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Temperature effect on *Fusarium oxysporum* f.sp. *melonis* survival during horticultural waste composting

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ABSTRACT

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Aims: The aim of this work was to study the effect of high temperatures generated during composting process, on the phytopathogen fungus *Fusarium oxysporum* f.sp. *melonis*. This investigation was achieved by both *in vivo* (semipilot-scale composting of horticultural wastes) and *in vitro* (lab-scale thermal treatments) assays.

Methods and Results: Vegetable residues infected with *F. oxysporum* f.sp. *melonis* were included in compost piles. Studies were conducted in several compost windrows subjected to different treatments. Results showed an effective suppression of persistence and infective capacity, as this process caused complete fungal elimination after 2–3 days of composting. In order to confirm the effect of high temperature during this process, *in vitro* experiments were carried out. Temperature values of 45, 55 and 65°C were tested. All three treatments caused the elimination of fungal persistence. Treatment at 65°C was especially effective, whereas 45°C eliminated fungal persistence only after 10 days.

Conclusions: The composting process is an excellent alternative for the management of plant wastes after harvesting, as this procedure is able to suppress infective capacity of several harmful phytopathogens such as *F. oxysporum* f.sp. *melonis*.

Significance and Impact of the Study: *Fusarium oxysporum* f.sp. *melonis* is a plant pathogen fungus specially important in the province of Almería (south-east Spain), where intensive greenhouse horticulture is very extended. High temperatures reached during composting of horticultural plant wastes ensure the elimination of phytopathogen microorganisms such as *F. oxysporum* f.sp. *melonis* from vegetable material, providing an adequate hygienic quality in composts obtained.

Keywords: composting process, *Fusarium oxysporum* f.sp. *melonis*, infective capacity, persistence, plant wastes.

INTRODUCTION

Greenhouse culture of horticultural plants is a major source of revenue in the province of Almería (south-east Spain) (Escobar 1998). There are about 30 000 ha of greenhouse culture distributed in this zone which yield a large amount of plant wastes (around 10^6 t per year) (Cara and Rivera 1998) and constitute a very serious environmental problem, as these vegetable residues are considered an important inoculum source of microorganisms and there are many reports showing that several soil-borne phytopathogens may survive on them (Conway 1996). Thus, management of crop residues in the field after harvesting is necessary to minimize these adverse effects, particularly if the pathogens involved can infect subsequent crops (Conway 1996).

The dumping or incineration of plant waste, or simply leaving it to decay in the vicinity of greenhouses are common practices, causing several undesirable agronomic and environmental effects.

The composting process is an adequate and profitable alternative for waste management. This process is an

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oxidative biotransformation that requires a rich and varied microbiota. Composting of green wastes has been shown to be a good system for the treatment and utilization of agricultural wastes, enhancing the preservation of the environment and improving soil fertility (García *et al.* 1993). Although the usefulness of compost for the control of phytopathogens was already known and practiced in the early stages of agriculture (Cook and Baker 1983), actually there are many reports related to the practical use of quality compost for plant health (Fuchs 2002). In this sense, after the discovery of some soil-borne plant pathogen micro-organisms, interest has grown in the composting process to take advantage of its plant protecting properties (Hoitink and Fahy 1986; Kostov *et al.* 1996; Suárez-Estrella *et al.* 2001, 2002).

Composting is proposed as an important means of plant disease control when applied to plant waste (Hoitink and Fahy 1986). Three main factors seem to be involved in the suppression activity of this composting process: (1) the high temperatures generated during thermophilic phase (above 40–50°C) (Yuen and Raabe 1984; Bollen 1985); (2) the production of antimicrobial compounds such as phenolics, generated during lignocellulosic material decay (Pérez-Torres 1988; Sáez 1989) and (3) the colonization of compost with many different organisms that either compete with pathogens for nutrients and/or produce general antibiotics that reduce pathogen survival and growth (Hoitink *et al.* 1997; Hoitink and Boehm 1999). Participation of all of these factors is desirable to ensure high-quality compost.

In this area, several important plant diseases caused by typical phytopathogens are known. Damping-off by *Pythium aphanidermatum* and/or stem rot caused by *Rhizoctonia solani* are diseases which are difficult to control (Gómez 1993). However, *Fusarium oxysporum* f.sp. *melonis* together with melon necrotic spot virus (MNSV) have become the most problematic and determinant disease agents in recent years (Gómez 1994).

Formae speciales of *F. oxysporum* cause vascular wilt diseases affecting a large number of hosts (Nelson 1981; Katan and Di Primo 1999). These pathogens are known to persist over long intervals among susceptible crops (Burgess 1981) and this persistence has been attributed mainly to the production of long-lived chlamydospores (McKeen and Wensley 1961; Garret 1970). There are seven *formae speciales* affecting cucurbits, being *F. oxysporum* f.sp. *melonis* one of the most important on a worldwide scale (Kim *et al.* 1993).

Once introduced into a field, *F. oxysporum* f.sp. *melonis* can remain indefinitely, even after rotation with non-susceptible crops (Gordon *et al.* 1989). This fungus survives in soil as chlamydospores, and is capable of colonizing crop residue and roots of most crops likely to be grown in rotation with melon (Gordon *et al.* 1989). It can enter the host through root tips, primarily in the area of elongation, and is aided by wounding (Martyn and Gordon 1996). As a result of the persistence of the pathogen in the soil, the disease is best controlled with cultivars that carry multiple resistance (Zink 1992).

Fusarium wilt caused by *F. oxysporum* f.sp. *melonis* is an important disease affecting melon production in the province of Almeria (Gómez 1994) giving rise to important economic loss in this area over the last 15 years. The suppression of this plant pathogen is therefore considered to be a need of modern agriculture.

This work proposes the composting process as a control method for *F. oxysporum* f.sp. *melonis* in which the great effect of temperature is involved in phytopathogen fungus elimination.

MATERIAL AND METHODS

Fusarium oxysporum f.sp. *melonis*

The phytopathogen fungus was *F. oxysporum* f.sp. *melonis* CECT (Spanish Type Culture Collection) 20474, a strain isolated from a diseased melon plant (*Cucumis melo* L.), showing typical symptoms of fusarium wilt and collected in June 1997 from a plastic greenhouse located in Almería (Spain).

Infected plant residues and experimental conditions

Flasks with 20 g of sterilized melon residues were inoculated with a 7-day-old fungal liquid culture (10^6 UFC g^{-1} of residue, approximately). After incubation at 30°C (15–30 days) residues were individually distributed and introduced into muslin bags. These were exposed to composting process.

For *in vitro* thermal treatments, 1 g of plant material was autoclaved (120°C, 20 min) in test tubes, inoculated in the same way above mentioned with *F. oxysporum* f.sp. *melonis* and finally exposed to different temperatures in incubation chambers. Temperature values independently assayed were 30, 45, 55 and 65°C.

In all cases, a propagule count of infected residues was carried out before the different processes were initiated. Furthermore, negative controls (untreated samples) were analysed simultaneously.

Fusarium oxysporum f.sp. *melonis* persistence and infective capacity during the composting process

Muslin bags containing infected residues were placed in the composting piles at a depth of 60 cm. One muslin bag was

taken from each pile at 0, 12, 24, 36, 48, 60, 108 and 204 h. These were subdivided into three subsamples. Residues were triturated with saline solution and serial dilutions were sown in potato dextrose agar medium (PDA). *Fusarium oxysporum* f.sp. *melonis* growth was analysed by colony forming units (CFU) count after 5 days and expressed as log of CFU per g of residue. The remaining triturated residue was used to inoculate melon plants which were kept in a greenhouse and observed for 40 days. After this time, *F. oxysporum* f.sp. *melonis* isolation in PDA was carried out from the stems of inoculated diseased plants (tissues treated with ethanol and cut into small pieces). In this case, infective capacity was expressed as infected plant percentage.

Fusarium oxysporum f.sp. *melonis* persistence and infective capacity during thermal treatments

Test tubes containing infected residues were incubated at 30°C. Five samples were taken at different time intervals. Infected and treated residues were suspended in 10 ml of potato dextrose broth (PDB) to confirm the *F. oxysporum* f.sp. *melonis* persistence after 5 days of incubation at 30°C with shaking. On the other hand, five samples were suspended and triturated in saline solution to inoculate melon plants which were used to confirm the infective capacity as cited above.

In this case, both persistence and disease incidence were expressed as the percentage of samples.

Semipilot-scale composting

Plant residues used to achieve the composting process were maintained inside open windrows. Windrows were 1.2 m × 1.5 m at the bottom and were up to 1.2 m high. Vegetable wastes were mainly constituted of pepper and C/N ratio of the mixture was 20–25 approximately. Air-forced composting of each pile was achieved through five perforated PVC tubes (5 cm diameter, 120 cm long) placed below a fine mesh screen near the bottom of the pile. Air flow of 90 m^3 for 1 h was applied by a blower (S&P CBB-60).

Piles were periodically turned and aerated while fluctuations in temperature values were observed. Temperature was monitored daily during the process using composting thermometers (Delta OHM, HD 9010; Caselle di Selvazzano (Padova), Italy). Turning was applied weekly from the fisrt 14 days of composting, until 42 days. After 40 days, temperature values inside the piles were stabilized at environmental levels. Water was added during turns to maintain the moisture content at 50–60% approximately. During the curing phase the piles were neither turned nor aerated.

A different combination of aeration factor and inoculation with a cellulytic fungus (*Trichoderma* sp.) was applied to each pile: Pile A (aerated, inoculated), Pile B (non-aerated, inoculated), Pile C (aerated, non-inoculated) and Pile D (non-aerated, non-inoculated).

Statistical analysis

Statistical analysis was carried out to analyse the fungal persistence and infectivity with respect to both different composting process and exposure time. In relation to thermal treatments, only the effect of temperature and exposure time were analysed. All experiments were designed as factorial treatments. Data were analysed by multifactorial analysis of variance (ANOVA) and compared using Fisher's protected least significant difference (LSD) test at 95% significance level. Variance components and correlation analyses were also carried out at 95% significance level.

RESULTS

Fusarium oxysporum f.sp. *melonis* persistence and infective capacity during composting process

Figure 1 shows the results obtained in relation to *F. oxysporum* f.sp. *melonis* survival in composting piles A, B, C and D. Results are shown as log of mean propagules per gram of residue against composting time (h). Analyses from non-composted samples are also shown in this graph (negative control). A sharp decrease in the number of fungal propagules was observed in all cases after 36–48 h of composting. However, the negative control showed stable behaviour over the whole assay period (Fig. 1). The total elimination of *F. oxysporum* f.sp. *melonis* was achieved after 96 h in all composting piles, although pile B showed a more gradual decrease in propagule number (Fig. 1).

Figure 2 shows the temperature evolution during the first days of the composting process, reaching values of nearly 70°C after 2 days. Statistical analyses showed that the

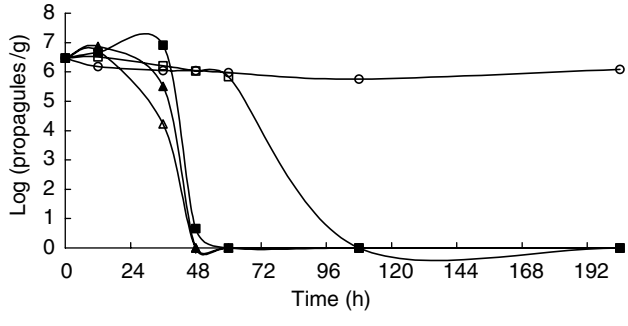

Fig. 1 *Fusarium oxysporum* f.sp. *melonis* persistence in vegetable residues during composting process. Log of mean propagules per gram against composting time (○, control; ■, Pile A; □, Pile B; ▲, Pile C; Δ, Pile D)

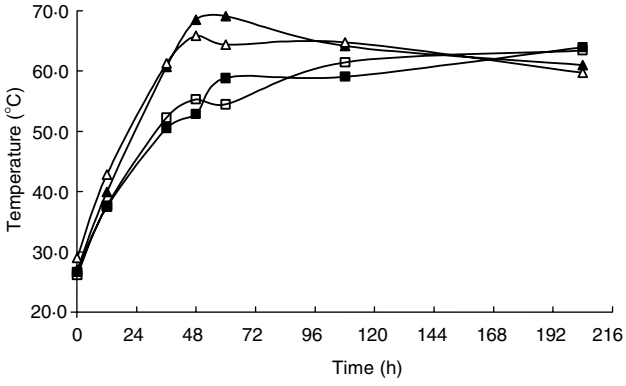

Fig. 2 Temperature evolution into composting piles (■, Pile A; □, Pile B; ▲, Pile C; Δ, Pile D)

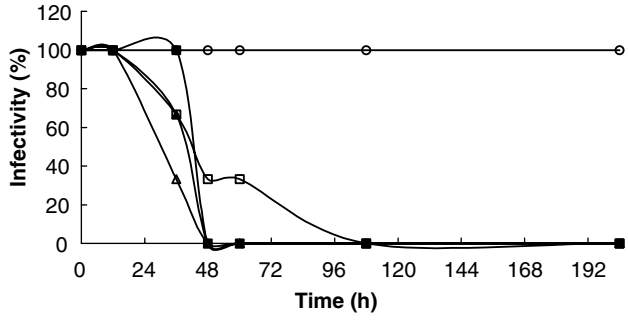

Fig. 3 *Fusarium oxysporum* f.sp. *melonis* infectivity from infected vegetable residues after composting process. Percentage of diseased melon plants against composting time (○, control; ■, Pile A; □, Pile B; ▲, Pile C; Δ, Pile D)

difference in temperatures observed between the four composting piles were not significant (data not shown).

Statistical analysis showed a great influence of aeration, inoculation, treatment (composted samples or negative control) and time factors (significant differences were observed at 95% significance level since $P < 0{\cdot}05$). Table 1 shows the different homogeneous groups with respect to the different factors.

Compost samples from aerated and inoculated piles showed the lowest persistence values (Fisher's protected LSD test, Table 1). On the other hand, the control samples (non-composted) and initial times of composting showed the highest persistence values during this process (Table 1).

Table 1 Effect of aeration, inoculation, treatment and time factors on *Fusarium oxysporum* f.sp. *melonis* persistence during the composting process. Mean values with a different letter differ significantly ($P < 0{\cdot}05$) according to Fisher's protected LSD test

Effect	Mean	Homogeneous groups
Aeration		
Yes	4·45357	a
No	4·77201	b
Inoculation		
Yes	4·33532	a
No	4·89025	b
Treatment		
Composted samples	3·14512	a
Control	6·08045	b
Time (h)		
108	2·87794	a
204	3·03959	a
60	3·71913	b
48	3·85860	b
36	5·87963	c
12	6·43751	d
0	6·47712	d

With respect to *F. oxysporum* f.sp. *melonis* infectivity, Figure 3 shows the results obtained during the composting process. Results are expressed as diseased plant percentage after the infection process against composting time (h). A decrease in fungal propagules was observed in parallel with persistence results. In all cases fungal infective capacity was eliminated between 2–4 days.

Statistical analysis showed a great influence of treatment factor (significant differences were observed at 95% significance level since $P < 0{\cdot}05$) and two homogenous groups were established (Table 2). Table 2 shows three different homogenous groups with respect to composting time also. Aeration, inoculation and repetition factors were not significant in any case.

In this case, the control samples (non-composted) and initial times of composting showed the highest values of infectivity (100% diseased melon plants) throughout the composting process (Table 2).

Table 2 Effect of treatment and time factors on *Fusarium oxysporum* f.sp. *melonis* infectivity during the composting process. Mean values with a different letter differ significantly ($P < 0{\cdot}05$) according to Fisher's protected LSD test

Effect	Mean	Homogeneousgroups
Treatment		
Composted samples	0·404762	a
Control	1·000000	b
Time (h)		
204	0·500000	a
108	0·500000	a
60	0·54167	a
48	0·54167	a
36	0·83333	b
12	1·00000	c
0	1·00000	c

Table 3 Correlation matrix between numeral variables analysed during the composting process

	Infectivity	Persistence	Temperature	Time
Infectivity	1·0000	0·9037	−0·8914	−0·3486
Persistence	0·9037	1·0000	−0·8375	−0·4352
Temperature	−0·8914	−0·8375	1·0000	0·2801
Time	−0·3486	−0·4352	0·2801	1·0000

Table 3 shows a correlation matrix between numeral variables analysed during the composting process: fungal persistence and infective capacity, composting temperature and exposition time. Data suggest a strong positive correlation between infectivity and persistence, while a negative correlation is observed between these parameters and composting temperature.

In vitro thermal treatment: *Fusarium oxysporum* f.sp. *melonis* persistence and infective capacity

Fungal survival as infected sample percentage against exposition time is shown in Fig. 4. The lowest temperature (30°C) was used as control treatment as fungal growth is best observed in this case. Figure 4 indicates the drastic effect of temperature on *F. oxysporum* f.sp. *melonis* persistence, as all tested values (45, 55 and 65°C) gave rise to fungal elimination. Thus, after 5 h, 4 days and 12 days of exposition at 65, 55 and 45°C respectively, fungal growth was not detected. The selected high temperatures, therefore, shown to be a determining factor in the fungal elimination.

Statistical analysis showed an important influence of temperature and time factors (significant differences were observed at 95% significance level, as $P < 0{\cdot}05$). Table 4 shows four and 10 different homogenous groups in relation with temperature and time factors, respectively.

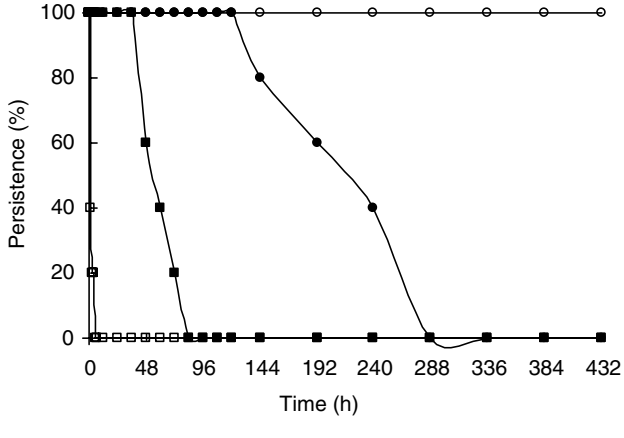

Fig. 4 *Fusarium oxysporum* f.sp. *melonis* persistence on infected vegetable residues after different thermal treatments. Percentage of infected samples against treatment time (○, control 30°C; ● 45°C; ■, 55°C; □, 65°C)

Table 4 Effect of temperature and time factors on *Fusarium oxysporum* f.sp. *melonis* persistence during different thermal treatments. Mean values with a different letter differ significantly ($P < 0{\cdot}05$) according to Fisher's protected LSD test

Effect	Mean	Homogeneous groups
Temperature (°C)		
65	0·185185	a
55	0·525926	b
45	0·807407	c
30	1	d
Time (h)		
432	0·25	a
384	0·25	a
336	0·25	a
288	0·25	a
240	0·35	b
192	0·4	bc
144	0·45	cd
120	0·5	de
108	0·5	de
96	0·5	de
84	0·5	de
72	0·55	ef
60	0·6	fg
48	0·65	g
36	0·75	h
24	0·75	h
12	0·75	h
6·5	0·75	h
5·5	0·75	h
4·5	0·8	hi
3·5	0·8	hi
2.5	0·8	hi
2	0·85	i
1·5	1	j
1	1	j
0·5	1	j
0	1	j

Samples from 30°C thermal treatment showed the highest persistence and infectivity values (Fisher's protected LSD test, Tables 4 and 5). On the other hand, samples from 65°C thermal treatment showed the lowest values of both parameters (Tables 4 and 5).

On the other hand, Fig. 5 shows the results obtained with respect to *F. oxysporum* f.sp. *melonis* infectivity during the different thermal treatments. Results are expressed as diseased plant percentage after infection process in relation to exposition time (h). The decrease in fungal propagules was consistent with the persistence results, i.e. after 4 h,

Table 5 Effect of temperature and time factors on *Fusarium oxysporum* f.sp. *melonis* infectivity during different thermal treatments. Mean values with a different letter differ significantly ($P < 0{\cdot}05$) according to Fisher's protected LSD test

Effect	Mean	Homogeneous groups
Temperature (°C)		
65	0·125926	a
55	0·422222	b
45	0·711111	c
30	1	d
Time (h)		
432	0·25	a
384	0·25	a
336	0·25	a
288	0·25	a
240	0·30	ab
192	0·30	ab
144	0·35	abc
120	0·4	bcd
108	0·45	cd
96	0·45	cd
84	0·45	cd
72	0·45	cd
60	0·5	d
48	0·5	d
36	0·65	e
24	0·65	e
12	0·65	e
6·5	0·7	ef
5·5	0·7	ef
4·5	0·7	ef
3·5	0·75	ef
2·5	0·75	ef
2	0·75	ef
1·5	0·8	f
1	1	g
0·5	1	g
0	1	g

3 days and 12 days of exposition at 65, 55 and 45°C respectively, diseased melon plants were not detected.

Statistical analysis showed an important influence of temperature and time factors ($P < 0{\cdot}05$). Table 5 shows the different homogenous groups in relation to the different factors.

DISCUSSION

During the composting process, the microbial transformation of organic materials leads to a stable by-product (compost) that provides several benefits to plants when applied to the soil. Owing to the use of compost as humic amendment in farmland or as substrate for culture in containers (Bollen 1985; García *et al.* 1993), phytopathogen destruction during composting is of great importance.

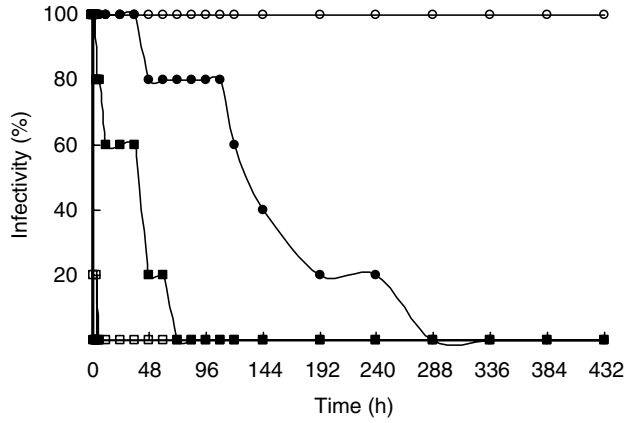

Fig. 5 *Fusarium oxysporum* f.sp. *melonis* infectivity from infected vegetable residues after different thermal treatments. Percentage of diseased melon plants against treatment time (○, control 30°C; ●, 45°C; ■, 55°C; □, 65°C)

The composting process consists of a first phase in which temperatures rise to 40–50°C and easily biodegradable susbstance are used by several microorganisms. A second thermophilic phase occurs in which less-biodegradable compounds are destroyed and several plant pathogens are killed by the high temperature generated (55–70°C) (Bollen 1993; Farrell 1993).

Results obtained with respect to *F. oxysporum* f.sp. *melonis* survival in composting piles pointed to a great cleaning capacity in the composting process of horticultural wastes, as elimination of this fungus was verified. Thus, no fungal survival was detected after 96 h in any case (Figs 1 and 3). Statistical analyses showed the high influence of the temperature factor in composting piles, establishing strong negative correlations between this factor and *F. oxysporum* f.sp. *melonis* persistence or infective capacity (Table 3).

Fungal inactivation was achieved during the bioxidative phase of composting (Figs 1, 2 and 3) suggesting the need for high temperatures over a period of time to destroy this pathogen. Thus, the US EPA (United States Environmental Protection Agency) recommends the maintenance of temperatures above 55°C for several days (US EPA 1979).

Overall, the results obtained support those of authors such as Hoitink and Fahy (1986), who pointed out the possibility of disease control by horticultural waste composting. Similar results were obtained in other processes reaching temperatures of up to 73°C (Wijnen *et al.* 1983) or 60°C (Hoitink *et al.* 1976) and eliminating phytopathogens such as *Phytophthora cinnamomi*, *Pythium irregulare*, *R. solani* and *Botrytis cinerea* or *Bortrytis allii*. Besides, there are several recent

reports showing the effect of composting process on phytopathogen bacteria such as *Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *syringae* and *Erwinia carotovora* pv. *carotovora* (Suárez-Estrella *et al.* 2001). In this case, bacteria showed a lower persistence capacity than *F. oxysporum* f.sp. *melonis*. On the other hand, the same authors observed the effect of this process on several viruses such as MNSV, tomato spotted wilt virus (TSWV) and pepper mild mottle virus (PMMV), being the last most resistant, as its complete suppression was achieved after 70 days (Suárez-Estrella *et al.* 2002).

Temperatures reached inside composting piles fluctuated between 60–70°C during the bioxidative phase (Fig. 2). These temperature values are considered the primary factor in the removal of phytopathogens from plant waste during composting (Ylimäki *et al.* 1983; Yuen and Raabe 1984; Bollen 1993). However, temperatures higher than 70°C are not recommended because they affect the microbial community involved in the composting process (Golueke 1992).

The effect of composting upon phytopathogen microorganisms has been explained on the basis of the combined action of several environmental factors (physical, chemical and biological) such as high temperatures, toxicity of several products in the decomposition of plant waste, microbial activity, etc. (Ylimäki *et al.* 1983; Bollen 1993). In this work, the results confirm the 'cleaner' effect of the temperature factor in the composting process, as *in vitro* 55–65°C thermal treatments produced total elimination of *F. oxysporum* f.sp. *melonis* after 4–5 days (Figs 4 and 5). Moreover, these results support those of authors such as Mansoori and Jaliani (1996), who confirmed the control of several soilborne pathogens of watermelon (*Fusarium solani, Phytophthora drechsleri* and *P. aphanidermatum*) by soil solar heating over 30 days.

Finally, according to the results obtained, it appears evident that the composting process is an excellent alternative for the management of plant waste after harvesting, as this procedure eliminates horticultural waste from the environment, and suppresses the infective capacity of several harmful phytopathogens such as *F. oxysporum* f.sp. *melonis*.

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