Neem-based oil-in-water (O/W) emulsion as a biopesticide

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Abstract. Neem tree (Azadirachta indica) is well-known to contain active components, such as azadirachtin (AZA), nimbin, and nimbidin that are potent to eliminate pesky insects. These active components can be found in large numbers in the neem oil. The favourable properties of neem oil to kill pests with minimum impacts to the ecosystem makes it veritably attractive to replace synthetic chemical pesticide. In this research, the neem oil is mixed with water and emulsifiers in the homogeniser to generate oil-in-water (O/W) emulsions. The droplet size, the size distribution, the stability, and the effectiveness of emulsion are analysed to determine the best formulation of neem-based biopesticide. The results showed that the formulation of 10%-w neem oil and 5%-w non-ionic emulsifier Lutensol® TO 6 produces the most stable emulsion with d32 of 0.50 μm. The fastest mortality rate for Musca domestica larvae is achieved at 20 ppm and 8 minutes with emulsion formulae of 10%-w neem oil and 5%-w non-ionic emulsifier Lutensol® TO 8.

1. Introduction

The synthetic pesticide has been integrated into agriculture to increase farming productivity. Even some local fishermen in Indonesia use synthetic pesticides to prevent flies (Musca domestica) infestation during the fish fermentation process [1]. Per contra of its purpose to eliminate pests, the synthetic pesticides are very toxic and require months and even years to degrade [2]. The residues left will affect negatively to the ecosystem, mostly if human consumes them.

The neem tree (Azadirachta indica) is classified as antifeedant, in which the active substances contained in the plant can exert a detrimental effect on the insect’s life cycle [3]. Azadirachtin (AZA) is the primary active components that can be mainly found inside the neem seeds. The amount of Azadirachtin in neem seed kernels is around 0.2–0.8%-weight, while neem oil can take up 30–50%-weight of the total seeds [4]. Azadirachtin is tetrannortriterpenoids plant limonoid and has a molecular structure of C35H44O16. Around 200 species of insects are liable to AZA [5].

AZA works as poison for insects as it can disrupt the production of hormone ecdysone, consequently wither the insects and make them die in the early stage. AZA also can block and suffocate the breathing pores of insects. Therefore, the contact activity of pesticides is essential [6]. The minimum lethal dose (LD) of AZA is varied depending on the type of insects. The LD50 of Spodoptera littoralis larvae is 1.00 ppm [7], while Schistocerca gregaria insects LD100 is 0.05 ppm [8]. Since the insects’ lethal dose is much smaller than the amount of AZA in the neem oil, where commercial neem oil can contain up to 10000 ppm of AZA, the emulsion can be attractive form to produce neem oil as a biopesticide.
An emulsion is a colloid form of two or more immiscible liquids. The neem oil is dispersed in the water to form oil-in-water (O/W) emulsion with the addition of emulsifiers. Thermodynamically, an emulsion is considered an unstable system due to its natural tendency for the mixture to minimize its interfacial tension [10]. The stability of the emulsion depends on the several properties, such as type of emulsifiers, formulation, and mixing procedures. Non-ionic emulsifier as emulsifiers can be more effective in term of emulsification and stabilization [11]. However, emulsifiers can also create another problem to the ecosystem, since some of the emulsifiers are not environmentally friendly, especially if it is available in large scale.

The formation of the emulsion system requires energy to reach the new thermodynamically stable condition. The devices to provide such energy are simple pipe flow, rotor-stator system, and high-pressure valve homogenizers [11]. Various researches are conducted to create the neem oil-based emulsion. High energy sonication was used to formulate nanoemulsion of 31.03 nm, and it was proven that the emulsions were potent for Cx. Quinquefasciatus [12]. Ultrasonic waves were also incorporated to produce nanoemulsion with a size of 50.21 nm [13]. Vortex method was used to formulate nanoemulsion from 40%-w neem oil using Tween 80. The emulsion has a droplet size in the range of 208 – 507 nm [14].

The recent study aims to determine the best formulation of neem-based O/W emulsion that gives the best stability and high mortality rates in eliminating Musca domestica larvae in the fish fermentation process. The emulsification process conducted in the room temperature using a homogenizer. Aside from the droplet size measurement, the droplet distribution of the emulsion was analyzed to relate its properties and effectiveness in eliminating Musca domestica larvae.

2. Material and methods

Neem oil with 1%-weight of Azadirachtin was procured from Bali, Indonesia and stored at room temperature. Emulsifiers used are non-ionic surfactant, Lutensol® TO 6, TO 8, and XL 70 from BASF (Jakarta, Indonesia). Both Lutensol® TO 6 and TO 8 are considered environmentally friendly and made from saturated iso-C13 alcohol. Luthensol® XL 70 is alkyl polyethene glycol ethers and made from C10 Guerbet Alcohol. Lutensol® TO 6 has an HLB value of 11, while both Lutensol® TO 8 and XL 70 have HLB value of 13. Demineralized water was used for all experiments.

Hexane and cyclohexane solutions were used as a solvent during neem oil analysis; NaOH, KOH 0.1 N solution, and phenolphthalein were used to determine neem oil’s FFA and saponification value; glacial acetic acid, KI solution, iodine monochloride solution and Na2S2O3 0.1 N solution were used to determine Iodine content.

2.1. Neem oil and emulsifier analysis

Neem oil was tested to determine the moisture content, specific gravity, iodine value, FFA, saponification value, and viscosity. Moisture content was measured using a gravimetric method at 130°C ± 2°C for 30 minutes. FFA and Iodine values were determined by titration method. Saponification value was determined by back titration of potassium oxide in the presence of phenolphthalein. Viscosity was measured by a rotary viscometer.
The emulsifiers were tested for its emulsifying power. The emulsifying power of emulsifiers was conducted to determine the performance of each emulsifier to decrease the surface tension of water. The capillary rise of emulsifier and water mixtures with a different concentration between 10 – 5000 ppm were measured by a capillary pipe at room temperature. The surface tension was calculated with equation 1.

\[ \gamma = \frac{\rho gh r}{2 \cos \theta} \]  

\( \gamma \) = surface tension, \( \rho \) = density of sample, \( h \) = the height of rise, \( r \) = radius of capillary tube, \( \theta \) = contact angle.

2.2. Emulsification of neem oil
Neem based O/W emulsion was prepared using high-speed Homogenizer D-500 from Wiggen Hauser, Germany. The 10%-w of neem oil was mixed with demineralized water and various emulsifiers (TO 6, TO 8, and XL 70) of 5%-w and 10%-w at room temperature for 30 minutes.

2.3. Droplet size and distribution of emulsion
The droplet size and the droplet size distribution were measured with an Olympus CX22 Microscope equipped with Optilab Advanced Camera. The formulated emulsion was diluted with demineralized water before experimentation to eliminate the viscosity effect. Each droplet diameter of the emulsion was measured with Olympus CX22 Microscope, and droplets mean diameter was calculated using Sauter Mean Diameter (d_{32}) equation, where mean volume droplets (V_{droplets}) divided by mean droplets surface area (A_{droplets}). The droplet distribution was measured by calculating the frequency of the droplets to emerge at the various size. The width of droplet size distribution determined the uniformity of emulsions.

\[ d_{32} = \frac{V_{droplets}}{A_{droplets}} \]  

2.4. Stability and viscosity test of emulsion
Emulsion stability is determined through creaming test. Samples are stored for two weeks at room temperature; emulsion is considered stable if there is no creaming occurred. The heights of a different phase are compared in cases of creaming. The viscosity of the emulsion is measured using Ostwald Viscometer at room temperature.

2.5. Spreading and dispersion time of emulsion
Spreading and dispersion time are conducted to determine the applicability of the biopesticide. Both tests are carried at room temperature. For spreading time measurement, a circle with 3 cm diameter is drawn in a glossy paper. A drop of an emulsion is placed in the middle of the circle. The time required for the emulsion to spread is measured by stopwatch.

For dispersion measurement, 1 mL of an emulsion is dropped into 100 mL of water. The time required for the emulsion to spread and mix uniformly in the water is measured by stopwatch.

2.6. Larvacidal activity of emulsion
Larvacidal activity is tested to see the effectiveness of neem-based O/W emulsion as a biopesticide. This test is conducted at room temperature on 25 Musca domestica larvae with different concentration of emulsion. The time required for 100% mortality is measured by stopwatch.

3. Results and discussion
Neem oil was analyzed to determine the moisture content, density, iodine value, FFA, and saponification value, as shown below. Through Table 1, it was noticed that neem oil has high viscosity.
around 40.7 cP at 40 °C, while water has a viscosity of 1 cP. The high viscosity of neem oil reduces the spreadability of neem oil.

| No. | Parameter                          | Results     |
|-----|------------------------------------|-------------|
| 1   | Color                              | Yellow brownish |
| 2   | Smell                              | Like red onion |
| 3   | Moisture content (%-w)             | 0.33        |
| 4   | Insoluble Impurities (%-w)         | 0.14        |
| 5   | RI                                 | 1.468       |
| 6   | Spesific Gravity (at 24 °C)       | 0.939       |
| 7   | Iodine Value                       | 69.31       |
| 8   | Saponification value               | 195.19      |
| 9   | Unsaponifiable value (%-w)         | 1.31        |
| 10  | FFA                                | 9.3580      |
| 11  | Viscosity (cP) (at 40 °C)          | 40.7        |

3.1. Emulsifying power of emulsifiers
The stability of the emulsion is associated with interfacial film mobility [15]; thus, the presence of emulsifier is greatly vital in the emulsion formation. The interaction between emulsifier and oil/water phase creates distinct interfacial film, resulting in the reduction of interfacial tension.

\[ \text{Surface Tension} = f(\text{Concentration}) \]

Figure 2. Emulsion performances to reduce surface tension at various concentration

In this research, the emulsifying power of emulsifier was measured by mixing each emulsifier with water at different concentrations. The surface tension of the water mixture at room temperature was analysed by capillary pipe method. Pure water initially has a surface tension of 0.0722 N/m at room temperature.

All emulsifiers have HLB value larger than ten and indicate hydrophilicity. It can be seen in Figure 2 that TO 6 could lower the surface tension of water mixture better than TO 8 and XL 70. At a concentration of 10 ppm TO 6 could decrease water mixture surface tension to 0.044 N/m; while TO 8 and XL 70 only achieved 0.065 N/m and 0.0644 N/m, respectively. This might occur due to TO 6 adsorption in the air-water interface is stronger than TO 8 and XL 70.

Increasing the concentration of emulsifier could even lower the surface tension of the water mixture. For TO 8 and XL 70, the surface tension was no longer has significant differences after reach 1000 ppm. It concluded that the critical micelle concentration (CMC) for TO 8 and XL 70 is at 1000 ppm.
ppm. CMC is described as the emulsifier concentration above which micelles are created spontaneously. The formation of micelles dramatically decreases the system's free energy by reducing the contact between the emulsifier and hydrophobic water groups. The increment of emulsifier concentration above the CMC ensues in more micelle formation but hardly decreases the system's free energy. As for TO 6, surface tension still decreased to 0.0285 N/m even after the emulsifier concentration was increased to 5000 ppm. It indicated CMC still not yet obtained.

3.2. Droplet and size distribution of emulsion

Emulsifiers reduce interfacial tension, causing droplet size to decrease. The droplet size and the droplet size distribution could also be the stability proxy parameter for O/W emulsion. If the droplets are too large, it will easily lead to de-emulsification process (such as creaming, flocculence, and coalescence). Emulsion with smaller droplets size tends to be kinetically stable.

The quantity of emulsifier required to produce the smallest droplet size will depend on its activity or concentration in the medium phase, as given by Gibbs free energy, which determines the reduction of surface tension [11]. In fact, this theory is in line with the outcomes obtained. It can be seen in Table 2 that adding 5%-w of TO 6 as emulsifier produced emulsion with a droplet size of 0.50 μm. Increasing the emulsifier concentration to 10%-w decreased the size into 0.44 μm. Both TO 8 and XL 70 also obtained smaller droplet size when the emulsifier concentration was increased from 5%-w to 10%-w. However, despite having the same profile, non-ionic emulsifier Luthensol® TO 6 achieved the smallest droplet size compared to TO 8 and XL 70. It is known in Figure 2 that TO 6 has the ability in reducing the tension in the air/water interface much better than TO 8 and XL 70. This might also imply that TO 6 performs better in oil/water interface. Due to TO 6 strong adsorption in the oil/water interface, it is able to increase the interfacial area by lowering the surface tension during the emulsification process. Hence, the energy to break the droplet is also reduced. The equilibrium is achieved, and emulsifier will prevent droplets coalescence.

| Emulsifiers | Sauter Mean Diameter (d_{s2}) (μm) |
|-------------|---------------------------------|
| TO 6        | 0.50 0.44                       |
| TO 8        | 3.18 2.47                       |
| XL 70       | 2.60 2.12                       |

Figure 3 shows emulsion droplet size with a different type of emulsifiers at 5%-w formulae. It shows the emulsion is polydisperse or has different size of droplets. At 100x magnification, it can be seen that emulsion with TO 6 as emulsifier has fine droplet size. The polydispersity of the emulsion can be seen in the droplet size distribution graphs in Figure 4 to Figure 6. The graphs show that all droplet size distribution is unimodal, where only one peak appears. It also can be seen that fine
emulsions generated by TO 6 have narrow distribution compared to TO 8 and XL 70 emulsions. The narrow distribution implicates that polydisperse emulsion can be considered as monodisperse. The distribution of the emulsion droplet size is a significant characteristic that affects emulsion stability. An essential feature is the size of particles dispersed in the emulsions as it can alter the rheological behaviours and emulsion stability [10].

![Figure 4](image1.png)

**Figure 4.** Particle size distribution of emulsion with: (a) 5%-w TO 6 and (b) 10%-w TO 6

![Figure 5](image2.png)

**Figure 5.** Particle size distribution of emulsion with: (a) 5%-w TO 8 and (b) 10%-w TO 8

![Figure 6](image3.png)

**Figure 6.** Particle size distribution of emulsion with: (a) 5%-w XL 70 and (b) 10%-w XL 70
3.3. *Stability and viscosity test of emulsion*

The stability of emulsion becomes very important when it comes to storage. A stable emulsion is achieved when there are repulsive forces within droplets [16]. The unstable emulsion will breakdown due to cohesion between droplets; resulting in creaming, sedimentation, flocculation, and coalescence.

In this research, the stability of emulsion was measured through creaming test. One millilitre of the sample was stored for two weeks, then the phase separation was observed. The emulsion is considered stable when no phase separation appears. From Table 3, it can be seen that TO 6 emulsion is stable, while creaming occurs in the other emulsions. Creaming is generated due to gravity, which happens when there is a different density between the droplets and medium phase. As gravity force is proportional to droplet diameter, Brownian diffusion may exceed gravity at small droplet size and creaming is avoided [11]. Thus, TO 6 emulsion with the smallest droplet size will be more stable, as it can be seen in Table 3.

For TO 8 and XL 70, at 5%-w concentration the creaming appeared around 0.3 mL and 0.5 mL, respectively. Increasing the emulsifier concentration apparently created more unstable emulsion. This might be due to a larger density difference in oil and water phases that leads to phase separation.

| Emulsifiers | Emulsifiers Conc. (%-w) | Stability | Sample |
|-------------|-------------------------|----------|--------|
| TO 6        | 5                       | S        |        |
|             | 10                      | S        |        |
| TO 8        | 5                       | 0.3 ml CL|        |
|             | 10                      | 0.4 ml CL|        |
| XL 70       | 5                       | 0.5 ml CL|        |
|             | 10                      | 0.7 ml CL|        |

S = Stable Emulsion; CL = Creaming Layer

The droplet size has a significant influence on the emulsion rheology. As can be seen in Figure 7, fine emulsions result in higher viscosity. TO 6 emulsion, which is the finest emulsion, had viscosity of 8.72 cP and 9.42 cP at 5%-w and 10%-w concentrations. While TO 8 and XL 70 emulsions had a viscosity less than two cP for both 5%-w and 10%-w concentration. The increase of viscosity could be caused by the reduction of the mean distance between the droplets and leads to higher hydrodynamic
interaction and viscosity [17]. Another possible reason is that smaller droplets have narrow droplets distribution; thus, polydisperse emulsion tends to be more monodisperse. A high concentration of the dispersed phase can generate high viscosity.

![Viscosity of emulsion with various type and concentration of emulsifiers](image)

**Figure 7.** Viscosity of emulsion with various type and concentration of emulsifiers

3.4. Spreading and dispersion time of emulsion

From the previous results, fine emulsion with Luthensol® TO 6 is very stable, but it is very viscous. The high viscosity can be the drawback of this emulsion in terms of applicability. Therefore, spreading and dispersion time are measured to determine, whether fine emulsions has serious practical implications as a biopesticide.

Figure 8 shows the spreading and dispersion time for each emulsion. It is noticed that the time to spread and disperse for fine emulsion with TO 6 (5%-w) was 33 seconds, which almost thrice and twice of TO 8 and XL 70 emulsions at 5%-w concentration. This delay was due to high viscosity of TO 6 emulsion. When emulsifiers’ concentration was increased to 10%-w, TO 6 emulsion could no longer spread and disperse in the water, since it became very viscous and formed a gel-like in the water.

![Spreading and Dispersion time of emulsion](image)

(a) Spreading time and (b) Dispersion time of emulsion with various type and concentration of emulsifiers

3.5. Larvacidal activity

The larvacidal activity was assessed to determine the potential of neem-based O/W emulsion as a biopesticide for *Musca domestica* larvae. The larvacidal activity was tested using TO 6, TO 8, and XL 70 emulsions at various concentration. From Figure 9, it can be seen that the 100% mortality was achieved from 5 ppm of neem-based emulsion. However, TO 6 emulsion requires a longer time to
achieve 100% mortality, despite being the most stable emulsion. It is due to the high viscosity of TO 6 emulsions that leads to longer dispersion time. Increasing the concentration of neem-based O/W emulsion will accelerate the mortality time of *Musca domestica* larvae.

![Figure 9](image)

**Figure 9.** Larvacidal effects of neem based O/W emulsion to *Musca domestica* larvae using TO 6 and TO 8 as emulsifiers.

### 4. Conclusion

Azadirachtin (AZA) is an active ingredient for pests control mostly found in the neem oil. Neem based O/W emulsion was generated by mixing neem oil, water, and emulsifiers. The type and concentration of emulsifiers play an important role in emulsion stability. Non-ionic emulsifier Lutensol® TO 6 gave the best emulsion stability with no creaming and smallest droplet size (0.44 μm). However, smaller droplets resulted in an increase of viscosity. Consequently, it generated longer spreading and dispersion time, thus lowering the larvacidal mortality rates. The fastest spreading and dispersion time increased larvae mortality rates. Further researches need to be conducted to find emulsifiers that can maintain emulsion stability without lowering the larvacidal activity.

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