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RESISTANCE STATUS OF *Anopheles gambiae s.l.* TO PUBLIC HEALTH INSECTICIDES AND PIPERONYL BUTOXIDE SYNERGIST IN MANGROVE VEGETATION OF RIVERS STATE, NIGERIA

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ABSTRACT – Present status of resistance, the susceptibility pattern to different insecticides by *Anopheles gambiae s.l.* and the efficacy of PBO-LLINs insecticides were investigated in the mangrove vegetation of Rivers State, Nigeria. Sample collection was done during the rainy season. Standard dipping method was used for larval collection. Mosquitoes tested were *Anopheles gambiae sensu lato.* Technical grade insecticides were used which included Organochlorine (Dichlorodiphenyltrichloroethane), Carbamates (Propoxur, Bendiocarb), Organophosphate (Primiphos-methyl) and Pyrethroids (alpha-cypermethrin, deltamethrin, permethrin, lambda-cyhalothrin). 25-three to five days old, non-blood-fed female adults were introduced into each of the four test and control bottles. Record was made of the number of dead and living mosquitoes at 15, 30, 35, 40, 45, 60, 75, 90, 105, 120 minutes. Piperonyl butoxide (PBO) synergist bioassay was carried out using standard methods. Percentage mortality of *An. gambiae s.l.* to bendiocarb and propuxur showed susceptibility (98% - 100%), in accordance with World Health Organization (WHO) recommendations. There was partial recovery of susceptibility against *An. gambiae s.l.* with PBO-permethrin and PBO-deltamethrin synergists. Analysis of variance was used to test for statistical differences in mosquito mortality to the insecticides used. Propoxur and bendiocarb are effective for indoor residual spraying (IRS). Recovery of susceptibility by PBO-pyrethroids makes them recommended over pyrethroids alone, for LLINs impregnation. The study makes available baseline information for monitoring the status of insecticide resistance in the mangrove ecological region of Rivers State, Nigeria.

Keywords: Anopheles gambiae s.l., insecticides, mortality, mangrove, piperonyl butoxide, resistance, Rivers State, Nigeria

INTRODUCTION

Malaria has for a long time plagued humans, most especially in the tropical world. Its plague has ranged from causing high level morbidity, mortality and severe poverty among the populace in its endemic regions. The African continent has been the most affected among the endemic regions in the tropics while

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Nigeria has topped the chart of the highest morbidity and mortality from malaria disease in the Africa sub-region WHO (2020). It is a disease of public health concern with an estimated 241 million cases and 627 000 deaths worldwide in 2020 (WHO, 2021).

In attempt to reduce the mortality, morbidity and poverty caused by malaria disease, a number of control measures aimed to reduce the population of the vectors of malaria disease has been tried. Some have been successful while others are still being experimented. These malaria vector control measures include: physical, cultural, genetic, biological and chemical methods. Public health insecticides as the name implies are insecticides that are used for the health interest of the public. Two major types of insecticides are used in public health, these include: residual insecticides which are stable, organic chemicals when applied to a surface. They remain toxic for sometimes, usually several months to insects resting on that surface. Examples of residual insecticides include organochlorines, organophosphates, carbamates. Non-residual insecticides do not possess a lasting effect and are unstable in light and air. Example is pyrethrum which is quick-acting with a knock down effect. It can be used as dusting powder, atomised space spray or in slow-burning coils which produce insecticidal smoke (WHO, 2020).

Biological and chemical methods have been applied in recent times in the control of malaria vectors. Such methods include the use of microbial larvicides and insect growth regulators in larviciding campaigns Ekerette and Ebere (2017; 2018), the use of chemical insecticides such as organochlorines, organophosphates, carbamates and pyrethroids, in indoor residual spraying (IRS) and pyrethroids in impregnation of long-lasting-insecticidal nets (LLINs).

Overtime, these chemical insecticides have been useful in the control of vectors of malaria including *An. gambiae s.l.* but in recent times, *An. gambiae s.l.* (with strains including *An. gambiae sensu stricto, An. arabiensis, An. melas/An. merus, An. coluzzi* and *An. quadriannulatus*) have been found to build-up resistance to some of them. Resistance to some WHO-approved public health insecticides by *An. gambiae s.l.* have been reported in some parts of Nigeria, these include: Ikot Ekpene, Akwa Ibom State, Oduoha-Emuhua and Port Harcourt, both in Rivers State, Oyo town, Oyo State, Misau, Bauchi State and Auyo in Jigawa State Opara et al. (2017); Ebere (2015); Ebere and Nwakama (2016); Adeogun et al. (2017); Umar et al. (2014); Abdu et al. (2017). In Africa, there have been records of resistance to insecticides by *An. gambiae s.l.* in Cameroon, Kenya, Tanzania, Cote d'Ivoire, Benin and Mali, Boussougou-Sambe et al. (2018); Omondi et al. (2017); Ochomo et al. (2015); Okara et al. (2010); Protopopoff et al. (2013); Kisinza et al. (2017); Camara et al. (2018); Yahouedo et al. (2016); Cisse et al. (2015).

The built-up resistance by *An. gambiae s.l.* to these chemical insecticides keeps rising overtime Ebere (2010; 2013; 2015); Protopopoff et al. (2013); Kisinza et al. (2017). These resistances put up by vectors of malaria to these insecticides have caused a colossal challenge in the control of malaria vectors. There is need for a regular routine monitoring of these gradual rising resistance in the *An. gambiae s.l.* to the few available public health chemical insecticides that have been approved by World Health Organization (WHO) Loroño-Pino et al. (2013). The lack of routine monitoring has resulted to a sudden realization of the rapid rise in the resistance to the insecticides across Africa, without adequate measures in place to tackle and manage such rise in resistance. According to Ebere and Nwakama (2016), early detection of insecticide resistance enables more rational selection of insecticides or may enable timely introduction of resistance management strategies.

The resistance management strategies could include, to find an alternative insecticide to the one that has become resistant by the *An. gambiae s.l.* so that the mosquito will again become susceptible to it or combine the insecticide with another chemical that will enhance its toxicity to a level that will cause the malaria vectors to become susceptible to the insecticide again.

This kind of combination is being used in piperonyl butoxide (PBO) synergist with pyrethroids. This has become necessary since the LLINs insecticides: permethrin and deltamethrin, both, of the pyrethroid class, had become resistant in parts of Nigeria and Africa, Nwankwo et al. (2017); Okorie et al. (2015); Awolola et al. (2014); Venter et al. (2017). The use of LLINs are known to provide important health benefits such as increase in protective efficacy, reduction in malaria incidence and child mortality in malaria endemic countries in sub-Saharan Africa, Matowo et al. (2015). Their synergist action with PBO has raised the susceptibility level of *An. gambiae s.l.* Incorporation of PBO synergist in LLINs, has been reported to restore the efficacy of pyrethroids in areas with high pyrethroid resistant population Kweka et al. (2017a); N'Guessan et al. (2010). The LLINs with PBO have proved to be safe and with no side effect for human beings (Beamand et al. (1996); Horton et al. (2011); Kweka et al. (2017b); Macedo et al. (2010). Therefore, there is need to monitor the susceptibility status of PBO synergist action with these pyrethroids: permethrin and deltamethrin, because currently, only pyrethroids are recommended by the WHO for use on mosquito nets, and alternatives are urgently required as insecticide resistance is compromising the performance of pyrethroid-only treated nets N'Guessan et al. (2007); Toe et al. (2014).

This research aims to come out with data on the current status of resistance of *An. gambiae s.l.* to public health insecticides in the mangrove vegetation and to investigate the level of effectiveness of the following LLINs synergists: PBO-permethrin and PBO-deltamethrin, on *An. gambiae s.l.*, in the mangrove vegetation of Rivers State, Nigeria.

MATERIALS AND METHODS

Study Area

Mangrove vegetation occupies the Niger delta region of Nigeria which comprises Rivers State and others and lies between latitude 4'45"N and longitude 6'50"E (Figure 1). The vegetation is bound by the Atlantic Ocean. Average temperatures are between 25 °C and 28 °C (77 °F and 82 °F). There are typically two seasons in the area: dry season from November to April and rainy season from May to October, with a short break in August. Fishing and subsistence farming are the main occupations in the area.

Mosquito Sampling

Potential breeding sites of *Anopheles* mosquitoes were sampled from May 2019 to December 2019 and also from May 2020 to October 2020. This was to ensure that sample collection was done in the peak of the rainy season, when there is abundance of larvae. Standard dipper (400ml) with one (1m) handle was used for larval collection from different breeding sites, WHO (2015) using the standard dipping method. The various sampling sites included borrow pits, vehicle tyre imprint, footprints, temporary sunlit pools, ditches in construction sites, puddles formed alongside lakes, drains, rain puddles, ponds and stagnant waters, among others. The various breeding sites from which samples were collected is shown on Figure 1.

Rearing of Mosquito Larvae

Larvae from the different breeding sites, were pooled together, kept alive and were placed in loosely capped plastic containers after which they were transported to the insectary of Malaria Vector Surveillance and Insecticide Resistance Monitoring Laboratory of the Department of Animal and

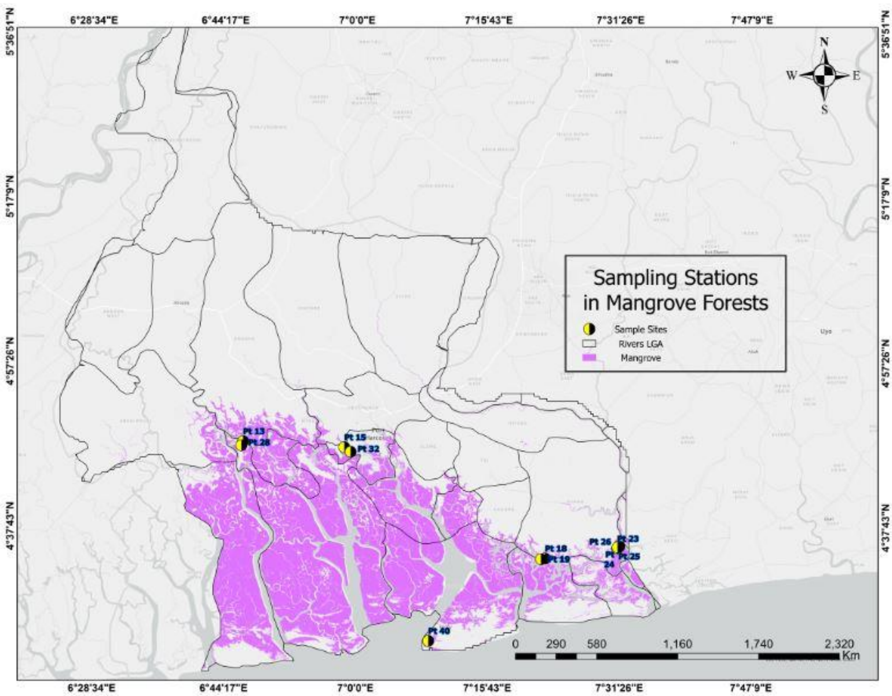

Figure 1. Map of Rivers state showing the mangrove vegetation zone and sampled breeding sites of *An. gambiae s.l.*

Environmental Biology, Rivers State University, Port Harcourt, Nigeria. The rearing of the larvae to adult following the methods of Gerberg et al. (1994) was carried out at the laboratory. The *Anopheles* larvae were put into plastic containers holding de-chlorinated water. The containers were covered with nets fastened with elastic bands and placed on platforms containing water below, to prevent crawling insects from invading the larvae. The larvae were fed with ground biscuits every two days and monitored till adult emergence. Newly emerged adults were separated into females and males. Adults were kept in screen cages and fed on 10% glucose solution provided continuously. Cages were held at 26°C - 29°C and 74% - 82% relative humidity. Only adult female mosquitoes were used for the test. The parental generation larvae as was collected from the field was used in the bioassay.

Morphological Identification of An. gambiae s.l.

Members of *An. gambiae s.l.* were morphologically separated from other anopheline mosquitoes using the morphological identification keys of Gillies and De-Mellion (1968) and Gillies and Coetzee (1987).

Insecticide Susceptibility Bioassays

Insecticide susceptibility bioassay was carried out using the standard Centers for Diseases Control and Prevention (CDC) protocol CDC (n.d.). The technical grade insecticides were supplied by the National

Malaria Elimination Programme (NMEP). The following technical grade insecticides were used: Organochlorine (Dichlorodiphenyltrichloroethane, DDT 100µg/ml), Carbamates (Propoxur 12.5µg/ml, Bendiocarb 12.5µg/ml), Organophosphate (Primiphos-methyl 20µg/ml), Pyrethroids (Alpha-cypermethrin 12.5µg/ml, Deltamethrin 12.5µg/ml, Permethrin 21.5µg/ml, Lambda-cyhalothrin 12.5µg/ml). The bioassay was performed with the Wheaton bottles (250ml). For the insecticide susceptibility bioassay, 3-5 days old non-blood fed female adult mosquitoes were used. Using an aspirator (length-60cm: half of it made of glass and the other half made of rubber, diameter-1cm), 25 mosquitoes were introduced into each of the four test bottles and the control bottle.

Record was made of the number of dead and living mosquitoes, at 15, 30, 35, 40, 45, 60, 75, 90, 105 and 120 minutes. It was not necessary to continue the bioassay beyond 2 hours. The data were recorded on the report form. A graph of the total percent mortality (Y axis) against time (X axis) for all replicates considered together was made using a linear scale.

There was no mortality in the control bottle after 2 hours (end of the bioassay). Consequently, there was no need to use Abbott's formula to correct results. Mosquitoes are considered dead if they can no longer stand. Mosquitoes that were alive at the end of the 120 minutes diagnostic time represented resistant mosquitoes to the insecticide being tested. The bottles were gently rotated while taking the count. Immobile mosquitoes that slide along the curvature of the bottle were easily categorized as dead. The number of dead mosquitoes were counted in the first readings of the bioassay, while the number of living mosquitoes were counted when few remained alive. In the end, the percentage of dead mosquitoes at the diagnostic time (dead mosquitoes/total of mosquitoes in the assay) is the most important value in the graph CDC (n.d.).

Piperonyl Butoxide (PBO) Synergist Test

PBO (100µg/ml) synergist bioassay was carried out using the standard Centers for Diseases Control and Prevention (CDC) protocol CDC (n.d.). Equal numbers of about 125 mosquitoes in each bottle was introduced into the synergist-control bottle and into the synergist-exposed bottle; the mosquitoes in the bottles were kept for 1 hour to allow the reaction of the synergist. After the 1-hour exposure was completed, the mosquitoes were transferred to two holding cages, one for the synergist-control mosquitoes and another for the synergist-exposed mosquitoes. This made it easier to transfer mosquitoes into the insecticide-treated bottles. The CDC bottle bioassay was performed using one set of insecticide-coated bottles (one control and four test bottles) for the synergist-control mosquitoes and another set (one control and four test bottles) for the synergist-exposed mosquitoes; the data for the two populations of test mosquitoes were compared.

Statistical Analysis

Two-way analysis of variance was used to test for significant differences in mosquito mortality to the insecticides. Significance was taken at a probability level of <0.0001. Tukey's multiple comparison test was used for mean separation.

RESULTS

Susceptibility of the Insecticides in Mangrove Vegetation

The results obtained showed that at 60 minutes (mid-time in the bioassay), percentage mortality of *An. gambiae s.l.* to the insecticides were: bendiocarb-99, propoxur-100, alpha-cypermethrin-72, lambda-cyhalothrin-71, dichlorodiphenyltrichloroethane-21, deltamethrin-76, primiphos-methyl-29 and permethrin-74, while at 120 minutes (end of bioassay), percentage mortality of *An. gambiae s.l.* to the insecticides were: bendiocarb-99, propoxur-100, alpha-cypermethrin-75, lambda-cyhalothrin-72, dichlorodiphenyltrichloroethane-48, deltamethrin-80, primiphos-methyl-34 and permethrin-82 (Figure 2).

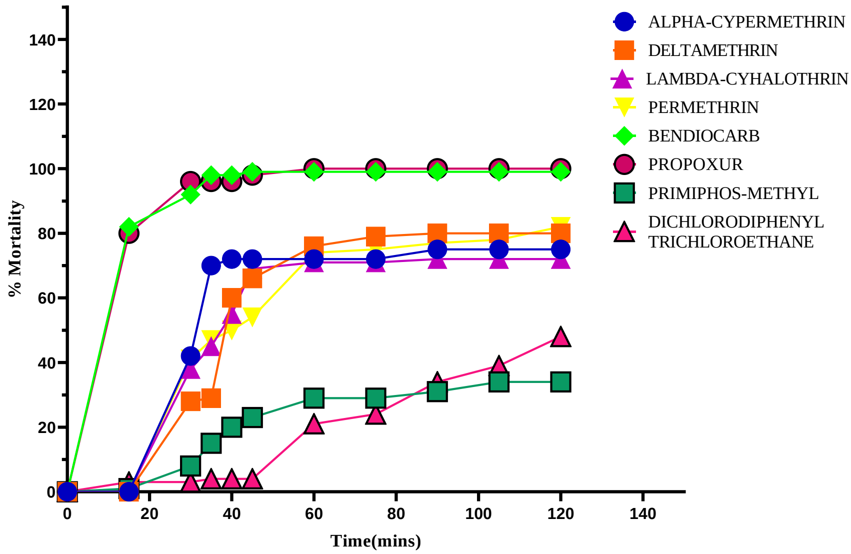

Figure 2. Susceptibility of the insecticides in mangrove vegetation

Tukey's multiple comparison test showed the mortality or susceptibility of the mosquitoes to the following insecticides were significantly different: Bendiocarb vs Primiphos-methyl; Bendiocarb vs DDT; Propoxur vs Primiphos-methyl; and Propoxur vs DDT (Table 1). Mosquitoes were more susceptible to Bendiocarb and Propoxur.

PBO-Deltamethrin comparison with Deltamethrin alone in Mangrove Vegetation

At 60 minutes (mid-time in the bioassay), percentage mortality of *An. gambiae s.l.* to the following insecticides were: PBO-deltamethrin synergist-92, deltamethrin alone-76, while at 120 minutes (end of bioassay), percentage mortality of *An. gambiae s.l.* to the following insecticides were: PBO-deltamethrin synergist-95, deltamethrin alone-80 respectively (Figure 3).

PBO-Permethrin comparison with Permethrin alone in Mangrove Vegetation

Comparing the susceptibility to PBO-permethrin and Permethrin alone, it was found that at 60 minutes (mid-time in the bioassay), percentage mortality of *An. gambiae s.l.* to the following insecticides were: PBO-permethrin synergist-80, permethrin alone-74, while at 120 minutes (end of bioassay), percentage mortality of *An. gambiae s.l.* to the following insecticides were: PBO-permethrin synergist-89, permethrin alone-82 (Figure 4).

Table 1. Analysis of variance of mortality based on type of insecticides in the mangrove vegetation.

Insecticide	Mangrove Vegetation
	Mean ± Standard Deviation
Alpha-cypermethrin (ACM)	14.2 ± 7.4
Deltamethrin (DM)	13.1 ± 8.0
Lambda-cyhalothrin (LCT)	12.8 ± 7.0
Permethrin (PM)	13.1 ± 7.4
Bendiocarb (BDC)	21.9 ± 7.4
Propoxur (PPX)	22.0 ± 7.4
Primiphos-methyl (PPM)	5.1 ± 3.2
Dichlorodiphenyltrichloroethane (DDT)	4.2 ± 4.3
P – value	<0.0001
Tukey's Multiple Comparison (P - Value)	
ACM vs DM	>0.9999
ACM vs LCT	0.9997
ACM vs PM	>0.9999
ACM vs BDC	0.1425
ACM vs PPX	0.1375
ACM vs PPM	0.0422
ACM vs DDT	0.0170
DM vs LCT	>0.9999
DM vs PM	>0.9999
DM vs BDC	0.0579
DM vs PPX	0.0555
DM vs PPM	0.1086
DM vs DDT	0.0490
LCT vs PM	>0.9999
LCT vs BDC	0.0440
LCT vs PPX	0.0422
LCT vs PPM	0.1375
LCT vs DDT	0.0641
PM vs BDC	0.0579
PM vs PPX	0.0555
PM vs PPM	0.1086
PM vs DDT	0.0490
BDC vs PPX	>0.9999
BDC vs PPM	<0.0001
BDC vs DDT	<0.0001
PPX vs PPM	<0.0001
PPX vs DDT	<0.0001
PPM vs DDT	>0.9999

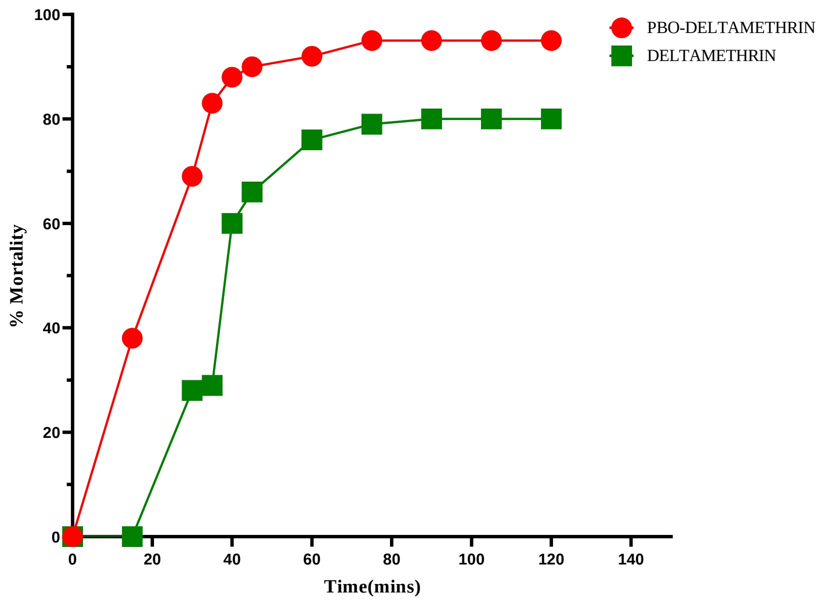

Figure 3. PBO-deltamethrin comparison with deltamethrin alone in mangrove vegetation.

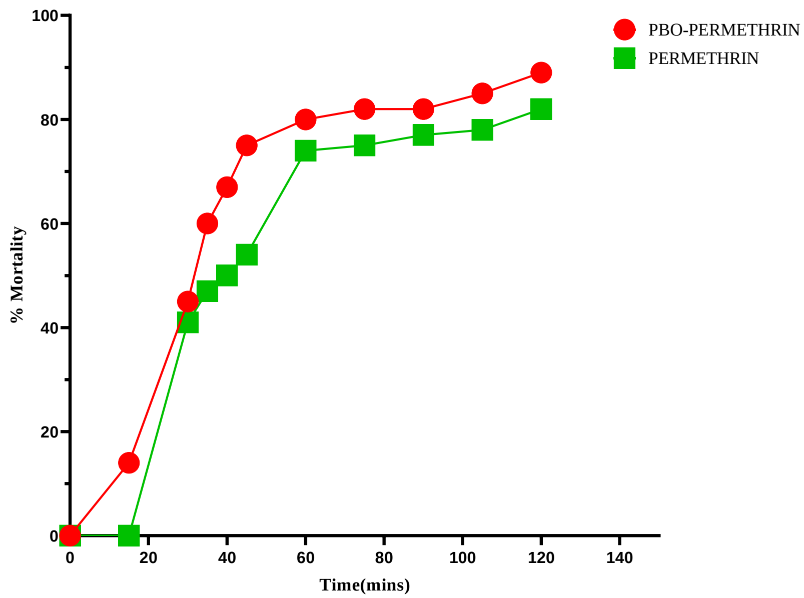

Figure 4. PBO-permethrin comparison with permethrin alone in mangrove vegetation.

PBO-Bednet-Insecticides Synergist Performance in Mangrove Vegetation

The results (Figure 5) showed that at 60 minutes (mid-time in the bioassay), percentage mortality of *An. gambiae s.l.* to the following insecticides were: PBO-permethrin synergist-80, PBO-deltamethrin synergist-92, while at 120 minutes (end of bioassay), percentage mortality of *An. gambiae s.l.* to the following insecticides were: PBO-permethrin synergist-89, PBO-deltamethrin synergist-95. The differences in susceptibility to both insecticides were not significant (Table 2).

Table 2. Analysis of variance of piperonyl butoxide (PBO) synergist.

Piperonyl Butoxide (PBO) – Insecticide in comparison with Insecticide alone in the Mangrove Vegetation		
	Deltamethrin	Permethrin
	Mean ± Standard Deviation (M±SD)	
PBO – Insecticide	19.1±7.6	15.4±7.5
Only Insecticide	13.1±8.1	13.1±7.4
P – value	0.0910	0.4786
t – value	1.776	0.7220
PBO – Deltamethrin with PBO – Permethrin		
P – value	0.2708	
t – value	1.133	

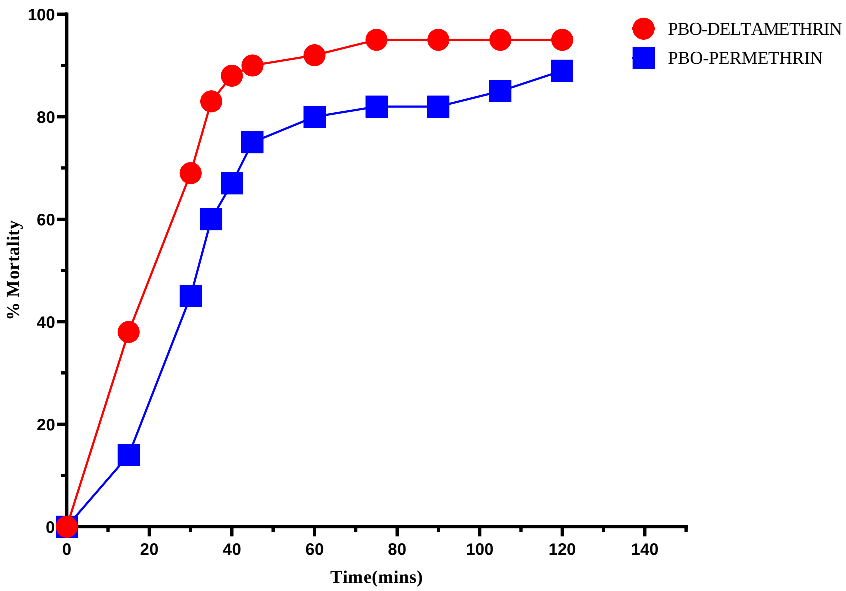

Figure 5. PBO-bednet-insecticides synergist performance in mangrove vegetation.

DISCUSSION

The mangrove vegetation of Rivers State, Nigeria covers the following local government areas: Asari-Toru, Akuku Toru, Degema, Bonny, Andoni, Opobo/Nkoro and Port Harcourt. Results showing the susceptibility status of *An. gambiae s.l.* in the mangrove vegetation of Rivers State to some public health insecticides, revealed that the insecticides tested were highly significant ($p = <0.0001$). 100% susceptibility was attained at 60 minutes (half of the time for the bioassay), with propoxur and 99% with bendiocarb. The highest mean mortality was 22.0 ± 7.4 for propoxur, closely followed by bendiocarb, 21.9 ± 7.4. The lowest mean mortality was 4.2 ± 4.3 for DDT, followed by 5.1 ± 3.2 for primiphos-methyl. Tukey's multiple comparison revealed that there was no statistical difference among the four pyrethroids tested which included: alpha-cypermethrin, deltamethrin, permethrin and lambda-cyhalothrin. In fact, they were highly insignificant (Table 1). From WHO recommendations, possible resistance is suspected (when mortality is between 80%-97%) in permethrin, which had 82% mortality at 120 minutes while resistance were suggested (when mortality is less than 80%) in primiphos-methyl, DDT, alpha-cypermethrin, deltamethrin and lambda-cyhalothrin. The level of resistance to DDT in the mangrove vegetation has been recorded in other parts of Nigeria Ebere (2015); Ebere et al. (2019); Umar et al. (2014); Adeogun et al. (2017) and of Africa like Mali, Cote d'Ivoire and Tanzania, Cisse et al. (2015); Camara et al. (2018); Kisinza et al. (2017).

Comparison of the carbamates showed *An. gambiae s.l.* was highly susceptible to bendiocarb and propoxur within short time periods which were less than 60 minutes. The mean mortality for bendiocarb was a bit less than that for propoxur and according to Tukey's multiple comparison, there was no significant difference between bendiocarb and propoxur. In Nigeria, propoxur resistance has been reported in Lagos with mortality rates ranging between 25-77%, Oduola et al. (2012), this is in contrast to the result obtained in this study in which susceptibility of *An. gambiae s.l.* to propoxur was 100% and the reason could be sustained exposure of mosquitoes to these insecticides, and agricultural activities and industrial pollutants, Reid and McKenzie (2016). Among the class of organo-compounds compared in the mangrove vegetation, similar observation to the carbamates and pyrethroids were made, as there was no significant difference between the organochlorine (DDT) with mean mortality less than the organophosphate (primiphos-methyl) (Table 1).

This shows that insecticides from the same family of chemical compounds might always act similarly without any significant differences in their actions, for example, from Tukey's multiple comparison the p-value for the carbamates was >0.9999, also among the pyrethroids the p-values range were 0.9997 to >0.9999 (Table 1). One reason that might be responsible for the existence of differences in insecticides from the same class could be that, they were not manufactured/produced or used at the same period of time. When *An. gambiae s.l.* has already started developing resistance to one, the other is very new to the mosquito in the field. This means the *An. gambiae s.l.* is yet to take time and develop resistance to the new insecticide that has been introduced, hence, the mosquito will remain highly susceptible to the new insecticide, for the time being till it develops resistance to the new compound. This same reason could be responsible for the insignificant differences observed among the pyrethroids mentioned earlier.

Resistance in multiple classes of insecticides has been observed from this study in four pyrethroids, one organochlorine and one organophosphate. Resistance to multiple classes of insecticides have been reported in other parts of Nigeria, Ebere et al. (2019); Oduola et al. (2010; 2012); Riveron et al. (2015). In Africa resistance of *An. gambiae s.l.* to multiple classes of insecticides have been recorded in the central and the north of Côte d'Ivoire, Benin and Burkina Faso, N'Guessan et al. (2003); Corbel et al. (2007); Djogbenou et al. (2008); Dabire *et* al. (2009). It is possible that the misuse and/or over use of agrochemicals by farmers could be instrumental to the multi-resistance selection in the mangrove vegetation. The indiscriminate use of agro-chemicals by farmers could have also generated high chemical residues and other environmental pollutants that are washed into the water bodies (mosquito breeding sites) generating several xenobiotics that exercise a resistance selection in mosquitoes at larval stage Akogbeto et al. (2006); Antonio-Nkondjio et al. (2011); Philbert et al. (2014); Tene Fossog et al. (2012). Resistance in multiple classes of insecticides could also be due to applications of local insecticides of unknown chemical composition and normal household insecticides may have contributed to the build-up of insecticide resistance in the local mosquito populations. This continuous exposure of mosquitoes to pyrethroids as well as other commonly used insecticides contributes to mosquitoes becoming strongly resistant to them Ebere et al. (2019). The detection of multiple resistances in mosquito population has serious consequences for the Nigeria malaria control programme as it has implications on the effectiveness of the LLINs and IRS currently used in the country. Resistance to multiple insecticides within a chemical class is commonly noted but rarely quantified and differences in resistance to different insecticides within a class are also seen, Hancock et al. (2018).

Piperonyl butoxide (PBO) is an organic compound used as a component of pesticide formulations and as synergist. Despite having no pesticidal activity of its own, it enhances the potency of certain pesticides especially for carbamates, pyrethrins, pyrethroids, and rotenone Bingham et al. (2011); Moores et al. (2009). Analysis of mortality of PBO-deltamethrin in comparison with deltamethrin alone in the mangrove vegetation showed no significant difference though the mean mortality for PBO-deltamethrin synergist was higher than that for deltamethrin alone (Table 2). It was observed that the PBO-deltamethrin synergist, in the present study did not indicate susceptibility (Figure 3) (when mortality is between 98%-100%), according to WHO recommendations but there was recovery of susceptibility against the *An. gambiae s.l.* when compared to when deltamethrin alone was used. PBO acts as an insecticide synergist by inhibiting the natural defenses of the insects. PBO inhibits enzymes present in insects, most important of which is the mixed function oxidase system (MFO) also known as the cytochrome P450 system, Young et al. (2005; 2006).

The improved difference in mortality between PBO-deltamethrin synergist and deltamethrin alone in this current study is an indication of the partial involvement of cytochrome P450 monooxygenase enzyme activity, of the resistant *An. gambiae s.l.* which targeted the pyrethroid, deltamethrin to detoxify the insecticide. This resistance has been inhibited by the synergist action of PBO-deltamethrin. The 95% mortality recorded by the PBO-deltamethrin in this present study also indicates that there are other categories of mechanism in *An. gambiae s.l.* that confer resistance to neurotoxic insecticides in malaria vectors such as, alterations to metabolic genes or pathways, target site mutation, and cuticular thickening. Metabolic resistance which result primarily from the amplification or up-regulation of detoxification enzymes beside P450 monooxygenases, are esterases and glutathione *S*- transferases, which occurs commonly and can confer high levels of resistance, Ibrahim et al. (2016). These other resistance mechanisms need to be investigated for complete susceptibility to be achieved in the present study. The present result agrees with the report of Awolola et al. (2014) in Nigeria, of the high efficacy of LLINs treated with deltamethrin plus PBO on resistant *An. gambiae s.l.* when compared with standard treated nets with no PBO. In Mozambique, deltamethrin plus PBO combination proved to be more effective against resistant *An. funestus* and *An. gambiae s.l.* Abilio et al. (2015); Riveron et al. (2018). In another report from Kolokope, Togo in West Africa, of resistance to *An. gambiae s.l.*, 14.8% mortality was recorded. When it was enhanced with PBO, the mortality with deltamethrin rose from 14.8% to 100% (Ketoh et al. (2018).

Analysis of mortality of PBO-permethrin in comparison with permethrin alone shows the mortality with PBO-permethrin synergist was higher than permethrin alone (Figure 4). There was no significant difference though the mean mortality for PBO-permethrin synergist was higher than for permethrin alone (Table 2). The improved susceptibility observed in PBO-permethrin synergist as against permethrin alone in this present study is an indication of the partial involvement of cytochrome P450 monooxygenase enzyme activity, of the resistant *An. gambiae s.l.* which targeted the pyrethroid, permethrin in order to detoxify the insecticide. This resistance has been inhibited by the synergist action of PBO-permethrin. The other resistance mechanisms need to be investigated for 100% susceptibility to be achieved. Another observation was that PBO-permethrin synergist in the present study did not indicate susceptibility (Figure 4) (when mortality is between 98%-100%), according to WHO recommendations. This result agrees with the recovery of susceptibility as reported by Ketoh et al. (2018) from Kolokope, Togo in West Africa, of resistance to permethrin by *An. gambiae s.l.*, mortality recorded was 7.5% but when it was enhanced with PBO, the mortality with permethrin rose from 7.5% to 92%. In another study carried out in Kpome, southern Benin Republic, when permethrin plus PBO was used on *An. colluzzi*, mortality rose from 19.27% to 69.67% Akoton et al. (2018).

Analysis of mortality of PBO-deltamethrin and PBO-permethrin synergists (two insecticides-PBO) used in LLINs-impregnation in the mangrove vegetation, showed that the mortality with PBO-deltamethrin synergist was higher than with PBO-permethrin synergist (Figure 5). The mean mortality for PBO-permethrin synergist was lower than that for PBO-deltamethrin synergist, though there was no significant difference (Table 2). According to WHO recommendations, possible resistance was suggested which needs to be confirmed (when mortality is between 80%-97%) for PBO-permethrin synergist and PBO-deltamethrin synergist in the mangrove vegetation. The closeness in the susceptibility range in this result would be expected since they are both from the pyrethroid class (Figure 5) and the level of resistance of *An. gambiae s.l.* to the pyrethroids synergist observed in this mangrove vegetation may have been due to the indiscriminate use of agro-chemicals by farmers, which could have also generated high chemical residues and other environmental pollutants that are washed into the water bodies (mosquito breeding sites) generating several xenobiotics that exercise a resistance selection in mosquitoes at larval stage, Akogbeto et al. (2016); Antonio-Nkondjio et al. (2011); Philbert et al. (2014); Tene Fossog et al. (2012).

CONCLUSION

Carbamates were highly effective on *An. gambiae s.l.* population in the mangrove vegetation of Rivers State, Nigeria, though there was complete susceptibility to propoxur when compared to bendiocarb. The malaria vectors were resistant to primiphos-methyl, DDT and the pyrethroids: alpha-cypermethrin, permethrin, lambda-cyhalothrin and deltamethrin. Metabolic resistance caused by P450 monooxygenases enzymes, was among the resistant mechanisms involved in the resistance shown by *An. gambiae s.l.* The regaining of lost susceptibility with PBO-pyrethroid synergist (though incomplete), attest to the effectiveness and recommendation for use, of the PBO synergist over using the pyrethriods alone, in LLINs impregnation. Other resistant mechanisms could have roles to play in the incomplete regaining of lost susceptibility by the pyrethroids. The use of propoxur and bendiocarb is recommended for indoor residual spraying in the mangrove vegetation region. This study provides baseline information for monitoring the status of insecticide resistance in Rivers State.

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STATEMENT OF AUTHORSHIP

Ekerette, I.B. designed and conducted the research. Ebere, N. supervised the research and proofread the initial manuscript.

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