

INSECTICIDE RESISTANCE STATUS OF THE MOST PREVALENT MOSQUITO SPECIES IN AWKA, ANAMBRA STATE

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ABSTRACT

Development of resistance in some local populations of mosquitoes has been reported as one of the major challenges faced in the control of mosquito borne diseases. This research therefore aimed to determine the insecticide resistant status of the most prevalent mosquito species in Awka Anambra State of Nigeria, using both cross-sectional and completely randomized designs. The research was conducted using 3 sentinel sites, 60 households and laboratory environment. Mosquitoes were collected, reared and identified using standard techniques. Insecticide susceptibility test was also carried out using standard procedures and test kits. Data collected were analyzed using Friedman test and ANOVA at 5% significance level. A total of 1,022 larvae of mosquito were collected from the sentinel sites, out of that, 785 (76.81%) adults successfully emerged in the insectary. Using Pyrethrum Knockdown Collection (PKC) and Human Landing Catch (HLC), 207 and 183 adult mosquitoes were collected respectively. Three genera of mosquitoes: *Anopheles*, *Aedes* and *Culex*, were identified. The most abundant species (60.6%; n = 709) collected was *C. quinquefasciatus* while the least abundant (3.42%; n = 40) was *Aedes vitattus* ($P = 0.000$). Knockdown responses of the *C. quinquefasciatus* exposed to different classes of insecticides was progressively highest (83.0% to 100%) with Deltamethrin (0.03%), from 10 to 60 minutes of exposure. The KDT50 (95% CI) and KDT95 (95% CI) for Deltamethrin (0.03%), were 3.99 (0.00-7.52) and 14.64 (8.43-44.74) respectively. Mortality responses of *C. quinquefasciatus* were 100% (25.00±0.00) for Pirimiphos-methyl (0.25%), Bendiocarb (0.01%), and Deltamethrin (0.03%); and 81.0% (20.25±0.96) for DDT. This study showed that *C. quinquefasciatus* abounds and could be of major public health importance in disease transmission in the study area. Individuals belonging to local population of *C. quinquefasciatus* that are able to survive the insecticide exposure have higher tendency of developing resistance to such insecticide. It therefore highlighted the need for ongoing monitoring and research into resistance development and the resistance status of local mosquito populations.

Keywords: Insecticide resistance, *Culex quinquefasciatus*, Deltamethrin (0.03%), Bendiocarb (0.01%), Pirimiphos-methyl (0.25%), DDT (4.0%), Awka, Anambra State

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1. INTRODUCTION

Mosquitoes are a diverse group of insects that belong to the phylum Arthropoda, class Insecta, order Diptera, and family Culicidae. The family Culicidae includes over 3,500 species of mosquitoes worldwide, which are further divided into several Genera. The most commonly encountered Genera in Anambra State of Nigeria are: *Aedes*, *Anopheles*, *Culex*, *Mansonia*, *Eretmapodite* and *Coquilletidia* (Egbuchee *et al.*, 2021). Many mosquito species belonging to some of these Genera are capable of transmission of specific disease pathogens (protozoa, virus and helminths), causing mosquito-borne diseases. Examples of mosquito-borne diseases include: filariasis, yellow fever, malaria, dengue fever, rift valley fever, Zika virus disease, Chikungunya, and West Nile virus. Mosquito-borne diseases cause millions of deaths and illnesses, with malaria alone accounting for over 400,000 deaths annually (WHO, 2021). In addition, they also result in substantial economic loss, including healthcare expenses and lost productivity. Mosquitoes are also regarded as public enemies because they exhibit characteristic biting annoyance, noise nuisance, sleeplessness and allergic reactions (Onyido *et al.*, 2009). The bites sometimes cause minor localized itching and irritations to the skin. Therefore, they are of great importance and should be controlled.

Among the various methods for controlling mosquitoes, insecticides are the most widely used. They are either used in treated materials (curtains, bed nets), Indoor Residual Spraying in form of aerosol, and larvicides, depending on the target mosquito species and their life cycle stage. Four major classes of insecticides are used for mosquito control: organochlorines, organophosphates, pyrethroids and carbamate (WHO, 2021). However, there have been innumerable reports of resistance to insecticides in the four classes by major disease vectors across all regions of the world (WHO, 2021, Moyes *et al.*, 2017). Most reports of insecticide resistance [isare](#) majorly on malaria vectors (WHO, 2021, WHO, 2018, Moyes *et al.*, 2017), with little attention on *Aedes* and *Culex* mosquitoes (Egbuchee *et al.*, 2019, WHO 2022a). However, when an insecticide is applied, it does not actually discriminate among mosquito species to target in terms of function, but then certain species that survive increases in population and become prevalent. This study was therefore undertaken to determine the insecticide resistant status of the most prevalent mosquito species in Awka Anambra State of Nigeria. By examining the prevalent mosquito species and their resistance status, this study would provide valuable insights into the challenges faced in controlling mosquito-borne diseases in the study area. With the understanding of the susceptibility status of mosquitoes in this specific area, appropriate measures can be implemented to mitigate the development and spread of insecticide resistance and ensure the effectiveness of control interventions.

2. MATERIAL AND METHODS

Study Area

The study was conducted in three sentinel sites (Site A, Site B and Site C) of Awka, the capital city of Anambra State Nigeria (Figure 1). The geographical coordinates of Awka are: Latitude [6°12'25"N25° N](#) and Longitude [7°04'04"E04° E](#) which covers an area of about 164.2 square kilometres and has a population of approximately 430,200 people. Awka experiences

both dry and rainy seasons with climatic variables similar to temperature range of 26.2°C to 30.3°C, rainfall range of 0.0mm to 680.8 mm and humidity range of 64% to 88% obtained in Anambra East LGA of Anambra State (Egbuche *et al.*, 2020). Factors such as that favour the breeding of mosquitoes abound in Awka. They include: refuse dump (Eziheet *et al.* 2017), water collection in discarded containers, gutter, tyre, basins and swamps (Egbuche *et al.*, 2019).

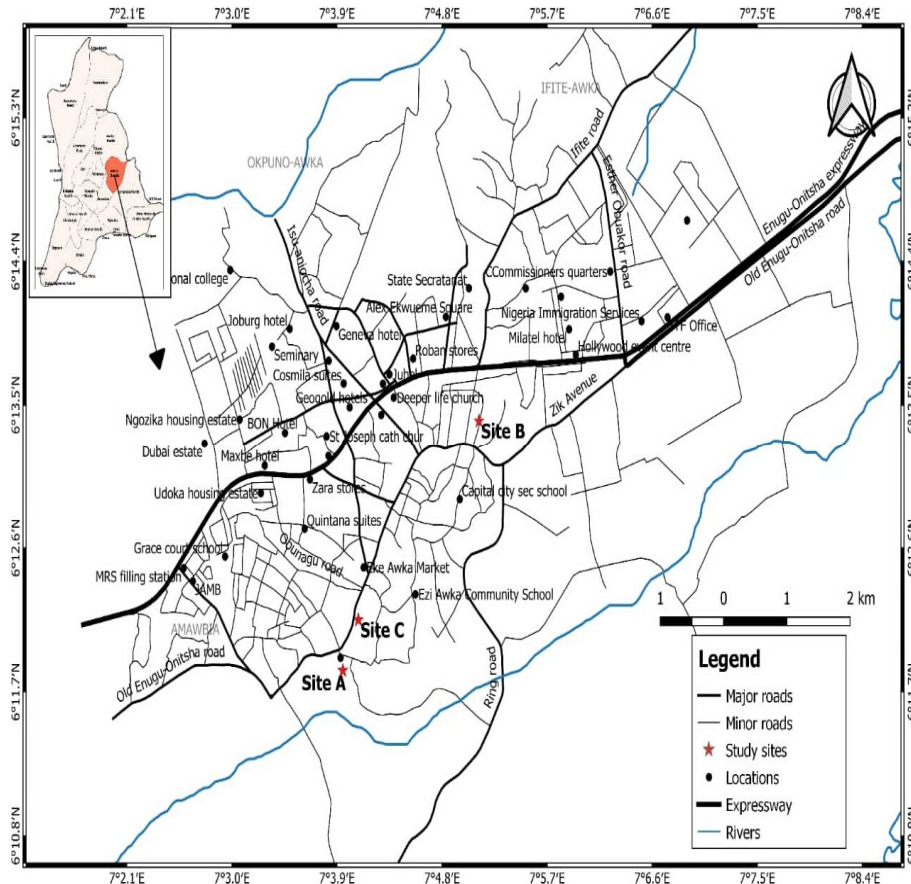


Figure1: Map of the study area showing the three sentinel sites marked Site A, Site B and Site C.

Source: Department of Geography, NnamdiAzikiwe University Awka.

Experimental Design

The research was carried out between August and December 2023. The study involved a cross-sectional design (which was employed in the survey of mosquitoes in the study area) and a completely randomized design (which was used for insecticide bioassay). These study designs are appropriate for this study because they allowed for data collection on multiple

variables at a single point in time, which is important for investigating the prevalent mosquito species and their insecticide resistance in Awka/Anambra State.

Sampling technique and Household selection for the survey of mosquitoes

The sentinel sites for this study were: Site A (Agulu/Awka), Site B (Amachalla) and Site C (Amikwo). Households were selected for this study in accordance to WHO (2016) guidelines for mosquito survey. Site A and Site C were randomly selected from the Ezinator section of Awka that comprises three groups, namely: Amikwo, Ezi-Oka and Agulu. Site B was randomly selected from Ifite section that comprises four groups: Ayom-na-Okpala, Nkwelle, Amachalla and Ifite-Oka. Each of these groups has a number of villages, altogether spanning 33 in Awka. At each sentinel site, twenty households were selected from each section using systematic sampling technique, making it a total of 60 households in all.

Ethical Consideration, Advocacy visit and informed consent

The study did not involve any invasive procedure, only mosquitoes were collected in and around selected households. Thus, Ethical approval letter with reference number 'MH/COMM/523/V.1/29' was obtained from Anambra State Ministry of Health. This alongside a letter of introduction from the Head of Parasitology and Entomology Department, Nnamdi Azikiwe University Awka was presented to the opinion leaders in the study area during advocacy visits. Verbal consents of the landlords / caretakers / lodge presidents and Heads of various households whose compounds and rooms were used in this study were equally obtained.

Indoor Collection of Adult Mosquitoes

This was carried out using pyrethrum Knockdown Collection Method as described by WHO (2003). A pyrethrum based insecticide available in the local market, Baygon® was used in this study. The collection was done in the early hours between 6:00 am-8:00 am from randomly chosen rooms where at least one person slept the previous night.

Outdoor Collection of Adult Mosquitoes

These were collected on human volunteers using a human landing catch (HLC). Materials used were torchlight, test tube, cotton wools, Ethanol (to suffocate the mosquitoes), wristwatches (for timekeeping), pens, papers (for recording the time of collection), cellophane bags (for collection of catches) and Low stool as described by Onyido *et al.* (2016). All catches were between 5:00 ~~pm~~ to 8:00 pm and recorded at quarterly-hour interval.

Collection of Immature Stages of Mosquitoes Using Dipping Method

Mosquito larvae and pupae were collected from different mosquito breeding habitats / sites such as groundwater pools, stills, and standing water, discarded tins and containers, polluted water in gutters around homes, and puddles made from vehicles as described by Egbuche *et al.* (2019).

Rearing of the immature stages of mosquitoes to adults for proper identification.

The mosquito larvae and pupae collected were reared into adult mosquitoes. The collected larvae were placed in white small bowl covered with nets and reared in the mosquito rearing cage. The larvae were fed with a mixture of yeast and biscuits. The adults that emerge from the larvae were held in the mosquito cage and were fed with glucose solution as described by Onyeka *et al.* 2020

Identification of the Collected Mosquitoes

Mosquito identification was carried out at the National Arbovirus and Vectors Research Centre, a vector research unit of the Federal Ministry of Health, located in Enugu, Enugu State, southeast Nigeria. Identification of the mosquitoes collected as adults or reared to adults from immature stages was done using the morphological keys described by Gillet (1972) and Gillies and Coetzee (1987). The most prevalent mosquito species was determined based on the relative abundance (also expressed in percentage) of different mosquito species identified in the study area.

Insecticide Susceptibility Test

The WHO standard procedures and test kits for adult mosquitoes were used to conduct the insecticide susceptibility test (WHO, 2022a,b,c). A maximum of 100 female *Culexquinquefasciatus* (as the most prevalent mosquito species in the study area) in four replicates (with 25 mosquitoes each) were tested for each insecticide. The adult mosquitoes used were reared from the immature stages since the larvae habitat of the main vector species (*Culexquinquefasciatus*) could be easily found in the study area. Another reason was that some of the mosquitoes collected as adults were dead and as such could not be used for the bioassay. The mosquitoes were 3-5 days old and were starved for 6 hours before the test. Four types of WHO bioassay test papers impregnated with the recommended diagnostic concentrations of the four insecticides were used for the bioassay. One control was lined with plain paper, while four exposure tubes contained 4% DDT, 0.05% deltamethrin, 0.1% bendiocarb, and 0.25% primiphos-methyl-impregnated paper strips. A pre-test was performed by introducing 20 female mosquitoes into the four holding tubes and allowing them to stand for one hour. The mosquitoes were then transferred to the exposure tubes and allowed to stand for one hour. Records of knockdown were taken at intervals of 0, 15, 20, 30, 40, 50, and 60 minutes. The mosquitoes were transferred back to the holding tubes and kept for 24 hours, during which they were fed with a 7% sucrose solution. Records of mortality were then taken after 24 hours, and the susceptibility status of the population was graded according to WHO-recommended protocol. Dead and survived mosquitoes from this bioassay were separately kept in clearly labelled 1.5 ml Eppendorf tubes containing silica gel for preservation. All susceptibility tests were carried out at 27°C \pm 2°C temperature and 75% \pm 10% relative humidity. The susceptibility status of tested populations was determined according to WHO (2022c) criteria. A resistant population is defined by mortality rates less than 90% after the 24-hour observation period while mortality rates greater than or equal to 98% are indicative of susceptible populations. Mortality rates between 90-97% suggest a possibility of resistance (suspected resistance) that requires confirmation.

Data Analysis

The knockdown and mortality for the test mosquitoes were calculated using the formulae below: $\text{Knockdown (\%)} = \frac{\text{Number of mosquitoes that were knocked down}}{\text{Total number of mosquitoes}} \times \frac{100}{1}$

$$\text{Mortality (\%)} = \frac{\text{Number of mosquitoes that died}}{\text{Total number of mosquitoes}} \times \frac{100}{1}$$

For each collection method as well as overall collection, Friedman test at 5% level of significance was used to compare the different populations of mosquito species. The ANOVA was used to compare the mortalities across the insecticides, and LSD was used to separate the means while the knockdown times for 50% (KDT₅₀) and 95% (KDT₉₅) of the test population were estimated by the log-time probit model (probit analysis) using the SPSS (Statistical Package for the Social Sciences) statistical software (version 25.0).

3. RESULTS

The result of the study showed that a total of 1,022 larvae of mosquito were collected from the different sites in Awka, Anambra State, out of that, 785 (76.81%) adults successfully emerged in the insectary (Table 1). The table showed that out of the three (3) sentinel sites sampled, the highest number (n = 421) of mosquito larvae was collected from Site A (421), followed by Site B (n = 345) while the least was collected from Site C (n = 256). However, adult emergence was highest, 83.20% from Site C, followed by Site A (81.24%) while the least was collected from Site B (66.67%). Using pyrethrum knockdown method (PKC), 207 adults were collected (Table 1); highest number of mosquitoes (n = 103) was collected from Site B, and least (n = 46) from Site A. Using Human Landing Catch (HLC), 183 adults were collected (Table 1); highest number of mosquitoes (n = 70) was collected from Site A and least (n = 53) from Site B.

Table 1: Population of mosquito collected from the study area.

Developmental stage	Sentinel site	Number of larvae collected	Number of adult emerged	Percentage value
Larvae	Site A	421	342	81.24
	Site B	345	230	66.67
	Site C	256	213	83.20
	Total	1,022	785	76.81
Developmental stage	Sentinel site	Number of adults collected	Percentage value	
Adult (PKC)	Site A	46	22.22	
	Site B	103	49.76	
	Site C	58	28.02	
	Total	207		
Developmental stage	Sentinel site	Number of adults collected	Percentage value	
Adult (HLC)	Site A	70	39.33	
	Site B	53	29.78	

Site C	55	30.90
Total	178	

Three genera of mosquitoes: *Anopheles*, *Aedes* and *Culex*, were identified in Awka Anambra State. Nevertheless, *Anopheles* Mosquito was not identified in the larvae collection. Four different mosquito species were collected as larvae (Table 2). The most abundant species collected was *C. quinquefasciatus* (n = 599) while the least abundant was *Aedes vittatus* (n = 7). There was significant difference in the populations of different species of mosquitoes collected as larvae ($P = 0.0000$). Using PKC, five different mosquito species were collected (Table 2). The most abundant species collected was *C. quinquefasciatus* (n = 70) while the least abundant was *Aedes vittatus* (n = 13). There was significant difference in the populations of different species of mosquitoes collected using PKC as a collection method ($P = 0.0000$). Using HLC, five different mosquito species were also collected (Table 2). The most abundant species collected was *Anopheles gambiae* s.l. (n = 75) while the least abundant was *Aedes albopictus* (n = 12). There was significant difference in the populations of different species of mosquitoes collected using HLC as a collection method ($P = 0.0000$). In overall, *C. quinquefasciatus* (n = 709) was the most abundant mosquito species collected in the study area, while *Aedes vittatus* (n = 40) was the least abundant. There was significant difference in the populations of different species of mosquitoes collected in the study area, regardless of the collection methods ($P = 0.0000$).

Table 2: Mosquito species found in the study area

Mosquito species	Abundance from different collection methods (%)			Total	Relative abundance (%)
	Larval collection	PKC	HLC		
<i>C. quinquefasciatus</i>	599(76.31)	70(38.65)	40(22.47)	709	60.60
<i>Ae. albopictus</i>	114(14.52)	32(15.46)	12(6.74)	158	13.50
<i>Ae. aegypti</i>	65(8.28)	34(16.43)	31(7.30)	130	11.11
<i>Ae. Vittatus</i>	7(0.89)	13(6.28)	20(11.24)	40	3.42
<i>An. gambiae</i> s.l.	-	58(28.02)	75(42.13)	133	11.37
Total	785	207	178	1170	

Insecticide Susceptibility (Knockdown and mortality responses) of *C. quinquefasciatus*

Knockdown responses of the *C. quinquefasciatus* exposed to different classes of insecticides used in the experiment is shown in Table 3. At 10 minutes, the highest (83.0%) knockdown was observed with Deltamethrin (0.03%) while the least (0.0%) was observed with DDT (4.0%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 10 minutes of exposure ($P = 0.000$). At 15 minutes, the highest (100.0%) knockdown was observed with Deltamethrin (0.03%) while the least (0.0%) was observed with DDT (4.0%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 15 minutes of exposure. At 20 minutes, the highest (100.0%) knockdown was observed with Deltamethrin (0.03%) while the least (0.0%) was observed with Primiphos-

methyl (0.25%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 20 minutes of exposure ($P = 0.000$). At 30 minutes, the highest (100.0%) knockdown was observed with Deltamethrin (0.03%) while the least (6.0%) was observed with Primiphos-methyl (0.25%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 30 minutes of exposure ($P = 0.000$). At 40 minutes, the highest (100.0%) knockdown was observed with Deltamethrin (0.03%) while the least (4.0%) was observed with Primiphos-methyl (0.25%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 40 minutes of exposure ($P = 0.000$). At 50 minutes, the highest (100.0%) knockdown were observed with Deltamethrin (0.03%) and Bendiocarb (0.01%) while the least (19.0%) was observed with Primiphos-methyl (0.25%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 50 minutes of exposure ($P = 0.000$). At 60 minutes, the highest (100.0%) knockdown was observed with Deltamethrin (0.03%) while the least (84.0%) was observed with Primiphos-methyl (0.25%). There was statistically significant difference in the knockdown effects of the different insecticide classes at 60 minutes of exposure ($P = 0.000$).

Table 3: Knockdown times (reported in percentages) of *C. quinquefasciatus* across the different classes of insecticides used.

Insecticide used	No. mosquito tested	Number (in percentage) of <i>C. quinquefasciatus</i> knocked down at different time interval								KDT50 (95% CI)	KDT95 (95% CI)
		10mi ns	15mi ns	20mi ns	30mi ns	40mi ns	50mi ns	60mi ns	24 hrs		
Primiphos-methyl (0.25%)	100	3	1	3	6	4	19	84	100	54.81 (*)	134.96 (*)
Bendiocarb (0.01%)	100	12	80	89	97	98	100	93	100	13.09 (5.20-18.06)	24.03 (17.58-229.3)
DDT (4.0%)	100	0	0	4	34	55	80	93	81	36.68 (33.21-40.30)	65.56 (56.74-82.84)
Deltamethrin (0.03%)	100	83	100	100	100	100	100	100	100	3.99 (0.00-7.52)	14.64 (8.43-44.74)

* means that the CI for KDT50 and KDT95 was not computed because of much irregularity in the knockdown effect of Pirimiphos-methyl

Mortality responses of *C. quinquefasciatus* mosquitoes are shown in Figure 2. There was no mortality recorded for the control after 24 hours recovery period. Highest mortality (100%) was recorded for Pirimiphos-methyl (0.25%), Bendiocarb (0.01%), and Deltamethrin (0.03%), each with mean±se mortality value of 25.00±0.00. DDT on the other hand had 81.0% mortality, with a mean±se value of 20.25±0.96. There was significant difference in the mortality effects of the different classes of insecticides on *C. quinquefasciatus* population ($P = \dots$).

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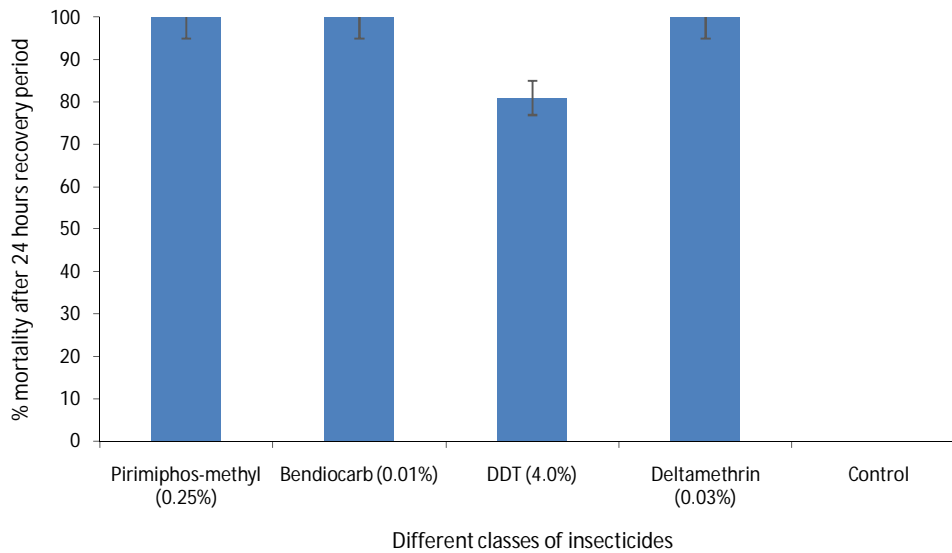


Figure 2: Mortality rate of *C. quinquefasciatus* to different insecticide classes

4.0 DISCUSSION

The findings from this study provide significant insight into the distribution and emergence patterns of mosquitoes in Awka, Anambra State. The rate of larval collection and subsequent adult emergence is critical for understanding the potential for disease transmission and for formulating vector control strategies. A total of 1,022 mosquito larvae were collected across the three sites in the study, with varying emergence success rates. This differential emergence rate has been observed in other studies and could be influenced by several factors including environmental conditions and larval habitat quality (Arévalo-Cortés *et al.*, 2022). The emergence rate of 76.81% observed in the current study is consistent with the findings of other studies in similar ecological zones, where emergence rates have ranged from 70 to 90% (Mohammed and Chadee, 2011; Ndenga *et al.*, 2017).

The uneven distribution of larvae and adults across the three sites (A, B, and C) may be attributed to variations in environmental factors that influence mosquito breeding and survival. For example, Site A, yielded the highest number of larvae but was second in adult emergence (81.24%). This could suggest that although larval habitats were more numerous or favourable in Site A, factors such as predation, larval competition, or potentially suboptimal conditions within the habitats may have adversely affected larval development into adults (Westby *et al.*, 2019; Egbuche *et al.*, 2016; Suh *et al.*, 2016). The relatively high emergence rate in Site C (83.20%) compared to the lower larval collection count may indicate more suitable conditions for larval development within this site. Prior research by Langerhans and Kern (2020) found that smaller bodies of water, which are less likely to be disturbed and may harbor fewer predators, can result in higher mosquito adult emergence rates. This finding

aligns with those in the current study, where despite fewer larvae being collected, the emergence rate was higher in Site C, suggesting a possible correlation between habitat suitability and emergence success. Site B had the lowest rate of adult emergence at 66.67%, which is lower than typically reported in other regions (Egbuche *et al.*, 2016).

The most prevalent mosquito collected in this study was *C. quinquefasciatus*. This suggests that the environment of the study area is most suitable for this species of mosquitoes than other species also collected. This is in line with the findings of Egbuche *et al.* (2021) who reported *C. quinquefasciatus* as the most abundant mosquito species in Anambra East LGA of Anambra State. Their larval population are the most abundant as well possibly because the breeding habitats present in this present study is suitable for their development. On the other hand, this study reported higher occurrence of *C. quinquefasciatus* indoors than in outdoor locations, though without any statistically significant difference. This suggests that *C. quinquefasciatus* is of great public health importance in disease transmission both in outdoor and indoor locations.

As part of control interventions, monitoring and evaluation of control efforts is required, especially interventions targeted against local populations of mosquitoes (Egbuche *et al.*, 2023). This study therefore also looked at the susceptibility status of the most prevalent mosquito species, *C. quinquefasciatus* in Awka Anambra State, to different classes of WHO recommended insecticides. The use of Primiphos-methyl, Bendiocarb, DDT, and Deltamethrin in mosquito control efforts has been studied extensively (Ononamadu *et al.*, 2020; Sikaala, 2014), and the present study's results both corroborate and expand upon previous work. Primiphos-methyl (0.25%) displayed a delayed mortality effect on mosquitoes, with 100% mortality observed only after the 24-hour recovery period. This pattern of delayed mortality suggests that the mosquitoes initially withstand the toxic effects but ultimately succumb (Furnival-Adams *et al.*, 2020).

In stark contrast, Bendiocarb (0.01%) exhibited rapid knockdown activity but showed a peculiar decrease from 100% to 93% at the 60-minute mark, later returning to 100% mortality after 24 hours recovery period. This could potentially be attributed to 'knockdown resistance,' wherein some mosquitoes recover from the initial lethal effect of the insecticide (Andreazza *et al.*, 2021). However, the temporary decline observed in this study is atypical, warranting further investigation, as generally, such a resurgence has not been widely reported. DDT (4.0%) demonstrated a surprisingly delayed action despite its historical positioning as a robust insecticide (Davis, 2014), as no knockdown was observed in the first 15 minutes. Knockdown did occur progressively over the 60 minutes exposure time, achieving a 100% mortality rate after 24 hours recovery period. This is reflective of alterations in mosquito susceptibility patterns and possible resistance mechanisms that have been emerging against DDT (Saber, *et al.*, 2022). Deltamethrin (0.03%) showed an exceedingly rapid and sustained impact, with a knockdown rate reaching 100% just after 15 minutes and maintaining this rate throughout the 60 minutes exposure period and 24 hours recovery period. Such high and immediate efficacy is consistent with previous studies demonstrating the potency of pyrethroids like deltamethrin (Lissenden *et al.*, 2021). The observed quick action suggests deltamethrin is an effective agent for immediate mosquito control, aligning with the findings from research by Salako *et al.* (2020), which highlighted the swift and consistent performance of pyrethroids in mosquito knockdown assays.

Comparing the susceptibility of mosquitoes to various insecticides in Awka, the result showed the superiority of Deltamethrin (0.03%) in terms of efficacy, with observed maximum mortality rates of 100%. The result is consistent with the findings of other studies that have reported high susceptibility of mosquito species to pyrethroids like deltamethrin (Goindinet *et al.*, 2017; Opondo *et al.*, 2019). Goindinet *et al.* (2017) stated that the neurotoxic mode of action of pyrethroids is highly effective against a wide range of mosquito species, which aligns with the high mortality rates observed in this study. Similarly, Opondo *et al.* (2019) found that deltamethrin elicited high mortality rates in mosquito populations in a Kenyan study, reinforcing the consistently high efficacy recorded in Awka. However, studies by Miller and Hamilton (2019) have warned of the potential for resistance development if pyrethroids are relied upon too heavily and without integrated pest management strategies (Smith *et al.*, 2016; Smith, 2015).

In the case of Bendiocarb (0.01%), an observed mortality rate of up to 100% was also noted, except for the fact that the knockdown effect was not progressive. This is comparable to the results obtained by Bamouet *et al.* (2019), who reported that bendiocarb remained effective against *Anopheles gambiae* in Cameroon, although with indications of reduced sensitivity. The relatively high mortality rates observed in Awka suggest that, at present, mosquito susceptibility to bendiocarb is maintained, although ongoing monitoring is recommended to detect potential resistance development (Brown and Pal, 2016). The relative quickness of knockdown by Bendiocarb compared to Primiphos-methyl could point toward differing mechanisms of action or resistance. For instance, a study by Rahimi *et al.* (2019) reported a quick action of carbamates in comparison with organophosphates, suggesting a higher vulnerability of some mosquito populations to this class of insecticides (Smith and Scott, 2017). Rahimi *et al.* (2019).

DDT (4.0%) demonstrated lower mortality rates in comparison to deltamethrin and bendiocarb, with a higher rate reported after longer exposure. Nevertheless, a study by Rahimi *et al.* (2019) found that DDT may still be effective in some settings, but resistance in mosquito populations is of increasing concern. This aligns with the trend in our study, where DDT demonstrated efficacy but was less effective than the other two insecticides. When *C. quinquefasciatus* was exposed to DDT, no knockdown was observed within the first 10 minutes, while the 24-hour mortality rate stood at 81%. This suggests a notable level of resistance to DDT, which is consistent with global trends of declining DDT efficacy due to long-term usage and selection for resistant genes within mosquito populations (WHO, 2021). The incomplete mortality could indicate a significant survival of resistant individuals, which can pose challenges for eradication programs (Smith *et al.*, 2016). The fact that DDT showed a peak mean mortality rate of 81.0% after a 60-minute exposure and 24 hours recovery periods suggests that resistance mechanisms may be present within the mosquito population in Awka, and it warrants detailed investigation into the resistance status of local mosquito species to DDT. The lack of mortality in the control group validates the experimental results and negates the possibility of natural mortality affecting the outcomes. This is an important consideration in experimental design, as highlighted by Cooke *et al.* (2017), who stressed the importance of incorporating controls to ascertain the true effects of the tested substances. After a 24-hour recovery period, the absence of mortality rebound in the control group and the sustained insecticide efficacy in deltamethrin (0.03%) and bendiocarb (0.01%), both at 25%, suggest no immediate recovery of affected mosquitoes. This

finding is in line with the work of WHO (2016b), who noted that recovery periods often do not significantly alter the initial mortality outcomes when dealing with susceptible mosquito populations.

The delayed action in the KDT50 and KDT95 across all the insecticides may suggest a resistance mechanism initially preventing knockdown, but not sufficient to avoid mortality at the tested concentration. The discrepancy between initial knockdown and final mortality aligns with observations by Rahimi *et al.* (2019), who noted similar patterns in mosquito populations subjected to organophosphates and hypothesized that metabolic resistance mechanisms could be initially at play (WHO, 2021; Rahimi *et al.*, 2019). Remarkably, the log-time probit model utilized in this study allowed precise estimation of the knockdown times for 50% and 95% of the populations tested. The significant differences between KDT50 and KDT95 metrics across all insecticides ($p < 0.05$) suggest important implications for understanding population dynamics and resistance levels within *C. quinquefasciatus*. The observation that the KDT95 was substantially higher than the KDT50 in all cases may indicate heterogeneous susceptibilities within the tested populations, which could be due to genetic variation, as evidenced by Wang *et al.* (2020), who reported significant genetic diversity in the resistance mechanisms among *Culex* populations (Wang *et al.*, 2020).

CONCLUSIONS

The study has found that different mosquito species exist within the study area, with *C. quinquefasciatus* as the most abundant species. It has equally reported that deltamethrin and bendiocarb were effective in the control of local population *C. quinquefasciatus* in Awka, while DDT showed lower efficacy, indicating the presence of resistance mechanisms. The exposure to Deltamethrin, a synthetic pyrethroid, resulted in a quick and high initial knockdown and complete mortality of *C. quinquefasciatus* population, making it the most effective insecticide. This study also alerts that mosquito populations that are able to survive the insecticide exposure have a higher tendency of developing resistance to such insecticide. It therefore highlighted the need for ongoing monitoring and research into resistance development and the resistance status of local mosquito populations.

Ethical Approval and Informed Consent

A letter of introduction was presented to the opinion leaders in the study area during advocacy visits and verbal consent of the Heads of various houses whose compounds along with the lodge president of the lodges where the rooms used for the study were subsequently obtained. An ethical approval letter with the number MH/COMM/523/V.1/29 was obtained from the Ministry of Health.

References

Akunne, C. E., Nwoke, B. E. B., Njokuocha, R. C., Eneanya, C. I., Ugochukwu, O. U., and Ogbu, K. I. (2019a). Diversity of mosquitoes (Diptera: Culicidae) and their larval habitats in Ihiala local government area of Anambra state, Nigeria. *Journal of Vector-Borne Diseases*, 56(3): 209-217.

Akunne, C.E., Ugonkwo, K.M., Ononye, B.U., Chidi, C.A., and Okafor, K.P. (2019b). The susceptibility status of *Anopheles gambiae* s.l populations in Ifite-Awka, Anambra State to standard insecticides. *The Bioscientist*, 7(1): 15-27

Andreazza, F., Oliveira, E. E., and Martins, G. F. (2021). Implications of sublethal insecticide exposure and the development of resistance on mosquito physiology, behavior, and pathogen transmission. *Insects*, 12(10): 9-17.

Aribodor, D. N., Ugwuanyi, I. K., Okeke, O. C., Obi, K. O., and Emecheta, B. C. (2016). Species composition and abundance of mosquitoes in NnamdiAzikiwe University, Awka, Anambra State, Nigeria. *Journal of Entomology and Zoology Studies*, 4(6): 717-720.

Bamou, R., Sonhafouo-Chiana, N., Mavridis, K., Tchuinkam, T., Wondji, C. S., Vontas, J., and Antonio-Nkondjio, C. (2019). Status of insecticide resistance and its mechanisms in *Anopheles gambiae* and *Anopheles coluzzii* populations from forest settings in south Cameroon. *Genes*, 10(10): 1-7.

Brown, A.W. and Pal, R. (2016). Insecticide resistance in mosquitoes: a pragmatic review. *Journal of the American Mosquito Control Association*, 32(2): 123-140.

Centres for Disease Control and Prevention (CDC). (2021a). Chikungunya. Retrieved from <https://www.cdc.gov/chikungunya/index.html>

Centres for Disease Control and Prevention. (2021b). Mosquitoes. <https://www.cdc.gov/mosquitoes/about/index.html>

Cooke, S. J., Birnie-Gauvin, K., Lennox, R. J., Taylor, J. J., Rytwinski, T., Rummer, J. L., ... and Haddaway, N. R. (2017). How experimental biology and ecology can support evidence-based decision-making in conservation: avoiding pitfalls and enabling application. *Conservation Physiology*, 5(1):1-4.

Cummings, C. L. (2018). Cross-sectional design. *The SAGE Encyclopedia of Communication Research Methods*. Thousand Oaks: SAGE Publications Inc. Retrieved.

Durner, E. F. (2021). The completely random design. In *Applied plant science experimental design and statistical analysis using SAS® OnDemand for Academics* (pp. 47-55). Wallingford UK: CABI.

Egbuche, C. M., Enyinnaya, J. O., Nwankwo, E. N. and Okonkwo, N. J. (2023). Screening of Locally Available Lemon Grass (*Cymbopogon citratus*) for Repellency Action against *Aedes aegypti*. *The Bioscientist*, 11(2): 180 - 193.

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Egbuche, C. M., Ezihe, C. K., Aribodor, D. N., and Ukonze, C. B. (2016). Survey of mosquitoes in open and closed larval habitats in Aguleri, Anambra East Local Government Area of Anambra State, Southeastern Nigeria. *Journal of Mosquito Research*, 1-6.

Egbuche, C. M., Onyido, A. E., Umeanaeto, P. U., Omah, I. F., Ukonze, C. B., Okeke, J. J., Ezihe, C. K., Irikannu, K. C., Aniekwe, M. I., Ogbodo, J. C and Enyinnaya, J. O. (2020). *Anopheles* species composition and some climatic factors that influence their survival and population abundance in Anambra East LGA, Anambra State, Nigeria. *Nigerian Journal of Parasitology*, 41(2):240 - 250.

Egbuche, C. M., Samuel, P. U., Ezihe, C. K., Ukonze, C. B., Chukwuzoba, O. A., Okoye, K. C and Onyido, A. E. (2019). Evaluation of Perma Net® 2.0 in the Control of *Culexquinquefasciatus* and *Aedesaegypti* from Awka, Anambra State, Nigeria. *Nigerian Annals of Pure and Applied Sciences*; 1 (1): 68 - 75.

Egbuche, C., Ezaka, E., Odoh, V., Omah, I., Okwubanego, C., Obi, C., Dashar, T., Egbobe, J. Olisakwe, S., Okafor, T. and Nwankwo, I. (2021). Mosquito Fauna of Anambra East LGA, Anambra State, Nigeria. *Journal of Entomology and Zoological Studies*, 9(3):32 -39.

Egbuche, C., Ezaka, E., Okwubanego, C., Omah, I., Odoh, V., Obi, C., and Nwankwo, I. (2021). Mosquito fauna of Anambra East LGA, Anambra state, Nigeria. *Journal of Entomology and Zoology Studies*, 9(4): 32-9.

Egwu, O. A., Ifeoluwa, A. O., and Fakorede, E. A. (2018). Review of mosquito-borne diseases and its control. *International Journal of Mosquito Research*, 5(1): 1-6.

Ezihe, K. C., Egbuche, C. M., Job, U., Friday, M. C., Anumba, J. U., Nwankwo, E. N., Chukwuzoba, A. O., Umenzekwe, C. C., Ejehu, Z. U. and Inya Agha, I. S. (2017). Mosquito species associated with refuse dumps within Enugu Municipal, Enugu State, Nigeria. *Journal Mosquito Research*; 7(6):1-5.

Goindin, D., Delannay, C., Gelasse, A., Ramdini, C., Gaude, T., Faucon, F., and Fouque, F. (2017). Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in *Aedes aegypti* mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). *Infectious Diseases of Poverty*, 6: 1-15.

Ikeh, E.I., Odu, G.E., Ezike, K.N., Emechebe, G.O. (2022). Prevalence and Awareness of West Nile Virus in Nigeria: A Review. *Journal of Tropical Diseases*, 10(2): 1-9.

Lissenden, N., Kont, M. D., Essandoh, J., Ismail, H. M., Churcher, T. S., Lambert, B. and Lees, R. S. (2021). Review and meta-analysis of the evidence for choosing between specific pyrethroids for programmatic purposes. *Insects*, 12(9): 8-26.

Mogbo, T. C. (2013). Comparative Study on the Abundance of Mosquitoes in Temporary and Permanent Habitats in AWKA, ANAMBRA STATE. *American Journal of Life Science Researches*, 1(4): 1-9.

Mohammed, A., and Chadee, D. D. (2011). Effects of different temperature regimens on the development of *Aedes aegypti* (L) (Diptera: Culicidae) mosquitoes. *Acta Tropica*, 119(1): 38-43.

Moyes, C.L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y. and Dusfour, I. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Neglected Tropical Diseases*, 11(7):5-10.

Ndenga, B. A., Mutuku, F. M., Ngugi, H. N., Mbakaya, J. O., Aswani, P., Musunzaji, P. S., ... and LaBeaud, A. D. (2017). Characteristics of *Aedes aegypti* adult mosquitoes in rural and urban areas of western and coastal Kenya. *PLoS One*, 12(12): 1-8.

Nwankwo, E.N, Ononye IP, Ogbonna CU, Ezihe EK, Onwude CO, Nwangwu, U.C.(2019) Insecticide Susceptibility Status of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Awka South Local Government Area, Anambra State, Nigeria. *Entomology, Ornithology and Herpetology*. 8:2-19.

Oke, O. E., Asenuga, E. R., Omonijo, A. G., and Adekunle, A. A. (2022). Mosquito-borne diseases in Nigeria: A review. *Journal of Parasitology Research*, 1-14.

Ononamadu, C. J., Datit, J. T., and Imam, A. A. (2020). Insecticide resistance profile of *Anopheles gambiae* mosquitoes: a study of residential and industrial breeding sites in Kano Metropolis, Nigeria. *Environmental Health Insights*, 14: 1-17.

Onyido, A. E., Ezeani, A. C., Irikannu, K. C., Umeaneto, P. U., Egbuche, C. M., Chikezie, F. M., and Ugha, C. N. (2016). Anthropophilic Mosquito Species Prevalence In Nibo Community, Awka South Local Government Area, Anambra State, Southeastern Nigeria. *Ewemen Journal of Epidemiology and Clinical Medicine*, 2(1): 14-20.

Opondo, K. O., Jawara, M., Cham, S., Jatta, E., Jarju, L., Camara, M., and D'Alessandro, U. (2019). Status of insecticide resistance in *Anopheles gambiae* (sl.) of The Gambia. *Parasites and vectors*, 12: 1-8.

Otu, A. A. (2019). Mosquito-borne diseases and vector control in Africa: A systematic review and meta-analysis. *PLUS, ONE*, 14(7): 2-9.

Rahimi, S., Vatandoost, H., Abai, M. R., Raeisi, A., and Hanafi-Bojd, A. A. (2019). Status of resistance and knockdown of West Nile vector, *Culex pipiens* complex to different pesticides in Iran. *Journal of Arthropod-Borne Diseases*, 13(3): 2-8.

Smith, L. B. (2015). Pyrethroid Resistance in African Anopheline Mosquitoes: What Are the Implications for Malaria Control? *Trends in Parasitology*, 1(1): 1-9.

Smith, L. B., Kasai, S., and Scott, J. G. (2016). Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. *Pesticide biochemistry and Physiology*, 133: 1-12.

Smith, L.B. and Scott, J.G. (2017). Rapid development of resistance to carbamate insecticides in mosquitos: A case study and review. *Environmental Health Insights*, 11: 1-17.

Wang, C. H., Lv, R. C., Zhu, C. Q., Ai, L. L., Lv, H., Zhang, B., ... and Tan, W. L. (2020). Genetic diversity and population structure of *Aedes aegypti* after massive vector control for dengue fever prevention in Yunnan border areas. *Scientific Reports*, 10(1): 1-7.

Westby, K. M., Sweetman, B. M., Van Horn, T. R., Biro, E. G., and Medley, K. A. (2019). Invasive species reduces parasite prevalence and neutralizes negative environmental effects on parasitism in a native mosquito. *Journal of Animal Ecology*, 88(8): 1215-1225.

WHO (2003). Malaria entomology and vector control: Learners guide, social mobilization and training. Available at www.malaria.org.zw
www.malaria.org.zw/vector/vc24.pdf. pp 109.

WHO (2021). Introduction. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions, 1: 1 -4

WHO (2022a). Planning and setting priorities for resistance monitoring. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions, 3: 6 -8

WHO (2022b). Testing a relevant and representative sample of mosquitoes. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions, 5: 15 -20

WHO (2022c). Standard Bioassay. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions, 6: 21 -44

World Health Organization (2019a). WHO recommended insecticides for indoor residual spraying against malaria vectors. Available online at https://www.who.int/malaria/areas/vector_control/lrssi/insecticides/en/
https://www.who.int/malaria/areas/vector_control/lrssi/insecticides/en/.

World Health Organization. (1998). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva.

World Health Organization. (2016a). Monitoring and managing insecticide resistance in *Aedes* mosquito populations: interim guidance for entomologists.

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

World Health Organization. (2019a). Vector-borne diseases. <https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases><https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases>

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World Health Organization. (2019b). Indoor Residual Spraying: An Operational Manual for Indoor Residual Spraying (IRS) for Malaria Transmission Control and Elimination. <https://www.who.int/publications/i/item/9789241550171>

World Health Organization. (2019c). Integrated vector management. Retrieved April 22, 2023, from <https://www.who.int/news-room/questions-and-answers/item/integrated-vector-management><https://www.who.int/news-room/questions-and-answers/item/integrated-vector-management>

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World Health Organization. (2020). Insecticide resistance. Retrieved April 22, 2023, from <https://www.who.int/news-room/q-a-detail/insecticide-resistance><https://www.who.int/news-room/q-a-detail/insecticide-resistance>.

World Health Organization. (2021). Dengue and severe dengue. Retrieved from <https://www.who.int/news-room/q-a-detail/dengue-and-severe-dengue><https://www.who.int/news-room/q-a-detail/dengue-and-severe-dengue>

Yayock, H. C., Osagiede, N. O., Bitrus, S., Gimba, H. S., and Shehu, R. (2021). Survey Of Mosquito Species, Composition, and Breeding Habitats In Four Communities Of Kaduna Metropolis, Nigeria. *Fudma Journal of Sciences*, 5(3): 122-129.