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Original Article

THE EFFECTIVENESS OF VARIOUS CITRONELLA OIL NANOGEL FORMULATIONS AS A REPELLENT OF *AEDES AEGYPTI* MOSQUITO

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ABSTRACT

Objective: The aim of this research is to determine the effectiveness of the repellency of various citronella oil nanogel formulations against *Aedes aegypti* mosquito bites applied to guinea pigs.

Methods: The Citronella oil nanogel was prepared by emulsification technique combined with gelling formation. The nanoemulsion formula consists of 6% Citronella oil, 10% propylene glycol, 6% combination of Tween 80 and Span 80 in variation to 4 formula, and deionized water until 100%. Nanoemulsions were then added to the gel base which is composed of Carbopol 940 and TEA. The nanogel formed was characterized physically and its effectiveness against *Aedes aegypti* mosquito using guinea pigs as an animal model. The repellent activity was determined by the amount of mosquito bites on the guinea pigs's skin.

Results: The organoleptic test displayed that all formulas comply with the requirements, except formula 1. The formula 2, 3, and 4 have particle size below 100 nm and the polydispersity index (PDI) less than 0.5. According to the repellent assay showed that all formulas have the effectiveness much higher than without the intervention. Formula 3 has the highest effectiveness of repellent activity against *Aedes aedgypti* in guinea pigs' skin. The repellent activity of Formula 3 showed a significant difference (p-value of 0.005) as compared to nanogel without Citronella oil.

Conclusion: The protective power of Citronella oil nanogel Formula 3 is the most effective as compared to the other formula.

Keywords: Citronella oil, Nanogel, Repellent, *Aedes aegypti*, Mosquito

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INTRODUCTION

Dengue Fever (DHF) is a viral infection spread by mosquitoes, especially the *Aedes aegypti* mosquito. The *Aedes aegypti* is very adaptive, in the summer this mosquito can spread to other countries in Europe and America. More than 100 countries have been endemic for 50 y, dengue fever has increased 30 times and total dengue infection in the world can reach 50–100 million cases per year [1]. The *Aedes aegypti* mosquito is also a vector of chikungunya, yellow fever and Zika viruses [2].

In Portugal there was a dengue fever outbreak in 2013. More than 2,100 cases were detected, and it spread to 13 other European countries [3, 4]. In Japan, 1512 cases of dengue fever were detected during 2006-2014. In 2014 there was a domestic outbreak of 162 cases and most of the cases occurred due to transmission from other countries. The countries sending the largest number of dengue fever cases in Japan are India 152 cases, Thailand 160 cases, the Philippines 230 cases, and Indonesia 317 cases [5, 6]. In Indonesia, during 2020, there were 108,303 cases of dengue fever recorded, 747 cases of which resulted in death. 11 Provinces in Indonesia have a high Case Fatality Rate. The high morbidity and mortality rates of dengue fever in Indonesia have the potential to spread to the entire population in the world, so attention is needed to prevent the spread or occurrence of dengue fever [7].

Mosquitoes find their food with their sense of smell. Interfering mosquitoes' sense of smell is one strategy to prevent direct contact or bites by mosquitoes on humans [8, 9]. Various methods of controlling the *Aedes aegypti* mosquito have been explored, one of which is repellent using synthetic materials (DEET, IR3535 or Picaridin)[9]. N,N-diethyl-m-toluamide (DEET) has disadvantages such as emitting an unpleasant odor, feeling oily and stimulate allergies and be toxic to the skin [10]. Utilize DEET repellent daily for a long time causes resistance in the mosquito population, and also harms the environment [11]. To avoid the negative effects of synthetic repellent, natural products are an alternative for mosquito control as they are considered safe and environmentally friendly [12]. Natural plant essential oils such as Citronella oil are natural ingredients that are used as a safe repellent for the *Aedes aegypti* mosquito [8]. However, the high volatility of essential oils has become a problem since it can only have a short-lived effect. Changing it into a controlled release repellent could be a solution [13].

Nanotechnology is believed to be able to improve the performance of active ingredients, commonly used for materials that have low stability, low solubility, low penetration and to control the release of active ingredients. Nanogel is a product form of nanotechnology which consists of an emulsion dispersed in a gel base, with the advantage of having an attractive shape that is easy to spread and apply to the skin and is not greasy [14]. Altogether, we performed this research to determine the effectiveness of the repellency of various formulations of Citronella oil nanogel against *Aedes aegypti* mosquito bites which were applied to guinea pigs.

MATERIALS AND METHODS

Instruments and materials

The UV-Vis spectrophotometer, pH meter, and particle size analyzer (PSA) were the instruments employed in this study.

Citronella oil was one of the primary ingredients used in this research, this compound was purchased from PT Zonakimia, Indonesia. Tween 80, Span 80, propylene glycol, Carbopol 940, triethanolamine (TEA), deionized water, and chemical repellent Product X were the materials employed in these experiments. Female adult *Aedes aegypti* mosquitoes, aged three to five days, were utilized as the experimental animals. They were raised in the Center of Health Research and Development Laboratory in Pangandaran, West Java.

Methods

Formulation of citronella oil nanoemulsion

Formulation of the Citronella oil nanogel for the experiments can be seen in table 1. We made 4 formulas by varying the concentration Tween 80 and Span 80.

Preparation of citronella oil nanogel

Before we make nanogel, we have to prepare the gel base first. The gel base was made by pouring 22.5 ml of distilled water into a mortar, adding 25 grams of Carbopol to the distilled water, mixing it, and adding 1.5 ml of TEA then homogenizing.

The nanoemulsions were prepared using the hot plate technique. The oil phase was homogenized at a temperature of 25 °C and a speed of 700 rpm for 30 min. Then the water phase consisting of deionized water and propylene glycol dropped into the oil phase and homogenized for 60 min [15].

After the gel base and the nanoemulsion are ready the next step is blending them to be nanogel. Insert the gel base into a 100 ml Beaker glass on the hot plate stirrer and turn on the magnetic stirrer at 100 rpm. Dripping dropwise the nanoemulsion to the gel base and adjust the speed stirrer at 300 rpm continuing the process for 30 min until the nanoemulsion and the gel base are mixed homogenously.

Table 1: Citronella oil nanogel formulations

Materials	Formula			
	F1	F2	F3	F4
Citronella oil	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Tween 80	0.975 g	1.95 g	2.925 g	3.9 g
Span 80	0.525 g	1.05 g	1.575 g	2.1 g
Propylene glycol	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Deionized water	19.5 ml	18 ml	16.5 ml	15 ml

Evaluation of citronella oil nanogel

The Citronella oil nanogel was evaluated for organoleptic, pH, and spreadability. These evaluations were carried out for 28 d and also with a freeze-thaw test to determine the physical stability as well. The physical characteristics observed are color, smell, transparency, and texture [16].

The size, the size distribution, and the zeta potential value of Citronella oil nanogel samples were measured by a particle size analyzer (PSA). About 50 µl of nanogel was added with 1 ml of distilled water then checked in the PSA [17].

We then performed the activity of Citronella oil nanogels as a repellent against *Aedes aegypti* mosquito as compared to X Product known as a branded repellent and empty nanogel (without Citronella oil).

Mosquito repellent activity

The Citronella oil nanogels were tested for protection by counting the number of mosquito bites on the guinea pig's skin. The guinea pigs as animal models in the experiment were bred in the Research and Development Centre of Health Pangandaran West Java Indonesia (Loka Litbangkes Pangandaran). Before the treatment, guinea pigs were acclimatized for 5-7 d, all the animals used must be specified normal values for the parameter to be tested [18]. The mosquitoes used were 25 per treatment, the interventions employed in this study were classified into 5 groups, including without any protection group; 3 nanogels formula groups i.e., formula 2, formula 3, and formula 4; X product group. The replication was performed 10 times. During the protection test, temperature and humidity measurements are conducted [19]. This experiment procedure has been approved by the Health Research Ethics Commission of Polytechnique of Health Ministry of Health Semarang no. 0316/EA/KEPK/2023.

The formula below can be used to determine the repellent's protective power (PP).

$$PP = \frac{C - R}{C} x\ 100$$

C = Number of mosquito bites on the control (untreated) hand

R = The number of mosquito bites on the treatment hands

Data analyses

A descriptive analysis was performed on the organoleptic data, pH, and spreadability. One-way ANOVA was used to analyze the mosquito repellent activity at a 95% confidence level. The Tukey HSD test is performed if the results show a significant difference in the data.

RESULTS AND DISCUSSION

The Citronella oil nanoemulsions were evaluated for physical characteristics for organoleptic and sensory using human senses. The physical evaluations of the Citronella oil nanoemulsions preparation observed are clarity, texture, color, and odor. The results of the physical evaluation of the Citronella oil nanogels can be seen in table 2.

Table 2: The results of the physical evaluation of citronella oil nanoemulsions

S. No.	Formula	Clarity	Texture	Color	Odor
1.	Formula 1	Cloudy as compared to Formula 2, 3, and 4	Liquid, lots of foam	White like milk	The distinctive smell of citronella
2.	Formula 2	Little bit cloudy compared to Formula 3	Liquid, not foaming	Transparent tends to be white	The distinctive smell of citronella
3.	Formula 3	Transparent	Liquid, not foaming	Transparent	The distinctive smell of citronella
4.	Formula 4	Cloudier compared to Formula 2 and 3	Liquid, a little bit of foam	Transparent tends to be yellowish	The distinctive smell of citronella

The physical evaluation results of nanoemulsion preparations with the best physical characteristics include non-foamy or slightly foamy liquid, transparent, and has the distinctive smell of Citronella oil, namely formula 2, formula 3, and formula 4. The physical characteristic of Formula 1 is unfavorable results including a cloudy whitish color like milk, having a lot of foam even though the smell still has the distinctive of Citronella oil. Referring to this result, Formula 1 was removed from the treatment group and no further tests were conducted.

The results of particle size, particle size distribution, and zeta potential value of Citronella oil nanoemulsions measurements can be seen in table 3.

Table 3: The particle size, particle size distribution, and zeta potential of Citronella oil nanoemulsions

S. No.	Formula	Z-average (nm)	PDI	Zeta potential (mV)
1.	Formula 2	27.3±0.17	0.16±0.00	-23.5±0.26
2.	Formula 3	24.4±0.44	0.16±0.02	-31.8±0.55
3.	Formula 4	46.7±0.49	0.48±0.01	-33.9±1.03

Data are reported as mean±SD, n=3

According to the nanoemulsions particle measurement results, the particle size of the three formulas determined can be stated as having a particle size in the nanoparticle category in the range of 24–46 nm. Nanoemulsion formulas can be categorized as nanoparticles when the formula has a particle size ranging from 10-140 nm [20]. Moreover, the three formulas of nanoemulsions have a uniform particle since their PDI values are under 0.5. The uniformity of the particle size will determine the stability of the mixture. The more uniform the particle size produced, the more stable the properties of the mixture [21].

The results of the pH measurements on the 4 Citronella oil nanogel formulas showed that the values do not meet the standards. Based on SNI 16-4946.2 the permitted pH for mosquito repellent gel is 4.5-7 [22]. However, we performed irritation testing using the observation method by dividing the guinea pigs' backs into 5 parts with the same area and then treating 4 parts with nanogel, and 1 part without treatment as a control. 0.5 g of each sample was smeared on the guinea pigs' backs, then covered with sterile gauze and fixed with plaster. After 24 h, the plaster was removed and left for 1 hour, then observed. After being observed, the part was covered again with the same plaster and observed again after 72 h. The observation demonstrated that none of the nanogels caused any irritation symptoms [23].

Confirming the irritation test of Citronella oil nanogels on the guinea pigs' backs, we continued to conduct the repellent activity test. The protection test was performed at the Research and Development Centre of Health Pangandaran West Java Indonesia (Loka Litbangkes Pangandaran), and the samples were adult female *Aedes aegypti* mosquitoes aged 3-5 d from rearing at the Loka Litbangkes Pangandaran. The experiment was carried out for 6 h and during the repellency or repulsion power test of the Citronella oil nanogels running, monitoring of temperature and humidity was done every 1 hour for 6 h [19]. The room temperature of the place for the running of the repellent activity test was recorded at about 26-27 °C. This temperature range is the optimal temperature for the *Aedes aegypti* mosquito for eating, namely 26-35 °C [24]. The relative humidity during the testing of the Citronella oil nanogels repellency activity test on guinea pigs was 59%-64 %. *Aedes aegypti* activity goes up with increasing humidity, the preferred humidity of this mosquito is more than 60% [25].

The results of the activity test of repellent of the Citronella oil nanogels against *Aedes aegypti* mosquitoes can be seen in fig. 1.

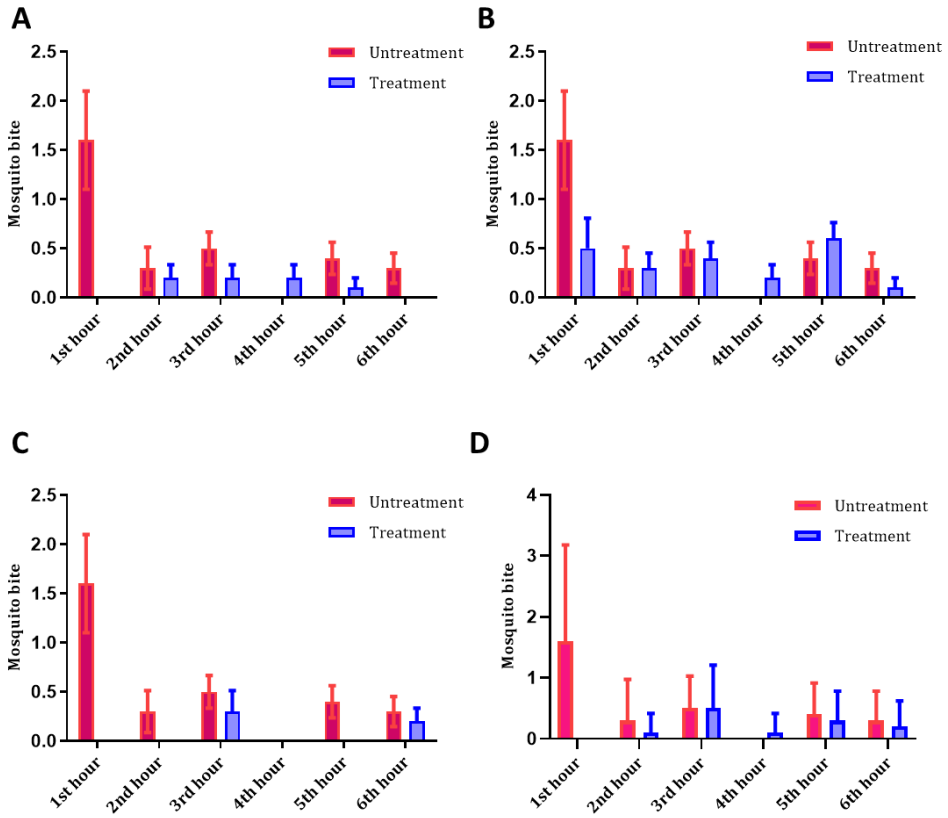

Fig. 1: The mosquito bite on the guinea pigs' skin, treatment with nanogel without Citronella oil (A); nanogel 2% of Citronella oil (B); nanogel 3% of Citronella oil; and nanogel 4% of Citronella oil (D). n = 10, all values represent mean±SEM

According to fig. 1, the least average number of mosquito bites in guinea pigs smeared with the Citronella oil nanogel formula 3, which is less than 1 mosquito or 0.5 with a variation of 1.197 with a range of 0-3. The results of the Anova test obtained a P-value of 0.0001, which means that statistically there was a significant difference in the average number of mosquito bites on guinea pigs' skin that were

treated with the Citronella oil nanogel. The data show that experimental animals smeared with Citronella oil nanogel tend to reduce mosquito bites. The main compound of Citronella is essential oil, citronellal, and geraniol which act as a mosquito repellent. The citronellal and geraniol in Citronella are not liked by insects, one of which is mosquitoes. The smell of citronellal and geraniol in Citronella can block carbon dioxide produced by humans so that it can affect mosquitoes' sense of smell [26].

Comparing the repellent activity of the Citronella oil nanogel formula 3 to the X product was very different as seen in fig. **2**. The X product is stronger than the Citronella oil nanogel formula 3.

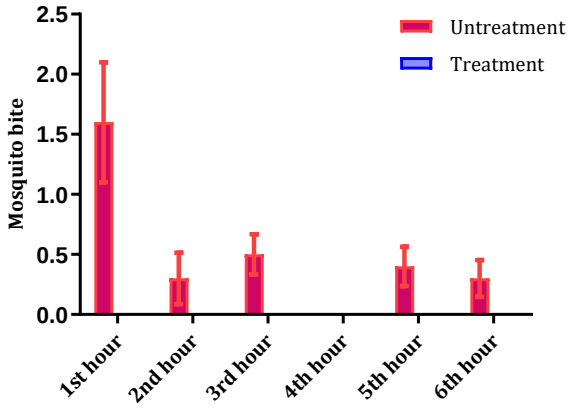

Fig. 2: The repellent activity of X Product consists of DEET as an active compound. n = 10, all values represent mean±SEM

However, enhancing the stability and efficacy of citronella oil as an active ingredient in nanogel can be used to create a mosquito repellent that is safer than DEET, which is well recognized to be harmful to human health.

CONCLUSION

The Citronella oil nanogel formula 3 with a composition of 1.5 ml of Citronella oil added with 2.925 g of Tween 80, 1.575 g of Span 80, 2.5 ml of propylene glycol, 16.5 ml of deionized water is the most effective formula in repelling *Aedes aegypti* mosquito. The development of a Citronella oil nanogel as mosquito repellent shows promise in terms of safety and environmental and human health.

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AUTHORS CONTROBUTIONS

AS: Conceived and designed the experiments, data analysis, writing. AF: AW: data analysis, reviewed the manuscript. IA: data analysis, writing and reviewing the manuscript. FWS: performed the research, data analysed, wrote manuscript draft. DWK: designed experiments, data analysed, reviewed, and finalized manuscript draft.

CONFLICT OF INTERESTS

There is no conflict of interest among the authors.

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