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Original Article

Evaluation of *Ocimum bascilicum* Oil and Diethyltoluamide Cream as Repellent Against Female *Anopheles* Mosquitoes in Sinnar State of Sudan (2022)

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Abstract

Introduction: Female *Anopheles* mosquitoes are the exclusive vectors of malaria parasites in Sudan, along with several metazoan parasites such as filarial worms and viruses. The sense of smell is one of the most important senses that mosquitoes use for long-range host-seeking. Carbon dioxide from the breath of humans and animals is a strong attractant of female mosquitoes.

Methods: This correlational study was performed in Al-Qaddal Center for Malaria Research in Sinnar State, Sudan. In this study, individual volunteers were topically applied *Ocimum bascilicum* oil to test its repellency activity. *O. bascilicum* oil was tested at four different concentrations (30%, 50%, 70%, and 100% v/v), respectively.

Results: Based on the obtained results, the oil exhibited the highest repellency activity at 100% concentration, and the average repellency rate was 100%. However, the lowest repellency activity was at 30% concentration, and the average repellency rate was 45%. Diethyltoluamide 13% (Soffell cream) was tested as a reference repellent cream and showed the same effect as *O. bascilicum* oil at 70% concentration, and the average repellency rate was 94%.

Conclusion: The results demonstrated that *O. bascilicum* oil had high repellency activity against female *Anopheles* mosquitoes, and thus can be used as mosquito natural repellent.

Keywords: Repellent Efficacy, *Ocimum bascilicum* oil, DEET, Female *Anopheles* mosquitoes, Sinnar, Sudan

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Introduction

Mosquitoes are undoubtedly one of the most harmful organisms to humans. In addition to being a source of nuisance, mosquitoes are the vectors of some deadly human diseases and are the first enemy of humans in the war against global infectious diseases since mosquito-borne diseases cause the death of millions of people worldwide (1). Out of about 35000 species of mosquitoes around the world, only some of them tended to bite humans, which made this small group a source of spreading infectious diseases. The study findings revealed that 44 African cities are highly adapted to crowding, and 126 million Africans are now facing a threat, especially in the tropics (1). Malaria constitutes a major public health problem in Sudan. Almost 75% of the population is at risk of developing malaria (2). Malaria transmission is unstable putting the whole country at risk of a malaria epidemic. The possibility of an epidemic increased with heavy rains, floods, and in case of interruption of control activities (2). In Sudan, the reported malaria cases represent 8.7% and 12.2% of the total outpatient attendance and hospital admissions, respectively. The disease proportional mortality was 4.3% in 2015, putting malaria as one of the main causes of death in Sudan (2). In Khartoum State, the West Nile virus was detected inside four species of mosquitoes (*Culex univittatus, Culex quinquefasciatus, Aedes vexans,* and *Aedes vittatus*) collected from different sites of soba west, Hellat kuku, Shambat, and Khartoum north central (1). Mosquitoes are responsible for the transmission of many medically important pathogens such as parasite viruses, bacteria, as well as serious protozoa-related diseases such as malaria, dengue virus, yellow fever virus, and encephalitis or filariasis (3). Mosquito repellents are widely used to avoid disease exposure (4,5). Repellents can never guarantee complete protection but can significantly lessen the chance of contracting vector-borne diseases (5-9). The sense of smell is one of the most important senses that mosquitoes use for long-range host-seeking (10).

Various mosquito attractants and repellents have

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been identified, many of which are produced by human metabolism or the bacterial degradation of sweat components. Lactic acid and 1-octen-3-ol are two components that act as strong mosquito attractants (11). Carbon dioxide from the breath is another strong attractant that sensitizes mosquitoes to other odorants (12).

There are several mosquito repellents for individual use in Khartoum state pharmacies. The most widely used insect repellent, DEET (N, N-diethyl-m-toluamide), has been in use for nearly 70 years. It is considered a highly safe repellent (13-15).

Ocimum bascilicum belongs to the family Lamiaceae vernacularly called Rehan; chemically, it contains a low percentage of essential oils, as well as volatile oils, linalool, lineol, geraniol, and polyphenolic acids (16,17). Rehan is used in traditional medicine to soothe pain, treat vomiting, and stress, and commonly as an insect repellant (16-18). Rehan is mentioned in the Noble Qur'an, in Surat Al-Rahman, the Almighty said "And love is the wind and the Rehan". In this study, *O. bascilicum* oil will be evaluated as a mosquito repellent against diethyltoluamide mosquito repellent commercially available in pharmacies in Khartoum State.

Materials and Methods

Study Type and Design

This was a correlation study, in which Pearson (2-tailed) analysis was used to evaluate the impact of concentration on the repellent activity of the *O. basilicum* oil and 13% diethyltoluamide (Soffell) ointment. The individual volunteers were topically applied *O. bascilicum* oil on the forearm. The repellent activity was tested, and 13% diethyltoluamide (Soffel) was applied as the reference repellent. All the individuals in the test group were topically applied the *O. bascilicum* oil at different concentrations (30%, 50%, 70%, and 100% v/v), respectively, and exposed to their forearms to female *Anopheles* mosquitoes inside cages for biting. The control group exposed their forearm to mosquito biting without applying any repellents. The repellent activities of the *O. bascilicum* oil at different concentrations and 13% diethyltoluamide (Soffel) ointment were evaluated finally.

Study Area

This study was performed at Al-Qaddal Center for Malaria Research in Sinnar State, Sudan.

Study Population

Female *Anopheles* mosquitoes were collected from several residential areas of Sinnar State. Laboratory classification for genus, species, and gender identification of mosquitoes was performed at Al-Qaddal Centre for Malaria Research Sennar State, Sudan.

Study Unit

Entomology Laboratory, Al-Qaddal Center for Malaria Research (Sinnar State, Sudan).

Collection of Plant Samples and Extraction of the Volatile Oil

The plant leaves of *O. bascilicum* were collected from the Bahari area north of Khartoum State. The plant leaves were manually separated from stems, washed with water to remove mud and dust particles, and dried at room temperature for three days. Then, 5 kg of dried leaves were treated for 4 hours in the Clevenger apparatus for hydro distillation to isolate the essential oil of *O. bascilicum*. The oil was collected in 20 mL containers, sealed, and stored at 4°C until tested (19,20). Different concentrations of *O. bascilicum* were made by dilution of the oil with ethanol alcohol as 30%, 50%, 70, and 100% v/v, respectively.

Repellency Tests

The essential oils were evaluated for their repellent activities against the *Anopheles* mosquito using the human-bait technique. For each test, 50 disease-free, laboratory-reared female mosquitoes were placed into separate laboratory cages (45 × 38 × 38 cm). Before each test, the volunteer's skin was washed with unscented soap, and the tested oil was applied from the elbow to the fingertips. In each cage, one arm was inserted for one test concentration. The treated and control arms were regularly interchanged to eliminate bias. Volunteers were asked to follow the testing protocol. Volunteers conducted their test of each concentration by inserting the treated and control arms alternatively into the same cage for one full minute.

Sample Size

Overall, 250 female *Anopheles* mosquitoes were collected and distributed into 5 cages including 50 mosquitoes in each cage.

Data Analysis

The data were analyzed by using the statistical program SPSS 2020 (Statistical Package for Social Science).

Results

Table 1 presents the number of attractant female *Anopheles* mosquitoes in treated and control groups and repellent activity rates obtained for every four concentrations of *O. basilicum* oil (30%, 50%, 70%, and 100% v/v), respectively. In addition, 30% (v/v) of the *O. basilicum* oil was applied to the forearm of the volunteer group. The number of attractant female *Anopheles* mosquitoes was 3-6, representing 40-50% repellent activity rates, and the average repellent activity rate was 45%. The correlation was significant at the 0.05 level with 70% *O. basilicum* oil and 13% diethyltoluamide (Soffell), respectively (Tables 1, 2, and 3). Further, 50% (v/v) of *O. basilicum* oil showed that the attractant

Table 1. Repellency Percentage Ranges and Average Repellency Rates of *Ocimum basilicum* and Diethyltoluamide

Oil or Lotion Name and Concentration (%)	Number of Attractant Mosquitoes		Repellent Activity Ranges	Average Repellent Activity Rates
	Treated Group	Control Group		
30% *O. basilicum oil*	3-6	5-9	40-50%	45%
50% *O. basilicum oil*	1-2	5-9	80-77.7%	78.8%
70% *O. basilicum oil*	0-1	5-9	100-88.8%	94%
100% *O. basilicum oil*	0	5-9	100%	100%
Diethyltoluamide	0-1	5-9	100-88.8%	94%

number of female *Anopheles* mosquitoes was 1-2, indicating 77.7%-80% repellent activity rates. The average repellent activity rate was 78.8%, and the correlation was not significant (Tables 1, 2, and 3). Moreover, 70% (v/v) *O. basilicum* oil demonstrated that the attraction range of female *Anopheles* mosquitoes was 0-1, implying 88.8100% repellent activity rates, and the average repellent activity rate was 94%. The correlation was significant at 0.05 and 0.01 levels with 30% *O. basilicum* oil and 13% diethyltoluamide (Soffell), respectively (Tables 1, 2, and 3). Additionally, 100% *O. basilicum* oil had the highest repellent activity rate (100%) and zero landed mosquitoes for all exposed tested groups, and the average repellent activity rate was 100%. In addition, 13% diethyltoluamide (Soffell) applied as the reference repellant to the forearms of the treated group showed 0-1 attractant female *Anopheles* mosquitoes, representing 88.8100% repellent activity rates. Further, the average repellent activity rate was 94%, and the correlation was significant at 0.05 and 0.01 levels with 30% *O. basilicum* and 70% *O. basilicum* oils, respectively (Tables 1, 2, and 3). Furthermore, 13% diethyltoluamide (Soffell) had the same impact of concentration on the repellent activity as that of the *O. basilicum* oil (70%). Based on the results, a 100% concentration of the *O. basilicum* oil with a 100% repellent activity rate was better than the reference repellant 13% diethyltoluamide (Soffell), showing a 94% average repellent activity rate (Table 1).

Table 2. The Means and SDs of 4 Concentrations of *Ocimum basilicum* Oil, Diethyltoluamide 13%, and Control Group

	N	SD	Mean
Control	50	1.414	42.80
30% *O. basilicum oil*	50	0.926	45.86
50% *O. basilicum oil*	50	0.479	48.34
70% *O. basilicum oil*	50	0.404	49.80
100% *O. basilicum oil*	50	0.000	50.00
Diethyltoluamide	50	0.404	49.80

Note. SD: Standard deviation.

Discussion

The results of this study confirmed that the *O. basilicum* oil had repellent activity against female *Anopheles* mosquitoes when topically applied at different concentrations (30%, 50%, 70%, and 100% v/v), respectively. Moreover, 30% v/v *O. basilicum* oil demonstrated only 45% repellent activity, whereas 100% concentration of *O. basilicum* oil showed 100% repellent activity with zero landed mosquitoes, which is better than reference repellent 13% Diethyltoluamide (Soffell) of 94% repellent activity. In the study of Aidaross et al, the *O. basilicum* oil represented only 58% repellent activity lesser than diethyltoluamide (Soffell) with 91% repellent activity (18,19). The study did not mention the concentration of the used *O. basilicum* oil. According to our results, the concentration of the applied *O. basilicum* oil in the above-mentioned study was between 35% and 40% (19,20). Fifty-one compounds of the *O. basilicum* oil were characterized and identified in the study by Garedaghi Y, Khaki (21) and Adam et al (22). Gas chromatography-mass spectrometry analysis 2.2 was employed to isolate the essential volatile ingredients of the *O. basilicum* oil, linalool, and geraniol which were reported as the main components of the oil and used as mosquito repellent at 2%, 4%, and 6% concentrations, respectively. The duration of the repellent activity of the oil through time was observed in the study instead of the repellent activity of the oil. According to our study, the 94% average repellant activity rate of 70% v/v *O. basilicum* oil was the same as the repellent activity of reference repellent 13% diethyltoluamide (Soffell). One limitation of this study was that the *O. basilicum* oil was topically tested for 1 minute only for each volunteer; this short period is not enough to observe whether the oil had an irritation impact on human skin. Further, the study was unable to measure the repellent activity duration of the volatile oil through the night hours. The study was performed in a laboratory environment, and perhaps the results may differ if the study had been performed outside the laboratory.

Conclusion

Overall, the oil extracted from *O. basilicum* has repellent activity. It is noteworthy that a higher concentration of oil can enhance the effect of repellent.

Recommendation

The oil of *O. basilicum* needs a comprehensive study for

Table 3. Pearson Correlation (2-Tailed) of 4 Different Concentrations of *Ocimum basilicum* Oil, 13% Diethyltoluamide, and Control

		Control	30% *O. basilicum*	50% *O. basilicum*	70% *O. basilicum*	100% *O. basilicum*	Diethyltoluamide 1 3%
Control	Pearson correlation	1	0.009	0.193	-0.071	-[a]	-0.071
	Sig. (2-tailed)	0	0.949	0.179	0.622	-	0.622
	Sum of squares and cross-products	98.000	0.600	6.400	-2.000	0.000	-2.000
	Covariance	2.000	0.012	0.131	-0.041	0.000	-0.041
	N	50	50	50	50	50	50
30% *O. basilicum*	Pearson correlation	0.009	1	0.018	0.360[b]	-[a]	0.360[b]
	Sig. (2-tailed)	0.949	-	0.904	0.010	-	0.010
	Sum of squares and cross-products	0.600	42.020	0.380	6.600	0.000	6.600
	Covariance	0.012	0.858	0.008	0.135	0.000	0.135
	N	50	50	50	50	50	50
50% *O. basilicum*	Pearson correlation	0.193	0.018	1	0.042	-[a]	0.042
	Sig. (2-tailed)	0.179	0.904	-	0.771	-	0.771
	Sum of squares and cross-products	6.400	0.380	11.220	0.400	0.000	0.400
	Covariance	0.131	0.008	0.229	0.008	0.000	0.008
	N	50	50	50	50	50	50
70% *O. basilicum*	Pearson correlation	-0.071	0.360[b]	0.042	1	-(a)	1.000[c]
	Sig. (2-tailed)	0.622	0.010	0.771	-	-	-
	Sum of squares and cross-products	-2.000	6.600	0.400	8.000	0.000	8.000
	Covariance	-0.041	0.135	0.008	0.163	0.000	0.163
	N	50	50	50	50	50	50
100% *O. basilicum*	Pearson correlation	-[a]	-[a]	-[a]	-[a]	-[a]	-[a]
	Sig. (2-tailed)	-	-	-	-	-	-
	Sum of squares and cross-products	0.000	0.000	0.000	0.000	0.000	0.000
	Covariance	0.000	0.000	0.000	0.000	0.000	0.000
	N	50	50	50	50	50	50
Diethyltoluamide 13%	Pearson correlation	-0.071	0.360[b]	0.042	1.000[c]	-[a]	1
	Sig. (2-tailed)	0.622	0.010	0.771	-	-	-
	Sum of squares and cross-products	-2.000	6.600	0.400	8.000	0.000	8.000
	Covariance	-0.041	0.135	0.008	0.163	0.000	0.163
	N	50	50	50	50	50	50

[a]Small (.1 to .3) [b]Medium (.3 to .5) [c]Large (.5 to 1.0)

its allergic activity, and the adverse impact in the form of rashes, irritation, pains, or other skin problems must be observed during an extended period of continuous use as the topical oil.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

Ethical clearance was obtained from the Ethical committee of AL Fajr College for Science and Technology Medical Laboratory Sciences program before commencing the investigation. Informed and free consent was obtained from the volunteers before they were recruited for the study.

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