

Original Research Article

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Effect of Different Abiotic Factors on the *in vitro* Growth of *Sarocladium oryzae* Causing Sheath Rot of Rice

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ABSTRACT

Studies on the varied physiological requirements of *Sarocladium oryzae*, which causes sheath rot of rice, were undertaken in the Department of Plant Pathology of the Annamalai University, Chidambaram. It was revealed that Potato dextrose agar medium, Czapek's Dox agar medium and Carrot agar medium supported significantly maximal radial growth (83.50mm, 78.50mm and 77.00mm, respectively) and with bio-mass production (774.09 mg; 734.87 mg and 710.89mg, respectively). Significantly, exposure of pathogen to acidic pH (4.0) recorded maximum mycelial growth (82.68 mm), mycelial dry weight (767.00 mg) which was followed by exposing the pathogen to pH 4.5 under *in vitro*. The highest radial growth of 66.10mm in 12 hr light and 12 hr darkness closely followed by 8 hr light and 16 hr darkness (59.50mm). The least mycelial growth was observed under exposing sunlight for 4 h (3.70 mm). *S. oryzae* experienced its greatest radial growth at 30°C was found to be more conducive for the mycelial growth of *S. oryzae* (73.00 mm) under *in vitro* recording the highest mycelial dry weight of 764.704 mg, which was followed by 35°C.

Keywords

Oryza sativa,
Oryza glaberrima,
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Introduction

Rice is a monocotyledonous annual grass and belongs to the family Graminae and the genus is *Oryza*. The genus *Oryza* includes two cultivated species and 20 wild species. The cultivated species are *Oryza sativa* (grown throughout the world) and *Oryza glaberrima* (grown only in Africa). In India, rice is cultivated in an area of 45.76 million ha

which is highest amongst all rice producing countries with an annual production of about 124.36 million tonnes with a productivity of 2.7 t ha⁻¹ (INDIASTAT, 2021). In Tamil Nadu, rice is cultivating in an area of 20.36 million ha with a production of 68.81 million tones and average productivity of k about 3.37 t ha⁻¹ (INDIASTAT, 2021). The crop is constantly subjected to various fungal, bacterial and viral diseases. Among the

fungal diseases, sheath rot is considered as one of the most important emerging diseases of rice, causing yield losses from 20–30% up to 85% (Pramunadipita *et al.*, (2020); Peeters *et al.*, 2021). *Sarocladium oryzae* was known to be the first major important pathogen of fungi that caused sheath rot disease of rice after been first isolated in 1922 in Taiwan (Mathur 1981; Bigirimana *et al.*, 2015). *S. oryzae* also is known to produce antimicrobial secondary metabolites such as helvolic acid and cerulenin (Ghosh *et al.*, 2002; Hittalmani *et al.*, 2016).

It develops well in rain-fed rice fields, and found in lowland and medium land environments (Pearce *et al.*, 2001; Sarangi *et al.*, 2019). This disease is mainly caused by the seed-borne fungus *S.oryzae* (Bigirimana *et al.*, 2015). Since its first description in 1922 in Taiwan as *Acrocylindrium oryzae*, the fungus has spread worldwide. *S. oryzae* can survive in seeds, plant residues, weeds and soil and it is transmitted by seeds, wind and insects. Wounds or natural damages may favours the pathogen to enter into the host (Sakthivel 2001; Bigirimana *et al.*, 2015; Mvuyekure *et al.*, 2017 and 2018). An investigation was carried out in the Dept. of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, for the various physiological requirements of *Sarocladium oryzae* causing sheath rot of rice.

Materials and Methods

Isolation and identification of pathogen

The pathogen was isolated from the diseased rice sheaths showing the typical lesions of sheath rot. The edge of the lesions were cut into small pieces by means of a sterile knife. Then the pieces were surface sterilized in 0.1 per cent sodium hypochlorite solution for 30 seconds and washed in three repeated changes of sterile distilled water and then plated into sterile Petri dishes containing PDA medium. The plates were then incubated at room temperature $28\pm 2^{\circ}\text{C}$. The tip of the hyphal growth radiating from the infected tissue was transferred

into PDA slants (Rangaswami, 1972). The fungus was purified again by single hyphal tip method and maintained on PDA slants for the further studies. Based on morphology characteristic the isolates were identified as *S. oryzae* (Bills *et al.*, 2004)

Effect of different solid media and liquid broth on the growth of *S. oryzae*

Solid media

Variation in the growth of *S. oryzae* in different media *viz.*, Beet root agar, Carrot agar, Corn meal agar, Czapek'sdox agar, Host leaf extract agar, Oat meal agar, Potato dextrose agar and Rose Bengal agar was studied. Fifteen ml of molten media were dispensed into each of 90 mm sterile Petri plates. Mycelial discs (6mm) taken from the advancing margins of 15 days old culture of *S. oryzae* by the aid of cork borer were separately placed each at the centre of the plate containing the above mentioned medium. The inoculated plates were incubated at room temperature ($28\pm 2^{\circ}\text{C}$) for 15 days and the linear growth of pathogen was measured in each case at 15 days after incubation. Each plate was replicated thrice.

Liquid broth

Eight liquid broth *viz.*, Beet root broth, Carrot broth, Corn meal broth, Czapek'sdox broth, Host leaf extract broth, Oat meal broth, Potato dextrose broth and Rose Bengal broth were prepared and 100 ml of the respective medium was dispensed in 250 ml Erlenmeyer flasks, autoclaved at 1.4 kg / cm for 20 min and cooled. The flask was inoculated separately with a 15 day old six mm culture disc of the respective isolate of *S. oryzae*. The flasks were incubated at room temperature $28\pm 2^{\circ}\text{C}$ for 15 days. Three replications were maintained for each isolate in each medium. After incubation the mycelial mat was filtered through a pre weighed Whatman No. 1 filter paper and then dried in hot air oven at 60°C till a constant weight was obtained. The mycelial dry weight was calculated by subtracting from the weight of the filter paper and recorded.

Effect of different temperature levels on the mycelial growth and biomass production of *S.oryzae* under *in vitro*

Solid medium

A quantity of 15 ml of the sterilized potato dextrose agar medium was poured into 90 mm sterile Petri plates. After cooling, they were inoculated with six mm mycelial disc of *S. oryzae* obtained from 15 days old culture grown on PDA and incubated in BOD at different temperature levels *viz.*, 5, 10, 15, 20, 25, 30, 35 and 40°C for 15 days in an incubator. The linear growth of the pathogen was measured in mm at the end of incubation period.

Liquid medium

Erlenmeyer flasks (250 ml) containing 100 ml of potato dextrose broth were sterilized, inoculated and incubated at different temperature levels *viz.*, 5, 10, 15, 20, 25, 30, 35 and 40°C for 15 days in BOD incubator. At the end of the incubation period, the mycelial mat was filtered through Whatman No. 41 filter paper of known weight. The filter paper with mycelial mat was dried in hot air oven at 105°C for 48 h. and the mycelial dry weight was calculated. In both the methods three replications were maintained for each treatment.

Effect of certain pH levels on the mycelial growth of *S. oryzae in vitro*

Solid medium

Potato dextrose agar medium of different pH levels were prepared *viz.*, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 by adding 0.1 N Sodium hydroxide and 0.1 N Hydrochloric acid and sterilized. A quantity of 15 ml of the sterilized potato dextrose agar medium was poured into 90 mm sterile Petri plates.

After cooling, they were inoculated with six mm mycelial disc of *S. oryzae* obtained from 15 days old grown on PDA. The plates were incubated at room

temperature ($28 \pm 2^\circ\text{C}$) for 15 days. The linear growth of the pathogen was measured in mm at the end of incubation period.

Liquid broth

Potato dextrose broth of different pH levels were prepared *viz.*, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 by adding 0.1 N Sodium hydroxide and 0.1 N Hydrochloric acid were prepared and sterilized. They were inoculated with six mm mycelial disc of *S. oryzae* obtained from 15 days old grown on PDA. The flasks were incubated for 15 days at $28 \pm 2^\circ\text{C}$ in BOD incubator. After incubation, the fungal biomass was separated through filtration in a previously dried and weighed filter paper (Whatman No. 41). The filter paper with mycelial mat was dried in hot air oven at 105°C for 48 h and the mycelial dry weight was calculated. In both the methods three replications were maintained for each treatment.

Effect of light on the growth of *S. oryzae*

The sterilized PDA medium was poured into the 90 mm sterile Petri plate and allowed to solidify. Such plates were inoculated with six mm mycelial disc obtained from the periphery of 15 days old culture of *S. oryzae* and incubated at different light intensities, namely, (1) 4hrsexposure to sunlight: The Petri plates were exposed to sunlight immediately after inoculation for 4 hrs and rest of time were kept on the laboratory (2) Continuous darkness: The inoculated Petri plates were kept inside the beaker and covered with black cloth for 15 days. (3) Continuous light: it was kept on the wooden table with 60 watts electrical bulb maintained 50 cm away from the top of the inoculated Petri plates. (4) 8hrs darkness and 16 hrs light: - the inoculated Petri plates were kept inside the black cloth covered beaker for 8 hrs and on the table for 16 hrs light. (5) 16 hrs darkness and 8 hrs light:- It was also maintained like above procedures. (6) 12 hrs light and 12 hrs darkness:- the alternate light and darkness was maintained by using black cloth covered beaker and electrical bulb. (7) 10

minutes exposed to UV light. (8) 20 minutes exposed to UV light. For these, the Petri plates were incubated in laminar air flow chamber and exposed to UV light for 10 and 20 minutes immediately after inoculation and kept on the laboratory table. (9) Natural diffused light under laboratory condition. The alternate light and darkness occurred automatically under laboratory condition. The observation was taken after 15 days of inoculation. Each treatment was replicated thrice.

Statistical analysis

The data were statistically analyzed using the Wasp version 2.0 developed by the Indian Council of Agricultural Research, Goa (Gomez and Gomez, 1984). Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Effect of different culture media on the growth and bio-mass production of *S. oryzae*

Results of the present study revealed that the mycelial growth and bio-mass production of *S. oryzae* on eight different culture media indicated that Potato dextrose agar and Czapek's Dox agar supported the maximum mycelial growth (83.50 mm; 78.50 mm, respectively) and bio-mass production (774.09 mg; 734.87 mg, respectively) of *S. oryzae* (Table 1).

It was followed by Carrot agar (77.00 mm; 710.89 mg, respectively) and Beet root agar (76.50 mm; 679.45 mg, respectively) whereas, the least mycelial growth was observed in Host leaf extract agar medium (58.00 mm; 519.76 mg, respectively) (table 1). Similar observations were made by Vinitha (2019); Panda and Mishra (2019); Pramunaditya *et al.*, (2020) recorded that among the medium tested, *S. oryzae* grow well on PDA at 25°C after 10 days.

The above results lend support to the present findings.

Effect of different temperature levels on the mycelial growth and mycelial dry weight of *S. oryzae*

Temperature plays an important role in reducing physiological deterioration and disease development. The metabolic activity of both host and pathogen depends on the action of enzymes that are extremely sensitive to temperature. Each pathogen has got its own cardinal temperature and understanding the temperature requirement of the pathogen will help to standardize the management strategies. In the present study, 30°C was found to be more conducive for the mycelial growth of *S. oryzae* (73.00 mm) under *in vitro* recording the highest mycelial dry weight of 764.704 mg, which was followed by 35°C (Table 2). The exposure of *S. oryzae* to high temperature i.e. 40°C was found to be highly detrimental to the growth of *S. oryzae* (table 2). While the exposure of pathogen to lowest temperature of 5°C recorded nil mycelial growth. Similarly, Panda and Mishra (2019) reported that maximum radial growth of *S. oryzae* was observed in 30°C (65.00 mm) followed by growth in 35°C (58.00 mm) significantly. This result is supported by findings of Kandhari (1996); Kumar and Singh 1995; Awoderu *et al.*, 1991; Mishra *et al.*, 1982. While at 10°C and 40°C *P. oryzae* did not grow, which is supported by Kandhari 1996; Kumar and Singh 1995. The above reports lend support to the present findings.

Effect of different pH levels on the mycelial growth and dry weight of *S. oryzae*

Hydrogen ion concentration (pH) is one of the most important factors influencing the growth of the fungi. The pH of the medium determines the rate and amount of growth and many other life processes of organism. The pH of growing media is also influenced on the growth and sporulation of fungi.

Table.1 Growth behavior of *S.oryzae* (So₅) in different solid media and liquid broth

S. No	Media	Linear growth of pathogen (mm)	Dry weight of mycelium (mg)
1	Beetroot agar	76.50 ^b	679.45 ^c
2	Carrot agar	77.00 ^b	710.89 ^b
3	Cornmeal agar	62.20 ^c	609.32 ^e
4	Czapek sDox agar	78.50 ^b	734.87 ^b
5	Host leaf extract agar	58.00 ^d	519.76 ^g
6	Oat meal agar	63.50 ^c	648.90 ^d
7	Potato dextrose agar	83.50 ^a	774.09 ^a
8	Rose Bengal agar	61.10 ^{cd}	559.84 ^f
	SE(d)	1.152	14.974
	CD(0.05)	3.525	30.443

Values are mean of three replications

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table.2 Effect of temperature requirement for the growth of *S.oryzae*

S. No	Temperature (°C)	Linear growth of pathogen (mm)	Dry weight of mycelium(mg)
1	5	0.00 ^h	0.00 ^g
2	10	29.00 ^g	389.65 ^f
3	15	38.66 ^f	493.89 ^e
4	20	44.33 ^d	568.98 ^d
5	25	50.00 ^c	696.32 ^c
6	30	73.00 ^a	764.70 ^a
7	35	66.00 ^b	734.52 ^b
8	40	40.33 ^e	513.89 ^e
	SE(d)	0.506	15.387
	CD(0.05)	1.627	22.516

Values are mean of three replications

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table.3 Effect of hydrogen ion concentration on the growth of *S.oryzae*

S. No	pH levels	Linear growth of pathogen (mm)	Dry weight of mycelium (mg)
1	3.5	77.23 ^b	695.30 ^b
2	4.0	82.68 ^b	767.00 ^a
3	4.5	78.98 ^a	744.70 ^a
4	5.0	76.12 ^b	655.30 ^c
5	5.5	64.50 ^c	578.70 ^d
6	6.0	61.34 ^d	523.30 ^e
7	6.5	58.86 ^d	510.00 ^{ef}
8	7.0	49.35 ^e	492.70 ^f
9	7.5	38.98 ^f	460.30 ^g
10	8.0	30.43 ^g	420.33 ^h
11	8.5	29.86 ^g	402.00 ^{hi}
12	9.0	19.56 ^h	380.66 ⁱ
SE(d)	-	0.882	11.654
CD(0.05)	-	2.997	29.771

Values are mean of three replications

Values in the column followed by same letters not differ significantly by DMRT(p=0.05)

Table.4 Effect of different light exposure on the growth of *S.oryzae* (So₅)

S. No	Light period	Linear growth of pathogen(mm)
1	Sunlight (4 h exposure)	3.70 ⁱ
2	Continuous dark light	55.60 ^d
3	Continuous light	38.60 ^f
4	8 h darkness & 16 h light	57.50 ^c
5	16 h darkness & 8 h light	59.50 ^b
6	12h light & 12 h darkness	66.10 ^a
7	10 min exposed to UV light	33.30 ^g
8	20 min exposed to UV light	31.00 ^h
9	Natural diffused light under laboratory	51.70 ^e
SE(d)	-	0.986
CD(0.05)	-	1.874

Values are mean of three replication

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

The results of the present study showed that among the pH levels (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) tested, exposure of pathogen to acidic pH (4.0) recorded maximum mycelial growth (82.68 mm), mycelial dry weight (767.00 mg) which was followed by exposing the pathogen to pH 4.5 under *in vitro* (Table 3). Increase in pH above 7.5 was not conducive for the growth of the pathogen.

Similarly, Panda and Mishra (2019) reported that the maximum mycelial mat was pH at 4.0 (769.0 mg) followed by pH 3.5 (746.6 mg). Contrarily, Tasugi and Ikeda (1956) also stated good mycelial growth of sheath rot fungus at pH 6.4. Mithrasena and Wijesundera (1992) observed maximum growth of *S. oryzae* in pH 3.5. These earlier reports lend support to the present findings.

Effect of different light exposure on the growth of *S.oryzae*

Light is also playing an important role in disease development. Rate of growth of *S. oryzae* was studied in different light exposure. Varied growth differences were observed, among all the light exposure with highest growth of 66.10mm in 12 hour light and 12 hour darkness closely followed by 8 hr light and 16 hr darkness (59.50mm). The least mycelial growth was observed under exposing sunlight for 4 h (3.70 mm) (Table 4). Results of the present study clearly revealed that exposure of pathogen to 12 h light and 12 h darkness showed maximum mycelial growth of *S. oryzae*. Similarly, Panda and Mishra (2019) reported that the highest radial growth was observed in 12 hour light and 12 hour darkness (68.1mm) closely followed by 8 hour light and 16 hour darkness (61.5 mm) significantly (table 4). Fungal growth was influenced by constant light and natural diffused light under laboratory condition. These findings are in agreement with the results of Oran (1975), Perez and Gonzalez (1982) and Lukose *et al.*, (2001). Maximum sporulation of the fungus was recorded in constant light and complete darkness which confirms the findings of earlier workers (Kato and Diamond 1966; Mathur *et al.*, 1967; Chakrabarti and Wilcoxan 1973; Chauhan *et al.*, 1987; Lukose *et al.*, 2001) are tallied with the results obtained in present investigations.

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