The toxicity of the extract of tobacco leaf *Nicotiana tabacum* L, marigold leaf *Tithonia diversifolia* (HAMSLEY) and citrus japansche citroen peel *Citrus limonia* against citrus psyllid (*Diaphorina citri* Kuwayama), the vector of citrus HLB disease

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Abstract. Potential insecticides generated from several plants or some plant wastes are used to control citrus psyllid *Diaphorina citri*. The aim of the study was to determine the toxicity of botanical insecticides from the extract of tobacco leaf, marigold leaves and Japansche citroen (JC) citrus peel against *D. citri*. The three plants, which were extracted with five kinds of solvents, namely acetone, distilled water, dichlormethane, hexane and methanol by maceration method. The test used leaf dipping method at 10% concentration with control and imidacloprid chemical insecticide as a comparison. The results showed that tobacco extract with all solvents was effective in controlling *D. citri*, starting at 1 day after treatment (DAT) with 44-92% mortality. The effective marigold leaf extract was those with the solvent of distilled water, dichlormethane and acetone. The mortality at 8 DAT was 100% and 70% and 74% respectively. Meanwhile, JC citrus peel extract with acetone, methanol and dichlormethane solvents were effective to control *D. citri* with the mortality at 8 DAT, the mortality reached 98%; 88% and 70% respectively. Thus, botanical insecticides of tobacco, marigold and JC orange peel with certain solvents can be used as controller of *D. citri*.

Keywords: Toxicity, Extract, *Nicotiana tabacum* L. *Tithonia diversifolia* (Hamsley) *Citrus limonia*, *Diaphorina citri* Kuwayama

1. Introduction

Citrus plants in Indonesia are very promising fruit commodities to be cultivated. However in the implementation of citrus agribusiness, several problems have been encountered, one of which is Huang Long Bing (HLB) disease, that has spread throughout the world [1, 2]. The disease is a major problem that can threaten the sustainability of citrus agribusiness. HLB disease is caused by the bacterium *Liberibacter asiaticus*, which can be detected by Polymerase Chain Reaction (PCR) using 16S rDNA primers (1). One of the causes of the spread of the disease was due to the presence of vector pests, namely the citrus psyllid *Diaphorina citri* [3]. The symptoms of HLB disease are irregular leaf spots (blotching), yellowing of primary and secondary leaf bones resembling a lack of certain nutrients. Plants that infected sometimes have sectoral symptoms, produce fewer fruits with small and asymmetrical sizes, and have bitter taste [5, 6].

*Diaphorina citri* significant as a pest, well as a vector to spread out of HLB disease. This psyllid transmits diseases caused by *L. asiaticus* through the puncture of the stylet. Because of its important
role as a vector, control must be carried out immediately if *D. citri* is found in endemic areas to HLB [7]. *D. citri* was found and attacks citrus plants at flushing period, so that the plant growth pattern needs to be considered in conducting the control because it can be used as an indicator of *D. citri*'s population [6, 7].

Control of citrus pests, especially *D. citri*, generally uses chemical insecticides that are known to have a negative impact on consumers and the environment. To reduce this, the alternative controls that are more environmentally friendly are needed, including using botanical insecticides. Indonesia's natural wealth which has a fairly abundant biodiversity can be explored, one of which was for botanical pesticides. Botanical pesticides can be made from plant parts either directly or by processes such as by extraction or by distillation in accordance with the desired formulation [8]. The use of extract material from plants for pest control is expected to be a substitute for the use of chemical pesticides. Such control can be cheaper because the materials used are easily available around the house or planting area.

There are so many types of plants that can be used as botanical pesticides, around 2400 species are included in 235 families [9]. These types of plants include neem, fragrant lemongrass, tuba root, clove, soursop, gadung, bengkoang, tobacco, lerak, *Jatropha curcas*, marigold, piretrum, citrus peel, and so on [8, 10-12]). Each type of plant has a specific type of bioactive compound that have function as a pest control i.e. insecticides, repellents or insect attractant. In this test the selection of tobacco plants, and citrus peels is based on the tests that have been previously carried out and have a role as insecticides [12-14].

Botanical insecticides from tobacco leaf materials have long been used by farmers as a pest and disease control material. As a botanical insecticide, it was proven effective against *Pophilla japonica*, *Tribolium castaneuna* (12) *Spodoptera litura*, *Nilaparvata lugens*, Helopeltis sp. and others [16-19]. Tobacco contains the main compounds of nicotine, which was toxic to the nerves of insects in which the reactions were very fast [20]. The marigold plant was a plant known as bush-shaped weeds which have quite a lot of distribution in the world. This plant is a fairly serious environmental problem in Africa [21, 22] thus the use of its leaves as a botanical insecticide would have a positive impact on weeds control. In addition, the flowers of the marigold plant also function as hosts and the source of nectar from beneficial insects around them. Marigold leaves containing compounds from terpenoids and fatty acids, were known to have insecticidal effects on several pests in the field and in the warehouse [11, 12, 23, 24]. Insecticides from citrus peel could suppress aphids and *D. citri* populations in citrus. Bioactive compounds in citrus peel detected as D-Limonen, linoleic acid and palmitic acid which have a role in controlling insects as insecticides and insect repellents [12, 24, 25].

Biopesticides and essential oils were a new trend in pest management in the modern agriculture and organic farming. The role of essential oil as an insecticide as an insect control has not reached its maximum potential because of its volatile nature and low residual activity. Such properties are considered safe and environmentally friendly which were compatible with biological control programs and their toxicity to low mammals [26].

2. Materials and methods

This research was carried out in the laboratory of Entomology at the Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI).

2.1. Plant extract preparation

The extracts were prepared from three kinds plant materials i.e. the foliage of tobacco (*Nicotiana tabacum* L.), marigold leaf (*Tithonia diversifolia* (Hamsley) and *Citrus* Japansche citroen peel (*Citrus limonia*). Test used dried plant materials and were made extract by maceration method. Tobacco was taken from the tobacco center area in Pamekasan, Madura, East Java. The marigold leaf was obtained from the garden at around ICSFRI and JC citrus peel was obtained from the fruit extracted for seedlings, so the peel was a waste. Each plant material was extracted by using some solvents, that were acetone, distilled water, diclorometan, hexane and methanol. The extract was made by soaking the
dried plant materials in the form of powder into the solvents with a ratio of 1:2 (v/v) and soaked for 24 hours. The soaked solution was then filtered and then evaporated with a rotary evaporator at 40°C.

2.2. Mass rearing of target pest
The tested insect was citrus psyllid *D. citri*. Tests carried out at the imago phase. Mass rearing was done to provide the tested insects in the amount suited to the need for treatment at the same age. The host plants used for mass rearing was jasmine citrus *Murraya paniculata*. Propagation was done by infesting the female of imago into jasmine citrus buds whose leaves had not yet opened, as a place for laying eggs. Imago infestation was performed onto the shoots that were given cages. After the eggs were laid, a week later, they hatched into nymphs and became imago for the next two weeks.

2.3. Plant extract toxicity test
The effectiveness testing of botanical insecticides from the three types of plants was carried out on citrus shoots that had been prepared previously. For each type of botanical insecticide, the test was carried out at a concentration of 10%, accompanied by control and imidacloprid insecticide as a comparison. The treatment was repeated 5 times and each replication consisted of 20 *D. citri* which were infested in each citrus shoot and given a cage. Data of *D. citri* mortality were assessed everyday until 8 Days After Treatment (DAT). Data were analyzed by Completely Randomized Design and the statistical analysis of these data was based on SAS analysis of variance (ANOVA) procedure. Means separation was done at p<0.05 by Duncan Multiple Range Test (DMRT).

3. Results and discussion
The test results showed that tobacco leaf extract, marigold leaf extract and JC citrus peel extract had the potential as insecticides. This was evidenced by the results obtained, as for tobacco extract, it was effective against the citrus psyllid *D. citri* in all solvents used, it give mortality faster than the other extracts. *D. citri* mortality at 1 DAT was 44-92% in the extract treatment, 58% on imidacloprid insecticide and 0% on control. The mortality continued to increase in the following day and started from 4 DAT of the extract treatment, the mortality was 94%-100%, it was significantly different from the control whose mortality was 0%, and was not significantly different from the treatment of comparative insecticide imidacloprid whose mortality was 94% (table 1).

| Treatment       | 1    | 2     | 3     | 4     | 5     | 6     | 7     | 8     |
|-----------------|------|-------|-------|-------|-------|-------|-------|-------|
| Control         | 0 d  | 0 d   | 4 d   | 4 b   | 4 b   | 4 c   | 4 c   | 4 b   |
| Aceton          | 92 a | 98 a  | 100 a | 100 a | 100 a | 100 b | 100 a | 100 a |
| Aquadest        | 88 a | 88 a  | 96 a  | 98 a  | 98 a  | 100 a | 100 a | 100 a |
| Dichlormethane  | 60 b | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |
| Hexsana         | 90 a | 86 a  | 88 a  | 94 a  | 98 a  | 98 a  | 98 ab | 98 a  |
| Methanol        | 44 c | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |
| Imidacloprid    | 58 bc| 66 b  | 78 c  | 94    | 94 a  | 98 a  | 92 b  | 100 a |

Number following by the same letter in each column was not significantly different at 5% DMRT.

Tobacco leaf extract contains nicotine, anabasin (alkaloids), n-Hexadecanoic acid, Oleic acid (Fatty acid) and 4,8,13-duvatrine-1,3-diol, Phytol, B Linalool (terpenoid) and other compounds known to have insecticidal [14, 25, 27-29]. Tobacco bioinsecticides have been shown to be effective against
several pests and act as synaptic poisons in insect nerves that mimic the neurotransmitter acetylcholin, which causes symptoms of poisoning similar to organophosphate and carbamate insecticides [30]. Nicotine is a nerve poison that works very quickly to insects and mammals. Nicotine Mode of action was binding to the acetylcholine receptor at nerve synapses. This disorder causes the failure of the body's system quickly due to the nervous system malfunction [31].

In the marigold leaf extract, not all the solvents used showed effective results for controlling D. citri. The treatments which showed the insecticidal effect were those extracted with distilled water and dichlormethane solvent. This was shown by the mortality at 4 DAT for extracts with distilled water and dichlormethane solvent, the mortality was 70% and 42%, respectively. The mortality increased with time, which were 84% and 56% in 6 DAT and continued to increase until the last observations at 8 DAT, where the mortality was 100% and 70% respectively (table 2). The marigold leaf T. diversifolia extract was identified to contain terpenoids and fatty acids which can function as insecticides. These compounds include Pinane, Pitole and phenols from terpenoid group [14, 27, 32] and stearic acid, oleic acid and hexadecanoic acid from fatty acid group [23, 25, 33]. In several experiments the ability of T. diversifolia extract had promising prospects as an insecticide because it had been shown to be potential as an insecticide by reducing pest populations and suppressing production losses, comparable to synthetic pyrethroid insecticides [10, 24].

| Treatment       | D. citri mortality in different day after treatment of DAT |
|-----------------|----------------------------------------------------------|
| Control         | 0 c 0 c 2 c 4 c 4 c 4 d 4 c 6 c                      |
| Acetone         | 4 bc 8 bc 14 c 20 bc 26 bc 36 cd 48 b 74 ab           |
| Aquadest        | 20 b 28 b 52 b 70 a 80 a 84 ab 88 a 100 a             |
| Dichlormethane  | 2 c 8 bc 22 c 42 b 48 b 56 bc 60 ab 70 ab             |
| Hexsana         | 0 c 4 bc 6,4 c 12 c 20 c 35 cd 44 bc 48 bc            |
| Methanol        | 4 bc 8 bc 10 c 22 bc 22 bc 28 cd 32 bc 46 b           |
| Imidacloprid    | 58 a 66 a 78 a 94 a 94 a 98 a 92 a 100 a              |

Number following by the same letter in each column was not significantly different at 5% DMRT.

JC citrus peel extract gave an insecticidal effect on the extract with acetone, dichlormetan and methanol. The mortalities with acetone, dichlormetan and methanol at 4 DAT were 66, 42, and 66% respectively (table 3). The mortality at 6 DAT became 82%; 60% and 86% were significantly different from the controls, and were not significantly different from the comparative insecticide - imidacloprid. The mortality at 8 DAT reached 98%; 70% and 88%. JC citrus peel extract with hexane solvent gave the mortality of 80% at the end of the observation, but at the beginning of the observation it was relatively small, that was 16% and 30%, respectively at 4 and 6 DAT, thus it was not recommended. In the previous study, it was stated that the use of bioactive compounds from citrus leaf extracts C. sinensis, T. cacao, T. diversifolia and A. accidentale in the laboratory and in the field was able to control termites [34].

From the three extracts used to control D. citri in this research, it was seen that tobacco leaf extract was the most effective compared to the others. This was in accordance with what was stated by [35] that tobacco with nicotine bioactive compounds was the most toxic compared to other types of plants and had a low LD50 of between 50-60 ppm. Nicotine was a nerve poison that worked quickly and in contact [32]. Botanical insecticides of tobacco leaf, marigold leaf and JC citrus peel with certain
solvents can be used as controller of citrus psyllid \textit{D. citri}. Further testing needs to be carried out in terms of the effective concentrations for each kind of the extracts.

\begin{table}
\centering
\caption{Toxicity of botanical insecticides from JC citrus peel extract to mortality of \textit{D. citri}.} \\
\begin{tabular}{lcccccccc}
\hline
Treatment & \multicolumn{8}{c}{\textit{D. citri} mortality in different day after treatment of DAT} \\
& 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 \\
\hline
Control & 0 c & 0 b & 0 d & 2 c & 2 c & 2 d & 6 d & 12 b \\
Aceton & 4 bc & 14 b & 54 b & 66 b & 80 a & 82 ab & 90 a & 98 a \\
Aquadest & 0 c & 0 b & 0 d & 4 c & 10 c & 16 cd & 22 cd & 22 b \\
Dichlormethane & 0 c & 16 b & 18 cd & 42 b & 52 b & 60 b & 62 ab & 70 a \\
Hexsana & 12 b & 12 b & 14 cd & 16 c & 20 c & 30 c & 50 bc & 80 a \\
Methanol & 4 bc & 16 b & 32 c & 66 b & 80 a & 86 a & 88 a & 88 a \\
Imidaclorpid & 58 a & 66 a & 78 a & 94 a & 94 a & 98 a & 92 a & 100 a \\
\hline
\end{tabular}
\end{table}

Number following by the same letter in each column was not significantly different at 5\% DMRT.

\section*{Conclusion}
Botanical insecticides of Foliage tobacco, marigold leaves and JC citrus peel with certain solvents can be used as controller of citrus psyllid \textit{D. citri}. Foliage extract of tobacco with all solvents was effective in controlling \textit{D. citri}. The effective marigold leaf extract was the extract with the solvent of distilled water, dichlormethane and acetone. JC citrus peel extract with acetone, methanol and dichlormethane solvents were effective to control \textit{D. citri}. Further research is needed to get the right concentration to control the citrus psyllid \textit{D. citri}.

\section*{References}
[1] Halbert S E and Manjunath K L 2004 \textit{Fla. Entomol.} 87 330
[2] Manjunath K L, Halbert S E, Ramadugu C, Webb S and Lee R F 2008 \textit{Phytopathology} 98(4) 387
[3] Alves G R, Diniz A J and Parra J R 2014 \textit{Journal of Economic Entomology} 107(2) 691
[4] Bove J M, Jogoueiix and Garnier M 1996 \textit{Molecular and Cellular Probes} 10 43
[5] Dwiastrutti M E, Triwiratno A, Endarto O, Wuryantini S and Yunimar 2011 \textit{Introduction and control of pests and diseases of citrus}. Indonesian Citrus and Subtropical Fruits Research Institute. Agricultural Research and Development Agency (In Indonesian Language)
[6] Bové J M and Huanglongbing 2006 \textit{J. Plant. Pathol.} 88 7
[7] Anonymous 2012 \textit{Botanical pesticides}. Center for Plantation Research and Development. Third printing. (In Indonesian Language)
[8] Wijaya N I, Adiartayasa N, Sritamin M and Yuliadhi K A 2010 \textit{J. Entomol. Indon.} 7 78 (In Indonesian Language)
[9] Kardinan A 1999 \textit{Natural Insecticide Sources}. In the Collection of Training Materials for Development and Utilization of Natural Insecticides. Center for Integrated Pest Management Studies. IPB. Bogor. (In Indonesian Language)
[10] Mkenda P A, Stevenson P C,Ndakidemi P, Farman D and Belmain S R 2015a \textit{Int. J. Trop. Insect Sci.} 35 172
[11] Pangihutan S J C, Rochman N and Mulyaningsih Y 2016 \textit{Agronid Journal} 2 1 (In Indonesian Language)
[12] Wuryantini S, Harwanto and Rizki A Y 2017 Response of citrus aphids Aphis gossypii to several botanical insecticides. \textit{Proceedings of the 2017 National Seminar and Perhorti Congress}. ISBN:970-603-720-094-8. (In Indonesian Language)
[13] Asmaliyah, Erna E W, Kusdi S U, Mulyadi, Fitri W and Sari W 2010 \textit{Introduction of plants producing vegetable pesticides and their traditional uses}. Center for Research and Development of Forest Productivity. Forestry Research and Development Agency, Ministry
of Forestry. ISBN : 978-602-98588-0-8. (In Indonesian Language)

[14] Harwanto, Wuryantini S, Endarto O and Yunimar 2015 Bioprocessing technology for botanical insecticides sourced from secondary metabolites produced by plants. Research report on the Indonesian Citrus and Subtropical Fruits Research Institute. (In Indonesian Language)

[15] Simarmata 1994 Guidelines for the introduction of botanical pesticides. (Jakarta: Agriculture department) (In Indonesian Language)

[16] Wiryadiputra S 2006 J. Pelita Perk. 22(1) 25 (In Indonesian Language)

[17] Sujak and Diana N E 2012 J. Agrovigor 5(1) 47

[18] Meikawati W, Salawati T and Nurullita U 2013 Utilization of tobacco plant extracts (Nicotiniana tobacum L) as an insecticide for controlling armyworm pests on chili plants. National Seminar Proceeding 2013. Towards a Sustainable and Civil Society (Semarang: Muhammadiyah University of Semarang) pp 455-460

[19] Tuti H K, Retno W and Supriyono 2014 J. Ilmu Pert. 29(1) 1724

[20] Affifah F, Rahayu Y S and Faizah U 2015 J. Lentera Bio. 4(1) 25

[21] Henderson L 2007 Bothalia 37 215

[22] Yang J, Tang L, Guan Y-L and Sun W-B 2012. Weed Sci. 60 552

[23] Ghosh G K 2000 Biopesticide & integrated pest management. A.P.H. Publishing. New Delhi

[24] Green P W C, Steven R B, Patrick A N, Iain W F and Philip C S 2017 Journal of Industrial Crops and Products 110 15

[25] Green A 2015 Hexadecanoic Acid. PPDB: Pesticide Properties DataBase University of Hertfordshire. Acces on http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/1336.htm

[26] Mossa A T H 2016 Journal of Environmental Science and Technology. ISSN 1994-7887 DOI: 10.3923/jest.2016.354.378.

[27] Pasdaran A, Nahar L, Asnaashari S, Sarker S D and Delazar A 2013 Pharmaceutical Sciences 19(1) 1

[28] Zhou J, Xie G and Yan X 2011 Encyclopedia of traditional Chinese medicines - molecular structures, pharmacological activities, natural sources and applications: Vol. 2: Isolated compounds D-G (Heidelberg: Springer Science & Business Media)

[29] Zammit M, Shoemake C, Attard E and Azzopardi L M 2014 International Journal of Biology 1 6 46

[30] Regnault-Roger C and Philogène B J R 2008 Pharmac. Biol. 46 41

[31] Nabil E El-Wakeil 2013 Gesunde Pflanzen 65 125

[32] Mustapha D, Stephen P F, Daniel S 2006 Insect Repellents: Principles, Methods, and Uses (Florida: CRC Press.)

[33] Paranjape K, Gowariker V, Krishnamurthy V N and Gowariker S 2015 The Pesticide Encyclopedia (Wallingford: The Centre for Agriculture and Bioscience International (CABI))

[34] Osipitan A A and Oseyemi A E 2012 Journal of Entomology 9(5) 257

[35] Cloyd R A 2004 Pesticide Review 17 3