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INSECTICIDES RESISTANCE PROFILES OF *ANOPHELES* MOSQUITO FROM RURAL AND PERI-URBAN COMMUNITIES OF GOMBE STATE, NORTH EAST, NIGERIA

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ABSTRACT

The emergence of insecticides resistance by malaria vectors poses a threat to the deployment of bed nets for malaria control in Nigeria and Gombe State specifically. This study aimed at evaluating the resistance status of insecticides recommended by World Health Organization Pesticide Evaluation Scheme for control of major malaria vector *Anopheles gambiae* sl. Immature stages of *Anopheles* were collected from ten (10) communities between January – December, 2022 and reared to adulthood. Twenty-five (25) batches of glucose fed female 2-5 days old *Anopheles* were exposed to each of the four (4) WHO diagnostic tubes (replicate) containing deltametthrin (0.05%), DDT (4%), bendiocarb (0.1%), pirimiohos-methyl (0.25%) impregnated paper for one-hour knockdown mortality and final mortality was recorded 24 hours' post-exposure. The enzyme that involve in breaking the resistance gene was also investigated using piperonyl butoxide (PBO). Death and alive mosquitoes were identified using morphological keys. All the test was conducted at the temperature 25-33˚C and relative humidity 60-80%. Resistance and susceptible mosquitoes was classified following World Health Organization criteria; mortality of 98-100 % indicating susceptible, 90-97 possible resistance that required confirmation and <90 indicating resistance. *Anopheles* were susceptible to pirimiphos-methyl (0.25%) and suspected resistant to bendiocarb (0.1%) (93-96%) but highly resistant with mortality (<90%) to DDT (4%) (3-74%) and deltamethrin (0.05%) (2 - 88%) in six of the study communities. Pre-exposure of *Anopheles* to PBO allow deltamethrin (0.05%) to restore its' susceptibility (≥98). The increased resistance to deltamethrin in *Anopheles* could endanger the efficacy of LLINs used for malaria vector control.

Keywords: Insecticide Resistance, *Anopheles gambiae*, Gombe State, PBO

INTRODUCTION

Malaria is a life-threatening disease transmitted through the bite of infected female *Anopheles* mosquitoes and caused by parasite species of the genus *Plasmodium.* In the tropical part of the world, the main malaria parasite is *Plasmodium falciparum* which is transmitted by the *Anopheles gambiae* complex (Service, 2004). Malaria is the most highly prevalent parasitic infection in sub-Saharan Africa, which results in high morbidity and mortality, economic and social impact, premature death, reduced productivity and huge medical cost (Adefioye, 2007; Kayode *et al.,* 2023). The World health organization (2022) reported that, about half of the world population is at risk of being infected with malaria. In 2021, there were approximately 247 million cases and 619, 000 deaths due to malaria worldwide. WHO African regions accounted for 95% of the global malaria case and Nigeria accounted for 27% of malaria cases and 31% death due to malaria (WHO, 2022).However, malaria prevalence among children age 6-59 months fell by 22% in Nigeria and Gombe State having 18% malaria prevalence, second behind Bauchi State with 32% in North East (NMIS, 2021). Moreover, malaria cases related mortality and morbidity among all age group in Gombe causes 11% maternal death, 60% general outpatient, 30% hospital admission, 30% under five years children death as well as 25% infant death has been reported despite the ongoing distribution of Insecticide Treated Nets (ITNs) and some pocket Indoor Residual Spray (IRS) since 2009 (The Sun, 8th September 2017, World malaria day commemoration speech).

Vector controls are important strategies in the reduction of disease transmission. Currently, mosquito vector control majorly depends on the use of long lasting insecticide treated mosquito nets (LLIN/ITNs) and indoor residual spray (IRS) in some rural communities. Although, WHO recommends universal coverage of Insecticide Treated Nets (ITNs) specifically, Long Lasting Insecticidal Treated Nets (LLINs) and Indoor Residual Spray (IRS) as core interventions in malaria-endemic countries to reduce global malaria burden in 2030 by 90% globally(Federal Ministry of Health, 2015). Yet, the spread of insecticide resistance is threatening the effectiveness and sustainability of this control method (Keita *et al.,* 2016; Thiawa *et al.,* 2018). In recent years the successes of these control tools is endangered by the spread of resistance to insecticide of malaria vectors in Nigeria (Awolola *et al.,* 2009; Ibrahim *et al.,* 2019; Oduala *et al.,* 2019; WHO, 2022; Kayode *et al.,* 2023).Resistance of *Anopheles* mosquito to pyrethroids, organochlorines and carbamate were also reported in Southern Nigeria with the exception of carbamate in South East (Chukwuekezie *et al.,* 2020; Oduola *et al.,* 2012). Additionally, high resistance to permethrin, DDT and the emergence of resistance to bendiocab in *An. Coluzzii* was reported in Northwest Nigeria (Ononamadu *et al.,* 2020). Studies conducted in Northern Nigeria shown high resistance of *Anopheles gambiae* to pyrethroids and DDT and less resistance to bendiocarb in *An. coluzzii* (Ibrahim *et al.,* 2019; Ahmed-Yusuf *et al.,* 2020) and widespread of *Anopheles* resistance developed against all the four WHO recommended classes of insecticide in Southern Gombe (Oduala *et al.,* 2019)). Therefore, the present study

was prompted by the paucity of information on the exact insecticide resistance status in Sudano-Sahelian of Gombe State.

MATERIALS AND METHODS

Study area

Gombe State is located in the North-Eastern region of Nigeria and it lies between latitude9°30′00″ and 12°30′00″ N and longitude 8°45′00″ and 11°45′00″ of the Greenwich Meridian. It covers a total land area of 18,768km² with projected population of 3,545,032 from 2006 census (Danjin *et al.*, 2020). It is characterized with annual rainfall of 850mm and temperature of 30 °C. The vegetation of Gombe State is Sudan Savannah and experience two distinct season, the dry season (November – May) and the rainy season (June –October) with an average rainfall of 850 mm. It is an agrarian State with about 60% of the population engaged in agriculture while some engage in business and few are civil servant. It shares boundaries with Yobe State to the North, Borno and Adamawa States to the East, Bauchi State to the West and Taraba State to the South. Based on climatic differences Gombe State can be divided into three zones that approximately squares with the three senatorial districts, namely; Southern Guinea Savanna (SGS) in Gombe South, Northern Guinea Savanna (NGS) in Gombe Central and Sudan Savanna (SS) in Gombe North (Msheliza and Bello, 2018).

Purposive sampling of two (2) LGA was carried out based on the presence of mosquito breeding habitats, farming activities, history of LLINs use, geographical location, zone and epidemiological importance including relatively high malaria infection rate. The study was conducted in two selected LGAs; Akko and Kaltungo of Gombe State.

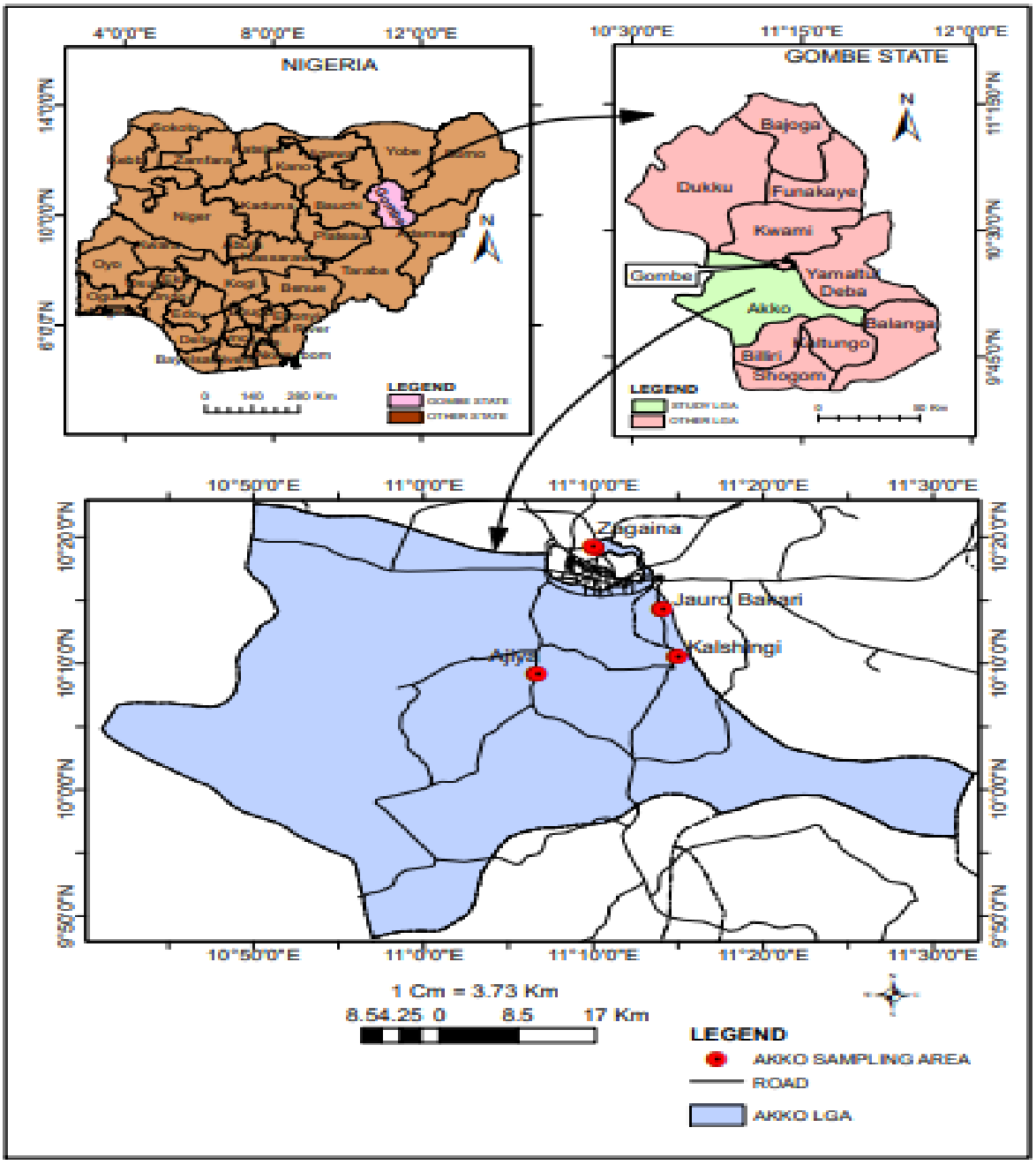

Figure 1: Map of Akko LGA sampling communities (Source: ZASTAL Kashere)

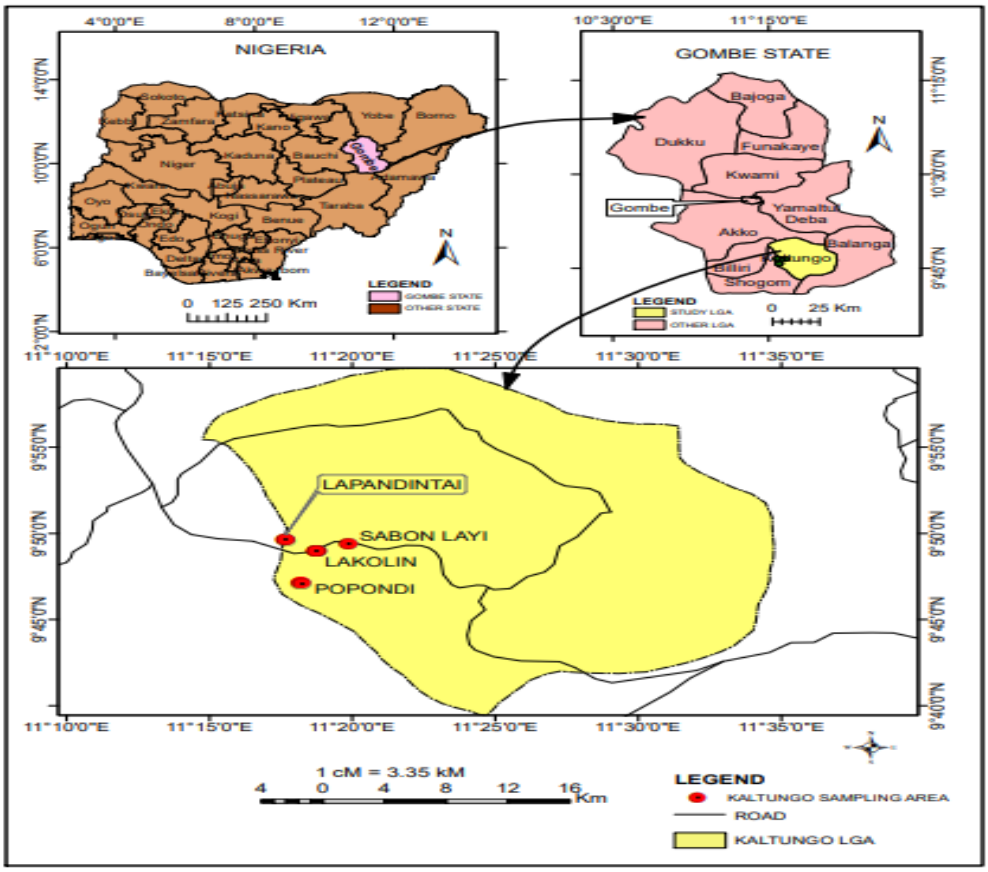

Figure 1: Map of Kaltungo LGA sampling communities (Source: ZASTAL Kashere)

Mosquito Larval Collection and Rearing

Immature (larvae and pupae) of *Anopheles* mosquito were identified in the breeding site using their resting position in the water and collected from eight (8) communities of the two Local Government Areas (Akko and Kaltungo) of Gombe, Nigeria. They were collected from their natural breeding sites such as rice farms, small pools, puddles, streams, construction site, trailer park, ditches, pond and potholes near residential houses (Umar and Nock, 2018) using the standard dipping method from January to December, 2022. A 350 ml dipper and Soup ladle were use depending on the volume of water in the breeding habitat and distance of the mosquito larvae in the water. Scooping techniques was employed to scoop the immature stages and emptied into a small transparent bowl. The bowl was scrutinized for the presence of predators. A pipette was used to remove all the predators. Coordinates of the breeding sites was established using the Global Positioning System (GPS) software (GPS Waypoint). Both the larvae and pupae of *Anopheles* mosquitoes that were collected are being transported to the Gombe State University, malaria control sentinel site laboratory, where they were reared to adulthood under control condition of temperature 28±3 °C, relative humidity 70±10% and 12L:12D photoperiodicity. The adult *Anopheles* mosquitoes that emerged from the larvae bowl were aspirated into the adult mosquito cages and fed with 10% glucose solution saturated in cotton wool till required for insecticides susceptibility test.

Morphological Identification *Anopheles* Species

All *Anopheles* mosquito collected indoor and those exposed to insecticides impregnated papers and ITNs were morphologically identified using Afrotropical Anopheline morphological taxonomic keys as updated by Coetzee, (2020a). The morphological features were observed with the aid of X10 Zeiss light stereo microscope and LCD digital microscope (1-1200x, China). The morphological feature includes; absence of lateral abdominal tufts of hair, speckled legs, 3- banded palps and pale interruptions on the 3rd main dark area of vein one of the wing. Individual identified mosquitoes were placed in 1.5 ml Eppendorf tubes containing silica gel plugged with tissue paper and kept at the Gombe State University Malaria Control Sentinel Site Laboratory.

Source of WHO test kits

The WHO insecticides impregnated papers; deltamethrin (0.05%), DDT (4%), bendiocarb (0.1%), pirimiphous-methyl (0.25%) representing each of the insecticide class, PiperonylButOxide (PBO) (4%) and WHO diagnostic test kit were obtained from the WHO/Vector Control Research Unit (VCRU) of the University of Sains Malaysia (Penang, Malaysia) supply and distribution channel through Global Fund and Nigerian Institute of Medical Research Vector Control Unit, Yaba, Lagos State, Nigeria.

Susceptibility bioassay

Three thousand and ninety-five (3,095) adult female *Anopheles* mosquitos from the two LGAs were tested against 0.25 % pirimiphos-methyl, 0.05% deltamethrin, 4% DDT and bendiocarb (0.1%) WHO insecticide impregnated papers. A total of 100 adult female *Anopheles* mosquitoes were exposed to each insecticide paper for 60 minutes at 28 ± 3◦ C and 70 ± 10% relative humidity. Batches of 25 sugar fed adult females *Anopheles* mosquitos aged 2-5 days was aspirated and exposed to each of the four tubes (replicate) containing

insecticide-impregnated paper for 1 hour. Knockdown rates were recorded at 5, 10, 15, 20, 30, 40, 50, and 60 minutes' post exposure. Test mosquitoes were then transferred back into holding tubes and feed with 10% glucose solution in moistened cotton wools for 24 hours mortality post-exposure period. All the bioassay was accompanied by two negative controls (non- insecticide papers) containing 50 adult female mosquitoes (World Health Organization, 2022). When the mortality in the control ranges between 5% and 20% Abbott's formula was used to correct the mortality (Abbott, 1987).

Piperonyl Butoxide (PBO) synergist bioassay

The Synergist bioassay, using WHO impregnated papers, was carried out with PBO (4%) against deltamethrin (0.05%). Four replicates of 25 non-blood fed female *Anopheles* mosquito were exposed to 4% PBO impregnated papers for 1 hour to suppress oxidase enzyme and then transferred to tubes containing deltamethrin (0.05%) for additional 1 hour (World Health Organization, 2016). Knockdown time was taken at intervals for 60 minutes. Exposed mosquitoes were transferred to holding tubes and provided with 10% glucose soaked in cotton wool and the mortality was recorded after 24 hours. Two control tubes were also run in parallel containing non- insecticide impregnated papers.

Data analyses

Two-way ANOVA was used to determine the significant differences in the means of insecticides. WHO criteria was used to classify mosquitoes as 'resistant' if less than 90% mortality was observed, resistance needing confirmation if mortality was between 90-97%, and susceptible if between 98-100% (World Health Organization, 2016). All the data collected was analyzed and tested at $\rho<0.05$ for significance.

RESULTS AND DISCUSSION

Susceptibility Profile of *Anopheles* Mosquitoes to Four Insecticides

A total of 3095 mosquitoes were tested for susceptibility against deltamethrin (0.05%), DDT (4%), bendiocarb (0.1%) and pirimiphous-methyl (0.25%) in all the study sites. Total of 2389 were susceptible and 706 were resistant to the four insecticides tested. This was mainly determined by the number of *Anopheles* mosquitoes available for use. A single susceptibility test should have sufficient mosquito numbers; i.e. 150 non-blood female mosquitoes constitute the recommended sample size (WHO, 2016). The negative control experiment with *Anopheles* mosquitoes exposed to untreated bioassay papers yield mortality; therefore, correction of natural causes of mortality by Abbott's formula was done.

The emerged non-blood fed female *Anopheles* mosquitoes from the larvae collected showed they were resistance to deltamethrin (0.05%) concentration with mortality ranging between 2% and 89% in all the study communities except in Popondi (mortality-90%) where the "possible resistance" status was recorded. DDT (4%) mortality obtained showed resistance in four (4) out of the eight (8) communities and possible resistance was registered in Kalshingi (mortality-94%) and Lapandintai (mortality-93%) while Susceptible status of the *Anopheles* was recorded against DDT (4%) in Popondi and Sabon Layi with mortality of 99% and 98% respectively. On the other hand, mosquitoes from the other study communities namely: Jaro Bakari, Kalshingi, Lapandintai, Popondi and Sabon Layi were susceptible to bendiocarb 0.1% (mortality >98%) and Possible resistance was registered in Zagaina (mortality-93%), Ajiya quarters (mortality-93%) and Lakolin (mortality-95%). All the mosquitoes tested against Pirimiphos-methyl (0.25%) were susceptible with a mortality >98% in the eight (8) study sites. However, there was significant difference in the mean mortality of *Anopheles* mosquitos tested in all the eight study communities ($\rho>0.05$) (Table 1).

Table1: Insecticides susceptibility pattern of *Anopheles* mosquitoes in relation to location

Study site	Insecticides tested	Number of mosquito exposed	24hrs Mean mortality ± S.D	24hrs Percentage mortality	Status	Sig
Ajiya quarters	Deltamethrin 0.05%	100	13.75±1.71^{c}	55	R	0.000
	DDT 4%	117	5.75±1.71^{c}	20	R	0.000
	Bendiocarb 0.1%	111	25.75±4.19^{b}	93	SR	0.000
	Pirimiphos-methy 0.25%	112	28.00±2.94^{a}	98	S	0.000
Jauro Bakari	Deltamethrin 0.05%	100	9.50±2.08^{c}	38	R	0.000
	DD 4%	81	9.50±2.38^{c}	47	R	0.000
	Bendiocarb 0.1%	104	26.00±0.82^{b}	100	S	0.000
	Pirimiphos-methy 0.25%	106	26.75±0.96^{a}	100	S	0.000
Kalshingi	Deltamethrin 0.05%	90	15.00±1.83^{c}	60	R	0.000
	DDT 4%	79	1.25±1.26^{c}	94	SR	0.000
	Bendiocarb 0.1%	78	19.25±4.27^{b}	99	S	0.000
	Pirimiphos-methy 0.25%	102	25.50±2.52a	100	S	0.000
Lakolin	Deltamethrin 0.05%	103	17.00±4.83^{c}	66	R	0.000
	DDT 4%	92	17.00±2.16^{c}	74	R	0.000
	Bendiocarb 0.1%	88	21.00±2.83^{b}	95	SR	0.000
	Pirimiphos-methy 0.25%	93	23.25±1.26^{a}	100	S	0.000

Lapandintai	Deltamethrin 0.05%	92	20.25±7.68^{cd}	89	R	0.000
	DDT 4%	82	19.00±4.55^{cd}	93	SR	0.000
	Bendiocarb 0.1%	90	22.50±5.74^{bd}	100	S	0.000
	Pirimiphos-methy 0.25%	100	25.00±3.^{74ad}	100	S	0.000
Popondi	Deltamethrin 0.05%	82	18.50±8.43^{cd}	90	SR	0.000
	DDT 4%	102	25.50±3.12^{cd}	99	S	0.000
	Bendiocarb 0.1%	96	23.50±1.29^{bd}	98	S	0.000
	Pirimiphos-methy 0.25%	96	24.00±0.00^{ad}	100	S	0.000
Sabon Layi	Deltamethrin 0.05%	89	14.50±0.58^{cd}	65	R	0.000
	DDT 4%	99	24.25±0.50^{cd}	98	S	0.000
	Bendiocarb 0.1%	86	21.00±0.82^{bd}	98	S	0.000
	Pirimiphos-methy 0.25%	89	22.25±2.06^{ad}	100	S	0.000
Zagaina	Deltamethrin 0.05%	100	0.50±0.58^{c}	02	R	0.000
	DDT 4%	119	0.75±0.96^{c}	03	R	0.000
	Bendiocarb 0.1%	117	27.25±2.50^{b}	93	S	0.000
	Pirimiphos-methy 0.25%	100	25.00±0.00^{a}	100	S	0.000

R = Resistant, S = Susceptible, SR= Suspected resistance. Mean mortality ±S.D with the same super script across column are not significantly different ρ<0.05

Metabolic resistance and involvement of P450 Cytochromes mono-oxygenase enzyme

To determine the contribution of PBO in the insecticide resistance mechanism. A total of 717 *Anopheles* mosquito species were exposed to PBO for one (1) hour along followed by deltamethrin for another one (1) hour in all the study sites. Exposure of deltamethrin along gave a susceptibility ranged between 2% and 90% after 24hours post-exposure in all the study site indicating resistance. A significant recovery from resistance to susceptibility was observed when mosquitoes were pre-exposed to the synergist piperonyl butoxide (PBO) followed by exposure to deltamethrin. Overall, the recoveries for deltamethrin were ranged from 18% and 100% indicating increase in susceptibility after 24 hour in all the study locations. Full recovery 100% susceptibility was observed in Lapandintai, Sabonlayi and Popondi and partial recovery 18% in Zagaina. This observation suggests that partial (not only metabolic but other resistance mechanism) and or full (metabolic resistance mechanism) involvement of cytochrome P450 monooxygenase enzymes in *Anopheles* mosquito deltamethrin- resistance in Zagaina, and JauroBakari Lapandintai, Sabonlayi and Popondi communities, respectively.

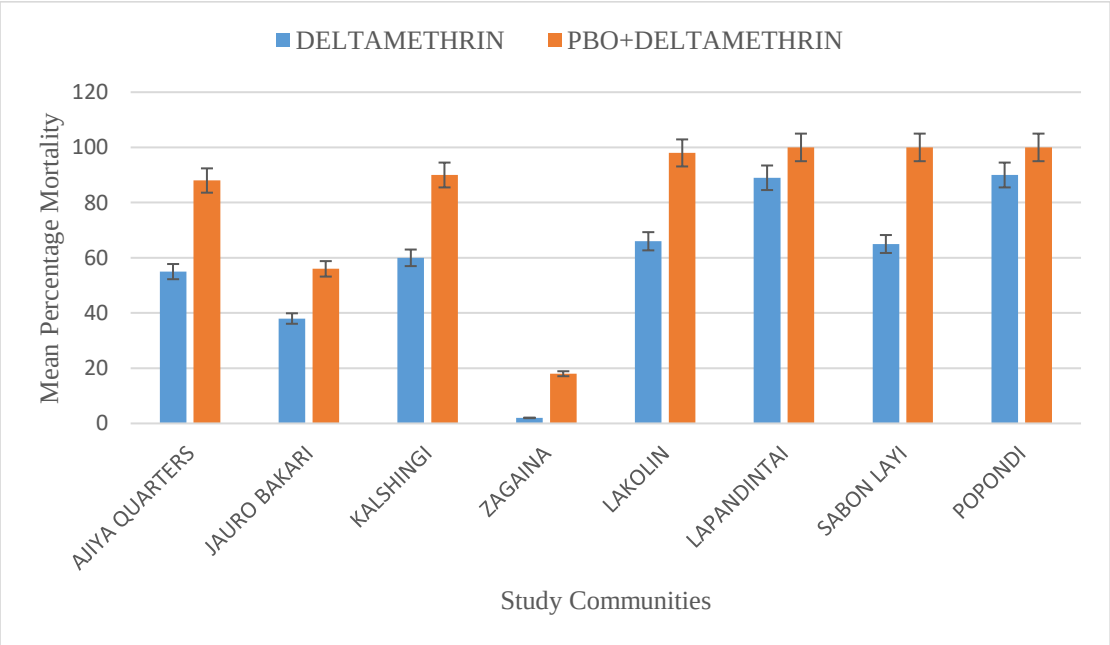

Figure 2: Mean mortality of Anopheles mosquitoes from the eight study communities after 24 hours exposure to deltamethrin alone and PBO plus deltamethrin

Discussion

Resistance to chemical insecticides use in public health for the control of vector borne diseases is a serious problem on the effectiveness of the current mosquito vector control interventions. The results from this study established deltamethrin 0.05%, DDT 4% and bendiocarb 1% resistance in *Anopheles gambiae* s.l. in Akko and Kaltungo LGAs of Gombe State. The study indicate highly deltamethrin resistance of 2% in Zagaina, Akko LGA and possible susceptibility of 90% in Popondi, Kaltugo LGA respectively and the later concurs with the finding of Ibrahim *et al.* (2014) that reported moderate resistant (78.4%) in Sudan Savannah of Northern Nigeria. Previously, deltamethrin resistance have been reported in Y/Deba where mortality was 56% (Ahmed-Yusuf *et al.*, 2020), Southern Gombe reveal mortality of 55% in deltamethrin (Oduala *et al.,* 2019), 40% mortality in Adamawa (Wahedi *et al*., 2021), two study site of Rivers State (Muhammad *et al.,* 2021) and in Ifite-Awka, Anambra State (Mortality, 30%) (Akunne *et al.,* 2019). Some level of resistance of *An. gambiae* s.l. to deltamethrin has been established in all ecozones of Nigeria (PMI, 2019). It also corroborates with the work of Zanga *et al*. (2022) who recorded highly resistance to deltamethrin in Kinshasha and Ellibou (NDri *et al.,* 2023) in Code'voire. In contrast, susceptibility to deltamethrin was reported in Nasarawa (Lama *et al*., 2021) North Central and 98.5% susceptibility in Ibadan, South West, Nigeria (Ibrahim *et al.,* 2013). The increase in the deltamethrin phenotypic resistance profiles among the *Anopheles gambiae* sl species tested might be attributed to the use of single pyrethroid insecticides in domestic and agriculture for vector borne disease control and elimination. Whereas the application of pesticides by the farmers for agricultural pest control is largely associated with level of education, socio-economic status and knowledge of pest in question (Kouamé *et al*., 2022). Over use of chemical pesticide for agricultural purposes for many year can exact huge selection pressure on the larval stages of mosquitoes which might resulted into development of insecticide resistance of the mosquito larvae (Piameu *et al*., 2021). Pyrethroid resistance in malaria vector was first reported in Nigeria as cited by Awolola *et al.* (2007) in 2002 since then, several studies have reported mosquitoes developing resistance to one or more insecticide classes (Oduala *et al.,* 2012; Oduala *et al.,* 2019; Ibrahim 2019; Fagbohun *et al*., 2020; Lama *et al.,* 2021; Owolabi and Ayankoya, 2023 Adeogun *et al.,* 2023).

Although, DDT (4%) was banned since the 80s due to long persist in the environment, residual efficacy on malaria vector and other vector-borne disease vectors (Jacob and Yoriyo, 2015) high level of resistant was established across all the ecological zone of Nigeria and Africa (Ibrahim *et al.,* 2014; Habibu *et al*., 2017; Oduala *et al.,* 2019; Ahmed-Yusuf *et al*., 2020; Adeogun *et al.,* 2023; NDri *et al.,* 2023) which is similar to our result from all the communities except Popondi (99%), SabonLayi (98%) where susceptibility was established and possible resistance was registered in Kalshingi (94%) and Lapandintai (93%). The susceptibility recorded might be as a result of mosquito use for the susceptibility bioassay in those study location were mostly other *Anopheles* species (*An. pretoriensis, An. maculpalpis* and *An. rufupes*) that are more fragile than the *An. gambiae* complexes. The resistance to DDT (4%) established in this study is an indication of cross resistance to deltamethrin because both the insecticides have similar mode of actions. The occurrence of both resistance and possible resistance to DDT revealed in this study may be linked to the use of organochlorine pesticides by subsistence farmers in storing their farm products as well as exposure of the mosquitoes to this insecticide in stores. The pesticides contaminate the adult mosquitoes resting indoor thereby leading to selecting pressure for resistance in this particular study communities. Currently, most organochlorine pesticides in Nigeria are not authorized for use especially in the public health sector. However, this chemical insecticide has a long residual effect which contaminates mosquito larval habitats. Studies have documented evidence on residues of DDT found in soil and water collected from the Indian Ocean (Lalah, 1993). Therefore, this characteristic residual effect of DDT cannot be ignored as a cause of this potential resistance in Gombe.

However, On the other hand mosquitoes from JaroBakari, Kalshingi, Lapandintai, Popondi and Sabon Layi were susceptible to bendiocarb (0.1%) with mortality >98%. This agree with the recent study done in Nasarawa (Lama *et al.,* 2021), Adamawa (Wahedi *et al.,* 2021), Umudike (Ekedo and Ukpai, 2020), 100% recorded in Niger Delta zone (Ekerette and Ebere, 2022) and 100% observed in Kinshasha (Zanga *et al.,* 2022). Possible resistance was registered in Zagaina (mortality 93%), Ajiya quarters (mortality 93%) and Lakolin (mortality 95%) respectively. This finding corroborates with the work carried out in four sentinel sites of Ondo State (Owolabi and Ayankoya, 2023) and Ononamadu *et al*. (2020) recorded possible resistance in *An coluzzii* from Northwest, Nigeria. Contrary to the finding of Habibu *et al.* (2017) in Bichi recorded high resistance in *An. gambiae* complex and recently, resistance to bendiocarb 0.1% was established in Southern Gombe (Oduala *et al.,* 2019), Y/Deba (Ahmed-Yusuf *et al.*, 2020) and Cote de'ivore (N'dri*et al.*, 2023). The susceptibility established may be due to less application of bendiocarb for both agriculture and domestic pest control.

All the mosquitoes tested against Pirimiphos-methyl (0.25%) were susceptible with a mortality >98% in the eight (8) study sites. Conversely, earlier studies in Southern Gombe suggests that, *An. gambiae* s.l. were resistance to pirimiphos-methyl (0.25%) (Oduala *et al.,* 2019), Ifite Awka, Anambra State with mortality of 15.31% (Akunne *et al.,* 2019) and suspected resistance in Ondo (Owolabi and Ayankoya, 2023). Additionally, resistance of *An. gambiae* s.l. to 0.25% Pirimiphos-methyl, DDT and pyrethroid has been reported in Niger Delta zone (Ekerette and Ebere, 2022) and Umudike (Ekedo and Ukpai, 2020). The susceptibility might be due to less application of pirimiphos-methyl (0.25%) in agriculture and domestic pest control. However, pirimiphos-methyl is known to be applied in IRS but not in the impregnation of LLINs which is the most currently deployed intervention for malaria vector control in Gombe. In a separate study, pirimiphos-methyl (0.25%) have been reported to exhibit high knock dawn and killing efficacy of *An. gambiae* s.l. (Kendie *et al*., 2023) and it was the only insecticide use in IRS with high efficacy than in pyrethroid and carbamate in *An. gambiae* s.l. resistant population from Mali (Keita *et al*., 2020). However, there was significant difference in the mean mortality of *Anopheles* mosquitos tested with all the four insecticides representing one from each classes in all the eight (8) study communities ($P<0.05$).

A significant recovery of susceptibility was observed after *Anopheles* were pre-exposed to the synergist Piperonyl But-Oxide (PBO) (4%) prior exposure to deltamethrin (0.05%) with mortality ranged from 18% and 100% after 24 hour in all the study locations. Full recovery 100% susceptibility was observed in Lapandintai, Sabonlayi and Popondi and partial recovery 18% in Zagaina. Moderate susceptibility was observed in Ajiya quarters, Kalshingi and Lakolin. Similarly, studies have reported increase in mortality in *An. gambiae* s.l. after pre-exposure to PBO-synergist with pyrethroid in

Southern Nigeria (Omotayo *et al.*, 2022; Ekerette and Ebere, 2022) and North West (Ononamadu *et al.,* 2020). This also agree with the finding of Zanga *et al.* (2022) who reported that, susceptibility was restored in resistance population of *An. gambiae* s.l. in Kinshasha. The increase in susceptibility might be due to breaking of the knockdown resistance gene in *Anopheles* mosquito population suggesting, partial and full involvement of cytochrome P450 monooxygenase enzymes in *Anopheles* mosquito resistance against deltamethrin in the study locations. Similarly, this could be reason behind low malaria prevalence among children aged 3-59 month in Gombe State after mass distribution campaign of deltamethrin-PBO nets and seasonal malaria chemoprevention that is ongoing since 2021 (NMIS, 2021). In Benin, better performance of PBO nets was observed after mass distribution campaigns in resistant population of *Anopheles gambiae* s.l. (Pennetier *et al*., 2013). Similarly, Dazie *et al.* (2017) reported high susceptibility of *Anopheles gambiae* s.l. from Ghana in the presence of pyrethroid resistant after LLINs mass distribution campaign. However, recent studies revealed less performance of deltamethrin-PBO net in Tanzania and Benin when compared with chorofenapyr Nets (Gleave *et al*., 2023). Although, non-PBO net provide protection against malaria vector; Pyrethroid-PBO performed better in enhancing less parasite prevalence via reduction in mosquito blood feeding rate and increase high mosquito mortality. Our finding suggests the involvement of P450 monoxygenase enzyme in the resistant (metablolic resistance mechanisms) population of *Anopheles* mosquitoes from Gombe State which is similar to the finding of Omotoyo *et al.* (2022). We also suggest that, the recovery of mortality from PBO pre-exposure indicate the role of metabolic resistance mechanism (WHO, 2021a; Soumaila *et al*., 2022). The synergist action of PBO (4%) plus deltamethrin (0.25%) in this study is as a result of hindering the activity of oxidase enzyme.

CONCLUSION

Anopheles mosquitoes collected from all the study communities were resistant to both deltamethrin and DDT insecticides except in Lapandintai and Popondi. Evidence of phenotypic resistance in *Anopheles* to bendiocarb was also seen in few of the communities. However, all *Anopheles* mosquitoes tested were susceptible against pirimiphos-mythyl in all the study community. It was established that, PBO used as synergy with deltamethrin (0.05%) increase the susceptibility of resistant *Anopheles* population in Gombe which implicate CYP450 enzyme complex in the resistance.

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