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Original Article

Leaf Extract of *Mirabilis jalapa* L. Induced Defense of Tomato Plant (*Lycopersicum esculentum* Mill.) Against Cucumber Mosaic Virus (CMV) Infection

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Abstract
Infection of Cucumber Mosaic Virus (CMV) could reduce the tomato production up to 50% depending on the age of the plant during infection and tomato varieties. Until now the control efforts against CMV infection have been made, but no consistent effective method was available. Recently, new approach by increasing the plant health to defense against plant disease was increased. It can be stimulated by inoculation of microbes associated with roots or by application of plant extract such as *Mirabilis jalapa*. In this study, the effect of *M. jalapa* leaf extract in different concentration to develop control against CMV infection in tomato plants was conducted. The results showed that the application of *M. jalapa* leaf extract could inhibit the development of CMV disease in tomato plants. The higher the concentration of *M. jalapa* leaf extract applied on tomato plant, the lower disease incidence as well as the longer incubation period of CMV disease in tomato plants. The applications of *M. jalapa* leaf extract elevated the activity of peroxidase and catalase enzymes. Thus, the inhibition of CMV disease development in tomato plant by application of *M. jalapa* leaf extract may related to the induction of peroxidase and catalase activity in tomato plants.

Keywords*: M. jalapa, CMV, plant defense, leaf extract, peroxidase, catalase*

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INTRODUCTION

Tomato is a horticultural commodity which has high economic value. One important obstacle on the cultivation of tomato plants is the disease caused by plant pathogenic virus. There are 18 types of viruses have been reported could infect tomato plants. Among them *Cucumber Mosaic Virus* (CMV) is the most harmful one (Crescenzi et al., 1993). CMV infection on tomato caused necrotic spots on fruit, leaf malformation, and in severe condition could lead to stunting and plant death (Crescenzi et al., 1993; Jorda et al., 1992). On several tomato production sites, the CMV infection caused substantial losses of production (Alonso-Prados et al., 1997).

In Indonesia, the virus that commonly infect tomato plants is *Cucumber Mosaic Virus* (CMV). Infection of CMV could reduce the tomato production up to 50% depending on the age of the plant during infection and tomato varieties. Until now the control efforts against CMV infection have been made, but no consistent effective method was available (Gallitelli, 1998; Jorda et al., 1992; Summers et al., 1995). CMV disease control efforts have been carried out, either by controlling the vector carrying the virus, as well as using a physical barrier, but the success of these two methods have not been consistent nor effective.

Recently, new approach by increasing the plant health to defense against plant disease was increased. It can be stimulated by inoculation of microbes associated with plant roots or by application of plant extract such as *Mirabilis jalapa* (Hersanti 2004). The ability of plant extracts in inducing plant defense against disease due to the presence of active compounds that could elevate plant defense related genes in the plant (Murphy et al,. 2001).

In this study the effect of *M. jalapa* leaf extract in different concentration was conducted to develop control against CMV infection in tomato plants

MATERIALS AND METHODS

Preparation of *M. jalapa* leaf extract

M. jalapa leaf extract was prepared by homogenizing the *M. jalapa* leaves in mortar pestle, and the phosphate buffer was then added. The *M. jalapa* leaf extracts at the concentration of 12,5%, 25%; 50%, and 100% were then prepared by adjustment of additional phosphate buffer.

Pot Experiment in Screen House

The experiment was conducted using completely randomized design (CRD) with 5 treatments and 5 replications. The composition of the treatments was as follows:

A0 = Without *M. jalapa* leaf extract (aquadest)
A1 = 12.5% concentration of *M. jalapa* leaf extract
A2 = 25% concentration of *M. jalapa* leaf extract
A3 = 50% concentration of *M. jalapa* leaf extract
A4 = 100% concentration of *M. jalapa* leaf extract

The data were analyzed using the ANOVA test with a significance level of 5%, and then the significant data were further analyzed by HSD test with a significance level of 5%.

M. jalapa leaf extract or aquadest (as control) was applied on two leaves above the cotyledons of 4 weeks old tomato plants. After 30 minutes the leaves were rinsed with water. Inoculation of CMV was done 24 hours after application of the extract by applying the sap of CMV infected leaves on the third and fourth leaves above the cotyledons.

The parameters measured were the symptoms shown on tomato test plant, incubation period, and the disease intensity.

The disease intensity was calculated as described by Abadi (2003) below.

$$I = \frac{\sum(nxv)}{NxZ} \times 100\%$$

Descriptions:
I = intensity of the disease per plant
n = number of leaves with each scale of disease severity
v = severity scale of each leaf
N = number of leaves per plant observed
Z = the highest scale of disease severity level

The scale of disease severity was as follow:
0 = healthy leaf
1 = mosaic lesion at ≤ 25% on leaf
2 = mosaic lesion on leaf at > 25% - ≤ 50% with blister
3 = mosaic lesion on leaf at > 50%, with blistering and malformation
4 = malformation, leaf scald and dwarf

The activity of catalase and peroxidase enzymes

The activity of peroxidase and catalase enzymes were measured using a method described by Reddy *et al.* (1995) and Luck (1974). The leaf samples of tomato plant were collected at 0, 7, and 14 days after inoculation of CMV.

RESULTS AND DISCUSSION

Symptoms and Incubation Period of CMV on Tomato Plant

CMV is a systemic viral disease which the symptoms spread to all parts of the plant. In this study the CMV symptom showed initially in young emergence leaves of tomato plants. The leaves infected by CMV showed mosaic symptom and on the later stage, leaves showed malformation to be wrinkle and smaller leaves. Further development of CMV symptom on severe condition showed yellowing, smaller leaves and stunting on all parts of tomato plant. These conditions were different with non infected tomato plant which showed healthy and green leaves of tomato plants (Figure 1). Lecoq et al. (1998) reported that plant leaves affected by CMV showed a mosaic, necrosis, and malformation. Further development showed that leaf size tends to shrink, leaves turn yellow and slightly thickened, and the fruits were changed in color and shape.

The observation of the incubation period of CMV symptoms on tomato plants was listed

in Table 1. Based on data shown in Table 1, the treatment of *M. jalapa* leaf extract significantly affected the period of incubation of CMV on tomato plant. Tomato plants without application of *M. jalapa* leaf extract showed the faster incubation period of CMV disease when compared to that of tomato plants treated with leaf extract of *M. jalapa*. The data also showed that the higher concentration of *M. jalapa* leaf extract application, the longer the incubation period of CMV disease. The longest incubation period of CMV was shown in tomato plants treated with 50% and 100% of *M. jalapa* leaf extract.

The result above showed that the treatment of *M. jalapa* leaf extract could delay the symptom of CMV on tomato plants. This result was similar to the result of Hersanti (2004) which has reported that leaf extract of *M. jalapa* was able to delay the incubation period of CMV.

(a) (b) (c) (d)

Figure 1. Symptoms of CMV infection in tomato plant : (a) mosaic on young leaves; (b) malformation; (c) leaf curling and stunted; (d) healthy leaf

Table 1. Incubation Period of CMV Symptom on Tomato Plants

Treatment	Incubation period (days)
No Treatment of *M. jalapa* leaf extract	8.8 a
12.5% of *M. jalapa* leaf extract	11.8 b
25% of *M. jalapa* leaf extract	13.8 c
50% of *M. jalapa* leaf extract	20 d
100% of *M. jalapa* leaf extract	20.4 d

Description: The number followed by the same letter in the same column indicates no significant difference based on HSD test (5%).

Table 2. The Disease Intensity of CMV On Tomato Plants

Treatment	Disease Intensity of CMV On Tomato Plants
No Treatment of *M. jalapa* leaf extract	50.158 d
12.5% of *M. jalapa* leaf extract	45.712 c
25% of *M. jalapa* leaf extract	41 226 b
50% of *M. jalapa* leaf extract	33 948 a
100% of *M. jalapa* leaf extract	31 646 a

Description: The number followed by the same letter in the same column indicates no significant difference based on HSD test (5%). Above the average figure has been transformed using arcsin.

The Disease Intensity of CMV on Tomato Plants

The data showed that the disease intensity of CMV on tomato plant without application of *M. jalapa* leaf extract was higher (50,2 % in average) than other tomato plants treated by *M. jalapa* leaf extract application. The data also showed that the higher the concentration of *M. jalapa* leaf extract application the lower the disease intensity of CMV. The disease intensity of CMV on tomato plants treated with *M. jalapa* leaf extract were ranging at 31-45% (Table 2).

The above results indicated that leaf extracts of *M. jalapa* decreased the disease intensity of CMV infected on tomato plants. In accordance with previous result that application of *M. jalapa* leaf extract could delay the incubation period of CMV disease, it can be suggested that the application of *M. jalapa* leaf extract showed to inhibit the development of CMV disease symptom. Dean and Kuc (1986) and Suganda et al. (2002) stated that the induction of resistance from external treatment did not make the plants become immune or not affected at all, but simply increase the degree of resistance and inhibit the progression of the disease.

The ability of *M. jalapa* leaf extract in inducing plant defense against plant disease is suspected to be an active compound contained in *M. jalapa* which induces defense related genes such as pathogenesis-related (PR) genes which encode pathogenesis-related proteins i.e peroxidase and catalase enzyme present in tomato plants (Murphy et al., 2001). In this study, we could not conclude whether the inhibition of CMV disease intensity was related to the inhibition of virus titer in planta, since we did not measure the CMV virus titer in tomato plants. According to Nicks (1993) the extent of damage or symptoms in plants is not always positively correlated with the level of concentration of viruses in plants.

Peroxidase Enzyme Activity

The data showed that the activity of peroxidase enzyme activity was higher on tomato plants treated with leaf extract of *M. jalapa* compared to that of without application of *M. jalapa* leaf extract (control) particularly at 1 and 14 days after application (Figure 2).

Van Loon et al. (1994) stated that peroxidase, a member of PR-protein from the group of PR-9, was accumulated at the time of plants infected by plant pathogen including virus. Gupta et al. (1990) stated that the resistant plants against plant disease tend to exhibit higher peroxidase activity compared to the susceptible plants. Zhou et al. (1992) also stated that increased expression of peroxidase activity caused by infection of plant pathogenic virus was correlated with the level of resistance to the virus.

Catalase Enzyme Activity

The data showed that the catalase enzyme activity was elevated on tomato plants treated with *M. jalapa* leaf extract particularly at 1 and 14 days after application. The average of the catalase enzyme activity in tomato plants was induced 1.3 - 1.5 times higher than in the untreated (control) tomato plants.

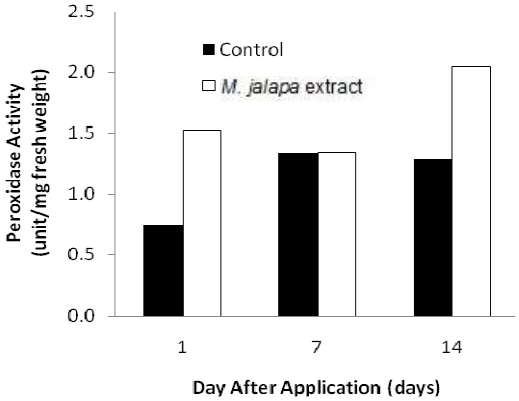

Figure 2. Peroxidase Enzyme Activity In Tomato Plant Leaves

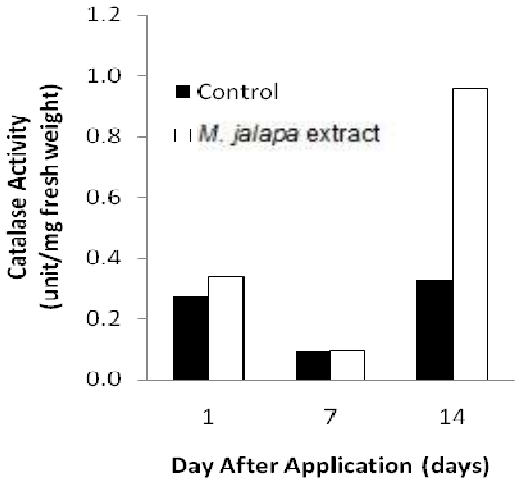

Figure 3. Catalase Enzyme Activity In Tomato Plant Leaves

Catalase enzyme has been widely known essential to destroy H_2O_2 formed in the peroxisomes via the oxidation reaction. H_2O_2 is known as oxidative agent that involved in the development of necrotic lesion as part of disease symptom development. Therefore, the increase of catalase activity will decrease the development of symptom caused by plant pathogen. Increased activity of catalase in tomato plants induced by leaf extract of *M. jalapa* suggested that the antioxidant induction effect of *M. jalapa* leaf extract help the defense reaction against CMV infection.

CONCLUSION

Based on the results of the study, it can be concluded that the application of *M. jalapa* leaf extract could inhibit the development of CMV disease in tomato plants. The higher the concentration of *M. jalapa* leaf extract applied on tomato plant, the lower disease incidence as well as the longer incubation period of CMV disease disease in tomato plants. The applications *M. jalapa* leaf extract elevated the activity of peroxidase and catalase enzymes. Thus, the inhibition of CMV disease development in tomato plant by application of *M. jalapa* leaf extract may related to the induction of peroxidase and catalase activity in tomato plants.

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