



KNOCKDOWN AND REPELLENT EFFECT OF MOSQUITO COILS FORMULATED FROM CITRONELLA (*Cymbopogon nardus*), PIGNUT (*Hyptis suaveolens*) AND AFRICAN BASIL (*Ocimum gratissimum*) AGAINST AEDES AEGYPTI

Nusaiba M. Sodangi¹, B.W. Tukura², Yusuf A. Yusuf³, Muktar I. Musa⁴ and M.N. Ezeunala³

- 1 National Biotechnology Development Agency, Abuja,
- 2 Nasarawa State University, Keffi,
- 3 National Institute for Pharmaceutical Research and Development, Abuja,
- 4 Ahmadu Bello University, Zaria, Kaduna.

Corresponding Author: nussyms@gmail.com

Abstract

Mosquito coils were formulated as insect repellents using Citronella (*Cymbopogon nardus*), African Basil (*Ocimum gratissimum*) and Pignut (*Hyptis suaveolens*). The mosquito coils were formulated using the crude leaves of the plants at three different concentrations (10 g, 15 g and 20 g). The leaves of the plants were formulated in combination with other ingredients which served as binders, fillers, diluents and preservatives. The efficacy of the repellents produced using the leaves of the plants was determined against *Aedes aegypti* at three different concentrations (10 g, 15 g and 20 g). The mosquito efficacy assessment test carried out on the formulated mosquito coils showed that the coil with *Cymbopogon nardus* had knockdown time (KT₅₀) of 40, 45 and 37 minutes for the 10 g, 15 g and 20 g concentrations respectively; *Ocimum g ratissimum* had KT₅₀ of 80, 69 and 55 minutes at 10 g, 15 g and 20 g concentrations respectively while *Hyptis suaveolens* had KT₅₀ of 12, 23 and 27 minutes at 10 g, 15 g and 20 g concentrations respectively. The coil with the active ingredient of 10 g of *Hyptis suaveolens* showed the best activity with KT₅₀ of 12 minutes. Analysis of variance (ANOVA) shows that there were significant differences (P<0.05) among the three different mosquito coils and control. There was also a significant interaction between the knockdown activity of the mosquito coils and time. These results suggest that these plants are potential mosquito repellents and can be used in insect repelling products. They may be alternatives to the commercial mosquito coils of synthetic source.

Keywords: Formulation, insect repellents, knockdown time (KT₅₀), *Aedes aegypti*.

Introduction

Mosquito repellents are substances that can be used to repel mosquitoes (vectors that transmit malaria). A typical example of a mosquito repellent is a mosquito coil which when ignited drives away mosquitoes. Mosquito coil contains active ingredients used to prevent the mosquito from biting particularly during the hours of sleep having been designed to burn for hours. These active ingredients have been known to cause a distasteful environment for the mosquito and act as agents of immobilization that disturbs the food-searching mechanism of the mosquito (Lokhande and Chapaitkar, 2022). Effective insect repellents can protect one from serious mosquito and tick-borne diseases (Filariasis, zika, yellow fever, dengue fever, etc.). Therefore, developing new ideas for overcoming these life-threatening sicknesses is one way of solving them. One of these illnesses carrying insects is the Mosquito which transmits parasitic and viral diseases to man (IUPAC, 2006). The most infectious protozoan parasite causing malaria infection in humans is *Plasmodium falciparum* (WHO, 1993). In 2010,

approximately 3.3 billion people were exposed to malaria resulting in 655,000 deaths (WHO, 2011). Dengue fever, yellow fever, chikungunya virus and zika virus are some of the diseases transmitted by *Aedes aegypti*, it is the principal vector in almost all chikungunya epidemics found in Africa, India and other countries in Southeast Asia (Mavale *et al.*, 2010) (Vega-Rua *et al.*, 2015). Historically, *Aedes aegypti* eradication programmes have been previously set up to control the outbreaks of dengue and yellow fever but they proved unsuccessful and have resulted in the re-occurrence of these diseases (Gubler, 1998). The species of *Aedes aegypti* were found in the Zika virus outbreak in Brazil recently (Ferreira-de-Brito *et al.*, 2016). *Aedes aegypti* are parasites that spend more time indoors than outdoors thereby rendering outdoor insecticidal sprays less efficient (Reiter, 2010). Nearly all insecticides have the potential to significantly alter the ecosystem, many are toxic to humans and others are concentrated in the food chain. It is necessary to balance agricultural needs with environmental and health issues when using insecticides. It is crucially important that all the

rural areas in Nigeria are being educated on the need to control insects, especially mosquitoes that might breed around the environment and transmit malaria to people living within the enclave. Many ideas and efforts are focused towards controlling malaria infestation both in urban and rural areas. A lot of measures are being taken to reduce mortality as a result of malaria. Historically, lethal effects have been the focus of the search for novel compounds to be used in vector control. Nevertheless, other effects such as repellency or irritancy may be used to reduce vector-host contact (Achee *et al.*, 2012). A compound is considered an irritant whenever insects move away after contact with it (Hilje & Mora, 2006). Compounds like pyrethroids or Dichloro-Diphenyl-Trichloroethane (DDT) reduce insect activity because of their irritant effect (White, 2007). Plants comprise compounds such as repellents, anti-feedants, and growth regulators preventing the attack from phytophagous insects, but some of these compounds are also repellent for haematophagous insects (Maia & Moore, 2011). Plants are used worldwide to protect people from haematophagous arthropods and numerous studies report repellent effects of essential oils (Maia & Moore, 2011; Amer & Mehlhorn, 2006; De Boer *et al.*, 2010; Moretti *et al.*, 2013). Citronella plant is popular as a 'natural' insect repellent. Research has verified its mosquito-repellent qualities (Kim *et al.*, 2005), including its effectiveness in repelling *Aedes aegypti* (dengue fever mosquito) (Trongtokit *et al.*, 2005). *Hyptis suaveolens* has both medicinal individualities as well as insecticidal properties. *Hyptis suaveolens* has been shown to be effective as an insecticide (Adda *et al.*, 2011; Conti *et al.*, 2010). *Ocimum gratissimum*, also known as clove basil, African basil, and in Hawaii as wild basil, is a species of *Ocimum* (USDA, 2009). The whole plant and the essential oil have many applications in traditional medicine, especially in Africa and India.

These natural compounds are biodegradable, environmentally friendly and readily available (Regnault-Roger *et al.*, 2012), and they generally have a low toxic effect to mammals (Isman, 2000). Moreover, in most tropical areas, traditional medicine which is largely plant-based (herbs or shrubs) is available at a low cost (De Boer *et al.*, 2010).

The use of plant products as insecticides is one of the important approaches of insect pest management and it has many advantages over synthetic insecticides (Weinzierl & Henn, 1992). Plant materials with insecticidal properties are locally available, biodegradable and inexpensive for the biological and agricultural control of pests. A mosquito coil made from natural mosquito repelling plants (Citronella, Pignut and Tulsi (African basil)) will remove the health problems caused by commercial mosquito repellents. The Mosquito Coil when burnt continuously emits smoke along with the active ingredient added to it which prevents the mosquitoes from biting (Phal *et al.*, 2012). This mode of

release of insecticide for the prevention of mosquito bites is an effective mosquito-repellent tool (Phal *et al.*, 2012). Recent studies showed that the smoke generated from burning mosquito coils is of certain health concern because of the chemical contents found in mosquito coils (Liu *et al.*, 2003), making a mosquito coil out of natural ingredients may remove these problems.

Emission rates of the product of incomplete combustion (PIC) depend on the types of organic fillers or base materials used and can be high enough to generate room concentrations markedly higher than health-based standards or references (Zhang *et al.*, 2010). However, using charcoal powder as base material not only reduces visible smoke, as officially labelled as smokeless coils, but substantially reduces emissions of PM_{2.5} mass, total particle number, Polycyclic aromatic hydrocarbons (PAHs), and aldehydes (Zhang *et al.*, 2010).

MATERIALS AND METHODS

Materials/Reagents/Apparatus

The raw materials used for the formulation of the mosquito coils were charcoal, starch, talcum powder, sodium benzoate and the leaves of the three different plants citronella (*Cymbopogon nardus*), African basil (*Ocimum gratissimum*) and pignut (*Hyptis suaveolens*). Charcoal served as the burning material, starch served as a diluent which aids in the bulkiness of the mosquito coils and serves as a binder that binds the other ingredients together, talcum powder serves as a hardener, lubricant, filler and an additive to the produced mosquito coil. Sodium benzoate aids in the preservation of the produced mosquito coil to last for a very long time, it also prevents the mosquito coil from spoilage and undesirable colour alterations. Apparatus used were: electrical oven, electrical blender (Phillips, HR 1707), weighing balance (OHAUS Analytical plus, England), sieve (4.75 mm), plastic bowls, rolling pin, stirrer, carton board, aluminium foil, and the Peet Grady Chamber.

Sample collection and preparation

One hundred kilogram (100 kg) each of *Cymbopogon nardus*, *Hyptis suaveolens* and *Ocimum gratissimum* plants/leaves was collected at a field in Suleja, Niger state. The leaves (100 kg each) of *Cymbopogon nardus*, *Hyptis suaveolens* and *Ocimum gratissimum* plants that were collected were spread out to dry. The dried plants were ground into a fine powder using a mortar and an electric blender (Phillips, HR 1707) and stored in a black polyethene bag. Charcoal (hardwood lump, 500 g) was sieved with a sieve (4.75 mm) and then it was blended into fine particles using an electric blender (Phillips, HR 1707). The starch (40 g) was mixed with cold water (70 cm³) (cold water starch) and stirred very well.

Mosquito coil formulation

The sieved charcoal samples (20 g, 15 g and 10 g) were weighed into plastic bowls and mixed with 10 g, 15 g and 20 g of the ground plants respectively. Talcum powder (15 g) was added to each of the mixtures and mixed properly.

Then Sodium benzoate (1 g) was added to the mixtures and stirred thoroughly using a porcelain pestle. The prepared cold starch was added to the mixture and was mixed properly to form a dough. The dough was then spread on a carton board covered with an aluminium sheet to form a smooth, thin paste (Kinball, 1999). An already fabricated metal in form of a coil was then placed on top of the smooth paste and carefully pressed in order to trace the shape of the coil. The coils were dried in an oven at 70 °C for 6 hours; removed from the oven and allowed to stand for 30 minutes (Phal et al., 2012).

Test for Mosquito Coils

Insect Culture: The laboratory evaluation of the repellence of the mosquito coil formulation was carried out at the Department of Animal Science of Bayero University Kano. The rearing of the *Aedes aegypti* mosquitoes used in the evaluation of the mosquito coils was done under controlled temperatures (28 °C-30 °C) and humidity (60-80 %). The dried and incubated mosquito eggs were placed in deoxygenated water and two to three drops of ground fish food solutions were added to feed the larvae. Pupae were removed from the larval pads as they appeared and were placed into mesh-covered paper cups. Following emergence, adult mosquitoes were tested over a period of six days (WHO, 2009). The adult mosquitoes were continually allowed to feed on a cotton ball soaked with 0.2 mol/dm³ sucrose solution. At 1-2 hours before testing began, the cotton balls were removed and the mosquitoes were preconditioned for the bioassay (WHO, 2009). **Bioefficacy test:** Peet Grady chamber was maintained under the temperature range of 28 - 30 °C and humidity range of 60 – 80 % for the entire experiment. The 2-3 day-old female *Aedes aegypti* mosquitoes were collected from the rearing cage and were released in the handling bottles. Before testing each product, the mosquitoes from the handling bottles were released into the Peet grady chamber and were kept in it for about 30 minutes. The coil was lit outside the chamber and was pushed inside the chamber up to the center. The coil was allowed to smoulder continuously for 120 minutes observation period. The knockdown count was taken after every 5 minutes for 120 minutes using a hand-counter. The knockdown insects were collected and kept for 24 hours for mortality observation in a jar with sucrose solution in it. Similarly, a test was done for the coil without herbal oils (WHO, 2009). The KT₅₀ values were assessed using a minimum of two replicates. Similar experiment was conducted for control (without coil) to find the mortality of mosquitoes in the chamber. **Statistical Analysis of Data:** The data generated were subjected to one-way analysis of variance (ANOVA) and correlation analysis with the help of MINITAB 14 software. Variability in the efficacy of the samples may be dependent on several factors such as plant species, season, location, the part for extraction and the preparation method (Nimet & Hasan, 2013; Kamal et al., 2011; ZhiSheng et al., 2013).

RESULTS AND DISCUSSION



Plate 1a: Formulated mosquito coil paste with *Hyptis suaveolens* as the active ingredient.



Plate 1b: Formulated mosquito coil paste pressed with the tracer.



Plate 1c: Already traced formulated coil.



Plate 2: Dried formulated mosquito coils.

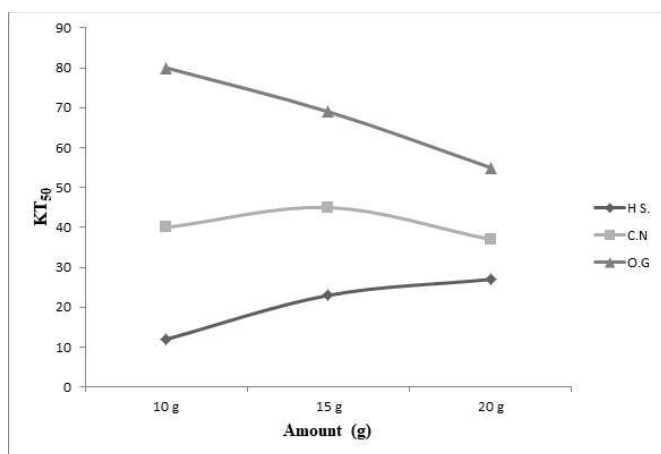


Figure 1: Effect of the amount of leaf extract against knockdown time. O.G- *Ocimum gratissimum*, C.N- *Cymbopogon nardus*, H.S- *Hyptis suaveolens*

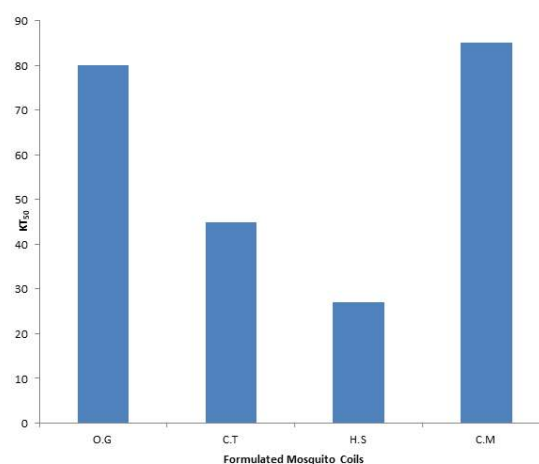
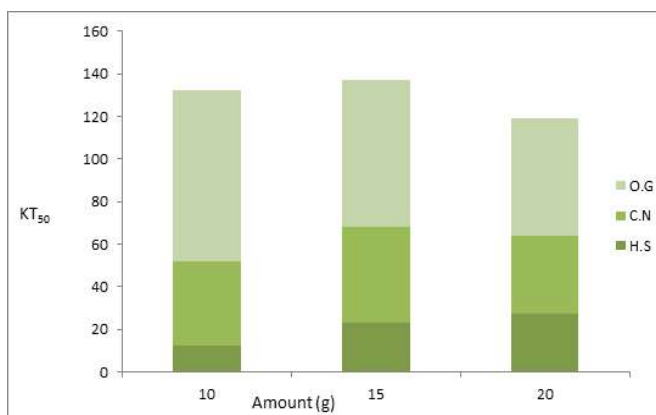


Figure 2: Efficacy of Formulated Mosquito Coil and Control. O.G- *Ocimum gratissimum*, C.T- *Citronella*, H.S- *Hyptis suaveolens*, C.M- Commercial mosquito coil

Table 1: Bioefficacy Variations in the Formulated Mosquito Coils

Coil source	Amount of leaves (g)	Percentage knockdown time (KT ₅₀ (minutes))
Ocimum gratissimum	10	80
	15	69
	20	55
Cymbopogon nardus	10	40
	15	45
	20	37
Hyptis suaveolens	10	12
	15	23
	20	27



O.G- *Ocimum gratissimum*, C.N- *Cymbopogon nardus*, H.S- *Hyptis suaveolens*

Figure 3: Efficacy of the Formulated Mosquito Coil at three different concentrations (10 g, 15 g and 20 g)

The concentration of active ingredients played an important role in determining the Bioefficacy of mosquito coils. The effect of concentration is optimized by determining the knockdown effect of mosquito coils at different levels of concentration ranging from 10-20 g. The efficacy of formulated mosquito coils has a significant impact on the bioefficacy of mosquito coils. The effect of the amount of leaf extract on the knockdown activity of the mosquito coils formulated from *Ocimum gratissimum*, *Cymbopogon nardus* and *Hyptis suaveolens* was studied by varying the amount of the leaf extracts in the range of 10-20g. The KT_{50} is the measure of the bio-efficacy of the mosquito coils. Based on the result obtained (Figure 1) for *Hyptis suaveolens*, the coil with the amount of 10g showed the highest knockdown activity with KT_{50} at 12 minutes. The knockdown activity of *Ocimum gratissimum* decreased with a decrease in the amount of leaf extract. A similar trend was observed for the mosquito coils using *Cymbopogon nardus* (Figure 3). The relation between the knockdown activity and the amount of *Hyptis suaveolens* was found to be markedly different compared to other samples; as there was a continuous increase in the knockdown activity with a decrease in the amount of *Hyptis suaveolens* in the mosquito coil for 10 g to 15 g.

Figure 2 shows the percentage knockdown of each mosquito coil at the optimum amount of the active ingredient. It can be seen that *Hyptis suaveolens* has the highest knockdown activity followed by *Cymbopogon nardus* and *Ocimum gratissimum*. The commercial mosquito coil containing 0.2 % allethrin which was used as a control was found to be less effective with the knockdown activity of more than 80 minutes.

CONCLUSION

The efficacy of formulated mosquito coils indicated that *Hyptis suaveolens* and *Cymbopogon nardus* were observed to be the most effective. The analysis of variance showed that there was a significant difference ($P < 0.05$) among the three different mosquito coils and control.

There is also a significant knockdown activity of the mosquito coils and time. The produced formulations can be used as substitutes for the more expensive synthetic formulations because they offer a unique way of protection against mosquitoes in the form of a great repellent effect and knockdown activity. These findings might open windows for formulating new insect repellents with longer durability and fewer side effect.

Recommendations

Based on the findings of this study, it is recommended that *Ocimum gratissimum*, *Cymbopogon nardus* and *Hyptis suaveolens* could be potentially used for the preparation of mosquito repellent products which could be in form of a coil, spray, cream, liquidator, candle and sticks, and could be prepared using suitable carriers/solvents/diluents, to get better protection from mosquito bites. These formulations could help in reducing the harmful effects of synthetic mosquito repellents on human health as they contain fewer harmful substances. Additionally, the production of locally made mosquito coils should be carried out in commercial quantities for economic purposes and for its usage, especially in rural areas.

Acknowledgement

Firstly, I am grateful to Almighty Allah for seeing me through this work. I am deeply grateful to my supervisor and the Head of the Department of Chemistry Prof. B. W. Tukura for his thorough supervision and guidance. I am also grateful to the coordinator of postgraduate program of the Department of Chemistry, Dr. S. I. Audu, the Dean of the Faculty of Natural and Applied Sciences, Prof. U. M. Gurku, Dr. B. O. Atolaiye and other staff of the university who directly or indirectly encouraged and supported me during the course of this research work. I am immensely grateful to Mal. Muktar Ibrahim Musa and also Mr. Yusuf A. Yusuf, Mr. Ibrahim, Dr. M. N. Ezeunala and Dr. M. I. Aboh of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja for their continuous support in the course of my laboratory work. I wish to express my profound gratitude to my late father, Mallam Mansur Sodangi, my mother, Hajiya Hadiza Adamu, and my sister Amina Mansur Sodangi, for their financial and moral support.

Declaration of conflicting interests

The author declared no potential conflicts of interest with respect to the research study, authorship, and/or publication of this article.

REFERENCES

1. Achee, N. L., Bangs, M. J., Farlow, R., Killeen, G. F. and Lindsay, S. (2012). Spatial repellents: from discovery and development to evidence-based validation. *Malaria Journal*, 11, 164–182.
2. Adda, C., Atachi, P., Hell, K. and Tamò, M. (2011). Potential use of the Bushmint, *Hyptis suaveolens*, for the Control of Infestation by the Pink Stalk Borer, *Sesamia calamistis*, on Maize in Southern Benin, West Africa. *Journal of Insect Science*, 11-33.

3. Amer, A. and Mehlhorn, H. (2006). Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitology Research*, 99, 478–490.
4. Conti, B., Canale, A., Cioni, P. L. and Flamini, G. (2010). Repellence of Essential Oils from Tropical and *Mediterranea lamiaceae* against *Sitophilus zeamais*, *Bulletin of Insectology*. 63 (2), 197–202.
5. De Boer, H., Vongsombath, C., Palsson, K., Björk, L. and Jaenson, T. (2010). Botanical repellent and pesticides traditionally used against hematophagous invertebrates in Lao People's Democratic Republic: a comparative study of plants used in 66 villages. *Journal of Medical Entomology*, 47, 400–414.
6. Ferreira-de-Brito, A., Ribeiro, I. P., Miranda, R. M., Fernandes, R. S., Campos, S. S. and Silva, K. A. (2016). First detection of natural infection of *Aedes aegypti* with Zika virus in Brazil and throughout South America. *Memórias do Instituto Oswaldo Cruz*, 111 (10), 655–658.
7. Gubler, D. J. (1998). Resurgent vector-borne diseases as a global health problem. *Emerging Infectious Diseases*, 4 (3), 442–450.
8. Hilje, L. and Mora, G. (2006). Promissory botanical repellents/deterrents for managing two key tropical insect pests, the whitefly *Bemisia tabaci* and the mahogany shoot borer *Hypsipylagranda* In: Rai M., Carpinella M. C., Editors. *Naturally occurring bioactive compounds*. Amsterdam: Elsevier Science, 379–403.
9. Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protect*, 19, 603–608.
10. IUPAC, (2006). Glossary of terms relating to pesticides (PDF). IUPAC.2123. Retrieved January 2014.
11. Kamal, G.M., Anwar, F., Hussain, A. I., Sarri, N. and Ashraf, M.Y. (2011). Yield and Chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *International Food Resource Journal*, 18, 1275–1282.
12. Kim, J. K., Kang, Chang, S., Lee, Jong, K., Kim, Young, R., Han, Hye Y., Yun and Hwa K. (2005). Evaluation of Repellency Effect of Two Natural Aroma Mosquito Repellent Compounds, Citronella and Citronellal. *Entomological Research*, 35 (2), 117–120.
13. Liu, W. K., Zhany, J., Hashim, J. H., Jalaludin, J., Hashim, Z., and Goldstein, B. D. (2003). Mosquito coil emissions and Health implication. *Environ Health prospect*, 111 (12), 1454–1460.
14. Lokhande G G and Chapaitkar N R (2022). Production of mosquito repellent insecticides (mosquito coil) using orange peels (*Cestrum*). *International Journal of Scientific Development and Research* 7(11): 1224–1228.
15. Maia, F. M. and Moore, S. J. (2011). Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal*, 10, 11.
16. Mavale, M., Parashar, D., Sudeep, A., Gokhale, M., Ghodke, Y. and Geevarghese, G. (2010). Venereal transmission of chikungunya virus by *Aedes aegypti* mosquitoes (Diptera: Culicidae). *American Journal of Tropical Medicine & Hygiene*; 83 (6), 1242–1244.
17. Moretti, A. N., Zerba, E. N. and Alzogaray, R. A. (2013). Behavioral and Toxicological Responses of *Rhodnius prolixus* and *Triatoma infestans* (Hemiptera: Reduviidae) to 10 Monoterpene Alcohols. *Journal of Medical Entomology*, 50(5), 1046–1054.
18. Nimet, K. and Hasan, B. (2013). Determination of lavender and *Lavandin cultivars* (*Lavandula* sp.) containing high quality essential oil in Ispart, Turkey. *Turkish Journal of Field Crops*, 8, 58–65.
19. Reiter, P. (2010). Yellow fever and dengue: a threat to Europe? *Euro Surveill*. 11, 15(10), 19509.
20. Regnault- Robert, C., Vincent C., and Arnasson T. (2012). Essential oils in insect control: low- risk products in a high-stakes world. *Annual Review Entomology*, 57, 405–424.
21. Trongtokit, Y., Rongsriyam, Y., Komalamisra, N. and Apiwatnasorn, C. (2005). Comparative repellency of 38 essential oils against mosquito bites. *Phytotherapy Research*, 19 (4), 303–309.
22. Phal, D., Naik, R., Deobhankar, K., Vitonde, S. and Ghatpande, N. (2012). Laboratory Evaluation of Herbal Mosquito Coils against *Aedes aegypti* Mosquito, *Bulletin of Environment, Pharmacology and Life Sciences*, 1(10), 16–20.
23. USDA Plants. (2009). PLANTS Profile for *Ocimum gratissimum*, Retrieved Jan. 7, 2022.
24. Van Emden, H. F., Peakall and David, B. (1996). *Beyond Silent Spring*. Springer.
25. Vega-Rua, A., Lourenco-de-Oliveira, R., Mousson, L., Vazeille, M., Fuchs, S. and Yebakima, A. (2015). Chikungunya virus transmission potential by local *Aedes* mosquitoes in America and Europe. *PLoS Neglected Tropical Diseases*, 9 (5), e0003780.
26. Weinzierl, R. and Henn, T. (1992). Alternatives in insect management: Biological and Biorational Approaches. University of Illinois, Urban-Champaign, North Central Regional Extension publication 401.
27. White, G. (2007). Terminology of insect repellents. In: Debboun, M., Frances, S., Strickman, D., editors. *Insect repellents: principles, methods, and uses*. CRC Press, 31–46.
28. World Health Organization. (2011). World malaria report 2011. WHO Global Malaria Programme. Geneva: WHO, 248.
29. World Health Organization. (1993). *Mise en oeuvre de la stratégie mondiale de lutte antipaludique*. Geneva: World Health Organization. Technical Report Series 839.
30. World Health Organization. (2008). World malaria

report 2008, WHO Global malaria programme.
Geneva: WHO, 190.

31. Zhang, L., Jiang, Z., Tong, J., Wang, Z., Han, Z. and Zhang, J. (2010). Using Charcoal as Base Material Reduces Mosquito Coil Emissions of Toxins. *Indoor Air*, 20(2), 176-84.
32. ZhiSheng, X., QunDi, L., ZhiKun, L., MingQian, Z., and XiaoXue, Y. (2013). The GC/MS analysis of volatile components extracted by different methods from *Exocarpium Citri Grandis*. *Journal of Analytical Method in Chemistry*, 918406.