

The Presence of Tobacco Mosaic Virus in the Compost Extract of Cigar Tobacco Debris

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Tobacco mosaic virus (TMV) is resistance to high temperature and able to survive over 10 years on dried leaves, and plant debris is considered as source of inoculums of TMV in the field. In order to inactivate TMV, TMV-infected cigar tobacco debris was composted at starting temperature of 50 °C for two to three days; however, TMV was still infective in the extract compost. If a half leaf cigar tobacco 'H877' was inoculated with compost extract, the symptoms appeared as a necrotic local lesion (NLL) and did not develop systemic lesions. The dilution end point of TMV in extract compost was 10⁻³. The number of lesion was higher in the glasshouse with average daylight temperature of 32 °C than in the field with average daylight temperature of 29-30 °C. The number NLL was lower and NLL size seemed to be smaller on the first and second inoculated leaves with extract than that of on the first and second inoculated leaves with TMV inoculums. There was a delay of time about 58-106 hours after inoculation of NLL from extract compost inoculums to appear than those of from TMV inoculums. These could be happened because of mineral nutrients of compost and also the temperature of maintaining tobacco plant which inhibited the infections, and of a thermal composting process which destroyed some TMV particles, particularly degraded its coat protein.

Key words: TMV, extract water compost, cigar tobacco debris

INTRODUCTION

Cigar tobacco is major commercial plant grows in Jember, Indonesia. Mostly, either Tobacco mosaic virus (TMV) or Cucumber mosaic virus (CMV) is still dominant infected on the cigar tobacco farms. TMV has many strains, i.e. strain U1, U2, B (bean), C (cowpea), S (soybean), P (peas), and vulgare, all those strains could induce vary symptoms on tobacco. Strains U1, S, B, and C induced necrotic local lesion on *Nicotiana glutinosa*, *N. tabacum* 'Burley 21', 'Xanthi nc.', and 'Samsun' (McDaniel *et al.* 1995).

Some cultivars resistance to TMV have been produced, i.e. 'H877' and 'H894'. They carry the N gene derived from *Nicotiana glutinosa*, the resistance gene to TMV which induce a necrotic local lesion (Hartana 1981). In 'H877', TMV in the glasshouse could develop systemic lesions or systemic necrotic burned, also after top pruning developed systemic necrosis on the stem, if the plants were exposed to the temperature of 36-40 °C (Wahyuni *et al.* 1999).

TMV is considered as the most heat resistant plant pathogen, stable *in vitro*, and able to survive over 10 years in dried leaves and cigarettes or cigars (Lucas 1975). Therefore, the infected dried leaves, cigar or cigarettes can be the source of inoculums of TMV to healthy plant. In the field, the most common source of virus inoculums is the debris of infected plants which serve as reservoirs for virus transmission to healthy plants, and the improper disposal of these plant

residues can contribute to recycling of virus as pathogens (Conway 1996).

In this paper, we wanted to observe whether processing compost of tobacco plant debris would inactivate TMV. Formerly, this extract was suggested can be used as pesticide because it contains nicotine. However, its nicotine concentration was low about 0.2-0.4%; so this extract could only be used for pursuing away small insects and this is not considered as pesticide (Wahyuni 2008, unpublished data).

MATERIALS AND METHODS

Compost and Compost Extract Preparation. TMV-infected 'H386' cigar tobacco debris was collected from tobacco government farms at Jember. The debris was cut into small pieces, placed in the plastic container and added with Effective Microorganism 4 (EM4) and rice bran, then incubated for 4-6 weeks. The starting temperature of composting process was 50 °C and during the composting process, the debris was stirred several times to maintain semiaerobic conditions. About 5 g CaCO₃ was added to 1 kg degraded debris to increase the pH during decomposition process, and the excess of decomposed debris solution was allowed to drain off. The matured compost was extracted with water (1:2) and the slurry was strained through muslin cloth.

Inoculate TMV from the Extract Compost to Tobacco 'H877'. The hypersensitive cigar tobacco 'H877' to TMV was used as a model to observe whether TMV was still survive in the compost extract. TMV inoculums was collected from cigar tobacco 'H382' at Jember, and used as the positive control. About 1 g dried leaves of TMV-infected tobacco was ground

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with 1 ml of 0.01M phosphate buffer pH 7.0, then diluted to 10^{-5} and inoculated on a half leaf of tobacco 'H877'. Compost extract was serial diluted at 10^{-1} to 10^{-7} with water and inoculated on a half leaf, each treatment was replicated six times. The development of symptoms and the number of local lesions were observed three times a day, on 7.00 a.m., 12.00 p.m., and 17.00 p.m. for 9 days after inoculation.

Distribute Local Lesion. To ensure that the necrotic local lesion (NLL) of TMV may develop systemically on the subsequence leaves of hypersensitive tobacco, the experiment was done by inoculating leaf with 1 ml extract at dilution of 10^{-2} , then after 7 days later the subsequence leaf was inoculated with 1 ml extract at dilution of 10^{-2} . For the positive control, the first inoculation was done with 1 ml TMV inoculums at dilution of 10^{-5} and the second inoculation was done after 7 days later with the same TMV dilution. For the negative control, the first inoculation was done with 1 ml H_2O , and second inoculation was done after 7 days later with the extract at dilution of 10^{-2} , as shown on Figure 1.

This experiment was repeated twice and the later was located in two different conditions of maintaining of plants which might effect to the development of local lesions. The first condition was under glasshouse, and the second condition was in the field. The development local lesions were observed on 1 to 10 days after the first inoculation of leaf with either compost extract at 10^{-2} dilution or H_2O .

RESULTS

Compost from TMV-Infected Tobacco Debris. During the composting process, the starting temperature in the plastic container increased up to about 50 °C for the first of two-three days, and after that decreased about 45 to 40 °C until plant debris well composted. The measurement of temperature might be was not accurate because done only during the daylight, but it should be also done during the night where the temperature outside the container was cooler. Plant debris composted well for two months processing, then extracted with water (1:3 w/v) and this was designated as compost extract.

Number Local Lesions Produced by Inoculation Leaf with Compost Extract in Serial Dilution. Inoculation of compost

extract in serial dilution on tobacco 'H877' leaf produced small NLL. It was stated that the number of small necrotic lesion expressed the virus amount in compost extract.

The number of local lesion was affected by the temperature in the experiment location and the dilution of extract (Figure 2). In the glasshouse with the average daylight temperature of 32 °C, the first symptoms was observed on 102 hours after inoculation (h.a.i.), while in the field with the average temperature of 29-30 °C, the first symptoms was observed on 150-169 h.a.i. The first NLL produced from TMV inoculums were observed at 44 h.a.i. (Figure 2). Sometimes during

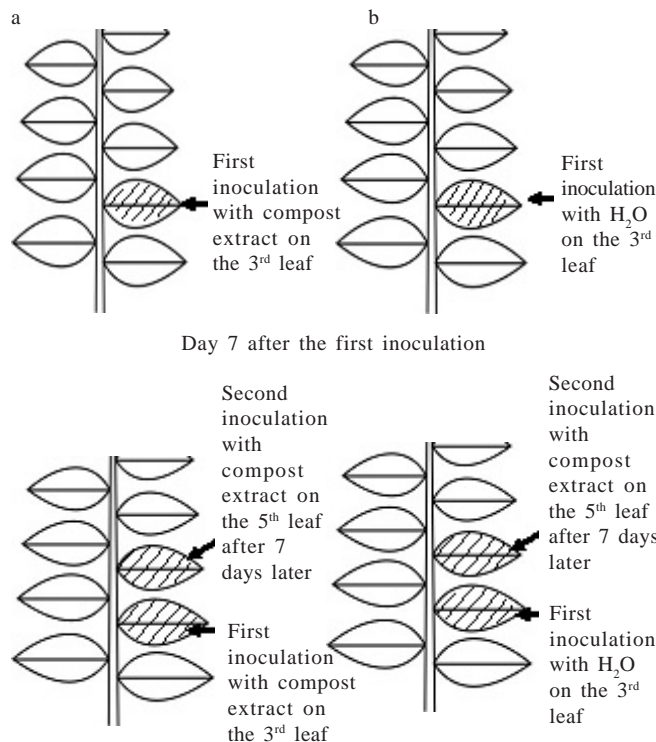


Figure 1. Inoculation of compost extract at dilution of 10^{-2} on the subsequence leaves of cigar tobacco 'H877'. a. First inoculated leaf with compost extract and second inoculated leaf with compost extract, b. First inoculated leaf with H_2O and second inoculated leaf with compost extract.

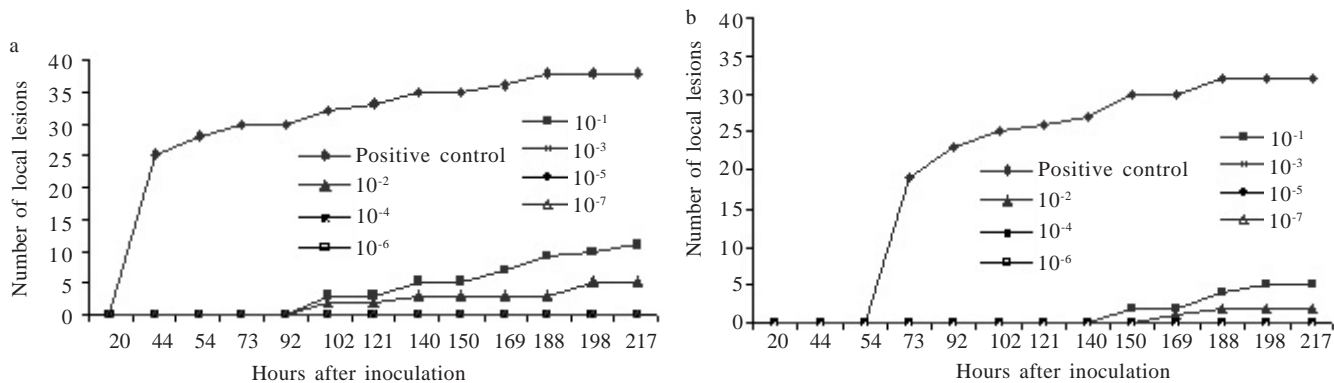


Figure 2. The number of local lesions produced by inoculated a half tobacco leaf with compost extract in serial dilutions, on 9 days after the first inoculation. a. The glasshouse experiment with average of daylight temperature 32 °C. b. The field experiment with average of daylight temperature 29-30 °C. All extract treatments were replicated six times. The positive control was inoculation with TMV inoculums at 10^{-5} dilution.

11.30 a.m.-1.30 p.m. the temperature in the glasshouse could increase to 35 °C. TMV in the compost extract was still infective at 10^{-3} dilution, although compost was stored for 4 months since compost was extracted. Thus, the dilution end point (DEP) of TMV in the compost extract of tobacco debris was 10^{-3} , in the field experiment was 2 NLL and in the glasshouse was 5 NLL (Figure 2).

Distribute Local Lesion. After 9 days inoculation in the glasshouse, the number of NLL was lower on the first inoculated leaf with H₂O and the second inoculated leaf with compost extract than those of on the first inoculated leaf with H₂O and the second inoculated leaf with TMV inoculums. The number of NLL was also lower on the first and second inoculated leaves with extract than those of on the first and second inoculated leaves with TMV (Table 1). At the same time of observation, the distribution of NLL on the second inoculated leaf was less than on the first inoculated leaf with either compost extract or TMV inoculums. The size of NLL was smaller in the second inoculated leaf than in the first inoculated leaf. The NLL of TMV inoculums on 65-70 h.a.i. was shown as individual NLL but on 75 h.a.i. some individuals NLL were joint together to form a larger necrotic lesion (Figure 3). However, this phenomenon did not shown with the compost extract, until 145 h.a.i. the necrotic lesions were still observed as individual NLL (Figure 3 & 4); with the maximum individual NLL diameter of 0.5-0.6 cm² and the minimum NLL diameter was less than 0.2 cm² (Table 1).

In the field experiment, there was a reduction in number of NLL followed with the smaller NLL size on the second

inoculated leaf. The NLL diameter on the first inoculated leaf was more than 0.4 cm² and on the second inoculated leaf with compost extract was less than 0.2 cm². The number NLL was lower on the second inoculated leaf with compost extract, followed with the smaller NLL size than that of on the first inoculated leaf, at the same time observation (Table 2).

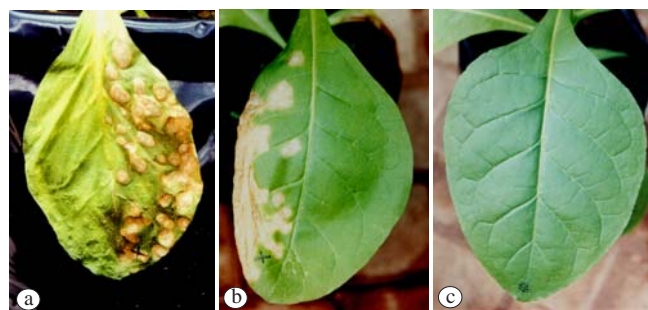


Figure 3. Development of TMV lesion on a half leaf inoculated with 1 ml TMV inoculums at dilution of 10^{-5} under the glasshouse conditions. a. Symptoms on 145 h.a.i. b. on 169 h.a.i. and c. healthy leaf.

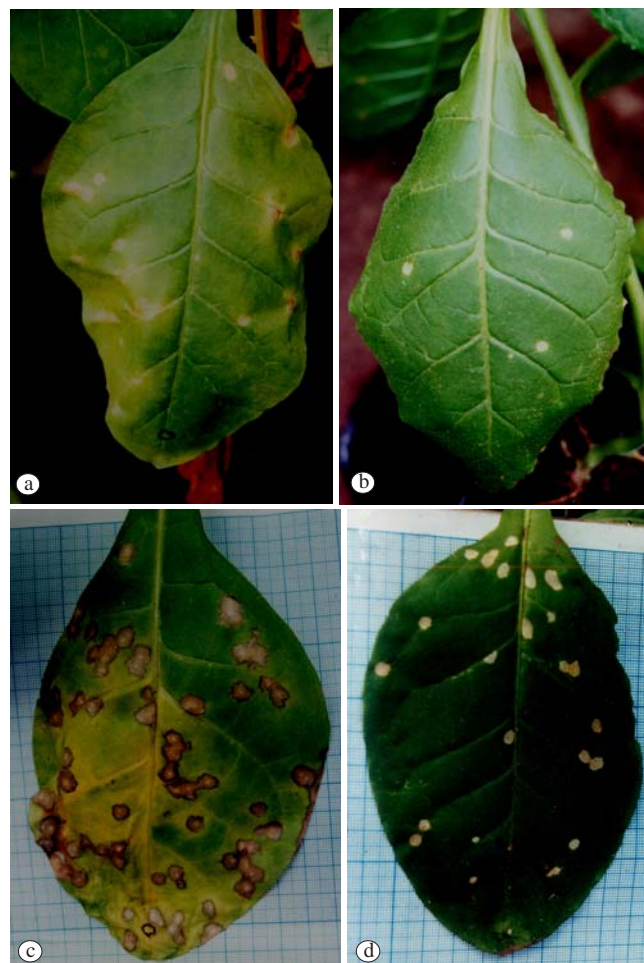


Figure 4. Comparison the number and size of NLL from a. compost extract of tobacco debris and b. TMV inoculums, in the glasshouse. The number of NLL on the second inoculated leaf (a and b) was lower than those of on the first inoculated leaf (a and c), and NLL size on b and d was smaller than those of on a and c.

Table 1. Number NLL and NLL size on the leaves inoculated with either compost extract or TMV inoculums in the glasshouse

Inoculation	NLL on the first inoculated leaf		NLL on the second inoculated leaf	
	Number NLL	NLL size	Number NLL	NLL size
(1) extract – (2) extract	11	A = 2, B = 5, C = 4	4	B = 2, C = 2
(1) H ₂ O – (2) extract	0	-	9	B = 7, C = 2
(1) TMV – (2) TMV	44	A = 16, B = 15, C = 13	17	B = 10, C = 7
(1) H ₂ O – (2) TMV	0	-	37	A = 2, B = 24, C = 11

(1) The first inoculation was done on the leaf number 3 from the base and (2) the second inoculation was on the leaf number 5 from the base, and this was done on 7 days after first inoculation. The first symptoms for TMV inoculums were observed at 44 h.a.i. and for the compost extract at 102 h.a.i. The extract volume was 1 ml and diluted at 10^{-2} . Experiment was done in the glass house with the average daylight temperature of 32 °C. The diameter of NLL A > 0.4 cm²; B = 0.2-0.39 cm²; C < 0.2 cm². Data was the average of six replications and was observed at the same time.

Table 2. Number NLL and NLL size on the leaves inoculated with either compost extract of tobacco debris or TMV inoculums in the field

Inoculation	NLL on the first inoculated leaf		NLL on the second inoculated leaf	
	Number NLL	NLL size	Number NLL	NLL size
(1) extract – (2) extract	5	A = 1, B = 2, C = 2	2	C
(1) H2O – (2) extract	0	-	3	B = 2, C = 1
(1) TMV – (2) TMV	27	A = 6, B = 15, C = 6	1	C
(1) H2O – (2) TMV	0	-	9	A = 3, B = 4, C = 2

(1) The first inoculation was done on the leaf number 3 from the base, and (2) the second inoculation was on the leaf number 5 from the base, and this was done on 7 days later. The first symptoms for TMV inoculums were observed at 65 h.a.i. and for the compost extract 150 h.a.i. The extract volume was 1 ml and diluted at 10^{-2} . The experiment was done in the field with the daylight temperature 29-30 °C. The diameter of NLL A > 0.4 cm²; B = 0.2 – 0.39 cm²; C < 0.2 cm². Data was the average of six replication and was observed at the same time.

DISCUSSION

Although tobacco plant debris was composted with EM4, the compost extracts still contained TMV. This was shown by NLL produced on cigar tobacco 'H877' which carries the N gene. This compost was processed at about 45-50 °C for two to three days, and the compost extract at dilution of 10^{-3} still contained TMV, it was shown by 2 NLL in the field experiment and 5 NLL in the glasshouse after 217 hours inoculation. However, Ghaly *et al.* (2006) found that compost of tomato debris infected with TMV which process in bioreactor with temperature of 63-65 °C for four days could inactivated TMV. A thermal process is capable of destroying TMV as other plant pathogens. They also found that under the same conditions, inoculation of that compost of TMV infected tomato which processed with temperature 63-65 °C reduced of 0, 0, 2, 12, 35 NLL on tobacco, and of 0, 0, 0, 2, 6, 15 NLL on tomato after 24, 48, 64, 96, 120, and 144 hrs of thermophilic treatments, respectively, while the untreated TMV produced 150 NLL on tobacco, and 22 NLL on tomato. It seemed there was a different host reaction on those treatments.

Inoculation a half leaf with either compost extract or TMV inoculums showed that NLL did not distribute systemically to adjacent half leaf, under daylight temperature of 30 °C in the field. But under glasshouse conditions, NLL from TMV inoculums seemed to develop as systemic lesions. As Hull (2002) mentioned that hypersensitive reaction (HR) of TMV which resulted in NLL occur at temperature above 28 °C. Because of the temperature in this experiment was 32 °C, NLL could develop to systemic necrotic lesions. In the other experiment, Wahyuni *et al.* (1999) found when the temperature in the glasshouse was above 34 °C, the NLL of TMV on tobacco 'H877' was firstly distributed locally on the inoculated leaf, and then this necrotic developed well, very fast, and distributed systemically to big necrotic lesions and caused some leaves dried. This was also determined by White and Antoniw (1991), if tobacco contains the N gene was maintained on above 34 °C, TMV firstly produced local lesion then developed to systemic lesions and sometimes to mosaic systemic.

Temperature was also effect the number and size of NLL. In the field experiment, the number and size of NLL was smaller than in the glasshouse. Besides it affected the number of NLL, the different temperature also effected the incubation period of TMV on tobacco 'H877' in producing NLL. In the glasshouse with the average temperature of 32 °C, the first

NLL produced from compost extract on 102 h.a.i. and from TMV inoculums on 44 h.a.i., while in the field with the average daylight temperature 29-30 °C, the first NLL from compost extract produced on 150-69 h.a.i. It seemed there was a delay of time (58-106 h.a.i.) for TMV from compost extract to appear as NLL compared to TMV from TMV inoculums. That delay of time could be caused by the mineral-nutrition contained in the extract that influenced on viral infection and it inhibited during the incubation period (Ghaly *et al.* 2006), and on the hypersensitive tobacco, symptoms of TMV was inhibited by the temperature more than 28 °C (Hull 2002).

In 1 ml of 10^{-2} dilution of compost extract produced about 5 to 11 NLL per leaf, while in 1 ml TMV inoculums which diluted to 10^{-5} produced 35 to 40 NLL. This was indicated that although tobacco debris was composted and the compost extract was stored for over 3 months, TMV was still present in the extract which diluted to 10^{-3} . As Lucas (1975) mentioned the DEP of TMV in leaf sap was about 10^{-7} , however, the DEP of compost extract was relatively high, 10^{-3} , and this difference could be caused by the heat during the composting process which inactivated some TMV particles.

The production of NLL indicates that the resistance tobacco 'H877' to TMV is a systemic acquired resistance (SAR). This SAR showed by the reduction in the number and size NLL (Hull 2002), and this could be caused by salicylic acid which activate the gene PR-1 protein to induce hypersensitive reaction to TMV (Linthorst 1991; Wahyuni *et al.* 1999).

TMV is considered as the most resistant plant pathogens to high temperature (Hull 2002). By observing the low NLL produced, some TMV particles might be destroyed by the temperature of composting process of 50 °C for about two-three days, particularly degraded its coat protein.

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