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PYRETHROIDS RESISTANCE IN *CULEX QUINQUEFASCIATUS* (SAY) (DIPTERA: CULICIDAE) POPULATIONS FROM BAUCHI STATE, NIGERIA

Umar Aliyu

Department of Biological Sciences, Faculty of Science, Bauchi State University, Gadau, Bauchi State, Nigeria.

*Corresponding authors' email: umaraliyu@basug.edu.ng

ABSTRACT

This study examines pyrethroid resistance in *Culex quinquefasciatus* mosquitoes collected from some selected Local Governments Areas of Bauchi State, North-Eastern Nigeria. Insecticide resistance to different phyrethroids was determined using WHO bioassay method. The mosquitoes were also pre-exposed to Piperonyl butoxide (PBO) using the WHO method as part of the synergist assay. The results obtained from this study indicated that *Culex quinquefasciatus* showed resistance to deltamethrin in Misau (70% mortality), permethrin resistance was detected in Bauchi (58% mortality) and Katagum (37% mortality). Phyrethroids resistance was not detected in Lamda-cyhalotrin and alphacypermethrin. Pre-exposure to PBO in Bauchi LGA has led to 100% mortality of culex mosquitoes exposed to deltamethrin and lambda-cyhalothrin while mortality to permethrin increased from 58% to 95%. In Misau, Pre- exposure to PBO led to 100% mortality of mosquitoes exposed to permethrin and alphacypermethrin while in deltamethrin exposed mosquitoes, PBO increased the mortality from 70% to 90% mortality. The study revealed high pyrethroids resistance of *Cx. quinquefasciatus* in Bauchi state which causes serious problems in the management of insecticides resistance and also diseases transmitted by this mosquito.

Keywords: Pyrethroids, Culex, Resistance, Vector

INTRODUCTION

Culex quinquefasciatus is a mosquito that belongs to the *Culex pipiens* complex Linnaeus and is maily found in Africa. This species usually bite at night and constitutes a major biting nuisance, particularly in cities where it is found to be breeding in wet pit latrines, cess pits and polluted puddles (Christopher *et al.*, 2012). In addition to causing discomfort due to its biting habit, *Cx. quinquefasciatus* is the primary vector of a number of illnesses such as West Nile virus and *Wulchereria bancrofti* (Lima-Camara, 2016). Because mosquitoes from the complex *Cx. pipiens* feed both on human and bird blood, they may transmit sylvatic arboviruses from migratory birds to man in urban territories (Ramon *et al.*, 2019). This mosquito also serve as a potential vector of the arboviruses responsible for the transmission Rift Valley fever (Tinto *et al.*, 2023).

The use of chemical insecticides is one of the best way for controlling medically important vectors like mosquitoes that transmit various diseases (Ukpai and Ekedo, 2018). These insecticides are very effective, but it has a lot of problems such water and environmental pollution, effects on non target insects and development of insecticides resistance (Ramos, 2020).

In mosquitoes, resistance to insecticides such as phyrethroids may be caused by either one of the following: target site mutation, behavioural changes, detoxifying enzyme metabolism and morphological modifications. These usually occurs occur in areas where diseases are endemic and subsistence remains a burden on communities, managing this resistance is a critical problem for vector control programs (Ibrahim *et al.*, 2023). Insecticides resistance in *Culex* spp. has been reported in many West African countries (Laura and Douglas, 2014). Poor management of insecticide resistance could lead to the failure of vector control efforts especially mosquito-borne diseases (Talipouo *et al.*, 2021). Furthermore, resistance to insecticides in *Culex* mosquitoes could be a problem if diseases caused by this mosquito re-emerge in areas where they were already eliminated. *Culex* mosquitoes resistance to insecticides could also be a problem for programmes to eliminate malaria if communities perceive reduced efficiency of IRS and ITN/LLINs and the usage rate of these tools decline (Ojianwuna *et al.*, 2022). Therefore, it has become necessary to study the susceptibility status of *Culex* spp. in regions such as Bauchi state where *Culex* spp. are abundant and has been implicated in the transmission of some diseases.

In Bauchi State, most of the studies focused on malaria vectors, information on the susceptibility status of *Culex quinquefasciatus* to the different classes of insecticides used in public health is lacking.

MATERIALS AND METHODS

Study Area

This study was conducted in Three (3) Local Governments Areas of Bauchi States, North Eastern Nigeria. Eggs and Immature stages of *Culex quinquefasciatus* were collected from Bauchi (10º28'01" N, 9º79'45"E), Misau (11º31'40"N, 10º46'52"E), and Katagum (11º67'26"N, 10º20'39"E).

Mosquito Collection and Rearing

Immature stages of *Culex quinquefasciatus* were collected from gutters and open ditches from September – December, 2023 from the three selected Local Governments Areas of Bauchi State. These larvae were reared at the insectary at Bauchi State University, Gadau. They were fed with ground biscuits and maintained at a temperature of 27˚C ± 2˚C and relative humidity of 78% ± 3% until the emergence adults. Adults mosquitoes were placed into cages and fed with 10% sugar solution prior exposure. *Aedes aegypti* were morphologically identified using morphological and pictorial keys according to Rueda, 2004.

Insecticides Susceptibility Bioassays

Bioassays were conducted with well fed Adult female *Culex quinquefasciatus* aged 3-5 days following the protocol described by WHO. The Pyrethroids tested were Deltamethrin (0.05%), Permethrin (0.75%), Lamda-cyhalotrin (0.05%) and Alphacypermethrin (0.05%). Four replicates each of 20 -25 3-

5 day old *Culex quinquefasciatus* females were exposed for 1 hour and the number on knockdown mosquitoes were recorded at intervals in accordance with WHO (2016) guidelines. Mosquitoes were then transferred to holding tubes and fed with sugar for 24 h before mortalities were recorded. For control, during each experiment, the mosquitoes were kept in holding tubes without insecticide exposure.

PBO synergist assays

Synergist bioassay were conducted to determine the role of metabolic enzymes in insecticides resistance in culex according to protocol described by WHO, 2016. These mosquitoes were first exposed to the synergist PBO (piperonyl butoxide) an inhibitor of P450 and esterase activity. Four replicates of approximately 20–25 mosquitoes 3-5 days old were first exposed to the synergist-impregnated papers for 1 h before they were transferred to tubes containing the insecticides impregnated papers. After one hour exposure, the mosquitoes were then transferred to holding tubes and fed with 10% sugar. Mortalities recorded after 24 hours.

Data Analysis

Data analysis was performed using Microsoft excel 2016. Insecticide Resistance Interpretation was done using WHO 2016 guidelines. The percentage mortality of the mosquitoes was calculated by summation of the total number of dead mosquitoes in all the replicates and then expressing it as a percentage of the total number of exposed mosquitoes. Mortality in the range of 98–100% = Susceptible, 90 -97% Possible resistance. A mortality of less than 90%, confirms the existence of resistant genes. Knockdown time (KDT50 and KDT95) was estimated using linear probit analysis (R Software version 4.3.1).

RESULTS AND DISCUSSION

Knockdown Time

Knockdown time of *Cx. quinquefasciatus* exposed phyrethroids is shown in Table 1. Analysis of the knockdown time showed that KDT values were lowest to lamda-cyohalotrtin and alphacypermethrin in Bauchi and Katagum Local Government. In Misau Local Governement, analyses of KDT values were highest to deltamethrin and lowest to alphacypermethrin (Table 1).

Table 1: KdT_{50} and KdT_{95} values of *Cx. quinquefasciatus* populations from Bauchi State exposed to Pyrethroids

LGA	Insecticide	KDT_{50} (95% CI)	KDT_{95} (95% CI)
Bauchi	Deltamethrin (0.05%)	63.7 (45.3 - 89.3)	213.862 (152.1 - 300.73)
	Permethrin (0.75%)	62.9 (42.3 - 93.64)	278.282 (186.9 - 414.1)
	Lamda-cyhalotrin (0.05%)	36.1 (24.5 - 53.6)	170.0 (155.5 - 250.2)
	Alphacypermethrin (0.05%)	35.795 (25.2 - 50.9)	146.4 (102.9 - 208.1)
Katagum	Deltamethrin (0.05%)	36.6 (25.2 - 53.9)	163.3 (112.42 - 237.6)
	Permethrin (0.75%)	107.334 (60.681 -189.857)	805.375 (455.313 -1424.3)
	Lamda-cyhalotrin (0.05%)	36.0 (24.5 - 53.0)	170.0 (115.5 - 250.2)
	Alphacypermethrin (0.05%)	36.0 (24.5,53.0)	170.0 (115.5 - 250.2)
Misau	Deltamethrin (0.05%)	46.9 (31.9 - 69.0)	214.4 (145.8 - 315.1)
	Permethrin (0.75%)	35.9 (25.3 - 50.9)	145.1 (102.3 - 205.4)
	Lamda-cyhalotrin (0.05%)	36.251 (25.4 - 51.7)	151.1 (105.1 - 215.6)
	Alphacypermethrin (0.05%)	35.9 (24.8 - 49.8)	142.6 (100.6 - 202.1)

WHO Resistant Bioassay

Insecticide Resistance Status of *Culex quinquefasciatus* from Bauchi Local Government is presented in Table 2. The result showed that Culex mosquitoes are highly resistant to Permethrin with 58% mortality and low resistance were observed towards deltamethrin with mortality of 83%. However, resistance Lamda-cyhalotrin and Alphacypermethrin was suspected in *Culex quinquefasciatus* with 90% and 95% mortalities respectively. In Katagum Local Government, the results indicated that *Culex quinquefasciatus* from Katagum Local Government were highly resistant to Permethrin with 37% mortality and low resistance was observed towards deltamethrin with 89% mortality. However, the population is susceptible to Lamda-cyhalotrin and Alphacypermethrin with 100% mortalities. In Misau Local Government, culex mosquitoes showed moderate resistant to Deltamethrin and permethrin with 70% and 89% mortality respectively. However, the population is susceptible to Lamda-cyhalotrin and Alphacypermethrin with 100% mortalities (Table 2).

Table 2: Insecticide Resistance Status of *Culex quinquefasciatus* from Bauchi Local Government

LGA	Insecticides (Conc)	Number Exposed	Number Dead	Mean Mortality (± S.E)	24h Mortality (%)	Susceptibility Status
Bauchi	Deltamethrin (0.05%)	100	83	20.70 ± 0.96	83	Resistant
	Permethrin (0.75%)	100	58	14.50 ± 3.70	58	Resistant
	Lamda-cyhalotrin (0.05%)	100	90	22.50 ± 0.58	90	Possible Resistant
	Alphacypermethrin (0.05%)	100	95	23.75 ± 0.96	95	Possible Resistant
	Deltamethrin (0.05%)	100	89	22.25 ± 0.96	89	Resistant
	Permethrin (0.75%)	100	37	9.25 ± 3.90	37	Resistant

Katagum	Lamda-cyhalotrin (0.05%)	100	100	25.00 ± 0.00	100	Susceptible
	Alphacypermethrin (0.05%)	100	100	25.00 ± 0.00	100	Susceptible
	Deltamethrin (0.05%)	100	70	17.50 ± 2.38	70	Resistant
	Permethrin (0.75%)	100	89	22.25 ± 1.75	89	Resistant
Misau	Lamda-cyhalotrin (0.05%)	100	100	25.00 ± 0.00	100	Susceptible
	Alphacypermethrin (0.05%)	100	92	23.00 ± 0.82	92	Susceptible

Values are Means ± S.D. WHO (2001) Criteria for scoring susceptibility/resistance: <90% = Resistant, 98-100% = Susceptible, 90 – 97% = Possible Resistance

Synergist Bioassay

Culex mosquitoes were first exposed to PBO before exposure to the insecticides. The result in Bauchi Local Government showed that there was a complete recovery of susceptibility in mosquitoes exposed to Lamdacyahalothrin and Deltamethrin after pre exposure to PBO increasing the mortality from 90% to 100% and 83% to 100% respectively. However, there was a partial recovery of susceptibility in culex exposed to permethrin after pre exposure to PBO increasing the mortality from 58% to 95% mortality (Figure 1).

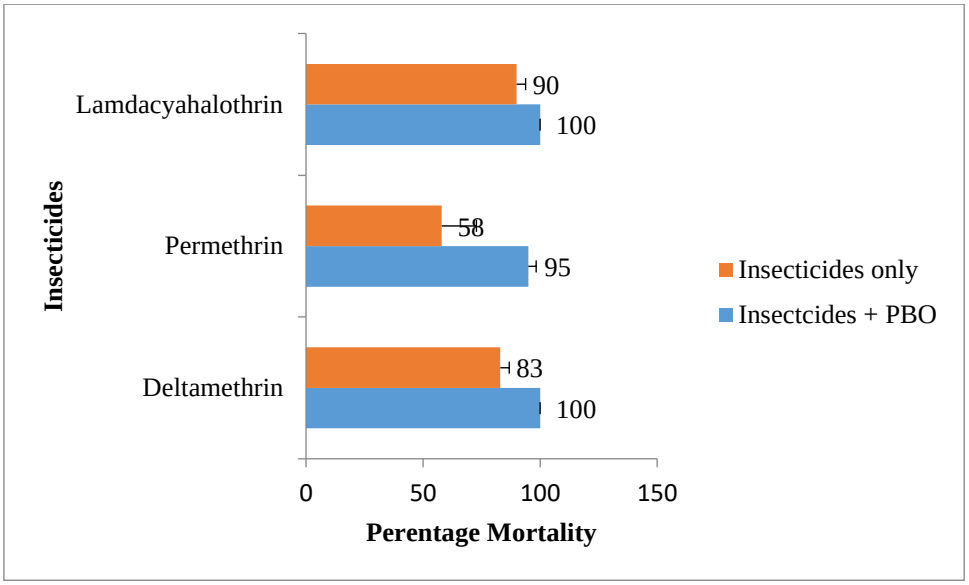

Figure 1: Effect of PBO in Insecticides Resistance in Bauchi LGA

In Katagum Local Government, complete recovery of susceptibility was achieved in mosquitoes exposed to Deltamethrin after pre-exposure to PBO (from 89% to 100%). However, partial recovery was observed in mosquitoes exposed to permethrin increasing the mortality from 37% to 66% (Figure 2).

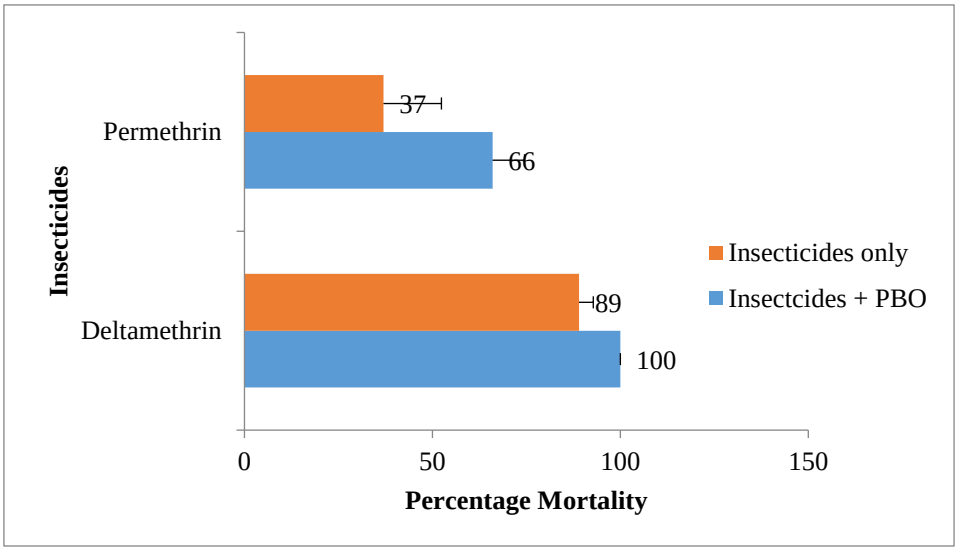

Figure 2: Effect of PBO in Insecticides Resistance in Katagum LGA

In Misau Local Government, complete recovery of susceptibility was achieved in mosquitoes exposed to Alphacypermethrin and Permethrin after pre-exposure to PBO increasing the percentage mortalities from 92% to 100%

and 89% to 100% respectively. However, partial recovery was observed in mosquitoes exposed to Delthamethrin increasing the mortality from 70% to 95% (Figure 3).

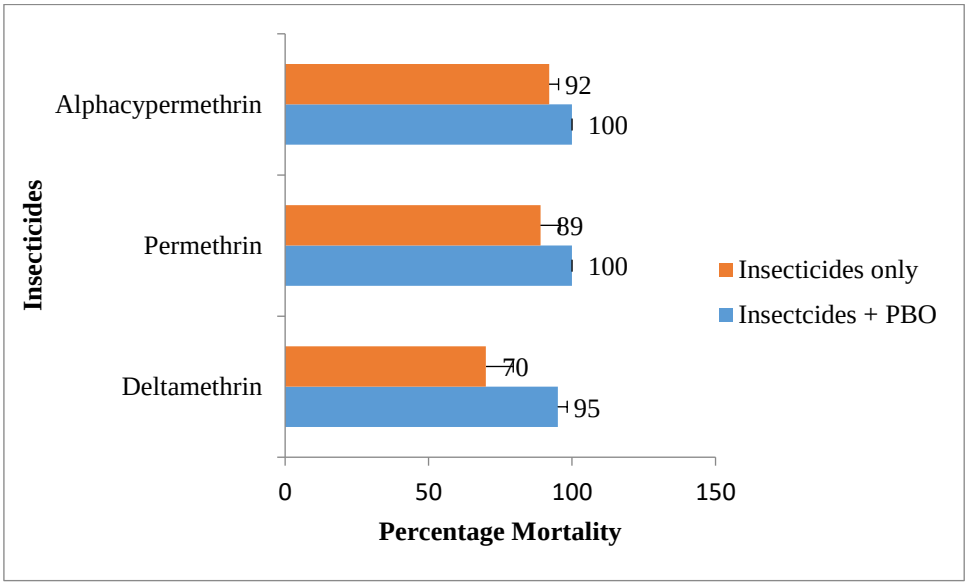

Figure 3: Effect of PBO in Insecticides Resistance in Misau LGA

Discussion

This study evaluates pyrethroids resistance in *Cx. quinquefasciatus* in Bauchi Stae, North eastern Nigeria. Monitoring insecticides resistance in Culex mosquitoes is not only vital to control of diseases transmitted by this mosquito but is paramount important in the control and prevention of all mosquito transmitted diseases.

For over decades, chemical insecticides such as pyrethroids have shown great promise for the use against mosquito vector control. This is due to their low toxicity to humans and high potency at low doses which quickly immobilizes and kill mosquitoes (Prasittisuk, 1994). However because of the indiscriminate use of these insecticides, resistance has been reported in several species of arthropods, including *Culex quinquefasciatus*, *Anopheles Gambiae s.l* and *Anopheles Funestus s.l* complex (Grieco, 2007).

From this study, *Culex quinquefasciatus* from Bauchi and Katagum Local Governments showed high resistance to permethrin (Table 2). This may be attributed to the increase in the usage of permethrin treated nets in Nigeria. In the case of susceptibility or suspected resistance in Deltamethrin, previous reports have indicated that same population of mosquito can be resistant and also susceptible to different insecticides from the same family. This may be due to cytochrome P450 (CYP6P4) up-regulated in this population and to be preferential to metabolized permethrin but not deltamethrin (Lawal *et al.*, 2019).

This study showed full susceptibility to Alphacypermethrin and Lamda-cyhalotrin in Katagum and Misau Local Governments and suspected resistance in Bauchi Local Government. Lamda-cyohalothrin. This is in agreement with the work of Abba et al., 2016 which reported 92 – 96% resistance of *Culex quinquefasciatus* to Alphacypermethrin.

Pre-xposure of the *Cx. quinquefasciatus* populations to PBO before exposure to the pyrethroids led to increased mortality in all the three Local Governments. This suggests the involvement of cytochrome P450 in resistance development in the *Cx. quinquefasciatus* populations from Bauchi States. This is in agreements with several reports in Nigeria and around the world (Omotayo *et al.*, 2022; Komagata *et al.*, 2010; Liu *et al.*, 2011).

CONCLUSION

This study revealed high resistance to *Culex quinquefasciatus* to permethrin and deltamethrin. With this pattern of resistance, it is showing the lack of effectiveness of type I and type II Pyrethroids against *Culex quinquefasciatus* in Bauchi State. However, Lamda-cyhalotrin and Alphacypermethrin may still be effective in controlling *Culex quinquefasciatus*. It can be concluded from this study that insecticides resistance surveillance should be conducted regularly in Bauchi State and also integrated vector control strategy should be considered.

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