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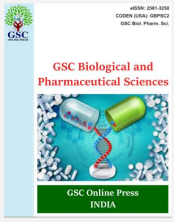

(Research Article)

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High prevalence of genus *aedes aegypti* resistance to carbamate, organochloride, and pyrethroid (COP) pesticides in Adonkolo Campus Federal University Lokoja, Kogi State, Nigeria

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Abstract

Arthropod-borne viruses pose a significant health challenge. Increased prevalence necessitates immediate attention to vector control and resistance management. WHO-approved insecticides to control these mosquitoes include Carbamate, organophosphate and pyrethroid. Adonkolo Campus, Federal University Lokoja metropolis have a disturbing presence of these vectors and therefore the research was aimed to detect the presence of resistance in genus *aedes aegypti* to those insecticides, DDT (organochloride, dichlorodiphenyltrichloroethane 4%), Bendiocarp (carbamate, 0.1%) and a type ii pyrethroid (Alpha cyhalothrin). The LC99 value was used on larval samples to with the help of probit and compared with the diagnostic value from approved WHO values. Increasing resistance to all three insecticides was obtained with Bendiocarb recording the highest rate at an average of 16 (64%) in all three insecticides while that of DDT stands at an average of 18 (72%) The mortality of less than 90% was declared as resistant. The insecticide susceptibility test for genus *aedes. aegypti* against these insecticides in Adonkolo showing resistance with a mortality rate at an average of 84% with Bendiocarb recorded the highest of 94%. The results showed that genus *aedes aegypti* were resistant to all three insecticides used for the WHO Bioassay and the larval test with tempho. Conclusions: The study reveals that the genus *aedes aegypti* population in the Adonkolo Campus tends to resist exposure to insecticide chemicals approved for vector control by WHO.

Keywords: *Aedes aegypti*; Bioassay; Insecticides; Resistance; Guinea savannah

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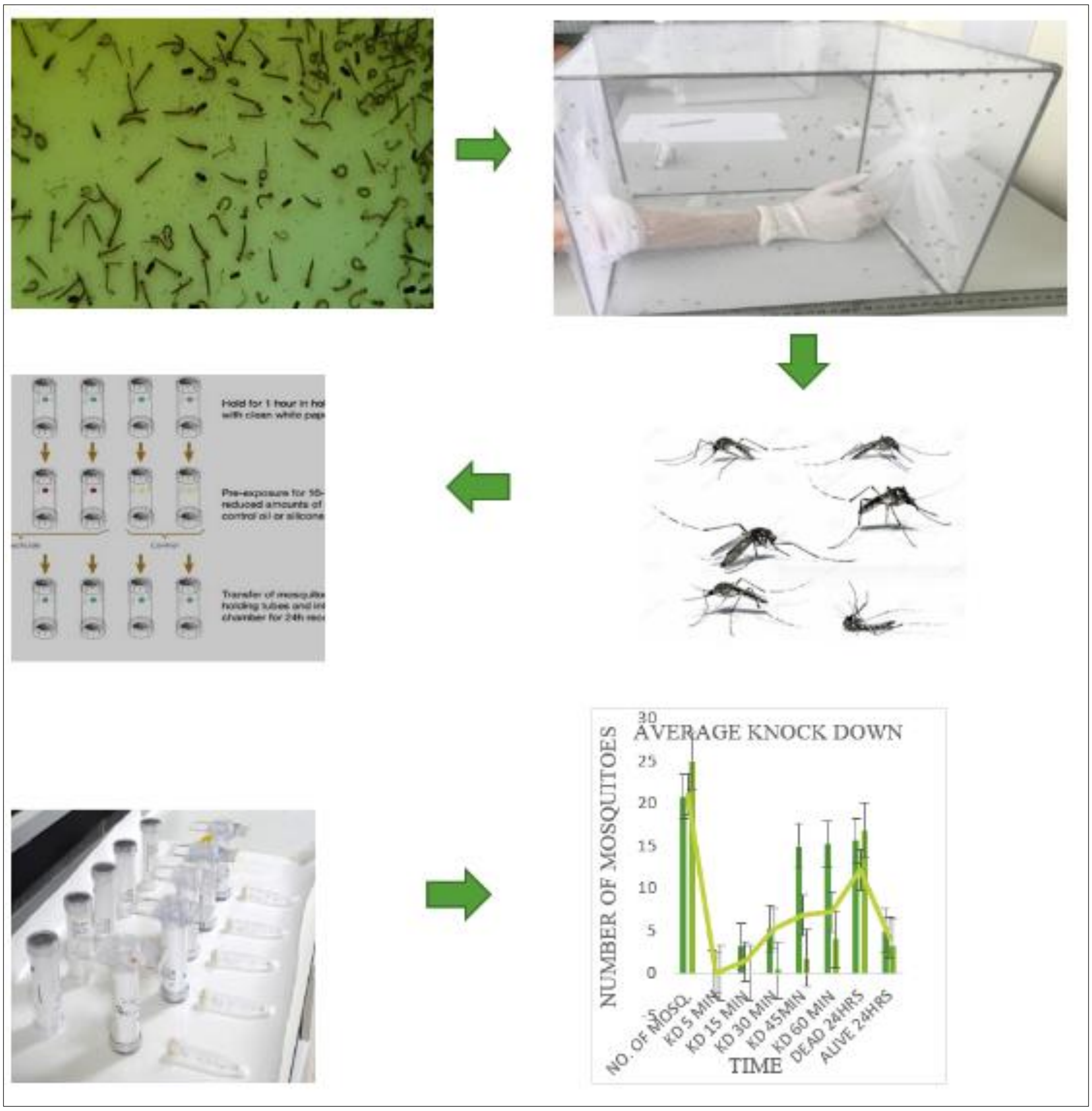
Graphical Abstract



1. Introduction

Aedes mosquitoes belonging to the subgenus *Stegomyia* mostly transmit viruses that are formed on arthropods, such as Dengue, Zika, and Chikungunya, primarily in urban and semi-urban environments. The three viruses are zoonotic in nature involving primates and mosquitoes (Asaga et al., 2022). Genus *aedes aegypti* and *aedes albopictus* are major vectors that usually coexist in reservoirs and transmit infection to humans in densely populated areas (Laure et al., 2016). Genus *aedes aegypti* is mainly found in the tropics and subtropics, while *Aedes albopictus* are invasive species found in virtually all continents. (Martins et al., 2020). Even though they are extremely cosmopolitan and active during the day, they have adapted to survive mostly by feeding on people. These traits have given them the ability to infect and spread disease to humans. (PNAD 2015). The mosquito genus *aedes aegypt*i is a primary vector for several viral diseases, including dengue, Zika, and chikungunya. Over the years, this species has developed varying levels of resistance to different classes of insecticides, complicating control efforts. Globally, insecticide resistance in genus *aedes aegypti* has been documented across various regions, particularly in areas where insecticide-based vector control strategies are heavily relied upon. (WHO 2020). There has been extensive reporting of resistance to pyrethroids, the most frequently used class of insecticides. For instance, genus *aedes aegypti* populations in the Americas, Southeast Asia, and some parts of Africa have shown significant resistance to pyrethroids. (Moyes and Vontas 2017). Nigeria, being one of the most populous countries in Africa, faces significant challenges in controlling genus *aedes aegypti* due to widespread insecticide resistance. Studies across various regions of Nigeria indicate a growing resistance to several classes of insecticides. (Ranson and Lissenden 2016).For instance, resistance to pyrethroids, particularly permethrin and deltamethrin, has been reported in genus *aedes aegypti* populations across different states. Moreover, there are indications of cross-resistance, where mosquitoes resistant to one class of insecticide also show resistance to others, such as organophosphates and carbamates. (Oduola and Olojede 2020 Research has demonstrated that human activity, weather, and climatic conditions, as well as ecology, all contribute to the large-scale spread and vectorial potential of viruses borne by arthropods in terms of both their abundance and geographic distribution. (Magalhae et al., 2020). Other factors that influence their abundance and transmission include international trade that involves movements of goods mostly used ones and people across different countries aids in the introduction of the arthropod-born virus to a new area that never existed before and these will likely result in a serious epidemics. (Do et al., 2020). Another factor that also influences the vectorial capacity of arthropod viruses is the lack of good design of towns and cities with poor installation of water pipes and inadequate sanitary systems. (Li et al., 2021); According to research, because of the continent's slow but steady population growth, arthropod-borne viruses are most common in Africa, where instances have been documented to occur in urban and suburban regions. (Abdullahi et al., 2019). A total of thirty-four (34) nations mostly in their capital states have reported several cases of Dengue virus (WHO 2020). Recent studies by (Asaga et al., 2022) have revealed evidence of seroprevalence in the country and increased resistance of the vectors to major insecticides (Mahe et al., 2022). This indicates that the infections were acquired locally, suggesting the virus is silently circulating in the population and there may be ongoing transmission (Figueiredo et al., 2010). The research was aimed at determining the species composition, abundance, and distribution of *Aedes* mosquitoes and estimating the risk of transmission of arboviruses in the Federal university Lokoja.

2. Material and Methods

2.1. Sampling Site

The larval was collected in an abandoned tyre inside Federal University Lokoja, Adankolo Campus in the metropolitan city of Lokoja, the capital city which lies on (6°31′0″N 3°23′10″E) Nigeria as shown in Figure 1. The state capital is characterized by southern Guinea Savannah. This vegetation type consists of grasslands, trees, and shrubs. It is common in the southern and central regions of the state. The inhabitants of the University campus are mainly medical students and staff offices and the campus is usually active during the day time. The inhabitant sleeps indoors during the night. The sources of water for both sites are boreholes and overhead tanks. During the rainy season, transient pools of water bodies, abandoned containers and a lot of drainages are available thus providing stagnant water bodies.

Figure 1 Sampling site access from Google and modified August, 2024

2.2. Outdoor collection

The larval collection was carried out in fresh stagnant water in an abandoned tyre using a cotton net and a plastic container. The larvae were put inside a well-aerated jerry can and transported to a new biology lab within the university campus in Adonkolo.

2.3. Mosquito rearing

The mosquitoes were maintained following standard insectary conditions (reared at 29 °C, -32 °C, and 70-80% relative humidity and fed with 10% sucrose solution soaked in cotton wool). The larvae were maintained in deionized water and fed daily with fish feed under the same conditions as the adults. The emerging F1 progenies (first filial generation) were used for insect susceptibility bioassays.

2.4. Morphological Species Identification

Mosquitoes were identified morphologically as genus *anopheles gambiae sensu lato* complex species using the procedure adopted for morphological identification keys (Gillies and Coetzee, 1987) and Reuda 2004 for *Aedes* identification.

2.5. Test for Sensitivity

Sensitivity tests to temephos were carried out. The sensitivity test began with the determination of the test concentration. A preliminary test has been carried out with a temephos concentration of 0 as negative control while 0.01 ppm; 0.1 ppm; 10 ppm; and 100 ppm of temephos as test concentration for diagnostic test. At each concentration, 20 larvae were put and each was done twice. Mortality rate was observed after 24 hours. In addition, probit analysis was carried out to determine LC10 and LC100. The concentration was in the range of LC10 and LC100 from the preliminary test. The concentration tests for temephos were 0 ppm as control; 0.02; 0.04; 0.06; and 0.1. At each concentration, 20 larvae were pushed and each was repeated twice. Larval mortality was observed after 24 hours. To determine the resistance status of genus *Aedes aegypti* to the organophosphate was analyzed using probit software. Furthermore, the LC99 value that was obtained was compared with the diagnostic value of resistance to temephos from WHO (≤ 0.02 ppm) (WHO 2016).

2.6. WHO Insecticide Susceptibility Bioassays

WHO Bioassays were carried out on 2-4 days F1 (First filial generation) non-blood fed adult female progenies detect and characterize resistance to DDT (organochloride, dichlorodiphenyltrichloroethane 4%), Bendiocarp (carbamate, 0.1%) and a type ii pyrethroid (Alpha cyalohtrin) for one hour knock down and 24 hours' mortality. (WHO, 2016). Four replicates of 25 mosquitoes per tube were exposed to these insecticides impregnated papers for 1 hour, and knock down rate was recorded for 5 minute, 15 minute, 30 minute, 45 minute and 60 minute and then transferred to the holding tubes and fed with 10% sucrose solution soaked in cotton wool and kept overnight under standard insectary conditions. One tube each was used as a control for each insecticide tested, with 25 females exposed to control papers impregnated only with the carrier oil (QC controls for Pyrethroids, OC control for DDT while OP and PY controls were used for bendiocarb and alpha cyalohtrin respectively). After 24 hours, Mortality rate was recorded for each insecticides and for each tubes. All bioassays were carried out at 27 °C, –30 °C, and 75–80% relative humidity. Resistance status of the population was established according to WHO criteria (WHO 2016) for which populations with mortality >98% are considered susceptible, populations with mortality of 90–98% are considered moderately resistant, and those with mortality >90% as resistant. Susceptible individuals are defined as individuals that did not survive a discriminating dose of the particular insecticide used (WHO 2016).

3. Results

3.1. Figure 2 Composition of the Mosquito Species Identified Morphologically

Out of the larvae collected, 1845 emerged to adult with a total of 1722 (93.33%) found to be predominantly genus *aedes egypti* and a mixed species of 13 (0.70%) genus *anopheles,* 110 (5.10%) *culex* found as shown in the Figure 2; below. Mosquitoes were identified morphologically as *genus anopheles gambiae sensu lato* complex species using the procedure adopted for morphological identification keys(Gillies and Coetzee, 1987) and (Reuda 2004) for *aedes* identification.

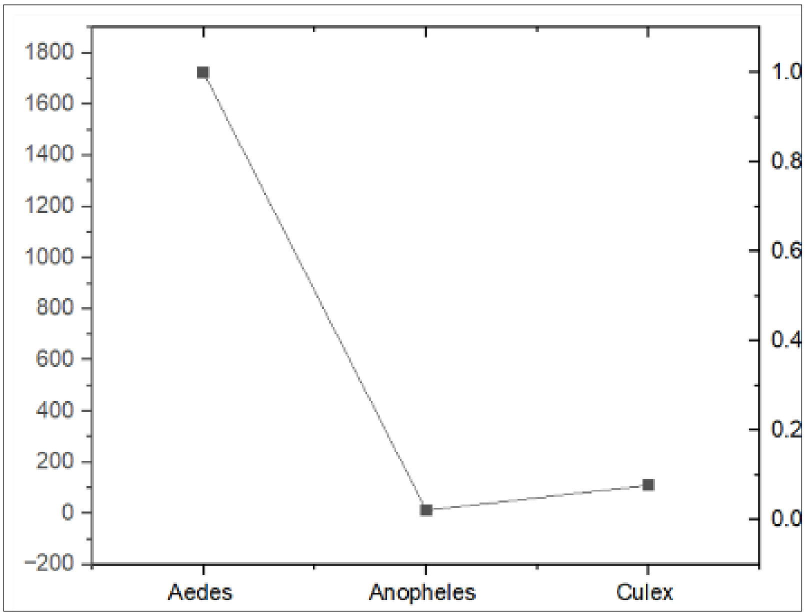


Figure 2 Summary of all vectors (mosquitoes species) Identified morphologically

3.2. Figure 3 and 4 Insecticide Susceptibility Bioassays and Percentage Mortality

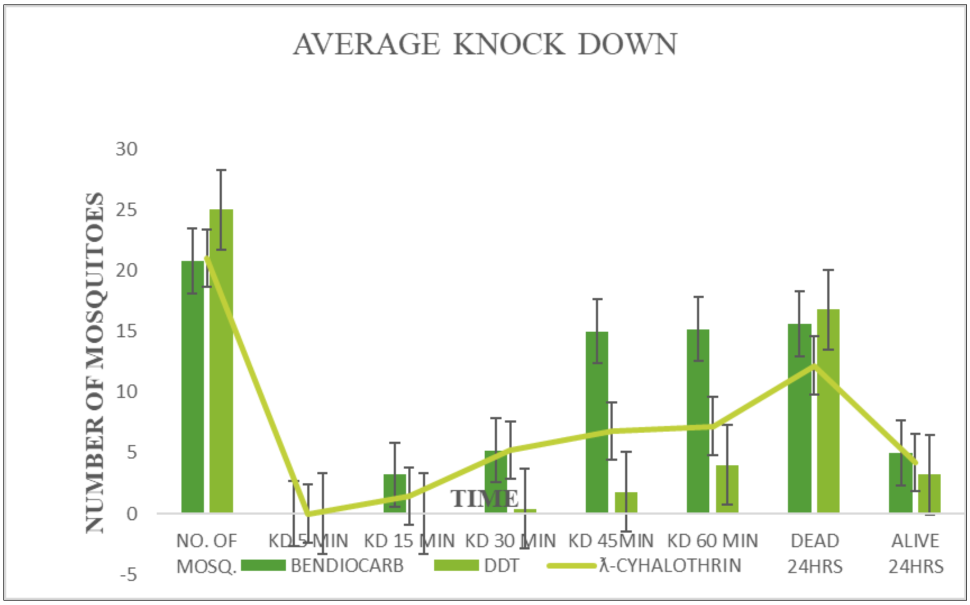


Figure 3 Average knock down rate with error bar representing data variability

WHO Bioassays were carried out on 2-4 days F1 (First filial generation) non-blood fed adult female progenies detect and characterize resistance to DDT (organochloride, dichlorodiphenyltrichloroethane 4%), Bendiocarp (carbamate, 0.1%) and a type ii pyrethroid (Alpha cyalohtrin) for one hour knock down and 24 hours' mortality. (WHO, 2016). Four replicates of 25 mosquitoes per tube were exposed to these insecticides impregnated papers for 1 hour, and knock down rate was recorded for 5 minute, 15 minute, 30 minute, 45 minute and 60 minute. The average knock down in the four replicate indicated that an increasing rate or resistance with time. There was a very low knock down rate even at 30 minute with less than 10 (40%). An increasing resistance to all the three insecticides were noticed at 45 minute to 60 minute. At exactly 60 minutes exposure, the knock down rate with Bendiocarb recording the highest rate at an average of (76%), DDT stands at an average of (20%) while that of alpha-cyahalothrin stand at (36%) as shown in figure 3. At 24 hours exposure, Mortality rate stands to be 74% alpha-cyahalothrin, 84% DDT while Bendiocarb recorded the highest with about 94% mortality rate. As shown in Figure 4.

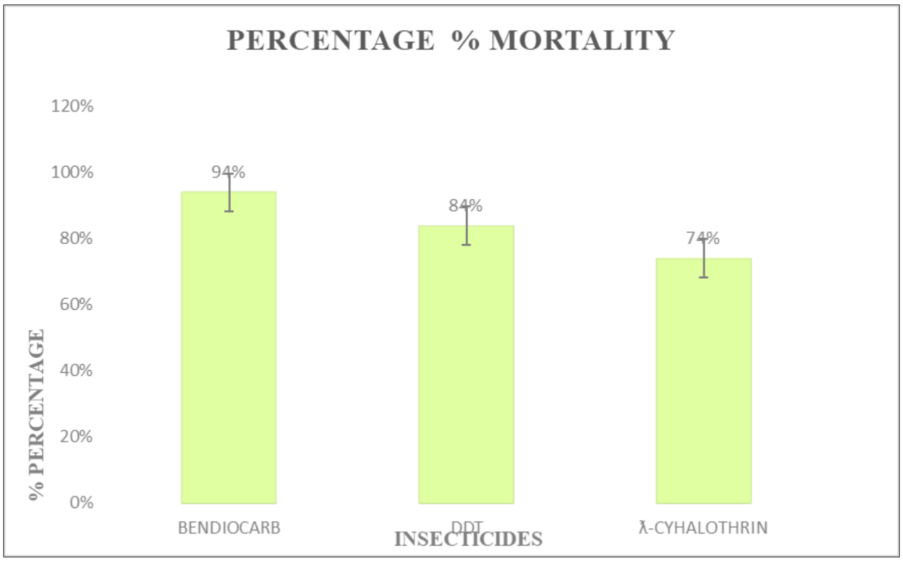


Figure 4 Percentage Mortality with error bar representing Data variability

3.3. Sensitivity Test to ascertain level of mortality to Temephos

After the larval test, the test concentration of LC0= 0 ppm, LC100= 0.1 ppm and LC10 was conducted with the aid of probit tests And found to at a concentration of 0.028 ppm. From probit analysis was found that the LC50 value was 0.074 ppm and the LC99 value was 0.199 ppm and these values were compared with the test value for resistance based on (WHO 2005) on 0.02 ppm and a value of 0.027 signifying resistance. An assessment using SPSS /POSTHOC/DUNCAN C ALPHA indicate the number of mosquitoes that knock down, died, moribund and came back to life after the knockdown at total time of 24 hours. The results of observations of mosquito mortality 24 hours after insecticide exposure can be seen in Table 1 with their mean Values among and in between groups with mean ± S.D. The mean with different letter superscript along the same column implies the mean difference is significant at the $P<0.05$ level as shown in Table 2. According to WHO criteria which is mosquito mortality 24 hours after insecticide exposure < 90% is resistance. Therefore, the susceptibility rate of from the three (3) insecticides stated as resistant.

3.4. Statistical analysis using Anova

Homogeneous Subsets

Table 1 SPSS /POSTHOC/DUNCAN C ALPHA Values among and in between groups with mean ± S.D

	Sum of Squares	**Df**	**Mean square**	**F**	**Sig.**
No of M Between group Within group Total	61.188 9.750	3 12	20.396	25.103	0.000
Dead 0 min Between group Within group	70.938 0.000	15 3	0.000 0.000	-	-
Dead 15 min Total Between group	0.000 0.000	12 15	14.396 0.896	16.070	0.000
KD 30 Min Within group Total	43.188 10.750	3 12	52.250 5.750	9.087	0.002
KD 45 Min Between group Within group	53.938 156.750	3	281.750 4.042	69.711	0.000

Total		12			
KD 60 Min Between group Within group Total	69.000 225.750	15 3	259.667 4.333	59.923	0.000
KD 24 Hrs. Total Between group	845.250 48.500	12 15	369.063 2.313	159.595	0.000
Alive Within group Total Between group	893.750 779.000	 3 12	 23.417 1.125	20.815	0.000

Table 2 Means for groups in homogeneous subsets are displayed as Knock down rate at 5, 15, 30, 45 and 60 min with mortality at 24 Hrs.

Knock down at 5 Minutes

Insecticides			***Subset for alpha = 0.05***	
		N	***1***	***2***
Duncan[a]	Bendiocarb	4	20.75	
	Alpha-cyhalothrin	4	21.50	
	DDT	4		25.00
	Control	4		25.00
	Sig.		0.262	1.000

a. Uses Harmonic Mean Sample Size = 4.000

Knock down at 15 Minutes

Insecticides			**Subset for alpha =**		**0.05**
		N	**1**	**2**	**3**
Duncan[a]	DDT	4	0.00		
	Control	4	0.00		
	Alpha cyhalothrin	4		1.75	
	Bendiocarb	4			4.00
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed;a. Uses Harmonic Mean Sample Size = 4.000.

Knock down at 30 Minutes

Insecticides			**Subset for alpha = 0.05**	
		N	1	2
Duncan a	Control	4	0.00	
	DDT	4	0.50	
	Bendiocarb	4		6.50
	Alpha-cyhalothrin	4		6.50
	Sig.		0.773	1.000

Means for groups in homogeneous subsets ;a. Uses Harmonic Mean Sample Size = 4.000.

Knock down at 45 Minutes

Insecticides			**Subset for alpha =**		**0.05**
		N	**1**	**2**	**3**
Duncan[a]	Control	4	0.00		
	DDT	4	2.25		
	Alpha-cyhalothrin	4		8.50	
	Bendiocarb	4			18.75
	Sig.		0.139	1.000	1.000

Means for groups in homogeneous subsets ;Uses Harmonic Mean Sample Size = 4.000

Knock down at 60 Minutes

Insecticides			**Subset for alpha = 0.05**			
		N	**1**	**2**	**3**	**4**
Duncan[a]	Bendiocarb	4				19.00
Sig.			1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets ;a. Uses Harmonic Mean Sample Size = 4.000.

Knock down at 24 hours

Insecticides			**Subset for alpha =**		**0.05**
		N	**1**	**2**	**3**
Duncan[a]	Control	4	0.00		
	Alpha-cyhalothrin	4		15.25	
	Bendiocarb	4			19.50
	DDT	4			21.00
	Sig.		1.000	1.000	0.188

Means for groups in homogeneous subsets ; a. Uses Harmonic Mean Sample Size = 4.000.

Mortality rate

Insecticides			**Subset for alpha = 0.05**	
		N	**1**	**2**
Duncan[a]	Control	4	0.00	
	Bendiocarb	4	1.25	
	DDT	4		4.00
	Alpha-cyhalothrin	4		5.25
	Sig.		0.121	0.121

Means for groups in homogeneous subsets; Uses Harmonic Mean Sample Size = 4.000.

4. Discussion

Several factors might contribute to resistance, such as frequent and prolonged use of pesticides. These insecticides have been in use for a long period by the National Malaria Control and Rollback Malaria Programmed for the control of mosquitoes including the genus *aedes aegypti* and have posed great for increasing resistance rate. Globally, insecticide resistance in the genus *aedes aegypti* has been documented across various regions, particularly in areas where insecticide-based vector control strategies are heavily relied upon. (WHO 2020). There have also been several reports of resistance to pyrethroids, the most frequently used type of insecticide. (Moyes and Vontas 2017). There is also increasing reportage of growing resistance to several classes of insecticides. (Ranson and Lissenden 2016) For instance, resistance to pyrethroids, particularly permethrin and deltamethrin, has been reported in genus *aedes aegypti* populations across different states. Moreover, the results tallies with the study of (Oduola and Olojede 2020) who show there are indications of cross-resistance, where mosquitoes resistant to one class of insecticide also show resistance to others, such as organophosphates and carbamates. In Northern Nigeria, the situation is particularly concerning as shown by the (Abdullahi et al., 2019) report. The region, characterized by its unique ecological and socio-economic factors, has seen a rise in insecticide resistance among *genus aedes aegypti* populations. Several studies have reported high levels of resistance to insecticides in states northern part of the country. These findings are alarming, as they suggest that conventional vector control methods should have alternatives to control *aedes* vectors. (Ibrahim and Riveron 2019). At exactly 60 minutes of exposure, the knockdown rate with Bendiocarb recording the highest rate at an average of (76%), DDT stands at an average of (20%) while that of alpha-cyhalothrin stands at (36%). At 24 hours of exposure, the Mortality rate stands to be 74% alpha-cyahalothrin, 84% DDT while Bendiocarb recorded the highest with about 94% mortality rate. Transmission and *aedes* vector prevalence is high and impacted by the environment. Not surprisingly, genus *aedes egypti* was established in different parts of the world by researchers in Nigeria (Asaga et al., 2022, Muhammad et al., 2022). The investigation shows that the genus *aedes aegypti* is probably the major *Aedes* species in northern Nigeria. Indeed, this species is known to be the most invasive species in many parts of the world Asaga et al., 2022, Muhammad et al., 2022). Several research from African and Asian countries reported similar patterns with genus a*edes aegypti* being the dominant species than *anopheles* in the suburban areas (Li et al., 2020, Ndosi et al., 2016). The introduction, establishment, and spread of this non-native species presented a serious threat to ecosystems, human activities, and public health in particular. (Akiner et al., 2016. This is because this species is the major vector for most arboviruses in Africa, and the world at large (Crawford et al., 2017. The invasions of this species also serve as an indicator for climatic changes in many tropical and subtropical regions of the world and environmental changes in response to socio-economic development (Liu-Helmersson et al., 2019)

5. Conclusion

Concern over genus *aedes aegypti*'s pesticide resistance is spreading around the world, and it has serious consequences for public health, especially in Nigeria and Africa. The situation in southern guinea Savannah, such as Lokoja, is dire and has to be addressed right once to prevent a comeback of arborial illnesses. To address this issue, efficient resistance management techniques such as consistent monitoring, the application of alternative insecticides, and integrated vector control are crucial.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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