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Factors affecting Competitive Saprophytic Ability of *Rhizoctonia oryzae sativae* (Saw.) Mord in Soil

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The effects of some factors, viz., duration and temperature of incubation, soil moisture, soil amendments and enrichment of baits, on the competitive saprophytic ability of *R. oryzae sativae* was studied in artificially inoculated natural soil by Cambridge method. The highest colonisation of substrate units (51.6%) occurred at 30°C and 50% soil moisture after 14 days of incubation. High (40°C and 100%) and low (20°C and 25%) incubation temperature and soil moisture reduced the saprophytic activity of the test organism. Soil amendment with mustard cake (1.0%) and enrichment of baits with single super phosphate (1.0%) increased saprophytic colonisation of baits (60.3% and 57.6% respectively) over unamended soil and unfortified baits.

Key Words : Competitive Saprophytic Alibity (CSA), *R. oryzae sativae*, substrate, rice, obligate saprophyte, Facultative parasite

INTRODUCTION

Rhizoctonia oryzae sativae, the incitant of stem and root rot diseases of rice (Mordue, 1974) is considered as a facultative saprophyte. The organism perpetuates the intercrop periods mainly by sclerotia (Hirayama *et al.*, 1981) or in stubbles and other crop residues as mycelium (Mordue, 1974). This saprophytic survival of a pathogen in the absence of a host plant is dependent on its ability to colonise moribund or dead host tissues in competition with

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other obligate saprophytes and is known as CSA, the competitive saprophytic ability (Garrett, 1956). Although some of the ecological factors affecting the survival and pathogenicity of this organism have been studied in the past (Das, 1986 ; Roy and Pan, 1989), but our knowledge about the conditions affecting the saprophytic life of this rice pathogen in soil is very meagre. In this article attempts have been made to present an account of the factors influencing the CSA of *R. oryzae sativae* in soil.

MATERIALS AND METHODS

The soil (pH=6.7 ; C : N ratio=8.6 : 1 ; organic carbon—0.69 ; MHC=47.0% and total microbial populations=11.2 x 10^5 fungi, 8.7 x 10^6 bacteria and 4.5 x 10^6 actinomycetes/g of soil) used was sandy loam collected from a double cropped rice field. The soil was air dried, ground, sieved through 2 0 mm mesh and finally stored at 10°C for subsequent use.

R. oryzae sativae, was isolated from infected rice stems and roots, purified and multiplied on PDA medium at 30 ± 1°C. The stock cultures were maintained on PDA slants at 5°C. The sclerotia of the fungus were raised on sand-maize meal medium and thus it was made possible to ensure identical nature of inoculum all though.

All experiments were carried out in natural soil (unsterilised) under controlled conditions of 30°C and 50% moisture holding capacity (MHC) of the soil other than those where effects of incubation temperature and moisture were studied. The soil moisture at a particular level was kept constant by daily weighing and watering, if required.

For determination of competitive saprophytic ability of *R. oryzae sativae* the method adopted was essentially the "Cambridge Method" developed by Butler (1953) and Lucas (1955). Single noded mature wheat straws (10 mm long) were used as baits. The inocula (air dry) @ 0, 0.5, 1.0, 2.0 and 4.0 g/100 g of soil were added to 200g of air dried natural soil in glass jars seperately and the moisture was adjusted as required with distilled water. The number of sclerotia/g of soil in each soil inoculum mixture was determined by wet seiving subsequently. In each set of experiment 40 stem pieces (baits) per replicate were buried and incubated at specific temperature usually at 30°C for 14 days. After 14 days the baits were recovered, washed thoroughly under running tap water, surface sterilised with 0.5% NaOCI and were subsequently placed on 2% water agar medium to determine the percentage of stem pieces colonised. Finally, the EID_{50} values were determined from the regression equation $\hat{y}=c+bx$ where, $\hat{y}$=calculated per cent colonisation of baits ; c=intercepting constant, and x=number of propagules per g of soil.

The effects of the different factors on CSA of *R. oryzae sativae* were studied in three replicated treatments.

Incubation periods : Colonisation of baits (%) were recorded after 3, 7, 14 and 28 days of incubation at 30°C and 50% MHC. Effects of different incubation temperature on colonisation of baits (%) were recorded at 20°, 30° and 40°C and at a fixed soil moisture (50%).

Effects of different soil moisture : It was studied by reconstituting it at 0 (air dry), 25%, 50%, 75% and 100%, MHC at 30°C.

Soil amendments : Soil was amended with three oil cakes viz., groundnut cake, neem and mustard at 50% and 1.0% (w/w).

Substrate enrichment : The substrates (baits) were fortified by keeping them evernight in solutions of urea, super phosphate and murate of potash at 1.0% and 0.1% before their burial in soil at 30°C and 50% MHC.

RESULTS

It was evident from Table 1 that with the gradual increase in incubation periods colonisation of baits by *R. oryzoe setivae* gradually increased and reached maximum (53.3%) after 21 days at 4% inoculum level but subsquently decreased after 28 days (46.9%). However, there was hardly any significant difference in per cent bait colonised after 14 and 21 days which was evident from the calculated EID_{50} value (213).

Results presented in Table 2 showed that the most suitable temperature for colonisation of baits was 30°C followed by 20° and 40°C irrespective of inoculum densities.

Soil moisture showed marked effect (Table 3) with highest colonisation (53.6%) with lowest EID_{50} value (225) occurred at 50% MHC. followed immediately by treatment at 75% MHC with EID_{50} value 239. Below (25%) and above (100%) soil moisture, colonisation of baits gradually decreased being minimum (10.3%) at 25% MHC. However, the organism totally failed to colonise baits in air dried soil. The difference between colonisation of baits at 50% and 75% MHC appeared insignificant.

Amendments of soils with groundnut and neemcake, regardless of dosages, 5.0 and 1.0%, (Table 4) reduced the saprophytic activity of *R. oryzae sativae* over that of unamended soil (Table 3) at the same incubation temperature and

Table 1. Effect of incubation periods on C. S. A. of *R. oryzae sativae* at 30°C and 50% MHC

Incubation periods (days)	Stem pieces colonised at different inoculum level (%)*	Regression equation	EID_{50} value	Correlation coefficient(r)
3	a) 0.0**	$\hat{y} = 13.59 + 0.062$	>300	0.876
	b) 4.3			
	c) 10.6			
	d) 15.3			
	e) 18.6			
7	a) 0.0	$\hat{y} = 21.897 + 0.084x$	>300	0.889
	b) 12.3			
	c) 21.6			
	d) 33.3			
	e) 37.6			
14	a) 0.0	$\hat{y} = 21.25 + 0.086x$	213	0.718
	b) 18.3			
	c) 44.6			
	d) 51.3			
	e) 52.6			
21	a) 0.0	$\hat{y} = 31.35 + 0.085x$	213	0.715
	b) 20.6			
	c) 42.3			
	d) 50.6			
	e) 53.3			
28	a) 0.0	$\hat{y} = 30.02 + 0.076x$	259	0.736
	b) 18.3			
	c) 35.6			
	d) 46.6			
	e) 46.9			

* % colonisation of baits was recorded after 14 days of incubation

** Each insertion is an average of 3 replications

a) = 0.0, b) = 0.5, c) = 1.0, d) = 2.0 and c) = 4.0 inoculum/100 g soil

Table 2. Effect of incubation temperature on C. S. A. of *R. oryzae-sativae* at 50% MHC

Incubation temperature (°C)	Stem pieces colonised at different inoculum level (%)*	Regression equations	EID_{50} value	Correlation coefficient(r)
20°	a) 0.0** b) 9.3 c) 28.3 d) 31.6 e) 32.3	$\hat{y} = 24.25+0.059x$	>300	0.690
30°	a) 0.0 b) 11.3 c) 49.3 d) 51.3 e) 51.6	$\hat{y} = 33.30+0.073x$	216	0,628
40°	a) 0.0 b) 8.3 c) 18.3 d) 18.6 e) 20.6	$\hat{y} = 13.32+0.039x$	>300	0.796

* % colonisation of baits was recorded after 14 days of incubation

** Each insertion is an average of 3 replication

a) = 0.0 ; b) = 0.5; c) = 1.0 ; d) = 2.0 and e) = 4.0g inoculum/100 g soil

and soil moisture while amendment with mustard cake increased it. The effect of soil amendment with 1.0% mustard cake on saprophytic colonisation of baits was most significant over the soil amended with the same cake at 5 0% irrespective of inoculum densities.

Similarly, enrichment of baits with 1.0% single super phosphate increased (57.6%) saprophytic colonisation of baits (Table 5). This was sequentially followed by enrichment of baits with 0.1% single super phosphate and 1.0% murate of potash. Baits enriched with 0.1% urea showed lowest colonisation (38.6%).

Table 3. Effect of soil moisture on C. S. A. of *R. oryzae-sativae* at 30°C

Soil moisture (% MHC)	Stem pieces colonised at different inoculum level (%)*	Regression equations	EID_{50} value	Correlation coefficient (r)
25	a) 0.0** b) 2.9 c) 5.6 d) 9.3 e) 10 3	$\hat{y} = 11.81+0.0319x$	>300	0.640
50	a) 0.0 b) 19.3 c) 48.3 d) 52.6 e) 53.6	$\hat{y} = 24.90+0.1093$	225	0.802
75	a) 0.0 b) 21.3 c) 49.6 d) 50.3 e) 51.6	$\hat{y} = 40.81+0.0347x$	239	0.762
100	a) 0.0 b) 8.3 c) 20.3 d) 29.6 e) 33.3	$\hat{y} = 10.94+0.128x$	>300	0.798
0	a) 0.0 b) 0.0 c) 0.0 d) 0.0 e) 0.0	—	—	—

* % Colonization of baits were recorded after 14 days of incubation

** Each insertion is an average of 3 replications

a) = 0.0; b) = 0.5; c) = 1.0; d) = 2.0 and e) = 4.0 g inoculum/100 g soil

(—) = Regression equiations, EID_{50} value and (r) could not be determined due to 'O' infection

Table 4. Effect of soil amendment with oil cakes on C.S.A. of *R. oryzae sativae* at 30°C and 50% MHC

Oil cakes with dose	Stem pieces colonised at different inoculum level (%)*	Regression equations	$EID_{\infty 0}$ values	Correlation coefficient (r)
	a) 0.0**			
Groundnut	b) 7.6			
cake	c) 14.6	$\hat{y} = 19.7+0\ 080x$	> 300	0.916
(5.0%)	d) 18.3			
	e) 29.3			
	a) 0.0			
Groundnut	b) 12.3			
cake	c) 18.6	$\hat{y} = 17.18+ 0.077x$	> 300	0.913
(1.0%)	d) 22.3			
	e) 32.6			
	a) 0.0			
Neem cake	b) 9,6			
(5.0%)	c) 17.3	$\hat{y} = 20.03+0.0614x$	> 300	0.820
	d) 26,3			
	e) 26.6			
	a) 0.0			
Neem cake	b) 14.3			
(1.0%)	c) 15.6	$\hat{y} = 20.89+0.0795x$	> 300	0.976
	d) 26.3			
	e) 35.3			
	a) 0.0			
Mustard cake	b) 24.6			
(5.0%)	c) 41.3	$\hat{y} = 38.82+0.060x$	263	0.965
	d) 40.3			
	e) 46.9			
	a) 0.0			
Mustard cake	b) 39.3			
(1.0%)	c) 46.6	$\hat{y} = 32.60+0.055x$	185	0.772
	d) 52.3			
	e) 60.3			

* % colonisation of baits was recorded after 14 days of incubation

** Each insertion is an average of 3 replications each containing baits a) = 0.0; b) = 0.5, c) = 1.0, d) = 2.0 and e) = 4.0 g inoculum/100 g soil

Table 5. Effect of enrichment of baits with different fertilizers on the C. S. A. of *R. oryzae-sativae* at 30°C and 50% MHC

Fertilisers with dose	stem pieces colonised at different inoculum level (%)*	Regression equations	EID_{50} value	Correlation coefficient (r)
	a) 0.0**			
Urea	b) 23.3	$\hat{y} = 28.98+0.083x$	251	0.975
(1.0%)	c) 31.6			
	d) 42.3			
	e) 50.6			
	a) 0.0			
Urea	b) 14.6			
(0.1%)	c) 26.3	$\hat{y} = 25.08+0.075x$	> 300	0.808
	d) 38.6			
	e) 38.6			
	a) 0.0			
Single super	b) 28.3	$\hat{y} = 32.91+0.084x$	204	0.933
phosphate	c) 39.3			
(1.0%)	d) 49 6			
	e) 57.6			
	a) 0.0			
Singlu super	b) 24.6			
phosphate	c) 37.3	$\hat{y} = 31.37+0.84x$	219	0.192
(0.1%)	d) 48.3			
	e) 53.6			
	a) 0.0			
Murate of	b) 25.3			
potash	c) 25.3	$\hat{y} = 24.15+0.110x$	239	0.998
(1.0%)	d) 47.6			
	e) 53.6			
	a) 0.0			
Murate of	b) 18.6			
potash	c) 24.6	$\hat{y} = 20.03+0.064x$	7300	0.820
(1.0%)	d) 31.3			
	e) 42.6			

* % colonisation of baits was recorded after 14 days of incubation

** Each insertion is an average of 3 replications

a) = 0.0; b) = 0.5, c) = 1.0; d) = 2.0 and e) = 4.0 g inoculum/100 g soil

DISCUSSION

The ability of a soil inhabiting pathogen to survive in soil depends upon its ability to colonise dead plant materials competitively with other facultative saprophytes which in turn is governed by various factors.

It appeared that substrate colonisation was low during the initial periods of incubation reaching its peak between 14-21 days and there after declined. Low substrate colonisation during the initial periods of incubation might have resulted from poor growth rate of *R. oryzae sativae* in natural soil (Basu, 1987) which is in aggrement with the observations made by Papavizas and Davey (1961).

Soil temperature and moisture appeared to be important factors determining the saprophytic activity of an organism in soil. They may act by modifying the behaviour of competitors in soil rather than by dircct effect on the growth of target organism. Optimum colonisation of wheat stem pieces, in the present study, was recorded at 30°C and reduced colonisation at 20° and 40°C. Sawada (1922) obtained optimum mycelial growth at 32°C for this organism. Roy and Pan (1989) reported highest sclerotial germination at 30°C. Dhar and Maity (1981) observed spread of the disease rapidly at 25° to 30°C under natural conditions. Thus, the present findings agree with the early observations that the organism has the optimum temperature around 30°C for its saprophytic and pathogenic activities.

R. oryzae sativae effectively colonised baits at 50-75% MHC that reduced at 25% and 100% MHC. Reduction in colonisation of baits at lower soil moisture (25%) may be from reduced germination of sclerotia (Roy and Pan, 1989). High soil moisture (100%) is known to favour development of microorganisms having high CSA and antagonistic bacteria which may cause lytic effects on mycelia (Kovoor, 1954). Besides, accumulation of CO_2 in the immediate vicinity of the substrates in poorly drained soils is known to prevent or redues the saprophytic activity of some organisms (Papavizas and Devey, 1961).

In the present study, poor saprophytic activity was recorded in soils amended with groundnut and neemcake. Saprophytic activity of many fungi reduces in soil during decomposition of organic substances. Reduction in viability of sclerotia of *R. solani* in soil amended with groundnut and neemcake has been reported (Laksman and Nair, 1984) and reduced saprophytic growth under the influence of different plant amendments is also known (Swardt and Paner, 1974) in addition to the antimicrobial activity of neem (Singh and Singh, 1981).

Baits when enriched with single super phosphate called for maximum saprophytic colonisation followed by murate of potash and urea. The present observations also coroborate the earlier observations made by Das (1986) and Basu (1989) who observed that single super phosphate and murate of potash not only

stimulated germination of sclerotia but supported better mycelial growth and prolonged lives of sclerotia in soil over urea.

So it appeared that from the present studies that *R. oryzae sativae* has a poor saprophytic activity in soil and which is markedly influenced by different physico chemical factors.

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