for the first time. Fawcett (1936) [7] observed stem-end rot due to *Colletotrichum gloeosporioides* in the form of firm to semi pliable small brown areas around the stem-end. As the area enlarges, a soft and pliable rot develops on the fruits. Cheema *et al.*, (1975) [4] reported that *Colletotrichum gloeosporioides* was predominantly associated with stem-end rot of sweet oranges. Pre-harvest stem end rot is caused by pathogen *Colletotrichum gloeosporioides* which also causes fruit rot, thereby seriously affecting the marketable fruit yield (Sawant and Bulbule, 2000) [15]. High inoculum of these fungi in the orchards build up due to the dead twigs on the bearing trees and honeydew secreting insects like black fly resulting in the development of sooty mold. This causes up to 22% pre-harvest drop in the orchards having 10% dead twigs of the canopy. The disease is common in all the citrus growing areas of the world and is more destructive in warm and humid regions, including India. The disease is prevalent on developing fruits of different age group and was more sever on the ripening fruits.

Among the various pathological constraints, pre harvest fruit drop (PFD) or anthracnose is a wide spread disease and every year it is leading to huge economic losses to sweet orange growers of Marathwada region. Disease appears as brown discoloration near stem-end of fruits. As the disease progress, this discoloration covers whole of the fruit and causes rotting.

A very limited research work have been carried out on pathological pre harvest fruit drop aspects in Marathwada region of Maharashtra, particularly in sweet orange. Therefore, considering the economical importance and keeping in view the serious nature of disease and past research work on controlling pre harvest fruit drop it was felt necessary to undertake the investigations on the pathogen associated with the pre harvest fruit drop or anthracnose of sweet orange with the following objective,

1) To isolate and identify pathogen associated with disease.
2) To study cultural and morphological characteristics of *Colletotrichum gloeosporioides* (Penz.)Sacc.

Material and methods

The present investigation is on pre harvest fruit drop of sweet orange (*Citrus sinensis* L. Osbeck) caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. A series of experiments were carried out in the laboratory at department of plant pathology, College of Agriculture, Badnapur. Field studies were carried out at the Sweet Orange Research Station (SORS), Badnapur as well as farmers field during 2016-2017 to fulfill the defined objectives. The details of the material used and methods employed for various experiments are described below:

Experimental site

The whole experiment was conducted in the department of Plant Pathology, College of Agriculture, Badnapur and Sweet orange research station, (SORS) Badnapur, Dist-Jalna (VNMKV, Parbhani) M.S.

Experimental materials

The various kinds of experimental materials *viz.*, fungicides, insecticides, fertilizers, seeds and other miscellaneous items required for conducting present investigation was obtained from the department of plant pathology, College of Agriculture, Badnapur and Sweet orange research station, (SORS) Badnapur.

Collection of samples

Sweet orange fruits showing the typical symptoms of pre harvest fruit drop were collected from the orchards of Badnapur [Sweet Orange Research Station (SORS), Badnapur], and farmer's sweet orange orchards of Ambadgaon (Dist- Jalna), Gadhejalgon (Dist- Aurangabad) during the *ambia bahar* in the months of September, October, November and December 2016. Fruits were classified into the following four categories on the basis of visual symptoms:

i. Showing rot on both stylar and stem-end.
ii. Brown soft and pliable rot on stem-end only.
iii. Firm dark brown rot on stem-end only.
iv. Fruits which became mummified and remained attached to twigs.

Field and laboratory facilities

All the field experiment were planned and conducted on the research farm of the SORS, Badnapur. *In-vitro* studies were conducted in the laboratory of department of Plant Pathology, College of Agriculture, Badnapur.

Methodology

Cultural and morphological characteristics

Colletotrichum gloeosporioides isolated from disease samples of experimental field were re-inoculated on different media *viz.*, Potato Dextrose Agar, Oat meal Agar, Richard's Agar, Czapak Dox Agar, Yeast extract Agar, Yeast mannitol Agar, Corn meal Agar, Asbhy' smannitol Agar, Host leaf extract and Plain Agar and the plates were incubated at 27 ± 2^0C for a week. The observations were recorded on all above mentioned media. The morphological characteristics of *viz.*, size of conidia and size of setae were recorded. The size of conidia was measured using ocular micrometer (calibrated using stage micrometer) under the compound microscope at 100 X magnification and size of setae measured at 40 X with the aid of ocular micrometer (calibrated using stage micrometer) in compound microscope.

Effect of Culture Media

Cultural characteristics *viz.*, mycelial growth, colony diameter, colony colour, 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)

Treatments details

T_1: Potato Dextrose Agar
T_2: Oat meal Agar
T_3: Richard's Agar
T_4: Czapak Dox Agar
T_5: Yeast extract Agar
T_6: Yeast mannitol Agar
T_7: Corn meal Agar
T_8: Asbhy' smannitol Agar
T_9: Host leaf extract
T_{10}: Plain Agar

Twenty ml of each medium listed above was poured into 90 mm diameter Petriplate. After solidification, 5 mm disc of

C. gloeosporioides from actively growing culture were cut using a cork borer and a single disc was placed upside down at the center of Petridish. Each set of experimental was replicated thrice and the plates were incubated at 27 ± 2^0 C. The measurement of colony diameter was taken when the maximum growth was attained in any of the media tested. The cultural characteristics *viz.*, colony colour, colony texture and sporulation were also recorded 15 days of incubation.

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

S. No.	Score	Grade	No. of conidia / microscopic field at 100X
1	++++	Excellent	70-50
2	+++	Good	30-50
3	++	Fair	21-30
4	+	Poor	10-20
5	-	No Sporulation	-

Statistical analysis

Statistical analysis was carried out as per procedure by Panse and Sukhatme (1967). Data on percentage were transformed in to arc sine values and analysis was done in CRD, also the statistical analysis for multiple, linear regression and correlation were done on the basis of average weekly date of per cent disease intensity and average weekly metrological data using MS

Result and discussion

Cultural Characters

Morphogenic diversity of *C. gloeosporioides* was studied based on the cultural and morphological characters. The observations were recorded for radial growth, colony colour, colony shape and sporulation etc. after specific period.

Effect of different media in supporting the growth of pathogen Mycelial Growth

The results (Table.2, PLATE I) revealed that all the culture media tested encouraged better growth except host leaf extract and variable sporulation of *C. gloeosporioides.* The mean colony diameter recorded with all the test media was ranged from 49.67mm (Host leaf extracts) to 90.00mm (Potato dextrose agar). However, significantly highest mean mycelial growth (90.00mm) was recorded on Potato dextrose agar (PDA) and found superior over rest of media tested. The second and third best media found were Czapackdox agar (89.82mm) and oat meal agar (88.43mm) and these three were statistically at par with each other. This was followed by Richard's agar (85.38mm), yeast extract agar (82.63mm), Ashby's mannitol agar (80.15mm), corn meal agar (76.48mm), yeast mannitol agar (72.50mm) and plain agar (64.65mm). The minimum mean mycelial growth of test pathogen was recorded on Host leaf extracts (49.67mm).

Colony Characteristics

Colony Colour

The colour of the colony was observed from bottom side of petri-dish. The observations on colony pigmentation were taken on 8 days after incubation. The data from Table 2 revealed that the cultures were initially whitish in colour, later on it became dark black in colour (Table.2, PLATE I). On PDA, it produced black colony, while on Oat Meal Agar, Czapak Dox Agar, Yeast Extract Agar, Yeast Mannitol Agar, Ashby's Mannitol Agar and Plain Agar, it produced white mycelium, while, Richard's Agar produced light black to white colour of colony, while on Corn meal Agar, it produced light yellowish to white mycelium.

Colony Texture

The pathogen was also observed for their colony texture. Slight difference was observed in appearance of colony (Table.2, Plate I). It has good growth with smooth margin to moderate growth with irregular margin on the medium, Potato Dextrose Agar (PDA), Oat meal Agar, Richard's Agar and Czapak Dox Agar, exhibited good growth, while, on Yeast extract Agar, Yeast mannitol Agar, Corn meal Agar, Plain Agar and Asbhy'smannitol Agar, it was moderate in growth. The slow growth of pathogen was observed on Host leaf extract medium as compared with other media test.

Sporulation Characteristics

The observations of cultural studies of present investigations are resembled with earlier reports of *viz.*, Chaudhari (1935) also reported that *C. gloeosporioides* grew well on Potato Glucose Agar, Czapek-Dox and Richards' agar medium whereas Kinnow Leaf Extract agar medium and Kinnow Twig Extract agar medium showed impaired mycelial growth of the fungus. Sudhakar (2000) [16] reported that, maximum radial growth of *C. gloeosporioides* was recorded in PDA and Oat Meal Agar. Lopez and Lucas (2010) observed that on PDA, aerial mycelium of *C. gloeosporioides* was velvety to densely floccose, with a gradual tendency from whitegreyish to dark green-moss pigmentation. On Oat Meal Agar, the colonies were less variable, with predominance of white-greyish floccose mycelium covering an abundant mucilaginous mass of orange acervuli and spores. The present findings are also in agreement with earlier reports of Pandey *et al*., (2012) [12], Kumar *et al*., (2015) [11], who reported the radial growth of *C. lindemuthianum* was highest on Potato Dextrose Agar as compared to other media tested. Zakaria and Bailey (2000) [18], Koche *et al*., (2011) [10] and Thangamani *et al*., (2011) [17], who reported high level of variation in cultural characteristics in isolates obtained from different solid media.

Morphological Characters

Mycelial Characters

Light microscopy studies revealed that mycelium of test pathogen were observed septate on all tested media. The mycelium was white initially, but developed dark black colour in advanced stage and grew rapidly.

Morphological characteristics of *C. gloeosporioides* on different media

Results depicted in (Table.3) that *C. gloeosporioides* exhibited a wide range of variability in respect of conidial and setae dimensions.

Conidial Dimensions

From the data presented in (Table.3), it is observed that conidial size of *C. gloeosporioides* was ranged from 18.21 to 26.62µm. However, large sized conidia (26.33-26.62 x 3.16-4.74µm) were produced on the Potato Dextrose Agar. It was followed by Richard's Agar (25.14-26.58x3.78-4.48 µm) and

Host leaf extracts (24.66-25.08 x 3.49-4.76 µm), Oat Meal Agar (24.18-25.47 x 3.65-4.08 µm), Corn meal Agar (21.74-22.90 x 3.08-4.12µm), Czapak Dox Agar (21.68-23.42 x 3.77-5.53µm), Yeast Mannitol Agar (20.87-21.34 x 3.96-5.60 µm) and Ashby's mannitol Agar (19.26-21.52 x 3.42-5.21 µm). Comparatively small sized conidia were noticed on Plain Agar (18.44-21.25 x3.84-4.31 µm) and Yeast extract agar (18.21-20.14 x 3.74-4.98µm) of *C. gloeosporioides.*

Setae dimensions

The data presented in (Table.3), revealed that the average setae length was maximum on Potato Dextrose Agar (164.11µm), followed by Yeast extract Agar (163.34µm), Corn meal Agar (126.23µm), Oat meal Agar (123.47 µm), Asbhy' smannitol Agar (64.59µm), Richard's Agar (61.34µm), Czapak Dox Agar (52.28µm), Yeast mannitol Agar (47.90µm), Plain Agar (47.76µm) and host leaf extracts (45.11µm) Whereas, measurement of the setae width was maximum on Asbhy'smannitol Agar (5.67µm), followed by Czapak Dox Agar (5.62µm), Potato Dextrose Agar (5.38µm), Oat meal Agar (5.23µm), Plain Agar (5.20µm), Yeast mannitol Agar (5.11µm), Richard's Agar (4.99µm), Host leaf extract (4.87µm), Corn meal agar Agar (4.73µm) and Yeast extract Agar (4.36µm). These present findings resembled with results reported earlier by *viz.*, Cai *et al.,* (2009) [2], Hyde *et al.,* (2009) [9], Gautam (2014) [8] and Kumar *et al.,* (2015) [11], who observed morphological features of *C. gloeosporioides*

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

S. No.	Media	Colony* Diameter (mm)	Colony characters		Sporulation
			Colony Colour	Colony Texture	
1.	Potato Dextrose Agar	90.00 (71.53)	Dark black colony	Good growth with smooth margin	++++
2.	Oat meal Agar	88.43 (70.09)	Whitish mycelium	Good growth with irregular margin	+++
3.	Richard's Agar	85.38 (67.50)	Light black to white colony	Good growth with smooth margin	+++
4.	CzapakDox Agar	89.82 (71.36)	Whitish mycelium	Good growth with irregular margin	+++
5.	Yeast extract Agar	82.63 (65.35)	Whitish mycelium	Moderate growth with smooth margin	+++
6.	Yeast mannitol Agar	72.50 (58.35)	Whitish mycelium	Moderate growth with irregular margin	+
7.	Corn meal Agar	76.48 (60.96)	Light yellowish to whitish mycelium	Moderate growth with smooth margin	++
8.	Asbhy'smannitol Agar	80.15 (63.52)	White to black mycelium	Moderate growth with smooth margin	+
9.	Host leaf extract	49.67 (44.79)	Yellowish mycelium	Slow growth with Smooth margin	-
10.	Plain Agar	64.65 (53.49)	Whitish mycelium	Moderate growth with Margin irregular	+
	SE ±	0.50			
	CD (P=0.01)	1.49			

*Avg. of three replications, Figures in parenthesis are Arc sine transformation values. (Dia. = Diameter) -: No; +: Poor (1-50 conidia/microscopic field 100x); ++: Fair (51-100); +++: Good (101-150); ++++: Excellent (>150)

Table 3: Morphological characteristics of *C. gloeosporioides* on different solid media

S. No.	Media	Conidial Dimensions		Setae Dimensions	
		Length (µm)*	Width (µm)*	Length (µm)*	Width (µm)*
1	Potato Dextrose Agar	26.33-26.62	3.16-4.74	164.11	5.38
2	Oat meal Agar	24.18-25.47	3.65-4.08	123.47	5.23
3	Richard's Agar	25.14-26.58	3.78-4.48	61.34	4.99
4	CzapakDox Agar	21.68-23.42	3.77-5.53	52.28	5.62
5	Yeast extract agar	18.21-20.14	3.74-4.98	163.34	4.36
6	Yeast mannitol Agar	20.87-21.34	3.96-5.60	47.9	5.11
7	Corn meal Agar	21.74-22.90	3.08-4.12	126.23	4.73
8	Asbhy'smannitol Agar	19.26-21.52	3.42-5.21	64.59	5.67
9	Host leaf extract	24.66-25.08	3.49-4.76	45.11	4.87
10	plain Agar	18.44-21.25	3.84-4.31	47.76	5.2

*Mean of ten observations

Conclusion

All the ten culture media tested encouraged better growth of *C. gloeosporioide*. However, Potato Dextrose Agar (PDA) was found to be most suitable medium for growth (90.00 mm). The second and third best medium found were Czapak Dox Agar (89.82 mm) and Oat Meal Agar (88.43 mm). Rest of the culture media recorded radial mycelial growth in the range of 49.67 mm (Host leaf extract Agar) to 85.38 mm (Richard's Agar). All the culture media tested, exhibited wide range of colony colour, colony texture, sporulation, conidial dimensions and setae dimensions.

Morphogenic characteristics of *C. gloeosporioides* was studied on different media and wide range of variability in respect of conidial and setae dimensions was observed. The conidial size *Colletotrichum gloeosporioides* ranged from18.21 to 26.62 µm. The large sized conidia (26.33-26.62x3.16-4.74 µm) were produced on the PDA which was followed by Richards agar, host leaf extracts, oat Meal Agar, Corn meal Agar, Czapak Dox Agar, Yeast mannitol Agar and Ashby's mannitol Agar. However, small sized conidia were noticed on plain agar and Yeast extract Agar. The maximum average setae length was recorded on potato dextrose agar while maximum setae width was observed on Ashby's mannitol Agar.

Plate 1: Effect of different culture media on growth and cultural characteristics of C. gloeosporioides (Penz.) Sacc.

T_1 Potato Dextrose Agar	T_6 Yeast mannitol Agar
T_2 Oat meal Agar	T_7 Conn's Agar
T_3 Richard's Agar	T_8 Asbhy's mannitol Agar
T_4 Czapak Dox Agar	T_9 Host leaf extract agar
T_5 Yeast extract Agar	T_{10} Plain Agar

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