Insecticidal activity of nanoemulsion of *Piper aduncum* extract against cabbage head cartepillar *Crocidolomia pavonana* F. (Lepidoptera: Crambidae)

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**Abstract.** Botanical insecticide nanoemulsion formulation is an insecticide with spontaneous emulsion system. Nanoemulsion formulation is an oil phase-in-water dispersion stabilized by a surfactant molecule which has size ranging from 50 nm to 500 nm. The oil phase consists of *Piper aduncum* extracts and bioethanol, while the water phase consists of aqudes and emulsifiers. The experiment was conducted in Insect Bioecology Laboratory, Faculty of Agriculture, Universitas Andalas Padang from October 2018 to March 2019. The purpose of this research was to obtain nanoemulsion from *P. aduncum* extract and to observe the activity of *P. aduncum* nanoemulsion against *C. pavonana*. Nanoemulsion formulation was prepared using spontaneous emulsification method then followed by toxicity test against *C. pavonana* larvae. Nanoemulsion formulations with high insecticidal activity were analyzed using Zetasizer Nano (ZS) Malvern for particle size and zeta potential. The results showed that particle size of AT.1 and BA.1 nanoemulsions were 141.1 nm and 172.5 nm respectively, and they were categorized as nano particles. AT.1 and BA.1 nanoemulsions caused mortality of *C. pavonana* larvae with LC95 value were 0.85% and 0.76%, respectively. Besides the toxic effect, the AT.1 and BA.1 nanoemulsions were also interfered growth and development of *C. pavonana* larvae.

1. Introduction

Cabbage (*Brassica oleracea* var. capitata L.) is high economic value commodities that face many obstacle in cultivation process. The main problem is pest attack from Order Lepidopteran, *Crocidolomia pavonana*. The damage due to *C. pavonana* larvae occurs from young plant to harvest. In severe attack the plants are fail to form crops and ultimately could not be harvested. Yield loss caused by this pest was around 70-100% [1].

Alternative control technique of *C. pavonana* attacks is the use of *Piper aduncum* extract as botanical insecticide [2, 3]. *P. aduncum* belongs to Piperaceae family group which can control insect pest because the presence of phenyl propanoid compound called dilapiol as the main insecticidal active compound [4]. Fruit extract of *P. aduncum* has strong insecticidal activity against *C. pavonana* [5], *Spodoptera litura* [6], and *Plutella xylostella* larvae [7].

Botanical insecticides should meet the effectiveness and efficiency criteria before mass production. Limitation on raw material for extract, easily degraded by sunlight and microorganisms, and unable to
reach pest within the plant tissue are the problems on using botanical insecticides. One technology that can solve those problems is nano technology, especially botanical insecticide nanoemulsion.

Nanoemulsion of botanical insecticide consists of oil and water dispersions stabilized by surfactant molecules, which the size ranging from 50-500 nm [8]. The small size of nanoemulsion serves to stabilize the nanoemulsion kinetic so that it prevents sedimentation and creaming if stored [9].

Nanotechnology also has several other advantages including, i.e less raw materials, photodegradation, increase the effectiveness and efficiency of insecticides by controlling the release and easily reaching targets due to their small size, easy and safe handling [10, 11].

Some basic information about the activity of P. aduncum is already known, but the information of insectidal activities of nanoemulsion is not yet widely available. The purpose of this study was to obtain nanoemulsion from P. aduncum extract and to observe the activity of P. aduncum nanoemulsion against C. pavonana.

2. Methods

2.1. Extraction
Extraction of P. aduncum was carried out by maceration method using ethyl acetate solvent. Fruits of P. aduncum were cut into 1 cm size and dried in room temperature without being exposed to direct sunlight for 3 wk. After dry, the materials were blended until it formed a fine powder. Then, 50 g of P. aduncum powder was put into an erlenmeyer flask separately and soaked in 500 ml of ethyl acetate solvent for 48 hr. Extract liquid was filtered using a glass funnel (diameter of 9 cm) which was applied with whatman filter paper No. 41 and then accommodated into an erlenmeyer flask.

Solvent was evaporated with rotary evaporator at 50 °C and pressure of 240 mbar. Ethyl acetate obtained from evaporation was used to re-soak the powder up to 3 times. Extract of P. aduncum was brown color. After that the extract was transferred into a storage bottle and covered with aluminium foil and plastic. The extract was stored in a refrigerator at 4 °C for further usage.

2.2. Preparation of Piper aduncum nanoemulsion
Nanoemulsion was prepared by spontaneous emulsion method. The nanoemulsions used 2 different types of emulsifiers with 2 concentrations. Nanoemulsion A was carried out by modifying the method of Diba [12] and Nanoemulsion B was carried out by modifying the method of Nuryanti [13].

In the nanoemulsion A, the organic phase was prepared by mixing extract and solvents as much as 10% of the total emulsion with a composition of 1:1. The water phase was prepared by mixing Tween 80 or Agristic as much as 3% or 5% and distilled water then stirred using a magnetic stirrer for 30 min at room temperature. Spontaneous emulsification technique was carried out by adding the organic phase into the water phase.

In the nanoemulsion B, the organic phase was prepared by mixing extract and solvents as much as 10% of the total emulsion with a composition of 1:1 then Tween 80 or Agristic as much as 3% or 5%. The water phase was distilled water. Organic phase was stirred using a magnetic stirrer for 30 min at room temperature. Spontaneous emulsification technique was carried out by adding the water phase into the organic phase

2.3. Nanoemulsion insecticide toxicity test
Toxicity tests of P. aduncum nanoemulsion were carried out with leaf-residue feeding method using randomized complete design. Each extract was tested at 5 concentration levels and 6 replications based on the preliminary test. Fresh broccoli leaves free of pesticides were cut into a size of 4 x 4 cm and dipped one by one into the solution with a certain concentration, including control solution until evenly wet, then left air-dried. After dry, each piece of treatment leaf and control leaf were placed in a petri dish that has been covered with tissue. Then, as much as 150f 2nd instar larvae of C. pavonana were added/petri dish. The leaf feeding treatment was carried out for 2 d, then the larvae were given untreated leaf feed until they reached the 4th instar. Larvae growth and mortality were recorded every
The larval mortality data was processed by probit analysis using the POLO-PC program (LeOra Software 1987). The larval development data was expressed as the mean value ± standard deviation. Statistik 8 was used to analyze the mortality data of larvae and duration of development of *C. pavonana* larvae and continued with the LSD (Least significant different) test at 5% level.

2.4. *Piper aduncum* nanoemulsion analysis

Nanoemulsion of *P. aduncum* was analyzed with Zetasizer Nano ZS Malvern for Particle Size Analyzer (PSA), polydispersity index, and zeta potential at Indonesian Center for Agriculture – Postharvest Research and Development (ICAPRD) in Bogor.

3. Results and discussion

Based on preliminary test to 8 nanoemulsions formula there were 4 nanoemulsion formula that showed 100% mortality of *C. pavonana* larvae including AT1, AA2, BT1 and BA1 (Table 1).

**Table 1.** Mortality of *Crocidolomia pavonana* larvae after treatment with *Piper aduncum* nanoemulsions in the preliminary test.

| Nanoemulsions | Emulsifier | Concentration (%) | Mortality (%) |
|---------------|------------|------------------|---------------|
| AT1           | Tween 80 3%| 0.0              | 0.0           |
|               |            | 0.5              | 70.00         |
|               |            | 1                | 100           |
| AT2           | Tween 80 5%| 0.0              | 0.00          |
|               |            | 0.5              | 53.33         |
|               |            | 1                | 90.00         |
| AA1           | Agristic 3%| 0.0              | 0.00          |
|               |            | 0.5              | 43.33         |
|               |            | 1                | 76.66         |
| AA2           | Agristic 5%| 0.0              | 0.00          |
|               |            | 0.5              | 93.33         |
|               |            | 1                | 100           |
| BT1           | Tween 80 3%| 0.0              | 0.00          |
|               |            | 0.1              | 36.66         |
|               |            | 0.5              | 100           |
|               |            | 1                | 100           |
| BT2           | Tween 80 5%| 0.0              | 0.00          |
|               |            | 0.1              | 20.00         |
|               |            | 0.5              | 40.00         |
|               |            | 1                | 66.66         |
| BA1           | Agristic 3%| 0.0              | 0.00          |
|               |            | 0.1              | 13.33         |
|               |            | 0.5              | 60.00         |
|               |            | 1                | 100           |
| BA2           | Agristic 5%| 0.0              | 0.00          |
|               |            | 0.1              | 30.00         |
|               |            | 0.5              | 80.00         |
|               |            | 1                | 100           |

Nanoemulsion AT1, AA2, BT1 and BA1 were further tested with 5 concentrations and 5 replications. Mortality observation of further tests was shown in Table 2, Table 3, Table 4, and Table 5. The results showed that nanoemulsions of *P. aduncum* had positive relationship between the
increasing of concentration and the number of mortality of *C. pavonana*. Nanoemulsion of *P. aduncum* was also interfered the development period of survival larvae.

**Table 2.** Mortality and growth development of *Crocidolomia pavonana* larvae after treatment with AT1 nanoemulsion.

| Concentration (%) | Mortality (%) ± SD | Development of larvae (days) |
|-------------------|--------------------|-----------------------------|
|                   |                    | Instar II – III ± SD        | Instar II – IV ± SD |
| 0.57              | 82.66 ± 17.38 a    | 4.35 ± 2.08                 | 7.00 ± 0.57 |
| 0.43              | 65.33 ± 31.76 ab   | 4.51 ± 2.89                 | 7.31 ± 0.27 |
| 0.33              | 50.66 ± 39.89 bc   | 4.03 ± 2.63                 | 7.05 ± 3.78 |
| 0.25              | 32.00 ± 2.98 cd    | 4.42 ± 0.27                 | 7.38 ± 3.87 |
| 0.19              | 14.66 ± 8.69 de    | 5.00 ± 0.32                 | 7.69 ± 2.70 |
| 0.00 (control)    | 0.00 ± 0.00 e      | 2.37 ± 0.04                 | 4.95 ± 0.10 |

**Table 3.** Mortality and growth development of *Crocidolomia pavonana* larvae after treatment with AA2 nanoemulsion.

| Concentration (%) | Mortality (%) ± SD | Development of larvae (days) |
|-------------------|--------------------|-----------------------------|
|                   |                    | Instar II – III ± SD        | Instar II – IV ± SD |
| 0.55              | 80.00 ± 24.95 a    | 4.52 ± 0.80                 | 6.84 ± 1.01 |
| 0.40              | 68.00 ± 44.07 ab   | 4.06 ± 0.79                 | 6.98 ± 1.18 |
| 0.29              | 45.33 ± 33.13 bc   | 4.02 ± 2.56                 | 6.34 ± 3.24 |
| 0.21              | 37.33 ± 13.82 bc   | 4.08 ± 3.33                 | 6.37 ± 4.27 |
| 0.15              | 22.66 ± 17.38 cd   | 4.47 ± 2.40                 | 6.53 ± 2.98 |
| 0.00 (control)    | 0.00 ± 0.00 d      | 2.52 ± 0.06                 | 4.89 ± 0.10 |

**Table 4.** Mortality and growth development of *Crocidolomia pavonana* larvae after treatment with BT1 nanoemulsion.

| Concentration (%) | Mortality (%) ± SD | Development of larvae (days) |
|-------------------|--------------------|-----------------------------|
|                   |                    | Instar II – III ± SD        | Instar II – IV ± SD |
| 0.40              | 80.00 ± 28.28 a    | 4.00 ± 0.48                 | 6.37 ± 0.40 |
| 0.24              | 68.00 ± 28.04 a    | 4.64 ± 1.06                 | 6.74 ± 1.19 |
| 0.14              | 36.00 ± 12.11 b    | 4.08 ± 0.58                 | 6.35 ± 0.73 |
| 0.08              | 26.66 ± 22.11 b    | 4.46 ± 2.43                 | 6.67 ± 3.06 |
| 0.05              | 17.33 ± 10.11 bc   | 4.33 ± 2.64                 | 7.00 ± 3.48 |
| 0.00 (control)    | 0.00 ± 0.00 c      | 2.44 ± 0.10                 | 4.92 ± 0.11 |

**Table 5.** Mortality and growth development of *Crocidolomia pavonana* larvae after treatment with BA1 nanoemulsion.

| Concentration (%) | Mortality (%) ± SD | Long development of larvae (days) |
|-------------------|--------------------|----------------------------------|
|                   |                    | Instar II – III ± SD             | Instar II – IV ± SD |
| 0.75              | 96.00 ± 8.94 a     | 3.55 ± 0.42                     | 6.31 ± 0.28 |
| 0.50              | 85.33 ± 15.91 a    | 3.37 ± 1.26                     | 6.26 ± 1.69 |
| 0.33              | 49.33 ± 25.21 b    | 3.40 ± 0.99                     | 6.05 ± 1.63 |
| 0.22              | 34.66 ± 25.12 bc   | 3.50 ± 1.28                     | 6.54 ± 1.91 |
| 0.15              | 14.66 ± 9.89 cd    | 3.67 ± 1.10                     | 5.67 ± 1.44 |
| 0.00 (control)    | 0.00 ± 0.00 d      | 2.55 ± 0.06                     | 4.85 ± 0.09 |
The results showed that the first nanoemulsion formula with the best toxicity and particle size criteria was nanoemulsion AT1 (3% Tween 80 emulsifier) with particle size of 141.1 nm and LC$_{95}$ of 0.95%. At the highest concentration (0.57%), the duration of growth development of C. pavonana larvae from 2nd instar to 3rd instar larvae stages was 1.12 d longer than the control and from 2nd instar to 4th instar larvae stages was 2.74 d longer than the control. The second formula was nanoemulsion BA1 (3% Agristic emulsifier) with particle size of 172.5 nm and LC$_{95}$ of 0.75%. At the highest concentration (0.75%), the duration of growth development of C. pavonana larvae from 2nd instar to 3rd instar larvae stages was 2.63 d longer than control and from 2nd instar to 4th instar larvae stages was 0.82 d longer than the control.

Dilapiol (phenyl propanoid) is known as the main compound of P. aduncum and has insecticidal and synergism activities [4, 14]. Dilapiol have methyl endosioxyphenyl group in its structure which is the characteristic of various synergies component block cytochrome P450 enzyme activity [15, 16]. Dilapiol works by blocking the activity of cytochrome P450 enzyme in Ostrinia nubilalis midgut [17]. The presence of insecticidal active compounds (dilapiol) in P. aduncum nanoemulsion caused the death of C. pavonana larvae. Healthy larvae was characterized by a body size that changed to greater extent, green color larvae with brown edges, and active moving larvae. Meanwhile, larvae that died from nanoemulsion were characterized by shorter body size, the color of the larvae become blackish brown, and the larvae were inactive.

Furthermore, detoxification mechanism by C. pavonana larvae against active compounds of P. aduncum was proven from previous studies [18]. They found that there were an increase of oxidative enzyme activity of cytochrome b5 and cytochrome P450 in at in-vivo and in-vitro test compared to control.

The results of the probit analysis (Table 6) showed the correlation between concentration and larval mortality. Probit analysis result was used as references to carry out the Particle Size Analyzer (PSA) test. As much as 2 formulas were selected from 4 formulas nanoemulsion which had the best lethal concentrate (LC) values, namely AT1 and BA1 nanoemulsion (Table 7).

### Table 6. P. aduncum nanoemulsion analysis based on probit analysis of POLO PC (LC$_{50}$ and LC$_{95}$ values (Lethal Concentrate)).

| Nanoemulsion | b ± SE (Standar Error) | LC$_{50}$ (%) | LC$_{95}$ (%) |
|--------------|------------------------|--------------|--------------|
| AT1          | 4.05 ± 0.44            | 0.33         | 0.85         |
| AA2          | 2.82 ± 0.36            | 0.28         | 1.09         |
| BT1          | 2.06 ± 0.23            | 0.16         | 1.02         |
| BA1          | 3.94 ± 0.35            | 0.29         | 0.76         |

### Table 7. Nanoemulsion analysis based on Particle Size Analyzer (PSA).

| Formulation     | Particle size (nm) | Polydispersity Index | Zeta Potential (mV) |
|-----------------|--------------------|----------------------|---------------------|
| Nanoemulsion AT1| 141.1              | 0.106                | -21.0               |
| Nanoemulsion BA1| 172.5              | 0.092                | -25.9               |

The size of nanoemulsion particles was also contributed to larval mortality. The smaller particle size of nanoemulsion was easier for active ingredient to enter the plant tissue and remain in there. The residue would affect C. pavonana larvae after entered the larvae bodies through feeding and movement process. The PSA results showed that both nanoemulsions had particle size of <200 nm. Such measures were included in the nano category presented by [8], that particle size in nanoemulsion systems ranged from 50-500 nm. The droplet size of the active ingredients of smaller nanoemulsion formula made the active ingredients easy to enter the plant tissue and immediately reached the target [11].
Polydispersity index indicated the quality of nanoemulsion dispersion. Small polydispersity index values showed a narrow particle size distribution, meaning the particle size was increasingly uniform [19]. In the results of table 4, the polydispersity index of AT1 nanoemulsion was 0.106 and BA1 nanoemulsion 0.092. The data showed that both formulas of BA1 and AT1 had good uniformity, BA1 nanoemulsion was more uniform than AT1 nanoemulsion.

Zeta potential produced an electrical repulsion force between oil particles that could inhibit agglomeration between particles. In general, particles with a zeta potential value more than +30 mV or less than -30 mV showed stability, because the electric charge of the particles was strong enough to resist the dominant particles in the nanoemulsion dispersion system [20]. The potential zeta value produced by AT1 and BA1 nanoemulsions were -21.0 mV and -25.2 mV, respectively. The zeta potential value less than -30 mV indicated that the 3 results of nanoemulsion were stable.

In general, both P. aduncum nanoemulsions could be used as alternative control of C. pavonana larvae, based on mortality, growth development of larvae, and characteristics of nanoemulsions (particle size, polydispersity index and zeta potential). In previous studies [2], P. aduncum single extract required 0.32% concentration (100% extract) to kill 95% C. pavonana. Whereas AT1 and BA1 nanoemulsions only need 0.85% and 0.76% concentration respectively (0.04% and 0.038% extract). Those indicated that P. aduncum insecticides in the form of nanoemulsion were only used 10% - 12.5% extract compared to single extract solution.

In a study [21], nanotechnology in organic pesticides could be done by developed toxic materials contained by plants or organic materials in the size of nanoparticles, hence it would be easier to reach the target and the amount of pesticides needed was much smaller. Other researchers [22] also reported that nanoemulsion with particle size of 31.03 nm had smaller LC50 value than nanoemulsion with a particle size of 251.43 nm. It was influenced by the size of the particles. The smaller the particle size of the emulsions was, the easier for the active ingredients to enter and be absorbed by the leaves. Meanwhile, the larger the particle size of the emulsions were, the harder the active ingredient to enter and be absorbed by the leaves.

4. Conclusion
The results showed that P. aduncum fruit had insecticidal activity in the form of nanoemulsion. AT1 and BA1 nanoemulsions were categorized as nanoemulsion because it had a particle size of 141.1 nm and 172.5 nm. Both nanoemulsions had toxic effects on C. pavonana with LC50 values of 0.85% and 0.76%. Aside from being toxic, nanoemulsions also caused the development of larvae that survived.

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