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Influence of temperature, pH and nutritional sources on mycelial growth and sporulation of *Pyricularia grisea*

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Abstract
Temperature, pH, carbon and nitrogen sources were tested against mycelia growth and sporulation of *Pyricularia grisea* causing blast diseases of rice. Among different temperatures ranges tested maximum mycelial growth and sporulation was observed at 25^0C (67.20 mm) with sporulation of $4.5x10^6$ conidia/ml after 144 hrs of incubation. Out of five pH levels, maximum mycelial growth (39.50 mm) and sporulation ($4.9x10^6$ conidia/ml) observed at 7 pH. Among five carbon sources tested maltose supported good growth (32.25 mm) followed by dextrose (26.20 mm) after 144 hr of incubation. Among five nitrogen sources tested barium nitrate supported good growth (61.20 mm) followed by Ammonium nitrate(45.80 mm). None of carbon and nitrogen sources induced the sporulation of *Pyricularia grisea*.

Keywords: *Pyricularia grisea*, pH, nutritional source, colony diameter, sporulation

Introduction
Rice blast, caused by *Pyricularia grisea* is one of the most destructive diseases of rice, because of its wide distribution and destructiveness under favourable conditions (Ou, 1985) [4]. It can cause severe yield losses where environmental conditions are favourable for disease development (Greer and Webster, 2001) [2]. In general, long periods of leaf wetness, high relative humidity, and temperatures of 17–28 °C favour rice blast development (Webster and Gunnel, 1992). In recent times, rice blast has become one of the prevalent and major disease on rice in Prakasam and Sri Potti Sreeramulu Nellore districts of Andhra Pradesh, causing heavy losses to the rice growing farmers. In view of this the present investigation was to study the influence of temperature, pH, carbon and nitrogen sources on growth and sporulation of *Pyricularia grisea* causing rice blast.

Material and Methods
Isolation of *Pyricularia grisea*
The diseased leaves of rice plants collected from farmers' fields were used for the isolation of the pathogen. The pathogen was isolated by following standard tissue isolation procedure (Tuite, 1969). Small bits of diseased leaves along with some healthy tissue were cut with help of a sterile scalpel and surface sterilized with one per cent sodium hypochlorite solution for 1 min. and rinsed aseptically in three changes of sterilized distilled water. Such surface sterilized leaf bits amended with streptomycin sulphate were transferred aseptically into sterilized Petri dishes containing solidified oat meal agar medium and incubated at 28 ± 1^0C for two weeks in a BOD incubator.

Purification of *Pyricularia grisea*
Fungus isolation techniques were used for getting pure culture of the fungus was transferred on sterilized PDA plates. The marginal mycelial growth that developed subsequently was picked-up aseptically for sub-culturing. The sub culturing was done at an interval 15 days and preserved at low temperature (5±10C) in refrigerator.

Results and Discussion
Influence of temperature on mycelial growth of *Pyricularia grisea*
The experiment was carried out to study the effect of temperature on mycelial growth. The results obtained are presented in Table 1. The variation in growth of fungus at different temperatures was found to be maximum at 25 ^{0}C (67.20) followed by 20^0C (31.53) after 144 hrs of incubation. It was observed that temperature below 30^0C or above 30^0C may suppress the growth of fungus and sporulation.

Further was observed that temperature 25 ^{0}C was found suitable for sporulation of fungus. *Pyricularia grisea* shows maximum growth at 25^{0}C temperature in potato dextrose agar medium with traces of sporulation. As the 20^{0}C and 30^{0}C temperature reduces the growth and sporulation. Similar observations were recorded by Awoderu et al. (1991) [1], Okeke et al. (1992) [3] on *Pyricularia grisea*.

They reported the favorable temperature for the growth and sporulation of *Pyricularia grisea* in the range of 20^{0}C and 30^{0}C. Studies revealed that with an increase or decrease in the temperature from the ambient of 30^{0}C, there was a steady decrease in the growth ability of *Pyricularia grisea*.

Influence of pH and nutrition on growth and sporulation of *Pyricularia grisea*

Mycelial growth of P. grisea was influenced by the pH (table 2). The mycelial growth was maximum at pH 7 (39.50 mm) with sporulation of 4.9x10^6 conidia/ml followed by pH 6 (38.20 mm) with 3.8x10^6 conidia/ml. The least mycelial growth was recorded at the pH 9 (31.65 mm) without any sporulation. However, low pH 4 had totally inhibited the growth of fungus. This clearly reveals that pH has a major role in mycelial growth of *Pyricularia grisea*. The highly acidic and alkaline ranges were found to be adversely affecting growth of Mycelia. It was observed that pH-6 and pH-7 was found suitable for both growth and sporulation.

Pyricularia grisea shows maximum growth in potato dextrose agar medium with traces of sporulation in pH 7 and pH 6 in potato dextrose agar amended medium. As the pH decreases the growth and sporulation reduce due to alkaline medium requirement for pathogenic fungi. The pH of the medium was changed up to 5 and 6 during the growth of the fungus (Thomas 1940 and Ramakrishanan, 1948) [7, 5].

Influence of carbon sources on mycelial growth and sporulation of *Pyricularia grisea*

The results on carbon sources (Table 3) reveal that among five carbon sources were evaluated against the *Pyricularia grisea* at different periods of incubation for mycelial growth and sporulation. Among the tested carbon sources, maltose gave optimum mycelial growth (32.25 mm) after 144 hr of incubation followed by dextrose (26.20 mm) and glucose (24.10 mm). It was noted that minimum mycelial growth was observed in Sucrose (20.30mm) and fructose (21.45 mm) over control (17.80 mm). None of carbon sources induced the sporulation of *Pyricularia grisea*.

Influence of nitrogen sources on mycelial growth and sporulation of *Pyricularia grisea*

The data presented in Table 4 reveals that barium nitrate supported maximum mycelial growth (61.20 mm) of *Pyricularia grisea* followed by ammonium nitrate (45.80 mm). It was noticed that minimum mycelial growth was observed in calcium nitrate (24.28 mm) followed by sodium nitrate (25.60 mm) and potassium nitrate (41.45 mm) over control (29.30 mm) at 144 hrs. None of nitrogen sources induced the sporulation of *Pyricularia grisea*.

Regarding carbon sources *Pyricularia grisea* shows maximum growth in maltose (32.70 mm) followed by dextrose (26.70 mm). While none of the tested carbon sources induces sporulation of conidia. These results were similar with the findings of Suryanaryanan (1958) [6]. Among the tested nitrogen sources, *Pyricularia grisea* shows maximum mycelial growth in Barium nitrate followed by Ammonium nitrate. In sodium nitrate find minimum growth followed by potassium nitrate. These results are similar with the findings of Vidhyasekeran (1971) [9].

Table 1: Influence of temperature on mycelial growth and sporulation of *P. grisea*

S. No	Temperature (^{0}c)	Mean colony diameter during incubation (mm)*			Sporulation
		96 hr.	120hr.	144hr.	No. of conidia/cm^2 (X 10^6)
1	20	18.30	26.50	31.50	Nil
2	25	22.25	40.60	67.20	4.5
3	30	14.10	18.70	24.40	Nil
4	Control (at room temperature)	27.60	38.25	54.55	Nil
SEm±		0.27	0.18	0.24	
CD at 5% level		0.68	0.44	0.54	

***** Mean of Fifteen Counts

Table 2: Influence of pH on mycelial growth and sporulation of *P.grisea*

S. No	pH	Mean colony diameter during incubation (mm)*				Sporulation
		96 hr.	120hr.	144hr.	168 hr	No. of conidia/cm^2 (X 10^6)
1	pH- 4.0	0.0	0.0	0.0	0.0	Nil
2	pH-6.0	20.10	26.40	35.80	38.20	3.8
3	pH-7.0	29.10	35.15	36.90	39.50	4.9
4	pH-9.0	25.30	27.10	29.00	31.65	Nil
5	Control	24.00	35.10	36.50	42.60	Nil
SEm ±		0.24	0.19	0.25	0.18	
CD at 5% level		0.57	0.40	0.53	0.37	

***** Mean of Fifteen Counts

Table 3: Influence of carbon sources on mycelial growth and sporulation of *P.grisea*

S.No	Carbon Source	Mean colony diameter during incubation (mm)*			Sporulation
		96 hr.	120hr.	144hr.	No. of conidia/cm^2 (X 10^6)
1	Glucose	14.20	22.45	24.10	Nil
2	Dextrose	16.50	24.65	26.20	Nil
3	Sucrose	12.45	19.30	20.35	Nil
4	Fructose	13.25	20.25	21.45	Nil
5	Maltose	18.60	26.45	32.35	Nil
6	Control	10.20	15.45	17.80	Nil
SEm ±		0.22	0.31	0.23	
CD at 5% level		0.54	0.69	0.51	

***** Mean of Fifteen Counts

Table 4: Influence of nitrogen sources on mycelial growth and sporulation of *P.grisea*

S. No	Carbon Source	Mean colony diameter during incubation (mm)*			Sporulation
		96 hr.	120hr.	144hr.	No. of conidia/cm^2 (X 10^6)
1	Ammonium nitrate	24.20	41.50	45.80	Nil
2	Potassium nitrate	23.45	38.36	41.45	Nil
3	Calcium nitrate	20.85	22.40	24.28	Nil
4	Sodium nitrate	18.25	21.45	25.60	Nil
5	Barium nitrate	34.30	57.50	61.20	Nil
6	Control	20.28	25.28	29.30	Nil
SEm ±		0.31	0.26	0.34	
CD at 5% level		0.78	0.64	0.77	

***** Mean of Fifteen Counts

Conclusions

Various sources of carbon, nitrogen, pH levels, different culture media and different temperature were evaluated *in vitro* for growth and sporulation of *Pyricularia grisea* under study. Out of three temperatures tested, maximum growth (67.20 mm) and sporulation ($4.9x10^6$ conidia/ml) recorded at 25^0C. It was noticed that pH 7 (39.50 mm) was most favourable for sporulation than lower and higher pH levels. Among the tested carbon and nitrogen sources, *Pyricularia grisea* showed maximum growth in Maltose (32.25 mm) and Barium nitrate (61.20 mm) and none of carbon and nitrogen sources induced the sporulation of *Pyricularia grisea*. Hence there is a need to conduct more research on influence of temperature, pH and nutritional sources for gowth and sporulation of *Pyricularia grisea* causing blast disease of rice.

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