Parasite Epidemiology and Control 18 (2022) e00259

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

# Insecticidal effectiveness of naphthalene and its combination with kerosene against the emergence of *Aedes aegypti* in Ika North East, LGA, Delta State, Nigeria

Chioma C. Ojianwuna, PhD, Victor N. Enwemiwe *

*Department of Animal and Environmental Biology, Faculty of Science, Delta State University, Abraka, Nigeria*

ARTICLE INFO

*Keywords:*
*Aedes aegypti*
Delta state
Insecticidal effectiveness
Kerosene
Larvicides
Naphthalene

ABSTRACT

Despite the substantial progress achieved in the search of nonchemical alternatives to insecticidal larviciding on mosquitoes, more work is still required to unravel the potency of viable substances in order to attend to several pest and disease problems. Insecticidal effectiveness of naphthalene and its combination with kerosene against the emergence of *Ae. aegypti* in Ika North East, LGA, Delta State, Nigeria was assessed. Immature stages of *Ae. aegypti* were collected and left to acclimatize for 6 h in standard laboratory conditions. Naphthalene measured in 2 g and its combinations with kerosene in 50:50 were emptied in 400 ml, 200 ml and 100 ml of water which resulted in 0.005%, 0.01% and 0.02% concentrations respectively. Water alone served as control for the experiment. Twenty third instar larvae and pupae were sorted into containers before exposure to treatments. Experiment was done in triplicates and observed for 10, 15, 20, 30, 40, 50, 60, and 80 min coinciding with WHO protocol for *Aedes* exposure. Mortality was highest in larvae exposed to 0.02% kerosene and naphthalene, and was also high in 0.02% naphthalene. Lowest mortality was recorded in pupae exposed to 0.005% of naphthalene. Significant differences in toxicity was recorded ($p < 0.05$). Mortality increased with time in larvae and pupae. Highest mortality in pupae and larvae was recorded in 0.02% kerosene and naphthalene mixture at 80 min post exposure time respectively. $LC_{50}$ and $LC_{95}$ of naphthalene exposed to *Aedes* larvae and pupae was between 0.002 and 0.018% and 0.021–0.051% respectively. Similarly, for naphthalene with kerosene was between 0.002 and 0.007%, and 0.015–0.035%. Pupae exposed to 0.005% naphthalene had more adult emergence than in others and the differences were significant ($p < 0.05$). Field trial is required with optimum concentrations.

## 1. Introduction

Interventions targeting mosquitoes and diseases they cause have been on the rise lately with the principal aim of reducing transmission burdens, particularly in places where vector resistance is on the increase. Even with these, resistance to interventions either in the form of vector or drug resistance threaten the successes achieved for mosquito-caused diseases in many areas (WHO, 2015). For instance, Mali and Uganda reported mosquito resurgence for the first time after many years of low abundance and distribution (Jagannathan et al., 2012; Coulibaly et al., 2014). Mosquito resurgence is a serious implication to wide range intervention

* Corresponding author.
*E-mail addresses:* ccojianwuna@gmail.com (C.C. Ojianwuna), enwemiwevictor@gmail.com (V.N. Enwemiwe).

https://doi.org/10.1016/j.parepi.2022.e00259
Received 3 January 2022; Received in revised form 1 May 2022; Accepted 21 June 2022
Available online 25 June 2022

since they now become ineffective due to vector changes caused by behavioral responses. Mosquito intervention involved using insecticides recommended and impregnated into bed nets and sprayed inside houses, even other forms of insecticidal impregnations such as insecticide-treated clothing, repellents, treated window screens and other insecticide treatable materials (Ekerette and Ebere, 2018).

The World Health Organization (WHO) recommended for use insecticides in different forms, after over 50 years of relying on dichlorodiphenyltrichloroethane (DDT) which is in the form of wettable powder. But with so many issues that arose from their use, many insecticides such as wettable powders, suspension concentrate, capsulate suspension and other forms of recommended insecticides within four classes of chemical insecticides were discovered, and recommended for trials in residual spraying of houses (WHO Pesticides Evaluation Scheme (WHOPES), 2007). Adult mosquito resistance to one or more of these recommended insecticides has been reported in WHO endemic regions. It is in considering this resistance that vector control intervention now clamours for not only newer technologies targeting mosquitoes but also the combination of recommended insecticides with other insecticide classes to boost the effectiveness and even the use of synergists (piperonyl butoxide) and integrated approaches (Ononamadu et al., 2020; Chukwuekezie et al., 2020; Ojianwuna et al., 2021a, 2021b). Other new control technologies include: the use of the transfer of genetic elements (Bourtzis et al., 2016), exploring the potentials of the bacterium *Wolbachia* through testing the compatibility of the mosquito cytoplasm especially *Aedes* (Lees et al., 2015; Yakob and Walker, 2016), eco-friendly sterile techniques (Lees et al., 2014) and their breeding sites through the introduction of mosquito-eating fishes; *Gambusia* sp. (Mischke et al., 2016), Pseudomugilid: *Pseudomugil signifer* and Perciformes: *Hypseleotris galii* and *Pseudogobius* sp., and the exotic *G. holbrooki* (Griffin, 2014).

It is quite unfortunate that some observations have been made on the ineffectiveness of synergist in some parts of Africa, probably due to many factors related to harsh environmental conditions that averted the possibility of vector susceptibility in these areas. These harsh environmental conditions due to oil spillage in breeding sites, agricultural pesticide run off, spraying insecticides indoors with windows opened leading to escape of mosquitoes intended to be killed and irregular use of recommended control options. *Anopheles* complexes, *Culex* complexes and *Aedes* complexes are the major genera reported with insecticide resistance in several studies (Ononamadu et al., 2020; Chukwuekezie et al., 2020; Ojianwuna et al., 2021a, 2021b). Majority of which are domicile in WHO African regions. Sibling species of these three mosquito genera with resistance and causing persistent diseases have been reported in most of *Anopheles gambiae* complex (Yakub, 2011). Mosquitoes could be urban or rural dwellers or rural species (Tikar et al., 2011). In total, up to 4000 of mosquitoes are known worldwide, over 500 are *Anopheles*, *Aedes* and *Culex*. In 2020, yellow fever outbreak was reported in Ika North East LGA, Delta State (WHO, 2017). This virus was reported in several communities in the Local Government Area. Yellow fever virus has a long history in Africa and their prevalence dates as far back the late 90's (Garske et al., 2014). Africa suffers over 400,000 cases with over 100,000 deaths and this extends to Nigeria where the virus occurs in every states in country's region (WHO, 2017). Case management and diagnosis of yellow fever is pricy. Interventions directed towards the vectors in their immature stages using locally available substances would be profitable.

Not much resistance has been reported in the culicine mosquitoes (Nelms et al., 2013). A few *Aedes* species include *Aedes aegypti, Ae. barbirostris, Ae. albopictus* amongst other subspecies. This mandated the search for bio-active alternatives that can be used to control the immature stages in their natural breeding sites before they emerge into adults. Different substances have been applied to control the larvae and pupae of mosquitoes in endemic areas. These including petroleum products on the growth and development of *Aedes* and *Anopheles* mosquitoes (Ekedo et al., 2019; Ojianwuna et al., 2021a), and many other methods involving the manipulation of


**Fig. 1.** Map of Ika North East (Google Earth Map).

physicochemical parameters of breeding sites as key in controlling adult abundance. Physical control through removal of breeding sites close to human dwelling has also been recommended (WHO, 2013). Some studies have outlined the effectiveness of insect growth regulators and bacteria agents (Msangi et al., 2011; Belinato et al., 2013; Mulamuli et al., 2016; Lawler, 2017). However, these control agents are pricy in terms of acquisition and are not locally available to local residents in endemic communities. One of the main reasons why mosquitoes may keep constituting problems may be that successful laboratories based control interventions are not translated for industrial application and use. This probably because, mass production may be very expensive and materials may be unavailable for purchase. Naphthalene and kerosene are two locally available substance globally and are quite affordable substances. They have been tried on other insects with great successes (Enwemiwe et al., 2020; Ojianwuna and Enwemiwe, 2021), hence their use for this study. Naphthalene is also a naturally occurring organic substance and kerosene is one vital mineral oil in Nigeria. These substances were chosen due to their availability, affordability and that they have been proven to cause toxicity in some insect species (Ojianwuna et al., 2021a; Ojianwuna et al., 2021b). Naphthalene acts by the activation of cytochrome P450 enzyme in neuronal channel which triggers acute toxicity of neural cells (Li et al., 2011). The mechanism of action of kerosene differs from naphthalene in that, they induce inflammation, suppress the insect immune system, reduced oxygen supply to tissues, imbalances in hormones and enzymes etc. (Maiyoh et al., 2015). Hence, this study reported the insecticidal effectiveness of using naphthalene and its combination with kerosene against the emergence of *Ae. aegypti* in Ika North East, LGA, Delta State, Nigeria.

## 2. Materials and methods

### 2.1. Study area

This study was carried out in the Insectary/Entomology unit, Department of Animal and Environmental Biology, Delta State University, Abraka. Wild mosquito larvae and pupae were obtained from four communities in Ika North-East Local Government Area; Owerre-Olubor (Lat. 6.287218$^0$N and Long. 6.340842°E), Umunede (Lat. 6.270005$^0$N and Long. 6.302945°E), Ute-Okpu (Lat. 6.094311$^o$N and Long. 6.182455°E) and Owa-Ofie (Lat. 6.208433$^0$N and Long. 6.177915°E) (Fig. 1). Mosquitoes were left in the laboratory to acclimatize at temperature of 28 ± 3 °C and relative humidity of 78 ± 5%.

### 2.2. Mosquito collection method

Immature stages of *Aedes* mosquitoes were collected from potential breeding sites including plastic containers, cellophane with ability to hold water, and abandoned tyres close to human habitation. WHO method of larval collection was applied using 350 ml deep ladles, scooping spoons, pipette, and buckets. Ladles and scooping spoons were used for immature stages collection, pipette was used for sorting larvae from pupae in the field and laboratory, as collections were kept in the transparent bucket to be transported to the laboratory. The *Aedes* larvae were cultivated in the laboratory under standard laboratory conditions to acclimatize and attain the third instar larval stage in larval holding trays which was properly netted. Mosquitoes in the larval tray was fed with low fat biscuit and yeast prepared in a ratio of 1 biscuit to 10 yeast tablets before exposure.

### 2.3. Toxicity assay

Naphthalene and its mixture with kerosene were used for the bioassay. Naphthalene was measured in 2 g (g) while its combinations was done in 50:50 (1 ml of kerosene in 1 g of naphthalene). The measured substances in single and mixtures were emptied in 400 ml, 200 ml and 100 ml of water which resulted in 0.005%, 0.01% and 0.02% of the test concentrations respectively. The effectiveness of these concentration in single and mixed forms were tried by introducing *Aedes* mosquitoes (third instar larva and pupae) into the concentrations. Mosquitoes exposed to water alone served as control for the experiment. Twenty third instar larvae and twenty pupae were sorted accordingly in separate plastic containers before exposure to treatments. The experimental set up was observed for 10, 15, 20, 30, 40, 50, 60, and 80 min respectively. The exposure time was adopted from the recommended WHO protocol for mosquito larviciding (WHO, 2013). The experimental set up was done using plastic containers in triplicates and were fed with biscuit and yeast as mentioned earlier before exposure.

### 2.4. Statistical analysis

Mortality and emergence inhibition data of *Aedes* mosquitoes were entered into MS Excel, 2013 and checked for possible errors. Total or mean emergence inhibition were calculated on the basis of the number of third instar larvae exposed. The overall emergence of adult mosquitoes were calculated using the formula by World Health Organization (2005):

$$\text{IE}\ (\%) = 100 - \left(\frac{Tx100}{C}\right)$$

Where T = percentage survival or emergence in treated batches and
C = percentage survival or emergence in the control.
Where adult emergence in the control was <80%, the test was discarded and repeated. Whereas, where the percentage was between 80% and 95%, the data was corrected using the Abbott's formula

$$\text{Mortality } (\%) = \frac{X - Y}{X} - 100$$

Where X = percentage survival in the untreated control and Y = percentage survival in the treated sample. ANOVA test was used to compare mortality, adult emergence and time mortality. Results were presented in mean ± standard error and in tabular forms. Significance was set at $\alpha = 0.05$. Tukey's test was used to separate means while 50% and 95% of lethal concentration and emergence inhibition was computed using Probit analysis. Mean and standard error, lower and upper bound confidence interval (CI), emergence inhibition and lethal concentrations were computed using XL Stat version 2020.

## 3. Results

### 3.1. Mortality records of Ae. aegypti

The acute toxicity of *Ae. aegypti* larvae and pupae exposure to naphthalene and its combination with kerosene at various concentrations is presented in Table 1. Mortality was highest in larvae of *Aedes* exposed to 0.02% kerosene and naphthalene, and this was closely followed by 0.02% naphthalene. The lowest mortality was recorded in pupae of *Aedes* exposed to 0.005% of naphthalene (Table 1). Significant differences in toxicity was recorded in the various concentrations of treatments exposed to pupa and larva of *Ae aegypti* ($p < 0.05$).

### 3.2. Mortality time record

The mean mortality time records of *Ae. aegypti* pupae and larvae exposed to naphthalene, and naphthalene with kerosene at different concentrations is presented in Tables 2 and 3. Generally mortality increased as time of exposure of larvae and pupae increased. In the pupae exposure group, the highest mortality time was recorded in 0.02% kerosene and naphthalene combination at 80 min post exposure time. Remarkably, mortality recorded in 0.02% naphthalene at 60 and 80 min equates 0.01% kerosene and naphthalene at 50, 60 and 80 min respectively. Within naphthalene exposure, no mortality was recorded in all concentration from 10 to 20 min. Furthermore, no mortality was recorded in *Aedes* pupae exposed to 0.005% of kerosene and naphthalene at 10 min. Within naphthalene and kerosene exposure, mortality observed in *Aedes* mosquitoes exposed to 0.005% was equal to the mortality recorded at 0.01% in 15 min, mortality recorded at 0.005% in 30 min was equal to the mortality recorded at 0.02% in 15 min while the mortality recorded at 0.01% was equal to the mortality recorded at 0.005% in 50, 60 and 80 min of post exposure period. The differences between the mortality time was significant ($p < 0.05$) (Table 2)

*Aedes* mosquito larvae exposed to 0.02% of kerosene and naphthalene recorded the highest mortality in 80 min and this was closely followed by the mortality recorded at 0.02% of naphthalene in 80 min exposure period. Across the treatments, mortality recorded at 0.02% of naphthalene in 20 min equaled mortality recorded in 0.01% kerosene and naphthalene at 40 min. Mortality recorded at 0.005% of naphthalene and kerosene in 50 min was equivalent to mortality recorded at 0.02% naphthalene in 15 min. Mortality recorded at 0.005% of naphthalene and kerosene in 60 min was equivalent to mortality at 0.005% of naphthalene in 50 min. The mortality recorded at 0.01% of naphthalene and kerosene in 80 min was equivalent to mortality at 0.02% of naphthalene in 40 min. Mortality recorded at 0.005% of naphthalene in 40 min was equivalent to mortality at 0.02% of naphthalene and kerosene in 40 min of exposure. No mortality was recorded in 10 min at all concentrations of naphthalene and 0.005% of naphthalene and kerosene in 10 min. Similarly, no mortality was recorded at 0.005% and 0.01% of naphthalene in 15 min and 0.005% of naphthalene and kerosene in 15 min. The differences between mortality were significant ($p < 0.05$) (Table 3).

**Table 1**
Acute toxicity of *Ae. aegypti* larvae and pupae exposure to naphthalene and its combination with kerosene at various concentrations.

| Treatment | Conc. (%) | Log dose | Mean ± SE | Lower bound 95% CI | Upper bound 95% CI |
|---|---|---|---|---|---|
| Larvae | | | | | |
| Water | 0.00 | 0.00 | 0.00 ± 0.0a | −1.87 | 1.87 |
| Naphthalene | 0.005 | −2.301 | 12.50 ± 0.88cde | 10.59 | 14.41 |
| | 0.01 | −2.000 | 14.50 ± 0.88def | 12.59 | 16.41 |
| s | 0.02 | −1.699 | 19.00 ± 0.88 fg | 17.09 | 20.91 |
| Kerosene and Naphthalene | 0.005 | −2.301 | 13.50 ± 0.88cde | 11.59 | 15.41 |
| | 0.01 | −2.000 | 16.00 ± 0.88efg | 14.09 | 17.91 |
| | 0.02 | −1.699 | 20.00 ± 0.88 g | 18.09 | 21.91 |
| Pupae | | | | | |
| Water | 0.00 | 0.00 | 0.00 ± 0.0a | −1.87 | 1.87 |
| Naphthalene | 0.005 | −2.301 | 5.50 ± 0.88a | 3.59 | 7.41 |
| | 0.01 | −2.000 | 7.00 ± 0.88ab | 5.09 | 8.91 |
| | 0.02 | −1.699 | 11.00 ± 0.88bcd | 9.09 | 12.91 |
| Kerosene and Naphthalene | 0.005 | −2.301 | 9.00 ± 0.88abc | 7.09 | 10.91 |
| | 0.01 | −2.000 | 11.00 ± 0.88bcd | 9.09 | 12.91 |
| | 0.02 | −1.699 | 15.50 ± 0.88defg | 13.59 | 17.41 |

Note: CI means confidence interval. Means of the same superscript letter do not differ significantly between treatments ($p < 0.05$) using Tukey's test.

**Table 2**
Mean mortality time records of *Ae. aegypti* pupae exposed to naphthalene, and naphthalene with kerosene at different concentrations.

| Treatment | Conc. (%) | Time mortality (minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 80 |
| Pupae | | | | | | | | | |
| Water | 0.00 | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a |
| Naphthalene | 0.005 | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 2.50 ± 0.88abcd | 3.50 ± 0.88abcde | 5.00 ± 0.88abcdefgh | 5.50 ± 0.88bcdefghi | 5.50 ± 0.88bcdefghi |
| | 0.01 | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 4.00 ± 0.88abcdef | 5.00 ± 0.88abcdefgh | 6.50 ± 0.88bcdefghij | 7.00 ± 0.88cdefghijk | 7.00 ± 0.88cdefghijk |
| | 0.02 | 0.00 ± 0.88a | 0.00 ± 0.88a | 0.00 ± 0.88a | 4.00 ± 0.88abcdef | 6.50 ± 0.88bcdefghij | 9.50 ± 0.88 ghijklm | 10.50 ± 0.88ijklm | 11.00 ± 0.88jklmn |
| Kerosene & Naphthalene | 0.005 | 0.00 ± 0.88a | 4.50 ± 0.88abcdefg | 4.50 ± 0.88abcdefg | 7.50 ± 0.88defghijk | 8.00 ± 0.88efghijk | 9.00 ± 0.88 fghijkl | 9.00 ± 0.88 fghijkl | 9.00 ± 0.88 fghijkl |
| | 0.01 | 1.50 ± 0.88ab | 4.50 ± 0.88abcdef | 6.00 ± 0.88bcdefghij | 9.00 ± 0.88 fghijkl | 10.00 ± 0.88hijklm | 10.50 ± 0.88ijklm | 11.00 ± 0.88jklmn | 11.00 ± 0.88jklmn |
| | 0.02 | 2.50 ± 0.88abcd | 7.50 ± 0.88defghijk | 8.50 ± 0.88efghijk | 9.50 ± 0.88 ghijklm | 12.00 ± 0.88aklmn | 14.00 ± 0.88lmn | 14.50 ± 0.88mn | 16.00 ± 0.88n |

Means of the same letter do not differ significantly between treatments ($p < 0.05$) using Tukey's test.

**Table 3**
Mean mortality time records of *Ae. aegypti* larvae exposed to naphthalene, and naphthalene with kerosene at different concentrations.

| Treatment | Conc. (%) | Time mortality (minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 80 |
| Larvae | | | | | | | | | |
| Water | 0.00 | 0.00 ± 1.59a | 0.00 ± 1.59a | 0.00 ± 1.59a | 0.00 ± 1.59a | 0.00 ± 1.59a | 0.00 ± 1.59a | 0.00 ± 1.59a | 0.00 ± 1.59a |
| Naphthalene | 0.005 | 0.00 ± 1.59a | 0.00 ± 1.59a | 2.00 ± 1.59abcde | 4.50 ± 1.59abcdefghi | 7.50 ± 1.59abcdefghijkl | 9.50 ± 1.59abcdefghijklm | 11.00 ± 1.59defghijklmn | 12.50 ± 1.59ghijklmn |
| | 0.01 | 0.00 ± 1.59a | 0.00 ± 1.59a | 3.50 ± 1.59abcdefg | 5.50 ± 1.59abcdefghij | 10.00 ± 1.59bcdefghijklm | 12.50 ± 1.59ghijklmn | 14.50 ± 1.59jklmn | 14.50 ± 1.59jklmn |
| | 0.02 | 0.00 ± 1.59a | 5.00 ± 1.59abcdefghij | 6.00 ± 1.59abcdefghij | 10.50 ± 1.59cdefghijklmn | 16.00 ± 1.59klmn | 16.50 ± 1.59lmn | 18.00 ± 1.59mn | 19.00 ± 1.59mn |
| Kerosene & Naphthalene | 0.005 | 0.00 ± 1.59a | 0.00 ± 1.59a | 1.00 ± 1.59a | 1.50 ± 1.59abcd | 2.50 ± 1.59abcdef | 5.00 ± 1.59abcdefghij | 9.50 ± 1.59abcdefghijklm | 13.50 ± 1.59hijklmn |
| | 0.01 | 1.00 ± 1.59a | 2.00 ± 1.59abcde | 2.00 ± 1.09abcde | 3.00 ± 1.59abcdefg | 6.00 ± 1.59abcdefghij | 11.50 ± 1.59efghijklmn | 14.00 ± 1.59ijklmn | 16.00 ± 1.59klmn |
| | 0.02 | 0.50 ± 1.59ab | 4.00 ± 1.59abcdefgh | 6.50 ± 1.59abcdefghijk | 7.00 ± 1.59abcdefghijkl | 7.50 ± 1.59abcdefghijkl | 12.00 ± 1.59fghijklmn | 16.00 ± 1.59klmn | 20.00 ± 1.59n |

Means of the same letter do not differ significantly between treatments ($p < 0.05$) using Tukey's test.

### 3.3. Toxic concentration assay

The summary of toxic concentration model of *Ae. aegypti* larvae and pupae exposed to naphthalene, and naphthalene with kerosene in Table 4. Naphthalene exposed to *Aedes* larvae and pupae showed that $LC_{50}$ and $LC_{95}$ values between 0.002 and 0.018%, and 0.021–0.051% respectively. Similarly, $LC_{50}$ and $LC_{95}$ values of naphthalene with kerosene were between 0.002 and 0.007%, and 0.015–0.035%. This suggests that the concentrations in part per thousand were effective in the mortality of the mosquitoes.

### 3.4. Adult emergence and inhibition

Adult emergence in pupae exposed to naphthalene, and naphthalene with kerosene at different concentrations is shown in Fig. 2. Apart from control where 100% emergence was observe, *Aedes* pupae exposed to 0.005% naphthalene had more adult emergence than in others and the differences between the adult emergence were significant ($p < 0.05$). Model prediction of emergence inhibition of pupae exposed to naphthalene, and naphthalene with kerosene at different concentrations is shown in Fig. 3. Mean emergence inhibition was 95.33 and standard deviation was 3.31. The likelihood of inhibition using Chi-square test was 7.77 and the difference was significant $p = 0.005$. Emergence inhibition for 50% and 95% ($EI_{50}$ and $EI_{95}$) was 0.001 and 0.008.

## 4. Discussion

This study examined the effectiveness of naphthalene and its combination against the activities of larvae and pupae of *Ae. aegypti* mosquitoes in Ika North East LGA, Delta State, Nigeria. We found a pattern of increased mortality and reduced adult emergence in the concentrations of naphthalene and its combination with kerosene, which is similar to that reported in their use on subterranean termites in laboratory conditions (Ojianwuna et al., 2021a, 2021b). Similar observation of high effectiveness was made in the study of Enwemiwe et al. (2020), where combination of kerosene and naphthalene was used in the treatment of flea lesions on the body of heavily, moderately and mildly infected persons in Igbokoda, Ondo State, Nigeria. This is the first study reporting the effectiveness of naphthalene and its combination on the activities of *Aedes* mosquitoes. However, one would expect that naphthalene which have a long term history in their use as insecticidal substance would have studies reporting their trials. This deficiency may be traceable to the toxic nature of this substance as perceived by many scholars. A study by Manoguerra et al. (2008) and Marwah and Marwah (2014) have amongst other studies highlighted the levels of fatality when naphthalene is mistakenly ingested. The consequences of naphthalene and kerosene may be severe when mistakenly ingested, as they have been reported as toxic substances (Venkatesh et al., 2011; Dayasiri et al., 2017). Ingestion is possible when these substances are used to fight against the activities of household insect pest where children are present.

In this present study, it was observed that mortality was highest in larvae of *Aedes* exposed to 0.02% kerosene and naphthalene, and followed closely by 0.02% naphthalene. The mortality recorded in this study could be explained following the mechanisms of actions as described by Li et al. (2011) and Maiyoh et al. (2015). This explanations are linked to the observations made during the exposures which pointed that the chemical constituents of these substances might have interrupted the functioning of the siphon, the respiratory organ of the larva. Thus, probably causing larvae not to feed. It was also reported in this present study that low concentration of naphthalene caused lowest mortality in pupae. Mortality of the *Ae. aegypti* pupae was generally lower compared to larvae in this study. This could be due to that pupae case shielded them from the effect of the chemical substance in treatments. Significant mortalities were recorded in the various concentrations exposed to pupa and larva of *Ae. aegypti* ($p < 0.05$). The effect of kerosene alone has also been reported in the study of Ojianwuna et al. (2021a) and their efficacy was high causing complete mortality in optimum concentrations. Naphthalene has been reported as potential substances against insects in several studies. Amongst these studies is the work of Fu et al. (2015), which reported the toxicity of oil of naphthalene plant against the activities of the workers of red imported fire ant and the toxicity occurred in a short time. Further to their use, the study of Obeng-Ofori et al. (1998) have shown the contact toxicity of naphthalene (concentrations between 100 µg and 100 mg) on stored product insect pest with mortalities above 70% in maize weevil, groundnut weevil, red flour beetle, and large grain borers. Growth and development activities of these insects were reportedly inhibited in treated grains. Naphthalene in combination with other substances caused high mortality in lesser grain borer (Bekele and Hassanali, 2001) and rice weevil (Rozman et al., 2006).

**Table 4**
Toxic concentration model of *Ae. aegypti* larvae and pupae exposed to naphthalene, and naphthalene with kerosene.

| Treatments | N | Regression line | Pearson $\chi^2$ goodness of fit (p-value) | $LC_{50}$ (95% CI) | $LC_{95}$ (95% CI) |
|---|---|---|---|---|---|
| Larvae | | | | | |
| Naphthalene | 40 | Y = 87.16–0.18× | 14.17 (0.000) | 0.002 (−0.009–0.006) | 0.021 (0.016–0.036) |
| Kerosene and Naphthalene | 40 | Y = 130.57–0.29× | 19.23 (<0.0001) | 0.002 (−0.006–0.005) | 0.015 (0.012–0.025) |
| Pupae | | | | | |
| Naphthalene | 40 | Y = 48.72–0.85× | 6.76 (0.009) | 0.018 (0.013–0.037) | 0.051 (0.034–0.176) |
| Kerosene and Naphthalene | 40 | Y = 59.20–0.44× | 9.52 (0.002) | 0.007 (−0.002–0.011) | 0.035 (0.025–0.080) |

N: Total number of mosquitoes assayed; 50% and 95% lethal concentration, $LC_{50}$ and $LC_{95}$, are in g for naphthalene and g/mL for naphthalene with kerosene; 95% confidence interval CI; $p > 0.05$ suggests a well-fitting model, $p < 0.05$ suggests an invalid model population.

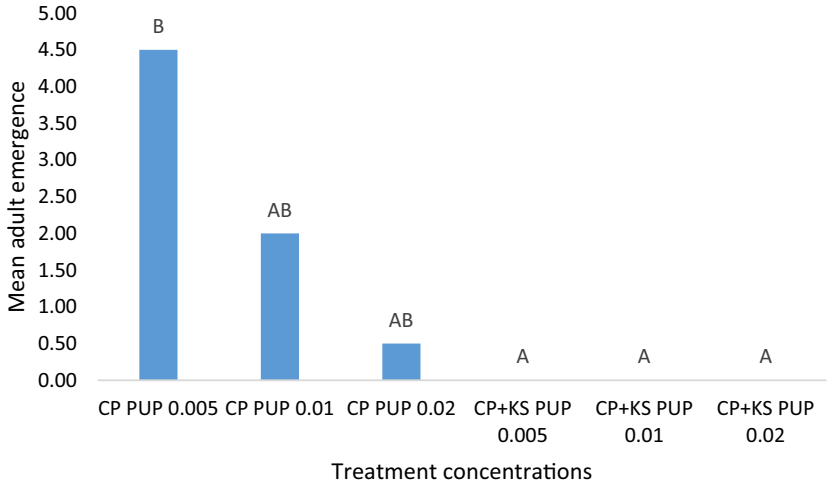


**Fig. 2.** Adult emergence in pupae exposed to naphthalene, and naphthalene with kerosene at different concentrations. (p = means of the same letter do not differ significantly between treatments ($p < 0.05$) using Tukey's test). Note: CP means naphthalene, PUP = pupae, and CP + KS = naphthalene and kerosene).

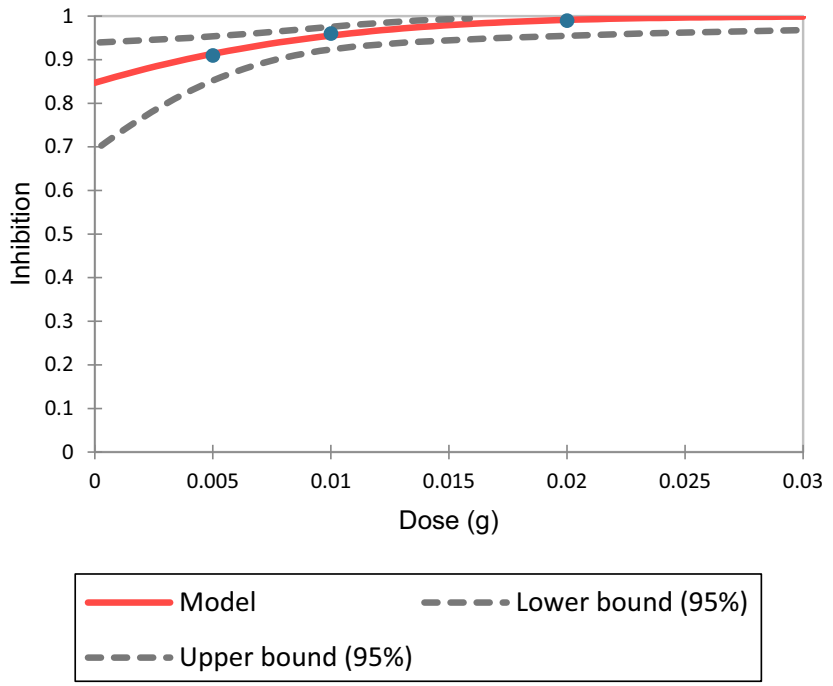


**Fig. 3.** Model prediction of emergence inhibition of pupae exposed to naphthalene, and naphthalene with kerosene at different concentrations.

Our findings in this present study revealed that mortality increased with reduced time, high concentration favoured higher mortalities and adult emergence were not common when naphthalene and kerosene was combined. This was reflected in the high mortality recorded in 0.02% kerosene and naphthalene combination at 80 min post exposure time with *Aedes* pupae. Remarkable correspondence was observed with mortality recorded in 0.02% naphthalene at 60 and 80 min and with 0.01% kerosene and naphthalene at 50, 60 and 80 min. This shows that the study of these substances are important in controlling populations of *Aedes* mosquitoes and in reducing their activities in as they breed in their various habitats. In the naphthalene group where adult emergence was observed, adults that came into contact with the floating clumps of naphthalene were suffocated to death and left floating with the clumps. This observation may be linked to the mechanism of action reported by Li et al. (2011). With reference to the residual effect of naphthalene and kerosene, the former had higher residual effect compared to the later. However, kerosene had quick action of

blocking siphon of larvae since they had higher tendency of spreading throughout the surface of water. The study of Djouaka et al. (2007) supported the observations made in this study. Another study by Maiyoh et al. (2015) explained in detail the mechanism of action which could be linked to the observation in this study. There was no corresponding increase in the activity of larvae and pupae exposed to the treatments as compared to the control. These observations suggest that while searching for alternative substance with great potentials of reducing larval and pupal activities, naphthalene could be the best candidate as issues of eco-toxicity could be reduced.

Furthermore, on the time mortality, no mortality was observed in larvae and pupae exposed to all concentration of naphthalene from 10 to 20 min. Both treatments were immiscible. However, that naphthalene did not result in immediate mortality showed that they lack the tendency of covering the surface of water. It also shows that these larvae maneuvered the clumps of floating naphthalene. More so, no mortality was recorded in *Aedes* pupae exposed to 0.005% of kerosene and naphthalene at 10 min. No larval mortality was equally recorded at 10 min in all concentrations of naphthalene and 0.005% of naphthalene and kerosene at 10 min. Similar trend was observed in 0.005% and 0.01% of naphthalene at 15 min and 0.005% of naphthalene and kerosene at 15 min. It could also mean that kerosene was not sufficient enough to cause siphon blockage and that pupal case interrupted effectiveness of treatment. Significant time mortalities was recorded in larvae and pupae exposed to the various concentration of treatments at other time.

Lethal concentration of mortality ($LC_{50}$ and $LC_{95}$) showed that naphthalene exposed to *Aedes* larvae and pupae was between 0.002 and 0.018%, and 0.021–0.051% respectively. Which predict that higher concentrations would cause complete mortality. Similarly, lethal concentration ($LC_{50}$ and $LC_{95}$) of naphthalene with kerosene was between 0.002 and 0.007%, and 0.015–0.035%. This showing that concentrations above 0.02 of naphthalene and kerosene mixture could be best to cause complete mortality in field trials. This did not correspond to the observations made in the study of Bekele and Hassanali (2001) and Rozman et al. (2006). This may be due to the fact that they were insect pests of stored products rather than mosquitoes and their resistant or susceptibility levels may be different. The finding of this study corresponded to the lethal concentration reported in Ojianwuna et al. (2021a). Considering the inhibition of emergence, *Aedes* pupae exposed to 0.005% naphthalene had more adult emergence than in other concentrations and the differences between the adult emergence were significant ($p < 0.05$). Emergence was 100% in mosquitoes exposed to water (control) after five to seven days in larvae and pupae. Emergence inhibition for 50% and 95% ($EI_{50}$ and $EI_{95}$) was 0.001 and 0.008. This suggests that concentrations between this predictions would favour adult emergence inhibition.

## 5. Conclusion

This study has shown that higher concentrations of naphthalene singly and their combinations with kerosene caused high mortality in larvae and pupae of *Ae. aegypti* mosquitoes. Larvae and pupae mortality being recorded highest after 80 min of post exposure. The oil smear made on water surface by kerosene and clumps of floating naphthalene were remarkable traits for mortality in this study. Therefore, adopting the treatment of potential breeding habitats of *Aedes* mosquitoes with these substances in field trials and implementing sensitization programs involving the application of these substances would be key to bringing immature stages of *Aedes* mosquitoes in this endemic area to reduction as well as the disease outbreaks.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and material

All the data are analyzed and presented in the article

## Funding

Not applicable.

## Authors' contributions

CC and VN conceptualized the study and curated the data, VN and CC carried out the investigation, designed the methodology, wrote the original draft, reviewed, edited and approved the final draft.

## Declaration of Competing Interest

The authors declare no competing interest

## Acknowledgements

Not applicable.

## References

Bekele, J., Hassanali, A., 2001. Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandscharicum* and *Ocimum kenyense* (Labiateae) on two post-harvest insect pests. Phytochemistry. 57, 385–391. https://doi.org/10.1016/S0031-9422(01)00067-X.

Belinato, T.A., Martins, A.J., Lima, J.B.P., Valle, D., 2013. Effect of triflumuron, a chitin synthesis inhibitor, on *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* under laboratory conditions. Parasit. Vectors 6, 83. https://doi.org/10.1186/1756-3305-6-83.

Bourtzis, K., Lees, R.S., Hendrichs, J., Vreysen, M.J.B., 2016. More than one rabbit out of the hat: radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. Acta Trop. 157, 115–130.

Chukwuekezie, O., Nwosu, E., Nwangwu, U., Dogunro, F., Onwude, C., Agashi, N., Ezihe, E., Anioke, C., Anokwu, S., Eloy, E., Attah, P., Orizu, F., Ewo, S., Okoronkwo, A., Joseph, A., Ikeakor, I., Haruna, S., Gnanguenon, V., 2020. Resistance status of Anopheles gambiae (s.l) to four commonly used insecticides for malaria vector control in south-East Nigeria. Parasit. Vectors 13, 152.

Coulibaly, D., Travassos, M.A., Kone, A.K., Tolo, Y., Laurens, M.B., Traore, K., Diarra, I., Niangaly, A., Daou, M., Dembele, A., Sissoko, M., Guindo, B., Douyon, R., Guindo, A., Kouriba, B., Sissoko, M.S., Sagara, I., Plowe, C.V., Doumbo, O.K., Thera, M.A., 2014. Stable malaria incidence despite scaling up control strategies in a malaria vaccinetesting site in Mali. Malar. J. 13, 374. https://doi.org/10.1186/1475-2875-13-374.

Dayasiri, M., Jayamanne, S.F., Jayasinghe, C.Y., 2017. Kerosene oil poisoning among children in rural Sri Lanka. Int. J. Pediatr. 2017, 8798610. https://doi.org/10.1155/2017/8798610.

Djouaka, R.F., Bakare, A.A., Bankole, H.S., Doannio, J.M., Kossou, H., Akogbeto, M.C., 2007. Quantification of the efficiency of treatment of Anopheles gambiae breeding sites with petroleum products by local communities in areas of insecticide resistance in Republic of Benin. Malar. J. 6, 56.

Ekedo, C.M., Okore, O.O., Obeagu, I.A., Okafor, C.C., 2019. Effect of some petroleum products on Anopheles gambiae S.L. larvae in Umudike, Ikwuano LGA Abia state, Nigeria. Int. J. Mosquito Res. 6 (3), 32–36.

Ekerette, I.B., Ebere, N., 2018. Efficacy of Selected Microbial Larvicides on Field-Collected Larvae of Mosquitoes from Port Harcourt Metropolis. European Journal of Biomedical and Pharmaceutical sciences 5 (3), 82–89.

Enwemiwe, V.N., Ojianwuna, C.C., Anyaele, O.O., 2020. Assessing the potentials of two local topical ointments as affordable treatment against tungiasis infestation: a self-experimentation in Igbokoda, Nigeria. Parasite Epidemiol. Control 11, e00168.

Fu, J.T., Tang, L., Li, W.S., Wang, K., Cheng, D.M., Zhang, Z.X., 2015. Fumigant toxicity and repellence activity of camphor essential oil from *Cinnamonum camphora* Siebold against *Solenopsis invicta* workers (Hymenoptera: Formicidae). J. Insect Sci. 15 (1), 129. https://doi.org/10.1093/jisesa/iev112.

Garske, T., Van Kerkhove, M.D., Yactayo, S., Ronveaux, O., Lewis, R.F., Staples, J.E., Perea, W., Ferguson, N.M., 2014. Yellow fever in Africa: estimating the burden of disease and impact of mass vaccination from outbreak and serological data. PLoS Med. 11 (5), e1001638.

Griffin, L., 2014. Laboratory evaluation of predation on mosquito larvae by Australian mangrove fish. J. Vect. Ecol. 39, 197–203.

Jagannathan, P., Muhindo, M.K., Kakuru, A., Arinaitwe, E., Greenhouse, B., Tappero, J., Rosenthal, P.J., Kaharuza, F., Kamya, M.R., Dorsey, G., 2012. Increasing incidence of malaria in children despite insecticide-treated bed nets and prompt anti-malarial therapy in Tororo, Uganda. Malar. J. 11, 435. https://doi.org/10.1186/1475-2875- 11-435.

Lawler, S.P., 2017. Environmental safety review of methoprene and baterially derived pesticides commonly used for sustained mosquito control. Mosquito 139, 335–343.

Lees, R.S., Knols, B., Bellini, R., Benedict, M.Q., Bheecarry, A., Bossin, H.C., 2014. Review: improving our knowledge of male mosquito biology in relation to genetic control programmes. Acta Trop. 132S, S2–S11.

Lees, R.S., Gilles, J.R.L., Hendrichs, J., Vreysen, M.J.B., Bourtzis, K., 2015. Back to the future: the sterile insect technique against mosquito disease vectors. Curr. Opin. Insect Sci. 10, 156–162.

Li, L., Wei, Y., van Winkle, L., Zhang, Q.Y., Zhou, X., Hu, J., Xie, F., Kluetzman, K., Ding, X., 2011. Generation and characterization of a Cyp2f2-null mouse and studies on the role of CYP2F2 in naphthalene-induced toxicity in the lung and nasal olfactory mucosa. J. Pharmacol. Exp. Ther. 339, 62–71.

Maiyoh, G.K., Njoroge, R.W., Tuei, V.C., 2015. Effects and mechanisms of kerosene use-related toxicity. Environ. Toxicol. Pharmacol. 40 (1), 57–70.

Manoguerra, A.S., Erdman, A.R., Wax, P.M., Nelson, L.S., Caravati, E.M., Cobaugh, D.J., Chyka, P.A., Olson, K.R., Booze, L.L., Woolf, A.D., Keyes, D.C., Christianson, G., Scharman, E., Troutman, W.G., 2008. Camphor poisoning: an evidence-based practice guideline for out-of-hospital management. Clin. Toxicol. 44 (4), 357–370.

Marwah, P., Marwah, A., 2014. Camphor poisoning: a rare but preventable cause of status epilepticus. Sci. Lett. 81, 956.

Mischke, C.C., Griffin, M.J., Greenway, T.E., Wise, D., 2016. Effect of mosquito fish, *Gambusia affinis*, on channel catfish *Ictalurus punctatus*, production ponds. J. World Aquacult. Soc. 44, 288–292.

Msangi, S., Lyatuu, E., Kweka, E.J., 2011. Field and laboratory evaluation of bioefficacy of an insect growth regulator (Dimilin) as a Larvicide against mosquito and housefly larvae. J. Trop. Med. https://doi.org/10.1155/2011/394541.

Mulamuli, M., Becker, P., Mudambo, K., de Jager, C., 2016. Field effectiveness of microbial larvicides on mosquito larvae in malaria areas of Botswana and Zimbabwe. Malar. J. 15, 586.

Nelms, B.M., Fechter-Leggett, E., Carroll, B.D., Macedo, P., Kluh, S., Reisen, W.K., 2013. Experimental and natural vertical transmission of West Nile virus by California Culex (Diptera: Culicidae) mosquitoes. J. Med. Entomol. 50 (2), 371–378.

Obeng-Ofori, D., Reichmuth, C.H., Bekele, A.J., Hasssanali, A., 1998. Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilimandscharicum*, against four stored product beetles. Int. J. Pest Manag. 44, 203–209.

Ojianwuna, C.C., Enwemiwe, V.N., 2021. Toxicity and repellency of scent leave (*Ocimum gratissimum*), kerosene and naphthalene in single form and in mixed form in termites (*Macrotermes bellicosus*). J. Biopestic. 14 (1), 41–49.

Ojianwuna, C.C., Enwemiwe, V.N., Ossai, P.I., 2021a. The use of deltamethrin, permethrin, their combination and synergist on female *Anopheles* mosquito in Isoko south, Delta state. Niger. J. Sci. Environ. 19 (1), 39–50.

Ojianwuna, C.C., Enwemiwe, V.N., Erhunmwun, S., 2021b. Effect of petroleum products on larvicidal activity of *Aedes* mosquitoes in Ika north-east LGA, Delta state, Nigeria. Open Ecol. J. 14, 24–30.

Ononamadu, C.J., Datit, J.T., Imam, A.A., 2020. Insecticide resistance profile of Anopheles gambiae mosquitoes: a study of a residential and industrial breeding sites in Kano Metropolis, Nigeria. Environ. Health Insight 14, 1–9.

Rozman, V., Kalinovic, I., Korunic, Z., 2006. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res. 43, 349–355. https://doi.org/10.1016/j.jspr.2006.09.001.

Tikar, S.N., Mendki, M.J., Sharma, A.K., Sukumaran, D., Veer, V., Prakash, S., Parashar, B.D., 2011. Resistance status of malaria vector mosquitoes, Anopheles stephensi and Anopheles subpictus towards adulticides and larvicides in arid and semi-arid areas of India. J. Insect Sci. 11, 1–10.

Venkatesh, C., Sriram, P., Adhisivam, B., Mahadevan, S., 2011. Clinical profile of children with kerosene aspiration. Trop. Dr. 41 (3), 179–180. https://doi.org/10.1258/td.2011.110093.

WHO, 2013. Larval source management: A supplementary measure for malaria vector control. In: An Operational Manual, p. 128. Geneva.

WHO, 2015. WHO Updates on Artemisinin Resistance. http://www.who.int/malaria/areas/drug_resistance/updates/en/. Google Scholar.

WHO, 2017. Global Vector Control Response 2017–2030. World Health Organization, Geneva.

WHO Pesticides Evaluation Scheme (WHOPES), 2007. WHO Recommended Insecticides for Indoor Residual Spraying against Malaria Vectors. www.who.int/malaria/cmcupload/0/000/012/604/IRSInsecticides.htm (accessed 12 February 2021).

World Health Organization, 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO Pesticides Evaluation Scheme: WHO/CDS/WHOPES/GCDPP/2005.13. WHO, Geneva.
Yakob, L., Walker, T., 2016. Zika virus outbreak in the Americas: the need for novel mosquito control methods. Lancet Glob. Health 4 (3), 148–149.
Yakub, L., 2011. Epidemiological Consequences of a Newly Discovered Cryptic Subgroup of *Anopheles gambiae*.