

RESEARCH PAPER

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# Effect of pathogenic, cultural variability and chemical management of *Sclerotium rolfsii*

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## ABSTRACT

Among the ten isolates of *Sclerotium rolfsii*, collected from major chilli growing areas of Uttar Pradesh found that the pathogenic variability of the pre germination disease incidence was highest (21.20%) in isolate of district Fatehpur and was lowest (9.5%) in Raibareilly isolates. The cultural variability on the radial growth of fungus and sclerotial production indicated that the radial growth of fungus was maximum in the case of SR1 (83.0 mm) isolate, followed by SR9 (82.00 mm) isolate which were statistically at par with each other. The colony characters was excellent, fluffy in SR1, SR2, SR3, SR4, SR8, SR9 and SR10 isolates and compact in isolates of SR5, SR6 and SR7. *In vitro* studies revealed that the fungicides like Taqat, Hexaconazole, Propiconazole, Tricyclazole, Copper-oxchloride and Vitavax were most effective against the pathogen. The effect of fungicides as seed treatment revealed Taqat and Hexaconazole were found most effective in seed germination (92.8 and 87.5%), respectively and minimum in disease incidence (5.3 and 7.1%), respectively.

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## INTRODUCTION

Chilli is one of the important export oriented crops, the chillies are good source of vitamin like A and C and minerals like Ca, P, Fe, Na, Cu in trace amounts (Thamburaj and Singh, 2001). India is the largest exporter of dry chilli. In India area, production and productivity of chilli were 792.10 million ha. 1223.40 million tones and 1.5 MT/ha, respectively (NHB, 2011). In India, chilli are grown in almost all states of the country and the major chilli growing states in terms of production are Andhra Pradesh (49%), Karnataka (15%),

Odisha (8%), Maharashtra (6%), West Bengal (5%), Rajasthan (4%) and Tamil Nadu (3%) (Kochi, 2005).

These diseases are responsible for lowering down the yield and quality both (Thamburaj and Singh, 2001). Among the many fungal diseases of chilli stem rot caused by *Sclerotium rolfsii* Sacc. is an important disease. This disease is also known as Foot rot/Southern blight/ white stem rot/stem rot in different places of the country. *Sclerotium rolfsii* Sacc. is well known polyphagous, omnivorous, ubiquitous and most destructive soil borne fungus. This is a wide spread sclerotial fungus that

causes leaf and stem blight of a large number of plants. Infrequently its teleomorph, *Athelia rolfsii*, has been found in nature or induced to form under laboratory conditions (Tu *et al.*, 1992). It produces oxalic acid for pathogenesis on many host plant (Bhoraniya *et al.*, 2002). It is a destructive plant pathogen with an almost unlimited host range. The severe stem rot of chilli causing 30-40 per cent seedling rot was observed in a 2.0 ha of atain farmer's field in Saurashtra (Gujarat) India. Infected seedlings exhibited brownish discolour of the leaf tip, wilting and whitish mycelia growth on colour region. Infected seedling showed rooting at the collar region with whitish mycelia growth. This is the first report of *S. rolfsii* causing colour rot in chilli from Gujarat (Lukose *et al.*, 2003).

## MATERIAL AND METHODS

Present investigation was carried out during 2012-13 and 2013-14 at the Department of Plant Pathology, C.S.A. University of Agriculture and Technology Kanpur. *Sclerotium rolfsii* affected samples plants roots were collected during the year 2012-13 from different chilli growing regions of Uttar Pradesh. Isolates of different isolations obtained from affected plants was tested in the same order to establish the pathogenic nature of fungi.

### Pathogenic variability of isolated pathogens:

The experiment was conducted under glass house conditions in pots. Sterilized loam soil, which was previously disinfected with 5 per cent solution of formalin was filled in pots and irrigated to provide adequate moisture. The inoculums was prepared by growing pure culture of *S. rolfsii* on paddy straw + corn meal for 10 days and soil inoculation was done 7 days before sowing of seeds by mixing of the soil with fungal culture in pots. The culture was added @ 5 per cent of weight of the soil in pots. Control pots were filled with soil without adding culture medium. Healthy and surface sterilized planting materials *viz.*, seeds of the chilli variety Chaman were first disinfected with 2.5 per cent sodium hypo-chloride solution for 3 minutes and then rinsed with sterilized water, dried and sown @ 20 seeds/pot (30 cm). The pots were then incubated in glass house at  $28 \pm 2^\circ\text{C}$  and irrigated regularly to maintain sufficient moisture. Three replications were maintained for each treatment.

The pots kept in glass house were observed critically for seedling emergence and appearance of symptoms on seedlings and adult plants upto 45 days of sowing. The pathogen was re-isolated from the internal tissues of infected plants on potato dextrose agar (PDA) medium for confirmation of Koch's postulates (1882). The percentage of pre-emergence and post-emergence infection was calculated from the number of seedlings that failed grow and the number of infected plants, respectively.

### Cultural variability among isolated pathogens:

To determine the cultural behaviour of different isolates, the prepared media was solidified by using two per cent Agar agar. Twenty ml sterilized solid medium was poured in sterilized Petri-dishes of 90.0 mm diameter. After the solidification of medium in each Petri dish, it was inoculated with 5.0 mm disc of 7 days old cultures grown in PDA medium and incubated at  $20 \pm 2^\circ\text{C}$  for 7 days. After completion of incubation period the radial growth was measured in mm in two directions at right angles to each other and average was calculated. Cultural characteristics *viz.*, colony type, colony and aerial mycelium were recorded.

### Effect of chemical fungicides against pathogen (*in-vitro*):

The efficacy of 14 fungicides, at the recommended doses were tested against the pathogen by poisoned food technique (Schmitz, 1930). The required quantities of the fungicides were incorporated into 100 ml of 2 per cent sterilized and solidified PDA and shaken well to make it homogenous. Medium was then poured in 90 mm Petri-dishes. Sterilized Petri-dishes with three replications of each treatment were allowed to solidify. The medium without any fungicides, poured and inoculated similarly, served as control. The Petri-dishes were incubated till the radial growth of the fungal colony in mm assessed the efficacy of various fungicides. All treatments were kept and incubated at  $27 \pm 2^\circ\text{C}$  till the growth of the colony touched the periphery in control plate and at upto one month for observation on sclerotia production. Sclerotia harvested after one month were tested for their viability on PDA and per cent viability was calculated. The per cent inhibition of mycelial growth was calculated by using the formula as given by Vincent (1927).

$$\text{Per cent inhibition (PI)} = \frac{C - T}{C} \times 100$$

where,

PI= Per cent inhibition

C= Growth in control,

T= Growth in treatment.

### Seed treatment with fungicides under glass house condition:

To see the efficacy of fungicides against pathogen *in-vivo*, the seeds of variety Chaman were coated with different test fungicides *viz.*, Taqat, Hexaconazole, Propiconazole, Tricyclozole, Copper oxychloride and Vitavax. The first set of soil was autoclaved to eliminate all the microbes from soil and then infested with pure culture of test soil pathogen (*S. rolfsii*) (@ 40-50 sclerotia) per pot. The other set for control used infested soil but seed were coated as normal distill water. For six treatments the 20 seeds treated with each fungicide were sown separately in 30 cm diameter earthen pots at equal distance and uniform depth. The pots were kept in net house and water was maintained time to time as per need. The experiment was conducted on Completely Randomized Design (CRD) with three replications under glass house conditions. Germinated seeds were counted twice after seven and thirteen days of seed sowing. Second observation was taken to see the persistency effect of fungicides control. The observation on seed germination and disease incidence was recorded. The experiment was carried out for two consecutive years 2012-13 and 2013-14. Disease incidence was calculated using following formula.

$$\text{Disease incidence (\%)} = \frac{\text{Total number of infected plant/pot}}{\text{Total plant population / pot}} \times 100$$

## RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

### Pathogenic variability among isolated pathogen :

The results (Table 1) revealed that there was no adverse effect of *S. rolfsii* isolates on the seed germination of chilli. The germination ranged from 70 to 90 per cent. The pre-emergence disease incidence was highest (21.20%) with the isolate from Fatehpur and was lowest (9.5%) in Raibareilly district. Similarly, seedling vigour after 30 days showed plant height from 17.0 to 18.8 cm with average means 18.01 cm in all *S. rolfsii* isolates tested. In post-emergence, the highest mortality (58.6%) was recorded in the isolates of district Kanpur and minimum 34.3 per cent in isolate of Raibareilly. There was no symptoms of stem rot appeared in un-inoculated plant from any isolates. Prabhu and Patil (2005) reported that pathogenic variation among the *S. rolfsii* isolates of soyabean. Leu *et al.* (1991) by pathogenicity of *Sclerotium rolfsii* on *Trapataiwanensis*.

### Cultural variability among isolated pathogen :

The different isolates under present study were grown on natural PDA media for their growth and sclerotial production. Each isolates was replicated thrice in 90.00 mm Petri-dishes. Observations regarding linear growth and colony characters were recorded after 7 days of inoculation and the sclerotia developed were recorded after 15 days of inoculation. The data were presented in Table 2 showed that the maximum mycelial growth (83.00 mm.) was obtained in SR1 isolate followed by SR9 (82.00

**Table 1 : Pathogenic variability of *Sclerotium rolfsii* isolates on chilli variety Chaman**

Isolate	Source	No of seeds/pot	Seed germination/pot	Germination %	Seedlings (cm)	Pre-emergence DI (%)	Post-emergence DI (%)
SR1	Kanpur	20	15	75.0	17.5	16.2	58.6
SR2	Kanpur Dehat	20	18	90.0	18.2	10.0	50.0
SR3	Fatehpur	20	14	70.0	18.0	21.2	48.4
SR4	Veg. Res. Farm Kalyanpur Kanpur	20	15	75.0	18.2	13.5	39.2
SR5	Hardoi	20	15	75.0	18.2	16.5	52.5
SR6	Raibareilly	20	18	90.0	17.0	9.5	34.3
SR7	Unnao	20	17	85.0	18.2	12.5	51.4
SR8	Aligarh	20	15	75.0	18.0	14.5	48.1
SR9	Hamirpur	20	14	70.0	18.0	19.5	49.8
SR10	Banda	20	15	75.0	18.8	16.2	52.5

mm) isolate which were statistically at par with each other. The lowest mycelial growth (74.0 mm) was recorded on SR6 isolate. The rest of the isolates (SR3, SR5 and SR4 isolates) showed their fungal growth in descending order which were not significantly different with each other (Savary, 1987).

The colony characters were quite distinct on different isolates. The colony was excellent, fluffy in SR1, SR2, SR3, SR4, SR8, SR9 and SR10 isolates and compact in isolates of SR5, SR6 and SR7. The colony colour was also dark brown which were same in all of them. Similarly, SR2 (420) and SR5 (420)

isolate was best for sclerotial productions which were followed by isolate SR6 (415). Least sclerotial production (250) was observed on isolate SR10 (Akram *et al.*, 2007).

The characteristics of the sclerotia were also studied and found that the shape of sclerotia produced by all isolates were round and the colour of these sclerotia were light brown in SR1, SR2, SR3 and SR4, brown in SR8, SR9 and SR10 and remains were dark brown (SR5, SR6 and SR7). The pathogenic and cultural variability of the pathogen agreed very closely with those described by Sharma *et al.* (2002) and Akram *et al.* (2008).

**Table 2 : Cultural variability of different isolates of the pathogen**

Isolates	Radial growth after 7 days (mm)	No. of sclerotia formed after 15 days	Colony characters		Sclerotial character		Pattern of sclerotial formation
			Colony appearance	Colour	Shape	Colour	
SR1	83	390	Fluffy	Dark brown	Round	Light brown	Bunch
SR2	80	420	Fluffy	Dark brown	Round	Light brown	Bunch
SR3	81	395	Fluffy	Dark brown	Round	Light brown	Bunch
SR4	80	400	Fluffy	Dark brown	Round	Light brown	Bunch
SR5	81	420	Compact	Dark brown	Round	Dark brown	Scattered
SR6	74	415	Compact	Dark brown	Round	Dark brown	Scattered
SR7	76	330	Compact	Dark brown	Round	Dark brown	Scattered
SR8	77	320	Fluffy	Dark brown	Round	Brown	Group
SR9	82	295	Fluffy	Dark brown	Round	Brown	Group
SR10	80	250	Fluffy	Dark brown	Round	Brown	Group
C.D. (P=0.05)	5.14	17.15					
S.E.±	1.73	5.77					

**Table 3 : Effects of various fungicides on the growth of *S. rolfsii*. (in-vitro)**

Fungicides	Average diameter of fungal colony (mm)	Per cent inhibition over control
Taqat (0.2%)	00	100
Hexaconazole (0.1%)	00	100
Propiconazole (0.1%)	00	100
Tricyclazole (0.1%)	00	100
Copper-oxychloride (0.3%)	00	100
Vitavax (Carboxin) (0.2%)	00	100
Matco (0.2%)	8.00	90.94
Roko (0.2%)	10.00	88.67
Capton (0.2%)	10.50	88.11
SAAF (0.2%)	20.00	77.35
Bavistin (0.2%)	25.50	71.13
Tebuconazole (0.1%)	35.40	59.92
Propanil (0.1%)	65.20	26.18
Mancozeb (0.1%)	80.30	9.09
Control	88.33	-
C.D. (P=0.05)	3.35	
S.E.±	1.15	

**Table 4 : Effect of seed treatment with fungicides on seed germination disease incidence under glass house conditions**

Fungicide	Seed germination (%)			Disease incidence (%)			% Disease incidence over control		
	2012-13	2013-14	Mean	2012-13	2013-14	Mean	2012-13	2013-14	Mean
Taqat (0.2%)	95.50 (79.12)	90.00 (71.92)	92.75	4.12 (11.62)	6.52 (14.69)	5.32	86.41	83.86	85.18
Hexaconazole (0.1%)	90.00 (71.80)	85.00 (67.37)	87.50	6.89 (15.17)	7.33 (15.62)	7.11	77.28	81.86	79.57
Propiconazole (0.1%)	90.00 (71.80)	80.00 (63.52)	85.00	9.52 (17.94)	13.70 (21.68)	11.61	68.61	66.10	67.35
Tricyclozole (0.1%)	85.00 (67.32)	80.00 (63.52)	82.50	15.66 (23.29)	16.64 (24.04)	16.15	48.36	58.83	53.59
Copper-oxychloride (0.3%)	80.00 (63.49)	80.00 (63.52)	80.00	25.47 (30.29)	22.53 (28.31)	24.00	16.02	44.26	30.14
Vitavax (Carboxin) (0.2%)	82.50 (65.34)	75.00 (60.05)	78.75	28.71 (32.38)	30.25 (33.34)	29.48	5.34	25.16	15.25
Control	70.00 (56.80)	65.00 (53.74)	67.50	30.33 (33.40)	40.42 (39.45)	35.37	-	-	-
C.D. (P=0.05)	7.38	6.68		1.99	2.71				
S.E. $\pm$	2.41	2.18		0.65	0.88				

### Chemical management under glass house conditions:

It is well known that any crop variety cannot sustain its resistance indefinitely and resistant varieties are recorded susceptible due to appearance of new virulent strains. Under such conditions it becomes necessary to fall back upon the chemical control of the disease in order to minimize yield losses. Therefore, the control of stem rot of chilli, the well recognized methods of chemical use and seeds treatment by fungicides was applied after ascertaining the efficacy of the chemical in bio assay test.

### Bio-assay of chemicals against the *S. rolfsii* (in vitro):

In order to find out the most effective fungicides for the control of stem rot of chilli, fourteen fungicides viz., Taqat (0.2%), Hexaconazole (0.1%), Propiconazole (0.1%), Tricyclozole (0.1%), Copper oxychloride (0.3%), Vitavax (0.2%), Matco (0.2%), Roko (0.2%), Captan (0.2%), SAAF (0.2%), Bavistin (0.2%), Tebuconazole (0.1%), Propanil (0.1%) and Mancozeb (0.2%) belonging to different groups were screened in laboratory by using poison food technique (Schmitz, 1930). It was found from the corresponding bar diagram that all fungicides were significantly superior over control in inhibiting the growth of the pathogen. Taqat, Hexaconazole, Propiconazole, Tricyclazole, Copper-oxychloride and Vitavax were most effective against the pathogen by checking 100 per cent growth of the pathogen. The fungicides which checked the radial

growth of the pathogen in their descending order were Matco, Roko, Captan, SAAF, Bavistin and Tebuconazole (Fouzia, 2006).

### Evaluation of seed treatment with fungicides under glass house conditions:

The fungicides which formed most effective in *in vitro* were further tested as seed dresser by their efficacy in pot experiment. The result (Table 4) revealed that by the using fungicides Taqat and Hexaconazole as a seed dresser were found most effective in seed germination (92.75 and 87.50%), respectively and also minimizing disease incidence by (5.32 and 7.11%), respectively. Among the treatment the poor seed germination (78.75%) and higher disease incidence (29.48%) was found by using of Vitavax (Carboxin) as seed dresser. The maximum per cent disease incidence over control (85.18%) and minimum over control (15.25%) in both years. These findings are closely related to the finding of Shahid *et al.* (1990) and Singh *et al.* (2005).

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