JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Synergistic Repellent Activity of Three Essential Oils Against Mosquitoes and Houseflies

Repellency of Three Essential oils

[1]Nwoko Christopher, [1]Nkwoada Amarachi, [1]Igbomezie Maryann, [2]Amakom Chijioke

[1]Reader, [1]Lecturer I, [1]Assisstant Lecturer, [2]Lecturer I

[1]Department of Chemistry, Federal University of Technology Owerri, Nigeria. PMB 1526,

[2]Department of Physics, Federal University of Technology Owerri, Nigeria. PMB 1526

Corresponding author's e-mail: chemistryfrontiers@gmail.comAuthor

Abstract : There is an increasing demand for less toxic repellents from natural products owing the indiscriminate use of noxious synthetic insecticides to control vector-borne diseases. Synergistic repellent activities of Cymbopogon citrates (CC), Allium Sativa (AS), and Palm pressed fiber (PPF) essential oils binary mixture against Musca domestica and Anopheles gambiae was performed. The essential oils were extracted by Soxhlet extraction from three tropical plants and analyzed via phytochemical screening having alkaloids, saponins, tannins, flavonoids, terpenes, and phenols as the main components of the plants. The essential oils blend was prepared as (50/50 % v/v) for double mixtures and (40/40/20 % v/v) for triad mixture. The efficacy levels of the oils can be described for mosquitoes as CC/PPF/AS > CC/AS > PPF/AS > CC/PPF, while the efficacy levels of the oils against houseflies are CC/PPF/AS > CC/AS > CC/PPF > PPF/AS. The CC/PPF/AS blend had the highest repellency time and lowest landing rate, while the protection time for CC/PPF blends against mosquitoes is short, but long against houseflies with low landing rates hence functions as a repellent and deterrent. The CC/AS blend showed low landing rates and high repellency times; and a good repellent for both vectors. PPF/AS blend had high landing rates and good repellency times; hence more of a deterrent than repellent for both vectors. In conclusion, the results indicated that CC/PPF/AS blend was the most promising for repellency and deterrence against mosquitoes and houseflies, and these oils could be used to develop a new formulation to control insects**.**

IndexTerms **– Mosquitoes, Houseflies, *Zingiber officinale*, *Allium sativum*, Essential oils**

I. INTRODUCTION

Female mosquitoes are blood-sucking insects that are parasites and responsible for the transmission of dengue fever, Chagas disease, rift valley fever, and malaria, inducing serious health challenges to persons regardless of age. As a nuisance, it slows down the socioeconomic activities of developing nations, with a significant impact on sub-tropical Africa [1,2]. The infected female anopheles' mosquitoes can transmit malaria and filarial worm with ease. In a research review study in Brazil, it was reported that the Amazonian system accounts for nearly 100% of all cases of malaria and mainly due to autochthonous P. vivax and P. falciparum transmission by mosquitoes [3]. For India, a current study found that malaria is endemic in the eastern and southeastern Indian regions of the nation, and malaria transmission is essentially dependent on climatic parameters [4]. Additionally, the continual challenge of anopheles as an infectious disease-causing vector has worsened travelers' concerns over popular destinations like Bali, Indonesia [5] While in Nigeria, they are notoriously known to be responsible for 25 % of global cases and 19 % mortality rate, moreover, there has not been a significant action to reduce the vector spread [6], and globally there are the recent surges of mosquitoes resistant to WHO-approved insecticides [7].

On the other hand, a study has reviewed that Musca domestica is also an environmental nuisance that negatively impacts humans and animals through pathogen transmission. They are favored by warm climatic conditions and are often found in slaughterhouses, restaurants, food centers, hospitals, food markets, and fish markets. Thus, it requires routine control measures as a critical component to house fly management [8,9]. Another study revealed that houseflies transmit helminthic eggs, protozoa cysts and trophozoites, bacteria fungi, and viruses by mechanical means through their vomits or excreta. Hence regular methods of control and prevention of houseflies should be implemented for effective reduction of the housefly population at a large scale [10]. A particular study in Khartoum State, Sudan reported a high rate of gastrointestinal parasites in the flies, after a total of 684 house flies were collected and examined for intestinal parasites [11]. Another interesting study sampled 400 flies from two different countries of Rwanda and Belgium in three specific locations: farms, homes, and hospitals. They observed that house flies internal bacterial community is very diverse but consistent with geographic location and habitat [12]. More importantly, because of its synanthropic nature, breeding behavior can be controlled by the use of repellents targeted at adult males at their resting sites, however, there is little or no action taken to reduce the spread and transmission of disease via locally sourced binary mixtures of essential oils [13]

Transmission of malaria and other vector-borne diseases (VBDs) like the house fly, therefore, is greatly influenced by environmental factors, due to the ectothermic nature of the insects and arthropod vectors [14]. More importantly, is the application of this factor in the development of repellents for preventive control of vector-borne diseases which aids effective vector reduction strategies. The repellents cause decreasing vectorial capacity and offer a more sustainable control strategy against vectors. The repellents (insecticide) approach is particularly effective because it can be locally tailored for more effective and sustainable vector control [15]. Although it seems that the most common insect repellent is DEET (N, N -Diethyl-M-Toluamide). However, like Picaridin and citronella oil, DEET also belongs to EPA toxicity category III and has its drawbacks. It provides about 2-5 hours of protection against mosquitoes but potential neurotoxicity if applied under sunscreen. It gives a greasy feel, unpleasant odor, and can also damage plastics. For this reason, many natural synthesized alternatives are increasingly gaining attention. The use of Lemon grass is one of them, which has the advantage of being odorless and not damaging to plastics, unlike DEET. The natural repellents also seem to provide a significant and comparable level of protection against a large range of VBDs [16,17]. Additionally, the issue of public concern on the use of synthetic repellents of many other chemicals has created a renewed interest in sourcing for natural product alternatives of plant origin against vectors. These essential oils from these natural products are non-toxic, environmentally friendly, biodegradable, relatively cheap, and can be locally sourced. In addition to their application as toxicants against mosquitoes, they are also been utilized as repellents against mosquitoes due to their phytochemical composition. In addition, plant essential oils have been generally accepted as insect repellents because they can either interrupt or interfere in the regular physiological, biochemical, metabolic, and behavioral functions of vectors [18,19]. Hence, the development of binary mixtures of essential oils as an alternative and investigating its synergistic properties is evolving to replace single essential oils as repellents.

Although it is commonly acknowledged that plant-based repellents are less hazardous than synthetic repellents, there is still the issue of biomass distillation that involves energy consumption, organic solvent extraction that must be carefully disposed of, and more worrisome is the use of edible cash crops that are sources of income to farmers. But there is a greater need to substitute through plant waste (traditional netting materials are made from raffia palm) with repellent properties that can give synergistic repellent activity (against mosquitoes and houseflies) with less impact to edible crops and reduced losses to small scale farmers income [20]. The present study, therefore, investigates for the first time, the synergistic repellent activities of binary mixtures of three essential oils from lemon grass; Cymbopogon citrates, palm pressed fiber and Allium sativa against Anopheles gambiae and Musca domestica.

2.0 MATERIALS AND METHODS

2.1 Plant Collection

The plants (*Cymbopogon citrates and Allium sativa*) were harvested from a horticulture garden along Owerri-Okigwe road in Imo State, Nigeria in January 2020. The plants were identified, by a plant botanist at the Federal University of Technology Owerri, Nigeria. About 200 g was washed, dried in the oven to a constant weight at 110 ^{o}C, and labeled. The palm pressed fiber was hand-picked from a local oil mill in Amaifeke-Orlu in Imo State, Nigeria, and dried in the oven at the same temperature for 1 hr. to a constant weight of 200 g.

2.2 Phytochemical screening

Cymbopogon citrates, Allium sativa, and palm pressed fiber extracts were analyzed for saponins (distilled water test); alkaloids (Wagner's test); tannins (lead acetate test); flavonoids (NaOH test); terpenoids and steroids (petroleum ether, chloroform, H_2SO_4, anhydrous Na_2SO_4, and CH_3COOH); phenols (ferric chloride test); and anthraquinones (Bontrager's test). All the chemicals used for phytochemical screening were analytical grade reagents from spectrum chemicals [21–23]

2.3 Essential oil extraction

Weighed plant matter of 200 g of each plant was ground to powder using a heavy-duty electric blender (Clarkson 1.1L; model UTI-4AL). Analytical grade reagents of n-Hexane (H1010) and Na_2SO4 (S1455) analytical from Spectrum chemicals were used throughout the analysis. A weighed amount of powdered plant matter was introduced into the Soxhlet extraction chamber and 250 mL of n-Hexane was used as the solvent for extraction and masses of the plants varied from 25/30 g. As the solvent boils, the oil is collected at the receiving bottom flask and dried over anhydrous Na_2SO4.

2.4 Laboratory trials

The repellent test for Musca domestica and Anopheles gambiae was based on WHO protocol (2009) with some modifications [23,24] Two constructed wooden cages (20 cm X 20 cm x 20 cm) were covered with a mosquito net before introducing each insect. Fifty female mosquitoes were supplied from the horticulture garden around where water hyacinths and water lettuce was planted and starved for 12 hrs. before the test. Similarly, sticky fly traps were placed at a slaughterhouse (abattoir) to trap 50 houseflies and starved for 12 hrs. Before each test, both arms of the volunteers were covered with rubber sleeves having no exposed area since the test is conducted simultaneously for houseflies and mosquitoes. On the first trial, both arms were used for control and had no essential oil applied to them. On the second trial, both arms had the essential oils applied on them and introduced into the cages. Each test was repeated by a different volunteer and the average was taken and reported. The essential oils were formulated as binary mixtures in the ratio of 1:1 (50/50 % v/v) for double mixtures to obtain 10 mL and 2:2:1 (40/40/20 % v/v) for triad mixtures to obtain 10 mL. The total number of mosquitoes and houseflies landing on control arms and the treated arms were recorded. If no insect landed after 2 mins, the arm was withdrawn from the cage, and a new rubber sleeve rubbed with essential oil is introduced again and the procedure repeated. The test was conducted for 6 hrs. to ensure the maximum duration and repellency exhaustion. The insect count was taken every 30 minutes beginning from the time of introduction (0.0hr), until the study was stopped (6 hr.) Also, percentage repellency was calculated using the formula and no mosquito bite was reported, also, free consent was obtained from the participants.

$$\% \text{ Repellency (landing)} = L/50 \times 100$$

Where L is the total number of insects (mosquito or housefly) that landed on the arm and remained for at least 30 sec, and 50 is the total number of insects introduced into the cage.

3.0 RESULTS

3.1 Phytochemical study

All the results of the phytochemical study are shown in table 1. In the present study, the saponins showed the formation of a foam layer in Cymbopogon citrates and Allium sativa. The alkaloid test showed the formation of intense brown/reddish precipitate in *Cymbopogon citrates* and *Allium sativa* respectively. The tannin test gave an intense yellow precipitate *in Cymbopogon citrates* and *Allium sativa* but less intense in PPF. Flavonoid was detected in all three samples giving a yellow coloration that turned colorless when a few drops of dilute acids were added.

Table 1. Phytochemical constituents of the three essential oils

Phytochemicals	***Cymbopogon citrates***	***Allium sativa***	Palm pressed fiber
Saponins	+	++	-
Alkaloids	+++	+++	-
Tannins	++	+++	+
Flavonoids	++	++	++
Terpenes	+	+++	-
Steroids	_	_	++
Phenol	++	-	++
Anthraquinones	+	-	-

Source: from this research study

On the other hand, the steroid was only detected in PPF, while terpenes were found in *Cymbopogon citrates* and *Allium sativa* owing to strong greenish color in the separation layer. Phenolic presence was found to be in Cymbopogon citrates and PPF, while on the contrary, only Allium sativa contained anthraquinones. The findings showed that Cymbopogon citrates, Allium sativa, and palm pressed fiber to have promising phytochemicals contained in the essential oil which can be released and serve as a repellent against disease vectors and provide a useful tool that promotes the localized application of Essential oil as repellents [25,26]. The repellency percentage for the essential oils after complete protection times elapsed is shown in table 2. trials were conducted from 30 — 360 mins. The control sample showed a minimum range from 61-70 % for houseflies at 30 mins to a maximum of 72 — 76 % for mosquitoes at 300 mins. This means that an average of 30—35 houseflies perched and remained for at least 2 mins during the 30 mins test, while an average of 35 — 38 houseflies perched and remained for at least 2 mins during the 300 mins test.

Table 2: repellency % of houseflies and mosquitoes after complete protection times elapsed

RepellencyTimes	Insects	Control (%)	CC/PPF (%)	CC/AS (%)	PPF/AS (%)	CC/PPF/AS (%)
30	Housefly	61-70	20-25	10-23	43-57	05-10
	Mosquito	65-68	26-32	22-24	43-50	08-15
60	Houseflies	63-70	33-35	15-20	40-42	01-07
	Mosquitoes	65-71	37-39	16-18	40-48	11-14
120	Houseflies	65-77	23-27	19-22	35-38	09-12
	Mosquitoes	70-72	26-29	13-20	48-52	11-15
180	Houseflies	65-69	27-33	11-18	55-57	15-18
	Mosquitoes	71-74	34-38	19-23	60-66	13-17
240	Houseflies	65-70	23-28	11-15	50-54	11-12
	Mosquitoes	70-72	22-28	16-23	49-53	10-13
300	Houseflies	65-77	29-33	16-25	52-62	12-17
	Mosquitoes	72-76	31-35	10-17	43-52	09-12
360	Houseflies	75-73	29-36	17-23	50-57	05-08
	Mosquitoes	65-68	27-32	14-18	40-47	06-11

AS: *Allium sativa***; CC:** *Cymbopogon citrates; PPF: palm pressed fiber* (Source: from this research study)

On the other hand, closer observation shows that PPF/AS binary mixture offered the least repellency percentage which ranged from 40—66 % that is an average of 20—33 mosquitoes of houseflies perched and remained for at least 2 mins. This is followed by a CC/PPF binary mixture of 20—39 % having of which is about 10—19 houseflies/mosquitoes perched and remained for 2 mins. The CC/AS essential oil mixture offered an improved repellency percentage when compared to CC/PPF and PPF/AS essential oils owing to 10—23 % of mosquitoes/houseflies landing after the mixture was applied on the rubber sleeves and introduced into their respective cages. The most effective essential oil was the CC/PPF/AS triad mixture that offered the lowest landing percentage of mosquitoes/houseflies after each experimental trial. The percentage ranged from 05—18 % which means about 2 — 9 insects landed on the volunteers' arms after experimental trials. Hence the efficacy of the blended essential oils towards the repellency of insects from the study is CC/PPF/AS > CC/AS > CC/PPF > PPF/AS. The repellency times are presented in the box and whiskers plot shown in Fig 1. Time range is seen as the first column with the data clustered in front of it showing 7 dot counts which represent 30, 60, 120, 180, 240, 300, and 360 mins. in figure 1. It can be seen that the control samples

remained at 0 times which means that insects landed on the arms almost within a minute or less of introducing the arms into the repellency box.

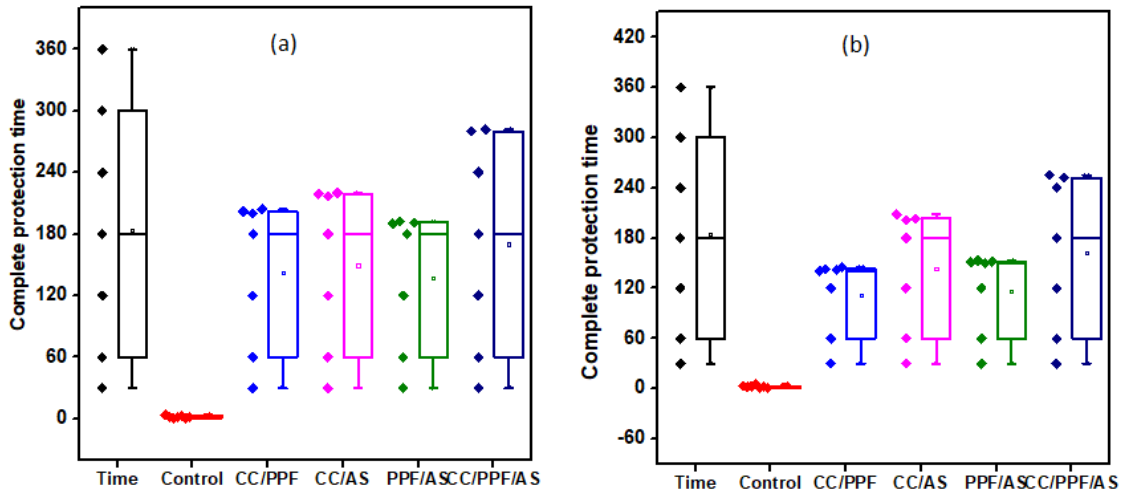

Fig 1. Box and whiskers plot showing of the binary mixtures of essential oils against and control sample, (a) houseflies repellency time; (b) mosquitoes repellency time (Source: from this research study)

Then Fig 1a shows the complete protection time for houseflies and showed that CC/PPF/AS offered the highest repellency times against houseflies up to 280 mins. Figure 1b similarly showed that CC/PPF/AS offered the best repellency against mosquitoes at 240 minutes maximum. Closer observation showed that CC/AS essential oils were more effective than CC/PPF and PPF/AS essential oils in both the houseflies and mosquitoes. Additionally, the essential oils offered more repellency times against houseflies than mosquitoes for all the four essential oil mixtures. The efficacy levels of the oils can be described for mosquitoes as CC/PPF/AS > CC/AS > PPF/AS > CC/PPF, while the efficacy levels of the oils against houseflies is CC/PPF/AS > CC/AS > CC/PPF > PPF/AS.

4.0 Discussions

When the complete protection time of a compound is long and the percentage of landing is low, the compound has a good efficiency in repelling disease-causing vectors. If the protection time is short but the percentage of landing is high, then the compound is more a feeding deterrent than a repellent. Conversely, if the protection time is long but the landing rate is high, then the compound is more of a biting deterrent [17,18,27]. From our study, the triad mixture offered the highest repellency time and lowest landing rate which suggests that it has a good efficiency in repelling vectors. On the other hand, the protection time for CC/PPF against mosquitoes is short but long against houseflies with low landing rates in both cases; hence suggested to be more of a repellent for houseflies and a deterrent for mosquitos. The CC/AS essential oils showed that there were low landing rates and high repellency times; hence it was suggested to be more of a repellent for both disease-causing vectors in this study. The PPF/AS showed high landing rates as seen in table 2 but good repellency times; hence it is suggested to be more of a deterrent than repellent for both mosquitoes and houseflies in this study [28,29].

Similarly, a study found that an essential oil mixture of *C. longa*, *Z. limonella*, and *P. heyneanus* was found to provide better protection against Aedes albopictus mosquitoes in the laboratory [30,31]. Another study also revealed that essential oil blends from *M. piperita, O. sanctum, E. globulus, and P. amboinicus repels Ae. aegypti* mosquitoes and also prevented feeding even at a lower concentration of 5% [32]. Another study utilized lemon grass essential oil prepared in the volume of 0.1 mL of 5 % in ethanol and studied via arm in cage test method showed that protection lasted around 30-105 mins [33]. The experimental study herein suggested that binary blends of Cymbopogon citrates with a palm pressed fiber and Allium sativa improved the complete protection times when compared to individual essential oils. Then again, another study on *Allium sativa* (garlic) essential oil was toxic to *T. molitor* larva, followed by pupa and adult [34]. In another assay, combinations were prepared from the *Allium sativa* and *Ocimum sanctum* concentration of each candidate at a 1:1 volume ratio and tested against the same stage of the mosquito. Findings revealed that the most effective treatment was as larvicide [35]. This also agrees with our findings showing that CC/AS combination was more effective against mosquitoes and houseflies than CC/PPF and PPF/AS essential oil combination. On the other hand, little is known on the use of pressed palm fiber waste as a repellent but palm kernel oil has been used as natural oil bases for *Ocimum. gratissimum* and was able to reduce human-mosquito contacts while palm fiber has been reported to impart repellency into the fabrics and netting materials [36,37]. Hence, the knowledge of traditional repellent plants is a substantial resource for the development of new products as an alternative to chemical repellents.

5.0 CONCLUSIONS

Several plant extracts and essential oils found in these studies are volatile, such as terpenoids, steroids, alkaloids, and aldehydes, which are repellent to insects for periods ranging from minutes to several hours. The research findings demonstrated the potential of Cymbopogon citrates (CC), Allium sativa (AS), and Palm pressed fiber (PPF) (binary mixtures) essential oil blends, repellency, and deterrence to *Musca domestica* and *Anopheles gambiae*, which showed their properties and capacity as potential oils for controlling vectors. Further studies on field applications should be conducted as well as their application in the new repellent formulation is necessary. Additionally, long-term studies need to be conducted with different concentrations and

formulations for determining the best protection time and effective dose of the synergistic repellent blend from the three plant essential oils.

REFERENCES

[1] Wellems T., Hayton K, Fairhurst R. The impact of malaria parasitism: from corpuscles to communities. Journal of Clinical Investigation 2009;119:2496–505. https://doi.org/10.1172/JCI38307.

[2] Sato A. Plasmodium—a brief introduction to the parasites causing human malaria and their basic biology. Journal of Physiological Anthropology 2021;40:1–13. https://doi.org/10.1186/s40101-020-00251-9.

[3] Bianca C, Lona L, Christophides G, Souza-Neto J. A comprehensive analysis of malaria transmission in Brazil. Pathogens and Global Health 2019;113:1–13. https://doi.org/10.1080/20477724.2019.1581463.

[4] Chaturvedi S, Dwivedi S. Estimating the malaria transmission over the Indian subcontinent in a warming environment using a dynamical malaria model. Journal of Water and Health 2020;18:358–74. https://doi.org/10.2166/wh.2020.148.

[5] Yoshikawa M, Kusriastuti R, Liew C. An interdisciplinary study: disseminating information on dengue prevention and control in the world-famous travel destination, Bali, Indonesia. Evolutionary and Institutional Economics Review 2020;17:265–93. https://doi.org/10.1007/s40844-019-00138-0.

[6] Aju-Ameh C. Mosquitoes are not the major culprits for the high burden of malaria in Nigeria: a commentary. Pan African Medical Journal 2020;35:1–10. https://doi.org/10.11604/pamj.2020.35.11.16972.

[7] Akuamoah-Boateng Y, Brenyah R, Kwarteng S, Obuam P, Owusu-Frimpong I, Agyapong A, et al. Malaria Transmission, Vector Diversity, and Insecticide Resistance at a Peri-Urban Site in the Forest Zone of Ghana. Frontiers and Tropical Diseases 2021;2. https://doi.org/10.3389/fitd.2021.739771.

[8] Khamesipour F, Lankarani KB, Honarvar B, Kwenti TE. A systematic review of human pathogens carried by the housefly (Musca domestica L.). BMC Public Health 2018;18:1049. https://doi.org/10.1186/s12889-018-5934-3.

[9] Gerry AC. Review of Methods to Monitor House Fly (Musca domestica) Abundance and Activity. Journal of Economic Entomology 2020;113:2571–80. https://doi.org/10.1093/jee/toaa229.

[10] Amoo EO, Adekeye O, Olawole-Isaac A, Fasina F, Adekola PO, Samuel GW, et al. Nigeria and Italy divergences in Coronavirus experience: Impact of population density. The Science World Journal 2020;1:1–9. https://doi.org/10.1155/2020/8923036.

[11] Ibrahim A, Ahmed H, Adam R, Ahmed A, Elaagip A. Detection of Intestinal Parasites Transmitted Mechanically by House Flies (Musca domestica, Diptera: Muscidae) Infesting Slaughterhouses in Khartoum State, Sudan. International Journal OfTropical Diseases 2018;1:1–5. https://doi.org/10.23937/ijtd-2017/1710011.

[12] Park R, Dzialo MC, Spaepen S, Nsabimana D, Gielens K, Devriese H, et al. Microbial communities of the house fly Musca domestica vary with geographical location and habitat. Microbiome 2019;7:147. https://doi.org/10.1186/s40168-019-0748-9.

[13] Zahn LK, Gerry AC. Diurnal Flight Activity of House Flies (Musca domestica) is Influenced by Sex, Time of Day, and Environmental Conditions. Insects 2020;11. https://doi.org/10.3390/insects11060391.

[14] Rocklöv J, Dubrow R. Climate change: an enduring challenge for vector-borne disease prevention and control. Nature Immunology 2020;21:479–83. https://doi.org/10.1038/s41590-020-0648-y.

[15] Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, et al. The importance of vector control for the control and elimination of vector-borne diseases. PLOS Neglected Tropical Diseases 2020;14:e0007831.

[16] Lee MY. Essential Oils as Repellents against Arthropods. BioMed Research International 2018;2018:6860271. https://doi.org/10.1155/2018/6860271.

[17] Maia MF, Moore SJ. Plant-based insect repellents: a review of their efficacy, development and testing. Malaria Journal 2011;10 Suppl 1:S11. https://doi.org/10.1186/1475-2875-10-S1-S11.

[18] Asadollahi A, Khoobdel M, Zahraei-Ramazani A, Azarmi S, Mosawi SH. Effectiveness of plant-based repellents against different Anopheles species: a systematic review. Malaria Journal 2019;18:436. https://doi.org/10.1186/s12936-019-3064-8.

[19] Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review. Bioresource Technology 2010;101:372–8. https://doi.org/10.1016/j.biortech.2009.07.048.

[20] Ofori-Boateng C, Lee KT. Sustainable utilization of oil palm wastes for bioactive phytochemicals for the benefit of the oil palm and nutraceutical industries. Phytochemistry Reviews 2013;12:173–90. https://doi.org/10.1007/s11101-013-9270-z.

[21] Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. TheScientificWorldJournal 2017;2017:5873648. https://doi.org/10.1155/2017/5873648.

[22] Pochapski MT, Fosquiera EC, Esmerino LA, Dos Santos EB, Farago PV, Santos FA, et al. Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from Ipomoea batatas (L.) Lam. Pharmacognosy Magazine 2011;7:165–70. https://doi.org/10.4103/0973-1296.80682.

[23] WHO. Guidelines for efficacy testing of mosquito repellents for human skin 2015:12–54.

[24] Mahato N, Sinha M, Sharma K, Koteswararao R, Cho MH. Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from Citrus Wastes. Foods 2019;8. https://doi.org/10.3390/foods8110523.

[25] Akintelu SA, Folorunso AS, Oyebamiji AK, Olugbeko SC. Mosquito Repellent and Antibacterial Efficiency of Facile and Low-Cost Silver Nanoparticles Synthesized Using the Leaf Extract of Morinda citrifolia. Plasmonics 2021;16:1645–56. https://doi.org/10.1007/s11468-021-01428-3.

[26] Nyasembe VO, Torto B. Volatile phytochemicals as mosquito semiochemicals. Phytochemistry Letters 2014;8:196–201. https://doi.org/10.1016/j.phytol.2013.10.003.

[27] Rehman JU, Ali A, Khan IA. Plant based products: Use and development as repellents against mosquitoes: A review. Fitoterapia 2014;95:65–74. https://doi.org/https://doi.org/10.1016/j.fitote.2014.03.002.

[28] He Z, Zhang J, Shi Z, Liu J, Zhang J, Yan Z, et al. Modification of contact avoidance behaviour associated with pyrethroid resistance in Anopheles sinensis (Diptera: Culicidae). Malaria Journal 2019;18:131.

https://doi.org/10.1186/s12936-019-2765-3.

[29] Nisar MS, Ismail MA, Ramzan H, Maqbool MM, Ahmed T, Ghramh HA, et al. The impact of different plant extracts on biological parameters of Housefly [Musca domestica (Diptera: Muscidae)]: Implications for management. Saudi Journal of Biological Sciences 2021;28:3880–5. https://doi.org/10.1016/j.sjbs.2021.03.070.

[30] Das NG, Dhiman S, Talukdar PK, Rabha B, Goswami D, Veer V. Synergistic mosquito-repellent activity of Curcuma longa, Pogostemon heyneanus and Zanthoxylum limonella essential oils. Journal of Infection and Public Health 2015;8:323–8. https://doi.org/10.1016/j.jiph.2015.02.005.

[31] Phasomkusolsil S, Soonwera M. Insect repellent activity of medicinal plant oils against Aedes aegypti (Linn.), Anopheles minimus (Theobald) and Culex quinquefasciatus Say based on protection time and biting rate. The Southeast Asian Journal of Tropical Medicine and Public Health 2010;41:831–40.

[32] Lalthazuali, Mathew N. Mosquito repellent activity of volatile oils from selected aromatic plants. Parasitology Research 2017;116:821–5. https://doi.org/10.1007/s00436-016-5351-4.

[33] Kim S-I, Yoon J-S, Baeck S-J, Lee S-H, Ahn Y-J, Kwon HW. Toxicity and synergic repellency of plant essential oil mixtures with vanillin against Aedes aegypti (Diptera: Culicidae). Journal of Medical Entomology 2012;49:876–85. https://doi.org/10.1603/me11127.

[34] Plata-Rueda A, Martínez LC, Santos MH Dos, Fernandes FL, Wilcken CF, Soares MA, et al. Insecticidal activity of garlic essential oil and their constituents against the mealworm beetle, Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae). Scientific Reports 2017;7:46406. https://doi.org/10.1038/srep46406.

[35] Mahanta S, Khanikor B, Sarma R. Allium sativum (Liliales: Asparagales) essential oil - based combinations – a potential larvicide for Culex quinquefasciatus (Diptera: Culicidae). International Journal of Tropical Insect Science 2020;40:837–44. https://doi.org/10.1007/s42690-020-00139-2.

[36] Anuar AA, Yusof N. Methods of imparting mosquito repellent agents and the assessing mosquito repellency on textile. Fashion and Textiles 2016;3:12. https://doi.org/10.1186/s40691-016-0064-y.

[37] Oparaocha ET, Iwu I, Ahanakuc JE. Preliminary study on mosquito repellent and mosquitocidal activities of Ocimum gratissimum (L.) grown in eastern Nigeria. Journal of Vector Borne Diseases 2010;47:45–50.