Design of nanoemulsion of *Tephrosia vogelii* extract as botanical insecticide to control cabbage pest

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Abstract. Nanoemulsion is a technology that can improve the performance of botanical insecticides. Nanoemulsion can increase the stability of active substances, prevent the decomposition of active ingredients by microorganisms, allows to activate ingredients directly on the target site, which aims to reduce damage. This research was conducted at the Sumatra Biota Laboratory, Faculty of Pharmacy and Insect Bioecology Laboratory, Faculty of Agriculture, Andalas University, Padang, from June to August 2018. The purpose of this research was to obtain *T. vogelii* formulations in the form of nanoparticles using spontaneous emulsification as a botanical insecticide that can control cabbage pests of *Crocidolomia pavonana* larvae. The result of this research showed the particle size nanoemulsion of formula A is 206.7 (nm) formula B is 432.6 (nm). The distribution of formula A nanoemulsion particles are classified as polydispersion, and Formula B is monodispersed. Mortality larvae instar 2 *C. Pavonana* in both formulas showed good results, namely >50%. This shows that nanoemulsion *T. vogelii* is very effective for controlling *C. pavonana* cabbage pests.

1. Introduction

Nanotechnology is the science or technology that studies very small particles (one by one billion meters) using a nanometer scale (nm), then manipulation to produce objects/substances with new functions of special character. Simply put, nanotechnology is the manipulation of matter on an atomic scale. Countries in Asia such as China, Korea and Thailand nationally have also established strategies for developing nanotechnology. Indonesia, with sufficient natural wealth and the fourth largest population in the world, should contribute actively to the development of nanotechnology. Nanotechnology is believed to provide added value in the agricultural sector (agro-industry). In the last decade, agricultural scientists have been very active in conducting research related to nanotechnology[1].

Botanical insecticides active ingredients derived from plants that are easily degraded in nature and are selective so that they are safe against non-target organisms and the environment. Botanical insecticides do not quickly cause resistance, can be combined with other pest control techniques, the application process simple to reduce reliance on synthetic insecticide products. This botanical
insecticide raw material can also be obtained easily and cheaply. Various types of Indonesian flora are abundant biological species that have insecticidal properties. Thus there is a great opportunity to get insecticidal products from plants [2].

Plants that have been known to have potential insecticidal activity are Tephrosia vogelii. The T. vogelii leaves contain isoflavonoid compounds such as rotenone, deguelin and tefrosin. Rotenon works as a toxic for cell respiration which inhibits electron transfer in NADH-coenzyme ubiquinone reductase (complex I) from the electron transport system in the mitochondria. This is will eventually lead to paralysis of various muscular systems and other body tissues in insects which eventually lead to death.

The formulation of botanical insecticides in the form of Emulsifiable concentrate (EC) and Wettable Powder (WP) has not had good physicochemical stability, because it is easily decomposed by sunlight and microorganisms. This application of botanical insecticide formulations must also be carried out continuously, so it is not efficient. Besides, more than 90% of the pesticides applied are not on the site; this does not only have an impact on the ecosystem, nor is it efficient in the application costs incurred by farmers. One technology that can improve the performance of botanical insecticide mix formulations is nanotechnology[3].

The development of nanotechnology in insecticides can improve the efficiency of the use of insecticides themselves. Some of the advantages of nanotechnology-based botanical insecticides that increase the stabilization of active substances, increase the surface area of the application, facilitate the systemic activity, reduce waste of organic solvents, protect active ingredients from decomposition by microorganisms and sunlight, improve solubility, prolong the persistence of active ingredients, and increase stability physic chemist formulations. Furthermore, the use of pesticides directly on the target will minimize the development of resistance mechanisms in pests and reduce the death of non-target insects. This certainly will have a positive impact on agricultural production, because many previous cases where certain pests explode due to inappropriate use of pesticides. Potential of botanical insecticides made from T. vogelii nanotechnology-based is very large, for that it is necessary to do research related to the development of botanical insecticide nanotechnology which is very effective and efficient in controlling pests[4].

2. Materials and methods

2.1 Extraction of T. vogelii
Extraction of T. vogelii was carried out by immersion or maceration method using ethyl acetate solvent. Ethyl acetate solvent was chosen because it refers to previous research that has been done by [Lina et al. 2014]. Powdered extract material taken from 100 grams was put into an Erlenmeyer flask, and ethyl acetate solvent was added as much as 1000 ml. The composition according to previous research. This soaking is left for 2 × 24 hours. Then the extracted liquid was filtered two times using a glass funnel (9 cm in diameter) with ordinary filter paper on filtering I and whatman nom or 41 filter paper in filter II. The sieve is collected in a gourd flask, then evaporated with a rotary evaporator at a temperature of 50ºC and a pressure of 240 mbar. The solution obtained from evaporation was used to re-soak the plant extract for four times. The extract obtained is then stored in the refrigerator at 4 ºC until it is used for testing and analysis of active compounds[5].

2.2 How to make nanoemulsion
Making nanoemulsions using spontaneous emulsification techniques. Emulsion system consists of an organic phase in the form of extracts of T. vogelii, surfactant, and (tween 80) 3% as an emulsifier. The first thing to do was: Water phase (sterile distilled water + tween80) was homogenized using a 1,500 rpm speed homogenator while stirring using a magnetic stabilizer for 35 minutes. Then the organic phase is prepared, in the form of extracts of T. vogelii + carrier material (1:1). After the water phase is ready in the stirrer, then do penetesan organic phase into the aqueous phase by hatching
(dropwise). When dripping the organic phase into the water phase, the water phase remains in the UK by using a magnetic stirrer. Next, the homogenization process was carried out for 45 minutes.

2.3 Zeta potential analysis and nanoemulsion particle size
Zeta potential values and nanoemulsion particle sizes were analyzed using Zetasizer Nano ZS Malvern. Two drops of nanoemulsion sample were dissolved in 20 mL of distilled water in a beaker. Several liquids are then put into the cuvette and placed into the PSA slot. The PSA device is then operated until particle size data is obtained. Zetasizer then measures the zeta potential value. From the PSA analysis, the average particle size and particle size distribution are stated in the polydispersity (IP) index. The best emulsion formula was determined based on the best characteristics (particle size, polydispersity index value, zeta potential) using light-scattering Particle Size TM Analyzer Delsa Nano C (Beckman Coulter, France).

2.4 Mortality of C. pavonana
Mortality of C. pavonana larvae was observed every day at 4:00 p.m. (depending on application time) until the larvae reach instar 4. Observation of larval mortality is recorded in the logbook. Observations were made by calculating larval mortality using the formula:

\[ M = \frac{n}{N} \times 100\% \]

Description:
M = Larval mortality (%)
\( n \) = Number of dead larvae
\( N \) = Number of larvae treated (10 tails)

3. Result and discussions

3.1 Nanoemulsion of botanical insecticides.
Making of nanoemulsion in this research is to use the Emulsion Inversion Point (EIP) method, which is classified as a low energy method. The EIP method is carried out by utilizing a catastrophic emulsion phenomenon which can change the emulsion from the water in oil (W/O) phase to the oil phase in air (O/W) and vice versa (Figure 1).

Figure 1. Schematic of the Emulsion Inversion Point method (Ostertag et al. 2012).

Figure 1. The manufacturing process involves the aqudest sterile+ tween80 water phase, the oil phase in the form of T. vogelii + ethanol extract, which is homogenized with a magnetic stirrer. The oil phase added little by little in the water phase will form an oil-in-water
(O/W) emulsion system. Along with the addition of the oil phase of the emulsion system will be turned into a water-in-oil emulsion (W/O).

Based on the research that has been done, the composition of nanoemulsion formulation is obtained as follows:
Table 1. Composition of Nanoemulsion Formulation

| Formula          | Formulation Composition |
|------------------|-------------------------|
|                  | Extract(gr)/25 ml | Ethanol(ml)/25 ml | Tween80(ml)/25ml | Water(ml)/25ml |
| A. T. vogelii 10%| T. vogelii 1.00     | 2.00              | 0.09             | 26.10          |
| B. T. vogelii 20%| T. vogelii 2.00     | 3.00              | 0.09             | 23.10          |

The composition of the organic phase plays an important role in nanoemulsion. The organic phase composition used in the study was formula A as much as 10% and Formula B 20%. The greater the amount of organic phase added, the more turbid the resulting solution will be cloudy (Figure 2). The emulsifiers used are Tween 80. Tween 80 is the best emulsifier that will produce a particle size smaller than in nanoemulsion with Tween 20 and Tween 85. The nanoemulsion particle size was measured with a particle size analyzer (PSA)[6].

![Nanoemulsion extract T. vogelii formula A and B](image)

3.2 Size and zeta nanoemulsion T. vogelii potential

Particle size analysis in this study includes the determination of particle size distribution (volume, number, intensity, distribution) and zeta potential, as follows:

Table 2. Potential particle and zeta sizes

| Formula          | Extract level (gr) | Average Particle size (nm) | Polydispersity Index | Average Zeta potential |
|------------------|--------------------|-----------------------------|----------------------|------------------------|
| A. T. vogelii 10%| T. vogelii 1.00    | 206, 7                      | 0, 370               | (-) 9, 19              |
| B. T. vogelii 20%| T. vogelii 2.00    | 432, 6                      | 0, 186               | (-) 11, 1              |

From the results of testing particle size distribution using Particle Size Analyzer it turns out, both formula A and formula B produce particle size in nanoparticle size ranges (1-1000 nm) and have met the requirements of nanoparticles. Increasing concentration in the extract of T. vogelii has an impact on particle size reduction and zeta potential (Table 2). Formula A with T. vogelii extract content of 10% (1.00 g) from the organic phase gives smaller particle size and zeta potential than Formula B. It also shows that the Emulsion Inversion Point (EIP) method is classified as an energy method low this can be used as a method for making extract nanoemulsion. The distribution of nanoemulsion particles is stated in the polydispersity index (IP). The IP value describes the particle size uniformity in nanoemulsion. This is also one of the advantages of measuring with PSA so that it can describe the overall condition of nanoemulsion. The IP value divides nanoemulsion into several types, namely mono dispersion (IP ≤ 0.3), polydispersion (0.3 < IP ≤ 0.7), and super dispersion (IP>
A small IP value states that particles in nanoemulsion have a uniform size (Nanocomposix 2015). Nanoemulsion formula A has an IP of 0.370 while formula B is 0.186. Formula A is included in the polydispersion range, i.e. particles in formula A nanoemulsion have various particle sizes. Formula B is included in the mono dispersion range, indicating that particles are uniform in size[7].

Zeta potential is used to characterize the nature of the surface charge of nanoparticles and is also needed to determine the stability of nanoparticles. The zeta potential value above (+) 30 mV or below (-) 30 mV shows a stable colloidal system because the magnitude of the particle charge can prevent particle aggregation based on the electrostatic repulsion. From the measurement results show formula A has a zeta (-) 28.15mV potential value, and formula B has a zeta (-) 29.35mV potential value. From these data, it can be concluded that the colloid system formed tends to be stable because it has a high zeta potential value, even though it does not exceed (±) 30 mV.

3.3 Mortality of C. pavonana

Based on the research has been done, the average mortality of larvae, instar two which has been treated with T. vogelii extract nanoemulsion, is as bellow:

| Type of treatment   | Mortality (%) |
|---------------------|---------------|
|                     | Formula A     | Formula B     |
| 0.0% concentration  | 0             | 0             |
| 25% concentration   | 67            | 100           |
| 50% concentration   | 82            | 100           |

Larval mortality on 0.0% concentration did not make C. pavonana larvae death, and this indicates insects were healthy, fit and fulfilled the criteria as test insects. On treatment using 25% concentration can turn off larvae by 67% for formula A, formula B is 100%. 50% concentration turns of larvae in formula A by 82%, formula B 100%. Treatment concentrations can be affected by larval mortality. The insect mortality rate is directly proportional to the concentration of the treatment, the greater the concentration given by the meal, the greater the mortality rate in the test insect, in other words, the percentage of larval mortality increases with the increase in extract concentration. The most significant test larval mortality was in formula B treatment. Larvae died on nanoemulsion treatment resulted in smaller larvae, then the larval body gradually drifted, brownish-black. This is thought to be due to the content of the rotenoid active compound found in T. vogelii extract. According to Hollingworth, 2001 auto none works as a respiratory toxin by inhibiting electron transfer in NADH coenzyme ubiquinone reductase (complex I) from the transport electron system in mitochondria. This barrier will reduce ATP production, then inhibit cell activity, resulting in paralysis due to muscle and other tissues lacking in energy, and ultimately lead to death. The poisoned body of C. pavonana larvae extracted from T. vogelii appeared black, which reflected the occurrence of cell and tissue death. This shows that the preparation of T. vogelii extract nanoemulsion effective as a controller of C. pavonana pests[8].

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