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Ekedo CM
Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Okore OO
Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Obeagu IA
Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Okafor CC
Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Correspondence
Ekedo CM
Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

Effect of some petroleum products on *Anopheles gambiae* S.L. larvae in Umudike, Ikwuano LGA Abia state, Nigeria

Ekedo CM, Okore OO, Obeagu IA and Okafor CC

Abstract
This study evaluated the effect of kerosene and premium motor spirit on the development of *Anopheles gambiae* s.l. larvae in Umudike. Anopheline larvae were collected monthly for a period of 3 months from June 2018 to August 2018. Larvae of *Anopheles gambiae* s.l. were exposed to 0ml, 20ml, 40ml, 60ml, 80ml and 100ml of premium motor spirit and kerosene respectively. Eleven larvae were introduced each into vials containing the various petroleum concentrations, each in four replicates, and their larvicidal activities were recorded and quantified. Mortality was observed and recorded after every 5 minutes interval of exposure for 30 minutes. Premium motor spirit was effective on the *Anopheles gambiae* s.l. mosquito larvae after treatments of breeding sites with concentrations between 20ml to 100ml. The highest concentration of premium motor spirit yielding no larvicidal activity (0% mortality) on *Anopheles gambiae* s.l. mosquito larvae was recorded at 20ml within 5 minutes of treatment while the lowest concentration of premium motor spirit yielding 100% mortality was recorded at 20ml within 20 minutes. The LC_{50} of premium motor spirit was lower at 51.29ml and LC_{90} was estimated to be 85.11ml. Kerosene was effective on *Anopheles gambiae* s.l. mosquito larvae at concentrations ranging from 0ml to 100ml within 30 minutes of treatment. The highest concentration of kerosene yielding no larvicidal activity (0% mortality) on *Anopheles gambiae* s.l. mosquito larvae was recorded at 60ml within 15 minutes of treatment, while the lowest concentration of kerosene yielding 100% mortality was recorded at 20ml within 30 minutes. The lethal concentration LC_{50} of kerosene was high at 75.86ml and LC_{90} at 199.52ml. In conclusion, *Anopheles gambiae* s.l. mosquitoes of Umudike were fully susceptible to premium motor spirit and kerosene, hence can be used as larvicides in this locality.

Keywords: Abia State, *Anopheles gambiae s.l*, kerosene, premium motor spirit, Nigeria, umudike

1. Introduction

Malaria has become a deadly vector-borne disease in the world giving rise to about 1.5 to 3 million deaths yearly. A vast majority of these deaths (more than 90%) resulting from malaria are found in Africa with its primary victims being those with low immunity such as children and pregnant women (Gachot *et al.*, 2001) [7]. WHO in 1992 set up sustainable strategies against malaria disease, which were focused on the treatment of malaria cases as well as vector preventive measures. The two main preventive measures against malaria vectors: Indoor Residual Spraying of Insecticides (IRS) and the use of Insecticide-Treated Nets (ITNs) have been very effective in the control of *Anopheles* mosquito over the years (Service and Davidson, 2004) [16]. However, with the emergence of very resistant populations of *Anopheles* mosquitoes, the efficacy of insecticide-based vector control tools is critically affected (Hemingway and Ranson, 2000) [9]. The failure of most of the currently available insecticides has necessitated the need for research focused on the development of new insecticides or the improvement of existing formulations of insecticidesfor humans, have damaging effects on environment, non-biodegradable and expensive [5]. The exploitation of native plants and plants based products to control the mosquito population is gaining much importance in the recent century. The larvicidal action of many local plants has been reported in different parts of the world [6]. Conventionally, plants and their products were used to destroy mosquitoes and other infectious. From ancient times petroleum products, such as petrol, kerosene, engine oil and waste oil, have been traditionally utilized as insecticides. Also in the recent past they have produced spectacular results as larvicides in several places and, have been advocated for as

Vector control tools by several National Malaria Control Programmes. Their insecticidal properties could be traced to their chemical buildup which has not yet been thoroughly investigated. Some early malaria control programmes kept the population of mosquitoes down by spreading kerosene on breeding sites (Burton, 1967 [3]; Thevagasayam *et al.,* 2003 [19]). In many communities that are vastly poor, this traditional vector control method is still in use, as they may not have the finance to obtain the conventional insecticides.

Many parts of the world consider larval control through source reduction and regular application of kerosene a key intervention in eradicating malaria (Kudom *et al.,* 2012) [11]. Larval control measures are aimed at reducing malaria the transmission of malaria by preventing breeding of mosquito vectors while reducing human vector pathogen contacts (Keiser *et al.,* 2005 [10]; Floore, 2006 [6]). The control of mosquito larval populations is often advantageous since the larvae are usually concentrated, relatively immobile, and often readily accessible. Moreover, it is impossible for mosquito larvae unlike the adults to change their habitat in a bid avoid control activities (Floore, 2006) [6].

Many environmental factors affect mosquito larval density and may ultimately influence the development and rate of survival of the mosquito larvae. Some of these characteristics are climatic, others include the physical and chemical characteristics of the laraval aquatic habitats, vegetation type, and also biological characteristics of the habitat (Gouagna *et al.,* 2012 [8]; Soleimani-Ahmadi *et al.,* 2013 [18]). A good understanding the larval ecology of malaria vectors is very important for the proper monitoring and control of malaria in Nigeria. Since the most effective method for controlling vector populations is to control the larvae in their aquatic habitats before they emerge as adults, the knowledge of larval vector ecology is a key factor in the establishment of effective control measures, (Diallo *et al.,* 2012) [4].

In Nigeria, recent studies have identified mosquitoes of the *An. gambiae* (principally *An. gambiae s.s.* and *An. arabiensis*) and *Anopheles funestus* complexes as the main vectors of malaria (Lenhart *et al.,* 2007 [12]; Sinka *et al.,* 2010 [17]). *An. melas* is found in the coastal areas and is involved in malaria transmission (Awolola *et al.,* 2002 [1]; Okwa *et al.,* 2009 [14]; Oyewole *et al.,* 2010 [15]). Many other studies have also looked into the control of these mosquitoes using conventional insecticides, with proven resistance in many areas. However, there are relatively few studies which have focused on the effect of petroleum products, such as petrol, kerosene, engine oil and waste oil on the development of *Anopheles* larva. Therefore, this study aims to support the empirical use of kerosene and petroleum motor spirit in areas of resistance by quantifying their lethal activities and their mode of action against *Anopheles gambiae s.l* [10, 12].

2. Materials and Methods

2.1 Study Area

The study was carried out at the Department of Zoology and Environmental Biology Undergraduate Laboratory, Michael Okpara University of Agriculture, Umudike, South-eastern Nigeria. Ikwuano, is located in the tropical rain forest zone of Nigeria (Latitude 05°26'-5°29'N and Longitude 07°34'-7°36'E). It has a mean annual rainfall of 2238 mm, minimum and maximum temperatures of 23 and 32°C, respectively, with a relative humidity range of 63-80% (NRCRI, 2003) [13].

Umudike is situated in Abia Central Senatorial district and is host to National Root Crops Research Institute, and Michael Okpara University of Agriculture both of which utilize agricultural pesticides.

2.2 Collection and Identification of Sample

Anopheline gambiae s.l. larvae collections were made for a period of 3 months from June 2018 to August 2018. Larval habitats were sampled for anopheline larvae with the aid of a standard 350 mL capacity mosquito dipper and or a white plastic pan with the same capacity as prescribed by WHO procedures (2005) [20]. In habitats positive for mosquito larvae, 10-30 dips were made at intervals along the edge. The number of deeps made per habitat was dependent on the size of the larval habitat. Larval collection was performed using plastic pipettes in small breeding places where dippers were not effective,. Samplings were done in the morning or late afternoon (early evening) for about 30 min at each larval habitat. All third and fourth instars of anopheline larvae were passed through a 100 mesh sieve. In the laboratory, each larva was identified to species by their morphological characteristics and based on its position on water (Azari-Hamidian and Harbach, 2009) [2].

2.3 Determination of Lethal Concentrations of Kerosene on Larvae of *Anopheles gambiae* s.l.

Larvae of *Anopheles gambiae* s.l. were exposed to 0ml, 20ml, 40ml, 60ml, 80ml and 100ml of premium motor spirit and kerosene respectively. The kerosene and premium motor spirit were obtained from a fuel station. Eleven larvae were introduced each into vials containing the various petroleum concentrations, each in four replicates, and their larvicidal activities were recorded and quantified. The Lethal concentrations of the premium motor spirit and kerosene were determined after treatment of *Anopheles* mosquitoes with the increasing doses of premium motor spirit and kerosene in the laboratory. For each dose of premium motor spirit and kerosene, the lowest concentration (LC_{100}) capable of inhibiting the development of larvae to the adult stage (100% mortality of exposed larvae) was determined as well as the highest concentration (HiC) not having any observable impact on the growth of larvae (0% mortality of exposed larvae). In between both concentrations, the LC_{50} (concentration leading to the mortality of 50% of exposed larval population) was determined for each dose of premium motor spirit and kerosene.

2.4. Data Analysis

The data collected was analyzed using IBM SPSS version 20 software to analyze descriptive statistic such as frequency and percentages, while probit analysis was used to analyze for $LC_{50 \text{ and}}$ LC_{90}.

3. Results and Discussions

3.1 Percentage mortality and Lethal Concentration of Premium Motor Spirit against the Larvae of *Anopheles gambiae* s.l.

Premium motor spirit was effective on *Anopheles gambiae* s.l. mosquito after treatments of breeding sites with concentrations between 20ml to 100ml. The highest concentration of premium motor spirit yielding no larvicidal activity (0% mortality) on *Anopheles gambiae* mosquito larvae was recorded at 20ml within 5 minutes of treatment while the lowest concentration of premium motor spirit yielding 100% mortality was recorded at 20ml within 20 minutes.

Table 1: Percentage mortality rate and lethal concentration of *Anopheles gambiae* larvae exposed to ranging concentrations of Premium Motor Spirit

Time interval	Percentage mortality (%) under different concentration						Lethal concentration		
	0ml	20ml	40ml	60ml	80ml	100ml	LC_{50} (95% FL)	LC_{90} (95% FL)	R^2
5min	0	0	9.1	72.7	81.8	100			
10min	0	45.5	9.1	72.7	90.9	-			
15min	0	63.6	18.2	90.9	100	-	51.29	85.11	0.92
20min	0	100	90.9	100	-	-			
25min	0	-	100	-	-	-			
30min	0	-	-	-	-	-			

11 larvae/replicate at each concentration; LC_{50}: lethal Concentration for killing 50 per cent of the treated larvae; LC_{90}: Concentration for killing 90 per cent of the treated larvae.

At 100ml concentration, premium motor spirit registered 100% mortality within the shortest time interval of 5 minutes of treatment on the larvae of *Anopheles gambiae* mosquito in the larvicidal bioassays. The premium motor spirit also showed 100% mortality against the larvae of *Anopheles gambiae* mosquito within 25 minutes of treatment. The LC_{50} of premium motor spirit was lower at 51.29ml and LC_{90} was estimated to be 85.11ml.

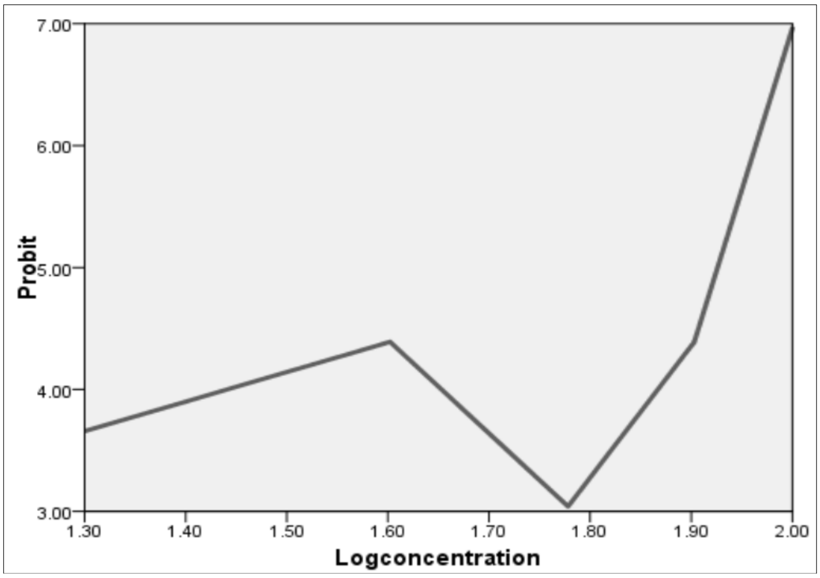

Fig 1: A plot showing the log concentration (ml) against probit of premium motor spirit used to estimate the LC_{50} and LC_{90} against *Anopheles gambiae* mosquito larvae

3.2. Percentage mortality and Lethal Concentration of Kerosene against the Larvae of *Anopheles gambiae* s.l.

The percentage mortality and median lethal concentration of the petroleum product, kerosene against *Anopheles gambiae* s.l. mosquito larvae is shown in table 2 below.

Kerosene was effective on *Anopheles gambiae* mosquito larvae at concentrations ranging from 0ml to 100ml within 30 minutes of treatment. Within 5 minutes of treatment, kerosene did not yield any larvicidal activity on *Anopheles gambiae* mosquito larvae. The highest concentration of kerosene yielding no larvicidal activity (0% mortality) on *Anopheles gambiae* mosquito larvae was recorded at 60ml within 15 minutes of treatment while the lowest concentration of kerosene yielding 100% mortality was recorded at 20ml within 30 minutes. The concentration of kerosene at 100ml registered 100% mortality within 15 minutes of treatment against the larvae of *Anopheles gambiae* mosquito in the larvicidal bioassays. At concentration of 80ml, kerosene showed 100% mortality at 20 minutes against the larvae of *Anopheles gambiae* mosquito.

Table 2: Percentage mortality rate and lethal concentration of *Anopheles gambiae* larvae exposed to ranging concentrations of kerosene

Time interval	Percentage mortality (%) under different concentration						Lethal concentration (ml)		
	0ml	20ml	40ml	60ml	80ml	100ml	LC_{50} (95% FL)	LC_{90} (95% FL)	R^2
5min	0	0	0	0	0	0			
10min	0	0	18.2	0	9.1	72.7			
15min	0	9.1	27.3	0	27.3	100	75.86	199.52	0.33
20min	0	27.3	81.8	72.7	100	-			
25min	0	63.6	90.9	100	-	-			
30min	0	100	100	-	-	-			

11 larvae/replicate at each concentration; LC_{50}: lethal Concentration for killing 50 per cent of the treated larvae; LC_{90}: Concentration for killing 90 per cent of the treated larvae.

Kerosine registered 100% mortality at 60ml concentration within 25 minutes while at 30 minutes interval, kerosene showed 100% mortality at two different concentrations of 20ml and 40ml respectively. The lethal concentration LC_{50} of kerosene was high at 75.86ml and LC_{90} at 199.52ml.

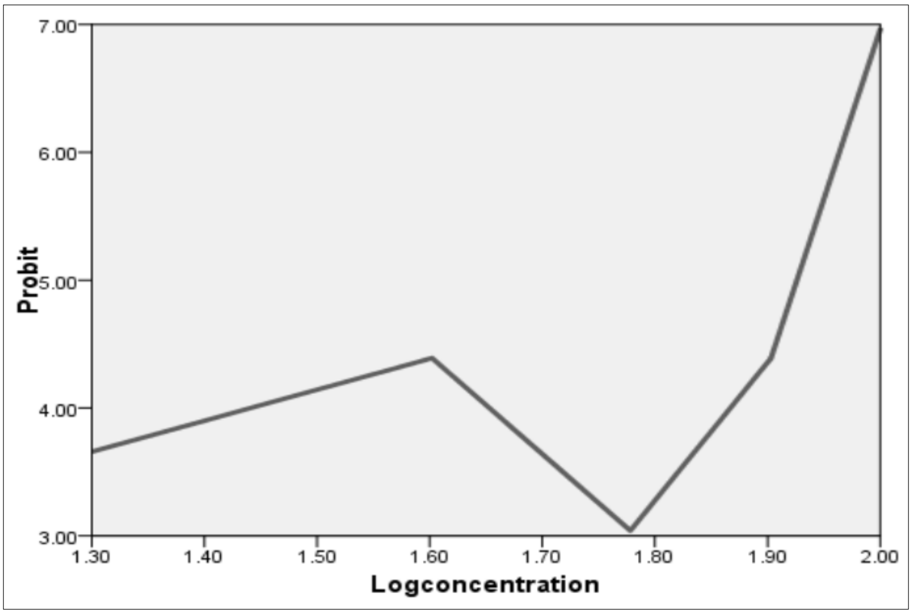

Fig 2: A plot showing the log concentration (ml) against probit of kerosene used to estimate the LC_{50} and LC_{90} against *Anopheles gambiae* mosquito larvae

This study has clearly established the larvicidal properties of premium motor spirit and kerosene, against *Anopheles gambiae* s.l. mosquito. Premium motor spirit otherwise known as petrol showed rapid response on its larvicidal activity against *Anopheles gambiae* s.l. mosquito larvae compared to kerosene which showed slower response in its larvicidal activity. This is depicted in table 1 and table 2 respectively which shows that at 100ml concentration, premium motor spirit registered 100% mortality on *Anopheles gambiae* s.l. mosquito larvae as compared with 0% mortality registered by kerosene within the same time of 5 minutes. Also, kerosene showed persistence in its larvicidal activity against the larvae of *Anopheles gambiae* mosquito. The high volatility of petrol or premium motor spirit may not allow its persistency in breeding sites inhabiting mosquito larvae. This low persistency may result in a low residual effect of this petroleum product in treated breeding sites. This observation made in this study correlates with the findings of Djouaka *et al.* (2007) [5] who found Kerosene to be highly effective on *Anopheles gambiae* Ladji at concentrations that were in the range of 11.8 × 10-3µl to 3,930 × 10-3µl/cm^2 on treated surface. They also conducted laboratory experiments on the larvicidal activities of petrol on the larvae of *Anopheles* Ladji. This yielded 100% mortality at 7,856 × 10-3µl/cm^2 treatment concentration in experimental bowls (Djouaka *et al.*, 2007) [5].
This study has revealed the mode of action of petroleum product (kerosene and premium motor spirit) on mosquito larvae demonstrates the active lethality of petroleum products. This lethality is most likely by contact-toxicity rather than suffocation. There is great possibility possible that some heavy metals from petroleum products dissolve diffuse into the water and are ingested by *Anopheles* larvae after treatment. There is therefore great need for In-depth analyses in order to identify and quantify these metals and to equally monitor their progression in the digestive system of *Anopheles* larvae after ingestion.

Conclusion
This study investigated the effect of premium motor spirit and kerosene on the development of *Anopheles gambiae* s.l. mosquito larvae and their larvicidal activities were recorded and quantified. Results from this study have pointed out that the high volatility of petrol or premium motor spirit does not allow its persistency in breeding sites inhabiting mosquito larvae. It was however found that premium motor spirit otherwise known as petrol showed rapid response on its larvicidal activity against *Anopheles gambiae* mosquito larvae compared to kerosene which showed slow response in its larvicidal activity against *Anopheles gambiae* mosquito larvae. In conclusion, *Anopheles gambiae* s.l. mosquitoes of Umudike were fully susceptible to kerosene and premium motor spirit. Hence these can be used as larvicides in this locality.

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