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Variation in Five Isolates of *Colletotrichum falcatum* the Causal Fungus of Red Rot of Sugarcane

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ABSTRACT

Variation among five isolates of red rot pathogen, *Colletotrichum falcatum* Went *(Physalospora tucumanensis* Speg), namely SRTI, NRS, RJSM, KCSM and RSM were determined studying the influence of temperature, pH and fungicide sensitivity on radial growth of colony, and pathogenicity on five host varieties. The isolates were grown on PDA at 20, 25, 30 and 35 ± 0.5°C maintaining pH levels of 5,6,7 and 8. To find out the fungicide sensitivity on colony growth, the isolates were grown on PDA amended with Vitavax-200 at 0,10,50 and 100 ppm and Nystatin at 0, 50, 100 and 1000 ppm. Pathogenicity test was made by inoculating the cane varieties, Isd 1/53, Isd 2/54. Isd 17, Isd 18 and Isd 22. Variation in colony diameter of each isolate at each level temperature and pH were remarkable. The optimum temperature for the isolate RJSM, NRS, KCSM and RSM was 30± 0.5°C but for isolate SRTI was 35±0.5°C. Above 30°C reduction in growth of RJSM, NRS, KCSM and RSM was observed. Similarly, range of pH for growth was notably wide, 5 to 8. All the isolates showed maximum growth at pH 7, above which growth was reduced except KCSM which had maximum growth at pH 8. Variations in colony diameter of the isolate on PDA amended with different concentrations of Vitavax- 200 or Nystatin were considered. Even at the same concentration of each fungicide, reduction in radial growth varied greatly. Prominent variation in pathogenicity and virulence among the isolates and reaction of cane varieties of the pathogen differed significantly.

INTRODUCTION

Red rot caused by *Colletotrichum falcatum* Went (Perfect stage: *Physalospora tucumanensis* Speg) is the most destructive disease of sugarcane (*Saccharum officinarum* L) in Bangladesh (Ahmed *et al.*1976, 1977). Red rot pathogen is known to be complex and variable but the degree of variability and complexity is known incompletely (Chona and Srivastava, 1960; Kirtikar, 1960; Kirtikar *et al.* 1965a,

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1965b; Singh *et al.* 1984; Talukder and Malek, 1985). The variability existing in the pathogen is the major factor for which many sugarcane varieties are unstable in their resistance to the disease (Abbott, 1953). Often a variety resistant to prevailing isolates of *C. falcatum* becomes susceptible due to the appearance of new isolate which is virulent towards that variety (Abbott, 1953; Kirtikar *et al.* 1965a, 1965b). Some isolates of the pathogen from different sugarcane varieties grown in different areas of Bangladesh were studied and distinct variations were recorded based on cultural and morphological characteristics and pathogenicity on some sugarcane varieties (Talukder and Malek, 1985). Variability so far recorded in red rot pathogen in other countries is also based on growth characters, morphology on a particular culture media and to some extent, pathogenicity on different sugarcane varieties (Rafay and Singh, 1957; Khirbat *et al.* 1980).

Attempt to study the variations in radial growth of different isolates of red rot pathogen on culture medium under different physical and chemical environment has not yet been reported. Such studies may be effecitve to determine the variability in *C. falcatum*. The present piece of work was undertaken to determine the variation in five isolates of *C. falcatum* due to influence of temperature and pH on radial growth of colony and their sensitivity to fungicide and pathogenicity on some sugarcane varieties.

MATERIALS AND METHODS

Collection of Isolates of red rot fungus: Pure culture of five isolates of *C. falcatum* were obtained from the Sugarcane Research & Training Institute (SRTI) Ishurdi, Pabna. They were isolated from red rot infected cane pieces collected from sugagcane growing areas of SRTI, Rajshahi Sugar Mills (RJSM), SRTI-Northern Regional Station (NRS), Kaliachapra Sugar Mills (KCSM) and Rangpur Sugar Mills (RSM). The isolates were named after abbreviated names of the places from where diseased specimens were collected. Pure cultures were prepared following single spore culture technique. The stock cultures of isolates were mainatined on oat meal agar in test tubes at 4± 1°C.

Culture medium and preparation of inocula: The medium used to grow the fungus and to study its growth in the laboratory was extra pure potato dextrose agar (PDA) manufactured by DIFCO. The dehydrated PDA was rehydrated in distilled water at 39 g/liter. Twenty millilitre of the medium per petridish was used. The pH of the cooked medium was adjusted to 6.5 for all experiments except the experiment on the effect of pH on radial growth of *C. falcatum* where the pH of the medium was adjusted to four different levels. To inoculate test plates under in vitro experiments and sugarcane varieties under pathogenicity test, inocula were prepared by cutting circular blocks of 20 d old culture on 3.9% PDA (DIFCO) containing mycelium and conidia with sterilized 6 mm diameter cork borer.

Influence of temperature and pH: To determine the variation in radial growth of the isolates at different levels of temperature, petridishes containing PDA medium, 20 m1/dish were inoculated with inoculum of individual isolate, one block/ dish and incubated at 20,25,30 and 35±0.5°C in computerized incubators.

After cooking, pH of the medium was adjusted to 5,6,7, and 8 using 0.1N HCl and /or 0.1N NaOH and sterilized in the autoclave for 20 min at 120°C under the pressure of 1.2 kg/cm^2. Sterilized medium was poured into 90 mm petridishes, after solidification petridishes were inoculated with the inoculum of individual isolate at one block per petridish and incubated at 25±1°C.

Fungicide sensitivity of Vitavax-200 and Nystatin: Stock sloution of the fungicides were prepared at 0, 200, 1000 and 2000 ppm for Vitavax-200, and 0,1000, 2000 and 20000 ppm for Nystatin in sterile distilled water,and passed through millipore filter (Toyo Roshikaisha Ltd made in Japan) under sterile condition for sterilization. Nineteen millilitre of autoclaved PDA medium with 50°C was poured into petridishes and 1 m1 of stock solution of each fungicide was added to have 0, 10, 50 and 100 ppm for Vitavax-200 and 0. 50,100 and 1000 ppm for Nystatin. The fungicidal solution was thoroughly mixed with the medium and distributed over the surface control by swirling notion on a Turn Table. Petridishes under control treatment were amended with sterilized distilled water only. After solidification the plates were inoculated with the inocula of individual isolate, one block/plate, and placed in an incubator at 25±1°C

Incubation of test plates and measurement of radial growth : Test plates in invitro experiments with different levels of temperature, pH and fungicide sensitivity were incubated in computerized incubators (Lo-Temp incubator, Yamato Model IL-67). Plates under the experiment with different levels of temperature were incubated in the incubators after setting the required level of temperature. Radial growth of colony was measured at 7-10 d of incubation when almost covered with growth of any of the isolates.

Pathogenicity test: Five sugarcane varieties such as Isd 1/53, Isd 2/54, Isd -17, Isd-18 and Isd 22 were planted in the month of December at the Experimental Farm of SRTI, Ishurdi, Panna. Twenty stalks of each variety inoculated artificially with 20 days old sporulatiog cultures of the isolates of *C. falcatum* by standard plug method (Chona. 1954) in the months of October of the following year. Two months after inoculation, canes were harvested from ground level. split into two halves and length and width of lesions as well as inoculated internodes were recorded. The index of virulence were calculated following a standard formula (Abbott, 1956; Anon., 1983).

The plates under individual experiment with temperature, pH, Vitavax-200 and Nystatin were placed in incubators following 4 X 5 factors factorial experiment in

completely randomized design (CRD). Each treatment under each experiment was represented by five replicated petri plates. The first factor (A) was temperature, pH, Vitavax-200, Nystatin and varieties of sugarcane and the Second factor (B) was isolates of *C. falcatum*. In case of pathogenicity test, the experiment was laid out in 5X5 factorial in CRD with 12 replications. Each replicatton was repersented by I cane stalk. Data were analyzed statistically for analysis of variance following standard procedures. Differences among means were evaluated for significance following least significant difference (LSD) test.

RESULTS AND DISCUSSION

Effect of temperature : The results presented in Figure 1 shows the effect of temperature, isolate and interaction of the two ractors (Temperature X Isolate) on the radial growth of five isolates of *C. falcatum* was significant ($P<0.05$). In case of isolates RJSM, NRS and KCSM colony diameter was significantly wider at 30°C compared to all other levels of temperature. Above this level. reduction in growth of those three isolates was observed . On the other hand, isolate SRTI showed maximum growth at the highest level and minimum at the lowest level. At 30°C radial growth of the isolate KCSM was significantly higher compared to other isolates. The second highest colony diameter was recorded under the isolate RSM followed by isolates NRS, RJSM and SRTI at 30°C. At 35°C the colony diameter of isolates RJSM and NRS were statistically similar, but significantly lower as compared to other isolates. Radial growth of the isolates SRTI, KCSM and RSM was significantly different from each other. Isolates SRTI and RJSM yielded maximum colony diameter at 25°C. At that level maximum agrowth was achieved under the isolate KCSM followed by NRS and RJSM. At 20°C the highest colony diameter was observed in the isolate RJSM. From this study it appears that suitable temperature for growth of colony on culture medium is 35°C for SRTI and 30°C for others (Figure 1). The observation are in agreement with the findings of Abbott (1938) who found maximum growth of the fungus at 30°C to 32.5°C and poor growth at 37°C. Ahmed (1969), Chona and Higorani (1951) and Chona and Sharma (1961) also reported similar results regarding mycelial growth of *C. falcatum*.

Effect of pH : Radial growth of each isolate at all levels of pH varied appreciably. Growth of the SRTI, RJSM, KCSM and RSM isolates increased gradually with increasing level of pH upto 7 and decreased there after (Figure 1). Growth of those four isolates was significantly ($P<0.05$) higher at pH 7 compared to all other levels. The fates of decrease in their growth above pH 7 were dissimilar, Maximum reduction was observed in the isolate RSM (37.4%) followed by isolate SRTI (35.4%), RJSM (22.5%) and KCSM (10.6%). colony diameter of the NRS increased gradually up to the highest level of pH. At pH 5 Colony diameter of RJSM, NRS and RSM were statistically similar but significantly lower than those of SRTI and KCSM. The different in growth of the two later isolates was not significant. At pH 6 maximum growth was recorded under KCSM followed by RSM, SRTI, NRS and RJSM. At pH 7 the highest growth was followed by RSM, SRTI, NRS and RJSM.

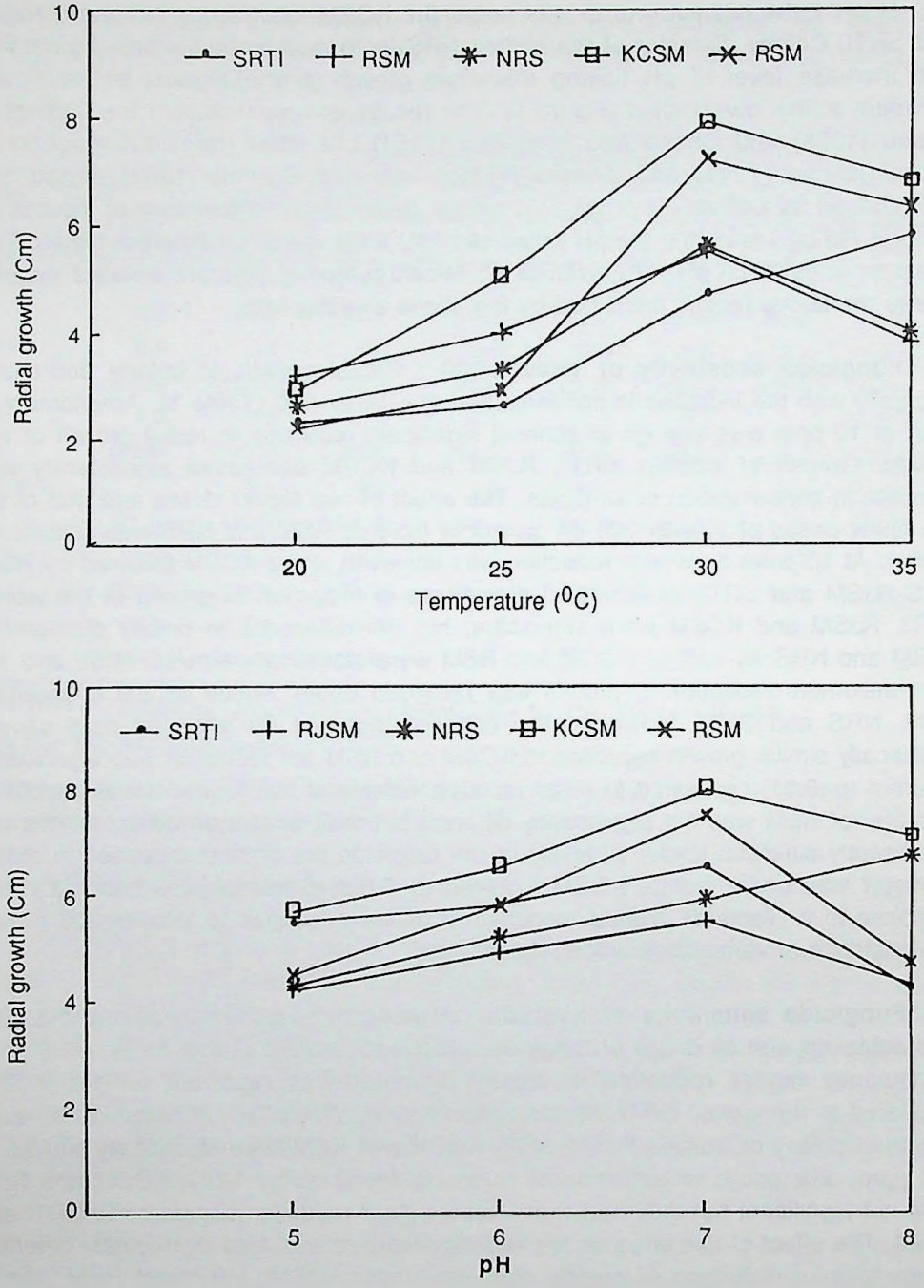

Figure 1. Effect of temperature and pH on radial growth of *Colletotrichum falcatum*.

At pH 7 the highest growth was under the KCSM followed by NRS and RJSM, and SRTI. Colony diameter of the isolate NRS increased gradually and significantly with increase level of pH having maximum growth at the highest pH level and minimum at the lowest level (Figure 1). The results corresponds with the findings of Abbott (1978) and Chona and Hingorani (1951) but differ from that reported by Ramakrishnan (1941) and Ahmed (1969). Lilly and Barnett (1951) stated that optimum pH as well as pH range of a fungus depends on temperature of incubation. Wolpert (1924) found that the pH range of many fungi varied on different media. This variation in optimum pH for growth of *C. falcatum* among different workers may be due to the above factors described by the above investigators.

Fungicide sensitivity of Vitavax-200 : Radial growth of colony decreased gradually with the increase in concentration of Vitavax-200 (Table 1). Amendment of PDA at 10 ppm was enough to achieve significant reduction in radial growth of any isolate. Growth of isolates SRTI, RJSM and KCSM decreased significantly with increase in concentration of fungicide. The effect of two higher doses and that of the two lower doses of Vitavax-200 on growth of isolates RSM and NRS was statistically similar. At 10 pmm maximum reduction was achieved under KCSM followed by RSM, NRS RJSM and SRTI. At this level differences in reduction in growth of the isolate SRTI, RJSM and KCSM were significant, but the difference in colony diameter of RJSM and NRS as well as KCSM and RSM were statistically similar. At 50 and 100 ppm maximum reduction in growth was recorded under isolate KCSM followed by RSM, NRS and SRTI. However, the concentrations of 50 and 100 ppm caused statistically similar growth reduction in KCSM and RSM but reduction was significantly different ($p<0.01$) compared to other isolates. Effects of two higher doses on colony diameter of RSM was not significantly different but their effects on other isolates was significantly different. Under all levels of the fungicide the highest reduction in colony diameter was under isolate KCSM followed by RSM. Other isolates showed varied response to it (Table 1). Varied response of different isolates to Vitavax-200 proved the existance of variabilities among the isolates.

Fungicide sensitivity of Nystatin : Significant reduction in colony diameter was achieved with all doses of fungicide under each isolate (Table 1). At 1000 ppm, significantly higher reduction in colony diameter was recorded for the KCSM compared to the isolate SRTI. At this concentration, differences in reduction of radial growth of colony of isolates RJSM, NRS, KCSM and RSM were statistically similar. At 100 ppm, differences in reduction of colony diameter under NRS, KCSM and RSM were not significant but they had significantly higher reduction compared to SRTI and RJSM. The effect of this dose on the two later isolates was also significantly different. Differences in reduction of colony diameter under RJSM, NRS and RSM due to amendment of PDA with 100 ppm Nystain were not significant but remarkably higher as compare to other two isolates. The lowest reduction was recorded under SRTI (Table 1). The result expresses that RJSM, NRS and RSM showed similar sensitivity to Nystatin while SRTI and KCSM showed dissimilar reaction.

Table 1. Reduction in radial growth of five isolates of red rot pathogen, *Colletotrichum* at different concentrations of Vitavax-200 and Nystatin.

Isolate	Reduction in Colony Diameter[1](%)							
	Vitavax-200 (ppm)				Nystatin (ppm)			
	10	50	100	Mean	50	100	1000	Mean
SRTI	4.5	6.7	8.6	6.7	2.7	4.1	8.2	5.5
	(19.5)	(43.5)	(72.1)	(45.0)	(6.5)	(15.9)	(66.2)	(29.5)
RJSM	5.8	7.3	8.5	7.3	(5.1	6.0	8.4	6.7
	(32.5)	(52.9)	(70.4)	(51.9)	(25.4)	(35.5)	(70.1)	(43.6)
NRS	6.0	6.5	8.6	7.1	5.1	7.7	8.8	7.4
	(35.4)	(40.9)	73.1)	(49.8)	(24.8)	(60.8)	(76.1)	(53.9)
KCSM	7.2	9.1	9.4	8.6	6.3	8.2	9.0	7.9
	(51.1)	(81.8)	(86.4)	(73.1)	(39.2)	(66.4)	(79.5)	(61.7)
RSM	7.0	8.9	9.0	8.3	5.2	6.8	8.8	7.1
	(47.7)	(78.3)	(79.7)	(68.6)	(25.8)	(45.0)	(76.1)	(49.0)
Mean	6.2	7.8	8.8	—	5.0	6.8	8.6	—
	(37.2)	(59.5)	(76.3)	—	(24.3)	(44.7)	(73.5)	—

At 1% level of probability LSD values for Vitavax-200= 0.2024, Isolate = 0.2613; Interaction-Vitavax-200 X Isolate = 0.4526.

At 1% level of probability LSD values for Nystatin = 0.3175, Isolate = 0.499; Interacation-Nystatin X Isolate = 0.7100.

1. Figures within parenthesis are transformed (X+1) values.

Pathogenicity test : Severeity of red not on sugarcane variety Isd 17 due to inoculation with the isolates SRTI and NRS was statistically similar but significantly higher compared to other three isolates. Disease severity caused by isolate KCSM was significantly higher compared to RJSM and RSM on the same variety. Index of virulence of the isolates SRTI, NRS or KCSM on Isd 17 was also significantly higher than the value recorded under all five isolates on other four varieties. Severity of disease due to isolates KCSM on Isd 22 was significantly higher than that caused by other 4 isolates on this variety or on Isd 1/53, Isd 2/54 and Isd 18 (Table 2).

If the mean of index of virulence under each isolate on each variety is considered, the virulence of SRTI and NRS are statistically similar but appreciably higher compared to isolate RJSM or RSM, both of which were equally virulent. The least virulent isolate was RSM. Difference in index of virulence under isolates RJSM and KCSM was not significant. The mean of disease index under all isolates on

variety Isd 17 was significantly higher than means on other four varieties. Differences among mean values of index of virulence on Isd 1/53, Isd 2/54, Isd 18 and Isd 22 were considerable.

Table 2. Variation in pathogenicity of five isolates of red rot pathogen, *Colletotrichum* of five sugarcane varieties.

Sugarcane Varieties	Index of virulence of five Isolates and Reaction of varieties to the Isolates[1]					
	SRTI	NRS	KCSM	RJSM	RSM	Mean
Isd 1/53	0.958 (VHR)	0.903 (VH)	0.657 (VHR)	0.703 (VHR)	0.762 (VHR)	0.796
Isd 2/54	0.712 (VHR)	0.764 (VHR)	1.038 (VHR)	1.237 (HR)	0.696 (VHR)	0.889
Isd 17	4.743 (VHS)	4.822 (VHS)	2.869 (INT)	1.036 (VHR)	1.393 (HR)	2.973
Isd 18	0.884 (VHR)	1.008 (VHR)	0.733 (VHR)	0.947 (VHR)	0.648 (VHR)	0.844
Isd 22	0.582 (VHR)	0.739 (VHR)	1.422 (HR)	1.268 (HR)	0.624 (VHR)	0.927
Mean		1.576	1.647	1.344	1.038	0.825

At 5% level of probability LSD values : Variety (A)=0.3379, Isolate (B) = 0.3379 and interaction (AXB) = 0.7556.

1. VHR=Very highly resistant. HR=Highly resistant, R=Resistant, VHS=Very highly susceptible, HS=Highly susceptible, S=Susceptible, INT=Intermediate.

The reaction of five varieties to five isolates varied. Variety Isd 17 was very highly susceptible to isolates SRTI and NRS, intermediate to KCSM, highly resistant to RSM and very highly resistant to RJSM. Isd 2/54 and Isd 22 showed highly resistant reaction to RJSM and KCSM respectively; while very highly resistant reaction to others. Isd 1/53 and Isd 18 were very highly resistant to all five isolates. The variation in susceptibility of sugarcane varieties to *C. falcatum* and in virulence of the pathogen have been reported by other investigators (Khirbat *et al.* 1980; Chona, 1980a;, 1980b; Singh *et al.* 1984; Talukder and Malek, 1985).

The findings of this investigation reveal that each test isolate of *C. falcatum* differs from others as regard to radial growth of colony on PDA at different levels of temperature as well as pH. The sensitivity to Vitavax-200 or Nystatin on growth on PDA amended with different rates varies remarkably. from pathogenicity test it is clear that each isolate has distinct variations in parasitic relationship with individual variety (Table 3). The results prove that isolates have different host specificity as regards parasitism. The findings of the study are in accordance with findings of other investigators who also recorded variations in growth and pathogenicity of isolates of *C*

falcatum (Chona and Srivastava, 1960; Rafay and Singh, 1957; Kirtikar, 1960; Kirtikar *et al.* 1965a; 1965b; Talukder and Malek, 1985).

Table 3. Comparison of five isolates of *Colletotricum falcatum in* respect of influence of different levels of temperature and pH and fungicide sensitivity of Vitavax-200 and Nystatin on radial growth and their virulence on host varieties.

Treatment	Rank of isolates (descending order)				
	I	II	III	IV	V
Temperature (°C)					
20	RJSM	KCSM	NRS	SRTI	RSM
25	KCSM	RJSM	NRS	RSM	SRTI
30	KCSM	RSM	NRS	RJSM	SRTI
35	KCSM	RSM	SRTI	NRS	RJSM
pH level					
5	KCSM	SRTI	RSM	NRS	RJSM
6	KCSM	RSM	SRTI	NRS	RJSM
7	KCSM	RSM	SRTI	NRS	RJSM
8	KCSM	NRS	RSM	RJSM	SRTI
Growth reduction (%) at different levels (ppm) of Vitavax-200					
10	KCSM	RSM	NRS	RJSM	SRTI
50	KCSM	RSM	RJSM	SRTI	NRS
100	KCSM	RSM	NRS	SRTI	RJSM
Growth reduction (%) at different levels (ppm) of Nystatin					
50	KCSM	RSM	RJSM	NRS	SRTI
100	RJSM	KCSM	NRS	RJSM	SRTI
1000	KCSM	NRS, RSM	NRS, RSM	RJSM	SRTI
Index of virulence on five cane varieties					
lsd 1/53	SRTI	NRS	RSM	RJSM	KCSM
lsd 2/54	RJSM	KCSM	NRS	SRTI	RSM
lsd 17	NRS	SRTI	KCSM	RSM	RJSM
lsd 18	NRS	RJSM	SRTI	KCSM	RSM
lsd 22	KCSM	RJSM	NRS	RSM	SRTI

From this study it appears that in Bangladesh considerable variations exist in red rot pathogen, *C. falcatum* with different pathogenicity and virulence. Such variability may be determinated on the basis of influence of temperature and pH, and fungicide sensitivity on radial growth of isolates of red rot pathogen.

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