EVALUATION OF INSECTICIDES RESISTANCE IN CULEX MOSQUITOES TO DDT, DELTAMETHRIN, PERMETHRIN AND PBO(SYNERGY) IN GOMBE METROPOLIS

ABSTRACT

Aims: To evaluate the resistance of Culex mosquitoes in Zagaina, Gombe State, to commonly used insecticides, providing insights for managing insecticide resistance in disease vector control.

Study Design: Cross-sectional susceptibility bioassay study.

Place and Duration of Study: This study was conducted Gombe State University Malaria Vector Sentinal Laboratory over three months (October-December).

Methodology: Approximately 600 Culex mosquito larvae and pupae were collected from blocked and improperly maintained drainages in Zagaina community using standard dipping techniques. The collected mosquitoes were reared to adulthood in an insectary at Gombe State University. Female Culex mosquitoes were separated and subjected to a time-course exposure bioassay using CDC bioassay tubes, following WHO guidelines for evaluating insecticide resistance in vectors.[8] WHO-impregnated papers were used to test susceptibility to four insecticides: DDT, Permethrin, Deltamethrin, and Piperonyl Butoxide (PBO). The knockdown rate was assessed followed by Mortality rate of 24 hours post-exposure, with no correction needed as control mortality was zero.

Results: High resistance was observed across all tested insecticides, with mortality rates of 15% for DDT, 7% for Permethrin, 65% for Deltamethrin, and 69% for PBO. These results indicate that Culex mosquitoes in Zagaina community exhibit significant resistance to DDT and Permethrin, moderate resistance to Deltamethrin, and lower resistance to PBO.

Conclusion: The insecticide resistance observed in Culex mosquitoes indicate an urgent need to investigate the genetic or metabolic mechanisms of resistance. Monitoring resistance patterns over time is essential for developing effective vector control strategies to combat mosquito-borne diseases.

Keyworks: Insecticides Resistance; Culex mosquitoes; DDT; Deltamethrin; Permethrin; PBO (synergy)

1.0 INTRODUCTION

Mosquitoes represent a critical danger for millions of people around the world. They are significant vectors of human diseases such as malaria, filariasis, yellow fever, dengue fever and encephalitis, besides allergic responses include local skin and systemic reactions such as angioedema, and urticaria[1,2,3]Mosquito-borne diseases, such as filariasis, and arboviruses, are major public health concerns worldwide, contributing significantly to morbidity and mortality, especially in tropical regions. Among the various mosquito species, Culex mosquitoes are key vectors of diseases such as lymphatic filariasis and West Nile Virus, posing a considerable threat to both human and animal populations[4]. *Culex pipiens* (Diptera: Culicidae) is one of the most common mosquito insects in rural and urban zones[5]. *Culex* mosquitoes are vectors of several pathogens causing infectious diseases of public health importance. These includes lymphatic filarial nematodes and arboviral infections. Blocked and improperly maintained drainages have been reported to serves as habitats for the Culex species and other disease vectors[6]. Globally, *Culex* mosquitoes have been implicated in the transmission of diseases, which has led to significant outbreaks in various parts of the world[7]

A large number of studies have shown that multiple, complex resistance mechanisms in particular, increased metabolic detoxification of insecticides and decreased sensitivity of the target proteins or genes are likely responsible for insecticide resistance. Gene overexpression and amplification, and mutations in protein coding-gene regions, have frequently been implicated as well. Six classes of insecticides namely, organochlorines, organophosphates (OP), carbamates, pyrethroids, pyrroles, and phenyl pyrazoles are recommended for use against adult mosquitoes[8]. Indoor residual spraying and long-lasting insecticidal nets treated with pyrethroids are the two most important measures for human protection from malaria-carrying mosquitoes[8]. In the past, massive sprayings of insecticides greatly limited mosquito-borne diseases and even eradicated lymphatic filariasis and malaria in a few areas. However, the widespread development of resistance in mosquitoes to the most commonly used insecticides is now causing serious problems in many areas[9]. This has resulted in a number of outbreaks of mosquito-related diseases in recent years[10]. The use of insecticides is the corner stone of mosquito-borne diseases control, either as indoor residual spraying (IRS) or the long-lasting insecticidal nets (LLINs). However, rampant insecticides resistance reported across the world[4], e.g. in Northern Nigeria[11] is threatening the success of VBDs control. The use of agricultural pesticides has led to the increasing insecticide resistance. The knowledge of the distribution of VBDs and their insecticides resistance profile is important before using any of the above control tools. Vector control is a very important part of the global strategy for management of mosquito associated diseases, and insecticide application is the most important component in this effort. However, mosquito-borne diseases are now resurgent, largely because of the insecticide resistance that has developed in mosquito vectors and the drug resistance of pathogens Numerous studies have shown that insecticide resistance in mosquitoes is often due to complex mechanisms, including increased metabolic detoxification and reduced sensitivity of target proteins. Gene overexpression, gene amplification, and mutations in coding regions of resistance-associated genes are common contributors[12]

Culex mosquito is known to be a major vector that transmit zoonotic diseases which affect humans and wild and domestic animals, such as lymphatic filariasis[13] commonly found in Gombe. Mosquito bite has been a major public health problem with malaria and lymphatic filariasis as major diseases in Nigeria and Gombe state in particular[14]. Available literature on the disease from both the North and Central parts of Nigeria and the report of a postal survey by the Nigerian Lymphatic Filariasis Elimination Programme (NLFEP) have shown that lymphatic filariasis is endemic[15]. Lack of information on the distribution and degree of risk of the disease in the country are the greatest challenges confronting the NLFEP. Despite extensive research on mosquito resistance, there is limited data on the resistance patterns of Culex mosquitoes in northern Nigeria, particularly in Gombe State. Understanding these patterns is crucial for developing localized mosquito control programs and for reducing the spread of mosquito-borne diseases. Previous studies have focused more on Anopheles mosquitoes, leaving a gap in the comprehensive understanding of resistance in Culex species. In Gombe State, the frequent use of insecticides for agricultural and domestic purposes may contribute to the development of resistance in Culex mosquitoes. The lack of updated, localized data on resistance levels hinders the development of effective vector control strategies in the region.[16].

The main objectives of this study, is to evaluate the insecticide susceptibility and resistance status of adult Culex mosquitoes to deltamethrin, permethrin and DDT. Additionally, it will assess the potentialsynergistic effects of piperonyl butoxide (PBO) on insecticide efficacy to inform public health interventions.Specifically, it will assess the resistance status of local mosquito populations using standard bioassay techniques.

2.0 MATERIALS AND METHODS

2.1Study Site

This cross-sectional research was conducted in Zagaina, Akko Local Government Area, Gombe State, Nigeria, within the period of November–December 2022. Akko is one of the 11 local governments of Gombe State. The state is bounded to the North by Yobe State, North-East by Borno State, South by Taraba State, South-East by Adamawa State, and in the West by Bauchi State. The state has an area of 20,265km2 and a population of around 1.8 million. The state is characterized by two distinct seasons, which are dry season (November to December) and wet season (April to October).[17]

2.2 Sampling Locations

Gombe State is located in the North Eastern part of Nigeria and lies in Sudan savannah vegetations with eleven (11) local governments. Samples were collected within 3 different breeding sites located at Zagaina, Akko local government of Gombe State with the following specification: Latitude: 10^0 61'60" N and Longitude: $10^0$57 59.99'E

2.3Sample Collection (Culex Larva)

Third to fourth instar larva and pupae of Culex mosquito larvae were collected from Zagaina community. Culex larvae were collected from natural breeding sites(small pools and puddles) using dipping method during the month of October to December, 2022. Coordinate of the study sites were established using global positioning system (GPS). The immature Culex mosquitoes collected were transported to Gombe State University Malaria Vector Sentinal Lab, they were reared to adult. The emerging adult Culex mosquitoes were placed in adult cage using mouth aspirator and fed with 10% sugar solution soaked in cotton wool.

2.4 Adult Feeding

10% sucrose was used to feed adult mosquitoes in the lab. The sugar solution was provided by soaking cotton with sucrose solution and then placed on top of a cage. Cotton was wet enough with sucrose and allowed the mosquitoes sucked it.

2.5 Insecticide Bioassays

Insecticide susceptibility bioassays were performed as per WHO standard guideline (WHO 2016). Three to five days old, non-blood-fed female Culex mosquitoes were exposed to insecticide impregnated papers with discriminating concentrations of deltamethrin (0.05%), permethrin (0.75%), DDT (4.0%) and POB synergy (4.0%) using WHO standard assays[8]

A total of 600 mosquitoes in 4 replicates were used for the insecticide bioassays. 25 unfed female Culex mosquitoes of 3-5 days old were introduced into four(4) CDC holding tubes coated with a white clean paper and four (4) control tubes coated with a clean white paper too. This were provided by Center for Disease Control (CDC) Atlanta Georgia as describe by the guidelines for evaluating insecticides resistance in vector using CDC tube bioassay([8]. The Culex mosquitoes earlier introduced into eight (8) holding tube(replicate) were exposed in the holding tube for 30mins, the expose temperature and the relative humidity were recorded both the maximum and minimum.

The Culex mosquitoes were then exposed CDC tubes coated with the insecticides (impregnated papers) and was exposed for 1 hour. The knockdown after the exposure was observed. The number of dead and live mosquitoes were monitored at different time interval (10, 15, 20, 30, 40, 50, & 60)mins. The mortality of test sample was calculated by summing the number of dead mosquitoes across all exposure replicates and then expressing this as a percentage of the total number of exposed mosquitoes.[8]

$$\text{Observed Mortality(\%)} = \frac{\text{Total number of dead mosquitoes} \times 100}{\text{Total sample size}}$$

The knockdown percentage for the test mosquitoes were calculated using the formula bellow

$$\text{Knockdown (\%)} = \frac{\text{Number of mosquitoes that died}}{\text{Total number of mosquitoes}} \times 100$$

The control mortality was zero (0), so there was no need for abbott's correction. This allow determination of total %mortality against time for all replicates. The tested mosquitoes were stored in microcentrifuge for further analysis.

2.6 Synergist bioassay

Synergist bioassays were performed to assess possible involvement of insecticide resistance mechanisms in field-collected, culex mosquitoesfrom study area. One synergy was used in this study, namely PBO (Sigma, USA) an inhibitor of monooxygenases. The synergist (4%)impregnated papers and insecticide-impregnated papers were procured from VCRU. Two treatments were compared for each test: insecticide alone and synergist + insecticide combination. In the insecticide alone, test mosquitoes were exposed to insecticide-impregnated papers for 1hr and during the synergist + insecticide combination assay mosquitoes were exposed for first 1hr to synergist impregnated papers followed by 1hr to the insecticide impregnated papers. After exposures mosquitoes were transferred to holding tubes for 24-h holding period and analyzed to determine percentage mortality.

2.7 Statistical Analysis

The percentage mortality of the mosquitoes exposed to each of the insecticides was calculated as the proportion of mosquitoes that died at the diagnostic time for each of the insecticides. Correction with Abbott's formula was not necessary as control mortalities was less than 5% throughout the test, mortality of replicates were determined for each insecticide. The WHO criterion for evaluating resistance or susceptibility was used mortality of less than 80% indicate resistance, while those greater than 98% indicate susceptibility. The Knock down data was subjected to probit analysis using statistical software (Statsdirect, 2013) to compute the KDT_{50} and KDT_{90} (Time taken to knock down 50% and 90% of the exposed mosquitoes. Analysis of Variance (ANOVA) was also used to compare the mortalities across the insecticide.

3.0 RESULTS

3.1 Susceptibility bioassay

The KDT_{50} and KDT_{90} results of female Culex mosquitoes exposed to DDT, Permethrin, Deltamethrin and PBO as well as the percentage mortality recorded after 24 hours exposure period are presented in Table 1 The table below presents the mortality rates and knockdown times (KDT) for different insecticides, with superscripts indicating significant differences between treatments. Lower letters (a, b, c, d) denote higher mortality or faster knockdown.

Table 3. Knockdown times (reported in percentages) of Culexmosquitoes across the different classes of insecticides used.

Time(mins)	Control(%)	Knockdown time DDT	Deltamethrin	Permethrin	PBO(synergy)
10	0	7	7	6	1
15	0	11	7	7	8
20	0	12	7	8	9
30	0	13	9	8	17
40	0	13	10	8	17
50	0	13	11	8	19
60	0	14	11	8	21
24hrs	0	15	65	7	69

Table 2. Mortality rates and Knockdown Time KDT for different insecticides

Insecticides	**Mortality after 24hours (%)**	**KDT_{50} mins**	**$KDT_{90\ mins}$**
DDT	15^{c}	19.11^{a}	52.95^{a}
Permethrin	07^{d}	1056.19^{d}	10804686.41^{d}
Deltamethrin	65^{b}	99.81^{c}	8083.52^{c}
PBO	69^{a}	26.50^{b}	65.00^{b}

Different superscript letter indicate significant difference (P < 0.05).

Note: Value followed by different letters in the same column are significantly different (P < 0.05)

Note: *98-100% mortality indicate susceptibility, 80_97% mortality implies is suggestive of the existence of resistance and further investigation is needed and <80% mortality implies resistance R– Resistant (WHO, 2013).*

The results of the susceptibility bioassay shows varying levels of insecticide effectiveness against Culex mosquitoes in Gombe Metropolis. Using the WHO (2013) criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24hour post exposure results indicate that Piperonyl butoxide (PBO) showed the highest efficacy, with a 24hour mortality rate of 69% and relatively fast knockdown times (KDT_{50} of 26.50 minutes and KDT_{90} of 65.00 minutes). Deltamethrin has also shown moderate effectiveness, with a 65% mortality rate, although its knockdown times were slower (KDT_{50} of 99.81

minutes and KDT_{90} of 8083.52 minutes), suggesting it may not act as quickly as PBO in controlling mosquito populations. DDT, despite its fast knockdown times (KDT_{50} of 19.11 minutes and KDT_{90} of 52.95 minutes), had only a 15% mortality rate while Permethrin showed the least effectiveness, with a very low mortality rate of 7% and extremely prolonged knockdown times (KDT_{50} of 1056.19 minutes and KDT_{90} of 10,804,686.41 minutes), indicating substantial resistance in the mosquito population.

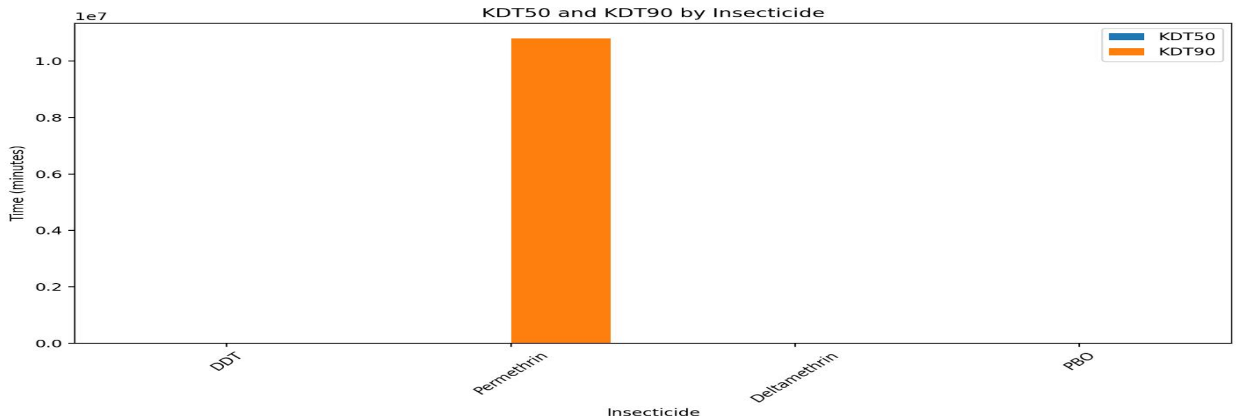

Fig.1 shows the knockdown rates at **KTD$_{50}$**mins and **KDT$_{90}$ mins**

DDT showed the fastest knockdown times (KDT50 = 19.11 min), while Permethrin showed very high KDT values, indicating significant resistance. PBO and Deltamethrin showed intermediate knockdown times, with PBO being more effective overall.

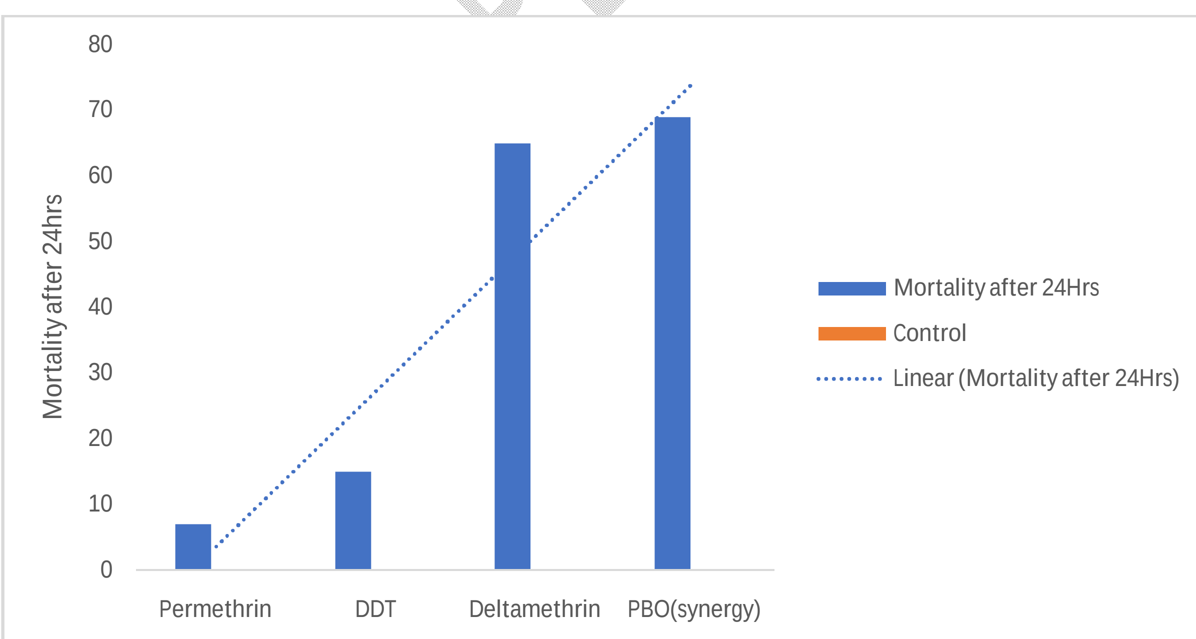

Fig 2. Percentage mortality rate of Permethrin, DDT, Deltamethrin and PBO (Synergy) after 24hrs of exposure

4.0 DISCUSSION

Many insecticides of the class organophosphate *(primiphos-methyl, malathion, fenthion),* organochlorine (DDT), and *pyrethroid* (*alphacypermethrin*, *deltamethrin*, *permethrin*, *lambdacyhalothrin*) have been used in mosquitoes control programme and for other vector control management. Checking the susceptibility status is a good indicator for the early detection of resistance[8]. The result of the knockdown assessment in Table 2. shows that the tested insecticidal papers induced knockdown of the adult culex mosquito, suggesting that knockdown mechanisms could be operating in the mosquitoes found in Gombe. This confirms earlier studies which indicates the knockdown effects of impregnated papers against mosquitoes in Nigeria[11,18]. The knockdown of the mosquitoes exposed to insecticidal papers indicates the presence of knockdown resistance (KDR) mechanism[19] operating in population of culex mosquitoes at Zagaina, Gombe metropolis. This could have been responsible for the level of resistance displayed by these mosquitoes to the insecticides evaluated.The results of the susceptibility bioassay shows varying levels of insecticide effectiveness against Culex mosquitoes in Gombe Metropolis. Using the[8] criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24hour post exposure results indicate that Piperonyl butoxide (PBO) showed the highest efficacy, with a 24hour mortality rate of 69% and relatively fast knockdown times (KDT_{50} of 26.50 minutes and KDT_{90} of 65.00 minutes). This indicates that PBO is the most effective insecticide among those tested, having both high mortality and reasonable knockdown speed. Deltamethrin has also shown moderate effectiveness, with a 65% mortality rate, although its knockdown times were slower (KDT_{50} of 99.81 minutes and KDT_{90} of 8083.52 minutes), suggesting it may not act as quickly as PBO in controlling mosquito populations.

Conversely, DDT, despite its fast knockdown times (KDT_{50} of 19.11 minutes and KDT_{90} of 52.95 minutes), had only a 15% mortality rate, indicating that while Culex mosquitoes are knocked down initially, they likely recover, showing signs of resistance. Permethrin showed the least effectiveness, with a very low mortality rate of 7% and extremely prolonged knockdown times (KDT_{50} of 1056.19 minutes and KDT_{90} of 10,804,686.41 minutes), indicating substantial resistance in the mosquito population. This is in agreement with other researchers who have lamented on the growing resistance of many mosquito species to DDT [20]. This is also in agreement with other documented evidence of Culex mosquitoes resistance to DDT [21] and deltamethrin[22]. The results from this study shows significant resistance to DDT and Permethrin in Culex mosquitoes in this region, with PBO emerging as a more viable alternative for effective mosquito control.

The delayed action in the KDT_{50} and KDT_{90} across all the tested insecticides may indicate a resistance mechanism initially preventing knockdown but not sufficient enough to prevent mortality at the tested concentration. This discrepancy between initial KDT and final mortality agrees with the observation by[23] who noted similar patterns in mosquitoes population subjected to organophosphate and hypothesized that metabolic resistance mechanism could be active initially. Remarkably the probit analyses using (statedirects) utilized in this study allowed precise estimation of the knockdown time for 50% and 90% respectively for the population tested. The significant difference between KDT of 50% and 90% across all insecticides are indicative of important implications with regards to understanding population dynamics and resistance level within Culex mosquitoes.

Summary of insecticide resistance cases and number of countries reporting this have constantly been on the rise. However, a consideration of factors (usage of ITN, IRS, agricultural pesticides) previously reported selecting for insecticide resistance[24] provided a reasonable clue of what could be the driver of insecticide resistance in Southern Gombe. Utilization of insecticidal nets by person in Gombe is 34%, which is just below the national average of 37%. Gombe state ranked 8th out of the 38 states with the highest percentage of households with at least one ITN[25]. There is a significant usage of Insecticide treated nets in Gombe state in Nigeria hence, these insecticide-based tools may have contributed to the insecticide pressure in selecting for resistance in the mosquitoes population[26]. The resistance might be explained by a long standing, massive use of DDT house-spraying in several districts of the country during the WHO malaria eradication in the 1950s. Moreover, the rapid expansion of urban agriculture couple with cotton production in west Africa could be one of the major factors that contributed to a large distribution of pyrethroids resistance in *Culex* mosquitoes. The resistance might also be caused by the massive free campaign of beds nets impregnated with permethrin and deltamethrin as the major control strategy against *Wuchereriabancrofti*transmitted by *Culex* mosquitoes[27] could also explain the resistance of Culex to pyrethroids. This hypothesis has been confirmed by the findings of[28] with increasing of *leu-phe*knockdown resistance mutation in *anopheles gambiae* from Niger following the Nationwide Long-lasting Insecticides-treated nets implementation, may be as a result of widespread utilization of chemical compound against mosquitoes and other domestic insects and pests. These chemicals tend to remain in the soil environment for month to several years and these mosquitoes become resistance to them by activating any of the two recent discovered major mechanisms that are involved in insecticide resistance: (a) **target-site insensitivity,** and (b) **increased metabolic detoxification** of insecticides. Target-site insensitivity result from structural modification or mutation(point mutation) of genes that encode target proteins that interact with the insecticides[29] while metabolic detoxification involves three major metabolic detoxification gene families: cytochrome p450, esterases, glutathione S-transferases(GSTs). A significant feature of the mosquitoes p450 and GSTs is their transcriptional upregulation which results in increased level of protein production and enzymatic activities. This in turn enhances the metabolic detoxification of the insecticides and plant toxins in the insects which leads to the development of insecticide resistance.

Moreover, Pyrethroid has been extensively used in agriculture since 1980's[30] particularly cotton and vegetable fields in Gombe. In fact, cultivation of vegetables and cotton requires intensive use of pesticides including insecticides belonging to the two main classes recommended for vector control in public health (organophosphate and pyrethroids) and which mostly were used indiscriminately to control vegetable and cotton pest. During treatment, insecticides residues in vegetables or cotton fields are washed into mosquitoes breeding sites, thus exerting a huge selection pressure on mosquito larvae populations, which result in the emergence of insecticides resistance in *Culex* mosquitoes[31]. This may most likely cause selection on strong resistance in Culex mosquitoes to DDT, permethrin, deltamethrin and PBO(Synergist) particularly in cotton and vegetable growing areas.

5. CONCLUSION

This study shows that there is high resistance to DDT, Permethrin, Deltamethrin and PBO by Culex mosquitoes. The results from the PBO synergistic assay carried out indicated an increase in percentage mortality yet resistance to culex mosquitoes. The current findings will help for decision making in National vector Control Program particularly in the choice of insecticides to use during campaigns of Indoor residual Spaying in Gombe. This information is essential in the monitoring of the development of insecticide resistance in Nigeria. There is a need for continued surveillance to find out the resistance factors present in these mosquitoes specie.

ETHICAL APPROVAL

This study received ethical approval from Gombe State university, Nigeria.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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