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Yahaya Muhammad Abdullahi
Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Sani Abdullahi Fana
School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Haliru Muhammad Bandiya
Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Usman Salisu Batagarawa
Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Muftau Zulkarnain
Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Halimat Lamide Uthman
Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Corresponding Author:
Yahaya Muhammad Abdullahi
Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Susceptibility status of *Aedes aegypti* to pyrethroids in Usmanu Danfodiyo University main campus, Sokoto, Nigeria

Yahaya Muhammad Abdullahi, Sani Abdullahi Fana, Haliru Muhammad Bandiya, Usman Salisu Batagarawa, Muftau Zulkarnain and Halimat Lamide Uthman

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Abstract
Study was conducted to assess the level of insecticide resistance in *Aedes aegypti* to different pyrethroids in four different locations within the Usmanu Danfodiyo University Sokoto (UDUS) main campus. Larvae and pupae of the mosquito were obtained from Biological Garden, Gidan Yaro, Adarawa and Gidan Yunfa communities. The collected larvae and pupae were taken to the Entomology Laboratory of Usmanu Danfodiyo University and reared on plastic containers. The newly emerged adults were aspirated into separate containers and fed with sugar solution. The 2-5 days old adult females of *Aedes* mosquitoes were subjected to WHO bioassays to assess the mortality effects of Alphacypermethrin, Deltamethrin, and Lambdacyhalothrin and Permethrin insecticides on *Ae. Aegypti* mosquitoes. The knockdown mosquitoes were counted and recorded for an hour. Final mortality count was done24 hours post-exposure. Results obtained revealed that the tested mosquitoes yielded different level of resistance to the insecticides. Maximum efficacy of 98-100% mortality was recorded on treatment with Alphacypermethrin across all the sites. Permethrin was susceptible to mosquitoes from Gidan Yunfa and Gidan Yero. Mosquitoes across all the sites were resistance to Lambdacyhalothrin and Deltamethrin with mortality ranging from 76-84%. The least mortality of 68% was recorded on mosquito population from Adarawa treated with Permethrin.

Keywords: Susceptibility, dengue vector, pyrethroids, bioassay, knockdown

1. Introduction

Mosquitoes as vectors can transmit several pathogenic organisms such as protozoans, arboviruses and filarial worms that cause diseases of public health importance [1]. These diseases include malaria, lymphatic filariasis, dengue and yellow fever and chikugunya. These diseases continue to impose a heavy burden on human societies and impede welfare and economic development [2].

The prevalence and incidence of mosquito-borne viral diseases are increasing globally [3]. Dengue is an important public health disease currently occurring in 128 countries [4, 5]. Several species of *Aedes* mosquitoes are involved in the transmission of arboviral diseases, responsible for mortality and morbidity in different countries [6]. Dengue is an emerging mosquito viral disease which is transmitted through the bite of an infected female *Aedes* mosquito, is public health threat in disease-endemic regions [7]. *Ae. Aegypti* is one of the main dengue virus vectors in Africa [8, 9].

The dengue vector, *Aedes aegypti* (Linnaeus) is responsible for dengue virus transmission [10]. It is widely distributed in the tropical and sub-tropical regions of the world and is well adapted to thrive in urban environments [11-13], where there is increased contact between mosquito vectors and human hosts, thus facilitating arbovirus transmission [6]. Major *Aedes* mosquito species in Nigeria include: *Ae. Aegypti*, *Ae. albopictus*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. Simpsoni* complex, *Ae. Vittatus* [14]. *Aedes* species are active and bite both humans and animals only during the daytime with early mornings and evenings as peak biting period [15].They are both endophilic and endophagic [16].

Despite the different control measures taken, mosquito borne diseases still remain a major cause of death and are increasingly threatening over 831 million (70%) people on the African continent [8, 17].

Nigeria is one of the high-risk countries for yellow fever transmission, with over 40,000 suspected cases reported between 1985-1991 [18]. Furthermore, there has been reported cases of yellow fever re-emergence in the Country with 341 suspected cases from 16 states with Kano, Kebbi, Kogi, Kwara, Nasarawa and Zamfara confirming the disease [19]. In 2016 there were reported cases of dengue outbreak in Sokoto State leading to outright and sudden death of some of the patients [20]. Although the cases were not confirmed but preliminary investigation conducted within the areas where cases were reported revealed higher density of *Aedes* mosquitoes.

The emergence and re-emergence of viral diseases transmitted by vectors raises global concerns about the causes of the emergence, threats to health, burden and the feasibility of prevention and control [21]. This phenomenon could be attributed to urbanization which was believed to have modified the ecology of *Aedes* mosquitoes by changing the composition and dynamics of the species, and increasing the abundance of their breeding sites, and thus contribute to arbovirus outbreaks [22]. This could be the reason for re-emergence of some of these diseases in different parts of Nigeria. It could also be due to breeding of the vector species which was observed to be in man-made containers such as cans, abandoned tires, earthen pots and others habitats associated with human dwellings [23, 24]. The construction and modernization projects going on in different parts of the country might add up to the breeding grounds for the vectors.

The habit of littering the environment with discarded containers among dwellers was also observed to provide good breeding sites for these mosquitoes [25]. This is also very common practice in some parts of Nigeria and might add to the causes of outbreak and reemergence of the *Aedes* vectored diseases. Earlier, it was reported that the threat of vector-borne diseases has risen due to the growth of cities, progression of climate change, and increase in globalization and international travel [6]. The ongoing constructions in the study area might have increases the availability of water holding containers which could have resulted in the abundance of *Aedes* mosquito.

The use of chemical insecticides is the most effective means of controlling mosquitoes, but its efficacy is being considerably challenged by widespread resistance to multiple classes of insecticides [26]. This is a serious problem toward effective implementation of vector control strategies. It was reported that development of resistance to the insecticides can hinder effective control of vector borne diseases efforts [26].This might also be the case in this part of the country as report indicated insecticide resistance as major problem in the control of yellow fever and other arboviral diseases [27]. Also multiple resistances to pyrethroids and organophosphates have been reported in *Ae. aegypti* populations from Asia [28], South America and The Caribbean [29, 30] and in a lesser extent in Africa [31]. However, there is no recorded information on insecticide resistance in *Aedes* mosquitoes from this part of Nigeria. Considering the reported cases of *Aedes* resistance to different insecticides, adequate attention needs to be focused on this vector species. This study aimed to assess the resistant status of adults *Aedes aegypti* mosquitoes to deltamethrin, Permethrin, alphacypermethrin and lambda-cyhalothrin from 4 different locations within Usmanu Danfodiyo University, Main Campus, and Sokoto, Nigeria.

2. Materials and Methods

2.1. Study Area

The study was conducted in Usmanu Danfodiyo University Sokoto permanent site which is located at latitude 13.1274^{O}N and longitude 5.2046ºE situated in Wammako Local Government Area, Sokoto. Different locations within the university were used for larval sampling.

2.2 Collection and Rearing of Larvae and Pupae

The study was conducted between the months of July to October, 2020. Larval and Pupal stages of *Aedes* mosquitoes were collected from discarded tires, cans, discarded clay pots and gutters from different locations within the study area. The samples were collected into white transparent plastic containers and taken to the Entomology Laboratory, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, for rearing. The containers were covered with small mesh size net and larvae fed a mixture of cabin biscuit and yeast tablets. The set up were kept under laboratory conditions and exposed to equal hours of light and darkness (12hour light and 12hour darkness). The newly emerged adult mosquitoes were transferred into the rearing cages and fed with sugar solution soaked in cotton wool. Adults *Aedes* 3-5 days old were used for susceptibility test using WHO procedure [32].

2.3 Bioassay Test

The Bioassays were carried out according WHO procedure in which mosquitoes were exposed to insecticide-impregnated papers [32]. Four replicates of 20-25 mosquitoes each were tested for susceptibility to each insecticide. The mosquitoes were acclimatized for one hour in holding tubes before the bioassay was conducted. The acclimatized mosquitoes were then transferred into different exposure tubes containing papers impregnated with Alpha-cypermethrin (0.05%), Deltamethrin (0.05%), Lambdacyhalothrin (0.05%) and Permethrin (0.75%) [33]. Knocked down mosquitoes were counted and the number recorded for one hour. After the exposure period, the mosquitoes were transferred back to the holding tube, and allowed stand upright for 24 hours. A piece of moist cotton wool was placed on the wire mesh end of the tube and place the tubes in a wooden box with large holes for ventilation and covered with a damp towel. After 24 hours after exposure, dead mosquitoes were counted. In addition, 2 batches of 20-25 mosquitoes were separately used as control for each of the insecticide. Control mosquitoes were exposed to insecticides free papers and mortality count taken as in test experiments. All bioassays were performed in Entomology Laboratory, Usmanu Danfodiyo University Sokoto, Nigeria. Temperature and relative humidity ranges from 27± 2^{0}C and 65-80% relative humidity.

2.4 Identification

The mosquitoes used in both the tests and control experiments were examined under the stereo microscope and identified to species level using morphological keys (Nelson 1986, Service, 2012) [34, 35].

2.5 Data analysis
The results of the bioassays were interpreted based on the overall percent mortality after 24hour of exposure to each insecticide across all four exposed replicates expressed as percentage of the total number of exposed mosquitoes. Susceptibility interpretation was done based on the WHO recommended criteria where 98–100% mortality indicates susceptibility; 90–97% mortality suggests possible resistance and required further confirmation and <90% mortality indicates the presence of resistance [32].

3. Results
The mortality of *Aedes aegypti* from different locations 24 hours after exposure to different pyrethroids is shown in Tables 1-4. The mortality rate showed that the different insecticides exhibited different degree of resistant. A total of 400 *Aedes* mosquitoes from Gidan Yunfa were tested. It is clear from Table 1 that the tested mosquitoes were susceptible to Permethrin and Alphacypermethrin with mortality values of 98 and 100% respectively indicative of the susceptibility nature of the insecticides. Mosquitoes exposed to Deltamethrin and Lambda-cyhalothrin showed resistance with mean mortality of 80% and 84% respectively. The mortality value of 2% was recorded on untreated control mosquitoes against Deltamethrin. It's also evident from Table 2 that 370 mosquitoes from Gidan Yero were exposed the insecticides. Mosquitoes treated with Alphacypermethrin and Lambdacyhalothrin showed susceptibility to the insecticides with 98% and 99% mortality respectively after 24hour exposure period. Deltamethrin and Permethrin revealed different level of resistance with 83% and 76% mortality respectively after same period of exposure. No mortality was recorded on the control after same exposure period.

Table 1: Mortality of *Aedes aegypti* from Gidan Yunfa 24 Hours after exposure to insecticides

Mortality Rate (%)						
Insecticides	**Number of Mosquito Tested**	**Number Dead**	**% Mortality**	**Number in Control**	**% Mortality in Control**	**Resistance status**
Alphacypermethrin	100	100	100	50	0	Susceptible
Deltamethrin	100	80	80	50	2	Resistance
Permethrin	100	98	98	50	0	Susceptible
Lambdacyhalothrin	100	84	84	50	0	Resistance
Total	400					

<90% means Resistance; 90% -97% means Possible Resistance; 98% -100% means Susceptible

Table 2: Mortality of *Ae. Aegypti* from Gidan Yero 24 Hours after exposure to insecticides

Mortality Rate (%)						
Insecticides	**Number of Mosquito Tested**	**Number Dead**	**% Mortality**	**Number in Control**	**% Mortality in Control**	**Resistance status**
Alphacypermethrin	80	78	97.5	50	0	Susceptible
Deltamethrin	100	83	83	50	0	Resistance
Permethrin	90	76	84	50	0	Resistance
Lambdacyhalothrin	100	99	99	50	0	Susceptible
Total	370					

<90% means Resistance; 90% -97% means Possible Resistance; 98% -100% means Susceptible

Similar trend was observed when mosquitoes from biological garden were exposed to the insecticides (Table 3). Mosquitoes exposed to Alphacypermethrin showed full susceptibility with 100% mortality while Lambdacyhalothrin yielded 90% mortality indicative of possible resistance. Permethrin and Deltamethrin recorded similar resistant status with 81% and 79% mortality respectively. No mortality was recorded on the control mosquitoes. Results on Table 4 revealed that Alphacypermethrin recorded 99% mortality against *Ae. Aegypti* which confirmed the susceptibility nature of the mosquitoes from Adarawa community. Deltamethrin and Permethrin recorded same level of resistance with 80% mortality each. Lambdacyhalothrin recorded low mortality result with 68% indicating resistance. Only 2% mortality was recorded on control mosquitoes against Permethrin treated samples. Correction of mortality results using Abbott's formula was not done since the recorded mortality was less than 5%.

Table 3: Mortality of *Ae. Aegypti* from Biological Garden 24 Hours after exposure to insecticides

Mortality Rate (%)						
Insecticides	**Number of Mosquito Tested**	**Number Dead**	**% Mortality**	**Number in Control**	**% Mortality in Control**	**Resistance status**
Alphacypermethrin	80	80	100	50	0	Susceptible
Deltamethrin	100	79	79	50	0	Resistance
Permethrin	100	81	81	50	0	Resistance
Lambdacyhalothrin	80	90	90	50	0	Possible Resistance
Total	360					

<90% means Resistance; 90% -97% means Possible Resistance; 98% -100% means Susceptible

Table 4: Mortality of *Ae. Aegypti* from Adarawa 24 hours after exposure to insecticides

Mortality Rate (%)						
Insecticides	**Number of Mosquito Tested**	**Number Dead**	**% Mortality**	**Number in Control**	**% Mortality in Control**	**Resistance status**
Alphacypermethrin	100	99	99	50	0	Susceptible
Deltamethrin	100	80	80	50	0	Resistance
Permethrin	80	54	68	50	2	Resistance
Lambdacyhalothrin	100	80	80	50	0	Resistance
Total	380					

<90% means Resistance; 90% -97% means Possible Resistance; 98% -100% means Susceptible

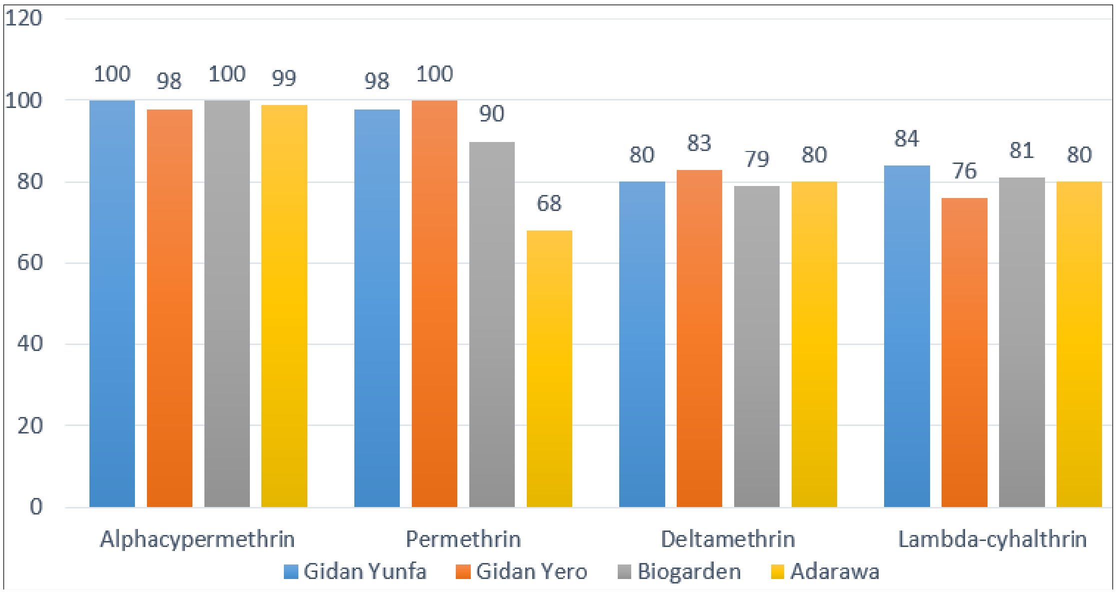

Fig 1: Susceptibility status of *Aedes aegypti* from the 4 locations within Usmanu Danfodiyo University, Sokoto.

4. Discussion

This study clearly revealed the effects of different pyrethroids on mortality of *Ae aegypti* in Usmanu Danfodiyo University main campus. The findings herein indicated that *Ae. Aegypti* from different locations within the Usmanu Danfodiyo University exhibited varying levels of resistance to deltamethrin, permethrin and lambdacyhalothrin. The exposure of the mosquitoes to alphacypermethrin yielded 98-100% mortalities indicative of the susceptibility nature of the insecticide. This means that among the insecticides tested, alphacypermethrin exhibited the highest mortality effect on the *Aedes* mosquitoes. Exposure to permethrin shows susceptibility to mosquitoes from Gidan Yero and Gidan Yunfa. Deltamethrin and Lambdacyhalothrin showed the lowest mortality rate and exhibited different level of resistance across all the locations. This further confirmed the reported cases of resistance to Lambda-cyhalothrin and DDT [36]. Thus, level of susceptibility varies according to insecticides and location.

The resistant status recorded in this study is not surprising as report have shown wide spread resistance of Pyrethroid in *Ae. Aegypti* [9]. Similar resistance to pyrethroid was reported in some parts of Nigeria [37-39]. Resistance to both beta-cypermethrin, deltamethrin, DDT, lambda-cyhalothrin, permethrin and malathion in *Ae. Aegypti* and *Ae albopictus* was earlier reported [40-42]. The findings of this study confirmed these findings and further agreed with reported case of possible resistance against *Ae. Aegypti* exposed to deltamethrin and permethrin in Jazzan region [36]. The results however, contradicted the full susceptibility recorded in *Ae. Aegypti* and *Ae. Albopictus* to deltamethrin in Central Africa [31] but further confirmed the reported case of resistance to Deltamethrin in in *Ae. Aegypti* from Senegal and Cape Verde [43]. Recent findings [44] demonstrated high levels of resistance to DDT, pyrethroids and organophosphates by *Ae. Aegypti* in Peru while, varying levels of resistance of *Ae. Aegypti* when exposed to deltamethrin was also reported [45]. The level of resistance recorded in this study against lambda-cyhalothrin also agreed with the level of resistance of *Ae. Aegypti* recorded when exposed to lambda-cyhalothrin [46, 47].

Resistance in young mosquitoes to pyrethroids was reported to be due to their strength and physiological activeness [48, 49]. This might explain the reasons behind higher resistance status recorded particularly against deltamethrin and lambda-cyhalothrin. Reports also revealed that increase in mortality due to pyrethroids exposure increases with increase in mosquito age in some vector species [49-51]. Even though *Aedes* mosquitoes exhibits different behaviour with malaria vectors same reason may applied as the bioassay was not conducted same day and some species are a day or two older. Thus, the differences in resistance status recorded could also be attributed to the differences in the age of the mosquitoes used.

The inhabitants of the area mostly engaged in farming activities. There is rampant and continuous usage of agricultural chemicals in the farms. These chemicals are sometimes washed deposited in the surrounding water bodies which serve as breeding ground for the mosquitoes. The developing larvae might get exposed to the chemical residues thereby enhancing development of resistant. Therefore, the continuous usage of the agrochemical in the field might be the reason for resistance status recorded. Agricultural and household use of insecticides in Nigeria has been implicated as the reason for the development of resistance in mosquitoes [52].

Although the present study had its limitations being confined only to Usmanu Danfodiyo University and larval collections were not done in all the available habitats, the information provided herein would form the basis for further studies on detailed *Aedes* mosquitoes. Also, the study being the first of its kind in Sokoto State, could serve as an essential tool for further investigation on the insecticide resistance and its mechanisms. The findings may also serve as a guide to the State Ministry of Health in planning and execution of its policies in vector monitoring, evaluation and control especially in areas where there were unconfirmed reports of dengue and yellow fever outbreaks in the state.

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6. Conflicting Interest

Authors declared that there was no conflicting interest

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