Botanical insecticide nanoemulsion of *Piper Aduncum* extract to control Cabbage Head Cartepillar *Crocidolomia pavonana* F. (Lepidoptera: Crambidae)

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**Abstract.** Botanical insecticide nanoemulsion is an insecticide with a transparent emulsion system, and it is an oil and water dispersion stabilized by a surfactant molecule, which has a size ranging from 50 to 500 nm. The experiment was conducted in Biota Sumatera Laboratory Faculty of Pharmacy and Insect Biocology Laboratory Faculty of Agriculture Andalas University Padang on from July to August year 2018. The purpose of this research was to obtain the nanoemulsion of *Piper aduncum* fruit as a botanical insecticide capable of controlling the pest of cabbage larvae *Crocidolomia pavonana*. The experimental design used Completely Randomized Design (CRD) that consists of control treatment and nanoemulsion treatment of *P. aduncum*. Observations were used PSA with Zetasizer Nano ZS Malvern tools. Insecticidal activity of nanoemulsion was tested against *C. pavonana*. As the study found, the nanoemulsion has the following characteristics; its size ranges from 18.56 to 216.7 nm, its polydispersity index ranges from 0.202 to 0.371, its zeta potential ranges from -0.410 to -28.7 mV, and its distribution width range from 8.11 to 5570 nm. It has insecticidal activity against *C. pavonana*.

**Keywords:** Nanoemulsion, Botanical Insecticide, Pest Insect

1. **Introduction**

Cabbage plants are one of the vegetable plants that have a high economic value and consumed a lot by many people. The need for vegetables increases every year, along with the increase in population. Cabbage plants are very easy to cultivate, but in practice, farmers often experience disturbances such as pests and diseases. This pest and disease attack can reduce the results both in quantity and quality; one of them is the *Crocidolomia pavonana* larvae.

The decrease in cabbage production due to the attack of *C. pavonana* can be reduced by integrated control methods, namely botanical insecticides. Botanical insecticides are plant-based insecticides that act as growth inhibitors and metamorphosis processes, antifeedant, repellent, and sterilants of insects [1]. Plant-based insecticides have advantages, extract components are synergistic, pest resistance does not occur, and can be combined with other integrated pest control components [2].

*Piper aduncum* is a plant that is used as a botanical insecticide. *P. aduncum* belongs to the Piperaceae family group which can control insects because the phenyl propanoid compound is dilapiol.
as the main active compound which is insecticidal [3]. *P. aduncum* fruit extract has a strong insecticidal activity against the larvae of *C. pavonana* [4], capable of mortality of *Spodoptera litura* larvae [5], and cause mortality of *C. pavonana* with a low concentration of LC$_{50}$ of 0.209% [6].

Botanical insecticides must meet the criteria of effective and efficient before mass-produced. Easily broken down by sunlight and microorganisms is one of the problems in applying insecticides. One technology that can correct this problem is nanoemulsion technology. Nanoemulsion is a transparent emulsion system, translucent and an oil dispersion and water stabilized by surfactant molecules, which has a size ranging from 50-500 nm [7] [8].

Nanoemulsion technology has several advantages, such as; strong against sunlight and microorganisms and being protected from photodegradation. It saves insecticidal raw materials and increases solubility, improves insecticide efficiency by controlled release and quickly reaches the target due to its small size. More importantly, it reduces environmental contamination by reducing the level of insecticide application [9] [10].

Nimba nanoemulsion oil has the ability as a larvacide against *Culex quinquefasciatus*. Besides that it can also be controlled by vector-borne diseases to be better alternatives than other pesticides [11]. Then, the form of botanical insecticide nano in neem oil can help provide maximum impact on the organizing target because the nature of nanotechnology is protected from early degradation in the environment compared to not nano (makro) [9] [10].

Some basic information about the activity of the plant part of *P. aduncum* is known, but information on the extract in the form of nanoemulsion is not yet widely available. Therefore, the author will conduct a study with title "Botanical Insecticide Nanoemulsion of *Piper aduncum* extract to Control Cabbage Head Caterpillar *Crocodoloma pavonana* F. (Lepidoptera: Crambidae)*. The study aims at; 1) obtaining insecticide nanoemulsion from *P. aduncum* fruit, and 2) determining the activity of the insecticidal nanoemulsion against *C. pavonana*.

2. Methodology

2.1. Extraction

*P. aduncum* extraction was carried out by the maceration method using ethyl acetate solvent [12]. The first stage, *P. aduncum* cut 1 cm in size and placed on a bamboo tray and paper lined up. Then let the air dry without direct sunlight for 3 weeks. After drying, the fruit is blended until smooth and sifted to obtain powder. Second stage weighed 50 g of *P. aduncum* powder then put into the erlenmeyer flask separately and soaked in 500 ml of ethyl acetate solvent for 2 x 24 hours. Then the extract liquid is filtered using a glass funnel (diameter 9 cm) which is lined with ordinary filter paper and accommodated in an erlenmeyer flask.

The first filter results are filtered again using a glass funnel (diameter 5 cm), the dialed filter paper Whatman No. 41, and accommodated in a steam flask. The results of the second filter were evaporated with a rotary evaporator at a temperature of 50°C and a pressure of 240 mbar. Ethyl acetate obtained from evaporation is used to re-soak the pulp of plant extracts up to 3 times soaking (2 subsequent soaking for 1 x 24 hours). The extract obtained in pumpkin in the form of a semisolid brown substance.

2.2. Manufacture of Insecticide Nanoemulsion

Nanoemulsion is prepared by spontaneous emulsification techniques [13], which occurs when the organic and water phases are mixed. The emulsion system consists of the organic phase (*P. aduncum* extract and ethanol 70%) and the water phase (water = sterile distilled water and nonionic surfactant = Tween 80). The organic phase was prepared by mixing *P. aduncum* extract and 70% ethanol solvent as much as 10% of the total emulsion (30 ml) with a composition of 1: 1. The amount of *P. aduncum* extract is 1.5 g, and ethanol 70% is 1.5 ml. The water phase was prepared by mixing sterile distilled water and Tween 80 (3%), then stirring using a magnetic stirrer for 30 minutes at room temperature.

Spontaneous emulsification technique is carried out by adding a dissolved organic phase into the water phase by dripping (drop by drop) and continued with magnetic stirring for 45 minutes. Then, the
nanoemulsion formula was filtered using Whatman® 47 mm with Pore Size 0.1 μm. The same way is also done on the mechanism of spontaneous emulsification using different solvents (organic phase) for comparison. These were acetone + methanol (3:1), 10% of the total emulsion (30 ml) and 20% of the total emulsion (30 ml), and ethyl acetate as much as 10% of the total emulsion (30 ml).

2.3. Insecticide Nanoemulsion Analysis
The prepared insecticide nanoemulsion then sent to Indonesian Center for Agricultural Post Harvest Research and Development, Bogor for analysis using Particle Size Analyzer (PSA). The tool used in the PSA is Zetasizer Nano ZS Malvern. We analyzed data to measure the particle size, polydispersity index, zeta potential, and distribution width of particles from nanoemulsion.

2.4. Nanoemulsion Insecticide Toxicity Test
The nanoemulsion formula is tested by prepared according to a predetermined concentration then the method is dissolved into distilled water. Pesticide-free fresh broccoli leaves are cut with a size of 4 cm x 4 cm and dipped one by one in the nanoemulsion formula with a certain concentration, including the control solution until it is evenly wet, then air-dried. After dry, each leaf piece treated and control leaves placed in a petri dish that has been covered with tissue. Then ten larvae instar II of C. pavonana were added/petri dish. Feeding the treatment leaves is carried out for 2 x 24 hours, then the larvae are fed with leaves without treatment until it reaches instar IV. This experiment was repeated three times. Data on larval mortality are recorded daily.

3. Result and Discussion
3.1. Insecticide Nanoemulsion Analysis
The results of the analysis of nanoemulsion using Particle Size Analyzer (PSA) with Zetasizer Nano ZS Malvern tools are varied, can be seen in Table 1.

| Nanoemulsion | Particle size (nm) | Polydispersity Index | Zeta Potential (mV) |
|--------------|--------------------|----------------------|---------------------|
| Nanoemulsion P. aduncum ethyl acetate solvent (EA) | 216,7 | 0,371 | -22,5 |
| Nanoemulsion P. aduncum acetone+methanol solvent 20% (AM 20) | 102,2 | 0,267 | -28,6 |
| Nanoemulsion P. aduncum acetone+methanol solvent 10% (AM 10) | 69,88 | 0,202 | -28,7 |
| Nanoemulsion P. aduncum ethanol 70% solvent (E) | 18,56 | 0,212 | -0,410 |
Based on nanoemulsion particle size, there is an influence of the type of solvent. Each nanoemulsion with different solvents has different particle sizes. The particle size of the solvents using EA, AM 20, AM 10, and E emulsions decreased from 216.7 nm, 102.2 nm, 69.88 nm, and 18.56 nm. It is estimated that the emulsion with smaller particle sizes, the solubility level of all the materials with the solvent is higher than the emulsion with larger particle size.

The polydispersity index indicates the quality of uniformity of an emulsion dispersion. Small polydispersity index values (≤0.5) show a narrow particle size distribution, meaning the particle size is increasingly uniform [14]. In the results of table 2, the polydispersity index values of the largest are 0.267 (AM 20), 0.212 (E), and 0.202 (AM 10). The evidence shows that all nanoemulsions produced have uniform particles.

Zeta potential has the produce an electrical repulsion force between oil particles that can inhibit agglomeration between particles. In general, particles with a zeta potential value exceeding +30 mV or less than -30 mV show stability, because the electric charge of the particles is strong enough to resist the dominant particles in the nanoemulsion dispersion system [15]. The range of potential zeta values produced by the four nanoemulsions above is -0.41 mV, -22.5 mV, -28.6 mV and -28.7 mV. Potential zeta values of less than -30 mV indicate that all nanoemulsions produced have less stable particles.

Table 2. Distribution width Analysis of the insecticidal nanoemulsion based on solvents

| Nanoemulsion                        | Dv 10 (nm) | Dv 50 (nm) | Dv 90 (nm) | Dv 100 (nm) |
|------------------------------------|------------|------------|------------|-------------|
| Nanoemulsion *P. aduncum* ethyl acetate solvent (EA) | 37,8       | 258        | 5570       | 7460        |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 20% (AM 20) | 49,1       | 172        | 5380       | 7460        |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 10% (AM 10) | 32,9       | 53,2       | 4700       | 7460        |
| Nanoemulsion *P. aduncum* etanol 70% solvent (E) | 8,11       | 12,1       | 20,5       | 7460        |

Based on Table 2 distribution width, it can be seen that the type of solvent with E code produces the lowest particle size distribution when compared to other solvent nanoemulsions, namely 8.11 nm (Dv 10), 12.1 nm (Dv 50), and 20.5 nm (Dv 90). The particle size distribution were compared to other
solvent nanoemulsions, namely 37.8 nm (Dv 10), 258 nm (Dv 50), and 5570 nm (Dv 90), respectively. This data is comparable to the data in Table 1, where the E solvent has the smallest particle size and EA solvent has the largest particle size. Most probably, the spontaneous emulsification technique in nanoemulsion processed with the highest distribution brought a lack of homogeneity, especially when the solution was stirred using a magnetic stirrer. It indicates that the formation of the nucleus had not run entirely [16].

3.2. Larval Mortality
The mortality of *C. pavonana* larvae was observed every day at the same time, every application time for approximately a week. Analysis of larval mortality data treated with several concentrations showed different results.

| Treatment | Mortality (%) |
|-----------|---------------|
| Nanoemulsion *P. aduncum* paralut et ethanol 70% solvent (E) concentration 100% | 100 |
| Nanoemulsion *P. aduncum* ethanol 70% solvent (E) concentration 50% | 96.67 |
| Nanoemulsion *P. aduncum* ethanol 70% solvent (E) concentration 25% | 96.67 |
| Nanoemulsion *P. aduncum* paralut ethanol 70% solvent (E) concentration 0.0% | 0 |
| Nanoemulsion *P. aduncum* ethyl acetate solvent (EA) concentration 5.0% | 100 |
| Nanoemulsion *P. aduncum* ethyl acetate solvent (EA) concentration 1.0% | 100 |
| Nanoemulsion *P. aduncum* ethyl acetate solvent (EA) concentration 0.5% | 100 |
| Nanoemulsion *P. aduncum* ethyl acetate solvent (EA) concentration 0.0% | 56.66 |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 20% (AM 20) concentration 1.0% | 100 |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 20% (AM 20) concentration 0.5% | 100 |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 20% (AM 20) concentration 0.0% | 0 |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 10% (AM 10) concentration 1.0% | 93.33 |
| Nanoemulsion *P. aduncum* acetone+methanol solvent 10% (AM 10) concentration 0.5% | 90.00 |
Table 3 presents pieces of evidence that the death of larvae is quite high due to the treatment of *P. aduncum* nanoemulsion insecticide. The average mortality of larvae is ≥ 90.00%, and at a concentration of 0.0% (control treatment) is 0% except for ethyl acetate nanoemulsion solvents. That was suspected because the ethyl acetate solution was powerful, so they can kill the larvae. Therefore nanoemulsion of ethyl acetate solvent is not recommended and not proceed to further testing. From Table 3 also presents that nanoemulsion treatment of insecticides provides a better role in suppressing the growth and development of *C. pavonana* larvae at high concentrations.

The presence of active compounds contained in the poisonous *P. aduncum* fruit nanoemulsion is the cause of death of *C. pavonana* larvae. Very significant difference with the control treatment (0.0% concentration), larvae look healthy and fit from day to day of observation until larvae reach instar IV. This is due to the absence of toxic active compounds in the control treatment (containing 70% ethanol and Tween 80, both ingredients also contained in *P. aduncum* insecticide nanoemulsion treatment).

Healthy larvae are characterized by large meals and larvae that change in size to become larger and active. While the larval mortality in nanoemulsion treatment was characterized by larvae not actively moving, the body color became blackish brown, until it finally died. Dilapiol compounds contained in *P. aduncum* fruit inhibit the action of cytochrome P450 polysubstrat monooxygenase (PSMO) enzymes, which function from reducing the toxic power of toxic metabolites in larval bodies. The inhibition of the function of the enzyme causes the insect to become paralyzed and eventually die [3].

The nanoemulsion particle size also affects the mortality of larvae. The smaller the emulsion particle size, it will be easier to enter and be absorbed by the leaves, and conversely the larger the emulsion particle size, it will be hard to enter and be absorbed by the leaves. The same thing was, which states that the size of droplets from active ingredients of smaller nanoemulsion formulas makes active ingredients enter the plant tissue and reach the target immediately [17]. The smaller nanoemulsion size has the function of stabilizing kinematic nanoemulsions. It prevents sedimentation and creaming when the nanoemulsion is in stored [18].

### 4. Conclusions and Implications

#### 4.1. Conclusion

Nanoemulsion from *P. aduncum* fruit has an size ranging from 18.56 to 216.7 nm, its polydispersity index ranging from 0.202 to 0.371, zeta potential ranging from -0.410 to -28.7 mV, and distribution width ranging from 8.11 to 5570 nm. It has insecticidal activity against *C. pavonana*.

#### 4.2. Implications

The implications of this preliminary can be continued at the advanced test stage so that it can be used as pest control for *C. pavonana* cabbage larvae.

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