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Identification and assessment of knock down rate of *Aedes* mosquitoes in northern Katsina State, Nigeria

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Abstract
This study was conducted to investigate insecticide resistance in *Aedes* mosquitoes in some Local Government Areas (LGAs) of northern Katsina State, Nigeria. *Aedes* mosquitoes larvae were collected from five LGAs namely Batagarawa, Daura, Kaita, Katsina, and Mashi. The mosquito larvae were then transported to the Postgraduate Research Laboratory of the Department of Biology of Umaru Musa Yar'adua University, Katsina (UMYUK), Nigeria for adult emergence. Four different WHO insecticide-impregnated papers namely Dichloro Diphenyl Trichloroethane (DDT 4%), pirimiphos-methyl (0.25%), Bendiocarb (0.1%) and Permethrin (0.75%) were used for this study. The impregnated papers were tested against the emerged adults of the collected *Aedes* mosquitoes using WHO standard operating procedure. The tested mosquitoes were identified and their knock down rates (kdr) to the test insecticides were determined. Both morphological and molecular identification revealed presence of two species of *Aedes* mosquitoes in the study areas namely *Aedes aegypti* and *Aedes albopictus*. *Ae. aegypti* was more abundant than *Ae. albopictus*. The kdr at 30 minutes was highest (39%) with pirimiphos-methyl, while permethrin had the least (1%). Similar trend in kdr at 30 minutes was recorded at 60 minutes after exposure. Pirimiphos-methyl was therefore recorded to be more effective against *Aedes* mosquitoes of the study area than the other insecticides. There is needed to investigate insecticide resistance mechanism in *Aedes* mosquitoes in Katsina State for proper control strategies.

Keywords: *Aedes aegypti*, *Aedes Albopictus*, Insecticides, Katsina, Knock down rate

1. Introduction
Nigeria as a country has been reported to be the most highly risked countries of the diseases named yellow fever in specific and one of the most susceptible country where many diseases resides [1, 2]. Furthermore, there was a major epidemiological threat in many major cities of the country where by the activities of the residence were hindered as a result of the risk they are facing [2]. Immunizing the urban populations in this high-risk country would require vaccinating almost 100 million people [3].
Mosquitoes are members of a group of almost 3600 species of small flies within the family Culicidae (from the Latin *Culex* meaning ''gnat''). A female mosquito is an insect that bites and feeds on the blood of humans and other animals, while male mosquito feed only on nectar and other substances, females need blood meals in order to produce viable eggs that will hatch [4, 5]. These mosquitoes are active from vespers until sun-up, but many other mosquito species are known as day feeders and are active during the day, especially around the dusk and dawn time frames. They find their host via heat, scent and exhaled carbon dioxide [6].
Control of mosquitoes is important because of their ability to spread disease such as malaria fever, filariasis, Yellow fever, chikungunya, west Nile, dengue fever and zika; their impact on local economics, and the low tolerance of the public to high number of biting mosquitoes [5]. In the absence of vaccine, vector control and personal protection remain the chief support for prevention of infection with pathogens transmitted by mosquitoes [5].
With the various control measures of controlling mosquitoes using synthetic chemical insecticides that are available to public, resistance has been developed to several insecticides. In Nigeria, *Aedes* mosquitoes have become serious species that have developed resistance and cross-resistance to a wide range in the public. However, little or no information can be found

on the resistance of *Aedes* mosquitoes in Katsina State.

An outbreak of yellow fever was observed in some Nigerian states [7]. In 2021, the outbreak was reported to reach a total of 2053 suspected cases in the country across all the states [8]. However, there was a drastic fall in yellow fever cases to a total of 31 in Nigeria [9]. Therefore, the present study was aimed at identifying species of *Aedes* mosquitoes and their knock down rate to some insecticides in some LGAs of northern Katsina State.

2. Materials and Methods

2.1 Study Area: The study was carried out in five Local Government Areas (LGAs) of Northern Katsina State, Nigeria. The selected LGAs include Batagarawa, Daura, Kaita, Katsina and Mashi (Fig. 1).

The inhabitants of these areas are predominantly subsistence farmers, civil servants, traders, students and others profession from different fields. The areas mostly experience rainy and dry seasons. The breeding peaks of mosquitoes are the wettest months of June, July, August and September but it is perennial around road-side drainage systems, tanks and other stagnant water bodies. They are and also found in ornamental plants.

Fig 1: Map of Katsina State Showing Selected LGAs for Collection of *Aedes* Mosquitoes

2.2 Collection and Rearing of Mosquito Larvae and pupae

Most of the samplings were carried out in the morning. On reaching the sites, water bodies such as swamps, ditches, water puddles, pools, drainages, domestic container, tree holes, domestic well and footprint were located. This was done with assistance of local guides from the communities after proper introduction to the village heads.

Also, for each sampling operation, records of Geographic location (GPS coordinates), Name of locality, Type of breeding sites, Source of the water, Nature of water collection, Temperature of the water and Water pH were taken. All specimens collected from a breeding sites were kept in one white plastic container and labeled appropriately with specific location of collection, time of collection, date of collection and initials of collector.

The collected larvae were transported to the insectary of Umaru Musa Yar'adua University, Katsina (UMYUK) for adult rearing and susceptibility tests. The containers were covered with muslin cloth to allow proper gaseous exchange for the larvae and pupae when transporting to the Insectary. The larvae were fed twice daily, with grinded cabin biscuit and yeast tablets (1 piece of biscuit with 20 yeast tablets grinded to powder form).

The larval containers were checked daily for emerged adults which were removed and placed into adult cages. The adults were fed with 10% glucose solution soaked into cotton wool and placed at the top of the cages. This was checked regularly to ensure availability of food at all times.

2.3 Morphological identification of mosquitoes tested

Rued (2004) pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with the transmission of Dengue virus, with the aid of dissecting microscope (Olympus, USA) was used for morphology identification of all the mosquitoes tested from the selected LGAs. The morphological identification of different species

of *Aedes* mosquitoes were carried out by studying the pattern of the antennae, the scales and color of the palps at the head region, the patterns of spots on the wings, thorax, terminal abdominal segments, scales of the legs, presence or absence of speckling in the hind tarsi and striations on the body using both compound and dissecting microscope following the taxonomic keys. Each of the tested mosquitoes was identified and recorded in the morphology identification form.
The identified mosquitoes were then carefully placed in well-labelled Eppendorf's tube containing silica gel using camel brush or forceps without damaging any part of the body. They were sent to the Department of Biochemistry, Bayero University, Kano (BUK), Nigeria, for molecular identification.

2.4 Determination of knock down rates of *Aedes* mosquitoes to some insecticides using WHO Tube Assay
This test was carried out according to WHO standard procedure (2016). Papers already impregnated with insecticide at the appropriate diagnostic concentrations were provided by the Katsina State Malaria Vector Sentinel Site located at the Department of Biology, UMYUK. The papers were impregnated with insecticides namely bendiocarb (0.1%), DDT (4%), permethrin (0.75%) and pirimiphos-methyl (0.25%).
Four replicates of 25 mosquitoes were acclimatized in the holding tubes for one hour before exposure to each of the insecticides. They were then transferred into different exposure tubes containing the aforementioned insecticides. Knocked down mosquitoes were counted and recorded at 0, 10, 15, 20, 30, 40, 50 and 60 minutes.

2.5 Morphological Identification of *Aedes* Mosquitoes
Mosquitoes used in the test and control tubes were examined under dissecting microscope and identified to species level using taxonomic keys by [10] pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with the transmission of Dengue virus, Male and female individuals were also distinguished by studying the pattern of the antennae, the scales and color of the palps at the head region, the patterns of spots on the wings, thorax, terminal abdominal segments, scales of the legs, presence or absence of speckling in the hind tarsi and striations on the body using both compound and dissecting microscope following the taxonomic keys.
The identified mosquitoes were then carefully placed in well-labelled Eppendorf's tube containing silica gel using camel brush or forceps without damaging any part of the body. They were sent to the Department of Biochemistry, Bayero University, Kano (BUK), Nigeria, for further molecular identification.

2.6 Molecular Identification of *Aedes* Mosquitoes
2.6.1 DNA extraction
Total genomic DNA was extracted using Qiagen DNA extraction kit following the manufacturer's protocol. Mosquitoes were homogenized using a battery-operated mortar and pestle (SIGMA) in 50 µl preheated grind buffers (this buffer is used to dissolve the DNA precipitate) in 1.5 ml Eppendorf's tube. The pestle was rinsed with a further 50 µl of the buffer to make a total of 100 µl. Homogenate mixture was incubated at 65 ºC for 30 minutes. The condensation was collected by brief micro-centrifugation and then 14 µl of 8 M of potassium acetate (help to precipitate the SDS detergent from the solution) was added and mixed by pulse vertexing. The mixture then incubated for 30 minutes on ice. The mixture was centrifuged for 20 minutes in 14,000 rpm at 4ºC after which the supernatant was transferred carefully to a clean new 1.5 ml Eppendorf's tube. At this point, 200 µl of 100% ethanol (for precipitation so we get good amount of DNA) was added and mixture were spun for 15 minutes at 14,000 rpm at 4ºC. The supernatant was discarded and the pellets was rinsed in approximately 100 µl ice cold 70% ethanol (Washing with 70% alcohol is to remove the excess of salts that might have come along with the extraction buffers). The pellets were air-dried for 2 hours and then re-suspended in 100 µl of distilled, sterile water and deionized water by incubation at 65 ºC for 10 minutes.

2.6.2 Polymerase chain reaction
The forward and reverse primers ITS1A: 5'-CCTTT GTACA CACCG CCCGT CG-3' and ITS1B: 5'-ATGTG TCCTG CAGT TCACA-3' were used to amplify the internal transcribed spacer 1(ITS) region of ribosomal RNA, with a fragment size of ~750 bp. The PCR reaction was carried out using KAPA Taq DNA polymerase in a total reaction volume of 15 µL. The reaction mix comprise 1 µL of gDNA, 1.5 µL of 10x Taq A Buffer, ~0.4 µM (0.51 µL) each of forward and reverse primers, 1.25 mM (0.75 µL) of $MgCl_2$, 0.25 mM (0.15 µL) of dNTP mixes and 0.12 µL of Taq DNA polymerase (KAPA Biosystems, Wilmington, MA, USA) in ddH_2O.
Amplification was carried out using standard conditions where initial denaturation of 5 min at 95 ºC was followed by 35 cycles each of 30 s at 94 ºC (denaturation), 40 s at 51 ºC (primer annealing) and 60s at 72ºC (extension). This was followed with 10 min final extension at 72 ºC. The PCR amplicons were separated in a 1.5% agarose gel stained with pEqGreen and visualized for bands, before digestion with a restriction enzyme, the "restriction site-associated DNA sequencing" Rsal (New England Biolabs, Ipswich, MA, USA). Reaction mixture for digestion comprised 5 µL of above PCR amplicons, 1 µL of NEBuffer™, 2U of *Rsa*I restriction enzyme and 3.5 µL of ddH_2O. The mixture was incubated at 37 °C, for 4 hrs and then size separated on 2.5% agarose gel stained with pEqGREEN. Sizes of the fragments on gel identified mosquitoes to species level with *Ae. Aegypti* typically producing bands of ~120, 180, and 400 bp, while *Ae. albopictus* produced bands of 170, 190, 200, and 290 bp.

2.7 Data Analysis
Knock down rate of the test mosquitoes was subjected to one-way analysis of variance (ANOVA) using SPSS version 20. Significantly different means were separated using LSD. All analyses were performed at $p< 0.05$ level of significance.

3. Results
3.1 Species composition of *Aedes* Mosquitoes in Some LGAs of Northern Katsina State
Two species of *Aedes* were identified from the selected LGAs of Katsina State namely *Ae. aegypti* and *Ae. albopictus*. Table 1 indicates that out of the 2000 mosquitoes tested, *Ae. aegypti* had the highest number (1821) while *Ae. albopictus* had the least (179).
Following morphological identification, the species were further identified and authenticated using molecular identification technique. Internal transcribed spacer 1(ITS1)

regions was used for the identification using universal primer for identification of *Aedes* mosquitoes. The PCR product amplified using the universal primers indicated that there was amplification which confirmed that the species belongs to *Aedes* mosquitoes (Plate 1).

Table 1: Species Composition of and Number of *Aedes* Mosquitoes from the Selected LGAs

LGAs	Number of Collected Mosquitoes	
	Aedes aegypti	***Aedes albopictus***
Batagarawa	344	56
Daura	388	12
Kaita	344	56
Katsina	365	35
Mashi	380	20
Total	1,821	179

Key: LGAs = Local Government Areas

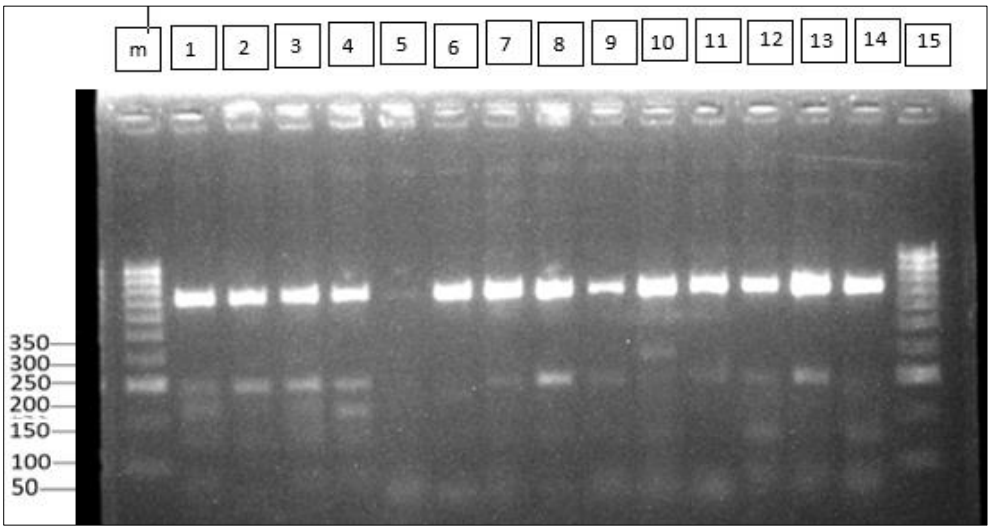

Plate 1: Agarose Gel Electrophoresis showing band of PCR products from primer region and amplification fragment showing 120, 180 and 400 pb. Lane M: 1kb ladder; *Aedes albopictus* 170, 190, 200, 290 and second profile 190, 200 and 290 pb: Lane 1-4 *Aedes aegypti;* Lane 6--14 *Aedes albopictus.* Lane 15 Negative control (distilled water).

3.2 Knock Down of *Aedes* Mosquitoes Treated with Some Insecticides

The kdr in the mosquitoes to bendiocarb (0.1%) was 23.0% from Katsina followed by those in Kaita (21%), 19% in Batagarawa and 13% in each of Daura and Mashi LGAs (Table 2). The result shows that there was significant difference (ANOVA: $F = 1.00$; $p = 0.02$) in kdr caused by bendiocarb to *Aedes* mosquitoes from the selected LGAs. Table 2 also reveals that DDT (4%) caused the highest knock down (10%) in mosquitoes from Mashi LGA followed by Batagarawa (9%), Daura (7%), Katsina (6%) and the least was found in Kaita (4%). The result showed that there was significant difference (ANOVA: $F = 0.200$; $p = 0.01$) in the kdr caused by DDT to the *Aedes* mosquitoes from the study area.

The kdr caused by permethrin (0.75%) varied among the *Aedes* mosquitoes from the study area. The highest (5%) was discovered in Batagarawa and Katsina LGAs, followed by 4% in Daura, 3% in Kaita and then the lowest kdr (1%) was in Mashi (Table 2). Statistically, the kdr caused by permethrin to *Aedes* mosquitoes was significantly different (ANOVA: $F = 0.01$; $p = 0.0001$) among the five LGAs selected. There was a general increase in kdr *Aedes* mosquitoes at 30 minutes after treatment with pirimiphos-methyl (0.25%). Table 2 shows that Katsina had the highest kdr with 39%, followed by Mashi (37%), Daura (36%), Kaita (28%) and finally the least (22%) was recorded in in Batagarawa LGA. Following similar pattern to that of the other insecticides, the kdr caused by pirimiphos-methyl was significantly different (ANOVA: $F = 0.880$; $p = 0.04$) among the mosquito samples collected from the selected LGAs at 30 minutes after treatment.

Table 2: Knock Down Rate (kdr) of *Aedes* mosquitoes exposed to some insecticides at 30 minutes after treatment

LGAs	kdr (% ± SEM) in *Aedes* Mosquitoes after 30 minutes of Exposure			
	Bendiocarb	**DDT**	**Permethrin**	**Pirimiphos-Methyl**
Batagarawa	19 ± 3.00^{ab}	9 ± 1.50^{a}	5 ± 1.50^{a}	22 ± 2.00^{c}
Daura	13 ± 3.00^{b}	7 ± 1.50^{ab}	4 ± 0.00^{a}	36 ± 2.00^{a}
Kaita	21 ± 3.00^{a}	4 ± 0.00^{b}	3 ± 1.50^{b}	28 ± 2.00^{b}
Katsina	23 ± 3.00^{a}	6 ± 2.00^{ab}	5 ± 1.50^{a}	39 ± 5.00^{a}
Mashi	13 ± 3.00^{b}	10 ± 2.00^{a}	1 ± 1.50^{c}	37 ± 3.00^{a}

Key: LGAs = Local Government Areas; kdr = Knock down rate

Values with different superscripts in the same column are significantly different at $p < 0.05$

Increase in exposure period from 30 to 60 minutes showed a general increase in kdr exhibited by the test insecticides against *Aedes* mosquito in the study area. Table 3 indicates that the kdr resulted by bendiocarb was highest (69%) in Katsina followed by Kaita (59%), Batagarawa (54%), Daura (53%) and the least in Mashi (51%). Statistically, there was significant difference (ANOVA: $F = 0.230$; $p = 0.003$) in kdr caused by bendiocarb in *Aedes* mosquitoes from the selected LGAs.

The kdr resulted by DDT in the mosquito samples ranged from 15% to 25% following the pattern: Batagarawa > Mashi > Daura > Kaita = Katsina (Table 3). The kdr was significantly different (ANOVA: $F = 0.890$; $p = 0.01$) at 60 minutes after treatment with DDT.

Permethrin caused kdr in *Aedes* mosquitoes ranging from 3 to 11% with the highest in Katsina and the lowest in Kaita (Table 3). Statistically, there was significant difference (ANOVA: $F = 0.100$; $p = 0.006$) in the kdr caused by permethrin in *Aedes* mosquitoes among the five selected LGAs.

Table 3 shows that pirimiphos-methyl, the highest kdr was recorded in Daura with 77% followed by 75% in Mashi, 74% in Kaita, 72% in Katsina and the least kdr was observed in Batagarawa (70%). There was significant difference (ANOVA: $F = 0.112$; $p = 0.01$) in kdr in *Aedes* mosquitoes exposed to pirimiphos-methyl from the five LGAs selected for this study.

Table 3: Knock Down Rate (kdr) of *Aedes* Mosquitoes Exposed to Some Insecticides at 60 Minutes after Treatment

LGAs	kdr (% ± SEM) in *Aedes* Mosquitoes after 60 minutes of Exposure			
	Bendiocarb	DDT	Permethrin	Pirimiphos-Methyl
Batagarawa	54±4.00^{b}	25±1.50^{a}	9±1.50^{a}	70±3.00^{b}
Daura	53±3.50^{b}	19±1.50^{ab}	6±2.00^{b}	77±5.00^{a}
Kaita	59±3.00^{b}	15±1.50^{b}	3±1.50^{c}	74±2.00^{a}
Katsina	69±3.00^{a}	16±2.00^{b}	11±1.50^{a}	72±2.00^{b}
Mashi	51±5.00^{b}	24±4.00^{a}	5±1.50^{b}	75±1.50^{a}

Key: LGAs = Local Government Areas; kdr = Knock Down Rate

Values with different superscripts in the same column are significantly different at $p < 0.05$

4. Discussion

This study provides a baseline data and assessment of knock down status of *Aedes* mosquitoes to four different classes of insecticides. *Aedes* mosquitoes, like other species, require a suitable, pleasing and conducive atmosphere and environment for their breeding and reproduction, the breeding site include disposable and used containers like tins of milk, soft drinks cans, disposable rubbers, spoiled cars, vans, etc. are the most productive and breeding site for *Aedes* species.

Aedes aegypti and *Ae. albopictus* were the two species of *Aedes* mosquitoes recovered in the selected LGAs. The dominance of *Ae. aegypti* in the study area is in line with some previous findings where it was reported as the major *Aedes* species found in some parts of Northern Nigeria [11, 12]. Similar findings were reported in some southern states of the country [13, 14]. Other researchers reported *Ae. aegypti* as the dominant species within the cities and in the sub-urban areas of African and Asia [15, 16, 17].

Tripet *et al*. [18] reported that, the association of *Ae. aegypti* and *Ae. albopictus* in the same micro environmental conditions makes them to share the same ecological niches that makes them to compete in the available resources leading to segregation of habitat. Another finding shows form of mating processes is called Satyrization was observed where the two species are able to mate in nature and that *Ae. albopictus* male effectively sterilized *Ae. aegypti* female [18, 19].

The kdr found in this study is in line with the finding of Nwankwo *et al*. [13] with 88.5% of kdr at 30 min and 98.75% at 60 min in pirimiphos-methyl while in DDT is was 27.5% and 60% at 30 and 60 minutes, respectively. Other findings by Konkon *et al*. [18] indicated kdr of 94.78, 90.93, 85.50 and 84.21% in mosquitoes exposed to permethrin.

5. Conclusions

The result of the study provides an insight of the current status of knock down of *Aedes* mosquitoes to four insecticides used in northern Katsina State. The study revealed the presence of *Aedes aegypti* and *Aedes albopictus* as the species found in the selected LGAs.

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