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Susceptibility status of female anopheles' mosquito to organophosphate and carbamate insecticides in market environs in Itu LGA, Akwa Ibom State, Nigeria

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Abstract

Malaria remains a serious public health problem and is still responsible for death of individuals globally with the female *Anopheles gambiae* established to be one of the major vectors which aid the transmission of this disease. This study examined the susceptibility and resistance of *An. gambiae* to carbamate and organophosphate which has not been frequently used in vector control in Nigeria. The CDC (Centres for Disease Control and Prevention) bottle bioassay was employed to measure the susceptibility of *An. gambiae* to insecticides. The procedure involved 2–5-day old *An. gambiae* adult mosquito populations starved from blood. In this study, we employed a diagnostic time of 30 minutes to administer doses of 12.5µg/bottle of Bendiocarb and Propoxur (Carbamates) and 20µg Pirimiphos-methyl (Organophosphate) to Anopheles spp. populations. The knockdown effect of the three insecticides indicated that bendiocarb and propoxur (carbamates) had a mortality of 98% while pirimiphos-methyl (organophosphate) had 81% at 30 minutes, regarded as the HUH for measuring resistance/susceptibility of insecticides. Further exposure at 60 minutes yielded 100% mortality for both bendiocarb and propoxur while 98% mortality was applicable in pirimiphos-methyl insecticides. *An. gambiae* was susceptible to bendiocarb and propoxur insecticides and resistant to pirimiphos-methyl. Invariably, vector control decision-making in the area will be influenced by the result of the study being that it is a baseline data/information. It is paramount that before any vector control strategies are implemented, sufficient susceptibility/resistance data be taken into thought

Keywords: Malaria; Anopheles gambiae; Mosquito; Carbamates; Organophosphates; Insecticide; susceptibility/resistance

1. Introduction

Malaria control in Africa, particularly in Nigeria, has seen to a greater extent some progress through vector control techniques such as Indoor Residual Spraying (IRS) and Long-Lasting Insecticide Nets (LLINs) [1,2]. A thorough assessment of the success achieved by these programs necessitates an understanding of vector composition and status in regions where these are implemented. In Nigeria, the major malaria vector *Anopheles gambiae* has been shown to be pyrethroid resistant [3]. Umar et al. and Opara et al. highlighted the importance of exploring other insecticidal classes as this will help closely monitor this vector and ensure the effectiveness of ongoing vector control programs [4,5].

Malaria is still considered a major problem of public health significance causing varying ill health symptoms and death in children and adults globally, with the last WHO report showing an increase in the global cases having an estimate of

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247 million cases in 84 endemic countries [6]. One major vector in sub-Saharan Africa, established by scientific reports is *An. gambiae* [7]. In most urban settlements of Akwa Ibom State, *Anopheles* mosquitoes are frequently seen laying their eggs abundantly in easily available stagnant pools of water caused by flooding due to multiple blocked drains or lack of drains during the rainy season [8]. The greatest extent of malaria with reference to the transmission dynamics typically correlates with the increased abundance of the female *Anopheles spp* and other malaria vector species during the most favourable season, the rainy season, which usually is observed although with climate variations from April to October in Nigeria.

In Akwa Ibom State, Itu LGA has two major markets in Itam, these are the Itam main market and the Nasarawa livestock market. The environs of these markets are abundant with breeding sites majorly small pools of stagnant water even though these are temporary breeding sites, they become very active during the rainy season, thus creating a dense population of these vectors and subsequent high transmission of malaria around this period.

Presently, the recommended methods and strategies for vector control include larviciding, indoor residual spraying (IRS) and the use of Long-Lasting Insecticide Nets (LLIN) [1]. These control strategies rely on the use of insecticides which to achieve success must be effective against the targeted population of the vectors. In recent times, the government of the state has scaled up the distribution of insecticide-treated bed nets to inhabitants of the state to help reduce the transmission of the parasite through the bite of infected female *Anopheles* mosquitoes [9]. As recommended by WHO, pyrethroid insecticides are suitable for treating bed nets whereas the other classes are applied for IRS [10, 11]. Pyrethroids are right now the most commonly used insecticides either for IRS or in application to bed nets. It is the insecticide of choice for controlling malaria vectors due to its proven effectiveness at low dosages, little risk not only on humans but also other non-target animals, as well as its excito-repellent, knock-down and long-lasting killing properties [11]. Another reason for pyrethroids being the choice insecticide in vector control programmes is the fact that it is less expensive to procure and apply when compared to carbamate [12]. In the 2022 WHO World Malaria report, there is a strong recommendation for an additional use of pyrethroid-chlorfenapyr insecticide-treated mosquito nets (ITNs) where pyrethroid resistance has been observed [6]. Given the prevalent pyrethroid resistance which has become an almost universal problem and may seriously impair the progress noticed in fighting malaria if drastic measures are not put in place [3, 13]. One potential solution for managing pyrethroid resistance is the use of organophosphate and carbamate insecticides, principally due to their distinct mode of action [14]. Given that research has shown pyrethroids and organochlorine insecticides to share an identical receptor site, hence it can be applied that resistance to pyrethroids may indicate resistance to organochlorines [2]. It is widely recognised in literature that *An. gambiae,* the most common malaria vector in Nigeria, is resistant to pyrethroid and organochlorine pesticides [15, 3, 16]. Scientific reports as it relates to the insecticide resistance status of the field strain of *An. gambiae* in Nigeria to carbamate and organophosphate insecticide however still remains limited [17].

To further reduce the spread of resistance, WHO recommends that all countries establish management and evaluation strategies for insecticide resistance in their malaria control programs [10]. Many African countries have now implemented entomological monitoring and susceptibility testing [6]. Hancock et al., [18] mapped the overall trend in insecticide susceptibility in African Countries covering Benin, Ghana, Guinea, Liberia, Mali, Nigeria and Senegal, and indicated an alarming general high resistance to Pyrethroids and DDT (organochlorine). However, 100% susceptibility of malaria vectors to pirimiphos-methyl has been reported across multiple sites in Nigeria [17].

This study shows the susceptibility status of *An. gambiae* in Itu, Akwa Ibom State, Nigeria to carbamate and organophosphate that has not been frequently used in vector control. It is hoped that the results of this study are intended to support and enhance efficient vector control decision-making in the country and on a global scale.

2. Material and methods

2.1. Study Area

The study was conducted in Itam in Itu communities of Itu Local Government Area of Akwa Ibom State, Nigeria (Fig. 1). The areas are located at longitude 5°3’0’’N and Latitude 7°5’5’’E. The inhabitants of these communities are mostly traders and subsistence farmers. These communities have a peak rainfall around April to October and less dry season from November to March. The Local Government is one of the populous in Akwa Ibom State, owing to the location of the popular Itam market which brings a large number of people into the area for marketing purposes, also the location of transport companies is considered to be a factor of high population in the area.

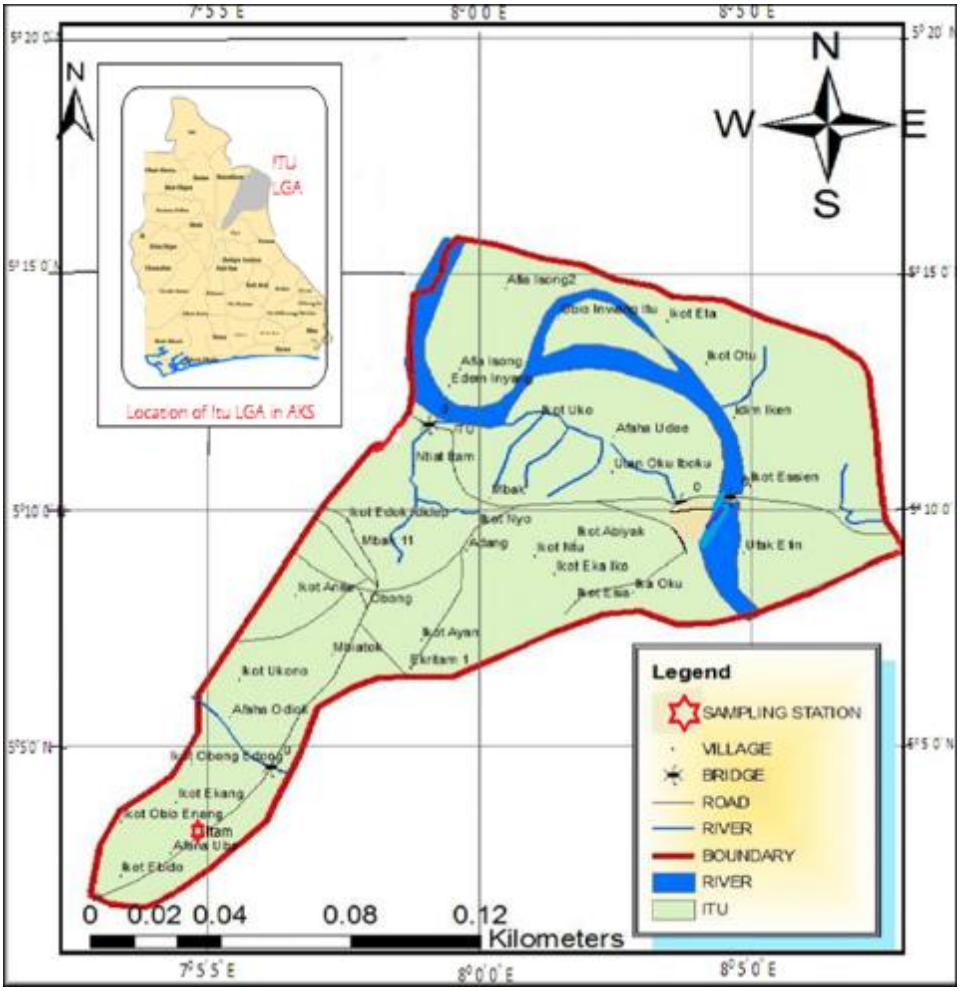

Figure 1 Map of Itu LGA showing Study Area

2.2. Mosquito Collection and Rearing

This study was conducted in October 2023. A survey was carried out to establish the existence of breeding habitats within the study areas, and different types of breeding habitats (gutters, ponds, hoof prints, roadside ditches, and stagnant water, among others) were identified. Larvae of *Anopheles* mosquitoes were collected from breeding sites within the study areas in the morning between 8 a.m. and 12 noon (due to harsh temperatures at noon which affect larval availability) using scoops (Fig. 2). With the aid of morphological keys of Gillies and DeMelion [19], Anopheline larvae were carefully differentiated from other genera and were collected while the others were discarded. Anopheles larvae were transferred with a robust ten-litre jerry-can to the insectary, Animal and Environmental Biology, University of Uyo and reared to adulthood at 28 ± 2ºc room temperature and 75-80% Relative Humidity (RH). At the insectary, the larvae were emptied into a breeding chamber and were covered with tender non-insecticidal net fastened with a band of rubber, this was done to prevent the hatched mosquitoes from escaping from the box and to prevent undesirable species from ovipositing in the water. After the emergence of adults, up to 5 days after collection depending on the instar, they were aspirated from the breeding chambers and kept in a mosquito cage made from a transparent white cylindrical bucket with a net tied to the rear end and fed with 10% glucose with a cotton wool pad.

Figure 2 Larvae of Anopheles Mosquitoe Collected During the Study

2.3. CDC Bottle Bioassay

The CDC (Centres for Disease Control and Prevention) bottle bioassay [20, 21] was used in measuring susceptibility to insecticides. Theoretically, the bioassay principle was similar to the WHO paper tests [22]. 2–5-day old *An. gambiae* adult mosquito populations, starved from blood were tested using the bottles. With 30 minutes as the diagnostic period, we administered diagnostic doses of 12.5µg/bottle of Bendiocarb and Propoxur (Carbamates) and 20µg Pirimiphos-methyl (Organophosphate) to *Anopheles spp* populations. The CDC bottles were washed with warm soapy water, rinsed three times and dried in an oven (45^0C) to ensure they were free from any insecticide residue. They were properly labelled at the cap as R1 to R4 (4 replicates for each insecticide) and control. The bottles were coated with 1ml of the prepared stock insecticides (equivalent to the diagnostic doses (12.5µg for bendiocarb and propoxur and 20µg for pirimiphos-methyl) and were at an inverted position and swirl to allow even distribution of the insecticides after which the lids were removed and the bottles were placed on their side with a cardboard at the opening (this was done to prevent sunlight penetration). The coating was done in four replicates (bottles) and a 2-replicate control treated with acetone for each of the insecticides. Using an aspirator, 25 batches of female anopheles' mosquitoes were introduced into the insecticide-coated bottles and were examined over an hour. The diagnostic time for measuring susceptibility and resistance was 30 minutes; consequently, the status of each insecticide was deduced at an exposure time of 30 minutes. Exposure to bottles with treatment for 30 minutes is seen as the standard critical value because it symbolizes the limit of tolerance between susceptibility and resistance. Dead mosquitoes were determined by physically observing when they became unable to stand, stopped movements of the legs that is motionless, and slid down the curved portion of the CDC bottle. The number of mosquito deaths was noted at intervals, every 0, 15, 30, 45 and 60 minutes.

2.4. Mosquito Identification

Individual adult *Anopheles* mosquitoes reared from the larval stage and used for the CDC bottle bioassay were identified using morphological keys adopted from Gillies and deMellion [19], Gillies and Coetzee [7] and Kent [23].

2.5. Data Analysis

Percentage knockdown to the insecticides and percentage mortality were calculated manually for mosquitoes from each of the study sites:

$$\text{Mortality} = \frac{Total\ number\ of\ Dead\ mosquitoes}{Total\ number\ of\ exposed\ mosquitoes} \times 100$$

According to the CDC standard [21], all dead mosquitoes during the diagnostic period intervals (30 minutes) when exposed to bottles with insecticide spray are susceptible to the tested insecticide. Mosquitoes surviving beyond the diagnostic time threshold are deemed to be partially resistant.

The critical parameter is the mortality within the diagnostic time, whereas bioassay monitoring is extended beyond this time point in order to determine the degree of mosquito resistance. The interpretation of the bioassay results at 30 min indicated that if the mosquito mortality is < 95%, this shows resistance [21]. By the CDC standard, where control mortality is > 10%, bioassay tests are discarded, however, where the mortality in the control group is up to 10 %, Abbott's formula was implemented [24].

KDT_{50} and KDT_{95} for all exposed mosquitoes from each study site were calculated using probit test [25]. Data were analysed using SPSS version 23 [26].

3. Results

The knockdown effect of the three insecticides indicated that bendiocarb and propoxur (carbamate) had a mortality of 98% while pirimiphos-methyl (organophosphate) had a mortality of 81% at the diagnostic time of 30 minutes regarded as the threshold for measuring resistance/susceptibility of insecticides. Further exposure at 60 minutes yielded 100% mortality for both bendiocarb and propoxur while 98% mortality was applicable in pirimiphos-methyl insecticides. The above information is shown in Table 1.

The findings from the present study revealed that *Anopheles gambiae* were susceptible to bendiocarb and propoxur (carbamate) insecticides with each having a mortality of 98%. However, resistance was observed in the population exposed to pirimiphos-methyl (organophosphate) with a mortality score of 81% at the diagnostic time required to determine susceptibility/resistance of insecticides (i.e., 30 minutes) (Table 1).

There was variability in KDT_{50} and KDT_{95} values calculated in minutes for each of the insecticides with their respective confidence interval. For bendiocarb (9.12 and 23.442); propoxur (6.28 and 19.906) and pirimiphos-methyl insecticide (13.091 and 46.773) for KDT_{50} and KDT_{95} respectively (Table 2). Propoxur had the lowest knockdown time closely followed by Bendiocarb and the highest was observed in pirimiphos-methyl. This indicates that Knockdown of mosquitoes to insecticides was more rapid in propoxur, closely followed by bendiocarb (both belonging to the carbamate class) whereas mosquitoes that were exposed to pirimiphos-methyl had a slower rate of knockdown (Tables 1, 2 and Figure 3).

Table 1 Mortality of Anopheles Mosquitoes Exposed to Insecticides

Insecticide	**No. exposed**	**No. of replicate**	**Threshold mortality %**	**KDT_{50}**	**95% CI**	**KDT_{95}**	**95% CI**
Bendiocarb	100	4	98	9.120	5.09- 11.72	23.442	21.99-30.08
Propoxur	100	4	98	6.28	1.176-8.12	19.906	9.97-21.60
Pirimiphos-methyl	100	4	81	13.091	9.52- 18.07	46.773	34.52- 52.4

Table 2 Percentage Mortality at 30 Minutes Diagnostic Time and Knockdown Time (KDT) No. of mosquitoes per Replicate = 25

Insecticide class	**Carbamate**				**Organophosphate**	
Time	**Bendicarb (12U/G)**		**Propoxur**		**Pirimiphos-methyl**	
	Replicate	**%Mortality**	**Replicate**	**%Mortality**	**Replicate**	**%Mortality**
15	4	79	4	90	4	61
30	4	98 *S	4	98 *S	4	81 *R
	Threshold for Measuring Resistance/Susceptibility					
45	4	100	4	100	4	95
60	4	100	4	100	4	98

The test was conducted with 4 replicates over an hour. Mortality during the 15, 30, 45 and 60 minutes were 79 and 98% for 15 and 30 minutes respectively and 100% at 45 and 60 minutes for Bendiocarb. At the same time internal above, mortalities were 90, and 98 for 15 and 30 minutes respectively and 100% for 45 and 60 minutes for Propoxur. The same time interval was repeated with pirimiphos-methyl in which the mortalities were 61, 81 and 95% and 100% respectively. Mortality rate ranged from 98% to 100% indicates full susceptibility while at 95% indicates resistance.

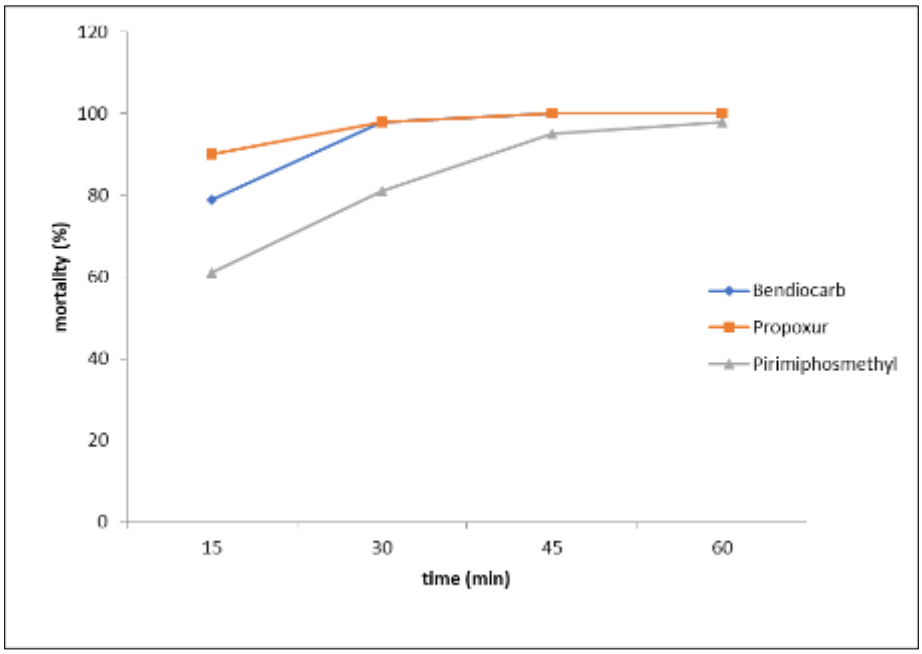

Figure 3 Graphical Representation of Knock-Down of Mosquitoes Exposed to Insecticides

4. Discussion

Vector control to a greater degree has remained a promising measure to curb the transmission of malaria globally attention focused on Nigeria. The continuous susceptibility of target vector population to insecticides of public health has a direct relationship with the success of the disease eradication. This study was conducted in Itu LGA, Akwa Ibom State, Nigeria in the month of October, 2023 to evaluate the susceptibility of *An. gambiae* to carbamate (bendiocarb and propoxur) and organophosphate (pirimiphos-methyl) insecticide. The CDC bottle bioassay previously described was adopted to determine the susceptibility/resistance for all tested insecticides [21, 20]. *An. gambiae* from the study site was susceptible to bendiocarb and propoxur having a mortality of 98% at the diagnostic time of 30 minutes. Conversely, Pirimiphos-methyl had a mortality of 81% at the diagnostic time, this warrant that exposed mosquitoes were resistant to it. However, further exposure was allowed till the 60th minutes (i.e., 1 hour) to assess the degree of resistance, at this instance, 100% mortality was observed in mosquitoes exposed to bendiocarb and propoxur while pirimiphos-methyl insecticide recorded 98% mortality. This suggests that resistance mechanisms (either target-site or metabolic) may be operating in the local Anopheles population in the area to pirimiphos-methyl insecticide. A similar observation has been documented by Okia et al. [27] research which reported 100% susceptibility of *An. gambaie* to carbamates but resistant to pyrethroids in eastern and northern Uganda with CDC bottle bioassay. The observation from this study differs from that of previous researchers who adopted the WHO tube bioassay [28, 29]. In the research conducted by Opara et al., [5] they reported the effectiveness of organophosphate as one of the insecticides against malaria vectors in Ikot Ekpene, Akwa Ibom State, Nigeria. Okorie et al. [30] reported resistance to pyrethroids and DDT but completely susceptible to organophosphates and carbamates in Ojoo and Bodija, Ibadan, Nigeria. The inconsistencies in results from researches using the CDC bottle bioassay and the WHO bioassay may be due to differences in test methodology, sample size and geographical location which could influence test outcome.

Several research groups have documented that frequent and extensive use of an insecticide in either the agricultural sector or in public health could result in resistance [31, 32, 33, 34]. Since the inhabitants of the study area are mostly traders and subsistent farmers, it is likely that the measure of use of insecticides in crop protection was not enough to justify selection pressure on local anopheles and other factors responsible for resistance to carbamate insecticide.

In observation of the effectiveness of the tested insecticide, propoxur proved to be at the peak, this is evident in the low KDT values obtained (6.28 and 19.906). The hierarchy is followed by bendiocarb (9.12 and 23.442) and pirimiphos-methyl had the highest KDT values in minutes (13.091 and 46.773) for KDT_{50} and KDT_{95} respectively making them least effective and having a slower knockdown effect. This is in line with the report of Umar et al. who reported that propoxur was more effective among the carbamate group of insecticides [4]. The implication of this study is that any vector control programmes (IRS and LLINs) employing carbamate (bendiocarb and propoxur) would not be compromised and going by the result of this study, it discourages the application of pirimiphos-methyl insecticide in any vector control measures

to prevent failure that may arise due to resistance of the target vectors. Additionally, Organophosphate insecticides have a greater toxic residue in the environment and man [11, 14]. Concomitantly, more research is needed on carbamate insecticides to reduce their high toxicity level to enable them to be safer for human use following its high susceptibility profile. Moreover, more surveillance is needed to monitor vector resistance to insecticides in the study area.

List of abbreviations

CDC: Centres for Disease Control and Prevention
DDT: Dichloro-Diphenyl-Trichloroethane
HUH: Humidity, Uniformity, and Homogeneity
IRS: Indoor residual house spraying
KDT: Knock-down Time
LLINs: Long-Lasting Insecticidal Nets
RH: Relative Humidity
WHO: World Health Organization

5. Conclusion

The findings from the study revealed the susceptibility of *An. gambaie* to bendiocarb and propoxur insecticides (Carbamate) and resistance to pirimiphos-methyl insecticide (Organophosphate). Invariably, vector control decision-making in the area will be influenced by the result of the study being that it is a base line data/information. It is paramount that before any vector control strategies are implemented, sufficient susceptibility/ resistance data be taken into thought.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare there are no competing interests.

Consent for publication

All authors have read and approved the research article for publication.

Availability of data and materials

All the data supporting the results of this study are included in the article itself and a supplementary appendix has been attached as an additional file.

Authors' contributions

UE, NU, NI, and NE conceptualized the idea and designed the study. All authors participated in the research experimentation study. Data analysis was carried out by UE and NE while interpretation of the results was carried out by all authors. The original writing was carried out by all authors. NI, UE, NE, and KO revised, proofread and prepared the final manuscript. All authors read and approved the final manuscript. Supervision of the research was done by NU, and NE.

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