Effects of Glucam P-20, Vanillin, and Fixolide on Mosquito Repellency of Citronella Oil Lotions

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ABSTRACT The objective of this study was to investigate the effects of three fragrance fixatives, Glucam P-20, Vanillin, and Fixolide, on the mosquito repellent property of citronella oil lotions. In the current study, two formulae (A and B) of oil-in-water citronella oil lotions were formulated using different ingredients (emulsifiers [Cremophors or Emulwax], stiffening agents, and emollients). Citronella oil was used at 10% wt:wt. The weight ratios tested between citronella oil and each fixative were 1:0.25, 1:0.5, and 1:1. Overall, 20 formulations, including one with no fixatives for both A and B, were produced, A1-A10 and B1-B10. The repellent activities of these 20 lotions against *Aedes aegypti* (L.) were tested using a human-bait technique. The types and concentrations of fixatives as well as the compositions of the formulations did affect the protection time of the citronella oil lotions. The lotion containing Emulwax and 5% vanillin (B6) was the most effective repellent. It provided the longest protection time of 4.8 h, which exceeded the minimum requirement of 2 h set by the National Institute of Health, Thailand. The shortest protection time (1 h) was observed in the lotion containing Emulwax and 2.5% Glucam P-20 (B2). It could be concluded that the tested fixatives affected the repellent activity of the citronella oil lotions.

KEY WORDS citronella oil, Glucam P-20, Vanillin, Fixolide, mosquito repellent

Mosquitoes are one of the main problems in several tropical countries including Thailand. It is generally recognized that mosquito bites not only cause allergic responses but also transmit several life-threatening diseases such as malaria and dengue (Chio and Yang 2008). As a result, both synthetic and natural insect repellents have been used for the prevention of mosquito bites. It is claimed that natural source repellents are safer and more environmentally friendly when compared with the synthetic ones (Katz et al. 2008). Various volatile oils possess mosquito repellent action (Thomas et al. 2009, Nerio et al. 2010). Citronella oil is a pale to dark yellow liquid with a strong citrus scent, and it creates a vapor barrier that can deter or repel mosquitoes from biting or coming into contact with human and animal skin. Generally, it is obtained by steam distillation of dried *Cymbopogon* plants such as *Cymbopogon nardus* L. and *Cymbopogon winterianus* (Lis-Balchin 2006). Two types of citronella oil are on the market: Sri Lanka (Ceylon) and Java types. Citronella oil is considered as generally recognized as safe (GRAS) by the U.S. Food & Drug Administration. For use as an insect repellent, the concentrations of citronella oil used can vary from 0.05 to 25% (wt:vol). As with many volatile oils, because of its high volatility, the crucial problem of citronella oil is its short-lasting ability to protect against mosquitoes. For mosquito repellent products sold in Thailand, the protection time against mosquitoes set by the National Institute of Health is 2 h. Unfortunately, the average protection periods of volatile based repellents are usually less than this minimum time limit (Choochote et al. 2007). Therefore, several strategies have been explored to promote the repellent activity of volatile oils including the use of microcapsules, nanoemulsions, and fixative substances (Nerio et al. 2010). In our previous study, the release of citronella oil was controlled by forming a complex with β-cyclodextrins. However, the longest protection time of the lotion incorporated with this citronella oil inclusion complex was only 1.8 h (Songkro et al. 2011). In an attempt to enhance the repellent efficacy of citronella oil lotion, fixatives have been selected in this current study. The use of fixatives to improve the protection time is the simplest method when compared with the other techniques. In general, only the mixture of volatile oils and fixatives were tested for mosquito repellency. Other ingredients necessary to produce proper dermatological formulations were not previously incorporated, but in this case they are accounted for.

Fixatives can be defined as substances which amplify the intensity and lasting qualities of the aromatic components of fragrance materials (Seldner 1982). Fixatives have lower volatility in comparison with the

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fragrances (Dobbs et al. 2009). They have the ability to slow the evaporation of volatile oils. Presently, a variety of fixatives have been marketed. There are two categories of fixatives based on their sources: natural and synthetic. The natural fixatives can be derived from botanical compositions and animal secretions such as civet and musk. The synthetic fixatives include Glucam P-20 (Flick 1991), Fixolide (Sell 1999), and 2,2,4-trimethyl-1,3-pentane diol (Dobbs et al. 2009).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a white or slightly yellow, crystalline powder that is derived from plants (i.e., *Vanilla planifolia* Andrews), bioconversion of related natural products or synthesis. Vanillin extracted from the native vanilla pods is the most expensive. Vanillin is soluble in alcohol, chloroform, and fixed and volatile oils. The molecular weight of vanillin is 152.15. It is widely used in foods, beverages, cosmetics, and pharmaceuticals (Walton et al. 2003, Sweetman 2005, Korthou and Verpoorte 2007). Vanillin has been widely investigated as a fixative for various mosquito repellent volatile oils. For example, Choochote et al. (2007) mixed selected volatile oils with 10% vanillin and tested for repellency against *Aedes aegypti* (L.) using a human-bait method. It was found that 10% vanillin could improve the repellent effects of certain volatile oils against *Ae. aegypti.* The average protection time obtained for *Zanthoxylum piperitum* oil mixed with vanillin was 2.5 h, which was longer than the 2 h minimum protection time for mosquito repellents sold in Thailand. Nevertheless, in another study (Thomas et al. 2009), 5% vanillin did not promote the repellent activity of volatile oil of *Kunzea ambigua,* an Australian Native Plant.

In addition to vanillin, Glucam P-20 and Fixolide were chosen as fixatives in an attempt to enhance the repellent property of citronella oil. Their renowned ability to increase the lasting power of volatile fragrances was the main reason for their selection. To our knowledge, both Glucam P-20 and Fixolide have never been tested for their ability to prolong the protection time of citronella oil against mosquito bites. Glucam P-20 is a trade name for polypropyleneglycol-20-methyl glucose ether or PPG-20- methyl glucose ether. Glucam P-20 is a pale yellow cosmetic liquid that is miscible with water, alcohol, and most organic oils and solvents. It has medium viscosity and molecular weight of 730.88. Glucam P-20 is practically odorless and has been used as a humectant and a perfume fixative (Seldner 1982, Flick 1991, Beering 1999). Fixolide is a synthetic polycyclic musk that was created to substitute for the more expensive, natural nitro musks in cosmetics and toiletries. Fixolide is the commercial name for 7-acetyl-1, 1, 3, 4, 4, 6-hexamethyltetralin (Sell 1999, Chittiteeranon et al. 2007). It is a white to pale yellow solid with a strong, sweet, fruity musk odor. Although it is the *S* enantiomer of Fixolide that produces the strong musk odor, only its racemate form is commercially available (Ciappa et al. 2004). The chemical structures of vanillin, Glucam P-20 and Fixolide are illustrated in Fig. 1. In the current study, these three fixatives in various concentrations were incorporated into the oil-in-water (o/w) lotion bases

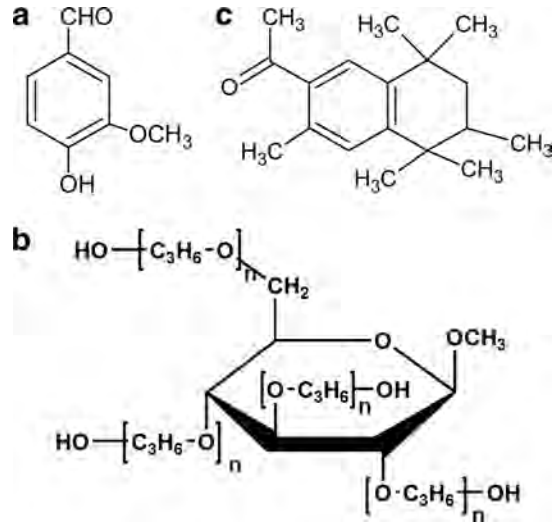

Fig. 1. Chemical structures of vanillin (a), Glucam P-20 (b), and Fixolide (c).

containing 10% wt:wt citronellla oil. The concentration of 10% was selected based on the concentration of a commercial product, Songkhla citronella mosquito repellent lotion (Songkhla, Thailand). Then, the physical properties and repellent activity of the formulations were evaluated.

The aim of the current work was to use a human bait technique (arm-in-cage-assay [Nerio et al. 2010]) to evaluate the effects of fixative type and concentration on the repellent activity of citronella oil lotions against *Ae. aegypti.* The lotions were prepared as o/w emulsions containing citronella oil at 10% wt:wt.

Materials and Methods

Chemicals. Citronella oil (Java type) and Fixolide were obtained from Thai-China Flavors and Fragrances Industry Co., Ltd. (Ayutthaya, Thailand). Cremophore A6, Cremophore A25, and other ingredients such as glycerin and Uniphen P-23 (preservative) were supplied by P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Glucam P-20 was a gift from Chemico Inter Corporation Co., Ltd. (Bangkok, Thailand). All chemical were of analytical grade and were used without further purification.

Evaporation of Citronella Oil. The evaporation of pure citronella oil and citronella oil mixed with fixatives at different weight ratios were investigated using a Mettler LP 16 Infrared Dyer and Moisture Analyzer equipped with Mettler PM 300 automatic balance (Mettler-Toledo AG, Switzerland). The ratios between citronella oil and each fixative were varied from 1:0.25, 1:0.5, and 1:1. The samples with equivalent amount of volatile oil were stored in an open-lid container and heated at 120°C for 50 min. Then, the loss of sample weight was recorded.

Preparation of Citronella Oil Lotions. A beaker method was used to prepare the o/w citronella oil

Table 1. Formulations of citronella oil lotions

Formulation code	Fixative	Concentration (% wt:wt)
A1	No	—
A2	Glucam P-20	2.5
A3	Glucam P-20	5.0
A4	Glucam P-20	10.0
A5	Vanillin	2.5
A6	Vanillin	5.0
A7	Vanillin	10.0
A8	Fixolide	2.5
A9	Fixolide	5.0
A10	Fixolide	10.0
B1	No	—
B2	Glucam P-20	2.5
B3	Glucam P-20	5.0
B4	Glucam P-20	10.0
B5	Vanillin	2.5
B6	Vanillin	5.0
B7	Vanillin	10.0
B8	Fixolide	2.5
B9	Fixolide	5.0
B10	Fixolide	10.0

lotions with or without fixatives. Citronella oil at 10% wt:wt was used as the active ingredient. Concentrations of each fixative were varied from 2.5, 5, and 10% wt:wt. Two formulae, A and B, were prepared containing different emulsifiers, stiffening agents, and emollients. Formula A contained Cremophor A6 and Cremophor A25 as emulsifiers, while formula B had Emulwax, which was specifically created by us, as an emulsifier. Details of the ingredients used for formula A have been described elsewhere (Songkro et al. 2011).

The ingredients for formula B are as follows: shea butter (1 g emollient); stearic acid (6 g stiffening agent); isopropyl myristate (5 g emollient); Emulwax (7 g emulsifier); glycerin (5 g humectant); Uniphen P-23 (1 g preservative); Citronella oil (10 g active ingredient); fixatives (0, 2.5, 5 or 10 g fixative); purified water to 100 g (vehicle).

Both formulae contained the same preservative (Uniphen P-23), humectant (glycerin), and the vehicle (purified water). Twenty formulations were prepared and designated as A1-A10 and B1-B10 for formula A and formula B, respectively (see Table 1). The number of samples in each for formulation was three ($n = 3$). These lotions were then kept in tight containers and stored at room temperature (31.0 ± 1.0°C).

Physical Property of Citronella Oil Lotions. The appearance, pH and viscosity of the citronella oil lotions were assessed by naked eyes, a digital pH meter, and a Brookfield viscometer, respectively. In the case of appearances, color, phase separation, creaming, and uniformity of the products were examined and recorded. The pH measurement was conducted in triplicate at room temperature (31.0 ± 1.0°C). For the viscosity measurement, the bob-cup Brookfield viscometer (model DV-III ultra, Brookfield Engineering Laboratories Inc., Stoughton, MA) and Brookfield Rheocalc software (version V 3.1–1) were used. Again, the determinations were carried out at room temperature.

Mosquito Repellent Test. The prepared lotions were tested for their repellent activity against *Ae. aegypti* using the human bait technique. This study was approved and carried out by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. The details of the test procedure have been published elsewhere (Tawatsin et al. 2006, Songkro et al. 2011). In brief, marked areas of the forearms of three human volunteers ($n = 3$) were thoroughly applied with the test formulation (0.1 g) then placed into a mosquito cage that contained host seeking female *Ae. aegypti* mosquitoes for 3 min of exposure at every 30 min interval. The protection time was defined as the time between application of the test formulation and the second successive bites.

Statistical Analysis. The statistical differences of physical properties of lotions were analyzed using one-way analysis of variance (ANOVA). A P value of <0.05 was considered significantly different.

Results and Discussion

Evaporation of Citronella Oil at 120°C. Figure 2 shows the evaporation of pure citronella oil in comparison with the oil mixed with each fixative at different weight ratios. The test temperature was fixed at 120°C, because the most noticeable differences in the evaporation behavior of samples were observed in the previous study (Songkro et al. 2011). At the first 5 min of experiment, the percentage of unevaporated oil in the presence and absence of fixative are similar. With increasing time, the differences in the amount of oil evaporation are clearly seen. The weight ratios of citronella oil to fixative influenced the evaporation behavior of citronella oil. Overall, a weight ratio of 1:1 (oil: fixative) provided the lowest evaporation rate for citronella oil, whereas the highest evaporation of oil was found in the pure citronella oil without any fixative. At 50 min, the rank order of percentage of unevaporated citronella oil was vanillin > Fixolide > Glucam P-20. The results indicate the ability of fixative to reduce the evaporation rate of the essential oil.

Citronella Oil Lotions. It should be pointed out that the prepared lotions contained the same weight ratio of citronella oil and fixative used in the evaporation study. These ratios were 1:0.25 (10:2.5), 1:0.50 (10:5), and 1:1 (10:10). All citronella oil lotions were homogeneous with the exception of the formulations A7 and B7, which contained 10% wt:wt vanillin as a fixative. Both formulations exhibited phase separations after a day of preparation. Thus, these two formulations were excluded from the current study. The lotions of citronealla oil with or without fixatives were milky white with a citrus scent. The appearances of the products were good. No phase separation and creaming occurred after 5 mo of storage at room temperature. The pH and apparent viscosity of the lotions are summarized in Table 2.

All formulations had an acidic pH ranging from 3.25 to 4.89. The highest and lowest pH values were obtained from formulation A1 and formulation A2, respectively. In general, the pH range of 5–8 is accept-

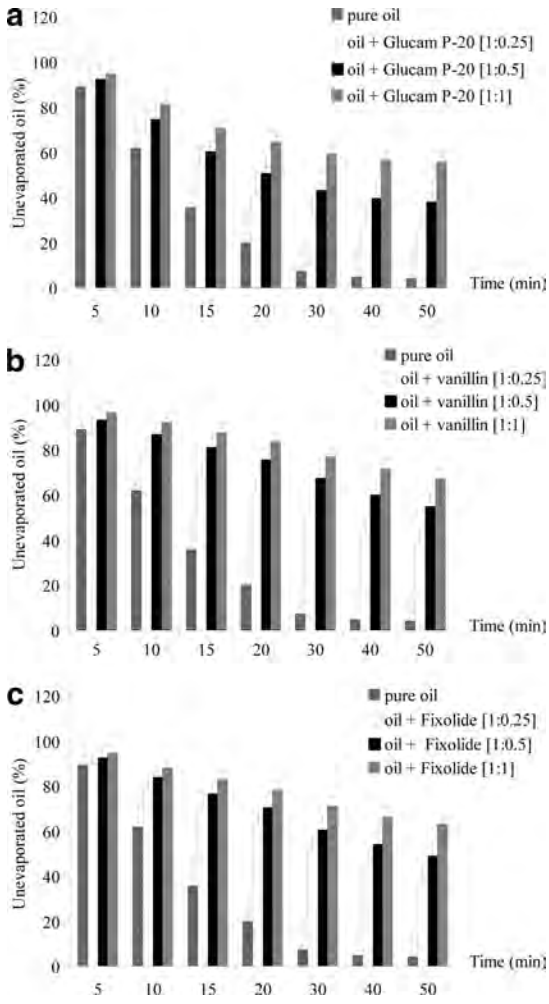

Fig. 2. Unevaporated citronella oil in the presence of fixatives at 120°C: (a) Glucam P-20, (b) vanillin, and (c) Fixolide. Each point represents mean ± SD ($n = 3$, where n is number of samples)

able for a topically applied product (Saraf et al. 2010). For safety reasons, possible skin irritation caused by the application of these lotions on the skin should be further investigated. All the prepared lotions had a low viscosity, which allows them to spread easily on the skin surface (Table 2). The viscosity of the formulation B1, which did not contain any fixatives, was significantly lower than that of the others ($P < 0.05$). The addition of fixatives into the formulations resulted in an increase in the viscosity of the preparations.

Mosquito Repellency of Citronella Oil Lotions. The protection times for the citronella oil lotions are summarized in Table 3. In the case of formulations A1-A10, it was found that the protection times of the lotions containing fixatives were generally longer than that of the formulation A1 that did not contain any fixatives. The rank order of protection times based on the type of fixatives was vanillin > Fixolide > Glucam P-20. The concentrations of vanillin did not seem to affect the

Table 2. Physical properties of citronella oil lotions at room temp (31.0 ± 1.0°C)

Formulation	pH (mean ± SD, $n = 3$)	Viscosity[a] (cp) (mean ± SD, $n = 3$)
A1	4.89 ± 0.38	80.93 ± 10.56
A2	3.25 ± 0.00	83.71 ± 0.00
A3	4.02 ± 0.12	131.97 ± 0.00
A4	4.18 ± 0.26	131.97 ± 0.00
A5	3.58 ± 0.17	131.97 ± 0.00
A6	4.62 ± 0.18	125.25 ± 11.63
A7	N/A	N/A
A8	3.34 ± 0.02	131.97 ± 0.00
A9	3.34 ± 0.02	131.97 ± 0.00
A10	3.46 ± 0.10	129.05 ± 5.06
B1	3.84 ± 0.01	61.82 ± 7.49
B2	3.69 ± 0.08	131.97 ± 0.00
B3	3.78 ± 0.03	116.41 ± 16.72
B4	3.82 ± 0.02	131.97 ± 0.00
B5	3.64 ± 0.15	87.87 ± 11.5
B6	3.58 ± 0.2	83.18 ± 31.31
B7	N/A	N/A
B8	3.43 ± 0.11	78.27 ± 21.47
B9	3.55 ± 0.17	112.9 ± 19.97
B10	3.93 ± 0.32	126.53 ± 9.42

n, no. samples; N/A, not applicable.
[a] Viscosity at shear rate of 85 s^{-1}.

protection time of the lotions, whereas increasing the amounts of Fixolide from 2.5 to 5% and 10% significantly reduced its ability to prolong the protection time against mosquitoes ($P < 0.05$). We suggest that 2.5% of Fixolide was its optimal concentration. The protection times for formulations A5-A10, which contained vanillin or Fixolide, were all longer than 2 h. Therefore, these formulations met the criteria set by the National Institute of Health, Thailand. For formulations B1-B10, the mean lowest protection time was observed in the formulations B2-B4, which contained Glucam P-20, whereas the formulations B5 and B6 that contained vanillin as a fixative provided the highest protection time against mosquito bites (longer than 4 h). Unlike vanillin, the addition of Glucam P-20 and Fixolide in the lotion bases resulted in a decrease of the protection times of the lotions when compared with the formulation B1 that contained no fixatives. Similarly to formula A (A8), the optimal concentration of Fixolide in formula B was obtained at 2.5% (B8). The formulations B1, B5, B6, and B8 met the requirement of the National Institute of Health because of their protection times were longer than 2 h. Overall, the longest protection time was obtained in the formulation B6 (4.8 h), whereas formulation B2 showed the lowest protection time of 1 h. When formulations A1 and B1 were compared, it was noted that the formula itself also affected the protection time of the citronella oil lotions. In conclusion, the promising prepared lotions with protection times of ≥2 h were formulations A5, A6, A8, A9, A10, B1, B5, B6, and B8, with B6 being the standout. These formulations had much longer protection time than that of the commercial lotion (1.3 h) that was previously investigated (Songkro et al. 2011).

Based on this current study, the type and concentrations of fixative as well as the compositions of the

Table 3. Protection times against *Ae. aegypti* of citronella oil lotions with or without fixatives

Formulation	Fixative (concn)	Average protection time (h) (mean ± SD, $n = 3$)	Protection time of each volunteer (h)		
			No. 1	No. 2	No. 3
A1	No	1.3 ± 0.3	1	1.5	1.5
A2	Glucam P-20 (2.5%)	1.7 ± 1.6	0.5	1	3.5
A3	Glucam P-20 (5.0%)	1.5 ± 0.0	1.5	1.5	1.5
A4	Glucam P-20 (10.0%)	1.8 ± 0.3	2	1.5	2
A5	Vanillin (2.5%)	2.8 ± 1.0	2.5	2	4
A6	Vanillin (5.0%)	2.7 ± 1.0	3	1.5	3.5
A7	Vanillin (10%)	N/A	N/A	N/A	N/A
A8	Fixolide (2.5%)	2.8 ± 1.3	3	1.5	4
A9	Fixolide (5%)	2.3 ± 1.0	2	1.5	3.5
A10	Fixolide (10%)	2.3 ± 0.6	2	2	3
B1	No	3.2 ± 1.2	2.5	2.5	4.5
B2	Glucam P-20 (2.5%)	1.0 ± 0.5	1.5	0.5	1
B3	Glucam P-20 (5.0%)	1.5 ± 0.0	1.5	1.5	1.5
B4	Glucam P-20 (10.0%)	1.5 ± 0.5	1.5	1	2
B5	Vanillin (2.5%)	4.3 ± 1.4	3.5	3.5	6
B6	Vanillin (5.0%)	4.8 ± 1.4	4	4	6.5
B7	Vanillin (10%)	N/A	N/A	N/A	N/A
B8	Fixolide (2.5%)	2.5 ± 0.5	2.5	2	3
B9	Fixolide (5%)	1.7 ± 0.8	1.5	1	2.5
B10	Fixolide (10%)	1.5 ± 0.5	1.5	1	2

n, no. human volunteers; N/A, not applicable.

formula played an important role in controlling the repellent properties of citronella oil preparations. In the current work, the mosquito test was investigated under laboratory conditions. To check whether the prepared lotions are suitable for practical use, the lotions that possessed protection times longer than 2 h should be further studied under field conditions.

Relationship Between Percentage of Unevaporated Citronella Oil and Protection Time. To assess this relationship, the percentages of unevaporated citronella oil with or without fixatives at 50 min (Fig. 2) were plotted against the protection times of their corresponding lotions (Table 3). As seen in Fig. 3, a linear relationship did not exist between these two parameters. For example, although the mixture of citronella oil and Fixolide at weight ratio of 1:0.25 gave the lowest percentage of unevaporated oil (37.8%), the formulation B8 that contained the same ratio had the longest protection time (2.5 h) when compared with the formulations B9 and B10. This indicates that there are other factors that influence the repellent activity of the citronella oil lotions. This could involve the other components of the formulations.

In conclusion, the current study has revealed the ability of fixatives, Glucam P-20, vanillin, and Fixolide, to reduce the evaporation rate of citronella oil at 120°C when compared with normal citronella oil. The most effective fixative was found to be vanillin, a natural fragrance fixative. At a 1:1 weight ratio of citronella oil and vanillin, the percentage of unevaporated oil was the highest (67%). Citronella oil was formulated in o/w lotions with the addition of three fixatives to prolong its repellent activity. The prepared lotions had a good appearance (pleasing color and uniformity) without any phase separations or creaming. The testing of the repellent properties of these citronella oil formulations by the human-bait technique revealed that vanillin could effectively increase the protection time of citronella oil lotions for both formulae A and B. Their protection times against mosquitoes were longer than 2 h, thereby meeting the minimum requirement set by the National Institute of Health, Thailand. The ability of the citronella oil lotions to repel mosquitoes appeared to be enhanced by the

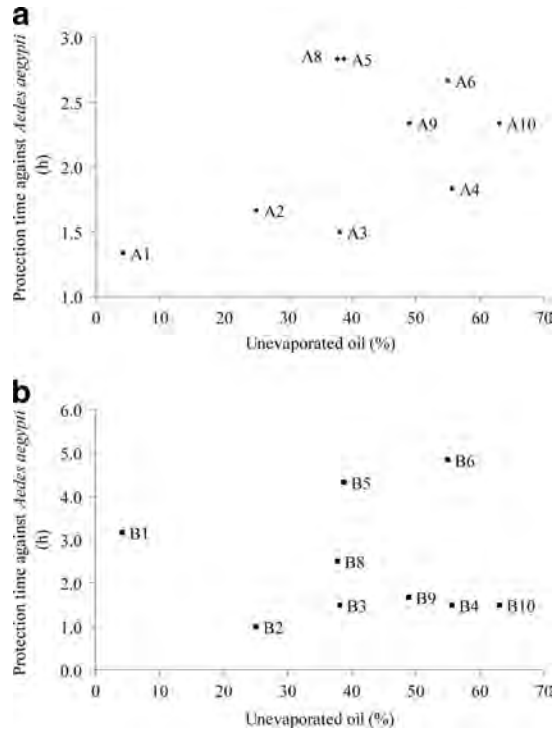

Fig. 3. Percentage of unevaporated citronella oil at 50 min and protection time of citronella oil lotions: (a) formulations A1-A10 and (b) formulations B1-B10.

addition of Fixolide, particularly in formula A. The optimal concentration for Fixolide was 2.5%. For Glucam P-20, the protection times of lotions containing Glucam P-20 at three concentrations were <2 h. Other than the fixatives, ingredients incorporated into the formulations also had some influence on the repellent efficiency.

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