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Screening of Suitable Culture Media for Growth, Cultural and Morphological Characters of Pycnidia Forming Fungi

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A B S T R A C T

Different agar media, including natural, Oat Meal Agar (OMA), Malt Extract Agar (MEA), V_8 Agar and Leaf extract agar of host plant, semi-synthetic media, Potato Dextrose Agar (PDA), carrot dextrose agar (CaDA) and synthetic media, Czapex Dox Agar (CDA), Richards Synthetic Agar (RSA), Asthana and Hawkers Agar (AHA)) were used to observed the mycelial growth rate, cultural and morphological characters of four fungal isolates after seven days of incubation at 26±1°C. The colony character including diameter, culture characteristics (texture, surface and reverse colouration, zonation) and sporulation of selected test fungi were greatly influenced by the type of growth medium used. On comparative study of higher mycelial growth of the four test fungi, the best performance where observed in PDA as well as moderate to heavy sporulation on this culture medium. These results will find use in fungal taxonomic studies.

Introduction

There are two million kinds of living organisms on the earth, of which fungi constitute approximately a hundred thousand species, and many more await discovery. No matter which aspect of fungi we look at, they are highly diverse and versatile organisms adapted to all kinds of environments and require several specific elements for growth and reproduction. Fungi are isolated on specific culture medium for cultivation, preservation, microscopic examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influences the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, the requirements for fungal growth are generally less stringent than for the sporulation. Nowadays, fungal taxonomy is in a state of rapid flux, because of the recent researches

based on molecular approaches, that is DNA comparisons of selected strains either isolated locally or obtained from culture collection centre, which has changed the existing scenario of fungal systematic and often overturn the assumptions of the older classification systems (Hibbett, 2006). Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological and reproductive stages are the classic approaches and baseline of fungal taxonomy and nomenclature that are still valid (Davis, 1995; Guarro *et al.,* 1999; Diba *et al.,* 2007; Zain *et al.,* 2009). It seems evident that in near future, modern molecular techniques will allow most of the pathogenicand opportunistic fungi to be connected to their corresponding sexual stages and integrated into a more natural taxonomic scheme. Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summer bell, 1996). Furthermore, findings for one species are not readily extrapolated to others, where significant morphological and physiological variations exist (Meletiadis *et al.,* 2001). With these perspectives, the present study was undertaken to observe the influence of nine different culture media on the mycelial growth, colony characters and sporulation patterns of four pycnidia producing phytopathogenic fungi.

Materials and Methods

Collection of disease samples

Collection of disease samples were done from Jaguli instructional farm, Mohanpur, (located at 22°43' N latitude and 88°34' E longitude with an elevation of 9.75 m above mean sea level) from Research farm of B.C.K.V., Mundouri, Nadia, West Bengal and also from 'C' block farm, BCKV, Kalyani Nadia, West Bengal.

Plants under study

The different crops used for the experiment includes:

Vegetable, Brinjal (*Solanum melongena*),
Flower, Tuberose (*Polianthes tuberosa*),
Medicinal, Mesta (*Hibiscus sabdariffa*),
Fruit, Jackfruit (*Artocarpus heterophyllus*)

The media

Different laboratory media including synthetic, semi-synthetic and natural mediawere used for isolation and maintenance of the pathogen. Different agar media, viz., Czapex Dox Agar (CDA), Richards Synthetic Agar (RSA), Asthana and Hawkers Agar (AHA), Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Malt Extract Agar (MEA), V8Agar, Leaf extract agar of the host plant.

Isolation of phytopathogenic fungi

Parts of diseased plants showing typical symptoms of the disease were collected from university experimental field. The diseased parts were made small pieces; surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 30-45 seconds followed by 5-6 times washing with sterilized distilled water. Thereafter, excess water on diseased parts was removed with sterilized blotting paper. Sterilized molten water agar medium of approximately 20 ml having temperature around 45 ^{0}C was poured into each sterilized (in hot air oven at 160 ^{0}C for 2 hours) Petri-plate, allowed to cool down and solidify. Then the surface sterilized diseased plant pieces (3 pieces per Petri dish) were transferred on to the solidified water agar medium in sterilized Petri plates. The Petri plates were incubated in

BOD at 28±1 ^{0}C temperature and observed periodically for the growth of fungus. When fungus just grows approximately 1.5-2.0 cm diameter then bits of fungal growth from such area ware transferred to PDA slants and incubated in BOD at 28 ±1^{0}C for few days. Then such pure cultured slants were preserved in a refrigerator at 5 ^{0}C for further studies and renewed once within 2-3 months. *Phoma sabdarifa*, *Phoma polyanthes*, *Phomopsis vexans* and *Phyllosticta artocarpina* were used from its pure cultures and 5 mm discs of each fungus were transferred at the centre of sterile Petri dishes (in triplicates) containing nine different growth media that includes natural (oat meal agar OMA, malt extract agar MEA, V_8A, and leaf extract agar LEA), semi-synthetic (potato dextrose agar PDA, carrot agar media CaDA) and synthetic media (czapek's dox CDA, Asthana and Hawkers agar AHA and Richard synthetic media (RSA).

Results and Discussion

All nine culture media supported the growth of test fungi to various degrees. Out of the four fungi, three fungi showed maximum mycelial growth on PDA and CaDA after 7 days of incubation period (Table 1), while *Phyllosticta artocarpina* showed maximum growth on LEA after 72 hrs (21.0 mm) whereas *Phoma sabdariffa, Phomopsis vexans* showed higher colony growth on PDA with 50.3mm, 61mm and *Phoma polyanthes* showed higher mycelial growth in CaDA of 78.9 mm and in PDA of 75.7mm respectively at 72 hrs.

In the present study, mycelial growth, pigmentation and zonations observed in fungal colonies were found to be influenced by the culture media used. In PDA, almost all tested fungi were characterized with regular mycelial growth and distinct circular zonations whereas in synthetic media comparatively lower mycelial growth is observed. In PDA, *Phoma sabdariffa* showed slightly fluffy mycelial growth with zonations, *Phomopsis vexans* showed good mycelial growth and pigmentation on the reverse side in PDA and thin mycelial growth with no zonation in case of synthetic media.

In case of *Phoma polyanthes*, fluffy thick mycelial growth were observed in PDA and semi synthetic media with dark pigmentation on the reverse side of the plate whereas lesser mycelial growth and lighter pigmentation in case of synthetic media except *Phyllosticta artocarpina* showed maximum mycelial growth in LEA, lesser in semi synthetic media, and least being in synthetic media.

PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth (Xu *et al.,* 1984; Maheshwari *et al.,* 1999; Saha *et al.,* 2008). Most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelial growth with ultimate loss of sporulation (UKNCC, 1998).

Our findings revealed the influence of culture media on the growth, colony character and sporulation of the test fungi differs based on the nature of the culture medium. Out of the nine media studied, OMA and MEA was found to be suitable for heavy sporulation while PDA reproduced most visible colony morphology.

From the investigation it can also be concluded that instead of using any single culture medium a combination of two or more media will be more appropriate for routine cultural and morphological characterization of fungi to observe different colony features and their responses (Fig. 1).

The compositions of different media used in the investigationare presented below:

a. Potato Dextrose Agar **Peeled potato (decoction) 200g** **Dextrose anhydrous 20g** **Agar agar 20g** **Distilled water1000ml**	**b. Oatmeal Agar** **White oat (decoction) 40g** **Agar agar 20g** **Distilled water 1000ml**	**c. Malt Extract Agar** **Malt extract 20g** **Agar agar 15g** **Distilled water 1000ml**
d. Czapek's Dox Agar **Sucrose 30g** **Sodium nitrate 2g** **Dipotassium hydrogen phosphate 1g** **Magnesium sulphate, 7 H2O 0.5g** **Potassium chloride 0.5g** **Ferrous sulphate, 7 H2O 0.01g** **Agar agar 20g** **Distilled water 1000ml**	**e. Richard's Synthetic Agar** Sucrose 50g Potassium nitrate 10g Potassium hydrogen phosphate 5g Magnesium sulphate, 7 H2O 2.5g Ferric chloride 0.02g Agar agar 20g Distilled water 1000ml	**f. Asthana and Hawker's agar** Glucose 5 gm KNO3 3.5 gm MgSO4, 7H2O 0.75 gm KH2PO4 1.75 gm Distilled water 1000 m Agar agar20 gm
g. Carrot Dextrose agar **Peeled and sliced carrot 200.00 g** **Agar-agar 20.00 g** **Distilled water 1000 ml**	**h. V8 agar media** 44.3 gm of V8 agar is dissolved in 1000 ml of distilled water andboiled to dissolve followed by sterilization in 15 psi at 161 degrees temperature for 15 degrees.	**i. Host Leaf Decoction** Agar Chopped leaf (decoction) 150g Agar agar 20g Distilled water1000ml

Table.1 Mycelia growth, colony characters and sporulation pattern of fungal isolates on nine culture media

Medium	Colony diameter (mm) in 72 hrs for *Phoma sabdariffa*	Colony character		Reverse Colour	Zonation	Sporulation
		Texture	Surface Colour			
PDA	50.3	Thin slightly fluffy	Whitish grey	Colourless	Concentric zones	Moderate
CaDA	42	Thin slightly fluffy	Whitish grey	Colourless	Concentric Zones	poor
OMA	40	Fluffy	Whitish grey	Colourless	Irregular concentric zones	moderate
MEA	42	Thin non Fluffy	Whitish grey	Colourless	Irregular concentric zones	heavy
V8A	41.3	Thick compact	Whitish grey	Colourless	Concentric zones	poor
LEA	38.2	Thin growth	Whitish grey	Colourless	None	moderate
AHA	20	Thin growth	Translucent	Colourless	None	poor
RSA	10	Thin growth	Translucent	Colourless	None	poor
CDA	8.7	Thin growth	Translucent	Colourless	None	poor

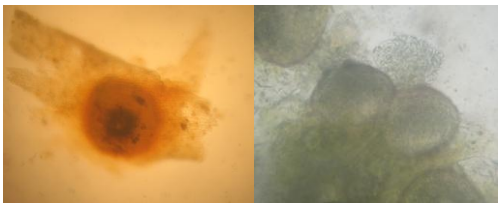
Medium	Colony diameter (mm) in 72 hrs for *Phomopsis vexans*	Colony character		Reverse Colour	Zonation	Sporulation
		Texture	Surface Colour			
PDA	61	Thick fluffy crust	Off white to light brown	Light lemon yellow	Concentric zones	moderate
CaDA	48	Thick fluffy crust	Pale white coloured to light brown	Deep yellow to brown	none	moderate
OMA	33.3	Flat suppressed mycelium	White to light brown	Deep yellow to brown	none	heavy
MEA	35	Fluffy cottony growth	Pale white coloured	Deep brown	concentric irregular zones	
V8A	31	Fluffy cottony	Light brown	Deep brown	Concentric irregular zones	moderate
LEA	20	Thin flat growth	Grey white	Colourless	Concentric zones	poor
AHA	34.6	Thin flat growth	Pale white	Light Lemon yellow	None	poor
RSA	38.3	Thin growth	Translucent	Light Lemon yellow	None	poor
CDA	20.6	Thin growth	Translucent	Colourless	None	poor

Medium	Colony diameter (mm) in 72 hrs for *Phoma polyanthis*	Colony character		Reverse Colour	Zonation	Sporulation
		Texture	Surface Colour			
PDA	75.7	fluffy	Light grey	Uniform black colour	Concentric zones	moderate
CaDA	78.9	fluffy	Light grey	Uniform black colour	None	moderate
OMA	78.3	Fluffy	greyish	Uniform black colour	concentric zones	moderate
MEA	56.3	Fluffy	Whitish grey	Uniform black colour	concentric zones	heavy
V8A	64	Thin growth	Deep grey	Uniform black colour	Concentric zones	poor
LEA	45	Thin irregular growth	Whitish grey	Uniform lighter coloured	Concentric zones	moderate
AHA	56.3	Thin growth	greyish	Uniform light coloured	None	Poor
RSA	63	Thin growth	Greyish	Uniform dark pigmentation	None	poor
CDA	60.3	Thin growth	Greyish	Uniform dark pigmentation	None	poor

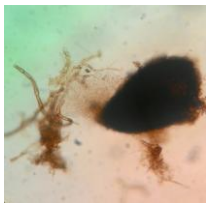
Medium	Colony diameter (mm) in 72 hrs for *Phyllosticta artocarpina*	Colony character		Reverse Colour	Zonation	Sporulation
		Texture	Surface Colour			
PDA	12.7	Irregularly formed Hard mycelium	light greyish	Uniform black pigmentation	none	moderate
CaDA	11.5	Hard mycelium	Deep black	Uniform black pigmentation	none	moderate
OMA	8	Hard mycelium	Whitish grey	Uniform black pigmentation	none	high
MEA	7.8	Hard mycelium	Whitish grey	Uniform black pigmentation		moderate
V8A	9	Hard thin mycelium	Whitish grey to dirty grey	Yellowish pigmentation	Concentric zones	poor
LEA	21	Hard mycelium	Deep black	Uniform black pigmentation	None	moderate
AHA	7.2	Hard mycelium	Greyish to dark grey	Uniform black pigmentation	None	poor
RSA	7.5	Hard mycelium	Whitish grey	Uniform dark pigmentation	None	poor

Pycnidia and Spores of the four test fungi

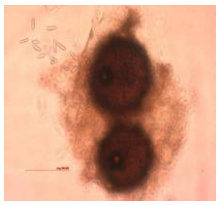
Fig.1 Colony growth, colour and pycnidia with spores of the test fungi on different culture agar media (PDA; CaDA, OMA, MEA, V8A, LEA, AHA, RSA, CDA)



Phomopsis vexans



Phoma sabdariffa



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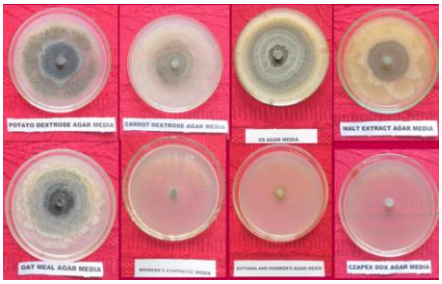

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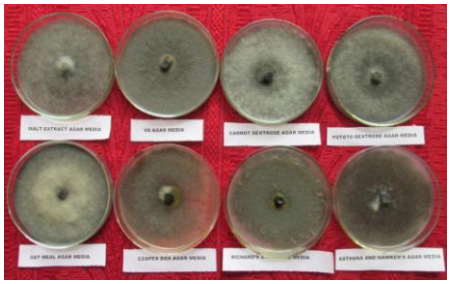
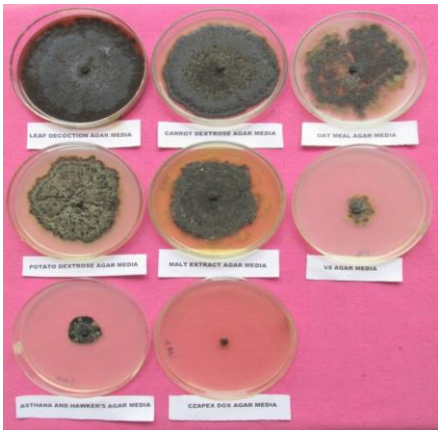


Phoma polyanthis



Phyllosticta artocarpina

Phomopsis vexans

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References

Davis JI (1995). Species concepts and phylogenetic analysis.Introduction. Syst. Bot., 20: 555-559.

Diba K, Kordbacheh P, Mirhendi SH, Rezaie S, Mahmoudi M (2007). Identification of *Aspergillus* species using morphological characteristics. Pak. J. Med. Sci., 23(6): 867-872.

Guarro J, Josepa G, Stchigel AM (1999). Developments in Fungal Taxonomy. Clin. Microbiol. Rev., 12: 454-500.

Hibbett DS (2006). A phylogenetic overview of the Agaricomycotina. Mycologia, 98(6): 917-925.

Kuhn DM, Ghonnoum MA (2003). Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. Clin. Microbiol. Rev., 16(1): 144-172.

Kumara KLW, Rawal RD (2008). Influence of carbon, nitrogen temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L). Trop. Agric. Res. Ext., 11: 7-12.

Maheshwari SK, Singh DV, Sahu AK (1999). Effect of several nutrient media, pH and carbon sources on growth and sporulation of *Alternaria alternata*. J. Mycopathol. Res. 37: 21-23.

Meletiadis J, Meis JFGM, Mouton JW, Verweij PE (2001). Analysis of growth characteristics of filamentous fungi in different nutrient media. J. Clin. Microbiol., 39(2): 478-484.

Northolt MD, Bullerman LB (1982). Prevention of mold growth and toxin production through control of environmental condition. J. Food Prot., 6: 519-526.

Saha A, Mandal P, Dasgupta S, Saha D (2008). Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. J. Environ. Biol., 29(3): 407-410.

St-Germain G, Summerbell R (1996). Identifying Filamentous Fungi – A Clinical Laboratory Handbook, 1st Ed. Star Publishing Co., Belmont, California.

UKNCC (1998). Growth and Media Manuals. Strain databases (www.ukncc.co.uk/)

Xu SO, Yuan SZ, Chen XC (1984). Studies on pathogenic fungus (*Alternaria tennuis* Nees) of poplarleaf blight. J. North East For. Inst., 12: 56-64.

Zain ME, Razak AA, El-Sheikh HH, Soliman HG, Khalil AM (2009).Influence of growth medium on diagnostic characters of *Aspergillus* and *Penicillium* species Afr. J. Microbiol. Res., 3(5): 280-286.

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