The Genus Rhizoctonia in Relation to Soil Moisture

I. Studies on *Rhizoctonia solani* and *Rhizoctonia bataticola*

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INTRODUCTION

A variety of plant diseases have been attributed to the two species of *Rhizoctonia, viz. R. solani* and *R. bataticola* which are considered cosmopolitan in occurrence and plastic in their parasitism. Soil moisture and temperature are the two factors having paramount importance in the saprophytic as well as the parasitic phases of these organisms. Considerable information on their saprophytic nature has been reported in recent years. Blair (1943) demonstrated the free mycelial spread of *R. solani* in unsterilized soil. However, *R. solani* is a poor cellulose decomposer; the cellulose decomposing microflora has a depressing effect on this fungus. Kovoor (1947) and Zachariah (1949) recorded *R. bataticola* colonizing dead cotton tissue in the soil. Kovoor (1954) further reported the free mycelial growth of *R. bataticola* in soil, with the older hyphae falling a prey to bacterial antagonism at high soil moisture while the younger hyphae continue their growth, colonize vegetative debris and produce sclerotia which serve as a means of dispersal. The observations of Norton (1953) on *Sclerotium bataticola* indicate that the fungus is shy of the soil microflora and hence do not grow extensively into the soil away from the food base.

The present work was undertaken to investigate the effect of soil moisture on the growth and survival of *R. solani* and *R. bataticola,* found

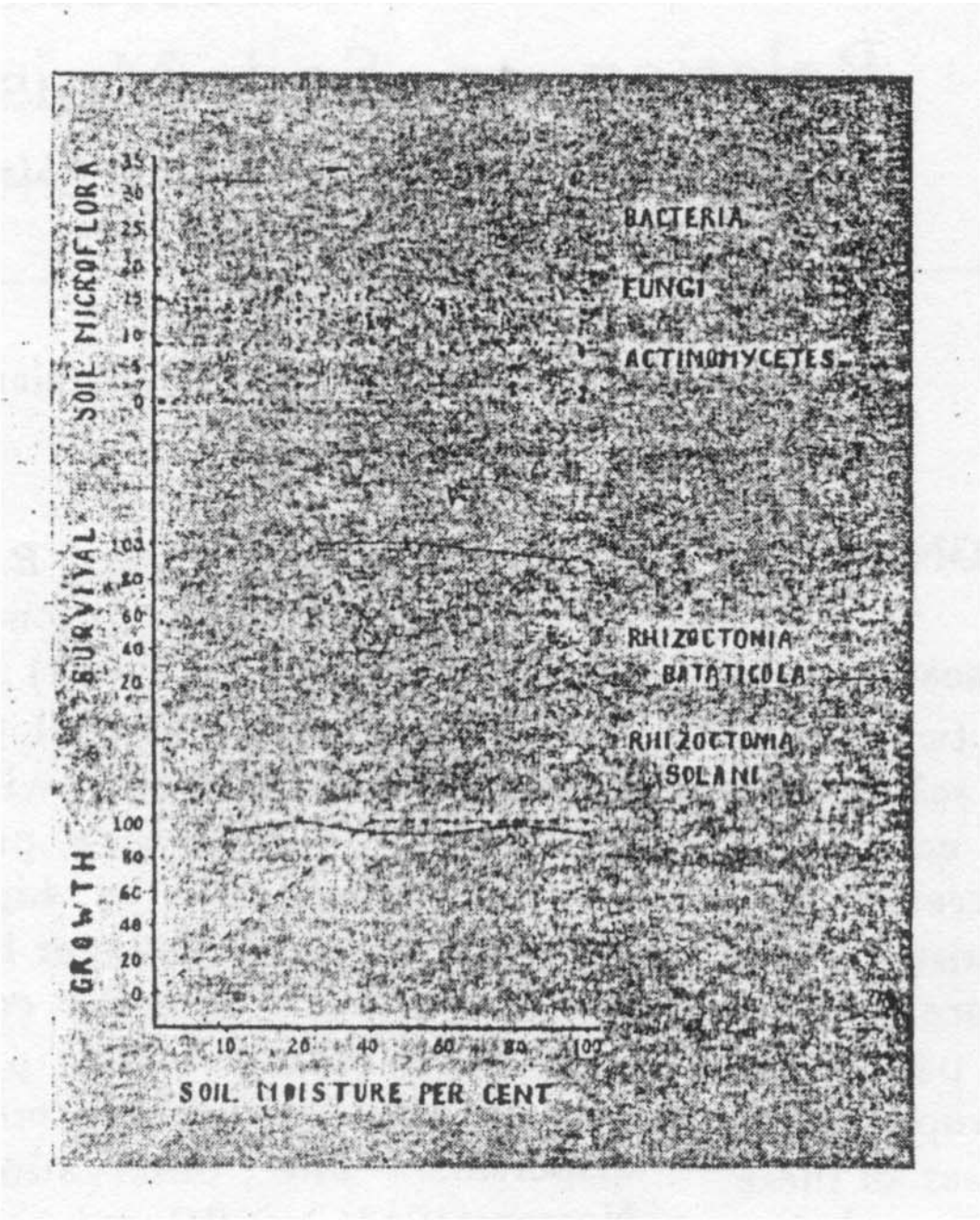

Figure showing growth (%) of *R. solani* and *R. bataticola* in sterile soil, survival (%) of these organisms in natural soil and the soil micro-flora, fungi in thousands and actinomyceles and bacteria in millions g. soils under different moisture conditions.

Plate I

frequently associated with the coconut root (wilt) disease.

MATERIALS AND METHODS

Fungal cultures

R. solani and *R. bataticola* isolated from roots of diseased coconut palms at the Research Station were used throughout.

Soil

The sandy soil of the Research Station garden was used for experimental studies. The moisture content of the soil was adjusted and maintained at 10, 20, 40, 60, 80 and 100 per cent of its water holding capacity.

Growth Studies

For studying the linear growth of *R. solani* and *R. bataticola* they were grown in soil contained in glass tubes 1 cm. in diameter and 45 cm. in length. After filling the tubes with the required quantity of soil with the moisture content adjusted, both the ends of the tubes were first plugged with cotton and then corked. Half the number of tubes were autoclaved at 20 lb. pressure for 30 minutes for the sterile soil series. All the tubes were inoculated with cork borer discs of the fungi cut from the growing edges of colonies on potato dextrose agar. The tubes were examined every three days and the linear growth of fungi recorded.

The growth of fungi through soil was also tested by using autoclaved coconut root pieces as colonizing medium for the fungi. 600 g. soil, both autoclaved and natural contained in glass jars had their moisture content adjusted to 10, 20, 40, 60, 80 and 100 per cent. Coconut root pieces were buried in the soil 5 per jar and the soil was inoculated on the top 1 cm. layer. The criterion of growth was the isolation of the test fungi from the coconut root pieces.

Survival of fungi

Healthy coconut root pieces artificially infected with the test fungi were buried in soil contained in glass jars, 10 per jar. The viability of the fungi was determined at regular intervals.

Soil microflora

For the evaluation of the soil microflora the dilution plate method was adopted using peptone dextrose agar (pH 4·0) with 30 ppm. Rose Bengal for fungi and the egg albumin agar (pH.6·5) for bacteria and actinomycetes (Waksman and Fred 1922).

Microbial antagonism

A modified Rossi Cholodny slide method was followed to study the extent of microbial antagonism towards the organisms under different soil moistures. The test fungi were grown on microscope

slides smeared with a thin layer of nutrient agar. The slides were examined at regular intervals after air drying and staining with Rose Bengal.

RESULTS

Growth

The linear growth of *R. solani* and *R. bataticola* in natural soil was very poor. Mycelial growth was limited to 0·5 to 1·0 cm. from the inoculum. In sterile soil, growth of *R. solani* was profuse under all soil moisture contents. Growth recorded after 21 days' incubation was 19·0 cm., 21·3 cm. and 21·1 cm. at 100, 50 and 25 per cent soil moisture respectively. Under similar conditions the growth of *R. bataticola* was 6·1 cm., 10·2 cm. and 8·3 cm.

Growth of both the organisms as determined by their saprophytic colonization on coconut root pieces was found to be good, when the soil was sterile (Table I and Fig.). In a preliminary trial when the soil moisture was maintained at 25, 50 and 100 per cent, *R. solani* made better growth than *R. bataticola*; however, *R. solani* was inhibited by high soil moisture. When the experiment was repeated, the soil moisture was maintained at 10, 20, 40, 60, 80 and 100 per cent. In this series *R. solani* failed to grow at 10 and 20 per cent soil moisture. The data presented in Table I show that both the organisms were unable to grow out into the natural soil and colonize the root pieces.

Table I

Showing the growth of *R. solani* and *R. bataticola* as percentage colonization on coconut root pieces at different soil moisture contents.

Soil moisture%		10	20	40	60	80	100
R. Solani	S. S.	0	0	100	100	100	100
	N. S.	—	—	—	—	—	—
R. bataticola	S. S.	93	100	93	92	100	90
	N. S.	—	—	—	—	—	—

S. S:— Sterile Soil N. S:— Natural Soil

Survival

The viability of *R. solani* was considerably affected by the soil moisture. At 25 and 50 per cent soil moistures *R. solani* remained viable in 100 and 96 per cent of the root pieces after 24 weeks' incubation. At 100 per cent soil moisture its viability was adversely affected and after 4 weeks' incubation the percentage survival was only 44. The per-

centage survival of *R. bataticola* remained high in all the treatments throughout the experimental period. (Table II & Fig.).

Table II

Showing the percentage survival of *R. solani* and *R. bataticola* under different soil moisture contents (average of two trials).

Period of incubation in weeks.	Soil moisture per cent					
	R. solani			*R. bataticola*		
	25	50	100	25	50	100
4	100	96	100	97	100	92
8	100	92	48	97	100	90
12	100	96	40	100	100	89
16	100	100	30	100	100	93
20	100	99	38	100	92	94
24	100	98	39	100	100	93

The significant difference in the behaviour of the test organisms in natural and autoclaved soil under similar moisture conditions reveal that the growth and survival of these fungi are related more with the associated micro-organisms in the soil than to the soil moisture. High soil moisture in the absence of the soil microflora in sterile soil had no adverse effect on *R. solani* and *R. bataticola* but in the presence of the soil microflora in natural soil, growth as well as survival of the fungi were considerably affected. For an elucidation of the correlation of the soil microflora to soil moisture conditions and its ultimate effect on the activity of the test organisms, the following investigations were carried out.

EVALUATION OF THE MICROFLORA OF THE SOIL UNDER VARIOUS SOIL MOISTURES

Fungi

Quantitatively, soil fungi increased on incubation of the soil, the maximum increase was recorded at 10 per cent moisture content of the soil after 30 days' incubation. At other moisture levels, the fungal number decreased on long incubation (Table III; Fig.).

Bacteria

After 15 days' incubation, soil bacteria were considerably reduced at 80 and 100 per cent soil moistures whereas in the other series no significant difference was evident. On further incubation bacterial numbers increased in all the series,

the highest increase being at 20 per cent soil moisture (Table III; Fig.).

Actinomycetes

The behaviour of the actinomycete flora was similar to that of bacteria in all the treatments excepting at 10 and 20 per cent soil moisture. At these two moisture levels of the soil, their numbers decreased initially but on longer incubation higher values were recorded (Table III; Fig.).

The pH of the soil was 6·5 prior to the treatment and after 15 and 30 days' incubation the soil pH was 6·0 in all the treatments.

Table III

Soil microflora in relation to soil moisture — Fungi in thousands and bacteria and actinomycetes in millions per 10 g. soil.

		Prior to treatment	Soil moisture per cent					
			10	20	40	60	80	100
Fungi	a	7·75	19·75	13·3	12·24	14·1	12·08	13·22
	b		30·5	12·22	11·0	7·06	5·29	8·33
Bacteria	a	1·475	11·75	15·9	11·44	14·68	8·7	7·3
	b		14·0	19·89	15·7	14·45	18·35	15·1
Actinomycetes	a	1·75	1·75	2·3	1·3	0·81	1·4	0·28
	b		3·0	7·3	2·09	3·91	2·35	1·21

a—15 days' incubation.

b—30 days' incubation.

Microbial antagonism

Fungal mycelia remained intact on the Cholodny slides after one and two weeks' incubation at 10 and 20 per cent soil moistures. On further incubation the bacterial flora increased and consequently its antagonistic activity also increased. As a result of the microbial antagonism the fungal hyphae were found corroded at several places. In the case of *R. bataticola* the sclerotia were found free from bacterial activity; similarly some of the very young hyphae also remained intact. The culture of *R. solani* under study is a purely mycelial form and hence was more easily affected by microbial antagonism than *R. bataticola*. The intensity of microbial antagonism was rated as mild (+), severe (++) and intense (+++) (Table IV).

Table IV

Indicating the intensity of microbial antagonism *in situ* in relation to soil moisture.

Incubation period in weeks	*R. solani*		*R. bataticola*	
	10% soil moisture	20%	10%	20%
1	Nil	Nil	Nil	Nil
2	Nil	+	Nil	+
4	+	++	+	+
6	++	+++	++	+++

DISCUSSION

R. bataticola and *R. solani*, two of the soil inhabiting fungi make free mycelial growth in unsterilized soil (Kovoor, 1954; Blair, 1943). The data presented here, however, show that the isolates of these two organisms from coconut roots behave differently. Both *R. bataticola* and *R. solani* isolates tested by us made free mycelial spread only in sterile soil. They were shy of the soil microflora just as the *Sclerotium bataticola* reported by Norton (1953). In sterile soil no significant variation in growth was recorded in the case of *R. bataticola* under the different soil moisture conditions, whereas, low moisture contents of the soil, namely 10 and 20 per cent were not favourable for the growth of *R. solani* (Table I). In their survival, in natural soil *R. bataticola* was not affected by the different soil moistures; however, *R. solani* recorded as low as 28 per cent survival at 100 per cent soil moisture. This significant variation in the behaviour of these two organisms may be explained as due to their basic morphological difference, *viz.*, the absence of sclerotia in *R. solani* which is capable of tiding over the unfavourable soil condition.

Thus, both in their growth and survival *R. bataticola* and *R. solani* were affected by the associated soil microflora as evident from their behaviour in natural and sterile soils. That the soil moisture by itself exerts very little influence on their activity is also revealed by the data furnished above (Table I). All the same, the soil moisture is indirectly related to the activity of these organisms by virtue of its influence on the general soil microflora. The increased microbiological activity in the soil at different moisture conditions is the

factor deciding the development and survival of the test organisms, is indicated by the direct microscopic examination. To sum up, the activity of these two isolates of *R. solani* and *R. bataticola* is indirectly related to the moisture content of the soil and is directly correlated with the soil microflora.

SUMMARY

The saprophytic activity of *R. solani* and *R. bataticola*, the organisms associated with the Coconut Root (wilt) disease was investigated in relation to soil moisture. Both the organisms made poor mycelial spread in natural soil. In autoclaved soil the test fungi grew well under a wide range of soil moisture. Evaluation of the soil microflora and the microbiological activity in the soil under different moisture conditions revealed that the activity of the test fungi is correlated with the microbial status of the soil which is directly influenced by the moisture content of the soil.

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