

GROWTH AND SPORULATION OF *SAROCLADIUM ORYZAE*, THE RICE SHEATH ROT PATHOGEN

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ABSTRACT

The sheath rot disease of rice caused by *Sarocladium oryzae* is becoming a problem next in importance to sheath blight in the wet zone of Sri Lanka. In this study the growth and sporulation characteristics of *S.oryzae* were investigated. Among the nitrogen sources tested, maximum growth was with ammonium nitrate, but urea induced very high sporulation. Optimum temperature for growth and sporulation varied with the medium. On potato dextrose agar it was 30°C and on the two host extract media it was 25°C. Sporulation was enhanced by exposure of colonies to 12 h light and 12 h darkness. The study showed that percentage of germination of conidia decreased with the increase in conidia concentration. The optimum temperature for spore germination was 25°C. Germination of rice seeds was unaffected by products of the fungal pathogen. Of the several fungicides tested Benlate was very effective at low concentrations in inhibiting conidia germination, growth and sporulation of the fungus.

KEY WORDS: Rice, *Sarocladium oryzae*, Sheath rot

INTRODUCTION

Sarocladium oryzae (Sawada) W.Gams and D.Hawkw. is the cause of the sheath rot disease of rice (Ou, 1972). The pathogen was originally named *Acrocylindrium oryzae* Sawada. Gams and Hawksworth (1975) compared some isolates of the fungus with Sawada's holotype material and determined that the fungus belonged to the genus *Sarocladium* and named it *S.oryzae*. They also found another species had passed under the name *A. oryzae* and named that fungus *S.attenuatum*. Hence, in the literature it is not always certain which report refers to which fungus.

The sheath rot disease was first reported from Taiwan in 1922. Since then it has spread to other Asian countries. The disease was first observed in Sri Lanka during the early part of the 1970's (Seneviratne, 1980). The disease, thereafter has intensified in Sri Lanka and has reached epidemic levels in certain parts of the country.

Even though it is apparent that the disease is fast becoming an important problem, there is very little published information on *S.oryzae* and the sheath rot disease in Sri Lanka. The purpose of this investigation was to study the biology of *S.oryzae* isolated in Sri Lanka and to acquire information on the pathogen which will be of value in studies on the epidemiology of rice sheath rot and its control.

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MATERIALS AND METHODS

The fungus

The isolate of *Sarocladium oryzae* used in this investigation was isolated from naturally infected rice plants obtained from the Regional Agricultural Research Centre, Bombuwela, Sri Lanka. Kochs' postulates were followed to prove pathogenicity. The identification of the fungus was confirmed by the International Mycological Institute, London (IMI no. 34526). Stock cultures of the fungus were maintained on potato dextrose agar (PDA) at 5°C.

Growth media

Rice sheath extract agar medium (RSA) was made using 50 g fresh, healthy rice sheath tissue, 20 g agar and 1 l distilled water. The rice tissue was macerated (MSE homogenizer, 4,000 rpm) in 200 ml distilled water, filtered through 2 layers of muslin cloth and made up to 1 l with distilled water. Thereafter the agar was incorporated.

Seed extract agar medium (SEA) was prepared using 50 g dried, healthy rice seeds, 20 g agar and 1 l distilled water. Preparation was as described under sheath extract agar medium.

The standard media, PDA, malt extract agar (MEA) and Czapek-Dox agar (CDA) were also used in several experiments.

Czapek-Dox medium without agar was used as the liquid medium for growth studies. Twenty five ml of the medium was dispensed in 150 ml Erlenmeyer flask. When necessary buffered Czapek-Dox medium was prepared by adding 100 ml citric acid-phosphate buffer to 100 ml double strength Czapek-Dox medium.

All media were autoclaved at 120°C under a pressure of 1.1 kg cm⁻² for 15 min.

Inoculation and Incubation

Randomly selected 0.5 cm² squares from 10-day old cultures of *S.oryzae* on PDA plates were used to inoculate both solid and liquid media. All inoculated media were incubated at 30°C. Incubation was always in constant temperature incubators.

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Assessment of growth and sporulation

Growth on solid agar media was assessed from the mean of five replicates of colony diameter measured on the under surface of 9.0 cm petri dishes having 15 ml medium. Sporulation on agar medium was assessed by pouring 10 ml sterile distilled water onto plates having the fungal colony. The colony surface was then scraped using a bent glass rod and the suspension was filtered through a muslin cloth. The number of conidia in the filtrate was estimated using a haemocytometer.

Growth in liquid media was calculated as the mean dry weight of mycelium after filtration through Whatman's No.1 filter paper. The mycelium was dried to a constant weight in a hot air oven at 80°C for 3.5 h. Sporulation was assessed by counting the conidia in the filtrate using a haemocytometer.

To study conidia germination, conidia suspensions were prepared from 10-day old cultures on PDA at 30°C by macerating with 10 ml distilled water (MSE homogenizer, 4,000 rpm) and filtering through muslin cloth. The suspension was standardized to the required concentration using a haemocytometer and 0.02 ml of the conidia suspension was incubated at 25°C on a clean microscopic slide in 15 cm diameter petri dishes lined with filter paper saturated with sterile distilled water. The period of incubation was 72 h.

Germination was assessed by calculating the percentage germination of 250 conidia and by mean germ tube length of 100 germ tubes.

Inhibitory effects of culture filtrate on seed germination

The fungus was grown in 25 ml Czapek-Dox liquid medium in 150 ml Erlenmeyer flasks at 30°C and harvested at 72 h intervals by filtration through Whatman No. 1 filter paper. The filtrates were used to soak seeds of variety Bg 94-1. Fifty seeds were soaked in each filtrate for 24 h. Uninoculated sterile liquid medium and sterile distilled water were used to soak rice seeds in the control experiments.

At the end of the soaking period, 30 seeds were selected randomly from each filtrate and spread on moist filter paper in 9 cm diameter petri dishes. Each petri dish had 10 seeds. After 7 d of incubation at 30°C the number of germinated seeds was determined.

Effect of fungicides

The following fungicides were used: Kasumin (2% Kasugamycin), Antracol (70% Propineb), Benlate (50% Benomyl), Monceren (25% Pencycuron), Dithane M-45 (80% Mancozeb) and Topsin (50% Thiophanate-methyl). The recommended dosages of these fungicides are, Kasumin 0.7 kg/ha, Monceren 0.15 kg/ha, Antracol 2.0 kg/ha, Benlate 0.7 kg/ha, Dithane 2.0 kg/ha and Topsin 0.7 kg/ha. Suspensions of each fungicide were made using sterile distilled water to contain 20, 40, 60, 80 and 100 ppm of the active ingredient. One ml of the fungicide suspension and 4 ml of sterile PDA at 45°C were mixed to prepare the fungicide amended media. Petri dishes of 5.0 cm diameter having 5.0 ml of the fungicide amended media were inoculated with the fungus. Growth and sporulation was assessed after incubation for 7 days at 30°C. In the control experiments a medium having sterile distilled water instead of the fungicide suspension and 4 ml PDA was used to grow the fungus.

To assess the effect of the fungicides on conidia germination 0.02 ml of a conidia suspension prepared as described elsewhere under conidia germination was mixed with 0.02 ml of each fungicide suspension and incubated at 25° C on clean microscopic slides in 15 cm diameter petri dishes lined with filter paper saturated with sterile distilled water. Germination was assessed at 24 h intervals. In the control experiments instead of the fungicide suspension, the conidia suspension was mixed with 0.02 ml sterile distilled water.

In the above experiment the percentage of inhibition was calculated using the following equation,

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

where, X = growth or sporulation or germination in the control; Y = growth or sporulation or germination in the presence of fungicide

Statistical analysis

All the experimental designs were complete randomized block designs and the data were analysed accordingly. Treatments were compared using LSD (Sokal and Rohlf, 1981).

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RESULTS

Experiments on growth and sporulation

Maximum growth was on the two host extract media (Table 1). However, there was no significant difference between growth on RSA and SEA. Growth was similar on both PDA and CDA. The highest sporulation was on PDA followed by the two host extract media. Growth and sporulation were poor on MEA.

Table 1. Growth and sporulation of *Sarocladium oryzae* on different media at 30°C, 7 days after inoculation

Medium	Growth* (Colony diameter, cm)	Sporulation* (No. of conidia/ml x 10 ⁶)
PDA	2.06 bc	19.04 a
CDA	2.25 b	4.24 c
MEA	1.64 c	4.08 c
RSA	3.02 a	7.12 b
SEA	2.89 a	8.88 b
CV (%)	15.2	16.6
LSD (P = 0.05)	0.47	1.90

* Mean of five replicates

Means in a column followed by the same letter are not significantly different at P = 0.05

PDA = Potato dextrose agar; CDA = Czapek-Dox agar;

MEA = Malt extract agar; RSA = Rice sheath extract agar;

SEA = Seed extract agar

To study the ability to use different sources of nitrogen, sodium nitrate in the Czapek-Dox medium was replaced with either ammonium nitrate or ammonium sulphate or potassium nitrate or urea. The medium with ammonium nitrate showed maximum growth followed by ammonium sulphate (Table 2). No significant difference in growth existed when the other nitrogen sources were used. Urea induced poor growth but it showed the highest sporulation. Sporulation in potassium nitrate and ammonium sulphate was very poor.

The effect of pH on growth of the fungus was investigated using buffered Czapek-Dox liquid medium at 30°C. Growth was maximum at pH 3.5 and

decreased with increase in pH (Table 3). Highest sporulation was at pH 5.5, but there was no significant difference between sporulation at pH 3.5 and 5.5.

Table 2. Growth and sporulation of *Sarocladium oryzae* on different nitrogen sources at 30°C, 14 days after inoculation

Nitrogen source	Growth* (Mycelial dry weight x 10 ⁻¹ g)	Sporulation* (No. of conidia/ml x 10 ⁶)
Sodium nitrate (5.8)	8.25 c	8.60 c
Ammonium nitrate (3.6)	15.56 a	14.50 b
Potassium nitrate (6.0)	5.76 c	2.70 d
Urea (8.5)	8.02 c	24.20 a
Ammonium sulphate(3.9)	11.49 b	4.30 d
CV (%)	16.5	4.7
LSD (P=0.05)	2.28	2.97

* Mean of four replicates

Mean in a column followed by the same letter are not significantly different at P = 0.05
Figure within brackets indicates the pH of the medium

Table 3. Growth and sporulation of *Sarocladium oryzae* * on different pH values at 30°C, 21 days after inoculation

pH of culture medium	Growth** (Mycelial dry weight x 10 ⁻² g)	Sporulation** (No. of conidia/ml x 10 ⁶)
3.5	17.15 a	12.68 a
4.5	14.71 b	5.28 b
5.5	13.68 bc	13.28 a
6.5	10.46 d	3.80 c
7.5	11.16 cd	2.10 d
CV (%)	8.3	9.6
LSD (P=0.05)	1.73	1.08

* The fungus was grown in Czapek-Dox liquid medium

** Mean of four replicates

Means in a column followed by the same letter are not significantly different at P=0.05

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Growth at 25°C and 30°C was similar and was significantly higher than growth at 35°C (Table 4). All four media used showed this significant difference in growth. The interaction between media and temperature was also significant. Optimum temperature for growth on PDA and CDA was 30°C, but for host extract media it was 25°C.

Table 4. Growth and sporulation of *Sarocladium oryzae* at different temperatures on different media, 7 days after inoculation

Temp. (°C)	Growth*					Sporulation*				
	(Colony diameter, cm)					(No. of conidia/ml x 10 ⁵)				
	PDA	CDA	RSA	SEA	Mean	PDA	CDA	RSA	SEA	Mean
25	1.93	1.38	2.37	2.68	2.09a	13.36	11.96	12.96	13.04	12.83b
30	2.01	1.59	2.27	2.37	2.06a	22.80	8.96	13.36	20.00	16.28a
35	1.56	1.39	1.39	1.60	1.48b	9.52	4.72	7.20	8.80	7.56c
Mean	1.83b	1.45c	2.01a	2.21a		15.22a	8.54c	11.17b	13.94a	
CV (%)			12.7			33.1				
LSD(P=0.05) Temperature			0.17			2.96				
LSD (P=0.05)Medium			0.15			2.56				

* Mean of five replicates

Means in a column or row followed by the same letter are not significantly different at P=0.05

PDA = Potato dextrose agar; CDA = Czapek -Dox agar; RSA = Rice sheath extract agar; SEA = Seed extract agar; Temp. = Temperature

In an experiment on the effect of day light and darkness on growth and sporulation of the fungus, a significant difference in mycelial growth under different periods of illumination (Phillips, MCFE 40 w/33) was not observed (Table 5). Interaction between light and medium for sporulation was highly significant. Sporulation was significantly low under darkness.

Conidia germination

At 24 h germinated conidia were observed only in the suspension having the lowest conidia concentration i.e. 2.5×10^5 conidia/ml (Table 6). The percentage germination decreased with increase in conidia concentration, and the difference was significant. Germ tube length also showed a similar pattern.

Table 5. Growth and sporulation of *Sarocladium oryzae* under different periods on different media, 7 days after inoculation

Period of illumination (h)	Growth* (Colony diameter, cm)					Sporulation** (No. of conidia/ml x 10 ³)				
	PDA	CDA	RSA	SEA	Mean	PDA	CDA	RSA	SEA	Mean
0	2.04	1.57	2.61	2.92	2.28a	14.0	6.0	11.0	12.0	10.8c
12	2.00	1.52	2.64	2.89	2.26a	127.0	18.0	24.0	22.0	47.8a
24	2.04	1.80	2.90	2.85	2.31a	97.0	19.0	19.0	26.0	40.3b
Mean	2.03b	1.63b	2.72a	2.89a		79.3a	14.3c	18.0b	20.0b	
CV (%)			10.5					14.9		
LSD (P=0.05) Illumination	NS							3.48x10 ⁵		
LSD (P=0.05) Medium	0.20							4.04x10 ⁵		

* Mean of four replicates; NS = not significant

Means in a column or row followed by the same letter are not significantly different at P=0.05

PDA = Potato dextrose agar; CDA = Czapek-Dox agar; RSA = Rice sheath extract agar;

SEA = Seed extract agar

Table 6. Effect of spore concentration on germination and germ tube length of *Sarocladium oryzae* at 30° C

Conidia concentration (No. of conidia/ml)	% Conidia germination*				Germ tube length(μ) **		
	24h	48h	72h	Mean	24h	48h	72h
2.5x10 ⁵	7.82	25.53	81.96	38.44a	36.57± 9.78	49.86± 8.47	29.29 ± 10.56
5.0x10 ⁶	0	4.04	19.93	7.99b	0	21.27± 4.16	18.30 ± 4.99
1.0x10 ⁷	0	1.96	12.23	4.76c	0	9.81± 2.73	7.83 ± 3.33
2.0x10 ⁷	0	1.08	9.49	3.52d	0	5.81± 1.60	7.86 ± 3.78
4.0x10 ⁷	0	0	1.97	0.65e	0	0	5.20 ± 1.72
Mean	1.56c	6.25b	25.13a				
CV (%)			0.002				
LSD (P=0.05) Spore germination	1.14						
LSD (P=0.05) Time	1.11						

*250 conidia were counted; ** Mean length of germ tube based on 100 conidia

ANOVA was done on log transformed percentages

Means in a column or row followed by the same letter are not significantly different at P = 0.05

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In a comparison on the effect of temperature on spore germination in distilled water, highest germination was observed at 25°C (Table 7). Germination was low at both 30°C and 35°C. Germ tube growth also followed a similar pattern (Table 7). For this experiment a conidia concentration of 2.5×10^5 conidia/ml was used.

Table 7. Effect of temperature on spore germination and germ tube length of *Sarocladium oryzae*

Temp. (°C)	% Conidia germination*				Germ tube length(μ) **		
	24h	48h	72h	Mean	24h	48h	72h
25	6.61	15.16	87.63	36.46a	46.89± 6.83	49.88±12.54	21.11 ± 5.01
30	1.99	6.55	12.32	6.95b	18.00± 2.75	25.19±6.25	36.86 ± 2.96
35	0	2.03	4.63	2.25c	0	14.11±3.06	13.75 ± 2.96
Mean	2.86c	7.91b	34.86a				
CV (%)				6.5			
LSD (P=0.05) Temperature				1.13			
LSD (P=0.05) Time				1.13			

* 250 conidia were counted; ** Mean length of germ tube based on 100 conidia

Temp. = Temperature

ANOVA was done on log transformed percentages

Means in a column followed by the same letter are not significantly different at $P = 0.05$

In both of the above experiments the few conidia which germinated at 24 h had long germ tubes (Tables 6 and 7). At 72 h a large number of conidia had started to germinate and the germ tubes of these conidia were much shorter.

Inhibition of seed germination

All seeds soaked in the culture filtrate, uninoculated medium and distilled water controls germinated equally well. The respective seedlings also showed no difference in their growth.

Effect of fungicides on growth and sporulation

All fungicides tested inhibited the growth of the fungus (Table 8). Benlate (Benomyl) was the most effective, causing 100% inhibition even at 40 ppm

concentration of the active ingredient. Topsin (Thiophanate methyl) and Kasumin (Kasugamycin) inhibited growth by 36% and 17% respectively at 40 ppm.

Table 8. Effect of fungicides on growth of *Sarocladium oryzae* on potato dextrose agar at 30°C, 7 days after inoculation

Fungicide	% Inhibition of growth *					Mean
	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	
2% Kasugamycin (Kasumin)	4.72	17.22	23.98	27.36	21.86	21.03c
70% Propineb (Antracol)	6.52	8.12	7.65	8.21	7.65	7.65d
80% Mancozeb (Dithane M-45)	3.14	5.40	6.41	9.91	9.91	6.95d
50% Benomyl (Benlate)	11.59	100.00	100.00	100.00	100.00	82.32a
25% Pencycuron (Monceron)	6.52	7.42	8.21	7.61	8.21	7.60d
50% Thiophanate Methyl (Topsin)	23.41	35.81	40.87	46.50	47.63	38.84b
CV (%)	7.7					
LSD (P=0.05)	1.17					

* Mean of three replicates

ANOVA was done on log transformed percentages

Means in a column followed by the same letter are not significantly different at $P = 0.05$ *

Benlate, Topsin and Monceren caused more than 90% inhibition of sporulation at 20 ppm while Dithane M-45 also caused about 88% inhibition (Table 9). The other two fungicides tested though less effective at 20 ppm, caused significant inhibition at higher concentration.

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Table 9. Effect of fungicides on sporulation of *Sarocladium oryzae* on potato dextrose agar at 30°C, 7 days after inoculation

Fungicide	% Inhibition of growth *					Mean
	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	
2% Kasugamycin (Kasumin)	61.0	96.0	96.6	100.0	100.0	90.7c
70% Propineb (Antracol)	59.0	96.0	100.0	100.0	100.0	91.0c
80% Mancozeb (Dithane M-45)	88.6	94.0	100.0	100.0	100.0	96.5b
50% Benomyl (Benlate)	98.6	100.0	100.0	100.0	100.0	99.7a
25% Pencycuron (Monceron)	94.6	98.3	100.0	100.0	100.0	98.5a
50% Thiophanate Methyl (Topsin)	98.0	100.0	100.0	100.0	100.0	99.6a
CV (%)	20.9					
LSD (P=0.05)	2.01					

* Mean of three replicates

ANOVA was done on log transformed percentages

Means in a column followed by the same letter are not significantly different at $P = 0.05$

All fungicides except Antracol and Dithane M-45 caused total inhibition of conidia germination at 20 ppm (Table 10). Dithane M-45 caused complete inhibition at 40 ppm and Antracol at 60 ppm.

Table 10. Effect of fungicides on conidia germination of *Sarocladium oryzae* at 30°C

Fungicide	% Inhibition of conidia germination *					
	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	Mean
2% Kasugamycin (Kasumin)	0	0	0	0	0	0
70% Propineb (Antracol)	61.3	12.6	0	0	0	14.7
80% Mancozeb (Dithane M-45)	6.0	0	0	0	0	1.2
50% Benomyl (Benlate)	0	0	0	0	0	0
25% Pencycuron (Monceron)	0	0	0	0	0	0
50% Thiophanate Methyl (Topsin)	0	0	0	0	0	0

* 250 conidia were counted

DISCUSSION

In this investigation the growth characteristics of *Sarocladium oryzae* were determined. In view of the confusion in nomenclature it will also be of interest to compare these characters with those reported for *Acrocylindrium oryzae* by a few authors (Mohan and Subramaniam, 1977; Kawamura, 1940; Kannian, 1979; Dutta and Purkayastha, 1978; Chung, 1975).

The fungus showed the best growth on the two host extract media and highest sporulation was on PDA. Among the nitrogen sources examined maximum growth was in the medium with ammonium nitrate. All these characters agree with those reported for *A.oryzae* (Mohan and Subramaniam, 1977). Urea which is widely used as a nitrogenous fertilizer in Sri Lanka, induced very high sporulation when used as the main source of nitrogen. However, mycelial growth in urea was rather poor, which may be due to the very high pH of the urea medium. This investigation showed that the optimum pH for growth was 3.5, growth decreased thereafter with increase in pH. However, the optimum pH for *A.oryzae* is 6.5 (Mohan and Subramaniam, 1977). Maximum growth and sporulation of *S.oryzae* on host extract media was at 25°C, while on PDA it was 30°C. An optimum temperature of 30°C for both growth and sporulation is reported for *A. oryzae* (Kawamura, 1940; Mohan and Subramaniam, 1977).

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Self inhibition of conidia germination was clearly seen in this investigation. At a conidia concentration of 4×10^7 conidia/ml the inhibition was 100%. Similar observations have been reported for *A.oryzae* (Dutta and Purkayastha, 1978). Chung (1975) has reported the inhibition of seed germination by the culture filtrates of the causative fungus of sheath rot of rice. This investigation failed to detect such inhibition.

Of the fungicides tested Benlate was extremely effective, inhibiting growth, sporulation and conidia germination at very low concentrations.

The results of this investigation established some factors which promote growth and sporulation of this fungal pathogen. The information acquired will be of value in further studies on the sheath rot disease of rice.

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