Properties of Soil Suppressiveness to Cucumber Wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* Owen

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朴昌錫·趙鏞涉 : 오이 덩굴쪼김병의 發病을 抑制하는 土壤의 特性에 關하여

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ABSTRACT It has been tried to find effective biological control measures involved in nature of soil suppressiveness to fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* Owen. Total 28 soil samples were obtained from Jinju, Haman, Namji, Milyang and Suncheon vinyl house area. The disease response of test soil was quantified in terms of DI50 value which caculated from log-probit transformation of diseases response curves. Soils designated 5 from Jinju, 7 from Suncheon, 22 from Namji were recognized as suppressive to fusarium wilt of cucumber. This suppressiveness was completely nullified after autoclave. The disease suppressiveness of tested soil did not indicate any consistency according to either chemical property or texture of soil. Conidial germination, induction and germination of chlamydospore were markedly inhibited in suppressive soil compared to those in intermediate or conducive soils, however, mycelial lysis did not appear to have direct relationship with disease suppressiveness of given soil. Population density of fluorescent Pseudomonads and Bacillus spp. in the soil originated from different degree of suppressiveness were not different significantly but the number of lytic bacterial plaques measured by triple layer agar method was remarkably higher in suppressive soil than that in intermediate or conducive soil.

INTRODUCTION

Cucumber wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* is well known world widely and leads to frequent heavy crop lossess[4,5,7,9,21]. Many variable information have been accumulated on the biological control of diseases caused by formae specialis of *Fusarium oxysporum*[1,18,29,31,35].

Particulary there has been so much interest in soil suppressiveness to fusarium wilt that the concepts and its possible implications in biological control become emphasized[6,7,15,23,24,35]. The machanism of suppressive soil are not clearly elucidated but most explanation for suppressiveness include microbial antagonism[2,15,20,26]. The physical and chemical properties of suppressive soil are not investigated intensively, but some works refer to soil texture and pH[28,30,35] Many workers have confirmed that the soil suppressiveness is eliminated by aerated steam treatment at 55~60 C for 30 minutes [11,16,28] or gamma radiation[1,11]. Burke[3] indicated that the factors responsible for suppression of bean root rot were not native to the suppressive soil but developed as a result of continuous cultivation of beans. He also suggested that the suppression could be due to a combination of physical and microbiological factors.

The inoculum density of *F. oxysporum* required to produce a given incidence of wilt was considerably greater in suppressive than in conductive soil [18,31] and the propagule density of the pathogen declined more rapidly in suppressive soil[11,18,18]. Alabouvette[1] reported that the total population of *F. oxysporum* established in suppressive soil is always lower than that in conducive soil. Ohh et al.[23] reported that the mycelial growth of *Fusarium solani* was markedly inhibited on the agar contained ginseng root rot suppressive soil extract. Furuya et al.[11,12] reported that a few of macroconidia of *F. solani* f. sp. *raphani* germinated in Kidamisoil (Suppressive) and most of macroconidia inoculated finaly lysed without chlamydospore production. Bukree[3] also found that lysis of the mycelia and germ tubes occurred within 6 days both in resistant and root rot soil but the chlamydospores were larger and more numerous in root rot soil then resistant soil.

Smith[31] reported that the germination of chlamydosporos and subsequent growth of hyphae of *Fu-*

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sarium f. sp. *vasinfectum* and *tracheiphilum* were less in suppressive soil than in wilt conducive soil, regardless in host rhizosphere or in response to added nutrient.

Number of bacteria are known to associate with inhibition of *F. oxysporum* in suppressive soil[22,28,31,33]. Among them, fluorescent pseudomonads and Bacillus species are most frequently reported. Scher and Baker[28] reported that a strain of *Pseudomons putida* isolated from California suppressive soil induced suppressiveness when added to conducive soil.

The objective of present study was to obtain some information on the biological control of fusarium wilt of cucumber through the investigations of soil suppressiveness. An attempt was made to analyze the disease suppressiveness of test soil quantitatively, tried to determine the differences of biological properties between suppesive and conducive soil and their effects on the growth of *F. oxysporum* f. sp. *cucumerinum*.

MATERIALS AND METHODS

Collection of test soil

The test soils were collected from 28 different sites of Jinju, Suncheon, Haman, and Milyang. The soil sampling sites were selected where cucumber had been grown under the vinyl house for more than three straight years at least once a year. The discription and designation of the collected soils was summarized in Table 1. Approximately 10 kilograms of soil was collected from the root growing zone at each sites. The soils were stored in cotton bag at room temperature after the soil was air dried and passed through a for millimeter screen.

Soil infestation and assessment of disease response

Air dried soil samples were evenly infested with the inoculum by hand. Inoculum levels were adjust-

Table 1. Designated numbers and descriptions of soil samples

Designated soil No.	Locality	Sampling site	Standing crop	Soil status	Soil texture
1	Jinju	Hadae-Dong	cucumber	paddy	loam
2	Jinju	Jangjae-Dong	cucumber	upland	sandy loam
3	Jinju	Chojoen-Dong	cucumber	paddy	loam
4	Jinju	Chojoen-Dong	pepper	upland	sandy loam
5	Jinju	Chojoen-Dong	pepper	upland	sandy loam
6	Jinyang	Geumsan-Myeon	cucumber	upland	sandy loam
7	Suncheon	Deugyon-Myeon	cucumber	paddy	clay loam
8	Suncheon	Injae-Dong	cucumber	paddy	clay loam
9	Suncheon	Injae-Dong	cucumber	paddy	clay loam
10	Kwangyang	Kwangyang-Eub	cucumber	paddy	clay loam
11	Kwangyang	Kwangyang-Eub	cucumber	paddy	clay loam
12	Kwangyang	Kwangyang-Eub	cucumber	paddy	clay loam
13	Suncheon	Samsan-Dong	cucumber	paddy	clay loam
14	Haman	Sanin-Myeon	melon	paddy	loam
15	Haman	Sanin-Myeon	water melon	paddy	loam
16	Haman	Sanin-Myeon	water melon	paddy	clay loam
17	Haman	Daesan-Myeon	water melon	paddy	sandy loam
18	Haman	Daesan-Myeon	water melon	paddy	loam
19	Haman	Daesan-Myeon	water melon	paddy	loam
20	Changyreng	Namji-Eub	pepper	paddy	sandy loam
21	Changyreng	Namji-Eub	pepper	paddy	sandy loam
22	Changyreng	Namji-Eub	cucumber	upland	sandy loam
23	Changyreng	Namji-Eub	cucumber	upland	sandy loam
24	Changyreng	Namji-Eub	cucumber	upland	sandy loam
25	Changyreng	Namji-Eub	cucumber	upland	sandy loam
26	Milyang	Sangnam-Myeon	cucumber	paddy	loam
27	Milyang	Milyang-Eub	pepper	upland	sandy loam
28	Milyang	Milyang-Eub	pepper	upland	sandy loam

ed to yield from 200 cfu/g to 1600 cfu/g soil. For some of the test soil, inoculum density was either increased dependent upon the variation of disease supressiveness. For 640g portions of each infested soil were distributed in plastic pots, and sowed four of one day old cucumber germlings seeds(*Cucumis sativus* L. cv. Cheong-Jang-Madi-Oi). The diseased plants were recorded daily based on the loss of turgidity, damping off at base, and yellowing. Typical wilt The number of plants showing wilt symptoms was recorded up to 25 days. From the results of this experiment, the inoculum dose inducing 50% disease incidence (D150) of each soil was calculated by using Probit analysis based on Probit-Log transformation.

Chemical analysis of soil

Chemical properties of collected soils were determined by routine soil-analyzing method modified by Institute of Agriculturl Sciences, Office of Rural Development. Total nitrogen contents was analyzed by Kjeldahl method and P_2O_5 was by Lancaster method. Organic matter was calculated from the carbon compound obtained by wet combustion method and contents of inorganic ions such as K, Ca, Mg and Fe were detected by Atomic Adsorption Spectrophotometer (Shimadzu, Model AA-630).

Microconidial germination in test soil

Purified microconidial suspension(10^9 conidia per m*l*) obtained from PDA broth was stored in refrigerator as stock solution. The spores sustained viability without germination for more than one month in refrigerated condition. The soil extracts were prepared by 1 : 5 dilution of test soil with distilled water. This dilutions were stored at room temperature for three hours with intermittent hand shaking and were filtered through Toyo-II filter paper. Point one milliliter of conidial suspension was added to 10m*l* of soil extract. One drop of conidia-soil extract mixture was placed on water agar surface. The conidial germination were examined under the light microscope at 200X magnification.

Mycelial lysis in test soil

Half length of sterile slide glass was placed on the week old culture of *F. oxysporum* on the PDA plates and let the mycelium grow under surface of slide glass for one week. To prevent moisture condensation, sterile glass rods were placed to support the slide glass on agar surface. Ten pieces of the slide glasses were buried in each test soil and observed the lysis of mycelium at given intervals for a period of time. The mycelium was stained with 0.3% of falcoflour white M2R solution and observed under the fluorescent microscope. Six criteria, from 0 to 6, were employed to generalize and to quantify the degree of mycelial lysis.

Chlamydospore formation and germination in test soil

Ten milliliter of homoginized mycelial mats was mixed into each of 50g of test soil and observed chlamydospore formation under the fluorescent microscope at five days interval. At the end of 35 days observation, the number of chlamydospores per one gram of test soil was counted. Chalmydospore germination was induced by adding 0.1% glucose and asparagine solution to the test soils containing chlamydospore in it. After 24 hours, germination rate and germ tube length were measured under the fluorescent microscope at 200X magnification as described previously.

Bacterial population analysis

The test tubes containing 10m*l* of 10^{-3} diluted soil suspension were placed in 90°C water bath for 15 minutes for isolation of Bacillus species. After heat treatment two m*l* of soil suspension was incorporated with 18m*l* of molten nutrient agar and pour into petri-plate. The colonies of bacteria fromed on the agar surface were examined through high power of phase contrast microscope to make sure the endospore forming. A selective media modified by-Sands[27] for isolation of fluorescent pseudomonads employed. One m*l* of serially diluted soil suspension was placed on the bottom of petri plates on which 15 to 20m*l* of molten selective agar medium was added and agitated gently. After 4 days of inubation at 28C, the colonies producing diffusible green fluorescent pigment were counted as fluorescent pseudomonads under UV light illumination at 366nm.

Modified triple layered agar method[17] were used to isolate the bacteria antagonistic to pathogenic fungus. The first layer of triple layered agar is 10 m*l* of water agar. This foundation layer is to support moisture for long period of incubation and

allow to even distribution of second layer. After water agar is solidified, the mixture of microconidia (10^6/m*l*) and 10m*l* of molten PDA were spreaded (seeding layer). The mycelium of fusarium started to appear on the surface of PDA in two days. Ten m*l* of molten 523 agar medium containing one m*l* of 10^{-3} dilution of soil suspension was overspreaded as test layer. The mycelium grew continuosely and penetrate through test layer (523 agar layer) and the bacteria from soil dilution also grew on the surface of test layer. Some of the bacteria definitely inhibit mycelial growth or lyze the fungal mycelium, which resulted bacterial plagues on the seeding mycelial lawn. The numbers of this plagues were counted.

RESULTS

Analysis of disease expressions

The disease incidence of test soils at given concentration of inoculum are summarized in Table 2. The response of the soils to the increasing density of inoculum was so diverse that the soil samples from given locality could not be generalized as to suppressiveness and/or conduciveness. The soil such as, 5 vs 6, 7 vs 10, and 22 vs 23 were the representatives of the soils that had the highest or lowest suppressive potential in each location of Jinju, Suncheon, and Namji. Generally, most of the test soils tended to yield about 50% disease incidence at inoculum concentrations between 400 to 800 cfu per gram soil.

The results of disease incidences corresponding to at given inoculum concentration of representation of representative six soils were plotted in upper parts of Figure 1. The arithmetic expression was not suitable for presenting wide incidences of the pair of soils within one figure. Therefore log-inoculum, probit-disease incidence transformation was introduced (lower part of Figure 1). The dots(suppressive soil) and circles(conducive soil) are showing the relationships between inoculum concentration and disease incidence. From the regression equation of log-probit transformation inoculum density required for 50% disease incidence(DI50) were calculated (Table 2). DI50 is very convenient to compare the suppressive potentials of test soil.

The selected six soils, representing the highest or the lowest suppressive potential, were autcolaved in order to manifest whether if the disease reducing property persists after destruction of biological factors. The disease incidence in either nature and autoclaved six selected soils at given dose of inoc-

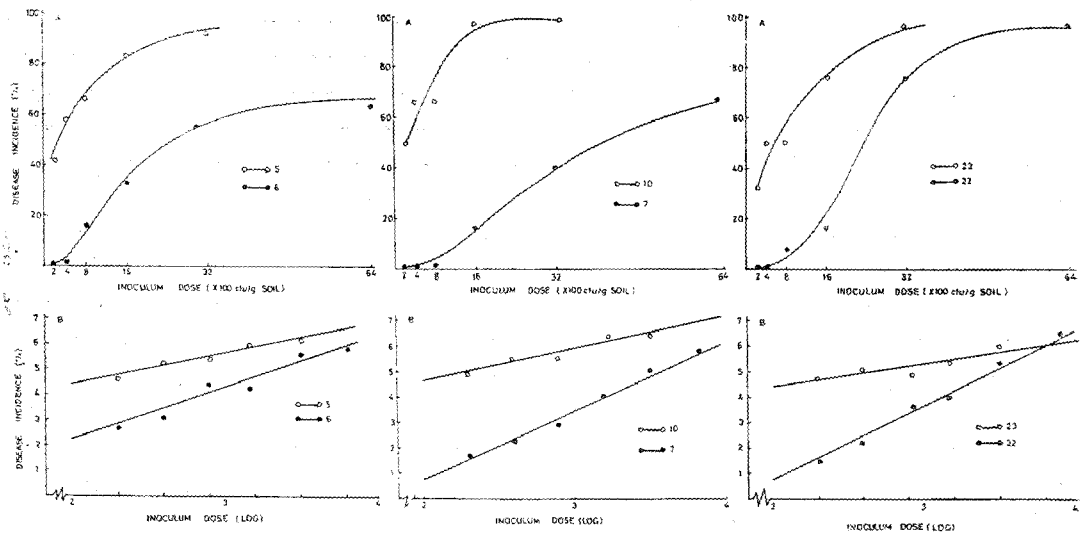

Fig. 1. Relationships between inoculum dose and disease incidence of fusarium wilt of cucumber subjected to suppresive soil (solid dark circle) and conducive soil (empty circle). A) arithmetic plot. B) log-probit transformation.

Table 2. DI50 values and percent disease incidence in the test soils infested with the pathogen of fusarium wilt of cucumber at given concentration

Designated soil No.	DI50[a] (CFU/g)	Percent of wilted plant at each inoculum concentration[b] 200	400	800	1,600	3,200	6,400
1	774	25	33	50	67		
2	1,142	0	17	25	67	67	83
3	1,031	8	17	33	67		
4	1,169	8	25	25	67		
5	2,249	0	0	16	33	55	67
6	295	42	58	67	83		
7	3,833	0	0	0	17	50	67
8	1,541	0	8	25	50	75	
9	618	17	50	50	100		
10	224	50	66	66	100		
11	562	25	50	42	83		
12	803	8	17	33	92		
13	1,622	17	8	33	25	67	
14	646	8	33	58	83		
15	877	17	8	50	67		
16	888	17	17	42	75		
17	1,111	8	33	33	67		
18	731	8	25	50	83		
19	750	8	8	50	92		
20	431	42	50	50	75		
21	1,232	8	17	33	67		
22	2,393	0	0	8	17	75	
23	421	42	50	50	83		
24	908	0	8	42	83		
25	1,092	0	0	42	67		
26	1,205	0	8	25	67		
27	1,453	0	25	17	58		
28	1,674	0	0	17	42	83	

[a] Inoculum dose requires 50% disease incidence calculated from regression equation log-probit disease.

[b] Inoculum concentration represents number of Colony Forming Unit(CFU) per one gram soil.

ulum are showen in Figure 2. In every location, the autoclaved soils showed high disease incidence even at lowest concentration of inoculum, regardless of high or low disease suppressive potential.

Chemical properties of test soils

The results of chemical analysis were summarized in Table 3. The soil pH ranged from 5.3 to 6.8 is not significantly different from the average pH of vinyl house soil in southern part of Korea[25]. Organic matter content of Suncheon soil was quite higher than any soils from other districts. However, the other chemical component of test soil indicated no consistency to sampling locations. In general, there were no significant relationship between chemical properties and soil suppressiveness represented in terms of DI50 values. Organic matter and total nitrogen were likely related to DI50, however, they were not significant statistically.

Germination of Microconidia in test soil

Germination rates of microconidia in extracts from suppressive soils were lower than those from conducive soils(Fig. 3). However, in soil 22, a suppressive soil from Namji, showed similar germination rates with soil 2, a conducive soil from Jinju that were higher than those of suppressive soils from Jinju and Suncheon, The other conducive soils from three location indicated higher germination rates but still lower than those in distilled water, the control.

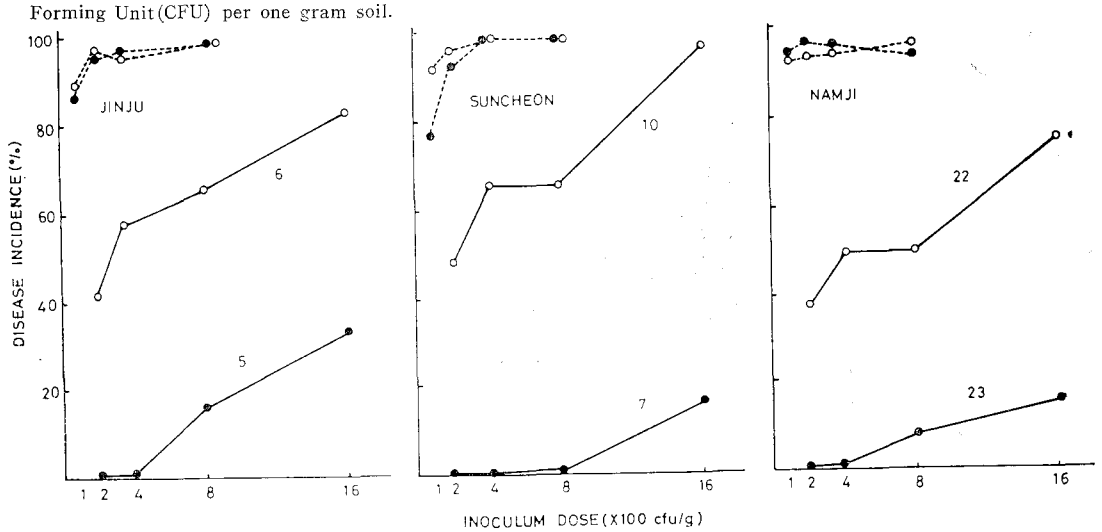

Fig. 2. Disease incidence in natural (solid lines) and autoclaved (dotted lines) soils collected from different locations in response to increase of inoculum density. (solid dark uircle indicates suppressive soil, empty circle represents conducive soil)

Table 3. Chemical properties of test soils and correlations between chemical component and DI50 value of test soils

Soil No.	Locality	DI50 (CFU/g)	Chemical properties of test soil							
			PH	Organic matter(%)	Total N(%)	P_2O_5 (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	CEC (me/100M)
2	Jinju	1,142	6.3	0.8	0.14	221	3.6	0.71	2.0	12.2
3	Jinju	981	5.4	1.8	0.20	255	6.0	0.74	1.8	13.4
5	Jinju	2,247	6.2	1.9	0.28	218	3.4	0.59	1.0	12.4
6	Jinju	295	5.4	1.9	0.21	235	4.6	0.82	1.5	10.5
7	Suncheon	3,833	5.8	3.9	0.77	252	5.5	1.78	2.3	13.5
9	Suncheon	618	4.8	2.9	0.30	249	3.0	1.26	1.3	9.4
10	Suncheon	224	5.8	3.4	0.52	267	5.2	1.64	2.0	14.1
13	Suncheon	1,622	6.1	3.3	0.39	247	6.3	1.90	2.4	12.8
14	Suncheon	646	5.7	3.4	0.67	244	6.9	1.66	2.4	15.8
15	Suncheon	977	6.8	2.5	0.33	186	8.2	0.83	3.6	16.8
16	Haman	888	5.4	1.3	0.13	112	3.4	0.42	1.3	8.4
18	Haman	731	5.7	1.6	0.17	218	3.8	0.57	1.2	8.7
21	Namji	1,111	6.7	1.8	0.18	255	6.3	0.27	3.6	12.2
22	Namji	2,393	6.5	2.0	0.27	272	6.1	0.99	3.3	13.9
23	Namji	421	6.5	0.9	0.15	238			1.6	8.4
25	Namji	1,092	5.3	1.4	0.23	272	5.5	0.97	2.1	12.3
Correlation* coefficient			.23 NS	.32 NS	.43 NS	.14 NS	.15 NS	.28 NS	.22 NS	.27 NS

DI50 : Inoculum dose requires for 50% disease.

* Correlation coefficient between DI50 and theSchemical properties.

NS : Non-significant at 5% level.

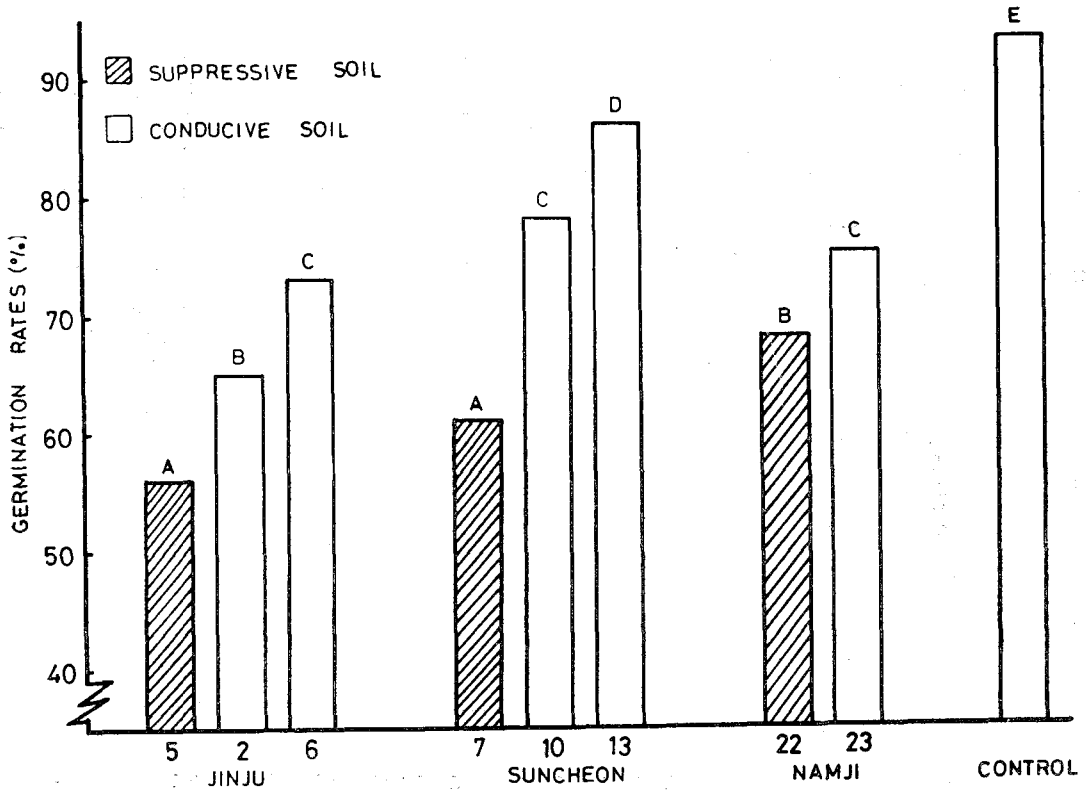

Fig. 3. Germination of microconidia of *Fusarium oxysporum* f. sp. *cucumerinum* in the soil extracts from suppressive and conducive soil. (germination rates belong to same letter do not differ significantly)

Lysis of mycelium in test soil

The mycerial lysis in the soil from Namji was rapid and higher than those in soils form elsewhere (Figure 4). In soil 23, the conducive soil, about half of the mycelia was lysed within 3 days and the most of the mycelia were lysed 15 days after treatment. In soil 22, the suppressive soil, the lysis was much slower that in soil 23 until 15 days. Thereafter, the degree of such a big difference was diminished leading eventually to complete mycelial lysis in 30 days. In Jinju soil, the progress of mycelial lysis in suppressive vs. conducive soil showed similar pattern until 20 days. In general, mycelium lysed more rapidly and completely in conducive soil. The progress pattern of mycelial lysis in suppressive vs. conducive soils from Suncheon was similar with those from Jinju and Namji up to 15 days. But there after the pattern was reversed; mycelial lysis in soil 7, the suppressive, progressed far ahead of that of soil 10, the conducive. Therefore the progress and degree of mycelial lysis, at this point, did not suggested any presumption regarding disease suppressiveness or conduciveness of given soil.

Chlamydospore formation and germination in test soil

As mycelium buried in soil was lysed, some portions of the mycelium converted to chlamydospore. The amount of chlamydospore formed in the test soil was presented in Table 4. The number of chlamydospores were less in suppressive soil than in

Table 4. Production and germination of chlamydospore converted from the mycelial fragment of *F. oxysporum* f. sp. *cucumerinum* buried in disease suppressive, intermediate and conducive soils

Designated soil No.	Disease response	No. chlamydospores[a] (×100000/g soil)	Germination rates(%)[b]
7	Suppressive	11.0+2.8	13.5 a[c]
5	suppressive	16.4+3.2	18.8 a
22	suppressive	14.6+2.4	23.1 a
2	intermediate	18.0+4.2	46.3 b
3	intermediate	25.2+4.5	82.0 d
27	intermediate	22.5+3.4	58.4 c
26	intermediate	21.0+3.8	43.3 b
10	conducive	22.8+4.8	45.0 b
9	conducive	18.2+3,4	76.2 d
23	conducive	15.2+1.8	39.7 b
6	conducive	34.2+6.4	85.7 d

[a] Number of chlamydospore was determinded by 10-ford serial dilution of test soil under ffuoresent microscope.

[b] Germination rates represent the averages of observation of 10 microscopic field at 200× magnification.

[c] Germination rates with same letters are not significantly different (p=0.05).

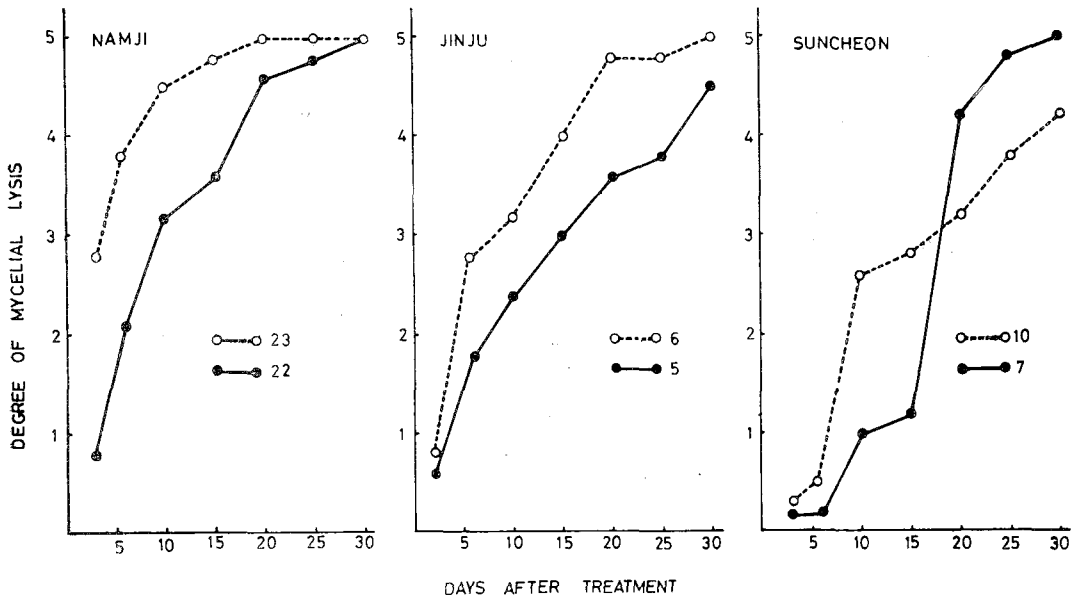

Fig. 4. Mycelial lysis of *Fusarium oxysporum* f. sp. *cucumerinum* in suppressive (solid lines with dark circle) and conducive soils (dotted lines with empty circle) collected from Jinju, Suncheon and Namji.

intermediate or conducive soil except in soil 23, however, there was no difference between intermediate and conducive soil. The chlamydospore germination induced by nutritional amendment in the test soil differ greatly from soil to soil (Table 4). In suppressive soil, the germination of chlamydospore was strikingly less than those in intermediate or conducive soil, less than 23% of germination was recorded. In either intermediate or conducive soil, the germination ratio ranged from 40 to 80%.

Analysis of antagonistic bacterial population

The populations of fluorescent Pseudomonads and Bacillus spp. which were assumed to antagonistic to *Fusarium oxysporum* were analyzed(Table 5). But it turned out that population density of these bacteria were not directly related to disease suppressiveness of test soil. Bacterial plaques formed in the middle of mycelial lawn of *F. oxysporum* f. sp. *cucumerinum* on triple layer agar were also counted. Such antifungal plaque was also observed in intermediate soil or even in conducive soil. However, the number of plaques originated from suppressive soil was more than either those from intermediate or conducive soil. Among those, soil 7 from Suncheon was especially prominant number of bacterial plaques. Antifungal bacterial population was determined to positively correlated to disease suppressiveness.

Table 5. Number of fluorescent pseudomonads and *Bacillus* spp. colonies and antifungal plaques counted from the suppressive, intermediate, and conducive soils

Designated soil number	Disease expression	No. of colonies[a] (×1000/g) Fluorescent pseudomonads	*Bacillus* spp.	No. antifungal plaques[b] (×1000/g)
7	Suppressive	39.3	312	58.1 a[c]
5	Suppressive	18.6	116	28.5 b
22	Suppressive	13.6	146	27.0 b
2	Intermediate	24.5	262	16.5 c
3	Intermediate	12.4	314	15.0 c
27	Intermediate	16.4	396	13.6 c
26	Intermediate	12.8	432	12.0 c
10	Conducive	12.0	645	8.1 d
9	Conducive	14.5	589	6.2 d
23	Conducive	11.8	512	5.8 d
6	Conducive	10.6	254	4.7 d

[a] Number of colonies represent average of 5 replication of each treatment.
[b] Number of bacterial plaques formed in the mycelial lawn of fer pathogenic fungus on triple layer agar.
[c] The averages with same letters are not significantly differ statistically at 5% level.

DISCUSSION

The pathogenic fungus, *F. oxysporum* f. sp. *cucumerinum* employed in this investigation showed strong pathogenicity to cucumber seedlings when the inoculum was infested into autoclaved soils even at concentration of 100 cfu/g. But in non-autoclaved soil, the disease incidence was significantly reduced depend upon the suppressive potential of test soils. In this experiments, no such things as the long term suppressive soil, described previousely in many investigations[1,15,19,29,32,35] have been found. But some of the test soil, such as 6, 7, and 22 suppressed fusarium wilt remarkably at given concentration of inoculum compared with other test soils.

Because inoculum concentration of soil-borne disease is less precisely defined the estimation of inoculum by a quantal response obscures the natural variation in the host pathogen system[8]. It is presumed that application of log-probit transformation to straighten the disease response curve is resonable. Because the susceptibility of individual plant to the disease at given inoculum concentration is distributed normally in a host populaton[8,13]. Accordingly, estimation of DI50 values from logprobit transformation is more appropriate than other type of transformation in comparing suppressive potential of test soil quantitatively.

Although some workers suggested that chemical components are related to soil suppressiveness, the results of chemical analysis of test soil in present study indicated none of chemical components of soil was related to soil suppressiveness (DI50 Value). Elimination of soil suppressiveness by biocidal treatment was also confirmed in this experiment. The suppression of disease incidence in suppressive soil was completely nullified after autoclaving. It is suggested that some biological entity is involved in soil suppressiveness.

It is generally considered that funngal mycelium is rapidly lyzed in suppressive soil[2,18,21,33] In this experiment, however, the mycelium in suppressive

soil was lyzed progressively and eventually lead to complete lysis in 30 days. The results of this experiment suggested that progress and degree of mycelial lysis is not suitable to presume the disease suppressiveness or conduciveness of given soil.

The mycelium of pathogenic fungus lyzed more lysis rapidly in conducive soil but chlamydospores were more abundant than those in suppressive soil, Burke[3] also reported that vegetative growth of *F. solani* f. sp. *phaseolina* was more extensive, but there were ultimately fewer and smaller chlamydospore in suppressive soil. It is presumed that mycelial lysis within first 15 days may be a normal physiological process contributing to generation of chlamydospore. After 20 days the mycelium lyzed without forming chlamydospores.

The germination rate of conidia of *Fusarium* spp. is often subjectcted to discuss in relation to soil suppressiveness[11,14,13]. In soil extracts from suppressive soil, the germination of microconidia was suppressed than that in conducive soil and the ungerminated conidia eventually lyzed. But the differences was not significant statistically.

Amount of chlamydospores converted from the mycelial fragment in suppressive soil was significantly less than those in conducive soil and germination rate was also lower in suppressive soil. Many workers reported that the germination of chlamydospores and subsequent growth of hyphae of *F. oxysporum* were less in suppressive soil than that in conducive soil[1,3,16,29,31,32]. It can be concluded that the test of chlamydospore formation and germination in the given soil be more reliabe to determine the soil suppressiveness than either mycelical lysis or conidia germination. Reduced proliferation, combined with the much lower germination of chlamydospores in suppressive soil may contribute to maintain a low density of pathogenic fusarium[1,18,21,35].

It is generally accepted that soil bacterial activities are related to fungistasis or soil suppressiveness [2,10,15,20,24]. Cook and Baker[7] indicated that bacteria are especially important as antagonists of pathogens such as *Fusarium* spp. and certain others that produce a germ tube and root rot by multiple infection.

Number of colonies of fluorescent Pseudomonads and Bacillus spp. examined in the present experiments indicated no tendency in relation to soil suppressiveness. It is presumed that bacterial numbers measured by ordinary soil dilution plate technique is not meaningful to determine soil suppressiveness. However, significant difference was found in the numbers of bacterial plaques fromed on the mycelial lawn in triple layer agar(TLA) between suppressive and conducive soil. TLA employed in this experiment allows the growth of all kinds of microorganisms such as, bacteria, fungi and actinomyces, but only bacteria formed lytic plaques on middle of mycelial lawn within 4 days. It might be attribute their rapidity of growth and promptness of inhibition. Baker and Cook[2] suggested that other antagonists can not match with bacteria intercepting sufficient numbers of pathogen germlings. TLA method is very useful not only for measuring anatagonistic population but also for screening antagonistic bacteria.

摘 要

오이 덩굴쪼김병의 발병을 抑制하는 토양의 特性과 病原菌에 대한 억제요인을 밝혀 내고자 진주, 함안, 남지, 밀양, 순천 등지의 28개 비닐하우스에서 토양을 채취하여 供試하였다. 접종한 병원균의 濃度에 대한 공시토양의 오이 덩굴쪼김병 發病曲線을 Log-Probit로 전환하여 토양의 발병억제 정도를 DI 50(50%의 공시식물을 발병시킬 수 있는 병원균의 농도)으로 數量化하여 공시토양의 발병억제 정도를 비교하였던 바, 같은 지역내에서도 채취장소에 따라서 抑制程度가 크게 달랐으며 진주 5, 순천 7, 남지 22 등이 발병억제능력이 큰 것으로 나타났다. 토양의 化學的 性分이나 物理性 등은 발병억제 능력과 일정한 相關을 나타내지 않았다. 발병억제 토양에서는 病原菌의 小型分生胞子와 厚膜胞子의 發芽率이 현저하게 억제되었다. 병원균의 菌系分解는 發病抑制型 또는 發病誘導型 토양에 따라서 일정한 傾向을 나타내지 않았으나 菌系片으로부터 形成된 厚膜胞子의 數는 발병억제형 토양에서 현저히 적었다. 토양 중의 형광성 Pseudomonads와 Bacillus spp.의 밀도는 발병억제형 토양과 유도형 토양간에 統計的有意差가 없었으나 병원균에 拮抗的인 溶菌斑 數는 발병억제형 토양에서 현저하게 많았다.

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