

Repellency Test of Wet Tissue Containing DEET (N,N-diethyl-3-metatoluamide) and Citronella Oil (*Cymbopogon citratus*) Against *Aedes Aegypti* Mosquitoes

Uji Daya Usir Tisu Basah yang Mengandung DEET (N,N-diethyl-3-metatoluamide) dan Minyak Serai terhadap Nyamuk *Aedes Aegypti*

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Abstract

Dengue hemorrhagic fever has been one of the major health problems in Indonesia. As the disease spreads out by *Aedes aegypti*, a variety of ways has been conducted to disconnect host, agent, and the environment chain including prevention of human contact with the vector using by various repellents. The present test compared the complete protection time of N,N-diethyl-3-metatoluamide (DEET) and citronella oil impregnated in wet tissue against *Aedes aegypti*. For this test, an ethanol-based 12.5% DEET and 20% citronella oil were prepared, into which dry tissue papers were immersed, drain in open air, and then stored in aluminum foil packs for one day and one week. Meanwhile, a number of disease-free adult female *Aedes aegypti* were placed in mosquito cages. The prepared one-day and one-week stored wet tissues were used to swab volunteer adult human hands (from elbow to wrist) which were then inserted into the mosquito cage in three replicates (25 mosquitoes each cage). Elapsed time from first hand insertion to the first mosquito bite was calculated and expressed as repellency time of the impregnated repellent. It was found that the average repellency times of one-day stored 12.5% DEET and 20% citronella oil wet tissues were 4 hour 26 minutes and 14.24 minutes, respectively, while for one-week stored were 4 hour 6 minutes and 12.57 minutes, respectively. Post Hoc test showed that the repellency time difference between one-day and one-week storage was not statistically significance ($p = 0.524$ for DEET and $p = 0.681$ for citronella oil).

Key words: *Aedes aegypti*, DEET, citronella oil, wet tissue

Abstrak

Demam berdarah merupakan salah satu masalah utama kesehatan di Indonesia. Karena penyakit ini disebarkan oleh nyamuk *Aedes aegypti*, berbagai cara telah dilakukan untuk memutus rantai pejamu, agen, dan lingkungan, termasuk mencegah kontak manusia dengan vektor dengan menggunakan bermacam-macam pengusir serangga. Uji ini membandingkan waktu proteksi N,N-diethyl-3-metatoluamide (DEET) dengan wak-

tu proteksi minyak sitronella yang terkandung dalam tissu basah terhadap *Aedes aegypti*. Untuk uji ini, dibuat larutan 12,5% DEET dalam etanol dan larutan 20% minyak sitronella. Sejumlah tissu kering dicelupkan ke dalam masing-masing larutan ini, ditiriskan dalam udara terbuka, kemudian dibungkus dengan lembaran aluminium dan disimpan selama satu hari dan satu minggu. Sementara itu, sejumlah nyamuk *Aedes aegypti* betina yang bebas penyakit ditempatkan dalam sangkar. Tissu basah yang telah disimpan selama satu hari dan satu minggu diusapkan pada bagian tangan (dari sikut sampai pergelangan tangan) relawan, kemudian tangan yang telah diusap itu dimasukkan ke dalam sangkar nyamuk dalam tiga replikasi uji (25 ekor nyamuk per sangkar). Waktu antara pemasukan tangan sampai dengan gigitan nyamuk pertama dinyatakan sebagai waktu usir tissu basah. Ditemukan bahwa waktu usir rata-rata tissu basah 12,5% DEET dan 20% minyak sitronella yang disimpan satu hari masing-masing 4 jam 26 menit dan 14,24 menit, sedangkan untuk tissu basah yang disimpan satu minggu masing-masing 4 jam 6 menit dan 12,57 menit. Uji *Post Hoc* menunjukkan bahwa penyimpanan tissu basah satu hari dan satu minggu tidak berbeda secara bermakna ($p = 0,524$ untuk DEET dan $p = 0,681$ untuk minyak sitronella).

Kata kunci: *Aedes aegypti*, DEET, minyak sitronella, tissu basah

Introduction

Dengue hemorrhagic fever (DHF) has been known in Indonesia since 1967. In the beginning, this viral disease mostly infected children living in the dense population cities but lately it also affects adults.¹ Increasing cases usually occurred in the beginning of the rainy season.²

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The main vector of DHF is *Aedes aegypti* mosquitoes.³ Neither drugs nor vaccines are available today to cure the disease. Therefore, a variety of control measure is done to disconnect the chain of host, agent, and the environment of the DHF vector transmission by eradicating adult mosquito and its larvae and eliminating the breeding nests.^{4,5} At community level, eliminating breeding places of the mosquito vector has successfully reduced DHF prevalence in many parts of Indonesia.⁶⁻⁹ Meanwhile, to prevent or reduce biting rate of the vector mosquito, the majority of people uses insect repellent since it is practical to apply and easy to obtain in the market.

Repellent is a variety of chemicals and natural plant substances that has an ability to release scents to which the insects are dislike and therefore they hinder from human where the skin is swabbed by these substances.¹⁰ There are a lot of synthetic and natural repellents commonly used in the society but mostly consist of 12,5% *N,N*-diethyl-3-metatoluamide (DEET).¹¹⁻¹⁴ The DEET is an effective synthetic substance, safe to apply on human skin, and mostly sold freely as a commercial product.¹⁵ When apply to skin, DEET-based repellent can effectively protect human from mosquito bites up to \pm 5 hours. However, DEET cannot be used under clothing or on damaged skin as it has irritant potency and may cause skin reactions.¹⁶

In addition to synthetic chemicals, there are at least 38 essential oils of plants' origin that have potential repellency against mosquitoes.¹² Of these, oil of citronella, patchouli (from *Pogostemon cablin*), clove (from *Syzygium aromaticum*), and Thai makaen (from *Zanthoxylum limonella*) were effective against *A. aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus*. Clove oil is the best repellent with the longest protecting time up to 2 to 4 hours against all three mosquito species.

Citronella oil is the main essential oil of an ordinary kitchen lemongrass *Cymbopogon citratus*.¹⁷ In South East Asia the plant is known as *oil grass* (Indonesia: *sereh* or *serai*). There are two chemotypes of citronella oil, Ceylon type from *Cymbopogon nardus* plant and Java type from *Cymbopogon winterianus* plant. Both types contain more than 80 constituents, about 50 substances make-up over 90% of the oil.¹⁸ The Ceylon type consist of geraniol (18-20%), limonene (9-11%), methyl isoeugenol (7-11%), citronellol (6-8%), and citronella (5-15%), while the Java type consists of citronella (32-45%), geraniol (11-13%), geranyl acetate (3-8%), and limonene (1-4%).¹⁹ It is well known that Java type is superior upon Ceylon type. Due to high essential oil content, the plant is usually used for scrubbing rheumatism and sore nerve, stop vomiting, and cholera remedy.²⁰ While the roots and stems can be used to remedy urine, sweat, relieve coughing, clean mouth, and warm body

temperature, the leaves are used for stomachache remedy, appetite increase, after birth recovery, and fever relief.²¹

Besides direct medicinal applications as described above, the citronella oil has been also tested as insect repellent against *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus* in different concentration and formula.^{11,12} Citronella oil has also been using extensively for more than 50 years as a source of important perfumery chemicals in soap, perfumery, cosmetic, and flavoring industries throughout the world. As insect repellent, it is commonly found as candles, lotions, gels, and sprays.²² Since also found in towelette wipes, the citronella oil may also be used as insect repellent by impregnating it into cleansing tissue. This form may provide comfortable, alternative application to liquid (lotion) repellent type which dries quickly and only leaves a thin layer of active substance. Yet, its retention time in wet tissue and its repellency time when wiped on the skin are unknown to what length of time the oil is still protective to mosquito bites. The present study compared the repellency time of DEET and citronella oil impregnated in the wet tissue for effective prevention of mosquito bites using *A. aegypti* as insect test.

Method

DEET of 99,5% purity was kindly provided by PT Gelpy Jakarta, while commercial product of 100% citronella oil was purchased from public outlet. Wet tissue papers was prepared by immersing the dry tissue in 12,5% DEET solution (prepared by diluting 99,5% liquid DEET with 60% ethanol) for ten seconds, dried in open air for 15 minutes, then wrapped immediately by aluminium foil packs (to prevent further oil evaporation) and stored for one day and one week separately before test. The same procedure was employed for tissue immersed in 20% citronella oil (prepared by diluted 100% lemon grass oil with 60% ethanol). Meanwhile, adult female *Aedes aegypti* mosquitoes (obtained from Department of Parasitology of Faculty of Medicine, Gadjah Mada University, Yogyakarta), were used as insect test that has been acclimatized for one day. The body surface object of the repellency test experiment was adult human hand area from elbow to wrist.

Test protocol for the efficacy of DEET and citronella oil follows Fradin and Day design as described elsewhere.¹⁷ In the present repellency test, laboratory mosquito cages of 30 cm by 22 cm by 22 cm dimension were constructed for three replicates. In each cage test, 25 disease-free, laboratory-reared female one-day acclimatized *A.egypti* were placed. Three different topical repellent application of DEET on hands were employed i.e. (1) positive control (direct application of 12,5% DEET), (2) one-day stored wet tissue containing 12,5%

Table 1. Elapsed Time of *Aedes aegypti* Biting to Human Hands Rubbed with One-day and One-week Stored 12,5% DEET and 20% Citronella Oil Wet Tissues

Test group	Complete Protection Time (second)			Average Complete Protection Time
	1	2	3	
<i>12.5% DEET</i>				
(+) Control (direct application)	8.700	10.620	8.700	9.340 s (2 h 26 m 20 s)
One-day stored wet tissue	14.460	17.160	16.320	15.980 s (4 h 26 m 20 s)
One-week stored wet tissue	13.440	15.360	15.480	14.760 s (4 h 6 m)
<i>20% Citronella oil</i>				
(+) Control (direct application)	3.850	616	2.911	3.227 s (53 m 47 s)
One-day stored wet tissue	1.084	744	735	854 s (14 m 14 s)
One-week stored wet tissue	764	745	756	754 s (12 m 34 s)

Table 2. Post Hoc Test Retention Time of One-day and One-week Stored 12,5% DEET and 20% Citronella Oil Wet Tissue Againsts *Aedes aegypti*

12,5% DEET			20% Citronella Oil		
Experiment I	Experiment II	P	Experiment I	Experiment II	P
One-day	(+) Control	0,00	One-day	(+) Control	0,00
	One-week	0,524		One-week	0,681

DEET, and (3) one-week stored wet tissue containing 12,5% DEET. The same applications were employed for 20% citronella oil i.e. (1) positive control (direct application of 20% citronella oil), (2) one-day stored wet tissue containing 20% citronella oil, and (3) one-week stored wet tissue containing 20% citronella oil. One-day and one-week stored repellent tissues were tested simultaneously (e.g. DEET was applied/rubbed on right hand while citronella oil was applied/rubbed on left hand).

For each group of insect repellency test, both hands (right and left) were wiped or rubbed with wet tissues as well as with direct topical application of DEET and citronella oil, then inserted into cage through its holes for 20 minutes. If within 20 minutes there was no mosquito bite, the hands were re-inserted into the cage for as long one minute in every 15-minute interval until the first bite. These procedures were repeated three times. The second and the third tests were done based on the first mosquito bite i.e. (1) if the first mosquito bite was within less than 20 minutes, the second and third experiments were conducted in one minute for every 5-minute interval, (2) if the first mosquito bite was between 20 minutes to four hours, the second and third experiments were conducted in one-minute for every 15 minute interval for as long four hours, (3) if the first mosquito bite was within more than four hours, the second and third experiments were conducted in one minute for every hour, (4) if there were no mosquito bites at all, the interval time were prolonged

as necessary. The elapsed time from first hand insertion to the first bite was calculated and recorded as the ‘complete protection time’ for the impregnated repellents in wet tissue. One-way Anova was used to analyze data followed by Post Hoc Test to figure out the level of means between treated groups.

Results

Table 1 presents the elapsed time of repellency test of two wet tissue types (i.e. those containing 12,5% DEET and those containing 20% citronella oil) for two separate storage times (one-day and one-week) against female adult *A. aegypti*, while Table 2 summarizes Post Hoc test results of different storage times of the wet tissues. The elapsed time is hereby designated as ‘complete protection time’ which directly indicates the repellency time of the topical repellent application. One-way Anova analysis for the repellency time data is not shown as it was employed directly into the Post Hoc test as presented in Table 2.

Discussion

Table 1 shows that generally the repellency time of the DEET is longer than the citronella oil, both impregnated in wet tissue. Surprisingly, however, direct topical application of 12,5% DEET lotion has shorter repellency time compared to the one-day and one-week storage of 12,5% DEET-containing wet tissues. Supposedly, the more concentrated the repellent on the skin, the longer the repellency time. Citronella oil follows this pattern where

direct topical application had longer repellency time compared to the citronella oil-containing tissues. However, as shown in Table 2, storage time of both repellents in wet tissues does not affect the repellency times as indicated by insignificant difference between one-day and one-week storage ($p = 0,524$ for DEET and $p = 0,681$ for citronella oil).

The present repellency test proves that the wet tissue containing 12,5% DEET can effectively repel away *A. aegypti* mosquito from the wiped human body. As shown in Table 1, the average repellency time of 12,5% DEET is more than four hours for both storage times. However, the application of wet tissue is peculiarly better than the direct lotion. The average repellency time of direct lotion application is approximately 2,5 hours, about 58% shorter than that of wet tissue. The shorter repellency time might be due to possible reaction of DEET with the skin.¹⁹ More concentrated DEET is likely more reactive to skin, resulting in lower repellency.

Contrast with the DEET, direct topical application of citronella oil has longer repellency time than that of the wet tissue of 20% citronella oil. This finding has good agreement with Fradin and Day efficacy test for citronella-based repellents.¹⁷ In their experiment, the protection times of repellent formula containing 10% and 5% citronella oil were 7 to 60 minutes and 5 to 30 minutes, about 20 and 14 minutes in average, respectively. In the present repellency test, 20% citronella oil formula has a repellency time of 14,24 minutes for one-day storage and 12,34 minutes for one-week storage wet tissue. The difference in protection time between the two tests might be due to the experimental condition and laboratory setting. Fradin and Day fully controlled the laboratory temperature and humidity strictly as well as human sensitivity variation to mosquito bites.

However, shorter repellency time of citronella-containing wet tissue against mosquito bites makes it less practical. Based on the present test, wet tissue needs to be continuously reapplied to the skin every 15 minutes. Repeated tissue applications can lead to even greater exposures to the citronella oil. Although US-EPA classifies it as low acute toxicant (Category III – slightly toxic with $LD_{50} > 4380$ mg/kg and skin irritation cleared within 7 days or less), recent study suggests that natural citronella oil may also contain methyleugenol, which has been shown to be carcinogenic in animal studies.^{22,23} At high dose levels it indicates potential reproductive and developmental toxicity as well as fetal sensitivity. Therefore, citronella oil cannot be applied in high doses. It might be in combination with other non toxic, cosmetic-grade substances, for example as moisturizer or sunscreen. So far, however, citronella oil has not been applied in wet tissue formula. Rather, it is commonly used directly as undiluted and diluted oil (lotion) or gel on the skin.

Conclusion

The wet tissue containing 12,5% DEET of either one-day or one-week storage can protect *A. aegypti* mosquito bites with 17 to 20 folds longer than the wet tissue containing 20% citronella oil. Application of citronella oil impregnated in wet tissue is less practical as it should be reapplied approximately every 15 minutes.

Recommendation

Since the application of impregnated repellent in wet tissues is less practical due to shorter repellency time, further study is suggested to prepare novel formula of citronella oil for direct topical use on the skin. It might be in combination with safe, cosmetic-grade substances in the form of gel or lotion.

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