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Effects of Different Culture Media, pH, Carbon, and Nitrogen Sources on the Growth of *Helminthosporium oryzae*, Causing Brown Spots on Rice

Sandeep Pandey
School of Environmental Biology, APS University, Rewa, Madhya Pradesh, India

ABSTRACT

Background and Objective: In order to study the fungus growth, pathogenicity, and development of control methods, it is essential to cultivate it in a controlled setting using nutritional media and different favorable sources. *Helminthosporium oryzae*, the causative agent of rice brown spot, was assessed in relation to different nutrient media, pH, nitrogen, and carbon sources. **Materials and Methods:** The bio-efficacy of the nutritional media against the fungus was evaluated using potato dextrose agar, cornmeal agar, carrot sucrose-agar, and rice polish agar. The growth of the brown spot pathogen was examined in relation to several pH levels (4, 5, 6, 7, 8, and 9), glucose, sucrose, maltose, fructose, lactose, and mannitol as carbon sources, and sodium nitrate, barium nitrate, lead nitrate, potassium nitrate, and ammonium nitrate as nitrogen sources. The laboratory study employed a Completely Randomized Design (CRD) to examine the experimental data. The one-way ANOVA test was used to evaluate the means' significance. Data analysis was conducted using the Statistical Analytical System (SAS) version 9.4 on the computer. **Results:** According to the study, the best media for mycelial development were potato dextrose agar and rice polished agar. The pathogen's growth was evaluated at different pH levels, and the results indicated that mycelial growth was most supported by pH values between 6 and 7, while pH 4 hindered mycelial growth seven days after inoculation. The best nitrogen and carbon sources that were determined to be appropriate for the mycelial growth were potassium nitrate and sucrose. **Conclusion:** These findings will help to improve the understanding of the pathogen's basic physiology and pave the way for the development of efficient methods for managing rice brown spot.

KEYWORDS

Rice, Brown spot, nutrient media, pH, *Helminthosporium oryzae*

INTRODUCTION

Brown spot of rice caused by *Helminthosporium oryzae* is a serious disease causing adverse impact in rice production in both upland and rainfed regions. It can cause 1 to 34% and, in more severe situations, up to 45% losses in grain yield in Asia and Africa[1-4]. Rainfall and relative humidity are the primary climate factors that influence the disease's incidence, while temperature reduces the occurrence of the brown spot

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pathogen[5]. Direct-seeded rice, low pH, moderate nitrogen, low accessible potassium, moderate to high phosphorus, and soils deficient in essential and trace elements are commonly associated with severe occurrence of disease. In addition, it is mostly caused by the dominance of native land races and is pandemic in regions with high rainfall[6-8].

The pathogen is known to cause damage during various stages, including vegetative growth, seed germination, seedling establishment, the reproductive phase, and storage. At different phases, the pathogen causes different types of damage. It has an impact on seed quality metrics like germination, viability, and growth vigour during storage. It impacts the root and shoot systems during seed germination, resulting in seedling blight, which reduces the survival of seedlings. The overall photosynthetic area decreases during the vegetative phase as a result of brown spots and blight symptoms developing on the leaves. Grain discolouration, inadequate grain filling, and yield loss are the types of damage that occur during the reproductive phase. The incidence of brown spots results in changes to the ionic composition, such as an increase in magnesium, sodium, total soluble sugar, and carotenoids, and a decrease in calcium, potassium, iron, chlorophyll a, chlorophyll b, and total chlorophyll[9].

The symptoms manifested as small, round, dark brown or purple brown dots for young spots and light reddish brown, grey, or whitish patches with dark to reddish brown edges for older spots[10-12]. On glumes and leaves, the lesions appear as light reddish patches with a dark or reddish brown border[13]. The affected spikelets have upright sporophores and prostrate hyphae on their black velvet mycelial mats. The pathogen is characterised by large conidia with 13 somewhat long septa. Conidia usually have a broad, curving distal part that tapers towards a hemispherical apex. Two polar germ tubes are used to germinate mature conidia, which have a thin peripheral wall and a brown colour[10]. According to the hyperspectral remote sensing analysis, during a severe infection, leaf reflectance increased at the ranges of 450 to 500 nm and 630 to 680 nm and decreased at the ranges of 520 to 580 nm, 760 to 790 nm, 1550 to 1750 nm, and 2080 to 2350 nm, respectively, as the percentage of infected leaf surface increased[14].

In order to cultivate fungi in a lab setting, the right culture media formulations and chemical growth factors including molecular, biological, or physical factors, are needed to promote mycelial development and spore germination. The fungi need technological advancements for culturing since they have a higher risk of cross-contamination between isolates in current *in vitro* or microfluidics culturing methods[15]. Mycological research and the development of cost-effective, practical, and environmental friendly techniques for a healthy management practice against fungal pathogens will benefit from an understanding of the pathogen biology of rice fungal pathogens[16-18]. It has previously been documented that the fungus uses several nutritional sources for development and reproduction[19-23]. Therefore, the present research aimed to determine the ideal media, pH, carbon, and nitrogen sources for the *in vitro* growth of the rice brown spot pathogen.

MATERIALS AND METHODS

Single spore isolation from leaf surface: Brown spot-infected leaves of the improved rice variety 'Basmati' were collected from the rice field in Rewa (24°31'50.62"N and 81°17'56.79"E), Madhya Pradesh, India, and brought to the laboratory for further investigation. The leaves were cleaned with water 2-3 times and finally with distilled water. The diseased tissue of the leaves was cut into 5×5 mm discs and then cleaned in 0.1% mercuric chloride for 1-2 min. These cleaned discs were transferred to potato dextrose agar in a Petri plate and then incubated at room temperature and kept for further study.

Culture media and study of mycelial growth: Four nutrient media viz., potato dextrose agar, cornmeal agar media, carrot sucrose-agar media, and rice polish agar media were tested[24]. The inoculation of fungal culture was done by the disc method[25]. Discs of 5 mm diameter of the twelve days old fungal

culture were cut by a cork borer from the margins and were placed centrally in the Petri dishes containing nutrient media. Fungal radial growth was measured after incubation at 25±2°C and 24 hrs intervals up to seven days.

Study of different pH levels: A PHILIPS digital pH meter was used to study the effect of different pH levels on the brown spot pathogen. The potato dextrose agar medium was adjusted to different pH levels 4, 5, 6, 7, 8, and 9 using 0-1 N hydrochloride acid on 0-1 N sodium hydroxide. The result was buffered with citrate phosphate (0.1 M citric acid solution, 0.2 M Na HPO•7H_2O) for the appropriate pH. Following inoculation, plates were incubated in the dark at 20°C. Three replicate plates for every pH treatment were used in the random full block experimental design. A 5 mm disc mycelial culture was seeded in the pH-adjusted media. Colony diameters were recorded every day following inoculation, and colonies with a pH higher than 7 were again measured 10 and 20 days later. Radial growth was computed daily.

Carbon and nitrogen sources: Potato dextrose agar media were used as basal media to study the effect of carbon and nitrogen sources. The carbon compounds viz., glucose, sucrose, maltose, fructose, lactose, and mannitol were tested at a concentration equivalent to the carbon present in an amount of dextrose 20 g/L in the basal media. The nitrogen compounds sodium nitrate, barium nitrate, lead nitrate, potassium nitrate, and ammonium nitrate were added at 2 g/L in the basal media. The mycelial diameter was recorded after incubation at 25±2°C and 24 hrs intervals up to six days.

Statistical analysis: A Completely Randomized Design (CRD) was used in the lab investigation to analyze the experimental data. Variance analysis was performed on all of the data. The significance of the means was assessed using the One-way Analysis of Variance (ANOVA) test. The computer's Version 9.4 of the Statistical Analytical System (SAS) was used for data analysis.

RESULTS AND DISCUSSION

Effect of culture media: The pathogen's radial mycelial growth rates exhibit variance in growth rates in several culture media with significant (p = 0.05) impact (Table 1). The potato dextrose agar (PDA) medium had the highest mycelia growth among the five media, followed by the rice polished agar medium. After five days of inoculation, the fungus developed a compact, round, colourless colony that extended to the plate's edge. Thin mycelium is shown during mycelial growth on carrot and maize meal agar media. According to the investigation, the fungus could not develop mycelially on Czapek-Dox Agar media.

The study supports the results of Arshad *et al*.[26], who found that after 96 hrs of incubation, the pathogen showed its maximum development on potato dextrose agar and malt extract. A study conducted by Billah *et al*.[27] observed that honey peptone agar, carrot agar, potato sucrose agar, and kauffman's agar were the best nutritional media for the growth of the tomato pathogenic seed-borne fungus *Curvularia lunata*. According to Johnson and White[28] the pathogen produces a higher percentage of anthraquinone pigments, including Cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone), trihydroxy-methyl, and dihydroxy-methyl anthraquinone per gram of growth in Czapek-Dox media. Valarmathi and Ladhalakshmi[29] also found that the pathogen grew most radially in potato dextrose agar medium, then in rice extract+PDA, and that rice polish agar medium improved sporulation five days after inoculation. However, Channakeshava and Pankaja[30] found that the radial growth was highest on paddy leaf extract agar (84.83 mm), and thereafter potato dextrose agar (61.33 mm). Likewise, after 144 hrs of incubation, Thakur *et al*.[31] noticed that paddy leaf extract exhibits the highest mycelium development and sporulation, followed by oat meal and maize meal agar media. According to Basavaraj *et al*.[32], fungal sporulation occurs five days after growing on rabbit food agar media.

Table 1: Effect of nutrient media, pH level, and different nitrogen and carbon sources on growth of *H. oryzae* after five days of incubation

Media	Mean colony diameter (mm)	pH	Mean colony diameter (mm)	Nitrogen sources	Mean colony diameter (mm)	Carbon sources	Mean colony diameter (mm)
Czapek-Dox	0.00	4	0.00	Sodium nitrate	24.85	Maltose	23.50
Potato dextrose	52.43	5	25.73	Barium nitrate	41.10	Sucrose	30.20
Corn meal	29.50	6	34.60	Lead nitrate	0.00	Fructose	22.20
Rice polished	51.00	7	35.50	Potassium nitrate	51.67	Lactose	20.20
Carrot	40.00	8	24.00	Ammonium nitrate	44.90	Dextrose	20.30
		9	28.83	Ammonium chloride	37.40	Mannitol	21.40
						Cellulose	21.30
SEM±	0.39		0.43		0.26		0.07
CD (5%)	2.16		2.03		1.24		0.33

Effect of pH: The fungus reacts significantly to the pH level variations. The pathogen's active mycelial development required a pH range of 6-7 (Table 1). After five days of incubation, the colony diameter averaged 35.50 mm, indicating optimal growth at pH 7. This was followed by pH 6. The fungus's mycelial development rises between pH 5.0 and pH 7.0 before beginning to decline; pH 9.0 showed the least amount of growth. It was discovered that pH 4 was unsuitable for fungal growth. The highest dry mycelial weight of 113.0 mg was recorded by Channakeshava and Pankaja[30] at pH 7.0, followed by 103.0 mg at pH 7.5 and 97.32 mg at pH 6.5. Thakur *et al.*[31] observed that after 144 hrs of incubation, pH 7.0 produced the highest mycelium growth (86.52 mm) and sporulation (6.7×104 CFU/mL). Singh *et al.*[33] found that the pathogen shows maximum radial growth at pH 7.0 (90.00) and least at pH 4.5 (44.89 mm). Similarly, Rao and Kumar[34] observed that pH 7 promoted maximum mycelial growth (39.50 mm) and sporulation (4.9×10^6 conidia/mL) of *Pyricularia grisea*, which causes blast in rice.

Effect of nitrogen sources: The pathogen's mycelial development varies remarkably depending on the nitrogen source (Table 1). The highest mycelial diameter was supported by potassium nitrate (51.67 mm), followed by ammonia nitrate (44.90 mm) and barium nitrate (41.10 mm), respectively. The least amount of development in mycelial diameter (25.20 mm) was observed in sodium nitrate (24.85 mm). The growth of the fungus was not fostered by lead nitrate. Channakeshava and Pankaja[30] noticed that the brown spot pathogen grows at its maximum at 2.0% ammonium peptone (115.24 mg). Singh *et al.*[33] observed that among the different nitrogen sources, potassium nitrate exhibited the greatest *H. oryzae* mycelium development, whereas ammonium chloride showed the least growth. The study is in accordance with Rao and Kumar[34] who reported that barium nitrate was found to sustain greatest growth (61.20 mm) among nitrogen sources, followed by ammonium nitrate (45.80 mm), without encouraging the sporulation of the rice blast fungus *Pyricularia grisea*. According to Murti *et al.*[35], rice plants containing urea as a nitrogen source had the highest percentage of sheath blight lesions (52.09%), followed by calcium nitrate (42.48%).

Effect of carbon sources: The findings showed that the growth of mycelial cells varied according to the carbon sources. The largest mycelial diameter (30.20 mm) was supported by sucrose, followed by fructose (22.20 mm) and maltose (23.50 mm) among the carbon sources (Table 1). In mannitol, cellulose, and dextrose, the mycelial development occurs in the following descending sequence. Lactose showed the least amount of fungal growth (20.20 mm). Among the various carbon sources, Channakeshava and Pankaja[30] observed that glucose had the brown spot pathogen's maximal growth (128.88 mg). The study also supports the findings of Murti *et al.*[35], who found that rice leaves fed with lactose, fructose, and sucrose medium as carbon sources had the greatest number and largest sheath blight lesions. The findings were in tune with Rao and Kumar[34], who found that maltose (32.25 mm) and dextrose (26.20 mm), after 144 hrs of incubation without producing sporulation were suitable carbon sources for enhancing the growth of the rice blast fungus.

CONCLUSION

This study demonstrated the impact of culture media, pH, and sources of carbon and nitrogen on *H. oryzae* mycelial growth. Mycelial growth was found to be more effective with potato dextrose agar, a culture medium with a pH of 6 to 7, potassium nitrate as a nitrogen source, and sucrose as a carbon source. In addition to helping create efficient methods for managing fungal diseases and mycotoxin contamination, these discoveries will offer valuable information for a deeper comprehension of the intricate and multi-level regulatory network pertaining to fungal development.

SIGNIFICANCE STATEMENT

In highland and rainfed areas across the world, brown spot of rice results in significant yield losses. Diseases are caused by a combination of environmental factors and fertilizer application, which provides nutrients. As a result, it is essential to evaluate the favourable environmental circumstances and essential nutrients for the fungus's sporulation and mycelial growth. This will aid in the development of an efficient plan for controlling the pathogen and rice brown spot infections.

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