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Control of Pythium Damping Off and Root Rot of Cucumber with S-H Mixture as Soil Amendment[1]

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ABSTRACT

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S-H mixture used as soil amendment at the rates of 0.5 to 2% (w/w) greatly or completely inhibited damping off and root rot of cucumber caused by *Pythium aphanidermatum* under the greenhouse and field conditions. Among the various combinations of the components of S-H mixture tested in laboratory, urea was the main factor for the inhibition of oospore survival and mycelial growth of the pathogen. Siliceous slag had some additive effect. Urea alone was almost as effective as S-H mixture in reducing the pathogen population and suppressing the disease, but the gained suppressiveness was lost after 25 days of incubation. Soil amended with urea plus siliceous slag remained suppressive at least for 28 days, the longest incubation period conducted in this study.

(Key words: Cucumber, *Pythium aphanidermatum*, soil amendment, disease control)

INTRODUCTION

The S-H mixture, developed as soil amendment by Sun and Huang[19] in 1983, has been used on many crops in Taiwan recently. It inhibited *Fusarium oxysporum* f. sp. *niveum*[19], *F. oxysporum* f. sp. *raphani*[20], *F. oxysporum* f. sp. *pisi*[12], *Plasmodiophora brassicae*[9,24], *Phytophthora melonis*[11], *Sclerotinia sclerotiorum* (Sun and Huang, unpublished), and *Pseudomonas solanacearum*[6] by different mechanisms. In a rather large scale experimental use, the S-H mixture

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improved root health and stand number of watermelon, cucumber, ginger, common bean, and Chinese cabbage[1,21].

Pythium aphanidermatum (Edson) Fitzp. is a plant parasite of warm regions[14]. It causes not only damping off, root rot, and fruit rot of many economical field crops, which are easily visualizable[23], but also diseases with obscure symptoms in rootlets of many field crops[16], and hydroponically grown spinich and leafy lettuce[3]. In previous experiments on control of Phytophthora blight of cucumber [11], we observed damping-off cucumber plants caused by *P. aphanidermatum* in the control plots, but not in those plots treated with S-H mixture. We, therefore, investigated the effect of S-H mixture on the cucumber damping off and root rot caused by *P. aphanidermatum.*

MATERIALS AND METHODS

Sources of seed and S-H mixture

Cucumber seeds of variety 'Joy' produced by Known-you Seed Company were obtained from market. The S-H mixture which contained 4.4% bagasse, 8.4% rice husks, 4.25% oyster shell powder, 1.04% potassium nitrate, 13.6% calcium superphosphate, 8.25% urea, and 60.5% siliceous slag[19] was made in the laboratory and used fresh.

Preparation of inoculum and infested soil

The isolate of *P. aphanidermatum* (PA-1) used in this study was originally isolated from a diseased cucumber from the field and maintained on corn meal agar (Difco Co.). A loamy soil (pH 7.2) collected from a cucumber field at Lukang of the Central Taiwan was found infested with *P. aphanidermatum* at 20 oospores/g soil estimated on a selective medium developed by Burr and Stanghellini[5]. The soil was used to grow 2 successive crops of cucumber in greenhouse, and the oospore density increased to 150 oospores/g soil at the end of the second cropping. A virgin soil was also collected from the same place and found to be free from *P. aphanidermatum.* Both infested and virgin soils were air dried, sieved through a 20-mesh screen and stored in greenhouse. After 2 months of incubation, both infested and intensified infested soils still retained an oospore density of 20 and 150 oospores/g soil, respectively, and the virgin soil had none.

Estimation of inoculum density and absolute inoculum potential

(1) Inoculum density: A 10-gram subsample of infested soil was placed in 90 ml of sterile distilled water and agitated for 3 min. Then, an aliquot (1 ml) was dispersed on the surface of a selective medium[5] plate (9-cm petri dish), with 10 replicates. After 48 hr of incubation at 36°C, soil was washed from agar surface and colonies originated from oospores (examined under compound microscope) were counted and reisolated for the pathogen.

(2) Absolute inoculum potential (AIP): The potato baiting technique of Stanghellini and Kronland[18] was used in this study. A 25-gram infested soil was placed in a petri dish and 10 ml of sterile distilled water added to bring the moisture to near saturation. Pieces of fresh potato tuber tissue (0.3 cm × 1 cm^2) were placed 1 cm apart on the soil surface. A water agar slice (0.3 cm × 0.49 cm^2) was then placed on the top of the potato tuber tissue and incubated for 42 hr at 28°C.

The agar slices were then removed, placed on the selective medium[5], and incubated at 36°C. The percentage of the water agar slices colonized by *P. aphanidermatum* was determined after 24 hr as the AIP[15,18].

Effectiveness of S-H mixture for control of Pythium damping off and root rot

In greenhouse (26 to 34 °C), soil infested with 20 oospores/g soil was amended with 0.5, 1, 2, or 5% (w/w) of S-H mixture and kept in a 15-cm pot for 10 days. The pots were sown with cucumber seeds which had been germinated in a moisture chamber at 28 °C for 16 to 24 hr. Non-amended infested soil was employed as control. The test plants were observed for damping off, and reisolation of *P. aphanidermatum* from rootlets of surviving plants was attempted 10 or 20 days after seeding. Three replicates were provided for the experiment.

Effect of S-H mixture and its components on the survival, mycelial growth and oospore production of *P. aphanidermatum*

In this study, various combinations of the components of S-H mixture were used at quantities correponding to the S-H mixture applied as soil amendment at 2% (w/w).

(i) Oospore survival: The soil infested with *P. aphanidermatum* (150 oospores /g soil) was mixed with S-H mixture or its component(s). The soil was then moistened to almost saturation and incubated at 28°C. The oospore density and AIP (% of baits colonized) were measured at different incubation time by the method described in the foregoing section.

(ii) Mycelial growth and oospore production: To measure the mycelial growth and oospore production of *P. aphanidermatum*, twenty-five gram of virgin soil (without pathogen) amended with the component(s) of S-H mixture was placed in a 9-cm petri dish and moistened to almost saturation. Pieces of fresh potato tuber tissue (0.5 cm × 1 cm²) inoculated with an agar disk of *P. aphanidermatum* were half buried in the amended soil. The pathogen occupied the potato tuber tissue and grew into the soil. After 3 days of incubation at 32 °C, the mycelial growth, hyphal width and oospore size were measured under a disecting or compound microscope. Also, the oospore density in the soil sample beneath the colony was determined by using a selective medium[5]. In another experiment, the influence of incubation time following addition of S-H mixture or its components was also determined. The inoculated potato tuber tissue was placed on the surface of the amended soil at the 0, 5, 10, 15, 20, or 25th day after amending. After 3 days, the mycelial growth of the inoculum was measured.

Disease control in field

Two field trials were conducted on the experimental farm of Taiwan Agricultural Research Institute from September to December, 1987, respectively. Cucumber fruits inoculated with *P. aphanidermatum* were incubated at 28 °C for 2 days, and then incorporated into the field soil. The field was irrigated immediately to keep the moisture at or above field capacity, a condition favorable to the growth and formation of oospores of *P. aphanidermatum*. After 7 days, the field (20 m × 25 m) was evenly divided into 4 subplots. Each subplot was 10 m × 12.5 m.

One subplot was amended with S-H mixture (1 Kg/m²); the diagonal one with urea (82.5 g/m²); and the remaining two were not amended to serve as check. Cucumber seeds (120 seeds/row, 10 rows/5 beds/subplot) were sown 14 days after amending. The inoculum density and AIP of the populations of *P. aphanidermatum* were determined at 10-day intervals by using a selective medium[5] and the potato baiting technique, respectively. The plant growth and yield were recorded at the end of the growing period.

RESULTS AND DISCUSSION

Effectiveness of S-H mixture on disease control

In greenhouse, the loamy soil collected from field was naturally infested with *P. aphanidermatum* at a density (20 oospores/g soil) high enough to cause damping off and root rot of cucumber under warm (28 °C) and wet (more than field capacity) conditions. S-H mixture used as soil amendment greatly reduced the incidence of the disease. At 0.5 or 1% (w/w), the soil amendment lowered the disease incidence to about half against the control. At 2 or 5% (w/w), damping off and root rot were completely inhibited. No pathogen could be recovered from amended soil and the rootlets of the plants (Table 1).

Table 1. Effect of S-H mixture on the damping off and root rot of cucumber caused by *Pythium aphanidermatum* in pot tests[1]

Dosage of S-H mixture (%, w/w)	Damping off[2] (%)	Reisolation from root[3] (%)
0 (CK)	70	65
0.5	40	42
1.0	30	25
2.0	0	0
5.0	0	0

1) The soil was naturally infested with *P. aphanidermatum* at 20 oospores/g soil, and thirty plants were used in each treatment.
2) The data were taken 10 days after seeding.
3) Fifty rootlets were isolated from five surviving plants for the pathogen, 20 days after seeding.

Effect of S-H mixture on oospore survival, mycelial growth and oospore production of *P. aphanidermatum*

(i) Oospore survival: The oospore density and AIP (% of baits colonized) of *P. aphanidermatum* were both greatly reduced in the infested soil (150 oospores /g soil) amended with different amounts of S-H mixture. No fungal propagules were detected in the soil treated with S-H mixture at or above 2% (w/w) (Table 2).

(ii) Mycelial growth and oospore production: A virgin soil was used instead of the infested soil to test the effect of S-H mixture on the mycelial growth and oospore production of *P. aphanidermatum*. The fungus was introduced to a disc of potato tuber tissue which was then half

Table 2. Effect of S-H mixture on the survival of *Pythium aphanidermatum* in naturally infested soil in laboratory[1)]

Dosage of S-H mixture (%, w/w)	Oospores/g soil[2)]		% bait colonized[3)]	
	Before tr.	10 days after tr.	Before tr.	10 days after tr.
0 (CK)	135	102	100	100
0.5	150	70	100	100
1.0	167	77	100	91
2.0	150	0	100	0
5.0	120	0	100	0

1) The soil was intensified infested with 150 oospores/g soil.

2) Oospore density was determined by the selective medium of Burr and Stanghellini[5].

3) The potato baiting technique of Stanghellini and Kronland[18] was used to determine the percentage of baits colonized as the absolute inoculum potential of *Pythium aphanidermatum.*

Table 3. Effect of S-H mixture on the mycelial growth and oospore production of *Pythium aphanidermatum* in a virgin soil amended with S-H mixture[1)]

Dosage of S-H mixture (%, w/w)	Mycelial growth[2)] (cm)	Oospores/g soil[3)]
0 (CK)	2.2	1500
0.5	2.2	730
1.0	0.7	40
2.0	0.2	0
5.0	0.15	0

1) *Pythium aphanidermatum* was introduced to a disc of potato tuber tissue which was then half buried in amended soil in petri dish.

2) Mycelial growth from the inoculated potato tuber tissue into the soil were measured after 3 days of incubation.

3) The oospore density in the soil sample beneath the colony was determined by using a selective medium of Burr and Stanghellini[5].

buried in amended soil in petri dishes. The results also showed that S-H mixture used at or above 2% (w/w) completely inhibited both the mycelial growth and oospore production of *P. aphanidermatum* from the inoculum. By contrast, in the check soil, the pathogen occupied the potato tuber tissue and grew into the soil to produce a lot of oospores (Table 3).

Effect of the component(s) of S-H mixture on the survival and mycelial growth of *P. aphanidermatum*

Greenhouse bioassays for certain root diseases to predict the disease potential of an infested soil usually require large quantities of soil, extensive greenhouse space, time and labor[4]. The estimation of the AIP[15] provides data on which the

maximum capacity of a patogen population to infect a population of fully susceptible host-plants under conditions optimum for infection could be assessed. In this regard, the potato baiting technique of Stanghellini and Kronland[18] is rapid and quantitative. Lin and Yang[13] reported that the AIP of *P. aphanidermatum* determined by the potato baiting technique has a postitive correlation with the disease incidence of cucumber damping off in greenhouse tests. Therefore, in studing the effect of S-H mixture and its components on the cucumber damping off caused by *P.*

Table 4. Survival of *Pythium aphanidermatum* in soil amended with S-H mixture or the combinations of its componets in petri dish test[1]

Treatment[2]	Oospores/g soil (% Baits coln.)[3]						
	Days after treatment						
	0	2	4	10	15	20	30
CK	115	80	75(100)	70(100)	65	60	60(100)
SH	115	23	0(0)	0(0)	0	0	0(0)
SH-K	115	0	0(0)	0(0)	0	0	0(0)
SH-P	115	0	0(0)	0(0)	0	0	0(0)
SH-S	115	30	35(40)	20(12)	3	3	8(10)
SH-U	115	83	68(100)	60(100)	70	78	70(100)
SH-O	115	0	0(0)	0(0)	0	0	0(0)
SH-R	115	0	0(0)	0(0)	0	0	0(0)
SH-B	115	0	0(0)	0(0)	0	0	0(0)
K	115	115	77(100)	105(100)	65	60	100(100)
P	115	70	50(100)	68(100)	33	40	40(100)
S	115	140	133(100)	48(100)	30	30	30(100)
U	115	70	18(45)	0(0)	0	0	0(0)
O	115	120	117(100)	55(100)	43	40	45(100)
R	115	65	75(100)	60(100)	55	40	40(100)
B	115	65	65(100)	70(100)	50	43	40(100)
U+K	105	45	45(100)	15	2(13)	0	0(0)
U+P	105	80	50(100)	5	0(0)	0	0(0)
U+S	105	0	0(0)	0	0(0)	0	0(0)
U+O	105	72	50(100)	2	0(0)	0	0(0)
U+R	105	70	60(100)	0	0(0)	0	0(0)
U+B	105	70	60(100)	0	0(0)	0	0(0)

1) S-H mixture and each of the combinations of its components were used at quantites corresponding to S-H mixture applied at 2%, w/w in the infested soil (150 oospores /g soil).

2) SH=S-H mixture; K=KNO_3; P=Calcium superphosphate; S=Siliceous slag; U=Urea; O=Oyster shell powder; R=Rice husks; B=Bagasse.

3) The oospore density was determined by using the selective medium of Burr and Stanghellini[5], and the potato baiting technique of Stanghellini and Kronland[18] was used to determine the percentage of baits colonized as the absolute inoculum potential of *Pythium aphanidermatum.*

aphanidermatum, we chosed to estimate the AIP of the pathogen instead of the tedious greenhouse plant bioassays.

Among the various components of S-H mixture and their combinations tested, urea was obviously the main factor contributing to the reduction on oospore survival, percentage of baits colonized (Table 4), mycelial growth, hypha width, and oospore size (Table 5). Siliceous slag had some additive effect. The few oospores of a reduced size produced from inoculated potato tuber tissue in amended soil did not germinate on the selective medium.

Effect of incubation time following amendment with S-H mixture on the inhibition of mycelial growth of *P. aphanidermatum*

Since urea alone was almost as effective as S-H mixture in suppressing *P. aphanidermatum* and siliceous slag had some additive effect, the time factor influencing the suppressiveness became very interesting in studing the mechanisms of S-H mixture on controlling *P. aphanidermatum*. The experiments were deviced to study the effect of incubation time on the suppressiveness. The results (Fig. 1) showed

Table 5. Effect of the components of S-H mixture in soil on the mycelial growth of *Pythium aphanidermatum*[1)]

Treatment[2)]	Mycelial growth (cm)	Hypha width (μm)	Oospore diameter (μm)
CK	2.2	5.4	21.5
SH	0.2	2.8	7.8
SH-K	0.2	2.8	8.3
SH-P	0.5	3.3	7.5
SH-S	0.2	2.5	8.6
SH-U	2.1	4.8	21.0
SH-O	0.2	3.2	8.1
SH-R	0.2	3.1	9.1
SH-B	0.2	3.2	9.5
K	2.2	5.6	25.0
P	2.2	5.6	24.0
S	2.1	4.5	26.0
U	0.3	3.1	8.9
O	2.2	4.8	24.0
R	2.2	5.1	24.0
B	2.1	4.8	24.0
U+S	0.15	2.9	5.6

1) S-H mixture and each of the combinations of its components were used at quantities corresponding to S-H mixture applied at 2% (w/w) in the soil. *Pythium aphanidermatum* was introduced to a disc of potato tuber tissue which was then half buried in amended soil in petri dish. The data were taken after 3 days of incubation.

2) SH=S-H mixture; K=KNO_3; P=Calcium superphosphate; S=Silceous slag; U=Urea; O=Oyster shell powder; R=Rice husks; B=Bagasse.

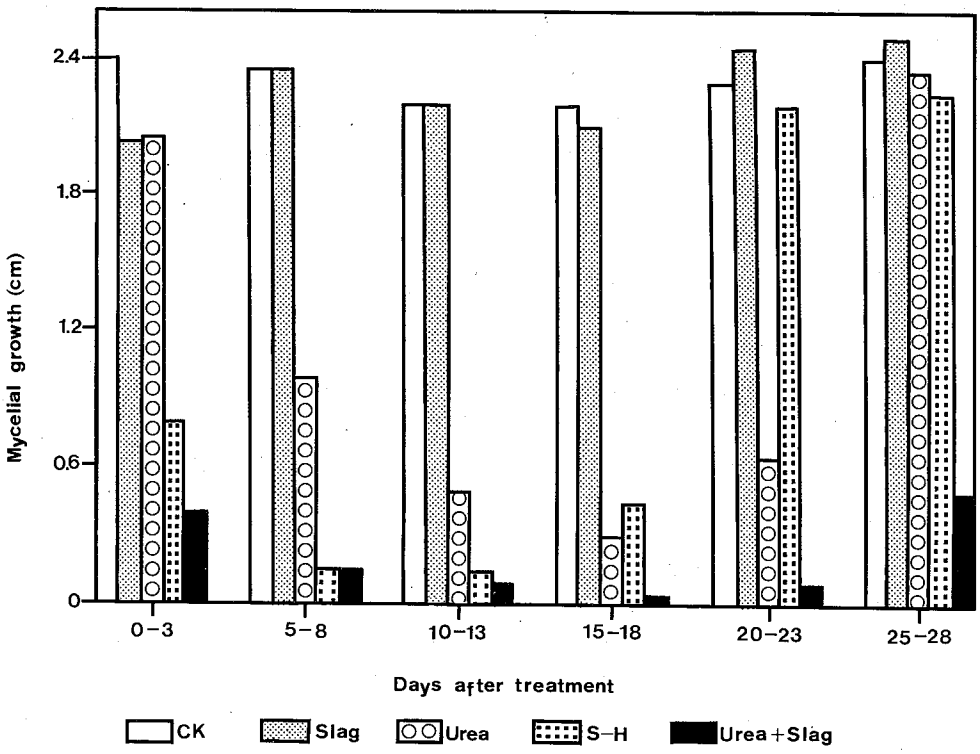

Fig. 1. Mycelial growth of *Pythium aphanidermatum* from an inoculated potato tuber tissue on soil treated with S-H mixture (2%, w/w), urea (0.16%, w/w), or urea (0.16%, w/w) plus siliceous slag (1.21%, w/w) for 3 days. The inoculated potato tuber tissue was put on the surface of the soil at different days after treatment.

that the reduction of mycelial growth of the pathogen occurred right after incorporating S-H mixture or its component urea into the soil. The reduction was greatest between 5 to 23 days. The gained suppressive effect of soil amended with S-H mixture or urea was lost after 20 or 25 days of incubation, respectively. The treatment of urea plus siliceous slag showed a similar trend of inhibition, and its effect lasted for at least 28 days in the amended soil, the longest incubation period conducted in this study. The treatment of siliceous slag alone had no inhibition effect at all. Possibly, the soil amended with urea plus siliceous slag may produce more inhibitors than those produced by using S-H mixture or urea alone. Urea can generate ammonia easily in natural soil and it had been reported responsible for inhibiting germination of propagules of several fungi[2,7,8,10,17,22]. Subsequent work has shown that toxic substance, principally ammonia, produced after amending S-H mixture or urea in soil, and ammonia with other inhibitors may be involved in the soil amended with urea plus siliceous slag. This work will be published in a separate communication.

Disease control in field

The daily average temperature recorded in the field by Taiwan Agricultural

Table 6. Effect of soil amendments on the survival of *Pythium aphanidermatum*, damping off, and yield of cucumber in a field trial[1]

Treatment	Oospores/g soil (% baits coln.)[2]					Damping off[3] (%)	Yield[4] (g/plant)
	Days after treating						
	0	10	20	30	40		
CK_1	123(98)	97(100)	68(100)	40(100)	40(100)	57 a[5]	33 a
CK_2	105(92)	76(100)	60(100)	35(100)	38(100)	89 a	42 a
Urea	112(92)	22(95)	5(58)	7(40)	3(25)	22 b	129 b
S-H mixture	98(90)	10(92)	10(48)	10(50)	7(50)	13 b	121 b

1) 120 cucumber seeds/row were sown 14 days after treatment on October 1, 1987.
2) See the footnotes of table 4.
3) Data were recorded 2 weeks after seeding and averaged from 6 rows of cucumber plants/treatment.
4) Data are the average of the first 4 times harvesting (from Nov. 10 to Dec. 2, 1987).
5) Data followed by the same letter in each column are not different significantly ($p=0.05$), according to Duncan's multiple range test.

Research Institute fluctuated between 26 and 29°C in September to October, and dropped to 22 to 26°C in November, 1987 (supplied by Dr. C. C. Chien, TARI). The higher temperature in September and October was favorable for *P. aphanidermatum* to cause damping off and root rot of cucumber. *P. aphanidermatum* was successfully introduced into the experimental plots and a density of about 100 germinable oospores/g soil was maintained. The population density of the pathogen in two check plots dropped to 30-40 germinable oospores/g soil within one month, but the AIP remained 100% within 40 days. By contrast, the populations of the pathogen in the plots treated with S-H mixture and with urea decreased quickly to 3 and 7 germinable oospores/g soil, respectively, and the AIP were 25 and 50%, respectively. The stand number, plant growth and yield were also improved significantly by using the S-H mixture or urea as soil amendment (Table 6). The nitrogen fertilizer used on cucumber by the farmers in Taiwan are 250 to 350 Kg/ha by 5 applications per season. The amount of S-H mixture (10,000 Kg/ha) or urea (825 Kg/ha) used in our experiments was a little bit excessive, because it was used in only one application. For commercial use in the field, the optimum rate of S-H mixture for control of *P. aphanidermatum* should be studied.

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S-H 混合物防治胡瓜猝倒及根腐病[1]

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摘 要

林益昇、羅朝村 (1988) **S-H 混合物防治胡瓜猝倒及根腐病** 植保會刊 30：223—234.

在溫室及田間的條件下，使用 0.5～2% (w/w) 的 S-H 混合物爲土壤添加物，能夠大量或完全抑制由 *Pythium aphanidermatum* 引起的胡瓜猝倒及根腐病。在實驗室測試 S-H 混合物各種成分之抑制效果，發現尿素是抑制病原菌卵孢子存活及菌絲生長之主要物質，而矽酸爐渣則有附加作用。單獨使用尿素和使用 S-H 混合物之效果相同，但只能維持 25 天，而將尿素和矽酸爐渣混合使用，其效果則至少可維持 28 天。

（關鍵字：胡瓜、猝倒病菌、土壤添加物、病害防治）

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