INOCULATION OF *Trichoderma harzianum* DURING MATURATION OF VINEYARD WASTE COMPOST TO CONTROL MUSKMELON *Fusarium* WILT

Agustina Bernal-Vicente, Margarita Ros,* and Jose Antonio Pascual

The utilization of compost vineyard wastes as suppressive growing media against *Fusarium* wilt is a good alternative for the disposal and recycling of these organic wastes. Inoculation of biological control agents (BCAs) enhances the biocontrol activity of compost. In this experiment, vineyard compost was sampled at different stages during the composting process, rating the values of acceptability for growing media. Under greenhouse nursery conditions, composts inoculated with *Trichoderma harzianum* T-78, (Th T-78*)* gave higher plant fresh weights, as well as lower pathogen incidence and disease severity than treatments with Th T-78 inoculated at muskmelon sowing and non-inoculated composts. Comparing the two composts inoculated with Th T-78 at different stages of the composting process, the one inoculated at the beginning of maturation phase showed lower pathogen incidence and disease severity than the one inoculated at the beginning of the composting process.

Keywords: Lignocellulosics; Fusarium oxysporum; Trichoderma harzianum; Melon seedling; Greenhouse nursery

Centro de Edafologia y Biologia Aplicada del Segura (CEBAS-CSIC), Department of Soil and Water Conservation and Organic Waste Management. P. O. Box 164, 30100 Espinardo, Murcia, Spain. Tel (+34) 968396200

** Corresponding author. Centro de Edafologia y Biologia Aplicada del Segura (CEBAS-CSIC), Department of Soil and Water Conservation and Organic Waste Management. P. O. Box 164, 30100 Espinardo, Murcia, Spain. Tel (+34) 968396200, Fax (+34) 968396213 Email: margaros@cebas.csic.es*

INTRODUCTION

In the region of Murcia (SE Spain), vineyards are widespread and cover large areas of land. The pruning of the vines is one of the most important procedures in the production of high-quality grapes. Therefore, the annual production of pruning residues is high.

Many alternatives for the disposal of these organic wastes have been proposed, composting being one of the most attractive on account of its low environmental impact and cost, as well as its capacity for generating a product valuable for increasing soil fertility (Pascual et al. 2002; Ros et al. 2005, 2006a; Masciandaro et al. 2000) or as a growing medium for horticultural purposes (Bustamante et al. 2008; López-Mondéjar et al. 2010). The main advantages of using composts as growing media are: 1) their high content of organic matter and nutrients, 2) their pathogen-free nature, and 3) their suppressive effect against phytopathogens (Ros et al. 2005; Segarra et al. 2007). Therefore, the application of compost may allow for reduced use of chemical fungicides

and/or fertilizers, thus reducing environmental problems and cutting the production costs in greenhouse nurseries.

During composting, organic matter is transformed into a rich humic product by the action of microorganisms and their enzymes (Garcia et al. 1992). Throughout the composting process, different microbial communities follow one another according to the nutritional and environmental conditions prevailing at each phase. Temperature is the most-influential parameter (Steger et al. 2007), although some other conditions also favour the presence of microorganisms with specific metabolic capabilities. The starting material is one of these conditions, since microorganisms in high concentration of the available carbon substrates will grow to a greater extent (Yogev et al. 2006).

The addition of specific microorganisms (biological control agents, BCAs) to compost is recommended to enhance disease suppression (Segall 1995; Trillas et al. 2006; Siddiqui et al. 2008), and further research, e.g. Postma et al. (2003), has revealed reduced severity of carnation diseases when using green compost inoculated with two different antagonists: *Verticillum bigatum* and non-pathogenic *F. oxysporum*, which are antagonistic to *R. solani* and *F. oxysporum* f. sp. *Dianthi*, respectively. *Trichoderma harzianum* is among the biological control agents (BCAs) most widely used against soil-borne pathogens (Lorito et al. 2010). Bernal-Vicente et al. 2009 have demonstrated the specific biocontrol effect of *T. harzianum* T-78 against *Fusarium oxysporum* f. sp. *melonis* under greenhouse nurseries. Different mechanisms are involved, such as antibiosis, mycoparasitism, competition for space or nutrients, and systemic induced or acquired resistance by a molecular cross-talk established between *Trichoderma* and the plant (Harman et al. 2004; Shoresh et al. 2010). A controlled inoculation strategy could improve efficacy and thus stimulate the incorporation of this biocontrol approach into everyday agricultural practices (De Ceuster and Hoitink 1999).

The objectives of this experiment were: 1. To study the feasibility of composting vineyard waste with and without Th T-78 inoculation, and the degree of Th T-78 establishment in the innermost part of the composting pile according to the time of inoculation: at the start of the composting process and at the beginning of the maturation stage. 2. To study the capacity of the composts inoculated with Th T-78 with regard to their use as suppressive growing media against *Fusarium* wilt of muskmelon seedlings compared to vineyard waste-based compost under greenhouse nursery conditions.

EXPERIMENTAL

Inoculum Preparations

The biological control agent (BCA) *T. harzianum* T-78 (Th T-78) was isolated from agricultural soil and preserved in the Spanish Type Culture Collection (CECT 20714). It was immobilized in bentonite following the protocol for its inoculation into compost described by Bernal-Vicente et al. (2009).

The phytopathogen *F. oxysporum* f. sp. melonis (FOM) was isolated from melon plants showing disease symptoms, using the method of Nash and Snyder (1962). Conidia of FOM were produced by growing one disc (5 mm diameter) of 7-day-old mycelia grown on potato dextrose agar (PDA, Scharlau (Spain)) in 100 mL of potato dextrose

broth (PDB, Scharlau (Spain). Both PDA and PDB were autoclaved at 121 ºC for 20 min and amended with 100 mg L^{-1} sterilised streptomycin. Culture was maintained at 25 ºC on a rotary shaker at 250 rpm for 5 days until the liquid medium was brown–pink in colour. Conidia were recovered by centrifugation (6000 g, 20 min), rinsed twice with sterile distilled water and filtered through 101 quartz wool (Panreac, Spain). Conidia concentrations were determined with a haemocytometer and diluted to the desired concentration.

Composting Process and Compost Characterisation

Three composting processes were carried out in the fields of the company Eisercorp (Hellin, Murcia, Spain). Open-air piles of 1 m^3 were prepared with vineyard wastes and inoculated with Th T-78 at the following stages of the composting process: Inoculation of Th T-78 (10^5 CFUs g^{-1}) at the start of the process (0 days) [**Pile–Th T-78 (0)**], at the beginning of maturation process (60 days) [**Pile-Th T-78 (60)]**, and non-inoculation of Th T-78 [**Control Pile**].

The composting processes were developed for 120 days. The moisture content was set initially between 40% and 50%, and it was maintained by watering. Piles were periodically turned to ensure aeration and the temperature evolution was monitored periodically.

Three samples from each of the three different piles were obtained by mixing sub-samples extracted from nine different locations in each pile. The sampling was performed at different times of the composting process (0, 26, and 120 days).

The physicochemical and chemical characteristics of the starting material (vineyard waste) and the final composts are shown in Table 1.

The electrical conductivity and pH of a 1/5 (w/v) aqueous extract were measured using a microconductivimeter (CM2002, Crison, Spain) and micro pH meter (pH 2002, Crison, Spain). The total organic carbon (TOC) was determined by the method of Yeomans and Bremner (1989), and total organic matter was measured by calcinations at 750 ºC. Nitrogen was determined by the Kjeldahl method, while total P and total K were determined according to Murphy and Riley (1962) and by flame photometry (JENWWAY PFP 7), respectively, after nitric–perchloric acid (1:1, v/v) digestion (Jackson 1958).

Table 1. Physicochemical and Chemical Properties of the Starting Material and the Mature Composts Obtained After Composting Process

Parameters	Vineyard	Pile-Th T-78 (0)	Pile-Th T-78 (60)	Control Pile
pH	5.56 c	6.71 ab	6.61 b	6.81 a
EC (mS cm^{-1})	2.0 b	1.89 bc	1.72 c	2.21 a
N total (%)	0.68 b	0.89 a	0.91 a	0.94 a
P total (%)	0.05 a	0.06 a	0.06 a	0.07 a
K total (%)	0.52 b	0.69 a	0.65 a	0.59 a
C/N	46.32 a	43.58 b	42.97 b	39.10 c

For each parameter, values with the same lower-case letter are not significantly different ($P < 0.05$).

Microbiological Analyses

The culturable microorganisms were estimated following serial dilutions of compost in sterile quarter-strength Ringer solution (OXOID, England), by plate counts. Potato dextrose agar (PDA, 39 g L^{-1} 50 mg L^{-1} rose Bengal; autoclaving at 120 ºC for 20 min, and amended with 100 mg L^{-1} of sterilized streptomycin) was used to estimate fungal growth after 3 days of incubation at 25 ºC and for estimating (Th-T-78). Tryptose soy agar (TSA, Oxoid: 3 g L^{-1}, 15 g L^{-1} agar, 50 mg L^{-1} nystatin) was used to estimate the bacterial population after 7 days of incubation at 28 ºC. The results are presented as the number of colony-forming units (CFUs) per gram of compost dried.

Greenhouse Experiment

The three mature composts obtained from the composting processes explained above were used as growing media in a greenhouse nursery. The treatments assayed were: vineyard waste compost inoculated with Th T-78 at 10^5 CFUs g^{-1} at the start of the composting process **[C-Th T-78 (0)]**, at the beginning of maturation process **[C-Th T-78 (60)]**, at the time of muskmelon planting **[C-Control +Th T-78]**, and vineyard waste compost without microbial inoculation **[C-Control].**

Muskmelon (*Cucumis melo* L. cv. Giotto) seeds were sown on the different growing media assayed as treatments, with a cover of vermiculite, in polystyrene trays with 150 wells, at one seed per well. The treatments of this experiment were established in a randomized design with six replicates per treatment (one tray per replication). Three of the six replicates were inoculated with FOM (3×10^4 CFUs g^{-1} growing medium), at 15 days after sowing.

Germination took place in a growth chamber at 28 ± 1 ºC and 90 to 95% relative humidity. Then, the trays were randomized on rails in a polyethylene-covered greenhouse with natural daylight conditions. The seedlings were irrigated daily and were harvested 30, 37, and 43 days after sowing. The aerial parts of all the seedlings were weighed to determine the fresh weight. Samples of the different growing media were stored at 4 ºC for analysis.

The % pathogen incidence was calculated for each treatment as: (FW- FWI/FW) *100. FW: fresh weight of seedlings without pathogen; FWI: fresh weight of seedlings with pathogen (Lopez-Mondejar et al. 2010). Disease severity of *Fusarium* wilt was rated according to a rating scale of 1-4. 1. Healthy; 2. Yellowing; 3. Stem wilting; 4. death.

Statistical Analysis

The data were analysed by SPSS 14.0 software, firstly with ANOVA. When the F-statistic was significant, Tukey´s post-hoc test ($P<0.05$) was used to separate means.

RESULTS AND DISCUSSION

Composting Process

Temperature fluctuations during composting followed a pattern similar to that of many other composting systems (Mena 2001; Ros et al. 2006b). The temperature ranged from 45 to 60 ºC during the first 40 days, while pile–Th T-78 (0) did not exceed 46 ºC

(Fig 1), staying around 40 ºC during the composting stage (60 days). These temperatures were reached as a consequence of the rapid breakdown of the readily-available organic matter and nitrogenous compounds by microorganisms (thermophilic stage). During maturation, beginning after 60 days of composting, the temperatures ranged between 30 and 35 ºC for all piles, where the organic matter became more stable - because the microbial activities and the organic matter decomposition rate decreased - and the temperature gradually declined (Ros et al. 2006b). The higher temperatures observed in Pile-Th T-78 (60) and the Control Pile 57 and 53 ºC respectively, compared to Pile-Th T-78 (0) 46 ºC, were probably due to Th T-78 inoculation at the beginning of the composting process, in which scenario *T. harzianum* did not permit the colonisation of ecological niches by other microorganisms (Benitez et al. 2004), thus slowing down the decomposition of organic matter.

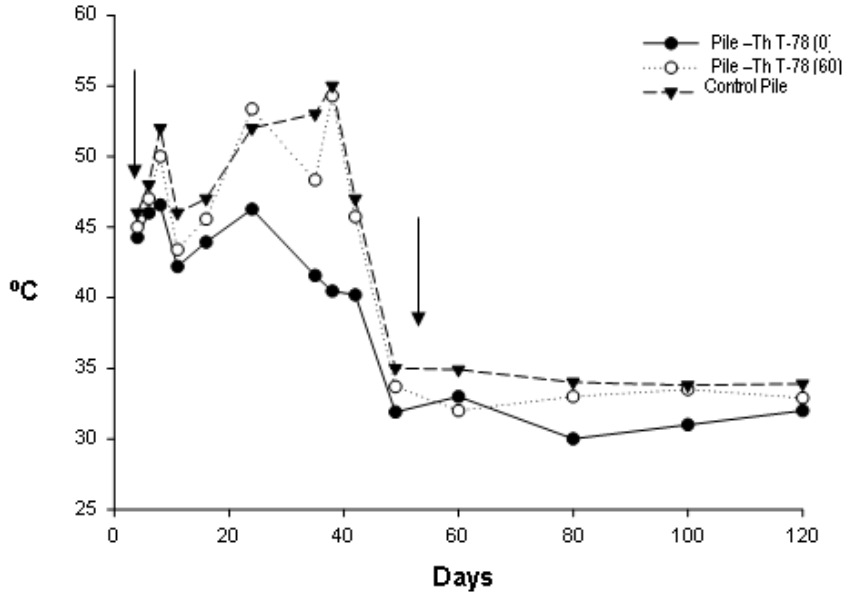

Fig. 1. Evolution of temperature during composting processes (↓ = Inoculation of the biological control, T. *harzianum* T-78 (Th T-78))

The maximum values of organic matter degradation over the composting process ranged from 7.0% to 14.5% (Table 2), lower than those reported in other composting processes (Benito et al. 2003; Garcia-Gomez et al. 2003). These differences might be due to the nature of the composting material, which was mainly composed of branches, showing a low content of readily-hydrolysable carbon (Komilis 2006). The incorporation of Th T-78 gave higher organic matter contents, and the decomposition was slower during the thermophilic stage for Pile-Th T-78 (0) 1% than for the other piles (Pile-Th T-78 (60) and the Control Pile, 8% respectively). In addition, the fungal and bacterial communities were maintained at the same level during this stage. These data do not agree with those of Singh and Sharma (2002), who observed an acceleration of organic matter breakdown with the inoculation of *T. harzianum,* a cellulolytic decomposer (Omar and Abd-Alla 1998).

Table 2. Total Organic Matter Content (%) at Three Time Points during Composting of Vineyard Waste

Piles	0 Days	26 Days	120 Days
Pile-Th T-78 (0)	93.43 a A	92.49 a A	86.80 b A
Pile-Th T-78 (60)	93.50 a A	85.90 b B	84.76 b A
Control Pile	93.55 a A	86.03 b B	80.00 c B

At each sampling time, values with the same capital letter are not significantly different ($P < 0.05$). For each treatment, values with the same lower-case letter are not significantly different ($P < 0.05$).

Differences in the microbial populations were expected as the temperature rose during the decomposition progress. The decomposition of organic matter in all piles was accompanied by a diminution of the CFUs of fungi and bacteria, due to the stabilization of the organic matter (Vargas-Garcia et al. 2007). The fungal communities diminished to a lesser extent than those of the bacteria over the composting process (Table 3). It is possible that lignocellulolytic microbial communities play a key role in the biotransformation processes that take place during composting, since lignocellulose is the main component of the organic wastes (Pérez et al. 2002). The population of fungi showed a reduction during the composting process, except in Pile–Th T-78 (0), where it was constant over time. The number of CFUs of the bacterial communities showed a significant reduction after the thermophilic stage. In Pile–Th T-78 (0), the number of bacterial CFUs at the end of the composting process was significantly higher than in other piles (Table 3).

Table 3. The Level of *T. harzianum* T-78 (Th T-78), Total Culturable Fungi and Total Culturable Bacteria at Three Time Points during Composting of Vineyard Waste

Th T-78 (log10 (CFUs g^{-1} compost))			
	0 Days	26 Days	120 Days
Pile-Th T-78 (0)	5.64 a A	4.72 b A	4.70 b B
Pile-Th T-78 (60)	<2 b B	<2 b B	5.34 a A
Control Pile	<2 a B	<2 a B	<2 a C
Fungi (log10 (CFUs g^{-1} compost))			
	0 Days	26 Days	120 Days
Pile-Th T-78 (0)	6.31 a B	6.46 a A	6.10 b A
Pile-Th T-78 (60)	6.47 a B	5.53 b B	5.20 b B
Control Pile	6.68 a A	5.73 b B	5.18 b B
Bacteria (log10 (CFUs g^{-1} compost))			
	0 Days	26 Days	120 Days
Pile-Th T-78 (0)	9.32 b B	9.75 a A	8.34 c A
Pile-Th T-78 (60)	9.79 a A	9.42 b B	7.78 c B
Control Pile	9.85 a A	9.69 b A	7.74 c B

At each sampling time, values with the same capital letter are not significantly different ($P < 0.05$). For each treatment, values with same lower-case letter are not significantly different ($P < 0.05$).

The number of CFUs of Th T-78 in Pile Th T-78 (0) diminished during the thermophilic stage, due to its low thermal resistance (Kredics et al. 2003), but it did not decrease more than one order of magnitude at the end of the composting process (Table 3). However, in Pile–Th T-78 (60), in which Th T-78 was inoculated at the beginning of maturation process, the final CFUs were of the same order of magnitude as at inoculation. In the Control Pile, the number of Th T-78 CFUs was below 100 CFU g^{-1} (Table 3). Vargas-Garcia et al. (2010) observed that the high temperatures favoured a high level of mesophilic microbial populations. Also, Zeilinger et al. (1993) found that the optimal pH for cellulose degradation with *T. Harzianum* enzymes was 4, while in our experiment the pH ranged between 6.8 and 6.6.

Greenhouse Experiment

The fresh weight of the aerial parts of the muskmelon seedlings in all the composts used as growing media increased during the greenhouse experiment (Table 4), independent of Th T-78 inoculation strategy. This was probably due to the 80-85% organic matter content, which is considered optimal for an ideal growing medium (Abad 2001), and also due to the nutrients available in the composts (Table 1). All the composts showed higher total N and total K than the starting material. Total P did not increase during composting. Although the nutrient values of the compost were low compared with other types of compost (Ros et al. 2003; 2006b; Yogev et al. 2006), the growth of plants with a low nutrient supply can produce lower pathogen incidence (Khan et al. 2004).

Table 4. Fresh Weight of Aerial Parts of Muskmelon Plants without FOM, at Three Different Time Points during the Greenhouse Nursery Experiment

Treatments	30 Days (g)	37 Days (g)	43 Days (g)
C-Control	1.37 b A	2.05 a A	2.01 a B
C-Control + Th T-78	1.26 c B	1.72 a B	1.55 b C
C-Th T-78 (0)	1.35 c A	2.00 b A	2.13a A
C-Th T-78 (60)	1.19 b C	2.08 a A	2.00 a B

At each sampling time, values with the same capital letter are not significantly different ($P < 0.05$). For each treatment, values with same lower-case letter are not significantly different ($P < 0.05$).

The pH values of the mature composts, ranging from 6.6 to 6.8, were higher than that of the starting material (vineyard waste) (Table 1). This could be attributed to the degradation of organic acids and the evolution of ammonia during the composting process. These values are slightly above the acceptable range for an ideal growing medium, pH 5.3 to 6.5, as defined by Abad et al. (2001). The electrical conductivity values of the composts obtained from piles inoculated with Th T-78 (Pile–Th T-78 (0) and Pile–Th T-78 (60)) were lower than those of the starting material and the other mature composts (Table 1). However, the mature composts showed electrical conductivity values above the limits established for ideal growing media, <0.5 dS m^{-1} (Abad et al. 2001), but below the upper limit of acceptability reported by Noguera et al. (2003), 3.5 dS m^{-1}. Mangal et al. (1988) showed the salt tolerance of muskmelon (the electrical conductivity at which growth is reduced significantly) to be 5.20 to 6.32 dS m^{-1}.

Also, the growth of muskmelon plants could have been influenced by the presence of hormone-like compounds acting as growth promoters (Bernal-Vicente et al. 2008). An increase of plant biomass in growing media containing composts also has been observed by other authors (Bustamante et al. 2008; Tittarelli et al. 2009; Bernal-Vicente et al. 2008).

Harman et al. (2004) suggested the use of *Trichoderma* spp. as plant growth enhancers, due to its production of growth hormones and enhanced transfer of minerals to the rhizosphere. However, in the greenhouse nursery assay, the inoculation of Th T-78 in the C-Control +Th T-78 treatment produced a significant decline in muskmelon fresh weight compared to other treatments, and a higher number of Th T-78 CFUs, which probably was because Th T-78 colonized the ecological niches of the composts and utilized nutrients and C sources from the composts.

The CFUs of Th T-78 in the C-Control treatment were below 100 UFC g^{-1} (Table 5). The number of CFUs in the C-Control +Th T-78 treatment was significantly higher than in C-Th T-78 (0) and C-Th T-78 (60). And this level was constant over the experiment. The treatment C-Th T-78 (60) gave higher numbers of Th T-78 CFUs than C-Th T-78 (0) during the experiment. The CFUs showed similar behaviour in the absence or presence of *F. oxysporum* (Table 5).

Table 5. Level of *T. harzianum* T-78 (Th T-78) at Three Different Time Points during the Greenhouse Nursery Experiment, with and without FOM

without FOM (Log10 (CFU/g compost))			
Treatments	30 Days	37 Days	43 Days
C-Control	<2 a D	<2 a D	<2 a C
C-Control + Th T-78	5.17 b A	5.89 a A	5.28 b A
C-Th T-78 (0)	4.20 a B	3.45 b C	4.00 a B
C-Th T-78 (60)	4.72 a C	4.66 a B	4.40 b B
with FOM (Log10 (CFU/g compost)			
Treatments	30 Days	37 Days	43 Days
C-Control	<2 a D	<2 a C	<2 a D
C-Control + Th T-78	5.20 c A	5.96 a A	5.48 b A
C-Th T-78 (0)	4.09 a C	3.41 b B	3.41 b C
C-Th T-78 (60)	4.87 c B	5.84 a A	5.12 b B

At each sampling time, values with the same capital letter are not significantly different ($P < 0.05$). For each treatment, values with same lower-case letter are not significantly different ($P < 0.05$).

The pathogen incidence and disease severity (Table 6) increased during the greenhouse assay in all treatments, reaching values ranged from 16% to 50% and 1.4 to 3.6, respectively. Seedlings grown on the C-Th T-78 (0) and C-Th T-78 (60) growing media showed lower pathogen incidence (0% to 22%) and disease severity (1 to 2.1) than the other growing media ((16% to 50%) and (1.6 to 3.6) respectively) over the experiment. The C-Th T-78 (60) treatment gave the lowest pathogen incidence and disease severity, ranging from 0% to 16% and 1 to 1.4, respectively. The inoculation of Th T-78 during composting [C-Th T-78 (0) and C-Th T-78 (60)], or at sowing [C-Control

+ Th T-78] showed that the pathogen incidence and disease severity can be reduced significantly in greenhouse nurseries. *Trichoderma harzianum* has been used widely as antagonistic fungal agent against *Fusarium oxysporum (*Celar 2003; Benitez et al. 2004). Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of the plant defence system are typical biocontrol actions of this fungus (Viterbo et al. 2001; Howell 2003). However, the C-Th T-78 (0) and C-Th T-78 (60) treatments, where Th T-78 was inoculated during the composting process, gave lower pathogen incidence and disease severity than C-Control + Th T-78, where inoculation was performed at sowing. However, in the latter case the CFUs of Th T-78 were one order higher (Table 5), indicating that the number of CFUs is important. The number does not reflect the real activity of fungi (Frankland 1990), due to the limitations of distinguishing between active forms (mycelia) and inactive forms (conidia, dead mycelia, etc.) of Th T-78 with such measurements (Lievens et al. 2006; Bridge and Spooner 2001).

Table 6. Pathogen Incidence and Disease Severity at Three Different Time Points during the Greenhouse Nursery Experiment

	Pathogen incidence (%) / Disease severity		
Treatments	30 Days	37 Days	43 Days
C-Control	26.80 / 2.8 b A	23.69 / 2.8 c A	50.36 / 3.6 a A
C-Control + Th T-78	18.10 / 1.7 b B	16.16 / 1.6 b B	29.63 / 3.0 a B
C-Th T-78 (0)	13.51 / 1.5 b C	12.30 / 1.5 b C	22.39 / 2.1 a C
C-Th T-78 (60)	0.00 / 1.0 c D	6.84 / 1.0 b D	15.95 / 1.4 a D

At each sampling time, values with the same capital letter are not significantly different ($P < 0.05$). For each treatment, values with same lower-case letter are not significantly different ($P < 0.05$).

The C-Th T-78 (60) treatment gave the lowest pathogen incidence and disease severity over the greenhouse experiment, probably due to the time at which Th T-78 was inoculated. The C-Th T-78 (0) treatment produced the second-lowest pathogen incidence and disease severity but the lowest number of CFUs, perhaps because *T. harzianum* activity was damaged during the thermophilic stage, due to its low thermal resistance (Kredics et al. 2003). The inoculation of Th T-78 during compost maturation probably increased its formation of complexes with organic matter, which increased its survival rate and activity, while avoiding loss by successive watering.

The pathogen incidence and disease severity for the C-Control treatment was higher (from 26.80% and 2.8, at 30 days, to 50.63% and 3.6 at 43 days, respectively) than those of the rest of the treatments over the greenhouse nursery assay. However, these values agreed with some reported works where suppression of pathogens by composts was obtained (Ntougias et al. 2008; Bernal-Vicente et al. 2008; Yogev et al. 2006). The suppression effect was strongly and repeatedly associated with microbial competition. Specific, antagonistic microorganisms that apparently re-colonize the compost following the thermophilic stage act as biocontrol agents, inducing plant resistance (Alabouvette et al. 2006; Hoitink et al. 1997; Perez-Piqueres et al. 2006).

CONCLUSIONS

1. Composting of vineyard waste is a good alternative for the disposal and recycling of these organic wastes, and the obtained compost can be used as a growing medium for muskmelon at greenhouse nursery conditions independently of the inoculation of *T. harzianum.*
2. The inoculation of *T. harzinum* at the beginning of maturation phase of composting process showed for this compost, lower pathogen (*F. oxysporum*) incidence and disease severity on muskmelon plants than the one inoculated at the beginning of the composting process, and at sowing time. This compost is a good suppressive growing media for muskmelon growth and to control *Fusarium* wilt.

ACKNOWLEDGMENTS

This research was supported by a national, competitive project funded by the Spanish Ministry of the Environment (Ministerio de Medio Ambiente Rural y Marino, MMARM (Reference 189 PC08 3-0.43). The author Agustina Bernal acknowledges the support of the FUNDACION SENECA in the funding of her research.

REFERENCES CITED

Abad, M., Noguera, P., and Bures, S. (2001). "National inventory of organic wastes for use as growing media for ornamental potted plant production: A case study in Spain," *Bioresource Technology* 77, 197-200.

Alabouvette, C., Olivain, C., and Steinberg, C. (2006). "Biological control of plant diseases: The European situation," *European Journal of Plant Pathology* 114, 329-341.

Benítez, T., Rincón, A. M., Limón, M. C., and Codón, A C. (2004). "Biocontrol mechanisms of *Trichoderma* strains," *International Microbiology* 7, 249-260.

Benito, M., Masaguer, A, Moliner, A., Arrigo, N., and Palma, R. M. (2003). "Chemical and microbiological parameters for the characteristics of the stability and maturity of pruning waste compost," *Biology and Fertility of Soils* 37, 184-189.

Bernal-Vicente, A., Ros, M., Tittarelli, F., Intringliolo, F., and Pascual, J. A. (2008). "Citrus composts and its water extract for cultivation of melon plants in greenhouse nurseries. Evaluation of nutriactive and biocontrol effects," *Bioresource Technology* 99, 8722-8728.

Bernal-Vicente, A., Ros, M., and Pascual, J. A. (2009). "Increased effectiveness of the *Trichoderma harzianum* isolate T-78 against *Fusarium* wilt on melon plants under nursery conditions," *Journal of the Science of Food and Agriculture* 89, 827-833.

Bridge, P., and Spooner, B. (2001). "Soil fungi: Diversity and detection," *Plant Soil* 232, 147-154.

Bustamante, M. A., Paredes, C., Moral, R., Agullo, L., Perez-Murcia, M. D., and Abad, M. (2008). "Compost from distillery wastes as peat substitutes for transplant production," *Resources, Conservation and Recycling* 52, 792-799.

Celar, F. (2003). "Competition for ammonium and nitrate forms of nitrogen between some phytopathogenic and antagonistic soil fungi," *Biological Control* 28, 19-24.

De Ceuster, T. J. J., and Hoitink, H. A. J. (1999). "Prospects for composts and biocontrol agents as substitutes for methyl bromide in biological control of plant diseases," *Compost Science & Utilization* 7, 6-15.

Frankland, J. C., Dighton, J., and Body, L. **(**1990). "Methods for studying fungi in soil and forest litter," In: Grigorova, R., and Norris, J. R. (eds.), *Methods in Microbiology: Techniques in Microbial Ecology,* Academic Press, London, 343-404.

García, C., Hernández, T., Costa, F., Ceccanti, B., and Ciardi, C. (1992). "Changes in ATP content, enzyme activity and inorganic nitrogen species during composting of organic wastes," *Canadian Journal of Soil Science* 72, 243-253.

Garcia-Gomez, A., Roig, A., and Bernal, M. P. (2003). "Composting of the solid fraction of olive mill wastewater with olive leaves: Organic matter degradation and biological activity," *Bioresource Technology* 86, 59-64.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. (2004). "*Trichoderma* species – Opportunistic, avirulent plant symbionts," *Nature Reviews Microbiology* 2, 43-56.

Hoitink, H. A. J., and Grebus, M. E. (1997). "Composts and the control of plant diseases," In: Hayes, M. H. B., and Wilson, W. S. (eds.), *Humic Substances in Soils, Peats and Waters: Health and Environmental Aspects*, Royal Society of Chemistry. Cambridge, UK, 359-366.

Howell, C. R. **(**2003). "Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts," *Plant Disease* 87, 4-10.

Jackson, M. L. (1958). *Soil Chemical Analysis*, Prentice-Hall, Englewood Cliffs, NJ, pp. 396-402.

Khan, J., Ooka, J. J., Miller, S. A., Madden, L. V., and Hoitink, H. A. J. (2004). "Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophtora* crown rot and leaf blight," *Plant Disease* 88, 280-286.

Komilis, D. P. (2006). "A kinetic analysis of solid waste composting at optimal conditions," *Waste Management* 26, 82-91.

Kredics, L., Antal, Z., Manczinger, L., Szekeres, A., Kevei, F., and Nagy, E. (2003). "Influence of environmental parameters on *Trichoderma* strains with biocontrol potential," *Food Technology and Biotechnology* 41, 37-42.

Lievens, B., Brouwer, M., Vanachter, A. C., Cammue, B. P. A., and Thomma, B. P. H. J. (2006). "Real-time PCR for detection and quantification of fungal and oomycete tomato pathogens in plant and soil samples," *Plant Science* 171, 155-165.

López-Mondéjar, R., Bernal-Vicente, A., Ros, M., Tittarelli, F., Canalli, S., Intrigliolo, F., and Pascual, J. A. (2010). "Utilisation of citrus compost-based growing media amended with *Trichoderma harzianum* T-78 in *Cucumis melo* L. seedling production," *Bioresource Technology* 101, 3718-3723.

Lorito, M., Woo, S. L., Harman, G. E., and Monte, E. (2010). "Translational research on *Trichoderma*: From 'omics to the field," *Annual Rev. of Phytopathology* 48, 395-417.

Mangal, L., Hooda, P. S., and Lal, S. (1988). "Salt tolerance of five muskmelon cutivars," *Journal of Agricultural Science* 110, 641-643

Masciandaro, G., Ceccanti, B., and García, C. (2000). " 'In situ' vermicomposting of biological sludges and impacts on soil quality," *Soil Biol. Biochem.* 32, 1015-1024.

Mena, E. (2001). "Composting of urban sewage sludges: A suitable strategy for their recycling in soil," Degree Thesis, Faculty of Chemistry, University of Murcia.

Murphy, J., and Riley, J. P. (1962). "A modified single solution method for the determination of phosphate in natural waters," *Analytica Chemica Acta* 27, 31-36.

Nash, S. M., and Snyder, W. C. (1962). "Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soil," *Phytopathology* 52, 567-572.

Noguera, P., Abad, M., Puchades, R., Maquieira, A., and Nogueras, V. (2003). "Influence of particle size on physical and chemical properties of coconut corrí dust as container medium," *Communications in Soil Science and Plant Analysis* 34, 593-605.

Ntougias, S., Papadopoulou, K. K., Zervakis, G. I., Kavroulakis, N., and Ehaliotis, C. (2008). "Suppression of soil-borne pathogens of tomato by composts derived from agroindustrial wastes abundant in Mediterranean regions," *Biology and Fertility of Soils* 44, 1081-1090.

Omar, S. A., and Abd Alla, M. H. (1998). "Biocontrol of fungal root rot disease of crop by the use of Rhizobia and Bradyrhizobia," *Molecular Microbiology* 43, 431-437.

Pascual, J. A., Hernández, M. T., García, C., Deleij, F. A. A. M., and Lynch, J. M. (2002). "Long-term suppression of *Pythium ultimum* in arid soil using fresh and composted municipal water," *Biology and Fertility of Soils* 30, 478-484.

Pérez, J., Muñoz-Dorado, J., de la Rubia, T., and Martínez, J. (2002). "Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview," *International Microbiology* 5, 53-63.

Perez-Piqueres, A., Edel-Hermann, V., Alabouvette, C., and C.Steinberg. (2006). "Response of soil microbial communities to compost amendments," *Soil Biology & Biochemistry* 38, 460-470.

Postma, J., Montanari, M., and van den Boogert, H. J. F. (2003). "Microbial enrichment to enhance the disease suppressive activity of compost," *European Journal of Soil Biology* 39, 157-163.

Ros, M., Hernández, T., and García, C. (2003). "Soil microbial activity alters restoration of a semiarid soil by organic amendments," *Soil Biology & Biochemistry* 33, 463-469.

Ros, M., Hernández, M. T., García, C., Bernal, A., and Pascual, J. A. (2005). "Biopesticide effect of green compost against *Fusarium* wilt on melon plants," *Journal of Applied Microbiology* 98, 845-854.

Ros, M., Klammer, S., Knapp, B., Aichberger, K., and Insam, H. (2006a). "Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity," *Soil Use and Management* 22, 209-218.

Ros, M., García, C., and Hernández, T. (2006b). "A full-scale study of treatment of pig slurry by composting: Kinetic changes in chemical and microbial properties," *Waste Management* 26, 1108-1118.

Segall, I. (1995). "Marketing compost as a pest control product," *Biocycle* 36, 65-67.

Segarra, G., Casanova, E., Borrero, C., Aviles, M., and Trillas, I. (2007). "The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plants," *European Journal of Plant Pathology* 117, 393-402.

Shoresh, M., Mastouri, F., and Harman, G. E. (2010). "Induced systemic resistance and plant responses to fungal biocontrol agents," *Annual Rev. Phytopathology* 48, 21-43.

Siddiqui, Y., Meon, S., Ismail, M. R., and Ali, A. (2008). "*Trichoderma*-fortified compost extracts for the control of *Choanephora* wet rot in okra production," *Crop Protection* 27, 385-390.

Singh, A., and Sharma, S. (2002). "Composting of a crop residue through treatment with microorganisms and subsequent vermicomposting," *Bioresour. Technol.* 85, 107-111.

Steger, K., Jarvis, A., Vasara, T., Romantschuk, M., and Sundh, I. (2007). "Effects of differing temperature management on development of Actinobacteria populations during composting," *Research for Microbiology* 158, 617-624.

Tittarelli, F., Rea, E., Verrastro, V., Pascual, J. A., Canali, S., Ceglie, F. G., Trinchera, A., and Rivera, C. M. (2009). "Compost-based nursery substrates: Effect of peat substitution on organic melon seedlings," *Compost Science & Utilization* 17, 220-228.

Trillas, M. I., Casanova, E., Cotxarrera, L., Ordovás, J., Borrero, C., and Avilés, M. (2006). "Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings," *Biological Control* 39, 32-38.

Vargas-Garcia, M. C., Suarez-Estrella, F., López, M. J., and Moreno, J. (2007). "Effect of inoculation in composting process: Modifications in lignocellulosic fraction," *Waste Management* 27, 1099-1107.

Vargas-Garcia, M. C., Suarez-Estrella, F., Lopez, M. J., and Moreno, J. (2010). "Microbial population dynamics and enzyme activities in composting processes with different starting materials," *Waste Management* 30, 771-778

Viterbo, A., Haran, S., Friesem, D., Ramot, O., and Chet, I. (2001). "Antifungal activity of a novel endochitinase gene (chit 36) from *Trichoderma harzianum* Rifai TM," *FEMS Microbiology Letters* 20, 169-174

Yeomans, J., and Bremner, J. M. (1989). "A rapid and precise method for routine determination of organic carbon in soil," *Communications in Soil Science and Plant Analysis*19, 1467-1476.

Yogev, A., Raviv, M., Hadar, Y., Cohen, R., and Katan, J. (2006). "Plant waste-based composts suppressive to diseases caused by pathogenic *Fusarium oxysporum*," *European Journal of Plant Pathology* 116, 267-278.

Zeilinger, S., Kristufek, D., Arisan-Atac, I., Hodits, R., and Kubicek, P. C. (1993). "Condition of formation, purification and characterization of an alpha-galactosidase of *Trichoderma reesei* RUT C-30," *Applied and Environmental Microbiology* 59, 1347-1353.

Article submitted: October 21, 2011; Peer review completed: February 12, 2012; Revised version received: March 2, 2012; Accepted: March 4, 2012; Published: March 12, 2012.