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Insecticide Resistance Status of the Most Prevalent Mosquito Species in Awka, Anambra State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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 reponses 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 can 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; bendiocarb; pirimiphos-methyl; DDT.

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* [1]. 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 [2]. 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 [3]. The bite sometimes causes 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 or 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 [2]. However, there have been innumerable reports of resistance to insecticides in the four classes by major disease vectors across all regions of the world [2,4]. Most reports of insecticide resistance are majorly on malaria vectors, with little attention on *Aedes* and *Culex* mosquitoes [2, 4-6]. 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. MATERIALS AND METHODS

2.1 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 (Fig.1). The geographical coordinates of Awka are: Latitude 6º12'25"N and Longitude 7º04'04"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 26.2ºC to 30.3ºC, rainfall range of 0.0mm to 680.8 mm and humidity range of 64% to 88% obtained in Anambra East LGA of Anambra State [7]. Factors such as that favour the breeding of mosquitoes abound in Awka. They include: refuse dump [8] water collection in discarded containers, gutter, tyre, basins and swamps [5].

2.2 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.

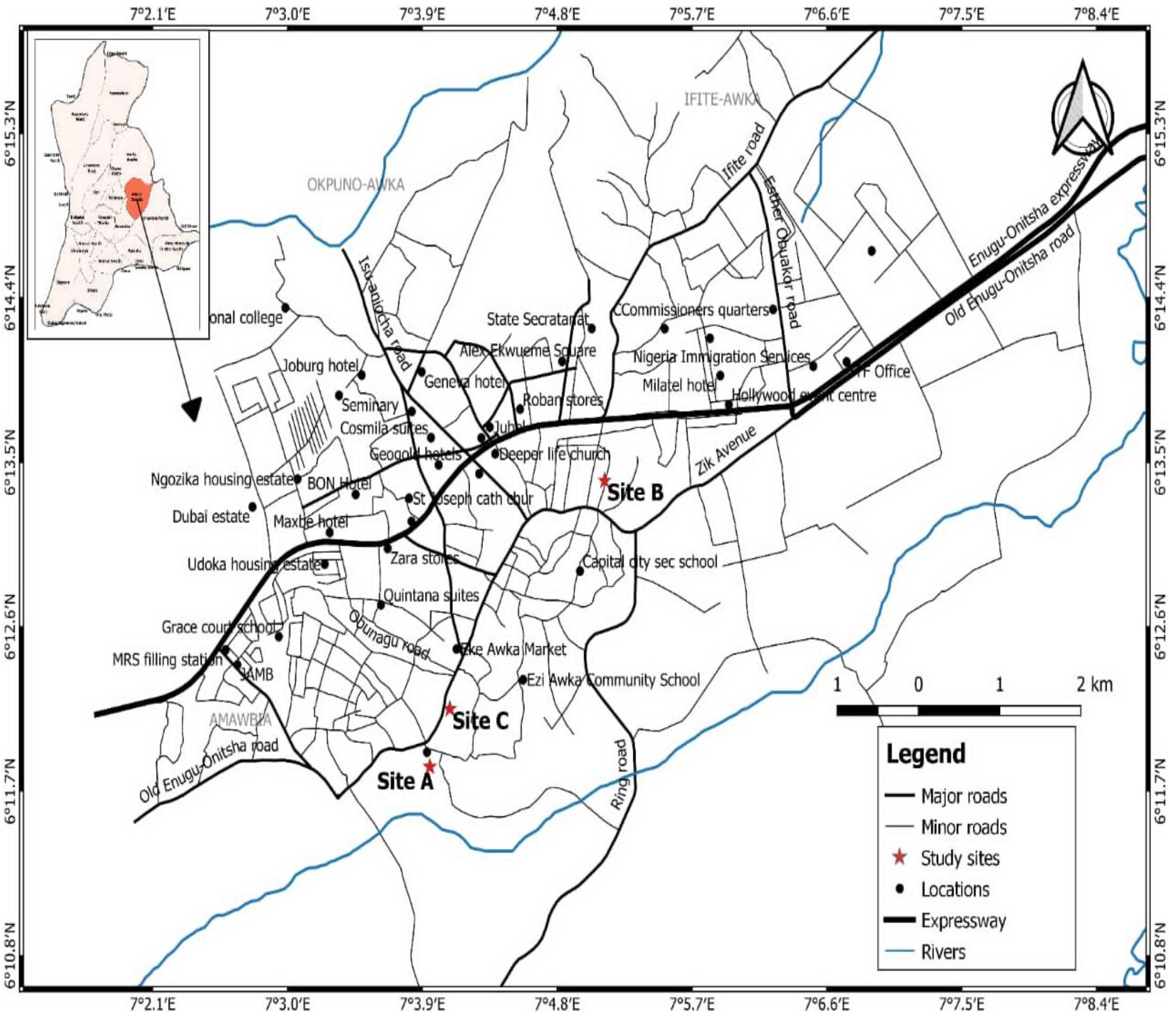

Fig. 1. Map of the study area showing the three sentinel sites marked Site A, Site B and Site C.
Source: Department of Geography, Nnamdi Azikiwe University Awka.

2.3 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 [9] guidelines for mosquito survey. Site A and Site C were randomly selected from the Ezinator section of Awka which 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.

2.4 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.

2.5 Indoor Collection of Adult Mosquitoes

This was carried out using pyrethrum Knockdown Collection Method as described by [10]. 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.

2.6 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 [11]. All catches were between 5:00 pm to 8:00 pm and recorded at quarterly-hour intervals.

2.7 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 [5].

2.8 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 emerged from the larvaed were held in the mosquito cage and were fed with glucose solution as described by [5].

2.9 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 [12] and [13]. 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.

2.10 Insecticide Susceptibility Test

The WHO standard procedures and test kits for adult mosquitoes were used to conduct the insecticide susceptibility test [6, 14, 15]. A maximum of 100 female *Culex quinquefasciatus* (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 (*Culex quinquefasciatus*) 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 ±2°C temperature and 75%±10% relative humidity. The susceptibility status of tested populations was determined according to [15] 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.

2.11 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% (KDT_{50}) and 95% (KDT_{95}) 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 the 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.

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 vitattus* (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 vitattus* (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 *Aeded 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).

3.1 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 %) 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 was 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 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**		

Table 2. Mosquito species found in the study area

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

Mortality responses of *C. quinquefasciatus* mosquitoes are shown in Fig. 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 = 0.000).

4. 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 [5]. 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% [16,17].

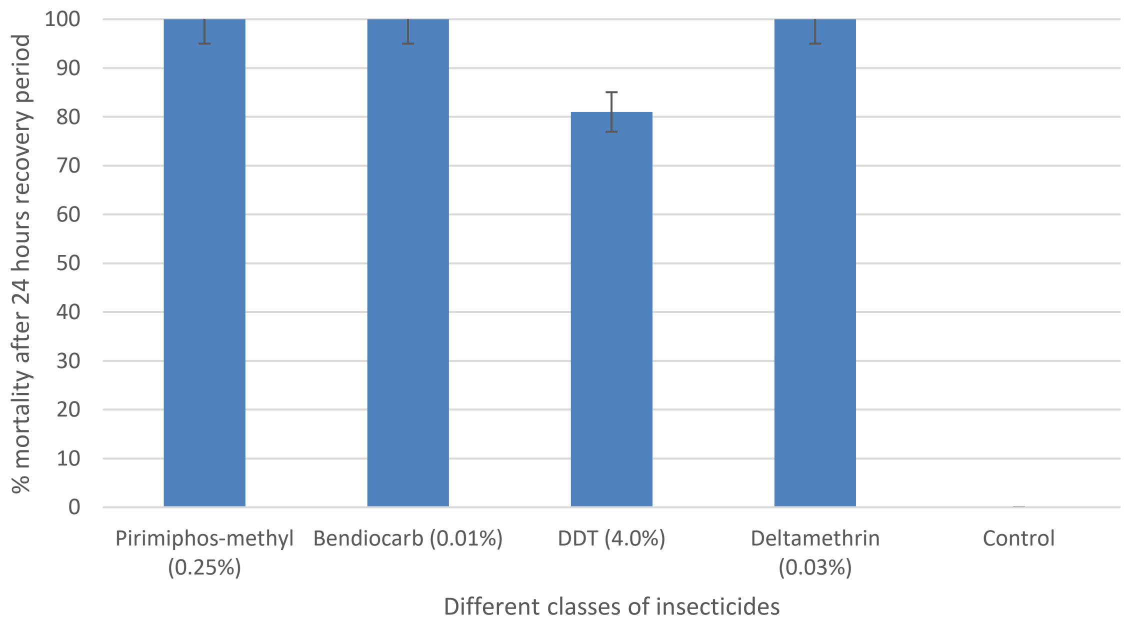

Fig. 2. Mortality rate of *C. quinquefasciatus* to different insecticide classes

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 *C. quinquefasciatus knocked down at different time interval*								KDT50 (95% CI)	KDT95 (95% CI)
		10mins	15mins	20mins	30mins	40mins	50mins	60mins	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*

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 [18,19]. 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 [20] 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 [19].

The most prevalent mosquito collected in this study was *C. quinquefasciatus.* This suggests that the environment of the study area is more suitable for this species of mosquitoes than other species also collected. This is in line with the findings of [1] 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 [21]. 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 [22]. 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 mosquitos initially withstand the toxic effects but ultimately succumb.

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 [23]. 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, 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 emerged against DDT. 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 [24]. The observed quick action suggests deltamethrin is an effective agent for immediate mosquito control, aligning with the findings from researches that 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 [25,26]. [25] 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, [26] found that deltamethrin elicited high mortality rates in mosquito populations in a Kenyan study, reinforcing the consistently high efficacy recorded in Awka. However, studies have warned of the potential for resistance development if pyrethroids are relied upon too heavily and without integrated pest management strategies [27,28].

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 [29] 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 [30]. 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 [31] reported a quick action of carbamates in comparison with organophosphates, suggesting a higher vulnerability of some mosquito populations to this class of insecticides [31,32].

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. [31] 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 [2]. The incomplete mortality could indicate a significant survival of resistant individuals, which can pose challenges for eradication programs [27]. 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 [33] 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 [34]), 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 [31] who noted similar patterns in mosquito populations subjected to organophosphates and hypothesized that metabolic resistance mechanisms could be initially at play [2,31]. 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. [35] who reported significant genetic diversity in the resistance mechanisms among *Culex* populations [35].

5. CONCLUSION

The study has found that different mosquito species exists 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 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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 writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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