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Cultural characterization of *Colletotrichum gloeosporioides* causing leaf spot of ginger on different solid media

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Abstract
Ginger leaf spot disease is one of the major fungal diseases of ginger. In the present study, the rate of growth of leaf spot (*C. gloeosporioides*) has been compared with different solid culture media types *viz*., Potato Dextrose Agar, Ginger Leaf Extract Agar, Czapek's Dox Agar, Potato Carrot Agar, Richard's Synthetic Agar etc. Among the various solid media tested, maximum mycelial growth was observed in Richard's Synthetic Agar (90 mm), Potato Dextrose Agar (90 mm) followed by Czapek's Dox Agar (89 mm) and lowest growth was observed in Potato Carrot agar (72.50 mm) at 27±1 °C. Colour of colony varied from grayish white to white. Texture of colony varied from velvety to cottony in nature, margin of colony varied regular to irregular and excellent sporulation was recorded in Potato Dextrose Agar and least sporulation was recorded in Czapeck's Dox Agar and Oat Meal Agar.

Keywords: Colletotrichum gloeosporioides, leaf spot of ginger, colony diameter, *Zingiber officinale*, *Solid media*

Introduction
Ginger (*Zingiber officinale* Rosc.) Commonly called as spice crop, is the most important tropical and subtropical spice crop. It belongs to family *Zingiberaceae*. It is indigenous to South-East Asia. Leaf spot caused by *Colletotrichum gloeosporioides* is one of the most threatened diseases, causing huge losses in field condition. The symptoms of leaf spot disease cause huge economic losses up to 13-66 per cent (Sarma *et al*., 1993) [9]. In the recent years the disease noticed on young leaves, symptoms appear as water soaked spots and later turns as white spots surrounded by dark margin and yellow halo (Fig. 1). Pathogen present on the infected leaves serves as the major source of infection and spreads by wind and rain splashed conidia. Sunken, dark colored, necrotic lesions were found. As disease progresses, lesions enlarge and adjacent lesion coalesce to form necrotic patches (Sundaraman, 1922) [11]. 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) [12]. Therefore studies were conducted in different suitable media to identify surface medium for the growth of *C. gloeosporioides*.

Material and Methods
Infected ginger leaves were collected from the Haveri, Uttara Kannada and Shivamogga districts. Infected leaves collected in brown paper bags and brought into the Laboratory kept in the refrigerator at 4 °C for further studies.

Isolation of *Colletotrichum gloeosporioides*
C. gloeosporioides isolate, isolated from the ginger leaves collected from the Haveri, Uttara Kannada, and Shivamogga district was used in this study. This isolate was isolated from the infected ginger leaves showing particularly leaf spot symptoms by standard tissue isolation method on Potato dextrose agar. 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 1 minute followed by 3 times washing in sterilized distilled water. The small pieces of leaves were further transferred to sterile blotting paper. The dried pieces after that transferred to Potato Dextrose Agar (PDA) under aseptic conditions.

The Petri plates were incubated at 27±1 °C for 8 days for the growth of the fungus. The developed fungal colonies were purified by single spore isolation method. The pathogen was identified as *C. 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). The stock culture was maintained on PDA medium at 4 °C and sub cultured 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 Maintenance. In this experiment 8 media *viz.*, Potato Dextrose Agar (PDA), Capek's Dox Agar (CDA), Oat Meal Agar (OMA), Potato Carrot Agar (PCA), Richards Synthetic Agar (RSA), V8 Juice Agar, Sabouraud's Dextrose Agar (SAM) and Ginger Leaf Extract Agar (GLAE) were used. All these were autoclaved at 121 °C under 15 psi for 20 min. Twenty ml of each medium was poured aseptically into 90 mm diameter Petri plates. After solidification, 5 mm discs from an actively growing zone using a cork borer of 10 days old culture was placed upside down at the centre of the solidified medium and were incubated at 27±1 °C. Each treatment was replicated Three times. Colony diameter was measured every day until the colonies reached the edges of the Petri plates. The various cultural characters like radial mycelial growth, colony color, type of growth was recorded.

Statistical Analysis: All treatments were designed in Completely Rndaomized Design (CRD) with three replication. Experimental data was statistically Analysed using Opistat Analysis Software

Results and Discussion

The present investigation exhibited that the colony characters and growth of *C. gloeosporioides* varied on different solid 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 zinger plant (*Colletotrichum gloeosporioides*) result showed that better growth in Richards synthetic agar and Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Oat meal agar (OMA) Sbouraud's Dextrose Agar (SBA), V8 Juice, Ginger Leaf Extract Agar (GLE), Potato Carrot Agar (PCA), respectively. Similar results were obtained by (Priya L., 2021, Abera Amsalu *et al.*, 2015) [7, 1]. All the media are not equally good for all fungi, nor there can a universal substrates or artificial medium upon which all fungi grow (Jagana Divya, Hegde Yashoda & Lella Rajasekhar Lella, 2017) [5]. Therefore different media were tried to examined the variant form of growth and cultural characteristics of *C. gloeosporioides*. White colony in all Media and Grayish white, 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) [13] was used PDA, GLEA, OMA, and SBA for *C. gloeosporioides* study. The results on cultural studies on solid media indicated that (Table 1 and Fig 2 & 3) the colony diameter of *C. gloeosporioides* was maximum on Richrds Synthetic Agar (90 mm) and Potato Dextrose Agar (90 mm) and Czapeck's Dox Agar (89 mm) which was significantly superior over all other tested medium followed by Oat meal agar (88 mm) and Sabouraud's Agar Medium (87 mm), Ginger Leaf Extract Agar (80 mm). In the present investigation better performance of *C. gloeosporioides* on PDA and Richard's Synthetic Agar may be attributed to inherent complex nature of material supporting good fungal growth. In this Investigation Oat meal agar and Czapeck's Dox media also shown Grayish white excellent growth after the PDA and RSA which is similar in (Shivakumar *et al*., 2016) [12] observed that growth characters of *C. gloeosporioides* studied in different solid media indicated that Potato Dextrose Agar, Richards's Agar and Czapek's Dox Agar and Oat Meal Agar, supported good growth of fungus colony. Pathogen growth was initially white later turn to black color 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 RSA 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 eight solid media PDA, RSA, and OMA are more superior to others. On PDA very luxuriant growth (90 mm) has been occurred on 8th day whereas, RSA (90 mm) and CDA (89 mm) has been showed moderate growth of the colony and very thinly spread in middle and dense at periphery. OMA and SDA completed their growth on 9th day. In between them CDA (89 mm) and GLEA (80 mm) growth were recorded. CDA has been showed white colony with excellent growth after the PDA. Remaining solid media were completed their growth on 9th day. V8 juice agar medium (85 mm) showed good growth with raised, white mycelium but it has been taken longer growth period than the PDA and CDA has been showed white cottony raised colony with excellent growth after the PDA and CDA where as GLEA and PCA has been showed very poor growth and flat elevation. Overall although all media has been completed their growth within 12 days, the major difference among them are growth type and elevation. Among all media PDA, CDA and RSA showed excellent growth with raised colony which is the indication of a most suitable media.

Conclusion and Acknowledgement

Potato dextrose agar supported the maximum radial growth (90.00 mm) of *C. gloeosporioides* whereas, higher sporulation was observed in potato dextrose agar, oat meal agar, richards's synthetic agar and potato carrot agar. In potato dextrose agar fungus showed white colour with regular margin and uniform growth of mycelium with distinct concentric rings and produced numerous conidia.

Table 1: Effect of different solid media on cultural characteristics of Colletotrichum gloeosporioides

Sl. No.	Media	Mycelial growth (mm)	Color of colony	Texture of colony	Margin	Sporulation
1	Czapeck's Dox Agar	89.00 (70.67)*	Grayish white	Cottony	Irregular	+
2	Potato Dextrose Agar	90.00 (71.60)	White	Cottony	Regular	+++
3	Potato Carrot Agar	72.50 (58.40)	White	Cottony	Regular	++
4	Oat Meal Agar	88.00 (69.77)	Grayish white	Cottony	Irregular	+
5	V8 Juice Agar	85.50 (67.65)	White	Velvety	Irregular	+
6	Sabouraud Dextrose Agar	87.00 (68.90)	White	Cottony	Irregular	+
7	Richard's Synthetic Agar	90.00 (71.60)	White	Cottony	Regular	++
8	Ginger Leaf Extract Agar	80.00 (63.47)	Grayish white	Cottony	Irregular	+
	S.Em.±	0.78				
	C.D. at 1%	2.51				
	C.V (%)	2.12				

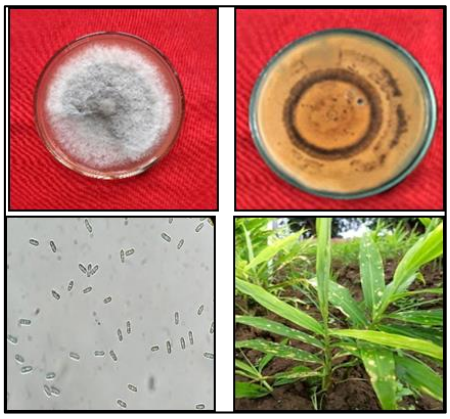

Fig 1: Isolates of *Colletotrichum gloeosporioides* based on colony and conidia characteristics. Plates in column A) aerial view: B) reverse view: C) Typical conidia shape in microscopic view and D) Infected plant parts from which the pathogen (*C. gloeosporioides*) was isolated

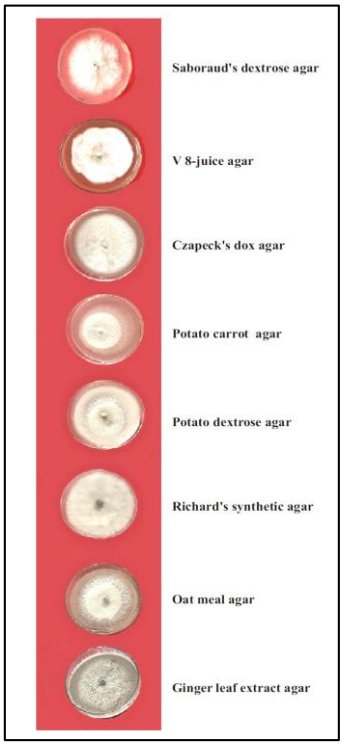

Fig 2: Effect of different solid media on radial growth and development of *Colletotrichum gloeosporioides*

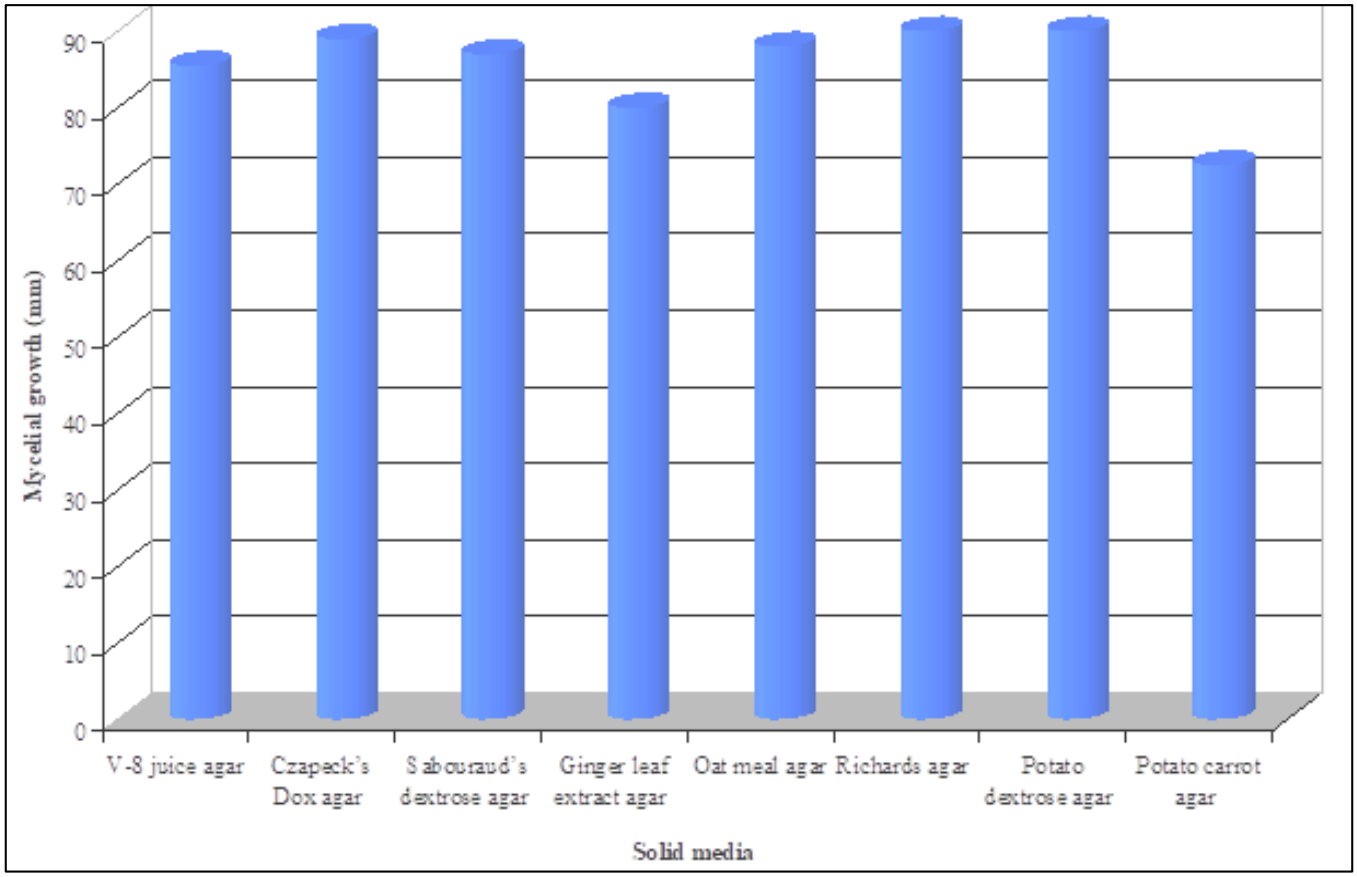

Fig 3: Effect of different solid media on mycelial growth of *Colletotrichum gloeosporioides*

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