Saudi Journal of Biological Sciences 30 (2023) 103524

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Insecticide susceptibility status in two medically important mosquito vectors, *Anopheles gambiae,* and *Culex quinquefasciatus* to three insecticides commonly used in Niger State, Nigeria

Ibrahim Kura Shehu [a,b], Hamdan Bn Ahmad [a], Israel Kayode Olayemi [b], Danjuma Solomon [c], Abu Hassan Ahmad [a], Hasber Salim [a,*]

[a] *School of Biological Sciences, Universiti Sains Malaysia, 11 800 Pulau Pinang, Malaysia*
[b] *Department of Biological Sciences, Federal University of Technology, Minna P.M.B. 65, Nigeria*
[c] *Department of Crop Production, Ibrahim Badamasi Babangida University Lapai, P.M.B. 11, Nigeria*

ARTICLE INFO

Article history:
Received 29 October 2021
Revised 5 November 2022
Accepted 1 December 2022
Available online 27 December 2022

Keywords:
Anopheles gambiae
Culex quinquefasciatus
Insecticide susceptibility
CDC Bioassays
Niger State
Nigeria

ABSTRACT

High resistance ability on insecticides among major mosquito vectors of diseases in Nigeria is of growing concern for severe control strategies. The objective of this study was to assess the susceptibility status of females Anopheles gambiae and Culex quinquefasciatus complexes mosquitoes to permethrin (21.5 µg/bottle-pyrethroids), propoxur(12.5 µg/bottle-carbamate) and malathion (50 µg/bottle organophosphate), in Niger State, North-Central, Nigeria. Anopheline and Culecine larvae were collected from the larval habitats of the studied sites (Bosso, Katcha, Lapai, and Shiroro) larvae and pupae were identified guided by standard keys and reared to adults in troughs. Insecticide susceptibility bioassays were performed according to the CDC bottle bioassay standard operating procedures on 3 days old, sugar-fed female Anopheles and Culex mosquitoes. Post-exposure mortality after 24hr and knockdown values for KDT$_{50}$ were calculated. Knock-down at 1-hour insecticide exposure ranged (84–96 %) permethrin, (94–100 %) propoxur and (100 %) malathion for An. gambiae and (86–97 %) permethrin, (92–100 %) propoxur and (96–100 %) malathion for Cx. quinquefasciatus. Mortality, after 24hr post-exposure was 100 % in malathion, indicating the high effect of the insecticide. Tested samples were found potentially resistant to permethrin recorded against mosquitoes collected from all study sites, in two locations of the study sites to propoxur and one location site to malathion. All the tested mosquitoes were found to be potentially resistant to permethrin, however, mosquitoes tested in Katcha and Shiroro resist potentially to propoxur. Except, Culex quinqufasciatus from Lapai that partially resist malathion, all the tested mosquitoes were found to be susceptible to malathion, across the study sites.

1. Introduction

Undoubtedly, mosquitoes are the most important dipterans accountable for the spread of mosquito-borne diseases like malaria, dengue and filarial fevers, and several others (Shetty et al., 2013). Among these diseases, malaria in Africa is caused by *Anopheles gambiae* (Giles, 1902) while filariasis is caused by *Culex quinquefasciatus* (say, 1823) with Nigeria having highest global records of morbidity and mortality of 25 % and 24 % malaria, and 14.3 % and low of < 10 % filarial disease (Adamu et al., 2020; Fortuna et al., 2021). Since there are currently no viable vaccinations or treatments for several mosquito-borne diseases, such as malaria, vector control management employing insecticides remains the primary method of disease control (Francis et al., 2017; Vatandoost et al., 2019).

Chemical-based control using pyrethroids, organophosphates, and carbamates is still an essential strategy for managing the populations of these medically significant vectors (Ukpai & Ekedo, 2019). According to Fagbohun et al., (2020), Ramos, (2020), the effectiveness of these insecticides has been greatly hampered by the advent of insecticide resistance due to overreliance on public health and agriculture, which headed to the rapid expansion of

---

[*] Corresponding author.
E-mail address: hasbersalim@usm.my (H. Salim).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103524

resistance in these vector populations. An insecticide product's failure to achieve the required level of control when used as directed for a specific species at a specific time and place can result in pesticide resistance in mosquitoes, which is a natural and heritable trait in a species population. The volume and rate of pesticide applications against an insect population, as well as the inherent traits of the insect species involved, all influence the degree of resistance development (Pates & Curtis, 2005; Brooke & Koekemoe, 2010; Abate et al., 2020).

In general, insecticide resistance in mosquito vectors can be caused by one or more of the following: behavioural changes, morphological modifications, target site mutation, and detoxifying enzyme metabolism, and occur in places where diseases are endemic and subsistence remains a burden on communities, managing this resistance is a critical problem for vector control programs (Agumba et al., 2019). Despite years of national efforts, to curtail the mosquito's resistance situation, still *An. gambiae* and *Cx. quinquefasciatus*, are described to prolong resistance to insecticides in Niger State (Olayemi et al., 2011). Antilarval and adult operations using chemicals are the most common vector control approaches used by health organizations to prevent disease transmission.

However, these could not solve the problem because of the anthropogenic activities such as constructions, farming irrigation, fishing, and several others, coupled with the lack of environmental sanitation engaged by people causing the increase in the number of larval breeding habitat types (Olayemi et al., 2014). These chemicals insecticides (Larvicides and adulticides) are inadequate to target for all the breeding habitats and residential places during application, and because of this inadequacy, mosquito species such as *Anopheles* and *Culex* can grow and develop from larvae to adults and increase in number (Fagbohun et al., 2020). Female *An. gambiae* and *Cx. quinquefasciatus* mosquitoes that are carry the parasites, *leave* to obtain blood meals for eggs development and hatchability, transmitting diseases, at the same time develop resistance ability to commonly used insecticides. In the past, there are a lot of reports on the susceptibility status of *Anopheline* and *Culicine* mosquitoes to pyrethroids, carbamate, and organophosphates insecticides in some parts of Nigeria for examples, the research works conducted in Minna, Northeastern, Akwa Ibom, Sudan Savanna, North Eastern, Abia, Sahel Savannah Region of Northwest, and Lagos (Olayemi et al., 2011; Ibrahim et al., 2014; Umar et al., 2014; Opara et al., 2017; Yoriyo et al., 2018; Ukpai & Ekedo, 2019; Fagbohun et al., 2020).

Currently, there is an increase in the number of these prevalent mosquito diseases in various local government areas of Niger State, but no such study has been recorded. The current study is the first to examine the susceptibility of *An. gambiae* and *Cx. quinquefasciatus* mosquitos to three chemical insecticides: permethrin (pyrethroids), propoxur (carbamate), and malathion (organophosphates), although some these chemical insecticide ingredients like permethrin and deltamethrin of the pyrethroids were previously tested only on *Anopheles* mosquitoes and in few local government areas of Niger State, chemical insecticides ingredients such as propoxur and malathion were less consider, and because of resistance ability and prolong test trial, the insecticides were suggested to be considered for this present study. Therefore, the results will serve as a foundation for tracking the spread of insecticides resistance.

2. Materials and methods

2.1. Description of the study area

Nigeria is a country in West Africa with a population of about 200 million people lies between the Equator and the Greenwich Meridian, roughly between 4° and 14° north latitude and 2° 2′ and 14° 30′ east longitude. It covers 923,768.5 km^2 and is characterized by undulating topographic relief patterned by valleys formed by its river systems. The southern coastal plains have a mean elevation of around 150 m above sea level. The northern plains rise to around 600–700 m, with the Jos Plateau (over 1,500 m) in the geographic Centre of Nigeria and the Mambilla plateau (over 2,100 m) among the mountains on the Cameroon border. The temperature varies depending on the ecological zone (Chukwu et al., 2018).

Tropical at the coast (in Humid Forest and Derived savannas) with extremely low and high temperatures of 10 °C and 37 °C, sub-tropical inland (in Derived and Guinea savannas), and semi-arid in the far north (in Sudan and Sahel savannas) with extremely low and high temperatures of 6 °C and 44 °C. Normal monthly temperatures in the Jos and Mambilla plateaus' mid-altitude region range from 21 to 25°C. In the north, annual rainfall varies from 500 mm to 750 mm, while in the south, rainfall ranges from 1,200 mm to over 4000 mm.

Niger State is one of Nigeria's Middle Belt states, located between Longitude 60 33E and Latitude 90 37 N on a land area of 88 km^2 (representing 9.30 % of the country's total land area with 85 % arable land), with a population of 3950.249 people as of the 2006 census, however, by 2012, a projected population of around 4.8 million people was recorded, and in 2019, the projected population of Niger State people by National Beureau of Statistics (NBS) reached 6.2million (National Beureau of Statistics, 2020). It is divided into twenty-five local governments. The state has a mean annual rainfall temperature of 61 %, 30.20 mm, and relative humidity of 1334 mm, the region has a tropical climate. Kaduna State and the Federal Capital Territory to its north-east and South-East borders, respectively; Zamfara State borders the north, Kebbi State borders the West, Kogi State borders the South, and Kwara State borders the South-West; and the Republic of Benin, along Agwara LGA, its north-west border. (Ukubuiwe et al., 2012). The climate is divided into two seasons: a wet season from May to October and a dry season from November to April, with annual rainfall varying from 1,100 mm in the north to 1,600 mm in the south. The vegetation in this area is typically grass savannah with a few scattered trees Fig 2.1..

2.2. Experimental design

The study involves field and laboratory works, fourth-instar larvae were sampled together with larval water by dipping at the rate of 20 dips per sampling site using 350 capacity dippers from the three types of conventional mosquito breeding habitats (Large water bodies, Swamps, and Gutters) of the study areas. Certain members of these fourth instars of the two different mosquitoes i.e., *Anopheles gambiae* and *Culex quinquefasciatus* sampled preserved into 10 % formalin and categorized macroscopically based on the observation of the placement of the position of the respiratory tube by the larvae in the water. They were most certainly *Culex* if the respiratory tube was narrow, long, and angled at a certain angle to the water surface. Mosquito larvae that drifted horizontally to the water's surface were most likely *Anopheles.*

The identified strains were sorted into morph groups in the laboratory and further identified to species level based on visible characteristics such as (e.g., presence or absence of siphon, the position of hair tufts, length of the siphon, arrangement of comb scales and several others), with the aid of a dissection microscope and guided by morphological keys. Then the live fourth instar members of the identified strains were reared and fed with 0.32 ml yeast solution separately under laboratory conditions of temperature 24.2^{OC} and relative humidity 64 % until they emerged to pupae. Males and females pupae of the two different strains were recognized macroscopically based on the observation of their

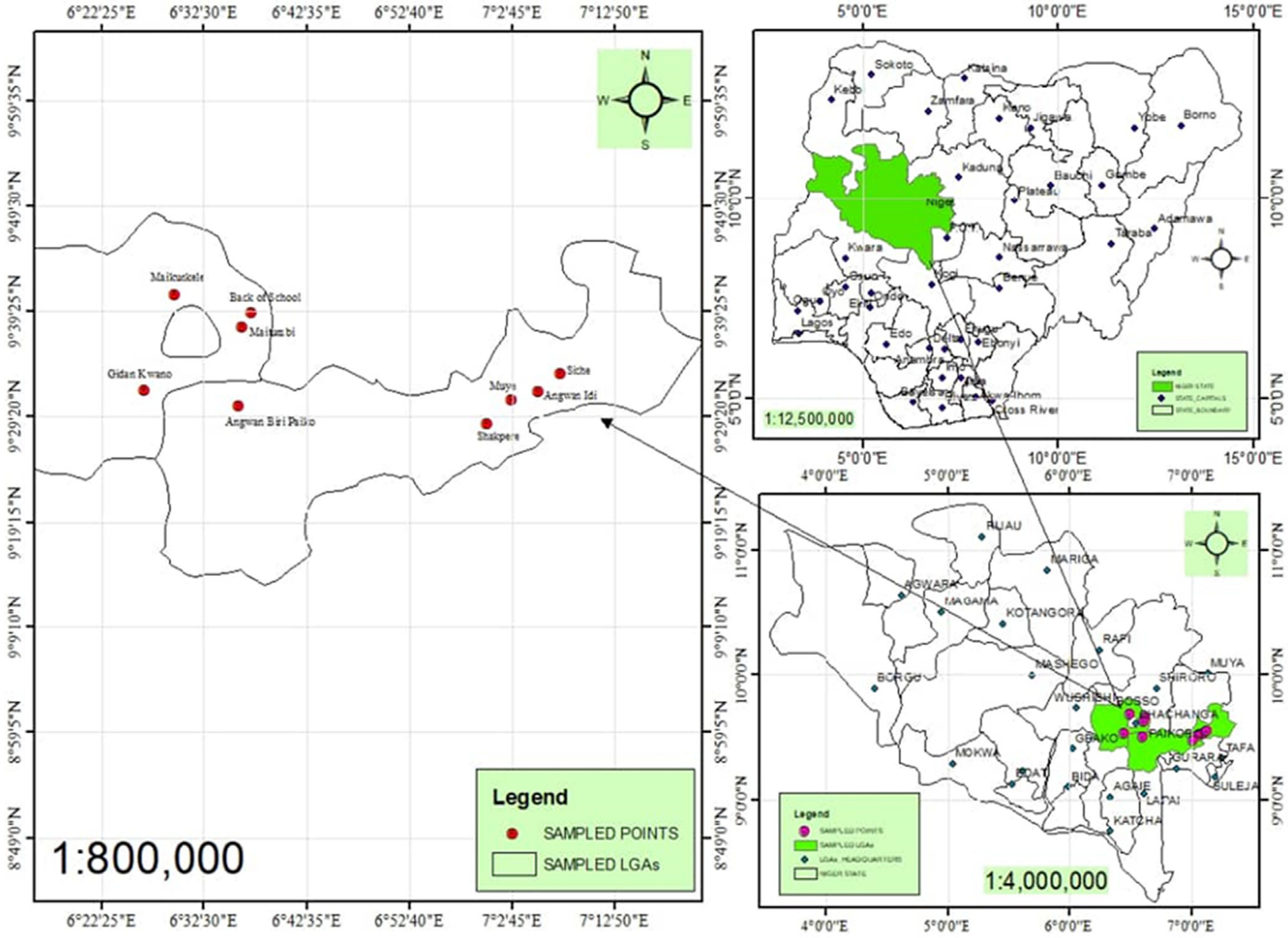

Fig. 2.1. Map of Nigeria showing geographical position of Niger State and local government areas (LGAs) studied.

size (usually, the female pupa is larger than the male pupa). The males pupae were then discarded using dropper and only the females pupae were allowed to growth to adult female mosquitoes. The emerged adult females mosquitoes were kept and fed with 10 % sucrose solution in the rearing trough until require for the insecticidal test (Wang et al., 2020).

The emerged F0 generations were then subjected to CDC bottle bioassays susceptibility test **(**Table 2.1**)**. A total of 1000 live mosquito samples that were morphologically identified at larval and pupal stages were separated into *An. gambiae* and *Cx. quinquefasciatus.* The female mosquitoes of these two strains were used for insecticides susceptibility test as described by (Olayemi et al., 2011).

2.3. CDC bottle bioassays

Female adult mosquitoes from *An. gambiae* and *Cx. quiquefasciatus* strains were tested for insecticide susceptibility to permethrin, propoxur, and malathion using CDC bottle bioassays (Aïzoun et al., 2014). Diagnostic dosages for the colony of mosquito species were measured; permethrin (21.5 μg/bottle), propoxur (12.5 μg/bottle), and malathion (50 μg/bottle) (Norris & Norris, 2011), the prepared stock solutions were stored in the refrigerator at 4^{oC} in light-proof bottles and removed 1hr before the CDC Bioassay was conducted. Into each of the four 250 ml Wheaton bottles i.e., replicate bottles (R_1, R2, *R*3, and R_4), diagnosis dose of insecticide to be evaluated was added using disposable pipettes, while the control bottles were treated only with acetone and tightly with caps and coated all over until all visible signs of the liquid were gone and bottles dried completely. Into each coated bottle (replicates and control), twenty-five (25), 3-day old females sucrose-fed individuals were introduced and the test conducted. no susceptible strain was used as a reference (Brogdon & McAllister, 1998; Zamora et al., 2009; CDC, 2016).

Knockdown was recorded every 5 min until 1hr; however, mortality was reported at 30 min critical time. The mosquitoes were taken from the bottles after an hour and separated into ''alive'' and ''knocked-down'' groups. Under insectary conditions, mosquito groups were kept in separate paper cups with a 10 % sucrose solution. To evaluate delayed mortality, they were scored as alive or dead after 24hr. The latest World Health Organization (W.H.O) standards (W. H O,2016) were used to classify the collections as resistant or susceptible: 98–100 % mortality shows susceptibility, 80–97 % mortality indicates resistance may be developing, and mortality<80 % indicates resistance (W. H.O, 2016; Francis et al., 2017).

Table 2.1
Collection sites with number of mosquitoes and description of the areas.

Sites	No of *An. gambiae*	No of *Cx quinquefasciatus*	Semi-rural/ City
Bosso	125	125	City
Katcha	125	125	Semi-rural
Lapai	125	125	Semi-rural
Shiroro	125	125	Semi-rural

2.4. Statistical analysis

Based on the data obtained the percentage mortality was calculated and subjected to one-way ANOVA. Probit method was employed for the adult bioassays data analysis (Sakuma, 1998), using a computerized program SPSS Statistical package (23 version). Based on the KDT$_{50}$ obtained from adult bioassays.

3. Result

3.1. Susceptibility status of Anopheles gambiae

The result of the efficacies of the three chemical ingredients namely, permethrin, propoxur, and malathion from selected three main classes of insecticides respectively, tested against the adult female *An. gambiae* sample collected from the four study sites is shown in (Table 3.1a**)**. Insecticide susceptibility levels in the mosquitoes varied from potentially resistant to susceptible among the different study areas. In each insecticide tested, the adult bioassay revealed considerable variations in susceptibility status of the adult female *An. gambiae* mosquitoes after 1hr minutes exposure and the 24hr recovery period. For permethrin, the highest mortality (96 %) of mosquitoes was obtained from Shiroro, while the lowest was recorded from Bosso (84 %). This highest mortality recorded of Shiroro's mosquitoes had no significant difference ($P > 0.005$) from the percentage mortality in the remaining mosquitoes collected from other study sites but significantly differed ($P < 0.005$) from the mortality rate of Bosso. Important to know, that permethrin showed potential resistance against the adult female *strain* that was obtained from other study sites (range = 8 4–96 %).

In the case of propoxur, the highest mortality rate was obtained from Lapai and Bosso (100 %), while the lowest was obtained from Katcha mosquitoes with a mortality value of 94 %. These values were significantly different ($P < 0.005$) from one another and the recorded value of Shiroro (96 %). Unlike permethrin, propoxur revealed potential resistance to susceptible status against strain in the study areas. Malathion elicited full susceptibility in mosquito populations, with 100 % mortality recorded in all the local government areas (LGAs).

Table 3.1b

Knockdown time (KDT$_{50}$ mg/L) profile of different insecticides tested on *Anopheles gambiae* mosquito population in different breeding sites.

	Permethrin	Propoxur	Malathion
Sampling Site	KDT$_{50}$ (mg/L) 95 % (CL)	KDT$_{50}$ (mg/L) 95 % (CL)	KDT$_{50}$ (mg/L) 95 % (CL)
Bosso	26.98 (19.67–32.59)	30.16 (22.07–33.88)	26.63 (22.90–29.51)
Katcha	20.46 (16.50–24.07)	34.61 (29.27–38.92)	24.96 (10.45–29.66)
Lapai	24.72 (17.25–29.86)	32.40 (27.85–35.92)	31.35 (25.86–35.28)
Shiroro	22.90 (18.01–27.15)	35.51 (31.24–39.09)	29.06 (22.75–31.19)

KDT50 is knockdown time of resistance to susceptible mosquito species tested.

The knockdown time (KDT$_{50}$) varied for the different insecticides and sampling sites (Table 3.1b**)**, permethrin recorded low and high mortality times ranging from 20.46 to 26.98 min in Katcha and Bosso, respectively**.** The mean Knockdown per time indicated that in less than 30 min of the exposure over 50 % mortality of these adult female mosquitoes collected from the study areas was recorded. For propoxur, the highest KDT$_{50}$ was obtained in Shiroro (35.51 min) and the least (30.16 min) in Bosso. The mean Knockdown time for propoxur in all the study sites was above 30 min of the exposed critical time for the adult. Correspondingly, the mean KDT$_{50}$ of malathion was highest (31.35 min) in Lapai with a ranged value of 25.86–35.28 min and lowest in Katcha with a value of 24.96 min and a range of 10.45–29.66 min. The mean Knockdown time for malathion revealed that below 30 min of the exposure time, over 50 % mortality of adult females' mosquito samples were collected from all the study areas.

3.2. Susceptibility status of Culex quinquefasciatus

A similar trend was observed in the bioassay's susceptibility status of these insecticides' chemical, tested on the adult females *Cx. quinquefasciatus* strain of the study sites detailed in **(**Table 3.2a**)**. Insecticide susceptibility level in adults' bioassays confirmed potential resistance to susceptible status. The result revealed substantial disparities in the susceptibility status of adult females of this strain after 1hr of exposure and the 24hr recovery period in all the study areas. Permethrin recorded the highest mortality (97 %) of mosquitoes from Bosso, the lowest was obtained in Katcha mosquitoes with a mortality rate of 86 %. This highest mortality had no significant difference ($P > 0.005$) from the percentage mortality of Shiroro but was significantly differed ($P < 0.05$) from the mortality values of Katcha and Lapai mosquitoes. Yet, the lowest mortality rate had no significant difference ($P > 0.005$) from the mortality rate Lapai. Highly to know, that permethrin showed potential resistance to this adult female mosquito species in all the studied sites. In the case of propoxur, the highest mortality rate was obtained from mosquitoes collected in Bosso (100 %), while the lowest was obtained from Katcha mosquitoes with a mortality value (92 %).

This highest mortality value had no significant difference ($P > 0.005$) from the recorded value of mosquitoes collected from Lapai (99 %) but significantly differed ($P < 0.05$) from the remaining sites. Shiroro significantly differed ($P < 0.05$) from the mortality rates of all the other sites. Disparate to permethrin, propoxur revealed potential resistance to the susceptible status of the same mosquitoes in all the sites. The same trend occurred with malathion that indicated potential resistance to susceptible status against the same mosquitoes with the highest mortality rates (100 %) in Katcha, Bosso, and Shiroro mosquitoes, respectively. While lowest mortality rate was recorded from mosquitoes of

Table 3.1a

Percentage mortality and susceptibility profile of *Anopheles gambiae* mosquito population in different breeding sites.

Sampling site	Permethrin		Propoxur		Malathion		Ctrl. Mortality%
	Mortality%	Status	Mortality%	Status	Mortality%	Status	
Bosso	84.00 ± 2.15^{a}	PR	100.00 ± 0.00^{b}	S	100.00 ± 0.00^{a}	S	0.00 ± 0.00
Katcha	92.00 ± 1.43^{b}	PR	94.00 ± 2.64^{a}	PR	100.00 ± 0.00^{a}	S	0.00 ± 0.00
Lapai	94.00 ± 1.79^{b}	PR	94.00 ± 2.64^{a}	S	100.00 ± 0.00^{a}	S	0.00 ± 0.00
Shiroro	96.00 ± 1.42^{b}	PR	96.00 ± 1.21^{b}	PR	100.00 ± 0.00^{a}	S	0.00 ± 0.00

Mortality values with the same superscript along the column were not significantly different at ($P > 0.05$). R is resistance, PR is potential resistance, Mortality% (ranges 98–100 %) is susceptible, (80–97 %) potential resistance, < 80 % is resistance, Ctrl, means control.

Table 3.2a
Percentage mortality and susceptibility profile of *Culex quinquefasciatus* mosquito population in different breeding sites.

Sampling site	Permethrin		Propoxur		Malathion		Ctrl. Mortality%
	Mortality%	Status	Mortality%	Status	Mortality%	Status	
Bosso	97.00 ± 1.91^{b}	PR	100.00 ± 0.00^{b}	S	100.00 ± 0.00^{a}	S	0.00 ± 0.00
Katcha	8.00 ± 2.58^{a}	PR	92.00 ± 3.65^{a}	PR	100.00 ± 0.00^{a}	S	0.00 ± 0.00
Lapai	89.00 ± 1.15^{a}	PR	99.00 ± 1.00^{b}	S	96.00 ± 1.63^{b}	PR	0.00 ± 0.00
Shiroro	$92.50 \pm 1{,}63^{b}$	PR	97.00 ± 1.91^{b}	PR	99.00 ± 1.13^{a}	S	0.00 ± 0.00

Mortality values with the same superscript along the column were not significantly different at ($P > 0.05$). R is resistance, PR is potential resistance, Mortality% (ranges 98–100 %) is susceptible, (80–97 %) potential resistance, < 80 % is resistance, Ctrl, means control.

Lapai with a value (96 %). The highest values did not differ significantly ($P > 0.005$) from the value obtained in the three areas but significantly differed ($P < 0.05$) from Lapai.

The knockdown time (KDT_{50}) varied for different insecticides and sampling areas KDT_{50} at 1hr of exposure and 24hr recovery period for permethrin (Table 3.2b). Except for mosquitoes from Katcha (30.74) minutes which had the KDT_{50} above 30 min critical time, the KDT_{50} of others were below the critical time with a ranged mean value of 19.53 min in Bosso to 25.28 min Lapai, for permethrin. Propoxur recorded the highest KDT_{50} Shiroro 35.71 min and the least of 30.16 min in Bosso. The mean Knockdown time for propoxur quantified that above 30 min of the exposure over 50 % mortality of adult females of the same mosquitoes was recorded in all the study areas. Similarly, KDT_{50} of malathion had variation in mortality time from 22.99 min as low time in Shiroro to 29.90 min as higher time in Lapai. The mean Knockdown time for malathion revealed that over 50 % mortality of adult females of the same strains were recorded in all the study areas below 30 min of the exposure.

Potential cross-resistance to fully susceptible of the three-chemical tested against the adult female of the two different strain mosquito populations in all the studied areas was observed (Table 3.2c). It is important to point out that potential cross-resistance occurred between permethrin and propoxur to both adult females *An. gambiae* and *Cx. quinquefasciatus* from Katcha and Shiroro with values of 86, 92, 94, 96, and 97 % mortality, respectively. These values are within the mortality % range value of 80–97 % of WHO, standard (WHO, 2016).

3.3. Comparison of the effectiveness of chemical insecticides tested in four LGAs

The result of the percentage (%) effects of the three chemical ingredients tested on mosquitoes in each site is detailed in (Figs. 3.3.1, 3.3.2, 3.3.3 and 3.3.4) respectively. The results showed significant variations between chemical insecticides, mosquitoes, and study sites. In Bosso site (Fig. 3.3.1), propoxur and malathion had significant highest effects on both female mosquitoes with a point value (100 %). Permethrin exacted much effect on *Cx. quinquefasciatus* (97 %) while compared to *An. gambiae* with a point value (84 %). In Katcha site (Fig. 3.3.2), a significant highest effect was observed in malathion (100 %) on both female mosquitoes. Permethrin and propoxur exacted greater effects on female *An. gambiae* with point values (92 and 94 % respectively) however, propoxur was insignificantly different ($p > 0.005$) from the point value of *Cx. quinquefasciatus* but significantly differed ($p < 0.005$) from permethrin with a point value (82 %) of the same species.

In Lapai site (Fig. 3.3.3), propoxur and malathion exacted full effect (100 %) on *An. gambiae*, however, this point value differed insignificantly ($p > 0.005$) from obtainable values (99 and 96 %) on *Cx. quinquefasciatus*. Permethrin exacted a greater effect on *An. gambiae* (94 %), this point value significantly differed ($p \leq 0.005$) from *Cx. quiquefasciatus* with a recorded value (89 %). While, in Shiroro study site (Fig. 3.3.4), malathion had a greater effect on *An. gambiae* mosquitoes (100 %), however, this recorded point insignificantly differed ($p > 0.005$) from *Cx. quinquefasciatus* mosquitoes with a point value of 99 %.

The highest effect of propoxur was recorded for *Cx. quinquefasciatus* (97 %), this highest value had no significant different ($p > 0.005$) from the recorded value on *An. gambiae* (96 %). Permethrin exacted its highest effect on *An. gambiae* with a value of 96 %, this recorded value significantly differed ($p < 0.005$) from *Cx. quinquefasciatus* mosquitoes (92.5 %).

Table 3.2b
Knockdown time (KDT_{50} mg/L) profile of different insecticides tested on *Culex quinquefasciatus* mosquito population in different breeding sites.

	Permethrin	Propoxur	Malathion
	KDT_{50} (mg/L) 95 % (CL)	KDT_{50} (mg/L) 95 % (CL)	KDT_{50} (mg/L) 95 % (CL)
Sampling Site			
Bosso	19.53 (05.74–328.11)	30.16 (22.07–33.88)	26.78 (20.88–31.06)
Katcha	30.74 (17.76–38.18)	32.41 (27.17–36.72)	26.77 (21.23–30.28)
Lapai	25.28 (10.68–33.42)	31.38 (26.65–34.72)	29.99 (23.61–35.98)
Shiroro	24.26 (19.00–28.01)	35.71 (31.54–39.19)	22.99 (20.67–30.54)

KDT50 is knockdown time of resistance to susceptible mosquito species tested.

4. Discussion

The sensitivity of *An. gambiae* and *Cx. quinquefasciatus* strains to three insecticides, permethrin, propoxur, and malathion, were examined in four LGAs of Niger State. From, the result of the investigation, *An. gambiae* and *Cx. quinquefasciatus* revealed possible resistance to permethrin in all the studied LGAs. It was also found that propoxur was effective in Bosso and Lapai areas towards the given mosquito species. Malathion was found to be very effective toward *An. gambiae* in all the study areas and agrees with the studies of Yusuf & Oshaghi, (2021) that found that malathion was the only insecticide found to be very active against the mosquito vector tested across the three Northern States of Nigeria. Amongst the strains of different areas, the greatest potential resistance was observed in the Bosso strain of *Cx. quinquefasciatus* followed by Shiroro of *An. gambiae* towards permethrin, *Cx. quinquefasciatus*, and *An. gambaie* strains from Shiroro had the highest potential resistance to propoxur, the highest potential resistance status to malathion was detected from the strain of *Cx. quinquefasciatus* from Lapai.

This current investigation found that the susceptibility status of these two mosquito strains harmonized the global pattern of susceptibility/resistance distribution. For example, the studies From parts of Nigeria, the report of susceptibility status of these mosquitoes to permethrin propoxur and malathion has been well docu-

Table 3.2c
Percentage mortality of potential cross-resistance in susceptibility profile of the two different mosquito strains population in different breeding sites.

Sites	***Anopheles gambiae***						***Culex quinquefasciatus***			
	Permethrin		Propoxur		Permethrin		Propoxur		Malathion	
	Mort %	Status	Mort%	Status	Mort%	Status	Mort%	Status	Mort%	Status
Katcha	92	PR	94	PR	86	PR	92	PR	–	–
Shiroro	96	PR	96	PR	92	PR	97	PR	–	–
Lapai	–	–	–	–	89		–		96	PR

Mortality values with the same superscript along the column were not significantly different at (P > 0.05). R is resistance, PR is potential resistance, Mortality% (ranges 98–100 %) is susceptible, (80–97 %) potential resistance, < 80 % is resistanceSSS.

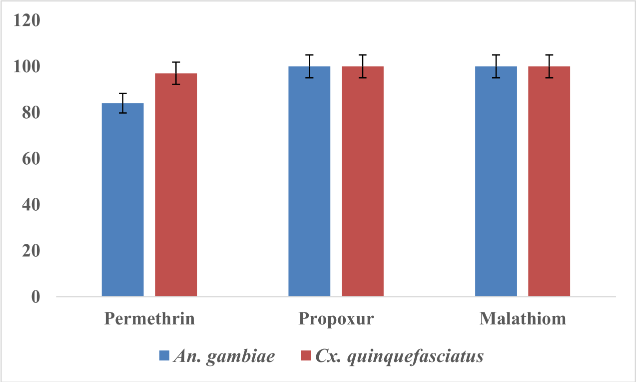

Fig. 3.3.1. Comparison of the effectiveness of the three chemical insecticides tested in Bosso logal government area of Niger State.

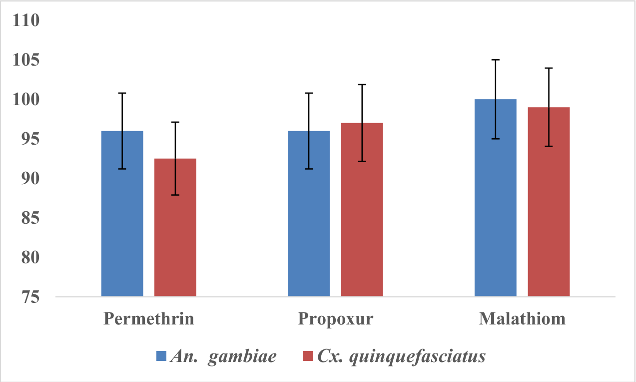

Fig. 3.3.4. Comparison of the effectiveness of the three chemical insecticides tested in Shiroro local government area of Niger State.

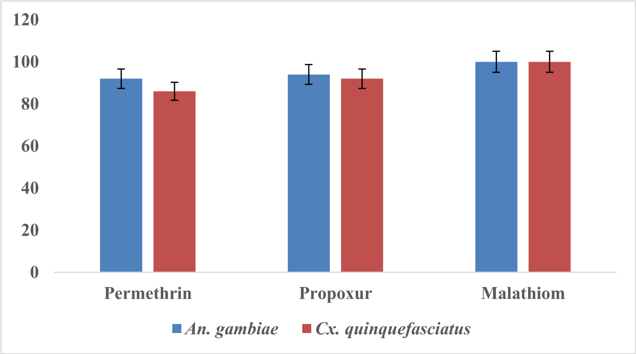

Fig. 3.3.2. Comparison of the effectiveness of the three chemical insecticides tested in Katcha local government area of Niger State.

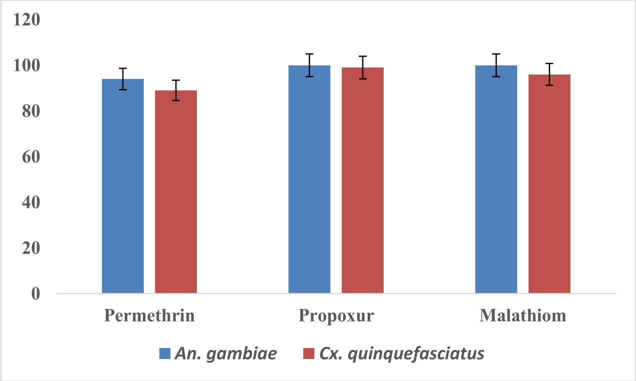

Fig. 3.3.3. Comparison of the effectiveness of the three chemical insecticides tested in Lapai local government area of Niger State.

mented in north-central, north-west, and southwest Awolola et al., (2005), Olayemi et al., (2011), Shettyet al., (2013), Umar et al., (2014), Reid & McKenzie, (2016), Opara et al., (2017), Yoriyo et al., (2018), Ukpai & Ekedo (2019), Fagbohun et al.,(2020), they reported *An. gambiae* and *Cx quinquefasciatus* species resistance to pyrethroids such as, cyfluthrin, deltamethrin, permethrin, lambda-cyhalothrin, and bifenthrin used for IRS and agricultural pests, on the other hand, insecticides like bendiocarb and propoxur were lethal to mosquitoes with a mortality rate of a minimum of 98 %, thus, justifying the application of carbamate insecticides (such as propoxur) which has a faster rate of knockdown effects, however, some species of *Anopheline* and *Culicine t*olerate higher dosages of these insecticides (Selvaraj et al., 2020).

There are several studies on the susceptibility status of different mosquitoes in other parts of the world. Wanjala et al., (2015), reported *An. gambiae* with high resistance to pyrethroids in western Kenya. Akiner (2014), reported an increased resistance of malathion and propoxur from Turkey. Propoxur resistance has also been documented in *An. gambiae* from Cote d'Ivoire and Senegal, both in West Africa. Djouaka et al., (2016), reported the susceptible status of *An. gambiae* to the group of organophosphates such as malathion, also a similar report from Chadi was recorded on malathion and pirimiphos-methyl (Kerah-Hinzoumbé et al., 2008). In the Benin Republic and Cameroon, susceptibility of *An. gambiae* to malathion had been recorded by (Corbel et al., 2007; Boussougou-Sambe et al., 2018). There are documented evidences of mosquitoes' resistance to deltamethrin (as pyrethroids) on adult and larval insecticide susceptibility status of *Cx. quinquefasciatus* by (Nazni et al., 2005, Low et al., 2013), who reported permethrin resistance in the populations of *Cx. quinquefasciatus* mosquitoes. Low et al., (2013), revealed resistance of *Cx. quinquefasciatus* mosquitoes against propoxur insecticide in Malaysia. The report by Bisset et al., (2006), shows that *Cx. quinquefasciatus* has proved resistant to malathion and carbamate insecticides in the Eastern, Central, and Western parts of Cuba. Also Tahir et al., 2013 reported the susceptibility of *Cx. quinquefasciatus* on malathion in Pkistan.

Similarly, in Brazil. Bracco et al., (1999) has shown that the mosquito is resistant to malathion, fenitrothion, and carbamate. In Iran, the research conducted by Rahimi et al., (2019), revealed the resistance status of all the insecticides plus propoxur tested both on *Cx. quinquefasciatus* in all field and lab strains (an especially susceptible strain that is kept for several years in insectary without any exposure to pesticides). The selection pressure for resistance, which is controlled by the length and frequency of pesticide application, the number of breeding sites treated, and the dosage used, determines the rate at which an insecticide loses its effectiveness. According to them (Ramos, 2020), insecticide resistance is described as "the ability of a species of insects to survive large doses of toxicants that are deadly to the majority of individuals in a typical population of that species." arises as a result of the excessive application of an insecticide and has become one of the main obstacles for the control of pests, this problem threatens the progress in the elimination and control of mosquito disease vectors. According to Olayemi et al., (2011), Wanjala et al., (2015). *Anopheline* and *Culicine* vectors of malaria and filarial disease are undoubtedly constantly under selection pressure due to the extensive and negligent use of insecticides for agricultural and public health purposes in our ecosystem, a development that is not encouraging for the long-term effectiveness of chemical mosquito control methods.

Despite none of the population was fully resistant against the tested chemical insecticide ingredients, the chemicals may encourage knock down effects due the presence of KDR resistance mechanism operating in the *An. gambiae and Cx. quinquefasciatus* populations of the study areas. Ibrahim et al., (2014), in their separate studies, noticed similar KDR situations in their studies areas. The mean total number of 3-day old adult females of these two different knocked down below and above critical time (30 mins) during the one-hour exposure to permethrin and the total death rates recorded after 24hr recovery exposure indicated that most KDT_{50} values recorded in this current study for these two mosquito species are within the range values (13.20 – 27.29 mins) and (21.65–31.48 mins) of KDT_{50} of pyrethroids such as cyfluthrin, lambda-cyhalothrin, and bifenthrin recorded separately by (Kumar et al., 2011; Umar et al., 2014) respectively, an indication of incipient potential-resistance to these insecticides.

This also conforms with the research works of Ndams et al., (2006), Djouaka et al., (2016), Fagbohun et al., (2020), that revealed the resistance status of *An. gambiae* although with relatively higher KDT_{50} range values (30.08–59.36) mins. This study is also in agreement with the work of (Kerah-Hinzoumbé et al., 2008), that reported the existence of resistance to permethrin with mortality rates 70.2 to 96.6 %, and deltamethrin (70.2 to 96.6 %) in several *An. gambiae* s.l. populations and the work of Skovmand &Sanogo, (2018), that reported permethrin resistance to be 20 times higher in larvae and 11 to 37 times higher in adult mosquitoes (measured by mortality or knock-down time).

Nevertheless, the result contradicts the work of Ndams et al., (2006), Selvi et al., (2007), Olayemi et al., (2011), Yoriyo et al., (2018), in separate research works on the KDR status of pyrethroids such as permethrin and cyfluthrin to *An. gambiae* (60 min) and *Cx. quinquefasciatus* (12.7–30 min) mosquitoes in different regions of Nigeria and Malaysia found mosquitoes susceptible at a certain range of $KDT_{50.}$

The finding also revealed mosquito species with higher KDT_{50} support resistance while those with lower KDT_{5O} support susceptible status to propoxur tested. The lower KDT_{50} values recorded in this study are similar to the values reported by Akiner, (2014), Boussougou-Sambe et al., (2018), Yoriyo et al., (2018), that indicated *An gambiae* and *Cx. quinquefasciatus* were susceptible to propoxur bendiocarb with high mortality>90 % at low minutes of 20.9 mins KDT_{50} and after 24hr post-exposure. The higher KDT_{50} values recorded in this study are similar to the values reported by (Ukpai & Ekedo, 2019), that *Cx. quinquefasciatus* was resistant to all the insecticides, with 24hr post-exposure percentage mortalities of 39.20, at 50 mins KDT50 for bendiocarb (carbamate).

The 24hr of (100 %) mortality recorded for the adult females *An. Gambiae and Cx. quinquefasciatus* in this study, is similar to the research findings of Corbel et al., (2007), Tahir et al., (2013), Opara et al., (2017), Boussougou-Sambe et al., (2018), Fagbohun et al., (2020), in West Africa, Nigeria, South-east Asian, and Malaysia, their reports revealed the susceptible status of these mosquitoes to malathion with KDT_{50} lower mortality time range values 21.50–23.30 mins while comparing to this current study. Nevertheless, this result is contrary to the report by (Norris & Norris, 2011), which showed resistance to malathion in Macha, Zambia after 24hr exposure period. A high level of malathion resistance has also been reported in *Cx. quinquefasciatus* larva in West India, (Delannay et al., 2018).

In the present study, potential cross-resistance occurred between permethrin and propoxur to both adult females *An. gambiae* and *Cx. quinquefasciatus* from Katcha and Shiroro with values of 86, 92, 94, 96, and 97 % mortality, respectively. Likewise, potential cross-resistance occurred between permethrin and malathion against the adult females *Cx. quinquefasciatus* from Lapai with 89 and 96 % mortality. These values are within the mortality percentage range value of 80–97 % of W.H.O standard. Also, the two different strains from Lapai and Bosso were susceptible to propoxur and malathion by presenting percentage mortality (100 %) during the study period.

This is the first report of cross-resistance of pyrethroids, carbamates, and organophosphates in adult females of these different strains in the State, which is evidence of regular usage of the three insecticides for malaria and filarial disease vectors control and crop protection in the study areas. This situation is in accord with the research results of Kerah-Hinzoumbé et al., (2008), Akiner, (2014), Samb et al., (2020) that indicated cross-resistance between pyrethroids, such as permethrin, deltamethrin, and carbamates such as bendiocarb and malathion. Also, in line with the findings of (Low et al., 2013), that demonstrated *Cx. quinquefasciatus* cross-resistance in Malaysia. Equally, cross-resistance between organophosphates and carbamates in *Cx. quinquefasciatus* has been documented frequently (Samb et al., 2020), indicating cross-resistance of pyrethroids, such as permethrin, deltamethrin, and carbamates such as bendiocarb and malathion. Cuamba et al., (2010), also revealed cross-resistance between pyrethroid and carbamate but in *An. funestus.*

For this connection, it may be possible of a strong physiological and ecological relationship of mosquito species between Katcha, Lapai, and Shiroro that warrants their unique physiological, structural, and behavioural adaptations. Another possible reason is that factors like agricultural uses of these insecticides can lead to selection pressure for the resistance, since members of communities of the study areas are local farmer involve on the use of chemical insecticides.

5. Conclusion

The percentage mortality and KDT50 range values obtained from the tested insecticides, *An. gambiae* and *Cx. quinquefasciatus* in the research areas are susceptible to potential resistance as well as multiple and cross-resistance. The ability to achieve such status is a result of residents in these areas' excessive reliance on insecticides to control agricultural pests and mosquitoes. These findings re-emphasize the on-going development of this species resistance to the tested insecticides used in impregnating Long Lasting

Insecticidal nets (LLINs) in the study areas. Thus, other alternatives as control strategies should be recommended.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to express their gratitude to the local communities in the study areas for enabling us to collect mosquito samples. We thank all our field assistants, as well as Mr. Musa Bulus, our laboratory technologist, for their support and assistance in the field and laboratory. We expressed our gratitude to the Federal University of Technology Minna in Niger State for allowing us to use their laboratory and equipment. Tetfund, which is part of the Nigerian Federal Ministry of Education, sponsored the study.

References

Abate, A., Getu, E., Wale, M., Hadis, M., Mekonen, W., 2020. Impact of bendiocarb 80% WP indoor residual spraying on insecticide resistance status of *Anopheles arabiensis*. Ethiopian J. Sci. Technol. 13 (3), 215–228.

Adamu, H., Clement, I., Hussaini, R., Inegbenosu, C., Obasuyi, C., Ezekiel, S., 2020. The Burden of Bancroftian Filariasis in Nigeria: A Review. Ethiopia J. Health Sci. 30, 2.

Agumba, S., Gimnig, J., Ogonda, L., Ombok, M., Kosgei, J., Munga, S., Ochomo, E., 2019. Diagnostic dose determination and efficacy of chlorfenapyr and clothianidin insecticides against *Anopheles* malaria vector populations of western Kenya. Malar. J. 18 (1), 243.

Aïzoun, N., Azondekon, R., Aïkpon, R., Gnanguenon, V., Osse, R., Asidi, A., Akogbéto, M., 2014. Study of the efficacy of a Wheaton coated bottle with permethrin and deltamethrin in laboratory conditions and a WHO impregnated paper with bendiocarb in field conditions. Asian Pac. J. Trop. Biomed. 4 (6), 492–497.

Akiner, M.M., 2014. Malathion and propoxur resistance in Turkish populations of the *Anopheles maculipennis meigen* (Diptera: *Culicidae*) and relation to the insensitive acetylcholinesterase. Turkiye Parazitol. Derg. 38 (2), 111.

Awolola, T., Oyewole, I., Amajoh, C., Idowu, E., Ajayi, M., Oduola, A., Coetzee, M., 2005. Distribution of the molecular forms of *Anopheles gambiae* and pyrethroid knockdown resistance gene in Nigeria. Acta Trop. 95 (3), 204–209.

Bisset, J., Rodríguez, M.M., Fernández, D., 2006. Selection of insensitive acetylcholinesterase as a resistance mechanism in *Aedes aegypti* (Diptera: *Culicidae*) from Santiago de Cuba. J. Med. Entomol. 43 (6), 1185–1189.

Boussougou-Sambe, S.T., Eyisap, W.E., Tasse, G.C.T., Mandeng, S.E., Mbakop, L.R., Enyong, P., Awono-Ambene, P.H., 2018. Insecticide susceptibility status of *Anopheles gambiae* (sl) in South-West Cameroon four years after the long-lasting insecticidal net mass distribution. Parasit. Vectors 11, 391.

Bracco, J.E., Barata, J.M.S., Marinotti, O., 1999. Evaluation of insecticide resistance and biochemical mechanisms in a population of *Culex quinquefasciatus* (Diptera: *Culicidae*) from São Paulo. Brazil. *Memórias do Instituto Oswaldo* Cruz 94 (1).

Brogdon, W.G., McAllister, J.C., 1998. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. J. Am. Mosquito Control Assoc. 14 (2), 159–164.

Brooke, B.D., Koekemoer, L.L., 2010. Major effect genes or loose confederations? The development of insecticide resistance in the malaria vector Anopheles gambiae. Parasit. Vectors 3, 74.

Chukwu, O., Ezenwenyi, J.U., Mebude, K.O., 2018. Spatial distribution of Nigerian universities offering forestry education using geographic information system. World News Nat. Sci. 20, 226–237.

Corbel, V., Nguessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Rowland, M., 2007. Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. Acta Tropica 101 (3), 207–216.

Cuamba, N., Morgan, J.C., Irving, H., Steven, A., Wondji, C.S., 2010. High level of pyrethroid resistance in an *Anopheles funestus* population of the Chokwe District in Mozambique. PLoS One 5 (6), e11010.

Delannay, C., Goindin, D., Kellaou, K., Ramdini, C., Gustave, J., Vega-Rúa, A., 2018. Multiple insecticide resistance in *Culex quinquefasciatus* populations from Guadeloupe (French West Indies) and associated mechanisms. PLoS One 13 (6), e0199615.

Djouaka, R.J., Atoyebi, S.M., Tchigossou, G.M., Riveron, J.M., Irving, H., Akoton, R., Wondji, C.S., 2016. Evidence of multiple insecticide resistance in the malaria vector *Anopheles funestus* in southwest Nigeria. Malar. J. 15 (1), 1–10.

Fagbohun, I.K., Idowu, E.T., Otubanjo, O.A., Awolola, T.S., 2020. Susceptibility status of mosquitoes (Diptera: Culicidae) to malathion in Lagos, Nigeria. Animal Res. Int. 17 (1). 3541–3549-3541–3549.

Fortuna, C., Montarsi, F., Severini, F., Marsili, G., Toma, L., Amendola, A., Capelli, G., 2021. The common European mosquitoes *Culex pipiens* and *Aedes albopictus* are unable to transmit SARS-CoV-2 after a natural-mimicking challenge with infected blood. Parasit. Vectors 14 (1), 1–6.

Francis, S., Saavedra-Rodriguez, K., Perera, R., Paine, M., Black IV, W.C., Delgoda, R., 2017. Insecticide resistance to permethrin and malathion and associated mechanisms in *Aedes aegypti* mosquitoes from St. Andrew Jamaica. PloS One 12 (6), e0179673.

Ibrahim, S.S., Manu, Y.A., Tukur, Z., Irving, H., Wondji, C.S., 2014. High frequency of kdr L1014F is associated with pyrethroid resistance in *Anopheles coluzzii* in Sudan savannah of northern Nigeria. BMC Infect. Dis. 14, 441.

Kerah-Hinzoumbé, C., Péka, M., Nwane, P., Donan-Gouni, I., Etang, J., Samè-Ekobo, A., Simard, F., 2008. Insecticide resistance in *Anopheles gambiae* from south-western Chad, Central Africa. Malaria J. 7 (1), 192.

Kumar, K., Sharma, A.K., Kumar, S., Patel, S., Sarkar, M., 2011. Multiple insecticide resistance/susceptibility status of *Culex quinquefasciatus,* principal vector of bancroftian filariasis from filaria endemic areas of northern India. Asian Pac. J. Trop. Med. 426, 429.

Low, V., Chen, C.D., Lee, H., Lim, P., Leong, C., Sofian-Azirun, M., 2013. Current susceptibility status of Malaysian *Culex quinquefasciatus* (Diptera: *Culicidae*) against DDT, propoxur, malathion, and permethrin. J. Med. Entomol. 50 (1), 103–111.

National Bureau of Statistics, 2020. Demographic statistics bulletin 2020.

Nazni, W., Lee, H., Azahari, A., 2005. Adult and larval insecticide susceptibility status of *Culex quinquefasciatus* (Say) mosquitoes in Kuala Lumpur Malaysia. Tropical Biomed. 22 (1), 63–68.

Ndams, I., Laila, K., Tukur, Z., 2006. Susceptibility of some species of mosquitoes to permethrin pyrethroid in Zaria Nigeria. Sci. World J. 1 (1).

Norris, L.C., Norris, D.E., 2011. Insecticide resistance in *Culex quinquefasciatus* mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. J. Vector Ecol. 36 (2), 411–420.

Olayemi, I., Ande, A., Chita, S., Ibemesi, G., Ayanwale, V., Odeyemi, O., 2011. Insecticide susceptibility profile of the principal malaria vector, *Anopheles gambiae* sl (Diptera: I), in north-central Nigeria. J. Vector-Borne Dis. 48 (2), 109.

Olayemi, I., Ukubuiwe, A., Oyibo-Usman, K., 2014. Mosquito species occurrence and diversity in conventional larval breeding sites in Minna metropolis, Nigeria. Int. J. Innovation Sci. Res. 9 (1), 86–93.

Opara, K., Ekanem, M., Udoidung, N., Chikezie, F., Akro, G., Usip, L., Igbe, M., 2017. Insecticide Susceptibility Profile of *Anopheles gambiae* sl from Ikot-Ekpene, Akwa Ibom State, Nigeria. Ann. Res. Rev. Biol., 1–9

Organization, W. H. (2016). Test procedures for insecticide resistance monitoring in malaria vectormosquitoes.http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1.

Pates, H., Curtis, C., 2005. Mosquito behavior & vector control. Annu. Rev. Entomol. 50, 53–70.

Rahimi, S., Vatandoost, H., Abai, M.R., Raeisi, A., Hanafi-Bojd, A.A., 2019. Status of Resistant and Knockdown of West Nile Vector, *Culex pipiens* Complex to Different Pesticides in Iran. J. Arthropod. Borne Dis. 13 (3), 284.

Ramos, A.G.H., 2020. Evaluation of the resistance to insecticides in *Aedes aegypti,* transmitter of dengue, in Latin America. Mexican J. Medical Res. ICSA 8 (15), 23–28.

Reid, M.C., McKenzie, F.E., 2016. The contribution of agricultural insecticide use to increasing insecticide resistance in African malaria vectors. Malar. J. 15 (1), 1–8.

Sakuma, M., 1998. Probit analysis of preference data. Appl. Entomol. Zool. 33 (3), 339–347.

Samb, B., Diagne, C.T., Diouf, M., Konaté, A., Dia, I., Faye, O., Konaté, L., 2020. Multiple insecticide resistance target sites in adult field strains of *Anopheles. gambiae* (sl.) from southeastern Senegal. Parasit. Vectors 13 (1), 1–10.

Selvaraj, P., Wenger, E.A., Bridenbecker, D., Windbichler, N., Russell, J.R., Gerardin, J., Nikolov, M., 2020. Vector genetics, insecticide resistance and gene drives: an agent-based modeling approach to evaluate malaria transmission and elimination. Plos Computational Biol. https://doi.org/10.1371/journal.pcbi.1008121.

Selvi, S., Edah, M., Nazni, W., Lee, H., Azahari, A., 2007. Characterization on malathion and permethrin resistance by bioassays and the variation of esterase activity with the life stages of the mosquito *Culex quinquefasciatus*. Trop. Biomed. 24, 63–75.

Shetty, V., Sanil, D., Shetty, N.J., 2013. Insecticide susceptibility status in three medically important species of mosquitoes, *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus,* from Bruhat Bengaluru Mahanagara Palike, Karnataka, India. Pest Manage. Sci. 69 (2), 257–267.

Skovmand, O., Sanogo, E., 2018. Resistance of *Culex quinquefasciatus* to selected chemical and biological pesticides. Medical Res. Arch. 6 (4).

Tahir, H.M., Hussain, K., Khan, A.A., Naseem, S., Malik, H.T., Butt, A., Yaqoob, R., 2013. Susceptibility of *Culex quinquefasciatus* (Diptera: *Culicidae*) to malathion in Sargodha district, Pakistan. J. Animal Sci. 3 (4A), 1–4.

Ukpai, O., Ekedo, C., 2019. Insecticide susceptibility status of *Culex quinquefasciatus* [Diptera: *Culicidae*] in Umudike, Ikwuano LGA Abia State, Nigeria. Int. J. Mosquito Res. 6 (1), 114–118.

Ukubuiwe, A.C., Olayemi, I.K., Omalu, I.C.J., Odeyemi, M.O., Jibrin, A.I., Oyibo-Usman, K.A., 2012. Comparative assessment of immature survivorship and developmental duration of *Culex pipiens pipiens* (Diptera: *Culicidae*) populations in north-central Nigeria. *Biomed Central*. Epidemiology 3 (10), WMC003753.

Umar, A., Kabir, B., Amajoh, C., Inyama, P., Ordu, D., Barde, A., Kobi, M., 2014. Susceptibility test of female *Anopheles* mosquitoes to ten insecticides for indoor residual spraying (IRS) baseline data collection in Northeastern Nigeria. J. Entomol. Nematol. 6 (7), 98–103.

Vatandoost, H., Abai, M.R., Akbari, M., Raeisi, A., Yousefi, H., Sheikhi, S., Bagheri, A., 2019. Comparison of CDC bottle bioassay with WHO standard method for assessment susceptibility level of the malaria vector, *Anopheles stephensi* to three imagines. J. Arthropod. Borne Dis. 13 (1), 17.

Wang, Y., Cheng, P., Jiao, B., Song, X., Wang, H., Wang, H., Gong, M., 2020. Investigation of mosquito larval habitats and insecticide resistance in an area with a high incidence of mosquito-borne diseases in Jining, Shandong Province. PloS one 15, 3.

Wanjala, C.L., Mbugi, J.P., Ototo, E., Gesuge, M., Afrane, Y.A., Atieli, H.E., Yan, G., 2015. Pyrethroid and DDT resistance and organophosphate susceptibility among Anopheles spp. mosquitoes, western Kenya. Emerg. Infect. Dis. 21 (12), 2178.

Yoriyo, K., Samdi, L., Wanah, B., Edward, E., Kela, S., 2018. Susceptibility of *Culex quinquefasciatus* Say, 1823 (Diptera; *Culicidae*) to cyfluthrin, propoxur and malathion in the Sudan Savanna, North Eastern Nigeria. Nigeria J. Entomol. 34, 19–24.

Yusuf, M.A., Oshaghi, M.A., 2021. Current Status of Insecticide Susceptibility in the Principal Malaria Vector, *Anopheles gambiae* in the Three Northern States of Nigeria. J. Arthropod. Borne Dis. 15 (2), 196–206.

Zamora Perea, E., Balta Leon, R., Palomino Salcedo, M., Brogdon, W., Devine, G., 2009. Adaptation and evaluation of the bottle assay for monitoring insecticide resistance in disease vector mosquitoes in the Peruvian Amazon. Malar. J. 8, 208.