Original Article

Current Status of Insecticide Susceptibility in the Principal Malaria Vector, *Anopheles gambiae* in Three Northern States of Nigeria

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Abstract

Background: Malaria is a major public health problem in Nigeria with 97% of its population with high morbidity and mortality. Mosquitoes play an important role in the transmission of malaria parasites. This study was conducted to evaluate the current resistance status of *Anopheles gambiae* to insecticides.
Methods: Larvae of *An. gambiae* was collected from three zones; A, B and C differentiated on the basis of variation in agricultural ecosystems between August and November, 2018 in the northeast and northwestern parts of Nigeria. They were carefully reared to adult stage and insecticidal susceptibility tests were conducted.
Results: The mosquitoes tested showed high levels of resistance to all the insecticides used with the exception of malathion. Study zone A, recorded 74% mortality after 24h to deltamethrin compared to 81% from zone B and 82% from zone C, respectively. Mosquitoes from zone B exposed to DDT had the highest level of resistance at 37% compared to 40% and 53% from zones A and C, respectively. Resistant to bendiocarb was also observed, with zone A having the lowest mortality of 44% compared to 48% from zone C and 55% from Zone B, respectively. According to the results of knockdown tests, mosquitoes from Zone A exposed to deltamethrin recorded the lowest knockdown across the study locations while zone B recorded the lowest knockdown for DDT.
Conclusion: The results of the study provide an insight into the current status of *An. gambiae* to four major insecticides in northern Nigeria as guideline for mosquitocontrol.

Keywords: Malaria; *Anopheles gambiae*; Insecticide resistance; Susceptibility; Northern Nigeria

Introduction

Malaria is a life-threatening parasitic vector-borne disease troubling many countries in the tropical and subtropical regions of the world (1). With over 200 million new cases between 2010 and 2017, Africa still carries the highest burden (92%) worldwide compared to South-East Asia (5%) and the Eastern Mediterranean regions (2%), respectively (2-3). Nigeria, Democratic Republic of Congo, Mozambique, India and Uganda account for nearly half of all

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malaria cases worldwide (3). In 2017 alone, there were reported cases of increased malaria transmission (more than half a million cases) from countries with highest burden in Africa (Nigeria, Democratic Republic of Congo and Madagascar) compared with the year 2016 (3). Malaria is a serious problem in Nigeria with approximately 100 million cases and over 200,000 deaths annually (4- 5). It also accounts for nearly 60% of outpatients visits, 30% of under-five hospitalization and contributes to 11% maternal mortality, making Nigeria the country with the highest burden in the world (4-5).

The malaria parasite is mainly transmitted in Africa by the *Anopheles gambiae* s.l. and the major vectors are *An. gambiae* and *An. funestus* species complexes (6-9). *Anopheles gambiae* forms a species complex comprising of eight morphologically indistinguishable, i.e., identical sibling species in the series Pyretophorus in the *Anopheles* subgenus Cellia across sub-Saharan Africa (6, 10). The individual species of the complex exhibit similar traits and are closely related, making it very hard to be identified morphologically except for some few larvae and adult females (11). The *An. funestus* complex is comprised of nine sibling species of which *An. funestus* sensu stricto (s.s.) is the principal vector with very high density and found across different geographical regions (12). However, the *An. gambiae* s.l. complex is the most widely distributed *Anopheles* mosquitoes in Nigeria (65.2%) followed by the *An. funestus* (17.3%), respectively (13).

The main control measures adopted in Nigeria for malaria vectors are insecticide-treated nets (ITNs)/long-lasting insecticide-treated nets (LLINs) (14). These methods when properly used against insecticide susceptible mosquito populations contribute significantly in the reduction of malaria cases (15-16). Other vector control measures used for malaria prevention in Nigeria include personal protective measures such as the use of repellents and house screening (14). There is a serious threat to these control measures as a result of progression of insecticide resistance to major malaria vectors across malaria endemic countries including Nigeria (14, 17).

Insecticide resistance is defined as the ability of some organism to tolerate a specific dose of a toxic substance that will be deadly to other organisms of the same species and from the same environment (18). Over the years, the problem of insecticide resistance is progressing and involving more classes of insecticide, and this can significantly affect the strength of vector control programs leading to failure, thus resistance management is designed to delay or prevent insecticide resistance (19). An important part in the resistance management strategies is identifying the resistance and mechanisms involved by obtaining the baseline information about the vector susceptibility, detection of resistance in the early stage and monitoring its frequency levels over time (20-21). An integrated approach where two or more methods are employed in the vector control programs could help in delaying the sustain progression of the resistance (20).

A study conducted in 2013 at Bichi (Northern Nigeria) reported a high resistance of *An. gambiae* with significant elevation of detoxification enzymes in deltamethrin and bendiocarb resistance strains compared to susceptible species from agricultural and residential areas (22). A similar study from Bichi was conducted in 2015 to assess the level of resistance against bendiocarb, permethrin and DDT. Very high resistance to permethrin and DDT was reported with less resistant to bendiocarb (14). Resistance to permethrin and DDT exposed *An. gambiae* s.s across all the geographical zones of Nigeria with the highest level of the resistance in the forest savannah, Mosaic and Guinea savanna has been reported (18). The resistance profile and kdr mutation of *An. gambiae* s.l. populations was also reported from two locations (Auyo and Bunkure) in northern Nigeria (13). Other studies have also reported resistance to commonly used insecticides from

Nigeria (6-7). Also insecticide resistance was reported in this species from the neighboring country Ghana (23-24).

Periodic monitoring of susceptibility status of *An. gambiae* to insecticides used in public health practice is vital and will guide stakeholders towards the procurement and strategy used in vector control programs. The aim of this study was to examine the current situation of insecticide susceptibility in the principal malaria vector, *An. gambiae* s.l. in northern Nigeria.

Materials and Methods

Study area

The study was conducted across three different locations within three states (Gombe, Jigawa and Kano) in northeast and northwest Nigeria from August to November, 2018 (Fig. 1). The locations were designated as study zones A, B and C differentiated by the type of vegetative zone, i.e., A: lies within the Sudan, Northern and Southern Guinea savanna; B: is found within the Sudan, Sahel and Northern Guinea savanna; and C, lies within the Sudan and Northern Guinea savanna (6, 13).

Zone A, Yamaltu Deba (10° 13′ 0″ N, 11° 23′ 0″ E) is one of the 11 Local Government Areas in Gombe State, Nigeria. It has a population of 255,248 and an area of 1,981 km². Gombe State (10° 15′ 0″ N, 11° 10′ 0″ E) is situated in the north-eastern part of Nigeria (25, 26). The state shares common borders with Borno, Yobe, Taraba, Adamawa and Bauchi states. The state has two distinct climates, the dry season (November–March) and the wet season (April–October) with average annual rainfall of 850mm (27).

Zone B, Auyo (12°21′N, 9°59′E) is a locality in Jigawa State, north-western Nigeria within the Sudan and Guinea savannah with pockets of Sahel savannah. The town is known for its irrigation activities in which rice and vegetables are produced. It has a total population of 132,001 and estimated land mass area of 512km (13, 26).

Zone C, Kumbotso (11°53′17″N, 8°30′10″E) is situated in Kano state, north-western Nigeria in the Sudan and Guinea savanna with a population of 409,500 and an area of 158km^2 (18, 26). The temperature is generally warm, and the annual rainfall is about 1,300mm between April and September (28).

Study Sample

Sampling was conducted from different breeding places in the study areas using dipping method as described by Habibu, 2017 (14) in order to provide laboratory stock of mosquitoes. The samples were transferred to the insectary at Bayero University, Kano with a rearing condition of 28±2 °C temperature, 65±5% relative humidity (RH) and 12:12h D: L. Two to three days old female sugar-fed mosquitoes were used for susceptibility tests (29).

The inclusion criteria used for the selection of the sampling areas include:
history of pesticide and herbicides use on the land from agriculture activities, vector control and irrigation activity, availability and high density of target species, high intensity of malaria transmission, and paucity of data on susceptibility profile of the malaria vector *An. gambiae* s.l.

Susceptibility test papers

The impregnated test papers recommended by WHO used in this study were purchased from the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia 1800 Minden, Penang, Malaysia.

WHO susceptibility test

The current WHO susceptibility bioassay guideline (21) was followed. At least 120–150 female mosquitoes were aspirated from the mosquito cage into six holding tubes giving six replicate samples of 20–25 mosquitoes per tube. With the mosquitoes in the tubes, the slide unit was immediately closed and the holding tubes set in a vertical position for one hour. Damaged mosquitoes were removed at the end of the one hour exposure time. The exposure tubes were lined with a sheet of insecticide-impregnated paper, while the plain control tubes were lined with oil-impregnated papers, provided for each group of insecticides by WHO, fastened into position with a copper spring-wire. The plain holding tubes containing the mosquitoes were attached to the vacant position on the slides and with the slide unit opened, the mosquitoes were blown gently into the exposure tubes containing the following insecticide treated papers with discriminating dosages: DDT 4%, bendiocarb 0.1%, malathion 5% and deltamethrin 0.05 %. The exposure tubes containing all the mosquitoes were set aside in a vertical position for one hour after which they were transferred back to the holding tubes by reversing the procedure outlined. Knockdown was recorded on deltamethrin and DDT from all the study zones. A pad of cotton-wool soaked in sugar solution 5% was placed on the mesh-screen end of the holding tubes and the mosquitoes were kept in the recovery period for 24 hours. During this time, the holding tubes were kept in the insectary. After the recovery period, the mortality was scored and recorded. A mosquito is considered alive if it is able to fly, regardless of the number of legs remaining (21).

Data analysis

The 24h mortality was accessed manually, while the susceptibility was defined as: 98–100 % mortality indicates susceptibility, 90–97% mortality requires confirmation of resistance and between 0–89% suggests resistance (21). Microsoft office excel, version 2013 was used to create charts, sort and clean the data. While Statistical Package for the Social Sciences (SPSS) version 16 was used to calculate the 95 % confidence interval and the means of the variable using the Student's t test. Abbott's formula (30) was used to correct for natural mortality if the control mortality was between 5 and 20%. The results of the tests with >20% mortality in controls were discarded and the test repeated (31).

Results

A total of 1800 F_0 2–3 days old adult female *An*. *gambiae* s.l. mosquitoes were used for the bioassays. *Anopheles* mosquitoes from all the three zones were only susceptible to malathion and resistant to DDT, bendiocarb and deltamethrin (Table 1, Fig. 2). A mortality of 74% (95%, CI: 68–79) was recorded in zone A to deltamethrin compared to 81% (CI: 72–89) from zone B and 82% (CI: 76–87) from zone C (Fig. 2). This study area had a comparatively lower mortality to deltamethrin, indicating a relatively higher resistance to this insecticide. Resistance by *An. gambiae* to bendiocarb in Nigeria is usually moderate but was found to be very high in all the three locations with zone A having the lowest mortality of 44% (CI: 38–49) compared to 48% (CI: 44–51) from zone C and 55% (CI: 53–56) from zone B, respectively (Table 1). The organophosphate, malathion was the only insecticide mosquitoes from all the study locations were susceptible to, with 100% mortality both in zones B and C, while zone A recorded 99% (Fig. 2).

According to the results of knockdown tests, mosquitoes from Zone A exposed to deltamethrin recorded the lowest knockdown at 30 minutes 32% (Standard Error, SE= 0.333), compared to 37% (SE= 0.344) and 48% (SE= 0.355) from zones B and C, respectively (Fig. 3). Similarly, at 60 minutes zone A mosquitoes still recorded the lowest knockdown 44% (SE= 0.354) compared to 51% (SE= 0.357) and 56% (SE= 0.355) from zones B and C, respectively (Fig. 3). Comparatively, from the deltamethrin knockdown result observed, zone A recorded the lowest knockdown rate at various time intervals shown above, which is in line with the low percentage mortality observed to deltamethrin after 24h 74% (CI: 68–79) from this study area (Figs. 2, 3). The highest percentage knockdown to deltamethrin was observed with mosquitoes from zone C, also in keeping with the high percentage mortality to this insecticide after 24h 82% (CI: 76–87) recorded from this locality (Figs. 2, 3).

The highest level of DDT resistance was 37% (CI: 34–39) and seen with mosquitoes from zone B compared to 40% (CI: 37–42) and 53 % (CI: 49–56) from zone A and zone B, respectively (Fig. 4). Comparatively, mosquitoes from zone B recorded the lowest mortality to DDT, signifying that they are highly resistant to DDT. Similarly, in confirming the aforementioned statement, these mosquitoes recorded the lowest knockdown of 2% (SE= 0.1) at 30 minutes and 9% (SE= 0.204) at 60 minutes compared to 3% (SE= 0.122) and 17% (SE= 0.268) from zone C and 8% (SE= 0.2) and 8% (SE= 0.274) from zone A, respectively (Fig. 4). The high knockdown observed to DDT with mosquitoes from zone A was in keeping with the high percentage mortality observed with mosquitoes from this study area to the organochlorines (Figs. 2, 4) and was statistically significant ($P< 0.05$).

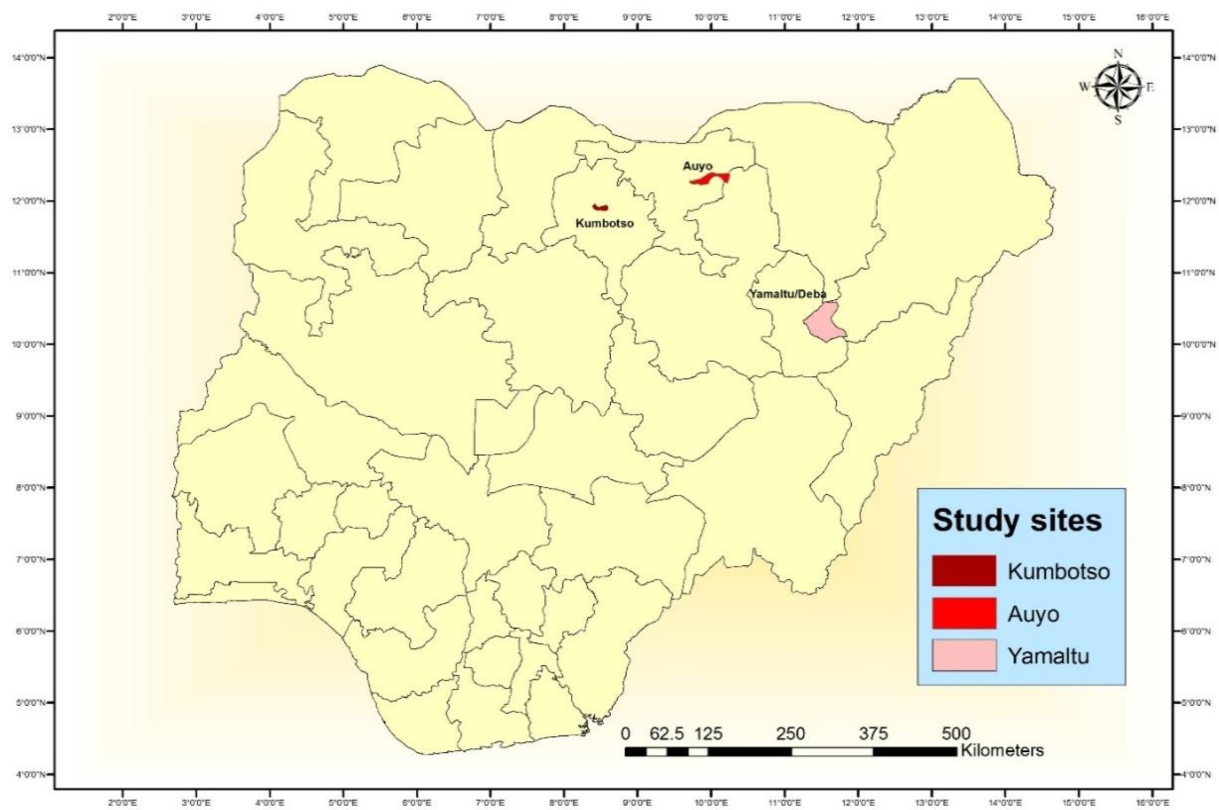

Fig. 1. Map showing the geographical locations of the study sites in Nigeria, 2018

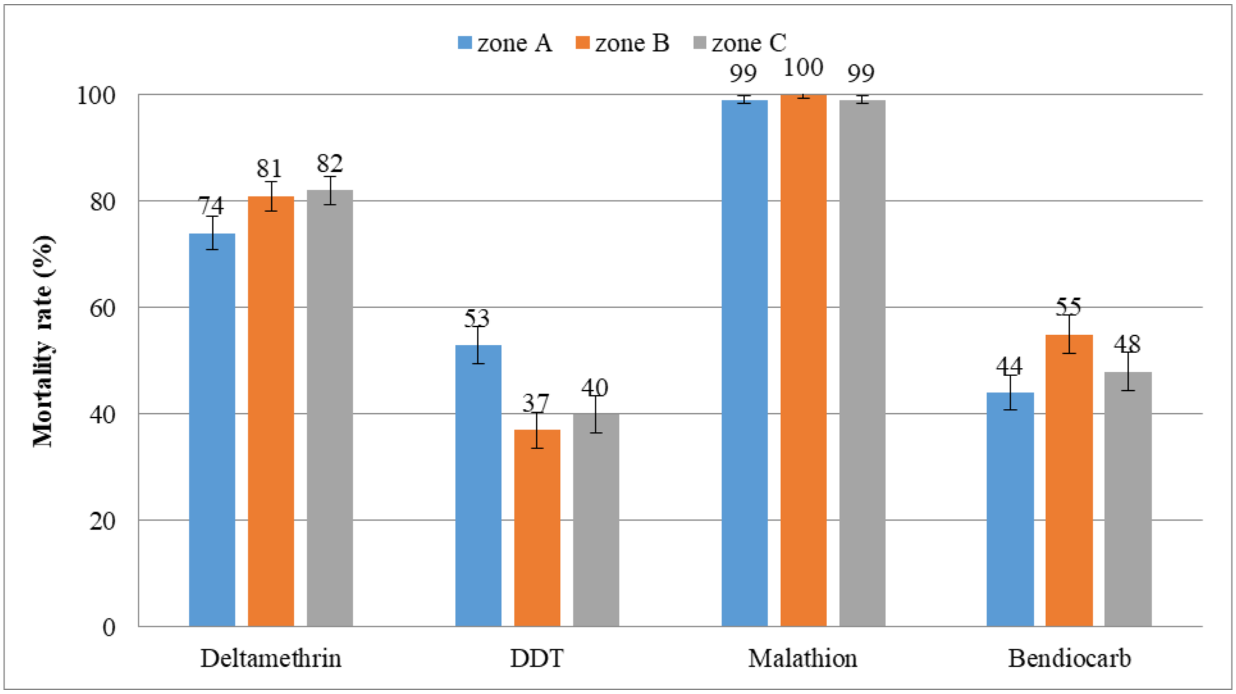

Fig. 2. Percentage mortality of *Anopheles gambiae* s.l. collected from some localities in Nigeria after 24h exposure to insecticides, 2018

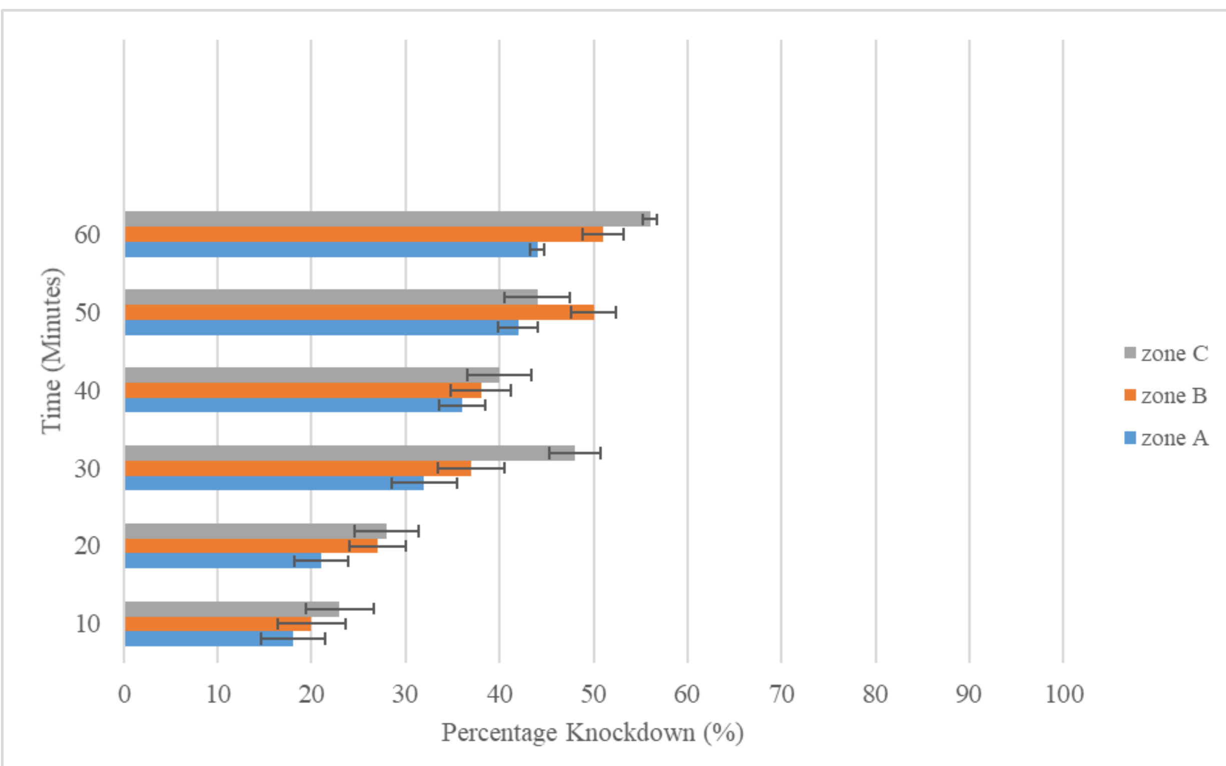

Fig. 3. Knockdown profile of *Anopheles gambiae* s.l. mosquitoes collected from Northern Nigeria exposed to Deltamethrin 0.05% after 60 minutes, 2018

Table 1. Susceptibility status of *Anopheles gambiae* s.l. at 95% confidence interval with the mean and standard deviation collected from the study zones in Nigeria after 24h exposure to different insecticides, 2018

Locality	Insecticide	Exposed	Mortality (%) Mean ± SD	Mortality (%) Control	95% confidence interval	Resistant Status
Zone A	Deltamethrin 0.05%	100	74.00±3.13	4.00±0.65	68–79	R
	DDT 4%	100	53.00± 3.56	0.00±0.00	49–56	R
	Malathion 5%	100	99.00± 0.71	0.00±0.00	97–100	S
	Bendiocarb 0.1%	100	44.00± 3.21	0.00±0.00	38–49	R
Zone B	Deltamethrin 0.05%	100	81.00±2.80	4.00±0.65	72–89	R
	DDT 4%	100	37.00±3.44	0.00±0.00	34–39	R
	Malathion 5%	100	100.00±0.71	0.00±0.00	100	S
	Bendiocarb 0.1%	100	55.00±3.55	0.00±0.00	53–56	R
Zone C	Deltamethrin 0.05%	100	82.00±2.74	2.00±0.48	76–87	R
	DDT 4%	100	40.00±3.49	0.00±0.00	37–42	R
	Malathion 5%	100	100.00±0.71	0.00±0.00	100	S
	Bendiocarb 0.1%	100	48.00±3.56	0.00±0.00	44–51	R

R: Resistant; S: Susceptible

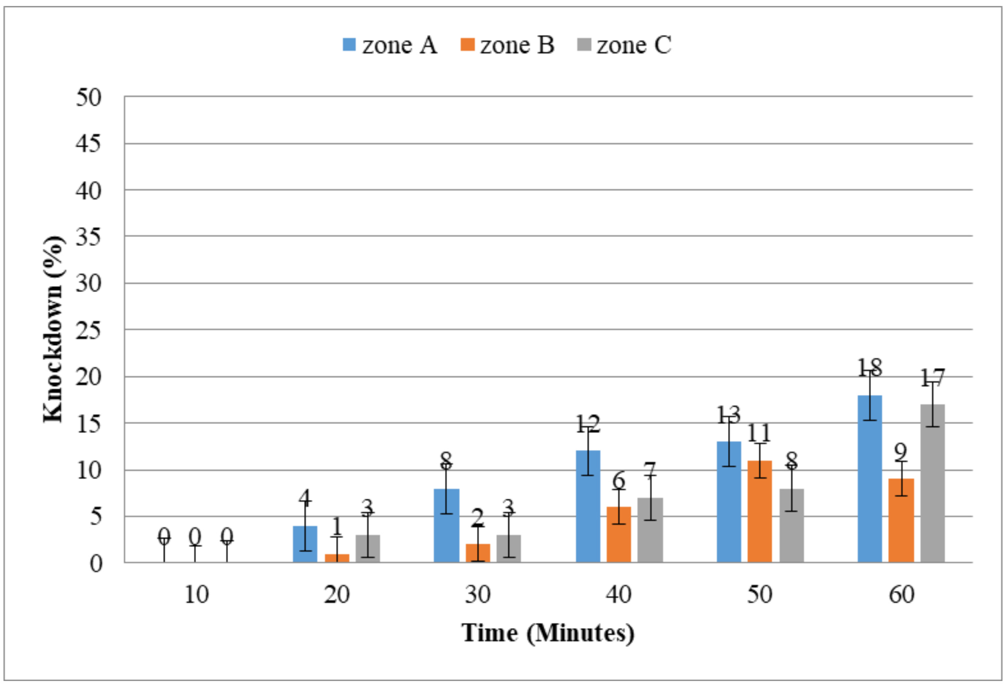

Fig. 4. Knockdown profile of *Anopheles gambiae* s.l. mosquitoes collected from Northern Nigeria exposed to DDT 4% after 60 minutes, 2018

Discussion

In the assessment of the resistance level to four insecticides viz; deltamethrin, DDT, malathion and bendiocarb by *An. gambiae* mosquito during the present study, bendiocarb showed resistance across all the study area, with zone A having the lowest percentage mortality followed by zone C and B respectively. This finding agrees with studies from the north-eastern state of Gombe in the Sudan savanna of Nigeria (7), where they reported a percentage mortality range of 2.3–100%. Similarly, a study from the forest zone vegetation of Kumasi in Ghana reported 38–56% mortality to bendiocarb (24). Another study from the coastal savanna vegetation of Ghana reported a mortality rate of 12.3 % to bendiocarb (23). Most of the studies conducted in the Sudan, Guinea, Sahel savanna and humid forest reported moderate resistance to full susceptibility to bendiocarb (13-14). The major challenge arising from this finding is that the initial thought of using carbamate as an alternative to the rapid spread of pyrethroids resistance may no longer hold (23-24, 32).

This study also reports resistance to the pyrethroids (deltamethrin) from the study sites with zone A having the lowest percentage mortality followed by zone B and C, respectively. This finding is in agreement with studies conducted in the northern Guinea savanna of Nigeria (33) where they reported percentage mortality of 83%, and from the Sudan through Guinea and some parts of Sahel savanna (13) where 78% mortality to deltamethrin was reported. However, a study conducted in the Sudan savanna disagrees with our finding where a very high resistance of 38% mortality to deltamethrin was published (22). Also, another study from the forest zone vegetation of Kumasi in Ghana reported very high resistance in the range of 15–46% (24). Recently, high resistance to deltamethrin in the range of 1–70% from the Sudan and Sahel savanna was reported in Nigeria (6-7). A rise in pyrethroids resistance by mosquitoes is becoming a big threat to the vector control strategy in Nigeria, thus necessary and urgent steps need to be taken to avoid complete failure of the measures (18).

The resistance profile of the mosquitoes from all the three study locations to DDT shows a high level of resistance, with zone B having the highest level followed by C and A. This finding is in agreement with previous studies from Sudan, Guinea and Sahel savanna (6-7, 13-14, 16, 33). Similarly, very high resistance in the range of 12–46% was reported from the forest zone and coastal vegetation of Ghana (23-24). In this study, malathion was the only insecticide found to be very active against the mosquito vector tested across all the study locations and agrees with previous studies from within and outside Nigeria (6, 13, 24, 32, 34).

A slow knockdown to deltamethrin, that increased with increasing time of exposure was observed from all the zones with mosquitoes from zone A having the lowest knockdown followed by zone B and then C, respectively. This finding agrees with previous studies reported (35). DDT also recorded very low knockdown from all the study locations. This finding is similar to previous reports (13, 35). However, a previous study conducted at Bichi in Kano-Nigeria reported high knockdown to DDT (14).

The high level of insecticide resistance by *An. gambiae* observed may be associated with increased use of pyrethroids treated bed nets (22). This is because farmers in these study locations use a wide range of pesticides and herbicides to protect their crops, and these pesticides marketed under different trade names belong to all the chemical classes including organophosphates, organochlorine, pyrethroids and carbamates (16). The insecticide resistance observed in this study along with previous reports of resistance from these locations involving three major classes (carbamate, organochlorine, pyrethroids) of insecticides used in public health practice is disturbing and may have a negative effect on the malaria control programme which ultimately can lead to failure of the vector control programme (6-7, 14, 33). Resistance management consisting of all available measures designed to delay or prevent resistance should be implemented in all the malaria endemic zones as soon as possible (18, 24). Some methods that can also limit the progression of insecticides resistance to mosquito vectors of malaria include: altering the dose and frequency of pesticide application in areas with high seasonal transmission; applying different formulations; avoidance of slow release formulations and identify new pesticides with an alternate mechanism of action (18).

Conclusion

The results of the study provide an insight into the current status of *An. gambiae* to four major insecticides in northern Nigeria. This may form a new baseline data for further studies on other classes of insecticides that can be adopted to guide the control of mosquito vectors of malaria in Nigeria.

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