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Full Length Research Article

INSECTICIDE SUSCEPTIBILITY STATUS OF MOSQUITOES FROM FOUR SELECTED COMMUNITIES OF KAFANCHAN, SOUTHERN PART OF KADUNA STATE

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ABSTRACT
A study was carried out in Kafanchan metropolis to determine the susceptibility status of *Anopheles gambiae* s.l (23.66 %), *Aedes aegypti* (24.76 %) and *Culex quinquefascaitus* (51.57 %) to the four major classes of insecticides approved by W.H.O. Mosquito larvae were sampled bi-weekly using the standard (350 mL dipper) dipping method from behind and around high-density populated communities in Kafanchan metropolis. They were sorted into species using taxonomic keys and reared in the laboratory to adult stage. DNA was extracted from selected *Anopheles* for identification. The sucrose-fed two to three days old adult females were tested for susceptibility according to WHO Insecticide Resistance Bioassay guidelines. The mosquitoes were tested against four insecticides (Pyrethroid: Permethrin 0.75 %; Carbamates: Bendiocarb 0.1 %; Organochlorine; Dichloro-Diphenyl-Trichloroethane {D.D.T} 4.0 % and Organophosphate; Pirimiphosmethyl 0.25 %) using the W.H.O recommended insecticide impregnated papers as the diagnostic kits for one hour and kept in holding test tubes for 24-hour period against the insecticides. A total of 1272 mosquito samples were exposed to the W.H.O insecticides papers. Mortality counts showed that all mosquitoes were suspected to be resistant to all the insecticides (73.84 % - 92.24 %). DDT insecticide had least significant effect on *Anopheles* (82.50 %) and *Culex* (73.82 %), while Bendiocarb holds promise for greatest effect on all mosquito species. This could be attributed to the common use of different types of insecticides and pesticides for control of medical insects and agricultural practices that occur in urban settlements. The study recommends regulated use of different classes of insecticides / pesticides for the control of mosquitoes specially to reduce the increase of insecticide resistance.

Keywords: Kafanchan Metropolis, Insecticide resistance *Anopheles gambiae* s.l, *Aedes aegypti* and *Culex quinquefascaitus*.

INTRODUCTION
Mosquitoes are small slender-bodied insects which female species viciously hunt their hosts, especially man and other animals for blood meals in order to continue their life cycle (Oniyado *et al.,* 2008). They have worldwide distribution and are found in both tropics and temperate regions of the world (Service, 1993). They are important and serve as vectors that carry parasites of diseases that plague humans including malaria, lymphatic filariasis, yellow fever, dengue haemorrhagic fever, chikungunya, and zika (WHO, 2014).

Malaria, one of many devastating diseases; is caused by a protozoan parasite from the genus *Plasmodium* and transmitted by *Anopheles* species. In 2020, COVID-19 emerged as an additional challenge to the provision of essential health services worldwide. Despite the advent or outbreak of COVID – 19 disease in the world, World health organization reported a surge in malaria burden (WHO, 2020) and called on partners, countries and implementers to scale up the fight against malaria. Currently, according to World malaria report, there were approximately 241 million cases of malaria and about 627 000 deaths caused by malaria in 2020 (WHO, 2021).

Moreover, insecticide based interventions have been the core mosquito control interventions in Nigeria and it has been reported in several areas that there is a growing trend of resistance to insecticides used in public health for the control of vectors especially *Anopheles* in Nigeria (National Plan for Insecticide Resistance Monitoring in Nigeria 2017-2020). Going by the current trend, it is believed that these interventions may have effect on the development of resistant populations in the mosquito vectors of other diseases including *Anopheles, Aedes* and *Culex,* mosquitoes (NMSP, 2014).

Insecticide resistance information and available data in Nigeria includes, especially Kaduna State but scanty, hence, it is important to establish the distribution of other mosquito vectors and determine their insecticide resistance status in Kaduna State where little to no data is available on such. This would provide more data and template for future vector control activities and disease responses in Kaduna State.

Kaduna state, especially southern part of Kaduna (Kafanchan) has little information on mosquito vectors insecticide resistance or susceptibility status and characterization (Ndams *et al.,* 2006 and Sow *et al.,* 2007). However, there are studies on insecticide resistance in *Anopheles* mosquito in different parts of the Country (Ndams *et al.,* 2006; Awolola *et al*., 2007; Oduola *et al*., 2012; Ibrahim *et al*., 2013; Ayorinde *et al.,* 2015) to the neglect of mosquito species like *Aedes* and *Culex*. It therefore become pertinent that investigations on the insecticide resistance or susceptibility status to include also *Anopheles* species, *Aedes aegypti* and *Culex quinquefasciatus* be carried out.

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MATERIALS AND METHODS

Study Area:

This study was conducted in Kafanchan metropolis, Kaduna state Nigeria; (09° 34' 52.52" N to 09° 59' 16.1"N and 08° 17' 33.36"E to 08° 23' 09.34"E) Greenwich Meridian (Gazetta, 1965) with estimated population of 278,202; (NPC, 2018), (Fig.1). The locations and communities were purposely chosen due to the presence of mosquito larval when a pre-study survey was conducted; the locations were, Garaje, Takau, Zakwa & Zauru, (Table 1). The climatic conditions, human activities and vegetative cover of Kafanchan metropolis results to favorable mosquito breeding habitats and mosquito / vector-borne disease transmission. These environmental factors varies from the northern to the southern part of the state with an annual mean temperature of between 24 °C and 29 °C; amount of annual rainfall is between 1,500 mm to 2,000 mm within 150days to 190 days; also relative humidity ranging between 20 % and 30 % in January, rising to between 60 % and 80% in July (Al-amin & Garba, 2014). The vegetation cover is southern Guinea savannah in the south of the State (Al-amin & Garba, 2014). Human activities which includes farming, use of chemicals, unplanned housing/slums amongst others are the leading factors which causes insecticides resistance, especially mosquito borne diseases.

Table 1: List of Communities where mosquitoes were sampled

S/No	Community	GPS coordinates Lat. (N°)	Lon. (E°)
1	Garaje	9. 59161	8.29902
2	Takau	9.57626	8.30425
3	Zakwa	9.60395	8.31784
4	Zauru	9.57942	8.23934

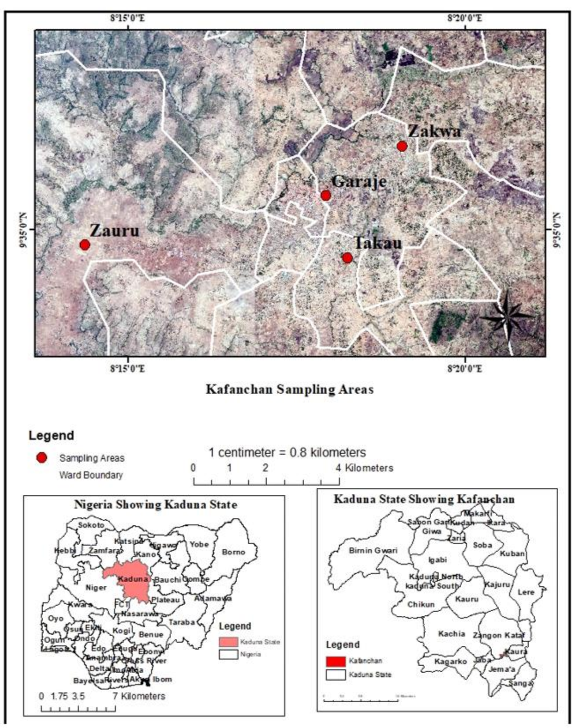

Fig 1. Kafanchan Metropolis: Sampling Locations (Source: Map Gallery, Geography Department, A.B.U Zaria)

Sampling Techniques, Identification and Handling of Mosquitoes.

Sample of mosquitoes immature larval stages was collected using the standard 350 ml plastic dipper of 7cm in diameter and a depth of 5cm with a 30cm long handle, (Service, 1976) from the natural habitats once every month from the period of six months from habitats that are positive for breeding of mosquitoes in the four selected communities within Kafanchan metropolis. The immature larval stages collected were carefully transported in suitable vials to the insectary at the laboratory of Department of Biological sciences, Faculty of Science Kaduna State University and were reared to adults in separate chambers under regulated optimum conditions at 25-28 °C. When adults emerged, they were fed with 10 % sucrose solution. Some adults were identified with the guide of the pictorial keys by Hopkins G.H.E (1952); Gillies & Coetzee (1987) and Reeds (2004) and molecular identification by DNA extracted from 40 female *Anopheles* mosquitoes. Only emerged and identified adult females were collected, sorted out and used for (bioassay) the insecticides resistance and susceptibility test amongst the 4 major classes of neurotoxic insecticide (carbamates, organochlorines, organophosphates, and pyrethroids).

Insecticide susceptibility test

Bioassays to detect resistance or susceptibility of adults were performed using sucrose fed, 2 to 3 day old reared adults. WHO Standard Insecticide- Impregnated Papers, test tubes and other kits were obtained from Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, (ACENTDFB) Ahmadu Bello University, Zaria, Nigeria. The procedure involves four batches of 20 – 25 each of reared adult were exposed to insecticide- impregnated papers in standard WHO test tubes lined with the papers. The mosquitoes were tested against four insecticides (Pyrethroid: Permethrin 0.75 %, Carbamates: Bendiocarb 0.1 %: Organochlorine; Dichloro-Diphenyl-Trichloroethane {D.D.T} 4.0 % and Organophosphate; Pirimiphosmethyl 0.25 %). For the control, 20 - 25 exposed to plain papers (non-treated papers). The mosquitoes were exposed to the diagnostic dosages at the respective exposure period as prescribed by the WHO Techniques to detect insecticide resistance mechanisms (field and laboratory manual), Hemmingway, (1998) and WHO, (1998 & 2016a). The test mosquitoes and the controls were held for 24 hours recovery period and the mortality recorded. For any Bioassay Control test that recorded mortality falls between 5 % and 20 %, the percentage mortalities were corrected by Abbott's formula (Abbott, 1925 & WHO, 2016a). All data were subjected to excel analysis computer program. The knock-down time after intervals of 10 minutes, 20, 30, 40, 50 and 60 minutes was expressed in percentages calculated using statistical packages and 30 minutes and 1 hour knock-down time (KDT_{30} & KDT_{60}) obtained (Raymond, 1985). Mean ratio and percentage mortality were calculated; thus the mortality rate was calculated as the percentage of within 24 hours post exposure. The susceptibility status was determined according to WHO guidelines; (WHO, 2016b) where 24 hours mortality rate from 0.00 % to 79.99 % is considered to be Resistance Established (RE) while Suspected Resistance (SR) is from 80.00 % to 95.99 % and Susceptibility Established is from 96 % to 100 %.

Molecular identification of *Anopheles* mosquitoes

The Quick-DNA™ Miniprep Plus Kit (D4069) product by ZYMO research company was used to extract genomic DNA (gDNA) from

40 mosquitoes according to the manufacturer's protocol. The genomic DNA was used in *Anopheles* mosquito identification to different species by the two protocols. PCR reagents, molecular graded chemicals and the primers used were obtained from New England BioLabs© retailed via Africa's Genomics company; INQABA Biotec West Africa Ltd (REF.No:NG2020/29193). The molecular identification reactions procedure were two steps. The protocols / steps were for PCR amplification reactions according to the protocol prescribed by Scott *et al.* (1993) which involved the use of three primers with two being species specific that identify *Anopheles arabiensis* and *gambiae* s.l (Table 2).

Table 2: Molecular Primers list distinguishing *Anopheles* species complex

Primer Name	Sequence (5' to 3')	Prod. Size (bp)	Source
IGS for *Anopheles* spp			
Universal (UN) F	GTGTGCCCCTTCCTCGATGT	Int control	Scott *et al.* (1993)
Arabiensis (AR) R	AAGTGTCCTTCTCCATCCTA	315	"
Gambiae (GA) R	CTGGTTTGGTCGGCACGTTT	390	"

The universal primer (UN) which is an internal control intergenic segment (IGS) anneals to the same position of the rDNA of all *Anopheles gambiae* species; thus referred to as universal primer. The GA primer anneals specifically to *An. gambiae* s.l and expresses a 390 bp in agarose gel. The AR anneals to *An. arabiensis* to show a 315 bp band.

RESULTS

A total of 1272 mosquitoes were encountered and species identified include the three major *Anopheles* species 301 (23.66 %), three major *Aedes* species 314 (24.76 %) and four major *Culex* species 656 (51.57 %) which comprised the majority of mosquitoes collected (Table 4). These mosquitoes were encountered mostly during the rainy seasons. *Anopheles* and *Aedes* mosquitoes were very rare during the dry season except for stagnant concrete gutters which were conducive for *Culex* species breeding. The knock-down time after intervals of 10 minutes, 30, 60 minutes and 24 hours was recorded and the summary mean knock-down time (30 minutes and 60 minutes) of the four insecticides as determined against adults that were sampled from Kafanchan metropolis as expressed (Table 3) showed that Pirimiphos-methyl insecticide had the lowest knock-down effect of 4.08±1.03 at 30 minutes and 10.50±1.67 at 60 minutes; while Bendiocarb had the highest knockdown effect at 30 minutes and 60minutes (Table 5).

Table 3: Mosquito species (%) sampled in the four selected communities of Kafanchan metropolis and environs, Kaduna state.

S/No	Mosquito species	No (%)
1	*Anopheles gambiae* (s.l)	193(15.17)
2	*Anopheles arabiensis*	61(04.80)
3	*Anopheles funestus*	47(03.69)
4	*Aedes aegypti*	233(18.32)
5	*Aedes vittatus*	73(05.74)
6	*Aedes simpsoni*	09(0.71)
7	*Culex quinquefasciatus*	573(45.05)
8	*Culex horridus*	39(03.66)
9	*Culex tigripes*	10(0.79)
10	*Culex univittatus*	34(02.67)
	Total number = 1272	

Table 4: The percentage Knock-down time (KdT) for the four major classes of Insecticides against *Anopheles gambiae* s.l, *Aedes aegypti* and *Culex quinquefasciatus* from Kafanchan metropolis, Kaduna state

Species	Insecticide (Conc)	No. Exposed	10 Mins	30 Mins	60 Mins	24 Hrs.
***Anopheles gambiae* s.l**	Permethrin (0.75%)	63	1.50±0.65a	4.25±0.48ab	10.00±0.41b	13.50±0.65b
	Bendiocarb (0.1%)	41	0.75±0.48a	3.75±0.63b	7.50±0.65b	9.25±0.63c
	D.D.T* (4.0%)	80	1.00±0.41a	5.50±1.04ab	13.50±1.19a	16.50±0.65a
	Pirimiphos methyl (0.25%)	70	2.00±0.54a	7.71±1.38a	14.14±1.37a	16.43±1.13a
		P-Value	**0.089**	**0.001**	**0.000**	**0.000**
Aedes aegypti	Permethrin (0.75%)	80	0.75±0.48a	5.75±1.11a	13.75±1.11a	18.25±0.85a
	Bendiocarb (0.1%)	75	1.00±0.41a	5.75±1.93a	13.75±1.11a	17.25±1.18a
	D.D.T* (4.0%)	80	0.00±0.00a	3.50±0.65a	14.00±1.35a	17.75±0.85a
	Pirimiphos methyl (0.25%)	80	1.00±0.41a	5.75±0.48a	15.50±0.87a	17.50±0.65a
		P-Value	**0.103**	**0.006**	**0.000**	**0.000**
Culex quinquefasciatus	Permethrin (0.75%)	80	2.25±1.31a	7.25±2.78a	12.75±2.14ab	19.50±0.29ab
	Bendiocarb (0.1%)	75	2.25±1.32a	7.00±2.27a	15.75±1.38ab	19.75±0.25ab
	D.D.T* (4.0%)	85	1.75±0.85a	5.25±1.44ab	15.00±1.78ab	21.00±1.00a
	Pirimiphos methyl (0.25%)	80	0.25±0.25a	2.25±1.93ab	9.75±4.54b	18.00±2.00bc
		P-Value	**0.310**	**0.046**	**0.000**	**0.000**

Keys: * D.D.T = Dichloro-Diphenyl-Trichloroethane **** KdT – 30, 60 & 24hr =** Knock-down time for 30, 60 minutes and 24 hours means at 5% level of probability (According to WHO guidelines; 2016: http://www.who.int/whopes/resistance/en/.)

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Table 5: Resistance status of *Anopheles gambiae* s.l. *Aedes aegypti and Culex quinquefasciatus* to insecticides in Kafanchan metropolis and environs

Location	Insecticide conc. (class/type)	*Anopheles gambiae s.l* Number tested (N)	% Mortality @24hrs / (Abbott)	Status	*Aedes aegypti* Number tested (N)	% Mortality @24hrs / (Abbott)	Status	*Culex quinquefasciatus* Number tested (N)	% Mortality @24hrs / (Abbott)	Status
Kafanchan	Permethrin 0.75%	63	85.71	SR	80	93.88	SR	80	92.24	SR
Metropolis	Alpha-cypermethrin 0.05%							74	95.95	SE
	Lambda-cyhalothrin 0.05%							80	93.49	SR
	Bendiocarb 0.1%	41	90.24	SR	75	84.91	SR	75	87.64	SR
	Propoxur 0.1%							69	89.86	SR
	D.D.T 4.0%	80	82.50	SR	80	88.75	SR	85	73.82	RE
	Pirimiphos-methyl 0.25%	70	82.86	SR	80	87.50	SR	80	84.74	SR

Keys:
* **D.D.T =** Dichloro-Diphenyl-Trichloroethane SR = Suspected Resistance (Mortality from 80.00% - 95.99%)
While S.E = Susceptibility established (Mortality from 96.00% - 100%); R.E = Resistance established (Mortality from 0.00% - 79.99%).
(According to WHO guidelines; 2016: http://www.who.int/whopes/resistance/en/.)

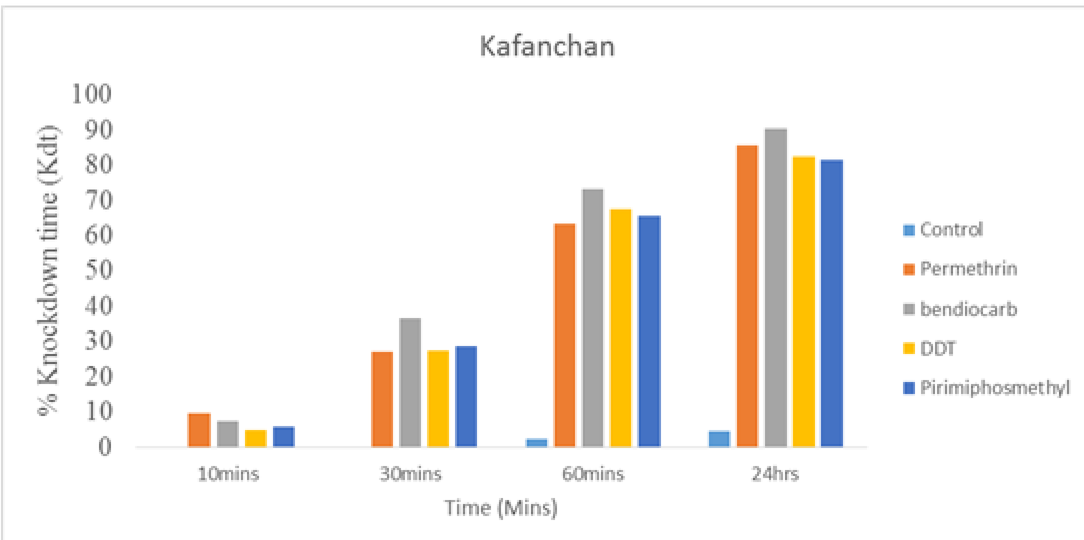

Fig 2: Kafanchan Metropolis. Mosquitoes Knock down profile.

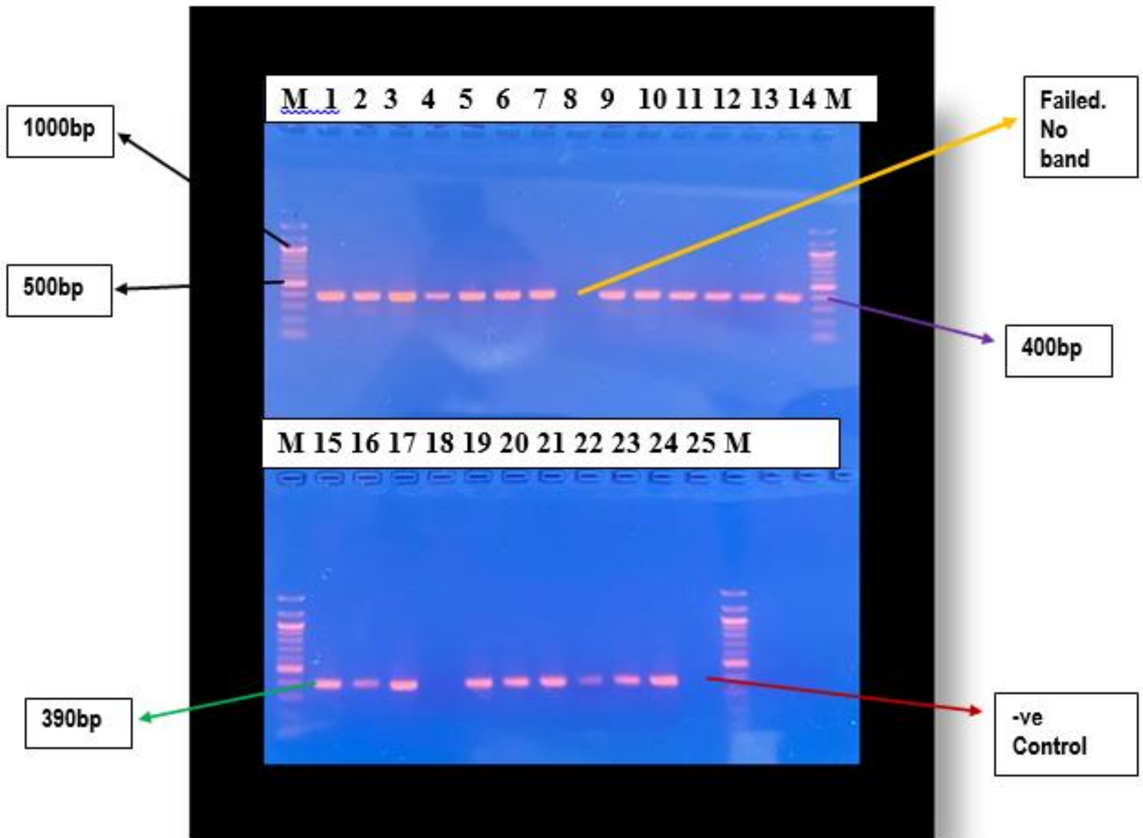

Plate I. Agarose gel picture of 1.5% for distinguishing *An. gambiae* s.l after PCR with primers (UN, AR, GA). Lane M = 100bp molecular weight marker. Amplicons on lane 1 to 24 are from selected high molecular weight DNA of *Anopheles* mosquitoes sampled in Kafanchan. Lane 25 is negative control. DNA band sizes of 390bp to authenticate species to be *Anopheles gambiae* s.l

DISCUSSION

The research reports the presence of three major mosquito genera (Anopheles, Aedes and Culex) that are commonly encountered within Kafanchan metropolis and environs. Anopheles, Aedes and Culex species were similarly reported by Anyanwu *et al.* (1999) in a study of Survey of Culicids in a Northern Guinea Savannah town of Zaria, Kaduna State; and Adebote *et al.* (2006); and Sow *et al.,* (2008) reported same in Malagum, Kwoi and Kafanchan environs and Yayock *et al.* (2014) in a survey on distribution of mosquitos species in Kaduna metropolis. Due to urbanization, climatic conditions, human activities like insecticide or pesticide use and pressure, there was an expected change in the population structure, diversity and abundance in the three different study areas. The summary mortality bioassay of the four major classes of insecticides as determined against adult mosquitoes that were sampled from the four selected communities of Kafanchan metropolis and environs was recorded (Table 5). The Malaria vector is of utmost importance in this study, thus the focus. Resistance of *Anopheles* mosquitoes to insecticides is common and wide spread in Nigeria and now Kafanchan in Kaduna state from the results of this study. *Anopheles gambiae* which is the principal vector for malaria was knocked down (9.25±0.63) least after 24 hours by bendiocarb insecticide and results revealed that suspected resistance (SR) was established in *Anopheles gambiae* s.l in all the class of insecticides, this agrees with study in Maiduguri by Umar *et al.* (2014) and Abdu *et al,* (2017) where a study on susceptibility status of *Anopheles gambiae* s.l. in Auyo, Jigawa state reported *Anopheles gambiae* s.l were resistant to DDT (9 % - 16 %) and permethrin (25 % - 35 %) but susceptible to bendiocarb (81% - 94%). A report by Oduola *et al.* (2016 & 2019) in south-western Nigeria and Gombe where surveillance on insecticide resistance in mosquito species were conducted, it revealed that there was resistance to especially pyrethroids type insecticides. Similar situation was encountered by Oyewole *et al.* (2018), in a study on susceptibility pattern of *Anopheles gambiae* s.l to different classes of insecticide in Osun, south-western Nigeria where *Anopheles gambiae* s.l was susceptible to four insecticides (permethrin: 55 %; Deltamethrin: 53 %; DDT: 19 % and lambda-cyhalothrin: 47%) but resistant to fenitrothion (99 %) and bendiocarb (98 %).

In the bioassay for *Aedes aegypti* and *Culex quinquefasciatus* suspected resistance was seen to all insecticides also but only Alpha-cypermethrin 0.05 % recorded high mortality; thus susceptibility established (SE). Resistance to the four major classes of insecticides were established in Kafanchan metropolis and environs; this agrees with studies carried out by Ibrahim *et al.* (2014); in Kano; Ndams *et al.* (2006) and Sow *et al.* (2007). The distribution of insecticides resistance of *Anopheles gambiae s.l* , *Aedes aegypti* and *Culex quinquefasciatus* in selected communities in Kafanchan did not vary significantly (P>0.023). Personal communications and localized interviews revealed that most communities and residents only use mechanical methods to control mosquitoes; example opening doors and windows to manually drive out adult mosquitoes. There is rarely usage of chemicals, except locally made smoke coils with lemon grass or dried orange peels.

Conclusion

The study deployed WHO standard Bioassay test using four selected insecticides to conduct *Anopheles gambiae* s.l susceptibility / resistance bioassay research. The overall insecticides susceptibility / resistance status of the known vector of malaria disease *Anopheles gambiae* s.l (77.76 %) to all the four selected insecticides paints a grim picture of suspected resistance. This suggests a serious setback to the effort to reduce malaria burden by 90 %, in line with WHO projection by the year 2030. However, this research showed that Carbamates (bendiocarb) holds good mosquitocidal abilities as a substitute for permethrin or D.D.T which are the commonly used and available insecticide or market-based control strategies against *Anopheles* mosquitoes that most members of the communities use. The research recommends continuous Mosquito Insecticide Resistance Mechanism and Surveillance. This will help in implementation of federal government 2030 malaria reduction goal.

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