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Effect of temperature, pH and media on growth and sporulation of *Helminthosporium oryzae*

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Abstract
Brown leaf spot of rice is found to be one of the most destructive diseases of rice. The study was carried out to determine the effect of different temperatures, pH and media on growth and sporulation of *Helminthosporium oryzae* under *in vitro* condition. Among different temperature range studied, maximum mycelium growth with 28.66 mm, 54.62 mm and 83.60 mm was observed at 30 °C on 48, 96 and 144 hours after inoculation with sporulation (6.1×10^4 cfu/ml) followed by 25 °C, 35 °C and 20 °C. Maximum mycelium growth 21.80 mm, 43.46 mm and 86.52 mm was recorded at pH7.0 after 48, 96 and 144 hours of incubation with recorded sporulation (6.7×10^4 cfu/ml) followed by pH 6.0. Among nine different cultures media, maximum mycelium growth recorded at Paddy leaf extract medium with 27.22 mm, 64.60 mm, 90.00 mm after 48, 96 and 144 hours with recorded sporulation of 7.2×10^4 cfu/ml followed by Oat meal medium and Corn meal agar medium.

Keywords: *Helminthosporium oryzae*, temperature, pH, media**,** mycelium growth

Introduction
Rice (*Oryza sativa* L.) is a cereal grain belonging to the poaceae family. It contains high amounts of carbohydrates, proteins, and fats. Rice serves as a primary caloric source for approximately one-fifth of total global calories consumed, according to Fukagawa and Ziska (2019) [6]. This makes rice the principal food for nearly 90% of the world's human population, especially in Asia. Statistics from 2021 indicate that worldwide, rice is cultivated across 165.65 million hectares, with total production reaching 515.08 million metric tons at productivity of 5090 kg per hectare (Anonymous, 2022) [2]. India stands as the second largest rice producer globally in terms of area and production, following China. Specifically in India, rice is grown on 45 million hectares, yielding a record 127.93 million tons of rice at a productivity of 2713 kg per hectare during 2021 (Anonymous, 2022) [2].

Despite a rapid increase in rice production, the crop confronts numerous biotic and abiotic stresses, leading to decreased productivity. Rice is vulnerable to over 70 diseases originating from pathogens like fungi, bacteria, viruses, and nematodes. Among these, *Helminthosporium oryzae* (Breda de Haan) Shoemaker, also known as *Cochliobolus miyabeanus*, is a highly destructive fungal pathogen responsible for rice brown spot disease. This ailment caused two significant famines - the Krishna-Godavari delta famine in 1918-1919 and the devastating Bengal famine in 1942, resulting in around 2 million casualties (Padmanaban, 1973; Chakrabarthi, 2001) [7]. Both rice seedlings and mature plants are susceptible to brown spot disease (Manandhar *et al.*, 2016) [10], with symptoms manifesting on leaves, panicles, glumes, and post-infection grains. Initial signs involve small circular brown to purple-brown spots. The disease is considered both seed-borne and air-borne. Spot size ranges from roughly 1/8 inch in diameter, varying from circular to oval based on environmental conditions.

Past investigations have determined that the most favorable temperature range for the growth and conidial germination of *H. oryzae* is 27-30 °C and 25-30 °C, respectively, accompanied by an optimal pH range of 6.8-7 (Ou, 1985) [12]. Fungal development is influenced by various factors like culture medium, temperature, and light exposure. Nutrients are essential for all living organisms, including fungi, to facilitate growth and reproduction. In laboratory settings, the growth medium must encompass all necessary elements and compounds to sustain life processes. However, no single medium suits all fungi universally. Thus, this current study aims to assess how different temperatures, pH levels, and culture media impact the growth rate

and sporulation of *H. oryzae.* This investigation focuses on measuring *Helminthosporium oryzae* growth rate and sporulation under varying conditions of temperature, pH, and culture media.

Materials and Methods

The *in vitro* experiments were carried out within the Plant Pathology laboratory at S.G. CARS in Jagdalpur, Bastar, (C.G.).

Effect of different temperature on growth and sporulation of *H. oryzae*

The effect of different temperature on the growth and sporulation of *Helminthosporium oryzae* was studied at six temperature levels *viz*., 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C. Petri plates were sterilized and 20 ml of sterilized PDA medium was poured. Using a sterilized cork borer, a 5 mm disc was cut from 12 days old pure culture of *H. oryzae* and inoculated in the center of each petri dish. Each treatment was repeated five times and mycelium diameter was recorded after incubation *i.e*., 48, 96, 144 hours interval.

Sporulation was noted after a 12-day interval, following which a spore suspension was prepared. A 5 mm mycelia disc was aseptically extracted using a sterilized cork borer, and the mycelia were delicately detached using a needle. Subsequently, the mycelia were softly blended in 10 ml of sterile distilled water. A 20 µl portion of the resulting spore suspension was placed onto a clean slide, covered with a cover slip, and the spore count per microscopic field was then observed.

Effect of different pH on growth and sporulation of *H. oryzae*

The sets of six different pH medium were adjusted from 4, 5, 6, 7, 8 and 9 levels to study the effect of pH on growth and sporulation of the *H. oryzae*. The pH of the culture media was adjusted prior to autoclaving by carefully adding either 0.1 N HCl and 0.1 N NaOH solutions while monitoring the pH value with an electronic pH meter. Petri plates were sterilized in hot air oven and 20 ml of sterilized PDA medium was poured. Using a sterilized cork borer, a 5 mm disc were cut from 12 days old culture and inoculated in the center of each petri dish. Each treatment was repeated five times and mycelium diameter was recorded after incubation (27±1 °C) *i.e.*, 48, 96, 144 hours interval and spore production was observed.

Evaluation of different media on growth and sporulation of *H. oryzae*

The effect of different media on the growth and sporulation of *Helminthosporium oryzae* was studied using nine media *viz*., Czapek dox agar medium (components per 1000 ml of water: sucrose (30 g), The components include: 2 g of sodium nitrate, 1 g of dipotassium phosphate, 0.5 g of magnesium sulfate, 0.5 g of potassium chloride, 0.01 g of ferrous sulfate, and 15 g of agar. Malt extract agar medium (Malt extract 20 g, Agar 20 g, Water 1000 ml), Sabouraud dextrose agar medium (Dextrose 40 g, peptone 10 g, agar 20 g, water 1000 ml), Oat meal medium (Oat meal 40g, agar 20 g, Water 1000 ml), Potato dextrose agar medium (Potato 200g, Dextrose 20 g, Agar 20 g, Water 1000 ml), Corn meal medium (Corn meal 50 g, Glucose 20 g, Agar 20 g water 1000 ml), Rice polish medium (Rice polish 200 g, dextrose 20 g, Agar 20 g, Water 1000 ml), Rose bengal agar medium (Dextrose 10 g, Rose Bengal 0.05 g, Agar 20 g, Water 1000 ml), and Paddy leaf extract medium (Rice leaf 200 g, Agar 20 g, Water 1000 ml). Petri plates were sterilized and 20 ml of sterilized medium was poured. Using a sterilized cork borer, a 5 mm disc was cut from 12 days old pure culture of *H. oryzae* and inoculated in the center of each petri dish. Each treatment was repeated five times and mycelium diameter was recorded after incubation (27±1 °C) *i.e*., 48, 96, 144 hours interval and spore production was observed.

Results and Discussions

Effect of different temperature on growth and sporulation of *H. oryzae*

The obtained result shown in (Table 1, Fig.1and Plate 1). The mycelial growth were recorded in different incubation period *viz.,* 48,96, 144 hours and observed the maximum mycelial growth (28.66 mm) in temperature 30 °C followed by 18.40, 14.60 and 12.20 mm recorded at temperature 25 °C, 35 °C and 20 °C respectively. Observation recorded after 96 hours of inoculation and observed the maximum mycelial growth (54.62 mm) in temperature 30 °C followed by 36.20, 30.40, 28.20 and 14.40 mm recorded at temperature 25 °C, 35 °C, 20 °C and 40 °C respectively.

Comparably, the mycelial growth of *H. oryzae* recorded after 144 hours of incubation showed that maximum mycelial growth was observed 83.60 mm at 30 °C followed by 67.58 mm at 25 °C, 47.60 mm at 35 °C, 42.56 mm at 20 °C and 24.64 mm at 40 °C and minimum mycelium growth was recorded at 15 °C (6.80 mm).

The results recorded of the sporulation after 12 days of incubation of *H. oryzae* in different temperature. The maximum sporulation was observed significantly higher at 30 °C (6.1 x 10^4 cfu/ml) which was followed by 25 °C (3.8 x 10^4 cfu/ml) and minimum sporulation was recorded 35 °C (1.4 x 10^4 cfu/ml) and the sporulation was not observed at 15 °C and 40 °C.

The present results of the investigation are in line with Channakeshava and Pankaja (2018) [5] who reported that temperature 30 °C, 25 °C were best for fungal growth with maximum radial growth of 70.67 mm and 62.83 mm.

The optimum temperature, growth for mycelial and sporulation of *H. oryzae* was found to be 30 °C. This is likely because 30 °C falls within the fungus's thermal tolerance range, allowing for optimal metabolic activity and physiological processes. Both lower and higher temperatures would have inhibited these processes, restricting growth and reproduction. A narrow temperature band is common for fungal pathogens, beyond which stresses can compromise their life cycle functions. Similar results were obtained from Arshad *et al.,* (2013) [4] who reported that best grown media for maximum growth of *Bipolaris oryzae* after 96 hours of incubation with optimum temperature at 28 °C.

Effect of different pH on growth and sporulation of *H. oryzae*

Six different pH *i.e.,* pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0, pH 9.0 was evaluated for mycelial growth and sporulation of *H. oryzae* under different incubation period *i.e.* 48, 96, 144 hours result shown in Table 2, Fig. 2, and Plate 2.

The data reported that the maximum mycelial growth of *H. oryzae* were recorded at pH 7.0 (21.80 mm) followed by pH 6.0 (19.48 mm), pH 8.0 (17.60 mm) and minimum mycelium

growth was significantly recorded at pH 4.0 (8.68 mm) after 48 hours of incubation. Similarly after 96 hours of incubation the maximum mycelial growth were observed in pH 7.0 (43.46 mm) which was followed by pH 6.0 (38.80 mm) and pH 8.0 (36.62 mm) whereas minimum mycelium growth was observed at pH 4.0 (16.60 mm).

Comparably, the mycelial growth of *H. oryzae* after 144 hours of incubation, was observed in pH 7.0 (86.52 mm) which was followed by pH 6.0 (57.20 mm) and pH 8.0 (53.20 mm) whereas minimum mycelium growth observed at pH 4.0 (22.40 mm).

The results were recorded for sporulation after 12 days of incubation of *H. oryzae* in different pH. The sporulation was observed significantly higher in pH 7.0 (6.7 x 10^4 cfu/ml) which was followed by pH 6.0 (3.8 x 10^4 cfu/ml) and minimum sporulation was observed pH 5.0 (1.3 x 10^4 cfu/ml) and the sporulation was not observed in pH 4.0 and pH 9.0.

The optimum pH for growth of mycelial and sporulation of *H. oryzae* was found to be pH 7.0. Fungal cells need a slightly acidic or neutral environment for their physiological processes to function properly. At pH 7.0, the metabolic activities and nutrient absorption of *H. oryzae* are likely optimal for vigorous reproductive growth. Very acidic (pH 4.0) or alkaline (pH 9.0) conditions can disrupt nutrient dynamics and cause cellular stress, hampering fungal colonization and sporulation. A circum neutral pH of 6.0-8.0 is common for most fungal pathogens to thrive best, which was observed for *H. oryzae* in this study as well. Similarly Ramesh *et al.,* 2021 also reported pH 6.0 to pH 7.0 were observed in ideal for radial mycelial growth and sporulation.

Evaluation of different media on growth and sporulation of *H. oryzae*

The data showed that in Table 3, Fig.3 and Plate 3 showed that after 48 hours of incubation the mycelium growth of *H. oryzae* was found significantly on different media. The result revealed that the maximum mycelial growth of *H. oryzae* was significantly recorded in Paddy leaf extract medium (27.22 mm) which was followed by Oat meal medium (26.40 mm), Corn meal agar medium (21.44 mm), Potata dextrose agar medium(17.60 mm) whereas minimum mycelium growth was recorded in Sabouraud dextrose agar medium (11.00 mm).

Result recorded after 96 hours of inoculation showed that the mycelial growth of *H.*

oryzae was maximum in Paddy leaf extract medium (64.60 mm) followed by Oat meal medium (62.40 mm), Corn meal agar medium (59.40 mm), Potato dextrose agar medium (44.80) whereas minimum mycelium growth was recorded in Sabouraud dextrose agar medium (24.20 mm).

Comparably, the data of mycelial growth of *H. oryzae* recorded after 144 hrs of inoculation revealed that maximum mycelial growth was in Paddy leaf extract medium, Oat meal medium and Corn meal agar medium (90.00 mm), which was followed by Potato dextrose agar medium (77.26 mm) and minimum mycelium growth was recorded in Sabouraud dextrose agar medium (35.22 mm).

The results recorded of the sporulation after 12 days of incubation of *H. oryzae* in different growth media. The maximum sporulation was observed significantly higher in Paddy leaf extract medium (7.2 x 10^4 cfu/ml) followed by Potato dextrose agar medium (5.6 x 10^4 cfu/ml) and the sporulation was not observed in rose bengal agar medium and sabouraud dextrose agar medium.

The optimum growth medium for mycelial growth and sporulation of *H. oryzae* was found to be paddy leaf extract medium. This is likely because it provided nutrients most similar to the natural habitat of the fungus. Paddy leaves would contain sugars, proteins, minerals and other compounds utilized efficiently by *H. oryzae* for its growth and reproduction. In contrast, defined media like Sabouraud dextrose agar lacked certain components, restricting fungal proliferation. Complex media mimicking the host environment enabled maximal physiological activity of *H. oryzae*. This underscores the importance of simulating natural conditions for pathogens to express their life cycle processes appropriately *in vitro*. According to findings of Channakeshava and Pankaja (2018) [5] it was found that the most efficient media was Paddy leaf extract with the maximum radial growth of 84.83 mm and the second-best media was by Potato dextrose agar with radial growth of 61.33 mm.

The findings aligned with the findings of Meghana and Hiremath (2019) [11], who noted that Potato Dextrose Agar exhibited the highest radial growth (89.33 mm) across all isolates, which was followed by Host Extract Dextrose Agar (87.25 mm).

Table 1: Effect of temperature on growth and sporulation of *H. oryzae*

Temperature °C	Incubation period (hrs)			Sporulation
	48 hrs	96 hrs	144 hrs	x 10^4 cfu/ml
	*Mean mycelium growth (mm)			
15 °C	0.0	0.0	6.80	0.0
20 °C	12.20	28.20	42.56	1.7 x 10^4
25 °C	18.40	36.20	67.58	3.8 x 10^4
30 °C	28.66	54.62	83.60	6.1 x 10^4
35 °C	14.60	30.40	47.60	1.4 x 10^4
40 °C	0.0	14.40	24.64	0.0
SEm±	0.255	0.243	0.298	
C.D. at 5%	0.750	0.714	0.874	
C.V.%	4.641	1.990	1.464	

*Mean of five replications

Table 2: Effect of different pH on growth and sporulation of *H. oryzae*

pH level	Incubation period (hrs)			Sporulation x 10^4 cfu/ ml
	48 hrs	96 hrs	144 hrs	
	*Mean mycelium growth (mm)			
pH 4.0	8.68	16.6	22.4	0
pH 5.0	17.2	32.2	51.6	1.3 x 10^4
pH 6.0	19.48	38.8	57.2	3.8 x 10^4
pH 7.0	21.8	43.46	86.52	6.7 x 10^4
pH 8.0	17.6	36.62	53.2	1.8 x 10^4
pH 9.0	13.4	22.82	34.6	0
SEm±	0.248	0.316	0.262	
C.D. at 5%	0.729	0.927	0.768	
C.V.%	3.393	2.224	1.148	

*Mean of five replication

Table 3: Evaluation of different media on growth and sporulation of *H. oryzae*

Name of culture media	Incubation period (hrs)			Sporulation 10^4 cfu/ml
	48 hrs	96 hrs	144 hrs	
	*Mean mycelium growth (mm)			
Czapek dox agar medium	16.20	33.60	47.40	1.4 x 10^4
Malt extract agar medium	20.16	43.60	57.60	1.1 x 10^4
Sabouraud dextrose agar medium	11.00	24.20	35.22	0.0
Oat meal medium	26.40	62.40	90.00	3.9 x 10^4
Potato dextrose agar medium	17.60	44.80	77.26	5.6 x 10^4
Corn meal agar medium	21.44	59.40	90.00	4.2 x 10^4
Rice polish agar	12.32	38.20	62.50	2.1 x 10^4
Rose bengal agar medium	11.56	31.20	39.40	0.0
Paddy leaf extract agar medium	27.22	64.60	90.00	7.2 x 10^4
SEm±	0.277	0.309	0.310	
C.D. at 5%	0.799	0.890	0.894	
C.V.%	3.406	1.547	1.060	

*Mean of five replication

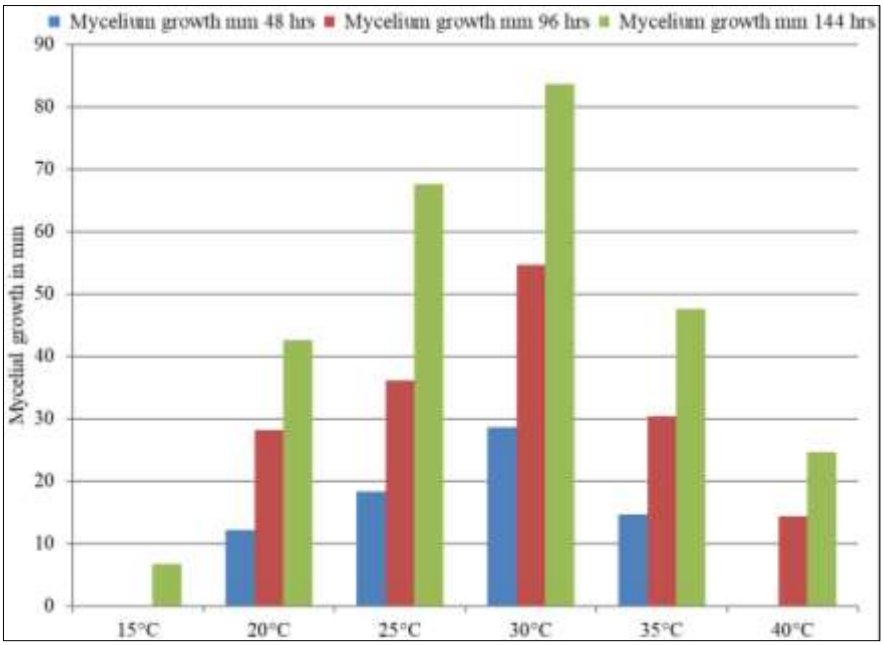


Fig 1: Effect of temperature on growth of *H. oryzae*

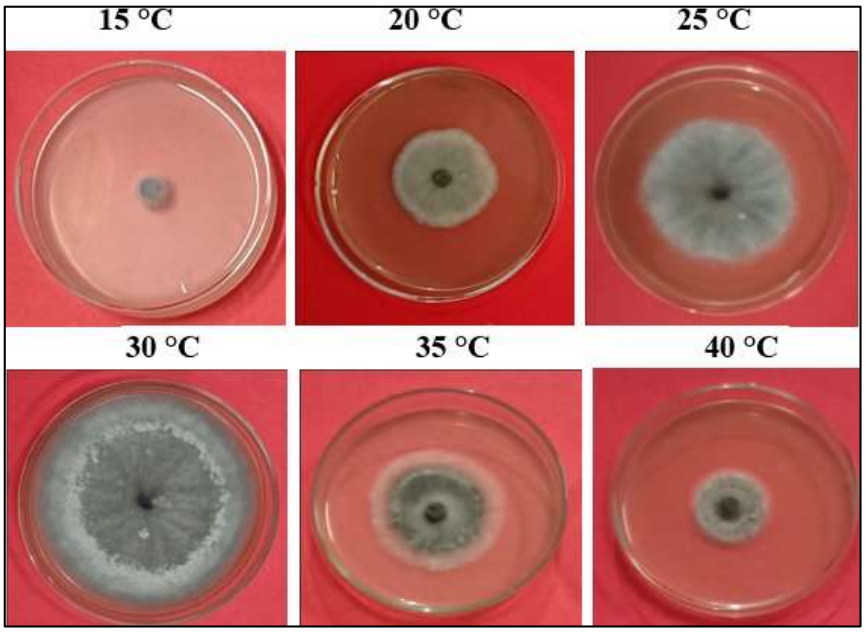


Plate 1: Effect of temperature on growth of *H. oryzae*

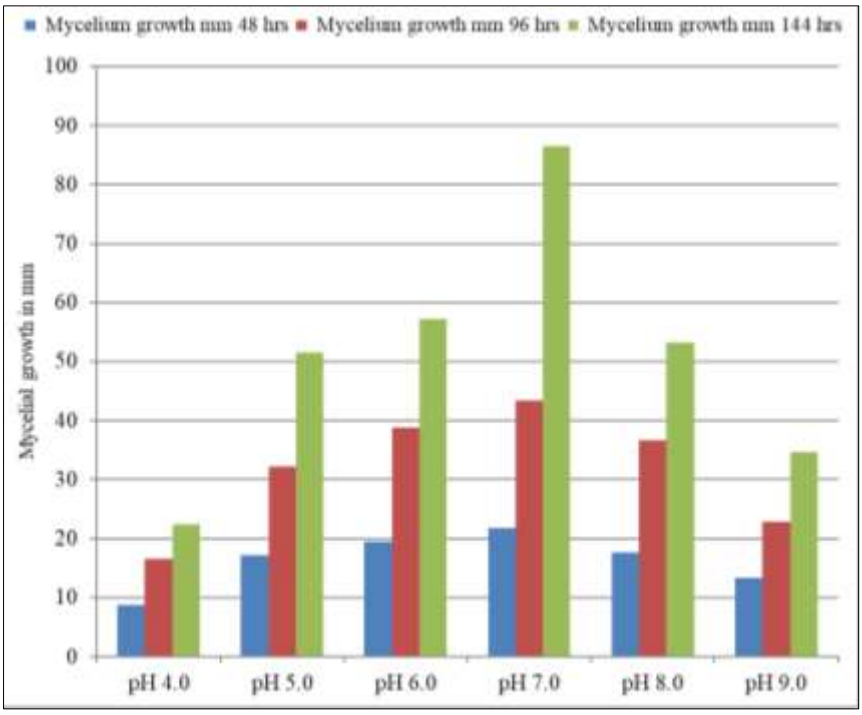


Fig 2: Effect of different pH on growth of *H. oryzae*

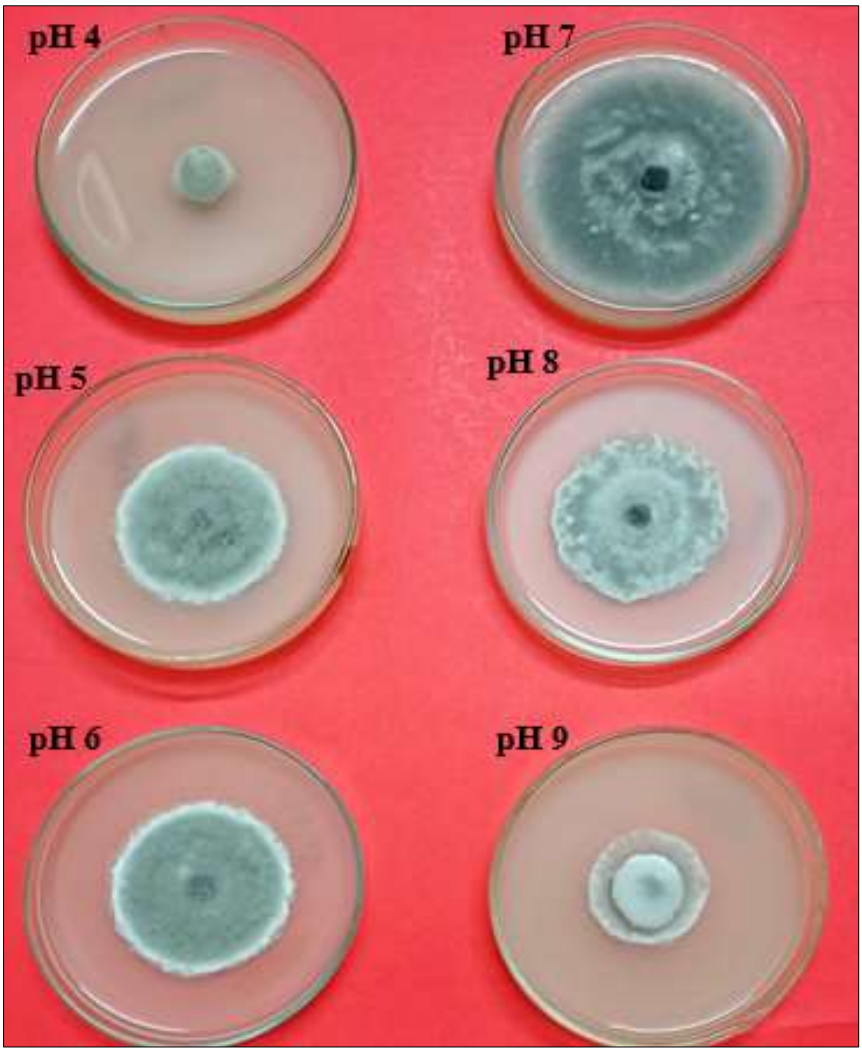


Plate 2: Effect of different pH on growth of *H. oryzae*

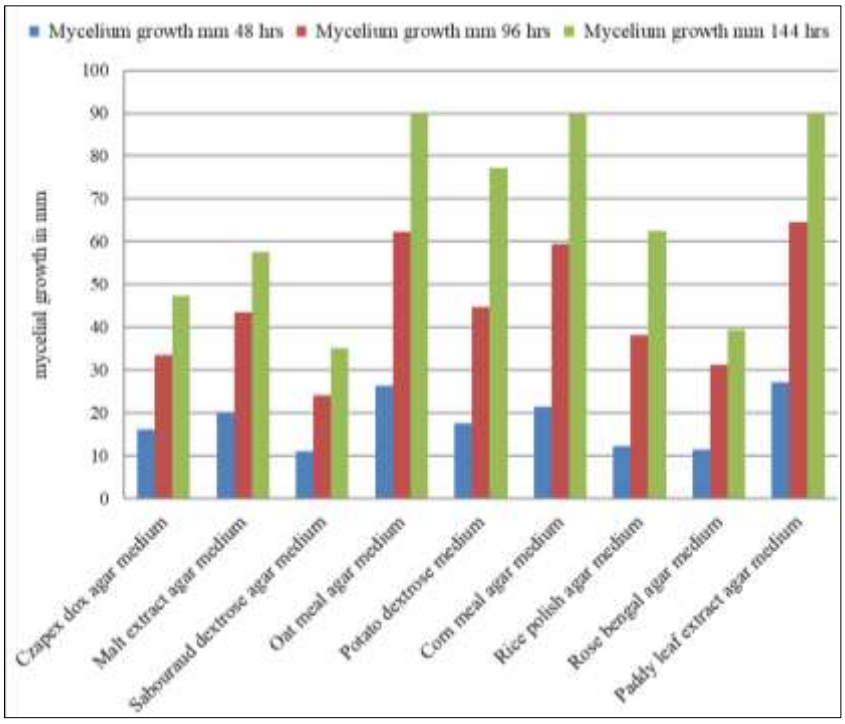
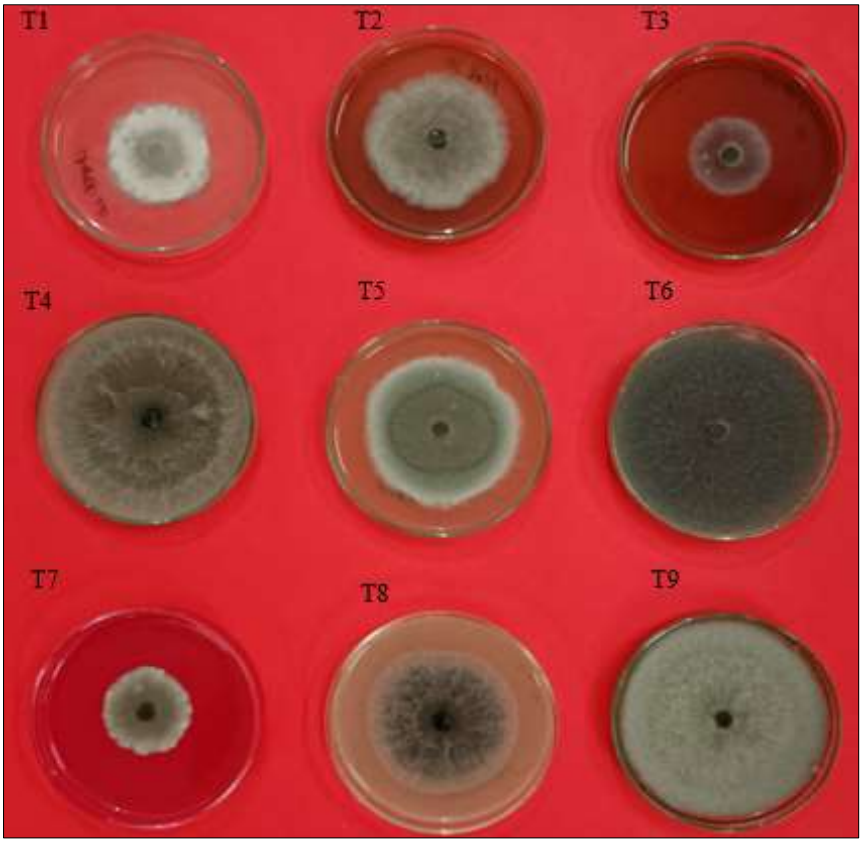


Fig 3: Evaluation of different cultural media on growth of *H. oryzae*



T_1-Czapex dox agar medium, T_2-Malt extract agar medium, T_3-Sabouraud dextrose agar medium, T_4-Oat meal medium, T_5-Potato dextrose agar medium, T_6- Corn meal agar medium, T_7-Rose bengol agar medium, T_8- Rice polish agar T_9-Paddy leaf extract media

Plate 3: Evaluation of different cultural media on growth of *H. oryzae*

Conclusion

The aim of the present investigation was to determine the effect of different temperature, pH and media on mycelia growth and sporulation of *Helminthosporium oryzae*. Among the different temperature, pH and growth media studied it was found that maximum mycelial growth and sporulation was observed at 30 °C and pH 7.0. The most effective media for the culture growth of the pathogen was Paddy leaf extract agar medium over the different media studied followed by Oat meal agar medium and Corn meal agar medium.

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References

1. Anonymous. Agricultural Statistics Report, Department of Agriculture & Farmers Welfare, Directorate of Economics and Statistics, Govt. of India, New Delhi; c2020 May, p.49.
2. Anonymous. World Agricultural Production, Foreign Agricultural Service, United States Department of Agriculture, Washington, D.C., United States, September; c2022. p.38.
3. Anonymous. Annual Report, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Govt. of India, New Delhi, August; c2022. p.8.
4. Arshad HMI, Hussain N, Ali S, Khan JA, Saleem K, Babar MM. Behavior of *Bipolaris oryzae* at different temperatures, culture media, fungicide and rice germplasm for resistance. Pakistan J Phyto pathol, 2013;25(01):84-90.
5. Channakeshava C, Pankaja NS. Effect of media, temperature, light, pH and nutrient source on growth and development of *Bipolaris oryzae* causing brown leaf spot of Paddy. Int. J Curr. Microbial. App. Sci. 2018;7(7):1713-1722.
6. Fukagawa NK, Ziska LH. Rice: Importance for Global Nutrition. J. Nutr. Sci. 2019;65:S2-S3.
7. Gangopadhyay S, Padmanabhan SY. Breeding for disease resistance in rice. Oxford and IHB Publication Co. Calcatta; c1987. P. 340.
8. Jaiganesh V,Kannan C. Studies on the cultural characters and pathogenicity studies of brwon leaf spot of rice caused by *Helminthosporium oryzae* (Syn: *Bipolaris oryzae*). Plant Archives. 2019;19(1):585-587.
9. Kumri S, Kumar A, Rani S. Morphological characterization of *Bipolaris oryzae* causing brown spot of paddy in Bihar. Int. Educ. Res. J. 2015;1(5):85-87.
10. Manandhar HK, Timila RD, Sharma S, Joshi S, Manandhar S, Gurung SB, *et al.* A field guide for identification and scoring methods of diseases in the mountain crops of Nepal. In Bioversity International; c2016.
11. Nayak MS, Hiremath SV. Cultural, morphological and molecular characterization of *Bipolaris oryzae* causing brown leaf spot of rice in Northern Karnataka. Journal of Pharmacognosy and Phytochemistry. 2019;8(2):1235-1239.
12. Ou SH. Rice disease (2nd ed.). Kew: Common wealth Mycological Institute Jabalpur. 1985;380:66.
13. Ramesh GV, Palanna KB, Kumar A, Chandra R, Bharath M, Ganiger PC, *et al*. Comparative evaluation of different media and pH for the culturing of *Bipolaris setariae* causing leaf blight in browntop millet in India. Journal of Entomology and Zoology Studies. 2021;9(1):2032-2039.