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SUSCEPTIBILITY AND RESISTANCE STATUS OF MALARIA VECTOR TO PYRETHROID AND ORGANOPHOSPHATE INSECTICIDES IN ONDO STATE, SOUTH WEST, NIGERIA.

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Abstract

Malaria is a major infectious disease that still affects nearly half of the world's population. In malaria control programs, understanding local resistance dynamics is crucial for effective vector management. Information on the susceptibility of malaria vectors in the study area is needed to improve malaria control efforts. This study investigates the susceptibility and resistance patterns of malaria vectors to pyrethroid and organophosphate insecticides in Ondo State, Nigeria. The research involved collection of mosquito larvae from six different locations conveniently selected from three senatorial districts in Ondo State, Nigeria. Mosquito larvae were collected and taken to the Laboratory insectary and reared to adults, and sorted. The results obtained indicates the female *An. gambiae* s. l. was the most predominant malaria vector in the state (41%). A batch of 25 adult *An. gambiae* s. l per location were subjected to four insecticides for susceptibility testing using CDC WHO methods. The results show the Anopheles species were more susceptible to deltametrin 0.05%, (100%), alphacypermerin 0.05%, (95%), primiphos-methyl 0.25%, (99%), and compared to permethrin, 0.75% (73%), but the difference was not statistically significantly different p=0.12. Intensity assay conducted on mosquitoes resistant to x1 concentration of permethrin shows 100% susceptibility to x5 concentrations of permethrin except in Ipogun having 100% susceptibility with x10 concentration (p >0.005). The findings highlight a growing resistance to pyrethroids with some populations also showing reduced susceptibility to organophosphates an indication of need for continuous monitoring and to diversify vector control strategies to sustain the effectiveness of malaria efforts in the region.

Key words: Susceptibility and Resistance, Malaria Vector, Pyrethroid and Organophosphate Insecticides, Ondo State, Nigeria.

INTRODUCTION

Malaria is a major infectious disease that still affects nearly half of the world's population (WHO, 2021).

Sub-Saharan Africa still bears the highest burden of malaria morbidity and mortality worldwide despite improvements in the detection of the pathogens and large-scale deployment of vector control measures, such as Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) (World Malaria Report, 2020).

Malaria vector control has been the most effective and promising strategy for malaria control (Killeen *et al.*, 2017). Control of malaria vectors has been an important strategy to prevent the disease, due to the no availability of an efficacy malaria vaccines (Alonso, 2021).

Information on spatial distribution of malaria vector species is needed to improve malaria control efforts generally.

The main malaria vectors in Sub-Saharan Africa are *Anopheles gambiae*, *An. coluzzii* and *An. arabiensis*. Amongst these, the two major species in West and Central Africa are *An. gambiae* and *An. coluzzii*, which occurs in sympatry (Koukpo *et al.*, 2019).

The potential distribution of *Anopheles gambiae* sensu lato and its siblings: *Anopheles gambiae* sensu stricto, and *Anopheles arabiensis* species occurrence in Nigeria from the data collected during the period 1900–2010 together with 19 bioclimatic, landuse and terrain variables shows that these species are currently widespread across all ecological zones where temperature fluctuation from mean diurnal temperature range, extreme temperature and precipitation conditions, high humidity in dry season from precipitation during warm months, and land use and land cover dynamics have the greatest influence on the current seasonal distribution of the *Anopheles* species (Godwin *et al.*, 2018).

In 2016, ninety-one (91) countries and territories in the world had ongoing malaria transmission with estimated 216 million cases of malaria and 445,000 malaria deaths (WHO, 2017). Fifteen countries accounted for 80% of all malaria cases and deaths globally. Sub-Saharan Africa region was home to 90% of malaria cases and 91% of malaria deaths, globally. Nigeria accounted for the highest proportion of cases globally (27%), followed by the Democratic Republic of the Congo (10%), India (6%) and Mozambique (4%) (WHO, 2017).

Malaria control relies heavily on the use of insecticide to reduce vectors populations however, the increasing resistance of malaria vectors to commonly used pyrethroid and organophosphate insecticides threatens the efficacy of these interventions (WHO, 2021). Globally 15 countries
still account for 80% of all malaria cases and deaths and Nigeria accounts for the highest proportion of malaria cases globally. All these still make malaria an important public health problems in Nigeria and presently in Ondo State.
There is decrease incidence of malaria cases in Nigeria as a whole but the trend in Ondo State is still on the increase from 2000 to 2021 report (WHO, 2022). In Ondo State, there is limited data on the current resistance and susceptibility status of malaria vectors, which is crucial for updating and refining control strategies (Smith *et al.,* 2022).
Understanding resistance patterns in this region is essential for designing targeted interventions and enduring the sustainability of malaria control efforts by providing up to date information on insecticide susceptibility and resistance.
It is against this background that this present study was hereby embarked upon to determine the susceptibility and resistance status of Anopheles mosquitoes to insecticides in the State.
This work will contribute to more effective vector control elimination goals and also to provide a more robust updated data on the distribution and susceptibility status of *An. gambiae s.l.* species, a major malaria vector in Nigeria. It will also provide information on the strength and synergy detected in pyrethroid resistant of *An. gambiae s.l.* mosquito in Ondo State, Nigeria.

Materials and methods

Study Design

The collection of mosquito larvae was conducted from January to July, 2004 in six different sites across Ondo State in Nigeria. Larvae of *Anopheles* were collected using standard method as described by Service (1977). Larvae of mosquito were collected from both natural and artificial habitats. They were reared to adult in the laboratory

Study Site

The study was carried out in Ondo State which falls within the latitude and longitude approximately 7.100005 and 4.841694. The sites were conveniently selected due to security situations in the country from each senatorial district in Ondo State, Nigeria (Ondo Central, Ondo West and Ondo East). The two sites from each senatorial district (one urban and one rural settings).

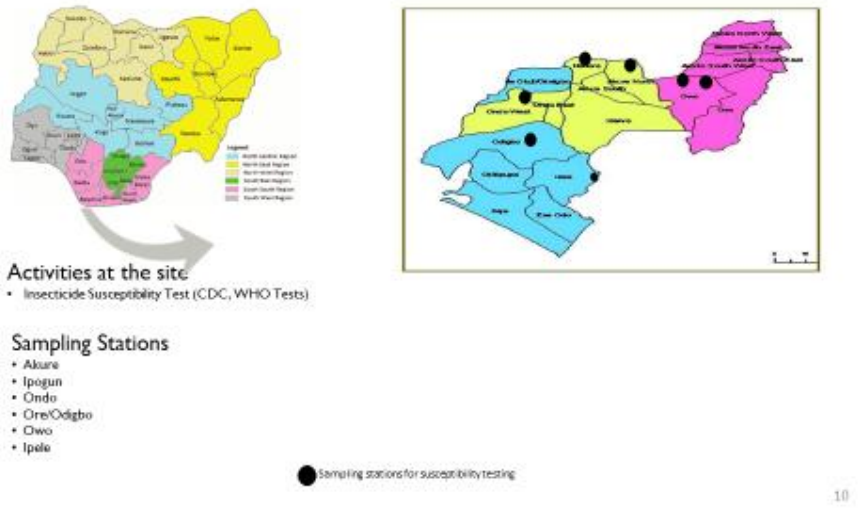

Figure 1: Map of Nigeria showing Ondo State and the study location within the States

Mosquito Larval Collection and Rearing

The larvae of *Anopheles* were collected from (between 9am in the morning to 12 noon) from the natural breeding sites according to method described in (Service, 1977). All conducive habitats were surveyed for the presence of mosquito larvae, different areas in the surveyed locations were sampled by using the "dipping" technique has detailed by Service, 1993. Larvae of mosquito were collected from both natural and artificial habitats. They were reared to adulthood under standard insectary condition (26–27 °C and 76–82 % relative humidity) in the larval bowls and fed with yeast daily, adult emergence were later transferred into the adult cage and fed with 10% sugar solution (sugar solution soaked in cotton wool) in the laboratory insectary.

Morphological Identification of Mosquito

The adult specimens were identified using systematic keys and catalogues. Identification of mosquito was by morphological differentiation as detailed by (Gillies and Coetzee, 1987). Anopheline mosquitoes was identified with their speckled legs, hind tarsus 4-5 entirely pale, abdominal segments without laterally projecting sufts of scales, 3 bands on the palps, 3rd main dark area of vein 1 with pale interruption and scaling on the abdomen on vein 7 and 8. **Insecticide Susceptibility Test**

Insecticide susceptibility tests with 1X diagnostic concentration were conducted on 976 adult female *Anopheles* mosquitoes (2-3 days old) using the standard WHO procedures (WHO, 1998). Each insecticide (permethrin and deltamethrin, alphacypermthrin and primiphos-methy) 25 mosquitoes were exposed to the insecticide impregnated tests papers with permethrin (0.75 %) and deltamethrin (0.05 %, alphapermethrin 0.05% and primiphos-methyl 0, 25%). Controls exposure to untreated papers were included. The knockdown effect of each insecticide was recorded every 10 minutes over the one-hour exposure period and final mortality recorded for 24 hours post exposure. The number of mosquito knockdown over the one-hour period was thereafter used to calculate time taken to knockdown 50 % of the population (Kdt_{50}) and time taken to knockdown 95 % of the population exposed (Kdt_{95}). All batches of insecticide-impregnated paper used has been pre-tested on the Kisumu laboratory strain of *An. gambiae* (Kisumu strain), which is known to be susceptible to pyrethroids (WHO, 2016).

Insecticide Intensity Assay

Based on the results of the susceptibility tests with 1X standard diagnostic concentration, intensity test using both 5X and 10X concentrations of permethrin was carried out on all the mosquitoes having percentage mortality less than 95 % (standard for susceptibility) with permethrin across all the six locations in the States(WHO, 2016). Mosquitoes were exposed for 30 minutes as described in the CDC bottle bioassay manual in the insectary for a further 24 hours before mortality was recorded.

Synergy Assay

A total of 25 female *Anopheles* mosquitoes were pre-exposed to 4 % piperonyl butoxide (PBO) for 24hrs. PBO-treated mosquitoes were exposed to permethrin (0.75 %) across the six locations. Knockdown rates of mosquitoes were recorded at intervals for one hour, then supplied with 10 % sugar meal for 24-hours recovery period after which mortality was recorded. All the bioassays were accompanied by negative control (WHO, 2016)**.**

RESULTS

Table 1: Average weather values from January-June, 2024

Locations	Akure	Ipogun	Ondo	Ore/Odigbo	Owo	Ipele
Temperature (^{o}C)	27.5	28.0	27.6	28.0	27.0	27.7
Pressure (%)	8.75	8.75	8.33	4.0	7.5	8.7
Humidity (%)	82.0	83.5	84.6	79.0	85.3	83.5
Wind speed (km/hr)	10	11	10.7	10.7	8.3	8.3

Table 1 shows the weather temperature conditions recorded in all the locations ranges from 27.0 ^{o}C in Owo to 28.0 ^{o}C in Ore /Odigbo and Ipogun in the State (Total average = 27.6 ^{o}C) while the average pressure, humidity and wind velocity are 7.6%,82.9% and 9.8km/hrs respectively.

Table 2: Distribution of mosquitoes collections from locations

Mosquito types	Akure	Ipogun	Ondo	Ore/Okigbo	Owo	Ipele	Total
Anopheles	138(17.6)	152(19.1)	109(13.6)	120(15.6)	159(19.9)	118(14.8)	796(40.6)
Culex	160(17.3)	154(16.6)	153(16.6)	152(16.5)	163(17.7)	139(15.1)	921(47.0)
Aedes	72(29.7)	48(19.8)	50(20.6)	22(9.1)	38(15.7)	12(4.9)	242(12.4)
Total	270(13.7)	354(18.1)	267(13.6)	294(15.0)	360(18.4)	269(13.7)	1959(100)

Table 2 shows the total number of 1,959 female mosquitoes collected out of which *Anopheles* species constituted seven hundred and ninety six (41%) and Culex and Aedes had 47% and 12% respectively.

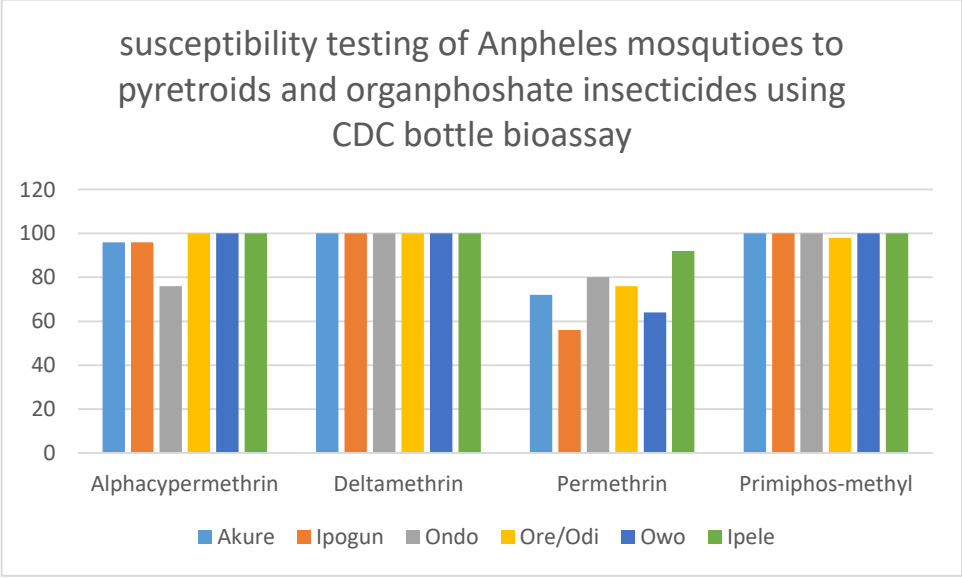

Figure 2 Susceptibility testing using CDC bottle bioassay

In this study the effect of the four insecticides used on the adult female Anopheles mosquitoes shows alphacypermethrin had a susceptibility of 96% in Akure, 76% in Ondo and 100% in Owo, Ore/Odigbo and Ipele($p>0.005$). Deltermethrin showed 100% susceptibility to all the insecticides from all the different

sites locations. The susceptibility of the *Anopheles* to permethrin shows a total resistance ranging from 52% in Ipogun to 92% in Ipele (p >0.005). The resistance and susceptibility range in Pirimiphos- methyl the only Organophospate insecticide was not significant (p < 0.07).

Anopheles species were more susceptible to deltametrin 0.05%, (100%), alphacypermerin 0.05%, (95%), primiphos-methyl 0.25%, (99%), and compared to permethrin, 0.75% (73%), but the difference was not statistically significantly different p=0.12

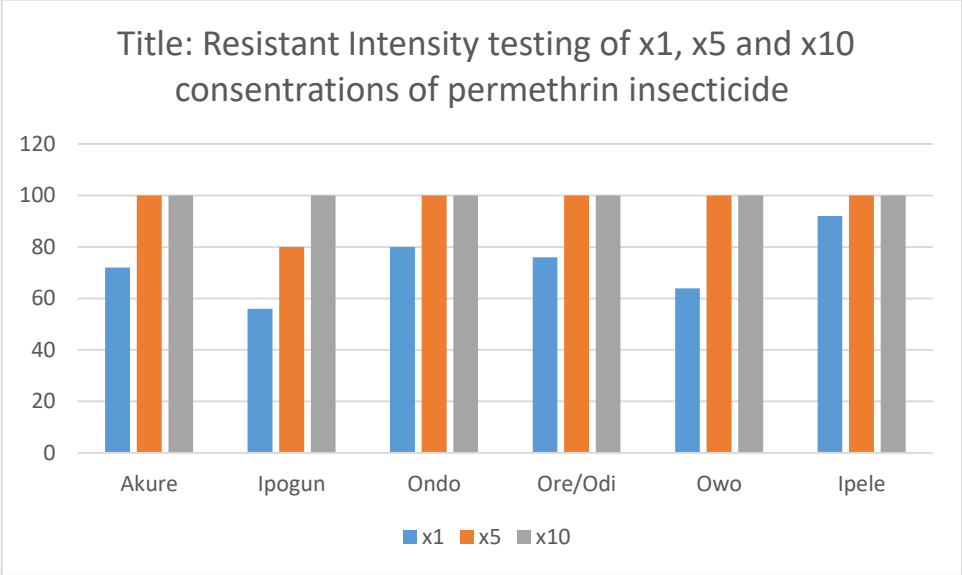

Figure 3: Resistant intensity testing of x1, x5 and x10 concentrations in the study areas
Intensity assay results on all mosquitoes resistant to X1 concentration of permethrin using X5 and X10 concentrations shows that there is significant difference in all the mosquitoes collected from all the locations with 100% susceptibility to x5 concentrations of permethrin with exception of Ipogun which only had 100% susceptibility to x10 insecticide concentration (p >0.005) as shown in figure 3.

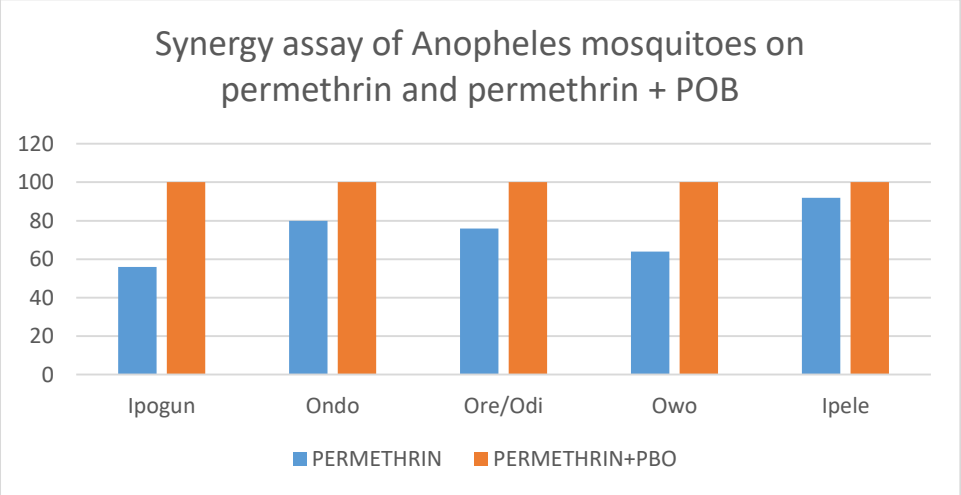

Figure 4: The Synergy assay conducted using POB plus Permethrin insecticide produced a significant result of 100% (p > 0.005).

DISCUSSION

This research work recorded cumulative average values of the temperature,

humidity, precipitation and velocity of winds of the study area was indicative of an environment conducive for mosquitoes. This is also in agreement with some studies conducted in the country and other African countries (Ajayi *et al.,* 2011, Godwin *et al.,* 2018 and Adedapo *et al.,* 2019).

Out of the total 1959 mosquitoes collected during the period of the study, it about 41% of these population were of the genus Anopheles, this might be one of the reasons for the high incidence of malaria report of WHO in 2021 in Ondo State. This is an indication of the availability of the malaria vector in the State (WHO, 2021). Also the abundance of this vector is in agreement with study carried out by Olabimbo *et al.*(2023) in Okitipupa, Ondo State and those carried out in other States in Nigeria (Awolola *et al.,* 2018; Adedapo *et al.,* 2019; Oduwole *et al.,* 2020).

The mosquitoes studied were seen to be highly resistance to insecticides. The susceptibility to permethrin ranges from 56-93%. This is a serious issue because this insecticide is the only one approved by WHO for bed net used during vector control programs.

The CDC bioassay revealed different levels of resistant and susceptibility of the *An. gambiaes s.l.* vector to different insecticides tested, alphacypermetrin was very susceptible in all the locations but shows resistance in Ondo township, this a new discovery in the State. The susceptibility and resistance ratio is statistically significant p >0.005.

Deltamerin insecticides had 100% susceptibility on all the mosquitoes and is an indication that this insecticide can be recommended for use generally in the State for the control of mosquitoes. Permethrin had a very high resistance rate in all the locations in the state (p >0.005), this was also discovered by Olabimbo *et al* in 2023 when he carried out a similar study in Okitipupa in the State, this is an indication that these insecticides must not be used in the State for control program. Primiphos-methly was seen to be very susceptible with resistance observed in Ore/ Odigbo area p-value=0.07. Furthermore, this study observe that the Anopheles mosquitoes were more susceptible to deltametrin 0.05%, this (100%), alphacypermerin 0.05%, (95%), primiphos-methyl 0.25%, (99%), and less susceptible to permethrin, 0.75% (73%), but the difference was not statistically significant, p=0.12. this results is very related to previous studies reported by Idowu et al., 2019 this is slightly different from the study of Olabimbo et al., 2023. The very high insecticides resistance is not unconnected to the farming activities and exposure to high agricultural chemicals in these locations. Resistance shown in this study by *An. gambiae* s.l. to the Pyrethoids tested agreed with previous reports (Awolola *et al.,* 2018; Adedapo *et al.,* 2019; Olabimbo *et al.,* 2023).

The resistance intensity rate to permethrin of 44% and 56% resistance observed in Ipogun in Ifedore Local Council area of the State is an indication of the total failure of the insecticide and must not be considered for use even in the entire State, p-value=0.002 .

The combination of PBO and permethrin was synergistic resulting in 100 mortality, this is a clear indication of the presence of cytochrome enzyme P450 which is known of playing detoxification effects on insecticides and therefore rendering them inactive to perform the desired effect on the mosquitoes. This hereby gives a direction on the type of insecticide combination to be used for vector control program in the State. This result can also be used to influence appropriate health policy in malarial control programme for capacity building on the type of trainings and the direction of advocacy to the public.

CONCLUSION

In conclusion this study present information on the distribution of female *Anopheles gambiae* s. l., predominance in Ondo State accounting for about 41% of the total mosquitoes collected and can be regarded as the local vector in the State that is responsible for the perennial transmission of malaria parasite disease in the State. The susceptibility to permethrin ranges from 56-92% in this study and the resistance intensity rate was at 44%. The most resistance was observed in Ipogun at 56%. The effect of piperonyl butoxide (PBO) and permethrin insecticides on mosquitoes resulting in 100 mortality indicated the presence of cytochrome enzyme P450 and possibly other enzymes, thereby confirming that the mosquitos' resistance profiles are not due to biological mutations.

Recommendations

The level of resistance to WHO approved insecticides is a threat to malaria control programs therefore, mosquito larval control should be used for supplementing pyrethroid-treated bed net in planning vector control activities in Ondo State.

This study demonstrated that Deltamethrin insecticide is greatly susceptible and it is hereby recommended for use in Ondo State.

Authors Contributions

OOA considered, designed and supervised the work, AMB developed the laboratory protocol, data analysis and wrote the draft of the manuscript, AJO and AFG did the field trips. SSD worked on and concluded the data analysis. OOA, OO and HAO review the manuscript. All authors read and approved the final manuscript.

Acknowledgement

We express our deep appreciation to community leaders who consented and permitted us to enter their environments to use for the collection of mosquitoes larvae and particularly my students that collected samples from their hostels in the Achievers University in Owo, Ondo State. Particularly our collaborators from the Malaria vector research team in the Nigerian Institute of Medical Research Yaba, Lagos, Nigeria.

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