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Screening of cultural media for the growth of foliar fungal disease pathogens associated with aromatic rice

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Abstract
Ten cultural media, namely Potato sucrose agar (PSA) medium, Potato dextrose agar (PDA) medium, Czapek's dox agar medium, Corn starch agar medium, Oat meal agar (OMA) medium, Rice straw agar medium, Rice grain agar medium, Rice leaf extract agar medium, Malt extract agar medium, and V8 juice agar medium, were used to observe the growth of three fungal pathogens of aromatic rice, viz., *Pyricularia oryzae, Bipolaris oryzae, and Rhizoctonia solani.* The radial growth and colony characteristics were greatly influenced by the medium used. In a comparative study of the higher mycelial growth of the three test fungi at ten different mediums, the best performance was observed in rice straw medium (*Pyricularia oryzae* and *Bipolaris oryzae*) and in rice leaf agar medium (*Rhizoctonia solani*). These results will find use in further fungal taxonomic *in vitro* studies.

Keywords: *Pyricularia oryzae, Bipolaris oryzae, Rhizoctonia solani,* culture medium, radial growth, colony character

Introduction
More than half of the world's population, including Asians, consumes rice (Oryza sativa L.), one of the most common staple foods. India, like many other south Asian nations, consumes a lot of cooked, puffed, and pounded rice. Due to its outstanding scent and superb grain quality, aromatic rice, which belongs to a distinct category of rice, is highly valued. The same diseases and pests that affect ordinary rice also affect aromatic rice. 50 diseases, including 6 bacterial, 21 fungi, 4 nematodes, 12 viruses, and 7 other disorders, attack the rice crop (Hollier *et al*., 1993; Webster and Gunnell, 1992; Jabeen *et al*., 2012) [7, 22, 9]. According to Ou (1985) [13], a multitude of diseases involving fungi, bacteria, viruses, and mycoplasma severely reduce rice crop yields. Blast (*Pyricularia oryzae*), brown spot (*Bipolaris oryzae*), and sheath blight (*Rhizoctonia solani*) are the common fungal foliar diseases of rice, according to Ghosh (1960). During the epidemic years, Blast resulted in significant yield losses of between 35 and 50 percent (Padmavathi *et al*., 2005). Around the world, there have been a number of rice blast epidemics that have reduced grain yields by 50% to 90% (Agrios, 2005; Yashaswini *et al*., 2017) [1, 2]. In particular, yield loss from Brown spot has been reported to be 90% (Ghose *et al*., 1960) [6], 3.7–29.1% (Singh *et al*., 1979) [17], 9.28–24.50% (Mia *et al*., 2001) [12], 26–52% (Chakrabarti, 2001) [4], and 18.75–22.50% (Kamal and Mia, 2009) [11], among other reports. According to the degree of infection, the Sheath blight disease reduces yield by 20 to 50% (Rao, 1995) [15]; Chahal (2003) [3] also observed a 54.3% yield loss. For large-scale investigations, particularly for the production of fungal spores when disease screening studies are concerned, it is imperative to comprehend the development of the fungus, particularly its vegetative growth and sporulation. To conduct research on the evaluation of pathogen virulence and genetic variation, analyses of cultural and morphological traits are crucial. Finding the optimal medium for pathogen development in order to keep them viable in lab settings for additional

Materials and Methods
The laboratory work was done in Laboratory, Department of Plant Pathology, College of agriculture, CAU, Imphal, Iroisemba, Manipur.

Isolation and purification of *Pyricularia oryzae, Bipolaris oryzae and Rhizoctonia solani*

The pathogens were isolated using rice plant diseased leaves showing the symptoms of Blast, Brown spot and Sheath blight from farmer's fields. By using the accepted tissue isolation technique, the pathogens were isolated (Tuite, 1969) [19]. With the help of a sterile blade, small pieces of diseased leaf tissue and some healthy tissue were separated, surface sterilised for one minute with a solution of 1% sodium hypochlorite, and aseptically rinsed three times in sterilised distilled water. These surface sterilised leaf fragments that had been added streptomycin sulphate were then aseptically put into sterilised Petri plates that contained solidified potato dextrose agar medium. These plates were then incubated at 28 ± 1 °C for two weeks in a BOD incubator. Following this, the fungi were purified using the conventional hyphal tip isolation method. A small amount of marginal mycelial growth was aseptically removed for sub-culturing. The sub culturing was done at an interval 15 days, after which the cultures were maintained on Potato dextrose agar slants and stored at 5 °C for use in all subsequent laboratory experiments. Laboratory research has been done on cultural traits such as colony characteristics, colony diameter, margin, and mycelia growth.

Effect of different media on the growth of *Pyricularia oryzae, Bipolaris oryzae and Rhizoctonia solani*

With three replications for each pathogen independently, the experiment was conducted using a completely randomised design. In Potato Dextrose Agar Medium (PDA), pure cultures were maintained. Ten cultural media namely Potato sucrose agar (PSA) medium, Potato dextrose agar (PDA) medium, Czapek's dox agar medium, Corn starch agar medium, Oat meal agar (OMA) medium, Rice straw agar medium, Rice grain agar medium, Rice leaf extract agar medium, Malt extract agar medium and V8 juice agar medium were used for the study and their compositions are listed in table 1. According to their compositions, the various media were prepared and autoclaved for 15 to 20 minutes at 121 °C and 15 psi. Fifteen ml of each medium (Table 1) was poured into each of sterilized petriplates. Each pathogen's culture disc (5 mm) was individually inoculated on ten different media, then incubated at 28 °C. On each medium, the colony diameter (mm), colony colour, colony type of margin, and colony growth rate were recorded as cultural characteristics

Table 1: Lists of 10 different media and their compositions

Sl. No	Medium	Compositions
1	Potato sucrose agar	Potato- 200 g, Sucrose-20 g, Agar-agar- 20 g, Distilled water- 1 lt
2	Potato dextrose agar	Potato- 200 g, Dextrose -20 g, Agar-agar- 20 g, Distilled water- 1 lt
3	Czapek's dox agar medium	Dipotassium phosphate- 1 g, Ferrous sulphate – 0.01 g, magnesium sulphate-0.50 g, Potassium chloride- 0.05 g, Sodium nitrate- 2 g, sucrose- 30 g, Agar-agar-20 g, Distilled water- 1 lt
4	Corn starch agar medium	Corn- 20 g, Dextrose- 30 g, Agar –agar- 20 g, Distilled water- 1 lt
5	Oat meal agar medium	Oat meal- 60 g, Agar – agar - 20 g, Distilled water - 1 lt
6	Rice straw agar medium	Straw- 100 g, Sucrose- 20 g, Agar – agar - 20 g, Distilled water - 1 lt
7	Rice grain agar medium	Grain - 20 g, Sucrose- 20 g, Agar – agar - 20 g, Distilled water - 1 lt
8	Rice leaf extract agar medium	Rice green leaf - 20 g, Sucrose- 20 g, Agar–agar - 20 g, Distilled water- 1 lt
9	Malt extract agar medium	Malt extract - 30 g, Peptone - 20 g, Agar –agar- 20 g, Distilled water- 1 lt
10	V8 juice agar medium	V8 agar (Hi-media)- 44.2 g, Distilled water- 1 lt

Results and Discussions

The experiment was conducted as detailed in "material and methods" to acertain the best suitable medium where the maximum growth of the three fungi could occur. Significant difference was observed.

The effect of different media on the radial growth and colony description of *Pyricularia oryzae* is presented in Table 2, Fig 1, Plate 1. Among all the media the highest mean radial growth of the *Pyricularia oryzae* was observed in Rice straw agar (55.67 mm) followed by Oat meal agar (51.00 mm), Rice grain agar (47.33 mm), Malt extract agar (45.33 mm), Czapek's dox agar (43.00 mm), Rice leaf extract agar (42.67 mm), Potato dextrose agar (39.00 mm), Corn starch agar (38.67 mm), Potato sucrose agar (37.67 mm) and the least radial growth was noticed in V8 juice agar (34.33 mm). Similar observations had been made by other researchers. Jagadeesh and Devaki (2020) [10] studied cultural morphology of 72 *Magnaporthe oryzae* isolates on OMA and PDA and observed significant growth. Teli *et al*. (2016) [18] reported that PDA and OMA were found to be the best media for radial growth of *Magnaporthe grisea* isolated from Ponnampet.

With respect to *Bipolaris oryzae*, the maximum growth was observed in Rice straw agar (84.67 mm) followed by Oat meal agar (84.33 mm), Rice grain (84.00 mm), Rice leaf (84.00 mm), Corn starch agar (81.00 mm), Czapek's dox agar (78.67 mm), Potato dextrose agar (78.00 mm), V8 juice agar (66.00 mm), Potato sucrose agar (60.00 mm) and the minimum radial growth was observed in Malt extract agar (55.33 mm). The radial growth and colony description of *Bipolaris oryzae* due to different media are presented in table 3, Fig 1 and Plate 2. These findings are in agreement with previous findings. Vinay Kumari *et al.*, (1997) [21] recorded maximum growth and sporulation of the *Bipolaris oryzae* on corn meal agar medium followed by potato dextrose agar (PDA) and rice leaves agar. Valarmathi and Ladhalakshmi (2018) [20] also studied the four different media Rice Extract, Potato Dextrose Agar, Oat Meal Agar and Malt Extract Agar and revealed these media supported the growth of the pathogen.

In case of *Rhizoctonia solani*, the maximum growth was observed in Rice leaf (84.00 mm), Czapek's dox agar (84.00 mm), Corn starch agar (84.00 mm) followed by Potato dextrose agar (83.33 mm), Potato sucrose agar (83.00 mm), Rice straw agar (81.67 mm), Malt extract agar (80.67 mm), Rice grain (76.33 mm), V8 juice agar (66.33 mm) and the minimum radial growth was observed in Oat meal agar (57.67 mm).(Table 4, Fig 1, Plate 3). The results are in accordance with the previous findings. Singh and Kaiser (1994) [16] reported that PDA supported the highest growth of the fungal isolates. Imran *et al.*, 2016 [8] studied four different media i.e., PDA, CMA, WA, PDA and revealed that PDA had shown highest growt

Table 2: Effect of different media on the growth of *Pyricularia oryzae.*

Media	*Radial growth (mm)	Colony description
Potato sucrose agar	37.67	Colony is dirty grey, concentric ring present, thin compact cottony aerial growth with irregular margin
Potato dextrose agar	39.00	Colony is dirty grey, concentric rings present, thin compact cottony aerial growth with regular margin
Czapek's dox agar	43.00	Colony is dark brown, concentric rings present, thin compact cottony aerial growth with regular margin
Corn starch agar	38.67	Colony is dark brown, no concentric ring, thin compact aerial growth with regular margin
Oat meal agar	51.00	Colony is creamy black, concentric rings present, thick compact cottony aerial growth with regular margin
Rice straw agar	55.67	Colony is dark brown, concentric rings present, thin compact cottony aerial growth with regular margin
Rice grain agar	47.33	Colony is dark brown, concentric rings present, thin compact aerial growth with regular margin
Rice leaf extract agar	42.67	Colony is dark brown, concentric rings present, thin compact aerial growth with regular margin
Malt extract agar	45.33	Colony is grey, concentric rings present, thick compact cottony aerial growth with irregular margin
V8 juice agar	34.33	Colony is dark brown, concentric ring absent, thin compact aerial growth with regular margin
S.E(d)(±)	2.02	
C.D	4.21	

*Mean of three replications, radial growth observed on the 12th day after inoculation

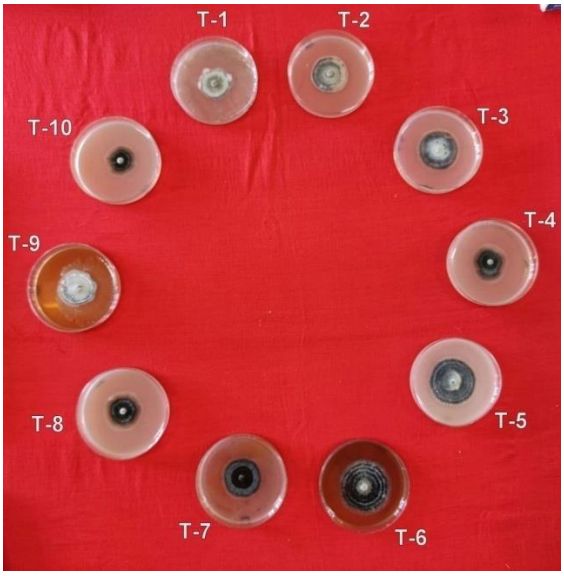


Plate 1: Growth of *Pyricularia oryzae in different media.* (T1- PSA, T2-PDA, T3- Czapek's dox, T4- Corn starch , T5-OMA, T6- Rice straw, T7- Rice grain agar, T8- Rice leaf, T9- Malt extract, T10- V8 juice agar medium)

Table 3: Effect of different media on the growth of *Bipolaris oryzae*

Media	Radial growth (mm)	Colony description
Potato sucrose agar	60.00	Colony is dirty black, concentric ring present, thick compact submerged growth with irregular margin
Potato dextrose agar	78.00	Colony is blackish green ,concentric ring absent, thick compact submerged growth with smooth margin
Czapek's dox agar	78.67	Colony is blackish white and fluffy, concentric zone present, thick compact submerged growth with irregular margin
Corn starch agar	81.00	Colony is dark brown, no concentric ring, thin compact aerial growth with regular creamy margin
Oat meal agar	84.33	Colony is dirty grey more fluffy, concentric ring absent, thick compact submerged growth with smooth and regular margin
Rice straw agar	84.67	Colony is light blackish fluffy, concentric ring absent, thin compact aerial growth with regular and smooth margin
Rice grain agar	84.00	Colony is light brown, concentric ring absent, thin compact aerial growth with regular and smooth margin
Rice leaf extract agar	84.00	Colony is dark brown, concentric ring absent, thin compact aerial growth with regular smooth margin
Malt extract agar	55.33	Colony is olive black and fluffy, concentric rings present, thick compact cottony submerged growth with irregular white margin
V8 juice agar	66.00	Colony is pinkish grey darker at the centre, concentric ring absent, thick compact submerged with irregular margin.
S.E(d)(±)	4.29	
C.D	8.95	

*Mean of three replications, radial growth observed on the 12th day after inoculation.

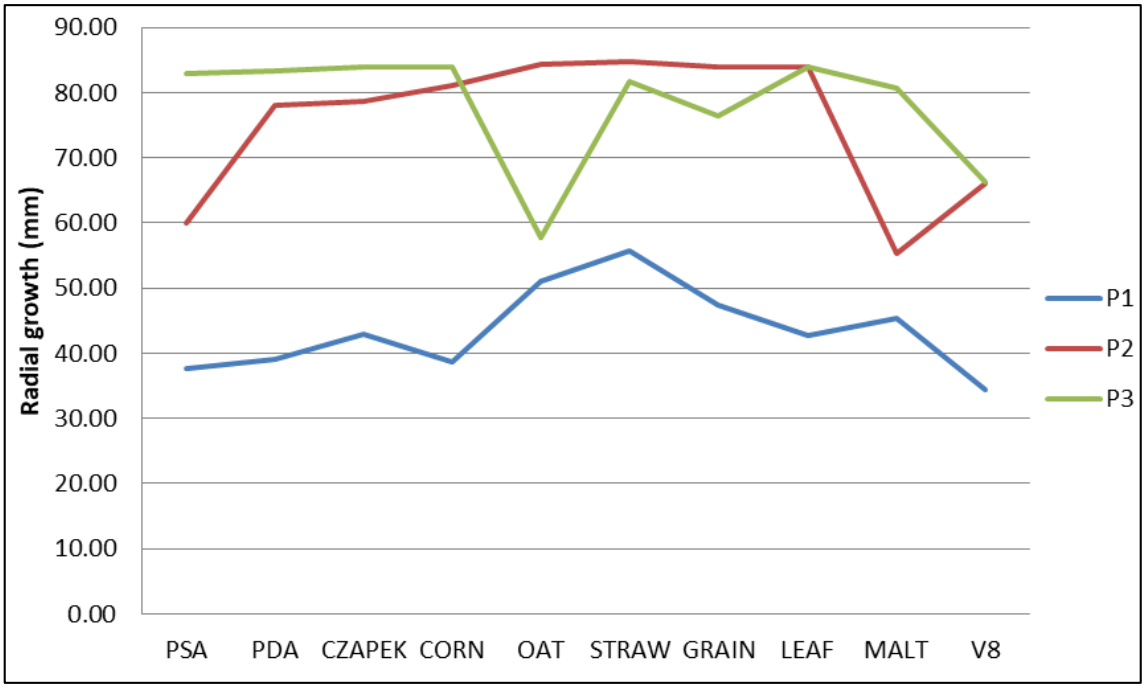
Plate 2: Growth of *Bipolaris oryzae* in different media. (T1- PSA, T2-PDA, T3- Czapek's dox, T4- Corn starch , T5-OMA, T6- Rice straw, T7- Rice grain agar, T8- Rice leaf, T9- Malt extract, T10- V8 juice agar medium)

Table 4: Effect of different media on the growth of *Rhizoctonia solani*

Media	Radial growth (mm)	Colony and sclerotia description
Potato sucrose agar	83.00	Colony is white, sclerotia with smooth border, honey dew present, clump formation more, size large, number less and manner of sclerotial formation is peripheral and centre,
Potato dextrose agar	83.33	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size small, number less and manner of sclerotial formation is peripheral and centre
Czapek's dox agar	84.00	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size small, number more and manner of sclerotial formation is centre
Corn starch agar	84.00	Colony is white, sclerotia with smooth border, honey dew present, no clump formation, size medium, number less and manner of sclerotial formation is peripheral
Oat meal agar	57.67	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size very small, number more and manner of sclerotial formation is peripheral
Rice straw agar	81.67	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size small, number less and manner of sclerotial formation is peripheral
Rice grain agar	76.33	Colony is white, sclerotia with smooth border, honey dew present, no clump formation, size small, number less and manner of sclerotial formation is peripheral on side wall
Rice leaf extract agar	84.00	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size medium, number less and manner of sclerotial formation is peripheral on side wall
Malt extract agar	80.67	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size small, number less and manner of sclerotial formation is peripheral
V8 juice agar	66.33	Colony is white, sclerotia with smooth border, honey dew absent, no clump formation, size small, number less and manner of sclerotial formation is peripheral
S.E(d)(±)	3.08	
C.D	6.41	

*Mean of three replications, radial growth observed on the 4th day and sclerotia observed on 10th day after inoculation

Plate 3: Growth of *Rhizoctonia solani* in different media. (T1- PSA, T2-PDA, T3- Czapek's dox, T4- Corn starch , T5-OMA, T6- Rice straw, T7- Rice grain agar, T8- Rice leaf, T9- Malt extract, T10- V8 juice agar medium)



P1- *Pyricularia oryzae*, P2- *Bipolaris oryzae* P3- *Rhizoctonia solani*

Fig 1: Graphical presentation of effect of different media on the growth of *Pyricularia oryzae*, *Bipolaris oryzae and Rhizoctonia solani*

Conclusion

The findings showed that the nature of the culture medium affects how the test fungi grow and behave as colonies. It was also found that the semi-synthetic mediums PDA, OMA, and Czapek's dox agar, which were previously thought to be the best medium for fungus growth, are comparable to the natural host extract mediums rice leaf agar medium, rice straw agar medium, and rice grain medium. In rice straw agar medium, *Pyricularia oryzae* showed its greatest growth. *Bipolaris oryzae* showed the greatest growth on rice straw agar, while *Rhizoctonia solani* showed the greatest growth on rice leaf medium. *Bipolaris oryzae* was found to grow at its fastest rate in rice straw agar, and *Rhizoctonia solani* was found to grow at its fastest rate in rice leaf medium. Further in future the research should focus on the enhancement of sporulation of the pathogens.

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