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Effect of Compost Amendment on Soil Microbial Community and Pathogen Cansing Fusarium Wilt Disease of Spinach

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The effect of different composts on soil microflora, pathogen and the development of Fusarium wilt of spinach caused by *Fusarium oxysporum* f. sp. *spinaciae*, was assessed under three successive cultivations in the greenhouse. Composts of wheat bran, wheat bran and sawdust, coffee grounds, chicken manure and the mixture of composts with or without 5% crabshell were applied into the infested soil at 5% (w/w) 30 days before the first cultivation and additional 2.5% after the second cultivation. The disease development was significantly suppressed by all compost amendments after the second cultivation, most notably in the compost mixtures. In most compost treatments, activation of microbial activity in the soil based on the hydrolysis of fluorescein diacetate was observed after each cultivation with a corresponding increase in the population of fungi, bacteria and actinomycetes determined by dilution plate technique. Particularly, bacterial population was enhanced more than the population of fungi and actinomycetes. Moreover, the bacterial community structure investigated using PCR-DGGE analysis clearly revealed that compost amendments diversified the bacterial community in the soil. Soil fungistasis measured by spore germination of the pathogen in compost treated soils, showed that composts strengthened soil fungistasis. From the findings reported, we suggest that the shift and modification of the microbial community structure induced by compost enhanced competition and/or antagonism among microbes and strengthened soil fungistasis leading to the decreased activity of the pathogen.

I. Introduction

The use of organic by products such as compost as soil amendment is one of the very attractive agronomic practices. The use of composts to reduce soilborne plant diseases is gaining the interest of plant pathologists, manufacturing and processing industries, regulators, consumers and growers. It is not only an attractive waste management strategy against the increasing problem dealing with the huge amount of organic matter/wastes. Compost additions to soil favors plant development and improve soil quality as well as suppressiveness against many soilborne pant diseases (Cotxarrera et al., 2002; Hointink, 1986; Hoitink and Boehm, 1999; Keener et al., 2000). Compost amendments therefore maintain and enhance the fertility and productivity of agricultural soils allowing sustainable land use.

Studies on the impact of compost on soil have mainly evaluated physical and chemical factors, potentially involved in plant productivity parameters. Evaluations performed both in pot and field experiments showed that compost amendments not only improve soil structure and act as a source of nutrients, but also strongly influence the soil microflora (Crecchio et el., 2001). Moreover, the addition of high quality composts may increase the soil microbial biomass and enhance microbial activity and soil enzyme activity (Albiach et al., 2000; Debosz et al). More interestingly, the effectiveness of composts in controlling soilborne plant diseases caused by *Pythium, Phytophthora, Rhizoctonia* or *Fusarium* spp. both in fields and in potting mixes in greenhouses is now well known (Schonfeld et al., 2003, Hadar and Mandelbaum, 1992; Hoitink et al., 1991; Hointink and Fahy, 1986). Suppressiveness was often related to biotic rather than abiotic factors (Reuveni et al., 2002; Garbeva et al., 2004). Abiotic includes physiochemical factors such as nutrient levels, organic matter, moisture and pH. Biotic factors on the other hand include microbial population in composts and/or soil, microbial competition for nutrients with pathogens, antibiotic production of lytic and other extra cellular enzymes, parasitism and predation and induction of host systemic resistance. Since compost amendments can cause a shift and modification of the microbial composition they potentially enhance the competition and/or antagonism among microbes, which leads to decreased activity of plant pathogen (Hointink and Boehm, 1999). The aim of the present study was to have a better insight into the relative effect of compost on disease development, pathogen and microbial community for the development of fungistasis.

II. Materials and Methods

Inoculum Preparation

F. oxysporum f. sp. *spinaciae* (Fos) gf-1 isolate obtained from wilted spinach plant was used. It was maintained in Potato Dextrose Agar (PDA) slant at 25℃. The isolate was incubated in an Erlenmeyer flask containing 100 ml of Potato Sucrose Broth (PSB) for 14 days at 25℃ on a rotary shaker. The fungal culture was passed through cheesecloth and centrifuged at 3000x g for 20 min. The bud cells in the resulting pellet were resuspended in sterile distilled water. Inoculum concentration was determined by counting cells ml^{-1} of suspension using a haemacytometer. Conidiospore was prepared using mycelial disks (5

mm) of Fos cut from the edges of a 7-day-old culture. The disks were transferred into PDA plates followed by incubation at 25℃ for 7-14 days for conidial spore production. Conidia were then scraped from the media and suspended in sterile distilled water. To remove mycelial fragments and germinated conidia, the sporangium was filtered through four layers of cheesecloth and washed twice by centrifugation at 5,000 rpm at 4℃ for 15 min. The concentration of conidia was adjusted to 2×10^5 conidia ml^{-1} by adding distilled water. Preparation of Fos chlamydospore was conducted following the method by Smith and Snyder (1971).

Suppressiveness of Compost to Fusarium Wilt Disease

The experiments were conducted in the greenhouse using a fresh field soil (Andisols; 58.0 % sand, 35.2 % silt, 6.8% clay) obtained from the Chiba University experimental field (Matsudo City, Chiba Prefecture, Japan). The assays were performed in soils amended with different composts prepared from a variety of feedstocks and organic materials such as: wheat bran (Wb), wheat bran and sawdust (WbSd), coffee grounds (Cf), chicken manure (Cm), and crab shell powder (Cs). A mixture of WbSd, Cf, and Cm at a ratio of 2:1:1 (w/w) with or without 5% Cs (Mix + Cs and Mix) respectively) was also used. Eight kilograms of conducive field soil was placed in a plastic containers (54 cm × 34 cm × 20 cm). Calcium carbonate ($CaCO_3$) was added to the soil to adjust the soil pH to 6.5. The soils were inoculated with the budding-cell suspension of Fos at a population density of 1×10^6 cells g^{-1}dry soil. Pathogen stabilization was given done by incubating the inoculated soil in a stable condition for 30 days at 25℃. Compost was then added into the infested soil and was mixed thoroughly. Initial compost added was at 5% (dry-wt basis), applied 30 days prior to spinach seeding followed by an additional application of 2.5% after two cultivation. Spinach (*Spinacia oleracea* cv. Parade) was seeded 30 days after compost amendment on the first cropping and 10 days after each cropping from the second and third croppings. Disease severity was evaluated at 30 days after seeding based on visual symptoms using the index of 0-3; where 0, healthy and asymptomatic plant; 1, first two leaves of the plant were cholorotic and wilted; 2, 2/3 of the total plant was cholorotic and wilted; 3, more than 2/3 of the plant wilted or dead. Disease severity was calculated according to the following formula:

Disease severity:

Σ (Disease index × corresponding number of plants) × (100/3) × total number of plants.

Microbial Activity and Populations

Soil samples for the assessment of total microbial activity and microbial populations were taken from 8 locations at a distance of 3 to 4 cm from the plant stem in each container using a soil sampler (4 cm in diameter, 8 cm in depth). Samples from each container were pooled, put into polyethylene bags and stored at 4℃ until use. The samples were mixed thoroughly prior to analysis. Total microbial activity was evaluated by measuring hydrolysis of fluorescein diacetate (FDA) (Sigma Chemical, Co., St. Louis, MO) as described by Schnurer and Rosswall (1982). FDA hydrolyzed to fluorescein by proteases, lipases and esterases. Each soil sample (5g) was put into a 50-ml Falcon Tube, and then 15 ml of 60 mM sodium phosphate buffer (pH 7.6) and 0.2 ml of FDA from1000 μg/ml stock solution was added to initiate the reaction. Each sample was replicated four times. For a blank, 0.2 ml acetone instead of FDA was added. These mixtures were shaken on a rotary shaker (1000 rpm) at room temperature for 20 min. Fifteen milliliter of chloroform-methanol (2:1) was added to each sample to stop the reaction. Soil in the mixtures was removed by filtration through a filter (0.45 μm, Millex®-GV). The amount of hydrolyzed FDA was determined by measuring the absorbance at 490 nm with a spectrophotometer (BECKMAN DU®-65 Spectrophotometer).

Three microbial groups including total fungi, bacteria and actinomycetes were enumerated to determine the effect of the different composts and compost mixtures on soil microflora. Each soil sample (25 g) was put into a sterile 500-ml Erlenmeyer flask, and suspended in 250 ml distilled water sterile water to 1×10^{-1} volume. Flasks were shaken on a rotary shaker (100 rpm) for 30 min, and dilutions of the samples were made before plating on semi selective agar media (fungi: Czapeck-Dox with Rose Bengal medium; actinomycetes: on soil extract medium; bacteria: Thornton's medium). Plates were incubated at 25℃ for 2-3 days. Counting of culturable fungi, bacteria and actinomycetes was done by counting the number of colony grown in each plate and expressed as CFU g^{-1} soil. Five replicate plates were used.

Extraction of DNA and PCR-DGGE Analysis

Soil samples were taken from all containers two months after compost amendment. Random samples (4-6 cores) from each container were collected using a sampling tube. Pooled soil samples from each plot were well mixed prior to analysis.

Total bacterial community DNA was extracted from 5 g soil sample using the Fast DNA® SPIN Kit for Soil (Qbiogene, USA). The amount of DNA and quality was assessed visually after electrophoresis in 2% agarose gel by ethidium bromide staining. Total extracted soil DNA at 100 μl dissolved with TE buffer was amplified in a PCR Thermal Cycler MP (Takara, Japan) with the universal primer set for 16S rDNA: PRBA338f-GC and PRUN518r (Muyzer et. al., 1993). The PCR reaction was conducted in 50 μl of the reaction mixture containing 2 μl of extracted DNA diluted at 1:250 as a template, 25 μl of Premix Taq, and 100 pmol of each primer. The reaction was programmed at an initial 5 min denaturation at 94 ℃ and was followed by 30 cycles

of 1 min at 95 ℃, 1 min at 56 ℃ and 1 min at 72 ℃. Thermal cycling was completed with an extension step at 72 ℃ for 5 min. PCR products were electrophoresed on a 2% agarose gel in 1 × Tris-acetate (TAE) buffer and stained with ethidium bromide.

DGGE was performed with the DCode System (Bio-Rad, USA). Two microliter of PCR product was loaded on polyacrylamide gels with a denaturing gradient of 40% (7% (w/v) acrylamide-bisacrylamide (37.5:1), 3.36M urea, 3.2% formamide) to 60% (7% (w/v) acrylamide-bisacrylamide (37.5:1), 5.04M urea, 4.8% formamide) with 1 × TAE and run for 4 h at 60℃ and 150 V. After staining of the gels with and ethidium bromide for 30 min, gels were scanned and examined then photograph under UV light.

Effect of Compost Amendment on Fos Spore Germination

Soil direct fungistasis of compost-amended soils was assayed by measuring the suppression of spore germination. Three experiments were carried out to determine the effect of compost amendment on the germination of conidiospores or chlamydospores as follows:

Germination of conidiospores in soil extract

The extract was obtained from field soil amended with compost (5% w/w), incubated for 1, 2, 3, and 4 weeks by adding distilled water to each soil (1:1 v/v). The soil-water mixtures were stirred thoroughly and allowed to stand for 4 hr. The supernatant was first filtered through Filter paper (Toyo, No. 2) and then aseptically though a Millipore membrane filter (0.2 μm). The extract was then pipetted into the cavity glass slide. Twenty microliter of Fos spores (2×10^5 spores ml^{-1}) was added into the cavity containing the extract. The concavity slides were then incubated in a petri-dish moist chamber for 12 hr at 25℃ and examined under the light microscope at × 1000. Five replicate slides were used for each treatment.

Germination of conidiospores on soil surface

Spore suspension (2×10^5 spores ml^{-1}) prepared as described previously was pipetted into a glass slide previously covered with Water Agar (2%) and allowed to dry for 4 hr. Five water agar slide adhering the Fos spores was then incubated up side down on the surface of the soil amended with composts. Each slide constituted one treatment and replicated five times. After 12 hr of incubation at 25℃ spores were examined by staining with lactophenol acid fuchsine staining solution.

Germination of chlamydospores buried in soil

Fos chlamydospores (2×10^5 cells ml^{-1}) obtained as previously mentioned were sealed in a cellulose tube (Wako Seamless Cellulose Tubing) and were introduced into 500 g of compost-amended soil and incubated at 25℃ for 12 hr. For light microscopy examination on the number of germinated chlamydospores in compost treated soil, the cellulose tubes were covered from each soil and stained with lactophenol cotton blue staining solution after opening the tube. The treatments consisted of five replicates each.

Statistical Analysis

Statistical analyses of data were performed on Statistical Analysis Systems Institute Inc. Ver. 4 (2000). The significant difference between biological properties of compost amended soils and disease severity at the end of each bioassay was assessed with analysis of variance and means were separated by least significant difference ($P = 0.05$) using Tukey-Kramer test.

II. Results

Suppressiveness and Biological Properties of Compost-Amended Soils

The severity of Fusarium wilt disease in spinach plants was not suppressed on the first cropping. However, it was effectively suppressed on the second and third croppings (Table 1). The most notably suppressive composts were chicken manure compost, wheatbran and sawdust compost and mixture of different composts with and without 5% crab shell powder, where the mixture of the different composts displayed to be the most suppressive and promoted plant growth (data not shown). Although there were no distinguishable differences among compost treatments, the composts displayed a certain degree of disease suppression that were distinguishable from that of the unamended soil. Total microbial activity measured by the hydrolysis of FDA per unit volume was higher in the compost-amended soils especially in the mixture of different composts than in the unamended control soils from the second cultivation onwards. Generally, higher number of culturable fungi, bacteria and actinomycetes were observed in the compost-amended soil compared to the unamended soil. Moreover, for most compost treatments population of bacteria and fungi were enhanced more than the number of actinomycetes. Populations of actinomycetes did not increase much by any treatment.

Molecular Structure of Bacterial Communities in Compost Amended Soil

To analyze the effect of the different composts and compost mixes on the bacterial communities, high molecular mass of DNA was extracted from samples obtained from random sampling from each of the soil amended with each compost treatment. Genetic fingerprinting by PCR-DGGE of bacterial 16S rDNA amplified fragments of soil DNA showed a complex DNA banding pattern, with some strong bands, some of lower intensity and a number of faint bands. Compost amend-

ments produced considerable shift or changes in bacterial community structure as illustrated in Fig. 1. The appearance of the different and complex bands in the amended soil compared to the unamended soil indicates an alteration of the abundance of the bacterial groups. Visual inspection of DGGE banding patterns revealed that at the start of the experiment the community structure of bacterial populations was represented by a large number of resolved weak but still distinguishable signals appearing from the smear in the background. This would indicate that many equally abundant populations initially represented the bacterial community. However an increasing diversity of banding pattern was evidenced over time accompanied by the appearing of more intense, dominant bands in compost amended soils, indicating a com-

Table 1. Disease severity, microbial activity and microbial population in compost amended soil

Compost	Cultivation[1]	Disease severity[2]	Microbial activity (hydrolyzed FDA $^{-1}$g $^{-1}$dry soil)	Microbial population (Log_{10} CFU $^{-1}$g soil)[4]		
				Fungi	Bacteria	Actinomycetes
Unamended soil	1st	98.6a	0.34a	5.5a	6.5a	5.8b
	2nd	98.5a	0.39b	5.7a	6.5a	6.0ab
	3rd	99.6b	0.39b	7.6b	7.2b	6.5a
Wheatbran (Wb)	1st	87.2a	0.48b	5.7a	6.6a	5.4a
	2nd	65.3b	0.45b	5.6a	7.3a	5.8a
	3rd	56.8b	0.48b	6.4a	7.9a	6.5a
Wheatbran & sawdust (WbSd)	1st	85.1a	0.33b	5.9b	6.2b	4.3b
	2nd	50.2b	0.42ab	6.7a	7.8a	6.0a
	3rd	40.3bc	0.47a	7.9a	8.1a	6.4a
Coffee grounds (Cf)	1st	90.3a	0.48ab	5.0a	6.3b	4.5a
	2nd	53.7b	0.50b	6.7a	7.7b	5.8a
	3rd	40.3bc	0.58a	6.9a	8.0a	6.3a
Chicken manure (Cm)	1st	86.1a	0.47ab	5.7ab	6.4b	5.6a
	2nd	50.5b	0.53a	6.4a	7.6a	6.0a
	3rd	27.5c	0.57a	7.5a	8.0a	6.8a
Crabshell (2.5%)	1st	88.7a	0.22a	5.3b	6.6ab	4.3b
	2nd	69.3b	0.39b	5.8b	7.9a	6.5a
	3rd	53.7b	0.41a	7.4a	8.0a	6.7a
Mixture (WbSd + Cf + Cm; 2:1:1)	1st	88.8a	0.42a	5.4b	6.0a	4.6a
	2nd	35.2b	0.41a	6.8a	7.3a	5.5a
	3rd	28.8c	0.49bc	7.6a	7.9a	5.9a
Mixture + 5% crabshell powder	1st	86.5a	0.25b	6.4b	6.7ab	5.4a
	2nd	38.2b	0.46a	7.5a	8.0a	6.0a
	3rd	28.5c	0.50a	7.9a	7.9a	6.5a

[1]Cultivation was conducted three times; soil was amended with 5% (w/w) compost before the first cultivation and 2.5% before the third cultivation.

[2]Average disease severity rating obtained after each cultivation evaluated on a scale of 0-3; where 0 = healthy and 3 plant wilted or dead. Numbers followed by the same letter are not significantly different at $P<0.05$ by Tukey-Kramer's test.

positional shift towards the dominance of bacterial populations after 2 months from compost application.

Effect of Compost Amendment on Fos Propagules

The results of the experiments revealed that the effect of soil extract on spore germination was very minimal when the extract was obtained at 1-2 weeks after compost amendment. Spore germination however showed to have been suppressed when soil-compost water extract was obtained 3 to 4 weeks after compost amendment (Fig. 2). On the other hand, incubation of Fos spore on surface of compost-amended soil did not affect much the propagule germination (Fig. 3). The effect was very small in all compost treated soil. None of the soil-compost mixes displayed strong suppressiveness all throughout the incubation. However, in an experiment using chlamydospores, germination was generally suppressed by the different composts except the crabshell powder which had a minimal effect, when propagules were buried into the soil (Fig. 4). The effect of compost on the germination of Fos chlamydospore was less when they were introduced into the soil compost mix immediately after compost application. The evident effect was observed when the chlamydospores were introduced four weeks after compost amendments. The germination of Fos chlamydospore was strongly suppressed in the coffee compost, chicken manure compost especially in the compost mixes with and without crabshell powder. In

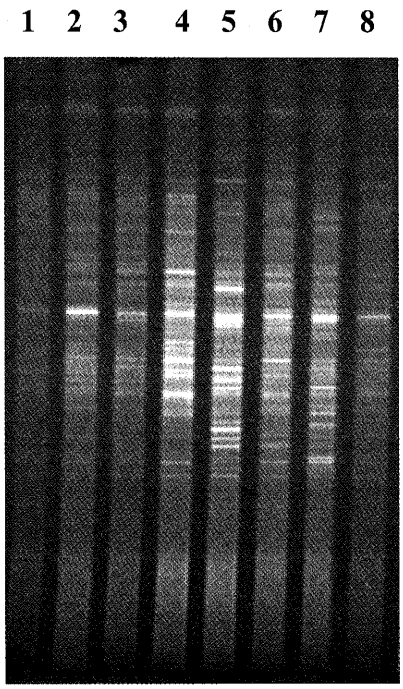

Fig. 1 Dynamics of total bacterial populations in soils amended with compost one month after compost amendment. DGGE fingerprints of 16S rDNA sequences amplified with the primer pair 338f-GC and PRUN518r from soil extracted community DNA. Lanel - unamended control soil, lane 2 - wheatbran, lane 3 - wheatbran and sawdust, lane 4 - coffee grounds c, lane 5 - chicken manure, lane 6 - crab shell powder, lane 7 - mixture of composts, lane 8 - mixture of compost with 5% crab shell powder

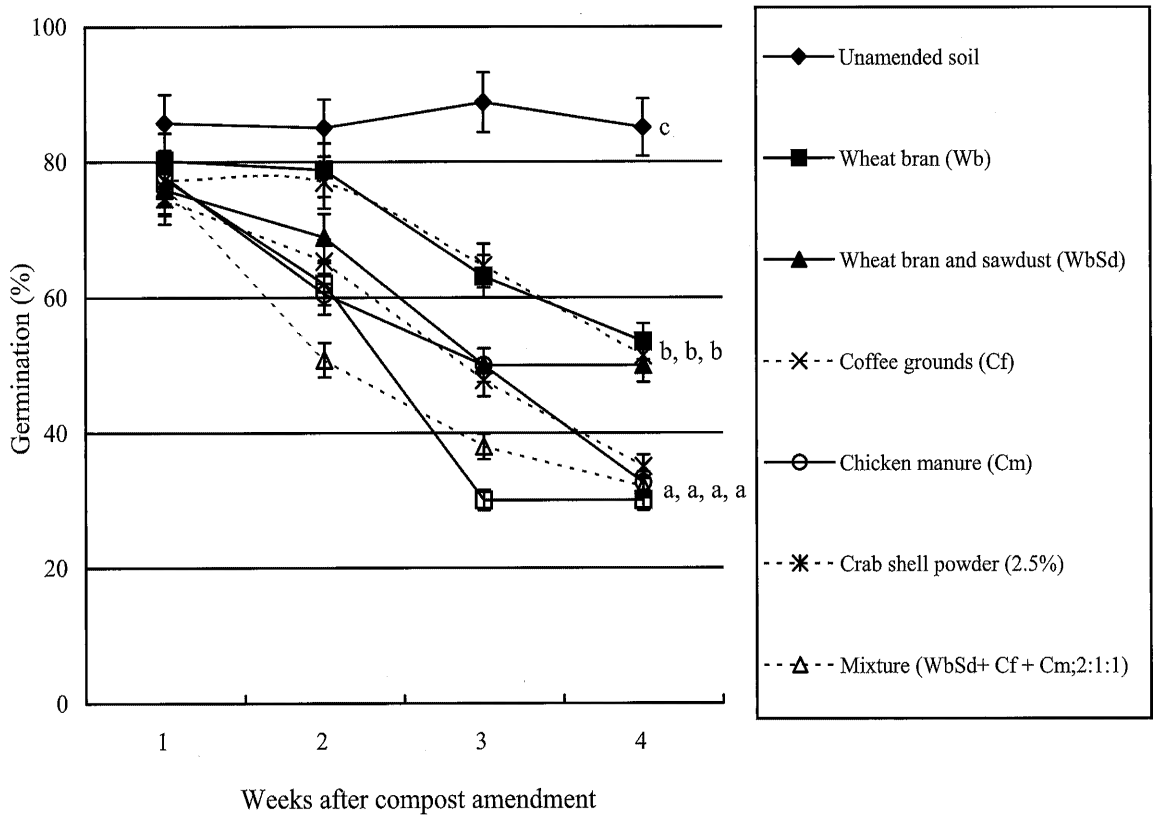

Fig. 2 Conidiospore germination of *Fusarium oxysporum* f. sp. *spinaciae* (Fos) in soil-compost water extracts after 12 hours of incubation. Water extract was filter sterilized (0.2 μm). Values with the same letters are not significantly different ($P = 0.05$) according to Tukey-Kramer's test.

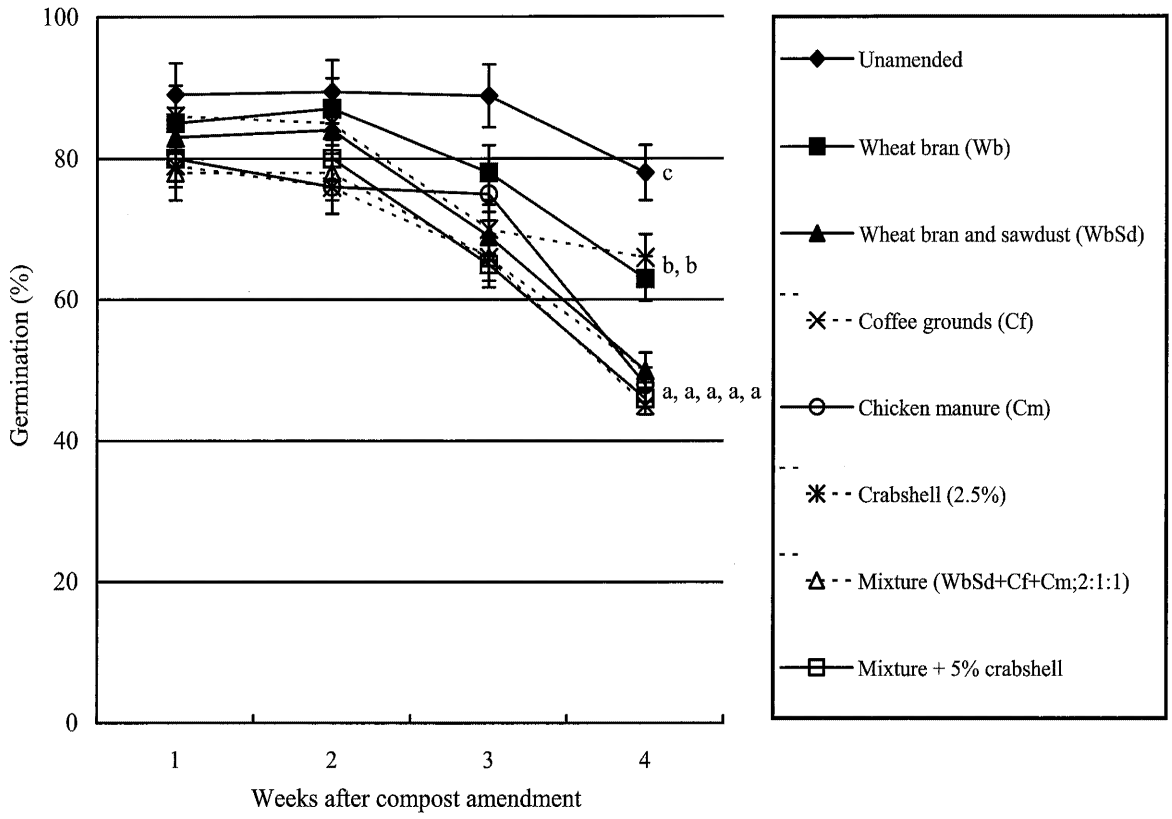

Fig. 3 Conidiospore germination *Fusarium oxysporum* f. sp. *spinaciae* (Fos) on surface of compost amended soil. Fos conidia were incubated as described in the materials and methods. Values with the same letters are not significantly different ($P = 0.05$) according to Tukey-Kramer's test.

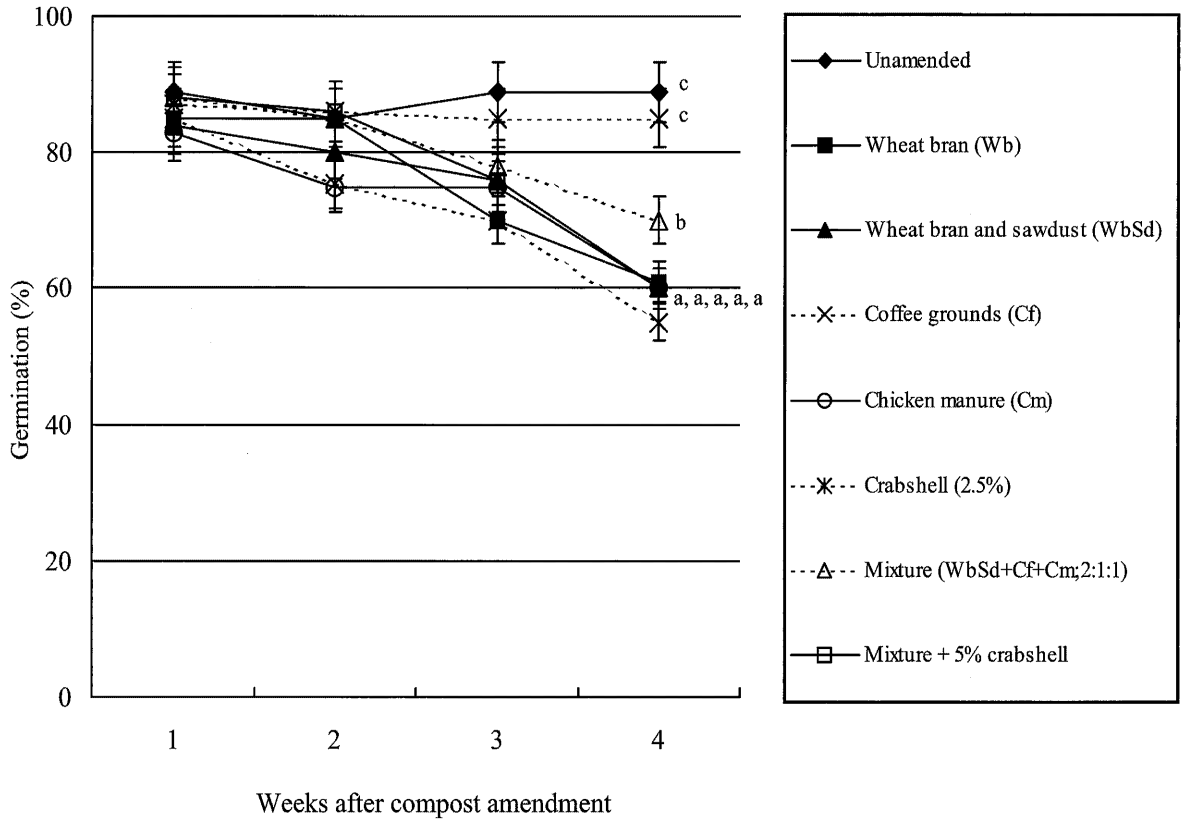

Fig. 4 Germination of *Fusarium oxysporum* f. sp. *spinaciae* (Fos) chlamydospore in compost amended soil. Values with the same letters are not significantly different ($P = 0.05$) according to Tukey-Kramer's test.

all experiments, none of the compost amendments increased the propagule numbers by enhancing germination of either spore or chlamydospore of the pathogen.

IV. Discussion

This study shows that compost amendments suppressed Fusarium wilt development when applied 5% (w/w) at 30 days before the first cultivation and 2.5% after the second cultivation. The results we obtained corroborates further with the report of Hoitink et al. (1997) that the suppressiveness of composts increased when composts are incorporated in the field or into the soil several months before planting and with composts of different compositions. Although disease suppression was not evident during the first cultivation, disease severity in compost-amended soils was lower and distinguishable from that of the unamended soil where microbial population and microbial activity was higher. This trend was particularly apparent in the composts mixes. This might be influenced by the diverse soil environment in which an innumerable soil microorganisms exists, that naturally inhabited the different organic materials and is attributed to increased disease suppression. Compost applications essentially enhance the population of the soil microflora and increase microbial activity of the soils (Bailey and Lazarovits, 2003). In our experiment, compost application increased soil microbial activity and microbial populations particularly of bacteria, fungi and actinomycetes. Among the microbial groups, bacterial population was affected much more than the number of fungi and actinomycetes and this was particularly pronounced in the compost mixes. This suggests that lower disease severity apparent in the suppressive composts is associated with the higher microbial population and activity in the amended soils. One possible explanation for this disease suppression is through competition between various microbial groups (fungi, bacteria and actinomycetes) and *F. oxysporum* f. sp. *spinaciae*. This was further documented by Nelson and Hointink (1983), who postulated that an increase in competition and antagonism among microbial populations and activity has been a mechanism of action for disease suppression in compost amended soils. Suppression of the disease by compost amendments mainly involved the highest microbial activity and microbial population.

All of the composts induced shifts in the bacterial community structures in the soil. Depending on the amended soil, these effects could be followed by an increase in microbial population and/or activities with corresponding decrease in disease severity. The bands in the DGGE profiles represent the dominant microbial population (Muyzer et al., 1993). The dominant bands showed by the DGGE banding patterns of 16S rDNA community profiles based on a general bacterial primer pair revealed complex profiles indicate the relative abundance of highly diversified bacterial groups in each compost-treated soil. From the distinct changes of the DGGE profiles observed, we suggest that there are several dominant groups of bacteria, which are relatively stable, independent of treatments and was found pronounced in the suppressive compost and compost mixes. The difference in the banding patterns between amended and non-amended soils suggested that shifts induced in the microbial community structures and microbial population were due to the stimulation of the naturally inhabiting soil microflora. Evident stimulation and high diversity of the microbial population particularly with the bacterial groups in the compost-amended soil was observed but the type of compost did not seem to influence the composition of the community. These results support the commonly held view that the use of composts boosts agricultural soil biodiversity. Moreover, it is known that the addition of different sources of organic matter may greatly affect soil microbes, and may alter the natural community of antagonistic microorganisms thus providing a potentially affective form of biological control of soilborne plant pathogens.

The observed suppression on the germination of Fos propagules in compost-amended soils demonstrates a strong fungistatic capacity of the soil. The suppressive effect of composts on propagule germination was observed from the second week after compos amendment and lasted for four weeks during the experiment. This displayed that spore germination essential for infection to the host and subsequent outbreak of the disease is kept under strong suppression in the compost amended soil. Thus, the mechanism of the suppression was considered to be due or in part to the improved soil fungistatic capacity and may be derived from competition of nutrients among microorganisms and antagonism by different groups of microbes. Therefore our studies demonstrate that composts strongly influence soil biological properties. It increased soil microbial population either through the stimulation of the soil microflora, an input of the compost microflora or a combination of both, leading to the activation of soil microbial activity. The activation of the soil microflora by composts consequently resulted in the induction of the diversification of the microbial population in the soil. It stimulates the indigenous microbial population and consequently affects pathogen development. This strong influence in microbial population and activity might have elicited the strengthened fungistatic capacity of soils leading to the suppression of germination and growth of the pathogen propagules essential for disease initiation and outbreak, which eventually resulted in plant disease suppression.

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コンポストの施用が土壌中の微生物群集およびホウレンソウ萎凋病菌に及ぼす影響

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摘　要

種々の有機質素材からなる堆肥を用い、*Fusarium oxysporum* f. sp. *spinaciae*によって引き起こされるホウレンソウ萎凋病の発病に及ぼす影響を評価するとともに、土壌中の微生物と病原菌に対する影響について調査した。堆肥はコムギフスマ、コムギフスマ･オガクズ、コーヒー粕、鶏ふん、およびこれらの混合物に５％のカニ殻粉末を無添加または添加したものを供試した。各堆肥を病原菌汚染土壌に５％（w/w）添加し、その30日後からホウレンソウを約１ヶ月ずつ連続して栽培し、2作後には堆肥をさらに2.5%追加施用した。その結果、各堆肥施用区とも２作目以降から発病が顕著に抑制されるようになり、特に混合堆肥施用区でその効果が高かった。FDA(fluorescein diacetate）分解活性を指標に土壌中の微生物活性を調べたところ、これらの堆肥施用土壌ではいずれの栽培時においても無処理の土壌に比べてその値が高く、希釈平板法で検出される糸状菌、細菌、放線菌の密度もそれに対応して増加していた。これら微生物群の中では細菌の増加が特に顕著であった。さらに、細菌を対象としたプライマーを用いてPCR-DGGE（denaturing gradient gel electrophoresis）解析を行った結果、堆肥施用に伴って土壌中の細菌群集構造が多様化していることが伺えた。また、堆肥を施用した土壌ではいずれも無処理の土壌に比べて病原菌の胞子発芽が抑制される傾向が見られた。以上の結果より、堆肥施用に伴う発病抑止機構の一つとして、多様化・活性化した土壌微生物の競合や拮抗など様々な機能の総合的作用によって土壌の静菌作用が強化され、病原菌の活動が抑制されることが考えられた。