International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 12-05-2021, Accepted: 27-05-2021, Published: 11-06-2021
Volume 6, Issue 3, 2021, Page No. 611-615

Effect of different solid media on the growth of *Colletotrichum gloeosporioides* (PENZ.) PENZ. & SACC. Causing anthracnose disease of mango (*Mangifera indica* L.)

Priya Lokare, Sumia Fatima
Pesticides and Plant Protection Research Laboratory, Dr. Rafiq Zakaria College for Women, Aurangabad, Maharashtra, India

Abstract
Mango anthracnose disease is one of the major fungal diseases of mango.
In the present study, the rate of growth of Anthracnose (*C. gloeosporioides*) has been compared in various solid culture media types *viz*., PDA (Potato Dextrose Agar), (WA) Water agar, (MLE) Mango leaf extract agar, (CDA) Czapek's Dox agar, (MEA) Malt Extract Agar etc. Among all the solid media tested, maximum mycelial growth was observed in Potato Dextrose Agar Medium (89mm) and in Czapek's Dox Agar Medium (89 mm), which was remarkably greater than the other culture media. It was followed by Sabouraud's Agar Medium (83mm), Mango Leaf Extract Agar Medium (83mm) and Asthana & Hawker's Media (85mm). All growth was recorded at room temperature (24ºC). Czapek's Dox Agar Medium and Potato Dextrose Agar Medium were found to be most suitable medium for growth. Rest of the culture media recorded colony diameter in the range of 83mm (Sabouraud's Agar Medium), 83mm (Mango Leaf Extract Medium), 75mm (Malt Extract Agar), 85mm (Asthana and Hawker's Medium), 78mm (Corn Meal Agar), 82mm (V8 Juice Agar Medium), and lowest growth 84mm on (Water Agar Medium).

Keywords: *colletotrichum gloeosporioides*, anthracnose disease of mango, colony diameter, *mangifera indica* L., solid media

Introduction
Mango (*Mangifera indica* L.) Commonly called as king of fruits, is the most important tropical and subtropical fruit crop (Sayiprathap, Ekabote, Narayanaswami, Ravindra & Adivappar Nagarajappa, 2018) [8]. It belongs to the flowering plant family Anacardiaceae. It is indigenous to India hence called as *Mangifera indica* L. which shows its Indian origin. Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most threatened disease causing huge losses in nursery condition. The symptoms of anthracnose will persist till flowering and fruiting period and results in the huge economic losses. In the recent years the disease becomes severe in nursery plants, on young leaves, symptoms appear as irregular black necrotic spots on both sides. Pathogen present on the infected leaves, twig and fallen leaves serves as the major source of infection and spreads by rain splashed conidia. Sunken, dark coloured, necrotic lesions were found. As disease progresses, small, sunken lesions coalesced to form large, necrotic patches (Islam et. al., 2015) [4]. The fungus derive food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon which all the fungi can grow and reproduce (Shivakumar, Palaiah, Raja & Mallesh, 2016) [9].
Therefore studies were conducted in different suitable media to identify surface medium for the growth of *C. gloeosporioides.*

Material and Methods
Collection of the infected leaves- Infected mango leaves were collected from the Abhijeet Jagtap Nursery, Vilad Ghat, located in an Ahmednagar district, Maharashtra. Infected leaves collected in air-tight plastic bags and brought into the Pesticides and Plant Protection Research Laboratory, Dr. Rafiq Zakaria College for Women, Aurangabad-431001, Maharashtra, India and kept in the refrigerator at 5ºC for further study.

Isolation of *Colletotrichum gloeosporioides*-*C. gloeosporioides* isolate (AJN10), isolated from the mango leaves collected from the Abhijeet Jagtap Nursery, Vilad Ghat, Ahmednagar district, Maharashtra was used in this study. This isolate was isolated from the infected mango leaves showing particularly anthracnose symptoms by tissue segment method on Czapek's Dox Agar Medium (CDA). Small portion of leaves were cut into the small pieces, in such a way that it contained both diseased and healthy portions and these pieces were surface sterilized in 0.1 per cent Mercuric Chloride ($HgCl_2$) for 20 seconds followed by 4 times washing in sterilized distilled water. The small pieces of leaves were further transferred to sterile blotting paper. The dried pieces thereafter transferred to Czapek's Dox Agar medium (CDA) under aseptic conditions. The petri plates were incubated at 24 ºC for 5 days for the growth of the fungus. The developed fungal colonies were purified by single spore isolation method. The pathogen was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Based on its mycelial and conidial characteristics according to (Darshan, Praveena, Ankegowda, & Biju, 2014; Sampath Kumar, Eswara Reddy, N. P. & Hariprasad Reddy, 2008) [3, 7]. The stock culture was maintained on CDA medium at 5ºC and subcultured after every 20 days. Pathogenicity of these isolates was also confirmed suggested by (Jayasinghe and Fernando, 2009) [6].

Preparation of different media and inoculation
The fungal pathogen (*C. gloeosporioides*) was inoculated on various types of solid media to identify the most suitable media for its growth and Maintainance. In this experiment 9 media *viz*., Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Malt Extract Agar (MEA), Water Agar (WA),

Corn Meal Agar (CMA), V8 Juice Agar, Asthana and Hawker's Medium (AHM), Sabouraud's Agar Medium (SAM) and Mango Leaf Extract Medium (MLE) were used. All these were autoclaved at 121 ºC under 15 psi for 20 min. Twenty ml of each medium was poured aseptically into 93mm diameter petri plates. After solidification, 4mm discs from an actively growing zone using a cork borer of 8 days old culture was placed upside down at the centre of the solidified medium and were incubated at 24ºC. Each treatment was replicated five times. Colony diameter was measured every day until the colonies reached the edges of the petri plates. The various cultural characters like radial growth, colony colour, type of growth, elevation were recorded.

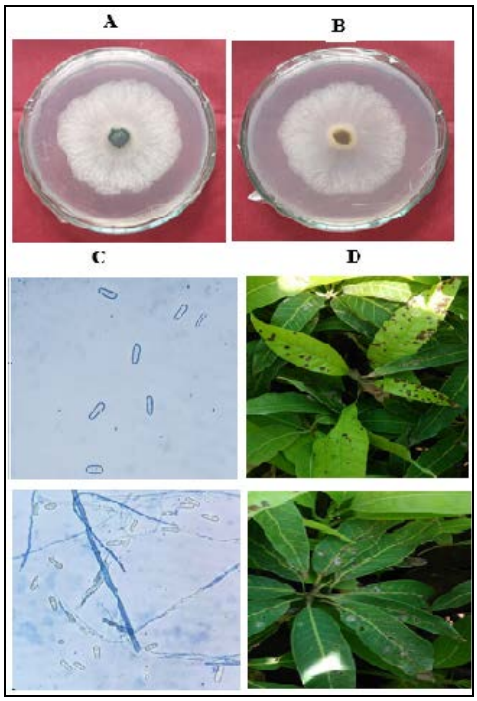
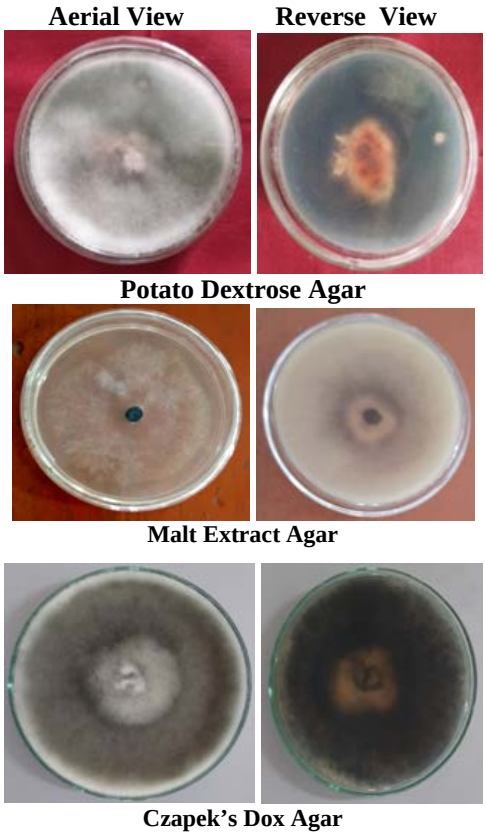


Fig 1: Isolate of *Colletotrichum gloeosporioides* (AJN10) based on colony and conidia characteristics. Plates in column A) aerial view: B) reverse view: C) Typical conidia shape in microscopic view and D) infected plant parts from which the pathogen (*C. gloeosporioides*) was isolated.



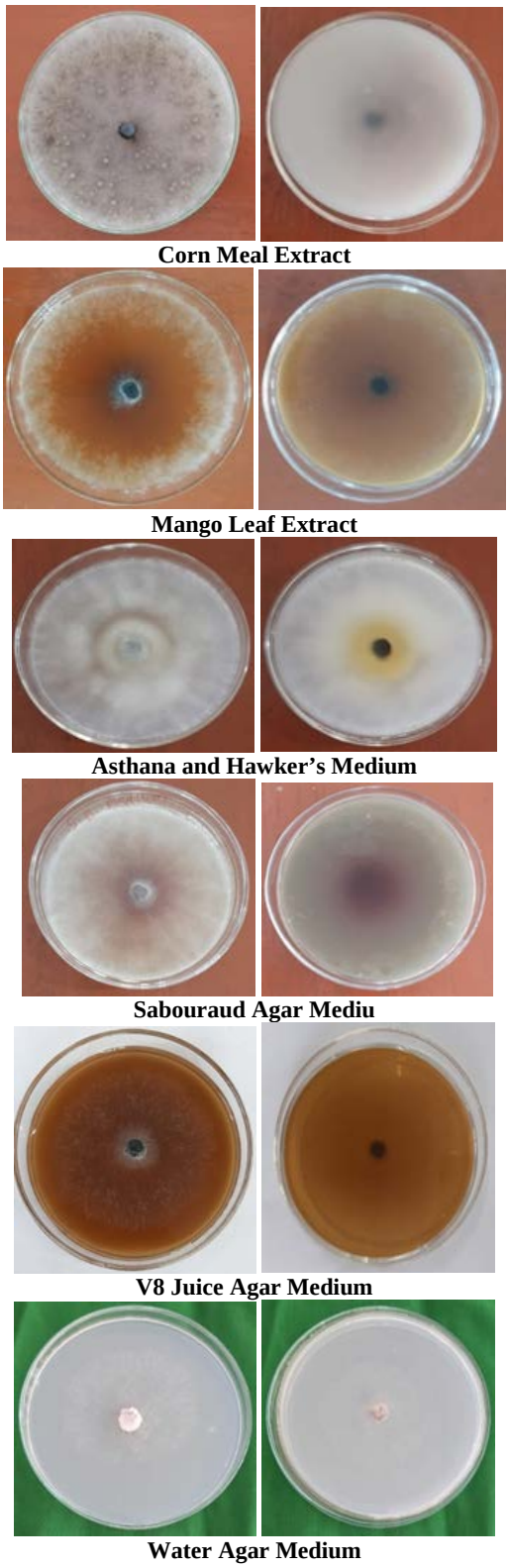


Fig 2: Effect of different solid media on growth of *C. gloeosporioides* with aerial view and revesre view.

Table 1: Effect of different solid media on cultural characteristics of *C. gloeosporioides*

Sr. No.	Different media	Colony diameter mm					Growth Characters		
		24h	48h	72h	96h	120h	Colour the colony	Types of growth	Elevation
1	PDA	2	33	89	-	-	White	Excellent growth	Raised, Fluppy
2	MEA	20	32	62	75	-	Whitish to gray	Moderate growth	Flat
3	CDA	12	44	83	89	-	Cream White	Excellent growth	Raised
4	WA	10	18	52	65	84	White	Poor and growth	Thinly flat
5	CMA	19	30	65	72	78	White	Poor and growth	Thinly flat
6	V8	19	55	62	76	82	White	Good growth	Raised, Fluppy

7	AHM	20	43	73	80	85	White	Excellent growth	Cottony,Raised
8	SAM	24	63	83	-	-	White	Moderate growth	Raised
9	MLE	26	61	83	-	-	White	Moderate growth	Raised

S. Em ± 9.22
C.D. at 1% 19.32
% C.V. 9.24%

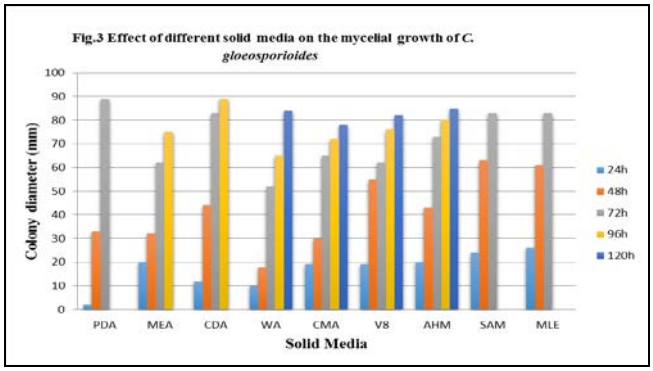


Fig 3: Effect of different solid media on the mycelial growth of *C. gloeosporioides*

Statistical Analysis: All treatments were designed in Completely Randomized Design (CRD) with five replications. Experimental data was Statisticaly analysed using NCSS 2020 Data Statistical Analysis Software.

Results and Discussion: The present investigation revealed that the colony characters and growth of *C. gloeosporioides* varied on different media. This might be due to the variation in the nutritional requirement of the fungus. Fungi secure food and energy from the substrate upon which they live in the culture. In order to culture the fungi in the laboratory, it is necessary to finish those essential elements and compounds in the medium which are required for their growth and other life process (Abera Amsalu, Lemessa Fikre & Adunga Girma, 2015) [1]. Among medias fungal pathogen from mango plant (*Colletotrichum gloeosporioides*) result showed that better growth on Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Sabouraud's Agar Medium (SBA), Mango Leaf Extract Agar (MLE), Asthana and Hawcker's Medium (AHM), V8 juice and Malt Extract Agar (MEA) respectively. Similar results were obtained by (Abera Amsalu *et al*., 2015) [1]. Not all the media are equally good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can grow (Jagana Divya, Hegde Yashoda & Lella Rajasekhar Lella, 2017) [5]. Therefore different media were tried to investigate the variation in growth and cultural characteristics of *C. gloeosporioides*. White colony in all Medias and pinkish, orange colour in the middle and white at the periphery region at the reverse view were observed. Media had significant effect on growth of pathogen which may be attributed to complex nature of media supporting good fungal growth. The pathogen (*C. gloeosporioides*) isolated from the Jagtap Nursery, Vilad, ahmednagar (AJN10) from leaf samples of mango. Anthracnose of mango caused by *C. gloeosporioides* is prevalent in nurseries where mango is grown. (Tasiwal Vinod & Benagi V.I., 2009) [10] was used PDA, MEA, CMA, SBA for *C. gloeosporioides* study. The results on cultural studies on solid media indicated that (Table 1 and Figure 2) the colony diameter of *C. gloeosporioides* was maximum on Potato Dextrose Agar (89mm) and Czapeck's Dox Agar (89mm) which was significantly superior over all other tested medium followed by Asthana and Hawker's media (85mm), Sabouraud's Agar Medium (83mm), Mango Leaf Extract (83mm),. In the present study better performance of *C. gloeosporioides* on PDA and CDA may be attributed to inherent complex nature of material supporting good fungal growth. In this study Asthana and Hawker's media also shown white fluppy excellent growth after the PDA and CDA which is similar in (Shivakumar *et al*., 2016) observed that growth characters of *C. gloeosporioides* studied in different solid media indicated that Potato Dextrose Agar, Richards's Agar and Asthana and Hawker's Agar supported good growth of fungus colony. Pathogen growth was initially white later turn to black colour on all media. Maximum colony diameter was could be due to good quality of nitrogen (Potassium nitrate) and carbon Sucrose, Dextrose) sources present in the CDA and PDA. These results are in agreement with (Ashoka, S. 2005) [2] reported that sporulation of *C. gloeosporioides* was excellent in Potassium nitrate supplemented as nitrogen source. According to the result, growth of *C. gloeosporioides* on different solid media has been significantly different from each other. Among 9 solid media PDA, CDA and AHM are more superior Than others. On PDA very luxuriant growth (89mm) has been occurred on 3rd day where as SAM (83mm) and MLE (83mm) has been showed moderate growth of the colony and very thinly spread in middle and dense at periphery. MEA and CDA completed their growth on 4th day. In between them CDA (89mm) and MEA (75mm) growth were recorded. CDA has been showed cream white raised colony with excellent growth after the PDA. Remaining solid media were completed their growth on 5th day. V8 juice agar medium (82mm) showed good growth with raised, fluppy mycelium but it has been taken longer growth period than the PDA and CDA. Asthana and Hawker's has been showed white cottony raised colony with excellent growth after the PDA and CDA where as WA and CMA has been showed very poor growth and flat elevation. Overall although all media has been completed their growth within 5 days, the major difference among them are growth type and elevation. Among all media PDA, CDA and AHM showed excellent growth with raised colony which is the indication of a most suitable media.

References

1. Abera Amsalu, Lemessa Fikre, Adunga Girma. Phenotypic charactericteristics of Colletotrichum species associated with Mango (Mangifera indica) in Southwest Ethiopia. International Knowledge Sharing Platform,2015:46:9-18. Retrieved from https://www.iiste.org.
2. Ashoka S. Studies on fungal pathogens of vanilla with special reference to Colletotrichum gloeosporioides (Penz.) Penz. And Sacc. M.Sc. (Agri) Thesis, Uni. Agric. Sci. Dharwad, (India), 2005.
3. Darshan CN, Praveena R, Ankegowda SJ, Biju CN. Morphological viability, mycelial compatibility and

fungicidal sensitivity of Colletotrichum gloeosporioides causing leaf spot of ginger (Zingiber officinale Rosc.). Journal Spices aromatic Crop,2014:23(2):211-223.
4. Islam MN, Poddar KK, Hossain I, Chowdhury MSM, Mehraj H, Jamal Uddin AFM. Seedling Diseases of Mango in Four Districts of Bangladesh. Int. J. Sustain. Crop Prod,2015:10(2): 55-61.
5. Jagana Divya, Hegde Yashoda, Lella Rajasekhar Lella. Cultural and physiological characterization of Colletotrichum musae, The causal agent of banana anthracnose. Inter. Jour. Of Appl. Biology and Pharmaceutical Technology,2017:8(2):22-30. http://dx.doi.org/10.21276/ijabpt.
6. Jayasinghe CK, Fernando THPS. First report of Colletotrichum acutatum on Mangifera indica in Sri Lanka. Ceylon Journal of Science (Biological Sciences),2009:38:31-34.
7. Sampath Kumar A, Eswara Reddy NP, Hariprasad Reddy K. Variations in cultural, morphological and nutritional characters of C. gloeosporioides Penz., isolates from mango. Geobiosciences,2008:3(5):86-90.
8. Sayiprathap BR, Ekabote SD, Narayanaswami H, Ravindra H, Adivappar Nagarajappa. Survey for the severity of anthracnose disease of Mango (Mangifera indica L.) on nurseries in Karnataka State during Kharif 2015-16. Int. J. Pure and App. Biosci,2018:6(1):1139-1141.
9. Shiva kumar KV, Palaiah P, Raja, Mallesh SB. Effectiveness of different cultural media on the growth and sporulation of Colletotrichum gloeosporioides causing anthracnose disease of Mango (Mangifera indica. L). Advances in Life Sciences,2016:5(3):793-796.
10. Tasiwal Vinod, Benagi VI. Studies on the cultural and nutritional characterictics of Colletotrichum gloeosporioides, the causal organism of papaya anthracnose. Karnataka. J. Agric. Sci,2009:22(4):787-789.