Mechanisms of Control of Fusarium Wilt Diseases by Amendment of Soil with S-H Mixture

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摘　　要

孫守恭　黃振文 (1985) **S-H 土壤添加物防治鐮胞菌萎凋病之機制**　(國立中興大學植物病理學系)

本研究中所用之蘿蔔黃葉菌 RS-S-3 菌株及西瓜蔓割病菌 FnC-B 菌株，在添加S-H混合物之土中，均有分生胞子及厚膜胞子發芽率低及發芽管易於瓦解之特性，以此特性可以測知 S-H 添加物之抑病機制。在鹼性營養液中，S-H添加物抑制胞子發芽之效力高。S-H 添加物中之礦灰及蚵殼粉爲分解蘿蔔黃葉病菌發芽管及抑制厚膜胞子發芽之主要成分，而礦灰爲分解西瓜蔓割病菌發芽管之主要成分。在礦灰之五種成分中，氯化鈣及氯化鐵能抑制蘿蔔黃葉病菌之胞子發芽，而氯化鐵可瓦解西瓜蔓割病菌之發芽管，也抑制厚膜胞子之發芽。土中施用 S-H 添加物後，眞菌及放線菌之密度增加，但細菌無增減。S-H 添加物之抑制胞子發芽效果可被添加 Rose Bengal, PCNB 或鏈黴素而全部或部份消失。土中施用 S-H 添加物，可減少西瓜根圈中蔓割病菌 14 倍，也減少西瓜根部被蔓割病菌之纏化達二倍多。在有病菌之土中，S-H 添加物增加西瓜根重三倍，根數也增加六倍。在無病菌之土中，S-H 添加物增加西瓜根重二倍，增加根數 4.7 倍。由以上研究結果得知，S-H 添加物防治鐮胞菌萎凋病之機制有數項因子，包括無機鹽類可直接抑制病原菌，間接的增高土壤 pH 及土中微生物數量以抑制病原菌，也能增加寄主根數及根重以及增強寄主之抗病力。

ABSTRACT

Isolate RS-S-3 of *Fusarium oxysporum* f. sp. *raphani* and isolate FnC-B of *F. oxysporum* f. sp. *niveum* were selected for the study because of their low percentage germination of conidia and chlamydospores and high percentage lysis of germ tubes on soil amended with S-H mixture. S-H mixture was more effective in inhibiting spore germination at high pH than at low pH in nutrient solution. Mineral ash and oyster shell of the component of S-H mixture caused

high percentage of germ tube lysis and strong inhibition of spore germination of *F. oxysporum* f. sp. *raphani* in soil. For *F. oxysporum* f. sp. *niveum*, mineral ash was the only component of S-H mixture capable of inducing germ tube lysis in soil. Among the five major components of mineral ash tested, $CaCl_2$ and $FeCl_3$ were inhibitory of conidial and chlamydospore germination of *F. oxysporum* f. sp. *raphani* and $FeCl_3$ was stimulatory to germ tube lysis and inhibitory to chlamydospore germination of *F. oxysporum* f. sp. *niveum* in soil.

Amendment of soil with S-H mixture increased the population of fungi and actinomycetes but not bacteria. Germination inhibition in soil amended with S-H mixture was nullified completely or partially by addition of rose bengal, streptomycin or PCNB. Amendment of soil with S-H mixture decreased the rhizosphere population of *F. oxysporum* f. sp. *niveum* more than 14 times and reduced colonization of watermelon roots by the pathogen more than 2 times. In the presence of the pathogen, S-H mixture increased root weight about 3 times and root number 6 times, whereas in the absence of the pathogen, it increased root weight about 2 times and root number 4.7 times.

Results of the study suggest that control of Fusarium wilt diseases by amendment of soil with S-H mixture is the result of a multitude of factors including direct pathogen suppression by its inorganic components, indirect pathogen suppression by raising the soil pH and by increasing the microbial population with its organic and inorganic components and increases in root number of the host and host resistant to infection by the pathogen.

INTRODUCTION

Although the possibility of using soil amendments to reduce soilborne diseases has been studied by many researchers from different countries, control of avocado root root rot caused by *Phytophthora cinnamomi* by annual incorporation of organic matter into the orchards in Australia appears to be the only case of successful application of this method in field scale[2]. Soil amendment has been a routine practice of farmers in the mainland China for hundreds or thousands of years mainly as a source of fertilizers. Recently it has been suggested that such practice might be the reason for the general absence of important root diseases on agricultural crops in China[2]. Soil amendment has been considered by many plant pathologists to be a promising method for controlling soilborne diseases in the future.

A soil amendment called S-H mixture consisting mainly of agricultural and industrial wastes has been developed recently for successful control of radish yellows[13] and watermelon wilt[5,16] in both greenhouse and field trials. We report here the possible mechanisms by which the S-H mixture suppresses the pathogens and the diseases caused by the pathogens in soil.

MATERIALS AND METHODS

Soil and S-H mixture

Loamy soil of a paddy field in Dali, Taichung was collected from depths

ranging from 0 to 15 cm after the surface litter was cleared. The soil was sieved through a 128-mesh screen, adjusted to about -5 bars moisture, and stored in closed plastic bags. It had the pH value of 5.3 and the composition of 35.2% sand, 38.8% silt, 26% clay and 1.4% organic matter. The S-H mixture (pH 8.0) consisted of 4.4% bagasse, 8.4% rice husks, 4.25% oyster shell powder, 8.25% urea, 1.01% KNO_3, 13.16% calcium superphosphate, and 60.5 % mineral ash[16]. Chemical composition of the mineral ash are 31% SiO_2, 44% CaO, 1.7% MgO, 18% Al_2O_3 and 1% FeO.

Selection of test organisms

To select isolates for bioassay, macroconidia of *Fusarium oxysporum* (Schl.) f. sp. *raphani* Kendrick & Snyder (isolate RS-0) and *Fusarium oxysporum* (Schl.) f. sp. *niveum* (E. F. Smith) Snyder & Hansen (isolate FnC-0) were obtained by growing the fungi on potato dextrose agar (PDA) under light (1900 Lux, 12 hr per day) at 24 C for 1 month, and placed on the surface of soil amended with 1% S-H mixture. After incubation for 16 hr at 24 C, conidia were scraped off the soil surface and suspended in 5 ml of distilled water in a petri dish. Non-germinated conidia were transferred to water agar and further incubated. After 16 hr, germinated conidia were each transferred to a PDA slant.

Germination and lysis of fungi

Macroconidia and chlamydospores of isolates of *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp. *niveum* were obtained by growing the fungi on PDA under light at 24 C for 15 and 65 days, respectively. Spores were suspended in 0.5 % glucose and 0.5 % asparagine solution for 1 hr before being placed on the surface of soil blocks as described by Ko and Ho[8]. After incubation, spores were stained with rose bengal [1% rose bengal, 5% phenol and 0.01% $CaCl_2$ in distilled water], scraped off the soil surface with a scalpel and destained on a glass slide with a drop of solution containing 5 N NaOH and 0.5 N NaCl[7]. Germination was determined after 16 hr incubation. For measuring length and estimating lysis of germ tubes, 20 hr incubation period was used.

Chlamydospore germination in liquid was tested in 100 ml of 0.5% glucose plus 0.5 % asparagine solution mixed with 1% S-H mixture in a 250 ml flask. Nutrient solution without S-H mixture was used as a control. The spore concentration used was about 10^5/ml. After incubation at 24 C for 12 hr, about 0.1 ml of the suspension from each flask was placed on a glass slide. Spores were stained with cotton blue and observed under microscope. At least 100 spores were examined for each treatment.

Determination of microbial population

Soil suspensions were prepared by grinding 10 g of soil with 90 ml of sterile distilled water. Serial dilutions (10^{-2}, 10^{-3} and 10^{-4} for fungi; 10^{-4}, 10^{-5} and 10^{-6} for actinomycetes; and 10^{-5}, 10^{-6} and 10^{-7} for bacteria) were plated on the following selective media: PDA plus 1000 ppm streptomycin for fungi, Chitin agar[11] for actinomycetes, tryptone agar[5] for bacteria, Nash PCNB agar[15] for *Fusarium* spp., and rose bengal-PNCB agar[12,14] for *Trichoderma* spp.

Pathogenicity test

About 2000 g of soil infested with 1.2 $\times 10^3$ propagules/g. soil of *F. oxysporum*

f. sp. *raphani* in a 20-cm pot was planted with radish seeds (cultivar Meiwa). Three replicates were used and the disease incidence was recorded after 30 days. The same procedure was used to test the pathogenicity of *F. oxysporum* f. sp. *niveum* on watermelon (cultivar Hong Niang).

Host response

About 2000 g of artificially infested soil containing 1.2×10^3 propagules of *F. oxysporum* f. sp. *niveum* per g of soil was mixed with 1% (W/W) S-H mixture, placed in a 20-cm pot and planted with 2 watermelon seeds in the greenhouse. Non-amended infested soil was used as a control. After 30 days, population density of the pathogen in the rhizosphere soil and root colonization by the pathogen were determined with the Nash PCNB medium[15] using the method described by Banihashemi and deZeeuw[1]. Five replicates were used for each treatment.

Analysis of data

Unless otherwise stated three replicates were used for each treatment and all experiments were repeated at least once. Student's t-test was used when comparisons were made between two treatments and Duncan's multiple range range test was used when more than two treatments were compared.

RESULTS

Selection of test organisms

Among the four isolates of *F. oxysporum* f. sp. *raphani* tested, isolate RS-S-3 was the best organism for assaying the inhibitory effect of soil amended with S-H mixture (Table 1).

Table 1. Comparison of spore germination and pathogenicity among isolates of *Fusarium oxysporum* f. sp. *raphani* on soils with and without amendment with 1% S-H mixture

Isolates	Conidial germination (%)		Chlamydospore germination (%)		Radish yellows (%)
	Amended soil	Non-amended soil	Amended soil	Non-amended soil	
RS-0	77 A[1]	92 A	58 C	74 C	75 a[2]
RS-S-B	48 B	91 A	41 C	59 C	45 b
RS-S-2	49 B	81 A	40 D	67 C	39 b
RS-S-3	36 B	82 A	43 D	74 C	82 a

1) Data followed by the same letter for each spore type of the same isolate are not significantly different at P=0.05 based on student's t-test.
2) Data followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

The isolate gave high percentage germination of conidia and chlamydospores on non-amended soil and low percentage germination on soil amended with S-H mixture. It was also strongly pathogenic to the host. Isolate FnC-B was the best among the four isolates of *F. oxysporum* f. sp. *niveum* for assaying the same effect (Table 2). Its germ tube showed only slight lysis on non-amended soil and

Table 2. Comparison of germ tube lysis, chlamydospore germination and pathogenicity among isolates of *Fusarium oxysporum* f. sp. *niveum* on soils with and without amendment with 1% S-H mixture

Isolates	Germ tube lysis (%)		Chlamydospore germination (%)		Watermelon wilt (%)
	Amended soil	Non-amended soil	Amended soil	Non-amended soil	
FnC-0	15 A[1]	2 A	48 D	81 C	89 a[2]
FnC-S-1	5 A	4 A	50 C	65 C	72 b
FnC-S-2	9 A	3 A	20 C	31 C	66 b
FnC-B	31 B	6 A	65 D	93 C	94 a

1) Data followed by the same letter for germ tube lysis or chlamydospore germination of each isolate are not significantly different at P=0.05 based on student's t-test.

2) Data followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 3. Chlamydospore germination of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) and *F. oxysporum* f. sp. *niveum* (FnC-B) in nutrient solutions with or without 1% S-H mixture

Treatment	pH value	Germination (%)	
		F. oxysporum f. sp. *raphani*	*F. oxysporum* f. sp. *niveum*
S-H mixture[1]	5.8	31 B[3]	24 B
	8.0	12 C	4 C
None[2]	5.8	81 A	74 A
	8.0	69 A	68 A

1) Original pH was 8.0.

2) Original pH was 5.8.

3) Data followed by the same letter in each column are not significantly different at P=0.05 according to Duncan's multiple range test.

extensive lysis on soil amended with S-H mixture. Chlamydospore germination of isolate FnC-B germinated completely on non-amended soil but only partially on amended soil. The fungus was also strongly pathogenic to its host. Isolate RS-S-3 of *F. oxysporum* f. sp. *raphani* and isolate FnC-B of *F. oxysporum* f. sp. *niveum* were, therefore, selected for used in this study.

Effect of S-H mixture on spore germination in nutrient solution

Chlamydospore germination of *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp. *niveum* were strongly suppressed in nutrient solution containing 1% S-H mixture (Table 3). S-H mixture was more effective in inhibiting spore germination at high pH than at low pH.

Effect soil amendment with S-H mixture and its components on the pathogens

Germination of *F. oxysporum* f. sp. *raphani* conidia and chlamydospores on soil was significantly decreased by amendment with S-H mixture, mineral ash, oyster shell, rice husks and urea, but was not affected by amendment with KNO_3, calcium superphosphate and bagasse (Fig. 1). S-H mixture was more effective in

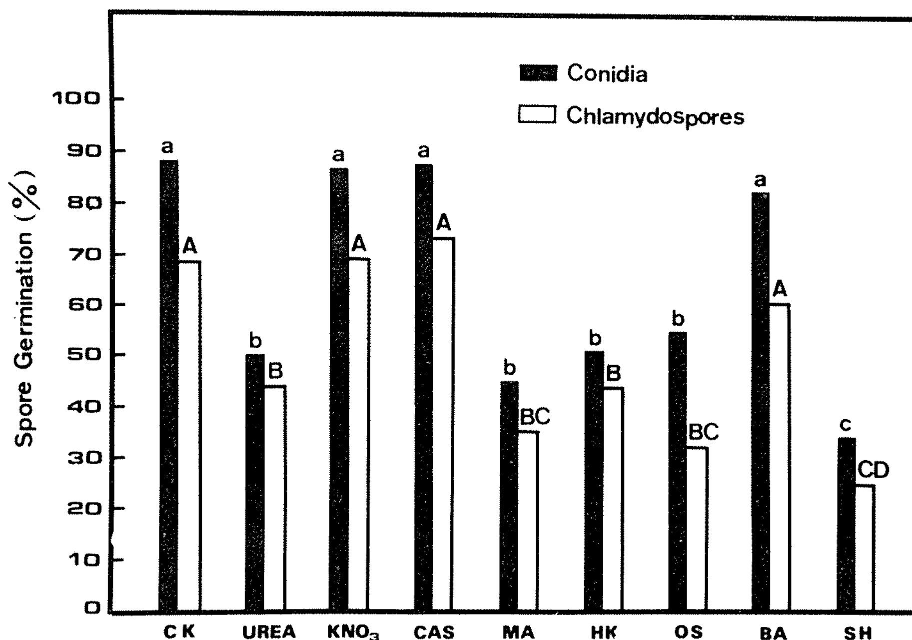

Fig. 1. Effect of amendments on spore germination of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) on the soils. SH: S-H mixture, CAS: Calcium superphosphate, MA: Mineral ash, HK: Rice husks, OS: Oyster shell, BA: Bagasse. Data with the same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

inhibiting spore germination than its individual component. Germ tube growth of the fungus on soil was strongly inhibited by amendment with S-H mixture, mineral ash, oyster shell, and urea and slightly inhibited by amendment with KNO_3, bagasse and rice husk, but was not affected by amendment with calcium superphosphate (Table 4). S-H mixture, mineral ash and oyster shell strongly enhanced germ tube lysis on soil. Amendment of soil with calcium superphosphate and bagasse also slightly increase lysis of germ tubes. Urea, KNO_3 and rice husk were not effective in inducing germ tube lysis. S-H mixture was more effective than any of its components in inducing germ tube lysis. Lysis of conidia and germ tubes of *F. oxysporum* f. sp. *niveum* on soil was enhanced by amendment with S-H mixture and mineral ash, but not with bagasse, rice husks, oyster shell, calcium superphosphate, KNO_3 and urea (Fig. 2).

Among the components of mineral ash only $CaCl_2$ and $FeCl_3$ significantly reduced germination of conidia and chlamydospores of *F. oxysproum* f. sp. *raphani* on soil (Table 5). $FeCl_3$ also enhanced lysis of germ tubes and

Table 4. Growth and lysis of germ tubes from chlamydospore of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) on soils amended with 1% of individual component of the S-H mixture

Treatment	Germ tube length (μm)	Germ tube lysis (%)
S-H mixture	56.2 A[1]	52.0 A
Mineral ash	50.4 A	34.2 B
Oyster shell	65.0 A	26.4 B
Urea	63.4 A	5.6 D
KNO_3	98.0 C	4.2 D
Calcium-superphosphate	105.0 CD	14.0 C
Bagasse	87.2 BC	15.7 C
Rice husk	92.0 C	12.4 CD
Check (none)	124.1 D	6.2 D

1) Data followed by the same letter in each column are not different at P=0.05 according to Duncan's multiple range test.

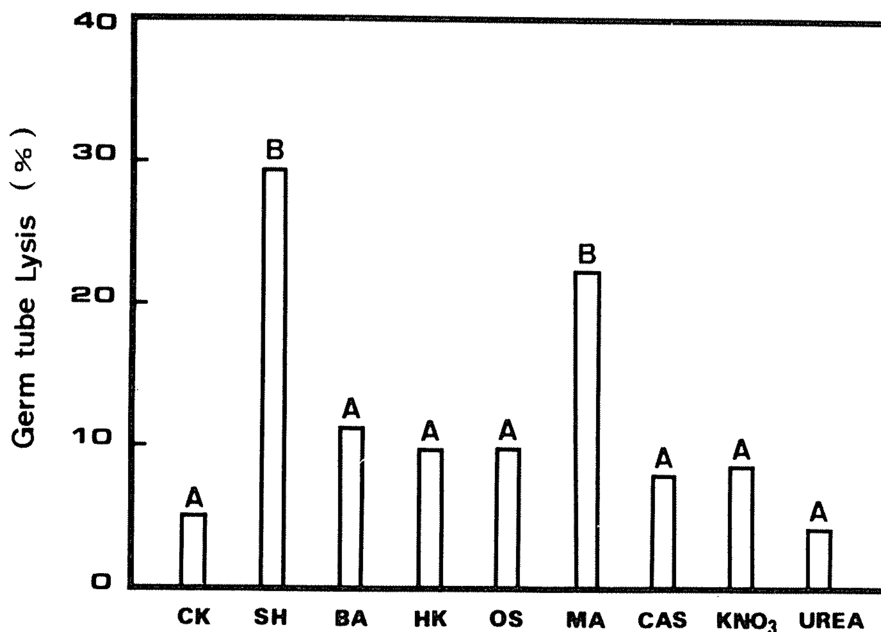

Fig. 2. Effect of amendments on germ tube lysis of *Fusarium oxysporum* f. sp. *niveum* (FnC-B) on the soils. SH: S-H mixture, CAS: Calcium superphosphate, MA: Mineral ash, HK: Rice husks, OS: Oyster shell, BA: Bagasse. Data with the same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

Table 5. Spore germination and germ tube lysis of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) and *F. oxysporum* f. sp. *niveum* (FnC-B) on soils amended with individual component of mineral ash

Treatment	Concentration (μg/g)	Soil pH	*F. oxysporum* f. sp. *raphani* Conidial germination (%)	*F. oxysporum* f. sp. *raphani* Chlamydospore germination (%)	*F. oxysporum* f. sp. *niveum* Germ tube lysis (%)	*F. oxysporum* f. sp. *niveum* Chlamydospore germination (%)
$AlCl_3$	150	4.9	83 A[1)]	79 A	5 A	87 A
$MgCl_2 \cdot 6H_2O$	200	5.3	92 A	87 A	8 A	84 A
H_2SiO_3	2000	5.4	81 A	84 A	2 A	91 A
$CaCl_2$	1000	5.3	54 B	57 B	9 A	75 A
$FeCl_3 \cdot 6H_2O$	100	5.0	58 B	61 B	18 B	56 B
Check(none)		5.3	79 A	77 A	4 A	85 A

1) Data followed by the same letter in each column are not different significantly (P=0.05) according to Duncan's multiple range test.

Table 6. Comparison of microflora in soils with and without amendment with 1% S-H mixture

Microflora	10^3 propagules/g. soil	
	Amended soil[1)]	Non-amended soil
Fungi	17.0 A[2)]	0.67 B[2)]
Actinomycetes	1010.0 A	480.0 B
Bacteria	34200.0 A	36500.0 A
Fusarium spp.	2.0 A	1.95 A
Trichoderma spp.	7.3 A	1.48 B

1) Microbial population was determined 30 days after amendment.
2) Data followed by the same letter for each group of microorganisms are not significantly different at P=0.05 based on student's t-test.

decreased germination of chlamydospores of *F. oxysporum* f. sp. *niveum* on soil.

Effect of S-H mixture on microbial population in soil

Thirty days after amendment of soil with 1% S-H mixture, the population of fungi increased about 25 times and that of actinomycetes increased about 2 times (Table 6). The population of bacteria in soil was not significantly affected by S-H mixture. Amendment of soil with S-H mixture increased the population of *Trichoderma* spp. about 5 times but did not affect the population of *Fusarium* spp.

Effect of microbial inhibitors on pathogen suppression by soil amended with S-H mixture

Inhibition of chlamydospore germination of *F. oxysporum* f. sp. *raphani* on soil amended with 1% S-H mixture was completely nullified by rose bengal and partialy nullified by streptomycin and

Table 7. Chlamydospore germination of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) and *F. oxysporum* f. sp. *niveum* (FnC-B) on the S-H mixture-amended soil and non-amended soil treated with microbial inhibitors

Microbial inhibitor	Concentration (μg/g)	Chlamydospore germination (%)			
		F. oxysporum f. sp. *raphani*		*F. oxysporum* f. sp. *niveum*	
		Amended soil	Non-amended soil	Amended soil	Non-amended soil
Rose bengal	100	78.6 A[1)]	75.6 A	69.0 B	83.2 A
Streptomycin	3000	65.6 B	82.0 A	85.0 A	83.0 A
PCNB	1000	68.6 B	77.9 A	57.0 C	87.6 A
Control		46.7 C	82.5 A	50.4 C	79.3 A

1) Data followed by the same letter in each column are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 8. Effect of S-H mixture on rhizosphere population of pathogen, root colonization by pathogen and root growth of watermelon in soil infested with *Fusarium oxysporum* f. sp. *niveum* (FnC-B)

Treatment	Infested with pathogen				Without pathogen	
	Rhizosphere population of pathogen (propagules/g. soil)	Root colonization by pathogen (%)	Dry wt. of root (mg/plant)	No. of roots/plant	Dry wt. of root (mg/plant)	No. of roots /plant
S-H mixture	218	36.2	349	16.0	593	21.0
None	3130	81.3	107	2.4	294	4.5

PCNB (Table 7). Germination of *F. oxysporum* f. sp. *niveum* on S-H mixture-amended soil was as high as non-amended soil when streptomycin was added. Rose bengal was slightly effective but PCNB was not effective in increasing germination of *F. oxysporum* f. sp. *niveum* on S-H mixture-amended soil.

Effect of S-H mixture on pathogen activity and growth of host in soil

Amendment of soil with S-H mixture decreased the rhizosphere population of *F. oxysporum* f. sp. *niveum* more than 14 times and reduced colonization of watermelon roots by the pathogen more than 2 times (Table 8). In the pathogen infested soil S-H mixture increased root weight and root number about 3 and 6 times, respectively. In the absence of the pathogen, S-H mixture increased root weight and root number about 2 and 4.7 times, respectively.

DISCUSSION

Amendment of soil with S-H mixture inhibited spore germination and induced germ tube lysis of both *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp.

niveum. Since S-H mixture in nutrient solution was inhibitory to spore germination of both fungi under sterile conditions, nonbiological factor apparently plays an important role in the pathogen suppression of S-H mixture-amended soil. Results show that mineral ash and oyster shell were the most important components of S-H mixture responsible for causing lysis of germ tubes and inhibition of spore germination of *F. oxysporum* f. sp. *raphani* in soil, although rice husks and urea may also contribute to the pathogen suppression to a limited extent. For *F. oxysporum* f. sp. *niveum*, mineral ash was the only component of S-H mixture capable of inducing germ tube lysis in soil.

Of the five major components of mineral ash, only $CaCl_2$ and $FeCl_3$ were inhibitory to conidial and chlamydospore germination of *F. oxysporum* f. sp. *raphani*, and only $FeCl_3$ was stimulatory to germ tube lysis and inhibitory to chlamydospore germination of *F. oxysporum* f. sp. *niveum* in soil. Oyster shell is rich in chitin and calcium. Its inhibitory effect in soil may be due to direct inhibition by calcium and indirect inhibition by chitin through stimulation of actinomycetes in soil(4). Calcium has been shown to increase resistance of tomato to infection by *Fusarium oxysporum* f. sp. *lycopersici*(3).

Being alkaline in nature the S-H mixture tends to increase soil pH(16). Since high pH soils disfavor the development of radish yellows caused by *F. oxysporum* f. sp. *raphani* and watermelon wilt caused by *F. oxysporum* f. sp. *niveum* (5,13), pH change may also contribute to the pathogen suppression in S-H mixture-amended soil although the extent of its contribution is still unknown.

Amendment of soil with S-H mixture also increased the populations of fungi and actinomycetes. It is considered possible that enhanced microbial activity may contribute to inhibition of sporangial germination(9) and lysis of germ tubes(10) in the S-H mixture-amended soil. The possibility is supported by the observation that addition of rose bengal, streptomycin or PCNB to soil amended with S-H mixture increased spore germination. Rose bengal and streptomycin are inhibitory to bacteria and actinomycetes but not to fungi(6), whereas PCNB is inhibitory to actinomycetes and certain fungi but not bacteria(4). This also suggests that the microorganisms involved are not specific.

S-H mixture also has profound positive effect on the growth of the host. Watermelon growing on S-H mixture-amended soil produced about 4.7 times more roots than that growing in non-amended soil. This may account at least in part for the lower rhizosphere population of the pathogen and lower percentage of root colonization by the pathogen in S-H mixture-amended soil than that in non-amended soil.

Results of this study suggest that a multitude of factors are contributing to the control of Fusarium wilt diseases by amendment of soil with S-H mixture. S-H mixture may suppress the pathogen directly with its inorganic components and indirectly by raising the soil pH and by increasing the microbial populations with its organic and inorganic components. The calcium content of S-H mixture may increase the resistance of the host to infection by the pathogen. S-H mixture

may also provide nutrients for vigorous root growth of the host which in turn may also reduce the damage caused by pathogen infection.

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