SUSCEPTIBILITY STATUS OF *Anopheles* MOSQUITO TO PIRIMIPHOSE METHYL AT RIVER UKE, NASARAWA STATE, NIGERIA

Supported by tetfund

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Received: September 12, 2020 **Accepted:** January 10, 2021

Abstract: This study was conducted to investigate the susceptibility and resistance status of *Anopheles gambiaes.l* to Pirimiphose Methyl at River Uke, Nasarawa State, Nigeria from May to July 2019. Anopheles mosquito larvae were collected from the upper and lower locations of the river usually between the hour of 9am to 2pm at the interval of two weeks by dipping and scooping methods. The samples were taken to Entomology Laboratory and Insectary of Nasarawa State University, Keffi and reared to adult stage. A total of three hundred (300) female *Anopheles* mosquito from each adult cage (Upper and Lower locations) were subjected to 20 µg does of Pirimiphose methyl for susceptibility test at a time interval of 0, 15, 30, 45 and 60 min for 24 h in which 25 mosquitoes were exposed to each of the 4-impregnated CDC bioassay bottles. The result indicate that susceptibility (98-100%) and possible resistance (97-98%) was observed at 60 min exposure time. Data analysis by ANOVA showed no significant difference between the Upper and Lower locations of the study area ($p>0.05$).

Keywords: Susceptibility, Pirimiphose-methyl, *Anopheles* mosquito, River Uke

Introduction

Malaria still remains a major health global challenge particularly, in tropical and sub-tropical countries like Nigeria, despite a constant scientific research on the effective intervention control measures to reduce its devastating effect caused to human lives and country's economy (WHO, 2014). It is estimated that malaria is responsible for about 110 million clinical cases with 300,000 deaths per year and the associated 11% mental deaths as well as ₦132 billion economic lost in Nigeria (USAID Health, 2005). It is generally a great cause of morbidity and mortality resulting in 25% infant and 30% childhood in Nigeria (FMH, 2013).

This growing concern of malaria burden occurred due to the constant resistance of malaria vector species against all forms of insecticides which allows for the detection of susceptibility status of *Anopheles* mosquito to different classes of insecticides like Organophosphate (WHO, 2013). Various factors including temperature, rainfall, vegetation and water condition such as fresh or polluted water greatly contributed to mosquito larvae distribution across the entire upper and lower breeding site locations (Das *et al*., 2007).

Hence, the need for integrated approaches to combat malaria transmission required the use of major test tools such as CDC bottle bioassay for assessing the susceptibility and resistance status among vector species to reduce its deadly negative effects mostly among children under five age of five, pregnant women and less immune persons (Brog don WG *et al*., 2010).

Materials and Method

Study area

This study was conducted in River Uke which is located in Nasarawa State in the North central part of Nigeria and lies between latitude 8^{o} 53^{1}N-8^{o} 56^{1} and longitude $7^{o}39^{1}$E- 7^{o} 46^{1}. Uke has a total land area of 875 sqkm and estimated population of 9664 (National population census, 2006).

Collection and identification of mosquitoes

Anopheles mosquito larvae were collected from the upper and lower areas of river Uke between 9am-5pm at interval of two weeks by dipping and scooping methods. The samples were taken to Entomology Laboratory and Insectary where they were sorted out with pipette into larval bowl and reared to adult mosquitoes using a mixture of fine grain powder of a piece of non-sugar biscuit and 10 pieces of yeast tablets at a temperature range of 27-31^{o}C and 61% humidity for 2-3 days. The emerged adult mosquitoes were carefully collected by aspirator and transferred into a labelled adult cages and fed with sugar solution for 1-2 days. Five (5) bottles were set up based on CDC bottle bioassay standard in which 4-bottles were coated with 20 µg dose of Pirimiphose methyl, while a control bottle was uncoated and left to dry for 24 h. Three hundred (300) female *Anopheles* mosquito from each adult cage were subjected to susceptibility status test with 25 mosquitoes exposed to each of the 4-impregnated bioassay bottles. The percentage knock-down (Death) according to the formula below:

Observed mortality = (Total number of dead mosquitoes)/ (Total sample size) x 100 and alive were recorded at the time interval of 0, 15, 30, 45 and 60 min and the test lasts for 24 h.

Data analysis

Analysis of variance (ANOVA) was used to analyze the efficacy monitoring data at 5% significance level. Descriptive statistical analysis was used to compare average mortalities and survivals.

Results and Discussion

Tables 1 and 2 showed the different exposure time of adult Anopheles mosquito to 20 µg dose of Pirimiphoes methyl during the period of the study. At a diagnostic time of 60-minutes, a total number of 100 adult mosquitoes were exposed to 20 µg dose of Pirimiphoes methyl. The results show that *Anopheles* mosquitoes were susceptible to the Pirimiphose methyl (98 - 100%) indicating 100% mortality at Upper location (Table 1). While at Lower location (Table 2), there was possible resistance (96-97%).

Table 1: Susceptibility and resistance status of female *An. gambiae s.l* at Upper Uke River

Month Exposure time (min)	Number Exposed (300)					
	May		June		July	
	% Death	% Survival	% Death	%Survival	% Death	% Survival
0	0(0)	100(100)	0(0)	100 (100)	0(0)	100 (100)
15	5(5)	95(95)	4(4)	96(96)	9(9)	91(91)
30	31(31)	69(69)	19(19)	81(81)	32(32)	68(68)
45	93(93)	7(7)	79(79)	21(21)	88(88)	12(12)
60	98(98)	2(2)	98(98)	2(2)	1 00(100)	0(0)
Total		**100**		**100**		**100 (300)**

NS: Not statistically significant at $P>0.05$ (ANOVA test)

Table 2: Susceptibility and resistance status of female *An. gambiae s.l* at Lower Uke River

Month Exposure time (min)	Number Exposed (300)					
	May		June		July	
	%Death	%Survival	%Death	%Survival	%Death	%Survival
0	0(0)	100(100)	0(0)	100 (100)	0(0)	100 (100)
15	24(24)	76(76)	37(37)	63(63)	25(25)	75(75)
30	42(42)	58(58)	55(55)	45(45)	48(48)	52(52)
45	79(79)	21(21)	80(80)	20(20)	76(76)	24(24)
60	96(96)	4(4)	97(97)	3(3)	96(96)	4(4)
Total		**100**		**100**	**100**	**(300)**

NS: Not statistically significant at $P>0.05$ (ANOVA test)

The use of pesticides is the principal pest control strategy commonly applied by farmers to protect their crop in the district. These products, if not used correctly, can have direct environmental and health consequences and indirect consequences through the selection of insecticide resistance in malaria vectors. This constitutes a real threat for insecticide-based vector control and can lead to vector-control intervention failures, as reported by Hargreaves *et al*. (2000) in South Africa. The findings of the study confirm with CDC Bottle bioassay test standard method. However, different studies have reported that the use of insecticides across all the various endemic areas such as households and agricultural settings haves generally increased selection pressure leading to the emergence of resistance in malaria vectors (Yadouleton *et al*., 2009). Therefore, testing for the resistance characteristics of malaria vectors to distinct class of insecticides is crucial to the development of area specific control measures (Corbel, *et al*, 2007). The present study has shown that practices of farmers in the area may favour the development of resistance in malaria vectors as indicated by Hemingway, (2014) and Yadouleton *et al.* (2009). The susceptibility test showed the levels of phenotypic resistance of *Anopheles* spp to agricultural insecticides.

Indoor residual spraying (IRS) strategy has a great impact on the transmission rate of malaria. IRS reduces the life span of mosquitoes, reduces the number of people bitten by mosquitoes and contributes to reduction of malaria transmission, mortality and morbidity (Steinhardt *et al.,* 2013). Student T-test analysis of bioassay results showed that statistically the average mortality (97.6%) on cement plastered surface was higher than the on mud-plastered surface (54.8%).

Acknowledgements

The author wish to express sincere gratitude to the entomology technicians who help in mosquito larval collections and rearing as well as Professor G. Amuga of the Department of Zoology Nasarawa State University, Keffi, for providing valuable advice in the preparation of this manuscript.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

Conclusion

The findings of the study revealed that 20 µg does of Pirimiphose methyl was effective against *Anopheles* mosquito at Upper Uke location but possible resistance was reported at lower Uke location affirming a CDC bottle bioassay test standard protocol (98-100%) and (96-97%). Therefore, additional studies are required to verify the susceptibility and resistance status of Pirimiphose methyl against *Anopheles* mosquitoes using higher dosage.

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