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Effect of a Soil Amendment for Controlling Fusarium Wilt of Cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum*

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오이 덩굴쪼김병(*Fusarium oxysporum* f. sp. *cucumerinum*) 방제에 대한 토양첨가제의 효과

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ABSTRACT: In order to find out effect on the inorganic and organic compounds as a soil amendment to control Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum*, this study was conducted during the last two years from 1993 to 1995. According to add 14 inorganic chemicals (1%, w/v) including $Al_2(SO_4)_3$ individually *in vitro*, these chemicals were confirmed their suppression, and especially Alum, CaO and $Al_2(SO_4)_3$ suppressed not only 20.9~25.0 percent on mycelial growth of the fungus, but also inhibited 72.8~97% on conidial germination. $Ca(NO_3)_2$ suppressed mycelial growth only, while KCl, K_2SO_4, NH_4NO_3 and Urea suppressed conidial germination. The 7 chemicals were finally seleced. Composted pine bark (CPB) suppressed definely more than 90% on conidial germination in the different extract concentration (2,5 and 10%), although mycelial growth on extract medium of CPB and milled alfalfa leaves (MAL) were not remarkable. The antagonist *Trichoderma* sp. (Tr-3) mixed with an amended soil (1%, w/w) containing composted pine bark showed a good mycelial growth to compete the causal fungus. And the antagonist *Pseudomonas* sp. (7-1-3) was also confirmed its antagonistic ability with culture filterate. It is known that a CPB soil amendment mixed with the two antagonists (1%, w/w) controlled almost completely Fusarium wilt of cucumber in greenhouse pots and a field experiment. It is therefore expected that biocontrol on Fusarium wilt of cucumber by a soil amendment can be applied to farmmer's fields.

KEYWORDS: *Fusarium oxysporum* f. sp. *cucumerinum*, Soil amendment, Biological control, Antagonists, *Trichoderma* and *Pseudomonas* sp.

Recently vegetables and ornamental crops have been intensively cultivated in plastic film house year round because vegetables are higher cash crop to pursue maximun profit. Structural culture fields are so limited that continuous cultivation has brought about serious root diseases such as Fusarium wilt of cucurbitaceous plants (Chung, *et al.*, 1986; Nam, *et al.*, 1988; Chung, *et al.*, 1994; Cho, *et al.*, 1989).

Garrett defined biocontrol that "disease is reduced through the activity of living organisms other than humans" (Baker *et al*, 1974; Baker, 1983). This approach is an essential method not only for maintaining sustainable agriculture without any serious yield loss, but also root disease can be effectively controlled by biocontrol including soil amendment.

Since Sanford (1926) attempted to control potato scab in some soils by apply green manure, studies on soil amendment have been intensively made after 1960s. Inoue in

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1964 conducted a control experiment on radish yellow by adding chitin to soil 7 weeks in advance before seed sowing. S-H mixture as organic amendment reduced the incidence of wilt and yellows caused by *Fusarium* spp., according to the report by Sun in 1985. Avocardo disease incited by *Phytophthora cinamomi* was fairly suppressed by using composted pine bark mixture (Hoitink *et al*, 1985; Hoitink, 1986). The effects of inorganic amendments on the plant and pathogen are promoted either direct, without the mediation of soil microorganisms, or indirect through biological transformation. In Korea, Park (1994) stressed that further studies on rhizosphere competence of biological agents on disease suppression and plant growth promotion intensively conducted, for the successful biocontrol of soil borne diseases.

An effective formulation of soil amendment applying to field conditions like natural suppressive soil is urgently needed in this country in the future. Therefore, the purpose of the study was conduced to screen inoranic and organic materials individually, and then found out effect of a formulated soil amendment mixed with the antagonist for controlling Fusarium wilt of cucumber under greenhouse pots and field conditions.

Materials and Methods

Isolation of pathogen and preparation of inoculum

Diseased host plants were collected from a cucumber field in the plastic film house, Cheongwon, Chungbuk. The causal fungus was purely isolated on Water agar, after disinfected the diseased tissue with sodium hypochlorite (0.5%) for 3 minutes. After the causal fungus cultured on wheat bran solid medium for two weeks, the inoculum was used for soil inoculation at rate of 2% (W/W).

Isolation and screening of antagonist

Rhizosphere soils collected from cucumber fields, and soil microorganisms were isolated by soil dilution plate method. Antagonistic microorganisms were screened by triple layers agar and dual culture method. Finally, *Trichoderma* sp. (Tr-3) and the antagonistic bacterium, *Pseudomonas* sp. (7-1-3) were isolated. Antagonistic activity was performed with inhibition of mycelial growth and suppression of conidial germination in the culture filterate.

Evaluation of inorganic and organic amendments

Inorganic chemicals were tested followed by Haung and Kuhlman (1991a), and 14 chemicals were $Al_2(SO_4)_3$, Alum($Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$), $CaCl_2$, $CaCO_3$, $Ca(NO_3)_2$, CaO, glycerine(10%), K_2HPO_4, KCl, K_2SO_4, NH_4NO_3, Urea, TSP (Triple superphosphate),and $MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$. Check soil did not recieve an amendment. Composted milled pine bark and milled alfalfa leaves were used as organic amendment.

Effect of the amendments on biocontrol of *F. oxysporum* f. sp. *cucumerinum* was tested in solutions. Solutions were prepared by dissoloving each (0.1%, W/W) of the 14 chemicals in deionized water. Deionized water alone was the check. Condia of the causal fungus were obtained from 10 days old Potato dextrose agar, and placing conidia in drop of chemical solution at 27°C. Then 9 hrs later conidial germination was estimated by compound microscope.

Soil extract medium was prepared by placing 1ml of the soil extract in 10 ml water agar after shaking 10 g of sterilized soil in 30 ml of each chemical 1%,w/w for 30 minutes (180 rpm). Then the mycelial growth was estimated by measuring mycelial growth 5 days after inoculation with 5 replicates. Check did

not recieve soil extract. Suppression rate of conidial germination was estimated from 300 conidia in the above soil extract solution. To compare the suppressive ability of $Al_2(SO_4)_3$, CaO and Alum at the concentration 0.5, 1.0, 1.5 and 2.0% (w/w), the chemicals were added separately to water agar and the suppression of mycelial growth was investigated. Further estimation of conidial germination was conducted as the above level.

To compare the suppressive activity in soil conditions, the chemicals (1.0%, w/w) were added separately to sterilized soil infested with *F. oxysporum* f. sp. *cucumerinum* (2%, W/W). After 2 weeks, the amount of inoculum survival was evaluated by the stem colonization method.

Colonization of the pathogen on cucumber stem was observed. The inoculum (2%, w/w), and each of the chemicals (1%, w/w) were mixed in sterilized soil in pot (10 cm diameter) for 7 days. Twenty cucumber stems (2cm length) were buried into the pot soil with 12% moisture content, the pots were kept for 10 days at 27°C. Then cucumber stems were plated out on water agar amended with 100 unit of streptomycin for observing colonized stems.

Effect of organic compounds

The organic compounds used were composted pine bark and milled alfalfa leaves. Pine bark was composted under natural outdoor conditions for one year and alfalfa leaves were used as forage purpose imported from the United States of America.

To find out suppressive effect of the fungus with the organic materials, 1ml of extract solution was separately mixed in water agar, after 2, 5 and 10 percent (w/v) of the materials boiled in 30 ml of deionized water by shaker (180 g/min) for 30 minutes. Suppression of mycelial growth was examined on water agar medium with five replicates. Conidial germination in the extract solution was also observed from 300 conidia.

The growth of pathogen and antagonist *Trichoderma* sp. (Tr-3) in the amended soil (1%, w/w)

According to soil plate method, sterilized soil was amended with the 7 effective chemicals plus composted milled pine bark (CPB) separately and then placed in petri dish. Application of $Al_2(SO_4)_3$, Alum, $CaCl_2$, CaO, KCl, K_2SO_4 and Urea the soil was followed by the formulated SF-21 used by Huang and Kuhlman (1991a).

Control effect on a soil amendment mixed with the antagonist:

Greenhouse test In a pot test, natural clay loam soil was sterilized. The causal pathogen was mixed in pot soil 2% (w/w) after pathogen cultured on wheat bran sand medium (wheat bran 5 g, sand 95 g).

One percent (w/w) of the CPM were mixed separately with sterilized and natural soils. Inoculum of the two antagonists was prepared. The antagonist, *Trichoderma* sp. (Tr-3) was cultured on wheat bran medium for 2 weeks, and the bacterium, *Pseudomonas* sp. (7-1-3) was cultured on nutrient medium for one week and the *Pseudomonas* suspension ($10^7 \times 10$ (cfu)) was inoculated on vermiculite and then air dried. The inoculum of the antagonists was mixed with soils and rate of concentration was 2% (w/w).

Details of treatments were as following. CPM plus the two antagonists, CPM plus *Pseudomonas* sp., CPM plus *Trichoderma* sp. alone, and pathogen alone without the amendment as control, respectively. One seedling sown per plastic pot (15 diameter x 20(H)), and 30 seedlings were sown in each treatment with three replicates. Seedlings

were cultured to follow a standard cultural practices and wilt symptom development was investigated every day up to 5 weeks after planting.

Field test Randomized block design was conducted under polyethylene film house, Chungbuk Univ. farm. Soils were sterilized and non-sterilized natural soils. Soils amended with the CPM (1%, w/w) before planting, and soil was not amended as control plots (non-amended). Treatments were same as greenhouse test. Seedlings were also kept to follow a standard cultural practices and watered by an overhead hand sprayer properly every day after the first symptoms of wilt appeared. The growing conditions and wilt development of seedlings were examined every day up to 8 weeks after planting.

Results

Screening inhibition ability of the antagonists to the fungus

According to triple layer agar method and dual culture, *Trichoderma* sp. and *Pseudomoans* sp. were finally selected. In dual culture, *Trichoderma* sp. showed not only antagonistic to the causal fungus, but also reacted its hyperparasitic and coilings into the mycelia of the pathogen (Fig. 1). The antagonistic bacterium *Pseudomonas* sp. (7-1-3) formed inhibition zones of 8.0 mm on dual culture plates and resulted in 1.2% of conidial germination of the causal pathogen con-

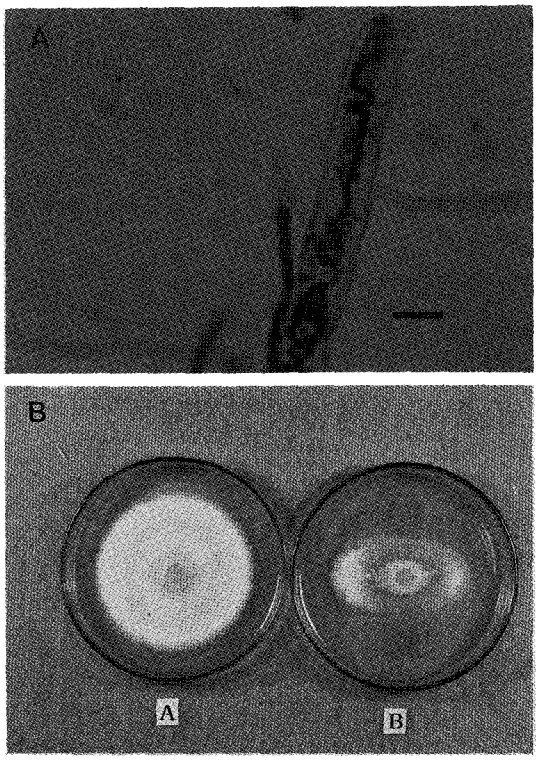

Fig. 1. A: Hyperparasitic penetration of the antagonist, *Trichoderma* sp. into the hypha of the causal fungus (Bar represents 10 μm) B: Inhibition zones produced by the antagonist, *Pseudomonas* sp.(B) and compared with causal fungus (A) *F. oxysporum* f. sp. *cucumerinum*

Table 1. Antagonistic ability of the antagonist *Pseudomonas* sp. to *Fusarium oxysporum* f. sp. *cucumerinum* on potato dextrose agar or in potato dextrose broth

Antagonist	Diameter of[a] inhibition zones (mm)	% of conidial[b] germination with culture filtrate	Dry weight (g dw/50 ml)
Pseudomonas sp. (7-1-3)	8.0	1.2	0
Untreated	0	92.1	0.19±0.01

[a]: Inhibition diameter zone was obtained from dual culture with 5 replication and readings were made 5 days after grown on P.D.A at 28°C.

[b]: Conidial germination of the fungus checked at 12 hrs after treated in the culture filterate of the antagonist grown on P.D.B for 72 hrs, and 300 conidia were investigated.

Table 2. Effect of various inorganic compounds on mycelial growth and conidial germination of *Fusarium oxysporum* f. sp. *cucumerinum* causing cucumber wilt

Chemicals	Suppression percent(%)	
	mycelial growth[a]	conidial germination[b]
$Al_2(SO_4)_3$	20.9 (28.5) ijkl[c]	98.3 (1.7)
Alum	25.0 (27.0) l	100.0 (0.0)
$CaCl_2$	17.5 (29.7) hij	80.7 (19.3)
$CaCO_3$	15.3 (30.5) defghi	65.5 (34.4)
$Ca(NO_3)_2$	13.9 (31.0) cdefgh	0.0 (100.0)
CaO	25.0 (27.0) l	72.8 (27.2)
Glycerine	9.2 (32.7) bcd	57.3 (42.7)
K_2HPO_4	9.8 (32.5) bcde	37.0 (63.0)
KCl	13.9 (31.0) cdefgh	78.4 (21.6)
K_2SO_4	11.2 (32.0) bcdefg	81.6 (18.4)
NH_4NO_3	6.4 (33.7) b	62.5 (37.5)
Urea	7.8 (33.2) bc	71.9 (28.1)
TSP	18.1 (29.5) hijk	7.6 (92.4)
$MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$	10.6 (32.2) bcdef	31.9 (68.1)
Check	0.0 (36.0) a	0.0 (100.0)

Alum: $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$

TSP: Triple superphosphate

a: Paraenthesis number means mycelial diameter of 5 replications, and records were made 4 days after treatment

b: Paraenthesis number means percent of conidia germinated.

c: Paraenthesis means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

trasted with 92.1% in control (Table 1).

Effect of inorganic chemicals on suppression of the fungus

Among the 14 chemicals, $Al_2(SO_4)_3$, Alum, CaO, $CaCl_2$, KCl, K_2SO_4 and Urea greatly inhibited conidial germination of the causal fungus, (Table 2) although inhibition of mycelial growth was not big difference. The remaining chemicals did not greatly suppressed conidial germination and mycelial growth. Ca $(NO_3)_2$ suppressed mycelial growth only, while KCl, K_2SO_4, NH_4NO_3 and Urea suppressed greatly conidial germination rather than mycelial growth. Three chemicals Al $_2(SO_4)_3$, Alum, and CaO inhibited more than 80% of conidial germination at different concentrations of 0.5, 1.0, 1.5 and 2.0%. In the case CaO, suppression mycelial growth was 3.7-36.0%, whereas, $Al_2(SO_4)_3$, and Alum were 63.9 to 83.2% for mycelial suppression (Table 3 & Fig. 2).

Effect on stem segment colonization

After the 14 chemicals (1%, w/w) individually added to the pot soil infected with fungus, stem segment colonization was examined. Stem segment colonization of the 7 chemicals including $Al_2(SO_4)_3$ was reduced by more than 42.3% in comparison with control. Especially colonization by the causal fungus with Urea occurred 29.3% suppression among the chemicals tested (Table 4). NH_4NO_3 was not effective at all and the remaining chemicals were not remarkable.

Effect on organic compounds

After composted pine bark and milled al-

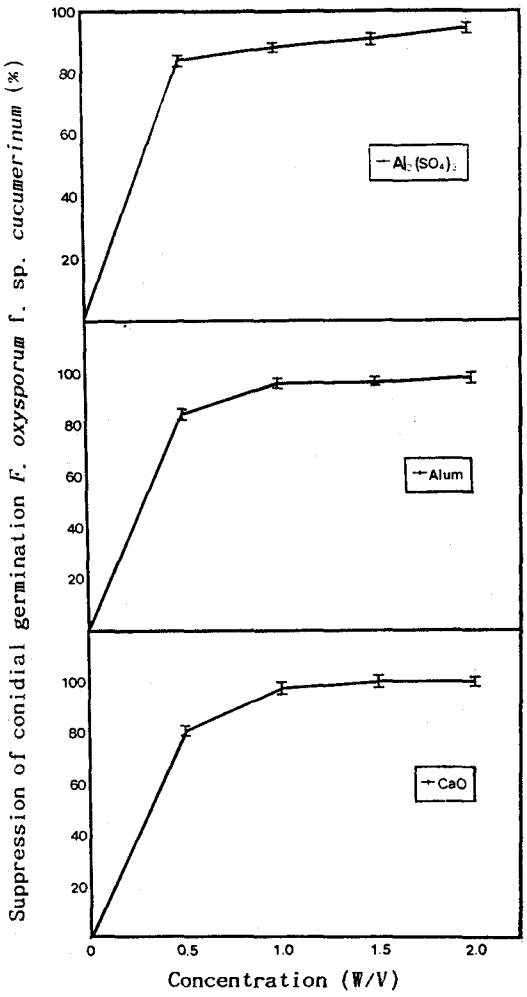

Fig. 2. Effect of CaO, $Al_2(SO_4)_3$, and Alum at different concentrations solution on conidial germination of *Fusarium oxysporum* f. sp. *cucumerinum* after 8 hours at 27°C. Alum: Al $_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$.

falfa leaves were boiled at 2, 5 and 10% (w/v) of deionized water volume, extract solution of CPB and MAL were prepared from composted pine bark (CPB) and milled alfalfa leaves (MAL) and at 2, 5, 10% (w/v) concentration of these extract solution were examined to control *F. oxysporum* f.sp. *cucumerinum* causing cucumber wilt (Table 5). Although mycelial inhibition of the fungus was not great compared to control, conidial

Table 3. Effect of different the concentrations of CaO, $Al_2(SO_4)_3$, and Alum on mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* on water agar

Chemical	Conc. (%)	Mycelial growth (mm)[a]	Suppression rate (%)
CaO	0.5	40.8±0.26[b]	3.7
	1.0	33.7±0.39	20.3
	1.5	30.0±0.51	29.2
	2.0	26.4±0.49	36.9
$Al_2(SO_4)_3$	0.5	9.3±0.3	78.1
	1.0	10.6±0.3	74.9
	1.5	8.7±0.17	79.5
	2.0	7.1±0.12	83:2
Alum	0.5	15.3±0.76	63.9
	1.0	10.3±0.29	75.7
	1.5	9.2±0.14	78.3
	2.0	8.3±0.25	80.2
Check		42.4±0.28	0

a: Radial diameter (mm) was obtained from mycelial growth on Water agar by preparing CaO, $Al_2(SO_4)_3$ and Alum concentration ((w/v). Reading were made at 4 days after treatment.
b: Values are average of 5 replicatios and standard deviation.
Alum: $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$

germination of the fungus was significantly inhibited by adding increasing proportions of CPB between 2 and 10% of water volume (Table 5). In the soil amended with CPM (1%, w/w), *Trichoderma* sp. (Tr-3) showed active growing to compete the causal fungus as a result of hyperparasitic reaction. In addition, pathogen was no active mycelial extension.

Disease incidence in greenhouse tests

The formulated CPM amendment in sterilized and non sterilized soils infected with *Fusarium oxysporum* f. sp. *cucumerinum* reduced significantly Fusarium wilt incidence (Table 6). The CPM (1%, w/w) plus antagonists was more effective than the other treatments regardless of sterilized and non sterilized soils. In addition, growing con-

Table 4. Effect of various chemicals on cucumber stem segment colonization by the causal pathogen *Fusarium oxysporum* f. sp. *cucumerinum*

Chemicals[a]	Stem-segment colonization(%)[b]
$Al_2(SO_4)_3$	42.3±0.45[c]
Alum	48.6±0.92
$CaCl_2$	53.2±0.65
$CaCO_3$	73.7±0.46
$Ca(NO_3)_2$	64.0±0.45
CaO	46.8±0.41
Glycerine	50.2±0.14
K_2HPO_4	80.0±0.52
KCl	48.4±0.4
K_2SO_4	49.0±0.55
NH_4NO_3	100.0±0.35
Urea	29.3±0.2
TSP	75.7±0.54
$MgCO_3 \cdot Mg(OH)_2 \cdot H_2O$	65.8±0.43
Check	100.0±0.44

a: 1%(w/w) of chemical was added to the soil infested with 3%(w/w) of Fusarium inoculum, and adjusted with 12% (v/w) water content.
b: Twenty stems were treated per chemicals, and investigation were made at 2 weeks after treatment.
c: Values are average of 5 replicatios and standard error
Alum: $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$, TSP: triple superphosphate.

ditions of seedlings were as good as healthy seedlings except pathogen inoculation pots (Table 6). With regard to wilt symptom development, readings on disease were made at 30 days after seedling transplanted. With regard to population densities of the antagonists associated with the CPM soil amendment, population densities of the bacterium (7-1-3) and *Trichoderma* sp. (Tr-3) was not significantly affected regardless of sterilized and non sterilized soils (data not shown).

Disease incidence in field under polyethylene film house

Cucumber seedlings amended with the CPM (1%, w/w) in sterilized and non sterilized soils infected with *Fusarium oxysporum* f. sp. *cucumerinum* (2%, w/w) were controlled almost completely Fusarium wilt incidence in comparison with 50% in sterilized and 30% in non sterilized control plots. Accordingly, growing conditions of cucumber seedlings were all healthy except pathogen inoculation control plots. The data was read upto 50 days after seedling translpanted.

Table 5. Effect of organic compounds[a] on mycelial growth and conidial germination of *Fusarium oxysporum* f. sp. *cucumerinum* on soil extract agar amended with decoction with each compound

Organic Compound	Con. (%)	*F. oxysporum* f. sp. cucumerinum	
		mycelial growth (mm)[b]	conidial germination (%)[c]
Composted Pine bark	2	27.8±0.25	10.4±0.07
	5	16.3±1.44	5.7±0.27
	10	12.3±0.48	4.6±0.29
Milled alfalfa leaves	2	37.0±0.71	37.0±0.13
	5	38.0±0.29	35.1±0.13
	10	39.0±0.41	31.5±0.31
Check		38.0±0.29	97.0±0.04

a: Different concentration of extract solution were prepared from the organic compounds after decoction for 30 minutes.
b: Values were average of 5 replications and standard deviation.
c: Mean percent of conidial germination was obtained from 300 conidia of the fungus and standard deviation.

Discussion

The seven inorganic chemicals including Al $_2(SO_4)_3$ of the 14 chemicals significantly suppressed more than 70% of conidial germination and 10% of mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* causing cucumber wilt (Table 2). These results were similar to the report on SF -21 formulated by Huaung et al on damping off of slash pine (1991a). SF-21 includes two ma-

Table 6. Control effect of composted pine bark mixture amendment (1%, w/w) on Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* in the greenhouse and field under polyethylene film house

	Soil amendment	Growing conditions of seedlings	% of Plants wilted	
			pot	field
Sterilized soil	CPM + the two[a] antagonists	+++[b]	10[e]	0
	CPM + *Pseudomonas* sp.[c]	+++	10	0
	CPM + *Trichoderma* sp[d]	+++	10	0
	None (Pathogen only)	-	50	50
Non-sterilized soil	CPM + the two[a] antagonists	+++b	0	0
	CPM + *Pseudomonas* sp.[c]	+++	0	0
	CPM + *Trichoderma* sp[d]	+++	20	0
	None (Pathogen only)	-	60	30

Readings were made at 30 days with pots and 50 days in field trial after seedlings transplanted.
[a]CPM: Composted pine bark and chemicals mixture together with the antagonist *Pseudomonas* sp. (7-1-3) and *Trichoderma* sp. (Tr-3)
[b]+++: Good growth; -:Poor growth with wilting and yellowing.
[c]Antagonist *Pseudomonas* sp. (7-1-3) was inoculated.
[d]Antagonist *Trichoderma* sp. (Tr-3) was inoculated.
[e]Percent of wilted plants is an average of 3 replications, and transeedling of each treatment.

jor ingredients : Pine bark and aluminum sulfate. Other researchers have reported that pine bark suppresses Pythium and Phytophthora root rot (Hoitink, *et al* 1985; Spencer, *et al* 1982).

Since control effect of soil amendment is known to be mutual phenomenon such as biological and chemical fators, Hoitink (1986) pointed out that composted pine bark suppresses soil borne fungi and to promote activity of indigenous thermophilic bacteria and *Trichoderma* spp. as antagonists. Additionally, tannin, resin, saponin, starch, other carbohydrates and ethylester contained in pine bark were toxic and fungistasis to the pathogen (Haung, 1991a). The factors were also interpreted as abiotic factor for inhibition of conidial germination in the extract solution of composted pine bark (Table 5). Davey and Papavizas (1960; Papavizas, 1968) reported that the suppression of *R. solani* in soil by composted organic amendment and supplementary nitrogen was related to the general microbial activity in the soil.

Aluminum sulfate, an acidifying agent controlied damping off of pine seedlings and oth-

er soil borne fungi (Huaung and Kuhlman, 1991a and 1991b). Naturally occurring soil aluminum reduced pathogenesis of sunflower pathogens *Verticillium albo-atrum* and *Whethzelinia sclerotiorum* (Orellana, *et al*, 1974). In our study, mycelial growth and conidial germination of the pathogen were significantly reduced by $Al_2(SO_4)_3$, Alum, and CaO (Table 2).

Since Papavizas (1985) mentioned that precaution in the use of a readily available substrate with *Trichoderma* or *Gliocladium*, these substrates also are the possible stimulation of a pathogen to colonize pine bark segments, and hence an increase grow in disease. With a formulated amendment (1%, w/w), *Trichoderma* sp. (Tr-3) was colonized in the CPM amended soil medium to compete the causal fungus. Urea suppressed more than 50% of stem segment colonization together with CaO, KCl, K_2SO_4 and glycerine (Table 4). Further investigation on the results is needed in the future, although urea had strong inhibition to pine bark segment with Rhizoctonia solani, Phytophthora and Pythium spp. (Huang *et al*, 1991a) and *Fusarium oxysporum* f. sp. *vasinfectum* (Chung, *et al.*, 1994). S-H mixture, a combination of bagasse, rice husks, oyster shells, mineral ash, and fertilizers controlled many soil borne disease in horticultural plants (Sun *et al*, 1985).

Trichoderma sp. reduced population densities of *Rhizoctonia* and *Pythium* spp. by secreting cellulase, β-(1-3)-glucanase and chitinase (Bin, *et al* 1991; Chet, *et al* 1981). The results on *Trichoderma* sp. (Tr-3) is coincided with a report by Chet in 1990 that a strain (T-35) of T. harizanum was an effective antagonist to *F. oxysporum* f. sp. *vasinfectum*. The indigenous *Pseudomonas* sp. *suppressed* also significantly occurrence of Fusarium wilt of cucumber in sterilized and non sterilized soils.

A different mechanism probobly explains the CPM amendment for controlling Fusarium wilt of cucumber. However our results were interpreted as a combined factor. We have shown that a formulated CPM amendment is almost completely controlled Fusarium wilt of cucumber through pots and a field experiment in sterilized and non sterilized soils. Therefore, this formulated CPM amendment is known to be the best formulated organic soil amendment (Table 6).

The future application of biocontrol with the CPM amendment, however, will require further resarch efforts to find out how widely applicable this treatment is in different soilborne pathogens, to learn how persistent the control is, and to learn to use them ecomically.

적 요

오이 덩굴쪼김병에 효과있는 새로운 토양첨가제의 조제와 방제효과를 구명코저 본 연구를 1993년부터 지난 2년간 수행하였다. 석회를 포함한 14종의 무기성분(1%, w/w)을 성분별로 공시하여 억제효과를 조사하였는데 특히 $Al_2(SO_4)_3$, Alum 및 CaO 등의 *Fusarium oxysporum* f. sp. *cucumerinum*에 대한 포자발아 억제율은 72.8-97%였고 균사생장 억제율은 20.9-25%이었다. 한편 $Ca(NO_3)_2$는 균사생장에만, KCl, K_2SO_4, NH_4NO_3와 Urea는 포자발아에만 억제효과가 컸으며 그 억제율은 37.0-71.9%이었다. 결과적으로 7종의 무기성분이 선발되었다. 부숙소나무 수피등 2종의 유기물을 2, 5 및 10% 농도별로 공시한 결과 부숙소나무 수피는 알팔파 잎가루에 비하여 병원균의 포자발아나 균사신전 억제효과가 우수하였다. 소나무 수피와 무기성분(1%, w/w)을 혼합한 토양첨가제 처리토양에서 길항균(Tr-3)의 생장은 양호하였을 뿐만아니라 병원균의 균사생장을 효과적으로 억제하였다. 그리고 길항세균(*Pseudomonas* sp.)여액에서의 병원균에 대한 길항력도 우수함이 확인되었다. 부숙 소나무수피와 알팔파 잎가루의 추출액배지에서의 균사생장 억제는 크지 않았으나 포자발아

는 부숙 소나무수피액 처리에서 뚜렷하였고 농도에 따라 억제율이 90% 이상이었다. 무기성분과 부숙 소나무 수피가루 및 2종의 길항균을 토양에 혼합한 (1%, w/w) 구에서는 오이 덩굴쪼김병에 대한 방제 효과가 폿트와 포장시험결과로 완벽함이 밝혀졌다. 따라서 토양 첨가제에 의한 오이 덩굴쪼김병의 생물학적 방제를 위하여 농가 포장에서의 그 활용이 기대된다.

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