**EVALUATION OF CULEX MOSQUITO SUSCEPTIBILITY TO ORGANOPHOSPHATE, CARBAMATE AND PYRETHROID INSECTICIDES IN MINNA, NIGERIA**

**Adefolalu, F.S1,3., Gigam, A.A., 1,3 Oluwadepo, T.J1., Olayemi, I. K2, 3 and Ukubuike, A. C2**

1 Department of Biochemistry Federal University of Technology, Minna, Nigeria.

2. Department of Animal Biology, Federal University of Technology, Minna, Nigeria.

3 African Centre of Excellence for Mycotoxin and Food safety (ACEMFS ) Federal University of Technology, Minna, Nigeria.

**Abstract**

Culex mosquito, an arboviral and filarial vector, is a common breed in Nigeria and other West Africa countries. The different classes of insecticides for this vector control are constantly prone to resistance problem. The present study assessed the susceptibility of culex mosquito to six World Health Organisation (WHO) recommended insecticides of three different classes. *Culex* mosquito larvae were collected from six different breeding sites within Minna, Niger state, Nigeria and were reared to adults in the insectary. The percentage knockdown and mortality for the adult mosquitoes were evaluated for bendiocarb and propoxur (carbamates); malathion and pirimiphos (organophosphates); permethrin and deltamethrin (pyrethroids) insecticides using WHO standard protocol. Total proteins of adult mosquitoes (dead and alive) were determined by Bradford method and Glutathione-s-transferase (GST) enzyme activity was evaluated using Randox assay kit. The highest breeding site for culex mosquito was the rice fields (36.0 %), while the lowest was in tyres (7.5 %). Gutters, dam, streams and rainpools had 21.6, 12.6, 11.6 and 10.7 (%) respectively. The percentage knockdown (30 minutes exposure time), with deltamethrin, permethrin, bendiocarb, propoxur, malathion and pirimiphos were 76.7, 91.9, 100.0, 72.6, 81.8 and 87.0 (%) respectively. The highest mortality (1hour after exposure) was achieved with bendiocarb (100 %), permethrin (95.1 %), pirimiphos (87.8 %), malathion (86.2 %), deltamethrin (79.0 %) and propoxur (73.1 %). The total proteins of dead mosquitoes were higher than in those alive after exposure to test insecticides. The highest GST activity of mosquitoes alive after exposure was with deltamethrin (728.0 µmol/min/mL), followed by permethrin (713.3 μmole/min/mL, malathion (546.3 μmole/min/mL), pirimiphos (447.4 μmole/min/mL), propoxur (272.2 μmole/min/mL) while dead mosquitoes after exposure to permethrin had the lowest activity (66.3 μmole/min/mL). In conclusion, *Culex* mosquitoes from the areas tested are resistant to the recommended insecticides except to bendiocarb also GST appears to be the detoxifying enzyme responsible for the resistance.

**Key words**: **Mosquito, Insecticides, Knockdown, Resistance, Enzyme**

**Introduction**

Mosquitoes are small flying insects comprising the *Culicidae* family with about 41 genera. Many of which are vectors of disease pathogens that have afflicted humans, domestic and wildlife animals. Mosquitoes species that are of medical or veterinary importance belongs majorly to 3 genera, C*ulex (Cx)*,  A*nopheles* and *Aedes* (Olayemi *et al.*, 2012)*.*

The *Culex (Cx*.) specie is the most widespread mosquito species across the world, found to thrive almost in every conceivable environment where water occurs, except for the extreme northern parts of the temperate zone (Bhattacharya and Bhattacharya 2016., Woodbridge *et al*, 2019).*Culex* highly opportunistic, feeding on humans and livestock, lives outdoors or near homes and prefers to bite birds but bite human when other animals are not nearby (Mohammed *et al*., 2021). *Culex* lay eggs in ppolluted water also are most activeat night, biting between dusk and dawn. They are zoonotic transmitting diseases to birds, humans, and other animals, though not known to be of major or significant epidemiological importance except for it biting nuisance, *Culex* cause allergic reactions, or significant blood loss when they occur in large numbers, due to their bites alone (Woodbridge *et al*., 2019). They are known to vector Rift Valley Fever and arbovirus such as West Nile virus fever, Japanese encephalitis, or St. Louis encephalitis, but also will transmit filariasis and avian malaria (Namias *et al.*, 2021).

Intensive vector control efforts is a very important part of the global strategy for management of mosquito and its associated diseases (Kaura *et al*., 2022). Strategies to eliminate or reduce mosquito populations and their disrupting pathogen transmission are diverse and continue to be active in the fields of research, which includes: habitat modification, insecticides treated bed nets, spray insecticides, drug treatment, predators and pathogens, genetic manipulation and sterile release (WHO, 2020). Incidence of mosquitoes borne diseases such as malaria, has reduced by 50 % since 2000, with 80 % of the reduction attributed to the use of insecticides in the form of: indoor residual spraying (IRS), insecticide-treated nets (ITNs) and long-lasting insecticide-treated bed nets (LLITS) which however, are currently under the threat of resistance (Kariuki and Kamau, 2022). Four classes of insecticides mostly used in mosquito control programs are: organophosphates, carbamates, pyrethroids and Organochlorines (WHO, 2016a). Yadouleton, A., Ahadji-Dabla, K. M., Chabi, C., Agolinou, A., Ahissou, F., Agbanrin, R. & Baba-Moussa, L. (2018).

Detoxifying enzymes such as Gluthathione- s- transferases (GSTs) in mosquito are one of the targets of the different classes of insecticides. GSTs can protect the mosquito either by increasing the rate of detoxification of insecticide into nontoxic products or protect against oxidative stress by direct peroxidase activity (Vontas *et al.,* 2001). Resistance is a form of self-defence and therefore, the overt expression of a mosquito's natural response to insecticides. Resistance is frequently attributed to increased levels of GST activity, thus when there is resistance GSTs are over-expressed (Ranson, & Hemingway, (2005). Metabolic resistance is due to loss of sensitivity of mosquitoes enzyme system (especially GSTs) to insecticides, thereby reducing the efficacy of the insecticides. This study aimed at evaluating the susceptibility of mosquito to three classes of insecticides (carbamates, organophosphates and pyrethroids) as an important component of any strong vector control or surveillance program in the intervention efforts to control mosquito population and disease transmission and outbreaks over time (Philbert *et al*., 2014) .

**Materials and Methods**

**Materials**

Wheaton glass bottles, microplate machine (German E-2500), mosquito-holding tubes, siphon tube, Bovine serum albumin (BSA), Coomassie Brilliant Blue G-250, WHO recommended insecticides, reduced glutathione (GSH) assay kit (Sigma -Aldrich) and Spectrophotometer.

**Mosquito Sample collection and rearing**

Mosquito larvae were collected from gutters which is one of the major breeding sites breeding sites at Bosso in Minna, Nigeria and identified morphologically (CDC, 2016 Olayemi *et al*., 2014).

Larvae were reared to adults under testing conditions (25 ± 2° C temperatures and 65 ± 5 % relative humidity).

**Mosquito Total Protein and Enzyme Activity Determination**

Total proteins and enzymes were derived from dead adult mosquitoes homogenized and centrifuged at 3000 rpm for 20 minutes.

**Total protein assay**

Total protein was determined by the method of Bradford (I976). Bovine serum albumin (BSA) served as standard protein. Coomassie Brilliant Blue G-250 was used as colorant. Absorbance values were taken at 595 nm in a Spectrophotometer.

**Glutathione s-transferase (GST) Enzyme Assay**

GST activity was assayed spectrophotometrically using Assay kit at 20 °C, using a UV Max Microplate Reader, to measure the rate of conjugation of GSH to CDNB (Vontas *et al.,* 2001). The reaction mixture (1.2 ml) containing 1 mM CDNB and 5 mM GSH in 0.1 M sodium phosphate buffer, pH 6.5, was placed in glass vials. Insect homogenate (60 μl) was added to the reaction mixture. Aliquots (210 μl) from each of the mixture were placed in microtiter plate, and the reaction rates were measured at 340 nm for 5 min. Blanks contain same reaction mixture with 60 μl of distilled water in place of homogenate. The GST activity was calculated as μmol CDNB conjugated/min/mL using the extinction coefficient (e = 9.6 mM−1cm−1) (Habig *et al*., 1974).

**Data analysis**

Results are presented as mean and standard error of mean; and one way ANOVA using SPSS version 23 software. Abbott’s formula was used to correct for the mortality observed in the bioassay where the mortality in the control was between 5-20 % (WHO, 2016).

**Results**

****

**Table 1: Percentage Mortality of *Culex* Mosquitoes Exposed to Selected Insecticides**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Class** | **Insecticides** | **% Mortality****Control**  | **Exposed**  | **Survived****Control**  | **Exposed**  |
| Pyrethroid  | Deltamethrin Permethrin  | 15.40 8.9012.15  | 79.00 95.1087.05  | 84.60 91.1087.85  | 21.00  4.9012.95  |
| Carbamate  | Bendiocarb Propoxur  | 8.90 8.208.55  | 100.0 73.1086.55  | 91.10 91.8091.45  |  0.00 26.9013.45  |
| Organophosphate  | Malathion Pirimiphos  | 9.60 12.4011.00  | 86.20 87.8087.00  | 90.40 89.8090.10  | 9.60 12.2010.90  |



**Table 2: Total Protein Concentration of *Culex* Mosquitoes Exposed to Selected Insecticides**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  **Class**  | **Insecticide**  | **Total****Control****Unexposed** **(Alive)**  | **Protein****Control****Unexposed** **(Dead)**  | **(mg/ml)****Exposed** **(Alive)**  | **Exposed** **(Dead)**  |
| Pyrethroid  | Deltamethrin Permethrin  | 0.185 ± 0.01b 0.196 ± 0.25b  | 0.152 ± 0.01b 0.191 ± 0.01b  | 0.100± 0.01a 0.088 ± 0.01a  | 0.253 ± 0.01c 0.110 ± 0.01a  |
| Carbamate  | Bendiocarb Propoxur  | 0.198 ± 0.14b 0.167 ± 0.01b  | 0.162 ± 0.07a 0.137 ± 0.01a  |  \*AD 0.127 ± 0.01a  | 0.477 ± 0.01c 0.250 ± 0.01c  |
| OrganoPhosphate  | Malathion Pirimiphos  | 0.236 ± 0.02b 0.139 ± 0.01b  | 0.277 ± 0.01b 0.261 ± 0.04c  | 0.109 ± 0.01a 0.113 ± 0.01ab  | 0.324 ± 0.01c 0.099 ± 0.01a  |

**Table 3: Mean Glutathion –S- Transferase Activity of *Culex* Mosquitoes exposed to selected insecticides**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  **Class**  |  **Insecticide**  | **GST****Control** **Unexposed (Alive)**  | **Activity (U/ml)** **Control Unexposed** **(Dead)**  | **GST Activity****Exposed** **(Alive)**  | **(U/ml)** **Exposed** **(Dead)**  |
| Pyrethroids  | Deltamethrin Permethrin  | 173.7 ± 6.7b 128.9 ± 9.7ab  | 178.3 ± 16.3b 153.0 ± 12.7b  | 728.0 ± 13.3c 713.3 ± 23.3c  | 91.0 ± 2.4a 66.3 ± 1.7a  |
| Carbamate  | Bendiocarb Propoxur  | 213.9 ± 8.6b 218.5 ± 12.7b  | 242.7 ± 20.0b 184.9 ± 1.5b  |  \*AD 272.2 ± 5.3d  | 79.6 ± 3.8a 128.07 ± 1.9a  |
| Organophosphate  | Malathion Pirimiphos  | 279.6 ± 7.6c 190.5 ± 15.9b  | 202.5 ± 8.5b 161.3 ± 5.6ab  | 546.3 ± 17.0d 447.4 ± 40.1c  | 91.5 ± 11.3a 74.9 ± 9.0a  |

It was observed with the six insecticides used for the study that there was a significant difference in the GST activity of mosquitoes that were susceptible to insecticides and those that are resistant. All six different insecticide susceptible mosquitoes showed very low levels of GSTs (p<0.05). No significant difference was observed in GST activities of resistant mosquitoes when insecticides of the same class were used; Pyrethroid Permethrin (713.27 ± 23.3 μmole/min/mg protein) and deltamethrin (727.97 ± 13.3 μmol/min/mg protein), Organophosphate Malathion (546.30 ±17.0 μmole/min/mg protein) and Pirimiphos methyl (447.37 ± 40.1 μmol/min/mg protein). In the case of Organocarbamates, there was no means of comparison between bendiocarb and propoxur resistant since no protein activity was recorded as there was no resistant mosquito population to bendiocarb because it caused 100 % mortality within a short time.

**Discussion**

The results of percentage knockdown in this study support the claim of Prapanthadara *et al.,* (2000) on the knockdown effects of insecticides against mosquitoes in Nigeria. There is a recorded level of resistance of *Culex* mosquitoes to five of the insectides used for this study which indicates the presence of knock down resistance (KDR) mechanism among *Culex* mosquitoes in Minna. This trend is similar to the report of knockdown resistance operating in populations of mosquitoes where the most of the WHO recommended insecticides have been extensively used except bendiocarb (Ibrahim *et al.,* 2016). The knock down mechanism could be responsible for the level of resistance displayed by these mosquitoes to the various insecticides evaluated in this study. The results of this study does not agree with that of Ukpai and Ekedo (2019) which indicated that *Culex quinquefasciatus* was not resistant to propoxur, bendiocarb, primiphosmethyl and permethrin. Meanwhile, it agrees with the report of Oduola *et al,* (2012) that *Culex* mosquitoes were highly resistant to deltamethrin, malathion and propoxur in lagos. In this study, *Culex* could not mount resistance against bendiocarb and this is in disagreement with a documented evidence of mosquitoes resistance to pirimiphosmethyl, bendiocarb and propoxur by Nazni *et al.* (2005). This is also similar to the report of Ibrahim in 2016, that the mosquito population in malaria, though resistant to deltamethrin has no resistance to malathion and contrary to Ukpai and Ekedo’s report in (2019) that all the mosquito populations were susceptible to bendiocarb. In addition, a total mortality percentage with bendiocarb and low mortality with propoxur that was observed in this study suggests that there was still a possibility of resistance against carbamates in the future. Gong *et al.,* (2013) reported that Mosquitoes were less resistant to permethrin than deltamethrin in this study. A reverse of this, occurred in Burkina Faso where samples of *Culex* quinquefasciatus collected were highly resistant to permethrin but to a lower extent, were resistant to deltamethrin. High levels of pyrethroid resistance were observed in *Cx quinquefasciatus* collected from Coˆte d’Ivoire and Burkina Faso, suggesting that they had been submitted to strong insecticide selection pressure under field conditions. Usually, when a population is selected with an insecticide, the resistance extends to other compounds of the same class of insecticides. In accordance with the report of Christiansen-Jucht *et al.,* (2014), the difference in the high susceptibility level of mosquito population to bendiocarb, Permethrin and the high resistance of mosquito to propoxur, deltamethrin, could have been governed by factors like testing temperature and frequent use of certain insectides. The high resistance of mosquito population to Pyrethroid- deltamethrin that was observed may have originated partly from dichlorodiphenyltrichloroethane (DDT) selection pressure during past decades when deltamethrin was the next option considered in chemical control. As was dichlorodiphenyltrichloroethane (DDT) resistance, pyrethroid resistance has been widely distributed throughout West Africa and this should be taken into consideration when planning the use of pyrethroid-impregnated materials for malaria vector control in Niger state and Nigeria as a whole will be disregarded by the local people. Nuisance mosquito control by impregnated bednets may not have the intended efficacy because of pyrethroid resistance that is gradually taking place. Therefore, it may be wise to supplement the implementation of bednet programmes by putting control measures based on larviciding and sanitation in urban areas. The biochemical studies agreed with the report of Zhu *et al.,* (2016) who found out that decreased temperature also increased the susceptibility of neurons of crayfish to pyrethroid insecticides making them more sensitive to excitation. *Culex* mosquitoes were exposed to bendiocarb at room temperature (25 oC) while propoxur was used at a temperature of 20 0C. The susceptibility of *Culex* mosquitoes increased as the temperature decreased. The observations agree with Pocquet *et al.,* (2013)*,* that temperature increase is often associated with decrease in toxicity of different insecticides against different insects. Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms. Also, larval breeding conditions considerably influence the susceptibility of *Culex* mosquitoes to different insecticides. Just as observed in this study, Pyrethroids and Organophosphates are known to exhibit a positive correlation between ambient temperature and mortality for many insect species, while carbamates are known to exhibit a negative correlation between ambient temperature and mortality for many insect species (Devries *et al.,* 1981). Environmental temperature has been shown to influence the outcome of insecticide exposure; temperature differences expected to occur naturally under field conditions can lead to notable variations in chemical efficacy (Akinwande *et al.,* 2021).

Mosquitoes exposed to bendiocarb had a low GST activity but a high protein level which suggests the inability of *Culex* to synthesize GST against bendiocarb. So it might be that the insecticide bendiocarb does not trigger the synthesis of GST. This is contrary to the report of McCaffery and Nauen, (2006), that GST is the point of attack for all organocarbamates class which bendiocarb belongs. Low protein versus low specific enzyme activity of an insect after exposure to insecticide could also be based on the fact that the enzyme has been inhibited by the insecticides. Enzymes are converted and utilized through metabolic process and thus can be found at low amount after the endothermic reaction. There is a higher protein level in *Culex* mosquitoes that were dead after 1 hour than that of unexposed (control group). The protein level was relatively lower in mosquitoes found alive after exposure when compared to that of the control group. The protein level of *Culex* mosquitoes found dead after exposure to bendiocarb was the highest not determined because bendiocarb gave a 100% mortality of culex before the diagnostic time was reached, thus there was no survivor. The mosquitoes found alive after exposure within diagnostic time have a lower protein compared to the mosquitoes found alive after exposure. The lower the protein level, the higher the enzyme activities and vice versa. According to the study of Mamai *et al.,* (2017), this principle of inverse relationship between protein concentration and enzyme activity is based on the fact that enzymes are protein and their level of secretion is based on the amount of protein present in a living system.

The results of the enzyme activity of tested mosquitoes might suggest that GST was involved in the resistance of *Culex* mosquitoes to deltamethrin, permethrin, propoxur and malathion and pirimiphos. Low GST levels obtained with mosquitoes that were exposed to propoxur and the least mortality that was observed indicate high resistance of culex to propoxur. Then there must have been a resistance mechanism other than GST which had been responsible for the low mortality observed with propoxur. Meanwhile, a very high GST levels were observed with delthamethrin-resistant individuals. The tested mosquito population were resistant to delthamethrin and permethrin. This is similar to the report of Hemingway and Ranson, (2000) that elevated GST activity in *Culex* is indirectly responsible for resistance to pyrethroids. The GST activity of alive mosquitoes after exposure to organophosphates differ in that GST level was higher with malathion than with pirimiphos despite the fact that the belong to same classes of insecticide and both possibly possess the same mechanism of action. One factor which could be responsible for this observation is age difference. The age differences among the tested mosquito groups influence the results of CDC bioassay and consequently the GST activity. This hypothesis was confirmed by the report of Kasai *et al.,* (2014) that the susceptibility of *Culex* to insecticides appears to increase with age as a result of decrease in the potency of mosquito detoxifying enzymatic system.

**Conclusion**

*Culex* mosquito specie collected in Minna were susceptible only to bendiocarb insecticide but displayed resistance to pyrethroids and organophosphates.

The highest total protein and glutathione enzyme activity was with the pyrethroid insecticides and also showed the highest resistance.

Bendiocarb did not show any developed resistance however, propoxur (a carbamate) insecticides had the lowest GST resistance activity.

It is therefore, necessary to carry out random insecticides susceptibility tests to monitor incidence of mosquito resistance to available insecticides if mosquito-borne diseases are to be prevented.

In addition further research to circumvent this insecticide resistance huddle continues to be of great and urgent necessity.

**Acknowledgements**

We thank the Mosquito Research team at the Federal University of Technology, Minna for providing the necessary facilities for this work.

We also wish to acknowledge staff and students of the Department of Biochemistry and animal Biology of Federal University of Technology Minna that participated in this study.

**References**

Akinwande, K. L., Arotiowa, A. R. & Ete, A. J. (2021). Impacts of changes in temperature and exposure time on the median lethal concentrations (LC50) of a combination of organophosphate and pyrethroid in the control of Culex quinquefasciatus, say (Diptera: Culicidae). *Scientific African*, *12*, e00743.

 Bhattacharya S, Basu P, Sajal Bhattacharya C.(2016). The southern house mosquito, Culex quinquefasciatus: profile of a smart vector. *Journal of Entomology Zoology Study*. 4(2):73–81

Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72, 248-254.

Center for Disease Control and Prevention (2016). *Vectors of lymphatic filariasis.* Retrieved from http://www.cdc.gov/parasites/lymphaticfilariasis/gen\_info/vectors.html.

Christiansen-Jucht, C., Parham, P. E., Saddler, A., Koella, J. C. & Basanez, M. G. (2014). Temperature during larval development and adult maintenance influences the survival of Anopheles gambiae ss. *Journal of parasites and vectors,* 7(1), 1-10.

DeVries, D. H. & Georghiou, G. P. (1981). Decreased nerve sensitivity and decreased cuticular penetration as mechanisms of resistance to pyrethroids in a (1R)-trans-permethrin-selected strain of the house fly. *Pesticide Biochemistry and Physiology*, *15*(3), 234-241.

Gong, Y., Li, T., Zhang, L., Gao, X. & Liu, N. (2013). Permethrin induction of multiple cytochrome P450 genes in insecticide resistant mosquitoes, Culex quinquefasciatus. *International Journal of biological science,* 9(9), 863.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249:7130–7139.

Haba Y. and McBride L. (2022). Origin and status of Culex pipiens mosquito ecotypes, *Review* [32, (5](https://www.cell.com/current-biology/issue?pii=S0960-9822(21)X0006-1)) 237 - 246|6, MARCH 14, 2022

Hemingway, J. & Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual review of entomology*, *45*(1), 371-391.

Ibrahim, S. S., Ndula, M., Riveron, J. M., Irving, H. & Wondji, C. S. (2016). The P450 CYP 6Z1 confers carbamate/pyrethroid cross-resistance in a major African malaria vector beside a novel carbamate- insensitive N485l acetylcholinesterase-1 mutation. *Molecular ecology*, 25(14), 3436-3452.

Jacob, W. & Joao, P. (2012). Training manual on malaria entomology for entomology and vector control technicians (basic level), Integrated vector management of malaria and other infectious diseases task order. *Publication of theUnited States Agency for InternationalDevelopment*, 2 (1), 33.

Kabula, B., Derua, Y. A., Tungu, P. K., Massue, D. J., Sambu, E., Mosha, F. W. & Kisinza, W. N. (2011). Malaria entomological profile in Tanzania from 1950-2010: a review of mosquito distribution vectorial capacity and insecticide resistance. *Tanzania journal of health research*, 13(5).

Kalaivani, A., Raja, D., Geetha, M. & Jegadeesh, R. (2015). Mosquito menace: A major threat in modern era. *Medical Journal of Dr. DY Patil University*, 8(3), 414-414.

Karunaratne, S. H. P. P., De Silva, W. A. P. P., Weeraratne, T. C. & Surendran, S. N. (2018). Insecticide resistance in mosquitoes: development, mechanisms and monitoring. *Ceylon J Sci*, *47*(4), 299-309.

Kasai, S., Komagata, O., Itokawa, K., Shono, T., Ng, L., Kobayashi, M. & Tomita, T. (2014). Mechanisms of Pyrethroid Resistance in the Dengue Mosquito Vector, Aedes aegypti: Target Site Insensitivity, Penetration, and Metabolism. *PLoS neglected tropical* diseases 8(6), 2948.

Keiser, J., Singer, B. H. & Utzinger, J. (2005). Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *The Lancet infectious diseases*, *5*(11), 695-708.

Kemabonta, K. A., Anikwe, J. C. & Adaezebiora, I. B. (2013). Bioefficacy of skaeter abate and spintor on anopheles gambiae and aedes aegypti mosquitoes from insecticides resistance areas of lagos and oyo states, Nigeria. *Journal of Agricultural Healthcare,* 3(3).

Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. & Fukatsu, T. (2012). Symbiont-mediated insecticide resistance procedures. *Proceedings of the National Academy of Sciences,* 109(22), 8618-8622.

Komagata, O., Kasai, S., Obara, I., Motoyama, N., Tanaka, I., Kobayashi, M. & Tomita, T. (2008). Concomitant identification of subspecies and insecticide resistance-associated mutations in the mosquito Culex pipiens complex by primer extension-based genotyping. *Medical Entomology and Zoology*, *59*(2), 33-46.

Kostaropoulos, I., Papadopoulos, A. I., Metaxakis, A., Boukouvala, E. & Papadopoulou-Mourkidou, E. (2001). Glutathione S–transferase in the defence against pyrethroids in insects. *Insect biochemistry and molecular biology*, *31*(4-5), 313-319.

Lazear, H.M., Stringer, E.M., & de Silva, A. M. (2016). The emerging Zika virus epidemic in the Americas. *Journal of America Medical Association* 315(18): 1945-1946.

Li, T., Zhang, L., Reid, W. R., Xu, Q., Dong, K. & Liu, N. (2012). Multiple mutations and mutation combinations in the sodium channel of permethrin resistant mosquitoes, Culex quinquefasciatus. *Scientific Reports,* 2 (1), 1-9.

Lin, Y., Jin, T., Zeng, L. & Lu, Y. (2012). Cuticular penetration of b-cypermethrin in insecticide-susceptible and resistant strains of Bactrocera dorsalis. *Pesticide Biochemistry and Physiology*, 103, 189-193.

Liu, N. (2015). Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annual Revision of Entomology*, 60, 537–559.

Low, V. L., Chen, C. D., Lee, H. L., Lim, P. E. & Leong, C. S. (2012). Nationwide distribution of Culex mosquitoes and associated habitat characteristics at residential areas in Malaysia. *Journal of American Mosquito Control Association*, 28 (2), 160-169.

Mamai, W., Bimbilé-Somda, N. S., Maiga, H., Juarez, J. G., Zinab, A., Ali, A., Lees, R.S. & Gilles, J. R. (2017). Optimization of mosquito egg production under mass rearing setting: Effects of cage volume, blood meal source and adult population density for the malaria vector, Anopheles arabiensis. *Malaria Journal,* 16(4), 41.

Marimo, P., Hayeshi, R. & Mukanganyama, S. (2016). Inactivation of anopheles gambiae glutathione transferase ε2 by epiphyllocoumarin. *Biochemistry research international*, *2016*.

Matowo, J., Jones, C. M., Kabula, B., Ranson, H., Steen, K., Mosha, F. & Weetman, D. (2014). Genetic basis of pyrethroid resistance in a population of Anopheles arabiensis, the primary malaria vector in Lower Moshi, north-eastern Tanzania. *Parasites & vectors*, *7*(1), 1-9.

McCaffery, A. & Nauen, R. (2006). The Insecticide Resistance Action Committee (IRAC): Public responsibility and enlightened industrial self interest. *Outlooks on Pest Management,* 2(2), 11-14.

McGraw, E. A. & O’Neill, S. L. (2013). Beyond insecticides: new thinking on an ancient problem. *Nature Reviews of Microbiology* 11, 181-193.

Mgbemena, I. C. & Ebe, T. (2012). Distribution and occurrence of mosquito species in the municipal areas of Imo State, Nigeria. *Analele Universită Ńii din Oradea - Fascicula Biologie Tom*. 14 (2), 93-100.

Mgbemena, I. C., Adjeroh, L. A., Opara, F. N., Ezeagwuna, D. & Ebe, T. (2012). Seasonal variation and relative abundance of drainage breeding mosquito species in Imo State, Nigeria. *International Journal of Biosciences*, 2(8), 23-35.

Mnzava, A. P., Knox, T. B., Temu, E. A., Trett, A., Fornadel, C. & Hemingway, J. (2015). Implementation of the global plan for insecticide resistance management in malaria vectors: progress, challenges and the way forward. *Malaria Journal*, 14, 173.

Monsuru, A.A., Wasiu, O.A., AbdulWasiu, O.H., Sunday, O.O., Ismail, O., Ganiyu, O. & Taiwo A. (2013). Larval habitats of mosquito fauna in Osogbo metropolis, Southwestern Nigeria. *Asian Pacific Journal of Tropical Biomedicine*, 3(9), 673- 677.

Moyes, C. L., Athinya, D. K., Seethaler, T., Battle, K. E., Sinka, M., Hadi, M. P. & Hancock, P. A. (2020). Evaluating insecticide resistance across African districts to aid malaria control decisions. *Proceedings of the National Academy of Sciences*, *117*(36), 22042-22050.

Muhammed, B.R., Yayo A.M., Ayamusi, O. J., and Lawa I.A (2021). Relative abundance and molecular identification of *Culex pipiens* complex (Diptera: Culicidae), in Kura Local:

Government Area, North-western Nigeria. *Parasite Epidiology and Control* 24:

e00213, ISSN 2405-6731, https://doi.org/10.1016/j.parepi.2021.e00213.

Namias, A., Jobe, N. B., Paaijmans, K. P. & Huijben, S. (2021). The need for practical insecticide-resistance guidelines to effectively inform mosquito-borne disease control programs. *Elife*, *10*, e65655.

Oduola, A. O., Idowu, E. T., Oyebola, M. K., Adeogun, A. O., Olojede, J. B., Otubanjo, O. A. & Awolola, T. S. (2012). Evidence of carbamate resistance in urban populations of Anopheles gambiae ss mosquitoes resistant to DDT and deltamethrin insecticides in Lagos, South-Western Nigeria. *Parasites & vectors*, *5*(1), 1-8.

Olayemi, I. K, Omalu, I. C., Abolarinwa, S. O., Mustapha O. M., Ayanwale, A. V., Mohammed, A. Z., Mohammed, Bello, I. M. & Chukwuemeka, V. I. (2012). Knowledge of malaria and implications for control in an endemic urban area of North Central Nigeria*. Asian Journal of Epidemiology,* 5(2), 42-49.

Olayemi, I. K, Ukubuiwe, A. C. & Oyibo-Usman K. A. (2014). Mosquito Species Occurrence and Diversity in Conventional larval breeding sites in Minna metropolis, Nigeria. *Journal of Entomology*, 9(1), 2351-8014

Olayemi, I. K., Idris, B., Omalu, I. C. J. & Odeyemi, M. O. (2012). Dry season refugia breeding ecology of mosquitoes (Diptera: Culicidae) in Minna, North Central Nigeria. *Journal of Biological Sciences,* 10 (38), 1200.

Philbert, A., Lyantagaya, S. L. & Nkwengulila, G. (2014). Review of Agricultural Pesticides use in the selection for resistance to insecticides in malaria vectors. *Publication of Advanced Entomology*, 2(2), 120-128

Pocquet, N., Milesi, P., Makoundou, P., Unal, S., Zumbo, B., Atyame, C. & Labbé, P. (2013). Multiple insecticide resistances in the disease vector Culex p. quinquefasciatus from Western Indian Ocean. *PloS one*, *8*(10), e77855.

Prapanthadara, L., Koottathep, S., Promtet, N., Suwonkerd, W., Ketterman, A. J. & Somboon, P. (2000). Correlation of glutathione S-transferase and DDT dehydrochlorinase activities with DDT susceptibility in Anopheles and Culex mosquitos from northern Thailand. *The Southeast Asian journal of tropical medicine and public health*, *31*, 111-118.

Rabi’u, H. M., & Ahmed, A. (2019). A preliminary study on the abundance and species composition of mosquitoes breeding in discarded automobile tyres in Minna, Niger State, Nigeria. *Int. J. Mosq. Res*, *6*(1), 119-123.

Ranson, H., & Hemingway, J. (2005). Mosquito glutathione transferases. *Methods in enzymology*, *401*, 226-241.

Tang, J. U. N., Rose, R. L. & Chambers, J. E. (2006). Metabolism of organophosphorus and carbamate pesticides. In *Toxicology of organophosphate & carbamate compounds* (pp. 127-143). Academic Press.

Ukpai, O. M., & Ekedo, C. M. (2019). Insecticide susceptibility status of Culex quinquefasciatus (Diptera: Culicidae) in Umudike, Ikwano LGA Abia state, Nigeria. *International Journal of Mosquito Research*, 6, 114-118

World Health Organization. (2016a*). Fact sheet; Dengue and severe dengue.* Retrieved from http://www.who.int/mediacentre/factsheets/fs117/en/.

World Health Organization. (2016b). *Fact sheet*; *Lymphatic filariasis*. Retrieved from http://www.who.int/mediacentre/factsheets/fs102/en/.

World Health Organization. (2016). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.

Yadouleton, A., Ahadji-Dabla, K. M., Chabi, C., Agolinou, A., Ahissou, F., Agbanrin, R. & Baba-Moussa, L. (2018). Establishment of baseline susceptibility data to various insecticides for Anopheles gambiae. *Journal de la Recherche Scientifique de l’Université de Lomé*, *20*(4), 1-9.

Zhu, F., Lavine, L., O’Neal, S., Lavine, M., Foss, C. & Walsh, D. (2016). Insecticide resistance and management strategies in urban ecosystems. *Insects Journal*, 7(1), 2-28.