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Niren Majumdar
Department of Plant Protection, Palli Siksha Bhavana (Institute of Agriculture), Visva Bharati, Sriniketan, Birbhum, West Bengal, India

Nakul Chandra Mandal
Department of Plant Protection, Palli Siksha Bhavana (Institute of Agriculture), Visva Bharati, Sriniketan, Birbhum, West Bengal, India

Correspondence
Niren Majumdar
Department of Plant Protection, Palli Siksha Bhavana (Institute of Agriculture), Visva Bharati, Sriniketan, Birbhum, West Bengal, India

Influence of culture media on mycelial growth, sporulation and spore size of quiescent pathogen *Colletotrichum gloeosporioides* isolated from banana

Niren Majumdar and Nakul Chandra Mandal

Abstract
Colletotrichum gloeosporioides is one of the most common fungal pathogen among different pathogens of banana which remains at quiescent state before maturity and rotting of fruits starts along with the ripening as it becomes active from quiescent state. Twelve different culture media were considered and significant variation in radial growth, sporulation and spore size of the fungus was noticed. The pathogen showed maximum radial growth on Potato Dextrose Agar (87.67mm), Malt extract Peptone Dextrose Agar (83.00mm) and Potato Sucrose Agar (81.33mm) media followed by Malt Extract Agar (79.00mm) and Yeast extract Dextrose Agar (76.67mm) whereas least growth observed on Czapek's Dox Agar (28.67mm). Highest sporulation recorded on Oat Meal Agar (26.26 X 10^6 and 45.70 X 10^6) irrespective of incubation period followed by PDA (24.66 x 10^6). No sporulation was noticed up to 7 days on Sabouraud's Agar (SA). Spore size also significantly varies among the media with longest spore on Yeast extract Dextrose Agar (16.64µm) and PSA (16.51µm). The breadth of the spore was more on Water Agar (5.08 µm), Glucose Peptone Agar (4.95 µm) and MPDA (4.95µm) as compared to others. Synthetic media poorly supported growth and sporulation of the pathogen as compare to natural and semi-synthetic media.

Keywords: Quiescent, banana, *colletotrichum gloeosporioides*, growth, sporulation, media, spore size

Introduction
Banana, an important tropical fruit has an international appeal, at postharvest phase is succumbed to more than 50 different pathogens causing enormous losses particularly during transport and storage. *Colletotrichum gloeosporioides* is one of the most common pathogen of banana which remains at quiescent state before maturity, although initially considered as latent or incipient pathogen [1] and rotting of fruits starts along with the ripening as it becomes active from quiescent state. Conspicuous symptom develops only at this stage and continues to over ripening stage. Therefore, a faster transport and modernized storage are essential for avoiding postharvest losses. *Colletotrichum* sp. the causal organism of anthracnose of banana fruit is a common postharvest disease in all tropical countries. Even though *Colletotrichum musae* is the most common species connected with anthracnose of banana, *C. gloeosporioides* has also been reported to be linked with banana anthracnose [2, 3, 4]. They cause substantial economic damage to crops in tropical, subtropical and temperate regions [5]. The genus *Colletotrichum gloeosporioides* and its teleomorph *Glomerella* has been associated with quiescent infections and post-harvest diseases on several other fruits such as avocado, mango, papaya, guava, citrus, apple and grapes [6, 7] and considered to be a major plant pathogens worldwide [8]. *Colletotrichum* sp. can infect banana fruits at any stage during the growing season in the field [9]. It is a serious problem when bananas are shipped as bunches for a long time and ripened under high temperature [10]. *Colletotrichum sp.* spreads from floral parts and senescent bracts to contaminate fruit in plantations [11]. Conidia reach the fruit surface in runoff rainwater or dew on the banana bunch [12]. They quickly germinate and form melanised appressoria, which are quiescent structures of the pathogen [13]. Therefore, mature fruits from the field look healthy and are sold to warehouse holders. The fact that all fruits were infested by ripening may derive from the latent infection structure of the fungi. It is documented that dormant appressoria from infecting fungi, germinate during fruit maturation and form infection hyphae that colonize the peel and then penetrate into the fruit pulp [14]. It is frequently necessary to use several media while attempting to identify a fungus in culture as mycelial growth and sporulation on artificial media are important biological characteristics [15].

Morphometric Parameters as well as cultural characteristics are very important and both of which have immense value in fungal identity and taxonomy [16, 17], despite standalone molecular characterization [18]. Vegetative growth, morphology and sporulation characters are greatly influenced by the content and composition of various media used [15, 19, 17] and change in the behavioral pattern by certain environmental factors particularly temperature, pH etc. [20-26]. Growth and development of pathogens are influenced by various external factors, of which media plays a significant role. A crucial and through knowledge of nutritional conditions and factors influence the mycelial growth of a fungi is the precondition for any study leading to the understanding of host-pathogen relationship. The present study was conducted to determine morphological character and influence of culture media on the growth, sporulation and spore size of *Colletotrichum gloeosporioides* causing anthracnose of banana. Fungal isolates from different geographical locations may behave differently for utilization of different media towards growth and sporulation, therefore, the present study with the local isolate with 12 different media was undertaken.

Materials and methods

Isolation of fungi

Typical anthracnose banana fruits (Champa) were collected from local market and washed with 0.1% mercuric chloride for 1 minute and serially washed with sterile distilled water twice. The samples were dried in aseptic condition. A small bit of diseased part (3-5mm) from the infected fruit was taken and washed with70% ethanol for 1 min followed by three serial wash with distilled water and then placed on to the solidified PDA medium supplemented with Streptomycin sulphate (100mg/l). The plates were incubated in BOD incubator at 27±1°C for 1-2 days until the mycelial growth develops on and around the diseased tissue bit. Small hyphal tip with medium was transferred aseptically to fresh PDA plate with the help of sterile inoculation needle.

Maintenance of the culture

The pure cultures of the fungi were sub-cultured on potato dextrose agar (PDA) slants and kept within BOD incubator of laboratory at 27±1°C for 10 days. These mother culture slants were preserved at 4°C in refrigerator. Further, these cultures were sub-cultured once in a month and used for other future studies.

Media preparation

Different natural, semi synthetic and synthetic culture media in solid form, were prepared to culture this fungus. The standard composition and technique for media preparation for Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Potato Sucrose Agar (PSA), Oat Meal Agar (OMA), Malt Extract Agar (MEA), Yeast Extract Dextrose Agar (YDA), Glucose Peptone Agar (GPA), Malt Extract Peptone Dextrose Agar (MPDA), Richard's Agar (RA) Sabouraud's Agar (SA), Czapek's Dox Agar (CDA) and Water Agar (WA) media were used as followed by (Dhingra and Sinclair [27].

Inoculation of the petri plates containing media

The pure culture of seven days old fungus was so obtained on PDA was used for inoculation of petri plates. A mycelial disc of 5 mm dia. was scooped out with a sterilized cork borer from the advancing margin of the colony and transferred at the center of the petri plates aseptically. Plates were incubated at 27±1^{0}C within BOD incubator for ten days.

Fungus growth measurement technique

Radial growth of the fungus was determined directly by measuring the diameter of colonies from underside the culture plate against light in the perpendicular axis at 24hrs intervals upto168hours of incubation. The radial growth was measured using mm scale.

Observation of growth characteristics and sporulation

The color, margin and topography of colony were observed by naked eye. For measuring sporulation on different media, the whole culture plates were washed thoroughly with 50ml of sterile distilled water and the spore suspension were made from it. Fungal sporulation were counted under hemocytometer.

Measurement of Spore size

Micrometric measurement of spores was done by standardized ocular micrometer. Average and range of 20 spores were considered.

The entire experiment was carried out during 2018 at the Department of Plant Protection, Palli Siksha Bhavana, Sriniketan, Visva Bharati, West Bengal.

Statistical Analysis

All experiments were conducted in a completely randomized design with three repetitions, for each treatment. The statistical analysis of the results was conducted by analysis of variance (ANOVA) in MS excel sheet.

Results

Growth characteristics on different solid media

The growth characteristics like color of colony, margin of colony, topography of mycelium (plate.1 and table.1) along with the sporulation and spore morphometry (table.4)of the test fungus was also studied on the above solid media. Wide range of media were used for isolation of different groups of fungi that influence the mycelial growth colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium [28].

Table 1: Growth characteristics of *Colletotrichum gloeosporioides* on different solid media

S. No	Media	Mycelial characters		
		Color	Growth	Type of margin
1	PDA	Initially white later saffron	raised fluffy	smooth, regular, circular
2	PCA	Hyaline	scanty	regular, circular
3	PSA	whitish saffron	raised fluffy	smooth, regular, circular
4	OMA	Bright orange to saffron	raised fluffy	smooth, circular
5	MEA	Greyish saffron	flat	regular, circular
6	YDA	whitish saffron	raised fluffy	regular, circular
7	GPA	white	light fluffy	regular, circular

8	MPDA	Greyish saffron	flat	regular, circular
9	RA	Brownish saffron	flat	regular, circular
10	SA	Light white	scanty	regular, circular
11	CDA	white color	Partially flat	regular, circular
12	WA	Hyaline to light white	santy	regular

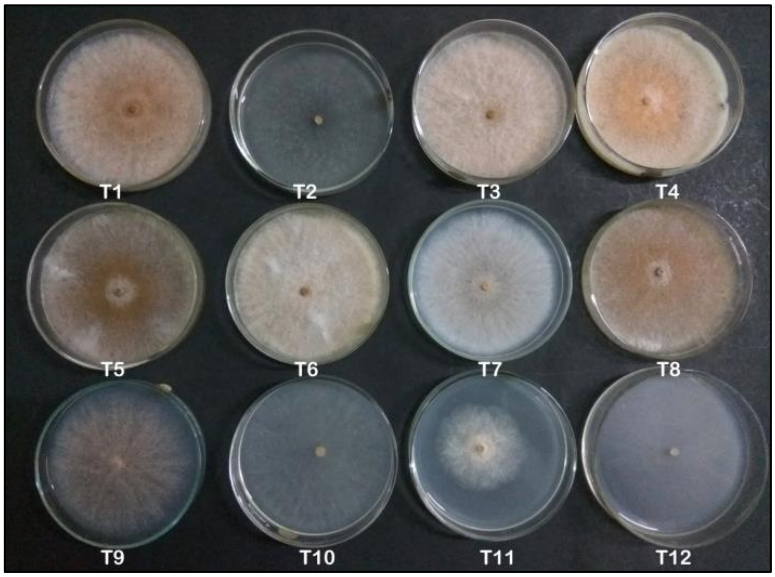

Plate 1: Growth character of *Colletotrichum gloeosporioides* at 7DAI (Days after Incubation) T1=PDA, T2=PCA, T3=PSA, T4=OMA, T5=MEA, T6=YDA, T7=GPA, T8=MPDA, T9=RA, T10=SA, T11=CDA, T12=WA

Radial growth of fungi on different solid media

In order to study the radial growth of *Colletotrihum gloeosporioides*, it was grown on all 12 different solid culture media and the data are presented in table 2. Data indicated that in all media supported the growth with statistically significant variation in radial growth. The data revealed that Potato Dextrose Agar (PDA) medium had significantly improved the growth (87.67mm) followed by Malt extract Peptone dextrose agar(MPDA) medium (83.00mm) and Potato Sucrose Agar (PSA), although both are statistically at par. PDA and MPDA always showed faster radial growth than other media tested. Minimum radial growth was recorded on CDA after 5th day of incubation.

Table 2: Radial growth of *C. gloeosporioides* in different culture media at time intervals

Media	Radial growth in mm					
	24hrs	**48hrs**	**72hrs**	**96hrs**	**120hrs**	**144hrs**
PDA	3.00	20.67	42.83	66.67	87.67	95.00
PCA	0.00	7.00	20.00	36.00	50.17	67.33
PSA	0.83	17.67	39.33	61.00	81.33	95.00
OMA	0.33	18.33	34.00	58.67	74.00	85.00
MEA	2.33	19.00	39.00	58.33	79.00	90.33
YDA	1.67	17.50	37.00	57.00	76.67	88.33
GPA	1.00	12.50	29.50	47.33	63.67	78.67
MPDA	3.83	22.33	44.67	67.00	83.00	94.33
RA	0.00	4.17	17.67	34.00	51.67	72.00
SA	0.50	12.50	26.67	43.00	57.67	66.67
CDA	0.33	4.00	10.00	19.33	28.67	36.33
WA	0.33	10.33	23.67	38.00	52.00	66.00
SEm	0.15	0.34	0.93	1.34	1.11	1.16
CD(p=0.05)	0.48	1.08	2.91	4.20	3.47	3.63

Growth rate of fungi on different solid media

There was an average higher growth rate between 120 hrs. To 144 hrs. of incubation considering all the media and thereafter growth rate decreases up to the end of incubation period. Highest growth rate was on MPDA (0.55mm/ hr) followed by PDA (0.54mm/hr) and least on CDA (0.18 mm/hr). (fig.1).

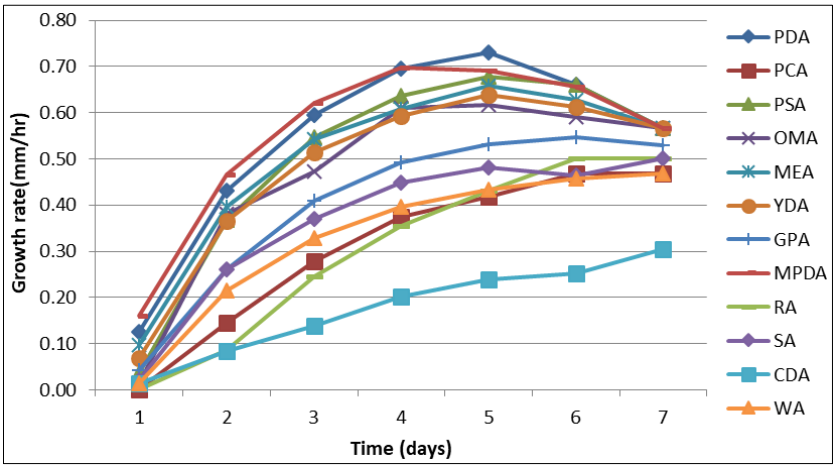

Fig 1: Growth rate of *Colletotrichum gloeosporioides* on different media

Sporulation on different medium

The sporulation of *C. gloeosporioides* was found to be supported by all the media tested. There were significant differences in sporulation between the days of incubation as well as media tested. The higher sporulation was recorded after 15 days of incubation irrespective of media except PDA. Maximum sporulation recorded on OMA irrespective of incubation period but least sporulation was on WA, PCA, and CDA at 7th and on PCA at 15th day of incubation respectively (table.3).

Table 3: Sporulation of *C. gloeosporioides* in different culture media at time intervals(X 10^6)

Media	Sporulation (X10^6)	
	7DAI	15DAI
PDA	24.66	22.36
PCA	0.22	2.27
PSA	14.13	22.59
OMA	26.26	45.70
MEA	9.67	17.82
YDA	13.07	11.40
GPA	5.00	8.16
MPDA	11.46	14.19
RA	3.01	11.40
SA	0.00	4.73
CDA	0.17	9.42
WA	0.03	6.27
SEm	0.91	1.47
CD (p=0.05)	2.62	4.23

Measurement of spore size of the fungi on different solid media

It is indicated in table.4 that there were significant variations in size of spore among all the media tested. YDA produced long (16.637µm) spore followed by PSA (16.51µm) although both are statistically at par but breadth was more in WA (5.08 µm) followed by MPDA (4.953µm), GPA(4.953µm) least width was in CDA (4.318µm).There was no sporulation in treatment SA at 7DAI.

Table 4: Spore size of *Colletotrichum gloeosporioides* on different media at 7DAI.

Media	*Colletotrichum gloeosporioides*			
	Length(µm)		Breadth(µm)	
	Range	Mean	Range	Mean
PDA	7.62-19.05	14.224	3.81-5.08	4.572
PCA	11.43-15.24	13.335	3.81-5.08	4.572
PSA	12.7-20.32	16.51	3.81-5.08	4.699
OMA	10.16-17.78	14.478	3.81-5.08	4.699
MEA	8.89-12.7	10.668	3.81-5.08	4.826
YDA	12.7-21.59	16.637	3.81-5.08	4.826
GPA	7.62-17.78	12.319	3.81-5.08	4.953
MPDA	7.62-12.7	10.033	3.81-5.08	4.953
RA	10.16-15.24	12.954	3.81-5.08	4.572
SA	0	0	0	0
CDA	12.7-17.78	14.605	3.81-5.08	4.318
WA	12.7-16.51	14.478	5.08-5.08	5.08
SEm		0.686		0.168
CD(p=0.05)		1.924		0.470

Discussion

The fungal systematic is still based mainly on morphological criteria as observable characteristics. Hence, fungi are recognized and identified basically by their phenotypes [17]. Conidia of the developed eight isolates were mostly monomorphic and exhibited cylindrical, hyaline conidia with size ranged between 14.5 and 19.1µm for length and 4.4 and 6.5µm for width [29, 30, 31, 32].

Rasangi Priyadarshanie and Vengadaramana [33]. Reported that Carrot Dextrose Agar and PDA were the best media for mycelial growth of *Colletotrichum musae*. Several researchers stated that PDA is the best media for mycelial growth which was the conformity of our study [34, 35, 36]. Maximum mycelial growth of *C. gloeosporioides* (mango) in Potato dextrose agar was also recorded by Pandey *et al*. [37]. According to the Deshmukh *et al.* [38] maximum mycellial growth of *C. gloeosporioides* from bean was noticed on PDA media followed by RA, OMA, MEA and Corn Meal Agar. Type of culture media and their chemical compositions significantly affect the mycelia growth rate and conidial production of *Phoma exigua* [19].We also noticed significant variation in growth and sporulation among different media.

C. gloeosporioides isolates of mango and it was further reported that the conidial size was 12.0- 17.0 x 3.5-6.0µm [39]. Das Gupta [40] reported the variation in the spore size (17.36-21.8µm x 2.66-2.88µm) among the isolates of *C. capsici* causing anthracnose of betel vine. Our findings also support the results of earlier worker.

In case of sporulation we got similar result as observed by Kumar and Dubey [22] while studying with *Colletotrichum dematium* var truncate. *Curvularia lunata* produces maximum growth and sporulation on PDA, Host extract, and Sabouraud's agar followed by Oat meal agar [41]. Okunowo *et al*. [42] also observed least sporulation and minimum mycelia growth of *Myrothecium roridum* on Czapek's Dox agar.

Conclusion

Culture media significantly influenced the growth, colony character and sporulation and spore size of the *Colletotrichum gloeosporioides*. Out of twelve test media used in the present study, OMA was found to be most suitable for heavy sporulation while PDA reproduced best mycelia and visible colony morphology. Least growth and sporulation recorded on synthetic media like CDA and RA. Natural and semi synthetic media were found to be more suitable for cultural and morphological studies of fungi as compare to synthetic media.

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