The Role of Leaf Extracts as Plant-activator to Enhance Salicylic Acid Production on Tomato Plant (*Lycopersicon esculentum* Mill.) Infected by CMV (Cucumber Mosaic Virus)

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Abstract — Plant will establish Systemic Acquired Resistance mechanism upon pathogenic infection in example, by inducing salicylic acid as a signal molecule. Resistance of plant against pathogen can be improved by addition of plant extracts as bioactivator. The objective of this study were to determine compound from *Clerodendrum japonicum* and *Catharantus roseus* leaf extract, which were potential as bioactivator and to evaluate the concentration of salicylic acid in tomato cultivars (*Lycopersicon esculentum* cv. Intan and cv. CL 6064) infected by CMV virus after application of plant extracts. The results showed that leaf extract of *C. japonicum* and *C. roseus* contained glyoxylic acid, phytol, and 1,2-benzenedicarboxylic acid which might be potential as plant activator. In tomato plant cv. Intan, leaf extract of *C. japonicum* was more potential to increase salicylic acid production averagely 36,91%, while in cv. CL 6064, leaf extract of *C. roseus* was more potential to increase salicylic acid production (averagely 27,47%).

Keywords — CMV, plant activator, salicylic acid, Systemic Acquired Resistance, tomato

I. Introduction

TOMATO plants (*Lycopersicon esculentum* Mill.) is one of the main horticultural crops cultivated in Indonesia [1,2]. However, cultivation of tomato plants in the field is often face obstacles, partly because of CMV (*Cucumber Mosaic Virus*) infection. In addition, CMV infection in Indonesia is often found in conjunction with other plant virus infections, so it can cause a decrease in tomato yield, approximately 50 to 100% [1,2,3,4].

Plants usually will establish Systemic Acquired Resistance (SAR) mechanism after virus attacked. This mechanism is initiated by accumulation of salicylic acid (SA) as a signal molecule for plant SAR mechanism, which then followed by expression of Pathogenesis Related (PR) protein. So, the presence of salicylic acid and PR protein in infected plants is considered as a response of plant immune system [5,6].

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SAR mechanism in plants can also be induced by application of bioactivator compound (plant activator). Plant activator is an agrochemical compound from plant which can work as a signaling molecule in the regulation of plants immune system. Plant activator compounds are expected to have similar characteristics which resembles SA (SA analog), so that the plant activator compounds have a role in inducing systemic resistance of plant against pathogen [6,7].

There were several studies on natural plant activator, for example: glycosides benzoxazinoid from *Zea mays*, *Triticum aestivum*, *Secale cereal*; avenacosides from *Avena sativa*; glycosides isoflavonoids from legumes; glucosinolate from *Brassicales*; active compounds from *Clerodendrum* as protein with molecular weight around 25-34 kDa, active compounds from *Catharanthus roseus* which are expected as natural plant activator [8,9].

The effectiveness of plant activator compounds can be evaluated through the plant resistance mechanism, first by observing its effect on visual symptoms, growth, and productivity. The second evaluation can be obtained by examining the content of salicylic acid and emergence of PR protein. This research was conducted to determine and measure concentration of salicylic acid, so that the role of plant extract as bioactivator can be evaluate.

II. Material & Methods

Plant Material

This study used CMV inoculum and tomato plant with two different cultivars: Intan and CL6064 from Indonesian Vegetable Research Institute (IVEGRI/BALITSA). Leaf extracts as a bioactivator inoculum were *Clerodendrum japonicum* and *Catharantus roseus*.

Preparation of Bioactivator and CMV (Cucumber Mosaic Virus) Inoculum

Two types of plant leaf extracts were used: *C. japonicum* and *C. roseus*, with the level of 50% concentration, respectively. Leaves were extracted by using 0.01 M buffer phosphate solution pH 7.0. Inoculation of plant extracts as bioactivator was performed on day 16th after sowing (16 DAS/16 HSS). Plant extracts were applied at the top of the

cotyledon using wet inoculation method, followed by rinsing with distilled water.

CMV inoculation used modified methods by Green [10], using wet inoculation method by mixing the Carborundum 600 mesh into a liquid inoculum (CMV solution). Solution was applied on the top of the true leaf blade at day 17th after sowing (17 DAS/17 HSS). Inoculated leaves were rinsed using distilled water. Once completed, the plant was ready for observation from week-1.

Secondary Metabolites Content Analysis in C. japonicum and C. roseus Leaf Extract

Secondary metabolites extraction was performed by dissolving freeze-dried *C. japonicum* and *C. roseus* leaf extract with 95% ethanol, then incubated overnight. The extract was then filtered by Whatman no. 1 filter paper to obtain fine secondary metabolites extract. Extract was then being analysed by gas chromatography–mass spectrometry (GC-MS) method.

Indirect ELISA Measurement

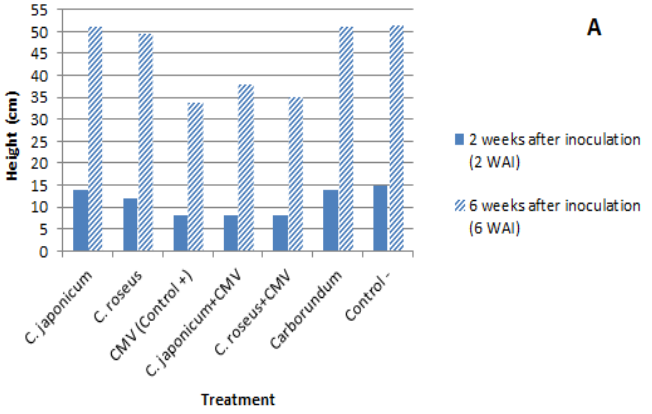
ELISA method was used based on Gunaeni & Duriat [11].

Salicylic Acid Content Analysis in C. japonicum Leaf Extract, C. roseus Leaf Extract, and Tomato Leaf

Extraction and analysis were conducted by modification of Tenhaken & Rubel [12] method. The concentration of salicylic acid was analyzed using HPLC (High Performance Liquid Chromatography). The eluents used were methanol : sodium phosphate buffer 50 mM pH 4.5 (40: 60) with a 1 ml/min flow rate. Extract and eluents was filtered by using a cellulose acetate membrane filter 0,2 um.

III. Result And Discussion

The results showed that leaf extract of *C. japonicum* and *C. roseus* as bioactivator tend to maintain growth of tomato plant when compared with tomato plants with no treatment (negative control). Similarly, when plants treated with a plant activator prior to CMV infection, *C. japonicum* leaf extract was more potential to increase the tomato plants height cv. Intan, while *C. roseus* leaf extract increased the tomato plants height cv. CL 6064 better (**Fig.1**).



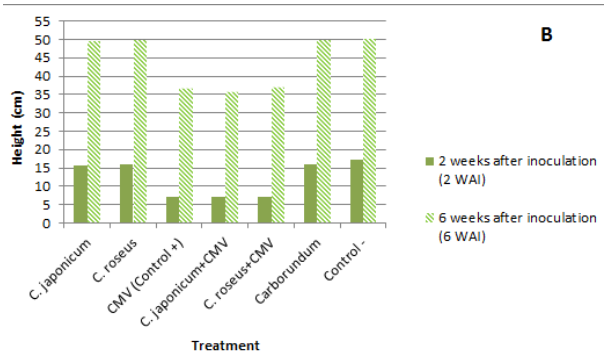


Fig. 1 Average tomato plants height (cv. Intan (**A**) and CL 6064 (**B**))

CMV content, shown by ELISA absorbance, in **Fig.2** showed that the use of leaf extracts as bioactivator could inhibit viral multiplication in two tomato cultivars. Therefore, secondary metabolites contents and protein profiles in both types of extracts (*C. japonicum* and *C. roseus*) need to be examined to proof that they play a role in inducing plant resistance [10,13,14].

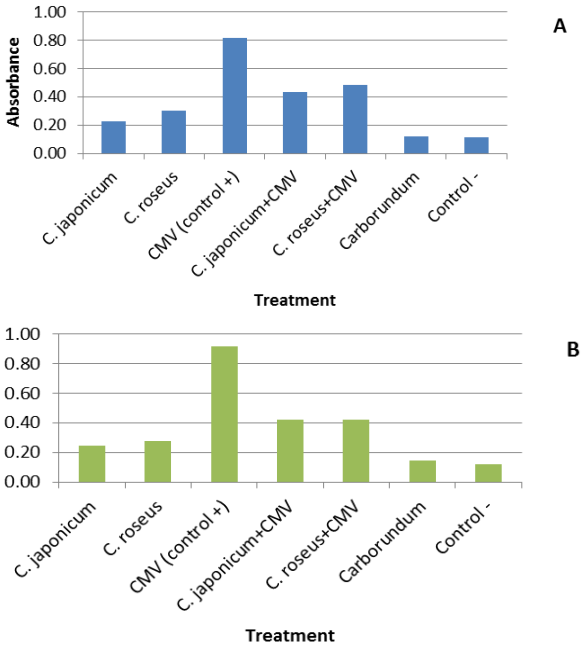


Fig. 2 Virus content on tomato plants (cv. Intan (**A**) and CL 6064 (**B**))

Result of GCMS analysis on extracts detected several compounds, shown in **Table1**. Measurement of the secondary metabolites content analogues to salicylic acid (or have similar characteristics) was conducted to determine the possible role of other compounds (other than salicylic acid) which could induce systemic acquired response on tomato plants infected by CMV [15,16].

The presence of glyoxylic acid in *C. japonicum* leaf extract might indicate that the salicylic acid derivative compounds play a role in inducing SAR mechanism by salicylic acid-phenyl propanoid pathway [17,18]. In both leaf extracts (*C. japonicum* and *C. roseus*) 1,2-benzenedicarboxylic acid and phytol were detected. The 1,2-benzenedicarboxylic acid is a

carboxylic acid group derivatives and has structural similarities with salicylic acid, while phytol is a diterpene compound group. Both of these compounds were also found in Wang, *et al*. [19] and Sermakkani & Thangapandian [20] research, which were known to have antimicrobial activity. So, there was a possibility that the two leaf extracts could activate the biosynthetic pathways of defense mechanism in tomato plant infected by CMV, or they had similar structures with salicylic acid which compatible with protein receptor in tomato plant, or the compound had a specific pathway downstream in the process which produced a similar defense compounds [17,18].

TABLE I
SEVERAL COMPOUNDS WHICH ARE DETECTED IN C. JAPONICUM AND C. ROSEUS LEAF EXTRACT

Compound	Group	Presence in *C. japonicum* leaf extract (%)	Presence in *C. roseus* leaf extract (%)
Glyoxylic acid	Phenol derivative – salicylic acid	0,86	
Phytol	Terpenoid	24,87	35,01
1,2-benzenedicarboxylic acid	Carboxylic acid	3,28	1,5

Salicylic acid content in *C. japonicum* leaf extract was measured about 1,3 ppm, while in *C. roseus* leaf extract was not detected. Protein profile analysis from *C. japonicum* and *C. roseus* leaves were conducted to determine the presence of defense protein in both types of plants. The results showed that both plants had a protein with molecular weight ~15 kDa which was suspected as defense protein. A protein with molecular weight ~25-34 kDa was not found on *C. japonicum*, which might be due to protein purification was not conducted beforehand to obtain the desired protein [8]. There was also a possibility that the expression of the protein with molecular weight ~25-34 kDa was very low relatively to the expression of other proteins.

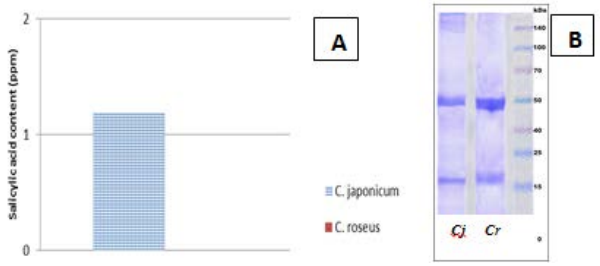


Fig. 3 Salicylic acid content (**A**) and protein profile (**B**) from *C. japonicum* (***Cj***) and *C. roseus* (***Cr***) leaf extract

In tomato plant cultivar Intan, the application of *C. japonicum* and *C. roseus* leaf extracts increased the salicylic acid concentration (*C. japonicum* application, averagely 36,91%) compared with plants that were not given the extract and untreated virus (control negative). Treatment of *Clerodendrum japonicum* leaf extract followed by CMV infection showed that salicylic acid levels on tomato plants tended to be higher compared to that in plants that were not treated by the extract but inoculated by virus (control positive). The results also showed that the accumulation of salicylic acid tend to decrease along with the increase of plant age (**Fig.4**).

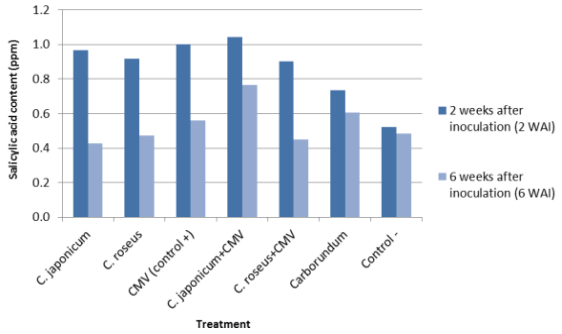


Fig. 4 Salicylic acid content on tomato plant cv. Intan

In tomato plant cultivar CL 6064, the application of *C. japonicum* and *C. roseus* leaf extracts increased the salicylic acid concentration compared with plants that were not given the extract and untreated by virus (control negative) (*C. roseus* application, averagely 27,47%). Application of *C. roseus* leaf extract followed by treatment of CMV infection suggested that salicylic acid levels on tomato plants tended to be higher compared to plants that were not given the extract but inoculated by virus (control positive). The results also showed that the accumulation of salicylic acid tend to decrease along with increase of plant age (**Fig.5**).

It can be concluded that *C. japonicum* leaf extract application on tomato plant cv. Intan give a better effect than the application of *C. roseus* leaf extract, while *C. roseus* leaf extract application on tomato plant cv. CL 6064 gives a better effect than the application of *C.japonicum* leaf extract.

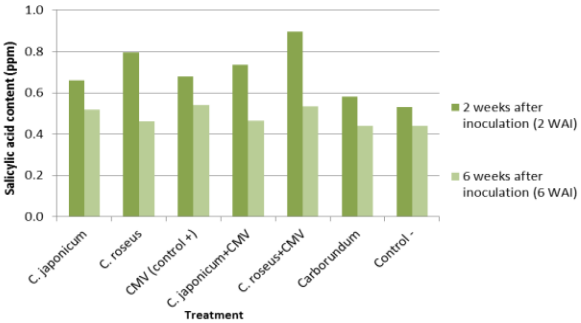


Fig. 5 Salicylic acid content on tomato plant cv. CL 6064

A similar study by Ratnawati [21] showed that different responses were shown on each tomato cultivars infected by CMV without any extract application. Salicylic acid concentrations tended to increase slowly in susceptible cultivars but it was not able to reduce the severity of symptoms (only delay the appearance of symptoms). Another study by Hersanti [22] showed that the application of extract on pepper plants which was infected by CMV might activate SAR mechanism, which was indicated from the increase level of salicylic acid. The same trend was also observed by Gunaeni, *et al*. [23].

IV. CONCLUSION

According to result of this study, *C. japonicum* and *C. roseus* were potential as a plant activator, but the ability of the *C. japonicum* was better in improving the salicylic acid content as indicator of SAR response in tomato plant cv. Intan,

while *C. roseus* was more potential improving the salicylic acid content as SAR response in tomato plant cv. CL 6064.

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