Suppression of the plant diseases, *Pythium* (damping-off), *Rhizoctonia* (root rot) and *Verticillium* (wilt) by vermicomposts

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ABSTRACT

Vermicomposts are stabilized, non-thermophilic materials produced from organic wastes by interactions between earthworms and microorganisms. Suppression of *Pythium* by vermicomposts was tested by substituting 0, 10, 20 and 40% of vermicomposts (by volume) into 100, 90, 80 and 60% of a soil-less plant growth medium Metro Mix 360 (MM360), inoculated with *Pythium* at a rate of 1:4000 by volume. Disease severity was rated after 10 days, from 1 (symptomless) to 4 (heavy). The disease severity rating was 3.4 in 0% vermicompost substitution, compared with 1.1 in 10% substitution, 1.3 in 20% substitution, and 1.1 in 40% substitution. A similar range of substitutions of sterilized or non-sterilized MM360 and vermicompost was tested in three experiments with radish seeds inoculated with 1:2000 or 1:667 by volume of *Rhizoctonia.* Suppression of *Rhizoctonia* by vermicomposts was correlated progressively with increasing rates of substitution of vermicompost in three experiments. In field experiments planted with strawberries, the incidence of *Verticillium* wilt was suppressed significantly by the application of 5 t/ha and 10t/ha of commercial paper and food waste vermicomposts.

INTRODUCTION

The biological degradation and stabilization of organic wastes by earthworms and microorganisms is termed vermicomposting, and vermicomposts are excellent plant growth media or soil amendments (Edwards, 1998). Suppression of various soil-borne diseases by traditional thermophilic composts has been reported (Chung, *et al*., 1988). Certain types of composted pine bark suppressed *Pythium* damping-off diseases when incorporated into planting mixes (Boehm, et al., 1993). Only a few studies have investigated the suppression of soil-borne plant pathogens by vermicomposts (Szczech, et al., 1993), or disease suppression in the presence of earthworms (Stephens & Davoren, 1997; Stephens, et al., 1994). Disease suppression by composts has been attributed to the activities of competitive or antagonistic microorganisms. The main objective of our experiments was to determine the potential of vermicomposts to suppress the incidence of the plant diseases *Pythium* and *Rhizoctonia* in cucumbers and radishes in the laboratory, and *Verticillium* in strawberries in the field.

MATERIALS AND METHODS

Suppression of *Pythium* damping off in cucumber plants by substitution of vermicomposts into a greenhouse soil-less bedding plant medium (MM360)

Pythium ultimum inocula were prepared by infesting an autoclaved (1h, 121 ºC) light peat mix with *Pythium.* The potting mixtures were prepared by substituting 0, 10, 20 and 40% food waste vermicompost (Oregon Soil Corporation, Oregon City, OR) into a soil-less growth medium Metro Mix 360 (MM360) (Scotts Co., Marysville, OH), with half of all treatments sterilized by autoclaving. All treatments were inoculated with 0.5 g/litre (1:4000 dilution) *Pythium* inoculum. For all treatments, involving 0, 10, 20 and 40% vermicompost substituted into sterilized or unsterilized media, eight cucumber seeds were planted in each of 5 replicate, 15-cm diameter pots. The seedlings were kept in a growth chamber at 20 ºC with 16-hour illumination, and watered daily. Ten days after germination, they were harvested and rated for disease severity, according to the following scale: 1, symptomless; 2, emerged but wilted; 3, post-emergent damping-off; and 4, pre-emergent damping-off. Completely randomized designs were used, and one way ANOVA was used with Minitab statistical software (Minitab Inc., State College, PA). Separations of means were based on the least significant differences ($P<0.05$).

Suppression of *Rhizoctonia* root rot in radish seedlings by substitution of vermicomposts into a greenhouse soil-less bedding plant medium (MM360)

Rhizoctonia solani inocula, prepared using the following method: 500 ml soil and 50 g finely-chopped potatoes, in 35 ml water, were placed in a 500 ml flask. The mixtures were autoclaved twice over 2 days, for 1 hour. After cooling, the soil/potato mixture was inoculated with agar plugs of *Rhizoctonia* and incubated for 2 weeks. It was dried overnight at room temperature and sieved through 2-mm and 1-mm sieves; the inoculum collected in between the 2-mm and 1-mm sieves was saved and the rest discarded. The potting media were prepared by substituting 0, 10, 20 and 40% of either autoclaved or unsterilized cow manure-based vermicompost into sterilized or unsterilized MM360. All treatments, except the controls, were inoculated with 0.5 g/litre (1:2000 dilution) *Rhizoctonia* inoculum, and 35 g of slow release fertilizer (14-14-14) was added per 2.2 litre of planting medium. For all treatments, 32 radish seeds were planted in each of 5 replicate, 10-cm diameter pots. The seedlings were kept in a growth chamber at 25^{o}C under continuous light. After 7 days, the seedlings were harvested and rated for disease severity, according to the following scale: 1, symptomless; 2, small lesion on the stem; 3, large lesion on the stem; 4, post-emergent damping-off; and 5, pre-emergent damping-off. Incidence of pre-emergent damping off was calculated by subtracting the numbers of seedlings that emerged from the numbers that emerged in the non-inoculated controls (both sterilized and unsterilized MM360). Completely randomized designs were used, and one way ANOVA was performed using SAS software (SAS Institute, Cary, NC). Separations of means were based on least significant differences ($P<0.05$).

In a second experiment, the same procedure was used, except that the concentrations of vermicompost used were: an uninoculated control, and 0, 20, 40, 60, 80 and 100% substitutions of vermicompost, which was not sterilized, into equivalent amounts of either sterilized or unsterilized MM360. For a third trial, the concentration of the inoculum added was increased to 1.5 g/litre (1:667 dilution), and the inoculum was incubated for 3 weeks. The vermicompost substitutions used in a third trial were 0, 10, 20, 40 and 70% of unsterilized cattle-based vermicompost substituted into 100, 90, 80 60 and 30% of unsterilized MM360.

Suppression of *Verticillium* wilt in strawberry plants in vermicompost-amended field soils

Six-week old strawberry seedlings var. ‘Chandler’ were grown in soils under high plastic tunnel hoop house structures measuring 9.14 x 14.6 x 3.6 m. Twenty-four plants were transplanted into each bed with 38 cm between plants with three rows spaced 38 cm between rows. Food and paper waste vermicomposts were surface-applied at 5.0 and 10.0 t/ha. Plastic mulch and drip irrigation systems were set-up over the raised beds after vermicompost and fertilizer applications. Disease severity was rated using the proportion of the total leaves and stems that showed *Verticillium* wilt symptoms. Treatments were replicated four times in a completely randomized design and differences between means were separated using least significant differences (P<0.05).

RESULTS

Suppression of *Pythium* damping off in cucumber plants by substitution of vermicomposts into a greenhouse soil-less bedding plant medium (MM360)

Substitution of equal amounts of unsterilized vermicompost into sterilized MM360 increased *Pythium* suppression in cucumber seedlings significantly at all substitution rates (Figure 1). Substitution of sterilized vermicompost into sterilized MM360 also increased *Pythium* suppression in cucumber seedlings significantly, but to a lesser degree, and only at 20% and 40% substitutions.

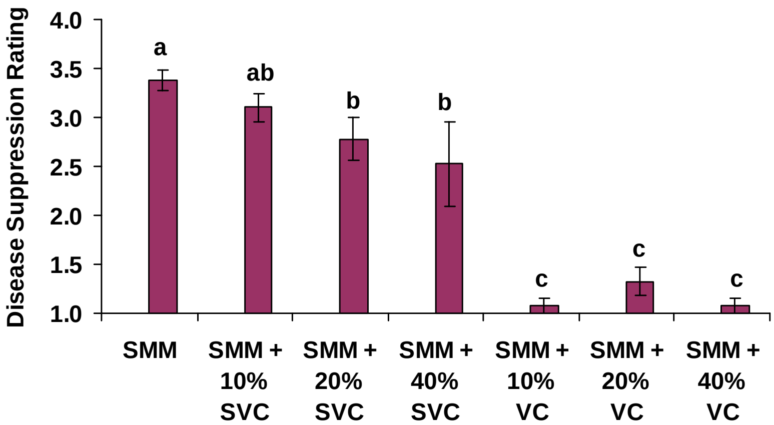

Figure 1. *Pythium* symptom suppression in cucumber seedlings planted in a soil-less medium (MM360) substituted with vermicompost, inoculated with 1:4000 dilution *Pythium* (mean ± standard error). SMM is sterilized MM360, VC is vermicompost and SVC is sterilized vermicompost. The disease scale is rated 1(symptomless) to 5 (severe). Columns followed by the same letter do not differ significantly (P<0.05).

Suppression of *Rhizoctonia* in radish seedlings by vermicompost-substituted greenhouse growth medium

Substituting unsterilized vermicomposts into sterilized MM360 increased disease suppression significantly at the rate of 40% vermicompost by volume (Figure 2). Substitution of sterilized vermicompost substituted into MM360 did not suppress *Rhizoctonia* significantly. In the second trial, vermicompost substituted into sterilized MM360 at concentrations of 40% and higher increased *Rhizoctonia* suppression significantly, compared with that in sterilized MM360 alone (Figure 3). In the third trial, when the inoculum concentration was increased threefold and incubated, unsterilized vermicompost substituted into unsterilized MM360 resulted in significantly increased *Rhizoctonia* suppression compared with that in unsterilized MM360 alone at 10%, 20%, 40% and 70% substitutions (Figure 4).

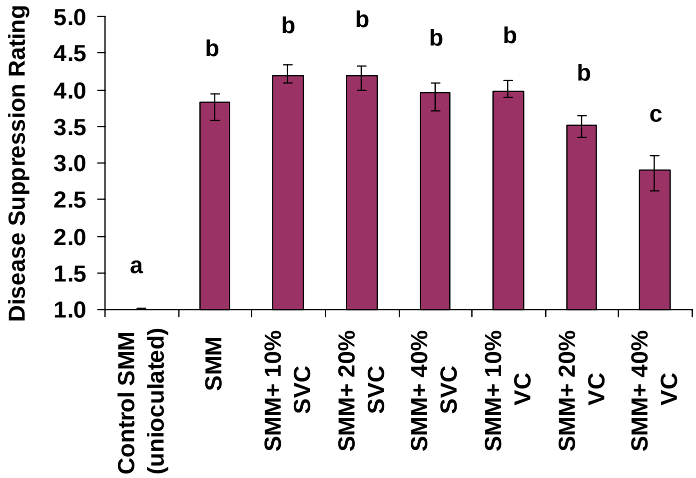

Figure 2. *Rhizoctonia* symptom suppression in radish seedlings planted in a soil-less medium (MM360) substituted with vermicompost, inoculated with 1:2000 dilution *Rhizoctonia* (mean ± standard error). SMM is sterilized MM360, VC is vermicompost and SVC is sterilized vermicompost. The disease scale is rated 1 (symptomless) to 5 (severe). Columns followed by the same letter do not differ significantly (P<0.05).

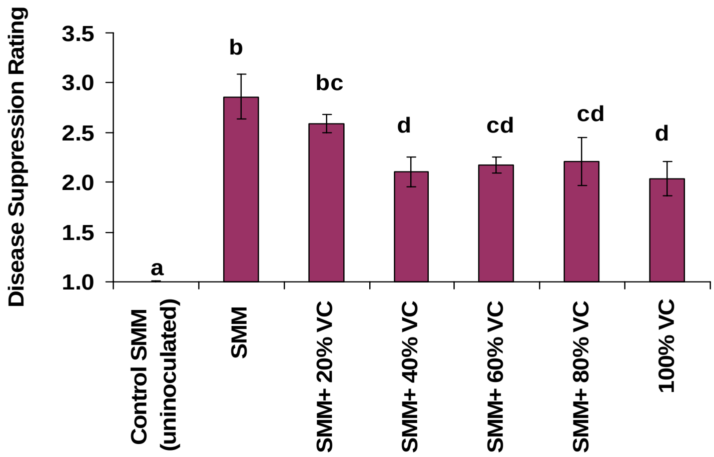

Figure 3. *Rhizoctonia* symptom suppression in radish seedlings planted in a soil-less medium (MM360) substituted with vermicompost, inoculated with 1:2000 dilution *Rhizoctonia* (mean ± standard error). SMM is sterilized MM360 and VC is vermicompost. The disease scale is rated 1 (symptomless) to 5 (severe). Columns followed by the same letter do not differ significantly (P<0.05).

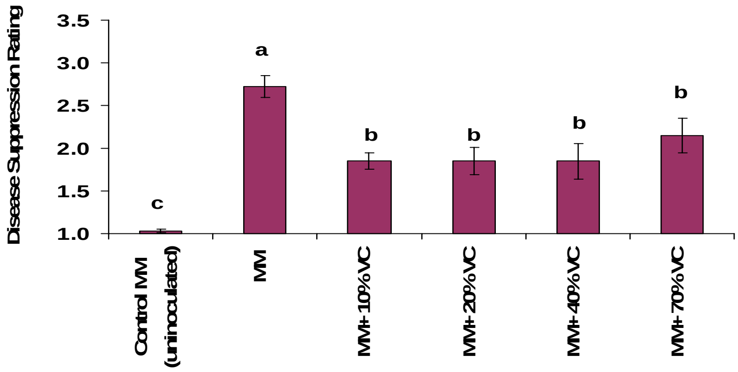

Figure 4. *Rhizoctonia* symptom suppression in radish seedlings planted in a soil-less medium (MM360) substituted with vermicompost, inoculated with 1:667 dilution *Rhizoctonia* (mean ± standard error). MM is unsterilized MM360 and VC is vermicompost. The disease scale is rated 1 (symptomless) to 5 (severe). Columns followed by the same letter do not differ significantly (P<0.05).

Suppression of *Verticillium* wilt in strawberry plants in vermicompost-amended field soils

Surface applications of 5 t/ha food waste, 10 t/ha food waste and 5 t/ha paper waste vermicomposts suppressed significantly the symptoms of *Verticillium* in field strawberries compared with the use of the inorganic fertilizer (Figure 5).

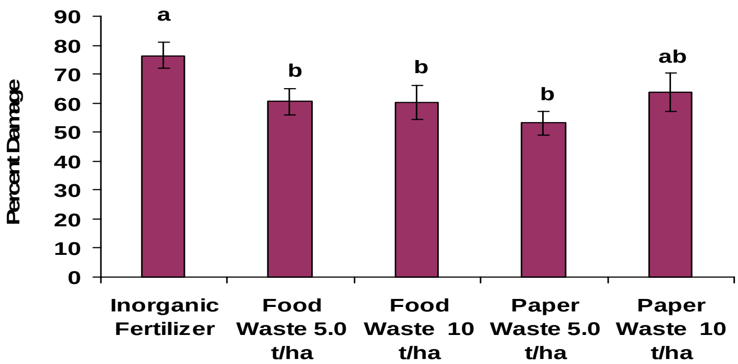

Figure 5. *Verticillium* wilt symptom suppression in strawberry field crops amended with topical applications of vermicompost (mean ± standard error). The percentage damage represents the proportion of plants per plot that were damaged by wilt. Columns followed by the same letter do not differ significantly (P<0.05).

DISCUSSION

Only small quantities (10% by volume), of vermicompost substituted into sterilized MM360, were required to induce *Pythium* suppression. This might be due to less aeration in the soil, leading to a greater competition between *Pythium* and beneficial microorganisms for resources, since *Pythium* is reported to be suppressed through general microbial suppression (Craft & Nelson, 1996). Unsterilized vermicompost substituted into sterilized MM360 increased *Pythium* suppression in cucumber seedlings to a significantly greater extent than sterilized vermicompost, evidence that the resistance was probably due to the high microbial activity of the vermicompost.

In the first *Rhizoctonia* trial, only the largest amount of vermicompost substituted into sterilized MM360 (40% by volume) suppressed *Rhizoctonia*. *Trichoderma sp.* has been identified as a biocontrol agent for *Rhizoctonia*, and might have been responsible for the suppression. The second trial indicated that increasing the vermicompost substitutions above 40% in sterilized MM360 did not increase the amount of disease suppression significantly (Figure 3). The decreases in disease suppression when sterilized vermicomposts were substituted into MM360 showed clearly the negative effects of vermicompost sterilization on its potential for disease suppression.

The use of vermicomposts suppressed significantly the diseases *Pythium* and *Rhizoctonia* in the laboratory and *Verticillium* in the field. However, the mechanisms of suppression cannot be identified in these exploratory experiments. It is well-established that traditional thermophilic composts can suppress the incidence of a number of plant diseases, probably by competition with or antagonism to non-pathogenic microorganisms (Craft & Nelson, 1996). There is good evidence that earthworms promote microbial activity and diversity in organic wastes to levels even greater than those in thermophilic composts (Edwards, 1998). Hence, there seems to be an even greater potential for suppression of plant disease by vermicomposts than by composts, probably due to stimulatory effects on soil microbial activity (thereby encouraging competing microorganisms). This conclusion seems to be supported by the results of our experiments.

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