

## Plant waste-based composts suppressive to diseases caused by pathogenic *Fusarium oxysporum*

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### Abstract

The suppressive ability of three plant residue-based composts that could serve as components of soilless media for several vegetable crops was tested on four different formae speciales of *Fusarium oxysporum*: *melonis*, *basilici*, *radicis-lycopersici* and *radicis-cucumerinum*. The composts were prepared under controlled conditions from a mixture of separated cow manure (SCM) with orange peels (OP), wheat straw (WS), or dried tomato plants that had been removed from the greenhouse after the end of the season (TP). Disease development in melon, tomato and cucumber seedlings growing in the three composts was significantly less than that observed in peat. Plant inoculation was achieved by conidia produced in culture, conidia naturally produced on infected stems and soil inoculum produced by enriching the soil with infected tissues. Pathogen colonization of the roots and stems of infected melon plants grown in TP-SCM and OP-SCM composts was significantly lower than that of peat-grown plants. Sterilization by gamma irradiation reduced the suppressive capability of TP-SCM and OP-SCM composts, whereas it did not affect the disease development and final disease incidence in peat. Tested formae speciales exhibited differing decline rates of the conidia incorporated in the composts, compared with the rate in the peat control, which suggests that different mechanisms may be involved in the suppression of the different pathogens. The present study shows that composts based on plant-waste residues suppress diseases caused by different formae speciales of *Fusarium oxysporum*.

### Introduction

In many parts of the world, arable soils are relatively infertile and crops may be infested with soil-borne pathogens. A potential solution to these problems is the use of soilless culture. However, in spite of the fact that most soilless media are initially free of soil-borne pathogens, infestation

often occurs during the course of the crop's growing cycle. Peat moss, the most widely used medium constituent, is especially conducive to a wide variety of soil-borne pathogens (Hoitink et al., 1977). In addition, the role of peat bogs in the assimilation of atmospheric CO<sub>2</sub> calls for replacement of peat with other, renewable organic substrates. Unlike peat, which is not a significant

source of plant nutrients, composts not only supply substantial amounts of nutrients (Raviv et al., 2002), but may also suppress soil-borne pathogens (Hoitink and Kuter, 1986; Raviv et al., 2005).

Compost is the product obtained from the aerobic decomposition of organic matter. Composts differ according to the raw starting material and the nature of the process. Composts that serve as components of container media must be stable, in order to avoid competition for oxygen and nitrogen between microorganisms and plant roots. They must have low salinity, low concentrations of phytotoxic substances, and be free of phytopathogenic organisms (Epstein, 1997; Inbar et al., 1993; Miller and Metting, 1992; Raviv, 2005). Composts serving as soilless media are produced from diverse organic wastes, such as sewage sludge, municipal solid waste, animal excreta, and food industry wastes such as rice hulls and corn cobs. Animal manures are especially valuable for both composting and co-composting, because they contain diverse populations of microorganisms, which accelerate the process. Manures are normally generated within agricultural areas where both the source of the raw materials and the users of the product co-exist, so that long-distance haulage is not required.

Various types of composts are known to suppress diverse diseases caused by soil-borne pathogens (Erhart et al., 1999; Gorodecki and Hadar, 1990; Hadar and Mandelbaum, 1986; Hoitink et al., 1993, 1997; Kwok et al., 1987; Trillas-Gay et al., 1986). The suppressive capacity of composts is clearly linked with their degree of maturity, although excessively stabilized composts tend to lose this quality (Hoitink and Grebus, 1997). The suppressive agents are complexes of microbial populations, which invade the compost pile during the curing stage. Sterilization largely negates the disease-suppressive capacity of composts (Larkin et al., 1993; Mandelbaum et al., 1988; Reuveni et al., 2002), which suggests that most of it is associated with microbial activity, although some residual activity is probably related to fungistatic compounds that are also present there (Hoitink and Fahy, 1986).

Several studies have addressed suppression of *Fusarium* pathogens by composts, but they have mostly focused on only one forma specialis (Chef et al., 1983; Cheuk et al., 2005; Kannangara et al., 2000; Raviv et al., 2005; Reuveni et al., 2002). The present study was designed to develop agricultural

waste-based composts that could serve as components of soilless media for several vegetable crops and that exhibit suppressive capacity toward four different formae speciales of *Fusarium oxysporum*. In addition, the survival of these four pathogens in the different media was studied.

## Materials and methods

### *Compost preparation*

Three carbonaceous amendments were added to fresh (up to 1 week-old) separated cow manure (SCM) in order to increase the C/N ratio of the mixture. The amendments were orange peels (OP), wheat straw (WS) and dried tomato plants removed from the greenhouse after the end of the season (TP). TP and OP were mixed with SCM at a volumetric ratio of 1:1, and WS was mixed with SCM at a volumetric ratio of 1:2. The three mixtures were composted in insulated 8 m<sup>3</sup> bins, with forced aeration to prevent overheating, as previously described (Raviv et al., 1998b). The aeration rate was 400 m<sup>3</sup> h<sup>-1</sup> per bin, and a temperature of 60 °C was used as a set point. This temperature is considered to be optimal for composting raw materials that do not contain human pathogens (Finstain and Hogan, 1993). When the compost temperature fell below the set point, the composts were aerated for 1 min h<sup>-1</sup> in order to maintain aerobic conditions at all times. The temperature was measured continuously by two sensors located at depths of 40 and 80 cm, and was recorded every 5 min. Daily temperature averages were calculated for each pile. The moisture content (MC) was measured twice weekly and maintained at 50–60% during the thermophilic period and at 40–50% thereafter, by adding water as necessary, in order to keep the soil wet but avoid leaching. The composts were turned twice with a front-end loader to improve their homogeneity. In general, temperatures rose to 60 °C 2–3 days after the start of composting and stayed at this level for about 80 days, during which time they rarely rose above 60 °C, as described by Raviv et al. (2005). The temperatures then started to decline, and reached ambient level in all piles 120 days from the start of composting. The composts were then left to cure for an additional 30 days.

### Compost characteristics

The organic matter, N, P and K contents and concentrations of several ions in compost and peat extracts were determined as described previously (Raviv et al., 1998a). Samples of peat, raw materials and composts were oven-dried at 70 °C and ground to pass a 20-mesh sieve. Samples were digested as follows: 200 mg were treated with 4 ml of 36 N H<sub>2</sub>SO<sub>4</sub> overnight at room temperature, after which 1 ml of H<sub>2</sub>O<sub>2</sub> was added to the reaction tubes, which were then incubated at 130–140 °C. After the tubes cooled to between 50 and 60 °C, the temperature was raised to 280 °C for 20 min. At the end of the process the solutions became clear, and after they cooled the concentrations of N, P, and K were determined in the digest by spectrophotometry (Hach, 1988) with Nessler reagent, molybdate, and tetraphenyl borate, respectively. All analyses were checked and calibrated against standard spectrophotometric solutions. The organic matter content was determined by weighing after the sample was ashed at 550 °C.

Concentrations of soluble ions (K<sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>-3</sup>) were determined in water extracts of the materials (1:10), which had been left for 4 h, with shaking once an hour. The potassium concentration was measured with a Corning Flame Photometer 410, nitrate with an RQflex Plus instrument (Merck, Darmstadt) (Merck, 2000), ammonium by the phenate method (Clesceri et al., 1998), and phosphate by colorimetry. The pH was measured directly in the solutions with a Radiometer PHM 95 pH meter (Copenhagen).

Biological oxygen demand was determined in the raw samples according to Raviv et al. (1998b). Moisture content of the samples was kept close to container capacity throughout the 2-week measurement process. The chemical characteristics of the composts and peat moss used in this study are presented in Table 1.

### Pathogens

Different inoculum types of the four formae speciales of *Fusarium oxysporum* were used in this study. Two types of conidia were used. For *F. oxysporum* f. sp. *melonis* we produced conidia on agar medium by growing the pathogen on yeast extract agar (Difco USA) at 27 °C for 7 days.

Table 1. Chemical characteristics of the studied composts and peat, and of their aqueous extracts<sup>a</sup>

|   | Peat | WS-SCM | OP-SCM | TP-SCM |
|---|------|--------|--------|--------|
| Organic matter (%)  | 91   | 53     | 57     | 40     |
| N (%)   | 1.11 | 2.39   | 2.84   | 1.92   |
| P (%)   | 0.11 | 0.74   | 0.86   | 0.87   |
| K (%)   | 0.27 | 2.11   | 4.12   | 1.2    |
| C/N ratio   | 45.4 | 13.1   | 11.9   | 12.2   |
| pH <sup>b</sup>   | 6.1  | 6.8    | 7.1    | 7.1    |
| EC (dS m <sup>-1</sup> ) <sup>2</sup>   | 0.75 | 7.74   | 4.43   | 5.62   |
| N-NO <sub>3</sub> <sup>-</sup> (mmol <sub>c</sub> l <sup>-1</sup> ) <sup>2</sup>  | 9.8  | 164.4  | 102.7  | 137.0  |
| N-NH <sub>4</sub> <sup>+</sup> (mmol <sub>c</sub> l <sup>-1</sup> ) <sup>2</sup>  | 3.56 | 19.5   | 18.8   | 3.0    |
| P-PO <sub>4</sub> <sup>-3</sup> (mmol <sub>c</sub> l <sup>-1</sup> ) <sup>2</sup> | 2.46 | 6.80   | 4.38   | 3.70   |
| K <sup>+</sup> (mmol <sub>c</sub> l <sup>-1</sup> ) <sup>2</sup>                  | 25.5 | 379    | 209    | 155    |
| BOD (g O <sub>2</sub> Kg DM <sup>-1</sup> day <sup>-1</sup> ) <sup>c</sup>        | 2.2  | 4.7    | 3.0    | 3.3    |

<sup>a</sup>WS-SCM = wheat straw + separated cattle manure;

OP-SCM = orange peels + separated cattle manure;

TP-SCM = tomato plants + separated cattle manure.

<sup>b</sup>In a 10:1 aqueous extract.

<sup>c</sup>Biological oxygen demand.

Conidia were scraped into sterile water with a scalpel, and filtered through eight layers of cheesecloth. Conidial suspension was centrifuged at 7500 rpm for 20 min. and the precipitate was resuspended in water. For *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum* f. sp. *basilici* and *F. oxysporum* f. sp. *radicis-cucumerinum* we used macroconidia that had been naturally produced on the lower parts of the stems of diseased tomato, basil and cucumber plants, respectively. These were scraped into sterile water with a scalpel. Conidial concentrations were determined with a haemocytometer and were diluted to the desired concentrations.

In some studies, natural inoculum was produced in the following manner: melon seedlings were inoculated with *F. oxysporum* f. sp. *melonis* and grown in pots containing peat or sand. The diseased plants were removed and new melon seedlings were planted, without additional inoculation. This procedure was repeated three times and in each case all melon seedlings became infected with *Fusarium*. After the fourth cycle, the sand and peat media were blended together. The resulting inoculum was added to the tested media at a volumetric ratio of 1:6. The final inoculum

density of the pathogen in the tested media was about 15,000 colony forming units per gram (CFU g<sup>-1</sup>).

#### *Evaluation of compost effect on disease suppression*

Disease suppression was evaluated by pot-based assays. Melon (*Cucumis melo* cv. Ofir), cucumber (*Cucumis sativus* cv. Kfir) and tomato (*Lycopersicon esculentum* Mill, cv. Hazera 5656) plants were used to evaluate disease suppression. Melon and cucumber seeds were sown in sand. Six days later, the seedlings were removed and their roots were washed and dipped for 2 min. in a conidial suspension containing  $3 \times 10^5$  conidia ml<sup>-1</sup>, and they were then transplanted into the tested medium. The tested media were mixed with perlite #4 (HaBonim, Israel) at a 1:1 volumetric ratio and introduced into 0.25 l pots. Five seedlings were planted in each pot; five pots of each group were planted with inoculated seedlings, and two were planted with non-inoculated seedlings. All greenhouse trials were arranged in a randomized-block design. Disease symptoms in melon and cucumber seedlings were manifested as wilt, yellowing and plant collapse. In the case of tomato plants, three 20 day-old transplants were placed in each 0.5 l pot. Discolouration of tomato stem diagonal cuts was evaluated after 50 days as an indicator for disease development. Another assay was carried out by planting the seedlings in naturally infested media.

Disease development was expressed as percentage of diseased plants, disease index, area under disease progress curve (AUDPC) (Campbell and Madden, 1990), and when indicated, percentage of the maximum value of the AUPDC. In all experiments, non-inoculated plants remained healthy.

#### *Medium sterilization*

Peat, TP-SCM and OP-SCM composts were sterilized by 2.5 Mrad of gamma irradiation in the Nahal Sorek nuclear reactor (Israel). Pots were filled with the substrates, mixed with perlite #4 at a 1:1 volumetric ratio, covered with plastic lead and sterilized. Roots of melon seedlings were dipped in *F. oxysporum* f. sp. *melonis* suspension and were then transplanted into these media, immediately after removing the pot covers, or 8,

16 or 24 h later, in order to assess recolonization of the previously sterilized media. Samples (5 g) were retrieved and general populations of fungi and bacteria were determined by the dilution method. Rose bengal agar was used for fungi and nutrient agar for bacteria (Dhingra and Sinclair, 1986).

#### *Pathogen survival*

Conidia of all four *Fusarium* pathogens were suspended in water and counted with a haemocytometer. Inocula were added to the media to a final macroconidial concentration of  $5 \times 10^5$  g<sup>-1</sup> at 90% container capacity. Fifty-gram samples from each medium were placed in 275 ml bottles, covered with polyethylene and incubated at 27 °C. Five replicates per treatment were analysed in this case. Water loss during incubation was negligible. Sub-samples of 5 g were periodically retrieved, and the pathogen population levels were determined by using the dilution method on a *Fusarium*-selective medium (Raviv et al., 2005). Survival percentage of the pathogen was determined by comparing the population level with that at the time of initiation.

#### *Colonization of roots and stems by F. oxysporum f. sp. melonis*

Root and stem sections (5 mm thick) were sampled from the melon seedlings at 4, 8 and 22 days after transplanting. Tissue sections were plated on a *Fusarium*-selective medium and were incubated for 5 days at 27 °C. Each value presented in Figure 6 is the average of 25 sections, i.e., five root or stem sections from each of five Petri dishes (replicates).

#### *Statistical analyses*

AUDPC and pathogen decline were expressed in an area under disease or decline curve, which was calculated as the integral by means of PSI-Plot software (Poly Software International, New York, NY). The colonization percentages were arcsin-transformed prior to the calculation of significance, in order to stabilize the variances. All values were subjected to the Tukey-Kramer Honestly Significant Difference Test at  $P = 0.05$ . All experiments were performed at least twice, yielding similar results.

## Results

### *Effect of compost on disease incidence*

Melon seedlings were artificially inoculated by dipping their roots in a conidial suspension of *F. oxysporum* f. sp. *melonis* and transplanted into different organic media. All three composts significantly suppressed disease (Figure 1A). In peat, wilting symptoms first appeared 10 days after inoculation, reaching 100% disease incidence 30 days after the inoculation, whereas in the OP-SCM, WS-SCM and TP-SCM media, disease incidence was less than 5–10%.

One week after the end of this experiment, melon seedlings were transplanted into the same

pots without the addition of new inoculum, to assess the persistence of the ability to suppress *F. oxysporum* f. sp. *melonis*. The seedlings were grown for 30 days and disease incidence was evaluated. This procedure was repeated three times, and disease incidence was significantly less than that in peat throughout the additional three planting cycles (Figure 1B).

Significant suppression of disease by OP-SCM and TP-SCM composts was also observed following the transplant of seedlings into medium infested with *F. oxysporum* f. sp. *melonis* (Figure 2A). This was again evident in repeated plantings (Figure 2B), similar to the suppression observed in artificially inoculated plants (Figure 1B). The suppression capability in the third and fourth cycles of

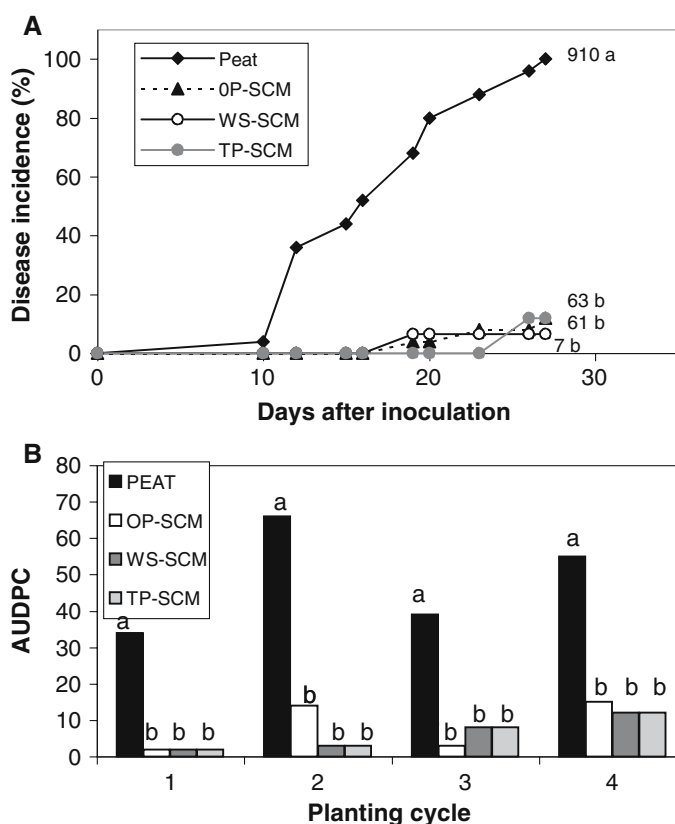


Figure 1. (A) Fusarium wilt suppression by composts. Melon seedlings were artificially inoculated with *Fusarium oxysporum* f. sp. *melonis*, and transplanted in: peat, OP-SCM (orange peel-separated cattle manure), TP-SCM (tomato plant-separated cattle manure), or WS-SCM (wheat straw-separated cattle manure). Numbers on each line indicate area under disease progress curve for the respective treatments. Different letters indicate a significant difference between treatments ( $P = 0.05$ ). (B) Fusarium wilt suppression in melon seedlings by composts during repeated plantings in the same pots. Bars indicate area under disease progress curve (AUDPC) values as percentages of the maximum. Data for the first planting were taken from Figure 1A. Each cycle lasted one month. Different letters above columns indicate significant differences between treatments ( $P = 0.05$ ).

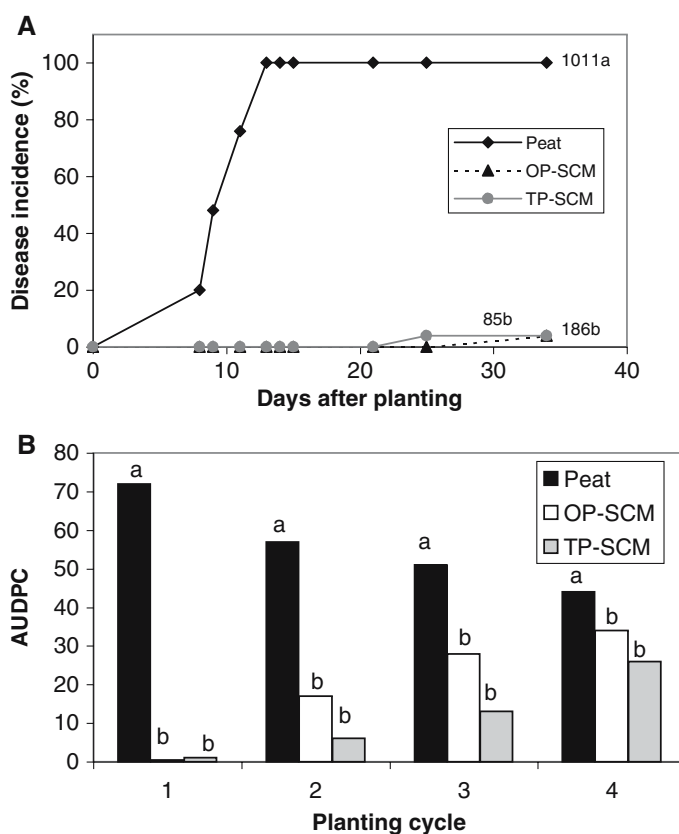


Figure 2. (A) Fusarium wilt suppression by composts. Melon seedlings were transplanted in *Fusarium oxysporum* f. sp. *melonis*-infested media: peat, OP-SCM (orange peel-separated cattle manure) or TP-SCM (tomato plant-separated cattle manure). Numbers on each line indicate area under disease progress curve (AUDPC) for the respective treatments. Different letters indicate a significant difference between treatments ( $P = 0.05$ ). (B) Fusarium wilt suppression in melon seedlings by composts in repeated plantings in the same pots. Bars indicate area under disease progress curve (AUDPC) values as percentages of the maximum. Data for the first planting were taken from Figure 2A. Each cycle lasted 1 month. Different letters above columns indicate significant differences between treatments ( $P = 0.05$ ).

transplanting into infested composts (Figure 2B) was less than that observed with artificial inoculation (Figure 1B).

Similar, albeit less clear patterns of disease suppression by the composts TP-SCM and OP-SCM were observed in the experiment with cucumber seedlings inoculated with *F. oxysporum* f. sp. *radicis-cucumerinum* (Figure 3). Both composts significantly suppressed disease development, compared with its rapid development in the non-suppressive peat, which reached 100% by the ninth day after inoculation. The OP-SCM compost was less effective than TP-SCM.

The suppression capabilities of WS-SCM and OP-SCM composts were also evaluated with *Fusarium* crown and root rot of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici*. The

discolouration indices of the stem cuts on the inoculated tomatoes transplanted into the two composts WS-SCM and OP-SCM were 0.6 and 0.3, respectively, compared with 4.5 in plants grown in peat (Figure 4).

#### Effect of sterilization on disease suppression

The effect of medium sterilization was evaluated in order to assess the role of the microbial activity in two of the composts (TP-SCM and OP-SCM) versus peat in disease suppression. Irradiation reduced the suppression capabilities of the two composts, whereas disease development and final disease incidence in peat were not affected (Figure 5). The reduction in disease suppression after irradiation was accompanied by elimination



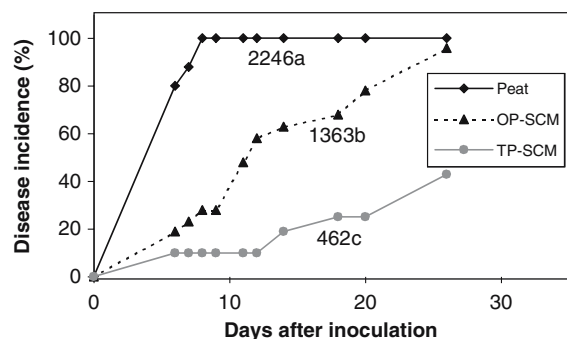


Figure 3. *Fusarium* wilt suppression by composts. Cucumber seedlings were artificially inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, and transplanted in: peat, OP-SCM (orange peel-separated cattle manure) or TP-SCM (tomato plant-separated cattle manure). Numbers on each line indicate area under disease progress curve (AUDPC). Different letters indicate significant differences between treatments ( $P = 0.05$ ).

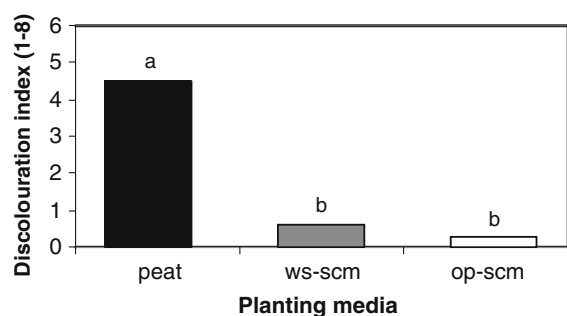


Figure 4. *Fusarium* crown and root rot suppression by composts. Tomato transplants were artificially inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici*, and transplanted in: peat, OP-SCM (orange peel-separated cattle manure) or WS-SCM (wheat straw-separated cattle manure). The discolouration index (on a scale of 1–8) of the stem cuts represents disease severity. Different letters indicate significant differences between treatments ( $P = 0.05$ ).

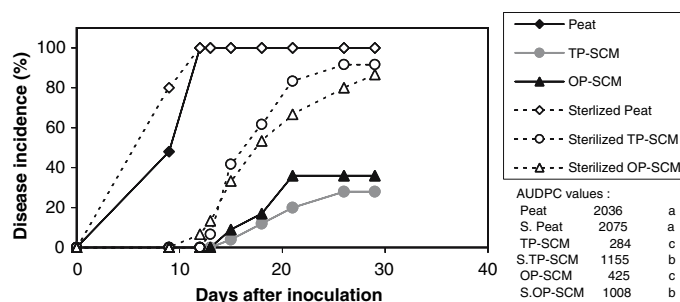


Figure 5. Effect of medium sterilization by gamma irradiation on its suppressive capacity against *Fusarium oxysporum* f. sp. *melonis*. Melon seedlings were artificially inoculated with *Fusarium oxysporum* f. sp. *melonis* and transplanted in: peat, OP-SCM (orange peel-separated cattle manure) or TP-SCM (tomato plant-separated cattle manure). Different letters indicate significant differences between treatments ( $P = 0.05$ ). AUDPC = area under disease progress curve.

Table 2. Disease incidence expressed as area under disease progress curve (AUDPC), and bacterial and fungal counts in tomato plant-separated cattle manure (TP-SCM) compost following gamma irradiation

| Incubation time <sup>a</sup> (h) | AUDPC             | Bacteria<br>g <sup>-1</sup> × 10 <sup>-4</sup> | Fungi<br>g <sup>-1</sup> × 10 <sup>-4</sup> |
|----------------------------------|-------------------|--|---|
| Non-irradiated control           | 96 a <sup>b</sup> | 310 a  | 10  |
| 0                                | 792 bc            | 0.01a  | 0   |
| 16                               | 462 ab            | 4000 b   | 0   |
| 24                               | 110 a             | 7400 c   | 0   |

<sup>a</sup>Numbers of hours of exposure to the open air, after terminating the gamma irradiation.

<sup>b</sup>Values in each column followed by different letters are significantly different ( $P \leq 0.05$ ).

of the fungi and a drastic reduction in the bacterial population (Table 2). Incubating the pots containing the irradiated TP-SCM compost in the greenhouse for 24 h after uncovering and irrigating them resulted in a significant increase in bacterial numbers and restoration of the suppression capability of the compost.

#### Root and stem colonization by *F. oxysporum* f. sp. *melonis*

Melon seedlings grown in infested medium (Figure 2A) were uprooted 4, 8 and 22 days after transplanting. Pathogen colonization of the roots from the composts was significantly lower than that of the roots from peat (Figure 6A). A similar trend was observed with stem colonization (Figure 6B). An unexplained reduction in stem colonization was observed 22 days after transplantation of the seedlings grown in peat

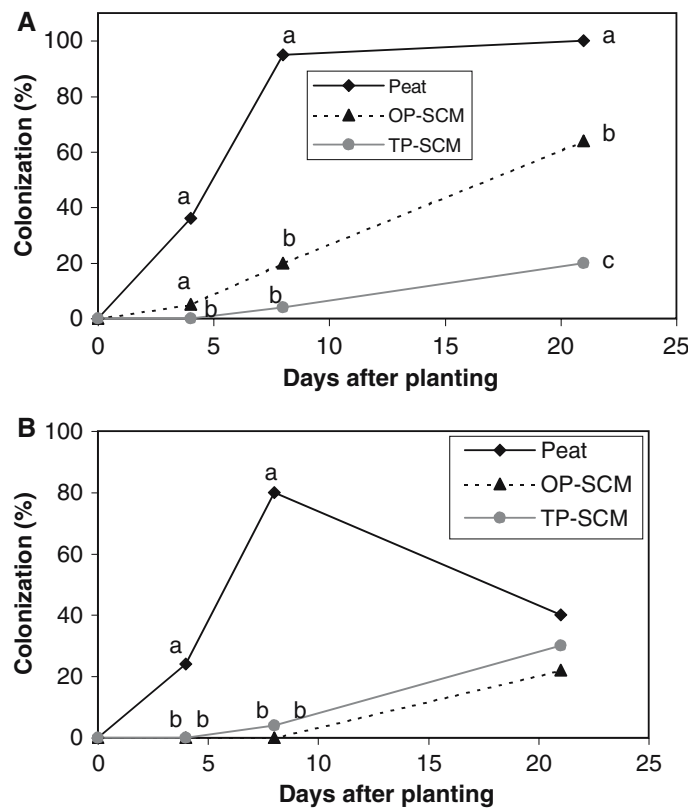


Figure 6. Colonization of melon seedlings by *Fusarium oxysporum* f. sp. *melonis*. Seedlings were grown in infested medium (Figure 2A). They were uprooted 4, 8 and 22 days after transplanting, and colonization by the pathogen was assessed. At each date, different letters indicate significant differences between treatments ( $P = 0.05$ ). A = root colonization; B = stem colonization.

(Figure 6B), as also observed in a previous study (Cohen et al., 2002).

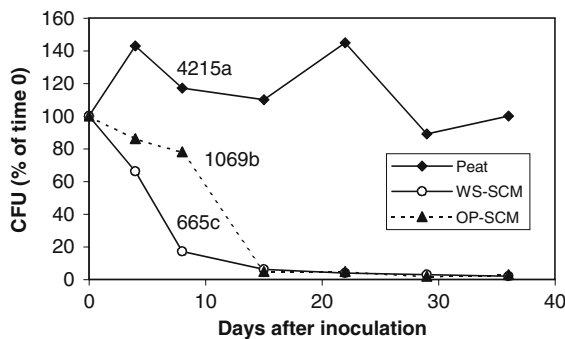


Figure 7. Decline of macroconidial populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* on stems in various organic media: WS-SCM (wheat straw-separated cattle manure), OP-SCM (orange peel-separated cattle manure), and peat. Numbers on each line indicate area under disease progress curve (AUDPC). Different letters indicate a significant difference between treatments ( $P = 0.05$ ).

# Effect of composts on pathogen survival

Conidia of four pathogens were mixed with peat and with the different composts. The populations of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *basilici* declined when mixed with the different composts, while peat did not affect their viability (Figures 7 and 8). Population declines were observed in *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *radicis-cucumerinum* in both peat and composts (Figures 9 and 10). In the experiment with *F. oxysporum* f. sp. *radicis-cucumerinum*, we compared the behaviour of macroconidia taken from the stem with that of microconidia from culture (Figure. 10), and found similar patterns of decline.

# Discussion

The potential of composts to suppress soil-borne pathogens has been demonstrated in a variety of



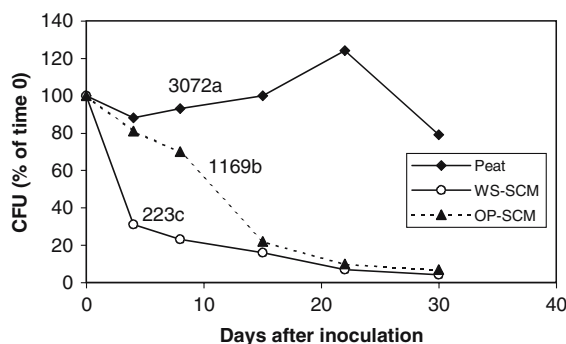


Figure 8. Decline of macroconidial populations of *Fusarium oxysporum* f. sp. *basilici* on stems in various organic media: WS-SCM (wheat straw-separated cattle manure), OP-SCM (orange peel-separated cattle manure) and peat. Numbers on each line indicate area under disease progress curve (AUDPC). Different letters indicate a significant difference between treatments ( $P = 0.05$ ).

studies. The present study showed three composts derived from plant residues suppressing diseases caused by *Fusarium* pathogens, as compared with disease development in the highly conducive peat (Figures 1A, 2A, 3 and 4). The composts clearly suppressed disease elicited by various types of inocula: conidia produced in culture, conidia naturally produced on infected stems, and soil inoculum produced by enriching the soil with infected tissues. Suppression was evident even when the severe root-dip inoculation method was used. In this sense, we consider that under normal production circumstances, when inoculum pressure is less drastic, or when inoculum is weakened (Freeman and Katan, 1988), compost-derived

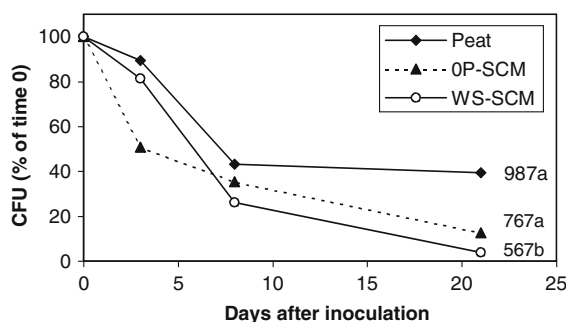


Figure 9. Decline of microconidial populations of *Fusarium oxysporum* f. sp. *melonis* in various organic media: WS-SCM (wheat straw-separated cattle manure), OP-SCM (orange peel-separated cattle manure), and peat. Numbers on each line indicate area under disease progress curve (AUDPC). Different letters indicate a significant difference between treatments ( $P = 0.05$ ).

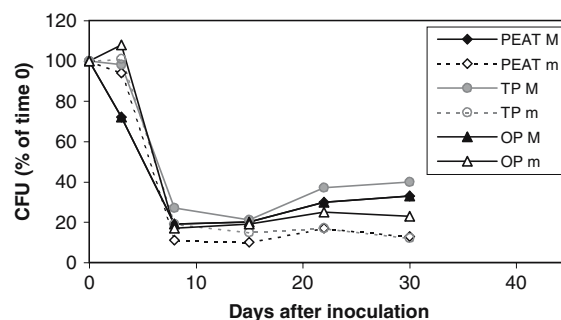


Figure 10. Decline of populations of macroconidia taken from plant stems (M) and of macroconidia from culture (m) of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* in various organic media: TP-SCM (tomato plant-separated cattle manure), OP-SCM (orange peel-separated cattle manure), and peat. Areas under disease progress curve (AUDPC) of the treatments do not differ from each other ( $P = 0.05$ ).

pathogen suppression may serve as a practical control tool. Previous reports of the suppression of *Fusarium* diseases by composts have been focused mainly on a single combination of compost and pathosystem, for example: coffee-waste composts for the control of *Fusarium* wilt in melon plants (Ros et al., 2005), pulp and paper mill (Pharand et al., 2002) or tomato residues (Cheuk et al., 2005) for the control of *Fusarium* crown and root rot in tomato plants, and separated cattle manure for the control of *Fusarium* root and stem rot in cucumber plants (Kannangara et al., 2004) and *Fusarium* crown and root rot in tomato plants (Raviv et al., 2005). In our present study, although different experimental designs were used for each pathosystem, all *Fusarium* pathogens were suppressed by exposure to the same composts, indicating a relatively broad spectrum of effectiveness for each of the tested composts. The TP-SCM compost also suppressed *F. oxysporum* f. sp. *radicis-cucumerinum* in a large-scale commercial experiment, when it was added to perlite at a rate of 25% (Yogev et al., 2006). In addition, WS-SCM and OP-SCM composts also suppressed *Meloidogyne javanica* (Raviv et al., 2005), as well as sclerotial germination and disease incidence with *Sclerotium rolfsii* (Danon and Hadar, unpublished). In a previous study (Reuveni et al. 2002), a compost based on a slightly different combination of raw materials (65% SCM, 20% WS and 15% chicken manure) suppressed *F. oxysporum* f. sp. *basilici*.

The disease-suppressive capability of composts is associated with microbial activity which, in our experiments, was nullified by sterilization (Figure 5). However, this activity resumed within 16–24 h of exposure to the open air, concomitant with bacterial recolonization (Table 2). Apparently, the microbial ‘vacuum’ created by sterilization enabled rapid recolonization by the few surviving bacteria, as well as by those from aerial contamination. Parker and Vincent (1981) found that certain bacterial populations, such as the myxobacteria, *Micrococcus* and *Arthrobacter*, survive gamma irradiation. Microwave treatment rendered a suppressive soil conducive to Fusarium wilt of watermelon (Larkin et al., 1993). The relatively long duration of the suppressive capability, which persisted through four replanting cycles in the present study (Figures 1B, 2B), is another indication of the involvement of microbial activity in this phenomenon. Induced suppressive capability, associated with microbial activity and pathogen suppression, has also been demonstrated in solarized soils (Greenberger et al., 1987).

The decline in pathogen populations (Figures 7–10) was manifested by a decrease in inoculum density. This could stem from a direct effect on the pathogen, e.g., by lysis or predation, and is a potential means of biocontrol by composts. Raviv et al. (2005) also reported a decline in the population of *F. oxysporum* f. sp. *radicis-lycopersici* in a suppressive compost. In the present study, a more pronounced decline of pathogen populations in suppressive composts than in peat was shown with *F. oxysporum* f. sp. *radicis-lycopersici* (Figure 7) and f. sp. *basilici* (Figure 8), but not with f. sp. *melonis* (Figure 9) or f. sp. *radicis-cucumerinum* (Figure 10), indicating that the mechanisms of suppression of different pathogens may differ. Hamid and Alabouvette (1993) showed that the suppressive capability of soil is not necessarily related to the destruction of pathogen in that soil. Thus, use of the pathogen decline to detect suppressive capability in a medium is relevant only when the suppression mechanism involves pathogen elimination. In the present study, disease reduction was accompanied by reduced colonization by the pathogen (Figure 6A, B), which may have resulted from the reduction in inoculum density, from induced resistance in the plant, or both. Reduced tissue colonization by a pathogen has been found by Zhou and Everts (2004),

Martyn and Netzer (1991) and Cohen et al. (2002). In another study (Gamliel and Katan, 1993), suppression of *F. oxysporum* f. sp. *vasinfectum* in a solarized soil was associated with a significant reduction in tissue colonization of cotton by the pathogen. However, Larkin et al. (1993) found colonization of roots by *F. oxysporum* f. sp. *niveum* to be similar in suppressive and non-suppressive mono-cultivated soils. Competition, which leads to a fungistatic effect, is another potential mechanism for suppression. Such a mechanism is not necessarily connected with inoculum decline.

The observations described here regarding suppression of Fusarium diseases associated with several different inoculation methods, over several consecutive growth cycles, as well as the decline in pathogen survival, suggest that several suppression mechanisms are active in these composts. The composting of plant residues not only provides suppressive composts but it also has sanitation value, since composting eliminates surviving pathogens from the infected tissues (Hoitink and Fahy, 1986). Composting of plant residues is also of significant environmental value in that it avoids the need for landfills. It should therefore be recommended as the treatment of choice for wastes such as tomato plants at the end of their growing cycle.

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