Utilization of plant waste as botanical pesticide for citrus pest control

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Abstract. Organic wastes from plants that contain certain secondary metabolite compounds can be used as botanical pesticides. In this study, the plant wastes used were chrysanthemum flower, JC citrus peel and foliage of tobacco. The botanical pesticides were tested for their effectiveness against the main pests of citrus that were citrus psyllid \textit{Diaphorina citri} and citrus aphids \textit{Toxoptera citricidus}. The study aimed to determine the effectiveness of botanical pesticides from plant wastes against both target pests. The three materials were extracted and processed by maceration method with methanol solvent. The test was arranged in a Randomized Block Design consisting of 12 treatments, that were the test concentration for each ingredient was 0.5 \%; 0.75 \% and 1 \%, comparative chemical insecticides dimethoate and imidacloprid, and control. The treatment consisted of two units for each target pest and repeated 4 times. The test results showed that the botanical insecticide from tobacco foliage was the most effective against both target pests compared to those from chrysanthemums flower and JC citrus peel. The insecticide tested was more effective against citrus aphids \textit{T. citricidus} compared to citrus psyllid \textit{D. citri}, as evidenced by the shorter time to achieve the mortality. For tobacco insecticides, further testing with lower concentrations is needed to determine the exact and effective test concentration, so that the use of materials is more efficient.

Keywords : plant waste, botanical pesticide, effectivity, citrus pest

1. Introduction

Plant waste is a byproduct of plants which the functions are not heeded. In the current era with the issue of the impact of global warming which is getting more serious, many efforts must be developed to solve the problem. One of the efforts made is to minimize the existence of trash or waste. Besides plastic waste, the utilization of organic waste from plants that have the potential to be used as other products is also being sought. Referring to previous studies [1], in this study, some plant materials which are waste and known to contain certain secondary metabolites will be tested for their effectiveness as botanical pesticides. Lots of potential plants as botanical pesticides include mimba (\textit{Azadirachta indica} Juss), marigold (\textit{Tithonia diversifolia}), soursop (\textit{Annona moricata}) chrysanthemum (\textit{Chrysanthemum cinerariaefolium}) tobacco (\textit{Nicotiana tobaccum}), lemongrass (\textit{Cymbopogon nardus}) and others [2; 3].

The main pests of citrus are citrus psyllid \textit{Diaphorina citri} and citrus aphids \textit{Toxoptera citricidus}. Both of these pests are not not only as pests, but also as the disease vectors of Huanglongbing disease (HLB) caused by the bacterium \textit{Liberibacter asiaticus} and the virus disease Citrus Tristeza Virus (CTV). Citrus psyllids in the nymph stage have close relation and develop in new shoots of citrus plants and their relatives of the Rutaceae family [4; 5; 6]. Psyllid attacks on buds cause stunted bud development, buds contract and wither, severe attacks can result in dry shoots, fall out and death [6; 7; 8; 9; 10]. The nymph of \textit{D. citri} secretion is white and spiral-shaped, which
contains sticky honey dew on the leaf surface. The presence of honey dew can cause secondary effects, that is the growth of sooty molds that cover the surface of the leaves, causing interference in the process of photosynthesis [10]. The adults can survive for a long time on old leaves [11; 9]. The adult role is very important in the transmission of HLB disease vectors from infected plants [2], HLB is known to be a very serious citrus disease and causes a decrease in citrus production in the world including in Indonesia [8; 13; 14 15]. Therefore, careful control needs to be performed, especially in endemic locations.

*T. citricidus* aphids attack plants by sucking the stylet and take the plant sap in phloem, causing direct and phytotoxic damage to the plants. Attacks are usually found in buds that are still developing and in flower buds. Buds that are attacked will become curly, curved. The indirect effect of aphid attack is that the honey dew produced and attached to the plants, especially leaves, causes the growth of sooty molds which can inhibit photosynthetic activity [16; 17]. Besides playing role as pests, psyllid also plays as a vector of diseases caused by viruses, namely the Citrus Trizteza Virus (CTV) [18].

Pest control using environmentally friendly materials continues to be developed in the current era. This is intended to reduce the negative impact of pesticide residues in the environment, and ensure consumers to obtain products that are safe for consumption. In this research, plant waste materials containing certain secondary metabolites were tested as botanical insecticides for controlling *D. citri* and *T. citricidus*. The ingredients used were chrysanthemums containing pyrethrin, JC citrus peels containing terpenoids and others, and tobacco shoots containing nicotine. Chrysanthemum flowers were waste from flower arrangement or bouquet and sorted flowers that were not harvested in the garden; JC citrus peel was a waste from the extraction of the fruit taken from the seeds which are going to be planted as rootstock in the production of citrus seeds; and tobacco shoots were parts of plants that were not harvested and usually left on the ground or thrown away. Several studies have even utilized cigarette butts as ingredients of botanical insecticide [19; 20].

Certain secondary metabolites are known to have toxic properties and function as components of plant protection against microorganisms and herbivores. Nicotine and terpenoids contain in tobacco extract are part of the biosynthesis of several alkaloids from plant secondary metabolites that function as chemical defence systems against pest attacks [21; 22]. Previous exploration and testing have been carried out, but the effectiveness of the concentrations used still needs to be ascertained to be recommendations for their use [1; 23].

Chrysanthemums containing pyrethrin have been used as botanical insecticides. Chrysanthemum extracts and lavender flowers tested against white lice *Bemisia tabaci* Genn have the power of repellence and are categorized as plant extracts with very high repellence properties [24]. Wuryantiniet al. [23] indicated that the contents of secondary metabolites in JC citrus peel extract with methanol solvents are monoterpenoids, coumarin, fatty acid flavonoids and phenolic compounds that function as anti-fungi, microbial queues, phytotoxics and insecticides; antifeedant; insecticides and acaricides' anti-microbial, anti-inflammatory; anti-microbial, analgesic, anti-oxidant, anti-inflammatory. The use of tobacco as a control of plant pest and disease is also widely reported. Tobacco plants have long been used as insecticides, and are known to be effective against *Spodoptera litura, Nilaparvata lugens, Helopeltis* sp. and others [25; 26] Tobacco extracts from cigarette cutters identified as containing alkaloids and terpenoids are effective for controlling pests in tomato plants [20]. Secondary metabolite content in tobacco also has bioactivity to insects as food inhibitors or antifeedants and inhibitors of spawning or oviposition deterrents in mosquito *Aedes aegypti* [27].

In this research, the highlighted plant material is waste that can be used for botanical pesticides. Tobacco and chrysanthemum have been widely used to control various plant pests and disease, but testing for citrus pests was still limited. JC citrus peel that has been known contain terpenoids, coumarin, fatty acids, flavonoids and phenolic that acts as an antifungal, antimicrobial, insecticides and acaricides, antifeedan, anti-inflammatory and antioxidant [23] has not been much used for pest and disease control agents.
The purpose of this study was to determine the control effectiveness of botanical insecticide of the three ingredients tested against the main pests, i.e., citrus psyllid *Diaphorina citri* and citrus aphid *Toxoptera citricidus*.

2. Materials and Methods
The study was conducted at the entomology laboratory and screen house of Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI) from January to March 2020. Plant waste materials used were obtained from the collection of plant material waste around the study site.

2.1. Preparation of target pests
Mass rearing of target pest to be tested was conducted at the screen house. Citrus aphids were obtained by taking pests from the citrus orchards in the field and then brought them to the screen house. Rearing was carried out on the buds of citrus plants by investing aphids obtained from the field, so that they multiplied in the shoots that have been provided. Aphids used for testing were 2-3rd nymph stages. The psyllid tested was the result of rearing that used the collection that was already existed in the screen house. Mass rearing of psyllids used the jasmine orange *Murraya paniculata* as their host. The female of imago invested on buds of host plants that were given a plastic cage to laid eggs on shoots. The eggs that were laid hatched about a week later. Within 18-21 days after the eggs laid out, *D. citri* became an imago, and the imago with an age of about 1 week that was used for testing. In mass rearing of target pests, time management must be done carefully so that the conditions of the tested pests were relatively uniform at the same time of treatment.

2.2. Preparation of plant extract
Extracted plant waste was in the form of dry powder from a process that has been done before. Each material, comprised of chrysanthemum powder, JC citrus peel powder and tobacco powder were prepared to be processed into extracts by maceration method using a methanol solvent in the ratio of 1: 2 v/v, soaked for 24 h then were filtered and evaporated until they became a thick extract and ready to be used for testing. Tests using a concentration of 0.5 %. 0.75 % and 1 % for each extract, so dilution was necessary.

2.3. Preparation Toxicity Test
The tests used citrus plants as the host of two target pests. Citrus plants that were used were atsprouting phase. Each plant consists of at least four shoots as a treatment arena and as a repetition. The test consists of 12 treatments, i.e., the concentration of each test material: 0.5 %, 0.75 % and 1 %; comparative insecticide treatment of dimethoate 2 mL / L (0.2 %) and imidacloprid and 5 mL / L (0.05 %) according to recommended concentration and control (without treatment). Each treatment consisted of 20 test pests that were invested on shoots and given a plastic cage so that pests did not come out of the testing arena. Plant extract according to the test concentration was sprayed on the shoots of citrus plants that had been invested with the target pest. The first observation was made 24 h after treatment and then observed every 24 h until the last observation at 168 h after treatment (HAT). Observation data were tabulated, the mortality was calculated for each treatment. Then the 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). Furthermore, the mortality data can be used to determine the lethal dose (LC 50) calculated using the Excel program.
3. Results and Discussion

3.1. Effectiveness against citrus psyllid *Diaphorina citri*

The results of research at the screen house showed that the potential of tobacco foliage extract, chrysanthemum flower extract and JC citrus peel extract as organic waste was able to kill *Diaphorina citri* psyllid on citrus plants. The mortality of target pests was observed at 24 h after application for up to 168 h. Even if compared to chemical insecticides with active ingredient dimethoate and imidacloprid, tobacco foliage extracts had higher results in killing target insects. Among the three organic wastes tested, the highest effectiveness in killing the target pests at 24 h after application was tobacco shoots at a concentration of 0.5% that reached 93% mortality, followed by chrysanthemum at a concentration of 1% with 45% mortality, and JC citrus peel at a concentration of 1% with 38% mortality. Tobacco extracts containing the active ingredient of nicotine in the alkaloid group proved better results from pyrethroids of chrysanthemums and limonoids from JC citrus peels. The consistency of the effectiveness of each organic waste tested was seen until observation day 7 (Table 1).

| Treatment          | % of mortality | 24 HAT | 48 HAT | 72 HAT | 96 HAT | 120 HAT | 144 HAT | 168 HAT |
|--------------------|----------------|--------|--------|--------|--------|---------|---------|---------|
| Control            |                | 0 a    | 0 a    | 0 a    | 1.25 a | 1.25 a  | 2.5 a   |         |
| Chrysantemum 0.5%  |                | 11.25 a| 12.5 ab| 13.75 ab| 18.75 b| 28.75 b | 40 b    | 47.5 b  |
| Chrysantemum 0.75% |                | 33.75 b| 51.25 cd| 53.75 cd| 63.75 d| 63.75 c | 70 cd   | 75 cd   |
| Chrysantemum 1%    |                | 45 b   | 58.75 d| 65 cd   | 77.5 d | 77.5 cd | 85 def  | 86.25 de|
| JC peel 0.5%       |                | 7.5 a  | 21.25 b| 25 b    | 38.75 c| 38.75 b | 46.25 b | 48.75 b |
| JC peel 0.75%      |                | 12.5 a | 37.5 c | 47.5 e  | 63.75 d| 63.75 c | 67.5 c  | 68.75 c |
| JC peel 1%         |                | 38.75 b| 53.75 cd| 60 cd   | 70 d   | 70 cd   | 75 cde  | 77.5 cd |
| Tobacco 0.5%       |                | 93.75 d| 95 e   | 96.25 e | 97.5 e | 100 e   | 100 f   | 100 e   |
| Tobacco 0.75%      |                | 100 d  | 100 e  | 100 e   | 100 e  | 100 e   | 100 f   | 100 e   |
| Tobacco 1%         |                | 100 d  | 100 e  | 100 e   | 100 e  | 100 e   | 100 f   | 100 e   |
| Dimethoate 0.2%    |                | 61.25 c| 67.5 d | 70 d    | 72.5 d | 82.5 d  | 88.75 ef| 88.75 de|
| Imidacloprid 0.05% |                | 90 d   | 97.5 e | 98.75 e | 98.75 e| 100 e   | 100 f   | 100 e   |

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

In general, it could be reported that higher concentration applied gave better results. Test results on both target pests proved that organic waste from tobacco, chrysanthemum and citrus peel could be used as botanical insecticides which were the alternative of integrated approach to organic farming systems. The growing development of organic farming in the community, by reducing or even suppressing the use of synthetic insecticides, provides an increasingly large market opportunity for botanical insecticides to become an alternative control. Therefore, the accurate formulation needs to be made for commercialization so that the application of plant-based insecticides in the field can be performed easily, effectively, and efficiently.
Pyrethrins contain six chemicals that function as pesticides found naturally in chrysanthemums. Generally, this pesticide is used to control mosquitoes, fleas, flies, moths and ants and other pests. The mode of action of pyrethrins is to disrupt the nervous system of insects that are affected or eat, resulting in paralysis and death. The use of pyrethrins as pesticides has been registered since 1950's [28]. In previous studies, JC orange peel extract was tested for its effectiveness against the citrus psyllid and citrus aphids *Aphis gossypii* at a concentration of 10 %, and the results showed the potential to be used as a biocontrol material [23]. However, more rational concentrations need to be tested to obtain recommendations for proper use. Alkaloid substances in tobacco plants were reported to have the potential as biopesticides to control armyworm pests on chilli [29] on tomato [20], mosquito attacks, *Aedes* sp. [27; 19] and *Helopeltis antonii* pests in cocoa [30].

### 3.2. Effectiveness against citrus aphids *Toxoptera citricidus*

Tests on aphids *T. citricidus* also proved the ability to kill the cocoa leaf by extracts of chrysanthemum leaves and JC citrus peel. The observation at 48 HAT showed quite high effectiveness that was above 90 % at a concentration of 0.5 % (Table 2). The higher concentration of tobacco shoot extract that was applied gave a higher mortality rate, indicating that its effectiveness was very good and can be used as a botanical insecticide to control citrus aphids. Sujak and Diana [31] reported that the active ingredient of nicotine was very effective in controlling *Aphis gossypii*.

| Treatment               | % of mortality |
|-------------------------|----------------|
|                         | 24 HAT | 48 HAT | 72 HAT | 96 HAT | 120 HAT | 144 HAT | 168 HAT |
| Control                 | 0 a    | 0 a    | 1 a    | 2.5 a  | 3.75 a  | 6.3 a   | 8.75 a  |
| Chrysanthemum 0.5 %    | 78.75 d| 93.75 d| 100 d  | 100 d  | 100 d   | 100.0 d | 100 d   |
| Chrysanthemum 0.75 %   | 95 e   | 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 100 d   |
| Chrysanthemum 1 %      | 97.5 e | 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 100 d   |
| JC peel 0.5 %          | 1.25 a | 35 b   | 44 b   | 48.75 b| 56.25 b | 63.8 b  | 66.25 b |
| JC peel 0.75 %         | 18.75 b| 32.5 b | 39 b   | 48.75 b| 58.75 b | 63.8 b  | 66.25 b |
| JC peel 1 %            | 37.5 c | 48.75 c| 64 c   | 71.25 c| 77.5 c  | 82.5 c  | 85 c    |
| Tobacco 0.5%           | 96.25 e| 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 100 d   |
| Tobacco 0.75 %         | 97.5 e | 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 100 d   |
| Tobacco 1 %            | 100 e  | 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 100 d   |
| Dimethoate 0.2 %       | 96.25 e| 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 0 a     |
| Imidacloprid 0.05 %    | 98.75 e| 100 d  | 100 d  | 100 d  | 100 d   | 100.0 d | 0 a     |

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

Biopesticide from tobacco, pyrethrum has been proven to be able to control several insect pests that attack cultivated plants. According to Ratnawati [32], the active ingredient of tobacco leaves enters the skin of target insects and causes death and reacts quickly paralyzing the nerves of insects [33]. Nicotine is an alkaloid found in tobacco plants. Nicotine is also found in other plants of the Solanaceae family such as tomatoes, potatoes, eggplant and green pepper at a very small level compared to tobacco. Alkaloid substances have also been known to have pharmacological properties.
Nicotine was first used as an insecticide in 1763, and its pure alkaloids were isolated in 1828 by Posset and Reimann, then synthesized in 1904 by Piclet and Rotsch. Nicotine alkaloids, nicotine sulfate and other nicotine compounds are used as contact poisons, fumigation, and stomach poisons. This insecticide is traded as Black Leaf 40R containing 40 % nicotine, to control soft-bodied insects. Nicotine is obtained from *Nicotiana tabacum* with levels of 2-5 % and *Nicotiana rustica* with levels of 5-14 %. Nicotine generally consists of 97 % alkaloids from tobacco [34]. The test results of chrysanthemum extract also proved the effectiveness to control psyllid and aphids on citrus. The higher concentration also proved the higher mortality from target insects. A research by Rinaldi et al. [24] reported that pyrethetamine from Chrysanthemum flower extract was effective in controlling the whitefly *Bemisia tabaci* Genn,

3.3. LC50 value

| Observed times | LC50 | Chrysanthemum | JC peel | Tobacco |
|----------------|------|---------------|---------|---------|
| 24 HAT         |      | 1.039 %       | 1.313 % | 0.238 % |
| 48 HAT         |      | 0.832 %       | 0.935 % | 0.219 % |
| 72 HAT         |      | 0.789 %       | 0.810 % | 0.195 % |
| 96 HAT         |      | 0.766 %       | 0.775 % | 0.127 % |
| 120 HAT        |      | 0.652 %       | 0.609 % | -       |
| 144 HAT        |      | 0.571 %       | 0.531 % | -       |
| 168 HAT        |      | 0.510 %       | 0.506 % | -       |

Based on probit analysis, it was known that the LC50 value for each kind of botanical insecticides tested against citrus psyllid was various (Table 3). The effectiveness of testing on the psyllid at 24 HAT, LC50 values for chrysanthemum flower, JC citrus peel and tobacco were 1.039 %; 1.323 % and 0.238 %, respectively, and the value decreased with increasing time. At subsequent observations up to 120 HAT, LC50 values of botanical insecticides from tobacco shoots were no longer detected because the mortality had reached 100 %, comparable to chemical insecticides dimethoate and imidacloprid. Boina et al. [35] stated that LC50 for *D. citri* exposed by imidacloprid insecticide was 0.09; 0.31 and 0.08, respectively at 17 °C, 27 °C and 37 °C. In contrast to pyrethroid insecticides which were suitable for cold conditions, the insecticides of organophosphate, carbamate, avermectin and neonicotinoid (Imidacloprid) were more effectively applied at relatively hot temperatures above 25 °C.

| Observed times | LC50 | Chrysanthemum | JC peel | Tobacco |
|----------------|------|---------------|---------|---------|
| 24 HAT         |      | 0.3143 %      | 1.0723 %| 0.4168 %|
| 48 HAT         |      | 0.2381 %      | 1.3150 %| -       |
| 72 HAT         |      | -             | 0.7468 %| -       |
| 96 HAT         |      | -             | 0.5852 %| -       |
| 120 HAT        |      | -             | 0.4590 %| -       |
| 144 HAT        |      | -             | 0.3581 %| -       |
| 168 HAT        |      | -             | 0.3475 %| -       |
In treatment against *T. citricidus* aphids, LC$_{50}$ values were generally lower than those of *D. citri* (Table 4). In this test, the reach of the mortality was also faster, indicated by no further value of LC$_{50}$ appeared on the treatment of botanical insecticide Chrysanthemum at 72 h because 100 % mortality had been achieved. Meanwhile, the treatment of botanical insecticide from tobacco shoots at 48 h resulted in 100 % mortality. For botanical insecticide of JC citrus peel, the LC$_{50}$ value at the beginning of the observation at 24 HAT was 1.072 %, at 72 HAT was 0.747 % and at the end of the observation, the mortality had not reached 100 % with an LC$_{50}$ value of 0.348 %. These results indicated that the botanical insecticide was higher in effectiveness against aphids. The highest effectiveness was obtained in the treatment with botanical insecticide from tobacco, followed by chrysanthemum and JC citrus peel. Furthermore, the use of concentrations of tobacco and chrysanthemum insecticides could still be rationalized again until the appropriate concentration was obtained for application recommendations.

4. Conclusions

The conclusions of this study were that the three types of botanical insecticides could control citrus psyllids *D. citri* and citrus aphids *T. citricidus*. The best results for controlling psyllids and aphids were tobacco, followed by chrysanthemums and JC citrus peels. Further testing is needed to determine the concentration that is effective and efficient, so that it can be used as a control recommendation.

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