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Control of Fusarium wilt of asparagus bean by organic soil amendment and microorganisms

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ABSTRACT

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Greenhouse experiments were conducted to evaluate 10 organic materials for control of asparagus bean wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum*. Results showed that amendment of shrimp and crab shell powder (SCSP) at 1% (w/w) in the pathogen-infested soil was the most effective in reducing population density of *F. oxysporum* f. sp. *tracheiphilum*. Treatment of pathogen-infested soil with SCSP at 1% (w/w) and 0.5% (w/w) reduced disease severity by 56% and 33%, respectively. These treatments also promoted seedling growth and formation of nodules of asparagus bean. Analysis of hydrolysis of fluorescein diacetate (FDA) showed that amendment of soil with SCSP at 1% (w/w) resulted in a significant increase of soil microbial activity. Among the seven strains of *Pseudomonas* spp. and 10 strains of *Bacillus* spp. tested in the pathogen-infested soil, *Bacillus* spp. strains PMB-028 and PMB-034 were the most effective strains caused in reducing disease severity by 36% and 47%, respectively. Meanwhile, treatment of PMB- 028 and PMB-034 also increased root fresh weight of asparagus bean by 20% and 46% and increased formation of root nodules by 30% and 31%, respectively. In advance studies, it revealed that combined treatment of SCSP at 0.5% (w/w) and *Bacillus* spp. strains PMB- 028 and PMB-034 was more effective than the treatment of SCSP or bacterial strain alone in reducing disease severity of Fusarium wilt and improving growth and nodule formation of asparagus bean. Further investigation are warranted on formulation and practical application of combine use of shrimp and crab shell powder and strains PMB-034 and PMB-028 of *Bacillus* spp. for control of Fusarium wilt of asparagus bean.

Keyword: *Vigna sesquipedalis*, Asparagus bean, *Bacillus* spp., *Fusarium oxysporum* f. sp. *tracheiphilum*, Fusarium wilt, microbial activity, organic amendment, shrimp and crab shell powder

INTRODUCTION

Fusarium oxysporum Schlechtend:Fr. f. sp. *tracheiphilum* (E. F. Sm.) W. C. Snyder & H. N. Hansen, is one of the most important pathogen limiting production of asparagus bean (*Vigna sesquipedalis* Liana) in Taiwan. Infected plants showed symptoms of marginal chlorosis or necrosis in leaves, discoloration of the stem vascular bundles of plants. Although synthetic fungicides have been used for effective control of Fusarium wilt of asparagus

bean in Taiwan, there are concerns of the harmful effects of these chemicals to environment, human health, and microbial ecology, including pathogen resistance to fungicides [32]. Other control methods such as the use of tolerant cultivar and cultural practices were often unsuccessful. Therefore, it is important to seek alternative methods that are effective and environmentally friendly for management of this disease.

Organic soil amendments have been used by organic growers in Taiwan to improve soil fertility by changing soil physical and chemical properties. Chinn [4] showed that soil with high levels of organic matter prevented spores of *Cochliobolus sativus* from germination. Total microbial populations were also higher in peat soils (40.3% organic matter) than in sandy soils (2.3% organic matter), and the cause of reduction of spore germination was attributed to an undetermined fungistatic principle. Changes in physiological and chemical properties of the soil after amendment were also unfavourable to pathogen. The suppressive effects of organic amendments on the diseases are primarily associated with a reduction in pathogen inoculum density in amended soils [34]. In addition to improving soil fertility, some soil amendments with inorganic and/or organic matter may affect population dynamic of soil microflora [9, 12], and thus, are used as effective methods for management of soilborne pathogens in crop production [6, 15]. For example, soil amendment with S-H mixtures was effective in reduction of apothecia of *Sclerotinia sclerotiorum* (Lib.) de Bary, the primary source of inoculum of Sclerotinia disease, in field of bean (*Phaseolus vulgaris* L.), canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) [10, 11]. Soil amendment with lettuce leaves was effective in the control of root and stem rot disease of cucumber (*Cucumis sativus* L.), caused by *Fusarium oxysporum* f. sp. *radicis-cucummerinum* [24].

A numerous of soil microorganisms have demonstrated activity in the control of various soilborne plant pathogens. Fusarium wilt of chickpea caused by *F. oxysporum* f.sp. *ciceris* has been controlled by antagonistic microorganisms such as *Trichoderma harzianum*, *Bacillus* spp., *Pseudomonas fluorescens* and *Pseudomonas* sp. NBRI9926P3 [19, 23, 26]. Jensen *et al.* [16] reported that seed treated with *B. subtilis* GBO3 reduced bean root rot (*F. solani* f. sp. *phaseoli*). There are sufficient data to reveal the effects of organic amendment and antagonistic microorganisms on suppression of soilborne plant diseases. However, there are few efforts have been made to evaluate the efficacy of organic amendments and microorganisms to control Fusarium wilt of asparagus bean. The objectives of this study were to evaluate the effect of organic materials and microorganisms for control of Fusarium wilt of asparagus bean.

MATERIALS AND METHODS

Soil, culture medium, and organic materials

The soil used in this study was sandy loam collecting from a vegetable field in Taichung, Taiwan. They were sieved through 4-mm-mesh screen, adjusted moisture to 10% (w/w) and stored in a plastic container. A commercial culture medium, BVB No.4 (Bas Van Buuren, Visser, the Netherlands) was also used in this study. The 10 organic materials used in this study were castor bean pomace, oyster shell powder, peanut pomace, rice husk, sea grass powder, sesame pomace, shrimp and crab shell powder, soybean meal, tea seed pomace and tobacco pomace bought from FwuSow Industry Co., Ltd., Taichung, Taiwan.

Inoculum preparation

The *F. oxysporum* f. sp. *tracheiphilum* PMF 05 used in these trials was isolated from a disease plant of asparagus bean grown in central Taiwan. Single spore cultures of this strain PMF 05 were maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) in Petri dishes (9 cm diameter) at 28℃ under 12h-photoperiod. Three agar discs (1cm diameter) containing mycelia mats were removed from 5-day-old single-spore cultures and placed in a 500 ml glass flask containing 300 g of autoclaved celery pieces. After incubation at 28℃ for 7 days, the infested celery pieces were thoroughly mixed with autoclaved soil. The moisture content of the soil was adjusted to 25% (v/w) by adding sterile distilled water. The soil infested with *F. oxysporum* f. sp. *tracheiphilum* was placed in plastic containers (55 cm×45 cm×15 cm, length×width×height) and kept at room temperature ranged from 28-30℃ for 30 days. The infested soils were then air dry for 10 days, placed in a refrigerator (6℃) and used as a stock inoculum for experiments. The air- dried

infested soil (stock inoculum) was ground and passed through a 1.7-mm screen before use. The infested soil for experiments was prepared by thoroughly mixing a stock inoculum with the growth substrate containing non-sterile BVB and soil (1:3 w/w). The inoculum density of *F. oxysporum* f. sp. *tracheiphilum* in the infested soil was adjusted to 10^4 cfu/g soil.

Screening organic materials for suppressive effects on *F. oxysporum* f. sp. *tracheiphilum*

Each of the 10 organic materials was incorporated into the soil infested with *F. oxysporum* f. sp. *tracheiphilum* at a rate of 1% (w/w). The pathogen-infested soil without organic amendment was used as a control. Amended or non-amended soils were placed in plastic pots (11cm in diameter and 8.5 cm in height), 500 g soil/pot and 4 pots/treatment. The moisture content of the soil was adjusted to 15% (v/w) by adding sterile distilled water. All pots were kept in a growth chamber at 28℃ under 12-h photoperiod for 7 days and then transferred to a greenhouse which temperature ranged from 28 to 32℃. Asparagus bean seedlings, Pai-pi cultivar, grown in BVB No. 4 for 7 days were transplanted into amended or non-amended soils, 3 seedlings/pot. The survival of the pathogen and development of microbial populations were estimated at 0, and 30 days after amendment. To estimate microbial populations, a 10 g (dry weight basic) soil sample, comprising three subsamples taken from each pot, was serially diluted in 100 ml of distilled water and mixed for 5 min with a Vortex stirrer. An aliquot (1 ml) of dilutions were plated on Nash- pentachloronitrobenjene agar (PCNB) for the pathogen, peptone-dextrose-rose bengal agar (PDRA) [20] for fungi, chitin agar (CA) [26] for actinomycetes and nutrient agar (NA, Difco) for bacteria. The cultural plates were incubated at 28℃ for 3-7 days to determine fungal and bacterial colonies developed on the media and for 10-14 days to determine population of actinomycetes. Representative colonies from each treatment were isolated, purified and kept at 2-8℃ for further study.

Effect of concentrations of shrimp and crab shell powder on suppression of Fusarium wilt of asparagus bean

The soil artificially infested with *F. oxysporum* f. sp. *tracheiphilum* PMF 05 (10^4 cfu/g soil) was amended with a commercial product containing shrimp and crab shell powder at rates of 0.1%, 0.25%, 0,5%, and 1.0% (w/w) and incubated at 28℃ under 12-h photoperiod. The pathogen-infested soil without shrimp and crab shell powder was used as a control. The amended or non-amended soils were placed in plastic pots (11cm in diameter and 8.5 cm in height), 500 g of soil/pot and 8 pots/treatment. The moisture content in each pot was adjusted to 15% (v/w) by adding sterile distilled water. After 7 days, asparagus bean healthy seedlings grown in BVB No.4 for 7 days were transplanted into pots, 3 seedlings per pot, and kept in the greenhouse. The plants were daily watered and applied 0.05% (w/v) urea 20 days after transplanting. Individual plants were rated for severity of Fusarium wilt at 20 and 40 days after transplanting, using a scale of 0 to 3, where 0 = healthy plants, no visible symptoms; 1 = weakly infected plant sowing vascular discoloration but no leaf yellowing; 2 = moderately infected plant sowing leaf yellowing and wilt of plants; 3 = severely infected plant sowing plant death. The disease severity for each replicate of each treatment was calculated by the following formula: $(\Sigma\ S_i \times N_i) \times 100 / (3 \times N_t)$, where S_i is the severity ratings 0 to 3, N_i is the number of plants in each ratting, and N_t is the total number of rated plants.

Plant height of asparagus bean seedlings was measured 40 days after transplanting. Populations of pathogen and rhizosphere microflora in 4 pots of each treatment were monitored at 0, 15, and 30 days after amendment by the method described above. Four of eight pots in this experiment were used for assessing disease severity, plant growth, and other four pots were used for analyzing microbial flora. The experiment was repeated twice.

Determination of fluorescein diacetate hydrolysis

The fluorescein diacetate (3', 6' - diacetyl fluorescein, FDA ; Sigma Chemical Co., St. Louis, Mo, U.S.A) was dissolved in acetone (analysis grade; E. Merck AG, Darmstadt, Germany) and stored as a stock solution (2 mg/ml) at -20℃ [34]. The non sterile field soil infested with *F. oxysporum* f. sp. *tracheiphilum* PMF 05 (10^4 cfu/g soil) was amended with the commercial product of shrimp and

crab shell powder at rates of 0.1%, 0.25%, 0,5%, and 1.0% (w/w) and incubated at 28℃ under 12-h photoperiod. The non sterile infested soil without shrimp and crab shell powder was used as control. There were three replicate pots for each treatment. The moisture content in each pot was adjusted to 15% (v/w) by adding sterile distilled water. To determine FDA hydrolytic activity, 2 g dry soil sample, comprising three sub-samples, were taken from each pot and mixed with 0.5 ml of FDA (final concentration, 10 μg/ml) and 100 ml of sterile 60 mM sodium phosphate buffer, pH 7.6 (0.2 M NaH_2PO_4 13 ml; 0.2 M Na_2HPO_4 87 ml, deionized water 100 ml). The mixture was incubated at 25℃ on rotary shaker (WISDOM 502 R, Apparatus MFG Co) at 100 rpm for 2 hours. The buffering capacity was sufficient to keep the pH at 7.6 for the duration of experiments. The soil mixture was centrifuged for 5 min at 5000 rpm (SIGMA 3K15, Nr. 11133, Germany), and the supernatant was filtered through a 3 μm membrane filter (Millipore Corp., Billerica, MA, U.S.A.). The intensity of hydrolyzed FDA was measured as absorbance at 490 nm on a spectrophotometer (JASCO V-530, Japan). The determinations of FDA hydrolysis in samples amended with shrimp and crab shell powder or non-amended controls were conducted at 0 day and 3-day interval for 18 days.

Screening trials of promising antagonistic bacteria

Seventeen bacterial strains isolated from rhizosphere soil of asparagus bean plants grown in Taichung were tested for control of Fusarium wilt of asparagus bean. Bacterial suspensions of each strain (10^8 cfu/ml) were prepared from cultures grown on NA at 30℃ for 2 days. Plug tray (72 cells per tray) were filled with the BVB substrate which was infested with bacterial suspensions of individual strains at approximately 10^8 cfu/g substrate. Seeds of asparagus bean were sown in the substrate, 1 seed/cell, and the plug trays were watered and kept in greenhouse. Two weeks later, seedlings were transplanted into pot (11 cm diameter) filled with soil infested with the pathogen (10^4 cfu/g soil), 3 seedlings/pots and 4 pots/treatment. The plants were watered daily and applied 0.05% (w/v) urea 20 days after transplanting. Seedlings sown in BVB substrates without bacterial suspension were used as control. After transplanting for 40 days, each plant was examined and scored for severity of Fusarium wilt, number of nodules and fresh weight of root. The experiment was repeated twice.

Control of Fusarium wilt of asparagus bean by combine treatment of shrimp and crab shell powder and *Bacillus* spp.

Seeds of asparagus bean were kept in moist paper towel at 28℃ for 4 days for germination prior to planting. Non-sterilized soil infested with *F. oxysporum* f. sp. *tracheiphilum* PMF 05 (10^4 cfu/g soil) were amended with shrimp and crab shell powder at rates of 0.5% (w/w), with or without *Bacillus* spp. strains PMB-034 and PMB-028 (10^8 cfu/g). For each treatment, germinated seedlings were planted in each treated soil in pots, 3 seedlings/pots and 4 replicate pots/treatment, and the pots were kept in growth cabinet at 28℃ under 12-h photoperiod. The pathogen-infested soil without the amendment of shrimp and crab shell powder and *Bacillus* spp. strains was served as the control. The moisture content in pots was brought to 15% (v/w) by adding sterile distilled water. After 7 days, all the pots were transferred to the greenhouse and plants were watered daily. After transplanting for 40 days, each plant was examined and scored for severity of Fusarium wilt, number of nodules and fresh weight of root. The experiment was repeated twice.

Statistical analysis

Data of disease severity, plant height, microbial population, fresh bean mass and root nodules collected from individual experiment were subjected to analysis of variance (ANOVA) for a complete randomization design. Means of the treatments in each experiment were compared using Duncan's multiple range test at the 5% level. All statistical analyses were conducted with SAS/STAT software (SAS Institute Inc. 1989). The colony-forming units (cfu) of microorganisms were transformed to $\log_{10}$ cfu/g soil.

RESULTS

Effect of organic amendment on survival of *Fusarium oxysporum* f. sp. *tracheiphilum*

Population of *F. oxysporum* f. sp. *tracheiphilum* PMF

05 were significantly ($P \leq 0.05$) reduced by the amendment of soil with organic materials such as castor bean pomace, oyster shell powder, peanut pomace, sea grass powder, sesame pomace, shrimp and crab shell powder, tea seed pomace and tobacco pomace at rate of 1% (w/w), compared to the control (Fig. 1). However, the treatments of 1% rice husk and soybean meal (w/w) were ineffective. Among the 10 organic compounds tested, the amendment of shrimp and crab shell powder was the most effective treatment and the survival rate of the pathogen was 56.8%, compared to 86.7% in the untreated control, representing appropriately 30% reduction of the pathogen in this organic amendment (Fig. 1). No significant reduction in population of the pathogen were observed in the treatment of rice husk and soybean meal, compared to the control.

Effect of different concentrations of shrimp and crab shell powder on suppression of Fusarium wilt of asparagus bean

The amount of shrimp and crab shell powder amended in the non sterile soil infested with *F. oxysporum* f. sp. *tracheiphilum* PMF 05 affected severity of Fusarium wilt and growth of asparagus bean (Table 1). Treatment of shrimp and crab shell powder in the soil at rates of 0.25 to 1.0% (w/w) significantly ($P \leq 0.05$) reduced severity of Fusarium wilt of asparagus bean plants recorded at 20 and 40 days after transplanting. For example, at 40 days after transplanting, the disease severity ratings were 38, 35 and

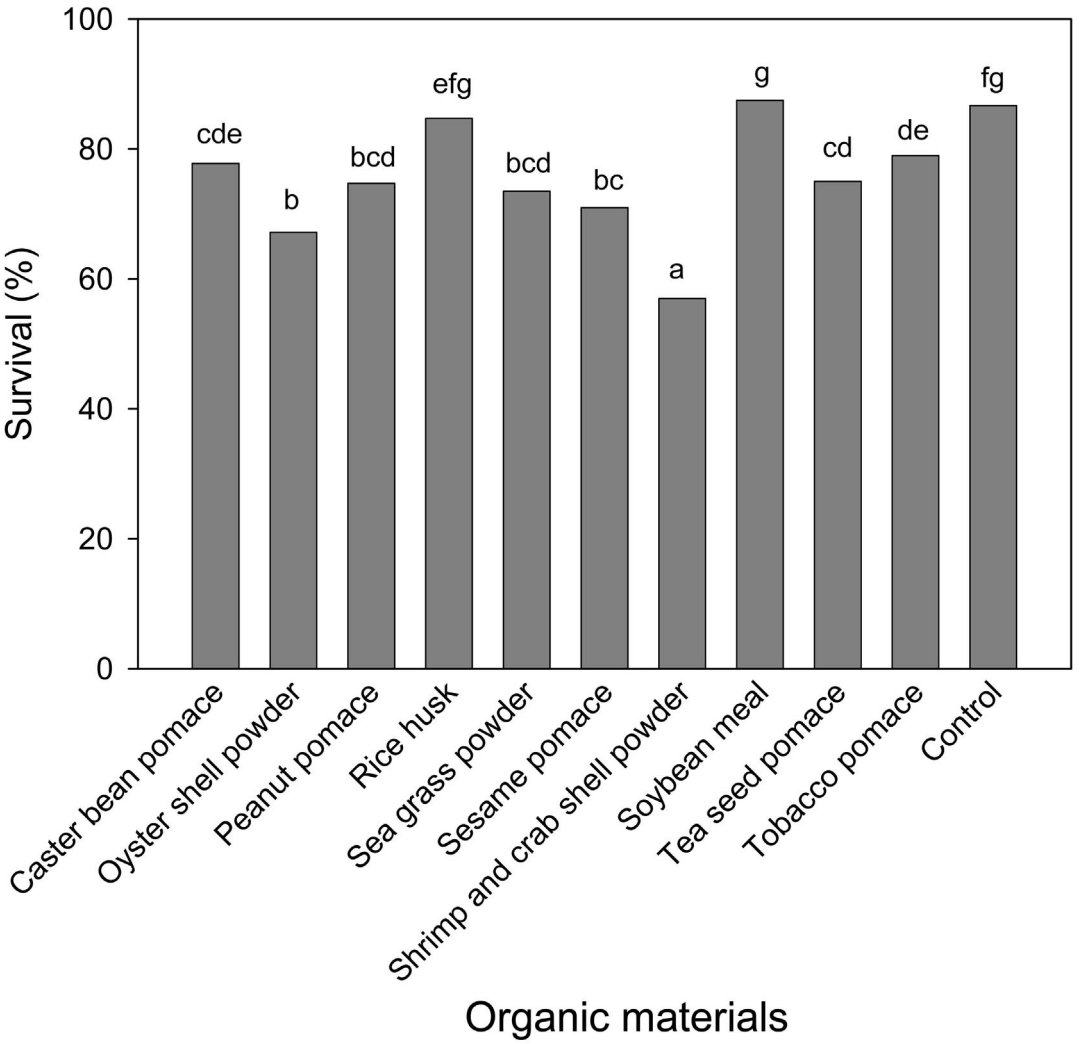

Fig. 1. Survival of *Fusarium oxysporum* f. sp. *tracheiphilum* PMF 05 in the soil amended with various organic materials at a rate of 1% (w/w) for one month. Value among treatments with the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 1. Effect of various concentrations of shrimp and crab shell powder on disease severity of Fusarium wilt of asparagus bean caused by *Fusarium oxysporum* f. sp. *tracheiphilum* PMF 05 (greenhouse experiments)

Concentration (%)[1]	Disease severity (%)[2]		Plant height (cm)
	20 days	40 days	
1.00	10	23	32.5
0.50	13	35	30.6
0.25	13	38	29.2
0.10	15	46	28.0
0.00 (control)	19	52	27.8
$LSD_{0.05}$[3]	5.9	13.3	2.5

[1] The soil collected from field in Taichung, was artificially infested with *Fusarium oxysporum* f. sp. *tracheiphilum* PMF 05. The pathogen-infested soils were respectively treated with various concentrations of shrimp and crab shell powder for 7 days prior to sowing of asparagus bean.

[2] Disease severity was recorded 20 and 40 days after transplanting, using the scale of 0 to 3: 0 = healthy plants, no visible symptoms; 1 = weakly infected plant sowing vascular discoloration but no leaf yellowing; 2 = moderately infected plant sowing leaf yellowing and wilt of plants; 3 = severely infected plant sowing plant death.

[3] Means (n=4) within each column are separated by Fisher's least significant difference ($P = 0.05$).

23% for the treatments of shrimp and crab shell powder at rates of 0.25%, 0.5% and 1.0%, respectively, compared to 52% in the control (Table 1). Amendment of shrimp and crab shell powder at rate of 0.1% was ineffective in reducing asparagus bean Fusarium wilt.

Amendment of pathogen-infested soil with shrimp and crab shell powder at 0.5% and 1.0% (w/w) resulted in a significant increase in plant height. The average plant height was 30.6 cm and 32.5 cm in the treatments 0.5% and 1.0% shrimp and crab shell powder, respectively, but was 27.8cm in control. At the lower concentration of 0.1% and 0.25% shrimp and crab shell powder, no significant (P>0.05) increase in plant height was observed.

Amendment of soil with shrimp and crab shell powder resulted in reducing populations of *F. oxysporum* f. sp. *tracheiphilum* PMF 05. At 30 days, the population of the pathogen in the control was 2.19×10^4 cfu/g soil, whereas the populations were decreased by 2.3 folds (0.96×10^4 cfu/ g soil), 1.67 folds (1.31×10^4 cfu/ g soil) and 1.43 folds (1.53×10^4 cfu/ g soil) for the treatments of shrimp and crab shell powder at 1.0%, 0.5% and 0.25 %, respectively (Fig. 2). Amendment of the growth substrate with shrimp and crab shell powder at 0.1% was ineffective in reducing population of the pathogen.

Adding shrimp and crab shell powder to soil at 0.25% to 1% significantly increased population density of actinomycetes and bacteria. For example, after incubation for 15 days, population densities of actinomycetes increased by 22.8 folds (54.1×10^8 cfu/g soil), 9.49 folds (22.5×10^8 cfu/g soil), 6.1 folds (14.4×10^8 cfu/g soil) and 2.3 folds (5.5×10^8 cfu/g soil), for the treatments of shrimp and crab shell powder at 1.0%, 0.5%, 0.25% and 0.1%, respectively, compared to the control of 2.37×10^8 cfu/g soil (Fig. 2). The actinomycetes populations in these treatments were declined after 30 days of amendment but remained higher than that of untreated control. Similar to actinomycetes, bacterial populations were increased by the treatment of shrimp and crab shell powder. For example, after incubation for 15 days, bacterial populations increased by 12.7 folds ($33,2\times10^8$ cfu/g soil), 8.7 folds (22.8×10^8 cfu/g soil), 6.9 folds (17.5×10^8 cfu/g soil), and 3.0 folds (7.9×10^8 cfu/g soil) for the the treatments of shrimp and crab shell powder at 1.0%, 0.5%, 0.25% and 0.1%, respectively, compared to the control of 2.62×10^8 cfu/g soil (Fig. 2). Fungal populations were also increased significantly by the treatments of shrimp and crab shell powder at 1.0% (7.7×10^4 cfu/g soil), 0.5% (7.1×10^4 cfu/g soil) and 0.25% (6.45×10^4 cfu/g soil), compared to the control (5.27×10^4 cfu/g soil) (Fig. 2).

Determination of the hydrolysis of fluorescein diacetate (FDA)

Amendment of soil with shrimp and crab shell powder significantly ($P\leq0.05$) increased microbial activities indicated by hydrolysis of fluorescein diacetate (FDA). After treatment for 15 days, the absorption value was highest (0.67) in treatment of 1.0% shrimp and crab shell powder, followed by treatment of 0.5% and 0.25% (0.62 and 0.6, respectively). The lowest microbial activities were observed in untreated control with

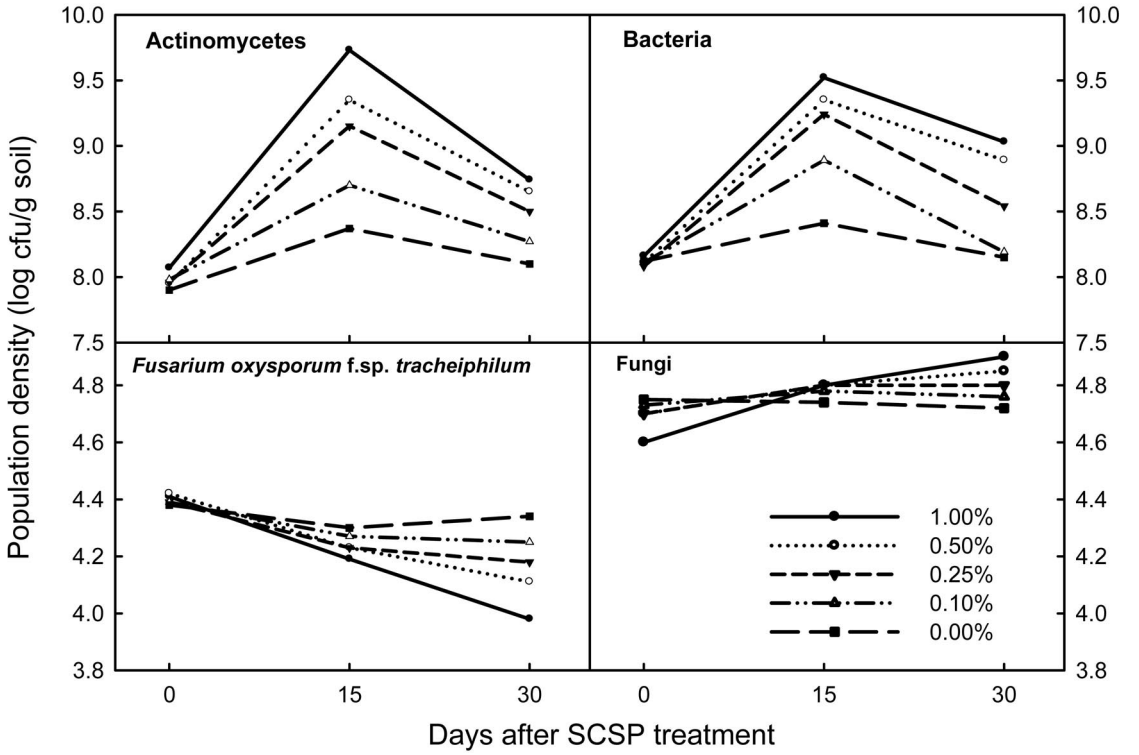

Fig. 2. Effect of concentrations of shrimp and crab shell powder (SCSP) on population densities of *Fusarium oxysporum* f. sp. *tracheiphilum* PMF 05, actinomycetes, bacteria and fungi at 0, 15 and 30 days in soil. The unamended soil was used as a control.

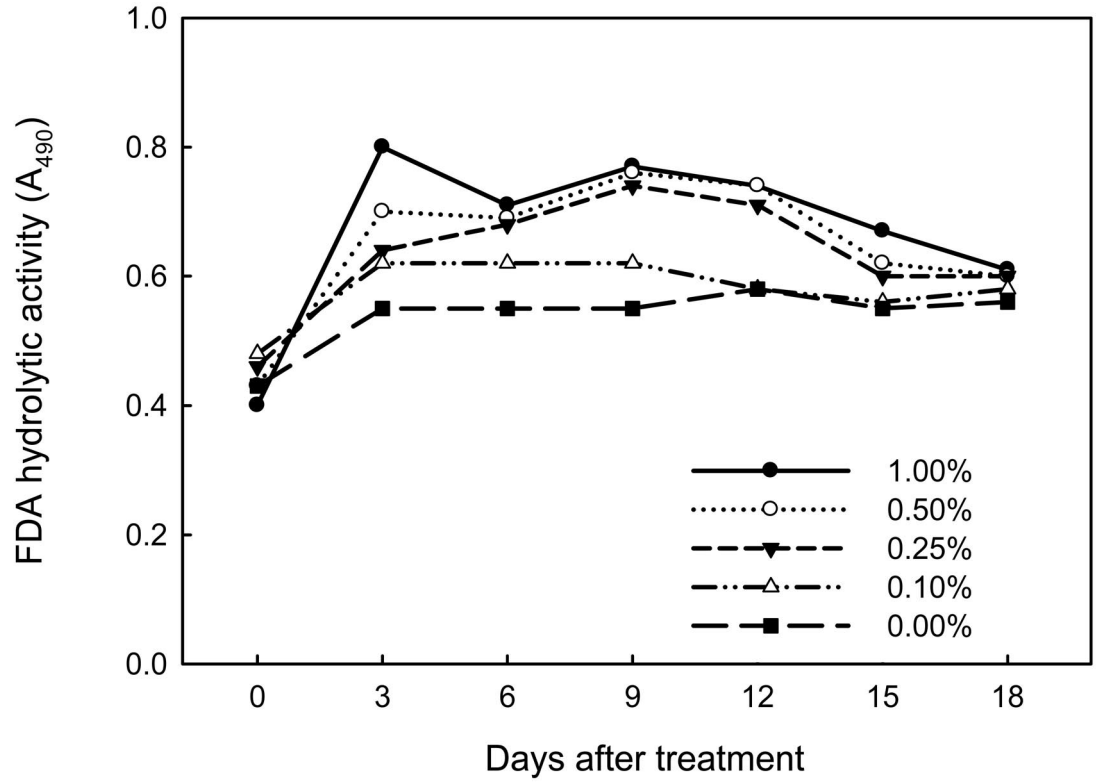

Fig. 3. Activity of microbial populations in soil amended with various concentrations of shrimp and crab shell powder. Total microbial activity was detected by spectrophotometer technique using the enzymatic hydrolysis of fluorescein diacetate for 18 days.

absorption value recorded at 0.55 (Fig. 3).

Screening of promising antagonistic bacteria

Among the 17 strains of rhizobacteria, 7 *Pseudomonas* spp. and 10 *Bacillus* spp., tested in the greenhouse, five strains of *Bacillus* spp., including stains PMB-018, PMB-021, PMB-023, PMB-028 and PMB-034 significantly ($P \leq 0.05$) reduced severity of Fusarium wilt of asparagus bean, whereas none of the stains of *Pseudomonas* spp. caused a significant reduction in disease severity (Table 2). Among the five effective strains of *Bacillus* spp., PMB-034 was the most effective strain with the disease severity of 29%, compared to 54% in the control.

Some of the bacterial strains were effective in increasing formation of root nodules and root fresh weight of asparagus bean. For example, root nodules produced on bean plants treated with *Bacillus* spp., strain PMB-034 and strain PMB-028 were 133 and 134, respectively, compared to 10^2 nodules in the control (Table 2). Fresh weights of roots of bean plants treated with strains PMB-034 and PMB-028 were 28.2g and 23.2g, respectively, compared to 19.3g in the control (Table 2).

Control of Fusarium wilt of asparagus bean by combine treatment of shrimp and crab shell powder and *Bacillus* spp. in the greenhouse

Amendment of soil with shrimp and crab shell powder, antagonistic bacteria, or combination of shrimp and crab shell powder and bacteria strains was effective in reducing Fusarium wilt of asparagus bean under greenhouse conditions. However, combined treatment of shrimp and crab shell powder and antagonistic bacteria was more effective than the treatment of shrimp and crab shell powder or antagonistic bacteria alone (Table 3). The most effective treatments were shrimp and crab shell powder (SCSP) 0.5% + *Bacillus* sp. strain PMB-034 + *Bacillus* sp. strain PMB-028 and SCSP 0.5% + *Bacillus* sp.

Table 2. Effect of antagonistic bacterial strains isolated from soil amended with shrimp and crab shell powder on control of Fusarium wilt asparagus bean and growth of seedling and formation of root nodules (greenhouse experiments)

Bacterial strain[1]	Disease severity (%)[2]	No. of nodules (per 10 plants)[3]	Root fresh weight (g/10 plants)
Pseudomonas spp.			
PMB-017	50 cd[4]	95 a	18.0 a
PMB-020	48 bcd	107 bc	18.5 ab
PMB-029	44 bcd	121 c	23.9 g
PMB-031	52 cd	114 cd	18.1 a
PMB-035	52 cd	124 ef	20.2 cd
PMB-036	69 e	108 bc	19.8 bc
PMB-037	54 d	102 ab	21.9 ef
Bacillus spp.			
PMB-015	48 bcd	114 cd	26.2 h
PMB-018	37 ab	130 fg	20.8 cde
PMB-021	39 abc	112 c	23.9 fg
PMB-023	38 abc	109 bc	19.2 ab
PMB-024	44 bcd	111 c	21.6 ef
PMB-028	35 ab	134 g	23.2 fg
PMB-032	46 bcd	121 de	21.6 ef
PMB-034	29 a	133 g	28.2 i
PMB-041	42 a-d	109 bc	19.3ab
PMB-045	42 a-d	122 e	21.3 de
Control	54 d	102 ab	19.3 ab

[1] Bacterial suspension (10^8 cfu/ml) of each strain was applied into soil artificially infested with *F . oxysporum* f. sp. *tracheiphilum* PMF 05 (10^4 cfu/g) and then seeded with asparagus bean .

[2] Fourteen-day-old seedlings were transplanted into pots, 3 seedlings/ pot. Disease severity was recorded 40 days after transplanting.

[3] Number of nodules and root fresh weight were observed immediately after recording the disease severity.

[4] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 3. Effect of concentrations of shrimp and crab shell powder (SCSP) and *Bacillus* spp. strains PMB-034 and PMB-028 on control of Fusarium wilt of asparagus bean and growth of seedlings and formation of root nodules (greenhouse experiment)

Treatment [1]	Disease severity (%) [2]	No. of nodules (per 10 plants) [3]	Root fresh weight (g /10 plants)
SCSP 0.5%	37 b [4]	115 ab	20.9 a
PMB-034	32 ab	125 abc	26.7 bc
PMB-028	38 b	130 bc	24.6 abc
SCSP 0.5% + PMB-034	25 a	137 c	26.8 bc
SCSP 0.5% + PMB-028	30 ab	127 bc	23.2 ab
SCSP 0.5% + PMB-034 + PMB-028	23 a	135 c	28.9 c
Control	56 c	110 a	21.6 a

[1] The soils collected from field in Taichung, were artificially infested with *F . oxysporum* f. sp. *tracheiphilum* PMF 05. The pathogen-infested soil was treated with 0.5% (w/w) shrimp and crab shell powder with or without *Bacillus* spp. PMB-034 or PMB-028 for 7 days prior to seedling of asparagus bean.

[2] Four-day-old seedlings were transplanting into pots, 3 seedlings/pot. Disease severity was recorded 40 days after transplanting.

[3] Number of nodules and root fresh weight were recorded immediately after rating of disease severity.

[4] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

strain PMB-034 with disease severity of 23% and 25%, respectively, compared to 56% in the untreated control, at 40 days after transplanting.

Treatments of soil with combination of shrimp and crab shell powder and bacteria also significantly increased formation of root nodules of bean plants (Table 3). For example, number of root nodules were 135 and 137 per 10 plants for the treatments of SCSP 0.5% + *Bacillus* strain PMB-034 + *Bacillus* strain PMB-028 and SCSP 0.5% + *Bacillus* strain PMB-034, respectively, but was 110 per 10 plants for the untreated control (Table 3). Moreover, the fresh weight of roots of bean plants was the highest in the treatment of SCSP 0.5% + *Bacillus* spp. strain PMB-034 + *Bacillus* spp. strain PMB-028 (28.9 g/10 plants), compared to the untreated control (21.6 g/10 plants) (Table 3).

DISCUSSION

This study reveals that amendment of soil with shrimp and crab shell powder at 0.5-1% (w/w) effectively reduced population of *F. oxysporum* f. sp. *tracheiphilum* and increased populations of antagonistic rhizosphere microorganisms including fungi, bacteria and actinomycetes; thereby reduced severity of Fusarium wilt of asparagus bean and promoted growth and nodule formation of this crop. These findings support previous reports that soil amendment with crab shell is effective in reducing population of various formae speciales of *Fusarium oxysporum* [30, 33] and improving control of Fusarium diseases of crops including Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Furthermore, amendment of soil with shrimp and crab shell powder 0.5-1% does not cause any phytotoxicity to plants of asparagus bean.

The shell of shrimps and crabs is rich in chitin which might be related to the effective control of Fusarium wilt of asparagus bean observed in this study. Benhamou and Theriault [2] reported that soil amendment with chitin and chitosan from crab shell effectively controlled Fusarium crown and root rot in tomato. Shrimp and crab processing wastes containing chitin, protein and calcium carbonate are generally pre-treated by the processes of size reduction, deproteination, and demineralization to obtain a chitin suitable for bioconservation or other use [1, 7, 8]. The shell of shrimp and crab is composed mainly of chitin impregnated with protein, which has been various modified with lipid and mineral salt. The impregnated mineral salts are primarily calcium carbonate with minor amounts of magnesium, phosphate, silica, and sulfur [31]. Studies on the antibacterial and antifungal effects of chitinase are scant and most of the discussion was focused on lysozyme activities from plant chitinase [18]. Further study is in progress on the role of chitin in the shrimp and crab shell powder for control of Fusarium wilt of asparagus bean.

This study indicates that soil infested with *Bacillus* spp. strain PMB-034 or strain PMB- 028 of *Bacillus* spp. was effective in suppressing Fusarium wilt of asparagus

bean, suggesting the possibility of using these selected strains of *Bacillus* spp. as biocontrol agents. The mode of action for *Bacillus* spp. strains PMB-034 and PMB-028 might be due to antibiosis. Landa *et al.*[20] reported that species of *Bacillus* isolated from the rhizosphere of chickpea inhibited conidial germination and hyphal growth of *F. oxysporum* f. sp. *ciceris* and suppressed Fusarium wilt of chickpea. Chen *et al.*[3] proved that expression of a chitinase gene increased the antifungal activities of an antibiotic-producing *B. subtilis* strain. Moreover, *Bacillus* spp. forms endspores that are resistant to unfavorable environmental conditions, and thus can be adapted to formulation and application in the field. Therefore, production of antibiotics and formation of endspores may be important features for using *Bacillus* spp. as biocontrol agents of plant diseases.

Although amendment of soil with shrimp and crab shell powder or strains of *Bacillus* spp. alone is effective in the control of Fusarium wilt of asparagus bean, results of this study indicate that the combined treatment of the organic matter at 0.5% and the bacterial strains *Bacillus* spp. strains PMB-034 and PMB-028 is more effective than the treatment of organic matter or bacterial agents alone (Table 3). The control strategy by organic soil amendment and biocontrol agents is environmentally sound. Thus, this combined approach of control of Fusarium wilt of asparagus bean is of practical importance. Further studies on formulation of shrimp and crab shell powder and *Bacillus* spp. strains PMB-034 and PMB- 028 for practical application are warranted.

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摘 要

何明清[1]、黃振文[1,2]. 2007. 利用有機土壤添加物與微生物防治長豇豆鐮孢菌萎凋病. 植病會刊16: 169-180. ([1]台中市南區402國光路250號 國立中興大學植物病理學系；[2]聯絡作者，電子郵件：jwhuang@dragon.nchu.edu.tw)

在溫室評估 10 種有機物防治長豇豆鐮孢菌萎凋病 (由 *Fusarium oxysporum* f. sp. *tracheciphilum* 引起) 的效果，結果發現 0.5% 或 1% (w/w) 蝦蟹殼粉可顯著促進土中放線菌與細菌量的增加外，亦可抑制 33-56% 長豇豆萎凋病的發生率。同時利用 fluorecein diacetate (FDA) 水解反應也發現土壤添加蝦蟹殼粉確可顯著提高土中的微生物活性。由長豇豆的根圈土壤分離本病原菌的拮抗微生物，測試 17 個細菌菌株中，發現 *Bacillus* spp. PMB-028 與 PMB-034 兩者可有效抑制 36-47% 罹病度，並可提高 33% 長豇豆的根鮮重與 30% 固氮根瘤數。進一步，研究發現 PMB-028 與 PMB- 034 兩菌株可顯著提高蝦蟹殼粉防治長豇豆鐮孢菌病害的效果，且又可促進植株的固氮根瘤數目。本研究證明利用土壤添加蝦蟹殼粉配合 *Bacillus* spp. PMB-028與 PMB- 034 防治長豇豆鐮孢菌萎凋病是一種頗具潛力的方法。

關鍵詞：長豇豆、枯草桿菌、鐮孢菌病害、有機添加物、微生物活性、蝦蟹殼粉