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The Repellency of Guava Shoots Extract to The Asian Citrus Psyllid (*Diaphorina citri)*

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Abstract.

CVPD or greening disease is the most devastating disease on citrus production in Indonesia and in the world. It is vectored by Asian citrus psyllid (Diaphorina citri). Guava leave extract is a prospective control means for reducing psyllid population. Research was conducted to investigate the repellent effect of guava shoots to psyllids. Repellent effect of grinded dried upper shoot (leaf number 1 and 2 from the top) of 50ºC and 80ºC of red, white, and non-seed guava shoots to ten adult psyllids of mixed gender were determined in Y-tube olfactometer. The result shows that guava shoots has repellence effect to psyllids adult. The effect is reduced as the increase of drying temperature. Highest repellence effect is found from red guava shoots, followed by non-seed guava and white guava. It is suggested that the highest repellent properties is in red guava shoots and the ability will increase in line with the increasing of drying temperature. However the effect of extraction temperature on psyllids attractiveness should be proved further.

Keywords: *Diaphorina citri,* guava, CVPD, disease vector, drying temperature

1. Introduction

Mandarin (*Citrus reticulata*) are widely grown in Indonesia. They are located mostly in North Sumatra and East Java. Production growth in 2011-2015 is decline in locations of outside Java but showed an increase in Java. Production of mandarin in Indonesia mostly comes from North Sumatra and East Java. Citrus production in Indonesia in 2020 is estimated 3.25 million tons, with the average increase 4.93% per year. Citrus consumption by household in 2016 was 3.41 kg/cap/year or 882,689 tons for Indonesian population. The demand of citrus fruit for households are projected to increase over the next five years (2016-2020) with an average of 3.73% [1].

The most serious problem in citrus production is the widespread of Citrus vein phloem degeneration (CVPD) or Huanglongbing or Greening disease. Besides impacting on the high mortality rate, CVPD also shortens the productive life of the citrus plants as well as decreases the productivity and product quality which in turn will weaken the competitiveness and fulfill the product needs [2]. The impact of CVPD is marked by a decrease in Indonesian citrus production from 2,467,632 tons in 2008 to 1,611,768 tons in 2012, and increased imports of citrus fruits from 138,000 tons with a value of USD117 million in 2008 to 256,000 tons with a value of USD247 million in 2012 [1].

The pathogen causes of CVPD are Gram-negative bacteria *'Candidatus Liberibacter asiaticus'* and *'Candidatus Liberibacter africanus'* for Asian and African types and *'Candidatus Liberibacter americanus'* for American type [3], [4]. Only the imago psylids and the 4-5 instar nymph are capable of transmitting the disease [5], [6]. Insect vector is able to transmit disease throughout its life [7], [8]. The control of CVPD is implemented through the application of four major components [9], namely (1) the use of disease-free seeds, (2) elimination of infected plants in the field, (3) vector insect control, and (4) quarantine. Control of CVPD disease vector insects (*D. citri*) still focuses on the use of synthetic insecticides that require a high enough cost to keep infected plants in production [10]. Alternative substitute for synthetic insecticides is the use of mineral oil [11], but the availability is still low and it is expensive. The presence of guava plants in citrus plantations was able to reduce the population of psylids and the incidence of CVPD disease [12], [13], but the capability variation among the varieties of guava plants have not been investigated.

2. Methods

2.1 Psyllid cultures

Culture of psyllids were obtained from Citrus and Subtropical Plants Research Station and were maintained on the ornamental orange jasmine plants (*Murraya paniculata* L.) in nylon mesh cages (600 mm long, 600 mm wide, 1000 mm high) in a greenhouse at 26±4°C and 60-80% relative humidity.

2.2 Leaf extract

Citrus (*Citrus reticulate*) shoots with 2 (two) fully open leave, and guava leave from the upper shoots (leaf number 1-2 from the top) were collected and then dried in oven at 50^0C for 24 h and 80^0C for 48 h. The dried leave were ground to powder with an electric grinder and then sieved to avoid unwanted granules from the powder. The leave powder was stored in airtight containers.

2.3 Olfactometer responses

Y-tube olfactometer was constructed from a 10.0 mm diameter (internal), 300 mm long, transparent glass tube, connected by a 5.0 mm diameter (internal) silicone tube to a sucking machine that was used to suck air into the olfactometer at 141 mL min-1, as measured with a flow meter (Model N 112-02G, Cole-Parmer Instrument Company, Illinois, USA). Each arm of Y-tube was connected to one of the two odour sources with silicone tubing. Air entering each olfactometer was filtered through activated charcoal and humidified by passing it through distilled water before it was passed through a transparent plastic container (50 mm diameter × 40 mm high) housing the odour source (treatment). Each odour source was 25 mg dried leave extract. Responses of adult psyllids of mixed gender were determined for the paired treatment comparisons listed in Table 1. Adult psyllids were collected in specimen tubes (31.5 mm internal diameter and 50 mm long). The specimen tubes were open at one end and covered by fine mesh at the other. The open end was immediately sealed with rubber plug after the psyllids were collected. The psyllids were then starved for 60 min before they were released into the distal end of the olfactometer used for the first of two tests (Table 1).

Table 1. Y-tube olfactometer comparisons: for each comparison, responses of 10 adult D. citri per replicate (n = 15) to odour from sources of leave extracts (1:1 w/w) dried at 50ºC for 24 h (50) and dried at 80ºC for 48 h (80) listed in the left hand and right hand column were recorded over 30 min intervals

Comparison	Odour sources		
I	Citrus + Red guava upper shoot (50)	Vs	Citrus + Non seed guava upper shoot (50)
II	Citrus + Red guava upper shoot (50)	Vs	Citrus + White guava upper shoot (50)
III	Citrus + Non seed guava upper shoot (50)	Vs	Citrus + White guava upper shoot (50)
IV	Citrus + Red guava upper shoot (50)	Vs	Citrus + Red guava upper shoot (80)
V	Citrus + Non seed guava upper shoot (50)	Vs	Citrus + Non seed guava upper shoot (80)
VI	Citrus + White guava upper shoot (50)	Vs	Citrus + White guava upper shoot (80)

The adults were left within this olfactometer for 30 min, then removed, kept within a covered specimen tube for 30 min and then released into a second olfactometer for 30 min for the second test. Ten adult psyllids were used for each replicate (n = 15 for each paired treatment). Responses to the volatile aroma sources were recorded as the proportion of adults

3. Result And Discussion

The results of the olfactometer experiments are shown in Fig. 1 up to 3. Variant response of adult psylids to the mixture of plant volatiles odour sources of citrus leave and guava leave extract (1:1 w/w) is observed in every set of Y-tube olfactory tests. It is also obviously seen that some adult psylids do not move to both of odour sources. Those phenomenon prove repellence effect of guava leave prevent adult psylids to choose citrus leave extract volatile as cue to their host plant (citrus). Barman and Zeng [14], [15] also found the same repellence effect of guava leave extract when sprayed on citrus leave. The presence of guava shoot odour are able to reduce adult psyllids population in citrus leave [16], [17]. Significantly less adult psylids move to the citrus dried leave with the addition of upper shoots of red guava dried leave than non-seed upper shoots guava dried leave which was dried at 50ºC for 24 h (P< 0.0001). The number of psylids move to citrus leave extract with the addition of upper shoots leave extract of red guava and non-seed guava is 2.53±0.22 and 3.80±0.31 respectively. The number of psyllids move to non-seed guava upper shoots are same with the number of the not moved psyllids.

Mixture of red guava dried upper shoot and citrus dried leave is also less attractive for psylids than mixture of white guava dried upper shoot and citrus dried leave (P< 0.0001). The number of psylids move is 2.60±0.13 and 5.47±0.22 for red guava and white guava odour sources respectively.

Psylids move in paired comparison test to the mixture of non-seed guava dried upper shoot and citrus dried leave is lower than mixture of white guava dried upper shoot and citrus dried leave. Repellent effect of non-seed guava dried upper shoot is significantly higher than white guava dried upper shoot (P< 0.0001). The number of psylids move is 2.63±0.32 and 4.80±0.23 for non-seed guava and white guava upper shoot odour sources respectively.

Based on the comparison responses of psyllids to the guava dried shoots (Fig 1, 2, and 3) drying at 50ºC for 24 h and 80ºC for 48 h, it can be stated that upper shoot (leave number 1 and 2 from the top) dried at 50ºC for 24 h has higher repellence effect (P< 0.0001) to psyllids adult than upper shoot dried at 80ºC for 48 h. The number of psyllids move to upper shoot dried at 50ºC for 24 h and at 80ºC for 48 h is 2.53±0.22 and 1.33±0.16, 3.80±0.31 and 2.07±0.18, and 5.47± 0.22 and 2.80± 0.11, for red, non-seed, and white guava respectively. It also suggest that the repellency is dose dependent [14] and higher repellence properties is in upper shoots of guava. It seem that the temperature and duration for drying of guava leave has a significant effect to the attractiveness of psyllids. However it should be proved further that the higher repellence effect is not caused by the lack of specific compound volatile from citrus leave as a cue for determining the host.

Based on the comparison of red, non-seed, and white guava dried upper shoot, it is suggested that aroma of red guava has highest repellent effect to psyllids adult, followed by non-seed guava, and white guava. Psyllid uses volatile compounds of specific species and intensities as a cue to determine the location of their host plants, and finds parts of plants that are still free of other competing insects [13], [18].

Fig 1. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + red guava upper shoots (CT + RGUS) and of citrus + non-seed guava upper shoots (CT + NSGMS), dried at 50ºC for 24 h (50) and at 80ºC for 48 h (80).

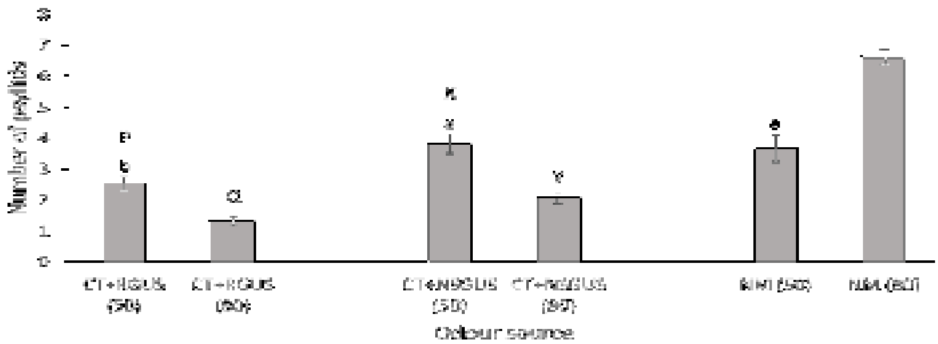


Fig 2. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + red guava upper shoots (CT + RGUS) and of citrus + white guava upper shoots (CT + WGMS), dried at 50ºC for 24 h (50) and at 80ºC for 48 h (80).

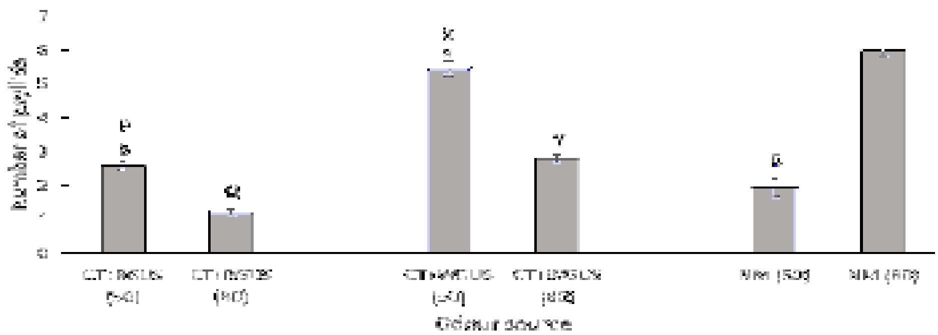
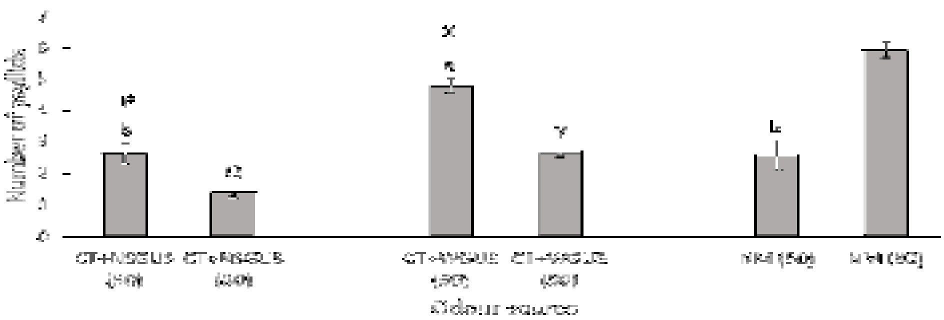


Fig 3. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + non-seed guava upper shoots (CT + NSGUS) and of citrus + white guava upper shoots (CT + WGMS), dried at 50ºC for 24 h (50) and at 80ºC for 48 h (80).



4. Conclusion

Guava leave has repellent effect to psyllids adult. Highest repellent effect is found from red guava leave, followed by non-seed guava and white guava. The repellent effect is also depended on the drying methods.

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