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Antagonism Between *Rhizoctonia solani* KÜHN and Certain Soil Saprophytes: A Laboratory study

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Antagonismus mezi *Rhizoctonia solani* KÜHN a určitými půdními saprofyty. Laboratorní studium

U tří saprofytů byl sledován projev antagonismu proti pathogenu *Rhizoctonia solani* v různých podmínkách kultivace. *Trichoderma viride* a *Chaetomium cochlioides* inhibovaly růst *R. solani* účinněji při inkubační teplotě 25 a 30° C. *T. viride* bylo antagonisticky aktivnější v prostředí kyselém, *C. cochlioides* v prostředí alkalickém. Je vysloven předpoklad, že půdní doplňky zvyšující aktivitu *C. cochlioides* by mohly být platným opatřením proti sledovanému pathogenu v alkalických půdách. Na rozdíl od předešlých zpráv byla kultura *R. solani* celulolyticky zaktivnější než *T. viride* a *C. cochlioides*, a to i při použití chloridu amonného jako droje dusíku.

Summary

Antagonism of three saprophytes to the pathogen, *Rhizoctonia solani* was studied in plate cultures under different conditions of temperature, pH, and nutrition. The antagonists *Trichoderma viride* and *Chaetomium cochlioides* were found more effective in the inhibition of the growth of *R. solani* at temperatures 25 and 20° C and this conforms to their growth-temperature relationship. *T. viride* was found more effective in acid media as reported by others, whereas other antagonists and especially *C. cochlioides* was more effective in the alkaline medium. Hence the suggestion is made that soil amendments increasing the activity of *C. cochlioides* might be a good control measure against the pathogen in alkaline soils.

The most interesting result of the investigation is that, contrary to the previous reports, *R. solani* appeared to be more cellulolytic than *T. viride* and *C. cochlioides* and this result was unaffected by the use of NH_4Cl as N-source.

Introduction

It is now a common knowledge that during the growth of pathogens in pure culture, contamination by other organisms frequently retards or prevents their growth. The discovery of this phenomenon has led to the study of antagonistic effects between organisms and also to the adoption of antibiotic therapy against human and plant diseases.

FAWCET (1931) was the first to emphasise the use of known mixtures of organisms in plant disease investigations and to suggest that the development of many plant diseases may be very much influenced by associated organisms. Thereafter, various workers like WEINDLING (1934), SANFORD (1946) and WOOD (1951) claimed in greenhouse studies that cultures of antagonists or their culture-filtrates added to the soil containing pathogens reduced or suppressed the development of disease. Although the success of the soil amendments or use of antibiotics for seed dressing is being realised, yet the value in understanding empirically the antagonistic phenomenon under different conditions cannot be overlooked. Hence in this paper the antagonism of saprophytic soil organisms, *Trichoderma viride, Penicillium clavariaeforme* and *Chaetomium cochlioides* against *R. solani* under different conditions of temperature, pH, and nutrition has been reported.

Material and Methods

Source of culture: *T. viride* and *C. cochlioides* were obtained from stock culture collection of the Imperial College, London and originally isolated from soil. *P. clavariaeforme* was isolated from a contaminated culture of *R. solani*. They were all maintained on potato dextrose agar (PDA) slants. *R. solani* was isolated from a diseased seedling of Swede.

Media: The following media were used:

(1) PDA: 20% peeled potato slices, 2% glucose & 2% agar. pH 5·9.

(2) Glucose, starch, cellulose powder or sodium carboxy-methyl cellulose: 1%, NH_4Cl or $NaNO_3$: 0·144% and 0·227% respectively; KH_2PO_4 : 0·1% and $MgSO_4 7H_2O$: 0·05% and agar 2%.

In order to prepare media distilled water was used. The initial pH of the glucose-NH_4Cl medium = 5·2; of glucose-$NaNO_3$ medium = 5·4, starch-NH_4Cl medium = 5·9, starch-$NaNO_3$ medium = 6·1, cellulose-NH_4Cl medium = 5·3, cellulose-$NaNO_3$ medium = 5·1, Na-carboxy-methyl cellulose (CMC) — NH_4Cl = 5·2, CMC — $NaNO_3$ 5·3.

An equal quantity (i.e. 15 ml.) of agar media was sterilised in tubes at 15 lbs. pressure for 20 minutes and plated in the sterile petri dishes.

Method of Inoculation: Spore suspension of the antagonists and 4 mm. disc from the 3—4 days old culture of the pathogen growing on PDA were used as inocula in all the tests.

Ringing Method: Three discs of culture of the pathogen were plated on each agar plate. A No. 12 cork-borer (12 mm. diameter) was dipped in spore suspension of the antagonists placed on the agar and then removed to leave a ring of spores around each disc. Observations were made every day to see under what conditions the pathogen could grow through the ring and to study the growth of antagonists. All treatments were in duplicate and the results after 4 days incubation are given below in the tables.

Table 1. Growth (mm./day) of the antagonists and the pathogen at different temperatures

Organism	Temperature °C					
	30	**25**	**20**	**15**	**10**	**5**
R. solani	1·5	16·5	13·0	12·6	7·0	4·5
T. viride	9·0	22·5	14·5	9·0	2·0	0·0
P. clavariaeforme	0·0	4·0	3·0	3·0	2·0	0·0
C. cochlioides	1·2	10·0	6·5	5·5	1·5	0·0

Table 2. Antagonism against *R. solani* at different temperatures

Antagonist	Temperature °C				
	25	**10**	**15**	**10**	**5**
T. viride	+	+	+—	— —	— —
P. clavariaeforme	—	—	—	— —	— —
C. cochlioides	++	+—	—+	— —	— —

++ : The pathogen confined inside the ring in all six colonies.
\+ : The pathogen penetrated the ring, and grown outside but the antagonist pursued it from behind and over-ran the pathogen in all six.
+— : More than three and less than six colonies attacked by the antagonist.
—+ : Less than three colonies attacked by the antagonist.
— : Pathogen affected inside and just outside the ring, but later escaped the attack.
— — : Pathogen unaffected.

Table 3. Growth of the antagonists and the pathogen at different pH values

Organism	pH				
	4·3	4·9	5·4	6·2	7·7
R. solani	14·0	15·3	16·6	18·0	10·3
T. viride	27·3	27·0	28·0	24·6	6·0
P. clavariaeforme	3·6	3·6	3·6	4·6	2·0
C. cochlioides	6·3	7·3	8·6	10·0	8·6

Table 4. Antagonism against *R. solani* at different pH values

Antagonist	pH			
	4·3	5·4	6·6	8·0
T. viride	++	++	+—	—+
P. clavariaeforme	+—	—+	—+	++
C. cochlioides	—+	+—	+	++

Results and Discussion

1. The growth of the pathogen and the antagonists is compared at different temperatures on PDA and results are given in Table 1.

In the preliminary experiments the optimum temperature for the growth of fungi under study was found. It corresponded to the temperature, of 25° C as reported in the Table 1. *T. viride* grows more rapidly at the temperature of 30°, 25° and 20° C. Below this temperature its growth is less than that of the pathogen. This indicates that in regions where the soil temperature is 20° C and above, this soil saprophyte might check the growth of the pathogen but below 20° C the pathogen might escape the attack of the antagonist. Regarding other antagonists their growth is too slow to check the growth of the pathogen at any temperature from 5 to 30° C.

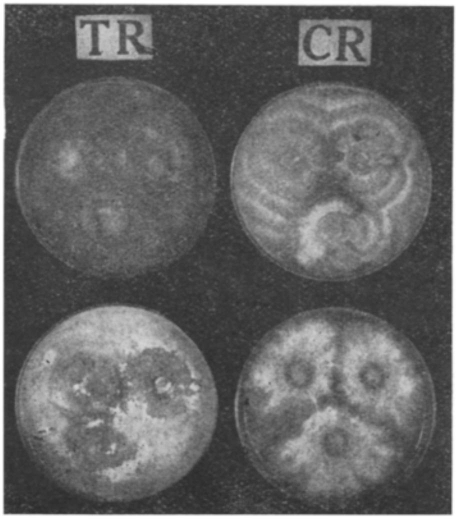

Fig. 1. Antagonism of organisms against *R. solani* at different pH values. — TR = *T. viride* + *R. solani*, CR = *C. cochlioides* + *R. solani*.

2. Next, antagonism at different temperatures was investigated and the results are expressed in Table 2.

From the above data it is clear that the antagonistic effect of *T. viride* appeared to be related to its growth at different temperatures in pure culture. But this was not the case with other antagonists. *C. cochlioides* was relatively slower in growth in pure culture but effectively checked the growth of the pathogen at 25° C and also at 20° C. This result conforms to its growth-temperature relationship. *P. clavariaeforme* as reported by WOOD (1951) seems to have a doubtful antagonistic effect. Inspite of the complete escape of the pathogen from attack by this fungus its spores, after germination, first deformed and then completely destroyed hyphae of the pathogen on both sides of the ring. As the spores of this antagonist are dispersed in the soil by means of various agencies, it may serve as a very effective antagonist against the pathogen.

3. The growth of organisms at different pH values was studied and the results are reported in Table 3.

They indicate that *T. viride* is potentially useful against *R. solani* under acid conditions whereas other antagonists are not, at all pH values.

4. Antagonism at different pH values was next investigated and the results are presented in Table 4 and Figs. 1 and 2:

The above result supports the view that *T. viride* may be effective in the control of pathogen in acid soils. As regards the antagonistic effect of *P. clavariaeforme* it is rather interesting to note its maximum effect at pH 8·0 and 4·3 and correspondingly less effect at other pH values. This result was quite unexpected in view of its growth in pure culture (Table 2 and Fig. 2). *C. chochlioides* in its effects behaves in an opposite way from *T. viride*. Although no previous information is available as regards the pH antagonism relationship of this fungus, it seems probable that it may be more effective in the control of the pathogen in alkaline soils.

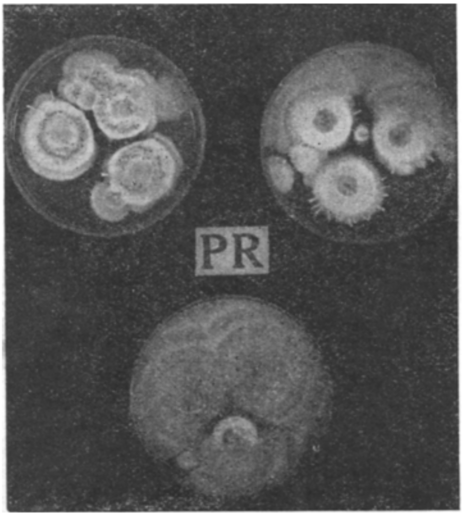

Fig. 2. Antagonism of *P. clavariaeforme* against *R. solani* (PR) at different pH values.

5. Growth of organisms in enriched agar media. It has been reported by Blair (1941) and Wood (1951) that addition of wheat straw or maize meal to the soil gave protection to the seedlings against the pathogen. Blair explained this as being due to

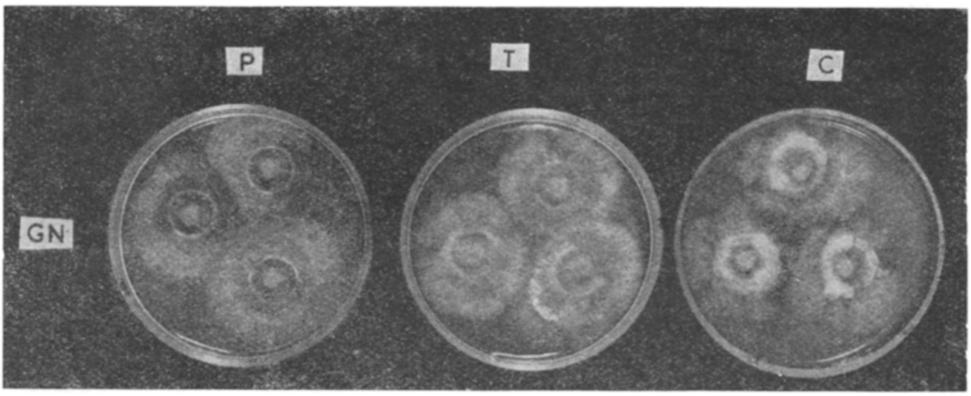

Fig. 3. Antagonism of organisms against *R. solani* on glucose with $NaNO_3$ as a nitrogen source. P = *P. clavariaeforme*, T = *T. viride*, C = *C. cochlioides*, GN (glucose + $NaNO_3$).

the stimulation of growth of cellulolytic saprophytes in the soil which, being more active cellulose-destroying fungi than *R. solani*, might have suppressed the pathogen. In view of these reports, experiments were conducted to study the effect of the carbon source like glucose, starch, cel-

Table 5. Growth of the organisms on different C-sources with $NaNO_3$ as a N-source

Organism	Cellulose	CMC	Starch	Glucose
R. solani	26·8	23·3	24·0	19·2
T. viride	15·5	14·6	18·2	19·2
P. clavariaeforme	1·0	1·0	2·0	3·0
C. cochlioides	1·3	8·0	3·3	3·3

Table 6. Growth on different C-sources with NH_4Cl as N-source

Organism	Cellulose	CMC	Starch	Glucose
R. solani	20·0	15·0	11·0	12·0
T. viride	16·7	13·0	15·0	17·0
P. clavariaeforme	1·0	2·0	1·0	1·0
C. cochlioides	1·3	7·0	1·3	1·0

Table 8. Antagonism against *R. solani* on different C-sources with NH_4Cl as a N-source

Antagonist	Cellulose	CMC	Starch	Glucose
T. viride	— —	—+	+	++
P. clavariaeforme	— —	— —	—+	+—
C. cochlioides	— —	+—	++	++

Table 7. Antagonism against *R. solani* on different C-sources with $NaNO_3$ as a N-source

Antagonist	Cellulose	CMC	Starch	Glucose
T. viride	— —	—+	+	++
P. clavariaeforme	— —	— —	+—	—
C. cochlioides	— —	—+	+—	—+

lulose and sodium carboxymethyl-cellulose (CMC), with $NaNO_3$ or NH_4Cl as nitrogen sources, on the growth of the antagonists and the pathogen and later to investigate the antagonistic effect under these nutrient conditions.

The result of their growth in pure culture is given in Table 5 and 6.

Contrary to the general belief that *T. viride* and other saprophytes are more cellulolytic than *R. solani* (BLAIR 1941), the results definitely suggest that at least the crucifer strain of the pathogen is more cellulolytic than the other organism. Another fact is that the replacement of $NaNO_3$ by NH_4Cl had some retarding effect on the growth

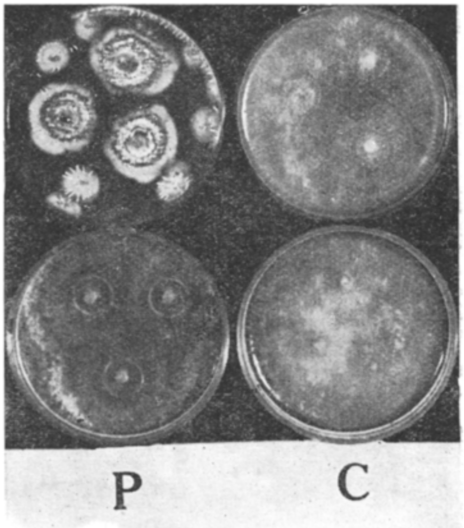

Fig. 4. Antagonism of organisms against *R. solani* on starch and cellulose with $NaNO_3$ as a nitrogen source. — P = *P. clavariaeforme*, C = *C. cochlioides*.

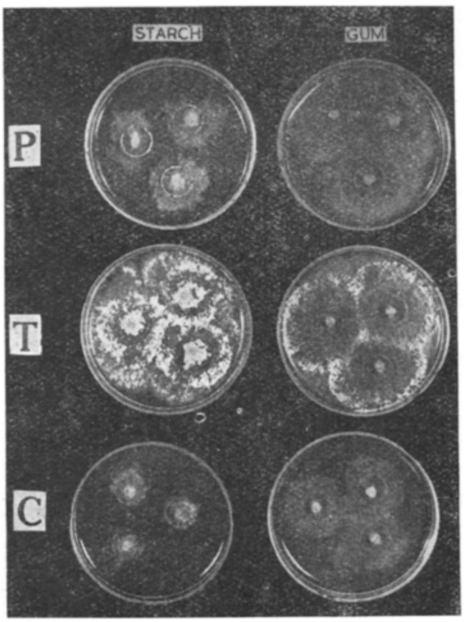

Fig. 5. Antagonism of organisms against *R. solani* on starch and sodium carboxymethyl cellulose (Gum) with NH_4Cl as a nitrogen source. — P = *P. clavariaeforme*, T = *T. viride*, C = *C. cochlioides*.

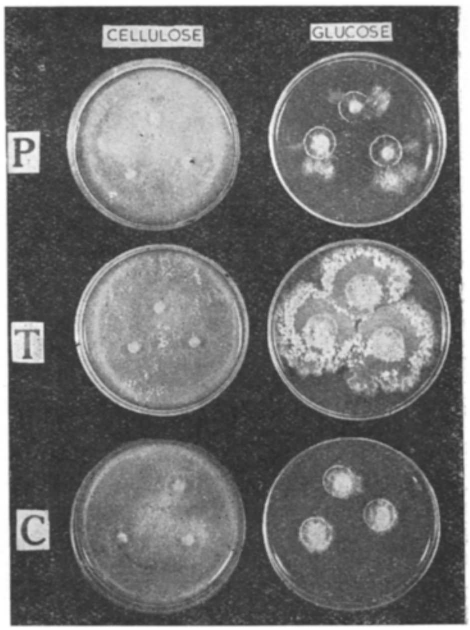

Fig. 6. Antagonism of organisms against *R. solani* on cellulose and glucose sources with NH_4Cl as a nitrogen source. — P = *P. clavariaeforme*, T = *T. viride*, C = *C. cochlioides*.

of the organisms in general. This effect is more pronounced on the growth of *R. solani* and this agrees with the results obtained in the cultural experiments (DESHPANDE, 1956).

6. Antagonism on different C-sources (Table 7 and 8, Figs. 3, 4, 5 & 6). In all cases cellulose did not support the antagonism of any organism against the pathogen. Glucose and starch appeared to be more favourable for the saprophytes to express their antagonism against the pathogen and the effect was more pronounced in the presence of NH_4Cl probably due to its inhibitive effect on the growth of the pathogen (DESHPANDE 1959). Although the growth in pure culture was an expression of the capacity to utilize the particular nutrient, yet it cannot be generally taken as an indicator of their antagonistic effect to the pathogen. In addition, the relatively poor growth of antagonists like *T. viride* and *C. cochlioides* and their weak antagonism to *R. solani* on cellulose was rather striking.

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Антагонизм между *Rhizoctonia solani* Kühn и некоторыми почвенными сапрофитами

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Изучалось явление антагонизма у трех сапрофитов против патогена *Rhizoctonia solani* в разных условиях возделывания. *Trichoderma viride* и *Chaetomium cochlioides* ингибировали рост *R, solani* эффктивнее при температуре инкубации 25 и 30 гр. Ц. *T. viride* антагонистически активнее в кислой среде, *T. cochlioides* в алкалической среде. Высказывается предположение, что почвенные добавки, повышающие активность *C. cochlioides* могут быть действенным мероприятием против исследуемому патогену в алкалических почвах. В различие от предыдущих данных культура *R. solani* целлюлотически активнее чем *T. viride* и *C. cochlioides*, что имеет место даже и в случае использования хлористого аммония в качестве источника азота.