BioPublisher®

Journal of Mosquito Research 2018, Vol.8, No.4, 29-33
http://jmr.biopublisher.ca

Research Report **Open Access**

Repellent Effect of Ethanolic Extract of Scent Leaf (*Ocimum gratissimum*) and Neem Leaf (*Azadirachta indica*) on Adult *Culex* Mosquitoes

Awosolu O.B. ✉, Obimakinde E.T., Adeleke A.B., Olusi T.A.
Department of Biology, Federal University of Technology, Akure, 23434, Nigeria
✉ Corresponding author email: awosconik@yahoo.co.uk; obawosolu@futa.edu.ng
Journal of Mosquito Research, 2018, Vol.8, No.4 doi: 10.5376/jmr.2018.08.0004
Received: 23 Apr., 2018
Accepted: 31 May, 2018
Published: 22 Jun., 2018

Preferred citation for this article:
Awosolu O.B., Obimakinde E.T., Adeleke A.B., and Olusi T.A., 2018, Repellent effect of ethanolic extract of scent leaf (*Ocimum gratissimum*) and neem leaf (*Azadirachta indica*) on adult *Culex* mosquitoes, Journal of Mosquito Research, 8(4): 29-33 (doi: 10.5376/jmr.2018.08.0004)

Abstract Mosquitoes control through the use of synthetic chemical compounds have been discovered to have toxic residual effect as it gets accumulated into the food chain, thus, there is a need for environmental friendly compounds such as botanicals. This study was carried out to determine the repellent effect of ethanolic extract of Scent leaf **(***Ocimum gratissimum*) and Neem leaf **(***Azadirachta indica*) on adult *Culex* mosquitoes. Mosquito larvae were acquired from stagnant pool of water around and within the Federal University of Technology, Akure. Leaves extract of *O. gratissimum* and *A. indica* was extracted using absolute ethanol as solvent. Different concentrations of the oil extract were prepared (5 ml, 10 ml, 15 ml, and 20 ml) and adult *Culex* mosquitoes were exposed to these concentrations for a period of 2 hours. The results showed complete protections time of 80 minutes at 20% concentration for *O. gratissimum* and 12 hours at 20% concentration for *A. indica*. It was observed that *A. indica* have higher protection time than *O. gratissimum*. The results from the bioassay of the extracts on the mosquitoes revealed that both leaf extracts have repellent effect on the *Culex* mosquitoes. The four different concentrations of the extract showed significant difference in their repellent effect ($p<0.05$). It is apparent that both *O. gratissimum* and *A. indica* oil extract have repellent activity against *Culex* mosquitoes.
Keywords Scent leaf **(***Ocimum gratissimum*); Neem leaf **(***Azadirachta indica*); *Culex* mosquitoes; Repellent

Background

Mosquitoes belong to family Culicidae and Order Diptera (Ralph, 2008; Molavi, 2013). There are about 3,500 species of mosquitoes, grouped into 3 sub-families; Toxorhnychitinae, Anophelinae, and Culicinae (Bayer, 2003; Reinert, 2008). Relatively few of these sub-families are significant vectors of human diseases. Mostly, the female of many species of mosquitoes are bloodsucking insects and they are important vectors of malaria, dengue fever, yellow fever and west Nile virus. Apart from their ability to transmit diseases, they are by far the most important of the bloodsucking arthropods causing annoyance and discomfort to human. They are found throughout the world except in places that are permanently frozen, and three quarters of all mosquito species live in humid tropics and sub-tropics where the climate is favorable for larval development and adult survival and diversity (Chadee, 2002).

Awad and Shimaila (2003) stated that the principal objective of vector control is the reduction in morbidity and mortality due to malaria and other diseases transmitted by mosquitoes; this is achieved by reducing level of transmission. Current control is based on the use of commercial insecticides which have potential toxic effect on public health and the environment. Various dipteran pests have been controlled in past decades using synthetic insecticides (Poopathi and Archana, 2010). Pesticides are indeed very effective in its use. But along with their useful effects, they also bring out serious harm to human health as well. Furthermore, these chemicals are expensive and are often toxic to human, natural enemies and other animals (Cartilla and Dela, 2012). The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in a reduced control and often to a negative impact on various non-target organisms and on the environment in general (Charles and Nielsen-Leroux, 2000).

Additionally, the use of chemical insecticides has been greatly impeded due to development of physiological resistance in the insect intermediaries, environmental pollution resulting in bio-application of food chain contamination and harmful effects on beneficial non-target animals. In recent years, the increasing information on

hazardous effects of synthetic insecticides on plant and animal health has alarmed scientists to seek some alternative ways such as insecticides which are eco-friendly (Montasser et al., 2011). Thus, the need for alternatives more effective and environment friendly control agent or insecticides is urgent (Poopathi and Archana 2010).

1 Results

1.1 Repellent effect of different concentration of *Ocimum gratissimum* extract against *Culex* mosquitoes

It was observed that the oil extract of *O. gratissimum* provided maximum protection time of 80 minutes at 20% concentration while the least protection time was observed at 5% concentration (Table 1). Generally, it was observed that, the protection time of the extract increases as the concentration increases. The complete protection time of the extract when compared with the control experiment were significantly different ($p<0.05$).

Table 1 Complete protection time of *Ocimum gratissimum* extract at different concentrations against *Culex* mosquitoes

Concentration (%)	Complete protection time (minutes)
5.0	50.00
10.0	56.00
15.0	65.15
20.0	80.00
Control 1 (Water)	02.00
Control 2 (Ethanol)	02.25

1.2 Repellent effect of different concentration of *Azadirachta indica* extract against *Culex* mosquitoes

The result showed that the oil extract of *Azadirachta indica* provided maximum protection time of 12 hours at 20% concentration while the least protection time of 5 hours was observed at 0.5% concentration (Table 2). Generally, it was observed that, the protection time of the extract increases as the concentration increases. The complete protection time of the extract when compared with the control experiment was significantly different ($p<0.05$).

Table 2 Complete protection time of *Azadirachta indica* extract at different concentrations against *Culex* mosquitoes

Concentration (%)	Complete protection time (minute)
5.0	59.00
10.0	75.80
15.0	105.0
20.0	120.0
Control 1 (Water)	02.00
Control 2 (Ethanol)	02.25

1.3 The repellent effects of all plant extracts

Figure 1 compared repellent effects of all the extracts at 5%, 10%, 15% and 20%. It was observed that the least complete protection time (59 mins) was recorded in *A. indica* at 5% concentration compared to the 50 mins recorded for *O. gratissimum* at 5% concentration. Similarly, the highest complete protection time (120 mins) observed in *A. indica* extract at 20% is significantly ($p<0.05$) different from the extract of *O. gratissimum* at the same concentration.

2 Discussion

From the results obtained, the study showed that the essential oils from both plants used as a repellent on *Culex* mosquito population are effective on the mosquitoes. The higher the concentration of the oil extract the higher the protection time, this may be due to an increase in the concentration of the active ingredient present in the oil extracts. This is supported by many studies conducted in recent time on *A. indica* oil extract. Sharma *et al.* (2008) reported the repellent activity of *A. indica* for 12 hours at 20% concentration which is in line with this result. Also, according to the reports of other authors, Abiy *et al.* (2015) stated that *A. indica* and Chinaberry (*Melia azedarach*) oil extracts have been recognized as mosquito repellents and they have shown quite interesting repellent activities

which was able to protect the skin for more than 2 hours. Also, Shyamapada (2011) showed that Neem oil extract had 180 minutes protection time from mosquito bites at 20% concentration which is in line with the result of this study. Thavara *et al.* (2002) also stated from his research that *A. indica* has 8 hours repellent effect on *Culex* mosquitoes at 20% concentration, Likewise, *A. indica* oil extract provided 7.4 hours repellent effect on *Culex* mosquitoes (Das et al., 2000), which all supported the result of this research.

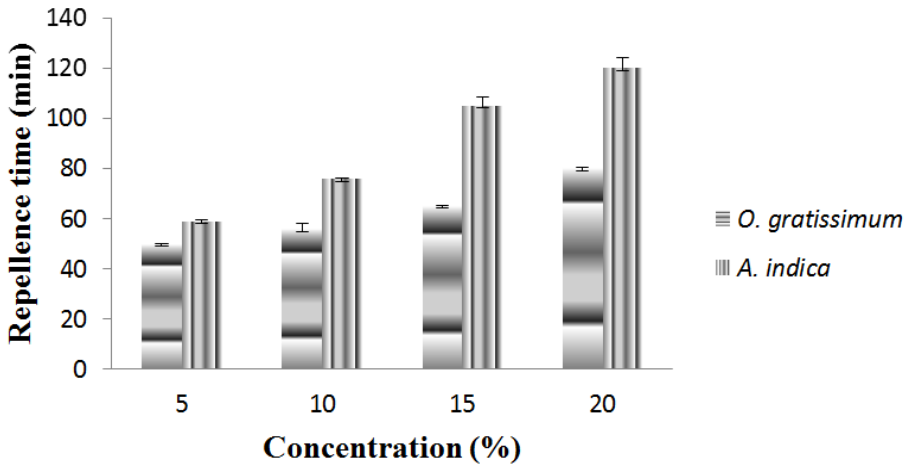

Figure 1 Comparison of the repellent time of ethanolic extract of the leaves of *Ocimum gratissimum* and *Azadirachta indica*

Scent leaf (*O. gratissimum*) oil extract equally has a repellent effect on *Culex* mosquitoes. The higher the concentration of the oil extract the higher the protection time, this may be due to an increase in the concentration of the active ingredient present in the oil extracts. This is supported by many studies conducted in recent time on Scent leaf oil extract. Kazembe and Chauruka (2012) reported *O. gratissimum* oil extract have 100% repellence on *Culex* mosquitoes for 30 minutes at 5% concentration which actually corroborates the result of this study. Also, Kweka and Prabhu (2012) reported that *O. gratissimum* produced 90 minutes protection time against *Culex* mosquitoes at 20% concentration which is in line with the result of this study.

3 Materials and Methods

3.1 Study area

The research was carried out at Federal University of Technology, Akure (FUTA). The University is located in Akure the capital city of Ondo State, Southwest, Nigeria. Akure is located between latitude 7°15'0"N and longitude 5°11'42"E. Akure has two seasons, which includes the rainy (April to October) and the dry season (November to March). The research was carried out during the rainy season, between the months of May to July, 2017.

3.2 Collection/culture of mosquito

Mosquito larvae were acquired from stagnant pool of water within the University environment. The water containing the larvae was poured into white transparent containers and was transported to the Biology Research Laboratory. The larvae were identified and separated into different genera. Of the different genera, *Culex* mosquito larvae were reared in the laboratory in a cage designed with mosquito net to prevent the escape of the adult mosquitoes during emergence. During the period of rearing, the larvae were fed with yeast and sucrose until the adult mosquitoes emerged. The emerged adult *Culex* mosquitoes were used for the experiment.

3.3 Collection and extraction of plant materials

The leaves of Scent leaves (*O. gratissimum*) and Neem leaves (*A. indica*) were collected in May 2017 around the University School premises and the taxonomic identification was done at the Biology Research Laboratory of the Federal University of Technology, Akure. The leaves were air-dried at ambient temperature (28±2) and pulverized into fine powder with an electric pulverizing machine in the Biology Research laboratory. Two hundred grams (200 g) of the powdered form of each plant were put in an Amber Winchester bottle and percolated with 1 L of ethanol in ratio 1:5 for three days with shaking at regular interval. The shaking causes the solvent to cover the leaf

samples inside the container. Muslin cloth was used to separate and filter the slurry from the solution. The solvent left in the solution was then concentrated to dryness on rotary evaporator (R110) at 40 ℃ in the Central Research Laboratory to give the crude ethanol extract labeled as EB1 (Colin and Cooke, 2000; Dreyer and Kragl, 2008).

3.4 Bioassay of extract

The mosquitoes used in this study were laboratory-reared *Culex* mosquitoes. The standard rearing protocol as described by Mullai and Jebanesan (2007); Dhanasekaran et al. (2013) were adopted. The repellent activity of the extract fractions obtained from *A. indica* and *O. gratissimum* were all assessed in the laboratory using a human bait technique (WHO, 1982). Five volunteers (age 21-34 years) participated in the laboratory tests, with each volunteer exposed to only one of the extracts at a time. The testing period lasted up to eight hours spread over 8 days, depending on the efficacy of repellent. The timing of the tests depended on the fact that *Culexs* are night-biters. Evaluations were carried out in a large cage made of mosquito-proof net at room temperature. An area of 3×10 cm on each forearm of the human volunteers was marked out with a permanent marker (Hang and Yang, 2008). Each extract was tested for repellence at three different concentrations. The test extracts 5%, 10%, 15% and 20% (extract/ethanol) for each plant extract were applied to the marked area of one forearm of each volunteer, while the other forearm was treated with only ethanol free from the extracts, to serve as a control. This procedure was repeated four times for each concentration and the average was taken. During the test, each volunteer put the test forearm in a mosquito cage (80×40×40 cm^3), containing 100 female mosquitoes (3-5 days old). However, before the start of each exposure, the bare hand, used as control area of each volunteer, was exposed for up to 30 seconds. If at least two mosquitoes landed on the bare hand, the repellency test was then continued. This was done to ensure that the mosquitoes were host seeking (Kalita et al., 2013). The number of mosquitoes probing the treated area of each volunteer was noted for half-hour (Karunamoorthi et al., 2008).

3.5 Statistical analysis

The data obtained were statistically analyzed using One-way Analysis of Variance (ANOVA) for mean, standard error (SE) and least significant difference (LSD) at 5% using 16.0 SPSS (Statistical Package for Social Sciences) software.

Authors' contributions

Awosolu O.B. conceived and designed the project and as well put up the manuscript; Olusi T.A. proofread while Obimakinde E.T. and Adeleke A.B. did the field and laboratory aspect of the project. All authors read and approved the final manuscript.

Acknowledgements

We sincerely appreciate those who assisted in sample collection and plant identification.

Competing interests

Authors declared that there exists no conflict of interests.

References

Abdullahi M., 2012, phytochemical constituents and Antimicrobial and grain protectant activities of Clove Basil (*Ocimum gratissimum), International Journal of Plant Research*, 2 (1): 51-58
https://doi.org/10.5923/j.plant.20120201.08

Abiy E., Teshome G.M., Meshesha B., and Girmay M., 2015, The Repellent efficacy of DEET, MyggA, neem (*Azadirachta indica*) oil and chinaberry (*Melia azedarach*) oil against Anopheles arabiensis, the principal malaria vector in Ethiopia, *Malaria Journal*, 34 (4): 23

Awad O.M. and Shimaila A., 2003, Operational use of neem oil as an alternative Anopheline larvicide, ParT A: Laboratory and field efficacy, *Eastern Mediterranean Health Journal*, 9(4): 637
PMid:15748061

Bayer E.F., 2003, Mosquitoes, the vector of malaria and filariasis, *Agricultural technology and International journal*, 2 (5): 633-637

Biu A.A., Yusufu S.D., and Rabo J.S., 2009, Phytochemical Screaning of *Azadirachta indica* (Neem) in Maiduguri, Nigeria Bioscience Research Communication, 21(6): 2

Cartilla P. and Dela Cruz J., 2012, Termiticidal Potential of *Stachytarpheta jamaicensis* (L), *Vahl Journal of Etnobiogy*, 1, 1-5

Chadee D., 2002, Indoor and Outdoor host seeking rhythms of *Anopheles albitarsis* (Diptera: Culicidae) in Trinidad and Tobago, *West Indies Journal of Medical Enthomology*, 29(3): 567-569

Charles J.F. and Nielsen-Leroux C., 2000, Mosquitocidal Bacteria toxins: Diversity, Mode of Action and Resistance Phenomena, *Journal of American Mosquito controls Association*, 95(1): 201-206

Colin, P., and Cooke M., 2000, Extraction, *Encyclopedia of Separation Science* 10:465-467

Das N.G., Nath D.R., Barvah I., Talukdar P.K., Das S.C., 2000, Field Evaluation of Herbal Mosquito Repellents. *Journal of Communication*, 31: 241-245

Dhanasekaran M., Karuppusamy S., Annadurai M., and Rajasekaran K.M., 2013, Evaluation of Phytochemical Constituents of Indian Medicinal Plant *Hydnocarpus alpina* WIGHT, Indian J. Pharm. Biol. Res. Vol. 1 (3): 23-18
https://doi.org/10.30750/ijpbr.1.3.5

Dreyer S., and Kragl U., 2008, Ionic Liquids for Aqueous Two-Phase Extraction and Stabilization of Enzymes, *Biotechnology and Bioengineering* 99(6):1416-1424
https://doi.org/10.1002/bit.21720
PMid:18023057

Hang C. E., and Yang E. C., 2008, A bioassay for natural insect repellents. *Journal of Asia-Pacific Entomology*, 11: 225-227
https://doi.org/10.1016/j.aspen.2008.08.002

Kalita B., Somi Bora S., and Sharma A.K., 2013, "Plant essential oils as mosquito repellent-A Review" *International Journal of Research Development. Pharmacology and Life Science*, 3(1): 741-747

Karunamoorthi K., Mulelam A., Wassie F., 2008, Laboratory evaluation of traditional insect/mosquito repellent plants against *Anopheles arabiensis*, the predominant malaria vector in Ethiopia, *Parasitol, Res*. 103(3): 529-534
https://doi.org/10.1007/s00436-008-1001-9
PMid:18493796

Kazembe T., and Chauruka D., 2012, Mosquito Repellence of *Astrolochii lepii*, *Cymbopogon citrates* and *O. gratissimum* Extracts and Mixtures, *Bulletin of Environment, Pharmacology and Life sciences*, 1(8): 60-64

Kweka E.J. and Prabhu K., 2012, Parasites and Vectors, *Asian Pacific Journal of Tropical Biomedicine*, 5: 189

Molavi L., 2013, "Africa's Malaria Death Toll Still "Outrageously High", National Geographic Society, 1(4): 192-210

Montasser S.A., Shamseldean M.M., Mamoud Y.A., Ibrahim S.A., and Sharaby A., 2011, Plant Extracts as Alternative Botanical Insecticides For controlling the Grasshopper Heteracris littoralis Ramb. (Orthoptera: acrididae) with reference to Histological Changes on the reproductive System, *Journal of Basic Applied Science Research*, 1(9): 1031-1038

Mullai K., and Jebanesan A., 2007, Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitacious plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae), *Tropical Biomedicine* 24(1): 1-6
PMid:17568371

Poopathi S., and Archana B., 2010, Mosquitocidal Bacteria toxins (*Bacillus sphaericus and Bacillus thuringiensis serovar israelensis*), Mode of action, Cythopathological effects *and mechanism of resistance*, 50(1): 65-71

Ralph H., 2008, Family Culicidae meigen, Mosquito Taxonomic inventory

Reinert W.F., 2008, New classificatn of composite grain, *Journal of America, Malaria control Association*, 16, 175-188

Sharma V.P., Ansari M. A., and Razdan R. K., 2008, Mosquito repellent action of neem (*Azadirachta indica*) oil, Journal of the American Mosquito Control Association, 9(3):359-360

Shyamapada M., 2011, Repellent Activity of Eucalyptus and A. indica seed oil Against the Filarial Mosquito *Culex quinquefasciatus* Say. (Diptera: Culicidae) in India, *Asian Pacific Journal of Tropical Biomedicine*, 2: 34-35

Thavara U., Tawatsin A., Chompoosri J., 2002, Phytochemicals as Repellents against Mosquitoes in Thailand, *Proceedings International Conference Biopesticides*, 3: 244-250

World Health Organization, 1982, Manual on Environmental Management for Mosquito Control (with special emphasis on malaria vectors), WHO Offset Publication: 66