Original Research Article

Journal of Biological Research

& Biotechnology

Bio-Research Vol. 21 No.3; pp. 2099-2112 (2023). ISSN (print):1596-7409; eISSN (online):2705-3822

Repellence of *Aedes aegypti* with oils from *Citrus sinensis* and *Citrus paradisi fruit peelsi* from Nsukka

[§,1]Ugwu Francis Stephen Ogbonna and [2]Chime Chidiebere Divine

[1]South East Zonal Biotechnology Centre/Zoology and Environmental Biology Department, University of Nigeria, Nsukka, Enugu State, Nigeria

[2] Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria

[§]**Corresponding author:** Ugwu Francis Stephen Ogbonna., Email: francis.ugwu@unn.edu.ng

Abstract

Aedes aegypti spread yellow fever, dengue fever, Zika virus and Chikungunya that can be prevented through vector control. Chemical insecticides are resisted by vectors, harm humans and the ecosystem thereby making their use opprobrious. Plants essential oils are safer alternatives. Oils of *Citrus sinensis* and *Citrus paradisi* fruit peels are readily available in Nsukka but their effect on *Aedes aegypti* remain uncertain so it became imperative to verify the effect of these oil extracts on them. The cold maceration method was used to extract essential oils from their peels. They were used singly and in various formulations to evaluate the behaviour of starved *Ae. aegypti* adults. WHO protocols were applied in obtaining the effective doses and complete protection time. Data collected were analysed using SPSS version 23.0. Various formulations repelled mosquitoes in the magnitude of 100 % > 75 % > 50% > 25% and significant differences ($p < 0.05$) noted between formulations. Formulations B and H showed highest repellence effects with 0 – 3 number of mosquito landings during the three minutes of test period. Probit analysis showed that 1.14 ml of formulation G was required to achieve 99% repellence. Formulation B demonstrated synergism of the oils attaining the highest complete protection time of 150 minutes. This study showed that oil extracts of *C. sinensis* and *C. paradisi* would prevent female *Aedes aegypti* adults from landing on or feeding from skins smeared with each of the oil or their combinations. The oils exhibited synergism when combined hence could be used to control this mosquito.

Keywords: *Citrus*, Essential oil, *Aedes aegypti*, *Citrus paradise, Citrus sinensis,* Repellence, Complete protection time.

Received January 16,2023; **Revised** August 5, 2023; **Accepted** August 7, 2023

https://dx.doi.org/10.4314/br.v21i3.4
Journal Homepage: http://www.bioresearch.com.ng.
Publisher*: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*

INTRODUCTION

Aedes are insects that belong to the order Diptera which consist of all two-winged insects such as mosquitoes, house fly, sand fly, etc. Mosquitoes are of the family Culicidae that are characterised by structural segmentation of the body, three pairs of legs, possession of a pair of wings and halters. The mouth parts are elongated and the sexes are separate with extended mouth parts used for uptake of fluids. Further molecular characterization, biome analysis and differences between different species are provided by Soni *et al*., (2018) and Soares *et al*. (2022). The female mouth parts are adapted further for piecing animal skins for sucking blood. Mosquito genera encompass numerous types; however, their consideration from public health perspective limit us to few genera where the genus, *Anopheles* and *Aedes* are best known for vectoring diseases of medical importance. *Aedes* encompass many other species. Here, we are limited to *Aedes aegypti* which is easily identifiable because its legs and body has successive bands coloured black and white as well as the possession of a lyre-like mark on the dorsal side of the thorax (Soni *et al*., 2018). This vector seems to have everything working in its favour: it has efficient capacity to vector arboviruses (Srivastava and Kachhwaha, 2019), global warming, generation of immense number of refuse tyres and containers (Odo *et al*., 2015), rapid urbanization and international travels enable the vector to spread fast across the globe with the attendant risk of spreading diseases of epidemic and pandemic proportions (Dickens *et al*., 2018; Elsey *et al*., 2019). Owing to the fact that the predominant occupation of our people is farming, they are professionally predisposed to the menace of mosquito bites.

Aedes eagypti and its cousin *Aedes albopictus* are mosquitoes closely associated with man (Odo *et al*., 2015), irritant pests and vectors of life threatening arboviral diseases such as chikungunya, Japanese encephalitis, West Nile, dengue, yellow fever, Zika and others (Paixão *et al*., 2018; Marques *et al*., 2022). These diseases impose great health/economic stress on people globally. Yellow fever, which could be fatal in 20 to 60% of cases currently, has no drug treatment. It is caused by a Flavivirus that has equatorial forest monkeys as the biological amplifying host but vectored in man by *Aedes aegypti* (Bessimbaye *et al*., 2021). Dengue (referred to as backbone fever) is prevalent in Asian countries and accounts for 70% of all cases globally and they may have antigenic cross reaction with Zika (Srivastava and Kachhwaha, 2019). Hussain *et al*. (2022) reported the following symptoms: severe headache, pain behind the eyes, muscle/joint pains and nausea during the febrile phase. Other symptoms include haemorrhagic fever, vomiting, swollen glands and rashes as medical signs of the condition that could result in death if not quickly treated. The resulting thrombocytopaenia could induce intracranial haemorrhage and Terson's syndrome that provoke impaired vision (Belenje *et al*., 2021). Hussain *et al*. (2022) consider encephalopathy as the most common dengue neurological sequela. Thompson *et al*. (2020) estimated that the economic cost of yellow fever virus and dengue virus globally vary from 2.1 to 57.3 billion USD and that the expenditure devoted to mosquito vector control (which is the best method of controlling arboviral infections (Paixão *et al*., 2018)) was between 5.62 and 73.5 million USD. According to Canali *et al*. (2017), the chikungunya epidemic that happened in 2007 in Emilia-Romagna region (Northern Italy), expenses incurred controlling the *Aedes albopictus* vector was put at €1.3 per resident. If the peoples of Sub Sahara Africa are further encumbered with the risk of increasing prevalence of arboviral diseases, the poverty in the region will certainly exacerbate.

The genera *Citrus* are trees that bear flowers and belong to the Rutaceae family (Favela-Hernández *et al*., 2016). Other approaches to its classification are provided by Akhtar *et al*. (2021) and Singh *et al*. (2021). *Citrus* is a native of South East Asia (Wu *et al.,* 2018) and now grown globally in the tropics and sub-tropics because of its immense economic, nutritional, phytochemical, pharmacological, antimicrobial, antiparasitic significance (Othman and Fadzil, 2021; Akarca *et al*., 2021). *Citrus* fruits consist of the exocarp and the endocarp. The latter is the edible part. The former is the peel which has two layers respectively called favedo (the outermost) and the albedo (the innermost) where the pigments and the oil glands are located (Prabasari, 2014). Our interest in this paper is the peel which is loaded with poorly explored components but regarded as waste in our locality. Available information suggests that the peels are highly endowed with biochemical components which number up to 200 (Paoli *et al*. 2016;

Khanikor *et al*., 2021; Essombe Malolo *et al*., 2023). The peel extract is toxic to *Aedes* in their various stages (Meisyara *et al*., 2021; Gomes *et al*., 2020). There are many species in this genus but we are interested in *Citrus sinensis* (Sweet orange) and *Citrus paradisi* (grapefruit) which has bitter taste. C. *paradisi* is a cross between *C*. *sinensis* and *C. maxima* from imported parents in the Caribbean (Essombe Malolo *et al*., 2023).

Citrus peels contain essential oils (CPO) that include some open chain hydrocarbons, naringin, alcohols, aldehydes, ketones, esters, alpha-terpenoids, monoterpenes, pectins, and many other volatile compounds (Favela-Hernández *et al*., 2016; Khanikor *et al*., 2021; Gomes *et al*., 2022). Mosquito repellence is a veritable property of CPO that had been documented (Heidari *et al*., 2017). In our locality, *Citrus* fruits abound but often, truckloads of them were often abandoned or allowed to rot in refuse dumps indicating that our people are not sufficiently informed of their usefulness. Incidentally, access to information is creating awareness of the fast development of resistance to chemical insecticides by this insect (Karunamoorthi and Hailu, 2014; Marques *et al*., 2022). In addition, use of insecticides is opprobrious because of the increasing hazard they pose to public health and ecosystem stability (Ugwu and Oyeagu, 2023). People are now turning to the use of botanicals to deal with insect pest because children highly exposed to N, N-diethyl-3-methylbenzamide (DEET) had suffered seizures, bradycardia and other conditions (Wu *et al*., 2018). It therefore becomes imperative that more options ought to be found to mitigate the combined threat of *Aedes* mosquitoes and arboviruses they vector in our clime. The task here is to explore the valorisation of *Citrus* fruit peels from Nsukka to ascertain if they can be part of tools that can protect people working outdoor from exophagic mosquitoes. We therefore set out to determine if repellence of *Aedes aegypti* could be attributed to *Citrus sinensis* and *Citrus paradisi* oils; and if so, ascertain the duration of this protection. The introduction is unnecessarily long and should be reduced

MATERIALS AND METHODS

Sourcing, breeding and maintenance of *Aedes aegypti*

Aedes mosquito eggs were sourced from the Entomology Unit, National Arbovirus and Vector Research Center, Enugu, Enugu State, Nigeria*.* About 1000 eggs were packaged in white cotton cloth measuring 10 cm long and 5 cm width, transported to and bred at the Entomology Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka*.* The egg patch was carefully layered in about 1.5 litres of water and allowed to incubate at room temperature in a transparent 10 litres plastic bucket covered with a piece of fabric net and the sides fitted with pluggable windows to permit manipulation/feeding of larva/adult mosquitoes. Pallets of fish feeds were added to the water to serve as feed when the eggs began to hatch and balls of cotton soaked in 10% glucose were placed in the windows to maintain adult mosquitoes (Ugwu and Onwuzuruike, 2018). Similar breeding buckets were created to breed more mosquitoes via transfer of a few adult mosquitoes to virgin breeding buckets where they were fed blood and sugar solution (Ugwu and Onwuzuruike, 2018). A day before experimentation, female mosquitoes were selected and placed in clear covered plastic cup with pluggable vents for manual manipulation of *Aedes aegypti* with aspirators. This was placed in 'arm in cage' box constructed according to WHO (2009) specification. Feeding was halted 12 hours before repellence test (WHO, 2009).

Preparation of the *Citrus* fruit peel oils

From *Citrus* trees grown within the University of Nigeria, Nsukka, were acquired *Citrus* fruits and their twigs. They were taken to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, for identification and authentication. Their voucher specimen numbers were UNH 217[d] and UNH[e] respectively for *C. sinensis* and *C. paradisi* (were they deposited? If so state location) The fruits from the two *Citrus* species were separated accordingly and taken to the laboratory for processing. They were washed with clean water and dried with clean towel. The peels were removed, dispersed on clean white cardboard papers sheets and air-dried on laboratory benches for three weeks. They were thereafter ground into tiny pellets with a manual grinder and stored air-tight in transparent plastic container. Cold maceration method of Rajani *et al*. (2000) was used for oil extraction at the Pharmacology and Toxicology Laboratory of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka*.* Each of the oils was confirmed by placing 0.1g of the processed sample between filters and observing translucency of the filter paper (Chen *et al.*, 2015). The following was used to calculate the percentage of oil yield:

$$\text{Percentage yield} = \frac{\text{Weight of oil extract}}{\text{Weight of sample before extraction}} \times 100$$

Repellency bioassay

Ethical clearance was obtained from the Enugu State Ministry of Health and the Board of ZEB Department, University of Nigeria, Nsukka (what were the reference numbers of the ethical clearances obtained?). The WHO method (WHO, 2009) was used to perform repellency bioassay. Briefly, oils from the two *Citrus* fruits were used singly and mixed proportions (Table 2, see formulation column). From each of the proportions, the following strengths were prepared using tween 80 as diluents: 25%, 50%, 75% and 100%. Each test human volunteer, after confirming his/her participation by signing consent form raised for this purpose, was required to avoid the use of any form of fragrance/cosmetics 12 hours before and during the experiments. Their forearms were washed with fragrance-free soap and rinsed with water. Another rinse with 70% ethanol was done and dried with a clean towel. Thereafter tween 80 was evenly applied on the forearm and allowed to dry. The hands were covered up to the wrist with latex hand gloves. The arm was then inserted into the insect cage for 3 minutes and the number of mosquito landings was taken. This served as a negative control test (more than 10 landings in thirty 30 seconds shows the readiness of the mosquitoes to bite before the test is commenced). The forearm was then treated with the lowest concentration of a sample and inserted into the insect cage for three minutes. The number of landings was taken and the procedure repeated for all the concentrations (25%, 50%, 75% and 100%) of a given sample by the same volunteer using 20 female mosquitoes once. That is, formulation A (1ml of C. *paradisi* oil + 0 ml of C. sinensis oil) would be composed into 25%, 50%, 75% and 100% concentration. Each concentration would be tested 3 times with 20 fresh mosquitoes in each case.

Estimation of complete protection time

Oil samples which yielded ≥ 60% percent repellency were used to establish the repellent duration of the oil extracts. The negative control test was done as described above to make certain mosquitoes' capacity to bite. Seventy (70) blood and sucrose starved female mosquitoes of ≤ 5 days old were placed in each of two identical (hand in cage) boxes for test and control respectively. Twelve percent (12%) DEET from Sigma-Aldrich was used for the positive control test. The sample and DEET was each evenly applied on the respective forearm and allowed to dry for three minutes before inserting them into the cages for three minutes. The hand insertion was repeated at 30 minutes interval until the first landing and probing of mosquito was observed. The test was replicated thrice.

Statistical analysis

Data obtained were first entered into Microsoft Excel Spreadsheet. They were later organized and analysed using the Statistical Packages for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, USA). The percentage repellence (R%) in 3 minutes of arm exposure was computed using the formula:

$$R\ (\%) = \frac{\text{Number of mosquito landing in control} - \text{Number of mosquito landing in text group}}{\text{Number of Mosquito landing in control}} \times 100$$

The mean number of mosquito landings and percentage repellence were compared between experimental groups using one-way analysis of variance (ANOVA) and Duncan *post hoc* test to assess multiple between-group means. Effective dose (ED) at 3 minutes exposure was estimated by probit analysis (Finney, 1952) method. The total number of mosquitoes/tests was 20, therefore a landing of 20 and above was taken to mean 20 maximum likely landings. Number that did not land was derived by subtracting number of landings from 20. ED was estimated based on the sample concentrations (100%, 75%, 50%, 25% and 0%), the number of mosquitoes introduced and the number of mosquitoes repelled. The complete protection time was estimated as the mean time interval taken for each treatment to wane off. These intervals were compared between groups using one-way ANOV*A.* The threshold for decision on significant difference was 95 % probability level, thus $p < 0.05$ was considered significant.

RESULTS

Physical properties and yield value of *Citrus sinensis* and *Citrus paradisi* oil extract

The essential oils of *Citrus sinensis* and *Citrus paradisi* had golden yellow colour and were quite transparent. They were viscous and have fairly pleasant and strong persistent odour. Weight for weight, the percentage oil yield in *Citrus sinensis* (3.98%) was ≥ to a third of that of *Citrus paradisi* (6.60%) as shown in Table 1.

Table 1: Percentage yield of *Citrus sinensis* and *Citrus paradisi* oil extract

Item	*Citrus sinensis*	*Citrus paradisi*
Weight of sample before extraction	500g	250g
Weight of oil extract (weight of sample container and oil minus weight of container)	25.51 - 5.91 = 19.68	22.40 - 5.91 = 16.49
Percentage Yield (weight of oil extract divided by weight of sample before extraction X 100/1)	19.89/500 X 100/1 = 3.98%	16.49/250 X 100/1 = 6.60%

Table 2: Mean number of *Aedes aegypti* mosquito landings in three minutes of arm exposure (range) to formulations of C. *paradisi* (Cp) and C. sinensis (Cs).

Formulations (volume in ml)	Concentrations				
	Control	100%	75%	50%	25%
A (Cp 1.0 + Cs 0.0)	47.00±6.25[a] (40 – 52)	2.33 ±2.31[c] (1 – 3)	6.00 ± 4.58[bc] (1 – 10)	18.67±11.93[b] (9 – 32)	35.67±2.31[a] (33 – 37)
B (Cp 0.5+ Cs 0.5)	37.67± 3.06[a] (35 – 41)	1.00± 1.73[c] (0 – 3)	1.33 ± 1.53[c] (0 – 3)	4.33 ± 2.08[bc] (2 – 6)	8.33 ± 2.08[b] (6 – 10)
C (Cp 0.33 + Cs 0.67)	43.67±23.50[a] (20 – 67)	1.67 ±1.53[b] (0 – 3)	4.67 ± 1.53[b] (3 – 6)	8.33 ± 8.08[b] (1 – 17)	14.33 ±4.93[b] (11 – 20)
D (Cp 0.25 + Cs 0.75)	36.67±2.52[a] (34 – 39)	1.67 ±2.08[d] (0 – 4)	5.00 ± 1.00[cd] (4 – 6)	8.33 ± 1.53[c] (7 – 10)	14.00 ±3.00[b] (11 – 17)
G (Cp 0.0 + Cs 1.0)	36.00±17.52[a] (19 – 54)	0.67 ±1.16[b] (0 – 2)	4.00 ± 3.61[b] (1 – 8)	6.33 ± 4.16[b] (3 – 11)	10.00 ±2.00[b] (8 – 12)
H (Cp 0.67 + Cs 0.33)	23.00 ± 5.57[a] (17 – 28)	0.67 ±1.16[c] (0 – 2)	1.33 ± 1.16[c] (0 – 2)	2.33 ± 1.53[c] (1 – 4)	8.67 ± 0.58[b] (8 – 12)
I (Cp 0.75 + Cs 0.25)	34.33±5.51[a] (29 – 40)	3.67 ±3.51[d] (0 – 7)	6.67 ± 4.51[cd] (2 – 11)	11.67 ± 3.51[c] (8 – 15)	19.67 ±2.89[b] (18 – 23)

Columns under concentration: values as mean; ± Standard deviation. Values with different alphabet superscripts across a row were significantly different ($p < 0.05$). Keys: the alphabet A to I stands for different formulations. Respective formulation was constituted according to volumes of oil in bracket.

Effective doses of *Citrus sinensis* and *Citrus paradisi* formulations against *Aedes aegypti*

Data on minimum, maximum and mean numbers of *Aedes aegypti* landings on exposed arms for the different formulations of *Citrus sinensis* and *Citrus paradisi* are summarized in Table 2. Expectedly, the number of mosquito landings was higher in the control. The extract had generally a dose dependent effect on landing rate as this increased as the concentration of the formulations decreased. But there was exception which as can be gleaned from the Table 3 (see below). In 100% of each extract, the least number of landings were noted, followed closely by 75 %, then by 50 % and 25 %. These observations reflected in the significance noted when dilutions of each formulation were compared between themselves and the control. The numbers of landings at 100 % formulation A were significantly different from the landings at 50 % and 25 % ($p < 0.05$). Thus, for most of the formulations, the magnitude of repulsion of *A. aegypti* was in the order of 100 % > 75 % > 50 % > 25 %. Very low landing rates were observed at 100 % and 75 % formulations B and H, where 0 – 2 and 0 – 3 landings occurred for the 3 minutes duration of exposure repeatedly. Also, 100 % formulations C, G, and D also had between 0 and 4 landings.

The estimated effective doses of the formulations at 50%, 95% and 99% repellent capacity for three minutes duration is presented in Table 4. Formulation H had the most impressive repellence activity, followed very closely by formulation B and G. Only formulations H and B had the capacity to cause 99% repellence for 3 minutes exposure duration. Approximately, 114% of formulation H (approximately 1.14 ml) would be required to achieve a similar effect within the same time interval. Formulation C and D also performed relatively well. The worst performing are formulations A and I.

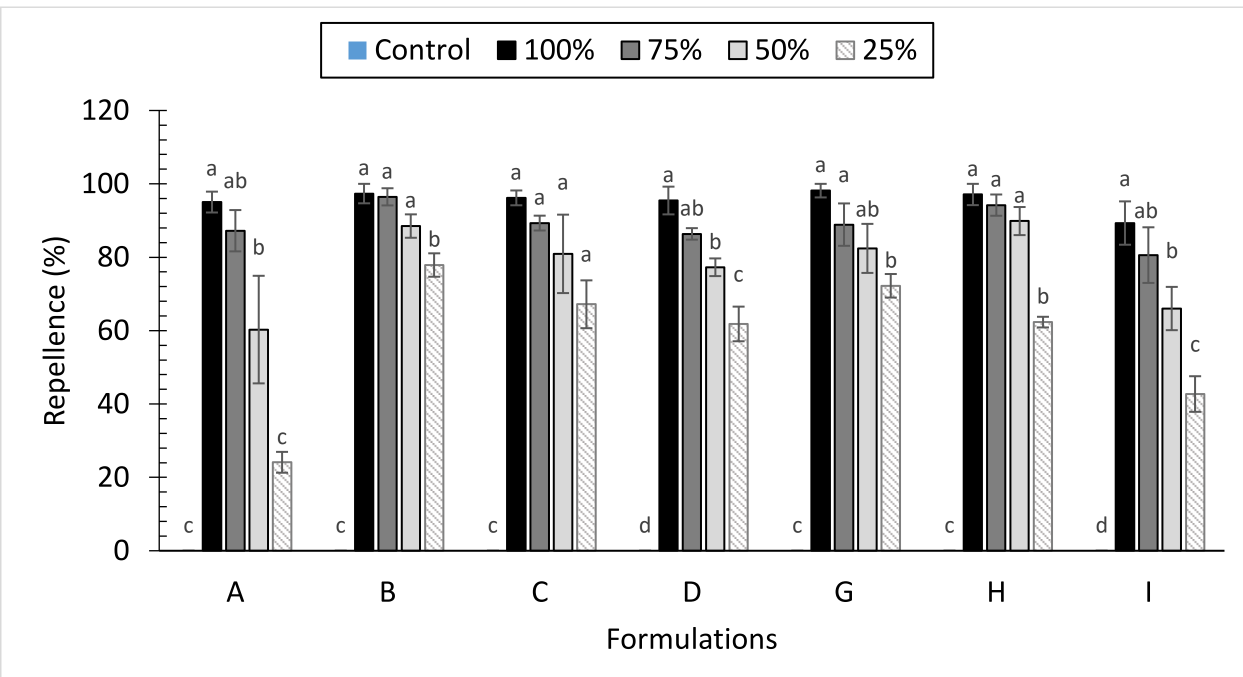


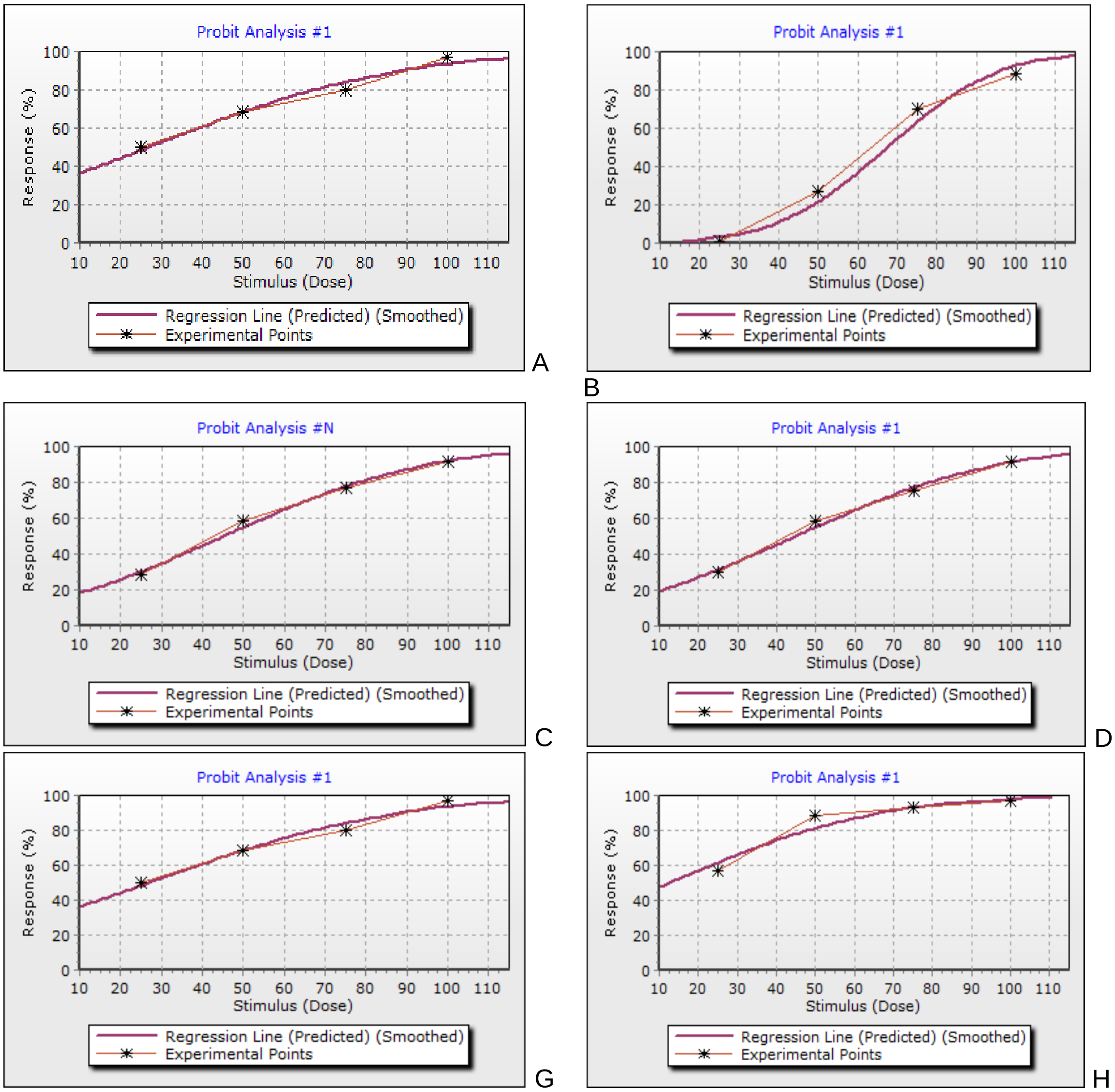
Figure 1: Repellence of *Citrus paradisi* and *Citrus* sinensis formulations against *Aedes aegypti* mosquitoes on three minutes exposure. Values as mean ± standard error of mean (SEM). Different alphabet labels indicate significant differences ($p < 0.05$). The repellence (%) of the control is zero; hence, it has no bar.

The probability (probit) response curve for estimations of the repellence effects of each of the formulations is provided as shown in Figure 2. Formulations A and I more obviously follow a sigmoid curve. From the curves, the repellence effect of formulations A and I approached zero more quickly than others as concentration of the formulation drops. Formulations B and H were more re-assuring *A. aegypti* repellents compared to the other formulations at similar low concentrations and both retained over 40% repellent effects even at concentration less than 10% of 1ml application over the skin for 3 min (Figure 2). No itching sensation was observed in the volunteers.

Table 3: Effective dose estimates for formulation of *Citrus* sinensis and *Citrus paradisi* against *Aedes aegypti* mosquitoes at three minutes exposure.

Formulations	Percentile	ED (95% Confidence limits)
A	50	66.994 (57.648 – 76.539)
	95	104.166 (91.295 – 129.767)
	99	119.567 (103.115 – 153.942)
B	50	31.765 (13.235 – 45.801)
	95	79.281 (61.612 – 123.047)
	99	98.968 (76.073 – 160.634)
C	50	49.365 (35.470 – 62.664)
	95	101.904 (83.427 – 142.486)
	99	123.672 (99.658 – 179.197)
D	50	49.334 (40.777 – 57.665)
	95	103 (89.730 – 125.290)
	99	125.514 (107.813 – 155.508)
G	50	38.369 (25.132 – 49.713)
	95	92.451 (75.887 – 125.756)
	99	114.858 (92.988 – 161.189)
H	50	27.732 (11.038 – 40.424)
	95	73.165 (57.031 – 110.466)
	99	91.988 (71.071 – 144.501)
I	50	64.607 (54.648 – 75.491)
	95	115.498 (99.171 – 146.591)
	99	136.582 (115.456 – 178.211)

ED = effective dose



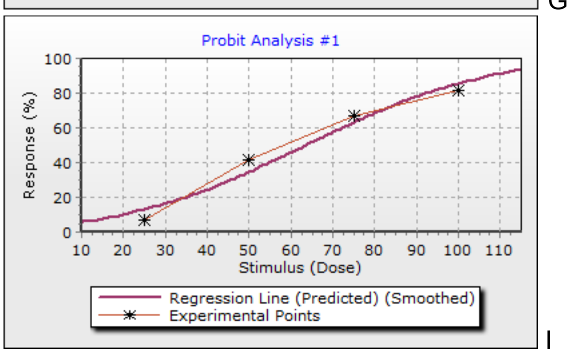


Figure 2: Dose response curve of *Aedes aegypti* repellence by formulations of *Citrus paradisi* and *Citrus sinensis* on three minutes exposure time. Key: the alphabets A-I stand for the formulations as shown in Table 2.

Complete protection time of *Citrus paradisi* and *Citrus sinensis* formulations against *Aedes aegypti*:

The mean complete protection time of the most effective doses of the formulations ranged from 5 to 150 min. (Table 5). Formulations B and G had the best protection time. Even though formulation H was effective as repellents, its mean protection time was less than 50 min. Though it could range from between 6 to 71 minutes depending on concentration used, but more likely it appeared to be below 50 min.

A graphical ordering of the complete protection time of the different effective doses of the formulations is presented in Figure 3. Formulation B75 (i.e. 75 % of formulation B) had the highest complete protection time of between 130 and 150 min. It was followed by formulation B100 and formulations G75, G50 and G100. Taken together, formulation B had the best complete protection time, and a comparatively similarly effective repellence dose as formulation H. Therefore, formulation B75 which had similar effective dose as B100 and a better complete protection time was the best. The DEET showed 100% repellence and CPT was 3 hours (mean). Furthermore, the topical application of the oil showed no adverse effect on the volunteers during and long after the tests.

Table 4: Protection time (min.) of formulations of *Citrus paradisi* and *Citrus sinensis* against *Aedes aegypti* mosquito

Formulations	Mean	S.D.	Minimum	Maximum
A100	58.67	17.90	38	69
A75	43.67	2.52	41	46
B100	104.33	31.01	73	135
B75	140.00	9.17	132	150
B50	6.00	1.00	5	7
C100	39.33	3.22	37	43
C75	46.67	14.22	37	63
D100	48.00	18.19	37	69
D75	72.00	33.51	39	106
G100	72.33	34.02	39	107
G75	89.00	32.05	70	126
G50	82.00	26.06	65	112
H100	37.67	1.528	36	39
H75	27.33	18.48	6	38
H50	48.67	19.40	36	71
I100	61.00	33.29	37	99
I75	57.33	36.09	36	99

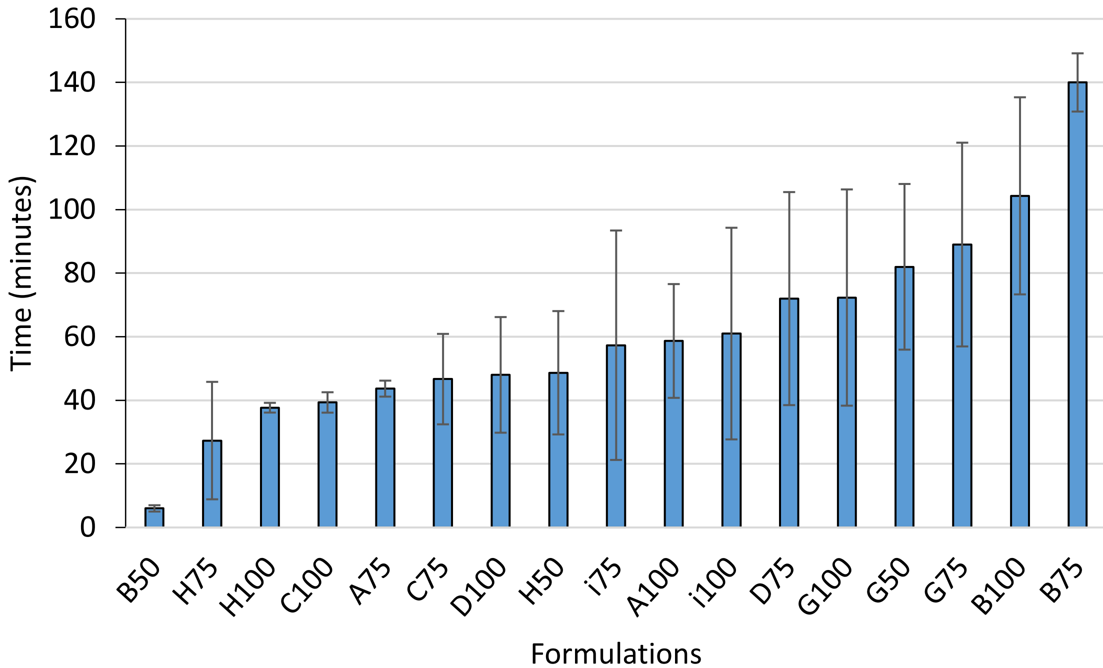


Figure 3: Complete protection time of formulation of *Citrus paradisi* and *Citrus sinensis* against *Aedes aegypti* ordered according to performance.

DISCUSSION

The threat from pathogenic insects and vector borne diseases is persisting especially from mosquitoes. Chemicals have been synthesized and applied to cope with them but their use is riddled with upbraiding. This arise following the biological endowments of the vectors themselves in their innate capacity to mount stiff resistance to insecticides and the latter constituting both ecological and public health risks. The scenario had compelled researchers to look up to plants phytochemicals to mitigate both vectors and associated diseases (Marques *et al*., 2022). This paper set out to confirm if *Citrus* plants around Nsukka could be employed to douse the threat from *Aedes* eagypti, a notorious vector of arboviruses. Topical applications of different formulations of essential oils extracted from *Citrus sinensis* and *Citrus paradisi* on bare forearms that were inserted into insect cages demonstrated repellence effects on the *vector.* The number of mosquito landings generally increased as the concentration of the different formulations decreased from 100 % to 25 %.

Of the two oils extracted via the maceration method from *Citrus* peels, *Citrus paradisi* oil obtained far exceeded those of *Citrus sinensis* per unit weight of respective peels (Table 1). This is consistent with the findings of Kim *et al.,* (2010) who had 4.17% for *Citrus paradisi* and 3.38% for *Citrus sinensis.* These results suggest that the source of *Citrus* and the method of oil extraction are comparatively satisfactory and could be relied upon for large scale production. Ibtehal and Sarah (2015) applied microwave-assisted steam distillation (MASD) and steam distillation technique to obtain essential oil from *C. sinensis* peels. Their percentage yields were 0.091% and 0.095% respectively. This implies that our extraction method is good enough and can be magnified for large scale extraction. The disparity in oil obtainable could arise because of the dissimilar extraction methods that researchers employ, the kind of *Citrus* employed (as observed in this study) and other prevailing conditions. Kamal *et al*., (2011) showed that *Citrus sinensis* had different values when processed fresh, oven-dried or air-dried. They indicated that oven-dried sample had the highest while fresh peels had the least yield.

The demonstration of the repellence of *A. aegypti* by application of *Citrus* oils with different formulations in this study had encouraging results (Tables 2 – 4; Figures 1 & 3) because it confirms that potent mosquito repellents can be sourced locally from common plant sources. Formulation H was effective as repellents, its mean CPT was less than 50 minutes. Formulation B (1:1 of *C. sinensis and C. paradisi*) had the highest protection time of mean 140 minutes when compared with the positive control, 12% DEET. Though this performance was far below that of the control, it could serve as a good starting point from where further improvement can be made. This raises hope that vector control can be accomplished by using locally sourced materials. Repellence to forestall biting will contribute to the multiple approaches contingent to halting the spread of *Aedes* spread diseases (Elsey *et al*., 2019). Investment in this area will further enable vulnerable peoples of Sub-Sahara Africa to regain confidence and capacity to solve our problems ourselves without going cap in hand to Europe and America soliciting for help. Utilizing the potentials inherent in *Citrus* will further mitigate embarrassment to the ecosystem as well as providing escape route to man from toxicity associated with chemical insecticides (Marques *et al*., 2022). The corollary is that locally sourced *Citrus* contain the equivalent contents that are responsible for the repellency as other *Citrus* sources in other climes. It has been confirmed that some constituents of *Citrus* species such as limonene is the basis for insecticide repellent qualities (Simon-Oke and Akeju, 2019; Khanikor *et al*., 2021). The complete protection time of the essential oil also improved with increase in concentration but differed in the two species of the *Citrus* extract. This confirms that *Citrus* have the capacity to be toxic to larvae, pupa and adults of different species of mosquitoes (Ivoke *et al.,* 2013; Favela-Hernández *et al*., 2016). *Citrus* fruits are cheap and readily available in our locality that so much of them are disposed as refuse. When the peels are turned to valuable products, they would contribute to safer environment that minimise vector breeding and development of species that are resistant to synthetic insecticides (Karunamoorthi and Hailu, 2014; Srivastava and Kachhwaha, 2019 Marques *et al*., 2022). Immunocompromised persons, pregnant women and children who are vulnerable to infections vectored by *Aedes eagypti* (Srivastava and Kachhwaha, 2019) could benefit from reduced incidences by applying repellents (Paixão *et al*., 2022). Moreover, besides affording natives accessible means of curbing nuisance from mosquitoes, the oil from the *Citrus* fruits could add to the list of oils used routinely for skin care. It could also source oil for other purposes. This dimension of course will increase the economic fortunes people from the area because oranges would cease to be wasted.

The foregoing glowing commentaries notwithstanding, there are obvious faux pas that must not be glossed over. Our results seriously contend with our earlier general observation that the data support “dose dependent” activity of *Citrus sinensis* and *Citrus paradisi*. Tables 2 – 4 and Figures 1 and 3 clearly show this disavowal. It is difficult to accept that a 75% concentration (Formulation B) will out-perform that of 100% and still hold on to the aphorism of “dose dependent” activity. Only further testing could unveil this conundrum. Meanwhile, formulation B eminently demonstrates synergism that most likely was the driver of the anti-mosquito activities demonstrated. Essential oil combinations had been reported to improve insecticidal activities (Marques *et al*., 2021); Gomes et al. (2020) indicate that this property may be due to other components of essential oil other than the oxygenated monoterpenes. Therefore, this study suggests that synergism between *C. sinensis* and *C. paradisi* could contribute to *A. aegypti* repellence. If the role of synergism is accepted as being responsible in Formulation B at 75% concentration, then the component oils must follow the trend in optimum curve where below or above the critical combination proportion, there will be significant reduction of anti-mosquito activity. Again, this is difficult to accept when you consider the rest of the data. On the whole, when the oil extracts were used singly (formation A and G), formulation G (*Citrus sinensis*) showed higher repellent effect and complete protection time than formulation A (*Citrus paradisi*) at various concentration as shown in Tables and Figures, particularly in Figure 3. The variability in the complete protection time may also suggest that the repellent effect is affected by the species and geographic origin of the plant used (Gillij *et al.*, 2008). Repellence observed in our study cannot be compared with effects observed in Meisyara *et al*. (2021) who obtained 100% repellence index for up to 7 hours with commercial oils. This might suggest that those manufacturers may have used undisclosed methods to concentrate inhibitory substances like limonene many times over or might have included components that inhibit evaporation of the oils from skins.

CONCLUSION AND RECOMMENDATIONS

This work confirms that the peels of *Citrus sinensis and Citrus paradisi* are no wastes because their essential oils could be profitably extracted for sundry purposes and as mosquito repellents to control *Aedes aegypti. Citrus paradisi* had more capacity to produce oil than *Citrus sinensis.* Their respective oil is active against arboviruses vector; however, *Citrus sinensis* oil out-performed *Citrus paradisi* in mosquito repellence. Their oils showed synergism when mixed in equal ratio and used at 75% concentration to sustain repellence time up to a mean of 140 minutes.

This research work showed that oil extracts from *C. sinensis* and *C. paradisi* provide the solution to deal with *Aedes* mosquitoes nuisance and as vector of life-threatening diseases among other uses. There is therefore the cardinal need to bring these findings to the end users. The way to go is that public health regulators, governments and donor agents could drive the access to information about these oils by advertising, deploying social networks tools online to present the oils of these fruit peels as routine tool that ordinary folks could use to protect themselves. Further studies are imperative to achieve the following: test the observation of Kamal (2011) that oven dried peels would yield more oils with local sourced *Citrus* fruits, identify additives which could futher increase their activities to improve the protective time to overtake those of DEET, induce pleasant fragrance and still not affect their activity when used as repellent cream.

Acknowledgements

We are grateful to Dr. Okolie, N. A. who assisted us with the extraction of oils from the *Citrus* peels. We thank Mr. Aguzie, I. O. N. for assisting us with the statistical analysis of data. We cannot thank enough many staff of the Department of Zoology and Environmental Biology and South East Zonal Biotechnology Centre, University of Nigeria Nsukka for their co-operation, advice and encouragements that made this work possible. We are also grateful to the University administration for availing us facilities and access to the internet.

Author's contributions

UFSO designed the study and CCD did the preliminary literature reviews. Both authors did the laboratory work and participated in writing the report, analysis and discussion. All authors approved the final draft of the manuscript.

Conflict of interest

Authors have no conflict of interest to declare.

REFERENCES

Akarca, G., and Sevik, R. (2021). Biological Activities of Citrus limon L. and Citrus sinensis L. Peel essential oils. *Journal of Essential Oil Bearing Plants*, **24**(6): 1415-1427.

Akhtar, N., Idrees, M., ur Rehman, F., Ilyas, M., Abbas, Q., and Luqman, M. (2021). Shape and texture-based classification of *Citrus* using principal component analysis. *International Journal of Agricultural Extension,* **9**(2): 229-238.

Belenje, A., Bose, R., and Jalali, S. (2021). Rare case of Terson's syndrome and viral retinitis due to dengue haemorrhagic fever in an infant. *British Medical Journal Case Reports*, **14**(5): e242274.

Bessimbaye, N., Mbanga, D., Moussa, A. M., Ferdinand, D. Y., Maxime, N. E. D., Barro, N., Tidjani, A and Ouchemi, C. (2021). Evaluation of yellow fever surveillance in Chad, 2015-2020. *African Journal of Microbiology Research*, **15**(3): 152-160.

Canali, M., Rivas-Morales, S., Beutels, P., and Venturelli, C. (2017). The cost of Arbovirus disease prevention in Europe: area-wide integrated control of tiger mosquito, *Aedes* albopictus, in Emilia-Romagna, Northern Italy. *International Journal of Environmental Research and Public Health*, **14**(4): 444.

Chen, G., Zhang, B., and Zhao, J. (2015). Dispersion process and effect of oleic acid on properties of cellulose sulfate-oleic acid composite film. *Materials*, **8**(5): 2346-2360.

Dickens, B. L., Sun, H., Jit, M., Cook, A. R., and Carrasco, L. R. (2018). Determining environmental and anthropogenic factors which explain the global distribution of Aedes aegypti and Ae. albopictus. *British Medical Journal*

Global Health, **3**(4): e000801.

Elsey, H., Agyepong, I., Huque, R., Quayyem, Z., Baral, S., Ebenso, B., Kharel, C.,Shawon,R.A., Onwujekwe, O.,Uzochukwu,B., Nonvignon, J.,Aryeteey, J.C., Kane,S.,Ensor,T and Mirzoev, T. (2019). Rethinking health systems in the context of urbanisation: challenges from four rapidly urbanising low-income and middle-income countries. *British Medical Journal Global Health*, **4**(3): e001501.

Essombe Malolo, F. A., Kouam, A. D. K., Nyobe, J. C. N., Ngah, L., Frese, M., Ndom, J. C., Langat., M.K., Lenta, B.N.,Mulholland,D.A.,Sewald,N. and Wansi, J. D. (2023). Coumarinolignoid and indole alkaloids from the roots of the hybrid plant *Citrus paradisi* Macfad (Rutaceae). *Molecules*, **28**(3): 1078.

Heidari, F., Sarailoo, M., Ghasemi, V., and Nadimi, A. (2017). Toxic and oviposition deterrence activities of essential oils from Citrus sinensis (L.) Osbeck and Citrus paradisi (Macfarlane) fruit peel against adults of Tribolium castaneum (Herbst). *Journal of Crop Protection*, **6**(1): 79-88.

Favela-Hernández, J. M. J., González-Santiago, O., Ramírez-Cabrera, M. A., Esquivel-Ferriño, P. C., and Camacho-Corona, M. D. R. (2016). Chemistry and Pharmacology of Citrus sinensis. *Molecules*, **21**(2): 247.

Finney, D. J. (1952). *Probit analysis: a statistical treatment of the sigmoid response curve*. Cambridge University Press, Cambridge.

Gillij, Y. G., Gleiser, R. M. and Zygadlo, J. A. (2008). Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresource Technology*, 99(7): 2507 – 2515.

Gomes, P. R. B., de Sousa, D. A., Everton, G. O., Fontenele, M. A., e Marques, J. N. L., de Freitas, A. C., ... and Mouchrek Filho, V. E. (2020). Chemical composition and larvicidal activity of Citrus limonia Osbeck bark essential oil. *Journal of Medicinal Plants Research*, **14**(7): 354-358.

Hussain, T., Rashid, Z., Kumar, J., and Kumar, D. (2022). Rare case of dengue encephalitis with extensive brain lesions from Pakistan. *BMJ Case Reports CP*, **15**(11): e250271.

Ibtehal, K. S. and Sarah, J. S. (2015). Extraction of essential oil from citrus by-products using microwave steam distillation. *Iragi Journal of Chemical and Petroleum Engineering*, 16(3): 11 – 22.

Ivoke, N., Ogbonna, P. C., Ekeh, F. N., Ezenwaji, N. E., Atama, C. I., Ejere, V. C., Onoja, S. U. and Eyo, J. E. (2013). Effects of grapefruit (*Citrus paradisi* MACF) (Rutaceae) peel oil against developmental stages of *Aedes aegypti* (Diptera: Culicidae). *Southeast Asian Journal of Tropical Medicine and Public Health*, 44(6): 970 – 978.

Kamal, G. M., Anwar, F., Hussain, A. I., Sarri, N. and Ashraf, M. Y. (2011). Yield and chemical composition of citrus essential oil as affected by drying pre-treatment of peels. *International Food Research Journal,* **18**(4): 1275 – 1282.

Karunamoorthi, K., and Hailu, T. (2014). Insect repellent plants traditional usage practices in the Ethiopian malaria epidemic-prone setting: an ethnobotanical survey. *Journal of Ethnobiology and Ethnomedicine*, **10**: 1-12.

Khanikor, B., Adhikari, K., and Rabha, B. (2021). Citrus essential oils: a suite of insecticidal compounds. Eds: Muhammad Sarwar Khan and Iqrar Ahmad Khan. *Citrus– Research, Development and Biotechnology,* IntechOpen: DOI: 10.5772/intechopen.95887

Kim, S. I., Yoon, J. S., Jung, J.W., Hong, K. B., Ahn, Y. J., and Kwon, H.W. (2010). Toxicity and repellency of *origanum* essential oil and its components against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults. *Journal of Asia-Pacific Entomology,* **13**: 369 – 373.

Marques, D. M., de Freitas Rocha, J., de Almeida, T. S., and Mota, E. F. (2021). Essential oils of caatinga plants with deletary action for Aedes aegypti: A Review. *South African Journal of Botany*, **143**: 69-78.

Odo, G. E., Agwu, E. J., and Haruna, A. S. (2015). Culicid forms distribution and breeding sites in Nsukka ecological zone of South Eastern Nigeria. *Journal of Parasitology and Vector Biology*, **7**(5): 94-100.

Othman, S. *A.*, and Fadzil, N. F. (2021). Utilization and valorization of *Citrus* Fruit by-products: A Review. *Enhanced*

Knowledge in Sciences and Technology, **1**(2): 170-176.

Paixão, E. S., Teixeira, M. G., and Rodrigues, L. C. (2018). Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. *British Medical Journal Global Health*, **3**(1): e000530.

Paixão, E. S., Fernandes, Q. H. R. F., Cardim, L. L., Pescarini, J. M., Costa, M. C. N., Falcão, I. R.,Brickley,E.B.,Santos, A.C., Souza, A.P., Carvalho-Sauer,R.C.O.,Smeeth,L.,Rodrigues, L.C.,Barreto, M.L and Teixeira, M. G. (2022). Socioeconomic risk markers of congenital Zika syndrome: a nationwide, registry-based study in Brazil. *British Medical Journal Global Health*, **7**(9): e009600.

Paoli, M., de Rocca Serra, D., Tomi, F., Luro, F., and Bighelli, *A.* (2016). Chemical composition of the leaf essential oil of grapefruits (*Citrus paradisi* Macf.) in relation with the genetic origin. *Journal of Essential oil Reearch*, **28**(4): 265-271.

Prabasari, I. (2014). Peel Ultrastructure During Developmental Stages of *Citrus Sinensis* (L.) Osbeck. *Planta Tropika: Jurnal Agrosains (Journal of Agro Science)*, **2**(1): 1-11.

Rajani, M., Shrivastava, N., and Ravishankara, M. N. (2000). A rapid method for isolation of andrographolide from Andrographis paniculata Nees (Kalmegh). *Pharmaceutical Biology*, **38**(3): 204 – 209.

Soares, I. M. N., Polonio, J. C., Zequi, J. A. C., and Golias, H. C. (2022). Molecular techniques for the taxonomy of *Aedes* Meigen, 1818 (Culicidae: Aedini): a review of studies from 2010 to 2021. *Acta Tropica*: 106694.

Simon-Oke, I. A. and Akeju, A. V. (2019). Laboratory evaluation of extract from peels and seeds of some Citrus species against Anopheles mosquitoes (Diptera: Culicidae). *Journal of Mosquito Research*, **9**(1): 1 – 8.

Singh, J., Sharma, V., Pandey, K., Ahmed, S., Kaur, M. and Sidhu, G. S. (2021). Horticultural classification of *Citrus* cultivars. *Citrus Research, Development and Biotechnology*: 1-24.

Soni, M., Bhattacharjee, C. K., Khan, S. A., and Dutta, P. (2018). DNA barcoding as a complementary approach for species identification from dengue endemic regions of North East India. *International Journal of Mosquito Research*, **5**: 46-52.

Srivastava, A., and Kachhwaha, N. (2019). A review: Recent outbreak of Zika viruses in India, its vectorology and virology. *Research & Reviews: A Journal of Microbiology and Virology*, **9**(2): 25-33.

Thompson, R., Martin Del Campo, J., and Constenla, D. (2020). A review of the economic evidence of *Aedes*-borne arboviruses and *Aedes*-borne arboviral disease prevention and control strategies. *Expert review of vaccines*, **19**(2): 143-162.

Ugwu, F. S. O. and Onwuzurike, J. C. (2018). Palm wines as potent attractant to mosquitoes. *Animal Research International*, **15**(3): 3055 – 3064.

Ugwu, F. S. O. and Oyeagu, U. (2023). Mediators of arrested development as attractive malaria vector control tools: The Azadirachta indica and azadirachtin routes. *Journal of Biological Research & Biotechnology*, **21**(1): 1805-1817.

World Health Organisation (2009). Guidelines for efficacy testing of mosquito repellents for human skins. WHO, Geneva, WHO/HTM/NTD/WHOPES/2009.4.

Wu, G. *A.*, Terol, J., Ibanez, V., López-García, *A.*, Pérez-Román, E., Borredá, C. and Curk, F. (2018). Genomics of the origin and evolution of *Citrus*. *Nature*, **554** (7692): 311-316.