

Suppression mechanism of Fusarium wilt of melon caused by
Fusarium oxysporum f. sp. *melonis* by compost

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Abstract

Several composts were prepared under controlled conditions from a mixture of separated cow manure (SCM) mixed with either orange peels (OP), wheat straw (WS), dried tomato plants (TP) or dried pepper plants (PP) that were removed from the greenhouses after the end of the season. The suppression ability of these composts was tested while serving as components of soil-less media for several vegetable crops using four different formae speciales of *Fusarium oxysporum*: *melonis*, *basilici*, *radicis-lycopersici* and *radicis-cucumerinum* (FOM, FOB, FORL and FORC respectively). TP-SCM, OP-SCM and WS-SCM significantly reduced disease development in melon, tomato and cucumber seedlings compared with the fast development of these diseases in conducive peat moss. Disease reduction ranging 41-94% by the composts was evident using 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. Pathogen colonization of the roots and stems of infected melon plants grown in TP-SCM and OP-SCM composts was significantly lower, compared to melon plants grown in peat. Sterilization of TP-SCM and OP-SCM composts by gamma irradiation reduced the suppression ability of the composts, while disease development and final disease incidence in peat were not affected. The populations of the four *Fusarium* forma speciales declined in 60-100% during 35 days when their conidia were mixed with composts. This could stem from a direct effect on the pathogen. The suppression effect of TP-SCM compost was observed during four consecutive replanting cycles of melon seedlings inoculated with FOM and three growing seasons in a commercial cucumber greenhouse, naturally infested with FORC.

TP-SCM and PP-SCM composts were also tested as suppressive media towards the pathogenic bacteria *Clavibacter michiganensis* sub sp. *michiganensis* (CMM) during two growing cycles of tomato transplants inoculated with CMM. The composts reduced CMM significantly (This is reported in this study for the first time).

These data show the great potential of suppressing severe plant diseases by TP-SCM compost. Previous studies on suppression Fusarium diseases by composts have focused mainly on a single combination of compost and pathogen while in the current study all Fusarium pathogens (and in addition CMM and *Botrytis*) were suppressed by exposure to the same compost, indicating a relatively broad spectrum of target pathogens.

The efficient suppression of FORC by TP-SCM compost along three growing seasons could not be explained only by a direct effect of the compost on the pathogen. FORC produces macroconidia which disperse in the air and can penetrate the plant through the foliage, without any contact with the growing medium. Therefore, we examined the possible involvement of induced resistance in this suppression phenomenon.

Suppression of a foliar disease caused by *Botrytis cinerea* served as a tool for assessing the potential involvement of resistance induced by TP-SCM compost in melon and cucumber plants before studying the main target, FOM. Diseased necrotic areas in both cucumber and melon grown on compost were significantly lower by 37-89% than the diseased areas in peat-grown plants both 48 hours and 72 hours after inoculation. A split root system, in which each half of the roots is grown in a different medium and treated differently, enables the evaluation of indirect effects of the medium on the disease through its effect on plant resistance. Assessing the association of induced resistance of *Fusarium* by compost in melon plants required developing a technique of side-grafting that creates two equally-developed undisturbed root

systems, as the traditional technique of separating the roots, failed in melon transplants. In addition, the side-grafting technique enables to evaluate the resistance induced by compost for the entire growing period, without the interference of wound effects. Using this technology we were able to show that 30% of Fusarium wilt reduction in melon plants by compost resulting of induced resistance. In order to understand the induced resistance mechanism the expression of the pathogenesis-related proteins PR-Q, chitinase1 and peroxidase was examined. The expression level of these three PR genes was not elevated when melon plants were grown in the suppressive compost, compared to conducive peat. It therefore appears that the induced resistance in our system is not associated with the three investigated PR genes, but it can be associated with other genes.

The verified observations of the suppression capability of composts demonstrated in this study, lead us to explore whether a sequential addition of compost to soils can elevate their suppressive ability. In organic farming, compost is applied annually in large quantities in order to supply nutrients and improve soil structure. The objective of this study was to assess the level of suppressiveness in soils with a history of organic farming. Fifteen pairs of soil samples were collected from various sites with a history of organic farming with parallel samples of soils farmed conventionally. Each pair was from the same site. The suppressiveness of the soils was evaluated using the melon-FOM pathosystem, following artificial inoculation, in both organically and conventionally managed soils sampled from the same site. In addition, the biological activity of the soils expressed by heat generation was measured by calorimetry. The results indicate suppression of the pathogen in 10 out of the 15 of the organically-managed soils. The biological activity was significantly higher in the organic soils compared with the conventional ones. The heat generation was found to correlate with

the suppression level, suggesting that it may serve to predict the suppressive capability of the soils. Although several significant differences were found between the chemical and physical parameters of the organic and conventional soils (most notably, their organic matter content), these characteristics showed no correlation to their suppressive ability.

The present study demonstrates compost-induced suppressiveness to a wide range of pathogens which is operated by several mechanisms, including induced resistance. It is still an open question whether these mechanisms are ubiquitous to all types of composts.

1 introduction

1.1 Compost

Compost is an organic matter that has undergone long, thermophilic, aerobic decomposition. In the composting process, a mixture of substrates is biodegraded by a mixed microbial community. The temperature rise during composting is a result of a heat generation concurring with microbial metabolic activity. It is determined by the degradability and energy content of the substrates, the availability of moisture and oxygen, and the mode of energy conservation (insulation, convective losses) (Finstein and Moris, 1975; Haug 1993). Many parameters influence the composting process, including: the raw materials and the proportion of each in the mixture, the moisture content of the mixture during the process, aeration, temperature, pH and composting technology. The composition of the microbial populations involved in this process is of special importance. Under optimal conditions, the composting process consists of four phases: (a) an initial warming-up phase (ambient-42°C), which may last only a few hours up to a few days; (b) a thermophilic phase (45-70°C), lasting a few days, several weeks (particularly in food wastes) or even months (particularly if wood wastes are component of the composting mixture); (c) a mesophilic phase, during which mesophile organisms, often dissimilar to those of the first warming-up phase, colonize the substrates; (d) a curing phase which normally last a few weeks (Hoitink and Boehm, 1999; Tuomela *et al.*, 2000). Different microbial populations predominate during the various composting phases. Each of them is adapted to a particular environment (Gray *et al.*, 1971). During curing, microbial activity reaches a steady state, and the rates of organic matter transformation decrease. These changes also reduce compost phytotoxicity (Chefetz *et al.*, 1996). It is important therefore that the composting procedure include a complete curing phase.

Composts can vary according to the raw material used and to the nature of the process. Composts serving as a component of container media must be stable in order to avoid competition between microorganisms and plant roots for oxygen and nitrogen. They must have low salinity, low concentration of phytotoxic substances, and be free of phytopathogenic organisms (Miller and Metting, 1992; Inbar *et al.*, 1993; Epstein, 1997; Raviv, 2005). Composts serving as soil-less media are produced from numerous organic wastes, such as sewage sludge, municipal solid waste, animal excreta, wastes from food industry, such as rice hulls, corn cobs etc. Animal manures are of special value for composting because they contain large, diverse populations of microorganisms, which accelerate the process. Manures are normally generated within agricultural areas where the supply of raw material and demand for the product do not require long-distance hauling (Mandelbaum *et al.*, 1998).

1.2 *Fusarium oxysporum* f. sp. *melonis*

Fusarium is a large class of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprophytes and are relatively abundant members of the soil microbial community. The fungus *Fusarium* belongs to the sub system of the Fungi Imperfecti (Deuteromycotina). A large diversity of formae speciales of *Fusarium oxysporum* causes vascular wilt diseases affecting a great number of hosts (Martyn and Gordon, 1996; Nelson, 1981) and producing serious economic problem. These pathogens can exist for a long time among susceptible crops and in soil with their persistence mainly attributed to the production of long-lived chlamydospores (Newcombe, 1960; Garret, 1970). The optimal soil temperature for penetration and infestation the host is 18-27°C. At higher soil temperature the pathogen can penetrate into the host without causing wilting (Martyn

and Gordon, 1996). There are seven formae speciales attacking cucurbits, and *Fusarium oxysporum* f.sp. *melonis* (FOM) is one of the most important on a worldwide scale (Kim *et al.*, 1993). Fusarium wilt caused by FOM is an important disease in the major melon growing areas (Katan *et al.*, 1994, 1996). *Fusarium* pathogen penetrates the plant in most cases through wounds in the roots zone. Afterwards it proceeds to grow towards the xylem tubes. While in resistant tomato and cotton plants the fungi stop penetrating a few millimeters up to three centimeters from penetrating point, in sensitive plants the fungi succeed in growing and spreading systematically in the plant by disturbing or delaying the resistance mechanisms (Beckman, 1987). In the first stages of the disease the fungi develop slowly by mycelium, which passes from one xylem tube to the next through pits. Later, fungal conidia are carried by the transpiration stream in the xylem tubes. In the next stage the fungi spread into neighboring tissues of the xylem tubes (Nelson, 1981). The disease symptoms are wilting with or without yellowing (Mas *et al.*, 1981). In the two cases, vascular browning can appear in the tubes which the pathogen has penetrated. The browning is a result of oxidation of phenols which are released into the tube as a part of the resistance mechanism against the fungal penetration (Beckman, 1987).

In the current study, three other *Fusarium oxysporum* formae speciales were tested in order to explore the generalist nature of the suppression phenomena: *basilici*, *radicis-lycopersici* and *radicis-cucumerinum*. These three *Fusarium* pathogens produce macroconidia in large quantities on the lower stem which are disperse in the air, in addition to the microconidia which are produced inside the plant tissue. They cause severe economic damage since they spread very quickly in the fields, as opposed to the slower spreading of FOM (Gamliel *et al.*, 1996; Jarvis, 1988; Katan *et al.*, 1997; Punja and Parker, 2000).

1.3 Suppression of soil-borne plant pathogens

Suppressiveness towards soil-borne plant pathogens was defined by Backer and Cook (1974). According to their definition, suppressive soil is a soil in which " the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil". Conducive soil is a soil in which the disease occurs and progresses. Disease suppression can occur in natural soils or can develop in soils or growing media as a result of growing management. Though some argue for a limiting use of the term disease suppressiveness to situations involving a clear biological component (Bruel, 1987), there are ample evidence of the role of both biotic and abiotic elements of the soil in disease suppression. Chemical and physical attributes of soil, including pH, organic matter and clay content can operate in the suppression of plant diseases directly or indirectly through their impact on soil microbial activity. Although these abiotic characteristics of soil can contribute to disease suppression, soil suppressiveness is, directly or indirectly, a function of the activity of soil microorganisms or microbial metabolites (Mazzola, 2002).

Most soils have some natural ability to suppress plant pathogens; this ability is nullified after sterilization, due to the importance of the presence and activity of microorganisms (Baker and Cook, 1974; Baker, 1987, Mazzola, 2002). The concept of disease suppressive soil has been described in terms of general suppression and of specific suppression. General suppression is generated by the overall activity of the microbial biomass, while specific suppression is generated by the activity of one or a few populations (Cook and Baker, 1983; Hoitink and Boehm, 1999; Weller *et al.*, 2002). General suppression is related to the level of microbial activity at critical stages of the pathogen's development, such as germination and pre-penetration growth

in the host rhizosphere. The total microbial biomass competes with the pathogen for carbon and nitrogen and possibly causes inhibition through more direct forms of antagonism (Cook and Baker, 1983). "Specific suppression operates against a background of general suppression but is more qualitative, owing to more specific effects of individual or select groups of microorganisms antagonistic to the pathogen during some stage in its life cycle"(Cook and Baker, 1983).

The microorganisms operating in pathogen suppression do so via diverse mechanisms, including competition for nutrients, antibiosis and induction of host resistance. Non-pathogenic *Fusarium* spp. and fluorescent *Pseudomonas* spp. play a critical role in soils that are naturally suppressive towards Fusarium wilt (Mazzola, 2002).

1.4 Suppression of plant diseases by composts

The suppressive ability of various types of composts in relation to soil-borne pathogens such as *Pythium* sp. and *Rhizoctonia solani* has been demonstrated in many studies (Erhart *et al.*, 1999; Gorodecki and Hadar, 1990; Hadar and Mandelbaum, 1986; Hoitink *et al.*, 1993, 1997; Kwok *et al.*, 1987). Fusarium diseases have been investigated as well (Borreo *et al.*, 2005; Borreo *et al.*, 2006; Chef *et al.*, 1983; Cheuk *et al.*, 2005; Kannangara *et al.*, 2000; Kavroulakis *et al.*, 2005; Raviv *et al.*, 2005; Reuveni *et al.*, 2002; Trillas-Gay *et al.*, 1986). However, the mechanism by which Fusarium is suppressed is not clear. In comparison to the numerous publications on suppressiveness of compost media towards soil-borne fungal pathogens, only a few studies have described suppressiveness towards bacterial plant pathogens (Abbasi *et al.*, 2002; Aldahmani *et al.*, 2005; Schönfeld *et al.*, 2003; Utkhede and Koch, 2004). The suppressive effect of compost as a growing medium on bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (CMM) has not been reported yet.

In many cases it was shown that the suppressiveness effect is the result of the activity of complexes of bacterial and fungal populations in the rhizosphere (Hadar and Mandelbaum, 1986; Hunter *et al.*, 2006, Krause *et al.*, 2003). Sterilization of compost considerably reduces or eliminates its suppressiveness, indicating that the mechanism of disease suppression is predominantly biological (Hadar and Mandelbaum, 1986; Noble and Roberts, 2004, Reuveni *et al.*, 2002; Trillas-Gay *et al.*, 1986). Rapid recolonization by diverse microbial populations soon after sterilization has resulted in resumed suppressiveness (Serra-Wittling *et al.*, 1996), further emphasizing the role of microorganisms in that suppressiveness. However, some residual level of suppressiveness is believed to be attributed to abiotic factors such as pH and C-source (Borrero *et al.*, 2004).

Various mechanisms are attributed to the phenomenon of disease suppression. Most of them are the result of interactions between antagonistic microorganisms and pathogens via competition, antibiosis and hyperparasitism (Hornby, 1990; Hoitink *et al.*, 1993; Messiha *et al.*, 2007; Malandraki *et al.*, 2008). However, according to certain studies, disease suppression by compost also resulted from induced resistance in the plant (Hoitink *et al.*, 1993; Pharand *et al.*, 2002; Kavroulakis *et al.*, 2005).

1.5 Induced resistance

Induced resistance is an activation of latent defense mechanisms that are expressed upon inoculation with a pathogen (Van Loon, 1997). However, it does not provide complete disease control, but rather, reduces lesion size and disease severity (Kuc, 1982). All plants possess active defense mechanisms against pathogen attack. A virulent pathogen can avoid triggering resistance reactions, disturbs them or evades the effects of activated defenses. The disease can be diminished if defense

mechanisms are triggered by a stimulus prior to infection by a plant pathogen. (van Loon, 1997). Induced resistance occurs naturally as a result of limited infection by a pathogen, as in hypersensitive reaction (van Loon *et al.*, 1998). Induced resistance can be triggered by certain chemicals, non-pathogens, avirulent forms of pathogens, incompatible races of pathogens, or by virulent pathogens under certain environmental conditions. Induced resistance can be expressed locally in the same tissue or organ that received the inducing treatment or systemically in a part of the plant spatially separated from the inducing treatment. There are at least three types of induced resistance: systemic acquired resistance (SAR), induced systemic resistance (ISR) and wound-induced resistance. SAR develops systemically in response to, for example, localized necrosis caused by a hypersensitive response or infection by a virulent pathogen. It can also be induced by treatment with certain chemicals, such as 2, 6-dichloroisonicotinic acid (Ryals *et al.*, 1996; Walters, 2009). SAR is mediated by salicylic acid (SA) and is dependent upon the systemic expression of pathogenesis-related-proteins (PRs) (Hammerschmidt, 1999, Pieterse and van Loon, 2007). In contrast, ISR develops as a result of colonization of plant roots by certain strains of plant-growth-promoting rhizobacteria (PGPR) and is mediated by a jasmonate and ethylene-sensitive pathway (Pieterse and van Loon, 2007). Wound induced resistance is typically elicited upon tissue damage, such as that caused by insect feeding (Stout, 2007).

Accumulation of SA occurs both locally and, at lower levels, systemically, accompanied by the development of SAR. Exogenous application of SA also induces SAR in several plant species (Ryals *et al.*, 1996; Van Loon and Antoniw, 1982). Several families of PRs are associated with SAR. Induction of PRs is regularly linked to necrotizing infections and is considered a marker of the induced state (Kessmann *et*

al., 1994; Vernooij, 1994). Some of these PRs are β -1,3-glucanases and chitinases which are capable of hydrolyzing fungal cell walls. Other PRs have more weakly characterized antimicrobial activities or unknown functions. The level of SAR can be modulated by ethylene and jasmonic acid (JA) (Sticher *et al.*, 1997; van Loon and Antoniw, 1982; Xu, *et al.*, 1994). These results suggest that induction and expression of SAR are regulated through interplay of several signaling compounds.

Induced systemic resistance is associated with certain strains of PGPR. ISR can be induced by several fluorescent *Pseudomonas* species or by various strains of *Bacillus*. Like SAR, it acts unspecifically against taxonomically different pathogens (Pieterse *et al.*, 2001; van Loon *et al.*, 1998; Walters, 2009). Pieterse *et al.* (2001) reported that rhizobacteria-mediated ISR follows a novel signaling pathway in which components from the jasmonate and ethylene response are engaged successively to trigger a defense reaction that, like SAR, is regulated by the NPR1 gene, but is independent of salicylic acid accumulation. Another characteristic of induced resistance that is common to both SAR and ISR is the phenomenon of priming, in which plant defenses are not directly activated by the inducing agent but instead are potentiated for enhanced expression upon a subsequent pathogen attack (Benhamou *et al.*, 2000; Beckers and Conrath, 2007; Walters, 2009). In the last decade many studies were carried out in order to understand the induce resistance mechanism due to the need for exploring environmentally friendly disease control agents. Such agents include an analogue of SA, acibenzolar-S-methyl (ASM), which is known to induce SAR in a range of plant species (Leadbeater and Stoub, 2007).

In order to unequivocally determine that induced resistance is involved with disease suppression, the site of treatment and the site of inoculation should be spatially separated. This is relatively easy with foliar diseases, but problematic with soil-borne

diseases, in which the pathogen and the root system are in close proximity in the rhizosphere. A possible solution to this problem is creating temporal separation. One approach to achieving temporal separation is growing the seedlings in a specific medium in the nursery and inoculating them later, after transplanting them into a uniform medium. Another approach is the use of a split root system, in which each half of the roots is grown in a different medium and treated differently, thus enabling to evaluate the indirect effect of the medium on the disease through its effect on plant resistance (Khan *et al.*, 2004; Lievens *et al.*, 2001; Zhang *et al.*, 1996; Zhang *et al.*, 1998).

1.6 Suppression of plant diseases in organically managed soils

Organic farming (OF) is characterized by using environment friendly pesticides and by improved soil traits as a result of annual application of compost. The addition of composted organic amendments to field soils has been shown to reduce the severity of soil-borne diseases (Chellemi *et al.* 2004; Lewis and Lumsden, 1992; Ros *et al.*, 2005; Rotenberg *et al.*, 2007a,b; Stone *et al.*, 2003 van Bruggen, 1995). The efficiency of a particular compost amendment is mediated by the complex interactions between biotic and abiotic factors; therefore, the utility of adding compost to soils is unpredictable (Rotenberg *et al.*, 2007b).

Van Bruggen (1995) indicated that there was lower incidence of corky root rot disease in organically managed farms. Sterilization of the organic soils by irradiation increased the disease level, suggesting the involvement of biological processes. Knudsen *et al.* (1999) found that organically-managed soils suppressed the incidence of brown foot rot of cereals caused by *Fusarium culmorum* in comparison with the level of the disease in conventionally-managed soils. Liu *et al.* (2007) reported that

soils from organic farms were more suppressive to southern blight caused by *Sclerotium rolfsii* than soils from conventional farms. Soil microbial respiration in the organic soils was higher than in the conventional soils, indicating that microbial activity was greater in these soils.

Van Bruggen and Semenov (2000) defined a healthy soil as a stable system with resilience to stress, high biological diversity and high levels of internal nutrient cycling. Van Diepeningen *et al.* (2005) found that on the average, organically managed soils are more stable systems with better soil health as defined by van Bruggen and Semenov (2000). Moreover, van Diepeningen *et al.* (2005) reported that organically-managed soils exhibited greater biological activity than conventionally-managed soils as was found before by Mäder *et al.* (2002). In contrast, the chemical and physical parameters showed only few differences.

So far, no studies were carried out in the special environmental conditions of Israel in order to explore the influence of organic management on the suppression ability of soils. The numerous evidence of the suppression capability of the composts tested in this study motivated me to test whether organic management contributes to soil suppressiveness.

1.7 Aims

The general objective of the present work was to corroborate the suppressive ability of waste-based composts towards soil-borne diseases caused by four different formae speciales of *Fusarium oxysporum* and to study the suppressiveness mechanism in the *Fusarium* of melon pathosystem. The study was expanded to assess the generality of suppressiveness: A. The suppressive ability of compost was tested in a commercial cucumber greenhouse with *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (FORC).

B. The suppressive ability of organically managed soils was tested in order to determine whether the annual addition of compost to the soils can contribute to their suppressiveness. C. The suppressive ability of compost was evaluated in the system of bacterial canker of tomato caused by *Clavibacter michiganensis*. The specific aims of the study are listed below and they were addressed in chapters 1, 2, 3, 4 and 5 respectively.

1. To characterize the suppressiveness of waste-based compost towards *Fusarium Oxysporum* f. sp.: *melonis*, *basilici*, *radicis-lycopersici* and *radicis-cucumerinum*.
2. To study the suppression mechanism of waste-based compost towards *Fusarium oxysporum* f. sp. *melonis*.
3. To study whether the suppression phenomenon can occur under commercial growing conditions with FORC in soil-less media.
4. To assess the level of suppressiveness and soil activity in soils with a history of organic farming.
5. To check whether the suppression phenomenon can occur in relation to the virulent bacterial pathogen *Clavibacter michiganensis*. subsp. *michiganensis*.

2 Methods

2.1 Suppression of Fusarium wilt pathogens by waste-based compost

2.1.1 Compost preparation

Three plant wastes: orange peels (OP), wheat straw (WS) and tomato plants residues (TP) that were removed from greenhouses after the end of the season, were chopped and mixed with separated cow manure (SCM) at a volumetric ratio of 1:1 and composted in controlled chambers. The three mixtures were composted in insulated 8-m³ bins, using forced aeration in order to prevent the piles from reaching excessively high temperatures, as previously described (Raviv *et al.*, 1998b). Aeration rate was 400m³h⁻¹ per bin. Temperature of 60°C was used as a set point, as it is considered the optimal temperature for composting raw materials not containing human pathogens (Finstein and Hogan, 1993). When the compost temperature fell below the set point, the composts were aerated for 1 minute per hour in order to maintain aerobic conditions at all times. Temperature was measured continuously and recorded every 5 minutes using 2 sensors located at a depth of 40 and 80 cm. Daily temperature averages were calculated for each pile. Moisture content (MC) was measured twice weekly. MC was maintained at 50 to 60% during the thermophilic period and at 40 to 50% afterwards by adding water as necessary, without reaching the point of leaching. The composts were turned twice using a front-end loader to improve their homogeneity. In general, temperatures rose to 60°C 2 to 3 days from the start of composting and stayed at this level for about 80 days; temperatures were rarely above 60°C during this phase. Temperatures started to decline afterwards and reached ambient level in all piles 120 days from the start of composting. The composts were then left for additional curing period of 30 days.

2.1.2 Preparation of plant growing media

OP-SCM, WS-SCM and TP-SCM compost were used. Peat (PlantoBalt, Riga, Latvia) served as a conducive control medium. Before testing, both organic components were mixed with perlite #4 (Habonim, Israel), at a 1:1 volumetric ratio, and were potted into 0.25 liter pots.

2.1.3 Evaluating compost effect on *Fusarium* diseases suppression

Four different formae speciales of *Fusarium oxysporum*: *melonis*, *basilici*, *radicis-lycopersici* and *radicis-cucumerinum* (from Prop. J. Katan collection) were used in the study. Disease suppression was evaluated using pot-based assays in a controlled greenhouse. Seedling inoculation was carried out by dipping their roots in conidial suspension at various concentrations as indicated for each experiment, or by planting them in inoculated media. As controls, peat and two of the composts (TP-SCM and OP-SCM) were sterilized by gamma irradiation in the nuclear reactor of Nahal Sorek, Israel at 2.5 Mrad. All greenhouse trials were arranged in a randomized block design and included at least 5 replicates. *Fusarium* wilt incidence was evaluated periodically. All experiments were carried out twice or more with similar results. In all experiments, non-inoculated plants remained healthy.

2.1.4 Evaluating compost effect on FORC suppression in soilless media in a commercial greenhouse

We used a commercial cucumber greenhouse in Baqa al-Gharbiyye, which was heavily infested (about 40% dead plants) with *F. oxysporum* f.sp. *radicis-cucumerinum* (FORC) in spring 2004. The greenhouse space was disinfected by solar heating while sealed for two months in summer from 1.7.04 to 1.9.04 without

ventilation. The temperatures measured inside the greenhouse were as follows: air temperature 22-62°C and media temperature 22-72°C while outside air temperature was 21-37°C. In addition to the solar treatment, the greenhouse space was sprayed with 2% NaOCl before the initiation of the experiment. Cucumber (*Cucumis sativus* cv. L. cv. *Seminis Turbo hybrid*) transplants were planted in 70 liter perlite #4 (HaBonim, Israel) boxes in two rows, 2400 plants per 1000 m³, in three consecutive seasons, without sterilization between seasons. The experiment included three treatments: 100% perlite, 50% TP-SCM compost: 50% perlite (volumetric) and 25% TP-SCM compost: 75% perlite. Each treatment included 35 plants in five replicates. The cucumber plants were planted in the same boxes in two consecutive seasons. In the second season we added a new treatment: infested perlite (about 30% dead plants, from the first season) was mixed with compost at a 1:1 volumetric ratio. The infection of the cucumber plants was natural, as FORC produces large amounts of macroconidia which spread out in the air. Dead plants were counted according to disease incidence.

2.1.5 Fusarium pathogens survival

Conidia of the above four *Fusarium* pathogens were suspended in water and were mixed with the tested media: three composts or peat. Assessment of *Fusarium* populations was carried out using the dilution method with a modified peptone PCNB medium (Gamliel and Katan, 1991).

2.1.6 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 modified peptone PCNB medium and incubated for 5 days at 27°C.

2.2 Studies of the compost suppression mechanism

2.2.1 Evaluating induced resistance mechanism by using a foliar pathogen:

Botrytis cinerea

Cucumber (*Cucumis sativus* cv. Kfir, "Zeraim Gedera", Israel) and melon (*Cucumis melo* cv. Ofir, "Zeraim Gedera", Israel) seeds were sown in peat or compost based media, in 0.5 L pots. When the plants reached the first leaf stage (ca. two weeks after sowing), detached leaves or intact plants were inoculated with 5 mm discs of four days old culture of *B. cinerea* **B16** grown on 0.5 standard concentration of potato dextrose agar. Two inoculum discs were placed on each leaf, one leaf per intact plant in five replicates of five plants. The intact plants were covered with polyethylene sheets to maximize the relative humidity and the detached leaves were kept in sealed boxes containing wetted paper sheets, eight leaves per box, in five replicates of one box each, at 25°C, for 2-3 days. The area of the expanding necrotic diseased tissue was measured. The experiments were carried out twice.

2.2.2 Induced resistance – the nursery transfer system

Melon seeds (cv. Ofir) were sown in peat or compost. Seven days later, the seedlings were removed, their roots were washed and dipped for 2 min in a conidial suspension containing 3×10^5 conidia ml⁻¹ and then transplanted into 0.25-L pots with a peat medium. The temporal separation between the germination-emergence stage, in which

the seedlings are grown in different growing media, and the inoculation stage, enables us to assess the carry-over effect of the tested growing media on disease suppression. Each treatment consisted of eight pots with five inoculated seedlings each. In addition, two pots with non-inoculated seedlings were used as control. This experiment was carried out three times with similar results.

2.2.3 Induced resistance - the side-grafting system

Twenty-five day old melon transplants were side-grafted and clipped, as described in Figure 2.2.1. Seven days later, one of the two stems was removed in order to obtain one plant with two intact, separate root systems. After seven additional days, the induced resistance test was initiated. The root systems of each plant were planted in two separate pots, containing either peat or compost, in three combinations: peat-peat, peat-compost and compost-compost. Ten days later, one root system of each plant was inoculated by pouring 10 ml of conidial suspension, at the various concentration (0.1×10^5 , 0.25×10^5 , 0.5×10^6 or 1.0×10^6 conidia, ml^{-1}), into each pot. Pots treated with 10 ml of distilled water served as non-inoculated controls. Each combination of a certain medium and inoculation concentration consisted of 30 plants. The plants were grown in a temperature-controlled greenhouse for 45 additional days. Disease incidence was periodically recorded.

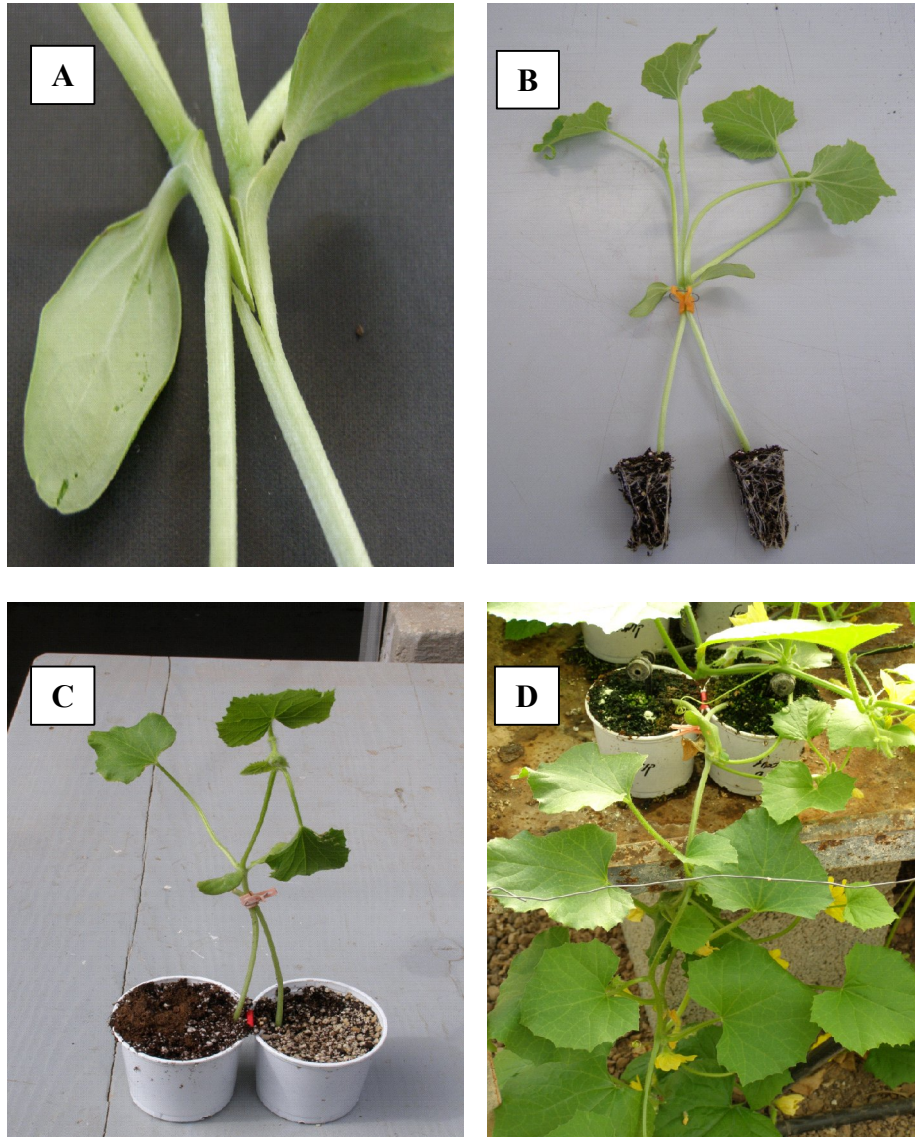


Figure 2.2.1. Side-grafting procedure and growing of grafted plant. **A.** Diagonal cuts on the stems of each seedling, 3-4 cm above the roots and combining the two stems. **B.** Clipping **C.** Planting into two separate pots. **D.** Grafted melon plant 45 days after grafting.

2.2.4 Isolation of RNA and expression of PR genes

Total RNA was extracted from 50 mg of 21 day old plants. Leaf discs were collected from leaf number 2 from the top, using Tri-reagent (Sigma) according to the manufacturer's protocol. RNA concentration and quality were determined spectrophotometrically measuring A_{260} and A_{280} . Equal amounts (5 μ g) of total RNA

served as templates for the RT reaction. RT-PCR analyses were performed to examine the expression of three PR genes in leaves of the healthy plants grown either on peat or on compost with an initial denaturation step of 94°C for 3 min followed by 35 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 55-64°C), and extension (45 sec at 72°C). After the last cycle, a final extension was carried out for 10 min at 72°C. The following primers, designed according to the publicly available melon database (<http://www.icugi.org/>), were used:

PR-Q 5'-GCAGGCCAATGGAGCTCT-3' and

5'-TCTTTAACTCTATCATCTGGGCCA-3', forward and reverse, respectively.

Chitinase1 5'-CGACCCTTTCCATCTTCTTCCT-3' and

5'-TCCTGAGCTAGTACATCCCAGAACT-3', forward and reverse, respectively.

Peroxidase 5'-CTCTTCCGCTCAACTCTCCG-3' and

5'-TCTCGATCGTCCAAATGTATGC-3', forward and reverse, respectively.

On the same sets of cDNA samples, another RT-PCR analysis was performed for the housekeeping gene *tubulin*, serving as an internal probe for mRNA level. This reaction was performed with primers 5'-CCGCAGACAAGCGTTCCAAAA-3' and

5'-TGATTCGGGATGAGGGAGA-3', forward and reverse, respectively.

2.3 Suppression of Fusarium wilt of melon in organically-managed soils

2.3.1 Sample collection

Soil samples were collected from pairs of 15 farms (Kibbutzim) that grow similar crops under both conventional and organic management in adjacent plots, having similar soil properties. The plots are located in various regions in Israel (Figure 2.3.1). The pedological definitions of the soils appear in Table 3.4.1. Ten soil samples of

about 1 kg each were collected randomly from each plot. The samples from each plot were mixed and checked for soil properties. All the organic fields had a history of at least three years after conversion to organic farming. Cropping history of at least five years is known for all the plots.

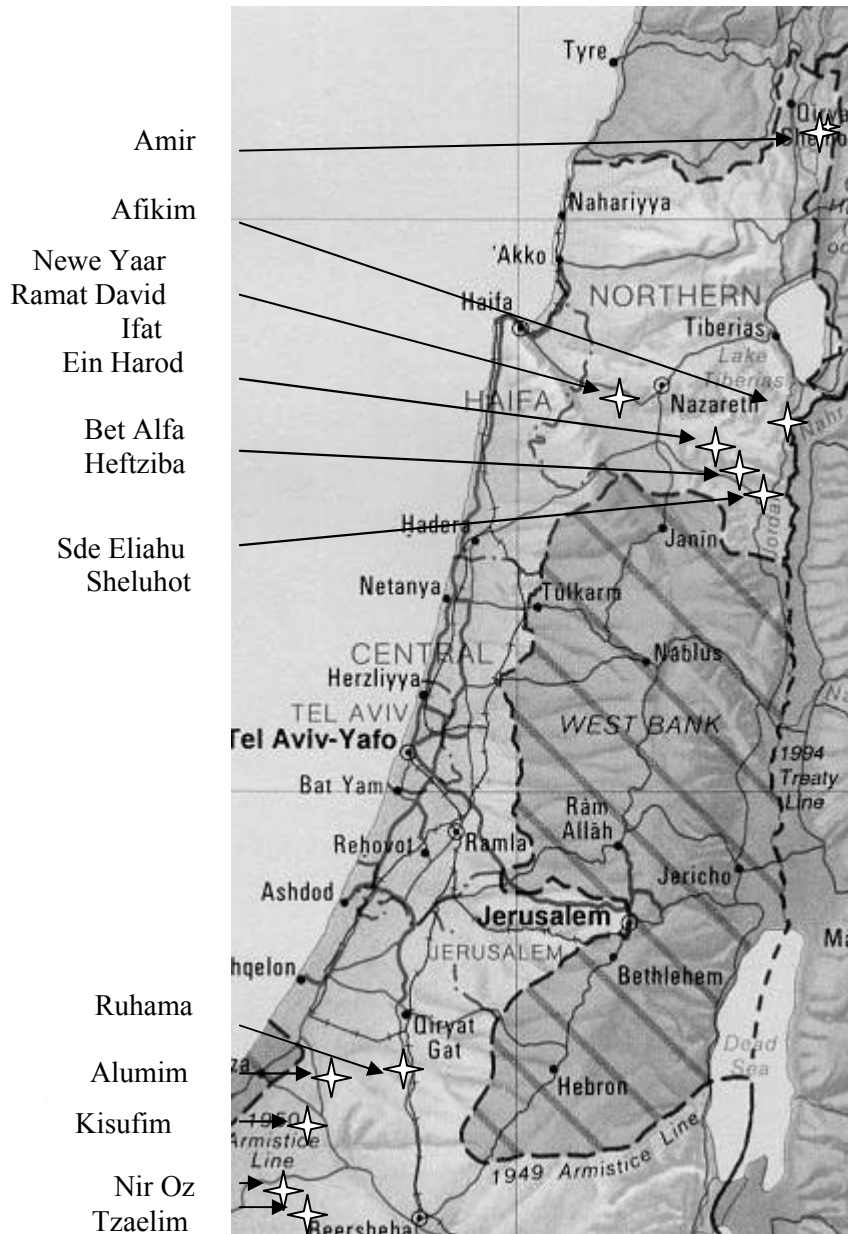


Figure. 2.3.1. Sampled field's location. Each site consists of similar organic and conventional plots.

2.3.2 Soil characteristics

The organic matter was determined by means of wet oxidation in potassium dichromate and sulfuric acid, the so-called Walkley and Black method (Allison, 1965). N-NH₄ was tested by extracting the ammonium from the soil with KCL, and using the colorimetric method (Mulvaney, 1996). N-NO₃ was measured in 1:10 water extract by UV difference (Mulvaney, 1996). P was determined colorimetrically according to the Olsen method, (Olsen, 1965). K content was determined by flame-photometric method from saturated soil paste extract, and EC was measured from a saturated soil paste extract too (Bower and Wilcox, 1965). pH was measured directly in the saturated soil paste using a special electrode with a single-pore capillary reference junction and a spear tip (Eutech instruments, EC-620-133). The distribution of soil particle size was determined by the hydrometer method (Day, 1965).

2.3.3 Evaluating disease suppression

Disease suppression was evaluated with pot-based assays using melon seedlings (*Cucumis melo* cv. Ofir). Seeds of melons were planted in sand, and 6 days later the seedlings were removed, their roots were washed, cut to 2 cm and dipped for 2 min in a conidial suspension that contained 1.5×10^5 conidia ml⁻¹. The seedlings were then transplanted into the tested soils or peat (Plantobalt, Riga, Latvia). The tested soils were mixed with perlite #4 (HaBonim, Israel), at a 1:1 volumetric ratio in order to improve water infiltration through the pots.

Ten 0.25 L pots were filled with each soil. Eight pots were planted with inoculated seedlings and two were planted with non-inoculated seedlings. Five seedlings were planted in each pot. The soils were tested in four trials and ten more pots with peat

served as a control for the virulence of the pathogen in each trial. The trials were arranged in a randomized block design.

Disease incidence was expressed as percentage of the diseased plants and as AUDPC. When indicated, it was calculated as percentage of the values of AUDPC of peat treatment in the same trial (peat was used for standardization). In all experiments, the non-inoculated plants remained healthy. In order to show the effect of the organic management, the suppression index was used:

$$\text{Fusarium wilt suppression index} = 1 - \frac{\text{AUDPC in organic soil}}{\text{AUDPC in conventional soil}} \times 100$$

Values above 0 indicate that organic management induced soil suppressiveness.

2.3.4 Calorimetric assay

Water content at field capacity of the soils was determined according to Cassel and Nielsen (1986). The soil samples were wetted to 70% of field capacity, 24 hours before use. The microcalorimeter used was CSC 4100, (Calorimetry Science Corp., Utah, U.S.A). The calorimeter temperature was set to 25°C. Fifty µl of water were added to the test ampoules while the reference ampoule was left empty at all times. After reaching a stable baseline, samples equivalent to 200 mg dry weight of wetted soil were added to the test ampoules. After reaching a stable heat release line, 0.2 mg glucose and 0.2 mg ammonium sulfate, dissolved in 50 µl water were added to the soil. The height line above the baseline of the soil and the time required for reaching the heat release peak after adding the solution were determined (Medina *et al.*, 2009). All the tests were carried out in at least 3 replicates. The values of the heat release express the biological activity of the soils. In order to show the effect of the organic management, the biological activity index was used:

$$\text{Biological activity index} = \frac{\text{Biological activity in organic soil}}{\text{Biological activity in conventional soil}}$$

Values above 1 indicate higher activity in the organic soils.

2.3.5 Statistical analyses

Disease development and pathogen decline were expressed by the area under disease progress curve AUDPC (Shaner and Finney, 1977), which was calculated as the integral of the area under the disease progress curve by using PSI-Plot software (Poly Software International, New York, NY). Disease development was also expressed as the percentage of diseased plants. The values of percentage of colonization were arcsinus-transformed prior to calculation of significance in order to stabilize the variances. All values were analyzed using Tukey - Kramer honestly significant difference test ($p = 0.05$). A Two Ways ANOVA test was used for the organic and conventional soils ($p = 0.05$).

3. Results

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

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 CO₂ 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 m³ bins, with forced aeration to prevent overheating, as previously described (Raviv et al., 1998b). The aeration rate was 400 m³ h⁻¹ 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 (Finstein and Hogan, 1993). When the compost temperature fell below the set point, the composts were aerated for 1 min h⁻¹ 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 H₂SO₄ overnight at room temperature, after which 1 ml of H₂O₂ 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⁺, NO₃⁻, NH₄⁺, PO₄⁻³) 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^a

	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 ^b	6.1	6.8	7.1	7.1
EC (dS m ⁻¹) ²	0.75	7.74	4.43	5.62
N-NO ₃ ⁻ (mmol _c l ⁻¹) ²	9.8	164.4	102.7	137.0
N-NH ₄ ⁺ (mmol _c l ⁻¹) ²	3.56	19.5	18.8	3.0
P-PO ₄ ⁻³ (mmol _c l ⁻¹) ²	2.46	6.80	4.38	3.70
K ⁺ (mmol _c l ⁻¹) ²	25.5	379	209	155
BOD (g O ₂ Kg DM ⁻¹ day ⁻¹) ^c	2.2	4.7	3.0	3.3

^aWS-SCM = wheat straw + separated cattle manure;

OP-SCM = orange peels + separated cattle manure;

TP-SCM = tomato plants + separated cattle manure.

^bIn a 10:1 aqueous extract.

^cBiological 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⁻¹).

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 3×10^5 conidia ml⁻¹, 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 5×10^5 g⁻¹ 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

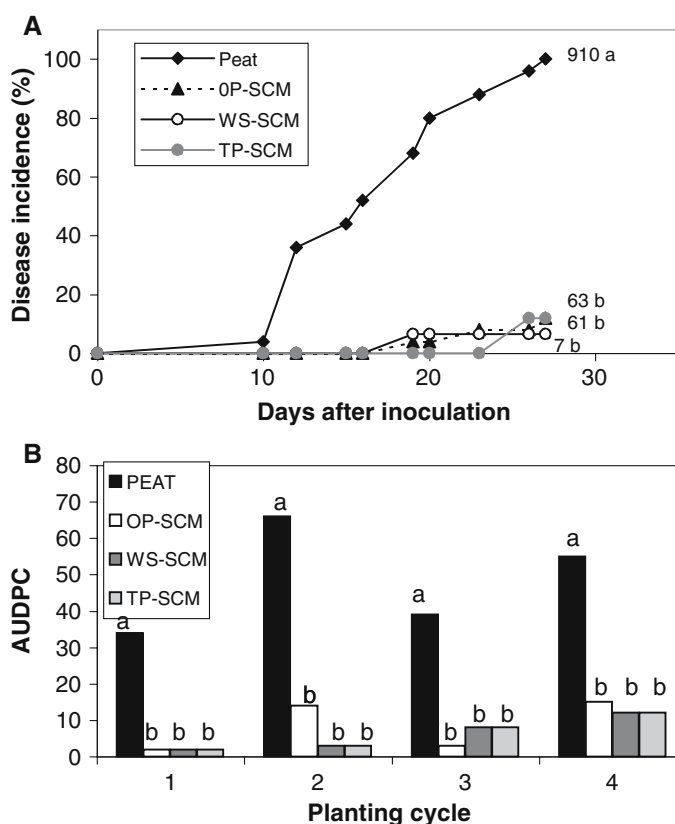


Figure 1. (A) Fusarium wilt suppression by composts. Melon seedlings were artificially inoculated with *Fusarium oxysporum* f. sp. *melonis*, and transplanted in: peat, OP-SCM (orange peel-separated cattle manure), TP-SCM (tomato plant-separated cattle manure), or WS-SCM (wheat straw-separated cattle 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 ($P = 0.05$).

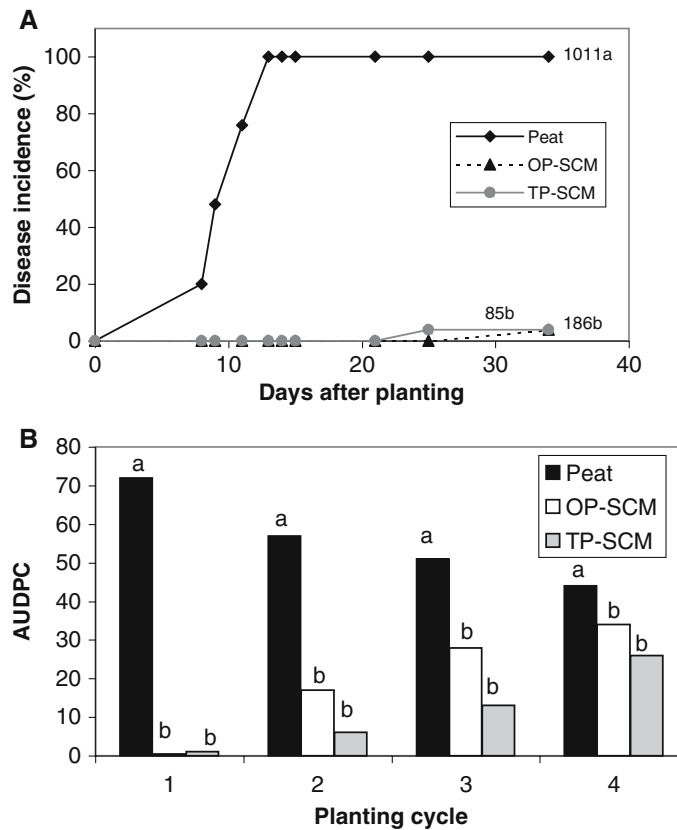


Figure 2. (A) Fusarium wilt suppression by composts. Melon seedlings were transplanted in *Fusarium oxysporum* f. sp. *melonis*-infested media: peat, OP-SCM (orange peel-separated cattle manure) or TP-SCM (tomato plant-separated cattle manure). Numbers on each line indicate area under disease progress 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

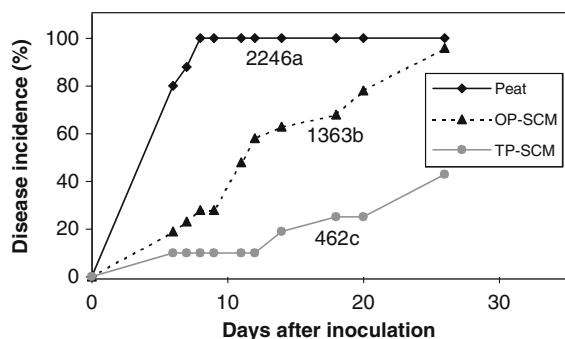


Figure 3. *Fusarium* wilt suppression by composts. Cucumber seedlings were artificially inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, and transplanted in: peat, OP-SCM (orange peel-separated cattle manure) or TP-SCM (tomato plant-separated cattle manure). Numbers on each line indicate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

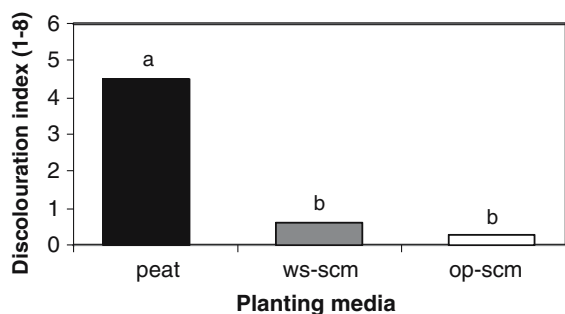


Figure 4. *Fusarium* crown and root rot suppression by composts. Tomato transplants were artificially inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici*, and transplanted in: peat, OP-SCM (orange peel-separated cattle manure) or WS-SCM (wheat straw-separated cattle manure). The discolouration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

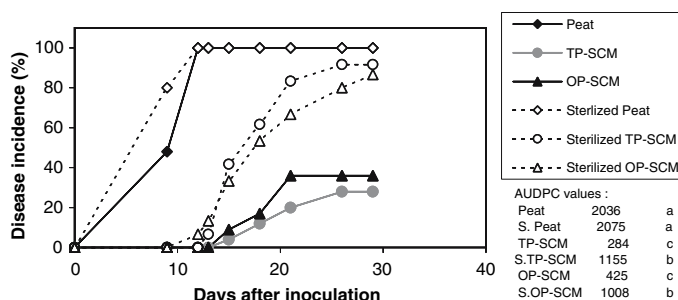


Figure 5. Effect of medium sterilization by gamma irradiation on its suppressive capacity against *Fusarium oxysporum* f. sp. *melonis*. Melon seedlings were artificially inoculated with *Fusarium oxysporum* f. sp. *melonis* and transplanted in: peat, OP-SCM (orange peel-separated cattle manure) or TP-SCM (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 ^a (h)	AUDPC	Bacteria $g^{-1} \times 10^{-4}$	Fungi $g^{-1} \times 10^{-4}$
Non-irradiated control	96 a ^b	310 a	10
0	792 bc	0.01a	0
16	462 ab	4000 b	0
24	110 a	7400 c	0

^aNumbers of hours of exposure to the open air, after terminating the gamma irradiation.

^bValues 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

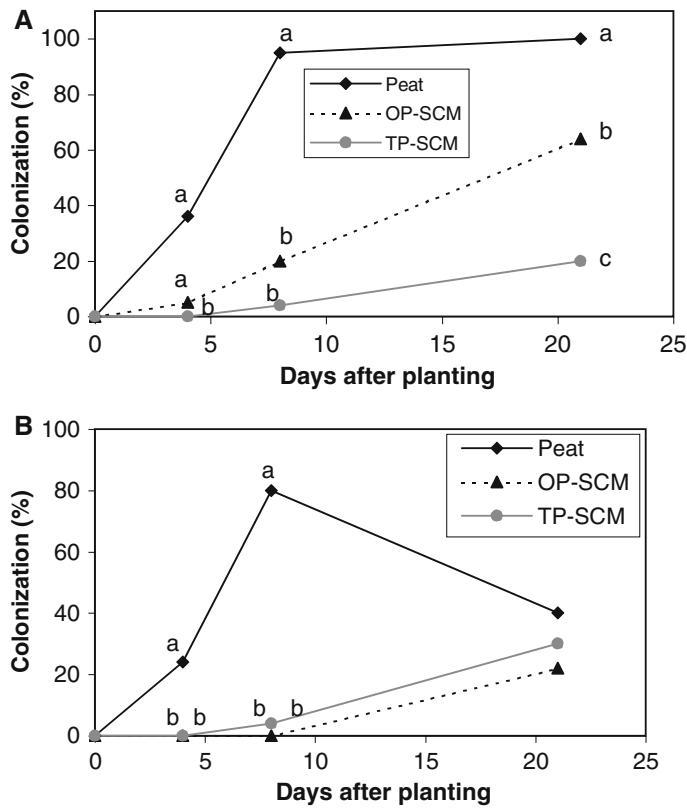


Figure 6. Colonization of melon seedlings by *Fusarium oxysporum* f. sp. *melonis*. Seedlings were grown in infested medium (Figure 2A). They were uprooted 4, 8 and 22 days after transplanting, and colonization by the pathogen was assessed. At each date, different letters indicate significant differences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

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

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.

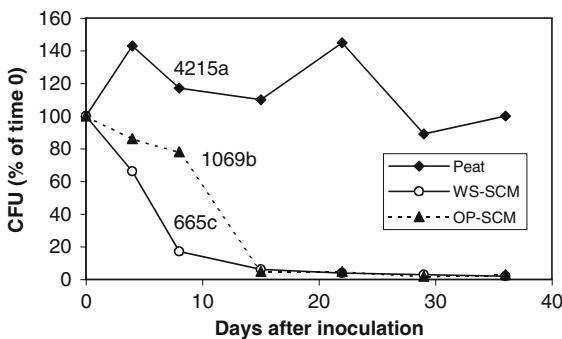


Figure 7. Decline of macroconidial populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* on stems in various organic media: WS-SCM (wheat straw-separated cattle manure), OP-SCM (orange peel-separated cattle manure), and peat. Numbers on each line indicate area under disease progress curve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

Discussion

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

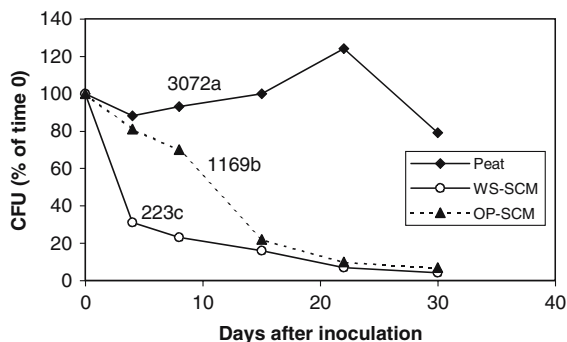


Figure 8. Decline of macroconidial populations of *Fusarium oxysporum* f. sp. *basilici* on stems in various organic media: WS-SCM (wheat straw-separated cattle manure), OP-SCM (orange peel-separated cattle manure) and peat. Numbers on each line indicate area under disease progress curve (AUDPC). Different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

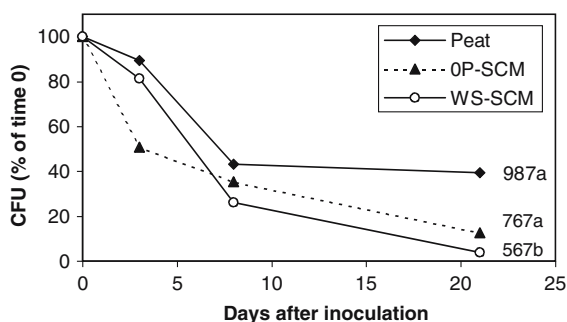


Figure 9. Decline of microconidial populations of *Fusarium oxysporum* f. sp. *melonis* in various organic media: WS-SCM (wheat straw-separated cattle manure), OP-SCM (orange peel-separated cattle manure), and peat. Numbers on each line indicate area under disease progress curve (AUDPC). Different letters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

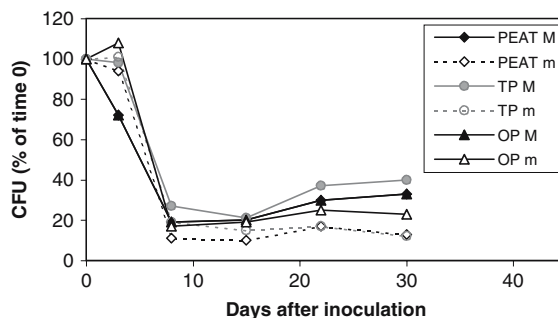


Figure 10. Decline of populations of macroconidia taken from plant stems (M) and of macroconidia from culture (m) of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* in various organic media: TP-SCM (tomato plant-separated cattle manure), OP-SCM (orange peel-separated cattle manure), and peat. Areas 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.

Acknowledgments

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3.2 Compost induces resistance against foliar and root diseases of Cucurbits: the grafting approach

Suppression of a foliar disease caused by *Botrytis cinerea* served as a tool for assessing the potential involvement of induced resistance by TP-SCM compost in melon and cucumber plants before studying the main target, *Fusarium oxysporum* f. sp. *melonis*. The method used for FOM was 'split root system', so that each half of the roots is grown in a different medium and treated differently. This enabled the evaluation of indirect effects of the medium on the disease through its effect on plant resistance (Khan *et al.*, 2004; Lievens *et al.*, 2001; Zhang *et al.*, 1996; Zhang *et al.*, 1998). Assessing the association of induced resistance to *Fusarium* by compost in melon plants required developing a technique of side-grafting that creates two equally-developed undisturbed root systems, as the traditional technique of separating the roots as reported above, was not effective in melon transplants. In addition the side-grafting technique enables to evaluate the resistance induced by compost for the entire growing period, without the interference of wound-induced effects. The expression of PRs, *PR-Q*, *chitinase1* and *peroxidase* was also examined in this study. These three genes were found in source leaves of CMV-infected melon plants (L. Gil and S. Wolf, unpublished). The aim of the work presented in this chapter was to confirm the involvement of the induced resistance mechanism of composts towards *Fusarium oxysporum* f. sp. *melonis* using the side-grafting technique and by examining the expression of three regulation genes.

3.2.1 Effect of growing media on severity of disease caused by *B. cinerea*

Leaves of cucumber and melon plants that were germinated and grown for 14 days on either a peat medium or a compost medium were inoculated with *B. cinerea* (Figures 3.2.1, 3.2.2). Diseased necrotic areas in both cucumber and melon grown on compost were significantly lower by 37-89% than the diseased areas in peat-grown plants, both 48 hours (Figures 3.2.3A, 3.2.3B) and 72 hours after inoculation (Figures 3.2.4A, 3.2.4B). Disease suppression by compost was evident both in detached leaves and in intact plants, in both cucumber and melon. A similar trend was obtained 96 hours after inoculation (Figure 3.2.2).

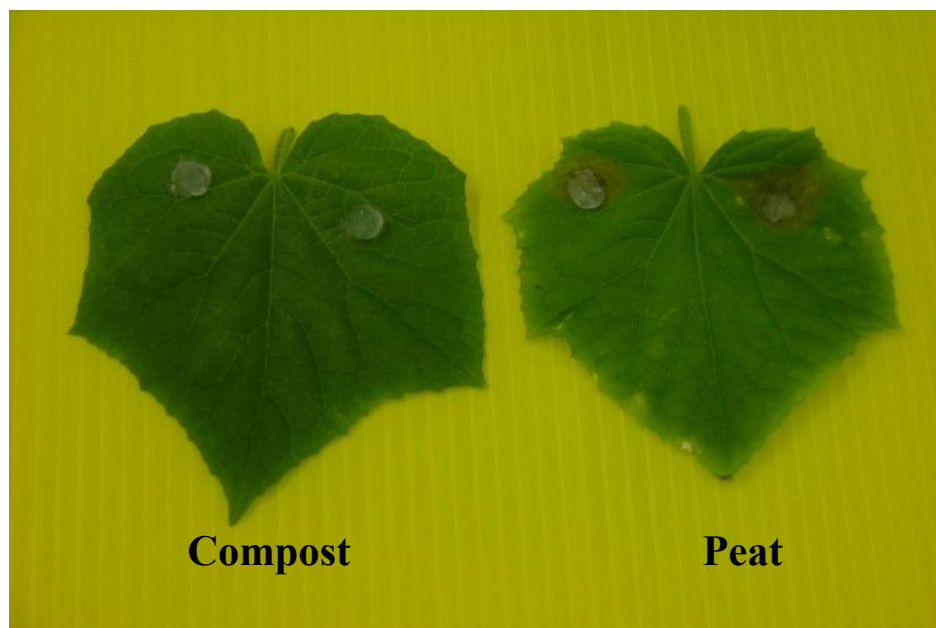


Figure 3.2.1. The effect of growing plants in compost (TP-SCM) or peat on the size of necrotic areas caused by *Botrytis cinerea*, on inoculated melon leaves. Disease evaluation was carried out 36 hours after inoculation.

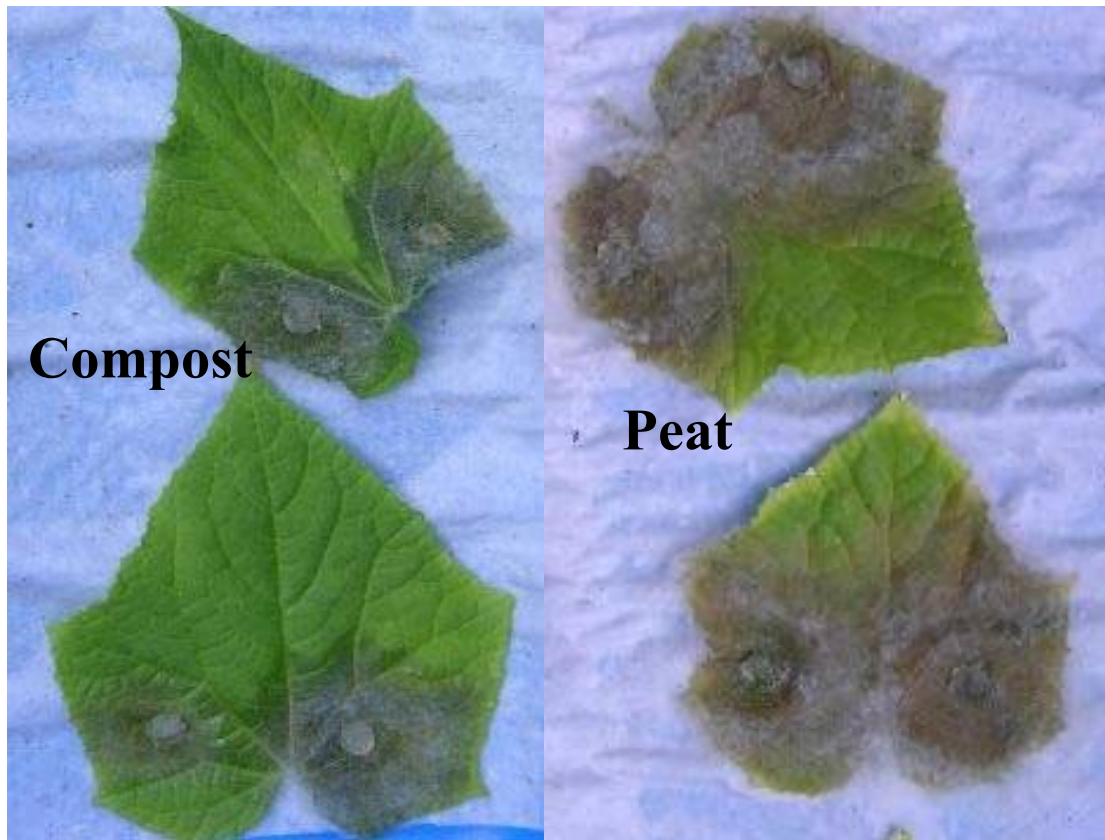


Figure 3.2.2. The effect of growing plants in compost (TP-SCM) or peat on the size of necrotic areas caused by *Botrytis cinerea*, on inoculated melon leaves. Inoculation was performed on detached leaves. Disease evaluation was carried out 96 hours after inoculation.

Figure 3.2.3A

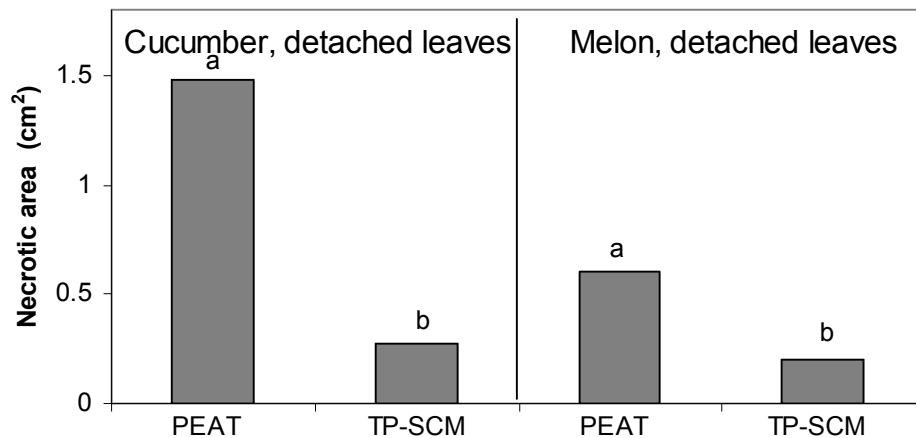


Figure 3.2.3B

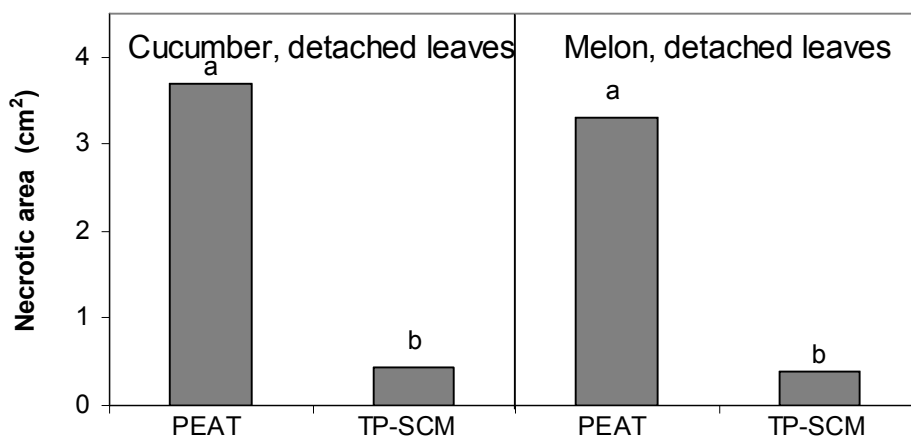


Figure 3.2.3. The effect of growing plants in compost (TP-SCM) or peat on the size of necrotic areas caused by *Botrytis cinerea* on inoculated detached leaves of cucumber or melon. Disease evaluation was carried out 48 hours (Figure 3.2.3A), and 72 hours (Figure 3.2.3B) after inoculation. All values were subjected to a Tukey-Kramer Honestly Significant Difference test at $P=0.05$. Different letters in each figure indicate a significant difference.

Figure 3.2.4A

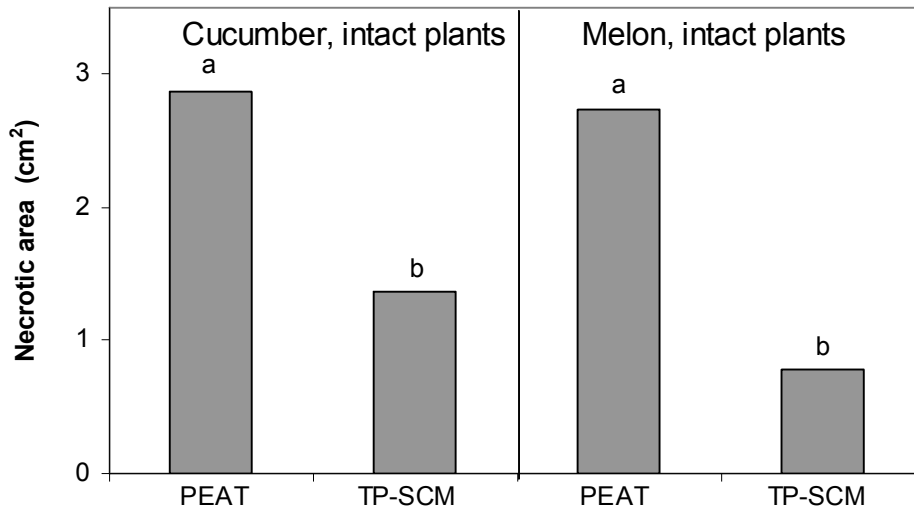


Figure 3.2.4B

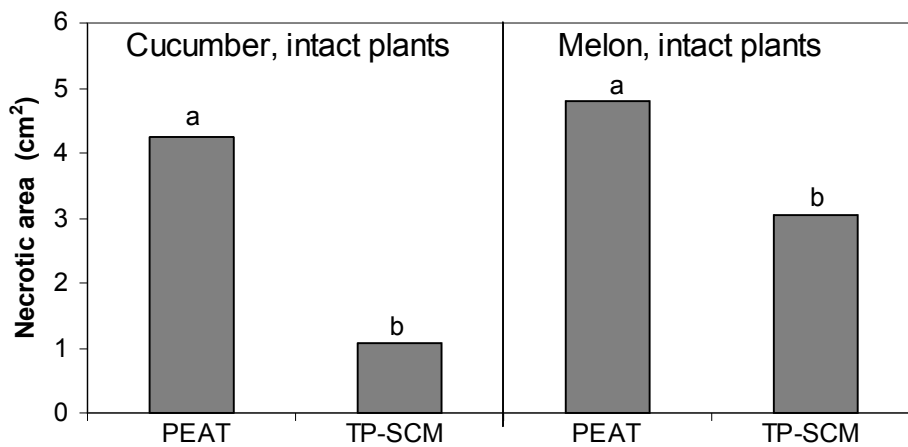


Figure 3.2.4. The effect of growing plants in compost (TP-SCM) or peat on the size of necrotic areas caused by *Botrytis cinerea* on inoculated leaves of cucumber or melon. Disease evaluation was carried out 48 hours (Figure 3.2.4A), and 72 hours (Figure 3.2.4B) after inoculation. All values were subjected to a Tukey-Kramer Honestly Significant Difference test at $P=0.05$. Different letters in each figure indicate a significant difference ($P=0.05$).

3.2.2 Induced resistance to FOM - the nursery transfer system

Melon seedlings were germinated in either peat or compost. Seven days after sowing, the seedlings were uprooted, washed, inoculated and transplanted into peat. We assumed that if a significant difference in disease level would be observed, this can be attributed to a carry-over effect of the exposure of the germinating seedling to the germination medium, and hence a decrease in disease incidence will indicate induced resistance. The two treatments (Figure 3.2.5) did not differ significantly from each other, suggesting that either the compost in the germination medium does not induce resistance to the transplants or that the germination period of 7 days' exposure to the compost was not sufficient to induce resistance after transplanting.

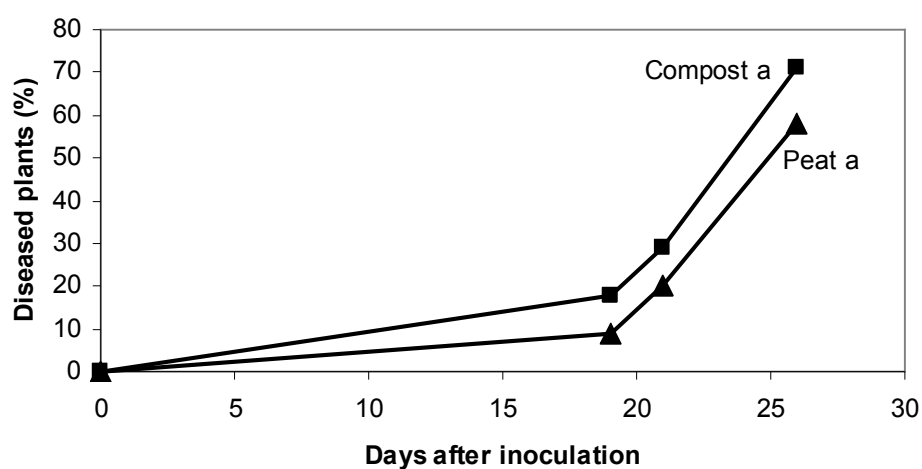


Figure 3.2.5. The effect of peat or compost as germination media on the development of Fusarium wilt of melons. Seeds were germinated either in peat or in compost. After seedling emergence, the plants were uprooted and inoculated at 0.3×10^6 conidia ml^{-1} and transferred to peat. No significant difference was observed between AUDPC values of the two treatments ($P=0.05$).

3.2.3 Induced resistance to FOM – the side-grafting system

In order to further address the induced resistance question, we used the split root system, which enables an exposure of the root system to the growing medium during the entire period of the experiment (55 days), under complete separation of the site of treatment from the site of contact with the pathogen. The preliminary experiments failed since melon seedlings produce only a small amount of roots which cannot be split adequately. We therefore produced side-grafted plants with two separated well – developed root systems which were grown under complete separation between the tested medium and the inoculated root-zone. The course of disease development expressed as AUDPC and disease incidence (Figure 3.2.6; Table 3.2.1 experiments 1 and 2). The results indicate that there is a significant reduction in disease incidence in plants in which one root system was grown and inoculated in peat while their other root system was grown in compost, as opposed to plants whose two root systems (inoculated and non-inoculated) were grown in peat. This occurred in two experiments, using the lower inoculum dosages, with both parameters indicates induced resistance by compost. In the following experiment, four combinations of inoculation of growing media were tested at four inoculation concentrations. The results, (Figures 3.2.7A, B; Table 3.2.1 experiment 3) show again that the compost treatment (compost-peat(I)), significantly reduced the disease incidence, as compared to peat (peat-peat(I)). The other two treatments which involved a direct effect by compost on both the plant and the pathogen were even more effective in disease reduction. The same trend was obtained at all inoculum concentrations.

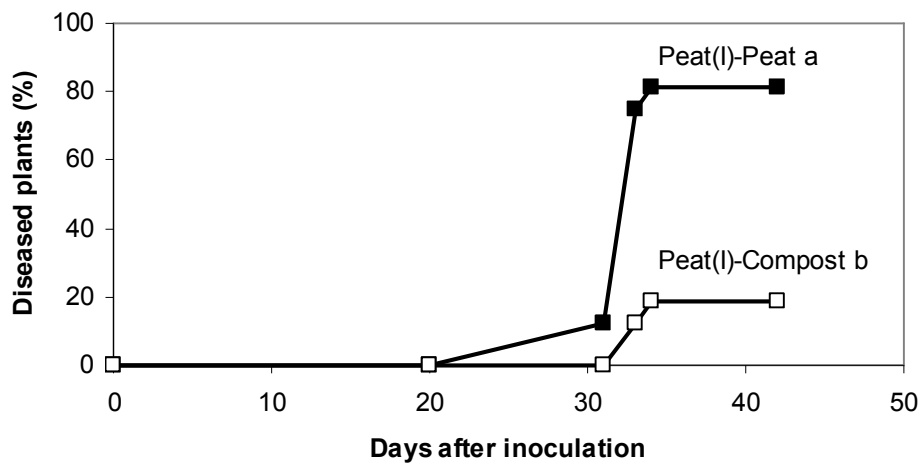


Figure 3.2.6. The effect of peat or compost on Fusarium wilt incidence in grafted melon plants with two root systems (Table 3.2.1, Experiment 1). In each plant, each of the root systems was grown either in peat or in compost, and the other root system was grown in inoculated (I) peat (0.5×10^6 spores, ml^{-1}). Different letters indicate a significant difference between AUDPC values ($P = 0.05$).

Table 3.2.1. Effect of peat or compost on the final disease incidence caused by *Fusarium oxysporum* f. sp. *melonis* and on disease development expressed by the area under the disease progress curve (AUDPC) in grafted melon plants with two root systems, in three different experiments. In each plant, each root system was grown either in peat or in compost, either non-inoculated or inoculated (I).

Experiment No.	Inoculum concentration (ml ⁻¹)	Treatment	Final disease incidence (%)	AUDPC
1	1×10 ⁶	Peat(I)-peat	81 a	546a
		Peat(I)-compost	56 a	425a
1 <i>a</i>	0.5×10 ⁶	Peat(I)-peat	81 a	351a
		Peat(I)-compost	19 b	28b
2	0.5×10 ⁶	Peat(I)-peat	85 a	1632a
		Peat(I)-compost	75 a	1080b
3	0.1×10 ⁵	Peat(I)-peat	83 a	617a
		Peat(I) -compost	47 b	332b
3	0.25×10 ⁵	Peat(I)-peat	100 a	1362a
		Peat(I)-compost	50 b	455b
3 <i>a</i>	0.5×10 ⁶	Peat(I)-peat	83 a	1140a
		Peat(I)-compost	60 a	563b
		Compost(I)-compost	40 b	195c
		Compost(I)-peat	37 b	194c
3 <i>a</i>	1×10 ⁶	Peat(I)-peat	100 a	2160a
		Peat(I)-compost	85 ab	716b
		Compost(I)-compost	75 b	475b
		Compost(I)-peat	70 b	399b

The statistical analysis was carried out for each inoculum concentration. Different letters in each experiment indicate a significant difference ($P=0.05$).

a – Results taken for Figures 3.2.6 and 3.2.7 for comparison.

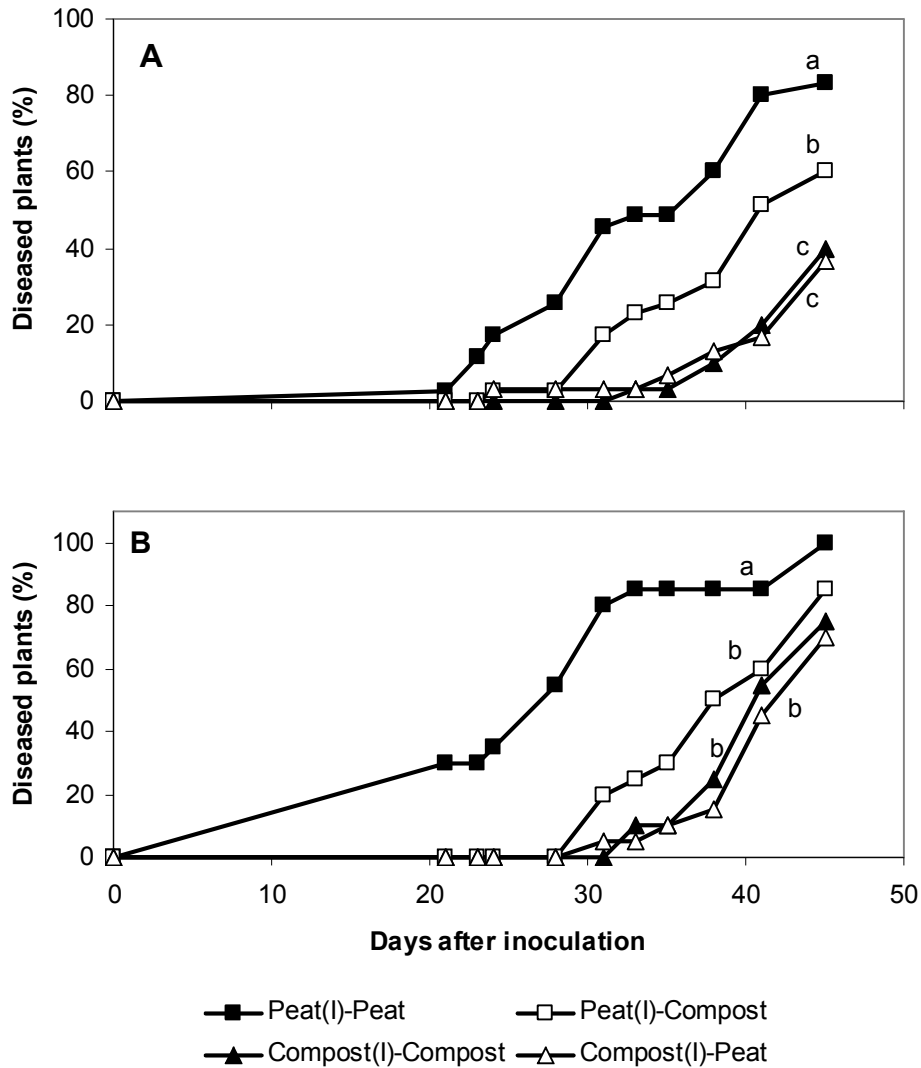


Figure 3.2.7. The effect of peat or compost on *Fusarium* wilt incidence using the side-grafted two root systems (Table 1, Experiment 3). In each plant, each root system grown in either peat or in compost, either in a non-inoculated medium or in an inoculated medium (I). The conidial concentrations used were 0.5×10^6 conidia, ml^{-1} (Figure 3.2.7A) and 1.0×10^6 conidia, ml^{-1} (Figure 3.2.7B). Different letters in each figure indicate a significant difference between AUDPC values ($P = 0.05$).

3.2.4 Expression of PR genes

To further explore the mechanism for compost-induced resistance, the expression of three different PR genes was examined in healthy plants grown in either compost or peat. The expression of genes coding for PR-Q, chitinase1 and peroxidase was evident in the leaves of all examined plants (Figure 3.2.8). Although RT-PCR analyses were not quantitative, no substantial differences in expression levels (as compared to the expression levels of *tubulin*) of the three genes were evident between plants grown on compost and plants grown on peat.

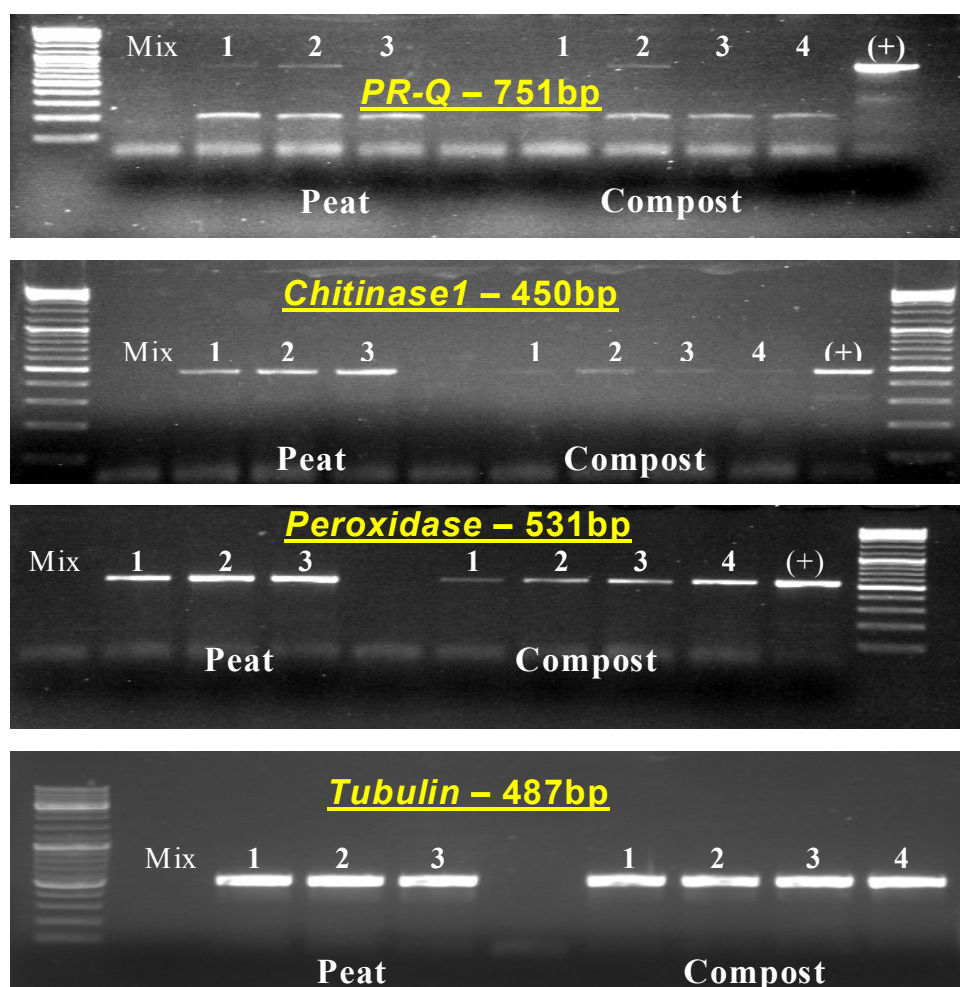


Figure 3.2.8. RT-PCR analyses of PR-Q, chitinase1 and peroxidase in leaves of melon plants grown on compost (4-5 replicates) or on peat (3 replicates). Analyses of PR-Q, chitinase1 and peroxidase were performed with primers to form fragments of 751, 450 and 531 bp. respectively. The housekeeping gene coding for tubulin served as a control for the RT-PCR reactions. Mix, PCR reaction mixture without the template (negative control); +, CMV-infected melon leaves serving as templates (positive control).

3.2.5 Summary and conclusions

When cucumber and melon plants were grown in suppressive compost, induced resistance was observed in the foliar disease caused by *B. cinerea* in both intact plants and detached leaves (Figures 3.2.1, 3.2.2, 3.2.3, 3.2.4). Growing plants in the compost medium resulted in decreasing the size of the necrotic areas even after 96 hours (Figure 3.2.2). The potential of the compost to induce significant resistance against a foliar disease lead us to proceed and explore the possible existence of this mechanism in soil-borne disease, although the mode of action is not necessarily identical in these two cases. The side-grafting technique, which creates two well developed, equal and completely separated roots systems, enabled us to study this issue in relation to *Fusarium oxysporum* f.sp. *melonis*. At all the conidial concentrations (0.1×10^5 , 0.25×10^5 , 0.5×10^6 or 1.0×10^6 conidia, ml^{-1}), disease incidence decreased when the root system which was growing in peat was inoculated, while the second root system of the same melon plant was growing in compost; this is in contrast to the treatment in which both root systems were growing in peat (Table 3.2.1). Moreover, except for the high conidial concentration (1.0×10^6 conidia, ml^{-1}) in the first experiment (Table 3.2.1), AUDPC of all the treatments in which one of the root systems was grown in compost were significantly lower in comparison to the treatments in which both root systems grew in peat (Table 3.2.1). These results suggest involvement of the induced resistance mechanism. However, this is not the exclusive mechanism. There has been additional significant reduction in disease incidence when both root systems were grown in compost (Figure 3.2.7A). This reduction is probably caused by direct influence of the microbial population of the suppressive medium. In the higher inoculum concentration (1.0×10^6), this additional reduction was not observed, probably due to high pathogen pressure (Zhou and Everts, 2007).

3.3 Suppression of FORC by compost in soil-less media in a commercial greenhouse

Compost efficiency in reducing disease caused by *Fusarium* pathogens was demonstrated in small scale experiments (Chapters 3.1 and 3.2). It was important to validate this phenomenon under natural infection condition of a commercial greenhouse. In this study we describe the effect of compost on the stem and root rot disease which has been causing heavy damage to cucumber crops in recent years in Israel. The cause of this disease is *F. oxysporum* f.sp. *radicis-cucumerinum* (FORC), which produces large quantities of macroconidia on the lower stem. This pathogen may spread out in the air and risk other plants in the greenhouse. The main damage occurs during spring and winter, while the pathogen affects the plant and causes wilting. Summer damage occurs only in soil-less media in which the total damage is more severe than in soil. Reduction in FORC by TP-SCM compost was observed in preliminary studies. Therefore, we tested addition of TP-SCM compost at two ratios to a conducive perlite during three growing seasons in a commercial greenhouse (Figure 3.3.1).

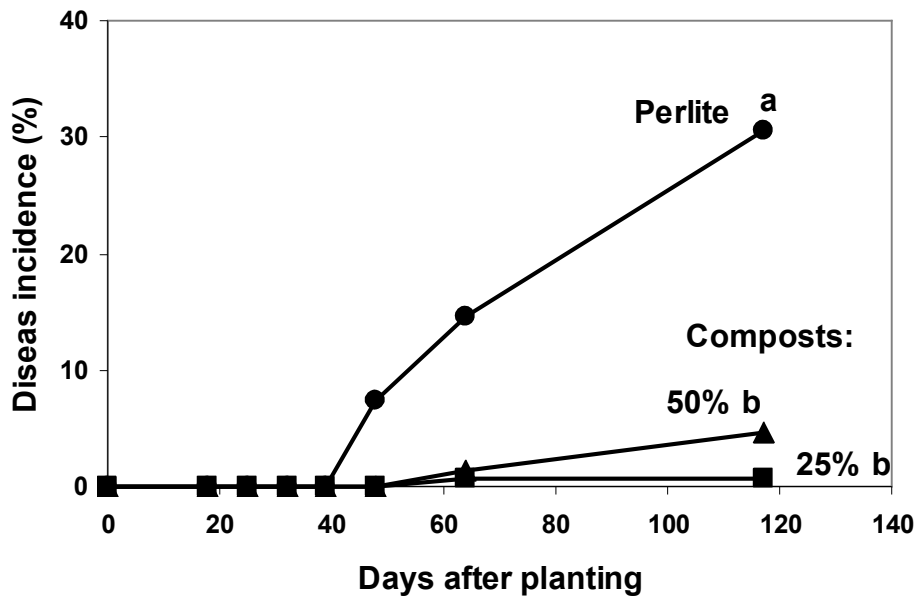


Figure 3.3.1. Fusarium wilt suppression by composts in a commercial greenhouse in autumn 2004. Cucumber transplants were planted in three media: 100% perlite; 75% perlite with 25% compost and 50% perlite with 50% compost. The inoculation by macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* was natural. Different letters indicate a significant difference between AUDPC values ($P = 0.05$).

3.3.1 Effect of growing media on severity of disease caused by FORC in a commercial greenhouse

The results of the first season autumn 2004 (Figure 3.3.1), showed a significant reduction in disease severity in the compost treatments, while 35% of the plants grown in perlite were dead. No significant difference was observed between the two concentrations of compost. The same disease level was observed in the following season (spring) in composts (without difference between the two concentrations), while there was a decline in disease level in perlite from 35% in the first season to 15%. (Figure 3.3.2). A difference between disease incidence in compost and in perlite

was still observed in the following season (summer) (Figure 3.3.3), but there was a 77% rise in disease incidence in the 25% compost treatment as opposed to the spring season, while no change was observed in disease level in the 50% compost and perlite. Adding 50% compost to the infested perlite medium did not reduce disease incidence (Figures 3.3.2; 3.3.3).

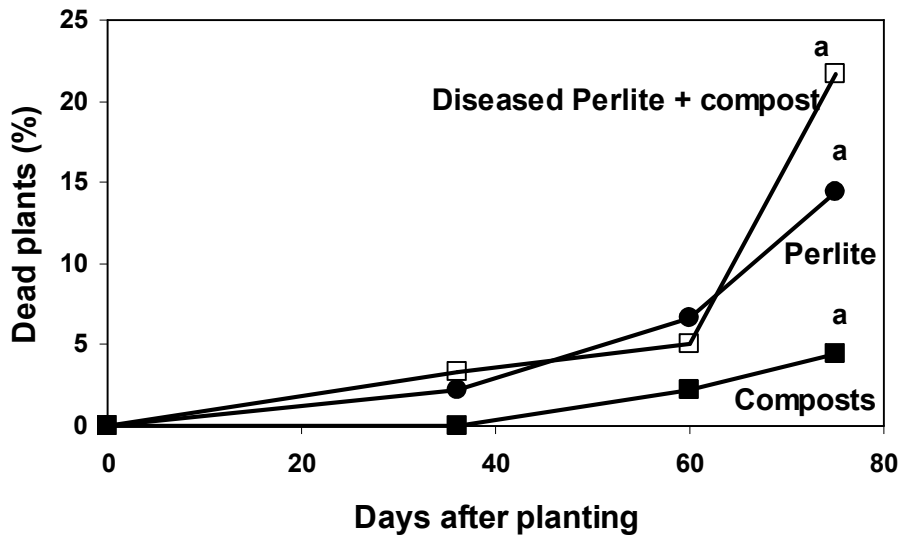


Figure 3.3.2. Fusarium wilt suppression by composts in a commercial greenhouse in spring 2005. Cucumber transplants were planted in the same three media as those of autumn 2004: 100% perlite; 75% perlite with 25% compost; 50% perlite with 50% compost and in an additional medium: 50% infested perlite from the previous autumn growing season with 50% compost. The inoculation by macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* was spontaneous. Same letters indicate no significant difference between AUDPC values ($P = 0.05$).

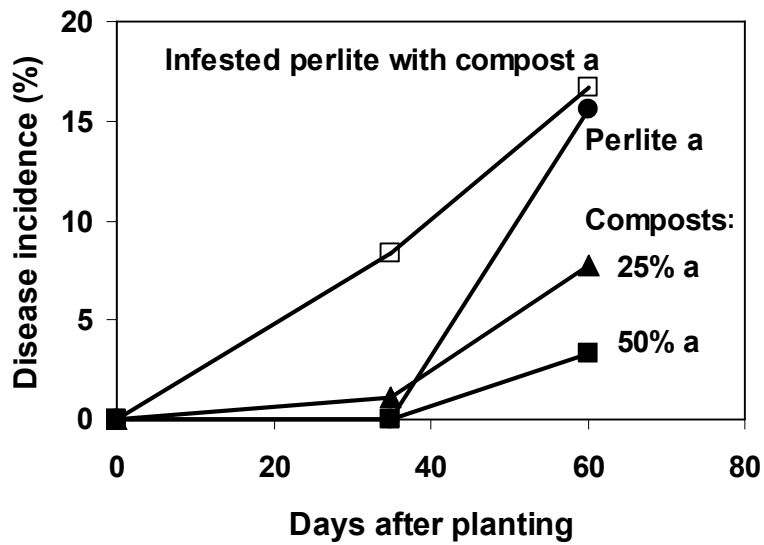


Figure 3.3.3. *Fusarium* wilt suppression by composts in a commercial greenhouse in summer 2005. Cucumber transplants were planted in the same four media as those of spring 2005: 100% perlite; 75% perlite with 25% compost; 50% perlite with 50% compost and 50% infested perlite with 50% compost. The inoculation by macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* was spontaneous. Same letters indicate no significant difference between AUDPC values ($P = 0.05$).



Figure 3.3.4. Wilt of cucumber plants caused by FORC in a commercial cucumber greenhouse in Baqa al-Gharbiyye.

3.3.2 Summary and conclusions

Suppressive compost is usually studied in small scale experiments involving artificial inoculation. This experiment was conducted with natural infection under commercial greenhouse conditions. TP-SCM compost efficiently reduced disease incidence caused by FORC, a virulent pathogen which causes severe damage, during three consecutive growing seasons while 15- 35% of the plants grown in perlite died. The fact that the experiment was carried out in a commercial greenhouse under common growing management and heavy natural infestation emphasizes the suppression potential of compost in growing media. The insignificant differences between the treatments in spring and summer 2005 could be attributed to the lack of uniformity of the natural infestation, which influenced the variability of the results.

TP-SCM compost was not efficient in preventing disease when added to previously infested perlite. This issue can be explained by the fact that suppression ability is influenced by inoculum density and decreases in heavy inoculum density, which was caused both by the infested medium and by the large amounts of air-borne macroconidia from the air.

3.4 Suppression of Fusarium wilt of melon in organically managed soils

Israeli organic growers amend their soils annually with 10-20 tons of compost per hectare. This compost is based mainly on cattle manure. It was found that this compost type can suppress several soil-borne diseases (Aryantha *et al.*, 2000; Reuveni *et al.*, 2002; Raviv *et al.*, 2005). It was therefore hypothesized that organically-managed, compost-amended soils may attain with time some level of disease suppressiveness (Abbassi *et al.*, 2002; Steinberg *et al.*, 2004; Darby *et al.*, 2006). The aim of this part of the study was to test this assumption and to determine its extent over a wide range of soil types and uses. In addition, we tried to test whether a relatively fast and accurate parameter, such as heat generation of the soil, can help to predict potential suppressiveness level.

3.4.1 Soil characteristics

Soil samples were taken from various sites located in the main agricultural regions of Israel (Figure 3.4.1). Three of the sites, Afikim, NeweYa'ar and Shluchot were planted with orchards - avocado, stone fruits and grapefruit, respectively. All the rest were planted with field crops grown in rotation, suited for the specific conditions of each location. Soil types are presented in Table 3.4.1, showing identical definition of

14 pairs of soils and only one case (Sheluchot) where there was a difference between the organic and conventional soil. Soil characteristics are presented in Table 3.4.2.

Table 3.4.1. Soils' pedological characteristics. Organic (or) and conventional (co) soils were sampled at each location. Data were provided by Dr. Pinchas Fine, Institute of Soil, Water and Environmental Sciences, the Volcani Center, ARO.

Field location	Soil type
Afikim or. and co.	Typic Haploxerert
Alumim or. and co.	Calcixerollic Xerochrept
Amir or. and co.	Typic Haploxerert
Bet Alfa or. and co.	Chromic Haploxerert
Ein Harod or. and co.	Chromic Haploxerert
Heftziba or. and co.	Chromic Haploxerert
Ifat or. and co.	Chromic Haploxerert
Kisufim or. and co.	Xeric Quartzipsamment
Newe Ya'ar or. and co.	Chromic Haploxerert
Nir Oz or. and co.	Xeric Quartzipsamment
Ramat David or. and co.	Chromic Haploxerert
Ruhama or. and co.	Xeric Quartzipsamment
Sde Eliahu or. and co.	Typic Haplargid
Sheluhot or.	Typic Haplargid
Sheluhot co.	Typic Haploxerert
Tzaelim or. and co.	Typic Torripsamment

Table 3.4.2. Soils' chemical characteristics. Organic (or) and conventional (co) soils were sampled at each location.

Field location	Organic Matter (%)	N-NH ₄ mg/kg	N-NO ₃ mg/kg	P mg/kg	K meq/l	EC dS/m	pH	Clay %	Silt %	Sand %	CaCO ₃ %
Afikim or.	10.4	40.2	11.8	297	0.9	1.6	7.1	25.1	32.5	42.4	23.2
Afikim co.	3.7	10.2	5.3	33.5	0.2	0.9	7.2	38.6	26.9	34.5	40.7
Alumim or.	1.4	9.5	29.6	68.1	2.2	3.8	7.3	13.7	31.2	55.1	12.7
Alumim co.	1.0	5.0	87.0	38.2	0.8	3.8	7.3	21.7	27.2	51.1	11.9
Amir or.	2.4	11.1	14.0	214	0.9	0.6	7.6	36.1	33.3	30.6	30.0
Amir co.	2.0	8.3	13.0	32.4	0.4	0.5	7.6	68.1	21.3	10.6	15.2
Bet Alfa or.	2.8	12	23.0	99.0	1.2	1.2	7.1	50.3	28.6	21.1	17.1
Bet Alfa co.	1.2	4.5	3.0	23.9	0.1	0.4	7.0	49.1	32.5	18.4	14.2
Ein Harod or.	1.6	16.7	5.6	39.0	0.3	0.5	7.0	54.3	28.6	17.1	15.4
Ein Harod co.	0.9	11.7	10.5	20.2	0.1	0.5	7.0	54.3	24.6	21.1	15.4
Heftziba or.	2.2	11.7	16.0	83.1	0.3	0.6	7.7	54.1	31.3	14.6	23.6
Heftziba co.	1.0	7.3	10.5	19.5	0.1	0.5	7.7	54.1	31.3	14.6	19.2
Ifat or.	1.6	7.2	5.2	45.1	0.2	0.4	7.0	53.1	24.5	22.4	10.2
Ifat co.	1.2	4.3	3.3	28.5	0.1	0.3	7.0	54.3	28.6	17.1	7.3
Kisufim or.	1.3	5.1	48.7	67.5	0.8	2.5	7.4	6.4	6.8	86.8	20.0
Kisufim co.	0.9	3.5	10.4	19.4	0.4	1.6	7.5	5.4	7.7	86.8	16.9
Newe Yaar or.	3.3	84.9	34.8	128	1.5	1.8	7.0	45.9	24.5	29.6	1.9
Newe Yaar co.	2.7	37.4	10.8	98.8	0.6	1.1	7.0	49.9	26.5	23.6	1.9
Nir Oz or.	0.5	6.4	11.3	23.5	0.9	1.4	7.5	3.7	5.2	91.1	4.6
Nir Oz co.	0.4	3.0	11.4	19.4	0.7	2.6	7.4	11.7	5.2	83.1	1.9
Ramat David or.	2.4	17.7	7.1	105	0.3	0.5	7.0	50.3	24.6	25.1	4.1
Ramat David co.	1.8	25.2	7.2	23.8	0.2	0.5	7.0	54.3	24.6	21.1	4.1
Ruhama or.	1.0	2.5	29.3	17.3	0.5	1.7	7.6	5.4	8.8	85.8	13.8
Ruhama co.	0.9	2.4	20.7	27.2	0.2	1.1	7.7	5.4	7.8	86.8	15.4
Sde Eliahu or.	2.5	4.8	8.9	97.7	0.5	0.6	7.2	34.3	32.6	33.1	48.8
Sde Eliahu co.	1.5	5.8	8.4	46.2	0.6	0.6	7.2	22.3	24.6	53.1	63.0
Shluhot or.	1.8	18.9	7.4	353	0.7	1.0	7.2	22.3	24.6	53.1	23.6
Sheluhot co.	1.9	5.5	5.5	42.0	0.3	0.8	7.1	50.3	28.6	21.1	25.2
Tzaelim or.	0.5	1.5	13.9	38.2	2.0	1.9	7.4	5.7	3.2	91.1	1.5
Tzaelim co.	0.3	2.6	9.3	12.1	0.6	0.6	7.7	7.7	1.2	91.1	3.8

3.4.2 Disease suppression

The suppressiveness of the soils was evaluated by comparing the development of Fusarium wilt in melon plants following artificial inoculation, in both organic and conventional soils from the same site (Table 3.4.3.). The course of the assay is demonstrated for the case of Ein Harod. Disease suppression in organic versus conventional soil is presented in Figure 3.4.1. In this case there is a significant difference between the two treatments and the organic soil is more suppressive to Fusarium wilt than the conventional soil. In ten out of 15 soil pairs tested, the organic soils were more suppressive than the conventional soils, as expressed by the suppression index (Figure 3.4.2). The statistical analysis conducted by a Two Ways ANOVA test showed a significant effect of higher suppression of the organic soils.

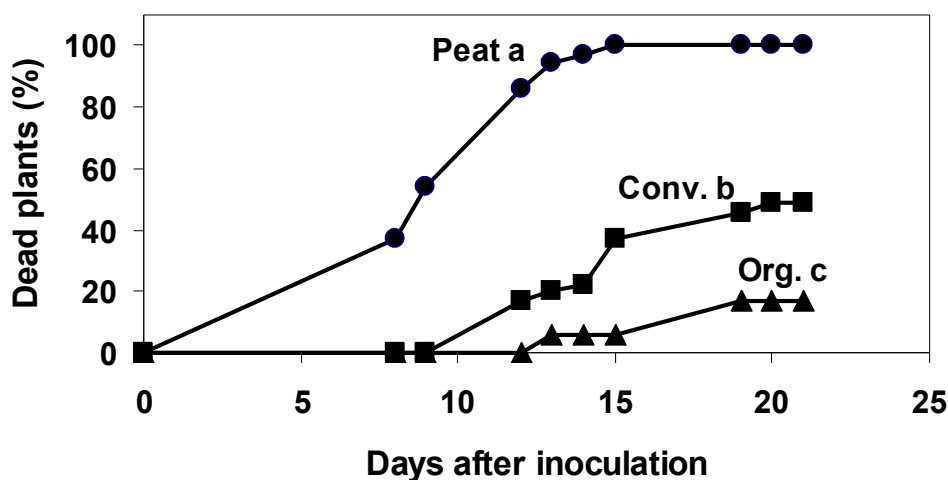


Figure 3.4.1. Fusarium wilt (artificial inoculation, for details see Materials and Methods) suppression in organic soil vs. conventional soil from Ein Harod and peat (one sample out of 15, studied in this research, Table 3.4.3). Same letters indicate no significant difference between AUDPC values ($P = 0.05$).

Table 3.4.3. Disease incidence in melon seedlings artificially inoculated in organic and conventional plots from 15 farms in Israel and heat generation of the same plots.

Plot location	Disease incidence Organic plot (%)	Disease incidence Conventional plot (%)	Heat generation Organic plot μW/mgDW	Heat generation Conventional plot μW/mgDW
Afikim	17.1 a	60.0 b	0.084 a	0.014 b
Alumim	48.1	55.8	0.028 a	0.019 b
Amir	47.1	52.9	0.017	0.011
Bet Alfa	48.6	48.6	0.025 a	0.014 b
Ein Harod	16.7 a	56.7 b	0.026 a	0.015 b
Heftziba	29.3	29.3	0.034 a	0.010 b
Ifat	45.7	52.8	0.009	0.011
Kisufim	62.5	32.5	0.021	0.015
Newe Yaar	2.9 a	27.5 b	0.090 a	0.053 b
Nir Oz	58.3	55.8	0.019 a	0.009 b
Ramat David	50.0	60.0	0.043	0.030
Ruhama	23.1	40.0	0.050	0.060
Sde Eliahu	22.9	20.0	0.041	0.054
Sheluhot	14.3 a	42.9 b	0.055 a	0.010 b
Tzaelim	49.4	86.7	0.004	0.002

Different letters in each location indicate a significant difference ($P=0.05$). A two Ways ANOVA test showed a significant effect of higher suppression and higher heat generation of the organic soils ($P=0.05$).

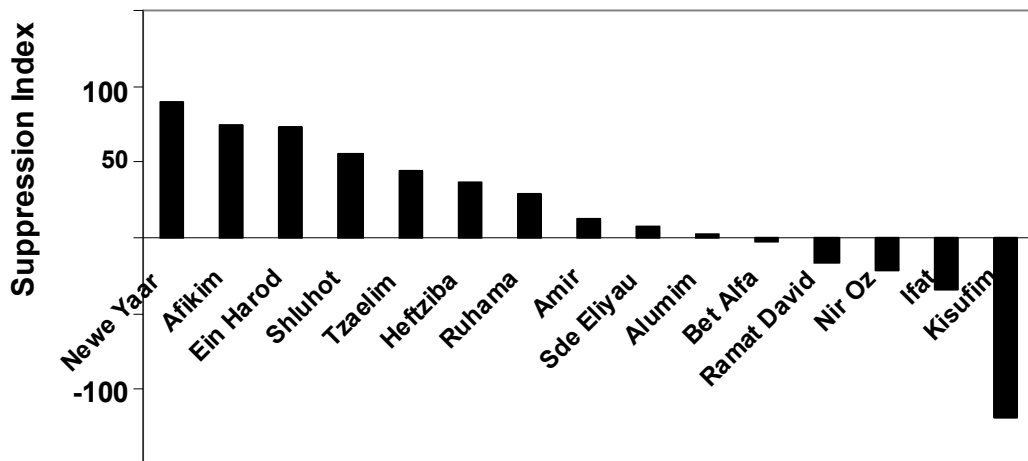


Figure 3.4.2. Fusarium wilt suppression index in the studied soils. Index value = $1 - (\text{AUDPC in organic soil} / \text{AUDPC in conventional soil}) \times 100$. Values above 0 indicate that organic management induces soil suppressiveness. Disease suppression by organic soils was higher than that of conventional soils, as tested by the Two Ways ANOVA Test ($P=0.05$).

3.4.3 Calorimetric assay

Heat generation, indicating the level of biological activity of the soils was measured using a calorimeter. The statistical analysis conducted by a Two Ways ANOVA test showed significantly higher activity levels of the organic soils (Figure 3.4.3). A significant correlation was found between the AUDPC of the soils and their heat release rate (figure 3.4.4). The possible effect of soil characteristics on disease suppression capacity and on soil activity was studied. Only one significant correlation between Fusarium wilt and N-NH_4 concentrations (Figure 3.4.5) was found.

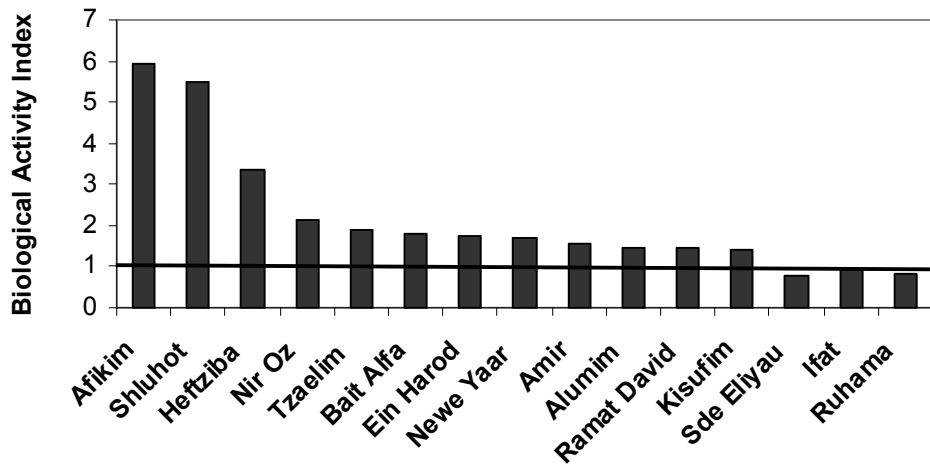


Figure 3.4.3. The biological activity index, as measured by calorimetry. Activity index = heat generation of the organic soil/heat generation of the conventional soil. Values higher than 1 indicate higher heat generation of the organic soil. Heat generation was significantly higher in the organic soils. (Tested using a Two Ways ANOVA Test ($P=0.05$)).

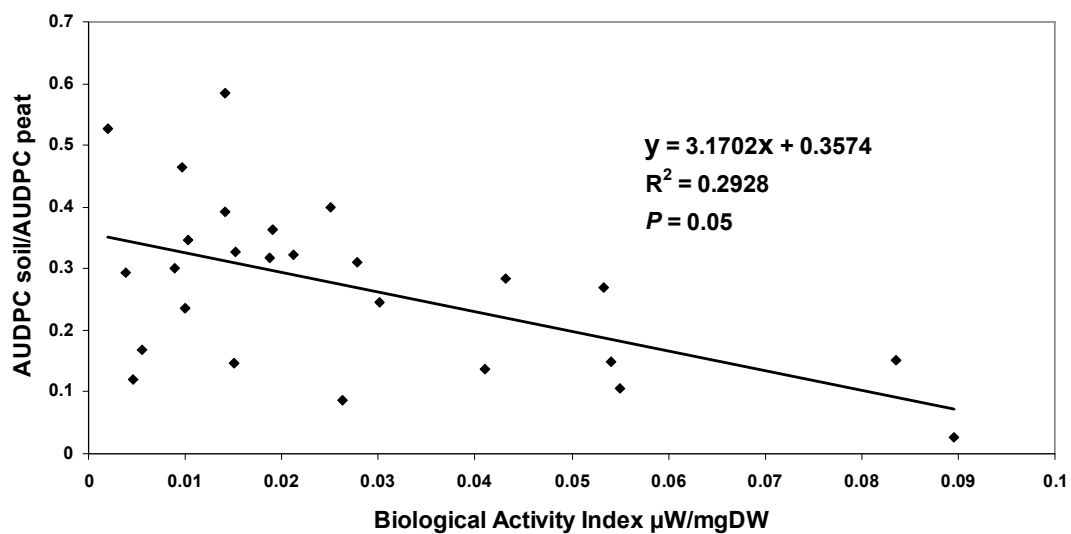


Fig 3.4.4. Relative incidence of Fusarium wilt (expressed in AUDPC values of each soil [organic or conventional]/AUDPC values of peat from the same trial) as a function of biological activity, as measured by calorimetry.

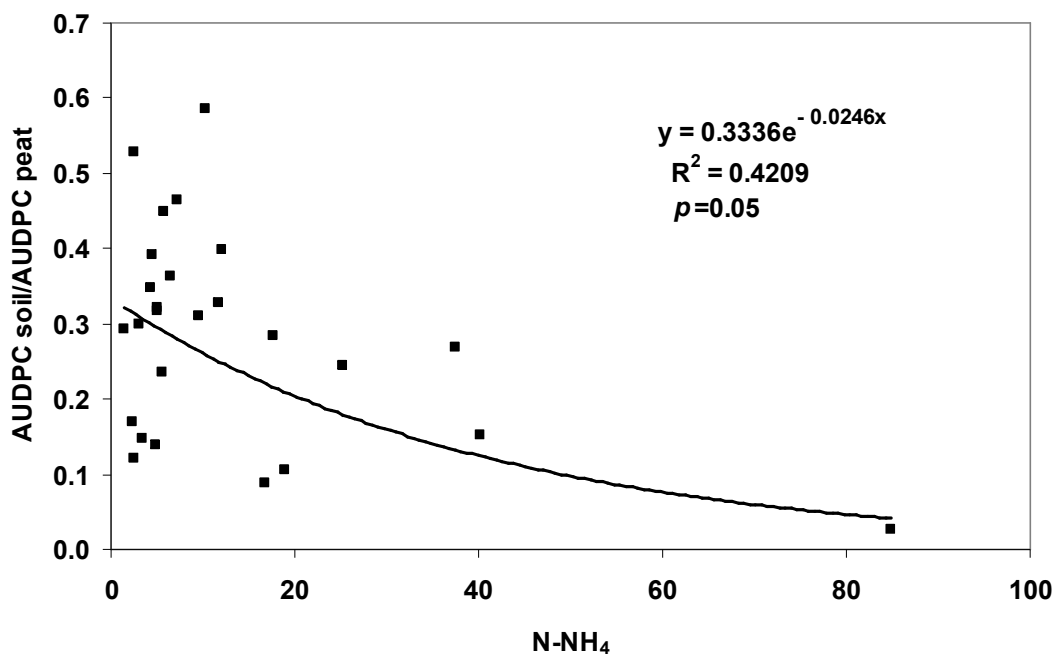


Figure 3.4.5. Relative incidence of Fusarium wilt (expressed in AUDPC values of each soil [organic or conventional]/AUDPC values of peat from the same trial) as a function of N-NH₄ concentration in the soils.

3.4.4 Summary and conclusions

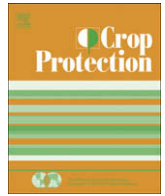
The majority of the tested soils showed a higher level of suppressiveness, as compared to peat moss, which is a substrate conducive to this disease (Figure 3.4.1). When the organic and conventional pairs were compared, the organically-managed soils were more suppressive against FOM than the conventional soils (Figure 3.4.2). A similar phenomenon was found by several other researchers (Liu *et al.*, 2007; Escudra and Amemiya, 2008). At present we do not have a convincing explanation for the outliers (such as Kisufim). Other researchers who sampled a limited number of soils received similar results, namely lower disease level in soils amended with organic matter (Messiha *et al.*, 2007), while in other cases no difference was found (Grünwald *et al.*, 2000). Additional research should concentrate on the microbial

communities that characterize each of the tested soils, which may explain such discrepancies (Saison *et al.*, 2006; Perez-Piqueres *et al.*, 2006; Benitez *et al.*, 2007).

The heat generation activity of the soil samples was loosely (although statistically significantly) correlated to their relative suppressive capacity (Figure 3.4.4). In general terms, the heat generation capacity of a soil sample can be determined by its content of OM and OM level of biodegradability. This, in turn, is affected by the length of time since the last application of compost and the maturity of the compost. Most of the soils pedological and chemical characteristics could not be correlated with their relative suppressive capacity (data not shown). The only notable exception is the content of ammonium ions in the soil solution (Figure 3.4.5). In most cases, high ammonium levels can be found shortly after the application of relatively fresh organic amendments. It is interesting to note that Darby *et al.* (2006) found a reduction of the suppressive capacity with time after the application of compost, which may point to a similar phenomenon: soil that contains fresh organic matter (and therefore can generate more heat and contains more ammonium) is more suppressive, and this capacity decreases with time, until another more organic matter is applied.

In conclusion, it appears that in some cases, repeated applications of organic matter can lead to a certain degree of soil suppressiveness against Fusarium wilt of melon caused by FOM, and that such suppression can be predicted to some extent based on the heat generation activity of the soil and its ammonium ion content.

3.5 Suppression of bacterial canker of tomato by composts



Suppression of bacterial canker of tomato by composts

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ABSTRACT

Suppression of *Clavibacter michiganense* subsp. *michiganensis* (CMM) by composts was studied in comparison to conducive peat. Composts based on tomato or pepper residues combined with cattle or chicken manure reduced disease caused by CMM by between 79% and 100% under both natural infection of mature plants and intentional inoculation. Populations of CMM in composts declined to undetectable levels within 15–20 days, while those in peat remained high for 35–40 days. Similarly, the colonization of compost-grown tomato-plant tissues by the pathogen was reduced (0–20% colonization), compared to plants growing in peat (53–90% colonization) or perlite (30–90% colonization). We conclude that the plant-residue composts suppress CMM and can therefore serve as a component in integrated-management programs.

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1. Introduction

Bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (CMM) is a serious disease of tomatoes. In recent years, the incidence of bacterial canker has increased, and the pathogen is now present in the world's main tomato-production areas. Tomato is the major host, but in some cases, natural infections have also been found on other solanaceous plants (Thyr et al., 1975). Several solanaceous and non-solanaceous plants have been identified as reservoirs for CMM epiphytic survival and spread (Chang et al., 1992). The significance of these epiphytic populations is not yet fully understood, although they seem to contribute to infections through pruning wounds (Carlton et al., 1994). The pathogen can survive several months in contaminated debris (Gleason et al., 1991). The bacteria can also persist under dry conditions on equipment, boxes and glasshouse constructions. Survival of the pathogen in the soil has become a serious problem in areas where intensive monoculture of tomatoes in fields and greenhouses is practiced. The efficiency of pesticides which are used for controlling

CMM is limited (de León et al., 2008). The pathogen can be present in commercial seed lots, and this is an important source of the primary seedling infection. Damage caused by bacterial canker can vary from none or minimal foliar injury to total (systemic infection), and is dependent on the source of the infection, weather conditions and cultural and disease-management practices.

Many compost types suppress a wide range of soilborne fungal diseases (Hadar and Mandelbaum, 1986; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Hoitink et al., 1975, 2001; Hunter et al., 2006; Raviv et al., 1998b; Reuveni et al., 2002; Schönfeld et al., 2003; Serra-Wittling et al., 1996; Termorshuizen et al., 2006; Yogev et al., 2006). Several composts were found efficient in suppressing soilborne fungal pathogens in tomatoes: *Fusarium oxysporum* f. sp. *lycopersici* (Borrero et al., 2005, 2006), *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Cheuk et al., 2005; Kavroulakis et al., 2005; Yogev et al., 2006) and *Pyrenocheta lycopersici* (Hasna et al., 2007). The causal agents of the suppression are complexes of bacterial and fungal populations (Hadar and Mandelbaum, 1986; Hunter et al., 2006; Krause et al., 2003). In many cases, compost sterilization considerably reduces or eliminates its suppressiveness, indicating that the mechanism of disease suppression is predominantly biological (Hadar and Mandelbaum, 1986; Noble and Roberts, 2004; Reuveni et al., 2002; Trillas-Gay et al., 1986; Yogev et al., 2006). In some cases, rapid recolonization by diverse microbial populations soon after sterilization has resulted in resumed suppressiveness (Serra-Wittling et al., 1996; Yogev et al., 2006), further emphasizing the role of microorganisms in that suppressiveness. However, some

Abbreviations: AUDPC, area under the disease progress curve; BOD, biological oxygen demand; CM, laying hens chicken manure; CMM, *Clavibacter michiganensis* subsp. *michiganensis*; NA, nutrient agar; OD, optical density; PBS, phosphate buffered saline; PP, pepper plant residues; SCM, separated dairy cow manure; TP, tomato plant residues.

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residual level of suppressiveness is believed to be attributed to abiotic factors (Borrero et al., 2004). Inclusion of compost in growing media as a method to suppress soilborne plant pathogens was first suggested by Hoitink et al. (1975) and is now a well-established commercial practice, corroborated by a large body of scientific evidence. This subject has been reviewed by various authors (Bonanomi et al., 2007; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Hoitink et al., 2001; Maher et al., 2008; Noble and Coventry, 2005; Noble and Roberts, 2004; Termorshuizen et al., 2006 and others). There are also examples of suppression of disease-causing nematodes in tomato (Everts, 2006; Oka and Yermiyahu, 2002; Raviv et al., 2005). In comparison to the numerous publications on suppressiveness of compost media towards soilborne fungal pathogens only a few studies have described suppressiveness towards bacterial plant pathogens (Abbasi et al., 2002; Aldahmani et al., 2005; Schönfeld et al., 2003; Utkhede and Koch, 2004). The objective of this work was to examine the potential of composts as plant-growth media in suppressing CMM, by assessing their effect on disease incidence, bacterial survival and plant colonization by the pathogen.

2. Materials and methods

2.1. Compost preparation

Two carbonaceous amendments were added to fresh (up to 1-week-old) separated dairy cow manure (SCM) and in two cases also to laying hens chicken manure (CM) (Table 1), in order to serve as bulking agents and to increase the C/N ratio of the mixture. The amendments were air-dried and chopped (<5 cm) tomato plant residues (TP) and air-dried and chopped pepper plant residues (PP). Both plant residues were taken from commercial greenhouses. TP and PP were mixed with SCM and CM at various volumetric rates (Table 1). The three mixtures (PP–SCM–CM, TP–SCM–CM and TP–SCM) were composted in insulated 6-m³ bins, equipped with forced aeration to prevent overheating, as previously described (Raviv et al., 1998a). The aeration rate was 400 m³ h⁻¹ per bin, and a temperature of 60 °C at a depth of 50 cm was used as the set point. When the compost temperature fell below the set point, the composts were aerated for 1 min h⁻¹ in order to maintain aerobic conditions at all times. Moisture content was measured twice weekly: it was maintained at 50 to 60% during the thermophilic period and at 40–50% thereafter, by adding water as necessary, but avoiding leaching. The composts were turned twice (days 5 and 42 from start of composting, when temperatures rose above 60 °C in some of the bins in spite of the activity of the blowers) with a front-end loader to improve their homogeneity. Compost temperatures are shown in Fig. 1. After about 80 days the temperatures started to decline and reached ambient levels in all piles 120 days from the

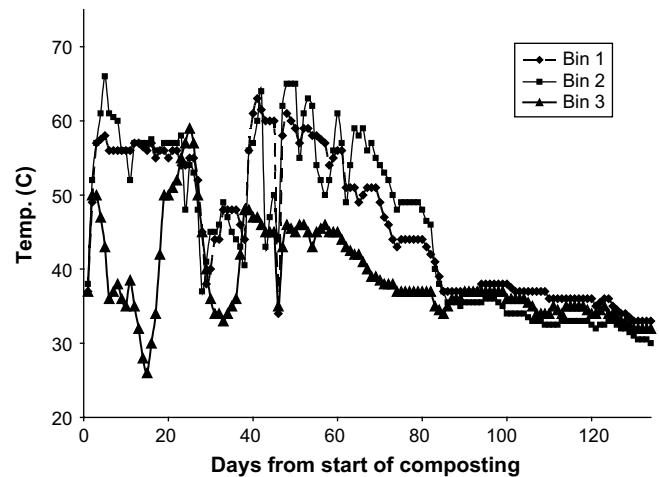


Fig. 1. Temperature curve with time of the three composts. Bin 1: 40% TP, 60% SCM; Bin 2: 44% PP, 24% SCM, 32% CM; Bin 3: 44% TP, 24% SCM, 32% CM. TP, tomato plants; PP, pepper plants, SCM, separated cow manure; CM, chicken manure.

start of composting. The composts were then left to cure for an additional 30 days. After the end of the curing process the composts were tested for phytotoxicity based on the cress bioassay (Emino and Warman, 2004) and found as non-phytotoxic (data not shown). In addition, no phytotoxic symptoms were observed in any of the tomato plants growing on any of the above media.

2.2. Compost and peat characteristics

The organic matter, N, P and K contents and the concentrations of several ions in the compost and peat extracts were determined as described previously (Raviv et al., 1998b). Samples of the peat (Plantobalt, Riga, Latvia) and of the composts were oven-dried at 70 °C and ground to pass a 20-mesh sieve. The samples were digested as follows: 200-mg aliquots were treated with 4 ml of 36 N H₂SO₄ overnight at room temperature, after which 1 ml of H₂O₂ was added to the reaction tubes, which were then incubated at 130–140 °C. After the tubes had cooled to between 50 and 60 °C, the temperature was raised to 280 °C for 20 min. At the end of the process, which lasted about 2 h, the solutions became clear and after cooling, the concentrations of N, P, and K were determined in the digest by spectrophotometry (Hach Co., 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⁺, NO₃⁻, NH₄⁺, PO₄³⁻) were determined in 1:10 medium: water extracts, which had been left

Table 1
Characteristics of the studied composts, peat, and their aqueous extracts.^a

Component	Peat	PP–SCM–CM 44:24:32		TP–SCM–CM 44:24:32		TP–SCM 40:60	
		Initial	Final	Initial	Final	Initial	Final
Organic matter (%)	91	74	45	68	42	66	37
N (%)	1.11	1.87	1.93	2.62	1.88	1.60	1.76
C/N ratio	45.4	22.0	13.7	16.6	13.0	22.6	12.4
pH ^b	6.1	7.2	7.8	6.9	7.2	6.7	7.0
EC (dS m ⁻¹) ^b	0.75	7.94	6.62	6.09	7.06	7.23	6.57
N-NO ₃ (mmol l ⁻¹) ^b	9.8	4.5	12.9	17.2	18.8	7.2	9.8
N-NH ₄ ⁺ (mmol l ⁻¹) ^b	3.6	6.0	0.2	6.8	0.3	3.0	0.2
BOD (g O ₂ kg DM ⁻¹ day ⁻¹) ^c	2.2	4.4	3.5	9.2	3.6	6.5	3.3

^a PP–SCM–CM = pepper plant residues + separated cattle manure + chicken manure; TP–SCM–CM = tomato plant residues + separated cattle manure + chicken manure; TP–SCM = tomato plant residues + separated cattle manure.

^b In a 10:1 aqueous extract.

^c Biological oxygen demand.

for 4 h and shaken every hour. The potassium concentration was measured with a Corning Flame Photometer 410, nitrate with the RQflex Plus instrument (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 PHM 95 pH meter (Radiometer, Copenhagen, Denmark). Biological oxygen demand was determined in the raw samples, whose moisture contents were kept close to container capacity, according to Raviv et al. (1998a). The chemical characteristics of the composts and peat moss used in this study are presented in Table 1.

2.3. Bacterial studies

Cultures of CMM from the collection of the Plant Pathology Department in ARO, Israel, were maintained on plates containing nutrient agar (NA; Difco, Sparks, MD, USA) and incubated at 28 °C for 48–72 h. Storage for short periods was carried out at 4 °C and for long periods at –80 °C in 40% glycerol. The following media were used for isolation of bacteria from plants and for quantitative estimation from the soil: D₂ANX (Hadas et al., 2005) containing (per liter) 10 g glucose (dextrose), 4 g casein acid hydrolysate, 2 g yeast extract, 1 g NH₄Cl, 0.3 g MgSO₄ · 7H₂O, 1.2 g Trizma base, 15 g agar (Difco), 3 mg nalidixic acid, 100 mg cycloheximide and 13 mg polymyxin B sulfate, adjusted to pH 7.4 after autoclaving; CNS (Gross and Vidaver, 1979) without daconil; NBY (Vidaver, 1967); PBS (phosphate-buffered saline 0.1 M) containing (per liter) 7.75 g Na₂HPO₄, 1.65 g KH₂PO₄, 0.5 g ascorbic acid, 8 g NaCl, pH adjusted to 7.4 after autoclaving. For each medium, heat-stable components were dissolved in distilled and autoclaved water. Heat-labile components were filter-sterilized using an FP 30/0.2 CA-2 filter (Schleicher & Schuell, Dassel, Germany) and added to cooled, molten, autoclaved medium before pouring into 9-cm plastic Petri dishes. Bacterial colonies that exhibited the typical appearance (yellow, raised and mucoid in appearance with round shape) of CMM on the various selective media were transferred to NBY agar plates for further identification. Infestation of plant-growth media with CMM was carried out as follows: a culture of CMM grown on NBY for 48–72 h was suspended in sterile distilled water and adjusted to an optical density (OD) of 0.67 at 480 nm to obtain 10⁸ cells ml⁻¹, and mixed with the growth medium to give a final concentration of 10⁶ cells g⁻¹.

2.4. Natural infection

Tomato seedlings (25 days-old, at the physiological age of three true leaves) of the indeterminate vine-type tomato cultivar 5656 (Hazera Genetics) were obtained from a commercial nursery (Hishtil Ltd., Afula, Israel). This cultivar is grown in greenhouses for fresh market consumption. Natural infection was observed during a test designed to compare the effects of several composts as peat-substitute growing-media components on crop development. Ten plants per treatment were grown in 10-l buckets, one plant per bucket, and were arranged in a randomized block design, in a temperature-controlled (18–26 °C) greenhouse during the period November 2003 to April 2004 under natural light conditions. Irrigation, fertilization and all plant-growing practices were conducted for all treatments according to common commercial practices in Israel. The major disease symptom was wilting which first appeared on January 11, 2004. All wilting plants were removed and taken for isolation and identification as described below.

2.5. Intentional plant inoculation

Tomato seedlings CV 5656 (Hazera Genetics; 25 days old), at the physiological age of three true leaves, were obtained from a commercial nursery (Hishtil Ltd., Afula, Israel). The inoculated

plants were maintained in a temperature-controlled greenhouse at 27 ± 2 °C with intervals of 12 h light and dark. The plants were grown in 1-l pots and irrigated daily. Plants were inoculated with 10⁴, 10⁶ or 10⁷ cells ml⁻¹ of CMM, by root dipping. The tomato seedlings were immersed for 60 min in the bacterial suspension, then transplanted to pots containing the different plant-growing media. Each treatment consisted of seven 1-l pots (replicates), with three tomato seedlings in each pot. The experiment was arranged in a randomized block design.

2.6. Bacterial survival studies

Plant-growing media were mixed with the bacterial suspension by means of a mixer to give a concentration of 10⁶ cells g⁻¹ and were incubated in the greenhouse in pots for 35 or 40 days as indicated and watered daily. Two experiments were conducted in five replicates. Samples of 10 g each were retrieved periodically and levels of bacterial populations were assessed as described further on. Sixty days after media infestation in the second experiment, tomato seedlings were transplanted into the same pots, without additional inoculum, in order to assess tissue colonization, as described below by the remaining CMM bacteria, as an additional tool for assessing bacterial survival. The estimation of CMM in plant-growing media was carried out as follows: 10 g samples of plant-growing media were added to 90 ml of sterile 0.1% (w/v) agar PBS in stomacher (Stomacher laboratory blender, Model 400, Type BA 7021, Seward Medical, UK) bags. The bags were sealed and shaken for 2 h on a rotary shaker at room temperature (24 °C). The bags were then mixed on the stomacher by gently shaking for 60 s. Then, 10-fold dilutions were performed from 10⁻¹ to 10⁻⁹, and each sample was applied (0.1 ml) on three media (two plates per media for each dilution): NBY (Vidaver, 1967), CNS (Gross and Vidaver, 1979), and D₂ANX (Chun, 1982), and incubated for up to 10 days at 27 ± 1 °C. The use of three media for CMM isolation is necessary due to the diversity in this pathogen population. All of the calculations were performed according to the medium that supported maximum colony-forming units of the pathogen population. Typical colonies were taken for further identification using the following three methods: Gram stain (Beveridge, 2001), PCR (Hadas et al., 2005) and plant inoculation for pathogenicity test (Fatmi and Schaad, 2002). Pathogenicity tests on tomato seedlings were performed by puncturing the stem four times with a needle that had been dipped in a suspension of bacteria containing 10⁷–10⁸ cells ml⁻¹. The inoculum was prepared from a culture grown overnight on nutrient agar and suspended in saline water (0.85% w/v NaCl in water). The plants were maintained at 28 °C and symptoms were recorded after 7–21 days. Every test was done in five replications.

2.7. Plant colonization by CMM

Three-week-old tomato plants were inoculated by root dipping (10⁵ CMM cells ml⁻¹), or by planting in infested medium (10⁶ cells g⁻¹), and were planted in TP-SCM compost, peat (Plantobalt) or perlite #4 (Habonim, Israel). The experiment was held under the conditions described above, but was continued for two growing cycles. The first cycle lasted 50 days from transplanting. Plants (symptomless) were uprooted and their stem tissue was assessed for colonization as described below. In the second cycle, tomato plants were transplanted into the same pots (without additional inoculation), 10 days later. The plants were grown for 54 days, uprooted and assessed for CMM colonization. Plants which were collected at the end of each cycle were washed and disinfected in sodium hypochlorite solution (0.3% a.i.) for 1 min, followed by three washes in sterile distilled water. Then, pieces of the stem were crushed and plated onto each of the following media: NBY (Vidaver,

1967), CNS (Gross and Vidaver, 1979), and D₂ANX (Chun, 1982), and incubated for up to 10 days at 27 ± 1 °C. Typical colonies were transferred from the different media to NBY to assess morphological characteristics. Further identification included: Gram staining, plant inoculation for pathogenicity test and PCR.

2.8. Statistical analyses

Area under the disease progress curve (AUDPC) and area under the decline of CMM population curve were calculated as the integral by means of PSI-Plot software (Poly Software International, New York, NY). The colonization percentages were arcsine transformed prior to the calculation of significance, in order to stabilize the variances. All values were subjected to a Tukey–Kramer Honestly Significant Difference test at $P = 0.05$.

3. Results

3.1. Compost and peat characteristics

The main chemical characteristics of the media used in these experiments are shown in Table 1. Although the organic matter content of peat is much higher than those of the composts (91% vs. 45–58%), peat is a more stable medium, which can sustain lower microbial activity, as reflected in its biological oxygen demand (BOD) value which is >33% lower than those of the composts. Composts have higher nutrients contents and their C/N ratios are much lower than that of peat.

3.2. Effect of plant-growing media on disease incidence and bacterial survival

Wilting of tomato plants by natural infection caused by CMM was observed in an experiment originally aimed at evaluating the effect of different growing media on tomato-plant growth (Table 2). The wilting phenomenon was evenly distributed among the experimental blocks, and appeared simultaneously in all blocks. Except for 10% wilting in compost TP–SCM–CM, none of the plants grown in the composts or in media containing 75% compost showed any wilting, while 60–100% of the plants grown in media containing peat or only 25% compost wilted. The compost therefore appeared to be suppressive to CMM. Average yield per plant followed closely ($R^2 = 0.951$, $P < 0.001$) the disease severity (Table 2). As compared to peat, the yield in the suppressive media was increased between 91% and 156%.

Table 2
Effect of growing media on yield and on incidence of wilting caused by *Clavibacter michiganensis* subsp. *michiganensis* in tomato plants.^w

Treatment ^x	Yield ^y (g plant ⁻¹)	Dead plants (%)
Peat moss	1852 c	70 a ^z
Peat (75%), TP–SCM (25%)	2445 bc	60 a
Peat (75%), PP–SCM–CM (25%)	830 c	100 a
Peat (75%), TP–SCM–CM (25%)	1717 c	70 a
Peat (25%), TP–SCM (75%)	4372 a	0 b
Peat (25%), PP–SCM–CM (75%)	4675 a	0 b
Peat (25%), TP–SCM–CM (75%)	4444 a	0 b
TP–SCM	4741 a	0 b
PP–SCM–CM	3769 ab	0 b
TP–SCM–CM	3536 ab	10 b

^w Plants were grown for 6 months, in the greenhouse.

^x TP–SCM = tomato plant residues + separated cattle manure; PP–SCM–CM = pepper plant residues + separated cattle manure + chicken manure; TP–SCM–CM = tomato plant residues + separated cattle manure + chicken manure.

^y Values followed by the same letter are not significantly different ($P < 0.001$).

^z Values followed by the same letter are not significantly different ($P = 0.05$).

In the subsequent experiment, we attempted to further validate this finding by using intentional inoculation. Tomato seedlings were inoculated by dipping their roots in one of three concentrations of CMM inoculum and transplanted to peat, perlite and TP–SCM compost. Disease incidence was significantly lower in the compost (79–97% reduction in AUDPC) than in the perlite and peat, at all tested inoculum concentrations ($P = 0.05$) (Fig. 2), thus further confirming the findings described in Table 2.

One of the mechanisms by which composts confer suppressiveness to soilborne pathogens relates to their effect on pathogen survival in the root zone (Aryantha et al., 2000; Raviv et al., 2005; Yogev et al., 2006). Survival of CMM in three compost media and peat was evaluated for 35 days (Fig. 3). Bacterial populations in the compost media were reduced to below detection levels after 20 days of incubation. In contrast, CMM level in the peat remained high during the entire period tested. The same trend was observed in an additional experiment in which TP–SCM was tested relative to peat and perlite (Fig. 4). Again, bacterial populations were reduced to below detection levels in the compost after 15 days of incubation while the CMM population remained high for 40 days in both peat and perlite, which served as organic and inert controls, respectively. Sixty days after inoculation of the media with CMM, tomato seedlings were transplanted into the same pots to examine whether the decline in the bacterial population would also be

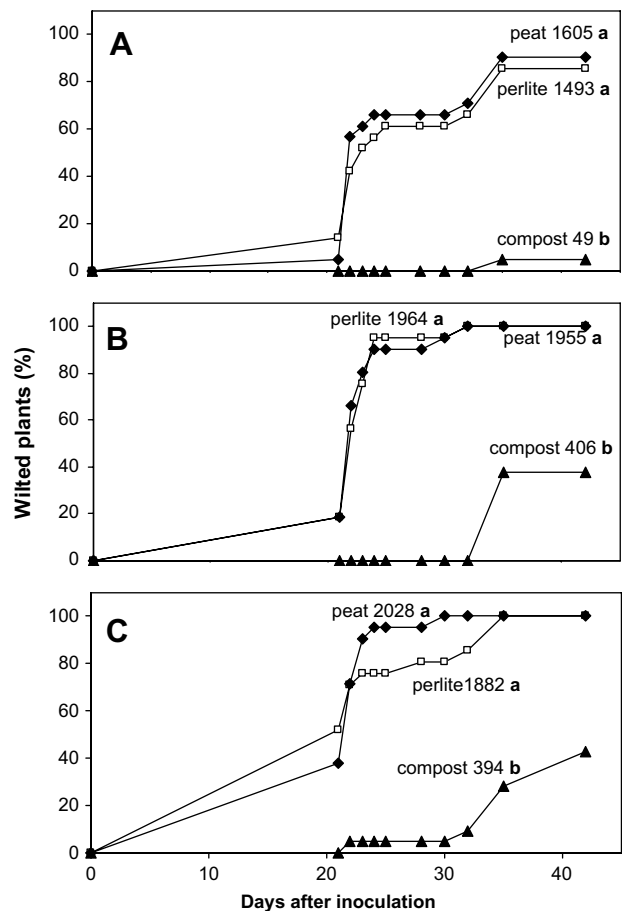


Fig. 2. Effect of growth media and inoculum concentrations on wilt incidence in tomato plants inoculated with *Clavibacter michiganensis* subsp. *michiganensis*, using the root-dipping method at three inoculum concentrations. Plants were grown in TP–SCM (tomato plant residues + separated cattle manure) compost, peat or perlite. Inoculum concentrations were: (A) 10^4 cells ml^{-1} , (B) 10^6 cells ml^{-1} and (C) 10^7 cells ml^{-1} . The statistical analysis refers to the area under the disease progress curve, which is shown by the numbers above or below the curves. Different letters at each inoculum concentration indicate a significant difference ($P = 0.05$).

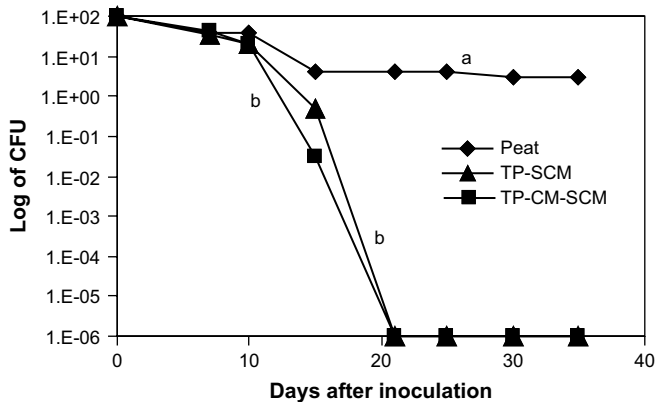


Fig. 3. Survival in time of *Clavibacter michiganensis* subsp. *michiganensis* populations in various organic media: TP-SCM (tomato plant residues + separated cattle manure compost), TP-SCM-CM (tomato plant residues + separated cattle manure + chicken manure compost), and peat, inoculated at 10^6 cells g^{-1} . The statistical analysis refers to the area under the decline of CMM population curve; different letters indicate significant difference ($P = 0.05$).

reflected in tissue colonization. Bacterial colonization of plants grown in TP-SCM compost (10%) was significantly lower than that of plants growing in peat (70%) (Fig. 4).

3.3. Effect of growth media and inoculation method on plant colonization

Using two inoculation methods, root dipping and planting in infested medium, TP-SCM compost significantly reduced colonization of the lower tomato stems, relative to peat and perlite (Table 3). When tomatoes were planted in the same media for a second growth cycle, a more pronounced and significant reduction in the colonization percentage of plants grown in the compost medium was observed. Tissue colonization by CMM was highest in plants grown in the peat medium during both cycles, in agreement with the data on CMM survival.

4. Discussion

The suppressive capacity of several plant-residue-based composts, including TP-SCM, has recently been described for four different formae speciales of *Fusarium oxysporum*—*melonis*, *basilici*, *radicis-lycopersici* and *radicis-cucumerinum*— using melon,

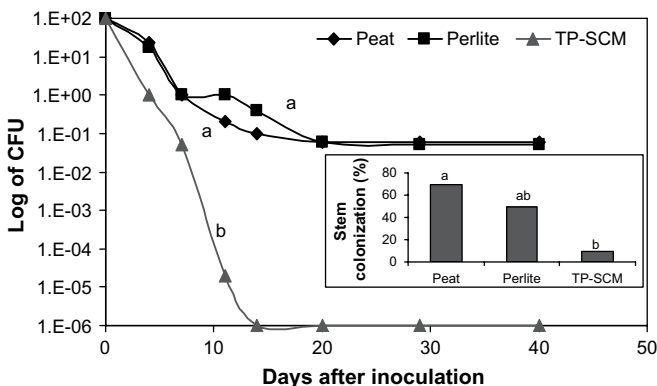


Fig. 4. Survival in time of *Clavibacter michiganensis* subsp. *michiganensis* populations in various organic media: TP-SCM (tomato plant residues + separated cattle manure compost), peat and perlite. Media were inoculated at 10^6 cells g^{-1} . The statistical analysis refers to the area under the decline of CMM population curve ($P = 0.05$). Inset: 60 days after medium infestation, tomato plants were transplanted into the same media and stem colonization was measured after 54 additional d. Different letters indicate significant difference ($P = 0.05$).

Table 3

Colonization of lower tomato stem by *Clavibacter michiganensis* subsp. *michiganensis* (CMM) growing on three growth media, using two inoculation methods.

Media and inoculation method	Colonization (%)	
	First cycle ^u	Second cycle ^v
Peat-MI ^w	53 a ^x	80 a
Perlite-MI	60 a	60 a
TP-SCM ^y -MI	20 b	0 b
Peat-RD ^z	90 a	80 a
Perlite-RD	90 a	30 b
TP-SCM-RD	30 b	10 b

Colonization (%) was evaluated at the end of each of the two plant- growth cycles.

^u First plant growth cycle lasted 50 days from transplanting. Afterwards, the plants were uprooted and their stem tissue was assessed for CMM colonization.

^v Ten days after the termination of the first cycle, tomato plants were transplanted into the same pots (without additional inoculation). The plants were grown for 54 days, uprooted and assessed for CMM colonization.

^w MI = Inoculation of the growth medium (10^6 cells g^{-1}).

^x Statistical analyses were conducted separately for each cycle and inoculation method. Values followed by the same letter are not significantly different ($P = 0.05$).

^y TP-SCM = tomato plant residues + separated cattle manure.

^z RD = Root dipping (10^2 cells ml^{-1}).

sweet basil, tomato and cucumber, respectively, as hosts (Raviv et al., 2005; Yogev et al., 2006). The ability of composts based on tomato plant residues to suppress CMM was also seen in the current study under commercial greenhouse conditions, where natural infection caused wilting of tomatoes (Table 2). The TP-SCM compost also caused a decline in the pathogen population (Figs. 2 and 3), reduced plant-tissue colonization (Fig. 4, Table 3) and reduced AUDPC for this disease between 79% and 97% (Fig. 2), even under severe intentional inoculation in which all plants in the peat collapsed. The fact that TP-SCM reduces two diseases of tomato—CMM and the fungal *Fusarium* crown and root rot (Raviv et al., 2005; Yogev et al., 2006), makes it an attractive component for tomato-growing media. Abbasi et al. (2002) concluded that compost amendments can play an important role in reducing economic losses from diseases, which should be of interest to tomato growers. Schönfeld et al. (2003) described another example of suppression of a soilborne bacterial pathogen, *Ralstonia solanacearum*. In that study, addition of compost to the soil resulted in an enhanced decline in the pathogen population and a significant reduction of disease incidence in tomato plants. The suggested mechanism was a shift in the composition of the soil microbial population due to the addition of the compost. In another example, infected radish and tomato plants grown in compost-amended substrates harbored significantly smaller populations of *Xanthomonas campestris* pv. *armoraciae* and *X. campestris* pv. *vesicatoria*, leading to reduced bacterial leaf spot via induced systemic resistance (Aldahmani et al., 2005). Utkhede and Koch (2004) reported CMM suppression under greenhouse conditions by vermicompost tea, applied as a spray, supporting the possibility of a mechanism of induced systemic resistance to CMM. In a previous study Yogev et al. (2006) found that the mechanism of *Fusarium* suppression by TP-SCM is microbial: medium sterilization resulted in nullification of the suppressiveness while under later bacterial recolonization, its suppressiveness was reinstated. The patterns of CMM decline in this compost (Figs. 2 and 3) and of the reduction in tissue colonization (Table 3) are similar to those with *Fusarium* (Yogev et al., 2006), possibly indicating a similar mechanism of suppression. However, this assumption has yet to be validated. The possibility that rhizobacteria are stimulated by composts and induce systemic resistance should be also considered.

Suppression of a pathogen by a growth medium or any other soil-management method, e.g. solarization (Greenberger et al., 1987), has a special benefit which is not seen with soil disinfestation by chemicals, or by steam disinfestations: adding a suppressive

compost to a growth medium not only reduces the incidence of disease caused by a native, pre-existing inoculum, as a chemical disinfectant does; it can also protect from reinfestation by invading inoculum, e.g. contaminated water or infected plant residues. Contamination from external sources is a common phenomenon in greenhouses with soilless media and this is especially relevant with CMM, which has several means of dissemination (Chang et al., 1991). Even if a pesticide is used for pathogen control, its dosage can be reduced by combining it with a suppressive growth medium which can be regarded as an additional measure. In addition to being much more suppressive than peat, composts based on plant waste material are also renewable resources and eliminate the need for landfilling and uncontrolled burning. They also have sanitation value since composting eradicates pathogens from the infected plant residues (Hadar and Mandelbaum, 1986; Hoitink and Fahy, 1986). Simultaneously, the tested composts had a beneficial effect on the yield (Table 2). We conclude that the tested plant-residue-based composts can suppress CMM and that this can become a component in integrated-management programs. There is a need, however, to ascertain that CMM-infected plant residues are exposed to high enough temperatures during the composting process so as to ensure complete eradication of any potential inoculum.

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4 General discussion

4.1 Suppression capability of composts

The suppressive ability of various types of composts in relation to *Fusarium* diseases has been demonstrated in several studies. (Bonanomi *et al.*, 2007; Borreo *et al.*, 2005; Borreo *et al.*, 2006; Chef *et al.*, 1983; Cheuk *et al.*, 2005; Escuadra *et al.* 2008; Kannangara *et al.*, 2000; Kavroulakis *et al.*, 2005; Pharand *et al.* 2002; Raviv *et al.*, 2005; Reuveni *et al.*, 2002; Ros *et al.* 2005; Trillas-Gay *et al.*, 1986). TP-SCM compost developed in the current study has a potential both to suppress several plant pathogens efficiently and to decrease problems of crop plant residues (distribution of seeds and diseases). This compost, which was produced several times under controlled conditions with the same characteristics during the current study, was excellent as a growing medium and kept its suppression ability for more than three years. In commercial compost production the results of the composting process may not be uniform. Compost may also be phytotoxic, if the process is unfinished and the compost is not mature (Widmer *et al.*, 1998). However, when TP-SCM compost was produced (twice) for this study by a commercial producer (Shacham, Givat Ada) under commercial conditions, but with proper turning regime and with a complete curing phase, the same level of suppression and physical and chemical properties as those of the controlled production compost were obtained. Several plant residues: tomatoes, wheat straw, orange peels and pepper plants served in this study as amendments to the coarse fraction of separated cattle manure in the controlled compost production process and no significant difference was observed in their suppression ability except in OP-SCM compost (Figure 3, Chapter 3.1); this compost was probably too old. Therefore, we can conclude that the quality of the composting procedure plays an important role in producing suppressive compost of cattle manure

with several additional plant wastes. The compost maintains its suppressive ability for a limited time. In other pathosystems, the "shelf-life" of compost's suppressiveness is much shorter: Danon *et al.* (2007) demonstrated that prolonged curing of biosolid compost resulted in the loss of suppressiveness towards *Sclerotium rolfsii*.

TP-SCM compost was found suppressive to *Fusarium oxysporum* f. sp. *melonis* and to three other virulent *formae speciales* of *Fusarium oxysporum*: *basilici*, *radicis-lycopersici* and *radicis-cucumerinum*, which may disperse very fast by aerial macroconidia. Moreover, this compost was also found suppressive towards the bacterial pathogen *Clavibacter michiganensis* subsp. *michiganensis* (CMM), which causes severe damage to tomatoes (reported here for the first time), and towards the foliar pathogen *Botrytis cinerea*. Horst *et al.* (2005), who reported the suppression of Botrytis blight in begonia by compost described it as an unusual phenomenon. TP-SCM compost has been also found suppressive to *Verticillium dahliae* in eggplants (Housam Kanaan 2009, personal communication). Most experiments were conducted on a small scale under controlled conditions, but the phenomenon was also verified in a commercial greenhouse under natural infestation.

The suppression effect was observed during four consecutive replanting cycles of melon seedlings inoculated with FOM, two growing cycles of tomato transplants inoculated with CMM and three growing seasons in a commercial cucumber greenhouse naturally inoculated with FORC. These data show the potential of suppressing severe plant diseases by TP-SCM compost. Previous studies on suppression of Fusarium incited diseases by composts have focused mainly on a single combination of compost and pathogen: coffee-waste composts for controlling 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 controlling Fusarium crown and root

rot in tomato plants, 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 the current study all *Fusarium* pathogens (and in addition diseases caused by CMM, *B. cinerea* and *V. dahliae*) were suppressed by exposure to the same compost, indicating a relatively broad spectrum of effectiveness. The disease-suppressive capability of TP-SCM and OP-SCM composts is associated with microbial activity as it nullified by gamma irradiation. Furthermore, rapid bacterial recolonization occurred within 16 hours of the exposure of the sterilized composts to the open air and was accompanied by increased suppressiveness (Table 2, Chapter 3.1). Similarly, Larkin *et al.* (1993) found that microwave treatment rendered a suppressive soil conducive to *Fusarium* wilt of melon, which shows the role of microorganisms in FOM suppression.

The decline of pathogen populations in the composts 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 mechanisms of bio-control by composts. In the present study, a more pronounced decline of pathogen populations in suppressive composts than in peat was shown in FORL, FOB and CMM but not in FOM and FORC. In these pathogen populations the decline occurred both in composts and in peat, indicating that 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 pathogens in that soil. Thus, the use of pathogen decline for predicting suppressive capability in a medium is relevant only when the suppression mechanism involves pathogen eradication.

The suppression of *Fusarium* wilt of melon by TP-SCM and OP-SCM composts and the suppression of CMM in tomato by TP-SCM and PP-SCM were accompanied by

reduced root and stem colonization by the pathogen, which may have resulted from the reduction in inoculum density in the growing medium, from induced resistance in the plant or from both. The reduction in tissue colonization accompanied by disease suppression was also found in melon plants with FOM by Cohen *et al.* (2002), and in plants with Fusarium wilt of watermelon by Martyn and Netzer (1991) and Zhou and Everts (2004). However, Larkin *et al.* (1993) found no difference in the colonization of watermelon roots by *F. oxysporum* f. sp. *niveum* in suppressive and non-suppressive mono-cultivated soils. Competition which leads to a fungistatic effect is another potential mechanism for suppression by composts (Serra-Wittling *et al.*, 1996). Such a mechanism is not necessarily connected with inoculum decline.

The efficient suppression of FORC by TP-SCM compost along three growing seasons could not be explained only as a direct effect of the compost on the pathogen. FORC produces macroconidia which disperse in the air and can penetrate plants through the foliage, without any contact with the growing medium (Gamliel *et al.*, 1996; Rekah *et al.*, 2000). Therefore, we concluded that induced resistance might be involved in this suppression phenomenon.

4.2 Induced resistance

Resistance induced by composts has already been reported for both foliar and root diseases (Zhang *et al.*, 1996, 1998; Pharand *et al.*, 2002). Reduction in disease intensity was demonstrated in several plant-pathogen systems such as *Septoria lycopersici* in tomato (Kavroulakis *et al.*, 2005) and bacterial leaf spot in radish caused by *Xanthomonas campestris* (Krause *et al.*, 2003). Suppression of Botrytis blight in begonia by compost has been reported by Horst *et al.* (2005). To the best of our knowledge, reduction of *B. cinerea* infection in cucumber and melon leaves by

compost, as exhibited in the current study, has not been reported previously. In the studies of foliar disease suppression, the site of treatment and the site of inoculation are spatially separated, thus induced resistance was suggested as a mechanism of suppression.

Unlike foliage diseases in which the pathogen and the root system are naturally separated, in soil-borne diseases the compost is in direct contact with the pathogen. Hence, if we hypothesize an induced resistance effect, we should separate the direct effect of the compost's microbial community on the pathogen from its indirect effect through the plant. The split root system allows separating the inoculation site from the treated site. This system was used by Zhang *et al.* (1996) and Lievens *et al.* (2001) to study induced resistance by compost to *Pythium* root rot. We failed to grow uniform and healthy melon plants with split roots using the method suggested by the above authors. Therefore, we side-grafted two individual melon plants, creating one strong plant with two equal and normally developed root systems. This plant was able to grow vigorously until fruit set (Figure 2.2.1). The side-grafting method allowed complete separation between the tested medium and the inoculated root-zone. Using this technology we were able to show significant resistance to *Fusarium* wilt in melon plants which was induced by the compost (Figures 3.2.4, 3.2.5). In addition, clear direct suppressiveness of the compost could also be noticed in the inoculated compost treatments. In contrast, induced resistance was not evident using the nursery transfer system, apparently since in this system the plant roots are exposed to the compost for only 7 days. Therefore, choosing an appropriate system is crucial for such a study.

The data regarding the mechanisms underlying resistance induced by compost treatment are sometimes contradicting. Zhang *et al.* (1996) found that compost induces resistance in cucumber to both *Pythium* root rot and anthracnose caused by

Colletotrichum orbiculare and that this phenomenon is negated by sterilization. They reported that the effect of compost on peroxidase activity in cucumber was most pronounced after plant infection. Similarly, high glucanase activity was found in *Arabidopsis* and cucumber plants grown in compost after infection, compared to plants grown in peat (Zhang *et al.*, 1998). These findings suggest that the microflora in the compost had an effect on these PR proteins in both plant types, but that much of the activation resulted from an infection by the pathogen. On the other hand, Alfano *et al.* (2007) found that *Trichoderma hamatum* 382 (which was isolated from compost) consistently modulated the expression of genes in tomato leaves without activation by the pathogen. However, except for one pathogenesis-related protein 5, the main markers of systemic induced resistance were not significantly induced. Yedidia *et al.* (2000) and Shores *et al.* (2005) studied the mechanism of induced resistance by *T. asperellum* 203 and found induction and accumulation of PR proteins in cucumber roots. The involvement of jasmonic acid and the ethylene signaling pathway were also verified (Shores *et al.*, 2005).

In the current study we have examined the expression of PR-Q, chitinase1 and peroxidase. PR-Q and peroxidase were found to be up-regulated in transgenic tobacco plants expressing viral movement proteins (Hofius *et al.*, 2001), and in squash plants infected by cucumber mosaic virus (CMV) (Teci *et al.*, 1996), respectively. Up-regulation of all three genes was found in source leaves of CMV-infected melon plants (L. Gil and S. Wolf, unpublished). However, the expression level of the three PR genes, chitinase1, peroxidase and PR-Q, was not elevated when melon plants were grown in the suppressive compost, compared to conducive peat. This is similar to the findings of Pieterse *et al.* (1996, 2002) on biocontrol bacteria in *Arabidopsis* and those of Alfano *et al.* (2007) on *T. hamatum* 382 in tomato. Alfano *et al.* (2007)

found that *T. hamatum* 382 induced systemic changes in plant physiology and disease resistance through systemic modulation of the expression of stress and metabolism genes. Similarly, van Loon *et al.* (1998) showed that beneficial microorganisms can induce systemic resistance (ISR). Pieterse *et al.* (1996, 2002) showed that biocontrol bacteria induced resistance in *Arabidopsis* independently of salicylic acid accumulation and PR gene expression. Zhang *et al.* (1998) concluded that compost induces systemic acquired resistance in a different way from that induced by pathogens or salicylic acid. On the other hand, Pieterse *et al.* (1998, 2002) and Verhagen *et al.* (2007) reported that rhizobacteria-mediated induced systemic resistance is controlled by a signaling pathway in which jasmonic acid and ethylene play key roles. It therefore appears that the induced resistance in our system is not associated with the three investigated PR genes, chitinase1, peroxidase and PR-Q, but it can be associated with other genes as was found by Alfano *et al.* (2007). The question of the mechanism of resistance induced by compost microbial populations is still open. The present study indicates that compost-induced suppressiveness operates by several mechanisms, including induced resistance. It is still an open question whether these mechanisms are ubiquitous in all types of composts.

4.3 Suppression of Fusarium wilt of melon in organically-managed soils

In Israeli organic farming, composts are applied to the soil annually. This is probably the main reason (coupled with the minimal use of pesticides) that organically managed soils exhibit greater biological activity than conventionally managed soils (Mäder *et al.*, 2002; van Diepeningen *et al.*, 2005). The addition of composted organic amendments to field soils has been shown to reduce the severity of soil-borne diseases

(Chellemi *et al.*, 2004; Lewis and Lumsden, 1992; Ros *et al.*, 2005; Rotenberg *et al.*, 2007a,b; Stone *et al.*, 2003 van Bruggen, 1995). Based on these observations it was decided to study the effect of organic management on soils' disease suppressiveness. The chosen pathosystem was melon and FOM. As far as we could verify, melon plants had not been grown in the tested plots which suggests that FOM was not a ubiquitous pathogen in these soils.

It was found that even under a high inoculation rate, when the percentage of dead plants in peat was 100% after only 15 days (Figure 3.4.1), we obtained suppression of the pathogen in 10 out of 15 organically-managed soils. This finding is compatible with the results of our previous experiments with composts (in soilless media) both on a small scale under controlled conditions greenhouse and in a commercial greenhouse. A similar phenomenon was found in organically-managed soils by several other researchers (Escuadra and Amemiya, 2008; Liu *et al.*, 2007; Messiha *et al.*, 2007).

The biological activity measured by calorimetry was significantly higher in the organic soils compared with the conventional ones (figure 3.4.3). Mäder *et al.* (2002) and van Diepeningen *et al.* (2005) also found higher biological activity in organic farming soils. The heat generation was found to correlate with the suppression level, suggesting that it may serve to predict the suppressiveness capability of the soils. However, we expected to find more pronounced differences between the suppression capability of the two differently-managed soils according to the many convincing evidences of suppression by composts reported in this study and the high quantities of compost applied annually to organically-managed soils. Nevertheless, in soils there are many other factors which may interfere with the results of any single assay. They include both biotic and abiotic factors.

Most of the above-cited studies were conducted under temperate climatic conditions (North Europe and North America). In these regions the soils are inherently richer in organic matter and their biological activity is higher than that of most of the soils in Israel, which belongs to the arid and semi-arid regions. The chemical and physical parameters of the soils exhibit only few differences between organic and conventional soils in other cases as well (van Diepeningen *et al.*, 2005). Although several significant differences were found between the chemical and physical parameters of the organic and conventional soils (most notably, their organic matter content), these characteristics showed no correlation with their suppressive ability.

More research should be conducted in order to elucidate the roles of these factors in the development of the suppressiveness phenomenon in organically-managed soils.

5 References

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בקרקות האורגניות נמצאה גבוהה יותר באופן מובהק מאשר בקרקות הקונבנציונאליות. נמצאה קורלציה בין כושר הדיכוי של הקרקות לבין שחרור החום מהן, עובדה המעידה על אפשרות של שימוש בבדיקה זאת כדי לחזות את כושר הדיכוי של הקרקות. למרות שנמצאו הבדלים בתכונות הכימיות והפיזיקאליות בין הקרקות האורגניות והקונבנציונאליות (ההבדל המשמעותי ביותר היה בתכולת החומר האורגני), לא נמצאו קורלציות בין תכונות אלו לכושר הדיכוי של הקרקות.

המחקר הנוכחי מראה שקומפוסט משרה עמידות למגוון רחב של פתוגנים באמצעות מנגנונים שונים שבהם יש מגע ישיר עם הפתוגן ובאמצעות השראת עמידות על הצמח. לא ידוע אם מנגנונים אלו מצויים בכל סוגי הקומפוסטים.

ישיר בין הפטרייה לגורם המחלה. הפטרייה FORC יוצרת כמויות גדולות של נבגים חיזוניים שנפוצים באוויר ויכולים לחדור לצמח דרך העלווה ללא קשר למצע הגידול. לפיכך, בדקנו את אפשרות מעורבותו של מנגנון השראת עמידות בתופעת דיכוי זאת.

מחלת הנוף עובש אפור שימשה ככלי ראשוני להערכת מעורבותו האפשרית של מנגנון השראת עמידות סיסטמית בצמחי מלון על ידי קומפוסט, לפני בדיקת הגורם העיקרי FOM. הוכח כי קומפוסט TP-SCM מעכב ב- 37-89% את התפשטות הכתמים הנקרוטים הנגרמים על ידי הפטרייה *Botrytis cinerea*. מערכת של פיצול שרשים שבה כל שורש גדל במצע שונה ומקבל טיפול שונה מאפשרת להעריך את ההשפעה העקיפה של המצע על הפתוגן באמצעות השפעתו על עמידות הצמח. בדיקת השראת עמידות לפוזריום בצמחי מלון על ידי קומפוסט, הצריכה פיתוח מערכת של צמחי מלון מורכבים בהרכבת צד, המאפשרת ייצור צמח עם שתי מערכות שורשים שוות ומפותחות זאת, מכיוון שהטכניקה הידועה של פיצול שרשים לא אפשרה התפתחות תקינה של צמחי מלון. בנוסף, מערכת הרכבת הצד מאפשרת לבחון את השראת העמידות במשך כל תקופת הגידול ללא השפעה של פציעת הצמח. באמצעות טכניקה זאת הצלחנו להראות ש 30% מהדיכוי של מחלת הפוזריום במלון על ידי קומפוסטים נובע מהשראת עמידות סיסטמית. כדי להבין את מנגנון השראת העמידות נבדקה התבטאותם של שלושה גנים הקשורים להשראת עמידות: PR-Q, chitinase I ו- peroxidase. לא נמצא ביטוי לגנים אלו בצמחי מלון שגדלו בקומפוסט המדכא לעומת כבול. ניתן להסיק, לפיכך, שבמערכת שלנו השראת העמידות אינה קשורה לשלושת הגנים שנבדקו, אך עשויה להיות קשורה לגנים אחרים.

מגוון הממצאים המוצגים במחקר זה בנושא יעילותם של קומפוסטים בדיכוי מחלות צמחים המוצגים במחקר זה הובילו אותנו לחקור האם הוספת קומפוסט לקרקעות תגרום לעליית כושר הדיכוי שלהן. בממשק גידול אורגני מוסיפים לקרקעות מידי שנה כמויות גדולות של קומפוסט כדי לספק חומרי מזון ולשפר את מבנה הקרקע. השווינו את כושר הדיכוי של קרקעות אורגניות ותיקות לקרקעות זהות המטופלות בממשק קונוונציונאלי. חמישה עשר צמדי קרקעות נדגמו ממשקים בכל הארץ, כאשר בכל צמד קרקע אורגנית וקונבנציונאלית דומות מאותו אתר. כושר הדיכוי של הקרקעות נבדק במערכת מלון - FOM באמצעות הדבקת נבטי מלון בפטריה ושתילתם בקרקעות השונות. בנוסף, נבדקה הפעילות הביולוגית של הקרקעות, כפי שהתבטאה בשחרור חום, באמצעות קלורימטר. התוצאות מראות ש 10 מתוך 15 הקרקעות האורגניות מדכאות את מחלת הפוזריום במלון יותר מאשר הקרקעות הקונבנציונאליות התואמות. הפעילות הביולוגית

תקציר

קומפוסטים מזבל בקר (SCM) מופרד בתוספת קש חיטה (WS), קליפות הדרים (OP) או שאריות צמחי עגבניה (TP) ופלפל (PP) יוצרו בתנאים מבוקרים ושמשו מרכיב עיקרי במצע גידול מנותקים על מנת לבחון את כושרם לדכא מחלות צמחים. נבדקה השפעת הקומפוסטים על דיכוי ארבעה תת מינים של הפטרייה פוזריום: *Fusarium oxysporum* f. sp. *melonis* (FOM),

F. oxysporum f. sp. *basilica* (FOB), *F. oxysporum* f. sp. *radicis-lycopersici* (FORL)

ו- *F. oxysporum* f. sp. *radicis-cucumerinum* (FORC).

הקומפוסטים TP-SCM, OP-SCM ו-WS-SCM דיכאו ב- 41-94% את מחלת הפוזריום במלון, עגבניה ומלפפון לעומת התפתחותן המהירה של המחלות בכבול. תופעת הדיכוי הוכחה תוך שימוש בסוגי מידבק שונים: נבגים מתרבית, מאקרוקונידיות שהוסרו מצוואר השורש של צמחי עגבניה ומלפפון חולים וקרקע נגועה בפטרייה. אכלוס שרשים וגבעולים ע"י פטריית הפוזריום בצמחי מלון שגדלו בקומפוסטים TP-SCM ו-OP-SCM היה נמוך יותר בהשוואה לצמחי מלון שגדלו בכבול. עיקור הקומפוסטים TP-SCM ו-OP-SCM בקרינת גמא ביטל את כושר הדיכוי של הקומפוסטים למחלת הפוזריום במלון לעומת הכבול בו לא השפיע העיקור על מהלך המחלה. נצפתה ירידה של 60-100% תוך 35 יום בכושר הנביטה של נבגי ארבעת תת מיני פטריית הפוזריום שהוספו לקומפוסטים. ירידה זאת יכולה לנבוע מהשפעה ישירה של הקומפוסט על הפתוגן. אפקט הדיכוי של קומפוסט TP-SCM נמשך במשך ארבעה מחזורי גידול עוקבים של שתילי מלון שהודבקו ב-FOM ושלוש עונות גידול רצופות של מלפפון בחממה מסחרית שהודבקו באופן טבעי ב-FORC. דיווחים קודמים על דיכוי מחלות פוזריום באמצעות קומפוסט התמקדו בעיקר בהשפעת קומפוסט על תת מין אחד של הפטרייה. במחקר הנוכחי הוכחנו כי ארבעה מיני פוזריום שונים דוכאו על ידי קומפוסט אחד (TP-SCM).

קומפוסט TP-SCM דיכא ביעילות גם את החיידק *Clavibacter michiganensis* subsp. *michiganensis*, שגורם לנבילה קשה של צמחי עגבניה, במשך שני מחזורי גידול. נתונים אלו מצביעים על הפוטנציאל הרב של קומפוסט TP-SCM לדכא מחלות צמחים קשות.

ההסבר ליעילות הדיכוי של מחלת הנבילה הקשה במלפפון, שנגרמת על ידי FORC, באמצעות קומפוסט TP-SCM במשך שלושה מחזורי גידול, אינו יכול להתבסס רק על דיכוי כתוצאה ממגע

עבודה זו מוקדשת באהבה ובהערכה

לבני משפחתי שעודדו תמכו וגילו

סבלנות רבה לאורך כל הדרך.

הבעת תודה

זכות גדולה היא להודות למדריכי פרופ' יצחק הדר ודר' מיכאל רביב על הנחיתם המקצועית, העידוד, העזרה והיחס החם שהעניקו לי בכל שלבי העבודה.

תודה מיוחדת לפרופ' יעקב קטן על הליווי המקצועי והתמיכה האין סופית, ולדר' רון כהן על העידוד המתמשך והעזרה בכתיבת העבודה והמאמרים.

תודה לפרופ' שמואל וולף וללידור גיל על נכונותם ועזרתם הפעילה בביצוע הבדיקות המולקולריות. תודה לדר' גיורא קריצמן על העזרה בביצוע הבדיקות הבקטריאליות.

תודה מיוחדת לדר' מנחם אדלשטיין על עזרתו המקצועית בתהליך הרכבת הצמחים.

תודה לפרופ' יגאל אלעד על אספקת פטרית הבוטריטיס ועצותיו המועילות.

תודות לדר' פנחס פיין על הנתונים הפדולוגים של הקרקעות. תודה לנביל גנאים על ההשקעה הרבה בניסויי החממה בבאקה אל גארביה.

תודה לתמר זכאי על הקדשת הזמן והסבלנות בעריכה הלשונית של עבודה זו.

תודה רבה לשרה ארז, לסוזן לוריא, לשלומית מדינה, לארקדי קרסנובסקי ולכרמלה חורב על העזרה הרבה לאורך כל הדרך. תודה לכל שאר החברים במחלקה למחלות צמחים ומיקרוביולוגיה בפקולטה לחקלאות, במחלקה לדלועים ובמחלקה לצמחי נוי במרכז המחקר נוה יער, על האווירה החברית וסביבת העבודה הנעימה.

עבודה זו נעשתה בהדרכתם של פרופ' יצחק הדר, המחלקה
למחלות צמחים ומיקרוביולוגיה, הפקולטה למדעי החקלאות,
המזון ואיכות הסביבה, האוניברסיטה העברית, רחובות, ודר'
מיכאל רביב, המחלקה לצמחי נוי, מינהל המחקר החקלאי,
מרכז מחקר נוה יער.

***Fusarium oxysporum* f. sp. *melonis* הפתוגנית**

על ידי קומפוסט

חיבור לשם קבלת תואר דוקטור לפילוסופיה

מאת

ענת יוגב

מוגש לסנט האוניברסיטה העברית בירושלים

יולי 2009