Mosquito Repellent Activity of Various Formulations of Scent Leaf Essential Oil

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Abstract— *Mosquitoes are the most deadly vectors of parasites that causes diseases such as malaria, yellow fever, dengue fever and philleriasis, in view of the recent interest of developing plant based mosquito repellant on a replacement to the synthetic repellence. Hence, this study aimed at evaluating the mosquito repellent activity of O.gratissimum with the objective of accessing its incense, spray and cream formulation. The plant material used in this study was sourced from Akanu Ibiam Federal Polytechnic Unwana and processed into crude extracts for incense and spray and also extraction of oil for cream formulation. The mosquitoes used in this study was cultured from stagnant water kept in the lab. The evaluation of incense burning and spray of the crude extract was done using 5 different household for 3 consecutive days. The landing time and percentage repellency was done using mosquito cages containing 30 female blood starved mosquito. ODOMOS was used as standard, petroleum jelly and essential oil as the test and petroleum jelly alone as the control. Each evaluation was done for 10mins and all in triplicate. The results obtained showed 4hours and 2hours for incense burning and spray respectively. The landing time for DEET is 6 minutes, 1 minutes and 3minutes for standard, control and test and total bite of mosquito is 1, 26 and 12 for standard, control and test and repellency of 96.6% 13.3% and 60% respectively. The result showed that Ocimum gratissimum has significant mosquito repellency. Therefore, more research attention is being invoked towards harnessing the potential of using this plants extracts as a replacement for synthetic repellant.*

Keywords— cream, incense, mosquito repellant and spray.

I. INTRODUCTION

1.1 Background of the Study

Female mosquitoes are one of the most disturbing blood sucking insects that afflicts human beings. They need blood meal in other to produce viable eggs that will hatch. Mosquito belongs to the family culcidea and order disptera (Ralph, 2008; Molavi; 2013). Mosquitoes are important primary host in the spread of malaria, yellow fever, and severe arboviral infections.

Malaria which is caused by plasmodium parasites transmitted through the bites of female Anopheles mosquitoes continue to impact a major disease burden on infants and young children in endermic region (WHO 2014; and Chaiyakunapruk; 2011) in 2012, there were about 207 million cases of malaria and an estimated 627,100 deaths all around the globe. (WHO; 2014). Malaria is among the biggest public health issues globally, especially in tropical Africa, in which Nigeria suffers the world's greatest malaria burden, with approximately 51 million cases and 207,000 deaths reported annually (approximately 30%of total malaria burden in Africa). While 97% of the total population (Approximately, 173 million) is at risk of infection (WHO; 2014). Therefore, the control of mosquitoes and prevention of their bites are important public health concern around the world. One

of the approach for control of these mosquito-borne diseases is the interruption of diseases transmission by either killing the mosquitoes or preventing them from biting individuals (Adeogun *et al.,* 2012).

Mosquitoe repellents generally functions by hindering the capacity of the female mosquito to recognize the external stimuli (for example, carbon-dioxide water vapor, and heat) that she utilizes to spot a host (Pears; and Granshaw 2000). The mosquito and other insects repellent properties of NiN-diethyl-3-toluamide also known as DEET, were discovered as the first DEET product was introduced in 1956. Since it became commercially available, it has generally been regarded as safe. However, toxic effects have been recorded, including encephalopathy in children, urticarial syndrome, anaphylaxis, hypotension and decreased heart rate . an alternative to repel mosquitoes could be plant-based natural materials like plant oils to prevent the adverse effects of synthetic repellents. In comparison with synthetic repellents, they are deemed safe and good for the environment (Azeem *et al.,* 2019).

Additionally, the use of chemical insecticide has been greatly impeded due to development of physiological resistance in the insects intermediary, environmental pollution resulting in bio-application of food chain contamination and harmful effects on beneficial non-target animals. However, people may be ignorant of the facts that overuse and injudicious application of such synthetic insecticides may result in resistance and unwanted toxic or lethal effects on a non-target organism as well as human and other environmental health challenges (WHO;2018).

This study therefore, aimed at determining the mosquitoes repellent activity of formations of scent leaf (*ocimum gratissimum*) essential oil in it various formulations.

II. MATERIALS AND METHODS

2.1 Collection of Plants:

Source: The scent leaf (*Ocimum gratissimum)* was obtained from the department of Science Laboratory Technology Herbarium in Akanu Ibiam Federal Polytechnic Unwana, Afikpo Ebonyi State, into a clean sack bag. The leaves was science and the unwanted leaves was removed. The leaves was taken to a plant taxonomist in the department of science laboratory technology to be authenticated.

2.2 Sample processing:

Crude Extraction (spray and incense burning): Under running tap water and was left to dry under room temperature and was grounded into a fine powder. The grounded leaves was divided into 2 portions, one portion was soaked in ethanol for 48hours and was filtered with a a Wattman No 1 filter paper. The filtrate was concentrated to a constant dry weight.

2.2.1 Essential oil extraction (cream):

The other portion was soaked in N-hexane for 36hours and then it was filtered. Ethanol was added and it was shake which forms two layers (N-hexane at the bottom and ethanol at the bottom).

2.2.2 Formulation of incense, spray (aerosol) and cream

Incense: A known quantity of the plant leaf *ocimum gratissimum* was allowed to dry in room temperature for about 2 - 3 days and it was burnt to generate the incense.

- ***Spray (aerosol) :*** A 40ml of the oil extracts was combined with a 40ml of the ethanol to form the spray.
- ***Cream :*** A 10% of the essential oil which was dissolved in acetone was mixed with a 10% quantity of the petroleum jelly to form a cream (Sofowara, 2018, and Ojo *et al*., 2010).

2.3 Experimental Design

The mosquito was gotten from a cultured larva from stagnant water, using physical characteristics of the larva for identification. The mosquito was maintained with 10% sucrose for 4 days at normal room temperature at 12 hours day light and 12hours darkness before use. (Karunamoorthy *et al.,* 2010).

2.4 Determination of Landing Time:

Landing time is the average time required for the first mosquito to land on the exposed area and attempt to take a blood meal. A untreated hand was exposed to the mosquitoes and the time of landing was recorded to determine the readiness of the mosquito to take blood. This procedure was repeated for about 3 times in each cage and the average landing time was calculated.

2.5 Percentage Repellency:

The repellency of the essential oil was evaluated by using arm-in-cage test. The student of Akanu Ibiam Federal Polytechnic Unwana Afikpo North Ebonyi State who volunteered themselves for this study was recruited. One of the creams was moderately rubbed on the dorsal part of the hand and the other hand un-rubbed serves as control.

Therefore % repellency = $\frac{C-T}{C} = \frac{100}{1}$

Where c = control

T = test

Oshagi, 2003 and Carrol *et al.*,2006)

2.6 Protection Time:

Five household was used for the incense, and spray for about 3 consecutive days. The response from them was obtained using structural questionnaires.

2.7 Statistical Analysis

The data obtained was analyzed and expressed as mean ± standard deviation. Comparism of mean was done by ANOVA Using SPSS 20.0 version.

III. RESULTS

TABLE 1
A TABLE SHOWING THE PROTECTION TIME OF SPRAY (AEROSOL) FORMULATION

HOUSE HOLD	DAY 1	DAY 2	DAY 3
1	2hrs 30 minutes	2hrs 30 minutes	1hrs 30 minutes
2	1hrs 30 minutes	1hrs 45 minutes	2hrs 50 minutes
3	2hrs 30 minutes	2hrs 30 minutes	1hrs 45 minutes
4	1hrs 45 minutes	1hrs 45 minutes	2hrs 30 minutes
5	1hrs 45 minutes	1hrs 30 minutes	1hrs 45 minutes

TABLE 2
A TABLE SHOWING THE PROTECTION TIME FOR INCENSE FORMULATION

HOUSE HOLD	DAY 1	DAY 2	DAY 3
1	4hours	3hrs 20 minutes	4hrs 30 minutes
2	3hrs 20 minutes	4 hours	4hrs 50 minutes
3	3hrs 25 minutes	3hrs 25 minutes	3hrs 20 minutes
4	4hrs 30 minutes	4hrs 15 minutes	4 hours
5	4hrs 15 minutes	4hrs 30 minutes	3hrs 25 minutes

TABLE 3
A TABLE SHOWING LANDING TIME, NUMBER OF BITES AND REPELLENCY % FOR CREAM FORMULATION.

	Landing time (minutes)	No of Bites	% Repellency (%)	No of mosquitoes
Standard	6 minutes	1	99.6	30
Control	1 minutes	26	13.3	30
Test	3 minutes	12	60	30

- **Key**: DEET formulation repellent is "Odomos" mosquitoe repellent control.
- Negative contains petroleum Jelly
- Test contains petroleum and essential oil from *Ocimum gratissimum.*

IV. DISCUSSION

Many mosquito-borne diseases, such as malaria, dengue fever, and yellow fever, are serious public health problems in tropical regions, especially in Nigerians. These diseases are transmitted to human beings through mosquito bite only. Prevention of mosquito bites is one of the main strategies to control or minimize the incidences of these diseases. The use of insect repellents can provide practical and economical means of preventing mosquito-borne diseases and death. It is important not only for local people in disease risk areas in Nigeria but also for travelers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from home countries. Many mosquitoes repellent formulations have been made containing plant materials (Andeniran *et al* 2012, WHO 2009). Efforts are been made to ensure safety and effectiveness of such repellants (Dickens *et al* 2013).

DEET (N,N- diethyl-3-methylbenzamytes) is grown to be the best chemical insects repellent over the years and at such as they widely applied in the control of insects vectors such as mosquito. Most of the modern mosquito repellants coming in diverse forms including repellent creams is made of DEET as most widely used. Through regarded as safe but toxic effects has been recorded including encephalophathy in children, utilized heart beat (Petersen, 2001).

Many mosquitoe formulation has been made containing oil of plants *O. gratissimum* (Esmonye et al 2011, Aderi and Fabiyi 2012, Dickens and Bohhot 2013). The limitation of these products is that they have short time of effectiveness became of their rapid volatility (Apywatt *et al* 2001).

In this study, the repellency of the O. gratissimum essential oil was tested in a various formulations of spray, incense burning and cream. For the incense burning and spray (aerosol), the repellency was measured in terms of protection time. This is the maximum length of time it take for mosquito presence and bite is observed after their application. The spray protection time of two hours was observed suggesting it takes about two hours for the active repellent ingredients in scent leaf essential oil to degrade upon exposure yo air. In similar manner, the burnt product of essential oil of scent leaf takes protection time of four hours to loose mosquito repellent activity. The lost of this activity may be due to oxidation on exposure to oxygen producing a non or less repellent products.

A similar studies done by Shankar et al 2013, Awosola et al 2018, shows that O.gratissimum has mosquitoe repellency by incense burning and spray. From the study also, the cream formulation presented with delay landing time. Protection from mosquito bite and consequently high repellency. The repellency of O.gratissimum is significant when compared with negative control but lower than that of the DEET standard. This implies that the essential oil O.gratissimum could to a great extend serve as a better competitive alternative to the synthetic DEET.

Similar study done by (Obeta et al 2021) on Moringas, scent leaf menthe, spicata, Agbalaka et al 2021 on dry scent leaf, and Shankar et al 2013 on Neem, lemon, curry leaf showed similar effect on repellency to the current study. Through the mechanism of action of the formulated repellent in this study are yet to be established, several lines of the evidences suggest that incense repellent molecules reduces mosquitoe host contact by interacting with odourance and odourant receptors, thereby ultimately affected olfactory- driven behavior (Chemo-attraction) (Bohbot et al., 2011). The first detailed mode of action of attraction was summarized by Davis 1985. Techniques were available for single cell recordings for olfactory Neurons from antinna of mosquito and number of repellent tested for activity on these cells Based mostly on these electrophysiologoical studies. Davis hypothesized that repellent had their effect by modified or blocking responses of olfactory receptor neurons. Normally sensitive to attractants. The same point was made by Dicksens and Bohbot 2013. In this study , one or more compound should be responsible for modifying or blocking responses by olfactory , receptors neurons, normally sensitive to attractive.

In humans, some of this attractants include (TMAO) in the sweat, odour of haemoglobin in RBC and heat emission from the living body. In conclusion, the essential oil of scent leaf in the various formulations of aerosol, incense burnig and cream exhibited significant observable mosquito repellent activity with highest effect associated with the cream. However, the would be side effectof it application on the skin is unknown and the oil components responsible for repellency unelucidated. To these, we finally recommend further studies in order to tap from this potential in scent leaf in providing organic based mosquito repellent.

REFERENCES

[1] WHO 2014. A global brief on vector–borne disease, Hpp://apps.Who.int/iris/bitstream/10665/111008/1/WHO-DCO-WHD 2014.1-eng.pdf.

[2] Ademiran, OI and Fabiyi E. 2012. A cream formulation of an effective mosquito repellent: a tropical product from lemon grass oil (Cymbopogon citratus) stapf. *J Nat prod plant resour.,* **2:**322-327.

[3] Adeogun AO Adewuyi, GO Etatuvie, SO Fawehinmi AB and Lawal, HO 2012. Herbal bassed mosquito Repellent formulated from the essential oil of plant. *J. Nat prod.,* **5:** 109-115.

[4] Apiwat, TD Steve, R Wratten, S Roderic, T Usavadee, T and T, Yenchit, T 2001. Repellency of volatile oils from plants against three mosquito vectors. *J. Vector Ecol.,* **26**, 76-82.

[5] Azeem, M Zaman, T Tahir, M and Haris, A 2019. Chemical composition and repellent activity of native plants essential oils against dengue mosquitoes , Acedes aegypti, ind crops prod-140:111609. http://doi.org/10.1016/j.indcrop.2019.111609.

[6] Bohbot, JD, Fu, L Le, LC Chauhan, KR Cantrell, RC and Dickens, JC 2011. Multiple activities of insects repellents on odorant receptors in mosquitoes. *Med. Vet. Entemol.,* **25**: 436-444.

[7] Carroll, SP and Loye, J 2006. Pmd, A Registered Botanical Mosquito Repellent With Deet-like efficacy. *Journal of America Mosquito Control Association*, **22**(3): 507-514.

[8] Chaiyakunapruk N Kongkaew, C Sakunrag, I and Tawatsin, K 2011. Effectiveness of Systematic *Review of controlled laboratory experimental studies tropical med int health,* **16**:802- 810.

[9] Dickens, JC and Bohbot, JD 2013. Mini review; mode of action of mosquito repellent . Pe*stBiochem Physiol.,***106**: 149-155.

[10] Esimone, CC Altama, AA Ngwu, G IIoabanafo,CA Momoh, MA and Onaku, LO 2011. Mosquito repellent activity of herbal oilments formulated with ocimum gratissimum oil. *J.Pharm res*., **4:** 3442-3444.

[11] Karunamoorthy, K Uango, K and Murugan, K 2010. Laboratory Evaluation of Traditionally Used Plant Based Insect Repellents Against the malaria vector anopheles arabunsispation. *Parasitol Research*, **106**(5): 1217-1223.

[12] Molavi, L 2013. "Africa's Malaria Death Joll Still 'outrageously high, natioanla geographic society, **1**(4): 192-210.

[13] Ojo, OO and Anibijuioon, H 2010. Studies on Extracts Of The Three Mecheal Plants of South-Western Nigerians: Hoslundia Opposita, lantana camera and cymbopogoncitratus. *Advance Natural Applied Science*, **4**(1): 93-98.

[14] Oshagu, MA 2003. Repellent Effects of Extracts and Essential Oil of Citrus Lemon (Rutaceae) and Mehssa Officinalis (Labiatae) Against main malaria vector anorpheles stephensi (Dipteria: Culicidae). *Iran Journal Public Health*, **32** (4): 23-29.

[15] Peterson C and Coats J 2001. Insect repellents-past, present and future, pesticides outlook, Plants profile for *ocimum gratissimum*/USDA **12:** 154-158 http://dx.doi.org/10.1039/bi06296b

[16] Ralp, H 2018. Family Culcidae meigen, Mosquito taxonomic inventory *J. Pharm Biol Arch.* (**3**): 357-362.

[17] Sharma, M Alexander, A Saraf, S Saraf, S Vishwakarma, UK and Nakhate, KT 2021. Mosquito repellent and Larvicidal perspectives of weeds lantana camera L. and *Ocimum gratissimum* L. found in central India. *Biocata Agric Biotechnol* ., **34:**102-104. http://doi.org/10.1016/j.bcap.2021.102040.

[18] Sofowora, A. (2008). Medicinal plants and traditional medicinal in Africa. 3rd Edition Ibadan: Spectrum Books.pp: 45-76.

[19] WHO 2018. Vector control for malaria and other mosquito borne disease, WHO Tech

[20] WHO World Malaria report 2014. Ceneva; World Health Organisation; 2014 .

[21] World health organization. Guidelines for efficacy testing of mosquito repelents for human skin WHO-HTM-NTD-WHOPES-(2009). 4 eng.geneva, Switzerland: World health organization.

[22] Obeta, MU Agbalaka, IP Ejinaka, RO and Dajok GD 2020. Environmental and Nutritional Health Awareness on Musca domestica as a Carrier of Parasites in Jos Metropolis, Plateau State-Nigeria". Acta Scientific Nutritional Health. **4**(3): 01-06.