Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 11, No. 1, January 2026

Research Paper

Optimalization Characteristics of the Formulation Essential Oil Nanobiopesticide: *Citrus hystrix*, *Melaleuca cajuputi*, and *Cymbopogon citratus* from West Aceh, Indonesia

Putri Mustika Sari[1*], Sumeinika Fitria Lizmah[1], Rayhan Amadius Weihan[1]

[1]*Agrotechnology, Agriculture, University Teuku Umar, West Aceh, Aceh, 23681, Indonesia*
***Corresponding author**: putrimustika@utu.ac.id

Abstract
Research on essential oils as biopesticides is very much, so in the development of essential oils as biopesticides there are weaknesses, namely the nature of essential oils as the main ingredient is volatile, and environmental factors are easy to degrade. So it takes a formulation that can maintain the level of the main compound in the long term, and this can be achieved through nanoemulsion. Based on that, this study aims to optimalize the characteristics of the essential oil nanoemulsion formulation derived from *Citrus hystrix* (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1) extracts so that it has properties worthy of being a biopesticide. So the method used is descriptive, by testing the stability of the formulation, transmittance, emulsion type, Particle Size Test, Polydispersity Index, and zeta potential. So the results of this study show that in the transmittance test J-1 (98.8%), K-1 (97.7%), S-1 (86.9%), pH test J-1 (5.94), K-1 (6.5), S-1 (6.68), viscosity test J-1 (4.63cPs), K-1 (4.34cPs), S-1 (4.39cPs), particle size test J-1 (10.9±0.05), K-1 (12.5±0.08), S-1 (12.6±0.15), polydispersity index test J-1 (0.563±0.04), K-1 (0.052±0.01), S-1 (0.635±0.08), zeta potential test J-1 (-18.9±1.51), K-1 (-19.9±1.41), S-1 (17.7±1.43). In the stability and emulsion type tests, the three formulation have the same characteristics, namely clear yellow color, distinctive odor, homogeneous, without sediment, without separation of 2 solution phases, and oil-in-water (o/w) emulsion type. Based on these data, it can be seen that the three formulation are stable and suitable to be biopesticides in further research.

Keywords
Biopesticide, Essential Oil, Nanoemulsion, Stability, Volatile

Received: 15 August 2025, Accepted: 3 November 2025
https://doi.org/10.26554/sti.2026.11.1.137-147

1. INTRODUCTION

Crop protection systems are part of a sustainable agriculture program that aims to reduce the impact of excessive pesticide use by farmers, thus preventing damage to the agricultural ecosystem (Soliman et al., 2023). Indonesia was one of the three largest users of synthetic pesticides in the world in 2021, using 283 kilotons (Diacono et al., 2016). The unwise use of synthetic pesticides causes long-term environmental damage, impacting plant growth, and increasing pest and disease resistance and resurgence, which can impact food security (Nath and Puzari, 2022).

Appropriate biopesticide formulation technology is needed to address the excessive use of biopesticides (Ghormade et al., 2011). Problems in the use of essential oil-based biopesticides include oil stability in storage, poor solubility in water and high degradation rates (da Costa et al., 2014), whereas biopesticides are expected to be durable enough to control pests (Noyes et al., 2009). One research topic that requires attention to overcome these obstacles is the use of nanotechnology. The use of essential oils requires the right formulation in the form of compounds that can protect essential oils from volatility and degradation, namely nanoformulation technology (Martín et al., 2010).

Nanotechnology encompasses various forms, including nano fibres, nanoparticles, nanotubes, and nanowires (Sari et al., 2025). In the formulation of nanoparticle, PEG is often used because it can improve the biocompatibility of nanoparticles and has high hydrophilic properties (Gupta and Curtis, 2004). PEG is highly soluble in organic solvents, has low toxicity, a low melting point (below 65°C), and the ability to dissolve several compounds (Koh et al., 2013). Nanoparticle formulations with active ingredients derived from plant extracts, with a sufficiently small formulation size, can target pests accurately without harming non-target organisms, as well as increasing the stability and resistance of the formulation when applied in the field against degradation due to weather. This allows pesticides to remain effective for longer periods of time and to be used more efficiently and in an environmentally friendly

(Bergeson, 2016).

The numerous problems experienced in the agricultural sector have given rise to polemics over pest resistance and resurgence, leading to the idea of reapplying the principles of environmentally friendly plant protection systems in agriculture by utilizing nanobiopesticides made from essential oils of eucalyptus, lemongrass, and lime leaves. Nanoparticle formulations have been extensively studied and have been shown to increase the effectiveness of essential oils (Pedro et al., 2013). Nanoemulsions and nanoencapsulation are among the most widely used and effective nanopesticide techniques. Nanoemulsions are transparent, light-transmitting emulsion systems consisting of water-in-oil dispersions stabilised by a film layer of surfactants or surfactant molecules, with droplet sizes ranging from 50 to 500 nm (Shakeel et al., 2008). The small droplet size of nanoemulsions makes them kinetically stable, creaming during storage, and preventing sedimentation (Solans et al., 2005). Furthermore, with the touch of encapsulation, the active ingredients are less volatile and more stable. So they can function as environmentally friendly and economically profitable pesticides (Ikawati et al., 2021).

This research has important significance in supporting sustainable food, where the goal is to apply a new method in the manufacture of essential oil biopesticides, namely with nanotechnology so that it is suitable for application on a field scale. There are many methods for obtaining nanoparticles, including the spray-drying method (Shaikh et al., 2006), complex coacervation (Guo and Zhao, 2008), interfacial polymerization (Zhu et al., 2009), and the emulsion diffusion method (Katas et al., 2009), but there are still many shortcomings in these methods (González et al., 2016). Meanwhile, the PEG polymer nanoparticle method for biopesticides containing essential oils from geranium and bergamot is one of the nanopesticide methods that has been widely used and is effective (Dewi et al., 2022).

In the development of essential oils as biopesticides, there are weaknesses related to the properties of essential oils as the main ingredient, which are insoluble in water, volatile, and easily degraded when applied to agricultural land. Therefore, the nanoemulsion method can overcome these problems. This is reinforced by literature from Ikawati et al. (2021) which states that PEG polymers have potential as nanoparticle materials in clove oil nanoformulations. Clove oil nanoformulations also show better stability than non-nanoformulated ones. Nanoformulations can prevent essential oils from degradation and evaporation, while enabling sustained release and maintaining high toxicity for 16 weeks. Some researchers focus on making essential oils into biopesticides, while research related to essential oil-based nanobiopesticides is still limited. Therefore, this study aims to convert essential oils into nanobiopesticides through the nanoemulsion method. The objective of this study is to optimalize the characteristics of the essential oil nanobiopesticide formula (*Citrus hystrix, Melaleuca cajuputi,* and *Cymbopogon citratus*) so that when applied to agricultural land, it does not volatile, and is more durable, while remaining environmentally friendly.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this study were *C. hystrix* leaves (J-1), *M. cajuputi* leaves (K-1), and *C. citratus* leaves (S-1), ethanol, Aquadest, virgin coconut oil, tween 80, PEG 400, methyl blue, beaker glass, aluminum foil, plastic wrap, measuring cup, filter paper Wathman No. 1, magnetic stirrer, sonicator (Elmasonic, S 300 H), UV-Vis spectrometer (SPECTROstar Nano, BMG LABTECH), viscometer (Brookfield NDJ-8S), pH meter (OHAUS), PSA and Zeta (Malvern), rotary evaporator (WIGGENS), centrifuge (Brenchmark Scientific), vortex (IKA Shaker), GC-MS (Shimadzu Model QP-2010).

2.2 Methods

This study used a descriptive method, which explains the optimalization of the characteristics of essential oil nanobiopesticides.

2.2.1 Making Essential Oils

Essential oil extract is made by cutting 500 grams of eucalyptus leaves into small pieces, then placing them in a container filled with ethanol as a solvent at a ratio of 1:2 to be macerated for 24 hours. Then, using a rotary evaporator (Suryani, 2024), pure essential oil extract is obtained. The use of a temperature of 60°C, a speed of 100 rpm, and a pressure of 80 mbar for 30 minutes is adjusted to the solvent used and the volume of extract being rotary evaporated. The extraction results were filtered using filter paper until 10 mL of pure essential oil extract was obtained.

2.2.2 Making Nanoemulsions from Essential Oils

Comparison of formulations for making nanoemulsions based on the results of research by Ma'arif et al. (2023), namely 1:1:8:1:50, which was modified in terms of formulation ingredients and essential oil volume. Thus, the nanoemulsion formulation was prepared by mixing the prepared pure essential oil extract with Tween 80 in a beaker using a magnetic stirrer at a speed of 100 rpm at 70 °C for 30 minutes. Virgin coconut oil Listyorini et al. (2018) was then added as the oil phase. PEG 400 Ikawati et al. (2021) was then added. Finally, distilled water was added as the aqueous phase gradually over 1 hour until a nanoemulsion was formed. After that, the formula was placed in a bath-type sonicator for 1 hour at a temperature of 30°C (Suryani, 2024) and a frequency of 37 Hz, while stirring occasionally until a nanoemulsion was formed.

2.2.3 Observation Parameters

2.2.3.1 Stability Test

Stability testing was conducted using the freeze-thaw cycle method, whereby the cycle was repeated six times with the formula stored at temperatures of 4°C and 40°C for 48 hours (Pratiwi et al., 2018). The formulation was observed using organoleptic test (visual), viscosity test (Brookfield NDJ-8S),

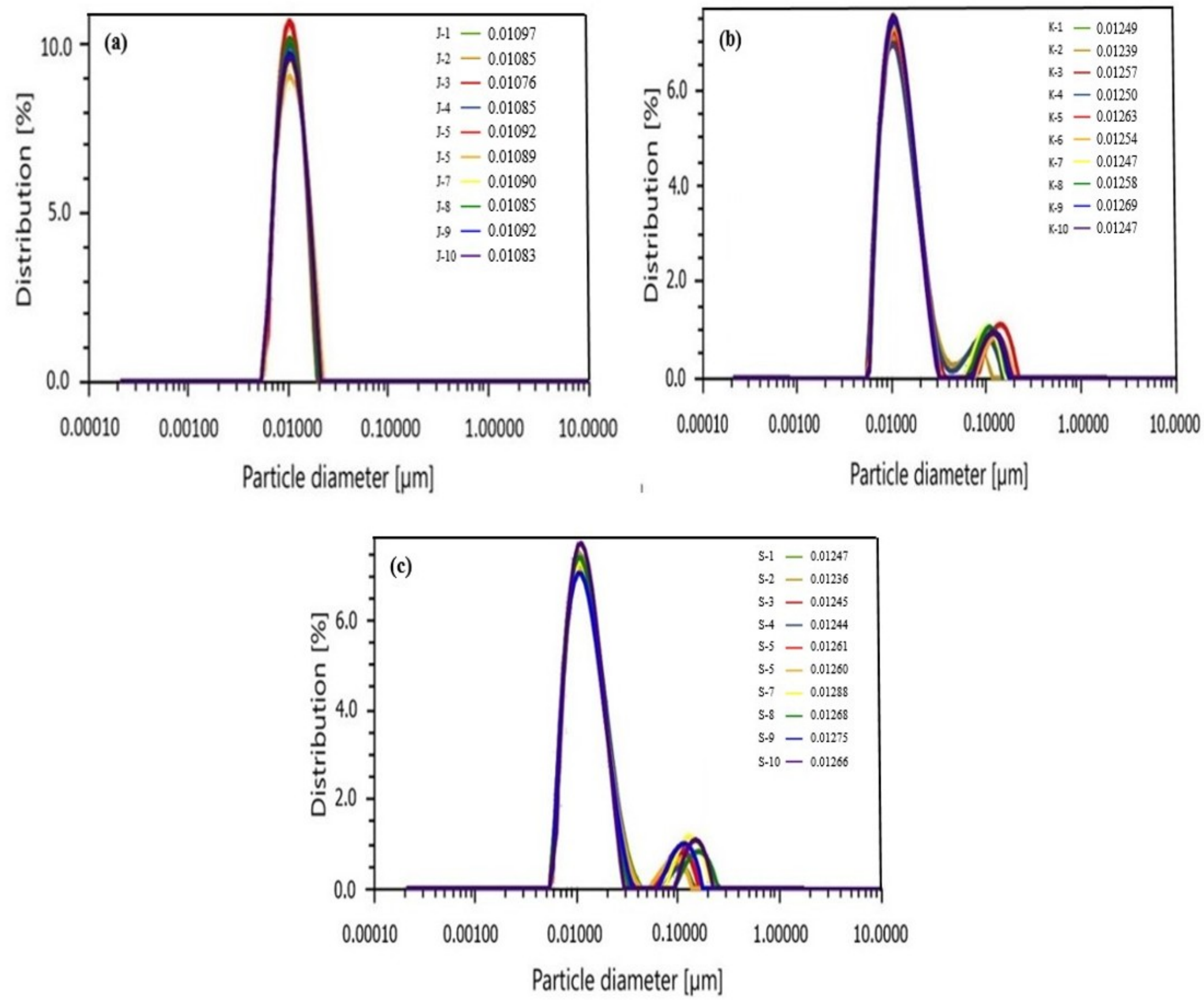

Figure 1. Particle Size Analysis and Dynamic Light Scattering (DLS) on 10 Replicates of (a) *C. hystrix*, (b) *M. cajuputi*, and (c) *C. citratus*

Table 1. Optimalization of Essential Oil Nanoemulsion Formulation

Material	Function	Nanobiopesticide Formulation		
		J-1	K-1	S-1
Essential oil	Active Ingredients	10	10	10
VOC	Oil Phase	10	10	10
Tween 80	Surfactant	80	80	80
PEG 400	Cosurfactant	10	10	10
Aquadest	Water Phase	500	500	500

Note: *Citrus hystrix* (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1).

pH test (OHAUS), and UV-Vis spektrofotometer test (SPECTROstar Nano, BMG LABTECH) to determine the stability of the preparation.

2.2.3.2 Emulsion Type Test

The nanoemulsion is mixed with methylene blue on a glass object, then stirred and observed visually. If the methylene blue dissolves in the preparation, it means the type of nanoemulsion is oil in water (o/w), but if the preparation has a water in oil (w/o) type, the methylene blue does not dissolve or clumps on the surface of the nanoemulsion (Ma'arif et al., 2023).

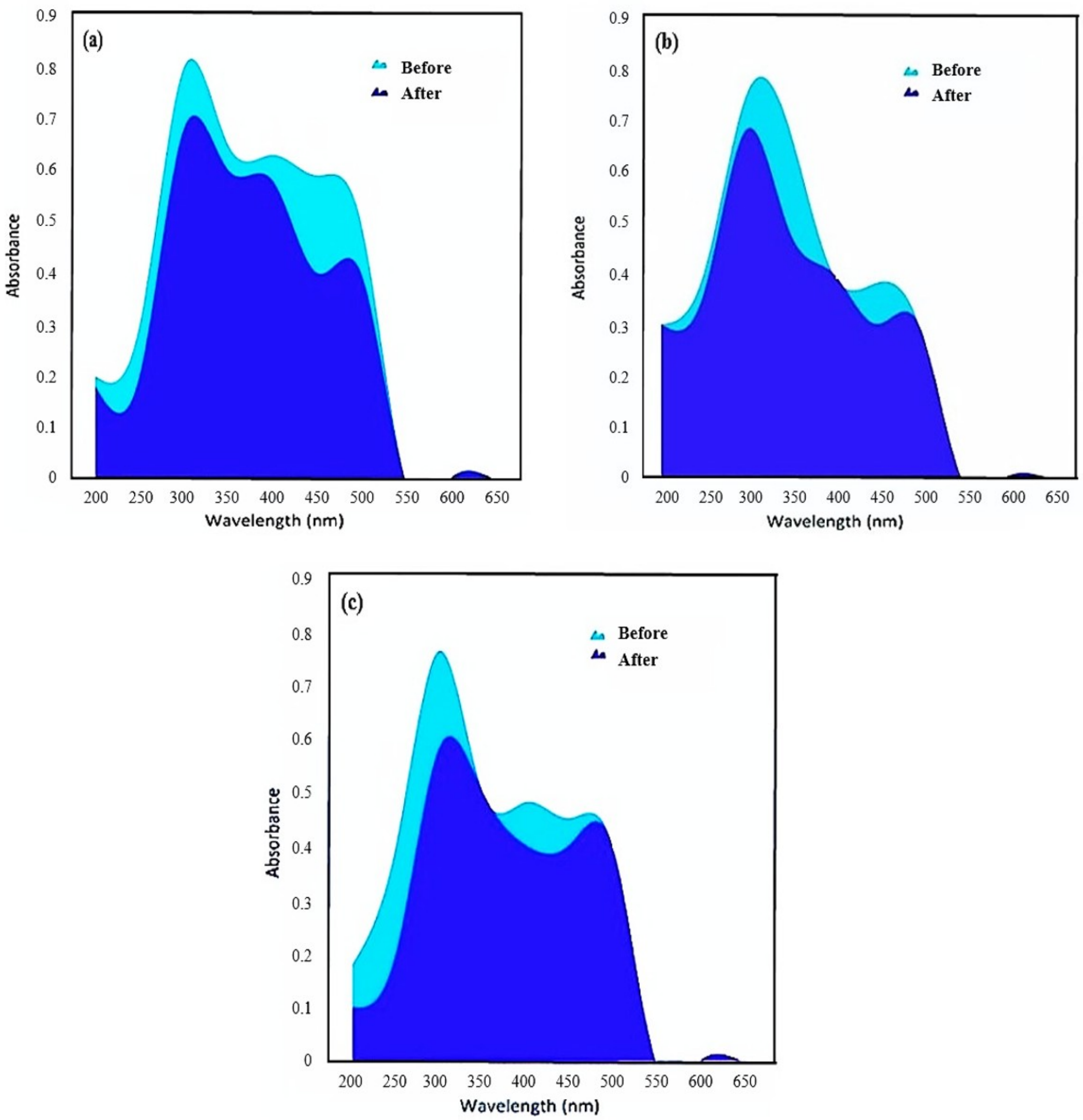

Figure 2. Stability Test of Essential Oil Nanobiopesticides Before and After Freeze-Thaw Cycle of (a) *C. hystrix*, (b) *M. cajuputi*, and (c) *C. citratus*

Table 2. Characteristics Test of Essential Oil Nanobiopesticides

Parameter	Nanobiopesticide Formulation		
	J-1	K-1	S-1
Transmittance (%)	98.8	97.7	86.9
pH	5.94	6.5	6.68
Viscosity (cPs)	4.63	4.34	4.39
Particle Size (nm)	10.9 ± 0.05	12.5 ± 0.08	12.6 ± 0.15
Polydispersity Index	0.563 ± 0.04	0.052 ± 0.01	0.635 ± 0.08
Zeta Potential (mV)	-18.9 ± 1.51	-19.9 ± 1.41	-17.7 ± 1.43

Note: *Citrus hystrix* (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1).

Table 3. Physical Test of Essential Oil Nanobiopesticides

Parameter	Nanobiopesticide Formulation		
	J-1	K-1	S-1
Color	Yellow transparan	Yellow transparan	Yellow
Scent	Typical	Typical	Typical
Homogenity	Homogeny	Homogeny	Homogeny
2-phase separation	–	–	–
Sedimentation	–	–	–
Turbidity	–	–	–

Note: *Citrus hystrix* (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1).
– = no
+ = yes

2.2.3.3 Particle Size and Polydispersity Index Test

1 mL nanoemulsion was mixed with 10 mL of distilled water using a vortex for 1 minute until homogeneous. Tested using a Malvern Particle Size Analysis (PSA) tool.

3. RESULTS AND DISCUSSION

The results of the optimalization of the nanobiopesticide formula for essential oils from *C. hystrix* (J-1), *M. cajuputi* (K-1), and *C. citratus* (S-1) leaves can be seen in Table 1.

In this study, essential oil nanobiopesticides were made using the sonication method, because this method does not require high temperatures so it does not damage the active compounds that are thermolabile. Through this method, the active substance is extracted by damaging the cells at 37 Hz waves. The damaged cells will easily release the active substance contained in the cells into the solvent. The spontaneous nanoemulsion method works by mixing surfactants from the oil phase into the oil-water phase without co-surfactants (Lefebvre et al., 2017). The formation of nanoemulsions is greatly influenced by the type of oil, surfactant, and cosurfactant used. The choice of virgin coconut oil as the oil phase is due to its properties as an emollient that is easily absorbed so it can help penetration. Tween 80 is used as a surfactant because it acts as an emulsifying agent (emulsifier) in the preparation of oil-in-water emulsions. PEG-400 is used as a cosurfactant because it has the potential to increase penetration by reducing surface tension. In addition, according to research by Sisak et al. (2017), propylene glycol as a cosurfactant can also cause an increase in viscosity and allow gel formation in nanoemulsion preparations.

The results of the characterisation of the nanobiopesticide formula of *C. hystrix* (J-1), *M. cajuputi* (K-1), and *C. citratus* (S-1) leaf essential oils against % transmittance, pH, viscosity, particle size, polydispersity index, and zeta potential can be seen in Table 2.

The mixture of 3 main components in the nanoemulsion formula, namely oil, surfactant-cosurfactant, and water, so that a clear mixture is obtained, characterized by a high % transmittance value. As seen in Table 2, the three formulas have a % transmittance of J-1 (98.8%), K-1 (97.7%), S-1 (86.9%). The % transmittance value is high because it is close to 100%. The higher the transmittance value, the clearer the solution, this indicates that the nanoemulsion droplets have reached a nanometer size. However, the transmittance test results, formula 3 (S-1) showed the lowest transmittance value among the three formulas, the color of the formula is not clear and transparent. According to Kaur et al. (2013), a transmittance value above 80% indicates the size of the emulsion particles in the nanometer range.

The results of viscosity measurements show that the three formulas have viscosities that meet the criteria, seen in the viscosity values of the three formulas of J-1 (4.63 cPs), K-1 (4.34 cPs), S-1 (4.39 cPs). This indicates that the viscosity of the nanoemulsion is easy to spread well on the surface. Liao and Huang (2022) state that higher viscosity values indicate a more concentrated solution, which means that the fluid resistance is higher. Zewude et al. (2021) also explain that nanofibres contain cationic surface charges under acidic pH conditions. The electrostatic repulsive force between nanofibres and cationic surface charges contributes to the stability of particle dispersion in water, which in turn affects the flow resistance of the solution or viscosity. Naiu et al. (2020) showed that nano particles <0.3 in solution are uniformly dispersed, reflecting ideal viscosity values (3.45 cPs - 6.73 cPs).

In Figure 1, it can be seen that the average size of the nanoemulsion in the three types of essential oils was ±0.01000 µm. The nanoparticle size distribution data was quite good, as it had a coefficient of determination value close to 10%. There was not much difference between the results of the nanoemulsion obtained through PSA analysis and dynamic light scattering (DLS) at 10 replocations. Several factors influence the final size of the nanoemulsion, namely the polarity of the solvent, the concentration of polymer in the organic phase, and the internal or external phase ratio (Ikawati et al., 2021). Figure 1 shows bimodal and unimodal size distributions. In statistics, a unimodal distribution is a probability distribution with a single peak, while a bimodal distribution has two peaks. For bimodal distributions, although they show two peaks, the intensity of the second peak is very low (below 10%) and the peak is a marginal distribution. This means that the second peak is an additional

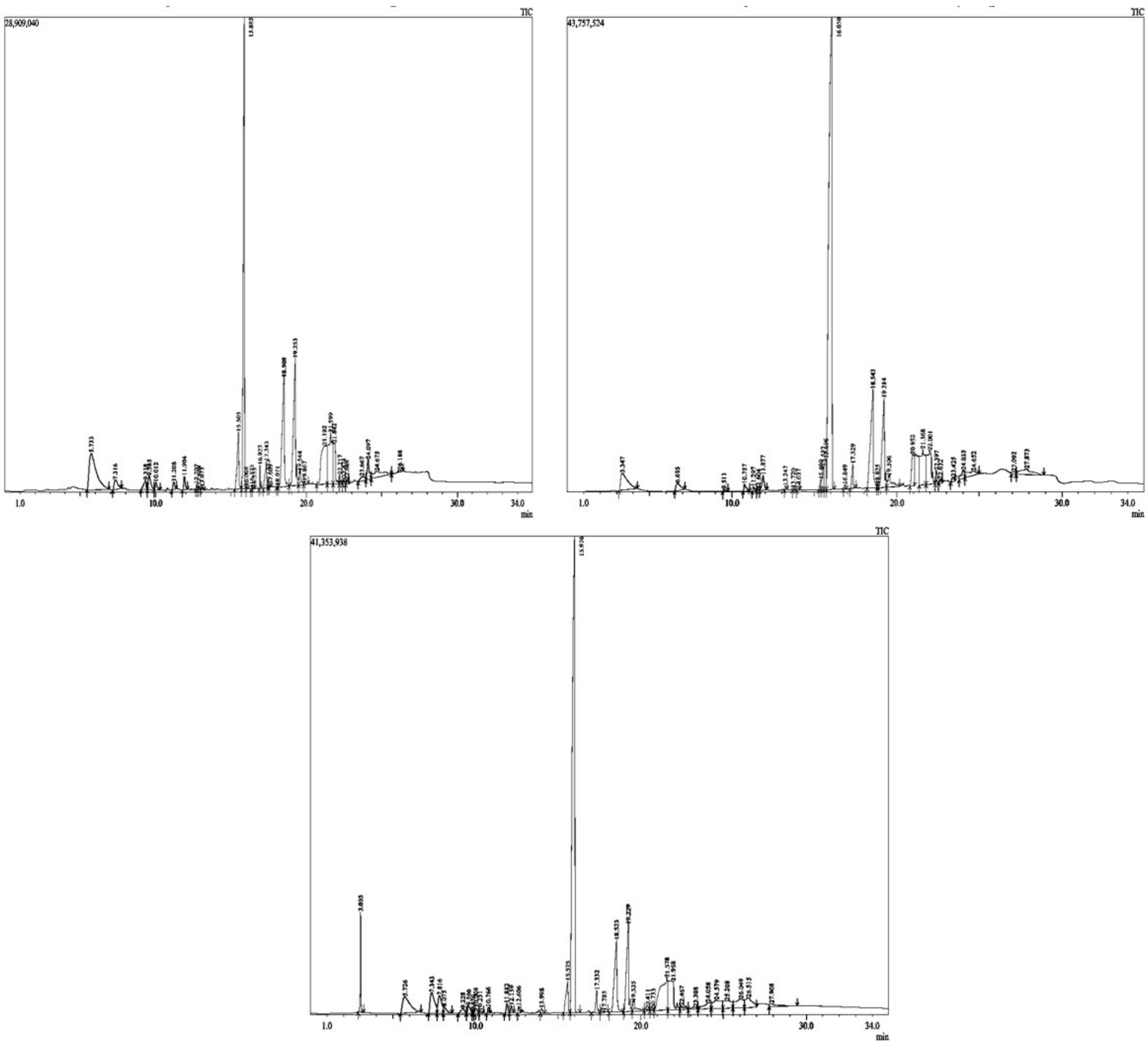

Figure 3. Chromatogram Analysis Results GC-MS Essential Oil of (a) *C. hystrix*, (b) *M. cajuputi*, and (c) *C. citratus*

Table 4. Stability Test of Essential Oil Nanobiopesticides Before and After Freeze-Thaw Cycle

Freeze Thaw Cycle	Parameter	Nanobiopesticide Formulation		
		J-1	K-1	S-1
Before	Transmittan (%)	98.8	97.7	86.9
	pH	5.94	6.50	6.68
	Viscosity (cPs)	4.63	4.34	4.39
After	Transmittan (%)	89.83	86.39	81.15
	pH	5.91	6.42	6.64
	Viscosity (cPs)	4.33	3.34	2.99

Note: *Citrus hystrix* (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1).

peak, an outlier peak at the edge of the distribution.

Particle size testing aims to determine the particle size in a sample. As seen in Table 2, the three formulas have particle size test J-1 (10.9±0.05) nm, K-1 (12.5±0.08) nm, S-1 (12.6±0.15) nm. This allows the nanobiopesticide to penetrate the outermost layer of the insect's skin, thereby causing insect

Table 5. The Content of Essential Oil Nanoemulsion Compounds as Biopesticides

Formulation	Main Constituents	Time Retention	Area (%)
S-1	β-Citronellal	7.343	8.5
	β-Citronellol	9.228	11.65
	Geraniol	9.850	62.83
	Geraniol Asetat	19.229	5.03
	L-Limonen	19.535	1.08
	Elemol & seskuiterpen	21.958	0.6
	Constituent unknown		10.31
J-1	β-Citronellal	7.316	56.42
	β-Citronellol	9.338	11.48
	Linalool	13.073	5.84
	Sabinen	18.807	4.13
	Geraniol Asetat	19.253	1.71
	Constituent unknown		20.42
K-1	Sineol (Eucalyptol)	3.347	46.46
	Terpineol	9.511	5.39
	Caryopilen	10.757	8.85
	Pinen	15.696	4.16
	Limonene	19.214	6.14
	Constituent unknown		29

Note: Citrus hystrix (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1)

mortalitas. The polydispersity index test aims to determine the homogeneity or uniformity of a particle size. All three formulas showed a good level of particle size uniformity, namely J-1 (0.563±0.04), K-1 (0.052±0.01), S-1 (0.635±0.08). This is because the polydispersity index values of the three formulas are in the range of 0.01-0.7. Nanoemulsion formulations have droplet sizes ranging from 2 to 500 nanometres, indicating a high level of kinetic stability (Suryani, 2024). Smaller nanoemulsion droplet sizes result in a larger surface area, thereby increasing solubility. Nanoemulsions with sizes <100 nm are more easily soluble (Hassanin et al., 2017). Polydispersity index value <0.3 indicates that the particle size has a narrow distribution, while a value >0.3 indicates a broad distribution distribution (González et al., 2016).

Zeta potential measurements were carried out to determine the surface charge of colloidal particles and the stability of nanoemulsions. The results of the zeta potential values for the three formulas showed that the values were in the range between -30 mV to +30 mV, as seen in Table 2. zeta potential test J-1 (-18.9±1.51), K-1 (-19.9±1.41), S-1 (17.7±1.43). This shows that the zeta potential value of the essential oil nanobiopesticide preparation shows a fairly low value from a good value, which is above +30μV or below -30μV. A low zeta potential value causes low interparticle repulsion so that particles are more easily flocculated. According to Zanela et al. (2018) said that Flocculation can cause separation between the oil phase and the water phase, thereby disrupting the stability of nanoemulsions.

The nanobiopesticide preparations of kaffir lime leaves (*C. hystrix*), eucalyptus leaves (*M. cajuputi*), and lemongrass leaves (*C. citratus*) that have been formed can be seen in Table 3.

Table 3 shows that all three formulas have a clear yellow color, a distinctive odor, are evenly dispersed, and do not experience phase separation. However, there are differences in color between the three formulas. This is due to the variations in the types of essential oil extracts used. Variations in essential oil types can produce different particle sizes in the nanoemulsions, which will affect color changes.

Nanoemulsion type testing was conducted to determine the nanoemulsion formed. Based on the test results for the three formulas, the methylene blue mixed in the preparation dissolved completely, producing a homogeneous blue color and the test did not clump. This indicates that all three formulas have an oil-in-water (o/w) emulsion type. Methylene blue is basically polar in nature, so it will dissolve well in o/w type nanoemulsion preparations because the nanoemulsion is dominated by hydrophilic components. In the water-in-oil (w/o) type nanoemulsion, methylene blue will tend to clump on the surface because the nanoemulsion is dominated by hydrophobic components (Ma'arif et al., 2023).

Based on the centrifugation test results for the three formulas above, there was no phase separation, sedimentation, or turbidity. This indicates that the nanoemulsion preparation is stable. The stability of this nanoemulsion preparation can be maintained as long as the oil concentration remains within the specified limits. When the oil concentration exceeds the limit, oil droplets can touch and merge with each other, thus causing phase separation, turbidity, and sedimentation in the formed nanoemulsion.

The stability test of the essential oil nanobiopesticide was conducted using freeze-thaw cycles. Based on the stability test results, after 6 freeze-thaw cycles, the results are shown in Table 4.

In Table 4, it can be seen that there was a decrease in % transmittance at (J-1 = 8.97), (K-1 = 11.31), (S-1 = 5.75), pH (J-1 = 0.03), (K-1 = 0.08), (S-1 = 0.04), and viscosity (J-1 = 0.3), (K-1 = 1), (S-1 = 1.4). The stress conditions given to the preparation are important to see the stability of the preparation. The freeze-thaw test is included in the thermodynamic stability test so it is suitable for determining the stability of thermodynamically unstable nanoemulsion preparations (Ullah et al., 2022). The test results showed that the essential oil nanoemulsion preparation was stable in the parameters of %transmittance, pH, and viscosity, because the size changes that occurred were still within the stable range after the freeze-thaw cycle. This is reinforced by the statement of Pratiwi et al. (2018) found that nanoemulsion stability can be improved by controlling the type and concentration of surfactants and cosurfactants, the type of oil used, the manufacturing method, process variables, and the addition of additives. The use of surfactant and cosurfactant

Table 6. Comparison of Formulation, Characterisation, Evaluation of Essential Oil Nanoemulsions

Natural Materials	Surfactant	Co-surfactant	Characterisation	Evaluation	References
Lime leaves, eucalyptus leaves, and lemongrass leaves	Tween 80	PEG 400	Particle size analysis, Stability	PSA J-1 (10.9 ± 0.05), K-1 (12.5 ± 0.08), S-1 (12.6 ± 0.15), PdJ (1.05 ± 0.04), K-1 (0.052 ± 0.01), S-1 (0.635 ± 0.08) for application as a biopesticide	This Research
Oil Palm Empty Fruit Bunches	Sodium hypochlorite (b/v) and acid hydrolysis	–	PSA (Particle Size Analyzer)	The use of physical and chemical methods has been proven effective on nanocellulose with an optimal size, namely an average diameter of 484.3 nm	(Sari et al., 2025)
Clove and lemon	Tween 80	–	Particle size analysis, Stability, Preparation of edible coating solution	Formulations with 4% LEO and formulations with 2% CEO can produce the smallest particle size and remain stable during storage	(Ghazali and Zakaria, 2024)
J. curcas and *M. fragarns*	Polysorbate	–	The droplet size analysis	Particle sizes below 200 nm, but both met the criteria for nanoemulsions	(Nuryanti et al., 2023)
Artemisia and *Rosemary*	Tween 80	–	The droplet size, polydispersion index and zeta potential	The stable characteristics of the rosemary EO formulation were obtained with a PSA value of 217±1 nm, a PdI of 0.36±0.01, and a zeta of 18.63±0.14 mV, and the artemisia formulation PSA of 199±2 nm, PdI of 0.42±0.02, and zeta of 12.27±0.26 mV	(Dunan et al., 2023)

concentrations in this method produces nano-sized droplets, so process optimalization can be an option in improving the stability of the formed nanoemulsions.

Figure 2 shows that the absorbance values before and after the freeze-thaw cycle indicate that of the three formulations, the essential oil nanobiopesticide has a significant absorbance value with an average of 0.6-0.8 at a wavelength of 300 nm, which means that the nanobiopesticide formulation remains stable. Thus, the essential oil nanobiopesticide formulation remains stable at a concentration of 1% during the freeze-thaw cycle. This is in line with the research by Elraies et al. (2023), which states that a nanoemulsion formulation can be considered stable if it has a relatively constant absorbance value with a small standard deviation. The absence of significant changes in absorbance indicates that the formulation is stable, enabling the nanobiopesticide to exhibit longer-lasting toxic properties when applied directly to agricultural land.

The results of the chemical compound analysis of three types of essential oils are shown in Table 5. Essential oil nanobiopesticides exhibit varying levels of compound content. The different content levels for each compound are due to the chemical composition and biological activity of the essential oils extracted from different plant species. This is reinforced by the literature from Avetisyan et al. (2017), which states that the essential oil content of each plant varies depending on the altitude, environment, cultivar variety, and cultivation techniques.

The results of GC-MS analysis in Figure 3 show several main compounds in the essential oils obtained from each plant extract. The main components in lemongrass essential oil are geraniol (62.83%) and β-citronellol (11.65%), in kaffir lime essential oil, they are β-citronellal (56.42%) and β-citronellol (11.48%), in eucalyptus essential oil, they are cineol (46.46%) and caryophyllene (8.85%). According to Regnault-Roger et al. (2012), essential oils contain many volatile and phenolic terpene compounds. Some plants can produce active secondary metabolites as a form of chemical defence against pests (Rajendran and Sriranjini, 2008). Monoterpene compounds can be an important factor in the biological activity of essential oils (Habtemariam, 2018). The content of secondary metabolite compounds in plants is volatile, such as monoterpenes, which act as repellents to insects, possibly due to their lipophilic chemical characteristics (Aliyu et al., 2015).

Previous research demonstrating the potential use of essential oils as biopesticides is evident in studies by Ikawati et al. (2021) on kaffir lime essential oil and Doumbia et al. (2014) on citronella, where essential oils exhibit toxic properties against certain types of storage pests. Essential oils can target more than one target site because they contain more than one active compound. This makes it difficult for insects to develop resistance. Different types of active compounds in essential oils typically have different attack pathways, such as through mitochondria, nerve membranes, or other pathways.

A comparison of the formulation, characterisation, and evaluation of essential oil nanoemulsion used in this study with other studies can be seen in Table 6. shows various natural ingredients such as *Citrus hystrix* (J-1), *Melaleuca cajuputi* (K-1), and *Cymbopogon citratus* (S-1) used as the main active ingredients in the manufacture of nanoemulsions with Tween 80 as the main surfactant. Co-surfactants such as PEG 400 are used to support emulsion stability by increasing the solubility of the active ingredients. Several parameters were tested, including organoleptic tests, pH, viscosity, and physical stability tests such as freeze-thaw cycles, centrifugation, and turbidity tests. These tests aim to ensure that the resulting nanoemulsion is physically stable, safe, and effective for application to the skin or target tissue.

PEG is generally known to have very few interactions with other components (Majumdar et al., 2010). The use of PEG can increase the bioavailability of substances such as bioinsecticides that are difficult to dissolve in water. By reducing particle size, this significantly increases bioavailability (Vasconcelos et al., 2007). The results of the study show that the nanoemulsion method using PEG can be used and is feasible for formulating essential oils as nanoemulsions.

4. CONCLUSIONS

Based on the results of the initial research that has been conducted, it can be concluded that essential oils can be made into nanoemulsions using the self-nanoemulsifying method with compositions such as virgin coconut oil, tween 80, PEG 400, and aquadest. Of the three formulas made, the particle size of the essential oil nanoemulsion is J-1 (10.9±0.05) nm, K-1 (12.5±0.08) nm, S-1 (12.6±0.15) nm, and polydispersity index test J-1 (0.563±0.04)%, K-1 (0.052±0.01)%, S-1 (0.635±0.08)%. The essential oil nanoemulsion formula with the best characteristics was obtained from *C. hystrix* (J-1). The characterisation results showed that all parameters met the requirements in the three formulas, so they are suitable as biopesticides for application in further research.

5. ACKNOWLEDGMENT

This research was funded by the Regular Fundamental Research Program (PFR) of the Ministry of Education, Science, and Technology, Indonesia. Based on Decree Number 0419/ C3/DT.05.00/ 2025, with Contract Number 117/C3/DT.05 .00/PL/2025.

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