

Survival and pathogenicity of *Rhizoctonia solani* causing sheath blight of rice

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Studies were undertaken on some factors affecting the viability of sclerotia and mycelia of *Rhizoctonia solani*, the causal organism of sheath blight of rice. Injury to sclerotia resulted in delayed germination. Sclerotia were found to be more resistant to heat than mycelia. Both mycelia and sclerotia remained totally viable after heat treatment (dry and wet) for 100 minutes at 40°C. At higher temperatures, viability was gradually reduced more prominently by dry heat than wet heat losing completely by dry heat at 60°C for 80 minutes or more. Sclerotia, on the other hand, were more affected by wet heat and viability was completely lost by wet heat treatment at 70°C for 80 minutes or more. Of the three moisture holding capacities of soil studied, survival was best at 50% MHC. Sclerotia were much more resistant than mycelia. The depth of soil to which the fungus was subjected was also found to have considerable effect on the viability. Inocula (mycelium and sclerotium) in deeper soil showed mycelia units retained their viability even after 180 days present on soil surface of 5 cm deep in the soil. At 80% MHC, however, the mycelia and sclerotia completely lost their viability at all the soil depths studied after 180 days. Viability was also very much reduced at 20% MHC. Maximum number of rice seedlings were affected when the inoculum was located at the surface soil and minimum number in deeper soil.

Key words : Rice, sheath blight, *Rhizoctonia solani*, viability-factors affecting

INTRODUCTION

With the introduction of high yielding cultivars of rice (*Oryza sativa* L.) in the sixties, widespread occurrence of sheath blight, caused by *Rhizoctonia solani* Kuhn [*Thanatephorus cucumeris* (Frank) Donk] has been noticed in different rice growing countries of the world. Since its first report in India in 1963 (Paracer and Chahal, 1963) sheath blight has become a major constraint in rice production in India. It is not unusual in tropical areas to find most of the leaves killed and total loss of the crop due to the disease, if a highly susceptible cultivar is grown in large continuous areas (Gangopadhyay and Chakraborty, 1982). The pathogen is known to perennate primarily as sclerotia in soil and initiate infection soil is, no doubt, affected by various soil factors to which it is exposed. Studies were undertaken on some factors affecting the viability of mycelia and sclerotia of *R. solani*, the results of which are presented in this paper.

MATERIALS AND METHODS

An isolate of *R. solani* (R2), isolated from blight infected sheaths of rice plants and rice cultivar "Ratna" were used for different experiments.

The fungus was grown in sterilized sand maize meal medium (fine sand 75 g, crushed maize 25 g, distilled water 100 ml) in 250 ml Erlenmeyer flasks for 10 days at 28°C. The sclerotia, which were produced in abundant numbers, were collected, washed in sterile distilled water, dried in folds of sterilized blotting papers and stored in airtight bottles at 4°C. The sclerotia were used within 7 days of collection.

For collection of mycelia the fungus was grown in potato dextrose broth for 10 days at 28°C in 250 ml Erlenmeyer flasks. The mycelial mat was collected by filtering through 2 folds of muslin cloth and washed thrice in sterile distilled water. The mycelial mat was then spread on a sterilized petridish and 6 mm diameter mycelial discs were cut by means of a sterilized cork borer, each disc being taken as one unit.

Viability of mycelia and sclerotia, after various treatments, was studied by placing them on 20 ml of 2% sterilized water agar in petridishes and the fresh growth/germination (%) of the mycelia (units) and sclerotia respectively were recorded after incubation at 28°C for 24 to 72 hrs.

Injury to the sclerotia was made (i) by pinpricks and (ii) by treatment with 0.5 and 1.0% hydrochloric acid for 1 minute followed by washing in sterile distilled water and drying on sterilized blotting papers.

Effect of temperature on viability of mycelia and sclerotia was studied in incubators at different temperatures. Sclerotia and mycelial discs were taken in watch glasses and placed at different temperatures for varying periods. For studying the effect of wet heat 10 ml of sterile distilled water, taken in culture tubes, were kept for 30 minutes at different temperatures. Mycelial discs and sclerotia were then placed separately in the distilled water in culture tubes. After different time intervals the mycelia discs or sclerotia were collected, dried in sterilized blotting papers and seeded on 2% sterilized water agar.

The effect of different soil moisture (% MHC of soil) at different soil depths was studied following Rana and Sen Gupta (1974).

For studying the effect of inoculum kept at different soil depths in initiating sheath blight of rice, polythene buckets (20 cm diameter, height 25 cm) with a small pore at the bottom were filled up with field soil. Blight affected rice sheaths, collected from the field, were cut into pieces of 4-5 cm and kept at different depths of soil (surface 6 cm and 10 cm deep). Three replications were taken for each depth and control was kept with healthy sheath pieces. The buckets were kept in natural condition in the field for 12 months. Surface sterilized seeds of cv. "Ratna" were then sown in soil in the buckets and watered regularly. Data were collected on percent germination of seeds and plants showing disease symptoms, as well as, root and shoot lengths in different treatments.

RESULTS AND DISCUSSION

While *Sclerotium rolfsii* and *S. delphanii* were reported to show enhanced germination in response to injury to the sclerotia (Chet, 1969) injury to the sclerotia of *R. solani* caused by pinprick or treatment with low concentration hydrochloric acid had some adverse effect, resulting in delayed germination of the sclerotia (Table 1). This is no doubt evidently due to differences in sclerotial morphology of *Sclerotium* and *Rhizoctonia*. Sclerotia of *Sclerotium* spp. have a typical hard cored rind acting as a barrier preventing uptake of

nutrients which was removed by mild injury. Such differentiation into rind is absent in *Rhizoctonia*. Therefore, injury retarded the germination of sclerotia of *R. solani* by damaging the outer mycelial cells of the sclerotia, deep seated mycelial cells, however, retaining their viability.

Table 1. Effect of injury on germination of sclerotia of *R. solani*

Treatment	Germination(%) after different* time intervals (h)				
	6	12	18	24	30
Hydrochloric acid (%)					
0.5	0	58	70	100	
1.0	0	0	38	66	
Pinprick	0	0	42	70	100
Control (uninjured)	0	70	100		

Higher temperature had some adverse effect on the viability of mycelia and sclerotia of *R. solani*, mycelia being much more affected (Table 2). Mycelia and sclerotia were unaffected at a temperature upto 40°C. At 50°C and above gradual reduction in viability was noticed with increased treatment period. The effect, in case of mycelia, was more pronounced when dry heat was used. Treatment of mycelial units at 60°C for 80 minutes (dry heat) and 100 minutes (wet heat) resulted in complete loss of viability. The sclerotia being comparatively more resistant bodies, small percentage of sclerotia retained their viability after treatment at 70°C for 60 and 80 minutes. Contrary to mycelia, sclerotia were more affected by wet heat. Perhaps when dry heat was applied, outer cells of the sclerotia were affected, the inner cells remaining unaffected. But in wet form, the heat penetrated inside affecting the inner cells also. The findings are in general agreement with the observations of Sherwood (1969).

On (1985) suggested that the sclerotia of *R. solani* are mostly present in the upper 2-3 inch (5-7 cm) below soil and float to the surface during land preparation (puddling, levelling etc.) for the season. These are carried away by water and when come in contact with a rice plant, initiate infection. Soil moisture and the depth of soil at which the pathogen, inocula are present play a very important role on the viability of the pathogen, as has been demonstrated in the results presented in this study (Table 3). A moderate moisture level (50% MHC) of soil was found to be most favourable for the survival of *R. solani*. At this moisture level hundred percent of the sclerotia retained their viability after 180 days on soil surface or at 5 cm depth of soil. Some amount (20%) of mycelial units also remained viable under such conditions. However, with an increase in soil depth the viability decreased. Reduced survivability after prolonged incubation at a comparatively low moisture level of soil (20% MHC) was apparently due to gradual desiccation. Exposure of the fungus at a very high soil moisture level (80% MHC) was also found to be highly detrimental to its survival, particularly in deeper soil. Reduced survivability of *R. solani* at high moisture level was attributed by Blair (1943) as due to decline in soil aeration. High soil moisture, on the otherhand, stimulates bacterial activity which may result in lysis of fungal mycelium. In a study of the growth of *Macrophomina phaseolina* in soil, Kavoov (1954) noted that at low moisture levels no bacterial action against the hyphae was

noticed, but at 80% moisture level, an active stage of bacterial attack was observed followed by lysis of mycelium. In the present study viability of sclerotia, of *R. solani* was completely lost when kept at 8% MHC of soil at a soil depth of 15 cm for 150 days and at all the soil depths studied after 180 days. Deleterious effect of higher depths of soil on the survival of sclerotia of *Sclerotium rolfsii* (Abeygunawardena and Wood, 1957; Rana and Sen Gupta, 1974; Punja and Jenkins, 1984) and *Sclerotium delphinii* was earlier reported. Such adverse effect of soil depth on sclerotial survival was attributed by most of the workers as due to inhibition by poor aeration and high CO₂ levels. Punja and Jenkins (1984) suggested that soil pressure over deeply buried sclerotia could also possibly account for the inhibition of their germination due to leakage of carbohydrates and amino compounds. Exudation of these compounds from sclerotia are reported to increase the activities of other soil microorganisms (Gilbert and Linderman, 1971; Coley-Smith *et al.*, 1974), which frequently resulted in sclerotial demolition.

Table 2. Effect of heat (dry and wet) on the viability of mycelia and sclerotia of *R. solani*

Treatment	Temperature(°C)	% of living units after treatment period (min.)					
		0	20	40	60	80	100
<i>Mycelia</i>							
Dry heat	30	100.0	100.0	100.0	100.0	100.00	100.00
	40	100.0	100.0	100.0	100.0	100.0	100.0
	50	100.0	86.7	82.3	53.3	40.0	33.6
	60	100.0	66.7	53.3	23.6	0.0	0.0
	70	100.0	50.0	33.6	20.0	0.0	0.0
Wet heat	30	100.0	100.0	100.0	100.0	100.0	100.0
	40	100.0	100.0	100.0	100.0	100.0	100.0
	50	100.0	90.3	85.0	73.3	53.6	33.6
	60	100.0	66.7	60.0	46.7	33.3	0.0
	70	100.0	53.3	33.7	33.7	10.0	0.0
<i>Sclerotia</i>							
Dry heat	30	100.0	100.0	100.0	100.0	100.0	100.0
	40	100.0	100.0	100.0	100.0	100.0	100.00
	50	100.0	100.0	100.0	86.6	80.0	66.6
	60	100.0	100.0	80.0	66.7	53.3	50.0
	70	100.0	80.0	66.7	43.1	20.0	0.0
Wet heat	30	100.0	100.0	100.0	100.0	100.0	100.0
	40	100.0	100.0	100.0	100.0	100.0	100.0
	50	100.0	100.0	80.0	66.7	53.3	33.3
	60	100.0	86.7	73.3	60.0	53.3	10.0
	70	100.0	73.3	60.0	33.3	0.0	0.0

Table 3. Effect of different soil moisture on the survival of *R. solani* at different soil depths

Treatment period (days)	MHC (%) of soil		Germination of mycelia and sclerotial units at different soil depths (cm)			
			Surface	5	10	15
30	20	M	40	40	30	20
		S	70	70	60	50
	50	M	80	80	80	70
		S	100	100	100	100
	80	M	80	70	60	40
		S	100	90	80	70
60	20	M	30	40	30	20
		S	60	60	50	40
	50	M	70	70	60	50
		S	100	100	100	100
	80	M	60	50	40	30
		S	70	70	60	50
90	20	M	30	30	20	10
		S	60	60	50	40
	50	M	60	60	50	40
		S	100	100	100	100
	80	M	50	30	20	10
		S	60	60	40	30
120	20	M	20	20	10	0
		S	40	50	40	20
	50	M	60	60	40	30
		S	100	100	80	80
	80	M	30	20	10	0
		S	50	50	50	20
150	20	M	10	10	0	0
		S	30	30	20	20
	50	M	40	40	20	10
		S	100	100	70	60
	80	M	10	10	0	0
		S	20	30	10	0
180	20	M	0	0	0	0
		S	10	10	0	0
	50	M	20	20	10	0
		S	100	100	60	40
	80	M	0	0	0	0
		S	10	0	0	0

M = mycelia units, S = sclerotia units.

The results presented above show the possibility of long period survival of the inoculum of *R. solani* in soil in absence of its host, rice plant. Papavizas and Davey (1962) observed that the surviving mycelium of *R. solani* in precolonized substrates was less sensitive to adverse microenvironment than the active saprophytic phase of the fungus. In the present study, rice seedlings, grown in soil inoculated about 12 months earlier with sheath blight infected plant parts at different depths of soil, were found to be variously infected with the disease (Table 4). Symptoms of seedling infection was not typical of that of sheath blight symptoms observed on older plants, but consisted of stunted plants, yellowing and later browning of the leaves and ultimate death of the seedlings. Significant reduction in seed germination as well as root and shoot elongation was also observed as a result of seedling infection by *R. solani*. Disease severity was related to the depth of soil at which the inoculum was located. It was lesser when the inoculum was present in deeper soil which could be due to (i) reduction of inoculum concentration after long incubation in deep soil and (ii) distance of the inoculum from the germinating seedlings.

Table 4. Perennation of *R. solani* at different soil depths and subsequent infection of the rice seedlings.

Depth of inoculum in soil	Seeds germination (%)	Infected plants (%) after (days)		Length (cm)	
		7	10	Root	Shoot
Upper layer (0.0 cm)	31.5 (34.14) ¹	27.62	100.00	1.35	3.95
Middle layer (6.0 cm)	48.5 (44.14)	27.62	100.00	2.70	7.62
Lower layer (12.0 cm)	70.5 (60.47)	0.00	70.17	6.00	17.70
Control (uninoculated)	90.0 (71.57)	0.00	0.00	7.50	18.00
L.S.D. (P = 0.05)	3.35			0.5	1.1

¹Figures in parenthesis are transformed angular values

The preceding discussion clearly showed the resistant nature of the sclerotia, of the rice sheath blight organism, *R. solani*, to various environmental conditions making it very difficult to control the disease by cultural means. However, destruction of the stubbles after harvesting of the crop and deep ploughing resulting in burying the fungal inoculum deep in the soil, followed by irrigation for increasing the soil moisture level may yield good control of the disease by reducing the inoculum density in the soil.

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