Ecologic and Taxonomic Studies on Pythium as Pathogenic Soil Fungi : III Population of Pythium ultimum and Other Microorganisms in Rhizosphere

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Ecologic and Taxonomic Studies on Pythium as Pathogenic Soil Fungi

III Population of Pythium ultimum and Other Microorganisms in Rhizosphere

By

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INTRODUCTION

The infection of plants by soil-borne fungi can be attributed to host-pathogen interaction occurring in the soil environment which is restricted or promoted by some physical, chemical and biological factors in the soil. Thus it has been considered that the growth of pathogen in pre-infection stage is promoted or suppressed by the competition, symbiosis and antagonism among soil microorganisms. Likewise, plant roots may favor or inhibit the development and multiplication of the pathogen in the soil. PARK (1) reported that the growth of pathogen is inhibited by the substances excreted from the root of host plant and that the soil microbial population inhibits the growth of pathogen in the soil.

The present paper aim to know the relative density of the mycelium of *Pythium*, of other soil microorganisms and of microorganisms antagonistic to *Pythium* in the rhizosphere of plants with different degree of susceptibility and the effect on *Pythium* of soil and root extracts obtained from the same rhizosphere.

EXPERIMENTAL METHODS AND RESULTS

1. Growth intensity of *P. ultimum* in the rhizosphere.

Twenty-four earthen pots (30 cm. diam.) were filled up with uniform amount of sieved and thoroughly mixed sandy-loam soil. Twelve pots were sterilized in the autoclave

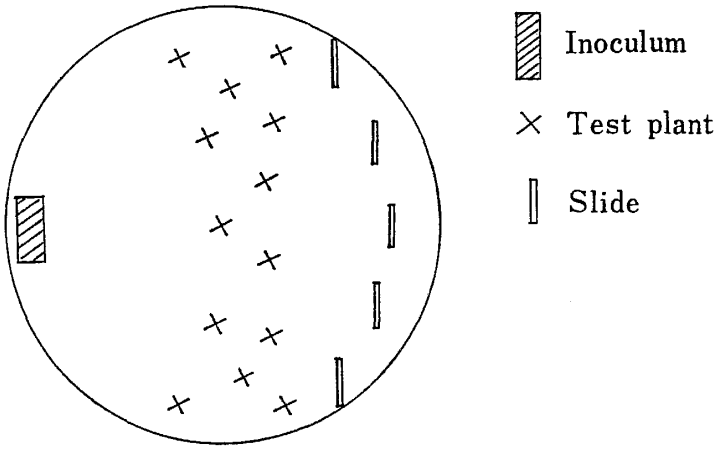

Fig. 1. Diagram of experimental pot.

at 25 psi for 2 hours. The other 12 pots remained unsterilized. The sterilized and non-sterilized pots were placed in separate but adjacent lots. The pots were arranged in randomized block method having 4 treatments with 3 replications per trial.

Seeds of the test plants, ranging from non-susceptible to highly susceptible were pre-germinated in sterile conditions under ordinary room temperature (22-28°C.) and carefully selected before planting in the pots. Thirteen seedlings were planted across the center of each pot. When the seedlings were about 3-5 cm. high, 15 grams of 3-day-old wheat bran culture of pathogenicity tested *Pythium ultimum* was buried 4 cm. deep in one side of the pot. After 4 days five clean glass slides one surface coated with a thin layer of water agar as modified by ISHIZAWA (2) were buried behind the seedlings close to the wall (Fig. 1). The glass slides were removed 5 days later.

The collected slides were dipped in water to remove the bigger soil particles and air-dried, fixed and stained with rose bengal for 1.5 minutes over steam bath. For each slide 100 microscopic fields were randomly selected and the percentage of non-septate hyphae is calculated.

Table 1. Population of *Pythium ultimum* in the rhizospheres of plants with different degree of susceptibility (%).

Test plant	Sterilized soil	Non-sterilized soil
Soy bean (N)*	4.0	4.5
Pumpkin (R)	8.2	11.1
Cucumber (S)	12.7	9.6
Control	4.3	10.1

*N=Non-susceptible
R=Resistant
S=Susceptible

Table 2. Comparative population of soil-infecting fungi in the rhizospheres of plants with different degree of susceptibility (%).

Test fungi	Test plant	Sterilized soil	Non-sterilized soil
Pythium ultimum	Soy bean (N)	4.0	4.5
	Pumpkin (R)	8.2	11.1
	Cucumber (S)	12.7	9.6
Fusarium oxysporum f. niveum	Pumpkin (N)	20.1	24.5
	Water melon (R)*	16.5	19.5
	Water melon (S)**	20.0	22.0
Pythium sp.	Spinach (N)	9.8	13.8
	Beet (S)	16.5	22.0
Rhizoctonia solani	Tomato (R)	8.5	8.5
	Pea (S)	20.0	17.2

*Asahi-yamato variety **Kaho variety

The data obtained from non-sterilized block were highly variable. On the other hand, there is a definite pattern in sterilized block. Non-susceptible plant has the least growth intensity while the susceptible plant has the highest. The resistant plant has moderate population (Table 1). This trend is manifested in both sterilized and non-sterilized blocks

by *Pythium* sp. and *Rhizoctonia solani* but was not the case in *Fusarium oxysporum f. niveum* (Table 2).

2. Microbial population in the rhizosphere.

From the control pots representing all the test plants and where *P. ultimum* was not introduced, the microbial population in the rhizosphere was determined.

With the use of cork borer (0.9 cm. diam.) 3 borings of 9.5 cm. deep were taken at 3 cm. from the base of each plant. The soil was pulverized, mixed thoroughly and aliquote samples of 1 gm. for moisture determination and 5 gm. for soil dilution and plate count were taken.

In soil dilution and plate count technique 2 kinds of media were employed. The THORNTON medium was used for bacterial count and WAKSMAN acid medium for fungal count. A final dilutions of 1:10,000 and 1:1,000,000 were used for fungi and bacteria respectively.

Table 3. Microbial population in the rhizospheres of plants with different degree of susceptibility (per gram oven-dried basis).

Test Plant	Sterilized soil		Non-sterilized soil	
	Fungi (T)	Bacteria (M)	Fungi (T)*	Bacteria (M)**
Soy bean (N)	10	30	33	30
Pumpkin (R)	530	60	100	20
Cucumber (S)	500	50	100	40
Control	200	60	200	30

*T=Thousand
**M=Million

As shown in sterilized block of Table 3, the non-susceptible plant has lower microbial population than the resistant and susceptible plants. In non-sterilized block only the fungal count follow the same trend.

3. Growth intensity of antagonistic microorganisms in the rhizosphere.

Using the remaining soil used in method 2 screening for antagonistic microorganisms against *P. ultimum* was conducted. The procedures given by HERR (3) in his Triple-Agar-Layer-Plate technique were followed.

No antagonistic microorganisms were found in sterilized soil. From the non-sterilized block the non-susceptible plant has the largest number next is the resistant plant. The susceptible host has the lowest (Table 4.).

Table 4. Number of antagonistic microorganisms to *P. ultimum* in the rhizospheres of test plants with different degree of susceptibility (%).

Test plant	Sterilized soil	Non-sterilized soil
Soy bean (N)	0.0	5.2
Pumpkin (R)	0.0	4.6
Cucumber (S)	0.0	0.4

4. Effect of soil extract on *P. ultimum*.

The whole soil of the control pots was used in this study. In getting the soil extract the displacement method by PARKER (4) with little modification was adapted.

The soil from each pot was pulverized and passed through 1 cm. diameter sieve. Sieving removed plenty of the roots and other plant parts which might interfere in this study. The moisture content of the soil was determined by oven drying.

The soil was properly packed in 1-liter capacity separatory funnel. Ethyl alcohol was used the displacing liquid. It took approximately 8 hours to obtain sufficient volume of displaced extract. The soil extract was concentrated by vacuum distillation and its effect on the conidial germination and mycelial growth on *P. ultimum* was made.

There was slight stimulatory effect of soil extract on conidial germination. Similarly little effect on mycelial growth was observed (Table 5).

Table 5. Effect of soil extract on conidial germination and mycelial growth of *Pythium ultimum*.

Test Plant	Sterilized soil		Non-sterilized soil	
	Germ. (%)	Mycelium (cm.)	Germ. (%)	Mycelium (cm.)
Soy bean (N)	68.3	1.2	52.1	1.3
Pumpkin (R)	59.6	2.2	38.3	1.6
Cucumber (S)	45.3	2.0	37.9	1.5
Water control	35.7	1.2	35.7	1.2

5. Effect of root extract on *P. ultimum*.

The roots of control plants with 13 healthy plants per pot were utilized in getting the root extracts following the method of KESSLER (5).

The distilled water extract from the roots was acidified with 1N HCI and shaken with n-butanol. Then the butanol extract was evaporated *in vacuo* to about 5 ml. Small amount of 1N HCI was added and centrifuged and finally the supernatant was neutralized with 1N NaOH. The resulting solution was used in the determination of the effect on the conidial germination and mycelial growth of *P. ultimum*.

Table 6. Effect of root extract on conidial germination and mycelial growth of *P. ultimum*.

Test Plant	Sterilized soil		Non-sterilized soil	
	Germ. (%)	Mycelium (cm.)	Germ. (%)	Mycelium (cm.)
Soy bean (N)	33.0	0.8	34.0	0.0
Pumpkin (R)	59.0	2.0	47.0	2.0
Cucumber (S)	86.0	1.9	30.0	1.6
Water control	85.0	0.0	85.0	0.0

The data on conidial germination obtained from non-sterilized block in Table 6 were highly variable. On the other hand, there is a significant difference between test plants in sterilized soil. It showed that the roots of non-susceptible plant has the highest inhibition while the susceptible host has practically nothing. Likewise, the non-susceptible plant shows no stimulation on mycelial growth while the resistant and susceptible stimulate.

DISCUSSION

The soil-borne disease will appear as a result of host-pathogen interaction taking place in the soil. The growth and development of both host and pathogen might be influenced directly by complicated factors in the soil and finally the infection of plants is determined by the susceptibility of the host to the pathogen. It is necessary, therefore, to clarify the interactions between soil and host, soil and pathogen and host-pathogen before we can discuss the infection of plants by soil-borne fungi.

The pathogen and microorganisms in the soil which are either in antagonism or symbiosis continue to grow and multiply and later become dormant. The pathogen attacks the host with different degree depending upon the susceptibility of the host. Some plants produce stimulatory substances for fungal penetration and others excret toxic substances through the roots. There is also much evidence which is reviewed by ROVIRA (6) that organic substances pass from the roots to the soil. Many of these substances can act as major elements for the soil microorganisms while others give vitamin-like effects. In either case such exudates may act selectively for organisms in the soil. Aside from producing the type of substance that favors microbial activities the roots may also produce substances inhibitory to other members of the soil microflora (7).

The inhibition of growth of pathogen by soil microorganisms is due to either toxin-like antibiotic substance (s) which is metabolized by microorganisms or to microorganisms which are parasitic on the mycelium or spores of the pathogen (8, 9, 10, 11). THORNTON (12) has shown that in soil certain actinomycetes were particularly associated with hyphae of *Rhizoctonia solani*. ROBERTSON (13) described a disease of oil-palm seedling in which typical symptoms in inoculation experiment could be obtained by double inoculation with *Pythium* sp. and *Rhizoctonia* sp. even though under laboratory conditons *Rhizoctonia* parasitize the hyphae of *Pythium*.

It is common to find that experimental series in sterilized soil behave very differently from series in normal soil. This may be due to the fact that some actinomycetes in non-sterilized soils were highly inhibitory to the pathogen (14). It is thought, therefore, that there are some microorganisms antagonistic to *Pythium* in the non-sterilized soil. On the other hand, it is clear that growth of *Pythium* in sterilized soil depends upon the susceptibility of the host. Growth of *Pythium* was lesser in the rhizosphere of resistant pumpkin than highly susceptible cucumber and the lowest in non-susceptible soy bean. This is commonly recognized (15, 16, 17).

Root extract of non-susceptible plant inhibited considerably conidial germination of *Pythium* while the susceptible did not. Non-susceptible plant did not promote mycelial growth but resistant and susceptible plants did. It can be considered that organic substances pass from the roots to the soil and it act selectively against the organisms in the soil. The result of these positive and negative effects on various elements of the soil flora is that plant's root tend to make the rhizosphere flora.

It is possible to say that microorganisms antagonistic to *Pythium* exist in non-sterilized soil and that resistant root excrets antifungal or fungistatic substances.

Soil extract to a large extent contains root extract plus other substances formed in the soil either chemically or biologically. In its entirety, soil extract can be considered as a dilute root extract. The root extract act as a germicide and soil extract as a growth stimulant.

From the results of experiment in sterilized soil, non-susceptibility of soy bean may be derived either from inhibitory substance(s) to the growth of *Pythium* being produce by the roots or the presence of antagonistic microorganisms in the rhizosphere. Resistance of pumpkin could be due to favorable increase of microorganisms which resulted to stiff competition among them wherein *Pythium* is also inhibited. The susceptibility of cucumber may be due to synergistic effect between the microbial population in the rhizosphere and *Pythium*.

The most important thing to be stressed here is that soil population should not be treated as a whole or as a single entity rather the interaction between them which might affect the over-all pattern where pathogen and host are involved.

SUMMARY

1. The growth intensity of *Pythium ultimum* in the rhizosphere of plant with different

degree of susceptibility decreases as the resistance of the hosts increases and this is also true to both *Pythium* sp. and *Rhizoctonia solani* but not with *Fusarium oxysporum f. niveum.*

2. The non-susceptible plant has lower microbial population than the resistant and susceptible plants.

3. No antagonistic microorganisms are found in sterilized soil. In non-sterilized soil, the non-susceptible plant has the most number of antagonistic microorganisms while the susceptible has the lowest.

4. Little stimulatory effect of soil extract on conidial germination and mycelial growth was observed.

5. The inhibitory action of root extract on conidial germination increases as the resistance of the hosts increases. Likewise, the non-susceptible plant shows no stimulation on mycelial growth while the resistant and susceptible show stimulation.

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