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Scientific Notes

Inhibition of *Pythium aphanidermatum* by Volatile Substances from Soil Amended with S-H Mixture

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The S-H mixture has a complex composition and the mechanisms of controlling soilborne diseases seem to vary with different diseases and involve multiple factors (13). The S-H mixture inhibited chlamydospore germination and caused germ tube lysis of *Fusarium oxysporum* Schl. f. sp. *niveum* (E. F. Sm.) Snyd. & Hans., and *F. oxysporum* Schl. f. sp. *raphani* Kendrick & Snyd. mainly by siliceous slag and enhances microbial activities in the amended soil (21). The suppression of *Plasmodiophora brassicae* Wor. is due to high soil pH and calcium content in the treated soil (7). Urea and siliceous slag were the main components of the mixture responsible for the suppression of bacterial wilt caused by *Pseudomonas solanacearum* E. F. Sm. (8). Lin and Lo (11) reported that amendment of soil with S-H mixture at the rate of 0.5 to 2.0% (w/w) effectively reduced the incidence of damping-off of cucumber caused by *Pythium aphanidermatum* (Edson) Fitzp. under greenhouse and field conditions. They also observed that amendment of soil with urea alone was as effective as S-H mixture in inhibiting the mycelial growth and oospore germination of the pathogen. The objective of this study is to elucidate the mechanism involved in the control of cucumber damping-off by S-H mixture or its ingredients.

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 (20) was made in laboratory and used fresh in this study at a rate of 2% (w/w) as soil amendment.

The isolate of *P. aphanidermatum* (Pa-1) used in this study was originally isolated from a diseased cucumber plant and maintained on corn meal agar (Difco Co.) (11). Oospores of *P. aphanidermatum* were obtained from 4-week-old cultures grown on V8 juice-cholesterol broth (clarified V8 juice 200 ml, calcium carbonate 2.5 g, cholesterol 30 mg, and distilled water 800 ml). Washed mycelial mats were comminuted in a blender for 10 sec and added to a loam soil (pH 5.7) collected from a cucumber field near Wu-feng, Taichung at the rate of about 2×10^4 oospores/g of soil. The infested soil, then, was dried in a greenhouse for 2 months or longer to break the constitutive dormancy of oospores (12), and to attain more than 80% germination when put on the selective medium (1).

Two grams of soil infested with *P. aphanidermatum* were moistened and placed on a glass which was kept on a stand on the surface of the moistened soil plate (9-cm diameter). Each soil plate contained 50 grams of the soil mixed with the S-H mixture (2%, w/w), urea (0.16%, w/w), siliceous slag (1.21%, w/w), or urea (0.16%, w/w) plus siliceous slag (1.21%, w/w), and moistened to near saturation. The petri dish was covered with lid and sealed with scotch tape. The *P. aphanidermatum* infested soil on the glass slide did not touch the amended soil, but it could receive the volatile gases from the amended soil in the petri dish. The soil plate was open for 10 min to get rid off the volatile gases, and the glass slide with infested soil was replaced with a new one in the soil plate at intervals of 5 days. Then, pieces of fresh potato tuber slice were placed on the surface of half of the removed soil samples for baiting *P. aphanidermatum* (14,19), and the other half samples were diluted and plated on the selective medium in petri dishes (1). The dishes were kept at 36 C for 20 hr and the germination of oospores were examined using a compound microscope. Besides, three 500-ml flasks were placed in row and connected with rubber tubes as a treatment set. The first flask had 100 g of soil amended with S-H mixture or its various combinations of the components; the second flask had 100 ml of 4% boric acid; and the last flask had 2 g of moistened infested soil on the surface of glass slide. The volatile gases evolved from the

amended soil in the first flask would be trapped when passing through the second flask of boric acid trap over the *P. aphanidermatum*-infested soil in the last flask. The sealed flasks were incubated at room temperature (28 ± 2 C) for 6 days, and the spore viability was determined on selective medium (1).

The results showed that the reduction of oospore germination occurred at first 5 days after incorporation of S-H mixture, urea, or urea plus siliceous slag into soil, and was lost gradually after 15, 15, and 35 days of incubation, respectively. The treatments of siliceous slag alone and control had no such inhibition effect (Fig. 1). Moreover, the volatile substances arising from amended soil lost the inhibition effect to the potato baits colonization and oospore germination immediately, when they passed through borric acid trap (Table 1.).

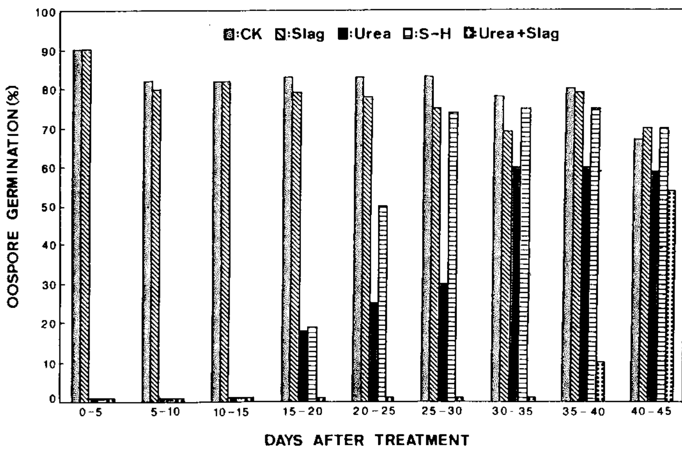

Fig. 1. Influence of volatile gases on the oospore germination of *Pythium aphanidermatum*. The pathogen infested soil was put above the surface of soil amended with S-H mixture (2%, w/w), siliceous slag (1.21%, w/w), urea (0.16%, w/w), or urea (0.16%, w/w) plus siliceous slag (1.21%, w/w), and replaced at 5 day's intervals.

TABLE 1. Effect of passing volatile gases from amended soil through boric acid trap over oospores of *Pythium aphanidermatum* on their subsequent germination

Soil amendment	% Oospore germination/% baits colonization[1]	
	With trap	Without trap
Control	82/100	82/100
Slag	77/100	79/100
Urea	80/100	0/0
S-H mixture	76/100	0/0
Urea+slag	75/100	0/0

1. The oospore germination and baits colonization were determined on the selective medium (1) and by potato baiting technique (19) 6 days after treatment, respecitvely.

The soil plates with soil and amendments were prepared as described in the foregoing item. The presence of ammonia vapors was detected by using 3 ml of 4% boric acid as trapping solution in glass vials placed on the prepared soil plates. The trapping solution was removed from the soil plate and added with 0.2 ml of an indicator prepared by dissolving 0.33 g bromoceresol green and 0.165 g methyl red in 500-ml ethanol (23). Then, the ammonia vapors absorbed in boric acid were titrated with 0.01 N sulfuric acid (2, 9, 16). The empty vial was replanished with fresh boric acid solution and immediately returned to the closed petri dish. The detection of ammonia vapors was monitored periodically at 5 day's intervals. The presence of ammonia vapors was also confirmed by a gas chromatography with a Tracor Model gas chromatography fitted with a detector specific for nitrogen and micro-diffusion titration method (2, 9). Non-amended soil was used as controls. All experiments were repeated once with 5 replicates for each treatment.

The results showed that ammonia vapors were consistently detected from amended soils by micro-diffusion titration method and gas chromatographic analysis of the trapping solutions. The soil amended with S-H mixture, urea, and urea plus siliceous slag produced high amounts of ammonia vapors within 15 days of treatment, and then declined to less than 27 ppm after 15 days of incubation. Only trace amount of ammonia vapors were detected in the treatments of blank check and siliceous slag alone (Fig. 2).

In another experiment, one ml of a 10 M sodium hydroxide was droped into 10 ml of a series of aqueous ammonium chloride solutions (0–0.2 M), and placed in a petri dish (9-cm diam.). The dish was covered and sealed immediately. These resulted in 45, 82, 108, 165, 294, 567, 838, 1411, and 2749 ug NH_3/ml air in the atmosphere of the petri dishes. Control treatments consisted of 10 ml of distilled water or distilled water droped with 1 ml of 10 M sodium hydroxide solution in the petri dishes. Two grams of moistened infested soil were placed in these petri dishes which contained the different concentrations of ammonia vapors, and removed after 6 days of incubation. The removed soil samples were air-dried, diluted in sterilized water, and plated on the selective medium (1). Germination of oospores was examined and recorded using compound microscope (12).

The results showed that the ammonia vapors arising from ammonium chloride plus sodium hydroxide solutions significantly reduced 32.5%, and 89.5% of oospore germination of *P. aphanidermatum* at the concentrations of 108 ppm and 294 ppm, respectively (Table 2).

Ammonia evolved from natural soil amended with urea has been reported responsible for the inhibition of

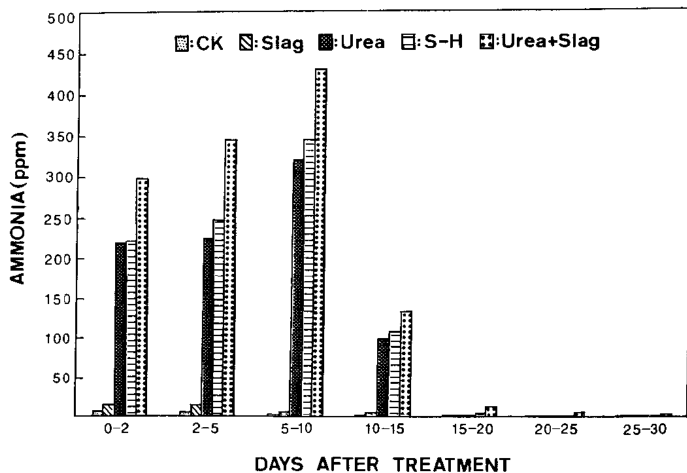

Fig. 2. Ammonia vapors produced from amended soil and detected at 5 day's intervals by a multicolumn gas chromatographic analysis of the trapping solution of boric aicd.

TABLE 2. Effect of ammonia vapors from ammonium chloride on oospore germination of *Pythium aphanidermatum*

NH_4Cl (M)	NH_3 (ppm)	Oospore germination (%)
0.000	0	86 a[1]
0.002	45	87 a
0.004	82	83 a
0.006	108	58 b
0.010	165	58 b
0.020	294	9 c
0.040	567	3 c
0.060	838	0 d
0.100	1411	0 d
0.200	2749	0 d

1. Data followed by the same letter in the column are not different significantly ($p=0.05$) according Duncan's Multiple Range Test.

mycelial growth and propagule germination of several fungi (3,4,5,6,17,18,21,22,23). Liming of acidic soils increased pH and volatile inhibitors were evolved, one of which was ammonia also (13, 18). Our data indicates that the volatile substances arising from the amended soils were responsible for the reduction of oospore germination of *P. aphanidermatum*, and that ammonia vapors were the main inhibitor. The actural quantities of urea used to incorporate the soil was 0.16% (w/w) which were corresponding to the S-H mixture applied as soil amendment at 2%, w/w. The amounts of ammonia vapors evolved from the amended soils maintained more than 100 ppm within 15 days after treatment, and declined rapidly thereafter. Although many fungi were very sensitive to ammonia, but some fungi, such as, *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans. (15) and *P. aphanidermatum* (10) were more resistant. The high amounts of ammonia vapors produced from these amended soils for 15 days was positively correlated with their effective inhibition of the oospore germination. However, besides ammonia, some unknown inhibitors might be produced from the treatment of soil amended with urea plus siliceous slag, because its inhibition effect could last for at least 35 days in the amended soil. The unknown inhibitor could be trapped in boric acid solution, and was volatile substance also (Table 1).

Key words: S-H mixture, urea, siliceous slag, ammonia, *pythium aphanidermatum*, oospore germination.

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摘　要

羅朝村、林益昇. 1992. 土壤添加S-H混合物產生揮發性物質抑制胡瓜腐霉菌. 植病會刊 1:206-209. (1.台中縣霧峰鄉　台灣省農試所植物病理學系; 2.台中市　國立中興大學植物病理學系)

土壤添加S-H混合物、尿素、或尿素加矽酸爐渣之後，釋放揮發性氣體抑制胡瓜腐霉病菌(*Pythium aphanidermatum*)的卵孢子發芽，並阻止其纏據新鮮馬鈴薯塊。惟抑制卵孢子發芽之有效時間則各分別在處理15、15、和35天後逐漸消失。而該揮發性氣體通過硼酸溶液後，立即失去其抑制效應。高量氨氣在處理後15天內均可測得，為主要抑制物質。另外，利用氫氧化鈉加氯化銨產生氨氣達108 μg/ml，即可有效抑制胡瓜腐霉病菌的卵孢子發芽。

關鍵字: S-H混合物、尿素、矽酸爐渣、氨氣、胡瓜腐霉病菌、卵孢子發芽。