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Cultural Characteristics of Phylloplane Fungi and Leaf Spot Pathogens Isolated from *Cajanus cajan*

Sravani Gogisetty, Mihira Kumara Mishra, Prabhat Ranjan Mishra

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ABSTRACT

Four different isolates of fungi were discovered from the phylloplane of Arhar. They were recognized and identified to be *Fusarium solani, Paecilomyces marquandii, Aspergillus niger* and *Aspergillus flavus.* Similarly, from the diseased leaves of Arhar two pathogens were isolated, characterized and identified to be *Curvularia lunata* and Sterile. These fungal isolates were cultivated on seven different culture media viz., Czapek's Dox Agar, Potato Dextrose Agar (PDA), Oat Meal Agar, Rose Bengal Agar, Richard's Synthetic Agar, Sabouraud's Dextrose Agar and Malt Extract Agar. Right after seven days of incubation at 25^0C, the mycelial growth rate, colony character and sporulation patterns of these fungi were evaluated. The type of growth media utilized had a significant impact on the diameter, cultural traits and sporulation of a few selected test fungi. On PDA and Richard's synthetic agar media, *Fusarium solani, Aspergillus niger* and *Curvularia lunata* recorded the superlative radial growth. In PDA and Sabouraud's dextrose agar *Aspergillus flavus* and Sterile recorded the highest growth. *Paecilomyces marquandii* recorded the maximal radial growth in Sabouraud's dextrose agar.

Keywords Arhar, Cultural characters, Different growth media, Leaf spot pathogens, Phylloplane fungi.

INTRODUCTION

Culture media are nutrient and mineral-rich mediums that facilitate the growth of microorganisms in the lab. It is impossible to develop microorganisms using only one type of culture medium because they differ in their natures, traits, habitats, and additionally in their nutritional requirements (Acharya 2021). In order to diagnose infectious diseases, extract antigens, produce serological tests for vaccines, conduct genetic research, and identify microbial species, microorganisms must be cultured. Additionally, it is necessary for preserving culture stock, investigating biochemical responses, detecting microbial contamination, determining the effectiveness of antimicrobial agents and preservatives, determining viable count,

Sravani Gogisetty[1]*, Mihira Kumara Mishra[2], Prabhat Ranjan Mishra[3]

[2]Professor
[1,2] Department of Plant Pathology, Odisha University of Agriculture and Technology, Odisha 751003, India

[3]Professor
Department of Entomology, Odisha University of Agriculture and Technology, Odisha 751003, India

Email: san.sravsgogisetty@gmail.com
*Corresponding author

and determining antibiotic sensitivity (Fatima 2022).

Fungi are worldwide in distribution and thrive on a variety of natural habitats. They need several distinct ingredients for development and reproduction. For cultivation, maintenance, microscopical analysis, biochemical and physiological characterization, these are isolated in the lab and placed on a specialized culture medium. Different groups of fungi are isolated using a wide variety of media, and the nature of the specific culture medium, light, temperature, pH, water availability, and neighbouring atmospheric gas mixture all have an impact on vegetative growth, colony morphology, pigmentation, and sporulation. However, the requirements for fungus growth are often simpler than those for sporulation (Kumara and Rawal 2008, Kuhn and Ghannoum 2003, Northolt and Bullerman 1982).

Physical and chemical variables significantly influence the diagnostic features of fungi. As a result, using a range of media is frequently necessary when trying to identify a fungus in a culture since mycelial growth and sporulation on synthetic media are important biological characteristics (St-Germain and Summerbell 1996). Furthermore, results from one species cannot always be extrapolated to other species, especially in filamentous fungi with significant physiological and morphological variations (Meletiadis *et al.* 2001). With this in mind, the present work was carried out to examine the effects of seven distinct culture media on the mycelial growth, colony characteristics, and sporulation patterns of four phylloplane fungi and two leaf spot pathogens isolated from leaves of pigeon pea (Sharma and Pandey 2010).

MATERIALS AND METHODS

Collection, isolation, and purification of phylloplane mycoflora from healthy leaves

Leaf samples from numerous Arhar plants were gathered from the campus farm at OUAT. A variety of healthy leaves alongside diseased leaf samples by diverse organisms were chosen and collected from each plant for pathogen isolation. All of those leaf samples were kept in labeled paper bags until the micro-organisms were isolated.

With a disinfected blade, the gathered leaf samples were divided into little pieces. These pieces were kept on PDA-filled petri plates. We took five samples on each plate. On the PDA, some samples have been kept on the adaxial side and some on the abaxial side. For seven days, the plates were incubated at 25°C.

The development of microorganisms in the plates was observed after incubation. Each plate's morphologically differentiated visible colonies of fungus have been moved to new PDA plates and purified through repeated subculturing. Pure cultures were given scientific names and transferred on PDA-containing cultural slants that can be preserved at 4^{o}C.

Collection, isolation, and purification of pathogens from infected leaves

Infected leaf parts of Arhar with distinctive spotted patches with elliptical brown spots were detected and collected from the campus farm of OUAT, Bhubaneswar.

In order to isolate the pathogen, leaves with more recognizable leaf spots were chosen. To get rid of dirt and other pollutants on the leaves' surface, sterile water was used to wash the chosen leaves. The leaves were then chopped into little pieces using a sterilized blade. With the aid of sterile forceps, these parts were submerged in a 0.1% $HgCl_2$ solution for 30 to 40 seconds. To remove any remaining $Hgcl_2$ traces, they were then thoroughly washed three to four times with sterilized water. These pieces were placed in the laminar airflow chamber under aseptic conditions on sterilized blotting paper to absorb excess moisture. Using sterile forceps, these pieces were afterward put on Petri dishes with PDA. For seven days, the plates were incubated at 25°C.

The hyphal tips were transplanted onto PDA-containing sterilized Petri plates as soon as mycelial development surrounding these areas was visible. To prevent contamination, frequent subculturing was done right away when the mycelium had grown properly. Following purification, the cultures were replicated, kept alive on 2% PDA slants, and stored at 4-6^{0}C for future research. Based on the pathogen's physical and cultural characteristics, a description of

it was provided.

Different culture media

Czapek's dox agar

Sucrose- 30 g, Agar- 20 g, Sodium Nitrate- 2 g, Potassium dihydrogen Phosphate (KH_2PO_4) - 1g, Potassium Chloride- 0.5g, Magnesium Sulfate- 0.5g, Ferric Chloride- 0.01g, Distilled water- 1000 ml.

All of the components listed were dispersed in 450 ml of sterile water, with the exception of KH_2PO_4 and agar. Separately, 500 ml of sterile water was used to dissolve the agar, which was then added to the aforementioned solution. The volume eventually increased to 950 ml. 50 ml of sterile water was used to dissolve potassium dihydrogen phosphate. Later, the two solutions were thoroughly combined before being autoclaved for 15 minutes at 15 psi.

Oat meal agar

Oat flakes- 40g, Agar- 20g, Distilled water- 1000 ml.

Oat flakes were heated up in 400 ml of sterile water for about 20 minutes before the mixture was strained through a double layer of muslin fabric. Later, 400 ml of sterile water was used to melt the agar separately. Both solutions were successfully mixed to create a final volume of up to 1000 ml. After that, the media have to be autoclaved at 15 psi pressure for 15 minutes.

Rose bengal agar

Dextrose- 10g, Papaic Digest of Soybean meal- 5g, Monopotassium Phosphate- 1g, Magnesium Sulfate- 0.5g, Chloramphenicol- 0.1g, Rose Bengal- 0.05g, Agar-15g.

Suspend 31.6g of powder in 1lt of sterile water. Warm up until it dissolves completely. Add 0.1g of Chloramphenicol per liter. Later solution was autoclaved at 121°C, 15 psi pressure for 15 min.

Richard's synthetic agar

Potassium Nitrate- 10g, Monopotassium dihydrogen Phosphate- 5g, Magnesium Sulphate- 2.5g, Ferric Chloride- 0.02 g, Sucrose- 50 g, Agar- 15 g.

Suspend 82.52 g in 1000 ml sterile water. Heat up until the medium has fully dissolved. Sterilize for 15 min with autoclaving at 15 psi, 121°C.

Malt extract agar

Malt extract- 30 g, Mycological Peptone- 5 g, Agar- 15 g, Final pH (at 25°C)- 5.4 ± 0.2.

Suspend 50 g in 1000 ml of sterile water and soak for 15 min. Sterilize by autoclaving at 115°C, 10 psi pressure for 10 min.

Sabouraud's dextrose agar

Dextrose- 40g, Peptone- 10g, Agar-agar- 15g, Distilled water- 1000 ml.

All the components were melted in 450 ml of sterile water, and in another 500 ml of sterile water, add agar separately. These two solutions were later effectively combined and their volume is made up to 1000 ml. Later the media was autoclaved for 15min at a pressure of 15 psi.

Potato dextrose agar

Potato pieces (peeled)- 200 g, Agar-agar- 20 g, Dextrose- 20 g, Distilled water- 1000 ml.

First 200 g of peeled potatoes were cut into pieces and then boiled in distilled water (400 ml). Later, 20 g of agar was dissolved in 400 ml of sterile water and also 20g of dextrose was put into the same flask. The two solutions have been mixed and the resulting volume is 1000 ml. It was autoclaved for 15 min at 15 psi pressure.

Cultural characterization of the pathogen and phylloplane mycoflora

Growth characters on different solid media

On various solid media, the cultural characteristics of the causative pathogen and the phylloplane mycoflora were investigated.

1 Czapek's dox agar
2 Oat meal gar

3 Rose bengal agar
4 Richard's synthetic agar
5 Malt extract agar
6 Sabouraud's dextrose agar
7 Potato dextrose agar

In sterile petri dishes, 20 ml of each media was poured and dried in laminar airflow. In petri dishes, 5mm disks from a germinating culture plate were mounted upside down in the midst of the solidified media. They are incubated at 27±1°C.

When the colony reached its maximal growth, its radial growth was calculated. On both sides, the colony's diameter was determined in the media.

RESULTS AND DISCUSSION

Identification, isolation, and purification of phylloplane mycoflora

The growth of various phylloplane mycoflora was monitored by collecting healthy leaf samples, cutting them into little pieces, and keeping them on a PDA. A light microscope was used to study the collected microorganisms. By using the fungal tip approach, the microorganisms were purified, and pure cultures have been developed for further research (Table 1).

Isolation, purification, and identification of leaf spot pathogens of Arhar

Samples of diseased leaves were carefully gathered, transferred to the lab, and examined under a light microscope to check for the pathogen's presence. In order to encourage the growth of microorganisms, certain leaf samples were surface sterilized with 0.1% Mercuric chloride and other samples with 1% Sodium hypochlorite. These samples were then stored on PDA plates. By using the fungal tip approach, the pathogens were isolated, and pure cultures were developed for additional research (Table 2).

Table 2. Colony characteristics of fungal pathogens from infected leaves of Arhar.

Pathogen	Name of the pathogen	Color of colony (Upper side)	Color of colony (Lower side)
1.	*Curvularia lunata*	Brownish black	Grey
2.	*Sterile*	Light brownish	Light yellow

Growth characters of phylloplane mycoflora in different solid media

Growth of *Fusarium solani* in different solid media

Significant different growth habit was observed in *Fusarium solani* with the maximum radial growth in PDA (90 mm), Richard's synthetic agar media (89.4 mm) followed by Czapek's dox agar (88.56 mm), Sabouraud's dextrose agar (85.26 mm), Oatmeal agar (80.6mm), Malt extract agar (76.73 mm). The least growth was observed in Rose bengal agar (68.86 mm) (Table 3).

Excellent sporulation was found in PDA. Good sporulation was observed in Oatmeal agar, Rose bengal agar, Malt extract agar. Fair sporulation was seen in Czapek's dox agar, Richard's synthetic agar, Sabouraud's dextrose agar.

Growth of *Paecilomyces marquandii* in different solid media

Significant different growth habit was observed in

Table 1. Colony characteristics of fungal isolates of Arhar phylloplane.

Isolate	Name of the phylloplane mycoflora	Color of the colony (Upper side)	Color of the colony (Lower side)
1	*Fusarium solani*	White	Pale yellow
2	*Paecilomyces marquandii*	Pale violet	Orange yellow
3	*Aspergillus niger*	Black	Pale yellow to colorless
4	*Aspergillus flavus*	Olive green	Cream to colorless

Table 3. Growth of *Fusarium solani* in different solid media.

Treatments	Different solid media	Radial growth (mm)	Sporulation
T1	Czapek's dox agar	88.56	+ +
T2	Oatmeal agar	80.6	+ + +
T3	Rose bengal agar	68.86	+ + +
T4	Richard's synthetic agar	89.4	+ +
T5	Malt extract agar	76.73	+ + +
T6	Sabouraud's dextrose agar	85.26	+ +
T7	Potato dextrose agar	90	+ + + +
SEm (±)		**0.251**	
CD (5%)		**0.769**	

+ + + +: Excellent, + + +: Good, + +: Fair, +: Poor.

Table 4. Growth of *Paecilomyces marquandii* in different solid media.

Treatments	Different solid media	Radial growth (mm)	Sporulation
T1	Czapek's dox agar	78.33	+ +
T2	Oatmeal agar	87.36	+ + + +
T3	Rose bengal agar	89.06	+ + +
T4	Richard's synthetic agar	77.03	+
T5	Malt extract agar	85.66	+ +
T6	Sabouraud's dextrose agar	89.70	+ + +
T7	Potato dextrose agar	86.76	+ + + +
SEm (±)		**0.405**	
CD (5%)		**1.242**	

+ + + +: Excellent, + + +: Good, + +: Fair, +: Poor.

Paecilomyces marquandii with extreme radial growth in Sabouraud's dextrose agar (89.70 mm) followed by Rose bengal agar (89.06 mm), Oatmeal agar (87.36 mm), PDA (86.76 mm), Malt extract agar (85.66 mm), Czapek's dox agar (78.33 mm). The least growth was observed in Richard's synthetic agar (77.03 mm) (Table 4).

Excellent sporulation was seen in PDA, Oatmeal agar. Good sporulation was observed in Rose bengal agar, Sabouraud's dextrose agar. Fair sporulation was noticed in Czapek's dox agar, Malt extract agar. Poor sporulation was noticed in Richard's synthetic agar.

Growth of *Aspergillus niger* in different solid media

Significant different growth habit was also observed in *Aspergillus niger* with the highest radial growth in

Table 5. Growth of *Aspergillus niger* in different solid media.

Treatments	Different solid media	Radial growth (mm)	Sporulation
T1	Czapek's dox agar	74.86	+ +
T2	Oat meal agar	86.9	+ + +
T3	Rose bengal agar	71.6	+ + +
T4	Richard's synthetic agar	90.0	+ + + +
T5	Malt extract agar	72.5	+ + +
T6	Sabouraud's dextrose agar	68.73	+ + +
T7	Potato dextrose agar	88.66	+ + + +
SEm (±)		**0.245**	
CD (5%)		**0.750**	

+ + + +: Excellent, + + +: Good, + +: Fair, +: Poor.

Richard's synthetic agar (90 mm) followed by PDA (88.66 mm), Oatmeal agar (86.9 mm), Czapek's dox agar (74.86 mm), Malt extract agar (72.5 mm) and Rose bengal agar (71.6 mm). The least radial growth was observed in Sabouraud's dextrose agar (68.73 mm) (Table 5).

Excellent sporulation was seen in PDA and Richard's Synthetic agar. Good sporulation was observed in Oatmeal agar, Rose bengal agar, Malt extract agar, Sabouraud's dextrose agar. Fair sporulation was observed in Czapek's dox agar

Growth of *Aspergillus flavus* in different solid media

Significant different growth habit was also observed in *Aspergillus flavus* with lavish radial growth in PDA (89 mm) followed by Sabouraud's dextrose agar (74.96 mm), Czapek's dox agar (74.2 mm), Malt extract agar (71.1 mm), Rose bengal agar (69.63 mm), Richard's synthetic agar (66.4 mm). The least radial growth was observed in Oatmeal agar (64.5 mm) (Table 6).

Excellent sporulation was observed in PDA. Good sporulation was seen in Czapek's dox Agar, Richard's synthetic agar, Malt extract agar Sabouraud's dextrose agar. Fair sporulation was found in Rose bengal agar. Poor sporulation was noticed in Oatmeal agar.

Table 6. Growth of *Aspergillus flavus* in different solid media.

Treatments	Different solid media	Radial growth (mm)	Sporulation
T1	Czapek's dox agar	74.2	+ + +
T2	Oat meal agar	64.5	+
T3	Rose bengal agar	69.63	+ +
T4	Richard's synthetic agar	66.4	+ + +
T5	Malt extract agar	71.1	+ + +
T6	Sabouraud's dextrose agar	74.96	+ + +
T7	Potato dextrose agar	89.0	+ + + +
SEm (±)		**0.268**	
CD (5%)		**0.821**	

+ + + +: Excellent, + + +: Good, + +: Fair, +: Poor.

Table 8: Growth of *Sterile* in different solid media.

Treatments	Different solid media	Radial growth (mm)	Sporulation
T1	Czapek's dox agar	80.96	-----
T2	Oat meal agar	76.56	-----
T3	Rose bengal agar	76.1	-----
T4	Richard's synthetic agar	82.3	-----
T5	Malt extract agar	76.7	-----
T6	Sabouraud's dextrose agar	85.26	-----
T7	Potato dextrose agar	90.0	-----
SEm (±)		**0.613**	
CD (5%)		**1.879**	

Growth of foliar pathogens of Arhar in different solid media

Growth of *Curvularia lunata* in different solid media

The foliar pathogen *Curvularia lunata* was grown in seven different media (Table 7). The fungus recorded the maximum radial growth in PDA (90mm) followed by Richard's synthetic agar (89.8 mm), Malt extract agar (82.33 mm), Oatmeal agar (75.67 mm), Czapek's dox agar (72.1 mm), Rose bengal agar (40.90 mm). The least growth was observed in Sabouraud's dextrose agar (31.67 mm).

Excellent sporulation was found in PDA. Good sporulation was observed in Oatmeal agar, Czapek's dox agar, Malt extract agar. Fair sporulation was seen in Rose bengal agar. Poor sporulation was noticed in Richard's synthetic agar, Sabouraud's dextrose agar.

Table 7. Growth of *Curvularia lunata* in different solid media:

Treatment	Different solid media	Radial growth (mm)	Sporulation
T1	Czapek's dox agar	72.13	+ + +
T2	Oat meal agar	75.66	+ + +
T3	Rose bengal agar	40.96	+ +
T4	Richard's Synthetic Agar	89.8	+
T5	Malt extract agar	82.3	+ + +
T6	Sabouraud's dextrose agar	31.6	+
T7	Potato dextrose agar	90	+ + + +
SEm (±)		**0.517**	
CD (5%)		**1.557**	

+ + + +: Excellent, + + +: Good, + +: Fair, +: Poor.

Growth of Sterile in different solid media

The foliar pathogen Sterile was also grown in seven different media (Table 8). The fungus recorded maximum radial growth in PDA (90 mm) followed by Sabouraud's dextrose agar (85.26 mm), Richard's synthetic agar (82.3 mm), Czapek's dox agar (80.96 mm), Malt extract agar (76.7 mm), Oat meal agar (76.56 mm). The least growth was observed in Rose bengal agar (76.1 mm). As the organism was Sterile it didn't produce any type of spores in different solid media.

Richard's synthetic agar (89.4 mm) and PDA (90 mm) were the best growth media for *Fusarium solani.* The optimal medium for the mycelial development and sporulation of *F. solani,* according to Bhagwat (1969), was PDA. Jhamaria (1972) found that Richard's synthetic agar and PDA were the best for the fungus's mycelial development.

On seven separate solid media, *Paecilomyces marquandii* was cultivated. Sabouraud's dextrose agar had the highest growth rate (89.70 mm), and rose bengal agar came in second. (89.06mm). Villanueva and Davide (1983) achieved comparable outcomes. Oat meal agar was shown by Julieta *et al.* (2012) to be the most effective medium for isolating *Paecilomyces* spp.

Different solid media were used to grow the fungus *Aspergillus niger,* and the two media with the strongest rate of growth were Richard's synthetic agar (90 mm) and Potato dextrose agar (88.66 mm). The same results were attained by Amritha and Richa (2014), Pathak (1993).

The *Aspergillus flavus* fungus was cultivated on various solid media, and PDA showed the best growth and good sporulation (89 mm). These outcomes are those that Raper and Fennel reported (1965).

PDA (90mm) and Richard's synthetic agar (89.8 mm) have been shown to be the best media for *Curvularia lunata* growth among the several types of media used. Due to the presence of several vitamins that are necessary for the development and advancement of the organism, these two media promoted superior growth than Malt extract agar (82.33 mm). Olufolaji (1983) and Mehi *et al.* (2014) also made observations of such phenomena. The fungus sporulated quite well in PDA. Mehi *et al.* (2014) also made a point of a similar nature.

The Sterile exhibited the maximum radial growth in PDA (90mm) followed by Sabouraud's dextrose agar (85.26 mm). On seven different solid media, the fungus didn't develop any spores since it was sterile.

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