Effect of Petroleum Products on the Larvicidal Activity of *Aedes* Mosquitoes in Ika North-East LGA, Delta State, Nigeria

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Abstract:

**Aims:**
The recent yellow fever outbreak in Delta State, especially in Ika North East local government, triggered the need for this study.

**Background:**
Diseases caused by *Aedes* are by far raising serious concerns in the world.

**Objective:**
To evaluate the larvicidal activity on the use of petroleum products in the control of *Aedes* mosquitoes.

**Methods:**
Mosquito species were collected using 350ml deep ladle and identified in the field using their resting positions in their local habitats. Larvae and pupae were separately exposed to 0.005, 0.01, and 0.02% of kerosene and petrol in single and mixed forms. The experimental sets were triplicated. Data was analyzed using ANOVA and Turkey’s test to compare mortality, and time of mortality. LC50 and LC95 were computed using Probit analysis.

**Results:**
Results revealed that all concentrations of treatment caused complete mortality in larvae except in 0.005%ml and 0.01%ml of kerosene alone and kerosene and petrol mixture at 50 minutes of exposure. All concentrations of treatment equally caused complete mortality in pupae except in 0.005%ml of kerosene (Mean= 10.00) at 30 minutes. The differences between mortality and time mortality records were significant (p< 0.05). Kerosene and petrol mixture and kerosene alone had the lower LC50 and LC95 0.0021 and 0.0088ml respectively in the larvae group. Kerosene and petrol mixture had lower LC50 and LC95 0.0037 and 0.0050, respectively.

**Conclusion:**
Therefore, scaling up this intervention on a large scale in endemic areas would reduce larvae density and disease outbreaks.

**Keywords:** Larvicidal activities, Petroleum products, *Aedes* mosquitoes, Ika North-East, Endemic, *Aedes* species.

1. INTRODUCTION

Mosquitoes are among the most important medical vectors transmitting diseases worldwide. This being that they drive pathogens responsible for disease organisms in prey coexistence. According to Powell (2018), a number of diseases are transmitted by mosquito species causing severe burdens in human and veterinary concerns. Mosquito-borne diseases such as those transmitted by the *Aedes* species include yellow fever, dengue fever, chikungunya fever, and Zika virus. The reported cases and mortality records as a result of *Aedes* mosquitoes attack are immeasurable. However, valid records of reported cases and mortalities are described in the bulletin of the World Health Organization (WHO, 2009). The WHO has in 2007, 2010 and recently marked Nigeria as a country with the highest risk of yellow fever in Africa with a greater percentage of non-immune human population been reported to be at high risk of...
outbreaks caused by mosquitoes in Africa (WHO, 2015; WHO, 2016b; NCDC, 2019). This is occurring in eleven countries in Africa with the exception of Nigeria where the implementation of routine immunization as well as frequent vaccination is on the rise (WHO, 2017).

Mosquito transmitted viruses are not only known to cause mortalities but also affect the economic standards of any nation. In 2013, the infection rate of yellow fever in Africa was over 380,000 cases, with death estimated at 80,000 (Garske et al., 2014). The cost of attending to these viruses is expensive compared to the cost of living of an average African. This calls for immediate intervention not only on the use of vaccines targeting the infected individuals but also the vector causing the diseases. Aedes aegypti and Aedes albopictus include the prominent vectors species causing the spread of viruses in the world. The WHO has in 2009 pointed out the first occurrence of Ae. albopictus in Nigeria in 1991, after the outbreak of sylvatic (jungle) fever in several rural villages in Delta state. There has been a major epidemic threat in Lagos, one of which is the major cities in Nigeria, where about one-third of the population is at risk as a result of species development due to the availability of suitable breeding sites (WHO, 2009). Re-emergence of yellow fever occurred in Nigeria after the last case in Kano State, in 2000. In 2017, the yellow fever virus resurfaced with 341 suspected cases reported from 16 states (Abia, Anambra, Borno, Edo, Enugu, Kano, Katsina, Kogi, Kwara, Kebbi, Lagos, Nasarawa, Niger, Oyo, Plateau, and Zamfara states). Of these states, 6 states (Kano, Kebbi, Kogi, Kwara, Nasarawa and Zamfara) reported confirmed cases of the disease (WHO, 2017). Presently, Delta State was included due to the outbreaks of the yellow fever virus and this calls for swift intervention on the vectors while other intervention is targeted on the disease.

Aedes mosquito species can breed in various habitats, including puddles, ditches, tree holes in forests, and other habitats associated with human dwelling, as reported by McBride et al. (2014). They normally breed in transient water-contained habitats. These include collections in tree cavities, leaf axils, bamboo stumps, rock pools and artificial containers (including tin cans, coconut shells, water storage containers, discarded vehicle tires, broken earthen and ceramic wares). These breeding habits keep them in close proximity with man, facilitating disease transmission. They lay their eggs just above the water level. As a result, water waves/ripples or increases in water volume led to the hatching of viable eggs. Aedes mosquitoes are characteristically dark black in color, with patches of white or silver markings on their bodies. They previously dominated the tropical and sub-tropical regions, but have over time spread to all continents, excluding Antarctica (Bonizzoni et al., 2013). According to Ezihe and Chukwuekezie (2018), the spread of the mosquito was aided by an unhealthy environment due to anthropogenic activities. Unlike other mosquitoes, the Aedes mosquitoes are diurnal, functioning at special hours of the daytime. Studies also revealed that with the extended urbanization around the world, the preference of mosquitoes for artificial containers for breeding has increased to a large extent, which implies that the distribution of larval mosquitoes is greatly influenced by human ecology (Chen et al., 2009; Li et al., 2014). Thus, many of the studies have been carried out to examine the mosquito larval diversity in artificial breeding sites. Studies have reported that the species of mosquitoes such as Aedes aegypti and Aedes albopictus generally breed in artificial containers (Chen et al., 2009; Adeleke et al., 2008; Suganthi et al., 2014). It has also been contemplated that in the urban areas, the environment has a major effect on the incidence of Aedes albopictus larvae (Li et al., 2014). In addition, studies have also observed the existing and disappearing species of mosquitoes along with their pattern of distribution (Asha et al., 2014). It has been reported that artificial containers provide a good habitat for the vector of Dengue (Rajesh et al., 2015).

Aedes mosquitoes are more adapted to urban environments, especially to human habitation. The control of vectors has been the recommended approach to combating disease spread which could come in several perspectives, including intervention targeting the immature stages (larvicide) and likewise from the adults (adulticides) as the case may be. The control has involved the use of chemical compounds to prevent the development of adult mosquitoes in aquatic environments (Yang et al., 2017). Vector control is the primary form of prevention of these frequently occurring diseases since this vector reproduces easily, where there are small collections of water at home and peri-domestic habitats of urban areas. Thus, the participation of the population in the daily care with stagnant water around or inside their houses is of fundamental importance to avoid the biological cycle of this vector (Savage et al., 1992; Trillia et al., 2016). The most effective control strategy for mosquito-borne diseases is to control the mosquito at either the larval or adult life stages (Carvalho et al., 2017). Controlling them at the larval stage is the best. Recently, studies on the control of mosquitoes have focused on their saliva as vaccine candidates (Jessica, 2018). It is well known that the saliva of mosquitoes houses various pathogens causing disease. Other control interventions targeting the larvae and pupa have focused on the use of industrialized insecticides, bacteria (Bacillus thuringiensis), understanding the biting cycle, the biological cycle, use of plants extracts, powders, and essential oils as viable control options of these vectors (Fillinger et al., 2009; Kandyata et al., 2012; Goellner et al., 2018; Mazigo et al., 2019).

The use of insecticides in the control of the vector that transmits these arboviruses is one of the preventive measures against the disease scourge (Jirakanjanakit et al., 2007). Four classes of insecticides which include organophosphates, carbamates, organochlorines, and pyrethroids are widely employed in these vector control programs (Marcombe et al., 2009; WHO, 2009). Resistance to multiple insecticides (pyrethroids and organophosphates) has been reported in Aedes aegypti in South-East Asia (Jirakanjanakit et al. 2005). This presents a serious problem that needs urgent attention on the next point of action. However, insecticide resistance to Aedes mosquitoes in Delta State has not been reported. The insecticide status of Aedes albopictus and Aedes aegypti is poorly documented in Nigeria (Ransom et al., 2010), especially Aedes albopictus, owing to its recent introduction in Africa. There is also a dearth of information on the insecticide susceptibility status of yellow fever virus in Awka South area of Anambra State, Nigeria, despite the dense population of
monkeys in this area as reported by Savage et al. (1992). In their study, Aedes aegypti and Aedes albopictus showed high strength of insecticide resistance, this presenting a major problem to the control of yellow fever and other arboviral diseases in Nigeria (Savage et al. 1992).

The control of mosquito larvae has commonly relied on the use of synthetic insecticides and repellents, but treatments with such chemicals are expensive, show scarce efficacy and have a strong environmental impact associated to relevant human health risks (Nkya et al., 2013). For these reasons, alternative controls using petroleum products, oils and repellents are now being appreciated. Although, the use of petroleum products in the control of Anopheles mosquitoes has been reported (Ekedo et al., 2019). But information on the use of essential control agents such as petroleum products in the control of Aedes mosquito larvae is lacking. Thus, the need for this study to determine the efficacy of the use of petroleum products in the control of Aedes mosquitoes. This enhances the relevance of mosquito vector control, especially in areas where the outbreak of diseases is occurring and on the rise, and helping policy makers in predicting the best control options for diseases caused by Aedes.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in the Insectary of the Entomology unit, Department of Animal and Environmental Biology, Delta State University, Abraka, Ika North- East Local Government area was being mapped out for the location and collection of Aedes mosquito larvae and pupae. Aedes mosquitoes were collected from Owerre-Olubor, Delta State. Owerre-Olubor is located in Ika North-East Local Government Area (Longitude 6.340842 and Latitude 6.257218). The Aedes mosquito was kept in the laboratory with a temperature set at 28±2°C and relative humidity of 78±3%.

2.2. Mosquito Collection Method

The larvae and pupa of the Aedes mosquito were collected at the early hours of the morning from temporary larval habitats such as puddles, ditches and water containers around the dwelling of humans. The collection of mosquitoes was done using 350 ml ladles, spoons, buckets and pipette. The ladle was used to thrust into the mosquito habitat, the pipette was used for sorting larvae from pupae in the field. The bucket was used to transport the collections from the field to the lab. At the laboratory, the sampled species were transferred into a larval holding tray properly covered with a net and were kept in laboratory with standard laboratory conditions. They were fed with low-fat biscuit and yeast prepared in a ratio of 1 biscuit to 10 yeast tablets before exposure.

2.3. Toxicity Assay

The larvae and pupae of Aedes were used for the bioassay of petroleum products. Kerosene and petrol were tested in their single and mixed forms for their efficacy on larvae and pupae of Aedes mosquitoes. 1 mL of petroleum products in their single forms were measured up and introduced differently into 200 mL, 100 mL and 50 mL of water to be used to expose the mosquitoes for toxicity studies which gave rise to 0.005%, 0.01% and 0.02% concentration. The ratio of 50:50 percent was adopted in the mixed forms. Petrol and Kerosene were measured up in the ratio of 0.5 ml: 0.5 ml and introduced separately into 200 mL, 100 mL and 50 mL of water to be equally used for toxicity assay which gave rise to 0.005%, 0.01%, and 0.02% concentration. Water alone served as control. Twenty larvae and twenty pupae were sorted accordingly in separate plastic containers before exposure to treatments. The experimental setup was observed for 10, 15, 20, 30, 40, 50, 60, and 80 minutes. The exposure time was adopted from the recommended WHO protocol for adult exposure of Aedes. (WHO, 2016a). The experimental setup was done using plastic containers in triplicates.

2.4. Statistical Analysis

Mortality data of Aedes mosquitoes were entered into MS Excel, 2013 and checked for possible errors. ANOVA test was used to compare mortality, and time mortality. Results were presented in mean ± standard error and in tabular forms. Significance was set at α= 0.05. The Tukey’s test was used to separate means while 50% of lethal concentration and 95% of lethal concentration were computed using Probit analysis. Mean and standard error, lower and upper bound confidence interval (CI), and lethal concentrations were computed using XL Stat version 2020.

3. RESULTS

3.1. Mortality Records of Aedes Aegypti Larvae

The mean mortality of Aedes aegypti larvae after 80 minutes of exposure to petroleum products at various concentrations is presented in Table 1. The highest mortality was recorded in the highest concentrations of all the treatments while reportedly low in 0.005% of kerosene and petrol respectively (Table 1). There was no significant difference in the mortality recorded in the best concentrations. Similarly, there was no significant difference in 0.005% of kerosene exposed to pupa and larva of Ae aegypti.

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Table 1. Mortality of Ae aegypti larvae and pupae after 80 minutes exposure to petroleum products at various concentrations.

| Treatment | Conc. (%) | Log dose | Mean ±SE | Lower bound 95% CI | Upper bound 95% CI |
|-----------|-----------|----------|----------|---------------------|--------------------|
| Larvae    |           |          |          |                     |                    |
| Control   | 0.00      | 0.00     | 0.00±0.0* | -3.221              | 3.221              |
| Kerosene  | 0.005     | -2.301   | 8.50±1.54* | 5.28                | 11.72              |
|           | 0.01      | -2.000   | 9.50±1.54* | 6.28                | 12.72              |
|           | 0.02      | -1.699   | 10.00±1.54* | 6.78                | 13.22              |
### Table 2. Time mortality records of *Ae. aegypti* exposed to petroleum products at different concentrations.

| Treatment | Conc. (%) | Log dose | Mean ±SE | Lower bound 95% CI | Upper bound 95% CI |
|-----------|-----------|----------|----------|--------------------|--------------------|
| Petrol    | 0.005     | -2.301   | 10.00±1.54 | 6.78               | 13.22              |
|           | 0.01      | -2.000   | 10.00±1.54 | 6.78               | 13.22              |
| Kerosene and Petrol | 0.005     | -2.301   | 8.50±1.54  | 5.28               | 11.72              |
|           | 0.01      | -2.000   | 9.50±1.54  | 6.28               | 12.72              |
| Pupae     | -         | -        | -        | -                 | -                 |
| Water     | 0.00      | 0.00     | 0.00±0.0  | -2.321            | 3.221              |
| Kerosene  | 0.005     | -2.301   | 7.00±1.54  | 3.78               | 10.22              |
|           | 0.01      | -2.000   | 10.00±1.54 | 6.78               | 13.22              |
| Petrol    | 0.005     | -2.301   | 7.00±1.54  | 3.78               | 10.22              |
|           | 0.01      | -2.000   | 10.00±1.54 | 6.78               | 13.22              |
| Kerosene and Petrol | 0.005     | -2.301   | 10.00±1.54 | 6.78               | 13.22              |
|           | 0.01      | -2.000   | 10.00±1.54 | 6.78               | 13.22              |
|           | 0.02      | -1.699   | 10.00±1.54 | 6.78               | 13.22              |

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

#### 3.2. Time Mortality Records

The time mortality records of *Aedes aegypti* exposed to petroleum products at different concentrations are presented in Table 2. In the larval exposure group, the highest mean time mortality was recorded in all concentrations of petrol, 0.02% of kerosene, and kerosene and petrol mixtures at 50 minutes, while 0.02% of kerosene and petrol mixture caused the highest mortality in *Aedes* pupae after 30 minutes of exposure. The lowest mean time mortality was recorded in 0.01% of kerosene, 0.005% of petrol and 0.01% of kerosene and petrol mixtures. The differences between the time mortality recorded in the highest and the lowest concentrations in this study were significant with notable exceptions (Table 2). It was observed that mortality in the highest concentrations was remarkable, revealing total mortality within 30 to 50 minutes of exposure.
Means of the same superscript letter do not differ significantly between treatments (p<0.05) using Tukey’s test.

3.3. Toxicity Assay

The toxicity assay of Aedes aegypti larvae and pupae to petroleum products is shown in Table 3. Kerosene and petrol exposed to Aedes larvae and pupae in single and mixed forms showed that LC₅₀ values range from 0.002 to 0.004 mL in the larvae exposed group and 0.004 to 0.005 mL in the pupae exposed groups. This finding showed that all the larvae and pupae exposed to the various treatments were susceptible. It was observed that larvae exposed to kerosene alone, and kerosene and petrol mixture, larvae exposed to petrol alone, and pupae exposed to kerosene and petrol mixture, pupae exposed to petrol alone, and kerosene alone followed the same regression line on the curve and reporting similar goodness of fit (Table 3).

4. DISCUSSION

The outbreak of arboviral diseases including Zika, Dengue, Yellow Fever, Chikungunya, among others, have always been associated with the Aedes mosquito species. The recent yellow fever outbreak in Delta State, Ika North East local government to be precise, has triggered the effort on controlling Aedes mosquitoes not only in the affected local government but in other areas where the mosquito exists. Despite the ongoing vaccination programme, the report of diseases is still on the rise. Thus, studies on the efficacy of using petroleum products as larvicides were conducted to add up to the existing treatments. A high mortality record was observed in all the highest concentrations of treatments. Although, 0.005% of kerosene and petrol recorded lowest mortality. The difference in the mortality recorded in the highest concentrations was not significant. Similarly, no significant difference was observed in 0.005% of kerosene exposed to pupa and larva of Aedes The mortality recorded in this study is in agreement with those reported by Lee et al. (2006) where complete mortality was recorded in Aedes mosquitoes exposed to various concentrations of Citrus bergamia, Cuminum myrrha, and Pimenta racemose. The mortality recorded could be due to the

petroleum products that block the respiratory tract of larvae and pupae. Residual effects of treatments may have led to the death of the resting stage, causing elongation of the pupae body.

The use of imidazolium salts was effective in causing mortality until the 15th day after larvae exposure (Goellner et al., 2018). But in this, high mortality was recorded from 30 to 80 minutes. This predicts effective vector control of the disease that may likely result from Aedes. Efforts on integrating adult control intervention on the species could be diverted to the immature stages (Fillinger et al., 2009). The time mortality recorded in this study is in agreement with those recorded in the study of Ekedo et al. (2019) where petroleum products caused complete mortality. The highest concentration in this present caused complete mortality compared to the lower concentrations, but the studies of Ekedo et al. (2019) observed that highest concentration of petrol had no larvicidal activity on Anopheles gambiae s.l. mosquito larvae at 20 mL within 5 minutes of treatment, while the lowest concentration of premium motor spirit yielding 100% mortality was recorded at 20 mL within 20 minutes. The differences in mortality may be ascribed to differences in mosquito species used. It may equally be due to that species of mosquitoes in their location were exposed to oil spilled habitats. Similarly, the studies of Ekedo et al. (2019) observed that the highest concentration of kerosene did not produce larval mortality at 60mL within 15 minutes of treatment, but the lowest concentration caused complete mortality at 20 mL within 30 minutes.

The Aedes Mosquito larvae and pupae exposed to the petroleum products were all susceptible. It is not certain if resistance may arise as a result of misapplication of the products if approved as a larvicidal agent. Over the years, the use of insecticides has recorded high resistance in adult Aedes mosquitoes (Moyes et al., 2017; Demok et al., 2019). Due to the high rate of resistance of the Aedes larvae and pupa, there has been a great increase in the infection and death rate of humans in Africa and all over the world. Potential breeding sites could be treated with petroleum products such as kerosene, petrol, kerosene and petrol to reduce larvae and
pupae abundance. According to Snr et al. (2011) abandoned and disposed tires can stay for a very long time in harboring the larvae and pupa of Aedes mosquito. Moreover, Bi et al. (2007) and Sripugdee et al. (2011) also stated that the weather conditions inside discarded tires and containers, such as cool temperature, humidity, and reduced light, create a suitable environment for Aedes larvae mosquito breeding. One of the best methods suitable for the control of the diseases caused by the Aedes mosquito is to control the immature stages of the Aedes mosquito through the introduction of larvicides to terminate the eggs, larva and pupa before their development to adults. Controlling the life stages of these species will lessen the distribution and abundance of the Aedes population. Hence, the reduction of the diseases they cause. Projecting the highest treatments 0.02% with high records of mortality in this study shows that for 1000L of water twenty times the one millilitre of kerosene alone, and kerosene and petrol used in this study will cause larvae and pupae mortality. Similarly, time mortality may fluctuate by 5 minutes in larger scale of treatment.

The LC$_{50}$ of kerosene alone, kerosene and petrol mixture on larvae was lower at 0.0021 mL and LC$_{90}$ was estimated to be 0.0088 mL, respectively. Kerosene alone and kerosene and petrol mixture were effective on Aedes mosquito larvae at concentrations ranging from 0.005 ml to 0.02 ml within 80 minutes of exposure. The lethal concentrations (LC$_{50}$ and LC$_{90}$) recorded in this study were lower than those reported by Ekedo et al. (2019) for Anopheles gambiae with similar treatments. In the pupae group, The LC$_{50}$ and LC$_{90}$ of kerosene and petrol mixture were lower at 0.0037 mL and 0.0050 mL, respectively. This present study shows that the larvae and pupa of the Aedes mosquito were susceptible when introduced into various concentrations of petroleum products. Kerosene and petrol are locally available products affordable by any household in Nigeria and effectively adopting these products for Aedes population control in their potential habitats such as plastic containers, abandoned tires, pots, gutters, ditches amongst others, would go a long way to lessen the threat posed by the vector and disease burdens.

CONCLUSION

This study has shown that the highest concentrations of kerosene and petrol in single and mixed forms caused complete mortality in Aedes mosquito larvae and pupae. Mortality of larvae and pupae was recorded within 30 to 80 minutes of exposure irrespective of concentration. Higher concentrations of kerosene alone, and kerosene and petrol were best at controlling larvae of Aedes while higher concentrations of kerosene and petrol mixture were best at controlling pupae. Therefore, adopting the treatment of potential habitats with these products and implementing house to house campaigns on their use to eliminate the immature stages of Aedes mosquito would reduce outbreaks of disease to a considerable limit.

ETHICAL STATEMENT

This study was approved by Research and Ethics Committee, Faculty of Science, Delta State University Nigeria.

CONSENT TO PARTICIPATE

Not Applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data sets used during the current study can be provided from the corresponding author [N.A], upon reasonable request.

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CONFLICT OF INTEREST

Not applicable.

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