

THE SUPPRESSIVE ACTIVITY OF THE COMPOSTING PROCESS ON PHYTOPATHOGEN BACTERIA AND VIRUSES

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1. ABSTRACT

One of the benefits ascribed to composting is the suppression of phytopathogens in disease control.

*The suppression of pathogenic bacteria and viruses during composting of horticultural wastes was studied. Plants infected with either bacteria (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *syringae* and *Erwinia carotovora* subsp. *carotovora*) or viruses (PMMV, TSWV and MNSV) were included in compost piles. Bacteria and TSWV were suppressed after 2-3 days composting. MNSV viral antigens were detected afterwards but infectivity tests were negative. PMMV was the most resistant pathogen since complete suppression at semi-pilot scale composting was achieved after 70 days, a period in which temperatures of 65-70°C were reached. This time was shortened in industrial scale composting (21 days).*

According to results obtained, the composting process was demonstrated to be effective for plant pathogen biocontrol. Many factors seem to be involved in pathogen survival during composting, temperature being the main factor, especially in the case of PMMV.

2. INTRODUCTION

Greenhouse horticultural cultures constitute the primary economical source in the province of Almería (southeastern Spain). This intensive agricultural practice yields annually a large amount of plant wastes, representing a serious environmental problem and constituting an important source of pathogens (Fletcher, 1984). Plant wastes are usually piled near greenhouses or burned. However, these practices cause several undesirable agronomic and environmental effects. Therefore, management of crop residues is necessary to minimize their adverse effects, particularly if the pathogens involved can infect subsequent crops (Conway, 1996). Several important plant diseases caused by typical phytopathogens appear in this area and their control is difficult. Plant diseases such as damping-off caused by *Pythium aphanidermatum* and/or *Rhizoctonia solani* or stem rot caused by *R. solani* have been reported by Gúmez (1993). *Fusarium oxysporum* f.sp. *melonis* together with MNSV (Melon Necrotic Spot Virus) have been the most critical and determinant phytopathogens (Gúmez, 1993) in the area. MNSV is a long-time persistent pathogen in soil because of its association with fungal spores of *Olpidium radicale* (Campbell, 1996).

This virus is distributed worldwide in melon and cucumber plants cultivated under plastic. The great stability of this virus and the resistance of *Olpidium radicale* spores could explain the existence of soils which remain infected for long periods. Other harmful viruses affecting pepper cultures in Almería are PMMV (Pepper Mild Mottle Virus) (Cuadrado et al., 1992) and TSWV (Tomato Spotted Wilt Virus) (Cuadrado, 1994; Cuadrado et al., 1991; Gea, 1996). Both viruses have been responsible for important damage to pepper crops in recent years. Their hosts are mainly Solanaceae, Compositaceae and Papilionaceae. Beside viruses, several phytopathogenic bacteria have also been reported in this area. *Erwinia carotovora* subsp. *carotovora*, *Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *syringae* or *Clavibacter michiganensis* subsp. *michiganensis* have been found to be responsible for damage to tomato, pepper and bean crops (Reche, 1991). Their capacity to remain in crop residues and several related problems make appropriate waste control necessary (Noval, 1991). Composting is proposed as a means of plant disease control when applied to the plant wastes (Hoitink and Fahy, 1986). Several reports show the elimination of *Fusarium* spp., *Rhizoctonia solani*, *Phytophthora cinnamomi*, *Pythium irregulare*, *Erwinia carotovora* var. *chrysantemi*, Tomato Mosaic Virus, Tobacco Necrosis Virus and Cucumber Mottle Mosaic Virus during composting (Avgelis and Manios, 1989; Avgelis and Manios, 1992; Hoitink et al., 1976; Hoitink and Fahy, 1986; Ylimäki et al., 1983; Lúpez-Real and Foster, 1985).

The suppressive activity of the composting process depends on three main factors: a) the high temperatures generated during thermophilic phase (above 40-50°C) (Bollen, 1985; Yuen and Raabe, 1984); b) the production of antimicrobial compounds such as phenolics generated during lignocellulosic material decay (S·ez, 1989); and c) 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). The use of compost in the control of phytopathogens has been known and practiced from early stages of agriculture (Cook and Baker, 1983). However, only recently, after the discovery of some soil-borne plant pathogenic microorganisms, interest

has grown with respect to the plant-protecting properties of compost (Hoitink and Fahy, 1986; Kostov et al., 1996; Lumsden et al., 1983). The reported research covers the composting of horticultural crop residues generated from greenhouse cultures located at Almería. Bearing in mind that composting could be the best alternative for waste management and owing to the absence of data about the inactivation of PMMV, TSWV, MNSV, *Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *syringae* and *Erwinia carotovora* subsp. *carotovora* by composting, the aim of this work was to investigate survival of these microorganisms during different composting trials.

3. MATERIALS AND METHODS

3.1 Collection of infected plants and residues and experimental conditions

Pepper plants (*Capsicum annuum* L. cv. California) infected with PMMV or TSWV and MNSV infected melon plants (*Cucumis melo*) were collected from several greenhouses located in Almería. For the bacterial study, sterilized melon residues were inoculated with bacterial liquid cultures. Bacterial strains were obtained from C.E.C.T. (Spanish Type Culture Collection). Infected plants and inoculated residues were individually distributed into samples of approximately 20 g of material to be composted and then placed muslin bags in the compost piles. These were exposed to composting processes at both semi-pilot and industrial scales.

3.1.a Semi-pilot scale

A preliminary trial at semi-pilot scale was developed in three piles (A1, B1 and C1) each 1.5 m long x 1.2 m wide x 1.2 m high. Piles were built up with a mixture of pepper, cucumber, and bean plant wastes. Wood chips were added as bulking agent. The C/N ratio of the mixture was 20-25 approximately. Piles were turned weekly and aerated every two days. Aeration 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. Aeration of 90 m³ air for 1 hour was supplied by a blower S&P CBB-60 type. The temperature was measured daily during the biooxidative phase. Muslin bags containing infected plants and residues were put 60 cm deep inside the composting piles. A muslin bag from each pile was taken at 0, 14, 28, 42, 56 and 70 days and was divided into five subsamples. Bacterial survival was determined by morphological and biochemical tests of isolates from plant material. Virus survival and infective capacity were determined by serological methods.

A second composting trial was undertaken using four piles (A2, B2, C2 and D2) composed of pepper wastes. Composting conditions were similar to those employed in the first trial. In this case, samples were taken at shorter intervals: 0, 12, 36, 60 and 132 hours. Each sample was analysed as for the first trial.

3.1.b Industrial scale

A study of composting at industrial scale was also undertaken. In this case, three samples were prepared with infected horticultural wastes and put 100 cm deep inside the composting pile (25 m long x 5 m width x 2 m height). Samples were taken at 0, 7, 21, 60 and 90 days intervals and analyses performed on each as for the other two trials.

3.2 Determination of viral survival

3.2.a Coat protein antigen detection

Viral antigen detection was performed by double-antibody sandwich (DAS) ELISA (Clark and Adams, 1977). Commercial ELISA kits for PMMV, TSWV and MNSV (Loewe Biochemica GmbH, Sauerlach, Germany) were used following the manufacturer's recommendations. Each plate also contained two known positive controls (infected samples), two negative controls (healthy samples) and two buffer controls (Hill and Moran, 1996). Extracts of test samples were obtained from the ground, leaves or stems and homogenized in a commercial extraction buffer at 1:15 (w/v) (TSWV) or 1:20 (PMMV and MNSV). Absorbance was measured after 1 hour (TSWV) or 1 and 3 hours (PMMV and MNSV) at 405 nm in a Bio-Rad 450 microplate reader (Bio-Rad Laboratories, Hercules, CA). A sample was considered positive if the optical density was greater than twice the mean of the healthy controls (Rodony et al., 1994; Culbreath et al., 1993; Chamberlin et al., 1992).

3.2.b Infectivity assays

Infective capacity was determined by mechanical inoculation of healthy pepper (for PMMV and TSWV tests) and melon (for MNSV test) plants. Viral saps were extracted from pepper and melon infected tissues (1.5 to 2 g) with 0.2 M sodium phosphate buffer pH 7.2 (PMMV), 0.01 M sodium phosphate pH 7 (TSWV) and 0.03 M sodium phosphate pH 8 (MNSV) (Pategas et al., 1989). 0.075 g charcoal and 0.075 g of carborundum powder were added to one ml of sap prior to inoculation. Between 2-4 seedlings were rubbed with each extract.

Inoculated plants were maintained in a greenhouse and observed over a two month period. Viral antigen detection was performed by ELISA on every plant. Young leaves or necrotic areas in the case of MNSV were used for this test. Negative (inoculated with buffer) and positive control plants were included and handled identically.

3.3 Determination of bacterial survival

Samples were suspended in Ringer's solution and inoculated into specific plate media after serial dilution. *Erwinia carotovora* subsp. *carotovora* (E.c.c.) detection was achieved following the methods of Dickey and Kelman (1988) and Noval (1991). In this case, Nutrient Agar (NA) and potato liquefaction medium were used. *Xanthomonas campestris* pv. *vesicatoria* (X.c.v.) detection was achieved by culturing on Yeast Malt medium (YM), selective medium of McGuire et al. (1986) and the methods cited by Schaad and Stall (1988). *Pseudomonas syringae* pv. *syringae* (P.s.s.) detection was carried out by culture on NA and King B media (Hildebrand et al., 1988; Noval, 1991). Positive controls from C.E.C.T. were included and handled identically.

4. RESULTS

4.1 Semi-pilot scale: preliminary trial

Antigen detection and infectivity results for PMMV and MNSV, as well as the evolution of the mean temperature in the composting piles are shown in Fig. 1. Results for TSWV and bacteria are not shown, since they were not detected in this trial. Therefore, these microorganisms were again tested in a second composting trial in which shorter sampling time was used.

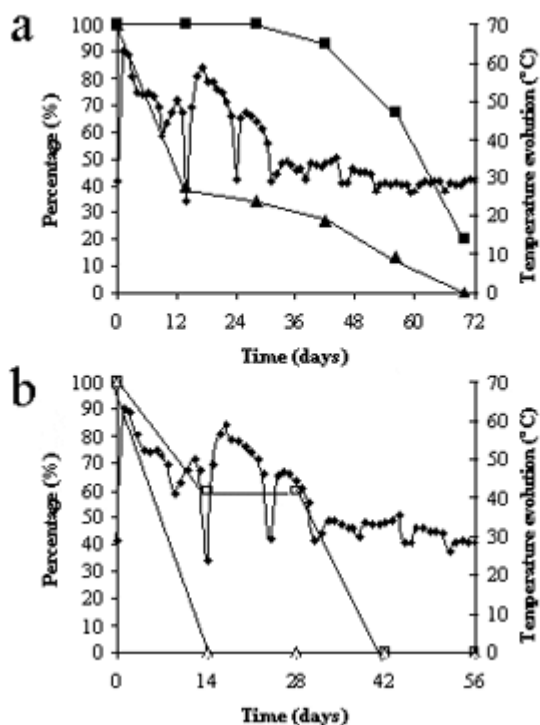


Figure 1. Effect of composting (semi-pilot scale) on a) PMMV persistence (■) and infectivity (●) and b) MNSV persistence (□) and infectivity (○). The mean temperature for the three piles is shown (▲).

Results showed an effective suppression of viral infective capacity of TSWV and MNSV after 14 days composting. However, MNSV viral antigens were detected up to 28 days (Fig. 1). After this time no positive samples were found. PMMV was the most resistant pathogenic virus; it remained in composted wastes up to 70 days (Fig. 1). Viral antigen percentages decreased with time and 20% ELISA positive samples were recorded at the end of composting period (Fig. 1). Virus infective capacity inactivation was achieved during the bioxidative phase of composting. Thus, after 56 days composting only a 13-14% of samples were infective but by the end of the trial, all samples were non-infective (Fig. 1).

4.2 Semi-pilot scale: second trial

The percentage of TSWV viral antigen at 36 hours composting was 75%, while infective capacity was maintained by only a 33% of samples. However, the composting process demonstrated an effective suppression of viral antigens and infective capacity before 60 hours (Fig. 2).

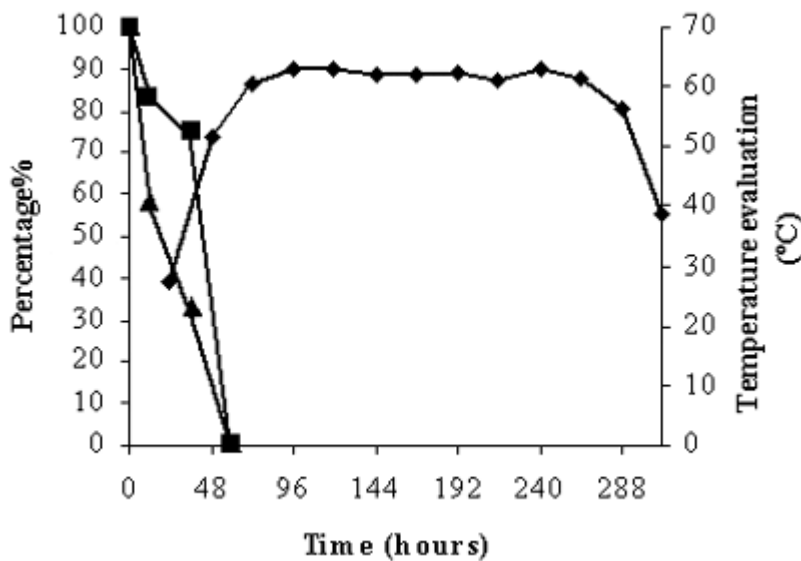


Figure 2. Effect of composting (semi-pilot scale) on TSWV persistence (■) and infectivity (▲). The mean temperature for the four piles is shown (◆).

Bacterial survival during composting is shown in Fig. 3. *Pseudomonas syringae* pv. *syringae* was not detected at any of the sampling times. *Xanthomonas campestris* pv. *vesicatoria* did not survive more than 24 hours. *Erwinia carotovora* subsp. *carotovora* was not isolated after 36 hours composting.

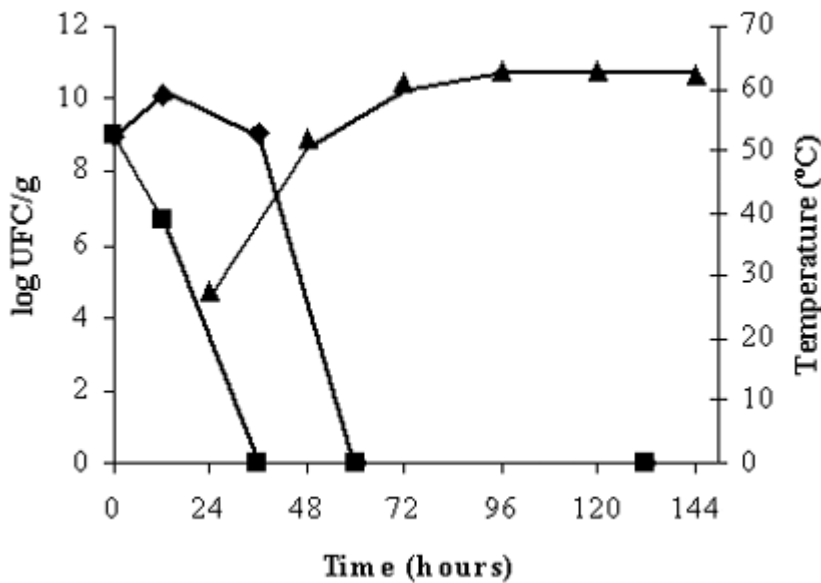


Figure 3. Effect of composting (semi-pilot scale) on *Xanthomonas campestris* pv. *vesicatoria* (■) and *Erwinia carotovora* subsp. *carotovora* (▲). The mean temperature for the four piles is shown (◆).

4.3 Industrial scale

PMMV was the only pathogen used with industrial scale composting, since according to results obtained at semi-pilot scale, this was the most persistent pathogen. Data are shown in Fig. 4.

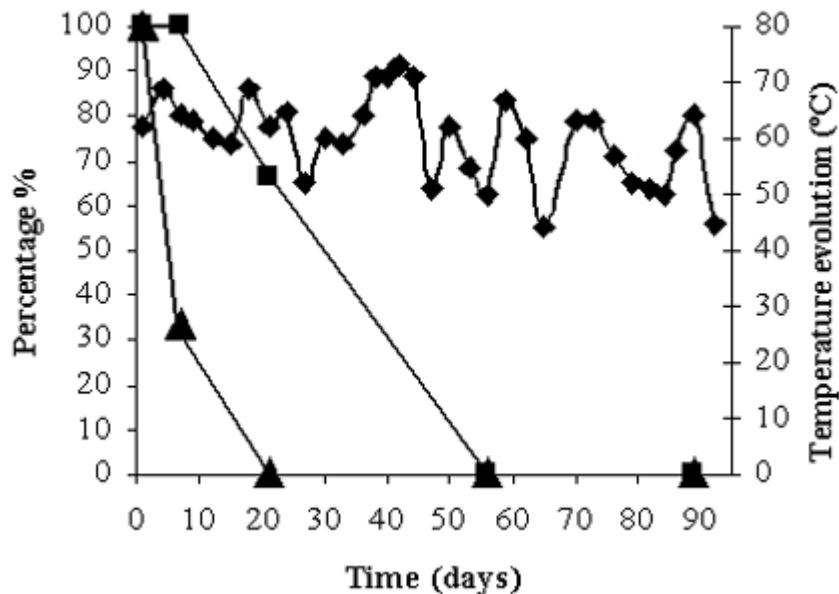


Figure 4. Effect of composting (industrial scale) on PMMV antigen detection (□) and infectivity (△). The mean temperature for the pile is shown (◇).

Elimination of PMMV viral antigens was achieved between 21 and 56 days composting (Fig. 4). Positive samples were not found afterwards. Elimination of viral infective capacity was achieved between 7 and 21 days composting. However, viral antigens were detected later than viral infectivity (after 50 days composting). Results obtained suggest that there was not a perfect correlation between viral antigen presence and infective capacity. That was the reason why both antigen detection and viral activity were analyzed (Figures 1, 2, and 4). Thus, the viral infective capacity was demonstrated to be the determinant factor to consider in relation with the potential contamination of horticultural wastes.

5. DISCUSSION

Pepper (*Capsicum annum* L.) constitutes the most important crop in Almería. Pepper plant residues can be considered to be a main source of wastes for composting. The application of pepper-compost to farmland depends on product quality and obviously, the absence of phytopathogens is a prime quality parameter.

Results obtained with respect to survival of phytopathogenic microorganisms inside composting piles either at semi-pilot or industrial scale showed suppressive capacity of the composting process. This composting suppressive capacity mainly affected TSWV and bacteria, since survival after 36 hours was not detected (Figures 2 and 3).

PMMV was the most persistent virus during the composting process. However viral PMMV activity decreased during composting. Viral inactivation was achieved during the biooxidative phase of composting, suggesting the need to use waste composting processes in which high temperatures are maintained for several weeks. The US EPA (Environmental Protection Agency of United States) requires the maintenance of these temperatures above 55°C for at least 15 days (US EPA, 1979).

Comparison of PMMV survival during composting at semi-pilot and industrial scales reflected a faster viral infectivity decrease at industrial scale (Figures 1a and 4).

This result can be explained by the higher temperatures reached and maintained in the industrial pile. In this sense, temperatures at industrial scale were about 67°C with peaks above 70°C and these were maintained for several days. In contrast, semi-pilot composting pile temperatures fluctuated between 60-65°C (Figures 1a and 4).

Although high temperature are considered to be the prime factor in removing phytopathogens from plant wastes during composting (Bollen, 1993; Ylimäki et al., 1983; Yuen and Raabe, 1984), the combined action with other factors such as

toxicity of several products from the plant wastes decomposition, microbial activity, etc. (Bollen, 1993; Ylimäki et al., 1983) is known.

Results obtained at both semi-pilot and industrial scales are promising since microorganism inactivation was achieved. However, temperatures higher than 70°C are not recommended because they affect the microbial community needed for the composting process (Golueke, 1992).

Results obtained agree with those reported by Hoitink and Fahy (1986) who carried out a general survey on plant disease control through horticultural waste composting. Similar results have been obtained in other processes reaching temperatures up to 73°C (Wijnen et al., 1983) or 60°C (Hoitink et al., 1976) in which several phytopathogenic fungi were eliminated. There are few results showing bacterial elimination during composting. López-Real and Foster (1985) reported *Pseudomonas phaseolicola* inactivation at 35°C after 4 days composting.

Results obtained in this work show that the composting process is an excellent solution for horticultural waste disposal, since the infective capacity of several harmful pathogens of pepper, melon, tomato and bean crops was suppressed. Viral and bacterial destruction during composting is of great importance due to the use of compost as humic farmland amendment or as substrate for culture in containers (Bollen, 1985).

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