Application of diethanolamide surfactant derived from palm oil to improve the performance of biopesticide from neem oil

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Abstract. The purpose of this research was to improve the performance of organic pesticide derived from neem plant using diethanolamide surfactant (DEA) derived from palm oil in controlling armyworms. The pesticide was made of neem oil. Neem oil is a neem plant product containing several active components, i.e. azadirachtin, salanin, nimbin, and meliantriol which act as a pesticide. DEA surfactant acts as a wetting, dispersing and spreading agent in neem oil pesticide. The neem oil was obtained by pressing neem seeds using a screw press machine and a hydraulic press machine. DEA surfactant was synthesized from methyl esters of palm oil olein. Pesticide formulation was conducted by stirring the ingredients by using a homogenizer at 5,000 rpm for 30 minutes. Surfactant was added to the formulation by up to 5%. Glycerol, as an emulsifier, was added in to pesticide formulations of neem oil. The efficacy of the pesticides in controlling armyworms fed soybean leaves in laboratory was measured at six concentrations, i.e. 10, 13, 16, 19, 22, and 25 ml/L. Results showed that the neem oil used in this study had a density of 0.91 g/cm³, viscosity of 58.94 cPoise, refractive index of 1.4695, surface tension of 40.69 dyne/cm, azadirachtin content of 343.82–1.604 ppm. Meanwhile, the azadirachtin content of neem seed cake was 242.20 ppm. It was also found that palmitic (31.4%) and oleic (22.5%) acids were the main fatty acids contained in neem oil. As the additive material used in neem oil in this study, diethanolamide surfactant had a pH of 10.6, density of 0.9930 g/cm³, viscosity of 708.20 cP, and surface tension of 25.37 dyne/cm. Results of CMC, contact angle, and droplet size analyzes showed that diethanolamide surfactant could be added into insecticide formulation by 5%. Results of LC tests showed that on Spodoptera litura the LC₅₀ and LC₉₅ values were 13 and 22 ml/L, respectively. Neem oil was found to inhibit the development of Spodoptera litura and its larval molting process.

1. Introduction
Surfactant is a compound which has an ability to reduce surface tension of a medium. It can be produced biochemically or in a synthetically chemical way. It is able to reduce surface tension because it has both hydrophilic (like water) and hydrophobic (like fat/oil) groups. These polar and non-polar parts of surfactant make it able to make water and oil mix well in a mixture. According to [1], in general, the functions of a surfactant are to reduce surface and interfacial tension, to increase the stability of dispersed globules, and to control the formation type of emulsion, such as oil in water (O/W) or water in oil (W/O). Today, surfactant is used as a component of adhesive material, coagulating agent, wetting agent, foaming agent, emulsifier, penetrating agent, and dispersing agent [2]. Diethanolamide surfactant is a surfactant whose molecules have no charges and its hydrophilic and hydrophobic prop-
properties are resulted from the existence of ester oxygen and hydrocarbon ester groups. Hydrocarbon groups consist of carbon-carbon and carbon-hydrogen bonds. In organic molecules, these bonds are non-polar bonds and hydrophobic. As these parts get longer, the solubility of the molecules in water is low. According to [3], alcohol and ester groups consist of polar molecules. This property makes ester oxygen groups hydrophilic. Diethanolamide can be produced in two ways, namely through (1) the reaction between methyl ester and diethanolamide and (2) reaction between fatty acid and diethanolamide.

Insecticide from neem oil has been long known and used by farmers to organically control pests in plants. Among other plant insecticides available in the market today, neem oil is the one which has low toxicity for beneficial organisms. This has made it a promising plant insecticide [4]. The target pests of neem oil include A. culicifacies [5], Ceraeochrysa claveri [6,7], Diaphorina ribesi [8], Mamestra brassicae [9], Nilaparvata lugens Stal [10], Pieris brassicae [11], Limnoperna fortunei (Dunker 1857) [13], and Spodoptera frugiperda [13].

Neem oil is extracted from seeds of neem plants. Neem plants belong to the family of Meliaceae and are found to be limited in Indonesia and the Philippines-Azadirachta excelsa Kack [14]. Today, neem oil is well known all over the world as a source of phytochemicals for human health and pest control. It contains at least 100 active components but triterpenoid, better known as limonoids, is the main component and azadirachtin which has a pesticide effect is the most important component. Azadirachtin has a molecular weight of 720 g/mol [4]. In Indonesia, neem plants are found alongside the north coast of Java, stretching from Indramayu to Banyuwangi. In addition, they are also found in Nganjuk, Jombang, Blitar, Ponorogo, Madiun, Bojonegoro, Bondowoso, Gianyar, Negara, and East Lombok [15].

One of the disadvantages of neem oil is that it is less stable under the field condition as a result of its high photodegradation level, short staying time, and slow killing power compared to non-plant pesticides [16, 17, 18]. Environmental factor and method of extraction gave significant effects on the composition of neem oil. As a result, there is no standard active material in the composition of this plant insecticide and this restricts the use of it in agricultural pest control [19, 20, 21]. Therefore, in its application, an additive material which can stick, wet, and disperse it to all parts of leaves, is required to make pest control more effective. One of the additive materials that can be used for this purpose is diethanolamide (DEA) surfactant. This study was aimed at improving the performance of organic pesticide made of neem seed oil by using DEA surfactant from palm oil in controlling Spodoptera litura pests.

2. Materials and Methods

2.1 The process of neem oil production and the analysis of its physicochemical properties
Dried neem seeds were pressed by using a screw press. The cake produced from this pressing process was repressed to take the remaining oil in the cake out. The crude oil obtained from this pressing process was left alone for 24 hours to separate the sediment which was still found in the crude oil. The sediment was then pressed by using a hydraulic press to take the oil out from the sediment. This crude oil was then analyzed for its azadirachtin content and physicochemical properties. Measurements were taken on azadirachtin content, refractive index, optical rotation, density, viscosity, surface tension, solubility, and contact angle. The analysis of physicochemical properties of neem oil was done at the laboratories of Surfactant and Bioenergy research Center of Bogor Agricultural University and Center of Agro industries, Indonesian Ministry of Industry.
2.2 The process of DEA surfactant production and the analysis of its physicochemical properties

DEA surfactant was produced by using an amidation reactor of 25 L/batch scale. The surfactant was synthesized by reacting methyl ester of palm oil with diethanolamide at the mole ratio of 1:2 by using 1% NaOH 30% as a catalyst at 140°C and the speed of 100-200 rpm for 3-5 hours.

2.3 Determination of optimum concentration of DEA surfactant as an additive material in the formulation of plan insecticide from neem oil.

This trial was done to obtain the concentration of DEA surfactant that could dissolve neem oil to form a stable emulsion as indicated by its droplet size analyzed by using a ICC 50 HD microscope (1000 x magnification), had small contact angle as analyzed by using a Contact Angle Analyzer Phoenix 300, and had a small surface tension as analyzed by a Critical Micelle Concentration (CMC) method. The common way of CMC determination is done by measuring the surface tension of surfactant solution as the function of concentration. DEA surfactant was dissolved in neem oil in the concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%. The surface tension of these surfactant solutions was measured by using a Spinning Drop Tensiometer at Surfactant and Bioenergy Research Center, Bogor Agricultural University.

2.4 Formulation of neem insecticide by using DEA surfactant

Insecticide formulation process was done by using a homogenizer. The formulation was made by mixing 90-95% neem oil, 1-10% DEA surfactant (in accordance with DEA surfactant concentration test results), and 1-5% supporting material to improve the stability of pesticide emulsion. The formulation process was done for 30-60 minutes at the speed of 5,000-6,000 rpm. Glycerol as much as 3% was used as the supporting material in this study.

2.5 Assessment of the effects of lethal concentration of neem formula on S. litura larvae

The resulted insecticide formula was tested at six concentration rates (10, 13, 16, 19, 22, and 25 ml/l) expected to cause death of 15-95% tested insects. Each insecticide formula was diluted with distilled water containing surfactant at the concentration similar to that of each neem oil formula. As a control, distilled water containing surfactant at the concentration similar to that contained in each formula was diluted to reach the concentration of 25 ml/l. For each treatment, 60 instar III larvae of Spodoptera litura were put into 6 petri dishes (10 in each petri dish). The tissue paper put in the lower and upper parts of each dish was wetted every day in order to maintain the moist of the leaves in the dishes. On day-2 and 4, treatment or control leaves were added. Observation on the mortality of tested insects was done on day-9.

3. Results

3.1 Neem oil production and the analysis of its physicochemical properties

The extraction process of neem oil was done by using screw and hydraulic pressing methods resulted in an oil yield of 12.3%. This analysis was aimed at assessing the properties of neem oil produced. The physicochemical properties of neem oil are listed in Table 1. It was shown that neem oil produced in this study had 0.91 g/cm³ density, 58.94 cPoise viscosity, and 1.4695 refractive index. As a comparison, at 25°C water and olive oil have viscosity of 0.890 and 81 cPoise, respectively.

Neem oil produced in this study was found to have a surface tension of 40.69 dyne/cm which was considered to be higher than those (25-35 dyne/cm) of commercial pesticides available in the market. High level of surface tension of neem oil has made the direct application of this oil in the field was less effective as neem oil could not dissolve in water nor spread evenly to the surface of leaves. High surface tension also made neem oil applied to the plants easy to fall down or evaporate as it could not attach to the leaves. This made the spraying of this oil, particularly during rainy season, a waste as the oil was easily washed away by the rain.
Table 1. Physicochemical properties of neem oil

| No. | Properties                             | Value                  |
|-----|----------------------------------------|------------------------|
| 1   | Density                                | 0.91 g/cm³             |
| 2   | Iodine number                          | 74.5 g Iodine/100 g    |
| 3   | Saponification number                  | 189 mg KOH/g           |
| 2   | Viscosity                              | 58.94 cPoise           |
| 3   | Surface tension                        | 40.69 dyne/cm          |
| 4   | Refractive index                       | 1.4695                 |
| 5   | Optical rotation                       | Not measurable/dark    |
| 6   | Azadirachtin content of neem oil       | 343.82—1,604 ppm      |
| 7   | Azadirachtin content of neem seed cake | 242.20 ppm             |
| 8   | Fatty acid composition (%)             |                        |
|     | Butyric (C4)                           | 0.98                   |
|     | Lauric (C12)                           | 7.29                   |
|     | Palmitic (C16-0)                       | 31.4                   |
|     | Stearic (C18-0)                        | 9.45                   |
|     | Oleic (C18-1)                          | 22.5                   |
|     | Linoleic (C18-2)                       | 10.2                   |
|     | Linolenic (C18-3)                      | 1.94                   |

Results of the HPLC analysis showed that the azadirachtin content of neem oil was 343.82–1,604 ppm while that of neem seed cake resulted from the pressing process was 242.20 ppm. These results were lower than the azadirachtin content found by [22], namely 0.1-0.5% of dried neem seed weight. Meanwhile, palmitic (31.4%) and oleic (22.5%) acids were found as the main fatty acids of neem oil.

3.2 Physicochemical properties of DEA surfactant
DEA surfactant produced in this study was found to have pH of 10.6, density of 0.9930 g/cm³, viscosity of 708.20 cP, and surface tension of 25.37 dyne/cm. These were in line with what was found by [23]. The pH level of diethanolamide used by Kirk-Othmer was 9-10. The value of surface tension of DEA surfactant produced in this study indicated that this surfactant could lower the surface tension of water by 61-65% from 72 dyne/cm.

3.3 Determination of optimum concentration of DEA surfactant as an additive material in the formulation of plant insecticide from neem oil

a. CMC Analysis on surface tension
Determination of optimum concentration of DEA surfactant by using a CMC analysis in order to assess the highest reduction in the surface tension of neem oil. It was shown that the addition of DEA surfactant at the concentration of 5 and 6% could lower the surface tension of neem oil from 40.69 to 23.82 dyne/cm. The addition of DEA surfactant higher than 6% was found to make the surface tension of neem oil increase back. According to [24], the addition of surfactant to a solution would lower the surface tension of the solution. After a certain concentration was reached, the surface tension would stay constant even though the concentration of surfactant was increased. The adding of surfactant to the amount that exceeded this concentration would make the surfactant aggregate to form micelles. The concentration when meselles start to form is known as the Critical Micelle Concentration (CMC). Surface tension will keep lowering down until CMC is reached. Once CMC is reached, surface ten-
sion will remain constant indicating that the interfacial is saturated and the formed micelles are in their dynamic equilibrium state with their monomers.

![Graph showing surface tension values of neem oil at various concentrations of DEA surfactant](image)

**Figure 1.** Surface tension values of neem oil at various concentrations of DEA surfactant

CMC is a standard parameter in the characterization of surfactant solution [25] as, in general, it indicates the minimum concentration needed to obtain surfactant association structure. A number of studies on the methods or techniques of CMC determination and factors affecting CMC of various types of surfactants have been done. The common methods or techniques used to determine CMC are based on surface tension [26], capillary electrophoresis [27] and optical density [28]. Theories have also been developed to explain the occurrence of CMC [29,30,31].

b. Analysis of Contact Angle

The analysis of contact angle was done to determine the contact angle of formulation which was diluted to 1%. This analysis was necessary to assess the ability of DEA surfactant in spreading neem oil on the surface of leaves. The graph of the decrease in contact angle of neem oil is depicted in Figure 2.

A contact angle is an angle formed between the surface of tested material (leaves) and neem oil dropped onto the surface of the tested material. The mean contact angles resulted from the addition of DEA surfactant to neem oil solution were about 17.82 to 52.52°. It is shown in the above graph that the addition of DEA surfactant at a concentration higher than 5% resulted in smaller contact angles. This indicated that the solution of neem oil was able to spread well on the surface of the leaves resulting in very small contact angles. Insecticide formulation solution was expected to have small contact angles as small as possible to make it able to attach to and spread over the surface of targeted objects. A contact angle close to 0° shows that neem oil droplets do not only attach to but also spread over well on the leaves. A contact angle close to 90° indicates that neem oil droplets are able to attach to the leaves but they do not spread well on the leaves. A contact angle close to 180° indicates that neem oil droplets do not attach to the leaves at all and even they directly slip down from the leaves.

The mixture of neem oil and surfactant with small contact angle indicated that the mixture spread over the leaves well when it is applied by farmers in the field. Therefore, DEA surfactant produced in this study could be utilized as a spreading and wetting agent in insecticide formula. Solution containing surfactant spreading all over the leaves could penetrate and spread over the fine hair on the surface through a capillarity force, while the droplets of solution containing solution with no surfactant did not spread but only attached to the surface of leaves [32].
c. Analysis of droplet size

This analysis was done to determine the droplet size of the emulsion formed by the mixture of neem oil and DEA surfactant diluted in water until the formula concentration was 1%. The analysis was done to ensure that the emulsion system of formulation product had a micro size. Small-sized droplets made the emulsion more kinetically stable so that sedimentation and creaming during storage could be avoided [33]. The graph showing the decrease in droplet size as a result of the addition of DEA surfactant produced in this study is depicted in Figure 3.

Emulsion is a preparation containing two phases, usually water and oil, which are not mixed. In an emulsion, a liquid is dispersed into droplets and the other liquid is stabilized with an emulsifier or a suitable surfactant [34]. An emulsion system is generally easy to get damaged by the addition of energy or by the passing by of time. This problem can be overcome by making the size of droplets smaller and by using a stabilizing agent such as surfactant. Small-sized droplets can be reached through the use of appropriate ratio of surfactant and oil and right temperature. In this study, it was found that the mixture of neem oil and DEA surfactant diluted in water to a concentration of 1% had mean droplet size of 1.61 to 8.17 µm. Smaller size droplets were obtained when higher concentration of DEA surfactant was added into neem oil.

As stated by [35], the length of hydrocarbon chains of a surfactant also affects the performance of surfactant in emulsion formation. A surfactant with long hydrocarbon chains increases the protective role of steric in minimizing the possibility of particle or droplet mergers. Olein, as a synthetic feedstock of DEA surfactant in this study, is composed dominantly of long chain fatty acids. Therefore, it was found in this study that the size of droplets formed was smaller as more DEA surfactant was added.

![Figure 2. Effects of DEA surfactant concentration in neem oil on contact angles](image-url)
Figure 3. Effects of DEA surfactant concentration in neem oil on droplet size

3.3 Lethal Concentration Test of Selected Insecticide Formula

Results of determination of optimum concentration of DEA surfactant as an additive material in the formulation of plant insecticide from neem oil showed that DEA surfactant concentration of 5% was the most effective in lowering the surface tension and contact angle of neem oil. This determination was made as no statistical differences were found in the effectiveness of DEA surfactant concentration of 5 and 6% in lowering the surface tension and contact angle of neem oil.

Results of the application test of insecticide formula of neem oil by using 5% DEA surfactant showed that the mortality rates of tested insects at 2, 4, 6, and 9 days after treatment (DAT) were about 0 - 1.8%, 5 - 15.2%, 6.8 - 55.8%, and 22.8 - 100%, respectively. The effect of neem formula treatment on mortality rates significantly increased at 9 DAT. This was caused by the existence of active substances, particularly azadirachtin, which inhibited insect development and this effect was observable long after the treatment was given. Larvae which fail to molt can live for significantly long time before they finally die [36].

Based on the values of \(LC_{50}\) and \(LC_{95}\) at 9 DAT and results of probit analysis of mortality rates of tested larvae, the neem formula by using DEA surfactant was \(y = -3.13 + 7.28 x\), with \(LC_{50}\) and \(LC_{95}\) values of 13 and 22 ml/L, respectively. This result was lower than [13] who found that neem oil of \(Azadirachta indica\) could kill \(Spodoptera frugiperda\) Smith at mortality rates of 83.33 +/- 0.83% and 89.58 +/- 0.90% at 4 and 6 DAT, respectively. Differences in efficacy might be caused by differences in types of pests and methods and techniques of insecticide production. Most neem product used in this study was neem oil which was obtained by pressing neem seeds. A solvent was then added to extract the active substances of the pesticide. Different methods and techniques applied in neem oil production resulted in different concentration of active substances and biological efficacy [37].
Table 2 Mortality of larva S. litura larvae treated with neem oil insecticide with dilution concentration of 10-25 ml/l at 2, 4, 6, and 9 days after application

| Formulation concentration (ml/l) | 2 days | 4 days | 6 days | 9 days |
|----------------------------------|--------|--------|--------|--------|
| 10                               | 1.7 (60) | 5.1 (59) | 10.5 (57) | 22.8 (57) |
| 13                               | 1.7 (60) | 12.3 (57) | 17.5 (57) | 43.9 (57) |
| 16                               | 0 (60) | 5.0 (60) | 6.8 (59) | 55.9 (59) |
| 19                               | 0 (60) | 11.9 (59) | 22.4 (58) | 79.3 (58) |
| 22                               | 1.7 (59) | 6.9 (58) | 29.3 (58) | 94.8 (58) |
| 25                               | 1.8 (55) | 15.2 (53) | 55.8 (52) | 100 (52) |

*Number of tested insects in each treatment was 60 instar III larvae of S. litura. Figures in bracket (n) show the number of larvae which were found back at a certain observation time. Figures less than 60 indicate that there are larvae which were not found back at the time of observation for unknown reasons.

Insecticide of neem oil is a systemic insecticide [38]. The work mechanisms of neem oil include inhibiting feeding, affecting hormone function in juvenile stages, reducing ecdysone, deregulating growth, altering development and reproduction, suppressing fertility, sterilizing, repelling oviposition, and disrupting molting processes [39]. Based on the complex active substances they contain, derivative products of neem oil can be used as antifeedants, growth regulators, sterilants, anti-oviposition agents, and repellents [40].

4. Conclusions
Neem oil used in this study had a density of 0.91 g/cm³, viscosity of 58.94 cPoise, refractory index of 1.4695, surface tension of 40.69 dyne/cm, azadirachtin content of neem seed oil of 343.82 – 1,604 ppm, and azadirachtin content of neem seed cake of 242.20 ppm. The main fatty acid composition consisted of palmitic (31.4%) and oleic (22.5%) acids. As an additive substance in neem oil insecticide, the ethanolamide surfactant used in this study had a pH of 10.6, density of 0.9930 g/cm³, viscosity of 708.20 cP, and surface tension of 25.37 dyne/cm. Based on the results of CMC analysis and the values of contact angle and droplet size, the most effective concentration of DEA surfactant used as an additive in neem oil insecticide was 5%. The LC₅₀ and LC₉₅ values on Spodoptera litura were 13 and 22 ml/l, respectively. Neem oil worked by inhibiting the development of Spodoptera litura and its larval molting process.

Acknowledgement
Financial support from Ministry of Research, Technology, and Higher Education through the National Strategic Excellence Research Scheme was acknowledged.

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