

## Insecticide resistance profile and sex ratio of *Culex quinquefasciatus* in Umuahia, Abia State, Nigeria

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### Abstract

A vast majority of neglected tropical diseases such as lymphatic filariasis, *Wuchereria bancrofti*, avian malaria and a number of other viral diseases are vectored by *Culex quinquefasciatus*. This study was carried out to ascertain the insecticidal susceptibility status and sex ratio of *Culex quinquefasciatus* in Umuahia, Abia State, Nigeria, from November 2022 to February 2023. Larvae and pupae of *Culex quinquefasciatus* were sampled from different areas in Umuahia, and were conveyed in properly marked containers to the insectaria in the Entomology Unit of Michael Okpara University of Agriculture, Umudike. The mosquitoes were sustained and grown at a temperature of 26±3°C and relative humidity of 74±4% to adult stage for World Health Organization (WHO) kit bioassays. Four kinds, of WHO bioassay test papers treated with recommended diagnostic concentrations of 4% DDT (organochlorines), 0.75% permethrin (pyrethroids), 0.1% bendiocarb (carbamates) and 5% malathion (organophosphates) bought from the vector control unit of Universiti Sain Malaysia, were used for the bioassay. Records of knockdowns were taken at intervals of 0, 5, 10, 20, 30, 40, 50 and 60 minutes, and then maintained for 24 hours post exposure in 10% sugar solution. The percentage mortality of each insecticide after 24 hours was thereafter calculated and all the adult mosquitoes collected were sorted into male and female to determine the sex ratio. The result showed that *Culex quinquefasciatus* mosquitoes in the study area were resistant to DDT (organochlorines), permethrin (pyrethroids) and bendiocarb (carbamates) but susceptible to malathion (organophosphates). The sex ratio (1.5: 1) was male-biased which favoured mosquito vector control programme. This study showed that malathion was the insecticide of choice against *Culex quinquefasciatus* in the area. In view of the above, the use of botanicals is recommended for future vector control programmes because they are environmentally friendly and less likely to develop resistance.

**Keywords:** Insecticide resistance, Sex ratio, *Culex quinquefasciatus*, Umuahia, Abia State

**Received** April 02, 2024; **Revised** September 16 2024; **Accepted** October 3, 2024

## INTRODUCTION

Mosquitoes contribute immensely to the transmission of many vector-borne diseases, as well as constitute enormous biting nuisance (Guntay *et al.*, 2018). At least 100 species from over 3,500 described mosquito species serve as vectors to diseases which includes but are not limited to malaria, yellow fever, dengue fever, chikungunya fever, filariasis, and many other viral encephalitis (Ukpai and Ekedo, 2019; Annadurai *et al.*, 2015). These disease vectors belong to three major mosquito genera: *Aedes*, *Culex* and *Anopheles* (Ukpai and Ekedo, 2019).

Poor urbanization within Africa has continuously created very suitable environments that have facilitated the wide spread of *Culex quinquefasciatus* within the region (Yadouleton *et al.*, 2015). Unfortunately, *Culex quinquefasciatus* has been incriminated as one of the major vectors of *Wuchereria bancrofti*, a filarial nematode responsible for lymphatic filariasis especially in the Western axis of Northern Nigeria (Hussaini *et al.*, 2020), as well as other parts of Nigeria. The two major tools approved by the WHO for mosquito control are indoor residual spraying and long-lasting insecticide treated nets (WHO, 2017). However, the success of these methods is grossly been mitigated by the increasing cases of resistance to insecticides reported in mosquitoes from various locations around the world (Ekedo, *et al.*, 2023; Chukwuekezie *et al.*, 2020; Ekedo and Ukpai 2019; Ukpai and Ekedo, 2019).

Increasing incidence of resistance will definitely translate to increase in mosquito-borne diseases, and therefore calls for regular evaluation of the resistance status of local mosquito populations, prior to deployment of any of the vector control intervention. This is particularly important because of the spatiotemporal variation of insecticide resistance across continents, countries, towns, communities and possibly within micro communities within a locality. This study will reassess the status of the work by Ukpai and Ekedo (2019) in Ikwuano, a neighbouring LGA to Umuahia North, and further

provide information on the mosquito's sex ratio unreported by their study.

Very important to the deployment of genetically modified mosquitoes in a bid to bring down vector population or to replace some trait, is knowledge of the sex ratio of the local mosquito population of interest alongside other baseline information (Alphey, 2014). A population with male bias would definitely present a positive outcome (Galizi *et al.*, 2014), since females are majorly incriminated as disease vectors. Scanty information exists within South Eastern Nigeria on the sex ratio and insecticide susceptibility/resistance profile of *Culex quinquefasciatus*, necessitating the need to contribute to this knowledge gap.

## MATERIALS AND METHODS

### Study area

The study was carried out in Umuahia, the capital territory of Abia State, South-eastern, Nigeria (Fig. 1). Umuahia is made up of Umuahia North and Umuahia South Local Government Areas. It lies within the tropical rain forest zone and has a minimum and maximum temperature of 23°C and 32 °C respectively, as well as an annual rainfall of 2238 (Ekedo and Ukpai, 2019).

*Culex* larva and pupae were sampled from different points (puddles, gutters, tyre tracks and ground water pools) within the study location as described by Ukpai and Ekedo (2019) from November 2022 to February 2023. A small plastic container was used to collect water with larvae from these points and poured into a large plastic bowl, whereas a plastic pipette was used to pick larvae from points where the plastic container could not access. Sorting was done to the larvae into different physiological age by the aid of a strainer, while predators and unwanted organisms were picked out using a pipette. The *Culex quinquefasciatus* larvae/pupae collected were transported in well labeled plastic containers to the Entomology Unit of Michael Okpara University of Agriculture, Umudike, and

were reared at  $26\pm 3$  °C and  $74\pm 4\%$  relative humidity to adult stage for World Health Organization (WHO) bioassays. Larvae were fed on ground biscuits and adults were provided with

10% sugar solution. The resulting adults were identified according to the morphological keys of Gillies and Coetzee (1987).

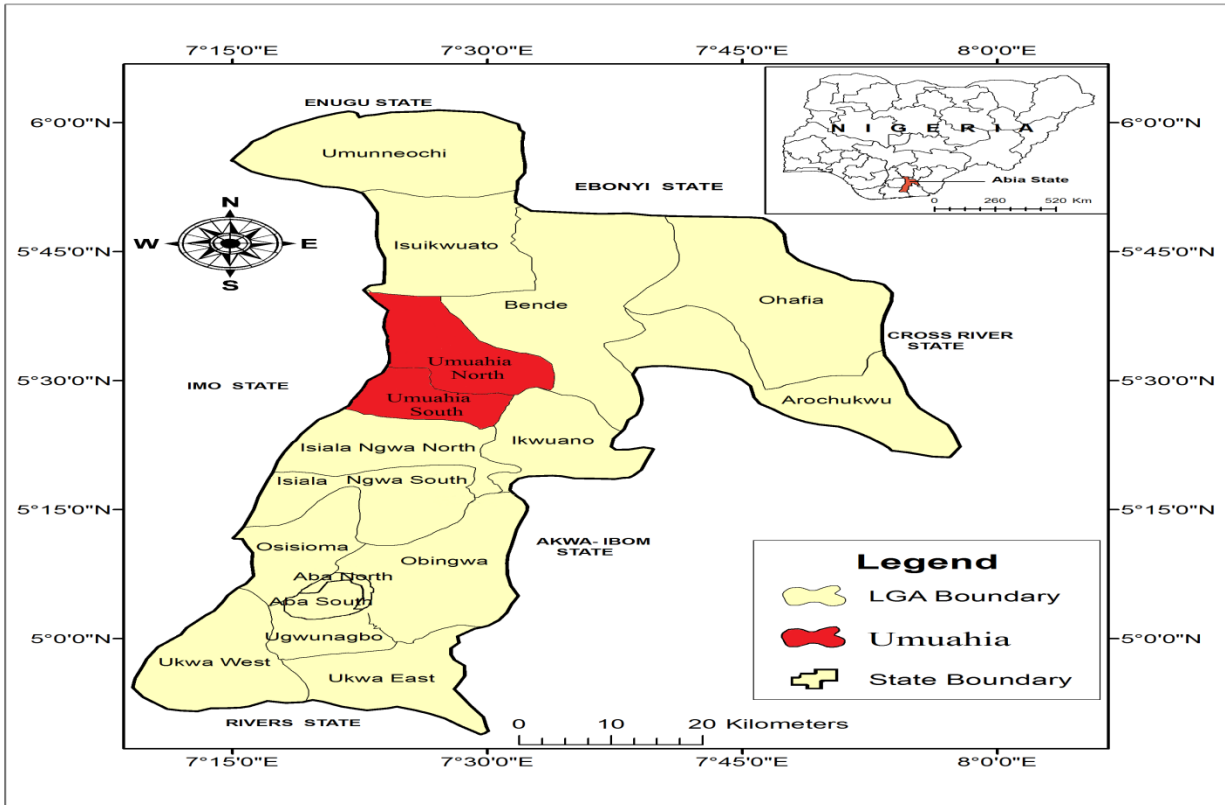


Fig. 1: Map of Abia State, showing Umuahia the study location

### Mosquito larval collection and rearing

### Insecticide susceptibility test

The WHO standard procedures and test kits for adult mosquitoes were employed for the insecticide susceptibility tests (WHO, 2022). Four types, of WHO bioassay test papers treated with appropriate diagnostic concentrations of 4% DDT (organochlorines), 0.75% permethrin (pyrethroids), 0.1% bendiocarb (carbamates), and 5% malathion (organophosphates) procured from Universiti Sain Malaysia, were employed for the bioassay. Sugar-fed female *Culex quinquefasciatus* mosquitoes of about 3-5 days old were used for the test. About 80 females were exposed to each insecticide in four replicates. Accordingly, 4% DDT (organochlorines); 0.75% permethrin (pyrethroids); 0.1% bendiocarb

(carbamates); and 5% malathion (organophosphates) treated strips of paper were put each into 4 exposure tubes and rolled to line with the wall of the tube and held into position by a metal clip for each of the insecticides, whereas a plain paper was used to line the control tube. As standard demands, an initial pre-test was carried out by using an aspirator to introduce 20 females *Culex quinquefasciatus* mosquitoes each into four holding tubes. These tubes were subsequently allowed to stand for 1 hour. The mosquitoes were after then moved into the exposure tubes through a hole on the lid that separates the holding tube and the exposure tube. The exposure tubes were then positioned to stand upright with the screen-end up and allowed to stand for 1 hour. Knockdown and mortality records were taken at intervals of 0, 15, 20, 30, 40, 50 and 60 minutes. This was followed up with

the transfer of the mosquitoes back to the holding tubes. The mosquitoes were kept for 24 hours in the recovery tubes and were fed with 7% sucrose solution during this period. Final records of mortality were taken after 24 hours and the susceptibility/resistance status of the population was determined according to WHO recommended standard (WHO, 2022). Dead and survived mosquitoes from this bioassay were carefully kept each in a clearly labeled 1.5 ml Eppendorf tubes containing silica gel, for preservation. All susceptibility tests were carried out at 26±3°C temperature and 74±4% relative humidity.

$$\text{Percentage mortality after 24 hours} = \frac{\text{Number dead}}{\text{Total Number}} \times \frac{100}{1}$$

### Sex ratio determination

The adult mosquitoes were sorted out into different species and sexes and counted as described by Khan *et al.* (2018) while the sex ratio was calculated by dividing the number of male mosquitoes by the number of females (Paaijmans *et al.*, 2009). Significant difference between the sexes was determined using Chi-square.

$$\text{Sex ratio} = \frac{\text{Number of Males}}{\text{Number of Females}}$$

### Data interpretation and statistical analysis

The percentage mortality of each insecticide 24 hours after exposure was calculated as the proportion of mosquitoes that died after 24 hours and the total number of mosquitoes exposed using 95% confidence intervals. Since mortality rate in the control tubes were less than 5%, there was no need for correction using Abbott formula (Abott, 1987). The WHO benchmark which states that mortality rates of less than 80% shows full resistance while those greater than 98% indicated full susceptibility, whereas mortality rates between 80-98% suggested the possibility of resistance that needs to be clarified served as a guide for result interpretation (WHO, 2022). Analysis of Variance (ANOVA) was also used to compare the mortalities across the insecticides

and Tukey pairwise was used to separate the means.

## RESULTS

### Percentage mortality after 24 hours exposure of *Culex quinquefasciatus* to four insecticides

The percentage mortality of *Culex quinquefasciatus* after 24 hours exposure to permethrin (pyrethroid), bendiocarb (carbamate), DDT (organochlorine) and malathion (organophosphate) is presented in Table 1. After 24 hours exposure, malathion recorded the highest percentage mortality (98.75%), followed by bendiocarb (60.00%), permethrin (25.00%) and DDT (7.50%). There were significant differences ( $p < 0.05$ ) in the percentage mortalities of the mosquitoes exposed to the different insecticides. The percentage mortalities for bendiocarb, permethrin and DDT were less than 80%: indicating resistance to these insecticides. However, percentage mortality in malathion was higher than 98% indicating susceptibility.

### Knockdown values of *Culex quinquefasciatus* within 60 minutes of exposure to permethrin, bendiocarb, DDT and malathion

After 60 minutes exposure of *Culex quinquefasciatus* to permethrin, the knockdown values (KDV) within the period and percentage mortality within 24 hours after exposure is presented in Table 2. The knockdown values of the mosquitoes at 10, 15, 20, 30, 40, 50 and 60 minutes of treatment ranged between 0.75 ± 0.25 and 20.00±0.41.

### Sex ratio

A total of 738 adults were collected, out of which 437 (59.2%) were male and 301 (40.8%) were female (Table 3). The male and female sex ratio was 1.5:1. Chi-square analysis showed that males were significantly higher than females ( $\chi^2_{(df = 1, 0.01)} = 16.77$ )

**Table 1: Percentage mortality after 24 hours exposure of *Culex quinquefasciatus* to four insecticides**

S/N	Insecticide	Mortality after 24 Hours	Status
1	Permethrin (Pyrethriod)	25.00 <sup>b</sup>	Resistant
2	Bendiocarb (Carbamate)	60.00 <sup>c</sup>	Resistant
3	DDT (Organochloride)	7.50 <sup>a</sup>	Resistant
4	Malathion (Organophosphate)	98.75 <sup>d</sup>	Susceptible

Key: a, b, c, d = means with different superscripts across the rows are significantly different at  $P < 0.05$ ;

**Table 2: Knockdown values (KDV) observed within 60 minutes of exposure to permethrin, bendiocarb, DDT and malathion**

Variables	Mortality (%)				
	Control	Permethrin	Bendiocarb	DDT	Malathion
Number of mosquitoes exposed	20.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00±0.00
Number KDT 10minutes	0.00	1.75 ± 0.25	0.75 ± 0.25	1.75 ± 0.25	3.75±0.25
Number KDT 15 minutes	0.00	2.75 ± 0.25	0.75 ± 0.25	2.75 ± 0.25	5.25±0.25
Number KDT 20 minutes	0.00	1.75 ± 0.25	1.75 ± 0.25	3.50 ± 0.29	7.00±0.41
Number KDT 30 minutes	0.00	1.75 ± 0.25	1.75 ± 0.25	2.75 ± 0.25	12.00±0.00
Number KDT 40 minutes	0.00	3.5 ± 0.29	1.75 ± 0.25	3.50± 0.29	14.75±0.25
Number KDT 50 minutes	0.00	3.5 ± 0.29	2.75 ± 0.25	3.75 ± 0.25	18.00±0.41
Number KDT 60 minutes	0.00	7.00 ± 0.00	3.00 ± 0.00	4.00 ± 0.00	20.00±0.41
Mortality after 24 hours	0.00	5.00 ± 0.41	12.0 ± 0.41	1.5 ± 0.65	19.75±0.25

**Table 3: Sex ratio of male and female mosquitoes collected in the study**

Sex	Number Caught	Percentage	Male: Female Ratio	$\chi^2$
Male	437	59.2	1.5:1	0.01
Female	301	40.8		
Total	738	100		

$$\chi^2_{(df = 1, 0.01)} = 16.77$$

## DISCUSSION

Sufficient knowledge of the insecticide susceptibility status of mosquitoes is a major factor with great capacity to make or mar the success of vector control strategies. For a long time pyrethroids and organochlorines proved to help in the control of mosquito menace primarily

due to their relatively low mammalian toxicity, ease in environmental detoxification and ability to kill mosquitoes at low concentrations. (Grieco *et al.*, 2007). Unfortunately, the constantly growing cases of resistance to these chemical compounds in mosquitoes have led to loss of these gained grounds (Nura *et al.*, 2020).

There is spatiotemporal variation of insecticide resistance across continents, countries, towns, communities and possibly within micro communities within a locality. A whole lot of factors may contribute to these variations and includes but are not limited to the mosquito species, life history and stage, physiological state, gut microbiota and symbiotic associations, climatic factors, socio cultural factors, agricultural practices and activities, as well as demographic and anthropogenic factors (Nkya *et al.*, 2013). *Culex quinquefasciatus* from the study were highly resistant to DDT (7.50% mortality) and permethrin (25% mortality). This agrees with Ukpai and Ekedo (2019), which recorded a lesser DDT resistance (10.48% mortality) in Umudike, a community in Ikwuano LGA area of Abia State, Nigeria. The report of Nura *et al.* (2020) in a similar vein showed high resistance to permethrin (15.39%) and DDT (18.12%) in Katsina State, Nigeria. Oke *et al.* (2022) reported also that *Culex quinquefasciatus* displayed high resistance to permethrin (61%) and DDT (41%) in Markudi, Nigeria. Omotayo *et al.* (2022) reported marginally high resistance to permethrin from Dutse LGA (12.2%) and Kafin – Hausa LGA (77.78%) of Jigawa State, Nigeria; though the study recorded susceptibility (100% mortality) in Ringim LGA. The report of Shehu *et al.* (2023) pointed out the insecticide resistance potentials of female *Culex quinquefasciatus* mosquito species sampled from 3 LGA's within Niger State, Nigeria. Cross-resistance occurs between permethrin and DDT insecticides primarily due to the fact they both have the same target site of insecticidal action which grossly depends on the sodium gate channels found in the nerve sheet (Wondji *et al.*, 2009). More so, the chemical contaminated nature of the mosquito breeding sites resulting from past use of insecticides for agricultural purposes may also serve as a contributing factor to this situation (Nura *et al.*, 2020). Sunaiyana *et al.* (2006) also reported DDT and permethrin cross resistance in Thailand. There is obviously no doubt that the over dependence on permethrin for the treatment of LLITNs in Nigeria may have contributed grossly to the continually growing resistance to permethrin and cross resistance to DDT commonly reported across Nigeria. (Omotayo *et al.*, 2022). In a similar manner reports have it that permethrin is also overly used for larviciding, indoor residual sprayings of adult mosquitoes, and for agricultural control purposes (Vasquez *et al.*, 2009). It has become glaring and indisputable that Permethrin and DDT may no longer be viable

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for the control of mosquito vectors (Nura *et al.*, 2020).

*Culex quinquefasciatus* was equally resistant to Bendiocarb (60% mortality). *Culex quinquefasciatus* showed suspected resistance to bendiocarb (90% mortality) in Markudi, Benue State, Nigeria (Oke *et al.*, 2022). Ehisianya *et al.* (2017) reported the resistance of *Culex quinquefasciatus* to Bendiocarb (48% mortality) within the University community of Michael Okpara University of Agriculture, Umudike, located in Abia State, South Eastern, Nigeria. The study explained that this resistance to bendiocarb, a highly effective carbamate insecticide could be related to the high and indiscriminate use of insecticides and fertilizers by farmers within the study area surrounded by agricultural activities owing to the agricultural research institute in its neighborhood. Two years after, Ukpai and Ekedo (2019) recorded 39.20% mortality for bendiocarb within the same Local Government Area of Abia State. Their study also attributed this to chemical based agrarian activities as observed by Ehisianya *et al.* (2017). However, Nura *et al.* (2020) reported susceptibility (96.4%) of *Culex quinquefasciatus* to bendiocarb in Sahel Savannah, Katsina State. The study attributed the susceptibility to down regulation of detoxification enzyme which allowed the insecticide get to the target site of action (Nura *et al.*, 2020). Bendiocarb belongs to a class of insecticides known as carbamate, and it came into full use as substitute for pyrethroids when resistance to pyrethroids became pronounced across Africa. (Aïkpon *et al.* 2013). Nchoutpouen *et al.* (2019) opined that bendiocarb resistance in *Culex quinquefasciatus* populations could be aided by the presence of knock down resistance (*kdr*) allele. Weetman *et al.* (2018) in his own opinion averred that the duo of *kdr* mutations and metabolic resistance mechanism is responsible for resistance in *Culex* species. Most of the larvae used in this study were collected from the Umuahia municipal sewage gutters, and Zayed *et al.* (2019) reported that larval samples picked from highly saline and acidic water bodies such as sewages will tend to be more resistant to insecticides than their fresh water counterparts.

Out of four insecticides used, only malathion was effective in controlling *Culex quinquefasciatus* in the study area; giving susceptibility of 98.75%. Nura *et al.* (2020) published susceptibility (96.4%

mortality) to malathion in Sahel Savannah area, Katsina State and also related it to the same effect observed in bendiocarb. Kanutcharee *et al.* (2008) published high susceptibility of *Culex quinquefasciatus* mosquitoes to malathion in Thailand while Yoriyo *et al.* (2018) reported a 100% mortality in Northeast Nigeria. Shehu *et al.* (2023) reported very high mortality values (100%) on exposure to malathion for mosquitoes from Bosso, and Shiroro LGA's and a slightly lower value for those sampled from Lapai LGA of Niger State, Nigeria, showing the high effect of malathion within the state. Guta *et al.* (2021) reported resistance (77%) of malathion to *Culex quinquefasciatus* in Jimma area, Ethiopia which is different from our result. Results of knockdown assessment from this study showed that the *Culex quinquefasciatus* mosquitoes were knocked down within first 10 minutes of exposure to the malathion impregnated papers, overly suggestive of the existence of knock down resistance mechanisms in the sampled population. This trend was also reported by Ehisiyanya *et al.* (2017) and Ukpai and Ekedo (2019). A number of researchers had reported inducement of Knockdown in mosquitoes by insecticide impregnated papers (Olayemi *et al.*, 2011; Ibrahim *et al.*, 2014; Umar *et al.*, 2014). This inducement of knockdown in these mosquitoes when exposed to insecticide impregnated papers is suggestive of the existence of knock down resistance (*kdr*) mechanism (Awolola *et al.*, 2007; Ibrahim *et al.*, 2014; Umar *et al.*, 2014) operating in populations of *Culex quinquefasciatus* mosquitoes within Umuahia. It is worthy to note that knock-down rates obtained from susceptibility tests are very important indicators that will assist in the early detection of resistance to an insecticide (WHO, 2022). It is therefore suggestive that the <100% knock-down rates observed at the end of 1 hour from this study is indicative of a currently ongoing malathion resistance development in populations of *Culex quinquefasciatus* within the study area. (Ser and Cetin, 2019). This could also account for the level of resistance to other insecticides (DDT, Bendiocarb and permethrin) evaluated. In addition, resistance to insecticides have been proved to be higher in areas where there is more usage of agricultural and public health insecticides as opposed to areas where usage is lower (Kioulos *et al.*, 2014; Gorouhi *et al.*, 2016). Sex ratio analysis showed that adult mosquitoes were dominated by males (59.2%) which were significantly ( $p < 0.05$ ) than the females. This contrasted with the female skewed ratio recorded *Bio-Research Vol.22 No.2 pp.2396-2405 (2024)*

by Okwa *et al.* (2007) in Lagos, Nigeria, Madara and Abdulraheem (2013) in Abuja, Nigeria and Mohammed *et al.* (2021) in Kura LGA of North-western Nigeria. However, Silva *et al.* (2023) recorded a sex ratio that was dominated by male in Brazil, which was considered a positive outcome, because male dominated sex ratios are strongly considered as an attractive method with great prospects of suppressing or eliminating the population of pests (Galizi *et al.*, 2014). Since only female mosquitoes serve as vectors to majority of the mosquito-borne pathogenic diseases, altering their reproduction to bias the sex ratio of offspring towards males is continuously torch lighted as a potential and emerging strategy powerful enough to manage the population of mosquitoes and in return bring down the burden of mosquito-borne diseases to a very bearable minimum (Simoni *et al.*, 2020).

## CONCLUSION

This study evaluated the insecticide susceptibility profile and sex ratio of *Culex quinquefasciatus* within Umuahia North LGA of Abia State, and showed that the *Culex quinquefasciatus* was highly resistant to DDT (organochloride), Permethrin (pyrethroid) and bendiocarb (carbamate) but susceptible to malathion (organophosphate). However, malathion was developing resistance because the mortality rate was < 100%. The population of the mosquito was male-biased which favoured mosquito control programme. Considering the high resistance to the pyrethroids, carbamate and organochloride, organophosphates (like malathion) is the recommended insecticide of choice for mosquito control within the study area. The major shortcoming of this study was inability to investigate the molecular basis behind the resistance observed, however we hope to look into this as a follow up to this study when more funds are secured.

## Conflict of interests

The authors have no conflict of interest to declare.

## Acknowledgements

The authors appreciate the laboratory staff of Entomology unit of Zoology and Environmental Biology Department, Michael Okpara University of Agriculture Umudike, for their support and for

granting us space. We also appreciate the staff of the Vector control unit of Universiti Sain Malaysia, for delivering the purchased kits in time. The authors also appreciate Mr. Utibe Usanga of Department of Geography, University of Uyo, Uyo, Nigeria for producing the study map.

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