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Physiological studies on *Fusarium moniliforme* Sheld, the causal organism of Bakanae disease of rice

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Abstract

Physiological studies on factors affecting the mycelial growth of *Fusarium moniliforme* Sheld., i.e., culture media, temperature, pH level, light duration and fungicide concentrations, were carried out. Potato dextrose agar medium (PDA) was found to be the best for the mycelia growth followed by Wakasman's agar, Basal medium, Czapeck's dox agar and Richards medium. The fungus showed significant growth within a range of 25-30^{o}C, the best growth was observed at 30^{o}C. Neutral pH (7) favoured the mycelial growth more as compared to other values. The effect of light and dark period on fungal growth was insignificant, however significant results were obtained when light was given for a continuous period of 24 hours. In vitro test of fungicides on mycelial growth of the fungus revealed that Benlate and Derosal inhibited the mycelial growth of the fungus completely, rather than other fungicides.

Introduction

Rice is an important Food and cash crop in Pakistan. It occupies second position to cotton with regard to export. It adds about 7-8% annually to the foreign exchange.In the year1991-92 it was grown on approximately 2096.9 thousand hectares of land with the production of about 3243.1 thousand tones (Anonymous 1992). Statistical analysis shows that during the last ten years, the area for rice cultivation has increased by 11% but the yield per hectare reduced to 12%, due to many factors. Among the various factors responsible for this yield reduction, insects, pests and diseases are the most important. Bakanae disease or Foot rot (*Fusarium moniliforme*) is a serious disease of rice causing great damage to the crops. This disease is widely distributed in all the rice growing areas of the world (Ou, 1985). In Pakistan disease incidence increased during 1989-90. Recently the fields of Basmati-385 were surveyed and even a single field was not free from the disease (Khan *et al*., 2000). In view of the overall economic importance of rice crop and losses caused by bakanae disease, it was thought essential to investigate the various physiological aspects of *F. moniliforme*, the the causal organism of bakanae disease of rice, in order to get an insight of the problem.

Material and Methods

Isolation of Pathogen: The pathogen *Fusarium moniliforme* was isolated from the stem of diseased rice plant by the method described previously (Aurangzeb *et al*. 1998). The isolated fungus was identified after reference to Booth (1971) and Nelson *et al*. (1983).

Physiological studies: Effect of different factors i.e. culture media, Temperature, pH levels & Light duration on the growth of the fungus were investigated. The sensitivity of the organism to various fungicides was also studied. In all the experiments 20 ml of the culture medium was poured into each petri plate (90 mm). The media were inoculated by placing 5mm disc of the fungus in the center taken aseptically with sterilized cork borer from 7 days old culture grown in petri plates and incubated at 30^{o}C. Mycelial growth was recorded by measuring the colony diameter (mm) along two axes at right angles after every 24 hours of incubation till in any one of the treatments the petri pates were full of fungal mycelium. All the experiments were run in triplicates and readings were taken after every 24 hours for 5 days.

Culture media: Five different culture media were employed to assess their suitability for fungal growth .The composition (g/L) of each of the medium is given below. Potato dextrose agar (PDA) Potato starch 20, Dextrose 20, Agar agar 20,Basal Agar medium (BA) Dextrose 20, KH_2PO_4 1.5, MgSO4.7H_2O 0.5, K_2NO_3 3.12, Agar agar 15. Wakasman's Agar medium (WA) Glucose 10, Peptone 5, KH_2PO_4 1, $MgSO_4.7H_2O$ 0.5, Agar agar 15. Richard's Agar medium (RA) KNO_3 10, KH_2PO_4 5, $MgSO_4.7H_2O$ 2.5 $FeCl_3$ 0.02, Sucrose 50, Agar agar 20. Czapeck's Dox Agar medium (CzA) Sucrose 30, $NaNO_3$, KH_2PO_4 0.1, KCL 0.5, $MgSO_4.7H_2O$ 0.5,

$FeSO_4.5H_2O$ 0.01, Agar agar 15. For this purpose 250 ml of each of the above mentioned media were prepared in distilled water and autoclaved at 121°C at 15 lbs. for 20 minutes.

Temperature: To asses the most suitable temperature for the colony growth, the fungus was grown on Czapeck's Agar medium and subjected to five different temperatures i.e. 20, 25, 30, 35, and 40°C for a period of 120 hours.

pH: The effect of various pH levels on the growth of *Fusarium moniliforme* Sheld was studied using Potato dextrose agar medium as the substrate . A quantity of 250ml of PDA was adjusted to each pH level i.e. 5, 6, 7, 8 and 9 pH, by the addition of appropriate volume of 0.1N NaOH and 0.1N HCL. The media for the respective pH was autoclaved, adjusted again for their pH and poured in petri plates.

Light duration: To determine the effect of light and darkness duration on mycelial growth, petri plates in triplicate after inoculation were incubated at 30°C under each set of different light regimes i.e. 24 hours continuous light, 16 hours light alternating with 8 hours darkness, 12 hours light alternating with 12 hours darkness, 8 hours light alternating with 16 hours darkness and 24 hours continuous darkness.

In Vitro evaluation of sensitivity of *Fusarium moniliforme* to various fungicides

Eight commercial fungicides i.e. Apron, Benlate, Derosal, Healthied, Ridomil, Score, Topase and Topsin-M were used in different concentration to check the sensitivity of the fungus. The concentrations used were 10, 30, 50 and 100ppm. Evaluation was made by using the Poisoned food technique of Ilyas *et al* (1982). Measured amount of stock solution was added to sterilized PDA medium in each flask for each concentration to make it 10,30, 50, and 100 ppm. The medium without fungicide served as control.

Statistical analysis

The data were subjected to statistical analysis and Duncan's Multiple Range Test for analysis of variance was applied for analysis of variance and Multiple comparison of means (Wright, 1972). Computer software package Costat version 3.03 was used for calculations.

Results and Discussion

Culture media effects: To determine the suitability, five different culture media (PDA, WA, BA, RA and CzA) were evaluated for their effect on mycelial growth. The growth of *F. moniliforme* Sheld, varied with the culture medium used and incubation period (Table 1). On each medium the growth increased with the increase in the incubation period. Themedium supporting the best growth was PDA which gave 73.0 mm colony growth at an incubation period of 120 hours as compared to Wakasman's Agar, Basal agar medium, Richards agar medium and Czapeck's Agar medium which showed 66.83, 58.0, 56.6 and 54.6mm diameter colony growth, respectively.. The least growth of the fungus on Czapek's agar medium may be attributed to the absence of suitable carbon source and lack of other nutrients. Walker (1957) also reported maximum colony growth of *Fusarium moniliforme* on the same medium.

Temperature effects: The fungi are unable to exhibit efficient growth in the laboratory unless they are incubated at proper temperature. In order to determine which temperature is best for the growth of *F. moniliforme*, a range of 20-40°C was employed, with a difference of 5°C.The fungus was grown on PDA. The mycelial growth was observed at all temperatures, but the best results were obtained at 30°C (Table 2). Above and below this temperature, the growth of the fungus was significantly reduced. According to Hawker (1950), the optimum temperature for the growth of *F. moniliforme* ranged from 25-30°C.

pH effects: One of the important factors which affect the growth of the fungus and many other life processes is the hydrogen ion concentration (pH) of the growth medium. To check this various pH values ranging from 5-9, with difference of 1 were evaluated using PDA medium. It was found that *F. moniliforme* preferred a neutral pH and showed maximum mycelial growth at pH 7. Both above and below this level the mycelial growth of the fungus decreased significantly (Table 3).

Effect of light duration: The duration of light to which fungus is exposed during the incubation period may affect several of the biochemical processes going on during the life of an organism. Continuous exposure of light for 24 hours was found to be the most suitable light duration for the mycelial growth of *Fusarium moniliforme* Sheld as compared to 16 hours light + 8hours darkness or 12 hours light + 12 hours darkness or eight hours light + 16 hours darkness. Continuous darkness resulted in least growth of the fungus (Table 4). Sun and Synder (1978) also reported that continuous light is essential for good mycelial growth.

Table 1: Effect of different culture media on mycelial growth (mm) of *Fusarium moniliforme*

Culture media	Incubation period (hrs)				
	24	**48**	**72**	**96**	**120**
Potato dextrose Agar	15.00a	26.38a	41.00a	57.16a	73.00a
Waksman's Agar	9.63bc	25.10a	37.83b	50.33b	66.83b
Basal Agar	11.16b	21.11b	34.05d	45.30c	58.00c
Richard's Agar	10.00b	23.55ab	31.80e	44.52c	54.67d
Czapeck's Agar	11.13b	25.37a	36.19c	51.40b	56.62cd

Means followed by different letters in each column are significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 2: Effect of different temperatures on mycelial growth (mm) of *Fusarium moniliforme*

Temperature (^{o}C)	Incubation period (hrs)				
	24	**48**	**72**	**96**	**120**
20	9.50b	22.61c	31.66c	41.38b	53.83b
25	12.58a	25.73b	38.52b	51.36a	62.65a
30	13.34a	28.51a	40.66a	52.30a	63.39a
35	12.55a	20.81d	29.83d	38.16c	43.47c
40	12.87a	1.33e	14.00e	16.32d	17.30d

Means followed by different letters in each column are significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 3: Effect of different pH levels on the mycelial growth (mm) of *Fusarium moniliforme.*

pH levels	Incubation period (hrs)				
	24	**48**	**72**	**96**	**120**
5.0	14.51a	28.16a	43.17a	55.32c	63.48c
6.0	15.16a	25.00b	40.83b	52.13d	63.34c
7.0	14.65a	29.83a	44.32a	59.67a	72.62a
8.0	12.63b	26.14b	40.00b	57.19b	70.00b
9.0	11.52b	20.59c	31.00c	40.00e	50.12d

Means followed by different letters in each column are significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 4: Effect of different intervals of light and darkness on mycelial growth (mm) of *Fusarium moniliforme*

Trearments	Incubation period (hrs)				
	24	**48**	**72**	**96**	**120**
Continuous light	10.00a	17.25a	32.50a	53.55a	71.52a
16 Hours light and 8 Hours darkness	9.00b	15.25b	30.32b	50.00b	67.75b
12 Hours light and 12 Hours darkness	9.00b	15.50b	30.00b	49.52b	66.00b
8 Hours light and 16 Hours darkness	8.75b	16.72a	19.60c	50.00b	66.13b

Means followed by different letters in each column are significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 5: Effect of different concentration (ppm) of fungicide on *in vitro* growth of *Fusarium moniliforme.*

Treatments	Fungicide concentration (ppm)				
	0	**10**	**30**	**50**	**100**
Apron	90a	89.70a	82.50a	72.25b	65.00a
Benlate	90a	*	*	*	*
Derosal	90a	*	*	*	*
Healthied	90a	20.00d	18.25de	13.75e	10.00e
Ridomil	90a	83.75b	73.45b	73.68a	50.75b
Score	90a	20.50d	19.56d	15.55d	12.50d
Topase	90a	36.32c	28.23c	20.15c	16.36c
Topsin-m	90a	16.51e	14.50e	8.00f	*

* No growth

Means followed by different letters in each column are significantly different at P = 0.05, According to Duncan's Multiple Range Test.

Effect of various fungicides on the mycelial growth: Host resistance is the best way for the plants to escape from the pathogen, in the absence of a durable host resistance the use of fungicide is another alternative. The sensitivity of the pathogen to various fungicides was evaluated using poisoned food technique (Table 5). It varied greatly with the fungicides used and its respective concentration. In general there was a significant decrease in the mycelial growth of the fungus with an increase in fungicide concentration. Derosal and Benlate completely checked the mycelial growth even at 10 ppm concentration, while Topsin-M, Healthied, Score and Topase were intermediate in their effect. Ridomil and Apron were found to be the least effective. Thakur et al (1978) reported that in vitro tests Benlate, Bavistain and Cupramar completely inhibited the fungal growth at 0.1% level.

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