

Elimination of tomato mosaic virus by composting tomato residues

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Abstract

Tomato plants infected by tomato mosaic virus (ToMV) were composted. Although the temperature in the stack of tomato residues did not exceed 47 °C during composting, ToMV was not detected in samples during composting. Moreover, tomato seedlings grown in pots containing a mixture of Russian peat and the composted material did not become infected. Because ToMV was detected in samples stored in an incubator at 47 °C up to the 75th day of the incubation, it was concluded that the biological degradation of ToMV seems to play a more important role than the heat inactivation of the virus.

Little information has been published on elimination of plant viruses during composting of infected plant residues (Hoitink, 1980; Lopez-Real and Foster, 1985; Hoitink and Fahy, 1986). Regarding tobacco mosaic virus (TMV), two rather contradictory reports have been published. Grushevoi and Levykh reported in 1940 that TMV was inactivated in compost piles where temperatures did not exceed 49 °C. On the contrary, more recently Hoitink (1980) reported that TMV-infected tobacco leaves and stems buried in piles of tree bark compost and exposed to 50-75 °C for six weeks retained their infectivity.

Cretan vegetable industry encompasses up to 1500 hectares of plastic houses. Tomato is one of the most important crops. Because of the low content of organic matter in soil, nutritional problems very often occur. Due to the high cost of peat and the lack of manure, compost production of plant residues and its local utilization in plastic houses might be profitable. Tomato residues from plastic houses, which are estimated to be approximately 40 000 tons yearly in Crete (Manios, unpublished data), could be used for preparing the compost. However, on a large number of holdings, tomato cultivars susceptible to tomato mosaic virus (ToMV) are grown and this virus is widespread in plastic houses in Crete (Avgelis, 1986). For this reason we have investigated the behaviour of ToMV during composting of infected tomato residues.

For compost preparation tomato cv. Dombo C₂VF₂, susceptible to ToMV, was transplanted at the fifth true leaf stage in a plastic house of 500 m² in early autumn. A purified virus preparation (0.01 mg ml⁻¹ nucleoprotein) of a local isolate of ToMV, belonging to strain 1 *sensu* Pelham (1972), was mechanically inoculated on celite-dusted tomato cotyledons at the first true leaf stage. Plants were grown according to the local system except that fruits were picked as soon as they were formed to favour production of vegetative mass. After seven months the plants were uprooted and allowed to dry. The dry residues were grinded with a hammer mill (Sumix). The size of the particles

ranged from a few mm to 7-8 cm. Samples were collected from the stack to determine C/N values and when it was 24/1, inorganic N was not added. Moisture content was about 60% and the stack was placed in a polysterol insulated box of 350 l previously used for composting of various vegetable residues (Manios, 1979). The composting process was monitored by determination of temperature, pH, EC, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and C/N values. In the context of the study on inactivation of ToMV, only temperature data will be discussed here. In the compost pile the temperature did not exceed 46 °C (Fig. 1). This value was recorded on the second day of the composting period and maintained on the same level for approximately two additional days. After the 9th day when the material was turned, the temperature again increased and reached 44 °C. Two successive turnings, at the 40th and the 70th day, did not result in any increase of temperature.

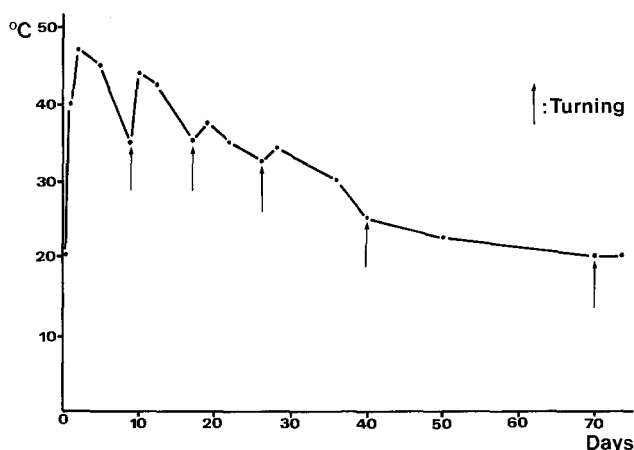


Fig. 1. Course of temperature in a compost pile of residues of tomato crops.

For virus isolation two samples of 50 g each were taken from the stack every ten days. The samples were homogenised in four volumes (w/v) of distilled water and the homogenate was filtered through three layers of muslin and centrifuged at 100 000 g for 1 h. The pellet was resuspended in 5 ml of 0.01 M potassium phosphate buffer, pH 7.0. The resuspended material was used undiluted at dilutions 10^{-1} , 10^{-2} and 10^{-3} , for a local lesion assay on *Nicotiana glutinosa*, each assay being tested on five leaves. ToMV was detected only in the samples taken from the stack prior to composting and caused 22 lesions/leaf of *N. glutinosa* in the 10^{-3} dilution. All further attempts to detect ToMV during composting were unsuccessful.

Upon completing of composting the final material was mixed up with Russian peat at different ratios to prepare five mixtures: 25% + 75%, 30% + 70%, 45% + 55%, 60% + 40% and 75% + 25% (v/v), respectively. Sand was added at a rate of 70 l m^{-3} . Seed of tomato 'Dombo' was sown in plastic pots of 0.1 l filled with the above mentioned substrate mixtures. A pathogen-free commercial substrate, Thiochum No 1, was used as a negative control. As a positive control the same substrate was used, but then inoculated by watering each pot with 20 ml of a purified ToMV preparation, con-

taining 0.01 mg ml⁻¹ nucleoprotein, prior to sowing. Treatments were arranged in a randomized block design with four replicates of eight pots each, in a greenhouse. The rate of root infection was assessed by determining the number of infected seedlings with symptoms and/or shown to contain virus by inoculation of *N. glutinosa* plants. Leaves and roots of the plants were examined two months after sowing. None of the seedlings grown in pots filled with substrate containing composted ToMV-infected residues showed mosaic symptoms. In addition, ToMV could not be detected in leave and root samples from the seedlings. On the contrary, 4 out of 32 seedlings grown in the pots watered with purified virus preparations showed mosaic symptoms and the virus was isolated from 18 root samples. The mixture of 75% tomato compost and 25% Russian peat was unsuitable as a substrate since tomato seeds did not germinate in it.

To determine the effect of incubation temperature on survival of ToMV in infected tomato tissue, dried samples from infected tomato plants 'Dombo' were stored in an incubator at 47 °C. At five-days intervals attempts were made to isolate ToMV from the samples as described previously. Testing infected tomato plant residues was positive until the 75th day. ToMV concentration decreased sharply after 20 days of incubation (6-10 local lesion/leaf at 10⁻¹ dilution) and it was maintained at low level (2-3 local lesion/leaf) until the 75th day. Failure to isolate ToMV from compost samples and the finding that tomato seedlings growing in that compost remained virus-free, indicates that ToMV did not survive composting. Elimination of ToMV in tomato plant residues during composting could result either from the biological degradation of the virus or from heat inactivation. However, the observation that tomato plant residues retained their infectivity following prolonged heating (up to two months) at 47 °C, may indicate that biodegradation of the virus is an important component of the process.

It has been shown that soil fumigants not only are unable to desinfect ToMV-infested soils, but may also cause an increase in virus infectivity (Broadbent, 1965; Van Winkel, 1974). This can be explained by the adverse effect of the fumigants on the soil microflora leading to delayed degradation of the virus (Broadbent et al., 1965; Van Winkel, 1974). Thus the biological factors must be considered to contribute largely to the degradation of ToMV, either in soil or in plant residues.

The difference between our results and those reported by Hoitink (1980) and Hoitink and Fahy (1986) might be due either to the host plant used or to the different method of composting. This preliminary work indicates that composting inactivates ToMV in tomato plant residues and suggests that tomato compost can safely be used in plastic houses. Nevertheless further work needs to clarify the mechanism of ToMV inactivation during composting as temperature alone did not reach lethal levels and therefore is inadequate to explain the above results.

Samenvatting

Inactivering van tomatemozaïekvirus (ToMV) tijdens compostering van afval van geïnfecteerde tomatplanten

Met tomatemozaïekvirus (ToMV) geïnfecteerde tomatplanten werden gecomposteerd. Hoewel de temperatuur in de composthoop tijdens de compostering niet hoger werd dan 47 °C, kon ToMV niet worden geïsoleerd uit monsters, die tijdens de compostering werden genomen. Bovendien werden tomatenzaailingen, die in een mengsel van pot-

grond met gecomposteerd materiaal waren opgegroeid, niet met het virus geïnfecteerd. Omdat ToMV tot 75 dagen na het begin van de incubatie bij 47 °C in de monsters kon worden aangetoond, kan worden geconcludeerd, dat de biologische afbraak van ToMV een belangrijker rol speelt dan de inactivering ten gevolge van de warmteontwikkeling.

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