The use of some pyrethroids (insecticides), their combination and synergist on *Anopheles* mosquitoes in Isoko South, Delta State

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Humans get infected with malaria parasites (*Plasmodium* species) through the bites of infected female *Anopheles* mosquitoes. Malaria incidence is rapidly increasing and insecticide resistance remains a global threat to vector control programmes. Considering the fact that susceptibility studies are lacking in Delta State, this present study evaluates the efficacy of using some insecticides (deltamethrin and permethrin), their combination and synergists (PBO) on the female *Anopheles* mosquitoes in Isoko South LGA. Larva and pupa of *Anopheles* mosquitoes were collected using 350 ml dippers. Two to five day-old emerged females were exposed to 0.75% deltamethrin and 0.05% Permethrin using the WHO bioassay and CDC bottle assay methods (combination) in four replicates. Percentage knock down time for 50 and 95% was determined using probit analysis. Mosquitoes' susceptibility to permethrin and deltamethrin was 72 and 87%, respectively and were termed resistant following WHO standard protocol. In the combined forms, mortality of 71 and 99% was recorded at 15 and 30 min, respectively. The differences between insecticides and combined forms were highly significant ($P<0.0001$). Synergized permethrin and deltamethrin recorded high susceptibility of 99 and 98%, respectively. The highest percentage knock down was recorded with synergized permethrin and deltamethrin at 60 min of exposure and the difference was significant ($P<0.05$). KDT_{50} and KDT_{95} ranged between 38.99 and 57.06 and 147.17 and 284.66 min, respectively. Synergized permethrin recorded the lowest (38.99 and 147.17 min, respectively). In conclusion, synergized forms of deltamethrin and permethrin could be adopted for the control of *Anopheles* mosquitoes' population. This could be used for impregnating bed nets as it would be effective against the spread of malaria in Isoko South Local Government Area.

Key words: Deltamethrin, permethrin, combined forms, synergist, *Anopheles* mosquitoes, Delta State.

INTRODUCTION

The female *Anopheles* mosquito spreads *Plasmodium* which causes malaria, a disease of public health importance worldwide and are endemic to several regions in Southeastern Asia, Sub-Sahara Africa, Eastwards Mediterranean, America, and Westwards Pacific (Agegenehu et al., 2018; Tesfa et al., 2018; Magaco et al., 2019). Nearly, all age groups of human are infected especially children under the age of five and this accounts for over 60% of reported cases (WHO, 2019), and estimated 25 to 30% mortality in infants and children (Federal Ministry of Health, 2006). Observation from a notable study by Tesfa et al. (2018) has shown that five different *Plasmodium* species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malaria*, *Plasmodium ovale* and *Plasmodium knowlesi*) cause human malaria; this leading to increased cases and sustained infection worldwide (Magaco et al., 2019; WHO, 2019). *P. falciparum* malaria infection is the deadliest causing high mortalities and morbidities when corroborated with Dengue, Chikungunya, Zika, HIV/AIDs amongst others while *P. vivax*, *P. malaria* and *P. ovale* malaria remain mild (WHO, 2014; Caraballo and King, 2014). However, the occurrence of *P. knowlesi*, a zoonotic pathogen, has been diagnosed in human (Oyewole et al., 2005). Infections caused by *Plasmodium* are prevalent and cosmopolitan almost in all communities in Nigeria. According to Calvo et al. (2009), *Anopheles* mosquitoes consist of over 400 species, of which 100 species are known to transmit malaria and only about 30 to 40 species infecting humans in various endemic settings. Mosquitoes thrive in close association with humans and animals, causing diseases of medical and veterinary concerns. Furthermore, observations made by Wang et al. (2011), showed that immature stages of *Anopheles* mosquitoes can stay up to 5 to 14 days while adult females can survive up to a month or more in laboratory culture. Though most do not survive more than 14 days in the wild, this length of time and vector potential is enough to transmit the malaria parasites from one host to the other (Mouchet et al., 2004). Different species of *Anopheles* such as *Anopheles gambiae, Anopheles funestus, Anopheles coustani, Anopheles nili, Anopheles mela* and *Anopheles moucheti* (WHO, 2016; Oyewole et al., 2010) were reported in several communities in Nigeria and coastal regions inclusive. *A. gambiae* (s.s), *Anopheles arabiensis* and *A. funestus* are the most prevalent and major cause of human malaria disease in Nigeria (Sinka et al., 2010).

The control of these species and the diseases they cause has been ongoing. Before now, mosquito control programme adopted the use of insecticide incorporated coils, indoor residual sprays (IRS), and treated clothing (Khairy et al., 2017). However, long lasting bed nets (LLBNs) and insecticide treated nets (ITNs) have equally been a highly valued tool frequently deployed for the protection of individuals from the bite of the female *Anopheles* (Lengeler, 2004; Tukei et al., 2017). More recently, the control of malaria in Africa using insecticide treated nets suffered setbacks (WHO, 2017) probably due to the lack of knowledge, unsustainable vector control practices, nonuse or distribution problems of ITNs (Mazigo et al., 2010; Aderaw and Gedefaw, 2013; Khairy et al., 2017). In same vein, the rise in the reports of insecticide resistance profile has directed the focus on determining the susceptibility status of the species to various grades of insecticides to guide the decision of policy makers as to what insecticides is best effective (IRAC, 2011; Oxborough, 2016; Ranson and Lissenden, 2016). The use of pyrethroid insecticides for the treatment of bed nets have been recommended by the WHO (WHOPES, 2012). However, the extended use of pyrethriod insecticides for agricultural crop protection has resulted in pyrethroid resistance in *A. gambiae* (Czeher et al., 2008; Ranson et al., 2011). The exposure of *Anopheles* mosquitoes to deltamethrin in Southeastern Nigeria showed that the mosquitoes were resistant to the insecticide (Chukwuekezie et al., 2020). In the same vein, *Anopheles* exposed to permethrin also showed some level of resistance of the mosquitoes to the insecticide in Peruvian Amazon (N'Guessan et al., 2007), and there are possibilities of pyrethroid resistance spreading to other areas. Due to this shortfall, several funders have risen to bring vector control to fruition in Nigeria through promotion of ITNs usage and IRS, and these funders include the Global fund, President's malaria initiative (PMI), World Bank, UNICEF, government and other local funders (Katz et al., 2011; Nigeria, 2011). In the past six years, mosquito surveillance in Nigeria has been in some selected states including Enugu, Niger, Kwara, Plateau, Rivers, Jigawa, Nasarawa, Lagos, etc. Delta among other states was recently included in the national surveillance.

Piperonyl butoxide (PBO-Synergist) has also been used in addition to insecticides to inhibit the metabolic defence causing resistance in species of mosquitoes (Vijayan et al., 2007; Dadzie et al., 2017). It is only when an understanding of the *Anopheles* species, vector biology, density diversity, biting rate, time, season, and effective use of the recommended vector controls options has been established that requisite actions on effective control would make more sense (WHO, 2011; Breman and Brandling-Bennett, 2011; Govella and Ferguson, 2012). Mosquito control programme has suffered several setbacks but their effective control has recently involved ascertaining the susceptibility status of mosquitoes to various grades of insecticides and incorporating the recommended in deployed bed nets. Since the year 2000, insecticide treated nets is one among the many highly valued and frequently employed methods adopted specifically to act as barrier to mosquito bites and for disease prevention (Corbel et al., 2004; Griffin et al., 2010; Monahan, 2017).

Currently, pyrethriod resistance is highly occurring and this not only makes the insecticide treated nets less active against target species, but also influences the efficiency of indoor residual sprays in houses. Resistance has been pointed out in area where the use of ITNs and intensive spray of insecticides is prevalent (WHO, 2016). There is little or no knowledge on the susceptibility status of deltamethrin, permethrin, their combination and synergist in Isoko South Local Government Area of Delta State. This study is important as it will provide baseline data required for establishing control measures of malaria in that locality and help policy makers in the choice of insecticide treated nets to be used. Hence, in this study, the use of deltamethrin, permethrin, their combination and synergists on the female *Anopheles* mosquito in Isoko South L.G.A of Delta State was investigated.

METHODOLOGY

Study area

The female *Anopheles* mosquitoes larvae were obtained from Olomoro and Oleh in latitude (5.436277° and 5.459901°) and longitude (6.142218° and 6.203129°), respectively (Figure 1).

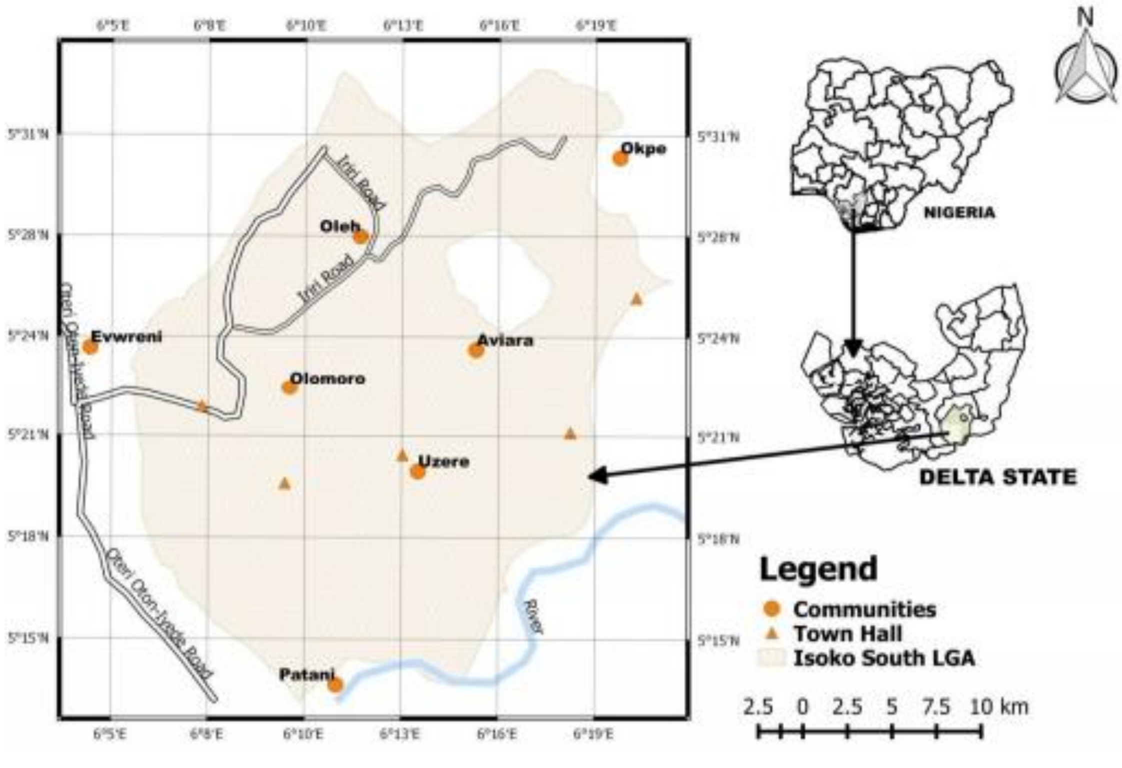

Figure 1. Map of Isoko South showing sampled locations.
Source: Ogeh et al. (2017).

Materials used

Materials such as adjustable laddles (350 ml dip) were used for scooping out larvae from their habitats; GPS mapping device was used to take the latitude and longitude reading of the villages; buckets and tray were used to transfer and rear larvae and pupae. Aspirators were used to transfer emerged adult mosquitoes to the adult net cages. WHO kits which comprise insecticide impregnated paper (deltamethrin of 0.75% concentration and permethrin of 0.05% concentration), four WHO exposure tubes marked with red dots, one control tube marked with yellow dot, four WHO holding tube marked with green dots lined with untreated paper, stopwatch for taking record of the time at intervals, WHO susceptibility test record form, thermo-hygrometer for measuring the humidity and temperature, and protective wears (lab coats, face masks and hand gloves).

Mosquitoes collection and rearing

Larva and pupae of *Anopheles* mosquito were collected from breeding sites (ponds, ditches and puddles) in Oleh and Olomoro. This was done at early hours of the day and during the raining season between July and September, 2020. Culturing of mosquitoes was done in the insectary of the Department of Animal and Environmental Biology, Delta State University, Abraka. The exposure was carried out in the laboratory with temperature 28±2°C and relative humidity of 80±4°C.

The larvae and pupae collected were kept in trays covered with net caps to prevent the emerging adults from escaping. Immature stages were fed with a mixture of 10 g of yeast to one piece of cabin biscuits. Emerged adult mosquitoes were transferred to the adult cages using an aspirator and the females were separated from the males using the proboscis and antennae as reference points. The females were used for the susceptibility test.

Susceptibility assay

This study adopted the WHO susceptibility test procedures as described by WHO (2020). The protocol of the WHO involved the use of insecticide impregnated papers in different diagnostic dose in order to assess the susceptible profile of *Anopheles* mosquitoes. Deltamethrin (0.75%) and permethrin (0.05%) were used in their single forms. Twenty five female *Anopheles* mosquitoes were introduced into four replicated treatments and control using an aspirator. Insecticide impregnated papers were lined in exposure tubes while untreated paper served as control in the holding tube. Then the mosquitoes were gently blown into the WHO exposure tubes containing the insecticide impregnated papers and control. Reading for knock down was taken for 10, 15, 20, 30 and 60 min. The mosquitoes (alive and knocked down) were gently transferred back into the WHO holding tubes and provided with food (cotton wool moistened with 10% sugar solution) and kept for 24 h. The knockdown at 60 min was recorded in the WHO susceptibility test record form while the mortality was recorded after 24 h exposure.

Combined efficacy assay

The combined efficacy was done using the CDC standard guidelines. 1 ml of permethrin and deltamethrin were measured in the proportion of 0.5 ml: 0.5 ml using calibrated pipette into four CDC bottles. The bottles were coated by swirled up, down and side to ensure the circulation of insecticide mixture. The bottles were left to dry for 24 h at room temperature. Then 25 female *Anopheles* mosquitoes each were introduced into four replicated treatments and control using an aspirator. Counts for mortality were taken for 15 and 30 min. After 30 min, mosquitoes were gently transferred into a holding cage where resistant species were separated from susceptible ones using an aspirator.

Synergistic assay

Twenty-five (25) female *Anopheles* mosquitoes were introduced into four replicated piperonyl butoxide (PBO) impregnated papers using an aspirator. The set up in WHO holding kit was left for 1 hour to allow for breakdown of metabolic enzymes. Thereafter, they were transferred to permethrin and deltamethrin impregnated papers for another 60 min. Untreated papers in WHO kits served as control in the holding tube. Counts were taken for knock down at 10, 15, 20, 30 and 60 min. The mosquitoes (both alive and knocked down) were gently transferred back into the WHO holding tubes and left on a water guarded tray for 24 h for mortality records. Sugar solution moistened in cotton wool was also used to feed the mosquitoes. Species were transferred to the holding cage as resistant ones were separated from susceptible ones before microscopic identification. This assay equally adopted the WHO standard procedure.

Mosquito identification and preservation

After mortality records were taken, the susceptible female mosquitoes were viewed and identified under the dissecting microscope while the resistant ones were killed in killing jar using ethyl acetate soaked in cotton wool. They were identified using standard manual by Gilles and Coetzee (1987) and Awono-Ambene et al. (2004). After identification, the female *Anopheles* mosquitoes were preserved in an eppendorf tube containing silica gel following WHO standard method.

Data analysis

Data were entered into MS Excel 2013 and checked for possible errors. Results of knock down were presented in percentages. ANOVA test was used to compare percentage mortality after 24 h. Significance was set at α=0.05. KDT_{50} and KDT_{95} were computed and modelled using the Pearson goodness of fit.

RESULTS

Anopheles spp. identification

The five hundred mosquitoes exposed to the insecticides and viewed under the dissecting microscope were identified as *A. gambiae* with the following morphological characteristics including three pale bands on palp, speckled legs, laterally projecting tufts at the abdomen, last two segment of the hind tarsi not being pale, and more than one pale bands at the proboscis.

Susceptibility status of *Anopheles* mosquitoes

The female *Anopheles* mosquitoes exposed to permethrin, deltamethrin, synergize permethrin and synergized deltamethrin in Isoko South Local Governments are shown in Table 1. The synergized permethrin exposure had the highest mortality followed by synergized deltamethrin. However, mosquitoes showed some level of resistance to permethrin (72%) and deltamethrin (87%) insecticides.

Table 1. Susceptibility status of *Anopheles* mosquitoes exposed to deltamethrin, permethrin and their synergist in Isoko South LGA, Delta State**.**

Insecticide	% Mortality (24 h)	Resistance status
Permethrin	72	Resistant
Synergized permethrin	99	Susceptible
Deltamethrin	87	Resistant
Synergized deltamethrin	98	Susceptible

≥98 shows susceptibility, 90 -97% shows suspected resistance, ≤90 shows resistance (WHO, 2016).

Knockdown of Anopheles

The knock down of *Anopheles* mosquitoes exposed to permethrin and deltamethrin, and their synergized forms in Isoko South LGA, Delta State is as shown in Figures 2 and 3. Considering the knock down of *Anopheles* mosquitoes in both exposures, an increase was observed with increasing time. Similar pattern of knock down was observed in permethrin and deltamethrin exposures. More species were knocked down in deltamethrin exposures from 20 to 50 min. Though at 60 min, percentage knock down of mosquito species was higher (71%) in deltamethrin compared to permethrin (60%). In the synergized forms, permethrin recorded the highest percentage knock down (78%) at 60 min compared to deltamethrin (59%). However, knock down increased with time. The differences between knock down of *Anopheles* mosquitoes exposed to permethrin and synergized permethrin were significant (F= 20.8, P<0.0001; F= 96.11, P<0.0001). Furthermore, the differences between knock down of *Anopheles* mosquitoes exposed to deltamethrin and synergized deltamethrin were significant (F= 40.88, P<0.0001; F= 49.05, P<0.0001).

Knock-down time records

The knock down time of *Anopheles* mosquitoes to deltamethrin, permethrin and their synergists is shown in Table 2. The mosquitoes from the various treatments showed remarkable knock down time of 50 and 95%. The exposure showed that KDT_{50} values range from 38.99 to 57.06 min while the KDT_{95} values range from 147.17 to 284.66 min. It was observed that the model in the treatments followed a well fitted curve according to the Pearson goodness of fit.

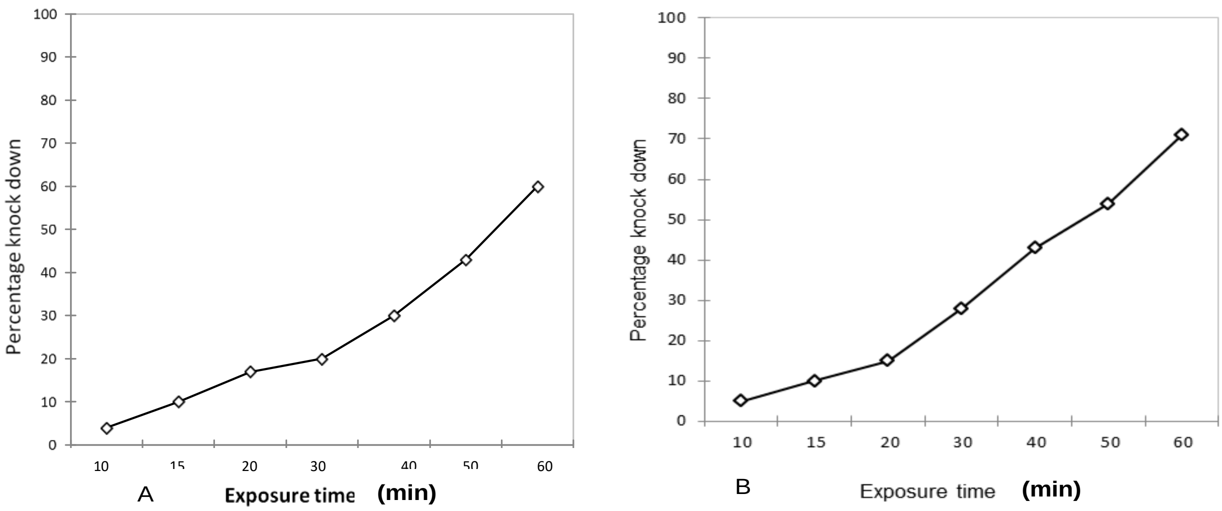

Figure 2. Showing the total number of *Anopheles* mosquitoes knocked down to permethrin (A) and deltamethrin (B) in Isoko South LGA, Delta State.

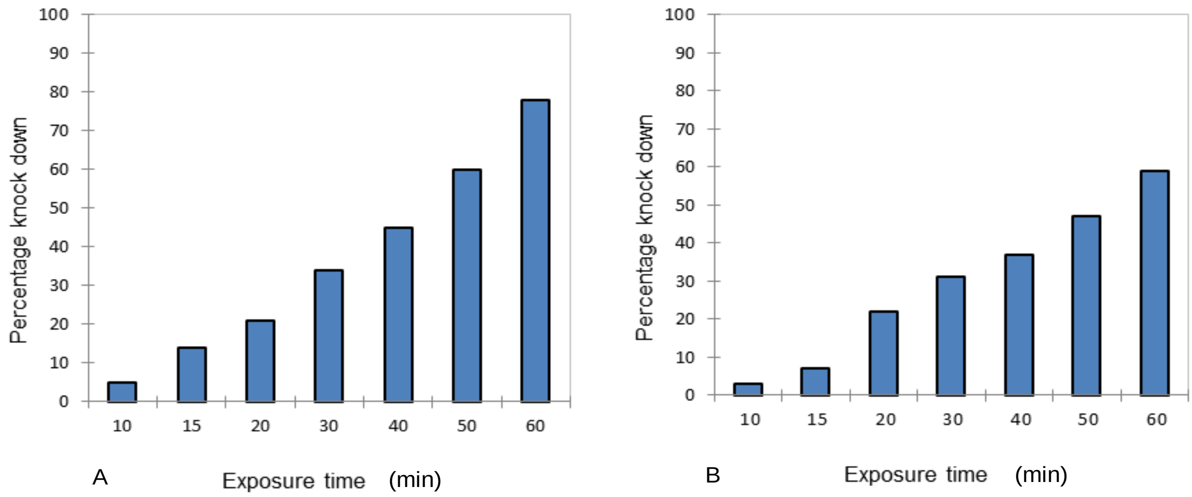

Figure 3. The total number knocked down of *Anopheles* mosquitoes to synergized permethrin (A) and deltamethrin (B) in Isoko South LGA, Delta State.

Table 2. Knock down time records of *Anopheles* mosquitoes to deltamethrin, permethrin and their synergists.

Treatment	N	Regression line	Pearson χ^2 goodness of fit	KDT_{50} (95% CI)	KDT_{95} (95% CI)
Permethrin	100	Y= 2.36X - 4.14	97.96	57.06 (49.97-68.11)	284.66 (196.99-490.26)
Deltamethrin	100	Y= 2.84X - 4.67	141.19	44.12 (40.23-49.21)	167.34 (131.10-233.96)
Synergized permethrin	100	Y= 2.85X - 4.54	153.16	38.99 (35.75-42.98)	147.17 (117.55-199.44)
Synergized deltamethrin	100	Y= 2.48X - 4.22	109.52	50.41 (44.96-58.30)	232.80 (168.79-370.15)

N: Total number of mosquitoes exposed; 50 and 95% knock down time, KDT_{50} and KDT_{95}, are in minutes; 95% confidence interval; $p > 0.05$ suggests a well-fitting model, $p < 0.05$ suggests an invalid model population.

Efficacy of combined insecticides

The mortality of *Anopheles* mosquitoes exposed to permethrin and deltamethrin in their combined form is as shown in Figure 4. At 15 and 30 min of exposure, mortality was 71 and 99% for permethrin and deltamethrin.

DISCUSSION

Resistance to pyrethroid has been the major challenge to vector control in West Africa and

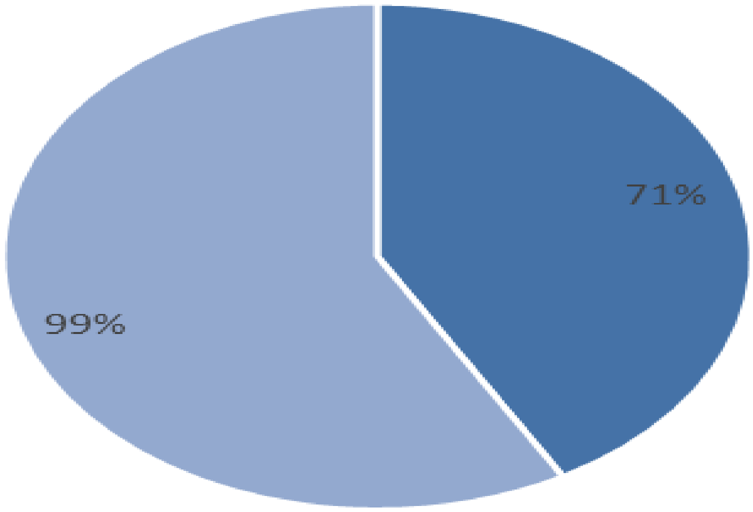

Figure 4. Mortality of *Anopheles* mosquitoes exposed to permethrin and deltamethrin in their combined form in Isoko South LGA, Delta State.

even with the challenges, there have been no alternatives to divert their use in recent times (Cisse et al., 2015; Chabi et al., 2016). As the fight against malaria progresses, it is imperative to determine the baseline efficacy of the insecticides used in the treatment of bed net which remain an effective option to vector control worldwide. This is to ensure that intervention is targeted on adequate monitoring of pyrethroid resistance in several localities. Following the widespread reports of pyrethroid resistance in *Anopheles* species throughout sub-Saharan Africa, studies on the efficacy of permethrin, deltamethrin, their combined form and synergists were carried out in Isoko South LGA, Delta State to determine the susceptibility status. The sustained pyrethroid resistance and lack of alternative for use particularly for treatment of long lasting nets would mean a great challenge to malaria vector control programme and thus resulting in deaths of approximately 120,000 (Ranson et al., 2011; WHOPES, 2012; WHO, 2012). It is still unsure as to whether excessive use of agricultural insecticides contributes to the difficulty in vector control (Philbert et al., 2014). The study of Abuelmaali et al. (2013) observed increased resistance in areas closer to agricultural farm lands where agricultural insecticides are frequently applied. It is probable that the resistance recorded with deltamethrin and permethrin in this study is due to associated agricultural practices within the location. In this present study, it was observed that permethrin and deltamethrin were resistant; thus, accounting for 72 and 87% mortality after 24 h, respectively. Dadzi et al. (2017) observed that deltamethrin singly was more effective to permethrin while synergized deltamethrin was more effective compared to synergized permethrin in Ghana. This observation was in the reverse form as synergized permethrin was more effective. This may be due to geographical differences in the location.

A study conducted in western Kenya reported mortality ranges from 24 to 96% as a result of permethrin exposures (Omondi et al., 2017). Furthermore, the study of Sow et al. (2010) reported complete knockdown and high susceptibility with permethrin. This is not in line with this study as 72% mortality was recorded, probably due to the fact that resistance is built with recurrent use without adequate monitoring. However, the findings of this study are in agreement with the study of Agossa et al. (2015) who reported higher resistance in Benin republic. In the two synergized forms, *Anopheles* spp. were susceptible after 24 h; thus, possibly showing that resistance to these insecticides was due to metabolic enzymes deactivating insecticide actions in species. The potentials of synergists have been known for various insecticides including permethrin and deltamethrin and were carried out to conquer the metabolic enzymes responsible for resistance in *Anopheles* spp. (Young et al., 2006). This study recorded 99 and 98% mortalities with synergized permethrin and deltamethrin, respectively, and this does not corroborate with the mortalities of 54.3 and 87.5% reported by Dadzie et al. (2017) for synergized permethrin and deltamethrin, respectively. It was equally observed that synergized deltamethrin caused high mortality in six *Anopheles* spp. (Vijayan et al., 2007). This is in support of this study.

Percentage knock down of *Anopheles* mosquitoes was highest in synergized permethrin and followed by deltamethrin. Knock down was lowest in synergized deltamethrin. The difference between knock down of mosquitoes to insecticides were highly significant (P<0.0001). It was observed that percentage knock down was higher from initial time of exposure in single forms of insecticides under study than in synergized form. The pattern of knock down in deltamethrin followed similar trend with synergized permethrin. Similar trend was observed for synergized deltamemthrin and permethrin. The percentage knock down recorded in this study was lower compared to that (84%) reported at 30 min by Omondi et al. (2017). Resistance was reported in single forms of insecticides where percentage knock down was observably high from the initial time of exposure. The findings of this study are in line with the reports of Habtewold et al. (2004) which showed that *Anopheles* mosquitoes were repelled from deltamethrin treated cattle, though most species alighted on host animals for less than 1 min, and insecticide shelf-life lasting for only one week. Nigerian-made Baygon reported lower mortality and Kenya-made raid reported low knock down (Makworo et al., 2017). It is probable that Nigerian-made pyrethroids produce high knock down rate than mortality in exposed species. The percentage knockdown recorded in this study is an evidence.

Percentage knock down for 50% (KDT_{50}) was between 38.99 to 57.06 min. It shows that it would take approximately 39 to 57 min for 50% of the mosquitoes to be knocked down. KDT_{50} of *Anopheles* mosquitoes was the lowest with synergized permethrin. This shows that synergized permethrin was effective compared to other insecticides. KDT_{95} was between 147.17 and 284.66 min. It shows that it would take approximately 147 to 285 min for 95% of the mosquitoes to be knocked down. KDT_{95} was equally the lowest with synergized permethrin. The study of Pemo et al. (2012) reported KDT_{50} of below 2 min for *Anopheles* spp. exposed to deltamethrin. The higher knock down time observed in this study is an indication that resistance has built up over time. Furthermore, mosquito species were equally susceptible to the combined forms of permethrin and deltamethrin after 30 min of exposure in CDC bottle assay. This portends that treating bed nets with either the combined and synergized forms of deltamethrin and permethrin would be effective against vector populations.

Conclusion

This study has shown that permethrin and deltamethrin exposure were resistant and susceptibility was recorded in synergized forms as well as combined forms. Percentage knock down effect was highly with synergized permethrin and deltamethrin at 60 min of exposure. Considering the effectiveness of synergized permethrin and deltamethrin, they can be adopted as insecticides for the control of *Anopheles* mosquito especially for the treatment of bed nets which remains a viable option for vector control today.

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