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Resistant status and intensity of resistance of *Anopheles gambiae* sensu lato to pyrethroid and organophosphate in Lafia, Nasarawa State Nigeria

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ABSTRACT: Monitoring and understanding the trend and dynamics of insecticide resistance are key to devising efficient control strategies. This study was carried out to investigate the resistance status of *Anopheles gambiae* sl mosquitoes in Lafia, Nasarawa State Nigeria to three pyrethroid (deltamethrin, Alphacypermethrin and permethrin) with concentration of 12.5 µg/bottle each and an organophosphate (pirimiphose-methyl 20 µg/bottle). Larvae and pupae of *Anopheles* mosquito were sampled within the metropolis and reared to adults in the insectary of Nasarawa State University, Keffi Nasarawa State. Emerged adults of 2 to 5 days old and non-blood fed were tested for resistance using CDC bottle bioassay susceptibility protocol. Results showed that the local mosquitoes species were resistant to all the three pyrethroid (deltamethrin, Alphacypermethrin and permethrin) but showed possible resistant to organophosphate (pirimiphose-methyl) with mortalities of 88, 84, 54, and 95% respectively. Exposure to piperonyl butoxide (PBO) as a synergist resulted in the susceptibility (100 and 98%) of mosquitoes to deltamethrin and Alphacypermethrin respectively but resistant (81%) to permethrin. The intensity of resistance of the vector population to pyrethroid was high especially with permethrin. The mosquitoes were only susceptible (100%) to deltamethrin and Alphacypermethrin at X_5 concentration and permethrin at X_{10} concentration. Insecticide based intervention strategy remain the principal vector control measure in malaria endemic countries like Nigeria. Based on the result of this study, future vector control programme should take into consideration the deployment of nets that are treated with alphacypermethrin or deltamethrin combined with PBO (PBO based nets) in the study area or increase the concentration of the pyrethoid insecticides used in this study on long lasting nets after their safety to human is assured.

Keywords: Insecticide, resistance, organophosphate, pyrethroid, Lafia,

INTRODUCTION

Mosquitoes are significant vectors of infectious disease-causing agents that greatly impact global health, with more than half of the world human population at danger of contacting mosquito-transmitted diseases (Famakinde, 2018). Malaria is a life-threatening disease caused by parasites that are transmitted to people, in most cases, by the bites of infected female *Anopheles* mosquitoes and it is preventable and treatable (WHO, 2019). *Anopheles gambiae* complex is the predominant vector of malaria in sub-Saharan Africa, but it is not the only vector in the field (Okwa, 2019).

The strength of malaria transmission is hinged on factors associated with parasite, vector, the human host, and the environment (WHO, 2019; Basing and Tay, 2014). Improvement in malaria control is as a result of expanded access to malaria vector control interventions which accounts for most of the malaria burden drops attained especially in sub-Saharan Africa in low and middle-income countries (LMICs) (WHO 2019; Killeen *et al*., 2017; WHO, 205a). Despite efforts to control the disease, malaria remains a universal puzzle mostly for sub-Saharan nations (Ebuka *et al*., 2020). Nevertheless, these improvements are threatened by evolving resistance to insecticides among *Anopheles* mosquitoes (WHO 2019). Resistance is an inherited ability of a population to stay alive in the presence of a fatal concentration of an insecticide that would normally kill a wild population. Regardless of significant and various efforts towards control of malaria through the use of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spray (IRS) which are highly effective, malaria remains a challenge in sub-Saharan countries (Epopa *et al*., 2019) and it is a threat to different control approaches used over many years in endemic areas (Hilary and Natalie, 2016).

The control of malaria through its vectors depends mostly on synthetic insecticides (Hilary and Natalie 2016). Owing to the persistence of malaria which is linked to numerous factors ranging from absence of a vaccine, resistance of malaria parasite to antimalarial drugs (Farooq and Mahajan, 2004; Cui *et al*., 2015), resistance of vectors to insecticides (WHO, 2015b; Alonso et al., 2011), and vectors behavior (Killeen *et al*., 2017), efforts to control malaria through vector control have intensified, so has the selection pressure on mosquitoes to develop resistance to insecticides (Hilary and Natalie, 2016). Malaria vector control remains one of the key pillars of malaria control and has contributed to the reduction of the spread of malaria (WHO, 2012). Unluckily, the effectiveness of Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS), which constitute the main tools of malaria vector control is threatened by the resistance of the vectors to insecticides (Reid and McKenzie, 2016; Cisse *et al.*, 2015; Philbert *et al*., 2014). The spread and strength of vector resistance have enlarged deeply and now threatens the achievement of control programs (Hilary and Natalie 2016; Sovi *et al*., 2020). Resistance to pyrethroid, one of the classes of insecticides available to treat bed nets, is now ubiquitous in African malaria vectors (Okechukwu *et al.,* 2020) and resistance to other insecticide classes used for adult mosquito control is increasing (Hilary and Natalie 2016; Edi *et al*., 2012; Djogb´enou, 2009). Some countries have reported mosquito resistance to at least one (pyrethroid) of the 4 commonly-used insecticide classes while others reported resistance of mosquito to all of the four main insecticide classes (WHO, 2019). Knowledge of mechanisms of resistance of mosquitoes to insecticides (Fodjo *et al*., 2018) and local entomological data are key to a successful control (WHO, 2015b). *Anopheles* mosquitoes are widespread in Nigeria and few have been implicated as vectors of malaria, the status of others are unknown resulting to resistance and making the control difficult. This study aims to assess insecticide susceptibility, intensity and resistance mechanisms of *An. gambiae* s.l. collected within Lafia metropolis.

MATERIALS AND METHODS

Study site

This study was carried out in Lafia which is the capital city of Nasarawa State in north central Nigeria. The city lies at latitude 8° 29' 38" N and longitude 8° 30' 55" E. According to 2006 population census, Lafia has inhabitant of 330,712 with a landmass of 2797.53 sq.km. One of the major economic activities of the inhabitants is farming (Figure 1).

Larval collection and susceptibility test

Larvae of Anopheline mosquitoes were sampled within Lafia metropolis, based on WHO guidelines (WHO, 2013). Immature *Anopheles* mosquitoes (larvae and pupae) were collected from different breeding sites using larvae dipper. Collected larvae were transported and reared to adult stage in the insectary of Nasarawa State University Keffi. Emerged adults were collected from the netted rearing pan with Aspirator, emptied into the adult cage and were fed with 10% sugar solution soaked in cotton wool.

Susceptibility test was performed according to Centre for Disease Control and Prevention (CDC) protocol (CDC, 2010). One hundred (twenty five each) of non-blood fed female adults *Anopheles* mosquitoes of 3 to 5-day old were exposed to four replicates of CDC bottles coated with 1 ml each of pyrethroid insecticides (Deltamethrin, Permethrin and Alphacypermethrin, 12.5 µg each) and an organophosphate (Pirimiphose methyl 20 µg) and another set of 25 adult female *Anopheles* mosquitoes were exposed to uncoated bottle (control) using glass aspirator. Similarly, intensity of resistance of the local *Anopheles* mosquitoes using different concentrations of pyrethroid (X_2, X_5 and X_{10}) was also determined using CDC protocol (CDC, 2010). Mechanism of resistance using synergistic assay was carried out too following CDC (2010) protocol. One hundred female *Anopheles* mosquitoes were first introduced to piperonyl butoxide (PBO) before exposure to pyrethroid insecticide. Susceptibility was determined from percentage of mosquitoes knocked down for half an hour or an hour for pyrethroid and organophosphate respectively. Mosquitoes tested were identified morphologically using keys from Gillies and De Meillon (1968) and Coetzee (2020). The mortality of the different tests achieved was interpreted according to the criteria proposed by World Health Organisation (WHO 2013) as follows: mortality between 98% and 100% implies that the

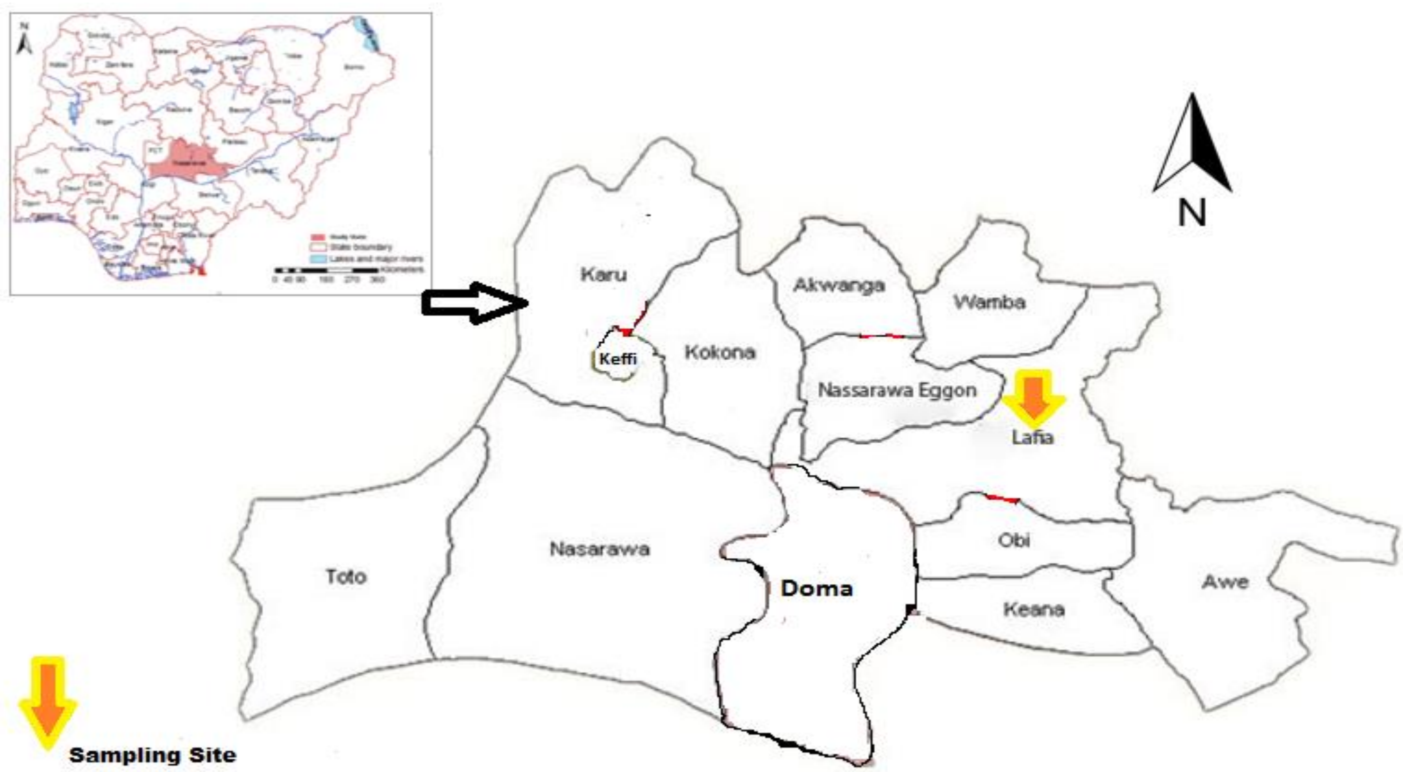

Figure 1. Map of Nasarawa State showing the study area (Lafia).

Table 1. Susceptibility status of Apheline mosquitoes exposed to pyrethroid and organophosphate.

Insecticide class	Insecticide tested	No. of mosquitoes exposed	No. of exposed mosquitoes dead	% mortality of exposed mosquitoes	Resistant status
	Deltamethrin	100	88	88	Resist.
Pyrethroid	Alphacypermeth-rin	100	84	84	Resist.
	Permethrin	100	54	54	Resist.
Organosphosphate	Pirimiphose Methyl	100	95	95	Possible resistance

vectors are susceptible, mortality between 90% and 97% indicates the presence of resistance genes in the vector population which must be confirmed, and mortality less than 90% confirms the existence of resistance gene in the test population.

RESULTS

Study of resistance status of malaria vector in Lafia, the capital city of Nasarawa State was carried out using CDC bottle bioassay presented in Table 1 showed resistance (88, 84 and 54% respectively) of insect vector to pyrethroid insecticides (Deltamethrin, Alphacypermthri and permethrin), the insecticides mostly used in public health for control of mosquito vectors (Table 1). Local mosquito species (spp) showed possible resistant (95%) to organophosphate (Pirimiphose-methyl) in the study area (Table 1).

Figure 2 showed the intensity of resistance of *Anopheles* mosquitoes to pyrethroid insecticides in the study Area. *Anopheles* vector spp in the study area were resistant to the three pyrethroids (Deltamethrin, Alphacypermthri and Permethrin) tested at X_2 concentration but susceptible at X_5 concentration of Deltamethrin and Alphacypermethrin whereas mosquitoes only become susceptible to Permethrin at X_{10} concentration (Figure 2).

Resistant mechanism of *Anopheles* mosquitoes was determined by exposing *Anopheles* vector to combination of piperonyl butoxide (PBO) and Pyrethroids. The result showed that local malaria vector spp were susceptible (100% and 98%) to synergized insecticides (PBO + Deltamethrin and PBO+ Alphacypermethrin) but resistant (81%) to synergized permethrin (PBO + Permethrin).

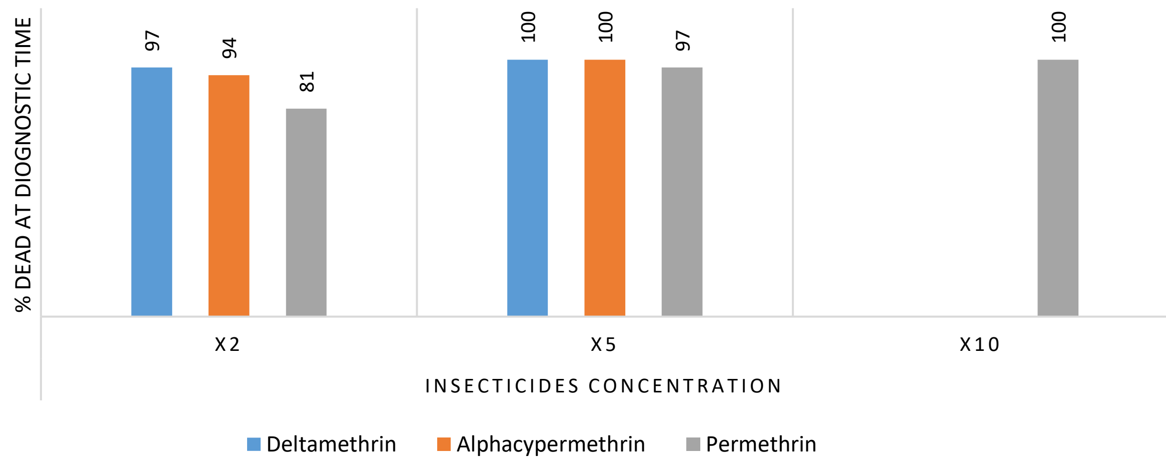

Figure 2. Intensity of resistance of Anopheles (larval to adult) exposed to different concentrations of three pyrethroid.

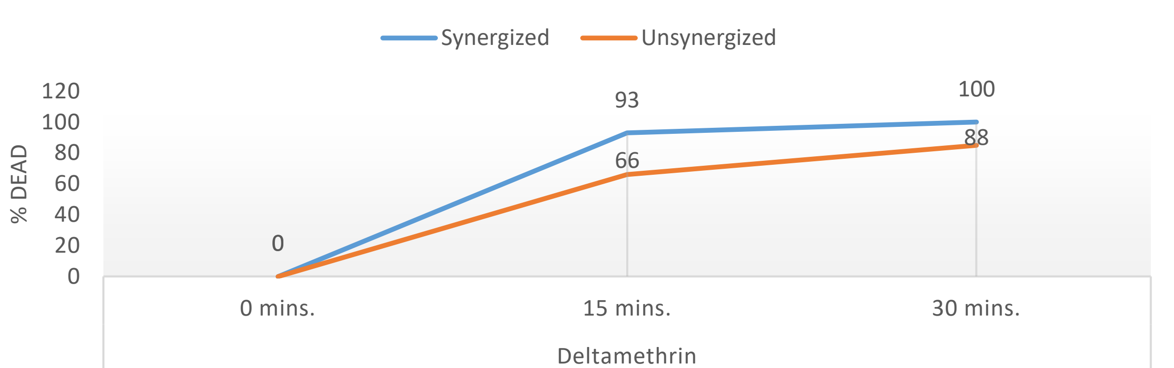

Figure 3. Synergistic assay test of mosquitoes exposed to deltamethrin alone (un-synergised) and Deltamethrin. + PBO (synergised).

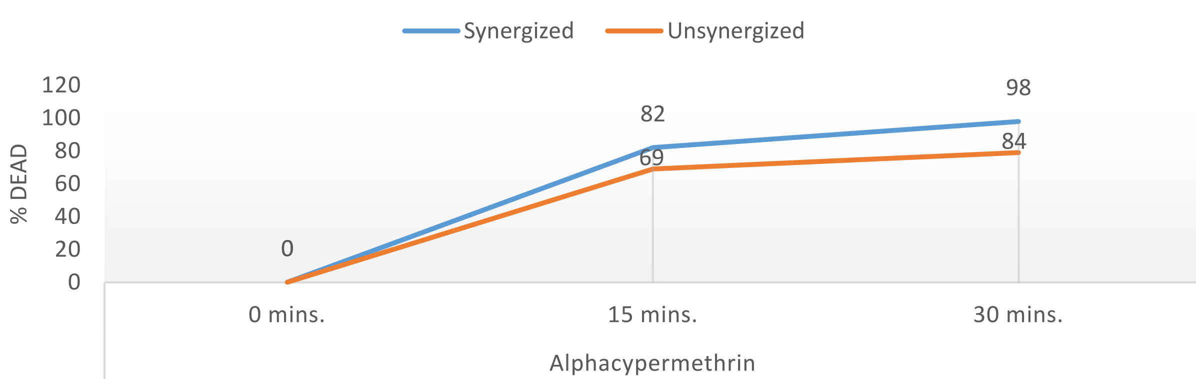

Figure 4. Synergistic assay test of mosquitoes exposed to alphacypermethrin alone (un-synergised) and alphacypermethrin + PBO (synergised).

However, mortality rate of the insects to unsynergized insecticides were lower compare to the synergised. (Figures 3 to 5).

DISCUSSION

Chemical control remains the most important and widely

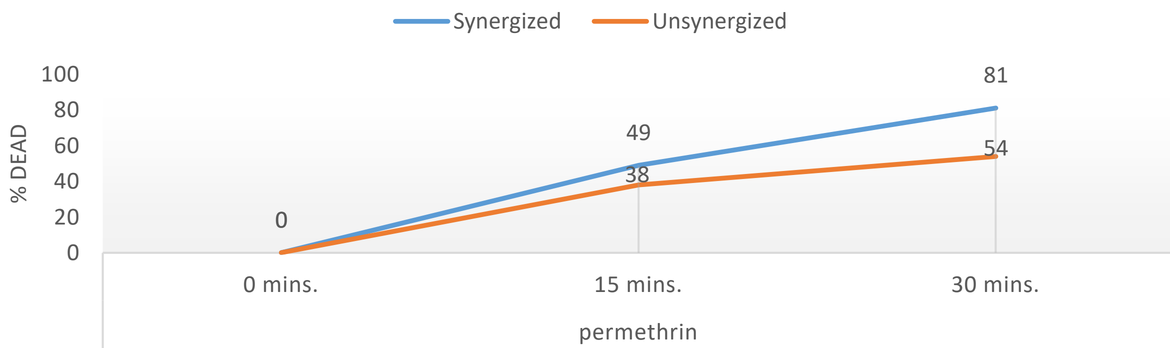

Figure 5. Synergistic assay test of mosquitoes exposed to permethrin alone (unsynergised) and permethrin + PBO (synergised).

used strategy against malaria vectors around the world. Insecticides are key components in public health and an agricultural toolbox in Nigeria. Pyrethroids, a supposed class of highly effective and extremely efficient neurotoxic insecticides are effective in the study area as suggested by the above result. The results showed that *An. gambiae* (*s.l.*) populations were resistant to pyrethroid (Deltamethrin, Alphacypermethrin and Permethrin) and suspected to be resistant to organophosphate (pirimiphos-methyl). This is in line with the findings of Awolola *et al*. (2002, 2007 and 2009), who reported widespread resistance of *An. gambiae* (*s.l.*) to pyrethroids in Nigeria. Okechukwu *et al*. (2020) also reported widespread resistance to pyrethroids and organophopsphate insecticides used in public health in South-East Zone of Nigeria.

Similar observations were made in other parts of Africa (Nkya *et al*., 2014; Gnanguenon *et al*., 2015; IRAC, 2011). Local *Anopheles* vector spp were susceptible to synergized insecticides (PBO + Deltamethrin and PBO+ Alphacypermethrin) but resistant to synergized permethrin (PBO + Permethrin) though percentage of *Anopheles* mortality to un-synergized Pyrethroid, (Deltamethrin, Permethrin and Alphacypermethrin alone) were lower in relation to the synergized. This indicates a metabolic resistant mechanism that occurs when increased or modified activities of an enzyme system prevents the insecticide from reaching its intended site of action by detoxifying foreign materials (WHO, 2012) resulting in the inevitable emergence of resistance in mosquito vectors. Resistance of malaria mosquito vector to pyrethroid which is as a result of metabolic resistant mechanisms may be largely due to cross-resistance, indiscriminate use and disposal of agricultural insecticides and/or use of pyrethroids in Long-Lasting Insecticide Nets (LLINs).

The result of this study also demonstrated high level resistance to pyrethroid insecticides used in public health in the study area. Although public health use of insecticide has an impact on the development of resistance in mosquitoes, one key source of resistance in malaria vectors remains the massive use of insecticides for control of agricultural pests and or indiscriminate disposal of insecticides container in the mosquito breeding sites. Local mosquito spp in the study area were suspected to be resistant (95%) to pirimiphose methyl. In other geopolitical zones of Nigeria, Okorie *et al*. (2015) reported similar observation. On the contrary, *Anopheles gambiae* (s.l.) susceptibility to pirimiphose methyl has been reported in some parts of South-West and South-East Nigeria (Nwankwo *et al*., 2017; Okechukwu *et al*., 2020). The resistance of the mosquitoes spp observed with pirimiphos-methyl could be because this insecticide was among the pesticides widely used in the previous years for crop control in agriculture as reported by Odeyemi *et al*. (2006) and could have exerted a high selection pressure on mosquito larvae.

High-level resistance of this local *Anopheles* mosquitoes to pyrethroid (especially permethrin), an insecticide used in public health for the control of malaria vector, could have huge implications in the control of malaria and its vectors in Lafia area, as most of the vector control interventions rely heavily on insecticides from this class. High resistance level to pyrethroid insecticides by this local malaria vector may lead to cross resistance or expand malaria vector resistance to other class of insecticide which can compromised insecticide-based vector control. Different resistance mechanisms have different strengths and possibly different capacity to drive control failure. Regarding operational impact, there is serious concern with both the degree to which insecticide resistance reduces the efficacy of an intervention and, at the extreme, the possibility that it will induce full ‘control failure.

Conclusion

Vector control using pyrethroid Insecticide-Treated Nets (ITNs) is a core strategy for malaria control because of their high toxic and fast acting properties against mosquitoes. The data of this study showed an increasing resistance to pyrethroid which is a serious threat to malaria vector control in this locality. High level of resistance of this local *Anopheles* mosquitoes to pyrethroid insecticides used in public health for the control of malaria vector, may have huge implications in the control of malaria through its vectors in this area as most of the vector control interventions rely heavily on insecticides from this class. Though, the *Anopheles* mosquito vectors showed high level of resistance to both organophosphates (pirimiphos-methyl) and pyrethroids especially (permethrin) in the study area, all hope is not lost on the pyrethroids because its combination with PBO yielded a promising positive result. It is therefore clear that new approaches of insecticides (pyrethroid + PBO) for LLIN are required if force towards malaria elimination is to continue.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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