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Effect of Different Cultural Condition on the Growth of *Fusarium moniliforme* Causing Bakanae Disease

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Abstract. In this study, *Fusarium moniliforme* causal organism of Bakanae disease has been isolated from infected rice seeds variety Pusa Basmati-1121 by using blotter technique. The effects of temperature, pH and carbon source on radial growth rate were assessed on potato dextrose broth medium. Precise characterisation of the growth conditions for such a fungal pathogen has an evident interest to understand and to prevent spoilage of rice crops. Study was carried out to check the effect of temperature (15–50 °C), pH (2-10), and different carbon sources (glucose, dextrose, sucrose, rice husk and sugarcane bagasse) on the growth *Fusarium molinifórme*. Optimum temperature and pH for growth was 20 °C and 5.0 with maximum dry mycelium weight and sporulation i.e. 2.168 gm 1.806 million spores / 100ml respectively. Maximum growth was observed when rice husk was used as sole carbon source (2.432 gm and 1.68 million spore/ 100 ml) however maximum sporulation (0.984 million spore/ 100 ml) was achieved when sugarcane bagasse was used as sole carbon source.

Keywords: *Fusarium moniliforme;* Basmati rice; Bakanae disease.

Introduction

Rice is the fastener diet for more than two billion people in Asia and for a few hundreds of millions in Africa and Latin America [1, 2]. One fifth of the total world area under cereals is comes under rice cultivation. The human population is rapidly approaching seven billion and more than one half depend on rice as their food staple [3]. In India, rice is grown in different agro climatic region ranging from Kashmir to Kanyakumari as upland, middle and low land rice. Rice is grown wide areas in India, especially is western Uttar Pradesh and Uttaranchal. The rice crop is highly sensitive and a potential host for several insect pests [4]. Total area coverage under rice is approximately 42.4 million hectare.

Rice crop suffers from the attack of various type of diseases caused by diverse type of pathogen. Out of 43 fungal diseases of rice 15 are worth coming (Table 1). Out of which foot rot / Bakanae is most important in basmati variety 1121. Foot rot of rice or Bakanae disease commonly known as foolish seedling disease caused by the fungus *Gibberella fujikuroi (Fusarium*

moniliforme anamorph)[5]. Fusarium species are the important pathogen cause significant looses in quality and concomitant with mycotoxins [6].

Table 1: Major diseases of rice and their causative organisms

S. No.	Disease	Casual organism
1	Black kernel	*Curvularia lunata*
2	Blast (leaf, neck [rotten neck], nodal and collar)	*Pyricularia grisea = Pyricularia oryzae*
3	Brown spot	*Bipolaris oryzae*
4	Downy mildew	*Sclerophthora macrospora*
5	Eyespot	*Drechslera gigantea*
6	False smut	*Ustilaginoidea virens*
7	Kernel smut	*Tilletia barclayana = Neovossia horrida*
8	Narrow brown leaf spot	*Cercospora janseana = Cercospora oryzae*
9	Pecky rice (kernel spotting)	*Curvularia* spp. *Fusarium spp. Microdochium oryzae Sarocladium oryzae*
10	Root rots	*Fusarium* spp. *Pythium* spp. *Pythium dissotocum*
11	Seedling blight	*Curvularia* spp. *Fusarium* spp. *Rhizoctonia solani* *Sclerotium rolfsii*
12	Sheath blight	*Rhizoctonia solani*
13	Sheath rot	*Sarocladium oryzae= Acrocylindrium oryzae*
14	Sheath spot	*Rhizoctonia oryzae*
15	Bakanae	*Fusarium moniliforme*

Fusarium moniliforme is an ubiquitous fungus distributed worldwide. Environmental factors such as temperature, water activity and pH have a great influence on fungal development. The fungus affects rice crop in Asia, Africa, and North America. In epidemic cases yield losses may reach up to 20% or more. A 2003 publication from the International Rice Research Institute estimated that outbreaks of bakanae caused crop losses that were 20% to 50% in Japan, 15% in Thailand and 3.7% in India [7]. Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum size have great influence on the growth of pathogen [8]. Present work depicts the role of different pH, temperature and media to understand ecological survival of pathogen which will be helpful in management strategy in the field.

Materials and methods

Isolation, Purification and Identification of *Fusarium moniliforme*

Isolates of *F. moniliforme* were isolated from diseased rice seed variety PB-1121 from Laboratory of Microbiology and Plant Pathology, Centre of Excellence for Sanitary and Phtyo Sanitary (SPS), Certification, Research and Training, Department of Plant Pathology during November 2013. Discolored seeds were placed on sterilized blotter plates and incubated at 20 ± 5°C in the dark / light for 14 days. Fungi were characterized based on their cultural, morphological and spore characteristics and identified by consulting various taxonomic monographs [9, 10, and 11]. A single micro conidial culture was prepared from each isolate. Studies of the following physiological aspects of *F. oxysporum* isolates were conducted in laboratory.

Optimization of Culture Conditions on Growth and Sporulation of *Fusarium moniliformae*

Effect of Temperature

The fungal strain *Fusarium moniliforme* was inoculated into potato dextrose broth and grown at range of temperatures varying from 10 °C to 50 °C for 12 days. Dry mycelium weight and sporulation at each temperature was determined [12].

Dry Mycelium Weight

The culture broth was centrifuged at 14,000 rpm for 20 min and the supernatant fluid was filtered through a filter paper (Whatman No.1). The mycelial biomass yield was estimated by washing with de ionized water and dried at 50°C for 48 h. The mean dry weight of the mycelium was determined as described by Prasad and Chaudhary [13, 4].

Sporulation

Sporulation was calculated with the help of haemocytometer using formula [14]–

Number of spores / 100 mL = V/NX100

N = Average number of spores per square of the four corner square of haemocytometer counted.

V = Volume of haemocytometer (0.256 x10-5) cc

Length of the spores was measured by calibrated ocular micrometer under compound microscope (10 x 45 x of magnification).

Effect of pH

To study the effect of pH, different pH values ranging from 2.0 - 10.0 were used after adjusting pH of the medium by using digital pH meter. Flasks of different pH were inoculated with fungi and incubated at 25 0 C for 12 days. Dry mycelium weight and sporulation were count after 4, 8 and 12 days of incubation.

Effect of carbon source

Glucose, starch, dextrose, sucrose, sugar cane bagasse and rice husk were used as carbon sources. Carbon sources were added separately into basal medium ($MgSO_4$- 0.45 g/l, KH_2PO_4 -5 g/l, NH_4NO_3- 1.85 g/l, ZnSO4.7H_2O -0.2 g/l , $CaCl_2.H_2O$- 0.1 g/l ,$CuSO_4$- 0.02 g/l, $CoCl_2$-0.02g/m, Na_2MoO_4-0.02g/l, $Na_2B_4O_7$-0.02 g/l $MnSO_4$- 0.02 g/l) at 10 g (w/v) and growth and sporulation rate was determined.

Result and discussion

Identification of *Fusarium moniliforme*:

On the basis of colony morphology and characteristics of macro and micro conidia, fungal isolates were identified as *Fusarium*. On further microscopic study, isolate was identified as *F. moniliforme* on the basis of micro conidia, produced on phialides, catenate, hyaline, oblong, 5-12 x 1.5-2.5 um, with (0-1) septum. Macroconidia produced in acervuli, hyaline, long fusoid, tapered to the ends, straight or curved, 25-60 x 2.5-4 um with 3-7 septa.

Amongst the fungal isolates, *Fusarium moniliforme* which is causal organism of Bakanae, was selected for further studies.

Effects of Temperature

Different temperature ranges (15-50 ^oC) were arranged in different BOD incubators. The *F. moniliforme* was inoculated into potato dextrose broth flasks. The flasks were incubated for 12 days and microbial bio mass and sporulation was recorded every day. Results are shown in Table 2.

Table: 2 Effect of different temperature on the growth of *Fusarium moniliforme*

S. No.	Temp ⁰ C	Dry mycelium weight (in gm)			Spores in millions/100mL medium
		After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	
1	15	0.055	0.099	1.282	0.088
2	20	0.479	1.569	2.168	1.806
3	25	0.305	1.516	1.446	1.421
4	30	0.281	0.411	1.002	0.980
5	35	0.110	0.286	0.311	0.056
6	40	0.006	0.014	0.021	1.39
7	45	0.00	0.00	0.00	0.00
8	50	0.00	0.00	0.00	0.00

Chi and Hansen (1964) [15] reported that *F. solani* isolates grew well at higher temperature of 28 ⁰C. The fungus grew at the temperature range of 15– 30 ⁰ C. However, growth of the fungus was drastically reduced below 15⁰C and started to decline above 30⁰C and become zero at 45 ⁰C, as these temperatures did not favor for growth of the fungus. It was observed that at 20 ⁰C, fungus attained the maximum growth and sporulation (2.168 gm and 1.806) while at 25⁰C, it was (1.446 gm and 1.421) after 12 days of inoculation. These studies are in confirmation with Anjaneya Reddy (2002) [16] who reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied from 20⁰C to 35⁰C. The aim of this work was to study the effect of temperatures ensure the elimination of *F. moniliforme*. Results are in confirmation with Imran Khan *et al.,* (2011) [17] showed the *F. oxysporum* f.sp. *ciceri* grew highest at 25 ⁰C.

Effect of pH

Table 3: Effect of Different pH on The Growth and Sporulation of *Fusarium moniliforme*

S. No.	pH	Dry mycelium weight (in gm)			Spores in millions/100mL medium
		After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	
1	2.0	0.046	0.082	0.282	0.33
2	3.0	0.123	0.196	0.388	0.81
3	4.0	0.205	0.665	1.134	1.34
4	5.0	0.488	1.411	2.432	1.68
5	6.0	0.410	0.997	2.289	1.56
6	7.0	0.346	0.801	1.971	1.39
7	8.0	0.141	0.621	0.879	0.93
8	9.0	0.068	0.191	0.226	0.18
9	10.0	0.002	0.048	0.032	0.00

Effect of pH are in confirmation with the findings of Jamaria (1972) [18] who reported that as the pH decreases or increases from the optimum, the rate of amount of growth gradually decreases. Gangadhara, *et al* (2010) [19] studied effect of pH levels on growth of *F. oxysporum* f. sp. *vanillae* isolates. Mean of the dry mycelium weight of the fungus and sporulation on different pH levels was calculated and shown in Table 3. Results showed that *Fusarium moniliforme* grew maximum in pH 5.0 (dry mycelial weight 2.289 gm and sporulation count 1.56). At high acidic range of pH, fungi showed very poor growth of mycelium. Growth of fungi increased with increase in pH up to

pH 5 and then decrease in growth was observed. Imran Khan *et al.,* (2011) [17] showed optimum pH for growth of *F. oxysporum* f.sp. *ciceri* ranged from 6.5 to 7.0.

Table 4: Effect of Carbon source on the growth and sporulation of *Fusarium moniliforme*

S. No.	Carbon Source	Dry mycelium weight (in gm)			Spores in millions/100mL medium
		After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	
1	Glucose	0.328	0.594	1.211	0.471
2	Starch	0.098	0.154	0.784	0.410
3	Dextrose	0.487	0.925	1.842	0.805
4	Sucrose	0.371	0.858	1.011	0.614
5	Sugarcane bagasse	0.294	0.520	1.220	0.984
6	Rice husk	0.657	1.381	2.754	0.826

Various carbon sources viz. sucrose, glucose and starch and agricultural residue such as rice husk and sugarcane bagasse were tested as sole carbon source for the growth of pathogen. Maximum growth was observed in medium containing rice husk (2.754 gm) after 12 days of incubation (Table-4) while maximum sporulations were achieved in medium containing sugarcane bagasse (0.984). Medium containing Dextrose and sucrose also showed effect on the growth and sporulation on *Fusarium moniliforme*. These results were found in proximity with the research findings of Kuhad *et al* 1998, found maximum growth and xylansae production when inoculated in medium containing wheat bran as sole carbon source.

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