Compatibility of citronella oil, clove oil, and cypermethrin 500 EC to control fruit sucker (Dasynus piperis) on pepper plant

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Abstract. Some botanical insecticides can be mixed with synthetic insecticides to reduce dependence on one type of insecticide and increase its effectiveness. This study evaluated combined formulations of botanical pesticides (citronella oil/CTO from Cymbopogon nardus and clove oil/CLO from Syzygium aromaticum) and synthetic insecticide (Cypermethrin 500 EC/Cyp) to improve their effectiveness against Dasynus piperis on pepper plants. This study was conducted in farmer’s fields, Central and West Bangka regencies in Bangka Island, from March 2013 to November 2015. This study consists of two steps; semi-field and field studies. All of the treatments used Randomized Block Design consisted of eight treatments and four replications. The treatments were CTO 5.0 ml l⁻¹; CLO 5.0 ml l⁻¹; CTO 2.5 ml l⁻¹ + CLO 2.5 ml l⁻¹; CTO 2.5 ml l⁻¹ + Cyp 1 ml l⁻¹; CLO 2.5 ml l⁻¹ + Cyp 1 ml l⁻¹; CTO 1.25 ml l⁻¹ + CLO 1.25 ml l⁻¹ + Cyp 1 ml l⁻¹; Cyp 2.0 ml l⁻¹; and control. The results showed that CTO, CLO, and Cyp formula was compatible, with CTO 2.5 ml l⁻¹ + CLO 2.5 ml l⁻¹ and CLO 2.5 ml l⁻¹ + Cyp 1.0 ml l⁻¹ were effective to control D. piperis.

Keywords: botanical insecticide, Cymbopogon nardus, formula, synthetic insecticide, Syzygium aromaticum

1. Introduction
The pepper plant (Piper nigrum L.) is a high-value spice. The world’s demand for pepper reaches 350 thousand tons in a year and around 29% is from Indonesia. In 2012, Indonesian pepper production reached 87,000 tons (white and black pepper), and approximately 62,600 tons were exported. Meanwhile, in 2015 the national pepper production reached 88,296 tons or only increased about 1% from national production in 2014 [1].

Pepper has a good opportunity as an exported commodity in Indonesia. Pepper is the seventh-largest foreign exchange for estate crop plantation [2]. Lampung black pepper and Muntok white pepper are two well-known Indonesian peppers in the world market. Originally, the development of the pepper area in Lampung and Bangka has spread in Kalimantan and Sulawesi [3]. However, the potential of foreign demand has not been supported by the high yield productivity of pepper in Indonesia. The potential for pepper production is approximately 2 tons ha⁻¹, while in 2021, the total yield produced by the farmer in Indonesia is around 85,527 tons [4].
One of the main factors for the decline in pepper production is pests and diseases from seeding to production stages [3]. One of the pests which attack the pepper is pepper berry sucker Dasynus piperis China (Hemiptera: Coreidae). Dasynus piperis is found almost in all areas of pepper plantations [5]. The fruit damage rate in Bangka Belitung has been reported to reach 16.00-36.80% [6; 7]. This pest attacks around 4.5 months old pepper fruit. This pest damages the pepper fruit by piercing the stylet and sucking the liquid from inside the fruit, and the pepper becomes empty and damage. The color of attacked fruit turns to black with symptoms spot as puncture marks. The attacked young fruit falls before getting older. When the D. piperis attacks the old fruit, the fruit is drying [3].

One of the efforts to control D. piperis is with pesticides. However, it has a drawback, such as resistance and resurgence of target pests, environmental pollution, and harm to human. A lot of botanical insecticides have been developed to resolve the bad effects of using synthetic pesticides. Cymbopogon nardus (L.) Rendle (citronella) is known to contain citronellol and geraniol that can be repellent to all pest insects [8]. Clove (Syzygium aromaticum L.) contains eugenol, which is known to irritate and weaken the nervous system of pest insects [9]. Citronellol and geraniol are the active ingredients that are lethal to pests because they have properties as contact poisons. High concentrations can cause death due to continuous fluid loss and stomach poisons [10].

Compatibility testing between botanical insecticide and synthetic insecticide is necessary to increase effectiveness and reduce toxicity to non-target organisms. Therefore, this study examined the effectiveness of citronellol oil, clove oil, and Cypermethrin 500 EC to control D. piperis.

2. Materials and methods
This research was divided into two steps, i.e., semi-field and field studies. The study was conducted in Sungkap Village, Simpang Katis, Bangka Tengah District (semi-field study), and Sadap Village, West Mendo District (field study), Bangka Island. This treatment used surfactant 0.2 ml l⁻¹ (alkylaryl polyglycol ether 400 g l⁻¹) to increase the sticky of the insecticide. In this research, a randomized block design was used, with eight treatments and four replications, as follows:

1. Citronella oil (CTO) 5.0 ml l⁻¹
2. Clove oil (CLO) 5.0 ml l⁻¹
3. CTO 2.5 ml l⁻¹ + CLO 2.5 ml l⁻¹
4. CTO 2.5 ml l⁻¹ + Cypermethrin 500 EC (Cyp) 1.0 ml l⁻¹
5. CLO 2.5 ml l⁻¹ + Cyp 1.0 ml l⁻¹
6. CTO 1.25 ml l⁻¹ + CLO 1.25 ml l⁻¹ + Cyp 1.0 ml l⁻¹
7. Cyp 2.0 ml l⁻¹ (contact and gastric poison)
8. Control

2.1. Semi-field testing
The study about botanical insecticide effectiveness was tested by sampling one tree each plot and in each tree was sampled two branches (east and west) that had fruit stem. Every stem was wrapped up with gauze (40 cm × 50 cm). In each cage, ten of D. piperis adults were reared and treated. Insect mortality observation was conducted in 1; 3; 6; 24; 48; 72 and 96 hours after application/HAA. To calculate Insecticide Effectivity (EI) using the following formula:

\[
EI = \frac{Ca - Cb}{Ca} \times 100\%
\]

Where:

| EI   | Insecticide effectiveness |
| Ca   | Mortality in treatment    |
| Cb   | Mortality in control [11] |

Efficacy criteria (EI) = (1/2 n + 1) ≥ 70%, n = total observation. Data population of D. piperis before and after application were analysed with variance analysis, while berries attack rate data (%), loss yield (%), net crops yield (g plant⁻¹), and mortalities of D. piperis were analysed with variance analysis. If
there were any significant differences between the treatments, the Duncan Multiple Range Test/DMRT analysis was used at a level of 5%. Yield loss percentage (berries damage intensity) was calculated using the non-parametric, Kruskal-Wallis test [12]. The observation data were tested for normality Mann-Whitney non-parametric test was used if data were not distributed normally, while variance analysis was used for normally distributed data. The DMRT at the 5% level was used for significantly different data.

2.2. Field testing
Each plot (treatment plot) consists of four trees. The distance between the plots was two lines of the tree. Insecticide application was conducted using a mini knapsack sprayer that has 4 atm of pressure. Each spray was applied by pointing the nozzle into the place of D. piperis adult and nymph. The first application was conducted since the pepper plantation had fruits (assuming there was no attack). After that, the application was conducted at intervals of two weeks for seven times applications. The control application was conducted at the place of D. piperis adult and nymph. The first application was conducted since the pepper plantation had fruits (assuming there was no attack). After that, the application was conducted at intervals of two weeks for seven times applications. The control was a plot without treatment.

Observation parameters consisted of (a) D. piperis population seen on the berries or perch of the observed plant was counted before and after the treatment; (b) berries’ attack rates (%) were calculated by counting the grains of each attacked branch; (c) yield result (g plant−1); (d) presentation of yield loss (berries damage intensity). To count berries attack rate and damage intensity, follow the formula below:

\[
\text{Berries attack rate} = \frac{\text{Total of damaged berries}}{\text{Total of berries}} \times 100\%
\]

\[
DI=\frac{\sum (n \times v)}{z \times N} \times 100 \%
\]

Information:
- DI = Damage intensity (%)
- n = Total attacked berries in a branch based on the attacked criteria (0, 1, 2, 3, 4, 5)
- v = Score in each category
- z = Scale value (score) in highest attack category (= 5)
- N = All total number berries that was observed (n₀ + n₁ + . . . + n₅)

Attacked criteria: 0 = No attack; 1 = 1-10% attacked berries in a branch; 2 = 11-25% attacked berries in a branch; 3 = 26-50% attacked berries in a branch; 4 = 51-75% attacked berries in a branch; 5 = 76-100% attacked berries in a branch.

3. Results and discussion
3.1. Semi-field testing
The cumulative mortalities of D. piperis were shown in Table 1. There was an increase in mortality with increasing time of exposure to insecticide. The highest cumulative mortality on D. piperis was CTO 1.25 ml l⁻¹ + CLO 1.25 ml l⁻¹ + Cyp 1 ml l⁻¹, equivalent with Cyp 2 ml l⁻¹ observed in 96 HAA, followed by CTO 5 ml l⁻¹ and CLO 2.5 ml l⁻¹ + Cyp 1 ml l⁻¹. Also, it showed that using Cyp with a concentration of 2 ml l⁻¹ can reduce the dose to 1 ml l⁻¹ with the addition of CTO 1.25 ml l⁻¹ + CLO 1.25 ml l⁻¹.

According to the assessment of the Pesticide Commission [4], the insecticide is deemed to be effective by following the EI formula. The CTO was more effective than CLO. It can be seen in Table 1. The CTO has an EI value of ≥70% from 5 of 7 observations, consisting of: 72.50%; 77.50%; 78.75%; 78.70%; and 86.25%. The effectivity of CTO in 5 ml l⁻¹ concentration was the same as Cyp 2 ml l⁻¹ and CTO 5 ml l⁻¹. The CLO was ineffective in controlling D. piperis because it only has an EI of ≥70% from three times observation, with the value of 71.25%; 76.25%, dan 75.00%. Those values did not meet the assessment criteria of the pesticide commission. However, the combination of CTO and CLO had the EI requirement by having a value ≥ of 70% from five times observation, with the values 71.25%; 85.00%; 85.00%; 85.00%, and 82.50%. It was indicated that CTO was effective in controlling D. piperis on pepper plantation when it was used single at a concentration of 5 ml l⁻¹ combined with CLO at a
concentration of 2.5 ml^{-1}, and mixed with Cyp at a concentration of 1 ml l^{-1}. Thus, CTO was compatible with CLO and Cyp.

Table 1. Percentage of cumulative mortality of *D. piperis* after pesticides treatments semi-field testing

| Treatment | Application-hours | EI (%) |
|-----------|------------------|--------|
| CTO 5 ml l^{-1} | 1 | 18.75 b |
|            | 3 | 16.25 |
|            | 6 | 42.50 |
|            | 24 | 80.00 a |
|            | 48 | 72.50 |
|            | 72 | 85.00 a |
|            | 96 | 86.25 ab |
| CLO 5 ml l^{-1} | 1 | 12.50 bc |
|            | 3 | 10.00 |
|            | 6 | 22.50 c |
|            | 24 | 15.00 |
|            | 48 | 26.25 b |
|            | 72 | 18.75 |
|            | 96 | 51.25 b |
| CTO 2.5 ml l^{-1} + CLO 2.5 ml l^{-1} | 1 | 17.50 bc |
|            | 3 | 15.00 |
|            | 6 | 66.25 b |
|            | 24 | 58.75 |
|            | 48 | 78.75 a |
|            | 72 | 71.25 a |
|            | 96 | 92.50 a |
| CTO 2.5 ml l^{-1} + Cyp 1 ml l^{-1} | 1 | 13.75 bc |
|            | 3 | 11.25 |
|            | 6 | 62.50 b |
|            | 24 | 55.00 |
|            | 48 | 87.50 a |
|            | 72 | 80.00 |
|            | 96 | 92.50 a |
| CLO 2.5 ml l^{-1} + Cyp 1 ml l^{-1} | 1 | 5.00 bc |
|            | 3 | 2.50 |
|            | 6 | 11.25 ed |
|            | 24 | 3.75 |
|            | 48 | 11.25 bc |
|            | 72 | 3.75 |
|            | 96 | 6.25 |
| CTO 1.25 ml l^{-1} + CLO 1.25 ml l^{-1} + Cyp 1 ml l^{-1} | 1 | 12.50 bc |
|            | 3 | 10.00 |
|            | 6 | 65.00 |
|            | 24 | 57.50 |
|            | 48 | 87.50 a |
|            | 72 | 80.00 |
|            | 96 | 100.00 |
| Cyp 2 ml l^{-1} | 1 | 52.50 a |
|            | 3 | 50.00 |
|            | 6 | 91.25 a |
|            | 24 | 83.75 |
|            | 48 | 92.50 a |
|            | 72 | 85.00 |
|            | 96 | 100.00 |
| Control | 1 | 2.50 c |
|          | 3 | 7.50 d |
|          | 6 | 7.50 c |
|          | 24 | 6.25 e |
|          | 48 | 7.50 c |
|          | 72 | 5.00 e |

Note: The numbers followed by the same letter in the same column showed no significant difference based on the Duncan Test of 5%.

3.2. Field testing

The population development of *D. piperis* treated with pesticides was in Tables 2 and 3. There was no consistent decrease pattern observed in the number of *D. piperis* population before and after treatments. Generally, the population development of *D. piperis* decreased after being treated with pesticides. A lower population of *D. piperis* was observed in applying single synthetic pesticides or a mixture with botanical pesticides. These applications also significantly decreased the population of *D. piperis* (Table 3). This study's low population of *D. piperis* was likely due to the very low *D. piperis* field population ranging from 0.13-4.88 individuals per tree. The previous study using single CTO and CLO (at a concentration of 2.5 – 5.0 ml l^{-1}) showed a decrease in *D. piperis* compared to control and was not significantly different from synthetic insecticides [7]. Another report [13] stating that CTO at a concentration of 2 ml l^{-1} was more effective than CLO to control *Crocidolomia pavonana* on cabbage with a mortalities rate of 76-88%.

Table 2. Population average of *D. piperis* before (B) and after (A) pesticides treatments

| Treatment | Application- | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-----------|--------------|---|---|---|---|---|---|---|
| CTO 5.0 ml l^{-1} | 0.69 | 0.38 | 0.38 | 0.88 | 0.81 | 1.00 | 0.69 | 0.19 |
| CLO 5.0 ml l^{-1} | 0.12 | 0.25 | 0.38 | 0.94 | 0.63 | 0.56 | 0.2 | 0.25 |
| CTO 2.5 ml l^{-1} + CLO 2.5 ml l^{-1} | 0.12 | 0.38 | 1.31 | 1.31 | 0.44 | 0.5 | 0.88 | 0.19 |
| CTO 2.5 ml l^{-1} + Cyp 1.0 ml l^{-1} | 0.63 | 0.38 | 1.13 | 0.44 | 0.69 | 0.25 | 0.31 | 0.19 |
| CLO 2.5 ml l^{-1} + Cyp 1.0 ml l^{-1} | 0.13 | 0.56 | 0.38 | 0.00 | 0.44 | 0.25 | 0.19 | 0.13 |
| CTO 1.25 ml l^{-1} + CLO 1.25 ml l^{-1} + Cyp 1.0 ml l^{-1} | 0.38 | 0.19 | 1.25 | 0.13 | 0.63 | 0.13 | 0.13 | 0.00 |
| Cyp 2.0 ml l^{-1} | 0.31 | 0.31 | 0.63 | 0.19 | 0.38 | 0.19 | 0.19 | 0.13 |
| Control | 0.69 | 0.63 | 1.00 | 1.00 | 0.63 | 0.94 | 0.44 | 0.88 |

Table 4 showed that the application of CTO was more effective compared to CLO. Even though the weight of total berries collected on CLO treatment was bigger than that on CTO treatment, CTO treatment generated less yield loss than CLO treatment, with 12.92%. Also, CTO treatment had lower yield loss compared to Cyp. It indicates that CTO was more effective than CLO to control the *D. piperis* population in pepper plantations. The CTO with the active compound of 34% citronella applied at 5 and 10 ml l^{-1} can control cacao fruit borer (*Conopomorpha cramerella*) generated a similar result to control in the first observation, with only a 7.78% difference. The percentage of cocoa yield loss was not significantly different between CTO and CTO (17.78%), neem oil (NEO) with azadirachtin active
compound (22.22-25.56%), and a combination of CTO, CLO, and NEO (25.55-32.22%) [14]. Insect from same species shows different sensitivity against particular bioactive compounds, which is likely due to the differences in the entry system of bioactive compounds into the insect’s body, for example, the difference in the thickness of the cuticle layer. Moreover, the sensitivity of adult insects to bioactive compounds is also influenced by sexual maturity, the aging process, and feeding habits [15].

Table 3. Population growth of D. piperis due to pesticides treatments

| Treatment                  | Application- |
|----------------------------|--------------|
|                            | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| CTO 5.0 ml l⁻¹             | 2.13 a | 3.88 a | 2.63 a | 1.00 bc | 1.88 a | 3.88 a | 2.75 a |
| CLO 5.0 ml l⁻¹             | 2.63 a | 2.75 ab | 2.00 a | 3.00 a | 1.25 b | 2.00 abcd | 2.75 ab |
| CTO 2.5 ml l⁻¹ + CLO 2.5 ml l⁻¹ | 2.00 a | 4.88 a | 2.00 a | 2.13 ab | 1.13 b | 2.75 ab | 1.38 bc |
| CTO 2.5 ml l⁻¹ + Cyp 1.0 ml l⁻¹ | 2.00 a | 3.13 ab | 1.88 a | 1.00 bc | 0.13 ab | 0.75 dc | 0.63 c |
| CLO 2.5 ml l⁻¹ + Cyp 1.0 ml l⁻¹ | 1.38 a | 0.75 b | 1.38 a | 0.63 bc | 1.13 b | 0.63 dc | 1.13 bc |
| CTO 1.25 ml l⁻¹ + CLO 1.25 ml l⁻¹ + Cyp 1.0 ml l⁻¹ | 1.75 a | 1.63 ab | 1.50 a | 0.25 bc | 0.88 ab | 2.13 abcd | 1.25 bc |
| Cyp 2.0 ml l⁻¹             | 1.25 a | 1.63 ab | 1.13 a | 0.63 bc | 0.38 ab | 1.50 bcd | 0.75 c |
| Control                    | 3.50 a | 4.00 a | 2.88 a | 4.00 a | 2.13 a | 2.75 abcd | 4.88 ab |

Note: The numbers followed by the same letter in the same column showed no significant difference based on the Duncan Test of 5%.

Table 4. Fruit damage intensity caused by D. piperis (%), total berries weight (g per tree), number of damaged berries, total berries number, and pepper yield loss (%)

| Treatment                  | Fruit damage intensity (%) | Total berries weight (g per tree) | Number of damaged berries | Total berries number | Yield loss (%) |
|----------------------------|----------------------------|----------------------------------|---------------------------|---------------------|---------------|
| CTO 5.0 ml l⁻¹             | 41.25                      | 946.88                           | 69.88                     | 164.13              | 12.92         |
| CLO 5.0 ml l⁻¹             | 36.09                      | 1006.56                          | 49.56                     | 121.31              | 16.19         |
| CTO 2.5 ml l⁻¹ + CLO 2.5 ml l⁻¹ | 45.57                     | 943.75                           | 75.63                     | 174.75              | 18.63         |
| CTO 2.5 ml l⁻¹ + Cyp 1.0 ml l⁻¹ | 37.62                      | 740.94                           | 44.00                     | 124.38              | 15.22         |
| CLO 2.5 ml l⁻¹ + Cyp 1.0 ml l⁻¹ | 36.35                      | 705.63                           | 45.81                     | 114.13              | 13.49         |
| CTO 1.25 ml l⁻¹ + CLO 1.25 ml l⁻¹ + Cyp 1.0 ml l⁻¹ | 35.42                      | 856.56                           | 43.38                     | 131.19              | 14.77         |
| Cyp 2 ml l⁻¹               | 35.13                      | 1226.25                          | 80.13                     | 197.56              | 15.23         |
| Control                    | 46.08                      | 920.63                           | 48.25                     | 140.69              | 16.86         |

The population of D. piperis with the single application of formulated CTO was lower than that of CLO. However, the population of D. piperis looked higher with applying either CLO and CTO or with synthetic pesticide (Cyp), indicating that a combination of CLO and CTO and other synthetic pesticides was less effective. A combination of CTO, CLO, and Cyp 2 ml l⁻¹ was more effective in reducing the D. piperis population and using synthetic pesticides around 50%.

The CTO is insect repellent [8, 16, 17, 18]. CTO can be used to prevent Chrysomya megacephala [19] and also has functioned as a neurotoxin [20] by inhibiting the acetylcholinesterase (AChE) enzyme. Exposure to neurotoxin causes central system disorder, convulsions, paralysis, and insect death [21]. CLO is also a neurotoxin. The phenolic compounds in CLO can decline function and disrupt the insect nervous system [9]. In addition, CLO was toxic to some storage pests [22, 23]. The synthetic pesticide used in this study is Cyp which is also a nerve and gastric poison. Cyp belongs to the pyrethroid group in the IRAC classification. The pyrethroid group works to prevent the closure of N+ channels in insect nerve axons, so that nerve impulses flow continuously [24]. It causes symptoms of seizures, tremors, and even death [21]. Cyp was compatible and synergistic with CTO and CLO, thereby increasing the toxicity of the mixture. (Table 2 and 3). The addition of CTO and CLO can reduce the use of Cyp insecticides. CTO and CLO, which contain more than one active ingredient that affects insects combined
with fast-acting Cyp, can increase the efficiency of insecticide performance, reduce dependence on one insecticide ingredient, and slow down the occurrence of insect resistance [13].

Production, damage level, and yield loss of pepper berries were not significantly different among the treatments due to the lower *D. piperis* population (Table 4). The pest population cannot be predicted in the location of the study.

4. Conclusion

The CTO was the most effective in controlling the development of *D. piperis* compared to CLO and CLO + Cyp. However, the combination of CTO and CLO was effective in reducing the population of *D. piperis* in the field. Population of *D. piperis* was lower in the effective treatments (CTO 5.0 ml l⁻¹, CTO 2.5 ml l⁻¹ + CLO 2.5 ml l⁻¹, dan CTO 2.5 ml l⁻¹ + Cyp 1 ml l⁻¹) that in the ineffective treatments (CLO 5.0 ml l⁻¹, and CLO 2.5 ml l⁻¹ + Cyp 1 ml l⁻¹). Moreover, the yield loss, attack rate, total healthy berries, weight, and the number of damaged berries were not significantly different between all treatments.

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