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EFFECT OF PHYSICAL AND CHEMICAL PARAMETERS ON GROWTH AND SPORULATION OF FUNGI ASSOCIATED WITH RICE COLLAR ROT OF MANIPUR

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Collar rot of rice of Manipur is found associated often with two types of fungi, *Pestalotiopsis versicolor* (Speg) and *Chaetomium globosum*. The physical and chemical parameters were studied on the growth sporulation of these fungi. There observed good responses of these parameters on the growth of these fungi with drastic differences among these fungi.

Keywords :Collar rot fungi; Factors effecting growth; Sporulation.

Introduction

Collar rot of rice caused by four species of *Ascochyta* was first reported by Hara[1]. The disease is reported to be caused by *Ascochyta oryzae* Catt. from Thailand[2], South East Asia[3], and Manipur[4]. However, in Manipur collar rot is also reported to be caused by *Pestalotiopsis versicolor* (Speg)[5] and by *Pyricularia oryzae* from Malays[6]. Not only these fungi as mentioned above, recently, *Chaetomium globosum* which can cause rice collar rot is also identified in Manipur. The paper discusses the effect of physical and chemical parameters on the growth and sporulation of these recently recorded two fungal species from Manipur causing rice collar rot.

Materials and Methods

Five mm of actively growing mycelia of these two fungi isolated from rice collar rot, viz., *P. versicolor* and *C. globosum* and grown in the culture media was taken for each experiment. It was then transferred to each of 100 ml Erlenmeyer conical flask containing 50 ml each of potato dextrose broth. Seven different culture media viz., Asthama and Hawker's medium, Potato dextrose, Oatmeal, Czapex dox, Richard, Corn meal and Sabouraud's conservation, were prepared[7]. 50 ml of medium was dispensed in each Erlenmeyer conical flask (100 ml). A 5mm mycelial plug taken out from the actively growing colonies of 3-day-old culture, was transferred aseptically to each flask. The inoculated flasks were then incubated at 25±1°C for 7 days. Each treatment was replicated for 8 times (5 for growth and other 3 for spore production studies). After incubation period, the mycelial mats were harvested by filtering through pre-weighed Whatman filter paper No. 1 and dried at 60°C for 72 hours in a hot air oven and then re-weighed to estimate fungal growth on dry weight basis. From the remaining three flasks, the extent of spore production was studied after 7 days of incubation. The number of spores per ml was calculated with the help of haemocytometer.

For the light treatment the inoculated conical flasks were exposed to three different conditions of light, viz., (i) complete light, (ii) alternate light and darkness (12 hr/12hr) and (iii) complete darkness for 7 days at 25±1°C. All the treatments were replicated 8 times (5 for growth and 3 for sporulation). Fungal growth was determined on dry wt. basis and the number of spores/ml was calculated with the help of haemocytometer (Table 1). For the temperature parameter the inoculated conical flasks were exposed to different temperatures, viz., 5, 10, 20, 30, 35 and

38°C for 7 days. Here also all the treatments were replicated 8 times. The measurement of the mycelial growth was done (Table 2).

Different relative humidities were prepared with the help of concentrated sulphuric acid and distilled water. The mixing ratios of sulphuric acid with distilled water as described by Horokawa and Kubota[8] were followed with little modification. The petri dishes were incubated at 25±1°C for 24, 48 and 72 hours to study the effect of different relative humidities on spore germination of the fungi associated with rice collar rot (Table 3).

In the chemical parameter Asthana and Hawker's medium was used as basal medium, however, glucose and potassium nitrate content in the basal medium were substituted by different carbon sources like, D(-) Arabinose, Dextrose, D(-) Fructose, Mannitol, D(+) Maltose, Sucrose, D(-) Ribose, D(+) Mannose and Cellobiose; and twentyone amino acids (Glycine, DL-Methionine, L-Glutamic acid, DL-Alanine, L-Cysteine, DL-Valine, L-Lucine, DL-non-Lucine, DL - Aspartic acid, DL-Ornithine, L-histidine, DL-Theonine, DL-iso-Lucine L-Hydroxyproline, L-Arginine, DL-ß-Phenylalanine, DL-Tryptophane, DL-Serine, L-Proline, DL-2 Aminobutyric acid and L-Lucine), by adding separately to 50 ml basal medium to give an equivalnet amount of carbon (2gC/1) and nitrogen (0.485 g N/1). All treatments were replicated 8 times (5 for growth and other 3 for sporulation). The media without carbon and nitrogen sources served as control. Fungal growth was determined on dry weight basis after 7 days of incubation. The number of spores was calculated with the help of haemocytometer (Table 4, 5).

Studies on the effect of pH were conducted on Richard's broth medium. Eleven pH values(2-12) were adjusted with pH meter (Systronics) using either KOH or HCl 50 ml of Richard's broth dispersed in each 100 ml Erlenmeyer conical flask and autoclaved at 121°C for 20 minutes. All treatments were replicated 8 times. Then flasks were inoculated with a 3-day old mycelial plug of 5 mm size and incubated at 25±1°C for 7 days. Mycelial mats were harvested on pre-weighed Whatman No. 1 filter paper and dried at 60°C for 72 hours using hot air oven and re-weighed. Fungal growth was determined on dry weight basis. The number of spores was calculated as before (Table 6).

Results and Discussion

The three different conditions of light showed different effects on growth and sporulation of *P. versicolor* and *C. globosum*. The maximum mycelial growth of both fungi was found in complete darkness but least in alternate light and darkness. These findings were supported by earlier workers[9, 10]. In the case of sporulation of *P. versicolor* complete light could induce sporulation after 4 days of inoculation. However, *C. globosum* failed to sporulate in this condition. It was also observed that alternate light and darkness and complete darkness failed to induce sporulation of both the fungi.

Percentage of germination of the spores two fungi was found less at the low RH but with the increase of RH the percentage increased. The maximum i.e. 100 percent germination was found at 100 p.c. RH.

The mycelial growth of the two fungi was favoured by a wide temperature range of 10-35°C. Optimum temperature for maximum mycelial growth of both fungi was recorded at 25°C, however, they fail to grow at 5.0 and 38°C. The behaviours of these fungi on temperature

Table 1. Effect of Light on Growth and Sporulation of Fungi Associated with Rice Collar Rot.

Sl. No.	Condition of light	P. versicolor growth(mg*)	C. globosum growth(mg*)	No. of spore/ml	
				P. versicolor	*C. globosum*
1.	Complete light	45.50	32.46	3.5×10^4	0
2.	Alternate light darkness (12h/12h)	57.00	40.65	0	0
3.	Complete darkness	70.00	49.94	0	0

Table 2. Effect of Temperature on Growth of Fungi Associated with Rice Collar Rot.

Sl. No.	Temperature (°C)	Growth (mg)	
		P. versicolor	*C. globosum*
1.	5	-	-
2.	10	111.00	68.24
3.	15	122.01	73.00
4.	20	381.00	114.03
5.	25	446.00	120.90
6.	30	403.20	102.00
7.	35	101.00	52.00
8.	38	-	-

Table 3. Effect of Relative Humidity (RH) on Growth and Sporulation of Fungi Associated with Rice Collar Rot.

Sl. No.	Relative Humidity (%)	Spore germination (%)		
		P. versicolor		*C. globosum*
		24h	*35h*	*24h*
1.	25	0	0	0
2.	30	0	0	0
3.	35	0	0	0
4.	50	0	2.0	10.0
5.	75	0	56.0	70.0
6.	90	0	80.0	85.0
7.	100	0	100.0	100.0

Table 4. Effect of Different Carbon Sources on Growth of Fungi Associated with Rice Collar Rot.

Sl. No.	Carbon Sources	*P. versicolor* growth (mg*)	*C. globosum* growth (mg*)
1.	Arabinose	52.50	16.82
2.	Mannitol	151.50	27.67
3.	Fructose	153.50	27.50
4.	Xylose	59.50	35.00
5.	Maltose	149.50	25.82
6.	Ribose	20.00	27.50
7.	Sucrose	129.00	33.00
8.	mannose	154.00	43.68
9.	Cellobiose	239.00	29.75
10.	Dextrose	39.27	27.00
11.	Control	5.00	5.00
* Mean of five replications			
	CD 5%	96.36	11.16

Table 5. Effect of Different Amino Acids on Growth of Fungi Associated with Rice Collar Rot.

Sl. No.	Amino Acid	*P. versicolor* growth (mg*)	*C. globosum* growth (mg*)
1.	DL-Methionine	30.08	3.00
2.	L-Glutamic acid	57.3	20.50
3.	DL-Alanine	87.18	12.33
4.	L-Cysteine	37.00	0.00
5.	DL-Valine	49.88	10.25
6.	L-Lucine	94.63	27.25
7.	DL-Non-Lucine	28.15	14.50
8.	DL-Aspartic acid	91.88	22.50
9.	DL-Ornithine	84.20	11.60
10.	L-Histidine	15.23	24.00
11.	DL-Theonine	16.50	16.00
12.	DL-iso-Lucine	22.75	14.25
13.	L-Hydroxy Proline	17.50	8.15
14.	Glycine	42.75	20.65
15.	L-Arginine	48.25	24.00
16.	DL-ß-Phenylalanine	60.50	14.00
17.	DL-Tryptophane	21.50	14.50
18.	DL-Serine	64.25	11.00
19.	L-Proline	60.00	15.75
20.	DL-2-Amino-Butyric acid	25.50	18.25
21.	L-Lucine	38.00	39.00
22.	Control	8.00	8.00
*Mean of five replications CD 5%		11.85	9.09

Table 6. Effect of Different pH on Growth of Fungi Associated with Rice Collar Rot.

Sl. No.	pH	*P. versicolor (Speg.)* growth (mg*)	*C. globosum* growth (mg*)
1.	2.0	250.83	44.67
2.	3.0	363.50	64.83
3.	4.0	405.83	73.50
4.	5.0	448.33	112.67
5.	6.0	412.33	113.13
6.	7.0	391.17	125.53
7.	8.0	384.00	152.30
8.	9.0	383.00	62.33
9.	10.0	376.00	43.67
10.	11.0	318.00	42.33
11.	12.0	0.00	0.00
*Mean of five replication CD 5%		36.27	38.22

Table 7. Effect of Different Culture Media on Growth of Fungi Associated with Rice Collar Rot.

Sl. No.	Media	*P. versicolor* (Speg.) growth (mg*)	*C. globosum* growth (mg*)
1.	Asthana & Hawker's Medium	231.60	166.63
2.	Potato dextrose broth	368.69	263.00
3.	Oat meal	52.80	47.88
4.	Czapek dox medium	32.38	33.63
5.	Richard's Medium	675.00	82.50
6.	Corn meal	317.50	258.00
7.	Sobouraud's Conservation	45.50	51.88
8.	Control	0.00	0.00
*Mean of 5 replications CD 5%		114.42	38.99

were similar.

The response of culture media on the growth and sporulation of these fungi was also different, P. versicolor showed maximum mycelial growth in Richard's medium followed by Potato dextrose and Czapek dox, however, the behaviour of *C. globosum* is quite contrasting, in the sense that maximum mycelial growth of this fungus was found in Potato dextrose followed by Corn meal and Czapex dox (Table 7).

The influence of pH on the growth of these two fungi was also different. A maximum mycelial gowth of *P. versicolor* was observed at pH 5 whereas in the case of *C. globosum* the maximum growth was observed at pH 8.

When different carbon sources were applied the maximum mycelial growth of *P. versicolor* was recorded in cellobiose and the least in ribose. There was no much difference in the mycelial growth of this fungus in other carbon sources. However, in the case of mycelial growth of *C. globosum* the maximum was recorded in mannose and the least in arabinose, but there were no significant difference in other carbon sources.

When the culture media were prepared mixed with different types of amino acids the maximum mycelial growth of *P. versicolor* was observed in L-Lycine containing media whereas the poor mycelial growth was observed in L-Histidine. However, the maximum mycelial growth of *C. globosum* was observed in L-Lucine containing media and the least in DL-Methionine containing media.

It is clearly shown in all the treatments mentioned above that there are drastic variations of responses of these two fungi. However, the behaviour of these fungi are nearly similar when treated with different ranges of temperatures. The variations might be due to the difference in genetic behaviour of these two fungi.

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