



Study on mosquito repellent effects and safety of basil essential oil (*Ocimum basilicum* L. Oil) on healthy volunteers

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SUMMARY

Objective: To test repellent effects of basil essential oil on *Aedes aegypti*, *Anopheles minimus* and *Culex tritaeniorhynchus* mosquitoes on healthy volunteers.

Subjects and methods: This testing was conducted according to WHO's guidelines. A total of 1,200 mosquitoes of individual species were selected and divided in different groups of 200 mosquitoes each. Six (6) healthy volunteers including 3 males and 3 females measured their forearms and exposed those skin areas with approximately 600 cm². The right forearms were smeared with the solvent while the left forearms were smeared with 2 ml of pure basil essential oil or a blend of essential oil - ethanol 700 (7:3). Each volunteer put their arms in the cage for 3 minutes, then counted the number of mosquitoes biting and touching the skin as well as estimated the mosquito repellent effect at the times of 0, 1, 2, 3, 4 and 6 hours after applying the testing samples. Additionally, the healthy subjects were observed and interviewed about adverse drug reactions (if any) within 6 hours of applying basil oil.

Results: The test samples revealed high repellent effects on three tested mosquito species. These effect values differ insignificantly after treating samples at 1, 2, 3, 4 and 6 hours ($p > 0.05$). However, repellent effects at 3, 4, and 6 hours significantly decreased compared to that after immediately treating testing samples ($p < 0.001$). The repellent effects of both samples on *Culex tritaeniorhynchus* at 6 hours were significantly lower than that on *Aedes aegypti* and *Anopheles minimus* (p values < 0.05). No adverse drug reactions were observed in all 6 patients.

Conclusions: Basil essential oil had high mosquito repellent effects on volunteers and no adverse drug effects.

Key words: Basil essential oil, mosquito, repellent effect, adverse drug reactions, healthy volunteers.

INTRODUCTION

Basil (*Ocimum basilicum* L.) has been considered a precious medicinal plant by a number of nations around the world. It is used in Traditional Medicine to treat diseases or symptoms such as vomiting, bloating, dysentery, colds, muscle spasms, allergic rhinitis, inflammation, pain, worm infections and is also used as a tonic. Additionally, basil essential oil has the effect of reducing mental fatigue, antibacterial, antifungal, insecticidal and is preferred to treat insect stings or snake bites. [1]

A few studies in the world and Vietnam have focused on the mosquito-repelling effect of basil and found that basil plants grown in pots can repel 40% of *Anopheles* mosquitoes while basil oil has a protective effect of 100% from *Aedes* mosquitoes within 6 hours [1]. Kiplang'at K.P. et al. (2013) announced that basil oil mixed in natural mineral oil at a rate of 3% was effective in protecting rabbit skin from 100% of *Aedes aegypti* [2]. Besides, Nguyen Thi Minh Thu and her colleagues (2021) realized that basil oil has good mosquito repellent

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Received: 9/8/2023

Reviewed: 22/8/2023

Accepted: 28/9/2023

effects against all three mosquito species consisting of *Aedes aegypti*, *Anopheles minimus* and *Culex tritaeniorhynchus* on guinea pigs [3]. Moreover, it did not irritate rabbit skin as well as did not affect experimental rabbits' heart rates, breathing rates and body temperatures [4]. To date, there have been no studies evaluating the mosquito repellent effect of pure basil essential oil on humans, especially the *Culex* repellent effect has not been evaluated.

With the goal of developing basil oil into a medicinal product to prevent mosquito-borne diseases, this study was conducted to evaluate the effectiveness of repelling *Aedes aegypti*, *Anopheles minimus* and *Culex tritaeniorhynchus* mosquitoes and the safety of basil oil in healthy volunteers.

MATERIALS AND METHODS

Time and location

This study was carried out between October and November 2021 at NIMPE.

Sample

Basil essential oil was extracted from the aboveground parts of fresh basil plants collected in Yen Xa, Tan Trieu, Thanh Tri, Hanoi in October 2021 which had been examined for species confirmation at the Department of Botany, Hanoi University of Pharmacy. After being extracted and purified by steam distillation, the resulting basil essential oil had a light yellow color, a characteristic aroma, a density of 0.942, and a refractive index of 1.502 at 20°C.

Mosquitoes

Female mosquitoes of 3 species comprising *Aedes aegypti*, *Anopheles minimus* and *Culex tritaeniorhynchus* with 1,200 mosquitoes of each species were selected for the study. Selection criteria included female ones at 5 - 8 days old that were healthy, had 6 legs, 2 wings, normal perching postures, and had not yet sucked blood. All mosquitoes were raised at

NIMPE under experimental conditions and fed with 10% glucose solution at a sufficient level that were not too full or too hungry.

Healthy subjects

Six healthy volunteers, ages 18 - 55, both male and female, were interviewed about their health status and allergy history before conducting the study.

Selection criteria: Volunteers signed an agreement to participate in the study and did not use any perfumes, chemicals, or skin oils within 12 hours before the test.

Exclusion criteria: Volunteers with a history of allergy to essential oils.

Appliances

Mosquito catching tubes made of glass and punctured at both ends had diameters of 1.2 cm and lengths of 18 - 20 cm.

Glass straw, outer diameters 1 - 1.2 cm, had a bent end to help catch mosquitoes from the plastic funnel into the resting cup easily. This straw was connected to a rubber hose or a flexible plastic tube with a length of 60 cm and was fitted with a suction nozzle.

Mosquito cages, size 40 × 40 × 40 cm, were made of tulle screen with hole size 32 - 36/ cm². The cage door has a sleeve to prevent mosquitoes from escaping.

A one-meter tape measure with an accuracy of 1 mm was utilized to determine the skin areas on the volunteers' arms that had been applied to essential oil.

Chemicals

Pure ethanol 70⁰ and unscented pure natural soap No Fragrance Soap 130 g.

Method

The experiment was carried out according to WHO guidelines (2009) [5]. Before testing, the volunteer's forearms were washed with unscented soap, rinsed with water, washed a second time with ethanol 70⁰ and then dried before carrying out the test. A tape measure was used to measure the forearms' lengths (L),



the wrists' circles (C) and the elbows' circles (K). After that, the exposed forearm areas were selected and calculated according to the formula $S = L \times (K+C: 2) \text{ cm}^2$ so that they were equivalent and approximately 600 cm^2 . On the contrary, the volunteer's hands and skin areas above their elbows were covered with rubber gloves and thick fabric, respectively, to prevent the volunteers from being bitten by mosquitoes in unwanted places. The tested skin area of each volunteer was measured and maintained the same at each experiment with pure essential oil and an essential oil - ethanol blend (7:3), as well as among tests with individual mosquito species.

All volunteers used their right forearms to apply the solvent (the control arms) and their left forearms to apply the test samples. When testing with pure essential oils, the right forearms were applied to nothing, and the left ones were smeared with 2 ml of pure essential oil to the prepared 600- cm^2 skin areas. Meanwhile, when testing with the mixture of essential oil - ethanol 70^o (7:3), the right forearms put 2 ml of ethanol 70^o on the open skin areas while the left forearms dropped the mixture. After that, the volunteers' arms were allowed to dry naturally for 2 - 3 minutes before being placed in the cage containing 200 of testing mosquitoes.

Each volunteer put her or his right arm in the cage for 3 minutes and counted the number of mosquitoes touching or biting the skin. Test results were considered satisfactory if there were 10 or more mosquitoes biting or touching volunteers' forearms in 3 minutes. On the other hand, if this number is < 10 mosquitoes, the test result was canceled. Next, the volunteers put their left arms into the cage and left them there for 3 minutes, then counted the number of mosquitoes touching and biting the skin before withdrawing their arms from the cage. Every hour, they did the same thing

as described above. The test was monitored at times 0, 1, 2, 3, 4 and 6 hours after application of the test samples. Accordingly, the cage was replaced with 200 new mosquitoes after every hour of monitoring and experimentation. The experiment was conducted similarly with three above mosquito species and the average values of mosquitoes touching and biting the forearms of 06 volunteers were calculated.

Evaluating mosquito repellent effects:

Mosquito repellent effectiveness was calculated as follows:

$$\% \text{ Mosquito bite reduction rate} = (NC - NT) / NC \times 100.$$

In which, NC and NT were the numbers of mosquitoes touching and biting the volunteer's arms without and with application of the test samples, respectively, at each studying time.

The rate of mosquito bite reduction from 90 to 100% was considered highly satisfactory.

Safety assessment:

Volunteers were followed and interviewed about unwanted effects like skin allergies, headaches, dizziness, or other unusual symptoms, if any, according to a set of prepared questionnaires. Then, basil essential oil was qualified as a mosquito repellent if the number of volunteers with undesirable reactions (such as unpleasant odor, sneeze, runny nose, headache, tiredness, itchy rash, skin rash, swells of the test specimen site, et cetera) was $\leq 2/3$ of the trial participants.

Data processing

Data expressed as mean \pm SD were processed by Excel program (Microsoft XP) according to the method of medical statistics, using Student's t-test and Fisher's exact test to compare the data before, during and after the test. Also, those data were compared among the control and treated arms. The difference was statistically significant when $p < 0.05$.

Research ethics

The study was approved by the Medical

Ethics Council of VUTM. Besides, volunteers had their rights to refuse and stop participating in the study whenever they want without explaining the reasons. Moreover, the mosquito species used in the test had never sucked blood, were healthy, and did not carry pathogens.

RESULTS

Mosquito repellent effects of study samples are shown in tables 1 - 6.

Table 1. Response of *Aedes aegypti* to basil essential oil after application (n = 200/hour)

Samples	Number of mosquitoes landing/biting at distinguish times (hour, mean ± SD)							p (hrs-control)
	The control	0 hour	1 hour	2 hours	3 hrs	4 hrs	6 hrs	
Pure basil essential oil (1)	175.3 ± 9.1	0.3 ± 0.5	0.2 ± 0.4	0.2 ± 0.4	2.2 ± 1.2	3.5 ± 1.1	4.8 ± 0.9	< 0.001
Basil oil-ethanol 70° (7:3) (2)	186.2 ± 8.0	0.2 ± 0.4	0.7 ± 0.5	0.5 ± 0.6	2.5 ± 0.5	4.0 ± 0.9	7.3 ± 0.8	< 0.001
p (1-2)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	

Both test samples had high effects of reducing the number of *Aedes aegypti* landing and biting exposed skin at 0, 1, 2, 3, 4 and 6 hours compared to those of the control (p values < 0.001). Moreover, there was no statistically significant difference in the number of mosquitoes touching/biting at 0,

1, 2, 3 and 4 hours after applying both samples (p values > 0.05). Nevertheless, at 6 hours after administration, pure basil essential oil had a notably better inhibitory effect on mosquito touch/bite than that of the mixture of essential oil - ethanol (7:3), p < 0.05.

Table 2. Effectiveness of the test samples in repelling *Aedes aegypti*

Samples	Mosquito repellent effectiveness (%) at different times (Hour, Mean ± SD)						p (hrs, i-0)
	0 hour	1 hour	2 hrs	3 hrs	4 hrs	6 hrs	
Pure basil essential oil (1)	99.8 ± 0.31	99.9 ± 0.23	99.9 ± 0.24	98.8 ± 0.70	98.0 ± 0.56	97.2 ± 0.57	p (3-0), p (4-0), p (6-0) < 0,01
Basil oil-ethanol 70° (7:3) (2)	99.9 ± 0.23	99.6 ± 0.28	99.7 ± 0.30	98.7 ± 0.31	97.9 ± 0.44	96.1 ± 0.49	p (3-0), p (4-0), p (6-0) < 0,01
p (1-2)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	0.03	

Both basil essential oil samples had high effectiveness in repelling *Aedes aegypti* and were not statistically different at times 0, 1, 2, 3 and 4 hours after application on volunteers' skin, but at 6 hours, the repellent effect of the basil oil-

ethanol mixture (7:3) decreased significantly compared to that of pure basil essential oil (p = 0.03). Both samples had remarkably reduced repellent potency at hours 3, 4 and 6 compared to that at 0-hour, p values < 0.01.



Table 3. Response of *Anopheles minimus* to basil essential oil after application (n = 200/hour)

Samples	Number of mosquitoes landing/biting at distinguish times (hour, mean ± SD)							p (hrs-control)
	The control	0 hour	1 hour	2 hrs	3 hrs	4 hrs	6 hrs	
Pure basil essential oil (3)	184.7 ± 7.5	0 ± 0	0.3 ± 0.5	0.5 ± 0.6	2.8 ± 0.8	6.3 ± 1.0	9.7 ± 0.8	< 0.001
Basil oil-ethanol 70° (7:3) (4)	185.5 ± 6.7	0.3 ± 0.5	0.3 ± 0.5	0.7 ± 0.5	3.0 ± 0.6	6.2 ± 1.2	10.3 ± 1.2	< 0.001
p (3-4)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
p (1-3), (1-4)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.01	

The number of *Anopheles minimus* touching and biting volunteers' arms at all study times decreased notably compared to that of the control (p values < 0.001). Nonetheless, the number of mosquitoes touching and biting

at each time was not statistically significant ($p > 0.05$). After 6 hours of application, the number of *Aedes aegypti* touching and biting was significantly lower than that of *Anopheles minimus* with both samples ($p < 0.01$).

Table 4. Effectiveness of the test samples in repelling *Anopheles minimus*

Samples	Mosquito repellent effectiveness (%) at different times (Hour, Mean ± SD)						p (hrs, i-0)
	0 hour	1 hour	2 hrs	3 hrs	4 hrs	6 hrs	
Pure basil essential oil (3)	100 ± 0	99.8 ± 0.27	99.7 ± 0.3	98.5 ± 0.42	96.6 ± 0.51	94.8 ± 0.44	p (3-0), p (4-0), p (6-0) < 0.001
Basil oil-ethanol 70° (7:3) (4)	99.8 ± 0.29	99.8 ± 0.28	99.7 ± 0.28	99.4 ± 0.35	96.7 ± 0.72	94.4 ± 0.54	p (3-0), p (4-0), p (6-0) < 0.001
p (3-4)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
p (1-3), (1-4), (2-3)	> 0.05	> 0.05	> 0.05	> 0.05	< 0.01	< 0.01	

Both test samples had similar *Anopheles minimus* repellent effectiveness at each study time ($p > 0.05$), but these values at 3, 4 and 6 hours decreased remarkably compared to that of the time immediately after application (p

values < 0.001). Also, the *Anopheles minimus* repellent potency of both reagents was statistically significantly lower than the *Aedes aegypti* repellent potency at 4 and 6 hours ($p < 0.01$).

Table 5. Response of *Culex tritaeniorhynchus* to basil essential oil after application (n = 200/hour)

Samples	Number of mosquitoes landing/biting at distinguish times (hour, mean ± SD)							p (hrs-control)
	The control	0 hour	1 hour	2 hrs	3 hrs	4 hrs	6 hrs	
Pure basil essential oil (5)	182.0 ± 11.7	0.3 ± 0.5	0.7 ± 0.5	1.0 ± 0.9	7.7 ± 1.0	10.8 ± 1.2	16.5 ± 1.6	< 0.001
Basil oil-ethanol 70° (7:3) (6)	179.8 ± 10.4	0.2 ± 0.4	0.3 ± 0.5	0.8 ± 0.8	6.5 ± 1.4	12.5 ± 1.1	17.3 ± 1.4	< 0.001
p (5-6)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
p (1-5), (1-6), (3-5)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.001	< 0.001	

Basil essential oil significantly reduced the number of *Culex tritaeniorhynchus* touching and biting forearms after applying the test samples at 0, 1, 2, 3, 4 and 6 hours (*p* values < 0.001). Besides, both samples had similar effects on reducing the number of mosquito

touches and bites (*p* > 0.05). However, at 4 and 6 hours after application, pure basil essential oil notably reduced effect in the number of *Culex tritaeniorhynchus* touching and biting compared to that of *Aedes aegypti* and *Anopheles minimus* species (*p* < 0.001).

Table 6. Effectiveness of the test samples in repelling *Culex tritaeniorhynchus*

Samples	Mosquito repellent effectiveness (%) at different times (Hour, Mean ± SD)							p (hrs, i-0)
	0 hour	1 hour	2 hrs	3 hrs	4 hrs	6 hrs		
Pure basil essential oil (5)	99.8 ± 0.27	99.7 ± 0.27	99.4 ± 0.53	95.8 ± 0.67	94.0 ± 0.93	90.9 ± 1.01	p (3-0), p (4-0), p (6-0) < 0.001	
Basil oil-ethanol 70° (7:3) (6)	99.9 ± 0.23	99.8 ± 0.27	99.5 ± 0.44	96.4 ± 0.63	93.0 ± 0.67	90.3 ± 0.98	p (3-0), p (4-0), p (6-0) < 0.001	
p (5-6)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05		
p (1-5), (1-6), (3-5)	> 0.05	> 0.05	> 0.05	< 0.001	< 0.001	< 0.001		

The repellent effects of both samples at different study times did not differ significantly (*p* > 0.05). However, the repellent potency was significantly reduced at 3, 4 and 6 hours after administering samples (*p* < 0.001). In addition, the repellent effect of both test substances on *Culex tritaeniorhynchus* was remarkably lower than that on *Aedes aegypti* and *Anopheles minimus*.

All 6 volunteers were interviewed and followed to detect unwanted effects, if any,

during the trial period. The results revealed that all of them found the delicate fragrance of basil oil and did not experience allergic reactions or any other adverse drug reactions (ADRs) during the testing period.

DISCUSSION

Evaluating the safety and testing the mosquito repellent effect of substances on humans is the next and necessary step after experimenting on



animals. In this study, the mosquito repellent effect of basil essential oil on humans was conducted according to WHO guidelines (2009) [5] and tested on 3 mosquito species which are causing serious diseases among mosquito species circulating in Vietnam [6]. The number of 6 participants was guaranteed to obtain statistical data and the balanced ratio between the number of men and women participating in the study was to eliminate gender bias that may affect the results of mosquito attraction. Additionally, the selected volunteers must ensure that they had not used perfume, lotions, ointments, or any other medications on their skin within 12 hours before the study as well as washed their arms with unscented soap to limit the errors caused by chemicals and body odor to attract mosquitoes.

The study results revealed that both test samples had high effectiveness against *Aedes aegypti* and the difference was not statistically significant after administration at distinct times of 0, 1, 2, 3 and 4 hours. However, after 6 hours of application, the mixture of essential oil - ethanol (7:3) reduced the effectiveness of repelling statistically significantly compared to that of pure essential oil ($p = 0.03$). Both samples reduced repellent potency at hours 3, 4 and 6 compared to that immediately after application (0 hour), p values < 0.01 . This proved that two samples had similar repellent effects on *Aedes aegypti* within 4 hours after administration, but after 6 hours, the pure essential oil had a notably higher repellent effect than that of the essential oil diluted with ethanol 70%. In particular, both test samples reduced their repellent effectiveness on *Aedes aegypti* after 3 hours compared to that at the initial time. This may be due to the evaporation of pure and diluted essential oil and led to reduced effectiveness.

Our above results are also consistent with that of Sritabutra D. et al (2013) [6] when

evaluating the repellent effects of basil oil - olive oil (1:9) and basil essential oil - coconut oil (1:9) on *Aedes aegypti* on humans. In which, basil essential oil - olive oil (1:9) and basil essential oil - coconut oil (1:9) mixtures had protective effects against *Aedes aegypti* for 41.25 ± 23.67 and 73.50 ± 12.37 minutes with repellent rates of 98.6% and 98.87%, respectively [7]. Our research results revealed that the protection time against *Aedes aegypti* of pure basil essential oil and diluted essential oils was up to 360 minutes (6 hours), much higher than that of Sritabutra D. et al. This may be because we used pure essential oil (100%) and diluted essential oil (70%), while the above authors only used 10% essential oil concentration and mixed it in olive and coconut oil as solvents.

Likewise, the test results on *Anopheles minimus* mosquitoes proved that the repellent effectiveness of both samples was like that at each time of the study ($p > 0.05$). However, at 3, 4 and 6 hours after application, the *Anopheles minimus* repellent effectiveness of both test substances decreased significantly compared to that of the time immediately after application (p values < 0.001). The *Anopheles minimus* repellent potency of both test substances was statistically significantly lower than the *Aedes aegypti* repellent potency at 4 and 6 hours after application ($p < 0.01$). This also demonstrates that both pure and diluted basil essential oil have a high effectiveness in repelling *Anopheles minimus* in the first 3 hours, then decrease remarkably in the following ones.

Similarly, the effectiveness of the test substance in repelling *Culex tritaeniorhynchus* at different study times was not statistically significant ($p > 0.05$). The reduction in repellent effect was notably at 3, 4 and 6 hours after applying the test substances ($p < 0.001$). However, both samples still had good effectiveness in repelling *Culex tritaeniorhynchus* mosquitoes

with more than 90% according to WHO regulations [5]. In addition, the repellent effect of both test samples on *Culex tritaeniorhynchus* was statistically significantly lower than that against *Aedes aegypti* and *Anopheles minimus* ($p < 0.05$).

These results on humans are also consistent with the results of experimental tests [3] and confirm that basil essential oil has good mosquito repellent effects. This is the first study to evaluate the mosquito repellent effect, especially the *Culex* repellent effect, of pure basil essential oil on humans.

Testing the samples' safety in humans is considered essential for drug development and widespread use. In this study, all 6 subjects had no allergic reactions, edema, erythema, burning papules or swelling in their skin areas where the test substances were administered. Additionally, all volunteers (100%) did not show any signs of fatigue, sneezing, runny nose, dizziness, or fatigue when inhaling essential oils and found these samples have a pleasant or normal fragrance. The above results demonstrate that basil essential oil was safe for volunteers during 6 hours of continuous exposure. These results are also consistent with folk experience of using basil essential oil to treat some gastrointestinal diseases or to reduce fatigue [1].

CONCLUSIONS

Both pure basil essential oil and the mixture of essential oil - ethanol 70^o (7:3) revealed good repellent effects against all three mosquito species comprising *Aedes aegypti*, *Anopheles minimus* and *Culex tritaeniorhynchus* on healthy volunteers at the study time (1, 2, 3, 4 and 6 hours) and these different values were not statistically significant ($p > 0.05$). The repellent effects decreased remarkably at 3, 4 and 6 hours compared to that of immediately after applying the test substance ($p < 0.001$). The repellent effects of the test samples

on *Culex tritaeniorhynchus* at 6 hours were notably lower than that against *Aedes aegypti* and *Anopheles minimus* (p values < 0.05). No adverse drug reactions were recorded in all 6 volunteers.

REFERENCES

- 1. Science direct.** Basil - an overview. *Science direct*. 2019, Retrieved from <https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/basil> on March 13, 2021.
- 2. Kiplang'at K.P, Mwangi R.W.** Repellent activities of *Ocimum basilicum*, *Azadirachta indica* and *Eucalyptus citriodora* extracts on rabbit skin against *Aedes aegypti*. *Journal of Entomology and Zoology Studies.*, 1 (5), 2013, pp.84-91.
- 3. Nguyen Thi Minh Thu, Nguyen Duc Thanh, Doan Minh Khiet,** Study on mosquito repellent effect of basil oil (*Ocimum basilicum* L. *Lamiaceae*) in experimental animals. *Journal of Vietnam Traditional Medicine and Pharmacy*, Vol. 20/11, 2021, pp. 5-12.
- 4. Nguyen Thi Minh Thu, Nguyen Duc Thanh, Do Thi Phuong.** Research on effects of basil oil (*Ocimum basilicum* L.) on skin and some vital functions of the experimental rabbits. *Journal of Vietnam Traditional Medicine and Pharmacy*, Vol. 6 (47), 2022, pp. 38-44.
- 5. WHO.** *Guidelines for efficacy testing of mosquito repellents for human skin*, WHO/HTM/NTD/WHOPE/2009.4, 2009.
- 6. Science direct.** Mosquito - an overview. *Science direct*, 2019, Retrieved from <https://www.sciencedirect.com/topics/immunology-and-microbiology/mosquito> on March 13, 2021.
- 7. Sritabutra D., Soonwera M.,.** Repellent activity of herbal essential oils against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.). *Asian Pacific Journal of Tropical Disease*, 3(4), 2013, pp.271-276.