Jurnal Perlindungan Tanaman Indonesia, Vol. 23, No. 1, 2019: 109–115
DOI: 10.22146/jpti.29656
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Potency of Salicylic Acid to Disrupt the Growth and Development of Papaya Mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae)

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Received October 27, 2017; revised March 19, 2018; accepted March 20, 2018

ABSTRACT

Mealybug is an important pest of papaya plants. Induction of plant resistance using elicitors, such as salicylic acid, might have the potency to reduce the extent of crop damage by mealybug. Therefore, a laboratory experiment was performed to determine the effect of salicylic acid on feeding preference, fecundity, oviposition period, and longevity of papaya mealybug adult, *Paracoccus marginatus*. The results showed that the application of salicylic acid increased total phenol content on papaya leaf (r = 0.57) hence decreased in feeding preferences and fecundity, slowed down the growth period of the nymph and pre-oviposition period, and prolonged the longevity of mealybug. The potency of using salicylic acid to control of mealybug on papaya in integrated pest management was discussed in this paper.

Keywords: mealybug papaya, *Paracoccus marginatus*, salicylic acid

INTRODUCTION

Mealybug, *Paracoccus marginatus*, is an important pest of papaya plants (Meyerdirk *et al*., 2004; Muniappan *et al*., 2008; Mastoi *et al*., 2011; Suartini *et al*., 2015). The attack of mealybug causes disruption of photosynthesis and fruit fall before ripe, thus potentially reduce plant productivity. The control technique using pesticides is relatively high, less effective, and unfriendly environmentally (Mariyono & Irham, 2001; Isenring, 2010; Tanwar *et al*., 2010; Krisnan *et al*., 2016). Elicitors compound, e.g. salicylic acid, is an alternative control technique to reduce the plant damage caused by pests (Thaler *et al*., 2002; Qiu *et al*., 2009; San Vicente & Placensia, 2011; Santamaria *et al*., 2013).

Salicylic acid is an elicitor inducing plant resistance to many pathogens (Chen *et al*., 1995; Suganda, 2001; Thaler *et al*., 2002; Martanto *et al*., 2003; Suryanti *et al*., 2009, Sujatmiko *et al*., 2012; Hoerussalam *et al*., 2013; Thakur & Sohal, 2013). Previous studies showed that spraying salicylic acid enhanced plant resistance to sucking pests, e.g. to control population of *Nezara viridula*, *Myzus persicae*, *Empoasca lybica*, and *Tetranychus urtica* (Thaler *et al*., 2010; Farouk & Osman, 2011; Mahmoud, 2013; Michael *et al*., 2013; Afifi *et al*., 2015; Elhamahmy *et al*., 2016; Rana *et al*., 2016), and to decrease the fecundity and the longevity of *Bemisia tabaci* (Shi *et al*., 2016). However, the effect of salicylic acid on sucking pest is inconsistent. For example, Zhang *et al*. (2011) and Zhang *et al*. (2015) reported that the application of salicylic acid to *Phenacoccus solenopsis* was not reduced the growth rate of nymphs on cotton and tomato plants.

The study about the use of salicylic acid to induce the resistance of papaya plants to *P. marginatus* is needed to be done. Therefore, this study aimed to determine the effect of salicylic acid on the growth and development of *P. marginatus*. The parameters used were the feeding preference of nymph, adult fecundity, the longevity of each instar of nymphs, pre-oviposition period, oviposition period, and adult longevity. The results of this study highly expected to provide information about the use of salicylic acid to control *P. marginatus*.

MATERIALS AND METHODS

The study was conducted at the green house and Laboratory of Plant Pest Sciences, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta in November 2016 to April 2017 with a daily temperature of 26−30ºC, and relative humidity of 44−60%. The study used a Randomized Complete Block Design (RCBD) with

a concentration of salicylic acid of 0, 50, 100, and 200 mg/l, respectively. Each treatment used five plants.

Propagation of Papaya Plants

Merah Delima variety was used as host plants, obtained from the Research Institute for Tropical Fruit Plants. Papaya nursery was carried out in a 250 ml volume plastic bag containing a mixture of soil and manure with a ratio of 2:1.

Mass Rearing of P. marginatus

P. marginatus adults were collected from papaya plants in Kaliurang area, Yogyakarta then infested to papaya plants aged 6 weeks old. Plants were put into wood-framed gauze cages (80×80×80 cm) to protect them from predator and parasite. *P. marginatus* was reared on plants until their number was sufficient for testings.

Salicylic Acid Test

Salicylic acid used was a pro-analyst salicylic acid from Merck. Salicylic acid was weighed according to the treatment concentration, dissolved in absolute ethanol (1−2 ml), and applied to 5-week-old plants. The method was repeated on the third and sixth day after the first application. 20 ml of salicylic acid was sprayed to the leaves surface using a hand sprayer. After 2 weeks, the leaves of the plants treated were used for the test of feeding preference, observation of insect biology, and analysis of leaf phenol content levels.

Feeding Preference Test

The feeding preference test was carried out based on the feeding preference test method by Zhang *et al*. 2011. *P. margaratus* were exposed to four leaves treated with salicylic acid in concentrations of 0 (water/control), 50, 100, and 200 mg/l, respectively. Those leaves were put in a rectangular box (24 × 24 × 8.5 cm) at four different edges, then 20 third instar nymphs were placed in the middle of the box. After 24 hours, the number of nymphs fed on each leaf (as a sign of feeding preference was present) was calculated. The leaf stalks were covered with moist cotton to maintain the vigor of the leaves. This test was carried out with 24 replications per treatment.

Fecundity, Longevity of Nymphs, Longevity of Female Adults, Preoviposition Period, and Oviposition Period

Fecundity was calculated daily based on the number of newly hatched nymphs. Observations on the longevity of the nymph and adult were employed by cutting the 2 upper leaves of the papaya plant treated in the 2nd week after the application of salicylic acid. Each leaf was placed in a glass bottle (5 ml in volume) filled with water, and the stem was covered with moist cotton to keep the vigor of the leaves. Five newly hatched nymphs were fed by papaya leaves. The longevity of nymphs until adults were observed daily. Instar of the nymphs was characterized by the presence of white exuvia. When the adults emerged until an egg sac formed was recorded as the preoviposition period. The oviposition period was calculated from the formation of the egg sac until the 1st nymphs hatched. The development of nymphs and adult was observed using a binocular microscope.

Analysis of Leaf Phenol Content

Analysis of leaf phenol content was carried out using the method by Senter *et al*. (1989) and conducted at the Laboratory of Chemistry and Biochemistry of Food and Agricultural Products, Department of Food and Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta. One ml of papaya leaf extract was diluted to 100 ml, then 1 ml of the dilution was taken and diluted again with 10 ml hence the total dilution became 1000× (df = 1000×). One ml of dilution was added with 5 ml of alkaline Na_2CO3 2%, and incubated for 10 minutes, then added 0.5 ml of Folin Ciocalteau reagent, vortexed, and incubated for 30 minutes. Absorptions were measured at a wavelength of 750 nm. The phenolic concentration was calculated based on the standard curve obtained from pure phenol solution using the following formula:

$$\%\ Phenol = \frac{x.df.100\%}{sample\ (mg)}$$

Note: df = dilution factor

Each treatment concentration has for replications. Leaf samples for analysis were harvested on the 12th day after application of salicylic acid.

Data Analysis

The effect of treatments on fecundity, the longevity of the newly hatched nymphs, 2nd instar, 3rd instar, preoviposition period, oviposition period, the longevity of adults and leaf phenol levels were tested by Variance Analysis (ANOVA). The post-hoc analysis using the LSD test was carried out at 5% when there

was significantly different from each treatment. Furthermore, the correlation and regression analysis between phenol levels and the growth parameters of *P. margaratus* was analyzed using the MS-Excel 2007 program.

RESULTS AND DISCUSSION

This research showed that the application of salicylic acid in papaya plants affects the biology of *P. marginatus* and increased the leaf phenol level thus reduce the feeding preference of 3rd instar nymphs. Although salicylic acid prolonged the longevity of the 2nd and 3rd instar nymphs, shorten the longevity of the 1st instar nymph was shorter. Therefore, the use of salicylic acid to papaya plants has the potential to reduce *P. marginatus* population. The results also showed that papaya leaves treated with salicylic acid had higher phenol level than untreated leaves (Table 1; $P < 0.05$). Nevertheless, the leaf phenol level was not significantly different from the concentrations of salicylic acid 50, 100 and 200 mg/l.

Salicylic acid, o-hydroxybenzoic acid, is a phenolic compound naturally exists in plants in low concentrations (Kawano *et al*., 2004, Janda *et al*., 2014, War *et al*., 2015). The function of salicylic acid is a phytohormone and has a role in physiological processes, for example in the management of resistance to pests. The synthesis of salicylic acid in plants is a complex mechanism. Plants synthesize salicylic acid from cinnamic acid produced by phenylalanine ammonia lyase with benzoic acid as an intermediate precursor, which takes place in the cytosol. Salicylic acid is also synthesized in chloroplasts from isochorismate acid produced from chorismic acid (Kawano *et al*., 2004, Hayat *et al*., 2010). Previous study stated that salicylic acid has a role in the plant resistance to diseases (Dempsey & Klessig, 2017). Lennon *et al*. (1997) also reported that salicylic acid is an inducer to increase the resistance of tobacco plants to Tobacco Mosaic Virus (TMV) through an increase in alternative oxidase proteins. Furthermore, War *et al*. (2012) stated that salicylic acid has a role in plant resistance to herbivorous insects, both piercing-chewing and biting-sucking pests.

Table 1. Effect of salicylic acid on total phenol content (%) of papaya leaves

Concentration (mg/l)	Phenol (%)
0	0.320 ± 0.04b
50	0.385 ± 0.03a
100	0.345 ± 0.01ab
200	0.381 ± 0.04a

Remarks: Means followed by the same letter were not significantly different according to BNT test (p = 0.05).

This study showed that salicylic acid was applied to papaya increased leaf phenol levels hence decreased feeding preference. The test showed that the number of 3rd instar nymphs fed on papaya leaves exposed to salicylic acid was smaller than those fed on untreated leaves (controls) (Figure 1). Other studies by War *et al*. (2011), Farouk & Osman (2011), and Damodaram *et al*. (2015) also stated that the application of salicylic acid can increase phenol levels. In the other hand, the study of Elhamahmy *et al*. (2016) showed that the use of salicylic acid did not increase phenol levels in canola leaves (*Brassica napus*). In general, the role of phenol compounds in developing plant resistance to herbivorous insects has also been studied in several types of herbivorous insects.

Other studies showed that salicylic acid is a deterrent for aphids and *B. tabaci* (Mahmoud & Mahfouz, 2015; Shi *et al*., 2016), hence those population in plants was lower than in control treatments. These studies proved that salicylic acid inhibited the feeding preference of piercing-sucking pests. Another study by Ollerstam and Larsson (2003) showed that salicylic acid has an important role in the resistance of *Salix viminalis* to gall midge (*Dasineura marginemtorquens*). However, Sekido and Sogawas (1976) reported that salicylic acid is a feeding stimulant on *Nilaparvata lugens*, but it reduced the feeding preference of *Laodelphax striatelus*.

The decrease in feeding preferences by mealybug on papaya leaves treated with salicylic acid may effect the growth and development of the longevity of nymphs and adults. The results showed that the use of salicylic acid prolonged the longevity of 2nd and 3rd instar nymphs, although shorten the longevity of 1st instar nymph (Table 2). In use to that, the exposure of salicylic acid had an effect on preoviposition and longevity of adults. In general, the application of salicylic acid in higher concentrations tends to prolong the preoviposition period and the longevity of adults. Meanwhile, the use of salicylic acid did not affect the oviposition period (Table 3; $P > 0.05$).

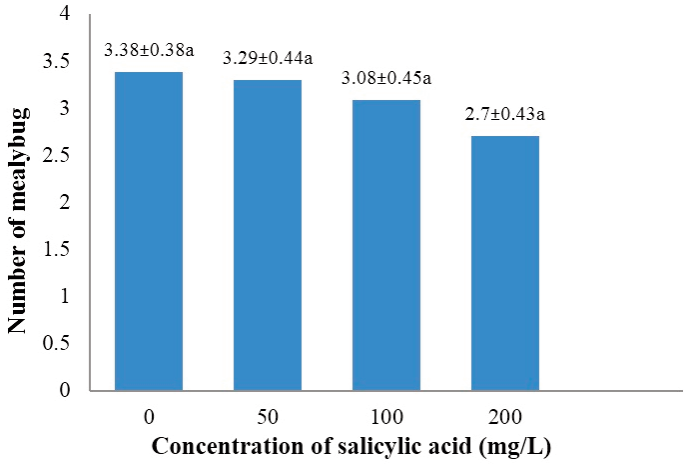

Figure 1. Feeding preference of *Paracoccus marginatus* on different concentrations of salicylic acid

Table 2. Effect of salicylic acid on the development of immature *Paracoccus marginatus*

Treatment	Longevity of nymph (day)			
	1st instar	2nd instar	3rd instar	Total Nymph
Concentration (mg/l)				
0	3.95 ± 0.17a	3.96 ± 0.17a	4.80 ± 0.32a	12.70 ± 0.35a
50	3.88 ± 0.09a	3.99 ± 0.21a	5.42 ± 0.35a	13.29 ± 0.47a
100	3.86 ± 0.14a	4.32 ± 0.15a	4.52 ± 0.22a	12.72 ± 0.19a
200	3.74 ± 0.11a	4.07 ± 0.21a	5.29 ± 0.27a	13.13 ± 0.48a

Remarks: Means followed by the same letter were not significantly different according to BNT test (p = 0.05)

Table 3. Effect of salicylic acid on the pre-oviposition period, oviposition period, and adult longevity of *Paracoccus marginatus*

Concentration (mg/l)	Pre-oviposition (day)	Oviposition (day)	Adult longevity (day)
0	4.23 ± 0.31c	6.78 ± 0.34a	14.36 ± 0.67b
50	5.00 ± 0.51bc	6.94 ± 0.29a	15.36 ± 0.99ab
100	6.83 ± 0.83a	7.22 ± 0.38a	17.73 ± 1.13a
200	6.42 ± 0.42ab	7.56 ± 0.74a	16.64 ± 1.04ab

Remarks: Means followed by the same letter were not significantly different according to BNT test (p = 0.05)

Table 4 showed that the use of salicylic acid to papaya leaves decreased fecundity of *P. marginatus* compared to controls (P <0.05). Nonetheless, the concentration of 50, 100, and 200 mg/l of salicylic acid to papaya leaves did not significantly affect the fecundity. Damodaram *et al*. (2015) reported that the application of salicylic acid increased plant phenol levels, thereby reducing the preference of *Bactrocera dorsalis* for laying eggs. Meanwhile, the application of elicitor compounds, such as salicylic acid, increased the levels of toxic compounds, e.g. phenol, peroxidase enzymes, polyphenol oxidase, and hydrogen peroxide (War *et al*., 2011; Damodaram *et al*., 2015), which potentially decrease the growth of larvae or nymphs, and fecundity.

The exposure of salicylic acid induces chemical compounds that affect the chemical and mechanical characteristics of plant tissue to insect growth (Cipollini *et al*., 2004; War *et al*., 2013). Phenol is a signaling molecule (precursor) to activate the resistance genes of plants to pests and diseases (War *et al*., 2012). Oxidation of phenol compounds is catalyzed by polyphenol oxidase and peroxidase produce quinone which is toxic to insects or inhibits insect appetite (War *et al*., 2012; Shoorooei *et al*., 2013), thus effecting insect growth and development.

Table 4. Effect of salicylic acid on fecundity of *Paracoccus marginatus*

Concentration (mg/l)	Fecundity
0	236.32 ± 24.63a
50	151.50 ± 12.48b
100	112.97 ± 8.02b
200	151.13 ± 16.03b

Remarks: Means followed by the same letter were not significantly different according to BNT test (p = 0.05)

This study showed the potency of an elicitor compound, salicylic acid, to manage herbivorous insect populations by affecting the chemical or mechanic-physic characteristics of plants (War *et al*., 2013; Damodaram *et al*., 2015). Nevertheless, several studies also explained that the production of elicitor compounds (e.g. phenol) for plant resistance was influenced by abiotic factors, such as light and soil nutrients (Dudt & Shure, 1994). In the other study, plants grow in different types of soil will have different phenol content (Doyle *et al*., 1978). Therefore, the plants would generally contain more defense compounds in soils with lower nutrient.

In this study, observations of the growth and development of *P. marginatus* were employed on leaves was not picked on fresh plants. This might affect the growth of *P. marginatus*. However, because all treatments, both controls and those applied by salicylic acid were carried out by the same method, the result of the effect of salicylic acid on the growth and development of *P. marginatus* was considered reliable. Moreover, this study will give additional information about pest management using a resistance induction technique. The use of inducing compounds, such as salicylic acid, has the potency to reduce the growth and development of *P. marginatus*. However, the concentration of elicitor compounds induced to plants and the influence of external factors, such as temperature, light, and humidity, is important to be considered.

CONCLUSION

The use of salicylic acid potentially can control the population of *P. marginatus*, through a mechanism of emphasis on feeding preference of nymphs and decreased fecundity of *P. marginatu*s.

ACKNOWLEDGEMENTS

We thank the Agricultural Research and Development Institute for funding this research. This paper is a part of the thesis for graduating from the Department of Plant Pest Science.

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