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Insecticide Susceptibility Profile of Malaria Vector Populations from the Coastal and Mainland Areas of Akwa Ibom State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IAA supervised the entire project, proof read and reviewed the entire manuscript. Authors NDE, MEA, BEB and MJA were involved in field work/ sample collection and wrote the literature review. Authors LPEU, PUI and LMS designed the study and performed the statistical analyses. All authors were jointly involved in the design of the work, laboratory analyses and writing the discussion and the first draft of the manuscript. All the authors were jointly involved in reading, and approved the final manuscript.

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ABSTRACT

Development of resistance by different malaria vector populations to insecticides has become a big threat to malaria vector elimination. This study evaluated the susceptibility of *Anopheles* mosquito populations in Akwa Ibom State, Nigeria to permethrin (0.75%), deltamethrin (0.5%), lambdacyhalothrin (0.5%), alphacypermethrin (0.75%), Dichlorodiphenyltrichloethane (DDT), propoxur, bendiocarb and pirimiphosmethylin in World Health Organization (WHO) test tubes following standard protocols. The mosquitoes were obtained as aquatic forms and reared under laboratory conditions to adults. The adults were subjected to WHO susceptibility bioassays following standard procedures. Malaria vectors across the study sites were resistant to permethrin,

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deltamethrin, lambdacyhalothrin and alphacypermethrin insecticides. Full susceptibility to propoxur and bendiocarb was recorded across the sites. Full susceptibility to pirimiphosmethyl was recorded in populations from three sites. Nevertheless, population of the malaria vectors collected from Oron was resistant to pirimiphosmethyl. KDT_{50} and KDT_{95} estimated for each insecticide using a log-time probit model revealed that knockdown was more rapid for deltamethrin, lambdacyhalothrin, alphacypermethrin, propoxur, bendiocarb and pirimiphosmethyl than for DDT and permethrin across the study sites. Morphological identification of all the mosquito samples used revealed that they were female *Anopheles gambiae* s.l. Sustained susceptibility of malaria vectors to pyrethriod is necessary for successful malaria control with insecticide treated nets and Indoor Residual Spraying (IRS). Emergence of focal points with insecticide resistance gives serious concern especially with the scale-up in distribution of pyrethriod treated nets to these areas. This may increase selection pressures due to overexposure. Further study to identify the exact resistance mechanism(s) of malaria vectors from these sites is recommended.

Keywords: Malaria; vectors; insecticides; susceptibility; KDT_{50}/KDT_{95}.

1. INTRODUCTION

Mosquitoes depict an almost worldwide scourge of humanity and undoubtedly cause the greatest suffering to both man and animals. They are capable of colonizing every conceivable type of water except fast flowing waters and rivers. Mosquitoes cause nuisance and can also kill by transmitting a number of diseases among humans and animals. They act as vectors for many deadly diseases such as malaria and filariasis in tropical and subtropical regions. Malaria which they transmit is the major public health problem in Nigeria, contributing a quarter of the malaria burden in Africa [1,2,3].

In Nigeria, up to 60% of out-patients attendance in health facilities and 30% of all hospital admissions are due to malaria. It is estimated that malaria is responsible for nearly 110 million clinical cases and an estimated 300,000 deaths annually [4,5]. A breakdown of the mortality rate indicate 25% infant mortality, 30% childhood mortality and 11% maternal deaths all attributed to Anopheles mosquitoes which spread the parasite responsible for the disease. Therefore, control strategies are needed to address this menace and curtail the death rate worldwide especially in malaria endemic countries such as Nigeria. Vector control is an effective way of reducing malaria transmission. It is a major component of the global strategy for malaria control, which aims to prevent parasite transmission mainly through interventions targeting adult *Anopheline* vectors [6].

Malaria control in Africa is mainly based on the use of indoor residual spraying (IRS) and insecticide-treated nets (ITN) with pyrethroid insecticides, these tools use insecticides from four chemical classes: organochlorines, pyrethroids, carbamates and organophosphates. Both interventions rely on the continuous susceptibility of *Anopheles* mosquitoes to a limited number of insecticides. Insecticide resistance by mosquito population to a vast range of carbamates, organophosphates, organoclorine and pyrethriod class of insecticides has been widely reported [7]. Nevertheless, data on insecticide resistance by adult population of mosquito is still very limited [8]. There is lack of information on the susceptibility status of Anopheles mosquito from the study sites to Pyrethriods, organophosphates and carbamate insecticides. This project is designed to address this lacuna.

2. MATERIALS AND METHODS

2.1 Study Sites

The study sites chosen for this work are the coastal areas of Oron and Itu as well as the inland areas of Mkpat Enin and Ikot Ekpene. Oron study location is between latitude 4.8246° North; longitude 8.2339° East. Oron LGA is bounded by Okobo, Udung Uko, Mbo, Urue Offong/ Oruko Local Government Areas and the Atlantic Ocean. Itu study location is between latitude 5.1745° North; longitude 8.05958° East and the LGA is bounded in the north by Odukpani LGA in Cross River State and Arochukwu in Abia State, in the West by Ibiono Ibom and Ikono LGAs. In the South and South-east, it is bounded by Uyo and Uruan LGAs, respectively. Mkpat Enin study location lies between latitude 4.7723° North; longitude 7.7385° East and the LGA is bounded by Ikot Abasi, Eastern Obolo, Oruk Anam, Onna and Etinan LGAs. Ikot Ekpene study location is between latitude 5.1752° North; longitude 7.7139° East and the LGA lies on the North-

Western flank of Akwa Ibom State, Nigeria. It is strategically positioned as an economic gateway of the State and a premier model Local Government administration in Nigeria.

2.2 Mosquito Larvae Collections

Water samples containing mosquito larvae and pupae were collected randomly and mainly from breeding sites, including gutters, ponds, small pools of stagnant water, muddy water, run-off from houses and irrigated vegetable farms. *Anopheles* larvae were identified from their horizontal position on the surface of the water while other species were identified by their angular position. They were carefully collected with a 350ml dipper (ladle) and transferred into 5000ml plastic containers, which were loosely capped to allow aeration. The water samples containing the aquatic stages of *Anopheles* mosquito were then transported to the Insectary/ Laboratory at the Department of Animal and Environmental Biology, University of Uyo, Nigeria and reared to adults.

2.3 Laboratory Rearing of Mosquitoes

The development of the larvae was monitored regularly and all those that pupated collected into shallow beakers using Pasteur pipettes, and then placed in appropriately labeled cages for adult emergence [9]. The larvae were fed with Oxford cabin biscuit and yeast while the adults were fed with ten percent (10%) sugar solution.

2.4 Insecticide Susceptibility Bioassay Tests

These tests involved the use of WHO insecticide susceptibility test kits with insecticide-impregnated papers. The test was carried out according to standard protocol outlined by WHO test procedures for insecticide resistance monitoring in malaria vector mosquitoes [10]. It involved the use of specially designed plastic tubes lined with insecticide impregnated papers including permethrin (0.75 percent), deltamethrin (0.05 percent), alphacypermethrin (0.75 percent) and lambdacyhalothrin (0.05 percent) papers. First, at least 150 non-blood-fed active adult female anopheles mosquitoes of 2-5 days-old were aspirated (in batches)from a mosquito cage into six holding tubes (prepared by lining the tube with clean sheets of white paper, 12 x 15 cm in dimension) to give six replicate samples of at least 25 mosquitoes per tube. The mosquitoes were allowed in the holding tube for one hour period of acclimatization. The content of four of the holding tubes were then transferred to four exposure tubes lined with a particular insecticide thereby forcing exposure to the insecticide for one hour [11]. Contents of the two holding tubes left were also transferred into two tubes labeled 'control experiments'. In this case, the mosquitoes were exposed to untreated papers impregnated with mineral oils for one hour also. During the exposure period, knock-down (KD) rates were recorded after 10, 15 20, 30, 40, 50 and 60 minutes, following the procedures of Bilali, et al. [12], and Niang et al. [13]. At the end of the I Hr. exposure period, the mosquitoes were transferred back to the holding tubes and kept there for 24 hrs. During this period, they were fed with 10% sugar solution; temperature as well as humidity were recorded and maintained at 27°C ± 2°C and 75% ± 10%, respectively [10]. At the end of the 24 Hrs. post-exposure period the number of dead mosquitoes were recorded. Abbott's formula was adapted for correction of cases where control mortality observed would have been between 5 and 20 percent. Abbott's formula:

$$\frac{(\%\ \text{test mortality} - \%\ \text{control mortality}) \times 100}{100 - \%\ \text{control mortality}}$$

Test results were to be discarded where control mortality was above twenty percent (20%) [14]. Each round of the test (conducted per site) involved four replicates each of the four different insecticides and two replicates for the control experiment. This implies that one set of control experiment was used for each round of the test involving four different insecticides. At least four hundred and fifty (450) non-blood-fed active adult female anopheles mosquitoes of 2-5 days-old were randomly sampled per site and subjected to this test at each round. The same procedure was repeated for all the four study sites selected within the study area.

In the end, the mosquitoes used for the tests will be preserved individually in Eppendorf tubes containing silica gel and labeled appropriately for identification.

2.5 Insecticide Susceptibility and Resistance Data Interpretation

The status of mosquito samples tested with the WHO tube tests was determined after twenty four hours (24 hrs) holding period according to the latest WHO criteria [10] as follows:

1. Mortality rates between 98 percent and 100 percent indicated full susceptibility.
2. Mortality rates between 90 percent and 97 percent required further investigation since these are described as suspected resistant populations.
3. Mortality rates < 90 percent, the population is considered resistant to the tested insecticide.

2.6 Identification of Anopheles Mosquitoes

After performing the bioassays, the mosquitoes used for the tests were preserved individually in Eppendorf tubes over desiccated silica gel and labeled with unique identification numbers for later identification. Morphological identification was done using morphological keys [15,16,17] and dissecting microscope (Olympus, USA).

3. RESULTS

Results presented in Fig. 1 shows that malaria vectors across the study sites were resistant to permethrin, deltamethrin, lambdacyhalothrin and alphacypermethrin insecticides (range of mortality: 55% - 71%). Full susceptibility to propoxur (100% mortality) and bendiocarb (100% mortality) were recorded across the study sites. Full susceptibility to pirimiphosmethyl (98% mortality) was recorded in populations from three study sites. Nevertheless, populations of the malaria vectors from Oron were resistant to pirimiphosmethyl (78% mortality).

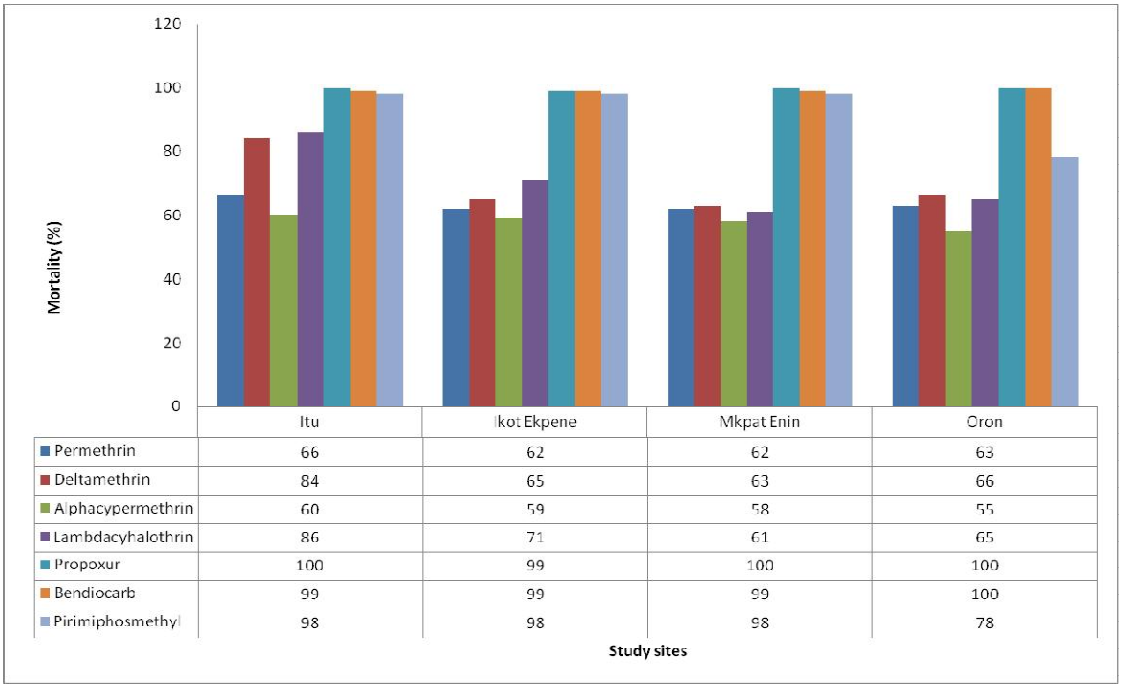
3.1 Knock down Effect

The results of knock down assessment determined over a one-hour exposure period of female anopheles mosquitoes from different study sites to eight different impregnated insecticide papers are presented in Figs. 2, 3, 4 and 5. Results indicated that in almost all the study sites knockdown was more rapid for deltamethrin, lambdacyhalothrin and alphacypermethrin followed by propoxur and was lowest in permethrin and DDT. However, in Oron study site, populations of vectors were seen to have been knocked down with propoxur as slow as was observed with permethrin and DDT. Across the study sites, knock down with DDT and permethrin was not more than 30% throughout the 60 min exposure period. Knock down was slowest with permethrin. Within the first 20 min of exposure, there was 0% knock down with permethrin. Exposure times which resulted in 50% and 95% knockdown (KDT_{50} and KDT_{95}) estimated for each insecticide using a log-time probit model (Table 1) indicated that KDT_{50} and KDT_{95} for permethrin ranged from 102.852 – 116.117 mins and 293.525 – 400.858 minutes, respectively. In deltamethrin where knock down seemed to be highest, KDT_{50} and KDT_{95} ranged from 17.494 – 31.247 minutes and 101.964 – 269.669 minutes, respectively.

4. DISCUSSION

Understanding the diversity of malaria vectors at local and regional levels is of utmost importance. According to Jan [18], not all female *anopheles* mosquito species are equal in vectorial capacity and susceptibility to chemical agents in their environment. In this present study, morphological analysis of the preserved mosquito samples showed populations of only *Anopheles gambiae s.l*. across the study sites. This malaria vector has been reported to be the principal vector of malaria in sub-Saharan Africa [16,19,20]. Although *Anopheles funestus* and *Anopheles gambiae* s.l had earlier been established as the major malarial vectors in Nigeria [21,22], this present study incriminated only *A. gambiae* s.l as the transmitters of malaria in the sites studied.

Sustained susceptibility of malaria vectors is necessary for successful malaria control with insecticide treated nets and IRS. Emergence of focal points with insecticide resistance gives serious concern especially with the scale-up in distribution of pyrethriod treated nets to these areas. This is because such scale-up may increase selection pressures due to overexposure. Long Lasting Insecticide Nets (LLINs) were deployed to Itu, Ikot Ekpene, Mkpat Enin and Oron LGAs for usage in protection against mosquitoes since 2010; and scale-up of the nets distribution in these areas has been implemented every four years, including the recent one carried-out in 2018. This implies that these pyrethriod treated nets have been in use till date in these areas. Previous studies revealed that use of LLINs could result in development of insecticide resistance in *Anopheles* mosquitoes to pyrethriods [9,23]. The possibility of these LLINs inducing changes in the adult mosquito populations thus contributing to the resistance status recorded in these study areas may not be ruled out.



	Itu	Ikot Ekpene	Mkpat Enin	Oron
Permethrin	66	62	62	63
Deltamethrin	84	65	63	66
Alphacypermethrin	60	59	58	55
Lambdacyhalothrin	86	71	61	65
Propoxur	100	99	100	100
Bendiocarb	99	99	99	100
Pirimiphosmethyl	98	98	98	78

Fig. 1. Bioassay results on susceptibility of malaria vector populations from different study sites in Akwa Ibom State, Nigeria, to pyrethriod, organophosphate and carbamate insecticides

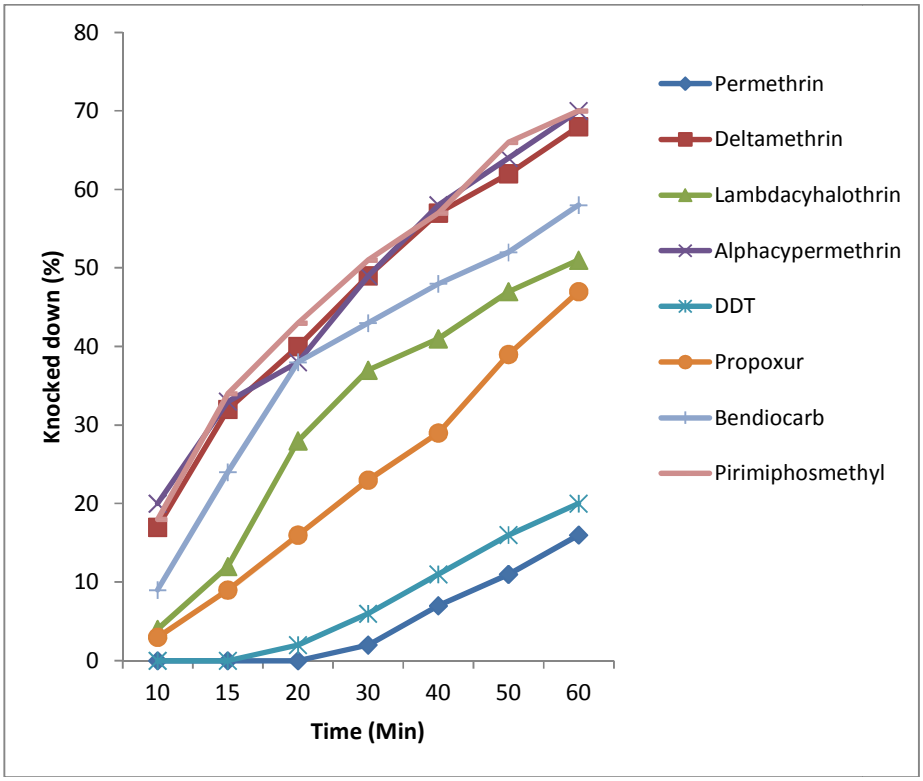


Fig. 2. Knock down rate of female *Anopheles gambiae* s.l. mosquitoes from Oron exposed to eight different insecticide treated papers

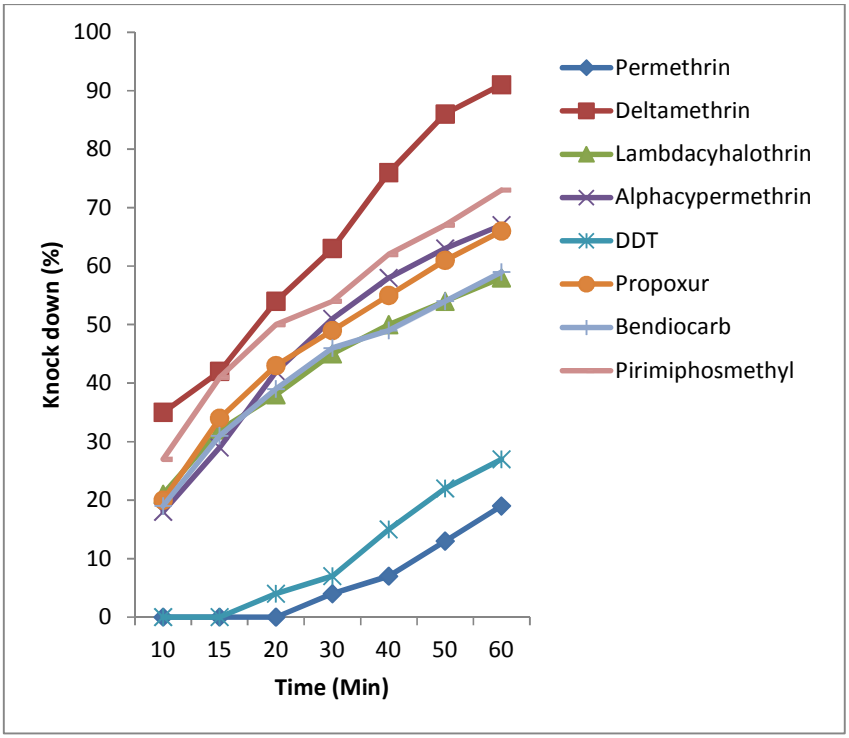


Fig. 3. Knock down rate of female *Anopheles gambiae* s.l. mosquitoes from Ikot Ekpene exposed to eight different insecticide treated papers

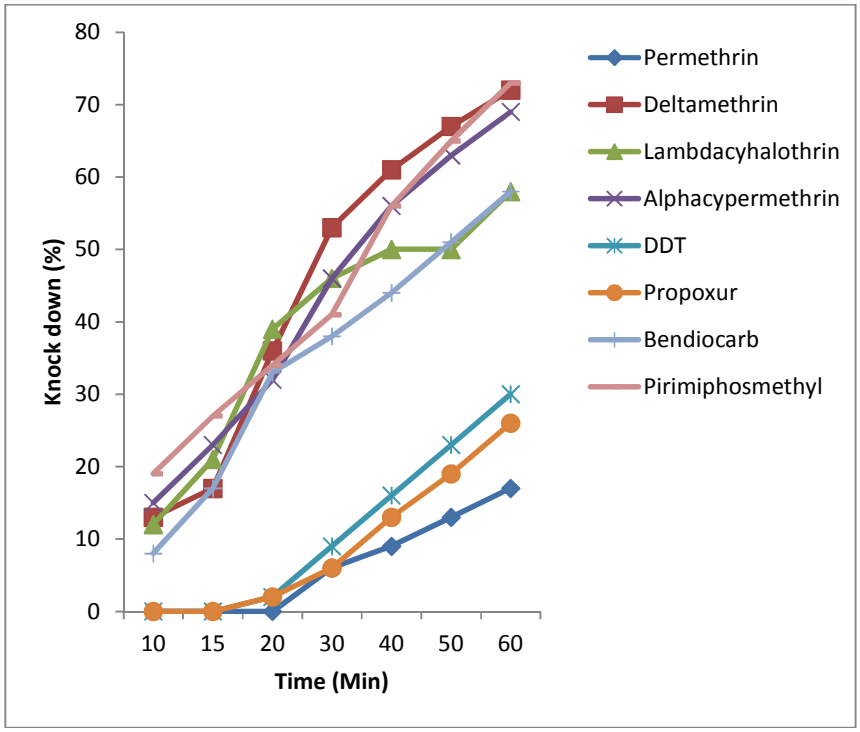


Fig. 4. Knock down rate of female *Anopheles gambiae* s.l. mosquitoes from Mkpat Enin LGA exposed to different insecticide treated papers

Table 1. Knock down times (KDTs) for *Anopheles gambiae* s.l. in the six different study sites after exposure to different insecticides

Study sites	Number exposed	Knocked down time (min)							
		Itu		Ikot Ekpene		Mkpat enin		Oron	
		KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)
Permethrin	100	108.298 (84.196-181.364)	335.884 (195.357- 1059.217)	102.852 (81.516-165.731)	293.525 (177.70-853.544)	116.117 (88.028-204.339)	400.858 (221.438-1400.261)	107.026 (83.192- 188.389)	294.706 (173.092-1012.503)
Deltamethrin	100	33.30 (27.257-41.935)	136.586 (88.309- 332.964)	17.494 (15.198-19.654)	101.964 (80.185-143.003)	31.296 (28.366-34.702)	158.971 (121.798-230.194)	31.247 (27.386-36.063)	296.669 (189.588-595.504)
Lambdacyhalothrin	100	31.192 (27.461-37.775)	423.493 (240.580-1081.435)	39.580 (32.827-51.166)	902.663 (396.045-4165.020)	41.616 (35.865-50.467)	444.064 (257.864-1069.139)	51.794 (44.965- 62.619)	384.073 (224.615- 679.636)
Alpha-cypermethrin	100	35.059 (31.516-39.504)	202.811 (147.448-320.544)	30.739 (26.958-35.390)	287.577 (185.243-568.715)	33.958 (30.323-38.530)	225.093 (158.50-375.880)	30.061 (26.326-34.606)	287.425 (184.545-572.315)
DDT	100	110.887 (85.497-175.337)	498.642 (276.571-1465.616)	92.604 (75.936-128.859)	331.826 (209.644-738.975)	83.094 (70.469-108.369)	261.108 (176.928-506.221)	112.676 (86.677-184.443)	425.973 (23.576-1305.137)
Propoxur	100	38.599 (32.992-47.040)	511.898 (279.594-1406.444)	31.146 (26.806-36.747)	410.805 (234.950-1035.854)	92.544 (76.166-129.467)	297.459 (190.741-662.888)	67.383 (56.890-85.970)	410.193 (256.005-855.631)
Bendiocarb	100	41.472 (35.154-51.748)	595.037 (310.607-1799.852)	39.118 (32.790-49.515)	745.430 (352.902-2848.273)	45.758 (39.859-54.769)	353.661 (224.475-711.894)	42.038 (36.380-50.663)	410.972 (245.325-935.389)
Pirimiphosmethyl	100	30.293 (26.436-35.042)	309.239 (194.057-644.142)	23.489 (19.760-27.384)	330.505 (194.771-797.061)	32.383 (28.756-36.898)	240.774 (165.109-421.978)	28.961 (25.383-33.187)	268.593 (175.161-519.989)

KdT_{50}: knock down time for 50% mosquitoes; KdT_{95}: knock down time for 95% mosquitoes; CL: Confidence limit

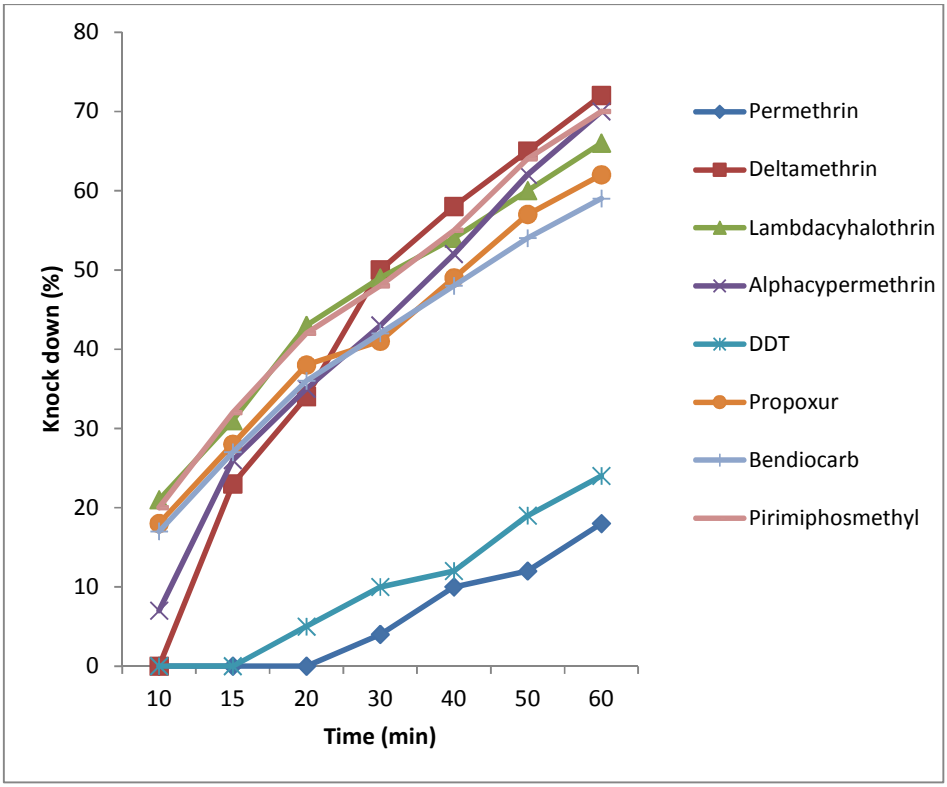


Fig. 5. Knock down rate of female *Anopheles gambiae* s.l. mosquitoes from Itu LGA exposed to eight different insecticide treated papers

This high level of resistance recorded was strongly corroborated by the trend in median knock down time (KDT_{50}) observed in the study areas. The carbamates, organophosphates and the type II pyrethroids (deltamethrin, alphacypermethrin, and lambdacyhalothrin) insecticides were more toxicity effective on the malaria vectors across the study sites than the type I pyrethriod, permethrin. This could be explained by the fact that the type II pyrethroids have a cyano group at the α-benzylic position (the α-carbon of the 3-phenoxybenzyl alcohol) and cause a pronounced convulsive phase that results in better kill because depolarization of the nerve axons and terminals is irreversible. In addition, the differing toxicity effects have been explained by the fact that the duration of modified sodium currents by type I compounds lasts only tens or hundreds of milliseconds, whilst those of type II compounds last for several seconds or longer. Higher KDT_{50} values in the field population have been suggested to give an early indication of the involvement of *kdr* mechanism of resistance [24,25]. The operational significance of resistance could be hinged on interplay between different resistance mechanisms in the vector population as ascribed by Awolola et al. [22]. Generally, these results are consistent with those of other studies conducted where *A. gambiae* s.l. has been reported to be resistant to pyrethroids [22] and DDT [26,27,28,29].

The development of resistance has also been linked to an increase in the activities of detoxification enzymes in mosquito populations that are challenged with environmental stressors in the breeding habitat [30]. Impact of the environment in this present study cannot be overlooked. Mosquito populations from this study sites may have developed detoxification enzyme machinery with abnormally high activities that allowed the aquatic stages of the mosquitoes to tolerate and thrive in this area. The results suggest that the population of mosquitoes in the study sites may have developed or are selectively being primed to develop resistance to insecticides. Several previous studies [31,32,33] have demonstrated the contribution of prior exposure to various environmental xenobiotics to the development of insecticides resistance by several insect species. In addition, other studies have also established a correlation between increase in tolerance to insecticides in many

insects and induction of detoxification enzymes as a result of prior exposure to environmental xenobiotics [34,35,36].

The organophosphates, carbamates and DDT are majorly deployed for Indoor residual Spray (IRS). This present study also revealed that pirimiphosmethyl-based insecticides may not effectively reduce the population of malaria vectors from Oron LGA as this study has revealed that the vectors in this study site have developed resistance to pirimiphosmethyl. It also indicated that the present populations of vectors across the study sites have developed resistance to DDT and as such, DDT-based IRS may not be effective in these areas. However, the results also showed that malaria vector populations across the study sites were susceptible to bendiocarb and propoxur insecticides. Consequently, vector control intervention employing any of these would be effective and successful since these will not be affected by resistance.

5. CONCLUSION

Given the growing threat of insecticide resistance, it is essential that up-to-date data on the magnitude and distribution of insecticide resistance be collected. This study was conducted to expand resistance monitoring to the coastal areas of Oron and Itu LGAs as well as the mainland areas of Mkpat Enin and Ikot Ekpene LGAs of the State. The present study presents for the first time, baseline data on the susceptibility status of *Anopheles* mosquitoes to pyrethriods, commonly used for bed nets treatment as well as carbamates and organophosphates from these study sites. This will guide in planning site specific integrated vector management project and programme.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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