



# Development of Gel for *Aedes aegypti* Repellent With Combination of Cinnamon Oil (*Cinnamomum burmannii* Blume) and Fennel Oil (*Foeniculum vulgare* Mill)

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## Abstract

**Background/Aim:** Aim of this study was to develop efficient mosquito repellent by combining fennel oil (*Foeniculum vulgare* Mill) and cinnamon oil (*Cinnamomum burmannii* Blume) in a gel preparation form.

**Methods:** Effectiveness of each oil and its combination as viral repellents was tested by the World Health Organization's Pesticide Evaluation Scheme (WHOPES) method. Repellent was prepared in gel form. Evaluation of gel preparation included organoleptic properties, homogeneity, pH, viscosity, flow properties, acute skin irritation test and effectiveness test as mosquito repellent.

**Results:** The mosquito protection test for cinnamon oil showed that cinnamon oil was most effective at concentration of 15 % (96.85 %), and fennel oil at 24 % concentration (79.26 %). Within 6 hours, gel made of 24 % fennel oil and 15 % cinnamon oil combination gave protection against 53.49 % mosquitoes.

**Conclusion:** The combination of cinnamon oil and fennel oil can be formulated into gel form with satisfying physical and chemical characteristics and effectiveness against *Aedes aegypti* mosquitoes for 6 hours.

**Key words:** Gel; *Aedes aegypti*; Cinnamon oil; Fennel oil; Repellent.

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## Introduction

*Aedes* mosquitoes are a type of mosquito that is usually found in the tropics. Its name is derived from the Greek word *aedes*, which means "unpleasant". These mosquitoes are more than unpleasant, since they spread some dangerous diseases such as dengue fever. This species can often be found in Asia. Its specific characteristic is black and white legs. *Aedes aegypti* mosquitoes are also known as dengue propagators.<sup>1-3</sup>

Currently, the most available mosquito repellents on the market contain of anti-mosquito spray and anti-mosquito lotion are mostly made from di-

ethyl-meta-toluamide (DEET) chemicals. Chemical insecticide has an impact on a residue and its active ingredients are difficult to decompose in nature. These negative effects need to be avoided by replacing chemical insecticide with natural insecticide.<sup>4</sup>

Based on the type form of mosquito repellent products on the market, mosquito repellents such as mosquito coil gel are classified as superior products. They can be used anytime and anywhere. In addition, gel formulations have many advantages when compared with other semi-solid prepara-

tions such as creams or ointments. Gelled preparations have good active absorption capability on the skin, it is easy washed it away with water, they are highly adhesive when used on hair and skin without clogging the pores.<sup>5,6</sup>

Plant ingredients such as fennel oil (*Foeniculum vulgare* Mill) contain anethole that can serve as a repellent. Anethole is a group of essential oil compounds that have a very distinctive aroma and flavour that mosquitoes do not like. Cinnamon oil (*Cinnamomum burmannii* Blume) has cinnamaldehyde compounds that are toxic to mosquitoes. This substance has a potential to be used to make mosquito gel preparations, because the smell and taste is not favoured by mosquitoes. Cinnamon oil exhibits antibacterial, antifungal, antioxidant and anti-thrombotic effects. The combination of both plants is expected to work synergistically as a mosquito repellent.<sup>7,8</sup>

## Methods

### Material

The material consists of eggs from *Aedes aegypti* L mosquitoes, Carbopol U21 (dope lubrizol), propylene glycol (The Dow Chemical Company), triethanolamine (Merck), phenoxyethanol (Clariant), *C burmannii* blume oil, *F vulgare* oil and aqua.

### Methods

The concentration of cinnamon oil and fennel oil was used in the formula that is determined based on preliminary test results, fennel oil was used in 2 %, 4 %, 6 %, 24 % concentration and cinnamon oil was used in 5 %, 10 %, 15 % concentration. In this research concentration of combination of 15 % cinnamon oil and 24 % fennel oil was used to assess the effective dose of cinnamon oil and fennel oil test effectiveness mosquito repellent performed following the procedure recommended by the current guidelines by the World Health Organization's Pesticide Evaluation Scheme (WHOPES). The effectiveness test of mosquito repellent using human subjects' method has been chosen according to actual conditions of use.<sup>9</sup>

### Maintenance of *Aedes aegypti* eggs

*Aedes aegypti* eggs were inserted into plastic cups or bowls containing water for 2-3 days to drip down the larvae. During the development period the larvae were fed in pellets until the pupa phase was formed. The larval stage usually lasted 6-8

days to become the pupa, then the pupa was transferred into a bowl of water and left in the cage for 1-3 days to develop into an adult mosquito. Female mosquitoes were separated from male mosquitoes and fed in the form of sugar water.<sup>9,10</sup>

### Preparation of gel formulation

Carbopol U21 was dispersed in distilled water with 500 rpm stirrer speed for 24 hours. Phenoxyethanol was dissolved in propylene glycol and stirred with a stir bar until the homogeneous mixture was produced. The developed Carbopol U21 was neutralised with triethanolamine stirred with a stir bar until homogeneity. All bases were stirred at 200 rpm for 30 minutes, then the fennel oil and the cinnamon oil were added and stirred until homogeneity. Then, evaluation of anti-mosquito gel was performed.<sup>11-13</sup>

### Evaluation of physical and chemical preparations gel

Physical and chemical properties such as organoleptic properties (colour and odour), homogeneity and pH of gel were determined. Determination of viscosity and flow properties were performed with a Brookfield viscometer at a rate of 0.5; 1; 2.5; 5; 10; and 20 rpm and turn 20; 10; 5; 2.5; 2; 1; and 0.5 rpm. The result obtained was plotted against shear stress (dyne/cm<sup>2</sup>) and shear rate velocity (rpm). Viscosity checks were performed for 3 months at 40 °C temperature storage, at room temperature and at temperature of 400 °C.<sup>14</sup>

### Effectiveness of gel protection

Before and after the experiment each test area of the forearm volunteers was washed with soap and rinsed with water, then dried. The left arm as control was inserted into the mosquito coil, observed and the number of mosquitoes perched within the brackets within 5 minutes were recorded. When mosquito was perched, the test had begun, and after 5 minutes the arm was carefully removed from the mosquito cage. Then the right leg was smeared with gel and placed into the mosquito coat to be observed for 5 minutes. Arms and legs should not move during the test. Number of mosquitoes perched on the volunteer arms and legs were recorded. Observation lasted for 6 hours, with continuous monitoring and recordings every hour (on start, 1st, 2nd, 3rd, 4th, 5th and 6th hour). The number of mosquitoes that alighted were recorded as well.

### Stability test

The gel stability test was performed and it includ-

ed organoleptic properties (colour and odour), homogeneity, pH, viscosity and flow properties. Evaluated at low temperature ( $4 \pm 2$  °C) and room temperature ( $25 \pm 2$  °C) for 3 months with evaluation every 2 weeks, and high temperature ( $40 \pm 2$  °C) for 4 weeks, with once a week evaluation.<sup>15-16</sup>

### Irritation test

Toxicity test was performed by acute dermal irritation test on rabbit. Rabbits used in test were white male rabbits, 4 - 6 months of age, weight between 1.8 - 2 kg. Regulation of the head of BPOM No. 7 Year 2014 concerning the non-clinical *in vivo* toxicity test was considered and it was complied with the regulation.<sup>17</sup>

## Results

The results of effectiveness test of *Aedes aegypti* mosquito cinnamon oil and fennel oil

The mosquito protection test for cinnamon oil is most effective at concentration of 15 % and its activity protection was 96.85 % (Table 1).

**Table 1:** Result of effectiveness test of protection power of cinnamon oil to *Aedes aegypti* mosquitoes

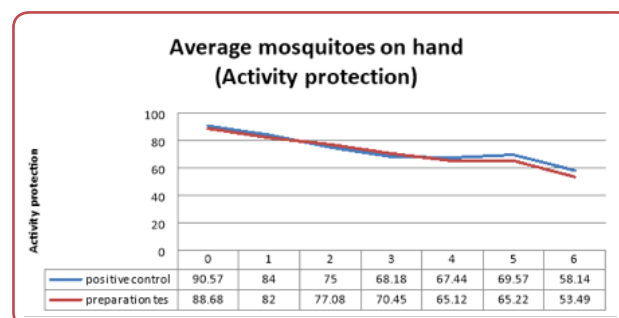
Days	Replication	Alcohol	Concentration		
			5 %	10 %	15 %
1	I	24	6	2	1
	II	24	4	3	2
	III	23	4	2	1
2	I	25	8	2	0
	II	23	8	2	1
	III	27	5	1	0
3	I	25	4	1	1
	II	27	6	2	1
	III	24	3	1	0
Average mosquitoes on hand		24.67	5.33	1.78	0.80
Activity protection			78.38 %	92.79 %	96.85 %

**Table 2:** Test results of effectiveness of fennel oil protection against *Aedes aegypti* mosquitoes

Days	Replication	Alcohol	Concentration			
			2 %	4 %	6 %	24 %
1	I	21	14	11	12	5
	II	20	14	11	10	3
	III	19	12	10	13	4
2	I	24	15	13	14	5
	II	22	16	12	11	4
	III	20	13	12	11	5
3	I	19	16	14	13	5
	II	22	14	13	11	4
	III	21	15	14	10	4
Average mosquitoes on hand		20.89	14.33	12.22	11.67	4.33
Activity protection			31.38 %	41.49 %	44.15 %	79.26 %

The mosquito protection test for fennel oil is most effective at 24 % concentration and its activity protection was 79.26 % (Table 2).

The test of mosquito protection from combination cinnamon oil and fennel oil for 6 hours compared with positive control (Figure 1).



**Figure 1.** Result of activity protection combination of cinnamon oil and fennel oil mosquito repellent

**Table 3:** Formulation design of the mosquito repellent gel

Materials	Concentration (%) (b/b)		Function
	Formula		
Fennel Oil	24		Active ingredients
Cinnamon Oil	15		Active ingredients
Carbopol U 21	3,01		Thickener
Propylene glycol	6,03		Humectan
Triethanolamin	0,5		pH adjuster
phenoxyethanol	0,2		Preservative
Aquadest	51,26		solven

### Formulation design

Table 3 contains the chemical composition of the repellent gel based on fennel oil and cinnamon oil.

## Evaluation of gel preparations

### Organoleptic

The mosquito repellent gel was made to show a homogeneous physical appearance, clear white colour and pleasant aroma.

### The results of viscosity testing and flow properties

The viscosity of gel on third temperature after storage for 12 weeks decreased. At 5 rpm speed using spindle No. 3 temperature 400 °C has viscosity of 7,050 centipoise (cP), temperature 250 °C has viscosity 3,600 cP, and temperature 40 °C has viscosity 2,550 cP (Figure 2).

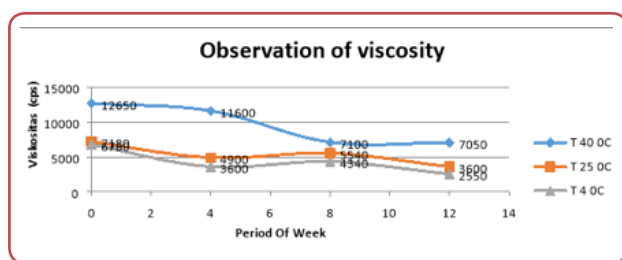


Figure 2. Graph of changes in viscosity values to three temperatures during 12 weeks.

### Storage flow properties

The pseudo plastic flow properties begin at zero and there is no yield value. Viscosity decreases with increasing rate of shearing. Increased shearing stress causes the regularity of the polymer thereby reducing the resistance and further increasing the rate of share in subsequent shearing stress (Figure 3).

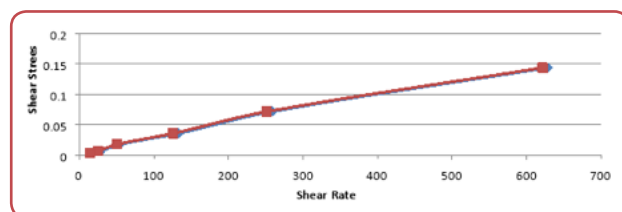


Figure 3. Rheology pseudo plastic

### pH

pH of gel was 6.00 - 6.25. The addition of a combination of cinnamon oil and fennel oil can cause a decrease in the pH.

### The result of mosquito repellent stability test

The mosquito repellent formulas are stable at temperature (25 ± 2 °C) and cold temperature (4 ± 2 °C) with homogeneous physical appearance, clear white colour, and aromatic viscosity of 7180 - 2550 cps, pseudoplastic flow properties pH 6.00 up to 6.25 (skin pH 4.5 to 6.5).

Table 4: Response of rabbits to irritation caused by mosquito repellent gel containing fennel oil and cinnamon oil administered over 72 h

Irritation response category in rabbits	Score
No Irritation	0
Very Low Irritation	1
Low Irritation	2
Middle Irritation	0
High irritation and exfoliation	0

### Result of irritation test

The combination of mosquito repellent oil gel and fennel oil resulted in mild irritation of the skin of experimental animals after application for 3 consecutive days (72 h) with a mild irritation index of 2 (Table 4).

## Discussion

With observation for 6 hours, combination of fennel oil in 24 % concentration and cinnamon oil in 15 % concentration, gave protection from 53.49 % *Aedes aegypti* mosquitoes. Chemical insecticides due to their longevity have a great impact on environment. If there is a possibility of finding a natural insecticide that is effective it is worth the effort.

This decrease in viscosity can be caused by storage packaging. It is assumed that it causes the gel to absorb water from the environment so that the volume of water in the formula increases. In addition, the arrangement of initially irregular molecules into long axes in the flow direction may result in some of the solvents binding to lose molecules, thereby causing a decrease in viscosity.

pH of gel was 6.00 - 6.25. The addition of a combination of cinnamon oil and fennel oil can cause a decrease in the pH which is welcomed since acid repels mosquitoes.

## Conclusion

Based on the observation and test of protection power it can be concluded that the combination of fennel oil and cinnamon oil are effective repellents against *Aedes aegypti* mosquitoes for 6 h. The combination of cinnamon oil and fennel oil can be formulated into gel preparations and meets the physical and chemical requirements.

## Acknowledgements

None.

## Conflict of interest

None.

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