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Effect of Different Media, Temperature, pH and Nitrogen Sources on Growth and Development of *Helminthosporium oryzae* Causing Brown Leaf Spot of Rice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript..

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ABSTRACT

The pathogen *Helminthosporium oryzae* was subjected to different cultural conditions viz., media, temperature, pH and nitrogen sources under *in vitro* conditions. The supreme radial progress of Czapeck Agar, OMA & PDA was recorded highest with mean radial growth followed by Richard's agar (88 mm), Water agar. The colony showed up as thick, leathery, slightly elevated, and abundant mycelia with brown-coloured conidia on this medium. Its colour ranged from greyish-white to dark brown. When exposed to constant light, the average mycelial growth of the fungus was measured at 58.60 mm, 51.41 mm at alternating light cycles, and 72.80 mm at total darkness. When the pathogen was assessed at various pH levels, the fungus's maximum radial growth was observed at pH 7.0 (90.00), and its least radial growth was observed at pH 4.5 (44.89 mm) and pH 5.0 (60.33 mm). Ammonium chloride showed the least growth among the various nitrogen sources, while potassium nitrate showed the greatest development.

Keywords: Radial growth; media; temperature; nitrogen; pH.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a important staple food grain for around 3 billion people across the world. Rice belongs to the family Poaceae and consist of 24 species of genus *Oryza sativa.* Of which only two major rice species *Oryza sativa* and *Oryza glaberrima* are under cultivation worldwide. It is a kharif season crop in India, 25ºC temperature and rainfall of more than 100 cm is appropriate for rice cultivation. An ideal food to include in a healthy diet is rice. It has a decent amount of fiber, protein (7.2%), carbohydrate (78%), vitamins and minerals such as thiamine (2.8 mg/g), iron (38 ppm), and riboflavin (0.5 mg/g) [1]. On some special occasions, rice flour is prepared to make a variety of traditional recipes like rice phirni, rice chakli, modak, etc. Rice is the second most important cereal crop after wheat, which feed about 45% of world population and provides 15% calories need [2]. It is the staple food crop of southern and eastern parts of India [3].

Like other crops, rice is also suffered from various biotic and abiotic stresses. Various biotic causes include the disease caused by fungi, bacteria, viruses, viroid's, nematodes, phytoplasma and also insects and weeds. Abiotic stress includes high and low temperatures, salinity, drought, flooding and nutritional deficiency like Khaira disease and White leaf disease. Various pathogenic microorganism includes fungi bacteria viruses causing disease which reduce crop yield. Approx 12–20% of crop losses are caused by fungi (Rajan, 1987). Major diseases are Blast (*Magnoporthe grisea* boriosis and cavara), Brown spot (*Helminthosporium oryzae* Brenda de haan), False smut (*Ustiloginoidea virens*), Bunt (*Neovossia horrible* padwick and khan), Sheath rot (*Sarocladium oryzae* sawada), and Stem rot (*Sclerotium oryzae* cattahea), Sheath blight (*Rhizoctonia solani)* Seedling blight (*Corticium rolfsii* curzi) Foot rot or the bakanae disease (*Fusarium moniliforme* sheld) and Bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*)**.**

Among these, Bipolaris oryzae-caused brown leaf spot is one of the most common illnesses affecting rice. Since 1900, rice brown leaf spot in Japan has been documented as a result of Bipolaris oryzae (Breda de Haan) Shoemaker (= Helminthosporium oryzae teleomorph = Cochliobolus miyabeanus). It is also referred to as "nai-yake," which includes Helminthosporiopsis, sesame leaf spot, and seedling blight. According to reports, the illness affects every nation that grows rice, including the Philippines, China, Japan, China, Myanmar, Sri Lanka, Bangladesh, Iran, Africa, South America, and Russia [4]. It is known to happen in most of the rice-growing states of India. In 1919, Sundraraman reported on it for the first time from Madras. In the states of Bihar, Chhattisgarh, and Madhya Pradesh, the condition is more severe when it comes to dry or direct-seeded rice. The disease is quite significant in many nations and has been known to result in significant losses in grain yield (up to 90%), especially when the leaf spotting phase takes on epiphytotic dimensions similar to those seen during the 1942 Great Bengal Famine [5].

2. MATERIALS AND METHODS

These experiments were conducted at Biocontrol laboratory, Department of Plant Pathology, College of Agriculture, S.V.P.U.A.T, Meerut, U.P. The pathogen was isolated from infected paddy

plants in the field CRC, S.V.P.U.A.T., Meerut, U.P., infected leaves exhibiting characteristic symptoms of brown leaf spot were collected. The brown spot pathogen was isolated and refined on Potato Dextrose Agar Medium. Ten solid media were used for examine morphological characteristics of the fungus. In order to investigate physiological properties like pH and temperature, eight pH levels 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 as well as six distinct temperature levels 15, 20, 25, 30, 35, and 40°C were examined. For every experiment, three replications were kept at each treatment level. Data was recorded for the radial growth and colony characteristics such as colour, topography, margin, and sporulation. To extract the fungus, drain the medium and pass it through Whatman No. 1 filter paper to weigh the dry mycelial biomass. The filter paper with the fungal mycelial mat was dried in a hot air oven at 60°C for 48 hours. After 48 hours, the pathogen's dry mycelial weight was determined.

In different amounts (1.0, 1.5, and 2%) the following nitrogen sources were added to the potato dextrose broth: proline, calcium nitrate, ammonium nitrate, ammonium chloride, and potassium nitrate. For both experiments, three replications were kept up at each source. Using the previously described method, the pathogen's dry mycelial weight was determined. As recommended by Panse and Sukathme [6], the laboratory research' Completely Randomised Design (CRD) was used to analyse the experimental data.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Morphological and Physiological Characters of the Pathogen

On 10 distinct media, morphological characteristics such as shape, colony colour, texture, edge, and radial growth were investigated (Table 1). Conidia had five to nine septations and a gently curved, broad centre section. When fully developed, conidia had septate mycelia and were reddish or fuliginous. The morphological characteristics of the mycelium and conidia were verified by Kumari et al. [7], who observed that, when seen via a 10X compound microscope, the spore size varied between 5.34-7.48 µm and 4.10-5.51 µm in different isolates grown in PDA media. The maximum fungal growth (90.00 mm) was observed in Czapeck (DOX) Agar, Oat Meal Agar, and Potato Dextrose Agar, followed by Richard's Agar (88 mm) and Water Agar among the ten solid media that were investigated. However, as Table 2 and Fig. 1 show, Rose Bengal agar had the lowest average radial growth of 25 mm after nine days of incubation. Arshad et al. [8] assessed the pathogen's maximal growth (57.80 mm) on potato dextrose agar, and their results were similar to ours. In comparison to the other two treatments under investigation, the fungus exposed to complete darkness for eight days had the highest average mycelial growth, measuring 72.80 mm, according to the results of physiological parameters including temperature, pH, and light regimes. (Table 3). The fungus's average mycelial growth was recorded at 58.60 mm in constant light, 51.41 mm in alternate light cycles, and 72.80 mm in total darkness. Similarly, Hau and Rush [9] discovered that sporulation responded well to a brief cycle of 12 hours of complete darkness. There were eight temperature readings, and the greatest radial development was observed at 30°C (80.12 mm), followed by 25°C (65.18 mm). The lowest radial growth of 35.56 mm was noted at 40°C. These results shared similarities with those of Ram Dayal and Joshi (1968), Ou (1985), Ahmed et al. [10], and Arshad et al. [8]. Arshad et al. [8] found that the temperature between 25°C and 30°C with 38–57 mm of radial growth on PDA medium was best for the development. The present investigation also proves that temperature levels ranging from 25°C to 30°C are best for the growth of the pathogen. Growth of the pathogen was evaluated at different pH levels and a maximum radial growth of the fungus was recorded at pH 7.0 (90.00) followed by 7.5 (89.34), 6.5 (88.70). The least radial growth of the fungus was recorded at pH 4.5 (44.89 mm) and pH 5.0 (60.33 mm) (Table 5). The outcomes matched those of Naresh et al. [11], who found that on potato dextrose agar, the pathogen Bipolaris sorokiniana grew and sporulated most effectively at a pH of 6.0–6.5 with a radial growth of 58.5–89.0 mm.

3.2 Evaluation of Several Nitrogen Sources on the Growth of Pathogen

Six different nitrogen sources were evaluted at three different concentrations (1, 1.5 & 2.0 per cent) in PDB, the growth of *H. oryzae* was observed. The results showed that potassium nitrate was significantly better than the other sources tested, with a highest average dry

mycelial weight of 103.39 mg, followed by peptone at 93.78 mg and proline at 89.17 mg. In contrast, ammonium chloride had the lowest average dry mycelial weight (Table 6). The current study's findings were similar with Naza et al. [12]. According to their findings, potassium nitrate showed the highest average radial growth of 90 mm among the four nitrogen sources examined for *Cochliobolus heterostrophus* [13].

Table 1. Cultural and morphology variability of *Helminthosporium oryzae* on growth media

Sr.No.	Media	Colony	Texture/Edge	Growth
1	Malt Extract Agar	Greyish brown	Leathery thick & raised colony	Profiuse mycelia with conidia
2	Yeast Extract Agar	Whitish Grey	Flat colony	Thin mycelium with no conidia
3	Oat Meal Agar	Greyish Brown	Waxy, thick with raised colony	Profise mycelia with conidia
4	Corn Meal Agar	Greyish to dark brown	Flat colony	Thin mycelium with no conidia
5	Water Agar	Light brown	Flat thread like colony	Thick mycelia no conidia
6	Czapeck (DOX) Agar	Greyish	Waxy, thick with raised colony	Thick mycelia no conidia
7	Sabouraud's dextrose Agar	Greyish at center & white at periphery	Leathery thick & raised colony	Scanty mycelia with conidia
8	Rose bengal Agar	Greyish to brown	Waxy, thick with slightlyraised colony	Thick mycelia no conidia
9	Richards Agar	Greyish at center & white at periphery	Waxy, thick with slightlyraised colony	Profise mycelia with conidia
10	Potato Dextrose Agar	Greyish at center & white at periphery	Waxy, thick with slightlyraised colony	Profiuse mycelia with conidia

Table 2. Effect of growth media on growth of *Helminthosporium oryzae*

Sr.No.	Media	Type of Media	Radial growth(mm)
1	Malt Extract Agar	Non-synthetic	63
2	Yeast Extract Agar	Semi-synthetic	62
3	Oat Meal Agar	Synthetic	90
4	Corn Meal Agar	Synthetic	67
5	Water Agar	Semi-synthetic	70
6	Czapeck (DOX) Agar	Synthetic	90
7	Sabouraud’s dextrose Agar	Synthetic	45
8	Rose bengal Agar	Non-synthetic	25
9	Richards Agar	Synthetic	88
10	Potato Dextrose Agar	Synthetic	90
		Sem (±)	**0.59**
		CD @ 1%	**1.77**
		CV (%)	**1.56**

Table 3. Effect of light regimes on growth of *H. oryzae* and its colony characters

Sr. No.	Light Regimes	Mean Colony Radial Growth (mm)	Colony Character
1	Alternate cycles of (12 hours of light & 12 hours of dark)	51.41	Light brown colour with moderate mycelium growth
2	Complete light (24hours)	58.60	Light brown colour with good mycelium growth
3	Complete dark (24hours)	72.80	Dark brown with good mycelial growth
	Sem (±)	**0.50**	
	CD @ 1%	**1.79**	
	CV (%)	**1.44**	

Table 4. Effect of temperatures on growth of *H. oryzae*

Sr. No.	Treatments	Mean Radial Growth(mm)
1	15	43.70
2	20	50.60
3	25	65.18
4	30	80.12
5	35	43.22
6	40	35.56
	Sem (±)	**0.71**
	CD @ 1%	**2.20**
	CV (%)	**2.32**

Table 5. Effect of pH levels on growth of *H. oryzae*

Sr.No.	pH	Mean radial growth (mm)
1	4.50	44.89
2	5.00	60.33
3	5.50	74.49
4	6.00	87.78
5	6.50	90.67
6	7.00	91.21
7	7.50	89.34
8	8.00	83.45
	Sem (±)	**0.81**
	CD @ 1%	**2.46**
	CV (%)	**1.81**

Table 6. Effect of different nitrogen sources on growth of *H. oryzae*

Sr.No.	Nitrogen sources	Average Dry Mycelial Weight (mg)			Mean
		1.00	1.50	2.00	
1	Calcium nitrate	76.51	82.92	79.71	79.71
2	Potassium nitrate	99.41	107.37	103.39	103.39
3	Ammonium sulphate	82.65	89.38	86.02	86.02
4	Ammonium chloride	55.20	63.15	59.18	59.18
5	Peptone	87.45	100.11	93.78	93.78
6	Proline	85.94	92.39	89.17	89.17
	Sem (±)	**0.43**	**0.36**	**0.64**	
	CD @ 1%	**1.37**	**1.15**	**2.03**	
	CV (%)	**0.92**	**0.72**	**1.14**	

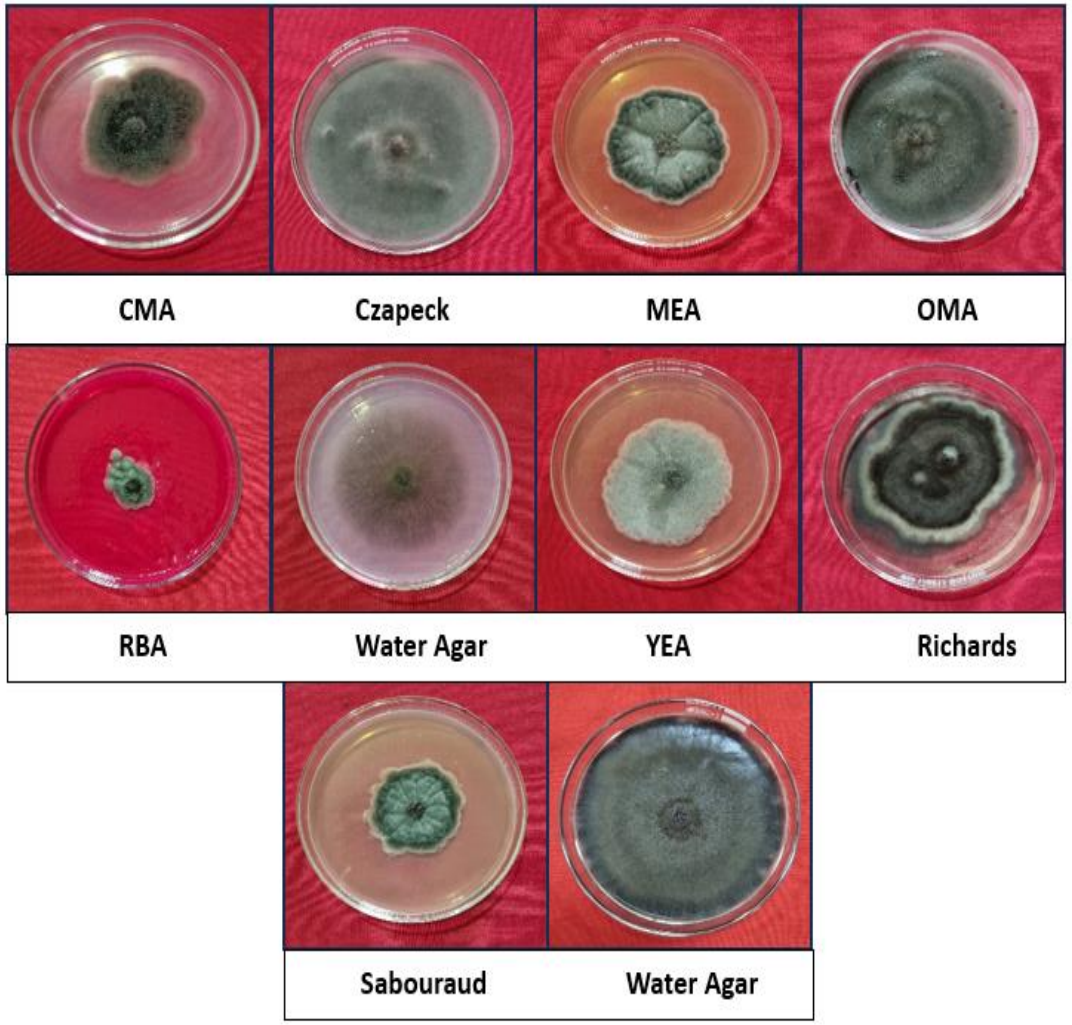

Fig. 1. Radial growth of different media of *H. oryzae*

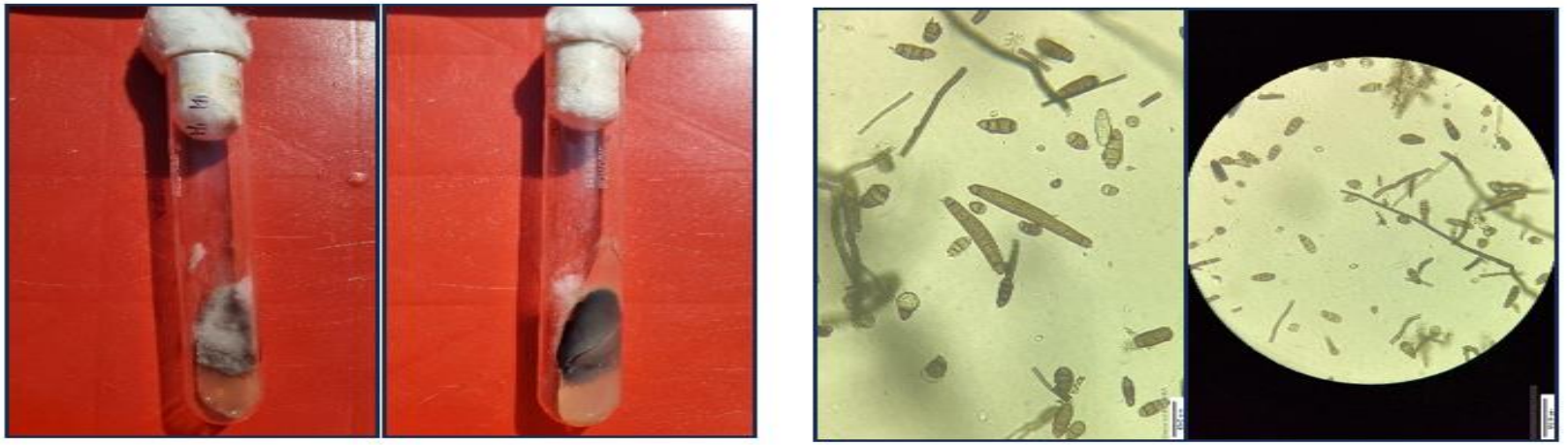

Fig. 2. Morphology & cultural characters of *H. oryzae*

4. CONCLUSION

Ten solid media that were tested, Czapeck Agar, OMA, and PDA was best for the fungus's growth followed by Richard's Agar and Water Agar. Rose Bengal Agar recorded the lowest average radial growth, at 25 mm. Highest radial growth of 80.12 mm among the eight temperature ranges, 30°C was shown to be the optimal temperature; 25°C followed with 65.18 mm, and 40°C recorded the lowest radial growth of 35.56 mm.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I, hereby declare that there is no generative AI technologies used such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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