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Potency of Guava Shoot Extract as a Control Means Against Diaphorina Citri

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Abstract. Citrus production in Indonesia and the world is facing a serious problem by CVPD attacks. Diaphorina citri is the vector of the disease. Guava leave extract is able to be proposed as prospective control means for the vector. Investigation was conducted to identify the repellent effect of guava leaves to adult of D. citri and the attractiveness to its predator, Menochilus sexmaculatus. Repellent effect of grinded dried of upper, middle, bottom shoot of non-seed, white, and red guava shoots to D. citri and M. sexmaculatus were determined in Y-tube olfactometer. The result showed that repellence effect to D. citri was on guava shoots odor sources. Repellence effect was highest on white guava shoots, followed by non-seed guava and red guava. Repellent effect was 80.7%, 72.7%, and 70.0% respectively. However they did not have any repellent effect to M. sexmaculatus. Guava shoots had the attractiveness effect to M. sexmaculatus adult. The effect was higher on upper, middle, and bottom shoot of red guava, and white guava, and middle shoot of non-seed guava than on upper shoot of citrus. Exception phenomenon was found on upper and bottom shoot of non-seed guava. The attractiveness on those shoots was lower than on citrus leaves. It was able to use guava shoots to repel D. citri and to attract M. sexmaculatus adult as a part of control means of D. citri vector of CVPD disease.
Keywords: CVPD, Diaphorina citri, M. sexmaculatus, Guava, Vector

1. Introduction

Citrus production in Indonesia currently increase by 6.22% or 159.46 thousand tons from 2019 with a total production of 2.72 million tons. The highest production of citrus in 2020 occurred in the second quarter, which increased by a total of 828.38 thousand tons produced by 19.71 million citrus plants. The increase was supported by the increase of the number of producing plants. It is reported by Indonesian Central Statistics Agency [1], citrus production in 2015 up to 2020 has increased successively as 2.13 million tons, 2.29 million tons, 2.51 million tons, 2.56 million tons and 2.72 million tons every year respectively.

Although citrus production has increased, the increase in production is mainly due to the addition of citrus plantations. Citrus productivity is still constrained by CVPD (citrus vein phloem degeneration) disease. The attack of CVPD has a devastating impact on citrus plantation. CVPD disease is caused by phloem-limited bacteria of 'Candidatus Liberibacter asiaticus' (CLas: α-Proteobacteria). It is a Gram-negative bacteria [2], [3]. The specific symptoms of the disease are: leaves with asymmetric, blotchy-mottling that seem like chlorosis symptom caused by mineral deficiencies; small upright leaves; vein-corking or yellowing; out-of-season flushing and heavy flowering; small, lopsided, bitter tasting fruit with aborted seeds; branch dieback; and death of trees [4]. It caused a high plants mortality. Citrus plant's life span will reduce to 7–10 years [5], [6]. CVPD also shortening the productive period and decrease the level of productivity and quality of citrus fruits [7]. Citrus plants will decrease their fruit productivity in 2–5 years after the first symptoms appear [5], [6]. The worst loss of citrus production in Indonesia was happened in 2008 up to 2012. It was from about 2,467,632 tons (2008) to 1,611,768 tons

(2012) and an increase in citrus imports from 138,000 tons with a value of USD117 million in 2008 to 256,000 tons with a value of USD247 million in 2012 [8].

The pathogen is transmitted by Diaphorina citri Kuwayama (Hemiptera: Psyllidae). Imago of D. citri or psyllid and the 4-5 instar nymph are the only stage that have capability of transmitting the disease [9], [10]. The vector can transmit disease throughout its life [11], [12]. Control of D. citri or psyllid still relay on the use of synthetic chemical insecticides that are expensive and not environmentally friendly [13]. An alternative to synthetic insecticides is mineral oil [14], but it is still rare and expensive.

In the process of searching for host plants, one of the factors that affect insects is volatile compounds released by plants. Volatile compounds are secondary compounds released by plants and evaporate quickly [15]. Each type of plant produce specific volatile compounds. The content and composition of these compounds have contributed to the distinctive character of the plant. Volatile compounds are produced by plants from the organs of leaves, flowers, or fruit. Volatile compounds secreted by plants can provide differences in the attraction of insects to plants. In addition to volatile compounds, physical and chemical properties can also affect the attractiveness of insects on their host plants. Intercroping of guava in citrus plantations was significantly decrease the infestation of D. citri and infection of CVPD disease. The presence of one guava plant among eight citrus plants succeeded in preventing the entry of D. citri populations and the occurrence of CVPD attacks on citrus plants [16] [17]. The volatile active compounds of guava leaves are a potential source of botanical insecticides, but the type and mechanism of the volatile compounds are unknown. D. citri in finding its host also relies on volatile odour of specific compounds from citrus shoots. Acetic acids and formic compounds have an important role in acceptance of host [18]. This research was aimed to identify the role of guava leaves extracts to D. citri and its predator as a prospective means for controlling D. citri.

2. Materials and Method

2.1. Insect Rearing

Rearing of D. citri was conducted inside a greenhouse. Disease free-cultured of adult of D. citri was obtained from research centre for citrus and sub-tropical fruit (BALITJESTRO), Malang, Indonesia. Orange jasmine (Muraya paniculata) plants were used as host plants. They were planted in plastic pots (Ø 25 cm, height 18 cm) in gauze cages (60 cm length, 60 cm width, and 100 cm height). Buds produced after pruning were used for eggs laying. The temperature of greenhouse was set at 26-30oC and the relative humidity was maintained at 60-70%.

Rearing of Menochilus (Cheilomenes) sexmaculatus was done using aphis as preys. First, aphis was reared on long bean plants. Seedlings of long beans planted in polybags (Ø 15 cm, height 30 cm) and were put in a cage with a gauze cover (100 cm length, 100 cm width, 100 cm height). After 7 days, the leaves were infested with aphis. Aphis were obtained from the field. Long bean plants were always replaced with new ones when they start to wither/dry due to being attacked by aphis. A pair of adult M. sexmaculatus were placed in a gauze cage containing long beans and aphis for laying eggs. Eggs will hatch after 3-4 days, and adult insects are obtained after 14-15 days.

2.2. Leaf extraction

Fully open leave of citrus (Citrus reticulate) upper shoots (leaf number 1 and 2 from the tip), and guava (Psidium guajava) upper shoots (leaf number 1 and 2 from the tip), middle shoots (leaf number 3 and 4 from the tip), and bottom shoots (leaf number 5 and 6 from the tip) were dried in oven for 24 hours at 50^{o}C. The dried leaves were then ground with an electric grinder and then sieved to extract the powder. Leaf powder were stored in airtight containers.

2.3. Repellence/attractant test

Choice test was performed for testing the repellence responses to D. citri and the attraction of M. sexmaculatus adult by using a modified olfactometer made of transparent glass tube (10 mm internal diameter, 300 mm long) with a "Y" shape. The main tube was A, the left branch was B and the right branch was C. Ten adult D. citri of mixed gender were placed at the end of tube A. Five gram of guava leaves extract according to treatment were put in an airtight container (Ø 150 mm, 150 mm high) and

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connected by a silicone tube (5 mm internal diameter) to the end of branch B, and 5 g of citrus leaf extract was connected to the end of branch C. Air was pumped through activated charcoal to clean the air and then through distilled water to clean unwanted particles and for humidifying the air. The cleaned air then move to the both odour sources containers with the air flow of 20 mL min-1. Air flow meter was used to measure the air flow. Next, the test insects were allowed to choose whether to go to the B end or the C end and their number was also recorded. The treatment was repeated 30 times. The same method was conducted on adult of M. sexmaculatus. The list of odour sources comparison is as shown in Table 1.

Table 1. Y-tube olfactometer test of 10 adults of D. citri or M. sexmaculatus for 30 minutes on the odour of citrus leaves extract compared to the upper, middle, and bottom shoots of red, white and non-seed guava (n = 30)

Comparison	Odor sources		
		D. citri	
I	Citrus leaf (CT)	Vs	Fresh air (FA)
II	Citrus leaf (CT)	Vs	Citrus leaf + Red guava upper shoot (RGU)
III	Citrus leaf (CT)	Vs	Citrus leaf + White guava upper shoot (WGU)
IV	Citrus leaf (CT)	Vs	Citrus leaf + Non-seed guava upper shoot (NSGU)
		M. sexmaculatus	
V	Citrus leaf (CT)	Vs	Citrus leaf + White guava upper shoot (WGUS)
VI	Citrus leaf (CT)	Vs	Citrus leaf + Red guava upper shoot (RGUS)
VI	Citrus leaf (CT)	Vs	Citrus leaf + Non-seed guava upper shoot (NSGUS)
VIII	Citrus leaf (CT)	Vs	Citrus leaf + White guava middle shoot (WGMS)
IX	Citrus leaf (CT)	Vs	Citrus leaf + Red guava middle shoot (RGMS)
X	Citrus leaf (CT)	Vs	Citrus leaf + Non-seed guava middle shoot (NSGUS)
XI	Citrus leaf (CT)	Vs	Citrus leaf + White guava bottom shoot (WGBS)
XII	Citrus leaf (CT)	Vs	Citrus leaf + Red guava bottom shoot (RGBS)
XIII	Citrus leaf (CT)	Vs	Citrus leaf + Non-seed guava bottom shoot (NSGBS)

3. Results and discussion

Y-tube olfactometer was an effective apparatus to test the response of insects to the odour of plants leaves extracts. Base on comparison I observation, It was showed that the population of adult insects of D. citri moving towards the odour source of the citrus leaf was significantly higher (7.93 ± 0.27) than the source of clean air (1.60 ± 0.13) (figure 1).

The presence of the odour of guava leaves reduces D. citri's attraction to the odour of citrus leaves. Only 3.0 ± 0.32 adult attracted to the odour source of citrus leave + red guava upper shoots (figure 2.A). It was significantly lower ($P < 0.0001$) than those was attracted to citrus leaves (7.00 ± 0.32). The same respond of adult D. citri was obtained on comparison IV, with the odour source of non-seed guava upper shoots. Most of the adult move to citrus leaves odour source (7.27 ± 0.32), and only 2.73 ± 0.32 adult move to the odour of citrus leaves + non-seed guava upper shoots (figure 2.C). The odour of guava shoots was also able to make D. citri confused to find the source of the odour of the host plant. Some of the adult (3.07 ± 0.55) were not moved to either citrus leaves odour source or to the odour sources of citrus leaves + white guava upper shoots (figure 2.B). Based on the comparison of white, non-seed, and red guava upper shoot, it is suggested that odour of non-seed guava has highest repellent effect to adult of D. citri, followed by red guava, and white guava. It is suggested that the highest repellent properties is in non-seed guava shoot. D. citri adults use specific volatile compounds of citrus leaves and with a certain intensity as a cue to find the location of the host plant, and find plant parts that are not occupied by other competing insects [17]. Gravid female use volatile compounds for feeding and for oviposition, while for male adult D. citri, the odour was used for finding feeding site and for locating the female in mating activities [19](Martini, at al, 2014). Volatile compounds was the most important factor in determining host plant, even though leaves colour of citrus plant also has a role to attract adult D. citri [20].

In comparison V (figure 3.A.) it was seen that the red guava shoots were significantly more attractive (P < 0.0001) than the citrus shoots. The same results were also found in comparison VI (figure 3.B.) with a population of 5.60 ± 0.49 and 4.80 ± 0.55, respectively. A different phenomenon was found in comparison VII (figure 3.C.), where citrus leaves were more attractive than non-seed guava shoots. As a polyphagous predatory insect, M. sexmaculatus eats various types of prey, especially fleas. This insect beetle uses the odours (compounds of volatile) of insect host plant to find its prey. Beetles were more attracted to the odour of citrus leaves than guava shoots, indicating that the insect that attacks citrus plants (D. citri) was also the prey of the beetle M. sexmaculatus [21]. It was suspected that the content of secondary metabolic substances that were repellent was very high, so the effect was stronger than the effect of the content of secondary metabolic substances that attract insects. Besides that, there was also mixing of odours of leaves from the two kind of plant which resulted in most of the beetle population (7.60 ± 0.14) not moving (NM) either to citrus or guava leaves odour sources. This indicated that the concentration of secondary metabolic substances that were attractive or repulsive in the shoots of white, red and non-seed guava were different. This phenomenon also showed that the repellence was dose dependent [22] and the highest repellence property is in the upper shoot of non-seed guava.

When the odour of the citrus leaves were compared with the odour of the middle shoots of white guava (figure 4.A.), red guava (figure 4.B.), and non-seed guava (figure 4.C.), the results were significant (P < 0, 0001). Middle shoots of guava were more attractive for the beetle. The populations of M. sexmaculatus attracted to the middle shoots of white, red, and non-seed guavas were 5.60 ± 0.27, 3.60 ± 0.28, and 4.40 ± 0.36, respectively, compared to the population attracted to the citrus leaves of 0.80 ± 0.18, 1.20 ± 0.29, and 1.60 ± 0.27. Herbivorous insects use volatile compounds with specific types and intensities as a source of chemical markers to determine the location of their host plants [23], and to find plant parts that are still free of other insects that are a source of insect's competitors [24]. Predatory insects also behave in the same way by using the same volatile compounds to search for habitats and host plants for their insect prey. Receptor neurons in the antennae will capture volatile compounds produced by plants. Insect response depends on the type and intensity of the captured compound. This response will increase with the higher intensity of volatile compounds in the environment that have been caught [25].

The presence of specific volatile compounds from citrus leaves is a mainstay for D. citri and its predator, M. sexmaculatus, in finding their hosts. Two types of compounds, acetic and formic acids, play an important role in host selection [18]. D. citri gravid female in laying its eggs on the shoots of citrus plants prefers citrus plants with CVPD symptoms. It is strongly suspected that this is related to the increase in plant-specific volatiles in citrus plants that have been attacked by CVPD [26].

The phenomenon of more attraction of the adult of M. sexmaculatus on the citrus leaves was also found on the older (bottom) leaves of non-seed guava (figure 5.C.). It is suspected that the odours of citrus leaves has a higher concentration than the odours produced by the older leaves of non-seed guava. This assumption was reinforced by the lower population of immobile beetles (NM). It was suspected that the dose or the volatility of attractant constituent reduced with the increase of the age of leaf shoots [27]. Guava leaves and fruit produce many kinds of volatile compounds, such as several sesquiterpenes [28], [29], alcohols and aldehydes [30]. The repellence of these volatiles was highly dependent on their concentration in the air [17]. The dimethyl disulphide compound produced when guava leaves were injured also has a repellent effect on D. citri [31].

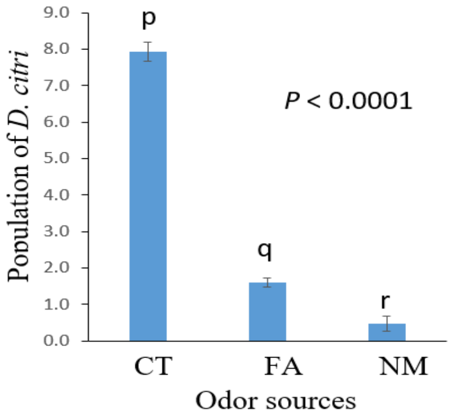

Figure 1. The mean (±SE) imago of D. citri that did not move (NM) or that moved to the odor of citrus leaves (CT) vs. clean air (FA) in Y-tube olfactometer

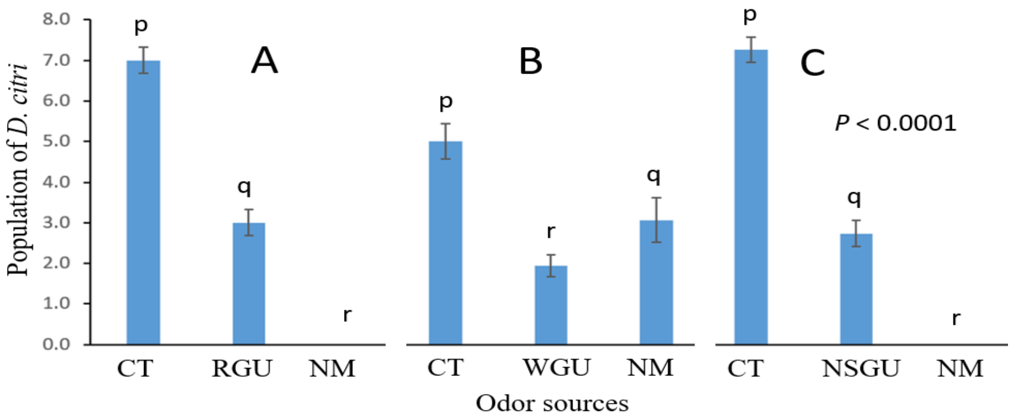

Figure 2. Mean (±SE) adult of D. citri that did not move (NM) or that moved to the odor of citrus leaves (CT) vs. red guava upper shoot (RGU) (A); white guava upper shoot (WGU) (B); non-seed guava upper shoot (NSGU) (C) in Y-tube olfactometer

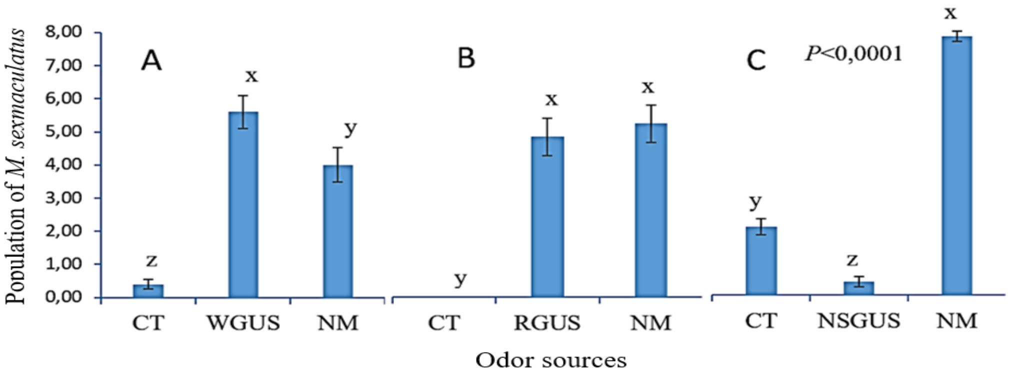

Figure 3. Mean (±SE) imago of M. sexmaculatus that did not move (NM) or that moved to the odor of citrus leaves (CT) vs. white guava upper shoots (WGUS) (A); red guava upper shoots (RGUS) (B); non-seed guava upper shoots (NSUS) (C) in Y-tube olfactometer

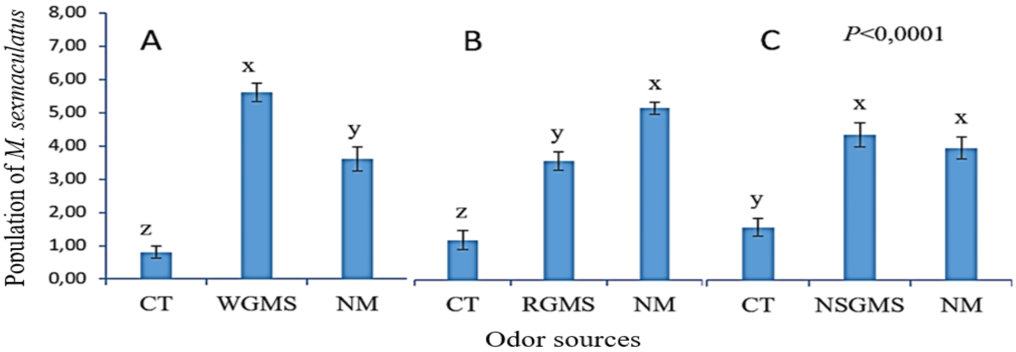

Figure 4. Mean (±SE) imago of M. sexmaculatus that did not move (NM) or that moved to the odor of citrus leaves (CT) vs. white guava middle shoots (WGMS) (A); red guava middle shoots (RGMS) (B); non-seed guava middle shoots (NSMS) (C) in Y-tube olfactometer

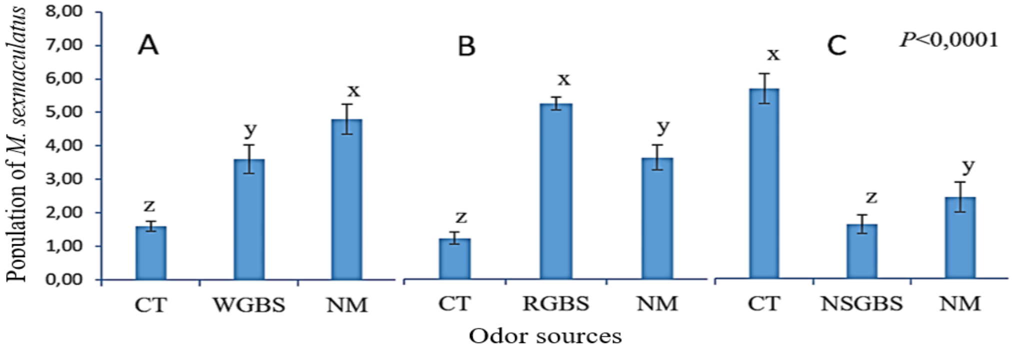

Figure 5. Mean (±SE) imago of M. sexmaculatus that did not move (NM) or that moved to the odor of citrus leaves (CT) vs. white guava bottom shoots (WGBS) (A); red guava bottom shoots (RGBS) (B); non-seed guava bottom shoots (NSBS) (C) in Y-tube olfactometer

4. Conclusion

Guava leaves were able to repel adult D. citri and attract the adult of M. sexmaculatus. The upper shoots of non-seed guava has the highest repellence effect to adult D. citri. The upper, middle, and bottom shoots of red and white guava, and middle shoots of non-seed guava were more attractive to M. sexmaculatus than the citrus leaves, except for the upper and bottom shoots of non-seed guava that less attracted to M. sexmaculatus compared to citrus leaves.

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