EFFICACY OF PIPERONYL BUTOXIDE (PBO) SYNERGIST ON PYRETHROID AND DICHLORODIPHENYL TRICHLOROETHANE (DDT) RESISTANT MOSQUITOES IN LEKKI, LAGOS STATE, NIGERIA

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ABSTRACT

Vector control using insecticide is an integral part of the global strategy for management of mosquito-borne diseases.. The development of insecticides resistance is a major concern in mosquito control. We evaluated the effect of piperonyl butoxide (PBO) synergist on dichlorodiphenyl trichloroethane (DDT) and pyrethroids resistant Anopheles gambiae s.l., Culex quinquefasciatus and Aedes aegypti in Lekki peninsula area of Lagos State, Nigeria. Mosquito larval collected from breeding sites in Lekki peninsula were allowed to emerge in the insectary and identified using appropriate morphological keys. Two-three days old female adults were subjected to susceptibility assays using WHO kits and insecticides impregnated test papers. Twenty (20) female adult mosquitoes of each genus were exposed to DDT (4 %) and permethrin (0.75 %) alone. Subsequently, another set of 20 of each genus were pre-exposed to PBO (4 %) for 1 hour before exposing them to permethrin and DDT, all assays were carried out in four biological replicates. The knockdown time was recorded as the time intervals for 60 minutes and mortality at 24 hour. Resistance to DDT was detected with percentages mortality of 55, 60 and 87.5 % for An. gambiae, Cx. quinquefasciatus and Ae. aegypti species respectively. Pre-exposure of mosquitoes to PBO significantly suppressed (p<0.05) resistance to both DDT and permethrin in all the mosquito species indicating the activities of P450 monooxygenase as a detoxifying enzymes mediating resistance to DDT and pyrethroids. Therefore, PBO should be incorporated in insecticide resistance management strategies in this area and others with similar mosquitos' resistance profile.

Keywords: Mosquitoes, Dichlorodiphenyl trichloroethane, Pyrethroids, Piperonyl butoxide, Insecticides resistance

INTRODUCTION

Mosquito control is largely dependent on the use of chemical insecticides, employing indoor residual spraying (IRS) and/or long-lasting insecticidal nets (LLIN) as the main strategy (WHO, 2018a; Fagbohun *et al*., 2019). Specifically, the pyrethroids are used for LLINs, while IRS utilize a wide array of insecticides approved by the World Health Organisation (WHO, 2015; 2018b). The use of insecticides based control measures has led to the global reduction in the transmission and spread of mosquito borne diseases (Maharaj, 2011; WHO, 2017a). However, the development and dissemination of mosquitoes that are resistant to these insecticides threatens the sustainability of their usage, as these control options are heavily dependent on effective insecticides (WHO, 2018a).

Moreover, a systemic review by Knox *et al.* (2014) on Anopheline mosquitoes insecticide susceptibility and resistance in the Afrotropical region between 2001 – 2003 and 2010 – 2012, reported a distinct increase in the prevalence of mosquitoes resistance to pyrethroids from 41 – 87 % and DDT from 64 – 91 % respectively throughout the Afrotropical region. The Global Plan for Insecticidal Resistance Management (GPIRM) was launched by WHO and charged with advisory role on insecticidal resistance preventive strategy (Mnzava *et al*., 2015). Many roadblocks to the fight against insecticidal resistance exist, however, financial constrain and inadequate infrastructural resources both at regional and national levels trumps other challenges (Chanda *et al*., 2016; WHO, 2017b). WHO has provided guidelines that is use in the monitoring of insecticide resistance: the Insecticidal Resistance Monitoring and Management Plan (IRMMP), which build a framework for which countries adhere to the recommendations of GPIRM (WHO, 2017c)

The potency of insecticides is enhanced by some synergistic compounds, their use of which has been outlined in the management of resistant mosquito in several areas. At non-toxic level, insecticidal synergists act by impeding certain enzymes which are normally found in insects and usually involve in the breakdown and detoxifying of insecticide molecules (Gleave *et al*., 2017; Fagbohun *et al*., 2019). Piperonyl butoxide (PBO) is one of such synergists that improves the activities of pyrethroids and organochlorines (Joffe *et al*., 2012). PBO's activity is usually through the inhibition of the P450 cytochrome and esterases enzymes in mosquitoes (Corbel *et al*., 2006). In sensitive insects, the expressions of the metabolic enzymes are at reduced levels whereas in non-susceptible ones, these enzymes reached elevated degrees. Hence, in those sensitive mosquitoes, insecticides will perform expectedly high and the utilization of synergist may provide only minimal or non-observable difference. Nevertheless, in certain resistant mosquitoes, synergists can tremendously improve the activities of insecticides due to the inhibition of the resistant insect's enhanced metabolic enzyme systems (IRAC, 2011). Thus, different synergists have been employed in vector control both in public health and agricultural purposes (Gleave *et al*., 2017; Protopopoff *et al*., 2018; Fadel *et al*., 2019).

In this study, the synergistic effect of PBO on pyrethroid and DDT resistant mosquitoes collected from Lekki, Lagos State, Nigeria was evaluated using WHO standard protocol.

MATERIALS AND METHODS

Study Area: This study was carried out in Lekki. Lekki is located at the eastern end of the Kuramo Waters, stretching eastward about 22 km in Eti Osa Local Government Area of Lagos State. It is bordered by; Lekki lagoon to the East, Cowries Creeks and Kuramo Waters to the West, Lagos Lagoon to the North and by Bight of Benin to the South. Lekki lies between latitude 6.46 North and longitude 3.58 East and has a land mass of 755 km^2 with a population of 390,800 as at 2016 (City Population, 2016).

Mosquito Larva Collection and Morphological Identification: The mosquito larva samples were collected from six sites in Lekki including Osapa, Ikate, Agungi, Elegushi, Admiralty and Lekki Phase 1 for three months from September to November 2019. This immature stage of mosquito was collected from different breeding sites such as abandoned tyres, undisturbed stagnant waters, gutters, drainage pipes or systems and abandoned containers. Larvae collection was done following WHO standard procedure (Service, 1993) and transferred into different well labelled plastic containers and subsequently transported to the insectary in Yaba College of Technology for culture until emergence. Identification to species level was done using morphological features as presented in relevant taxonomic keys (Gillet; 1972; Harbach, 2011; Gaffigan *et al.*, 2015).

WHO Insecticides and Synergistic Bioassay: Following adult mosquito emergence, twenty (20) mosquitoes each of *Anopheles, Culex* and *Aedes* species were used to conduct a single set of insecticide susceptibility tests in four replicates (WHO, 2016). The bioassays were conducted using WHO insecticides susceptibility kits, insecticides impregnated test papers (4 % DDT and 0.75 % permethrin) and 4 % PBO synergist, according to WHO standard procedures (WHO, 2016). Twenty (20) control mosquitoes were exposed to non-treated papers during the treatment exposure. After 60 minutes of exposure, all knocked down and surviving mosquitoes were transferred to the holding tubes and fed with 10 % sugar solution. After 24 hours, individual mosquitoes were recorded as either dead (susceptible) or alive (resistant).

Mortality Variables: The percentage mortality of mosquitoes for each insecticide was calculated as a proportion of mosquitoes that died after 24 hours from the total number of exposed mosquitoes using 95 % confidence intervals. The results were interpreted according to WHO criteria which is: mortality below 90 % is confirmed resistance, mortality between 90 – 98 % is suspected resistance and mortality over 98 % is susceptible (WHO, 2016). Knockdown times, both 50 % (KDT_{50}) and 95 % of tested population (KDT_{95}) by exposure to synergized and non-synergized bioassays were analyzed using log-time probit model (IBM SPSS, 2015).

Data Analysis: Chi-square was used to examine the similarities between the 24 hours' mortality of synergized and non-synergized bioassays. IBM SPSS statistical package version 23 was used to carry out all data analysis, p-value of < 0.05 was considered significant.

RESULTS

The 24 hours' percentage mortality of mosquitoes exposed to DDT and permethrin showed that *An. gambiae s.l.* (55 %), *Cx. quinquefasciatus* (60 %) and *Ae. aegypti* (87.5 %) from Lekki LGA were resistant (Table 1).

The estimated KDT_{50} ranges from 40.6 minutes in *Ae. aegypti* exposed to permethrin to 694.6 minutes in *An. gambiae s.l.*, while KDT_{95} ranged from 580.4 minutes in *Ae. aegypti* exposed to permethrin to 11332.4 minutes in *An. gambiae* exposed to DDT (Table 1).

Out of the three mosquito species exposed to the two insecticides (permethrin and DDT), *An. gambiae* had the highest estimated knockdown time of 11332.4 minutes for DDT, which was way higher than what was documented for the rest species. On the other hand, *Ae. aegypti* was the most susceptible species with 87.5 % mortality rate and a KDT_{95} of 927.1 minutes to DDT (Table 1).

PBO synergized assays for the DDT and permethrin resistant mosquitoes showed higher 24 hours' percentage mortality and reduced KDT_{50} and KDT_{95} values, though only *An. gambiae* exposed to PBO plus permethrin and *Culex* exposed to PBO plus DDT showed statistical significance difference ($p<0.05$) on comparison with non-synergized assay (Table 1). PBO synergized bioassays more potent and lethal on the mosquitoes when used with permethrin than it was with DDT as demonstrated by it increased suppression of resistance.

The progressive percentage knockdown of *An. gambiae*, *Cx. quinquefasciatus* and *Ae. aegypti* exposed to DDT and permethrin in comparison to PBO synergized bioassays are presented in Figure 1. PBO synergized bioassays consistently showed a higher knockdown rate at the different time intervals as compared to the non-synergized ones.

DISCUSSION

Insecticide usage is the most vital part of vector control strategy advocated by WHO against all mosquito transmitted diseases, the effectiveness of which has been undermined by development of resistance to most classes of insecticides in use (Wilson *et al.*, 2020).

The observed low percentage mortality in the three species of mosquitoes exposed to DDT and permethrin was similar to previous

Table 1: Percentage mortality and estimated knockdown time of mosquitoes exposed to insecticides and PBO + insecticides in Lekki LGA of Lagos State, Nigeria

Mosquito species	Insecticides	Number exposed (N)	KDT_{50} (Min)	KDT_{95} (Min)	Mortality (%)	P-value	Resistance status
Anopheles gambiae	DDT	80	694.6	11332.4	55	0.1	Resistant
	DDT + PBO	80	100.6	660.6	92.5		
	Permethrin	80	331.7	2584.3	72.5	0.002	Resistant
	Permethrin + PBO	80	44	174	93.8		
Culex quinquefasciatus	DDT	80	164.8	1133.5	60	0.006	Resistant
	DDT + PBO	80	133.2	3168.7	93.75		
	Permethrin	80	216.4	1998	90	0.5	Resistant
	Permethrin + PBO	80	61.5	202.5	100		
Aedes aegypti	DDT	80	175.8	927.1	87.5	0.7	Resistant
	DDT + PBO	80	129.1	980.8	92.5		
	Permethrin	80	84.9	580.4	90	0.6	Resistant
	Permethrin + PBO	80	40.6	114.5	97.5		

Note: KDT= Knock down time, DDT = dichlorodiphenyl trichloroethane, PBO = piperonyl butoxide

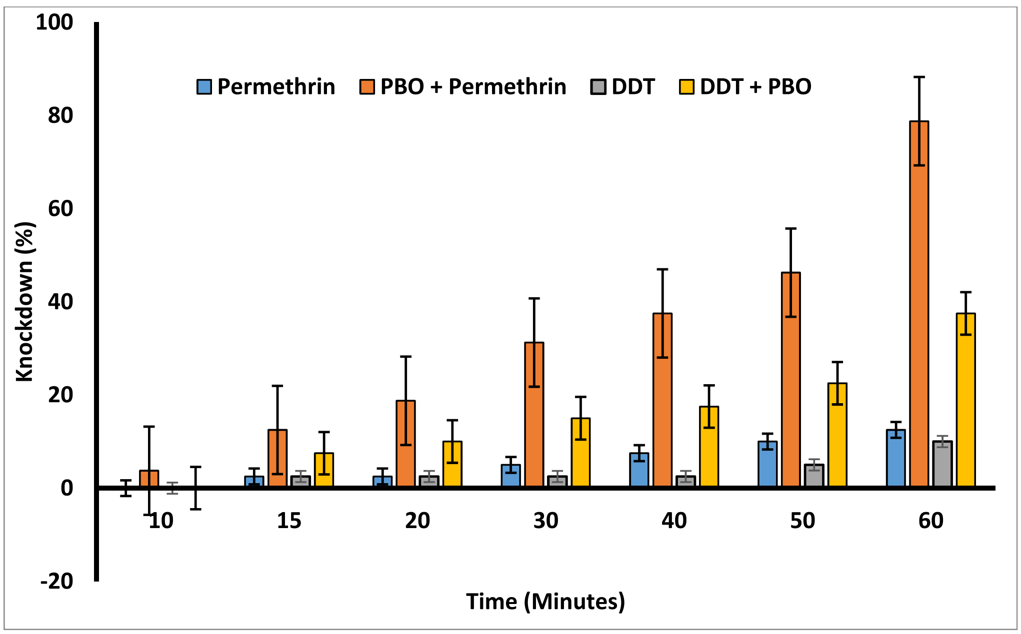

Figure 1: Progressive percentage knockdown time of *Anopheles gambiae s.l.* exposed to insecticides and PBO plus insecticides in Lekki LGA of Lagos State, Nigeria

reports for *Anopheles* from sub-Saharan Africa (Corbel *et al.*, 2007; Oduola *et al.*, 2010; Adeogun *et al.*, 2017), *Culex* (Corbel *et al.*, 2007; Tmimi *et al.*, 2018; Fagbohun *et al.*, 2019) and *Aedes* (Ayorinde *et al.*, 2015; Kamgang *et al.*, 2017). This is a troubling phenomenon since this means that, when these insecticides are used in this region, the mosquitoes will be able to thrive, breed and further spread their resistance gene to subsequent generations, thereby making insecticides based control efforts wasteful and enhancing the transmission of mosquito vector-borne diseases. *An. gambiae* remains a highly efficient and the major vector of *Plasmodium falciparum* malaria. Given its high resistance to different insecticides, it is important that continued surveillance be sustained in the endemic areas and usage of LLINs needs to be reemphasized.

Insecticides resistance in mosquitoes has been associated with target site mutation and increasing activities of detoxifying enzymes (Liu, 2015). This study, through the PBO synergist bioassay established the importance of detoxifying enzyme; cytochrome P450 monooxygenase in the resistance to DDT and pyrethroids in mosquitoes from this study location. Studies in Nigeria and other West African countries have reported similar results in different species of mosquitoes (Dadzie *et al.*, 2017; Badolo *et al.*, 2019; Fagbohun *et al.*, 2019). The quicker knockdown time rate recorded in PBO-synergised assays substantiate the need to intensify the use of PBO based insecticides control measures in Lekki and even other parts of Nigeria. Combination of PBO and pyrethroids in LLINs in several trials have proven to provide valuable improvements in the reduction of vector density and diseases transmission (Tungu *et al.*, 2010; Protopopoff *et al.*, 2018).

Conclusion: Taken together, this study has highlighted the insidious challenge that insecticides resistance might pose in the fight against mosquito borne diseases. Should Nigeria aim to meet the WHO targets of 90 % reduction in the prevalence of vector borne diseases by the year 2030, this current reality would have to be addressed deliberately in order not to hinder other control strategies. Hence, we recommend that PBO should be incorporated in insecticide resistance management strategies in areas with similar mosquitoes resistance profile for effective control of mosquito populations.

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