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Original Research Article

INSECTICIDE SUSCEPTIBILITY PROFILE OF *Aedes aegypti* IN A NIGERIAN UNIVERSITY ENVIRONMENT

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ABSTRACT

Resistance to insecticides is one of the major challenges to eradicating insect vectors and arthropod-borne diseases. *Aedes aegypti* is a major vector of several arboviruses, including yellow fever, dengue fever, chikungunya virus, and Zika virus. This study was designed to assess the insecticide susceptibility of *the Aedes aegypti* population at the University of Nigeria, Nsukka, to various commonly used pyrethroids. Ovitraps were used to collect eggs within the University environment. The collected eggs were reared to the adult stage at the insectary unit of the National Arbovirus and Vectors Research Centre, Enugu. Non-blood fed, 3 – 5-day-old, F1 of the female *Ae. Aedes* were exposed to the Centre for Disease Control (CDC) bottle bioassay using permethrin (15 µg/bottle), deltamethrin (10 µg/bottle), and α-cypermethrin (10 µg/bottle). Knockdown was recorded after 15 minutes and 30 minutes (diagnostic time). The results showed that the tested *Aedes* population was susceptible to the three pyrethroids: permethrin (100%), α-cypermethrin (100%), and deltamethrin (98%). This study indicates that pyrethroids can still be used in the control of *Ae. aegypti* in the study area. However, rational use of insecticides and an integrated vector control approach are recommended to prevent the development of resistance in the *Ae. aegypti* population.

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INTRODUCTION

Aedes aegypti has remained one of the most widely distributed mosquito species in tropical and subtropical regions of the world, where its resistance to insecticides poses a major challenge to control efforts [1-2]. It is highly anthropophilic and haematophagous, commonly inhabiting urban areas near human dwellings and showing a strong preference for biting in shaded outdoor areas. It is most active during the day, especially early in the morning and late in the afternoon before sunset [3]. Breeding in *Ae. aegypti* mostly occurs in artificial water-holding containers such as empty flowerpots, discarded tyres, discarded beverage and tomato cans, and swimming pools in or near human settlements [4].

Aedes aegypti is a nuisance pest and a vector of life-threatening diseases, including yellow fever, dengue fever, Zika virus, and chikungunya [5]. Over 100 countries worldwide recorded the highest number of dengue cases in 12 months in 2024, resulting in an all-time high of over 14.6 million cases and more than 12,000 dengue-related deaths [6]. In Nigeria, dengue fever is highly endemic across almost every state and is one of the predominant causes of febrile illnesses that are often misdiagnosed [7]. Also, yellow fever (YF) is an acute viral haemorrhagic disease transmitted by infected mosquitoes, primarily from the *Aedes*, *Haemagogus*, and *Sabethes* species. It can spread quickly

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and impose a substantial burden on public health [8-9]. It affects both humans and non-human primates in a sylvatic transmission cycle [10-11]. A greater percentage of patients with YF are asymptomatic, with about 15% – 25% of infected persons with symptoms entering the severe phase, which has a death rate of 20% – 60% [12]. Nigeria is endemic for YF outbreaks due to suitable environmental conditions such as favourable climate, vegetation, human activities (poor housing conditions, deforestation, indiscriminate disposal of artificial containers, overcrowding, etc.), which lead to increased proliferation of the vector and also low immunization programmes in many regions [13]. These trends highlight the importance of sustained surveillance and control of the *Ae.aegypti* populations in Nigeria and other endemic regions.

Different vector control methods have been tested to reduce *Ae. aegypti* population with varying degrees of success. In many developing countries, including Nigeria, *Ae*. *aegypti* control still relies heavily on chemical insecticides. However, due to their extensive and sometimes indiscriminate use, there have been reports of resistance to these insecticides in the Americas, Africa, and Asia [14]. The development and spread of insecticide resistance across all four major classes of insecticides poses a significant challenge to public health, as they reduce the effectiveness of chemical-based larvicidal or adulticidal control programmes [15]. The mechanisms responsible for insecticide resistance in *Aedes* worldwide usually involve target-site mutations (e.g., kdr mutations involving V410L, V1016I, and F1534C) and metabolic detoxification via cytochrome P450 monooxygenases, esterases, and glutathione S-transferases [16]. Pyrethroids are commonly employed in public health because of their human-friendly properties, high insecticidal effectiveness at low doses, low cost and quick knockdown effects [17]. Pyrethroids used in public health against *Aedes* mosquitoes include deltamethrin, alpha-cypermethrin, permethrin, cyfluthrin, and lambda-cyhalothrin [18]. Pyrethroid resistance in *Ae*. *aegypti* is mainly attributed to mutations in one or more amino acids of the voltage-gated sodium channel (VGSC), which prevent interactions with the pyrethroid molecules [19].

The emergence and re-emergence of dengue fever and other arboviral diseases in Nigeria require effective vector control programmes, including monitoring of vector population insecticide susceptibility. Therefore, resistance surveillance should be an important aspect of public health and vector control in Nigeria to ensure rational choices for vector control strategies.

MATERIALS AND METHODS

Study Area

The University of Nigeria, Nsukka is situated in Nsukka metropolis of Nsukka Local Government Area in Enugu State, Nigeria. It lies between longitudes 7°24'E and 7°26'E, and latitudes 6°51'N and 6°53'N, with an elevation of approximately 456 meters above sea level [20]. Nsukka has a tropical savanna climate with distinct wet and dry seasons. It is usually warm year-round, with the rainy season from April to November and the dry season from November to late March. Rainfall is the most predominant form of precipitation throughout the year. The average annual temperature and rainfall are 24.9ºC and 1,579 mm, respectively [21-22]

Collection and Rearing of the mosquito

Ovitraps were used to collect the *Ae. aegypti* eggs used in this study. The ovitraps used were plastic cups lined with white cotton strips and half-filled with water. These cups were placed at different locations within the University of Nigeria, Nsukka, in cool, shaded areas. The ovitraps were inspected every 48 hours for trapped eggs. The strips with eggs were retrieved and air-dried before transportation to the National Arbovirus and Vectors Research Centre, Enugu (NAVRC).

They were reared in a temperature-, light-, and humidity-controlled insectary. A temperature range of 27 ± 2 °C and a relative humidity of approximately 70%–80% were maintained in the insectary to provide favourable environmental conditions. A photoperiod of 12 hours of light and 12 hours of darkness was also maintained. The white strips containing the eggs were placed in larval bowls with water, and 0.2 g of yeast was added to stimulate egg hatching. The larvae were fed Tetra Cichlid Floating Fish Food (®/TM/©2019 Germany). On pupation, the pupae were picked using Pasteur pipettes from the larval bowls and transferred to plastic cups half-filled with water. The plastic cups containing the pupae were introduced into a 30 cm x 30 cm x 30 cm netted cage, which served as the holding cage for adult emergence. The emergent adults were maintained on 10% sugar solution soaked in cotton wool and placed in a petri dish. Identification was done using morphological taxonomic keys for *Aedes* species [23].

Susceptibility Testing

The CDC bottle bioassay protocol and kits [24] were used for this study. Technical grade of the pyrethroid insecticides (Permethrin – 15 µg/bottle, Deltamethrin – 10 µg/bottle and α-Cypermethrin – 10 µg/bottle) and 1 ml of acetone/bottle for the control were provided by the National Arbovirus and Vectors Research Centre, Enugu. Non-blood fed, 3 – 5-day-old, F1 of the female *Ae. aegypti* were used for the susceptibility tests. Twenty-five mosquitoes were gently aspirated into the coated bottle per replicate. For each insecticide, 4 replicates were tested alongside the control. The bottles were placed on their sides after the mosquitoes were introduced. A timer was started immediately after the mosquitoes were introduced into the bottle, and the number of knockdown mosquitoes was recorded after 15 minutes and then 30 minutes, which is the diagnostic time.

Data Analysis

The data were analyzed using IBM SPSS Statistics Version 23.0 (IBM Corp., Armonk, NY, USA). Mean mortality values across the pyrethroids were compared using One-way Analysis of Variance (ANOVA). The level of significance was set at $p < 0.05$. The results were interpreted using the WHO criteria [25]. According to this criterion, mortality rates between 98 % and 100 % indicate a susceptible mosquito population; rates between 90 % and 98% indicate possible resistance; and rates below 90% indicate resistance.

RESULTS

Knockdown effects of the pyrethroids against *Aedes aegypti*

In this study, α-cypermethrin, deltamethrin and permethrin were used to bioassay the *Ae. aegypti* collected from wild populations in the University of Nigeria, Nsukka. A total of 300 female *Ae. aegypti* were obtained from the population sample and morphologically identified for the test.

The mortality and survival rates of *Ae. aegypti* exposed to α-cypermethrin, deltamethrin and permethrin are summarized in Table 1.

Table 1: Percentage mortality of *Aedes aegypti* exposed to pyrethroids.

Insecticide	Mortality (%) within diagnostic time intervals			Survival (%) within diagnostic time intervals		
	0 m	15 m	30 m	0 m	15 m	30 m
Alpha-cypermethrin	00	89	100	100	11	00
Deltamethrin	00	92	98	100	08	02
Permethrin	00	96	100	100	04	00
Control	00	00	00	100	100	100

Diagnostic intervals are in minutes (m)

At 0 minutes of exposure, no mortality was noticed. At 15 minutes of exposure, 89%, 92% and 96% mortality of *Ae. aegypti* was recorded for α-cypermethrin, deltamethrin and permethrin, respectively. At the end of the 30-minute exposure, 100% mortality of *Ae. aegypti* was found to be susceptible to α-cypermethrin and permethrin, with 98% mortality observed with deltamethrin. However, no mortality was recorded in the control group.

Mortality at 15 and 30 minutes was comparable across the three insecticides. However, at 15 minutes and 30 minutes, mortality among the *Ae. aegypti* exposed to the insecticides was significantly higher than in the control group ($p < 0.05$) (Table 2).

Table 2: Mean mortality of *Aedes aegypti* exposed to pyrethroids.

Insecticide	Diagnostic time interval 0 m	15 m	30 m
Alpha-cypermethrin	0 ± 00	22.25 ± 3.40^a	25.00 ± 0.00^a
Deltamethrin	0 ± 00	23.00 ± 2.16^a	24.50 ± 1.25^a
Permethrin	0 ± 00	24.00 ± 0.82^a	25.00 ± 0.82^a
Control	0 ± 00	0.00 ± 0.00^b	0.00 ± 0.00^b

Values as mean ± Standard deviation. Values with different letter superscript along a column were significantly different ($p < 0.05$).

DISCUSSION

The record of 100 % mortality against the test population of mosquitoes using α-cypermethrin and permethrin, and 98% with deltamethrin shows that all the *Ae. Aegypti* tested were susceptible to the three pyrethroids used in this study, according to the WHO [25] criteria. In this study, α-cypermethrin and permethrin achieved 100 % mortality against the test population of *Ae. aegypti* within 30 minutes of exposure. No resistance was observed across the pyrethroids.

The susceptibility of the *Ae. aegypti* to deltamethrin obtained in this study is consistent with the findings of [15] in Awka, Nigeria, where a 100% mortality rate was recorded. Similar findings were also reported in Southern Afar, Ethiopia, for deltamethrin and permethrin, but resistance to alpha-cypermethrin in Awash Arba town, a part of the study area [18] . Both Nwankwo *et al.*[15] and Seid *et al*. [18] conducted studies in larger study areas, demonstrating the continued efficacy of pyrethroids for Aedes control. Studies conducted across larger areas demonstrated the continued efficacy of pyrethroids for Aedes control [15,18] . Our findings, using deltamethrin, partly contrast and partly agree with the report in both the nonfarm and farm areas in Lagos (26). Their report showed that *Aedes aegypti* collected from farm areas were susceptible to deltamethrin, whereas those from nonfarm areas were resistant. However, all the mosquito populations were susceptible to the permethrin they used. Reported resistance to deltamethrin at Umudike contrasts with our report [27]. Working across more diverse study areas increases the likelihood of detecting resistance [26,27]. Also, in another study in Sokoto, resistance of *Ae. aegypti* to deltamethrin was reported [28]. Resistance of *Ae. aegypti* to deltamethrin has also been reported in other African countries, including Ghana, Tanzania, and Benin [28–30].

Report of susceptibility of *Ae. aegypti* to permethrin in all farm and nonfarm site populations in Lagos is consistent with the 100% mortality recorded in this study [26]. A similar study also reported the susceptibility of *Ae. aegypti* to permethrin in Osun State, Nigeria [31]. However, varying degrees of resistance of *Ae. aegypti* to permethrin was reported in Sokoto [28], although some mosquitoes from parts of the study area were found to be susceptible. There were similar findings in Bauchi, where the *Ae. aegypti* populations from different study areas were treated with permethrin [32]. Suspected resistance to permethrin has also been reported in Makurdi, Nigeria [33]. These patches of resistance and susceptibility to permethrin in *Ae. aegypti* were also reported in Senegal [34], showing that improper handling of the insecticide might lead to an increasing problem of resistance in the future.

Our record for susceptibility to α-cypermethrin is consistent with that reported in Sokoto [28], where 100% mortality was also observed. However, susceptibility of the Aedes mosquito to all pyrethroid-based insecticides except α-cypermethrin (94%) was recorded in Osun state [31]. Also, varying levels of resistance and susceptibility among Aedes mosquitoes at different study sites in Ifangni, Benin Republic were reported [35].

The susceptibility of the *Ae. aegypti* mosquitoes to these insecticides suggests relatively low selection pressure, which may be attributed to the low use of these pyrethroid-based insecticides at the University of Nigeria, Nsukka, compared to more agriculturally intensive areas. The development of resistance in some of these regions could be due to heavy agricultural activities and urbanization, leading to uncontrolled and incessant use of these insecticides, which are sometimes washed into nearby water bodies, thereby exposing developing larvae to insecticide residues. This exposure could trigger the development of resistance, which might have accounted for the results recorded in those areas. Frequent applications in houses may also contribute to the development of resistance in these vector species, especially with insensitivity to recommended doses.

CONCLUSION

This study showed that the *Ae. aegypti* populations from the University of Nigeria, Nsukka, were susceptible to the three commonly used pyrethroid insecticides tested: α-cypermethrin, deltamethrin and permethrin. These findings suggest that pyrethroids may still be effective for controlling *Ae. aegypti* in the study area. However, proper use of insecticides, such as applying at recommended rates and doses, is essential to minimize the risk of resistance development. Also, regular and periodic surveillance should be conducted to monitor changes in susceptibility and provide evidence for selecting effective vector control measures. In addition, integrated vector control management strategies should be adopted to sustain the effectiveness of the existing pyrethroid insecticides in the *Ae. aegypti* population control.

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AUTHORS' CONTRIBUTIONS

This work was carried out through the collaborative efforts of the authors. The writers of this publication are solely responsible for any claims relating to its content. PNE handled the investigation, the original draft, and data analysis; GIN handled conceptualisation, supervision, and writing, while NCA handled manuscript validation and resource provision. References, manuscript validation, and provision of resources were handled by MIN; CMO provided technical support and resources.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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