

Susceptibility of *Anopheles gambiae* (Giles, 1902) to public health insecticides and efficiency of piperonyl butoxide in a Niger Delta Region

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Abstract

Susceptibility to some WHO-approved public health insecticides by female *Anopheles gambiae* sensu lato (malaria vector) and the efficiency of piperonyl butoxide (PBO)-bednet insecticides was tested in fresh water swamp vegetation of Rivers State, Nigeria. Larval samples using standard dipping technique were collected in rainy season. Technical grade insecticides used in the bioassay comprised of Pyrethroids (Permethrin, Deltamethrin, Lambda-cyhalothrin, Alpha-cypermethrin), Carbamates (Bendiocarb, Propoxur), Organochlorine (Dichlorodiphenyltrichloroethane) and Organophosphate (Primiphos-methyl). 25 non-bloodfed adults, three to five days old were introduced into each of the four-replicate and control bottles. Number of dead mosquitoes at 15, 30, 35, 40, 45, 60, 75, 90, 105, 120 minutes, was noted. PBO synergist test with deltamethrin and permethrin was done. ANOVA examined the differences in vector mortality. Percentage mortality of the vector to: bendiocarb-99 and alpha-cypermethrin-100, agreed with WHO recommendations. There was partial recovery of susceptibility with PBO-(permethrin and deltamethrin). Bendiocarb and alpha-cypermethrin are effective for indoor residual spraying (IRS). Recovery of susceptibility by PBO-pyrethroids gives them edge over lone pyrethroids, and they are recommended for impregnation of long-lasting insecticidal nets (LLINs).

Keywords: Mortality, fresh water swamp, resistance, recommend, Rivers State

Introduction

Malaria cases have been estimated at 2.2 billion and 12.7 million deaths have been averted since 2000, but the disease remains a serious global health threat, particularly in the WHO African Region. There were an estimated 263 million cases and 597 000 malaria deaths worldwide in 2023. This represents about 11 million more cases in 2023 compared to 2022, and nearly the same number of deaths. Approximately 95% of the deaths occurred in the WHO African Region, where many at risk still lack access to the services they need to prevent, detect and treat the disease (WHO, 2024) [49]. Distributions of malaria cases by country showed that 27 of the 29 countries that account for 95% of malaria cases globally are in the WHO African Region. Nigeria has 27% of the world cases, 23% of the world's total death rate (both data were the highest in the world for a single country) and 31 million Insecticide Treated Nets (ITNs) were delivered to the country in 2019 (WHO, 2020) [48].

Malaria vector control using either Long Lasting Insecticide Nets (LLIN) or Indoor Residual Spraying (IRS) relies on the continued susceptibility of Anopheles mosquito vectors to a limited number of insecticides approved for their control (Betson, Jawara, & Awolola, 2009) [6] and the efficacy in the use of microbial larvicides on mosquito breeding sites (Ekerette & Ebere, 2017; 2018) [17, 18] as a source reduction strategy. 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 the following classes of insecticides: organochlorines, organophosphates, carbamates. Non-residual insecticides do not possess a lasting effect and are unstable in light and air.

Example is the class pyrethroids 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) [48].

The emergence of resistance in *An. gambiae s.l.* populations from several settings is seen as a threat to ongoing efforts to prevent malaria transmission (Boussougou-Sambe *et al.*, 2018) [8] and has also posed a threat across the African countries, to effectiveness of the different mosquito control measures (Ranson *et al.*, 2011; Kolade, Kehinde, Oluwatobi, Adedapo, & Audu, 2013) [26, 38]. The successful implementation of IRS programme partly depends on availability of insecticide(s) susceptible *Anopheles* mosquitoes in the local environment. Therefore, it is imperative to periodically conduct bioassay tests to assess the susceptibility status of local mosquito species to IRS interventional insecticides (Umar *et al.*, 2014) [44].

Resistance to the four major classes of insecticides available for public health has indeed been reported in the primary African malaria vector, *An. gambiae s.l.* (Hargreaves *et al.*, 2000; N'Guessan, Corbel, Akogbeto, & Rowland, 2007) [22, 29]. In the Niger delta and some parts of Nigeria, resistance have been reported to these commonly used public health insecticides: pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin) (Ebere, 2010; 2015; Ebere, Atting, Ekerette, & Nioking, 2019) [13, 14, 15], organochlorine (dichloro diphenyl trichloroethane (DDT)), organophosphate (pirimiphos-methyl), and carbamates (propoxur and bendiocarb) (Nwankwo *et al.*, 2017) [31], where it has been incriminated with the use of insecticide in public health for mosquito control and in agriculture for pesticide control of agricultural pests (Yawson, McCall, Wilson, & Donnelly, 2004) [50]. Based on the World Health Organization (WHO) susceptibility assay and Center for Disease Control (CDC) bottles bioassay, insecticide resistance in malaria vectors

have been reported in 64 malaria endemic countries (Knox *et al.*, 2014)^[25].

The two major causes of insecticide resistance are through increased metabolic detoxification and decreased target site sensitivity (Hemingway & Ranson, 2000)^[23]. In metabolic detoxification, the insecticide is prevented from reaching its site of action by detoxification enzymes. The three main enzyme families responsible for metabolic resistance are cytochrome P450s, glutathione S-transferases and esterases (Hemingway & Ranson, 2000)^[23]. Decreased target site sensitivity on the other hand, reduces the rate at which the insecticide binds to its target site. The main target site mutation conferring resistance to pyrethroids and cross resistance to DDT in *An. gambiae* s.s. is the 'Knock-down resistance' mutation (kdr). Two amino acids changes in the sodium channel gene at codon 1014 are involved in kdr in *An. gambiae* s.s.: a leucine to phenylalanine substitution (1014F) (Martinez-Torres *et al.*, 1998)^[27] and a leucine to serine substitution (1014S) (Ranson *et al.*, 2000)^[37]. A single amino acid substitution of glycine to serine at position 119 in the ace-1 gene confers resistance to both organophosphates and carbamates in *An. gambiae* s.s. (Weill *et al.*, 2004)^[45].

A rapid and inexpensive alternative to assess resistance mechanisms is to carryout test with synergists. Synergists are enzyme inhibitors of insecticide detoxification enzymes. Synergists are available for the metabolic detoxification enzymes: esterases, oxidases, and glutathione S-transferases. Synergists act by stopping the apparent resistance observed in the susceptibility test, if a detoxification enzyme plays a role in that particular resistance mechanism (CDC, n.d.).

Piperonyl butoxide (PBO) is a chemical synergist that acts by inhibiting enzymes involved in the natural defense mechanisms of insects, which results in pyrethroid not being detoxified in the insect and the pyrethroid on the long-lasting insecticidal net remaining potent against mosquitoes despite resistance. Such PBO-pyrethroid-treated long-lasting insecticidal nets appear to have similar or better efficacy against resistant mosquitoes under controlled household conditions than standard long-lasting insecticidal nets that do not have PBO (Corbel *et al.*, 2010; Pennetier *et al.*, 2013; Protopopoff *et al.*, 2018)^[11, 34, 36]. Therefore, synergists such as PBO clearly have an important role to play in increasing the efficacy of pyrethroids when used against pyrethroid-resistant mosquitoes. Currently, only pyrethroids are recommended by the WHO for use on mosquito nets (N'Guessan *et al.*, 2007; Toe *et al.*, 2014)^[29].

Therefore, the essence of this study was to make available data that reflect the present level of resistance of *An. gambiae* s.l. to WHO-approved public health insecticides and to find out the level of efficiency of PBO-deltamethrin and PBO-permethrin synergists on the population of *An. gambiae* s.l. (since they have been used in the impregnation of LLINs), in the fresh water swamp area of Rivers State, a Niger delta region in Nigeria.

Materials and Methods

The Study Area

Rivers State lies between latitude 4°45" N and longitude 6°50" E and is a predominantly low-lying pluvial state in southern Nigeria, located in the eastern part of the Niger Delta on the ocean ward extension of the Benue Trough. The inland part of the state consists of tropical rainforest,

and towards the coast, the typical Niger Delta environment features many mangrove swamps. Rivers State has a total area of 11,077 km² (4,277 sq mi), making it the 26th largest state in Nigeria. It has a population of about 5,198,716 as of the 2006 census. The population grew to 7,303,900 with a population density of 755.4/km² as of 2016 estimate, with an annual population change of 3.5% from 2006-2016. Population estimate in the vegetations in 2016 were: lowland forest – 3,131,600, mangrove - 2,504,900, and fresh water swamp – 1,378,700 (Rivers State Government, 2021)^[41]. Surrounding states are Imo, Abia and Anambra to the north, Akwa Ibom to the east and Bayelsa, Delta to the west. In the south, it is bounded by the Atlantic Ocean. Its topography ranges from flat plains, with a network of rivers and tributaries. Average temperatures are typically between 25 °C and 28 °C. Some parts of the state still receive up to 150 mm (6 in) of rainfall during the dry period. Relative humidity rarely dips below 60% and fluctuates between 90% and 100% for most of the year (Rivers State Government, 2021)^[41]. The land surface of Rivers State can be divided into three zones: freshwater swamps, mangrove swamps and coastal sand ridges. The freshwater zone extends northwards from the mangrove swamps. This land surface is generally less than 20m above sea level. As a lower Niger floodplain, it contains a greater silt and clay foundation and is more susceptible to perennial inundation by river floods. The floodplain's total thickness rises to about 45 m in the northeast and over 9 m in the beach ridge barrier zones to the southwest. On coastal sand ridges, the soils are mostly sandy or sandy loams. Various crops are supported including coconut, oil palm, raffia palm and cocoyam. The drier upland region of Rivers State covers 61% of landmass while the riverine areas, with a relief range of 2m to 5m, take up 39% (Rivers State Government, 2021)^[41].

Rivers State has vast expanse of swamped areas which empty in the Bight of Bonny (ocean), hugely forested area, as well as a large area of mangrove cover, creating conducive environment for malaria vectors. There are two distinct seasons in the study area: a dry season from November to April and a rainy season which extends from May to October with a short break in August. Rivers State consist of urban and rural settlements, as well as, residential and industrial areas spread across the three ecological regions. The major occupation in the urban area is manufacturing and commercial industries while in the rural area, it is fishing and subsistence farming. The study area is also rich in crude oil mineral deposit, giving rise to extensive extraction and development activities cutting across the entire region. No extensive spraying or major vector control programmes have been carried out previously in this area until 2013 when LLINs were distributed by the National Malaria and Vector Control Programme and IRS was carried out in some communities in the study area (Rivers State Government, 2021)^[41].

There was ease of accessibility to the sampling sites since it lies within areas of human settlement (Hamza, EL Rayah, EL, & Abukashawa, 2014)^[21]. The major consideration of the study area, was based on the fact that, it's a unique ecological region, with different environmental conditions that may have varying effect on the distribution and the population genetics of the *An. gambiae* s.l., completely different from environmental conditions obtainable in the other ecological zones such as: mangrove and lowland

forest zones, all lying within the bounds of Rivers State, Nigeria.

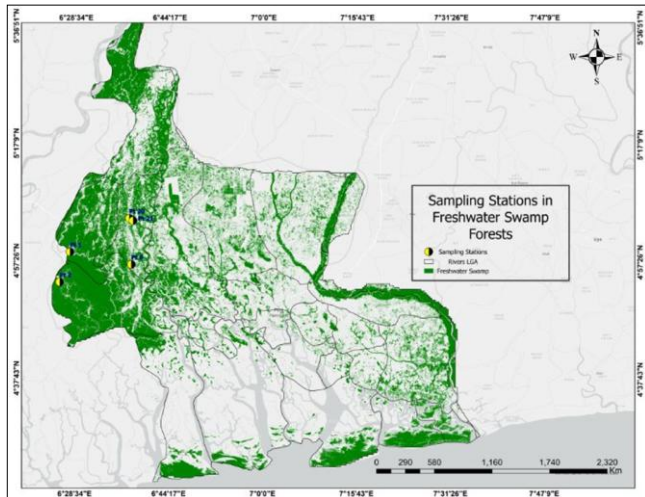


Fig 1: Map of Rivers State showing the fresh water swamp vegetation zone and sampled breeding sites of *An. gambiae s.l.*

Mosquito Sampling

Various potential sample breeding sites of *Anopheles* mosquitoes included borrow pits, vehicle tyre imprint, footprints, temporary sunlit pools, ditches in construction sites, drains, rain puddles, ponds and stagnant waters, among others, were sampled in the fresh water swamp vegetation. The sampling was carried out from May to November, this was to ensure that sample collection was done in the rainy season, when there was abundance of larvae, Standard dipper (400 ml) with one (1 m) handle was used for larvae collection from different breeding sites (WHO, 2015) [47]. The various breeding sites from which samples were collected are shown on Figure 1.

Rearing of Mosquito Larvae

Larvae from the different breeding sites were pooled together, kept alive and 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 Environmental Biology, Rivers State University, Port Harcourt, Nigeria. The samples were processed at the laboratory where the mosquitoes were reared to adults following the methods of Gerberg, Bernard, & Ward, (1994) [19]. In the laboratory the *Anopheles* larvae and the breeding water from the sampling sites, which were transported in a plastic container were gently poured into a white tray. A 3-ml rubber pipette was used to pick the larvae from the white tray into another plastic container. This method was used to sort the *Anopheles* larvae into plastic containers holding dechlorinated 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 finely ground biscuits every two days and monitored till adult stage for emergence. Newly emerged adults were separated into females and males, using aspirator to pick them individually from the adult cage. Females have non-plumose antennae with palp about as long as proboscis while the males have plumose antennae with palp about as long as proboscis and swollen at ends. Adult females were kept in screen cages

and fed continuously on 10% glucose solution. Cages were kept at 26°C - 29°C and 74% - 82% relative humidity.

Morphological Identification of *An. gambiae s.l.*

Members of *An. gambiae s.l.* were on the bases of physical features separated from other anopheline mosquitoes with non-similar features, with the means of identification put together by, Gillies & Coetzee (1987) [20].

Insecticide Susceptibility Bioassays

Susceptibility test was done with the use of CDC procedure (Centers for Diseases Control and Prevention) (CDC, n.d.). The methodological rated insecticides were made available by NMEP (National Malaria Elimination Programme). Subsequently, these community health methodological-rated insecticides were used: Organophosphate (Primiphos-methyl 20µg/ml), Organochlorine (Dichlorodiphenyltrichloroethane, DDT 100µg/ml), Pyrethroids (Alpha-cypermethrin 12.5µg/ml, Deltamethrin 12.5µg/ml, Permethrin 21.5µg/ml, Lambda-cyhalothrin 12.5µg/ml), Carbamates (Propoxur 12.5µg/ml, Bendiocarb 12.5µg/ml).

Test was done while the bottles laid on their sides. For the insecticide susceptibility bioassay, 3 to 5-day female mosquitoes that have not taken blood meal from any host, were used. With the aid of a mosquito aspirator, 25 *Anopheles gambiae s.l.* were put into each of the four bottles for the test and also the control bottle. A timer was started. The bottles were examined at Time 0 and the number of dead and/or live mosquitoes were counted and recorded.

Record was made of the number of dead and live mosquitoes, at (15, 30, 35, 40, 45, 60, 75, 90, 105 and 120 minutes) until all were dead, or up to the 2 hours of experimentation. In the absence of mortality in the control, the Abbott's formula was not used to correct results. Mosquitoes were considered dead if they could no longer stand. Mosquitoes that were alive at the time the experiment ended, are those that resisted the insecticide that was tested. "Dead" mosquitoes were mosquitoes that could not stand (CDC, n.d.). The cylindrical bottles were mildly spinned as count of the mosquitoes was in progress. Mosquitoes that were not moving nor flying, were observed at the bottom as the mild spinning was going on and they were counted as dead mosquitoes. Percentage of mosquitoes that are not able to fly in a coordinated manner or are dead at the time the experiment ended over the total number of mosquitoes that were involved in the experiment, is the most vital record in the graph (CDC, n.d.).

Piperonyl Butoxide (PBO) Synergist Test

CDC procedure was applied in the test for piperonyl butoxide synergist (CDC, n.d.). Same figures of 125 mosquitoes were put into the synergist-control bottle, as well as, the synergist-exposed bottle. They were left for 60 minutes so the synergist could permeate the body of the mosquitoes very well. Thereafter, they were moved into two different netted enclosures from where 25 each were put into the insecticide coated bottles. The piperonyl butoxide synergist test was done with a set of insecticide-treated bottles and alternative set of same insecticide-treated bottles for both synergists exposed and control mosquitoes, respectively. The recorded result from the control and exposed were kept side by side so comparison could be made for recovery of susceptibility to be observed in the synergist-exposed mosquitoes.

Data Analysis

A linear graph of replicates, with time on the x-axis and of the overall percent mortality on the y-axis was drawn. Outcomes from the insecticide susceptibility test were appraised in line with WHO (1998) [46] approvals for judging the implication of perceived resistance: 98%–100% death at the recommended diagnostic time point to susceptibility; 80%–97% death at the recommended diagnostic time proposes the likelihood of resistance that will warrant further investigation for the fact to be established; < 80% death at the recommended diagnostic time proposes resistance. Where < 95% death occurs at the diagnostic time in tests that have been conducted under best conditions and with a sample size of >100 mosquitoes, then resistance can be strongly suspected. 8.0.2 (263) version of Graphpad Prism software was used in computing percentage mortality, mortality mean and standard deviation, p-values, and Tukey’s multiple comparisons between two insecticides for significant differences.

Results / Findings

The result in Figure 2 showed that after 1 hour, the following were the percentage mortalities of the malaria vector to the insecticides: alpha-cypermethrin-96, bendiocarb-99, propoxur-95, lambda-cyhalothrin-93, permethrin-85, deltamethrin-70, dichlorodiphenyltrichloroethane-15, primiphos-methyl-34 while after 2 hours,

percentage death of the malaria vector to the insecticides were: bendiocarb-99 and alpha-cypermethrin-100 (both indicated susceptibility), propoxur-96, lambda-cyhalothrin-96, permethrin-89 and deltamethrin-84 (these four suggested possible resistance) however, dichlorodiphenyltrichloroethane-38 and primiphos-methyl-45 (confirmed resistance in them).

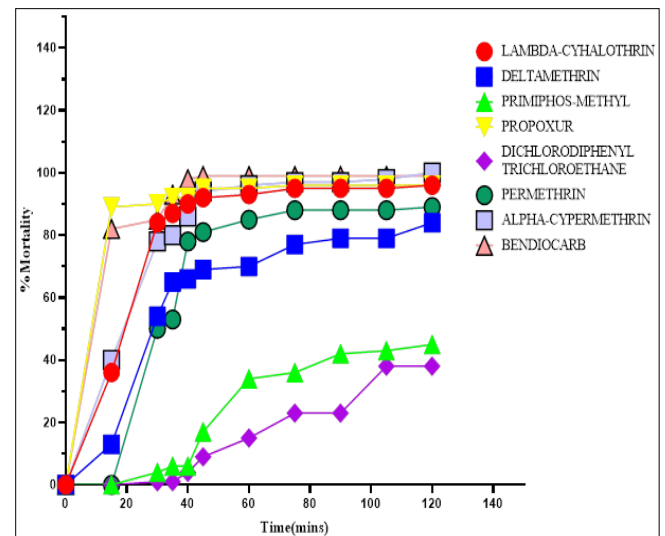


Fig 2: Susceptibility of *An. gambiae s.l.* to the insecticides in the fresh water swamp vegetation

Table 1: Analysis of Variance of Mortality Based on Type of Insecticides in the Fresh Water Vegetations

Insecticide	Vegetation
	Fresh Water Swamp
	Mean ± Standard Deviation (M±SD)
Alpha-cypermethrin (ACM)	21.6 ± 7.3
Deltamethrin (DM)	14.9 ± 6.9
Lambda-cyhalothrin (LCT)	19.6 ± 7.8
Permethrin (PM)	15.9 ± 8.6
Bendiocarb (BDC)	21.3 ± 7.1
Propoxur (PPX)	19.7 ± 7.8
Primiphos-methyl (PPM)	5.3 ± 4.7
Dichlorodiphenyltrichloroethane (DDT)	3.5 ± 3.7
P – value	<0.0001
Tukey’s Multiple Comparison (P - Value)	
Alpha-cypermethrin vs Deltamethrin	0.7387
Alpha-cypermethrin vs Lambda-cyhalothrin	>0.9999
Alpha-cypermethrin vs Permethrin	0.9042
Alpha-cypermethrin vs Bendiocarb	0.9977
Alpha-cypermethrin vs Propoxur	0.9993
Alpha-cypermethrin vs Primiphos-methyl	0.0001
Alpha-cypermethrin vs Dichlorodiphenyltrichloroethane	<0.0001
Deltamethrin vs Lambda-cyhalothrin	0.7524
Deltamethrin vs Permethrin	>0.9999
Deltamethrin vs Bendiocarb	0.3178
Deltamethrin vs Propoxur	0.3844
Deltamethrin vs Primiphos-methyl	0.0337
Deltamethrin vs Dichlorodiphenyltrichloroethane	0.0050
Lambda-cyhalothrin vs Permethrin	0.9122
Lambda-cyhalothrin vs Bendiocarb	0.9972
Lambda-cyhalothrin vs Propoxur	0.9991
Lambda-cyhalothrin vs Primiphos-methyl	0.0002
Lambda-cyhalothrin vs Dichlorodiphenyltrichloroethane	<0.0001
Permethrin vs Bendiocarb	0.5278
Permethrin vs Propoxur	0.6052
Permethrin vs Primiphos-methyl	0.0124
Permethrin vs Dichlorodiphenyltrichloroethane	0.0016
Bendiocarb vs Propoxur	>0.9999

Bendiocarb vs Primiphos-methyl	<0.0001
Bendiocarb vs Dichlorodiphenyltrichloroethane	<0.0001
Propoxur vs Primiphos-methyl	<0.0001
Propoxur vs Dichlorodiphenyltrichloroethane	<0.0001
Primiphos-methyl vs Dichlorodiphenyltrichloroethane	0.9984

PBO-Deltamethrin comparison with Deltamethrin alone in Fresh Water Swamp Vegetation

When a period of 1 hour had elapsed, percentage deaths of the malaria vectors in fresh water swamp vegetation to these insecticides were: PBO-deltamethrin synergist-84, deltamethrin alone-70, when a period of 2 hours had elapsed, percentage death of the malaria vector in fresh water swamp vegetation to the following insecticides were: PBO-deltamethrin synergist-91, deltamethrin alone-84 (Figure 3).

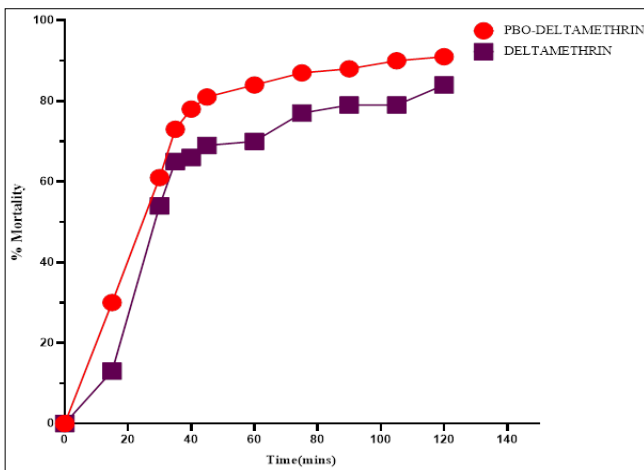


Fig 3: PBO-deltamethrin comparison with deltamethrin alone in fresh water swamp vegetation

PBO-Permethrin comparison with Permethrin alone in Fresh Water Swamp Vegetation

When a period of 1 hour had elapsed, percentage deaths of malaria vector in fresh water swamp vegetation to these insecticides were: permethrin alone-85, PBO-permethrin synergist-83, after a period of 2 hours had passed, percentage mortalities of the malaria vector were: permethrin alone-89, PBO-permethrin synergist-90 (Figure 4).

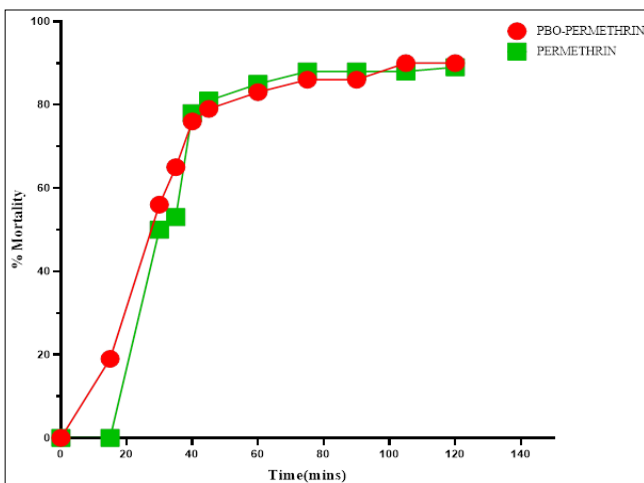


Fig 4: PBO-permethrin comparison with permethrin alone in fresh water swamp vegetation.

Table 2: Analysis of Variance of Piperonyl Butoxide (PBO) Synergist

Piperonyl Butoxide (PBO) –Insecticide in comparison with Insecticide alone in the Fresh Water Swamp Vegetation		
	Deltamethrin	Permethrin
	Mean ± Standard Deviation (M±SD)	
PBO-Insecticide	17.3±7.3	16.6±7.6
Only Insecticide	14.9±6.9	15.9±8.6
P – value	0.4308	0.8454
t – value	0.8042	0.1976
PBO – Deltamethrin with PBO – Permethrin		
P – value	0.8149	
t – value	0.2372	

PBO-Bednet-Insecticides Synergist Performance in Fresh Water Swamp Vegetation

When a period of 1 hour had passed, percentage deaths of malaria vector in fresh water swamp vegetation to these insecticides were: PBO-deltamethrin synergist-84, PBO-permethrin synergist-83, after a period of 2 hours had elapsed, percentage deaths of malaria vector to these insecticides were: PBO-deltamethrin synergist-91, PBO-permethrin synergist-90 (Figure 5).

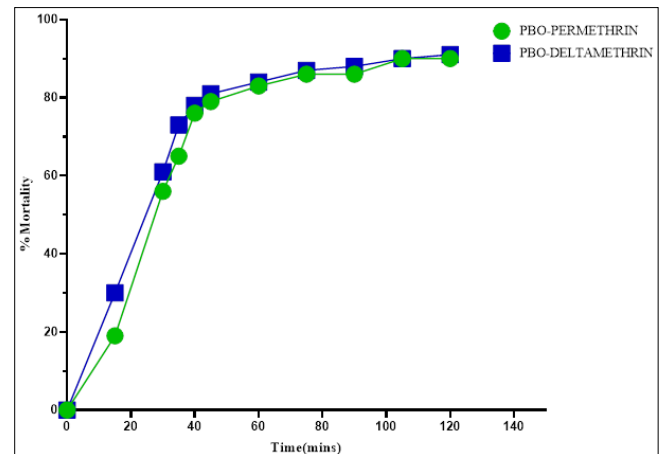


Fig 5: PBO-bednet-insecticides synergist performance in fresh water swamp vegetation. ss

Discussion

Ogba Egbema Ndoni, Ahoada West, Ahoada East and Abua/Odual local government areas (L.G.A.) are situated within the fresh water swamp vegetation. There was statistical difference in the results of the eight insecticides ($p = <0.0001$) in the bioassay of the malaria vector (Figure 2). Bendiocarb had the highest average mortality, closely followed by propoxur whereas, DDT recorded the lowest mean mortality, followed by primiphos-methyl (Table 1). From Tukey’s multiple comparison (Table 1), no significant difference existed between the following pyrethroids: lambda-cyhalothrin, permethrin, alpha-cypermethrin and deltamethrin. It was similarly observed among the carbamates that, there was no significant difference between bendiocarb and propoxur ($p > 0.9999$). The p-value for bendiocarb and propoxur implies that insecticides from the

same class (family) of chemical compounds could always act similarly without any significant difference in their actions. One reason responsible for the observed differences could be that, they were not formulated / produced and used at the same period. By the time *An. gambiae s.l.* develops resistance to one (in this case, propoxur), the other is still very new to the mosquitoes in the field (in this case, bendiocarb). This could be understood that the mosquito is gradually with time developing resistance to the novel insecticide which has been invented therefore the mosquito would still be very much susceptible to the novel insecticide, for now the pending when eventually it progresses in resistance to the novel insecticide.

In the current study, there was resistance of the malaria vector to several groups of insecticides (Table 1). Resistance was found in the malaria vector to carbamate (propoxur), pyrethroids (lambda-cyhalothrin, permethrin and deltamethrin), organochlorines (DDT), organophosphate (primiphos-methyl). Four classes were affected in multiple-insecticide class resistance to the malaria vector. This current research agrees with earlier findings in Oduoha-Emuoha community, in Emuoha L.G.A., Rivers State, where the malaria vector resisted pyrethroid, organochlorine, organophosphate and carbamate (Ebere *et al.*, 2019) [15] and in Misau, Bauchi state, northeast of Nigeria, the malaria vector resisted pyrethroid, carbamate and organochlorine (Umar *et al.*, 2014) [44]. Report from Mali (West Africa), shows that the malaria vector also resisted DDT, deltamethrin and lambda-cyhalothrin (Cisse *et al.*, 2015). Oduola *et al.* (2010; 2012) [32, 33] and Riveron *et al.* (2015) [39] have as well reported on resistance in multiple insecticide classes. This has great negative impact on the control of malaria vectors.

Analysis of mortality based on the piperonyl butoxide (PBO)-bednet insecticide (synergist) when PBO-deltamethrin and PBO-permethrin were used. The mean mortality for PBO-deltamethrin was 17.3 ± 7.3 . The mean mortality PBO-permethrin was 16.6 ± 7.6 (Table 2). Agrochemicals usage of these insecticides in Nigeria, was permitted by the National Agency for Food and Drug Administration and Control, NAFDAC, (NAFDAC, 2004) [30]. Probably, the various degrees of mismanagement and/or abuse of these chemicals by farmers might have reached a critical level for the multi-resistance selection to set in. The abusive use of agro-chemicals by farmers might have also produced high chemical (insecticides) remains and other environmental contaminants that are washed into the aquatic ecosystem (mosquito breeding sites), producing numerous xenobiotics (chemicals that an organism is unprotected from, that are external/strange to the normal metabolism of that organism) that demonstrate a resistance selection in malaria vectors at larval stage (Akogbéto, Djouaka, & Kindé-Gazard, 2006; Antonio-Nkondjio *et al.*, 2011; Tene Fossog *et al.*, 2012; Philbert, Lyantagaye, & Nkwengulila, 2014) [2, 4, 35, 42]. The two ecological factors (production of chemical pesticides and spilling of petroleum product) are frequently seen in Nigeria and can undoubtedly add to the reasons for local selection of insecticide resistance profiles (Djouaka *et al.*, 2016) [12]. Frequently used synergists that include piperonyl butoxide (PBO), prevents cytochrome P450 monooxygenase enzyme action directed at DDT and pyrethroid insecticides (Bingham, Gunning, Gorman, Field, & Moores, 2007; Moores *et al.*, 2009) [7, 28]. Conceivable resistance that requires to be established factually (when

recorded death is between 80% - 97%), was observed with PBO-deltamethrin and PBO-permethrin synergists, according to WHO recommendations.

Examination of death rate with the use of piperonyl butoxide (PBO)-deltamethrin in contrast with deltamethrin only (without the synergist) (Figure 3) revealed that, there was no statistical difference (Table 2). For the PBO-deltamethrin, the average death was 17.3 ± 7.3 and for deltamethrin alone was 14.9 ± 6.9 . The mean mortality for PBO-permethrin synergist was 16.6 ± 7.6 and for permethrin alone was 15.9 ± 8.6 (Table 2). This result agrees with the report of Awolola *et al.* (2014) [15] in Nigeria, of the high efficacy of LLINs treated with deltamethrin + PBO on resistant *An. gambiae s.l.* when compared with standard treated nets with no PBO. In Mozambique, deltamethrin + PBO combination proved to be more effective against resistant *An. funestus* and *An. gambiae s.l.* (Abilio *et al.*, 2015; Riveron, Watsenga, Irving, Irish, & Wondji, 2018) [40]. In a West African community of Kolokope, Togo, resistance by the malaria vector showed a recorded 14.8% death, as it was boosted with PBO, the death record with deltamethrin went up from 14.8% - 100% (Ketoh *et al.*, 2018) [24]. Between the lone deltamethrin and the deltamethrin with PBO, a statistical difference existed. Reports from previous findings agrees with the observations in this study.

Similar to that obtained with deltamethrin, investigation of mortality of permethrin only and permethrin combined with piperonyl butoxide (PBO) (Figure 4), shows there was no significant difference (Table 2). This result agrees with the report of Ketoh *et al.* (2018) [24] in West African community, Kolokope, Togo, permethrin resistance by the malaria vector showed death record of 7.5%, when boosted using PBO, the death with permethrin climbed from 7.5% - 92%. Alternative research done in the southern part of Benin Republic, Kpome community, where synergist of permethrin and PBO was used on *Anopheles colluzzi*, death went up from 19.27% - 69.67% (Akoton *et al.*, 2018) [3]. Reports from previous findings agrees with the observations in this study.

Observations from the two bednet insecticides (deltamethrin and permethrin) shows that, it was not only cytochrome P450 monooxygenase enzyme action directed at pyrethroid and DDT (Bingham *et al.*, 2007; Moores *et al.*, 2009) [7, 28], (which was inhibited by the PBO-pyrethroid synergist), that was involved in the malaria vector resistance to the insecticide in this present research. This was shown in the 95% mortality which would have been 100% if cytochrome P450 monooxygenase enzyme activity was the only resistance mechanism involved. This result agrees with Ekerette, Dimkpa, Ebere, & Nyenke, (2023) [16] report that, the KDR gene was positive in samples 2 and 4, establishing its' presence in the lowland forest vegetation, while samples 2 and 4 of the very same lowland forest vegetation were positive for ace-1r gene, as its presence was confirmed. This without doubt demonstrate that, at the same time, the same malaria vectors which were positive for KDR genes, were as well positive for ace-1r genes. Regaining of susceptibility over the malaria vector was achieved when contrasted with only when lone insecticide that was used with no PBO involved.

Investigation of death of the malaria vectors when permethrin-PBO synergists and deltamethrin-PBO (Figure 5), used in bednets-impregnation, showed that, there was no

existing statistical difference (Table 2). The mean death of malaria vector recorded in the use of the synergist of PBO-permethrin was 16.6 ± 7.6 and with PBO-deltamethrin synergist was 17.3 ± 7.3 (Table 2). The more efficient synergist was PBO-deltamethrin over PBO-permethrin, even though the regaining of susceptibility was recorded by both over the malaria vector when contrasted with the usage of lone insecticide without the synergistic combination with PBO.

Conclusion

The malaria vector is susceptible to alphacypermethrin along with bendiocarb. Suspected resistance of *Anopheles gambiae* s.l. are seen with lambda-cyhalothrin, deltamethrin, permethrin and propoxur while confirmed resistance of the malaria vector was observed in the use of DDT and primiphos-methyl. There was resistance of the malaria vector to multiple groups of chemically formulated insecticides. Recovery of susceptibility was seen in the synergist combination of piperonyl butoxide with deltamethrin and permethrin, respectively and both are used for bednet impregnation of long-lasting insecticidal nets (LLINs). The use of alphacypermethrin and bendiocarb is recommended for indoor residual spraying in the fresh water swamp vegetation. This research makes available baseline data for surveillance and monitoring of the status of insecticide resistance in Rivers State. Focus should be on periodical testing of the *Anopheles gambiae* s.l. susceptible insecticides (alphacypermethrin and bendiocarb) for possible resistance and the formulation of new insecticide compounds to replace the one that resistance has been confirmed (DDT and primiphos-methyl).

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