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Insecticides resistance profile of *Anopheles* mosquitoes from three different localities in Sudan savannah region of Kano state Nigeria

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Abstract
Malaria remains a significant public health and development challenge. The African region shoulders the heaviest malaria burden. Nigeria accounts for around 27% of the global burden of malaria. The majority of malaria endemic countries in sub-Saharan Africa have seen a rapid increase in insecticide resistance, which is expected to seriously jeopardise the effectiveness of vector control efforts and cause a rebound in disease cases. Larval collection was conducted in three localities in Kano south (Wudil, Garko and Bunkure) of Kano State, Nigeria between August, September and October 2019 and January, February and March, 2020 in rainy and dry seasons respectively. Following which, adult female mosquitoes were morphologically identified and utilised in insecticides bioassay using permethrin 0.75%, deltamethrin 0.05%, bendiocarb 0.1%, dichlorodiphenyltrichloroethane (DDT) 4%. This study provides information on insecticides resistance profile of *Anopheles* mosquitoes which may be considered in mosquito vector control strategy in Sudan savannah region of Kano state Nigeria**.**

Keywords: *Anopheles*, permethrin, deltamethrin, bendiocarb, resistance profile

Introduction
Malaria is the leading cause of death among vector-borne diseases (WHO, 2014) [21], having killed millions of people over the millennia (Gelband *et al*., 2004) [6]. *Plasmodium* is the deadly parasite that causes malaria, which is spread by the bite of female *Anopheles* mosquitoes carrying the infection. As of 2021, the WHO African Region accounted for 95% of all malaria cases (234 million cases) and 96% of all deaths (593,000 deaths), continuing to bear the brunt of the disease. Children under the age of five accounted for over 80% of malaria mortality in the African Region (WHO, 2022) [23]. Insecticides, mosquito nets, indoor residual spraying, and antimalarial medications can all be used to treat and prevent malaria. Vector control is essential part of plans for controlling and eradicating malaria because of its great efficacy in preventing infection and minimising disease spread. Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are the two main therapies. A growing resistance among *Anopheles* mosquitoes to insecticides is a danger to the progress made in the global control of malaria. Other threats to ITNs include inadequate access, net loss from daily stress outpacing replacement, and mosquito behaviour changes (apparently biting before people go to bed and resting outside, avoiding insecticide exposure (WHO, 2022) [23].
Malaria can be fatal particularly when caused by the *Plasmodium* species common in Africa. Most malaria deaths are caused by one or more severe complications including; cerebral malaria, breathing problems, organ failure, anaemia, low blood sugar etc. (Swan *et al*., 2005) [18]. A key element of plans for controlling and eliminating malaria is vector control, which is a very successful means of lowering the disease's spread. In the majority of malaria risk locations, the World Health Organisation currently advises the use of IRS or ITNS for the control of malaria vectors (WHO, 2021) [22]. Other therapies, such as larviciding, may be added to these two depending on the situation and the resources available.
When we take into account its entomological consequences, insecticide resistance poses a serious danger to vector management; nonetheless, its epidemiological impact is less evident than one might anticipate.

Insecticides may still be effective in lowering the percentage of elderly, possibly contagious vectors in regions where insecticide resistance has been found, hence halting the spread of malaria. Examining the ecological relationships among vectors, parasites, and their environmental elements indicates that the influence of insecticides on the spread of malaria is complex and could account for their continued effectiveness in spite of widespread resistance to insecticides (Alout *et al*., 2017) [2]. The use of pesticides in agriculture is thought to have contributed to the selection of insecticide resistance in mosquitoes, a growing body of research indicates that pesticide usage in public health is also a factor in the development of insecticide resistance in malaria vectors (Hemingway, 2014: Fodjo *et al*., 2018; Chouaïbou *et al*., 2016; Nkya *et al*., 2014) [9, 5, 3, 14]. Increased agricultural output due to growing human populations, especially in Africa, results to increased pesticide use (Ndo *et al*., 2019) [13].

Materials and Methods

The study was carried out in 3 selected local governments of Kano south, Kano State, Nigeria. Wudil local government Sudan savannah zone of West Africa with about a population of 185189 and lies between latitude $11^{O}49$'N and $8^{O}51$'E longitude, with an area of 362 km^2; Garko local government Sudan savannah zone of West Africa with about a population of 162500 and lies between latitude $11^{O}31$'N and longitude $8^{O}54$'E, with an area of 450 km^2; and Bunkure local government which is also located in Kano state within the Sudan savannah zone of West Africa about 840 kilometers from the edge of the Sahara desert (Ado-Kurawa, 2006) [1] with about a population of 170,891, and lies between $11^{O}42$'N latitude and $8^{O}33$'E with an area of 487km^2 . The vegetation of Kano State is semi-arid savannah sandwiched by the Sahel savannah in the north and the Guinea savannah in the south. The state has the largest irrigation projects in Nigeria, with six irrigation projects and more than 20 earth. Alongside the importance of these dams to the development of agriculture and provision of food comes the health implication of providing suitable breeding sites for vectors of diseases. Rice paddies in particular have been established and increase the risk of malaria by providing suitable sites for vector development The study was done during rainy (July to September, 2019); dry (January to March, 2020) seasons. The *Anopheles* species of mosquito larvae where collected *in situ* (naturally infested water bodies) where rice is produced during the rainy season and vegetables (tomatoes, carrots, garden egg, cabbage, spring onion, cress leaves, lettuce) were grown in dry season. Occupation include rice farming, fishing, irrigation based activities, food processing and animal rearing.

Fig 1: Co-ordinates of sampled study sites (Source: www.geoplanner.com)

***Anopheles* larval collection**

The larvae were collected using entomological ladles and transferred into plastic buckets (Robert *et al*., 2002) [16]. The larvae were separated from debris and water is filtered using sieve. Water from the breeding sites was taken to the insectary at Bayero University, Kano. The emerged adults, were maintained at standard insectary condition (25-28 ºC and relative humidity of ~70-80%, with a 12 hr. day/ night cycle (Das *et al*., 2007) [4]. Debris, worms and species of *Culicines* were sucked from the larval containers and discarded. The water was changed regularly and replaced with distilled, sterile and deionized water in some containers and water from the field in the other containers. The larvae were fed with ground baby fish food. The development of the larvae was monitored regularly and all the emerged pupae were sucked from the larval containers and kept in plastic cups and then transferred into white-plastic labeled mosquito cages a for adult emergence. All emerged mosquitoes were fed on 10% sugar solution imbibed in cotton wool. Only female mosquitoes were used for the experiments.

3.2.3 Mosquito species identification

Anopheles species were identified morphologically using the standard identification keys of Gillies and Coetzee (1987) [7]. The identification focused dark spot at the upper margins of the wings, which is common to all *Anopheles*. The number of palpis elongation and segmentations was also considered into three. The legs were checked for the presence of Speckles on the third preapical dark area on vein 1 and a pale interruption and tarsi 1-4 with conspicuous pale bands, which were common features for *Anopheles gambiae*. The mosquito larvae were identified by the presence of a head with mouth brushes for feeding, a thorax and a segmented abdomen. The presence of a respiratory siphon in *Anopheline* larvae was checked and the standing position of the *Culicine* larvae in the water surface was also checked. The *Culicine* must position them parallel to the water surface to breathe via spiracles on their abdomen. Larvae swim backwards near the water surface until contact is made with a solid object, which they then lie against. They dive below the surface when disturbed. They feed on microorganisms in the surface micro layer such as algae and bacteria. The shaped pupae were identified to be like a comma. The pupae head and thorax was not separated. Pupae must visit the water surface to breathe via a pair of respiratory trumpets on the cephalothorax. The pupal stage lasts from a few hours to a few days from which it turns to adult.

3.3. WHO Insecticide Susceptibility/ Resistance Bioassay Tests

Insecticide susceptibility assays were carried out on 2-3 days old adult *An. gambiae* mosquitoes using World Health Organization standard protocol for adult mosquitoes (WHO, 2013) [20], with four replicates of 20 to 25 non – blood fed adult female mosquitoes. Five sheets of clean white paper impregnated with oil were rolled into a cylinder shape, and are inserted into four holding tubes (one per tube) and fastened into position with a steel spring-wire clip. The tubes were attached to slides. 20–25 active-female mosquitoes were aspirated using aspirator from a mosquito cage into the four holding tubes through the filing hole in the slide that made four replicate samples per tube. The slide units were then closed and the holding tubes set in an upright position for one hour, after which all dead and knocked down mosquitoes were removed. Another four set of insecticide-impregnated papers exposure tubes were prepared in much the same way as oil impregnated and were all labeled. The insecticide-impregnated papers containing tubes were attached to the vacant position on the slides, and with the slide unit open the mosquitoes were blown gently into the exposure tubes, and the slide units is closed. The mosquitoes were kept in the exposure tubes for 1 hour and the number knocked down mosquitoes were recorded: 0min, 15min, 30min, 45min and 60min in succession. At exactly 1-hour exposure period, the mosquitoes were transferred back to the holding tubes, and maintained in the holding tubes for 24 hours during which the number of dead mosquitoes, temperature and humidity were recorded. Mosquitoes were classified as dead or knocked down if they were immobile or unable to stand or take off.

The following insecticides (classes) were used for the assay: (1) 0.75% permethrin (a pyrethroid: an inhibitor of closure of voltage – gated sodium channels, (2) 0.05% deltamethrin (a pyrethroid), (3) 4% dichlorodiphenyltrichloroethane (DDT), (4) 0.1% bendiocrb (carbamate)

Results

The results of average median knockdown time (KT_{50}) for the studied insecticides are presented in tables 1-3.DDT showed a significantly lower KT_{50} (145.02 and 194.57 minutes in Garko and Bunkure, respectively) compared to Wudil with a KT_{50} OF 503.85 minutes; Permethrin showed a significantly lower KT_{50} (154.64 minutes) in Wudil compared to (177.23 and 177.32 minutes for Garko and Bunkure respectively); Bunkure had a significantly lower KT_{50} (56.98 minutes) for Deltamethrin compared to 90.14 and 105.08 minutes for Wudil and Garko respectivey; Wudil and Garko had a significantly lower KT_{50} (44.23 and 38.51minutes respectively) compared to Bunkure (49.13 minutes)

Table 2 is the results of average median knockdown time (KT_{50}) based on breeding sites (agricultural and residential) which showed that bendiocarb has a statistically significantly lowest KT_{50} (42.63 and 48.04 minutes for agricultural and residential sites respectively) compared to other insecticides used in the study

From table 3 comparing rainy to the dry season, all the insecticides tested showed a statistically lower KT_{50} in the dry season compared to the rainy season except permethrin which was not statistically significant across the two seasons.

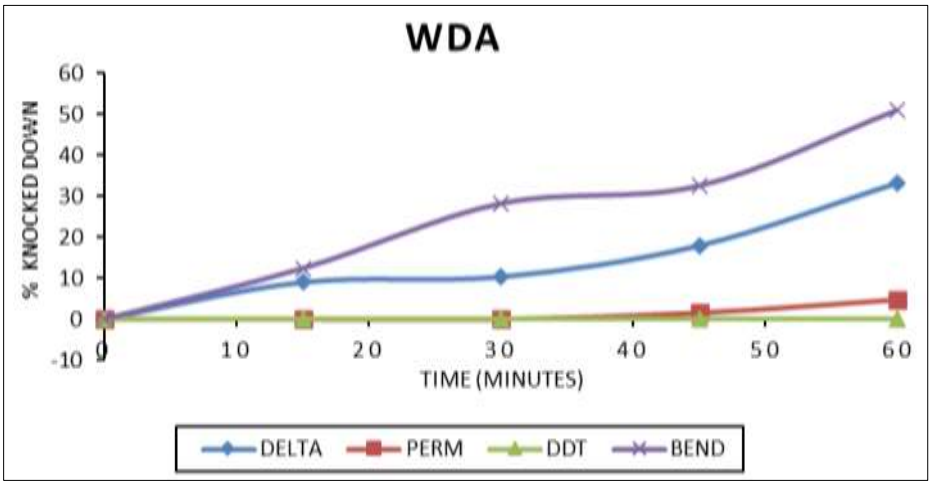

Fig 2: Percentage knockdown profile of *Anopheles* mosquitoes 'WHO bioassay collected from Wudil agricultural (WDA) site in rainy season.

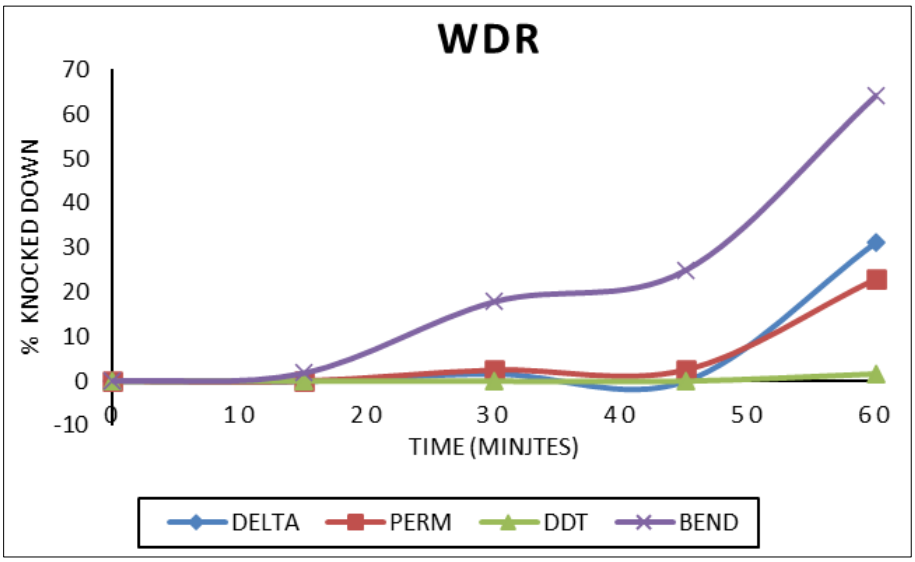

Fig 3: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Wudil residentiall (WDR) site in rainy season.

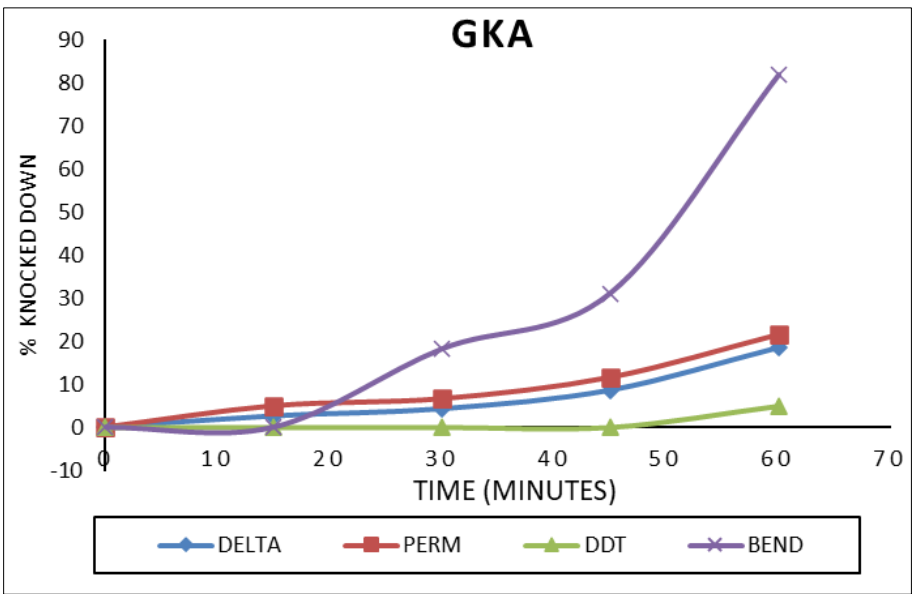

Fig 4: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Garko agricultural (GKR) site in rainy season.

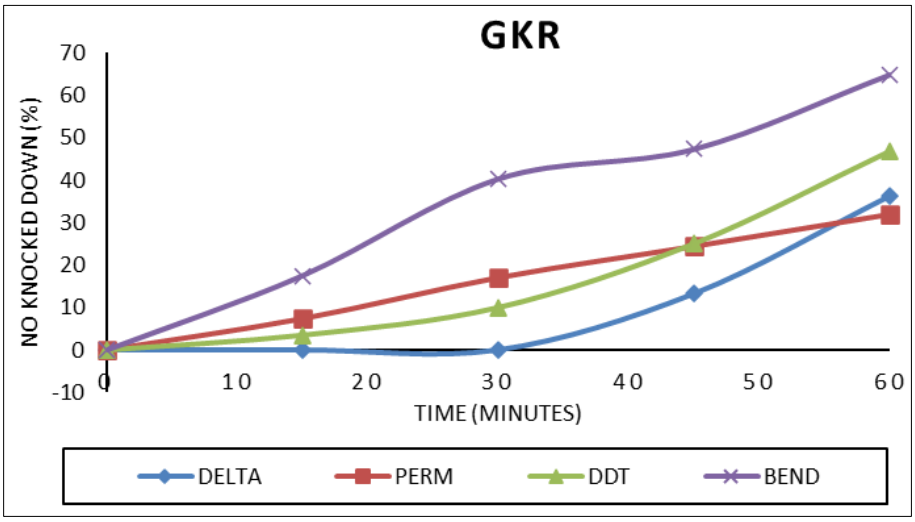

Fig 5: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Garko residential (GKR) site in rainy season.

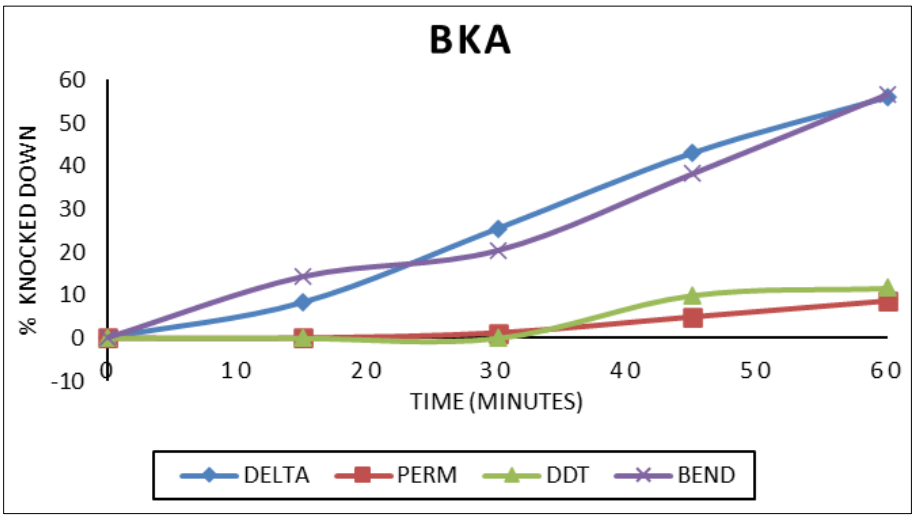

Fig 6: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Bunkure agricultural (BKR) site in rainy season.

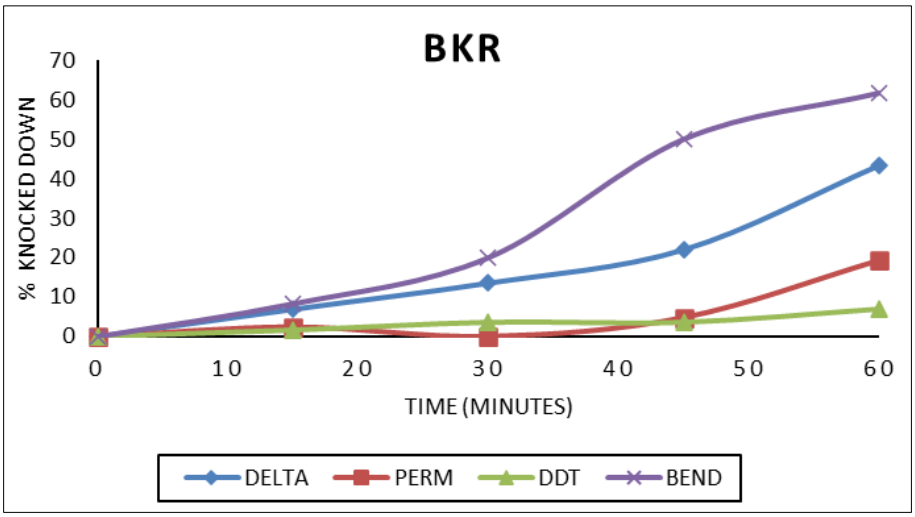

Fig 7: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Bunkure residential (BKR) site in rainy season.

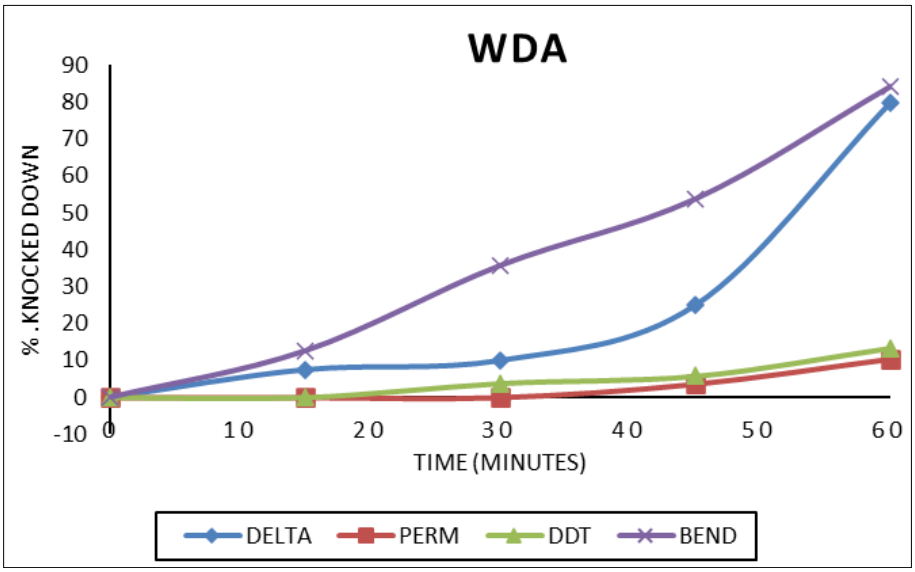

Fig 8: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Bunkure agricultural (GKR) site in dry season.

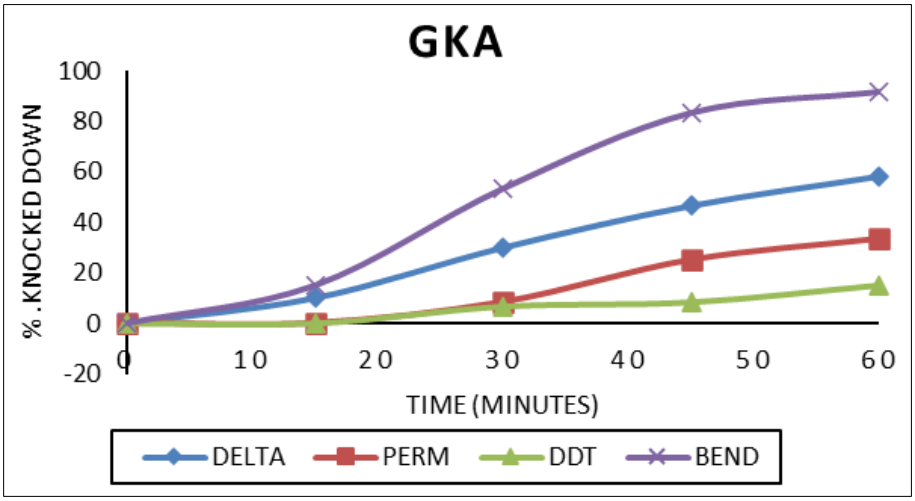

Fig 9: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Garko residential (GKA) site in dry season.

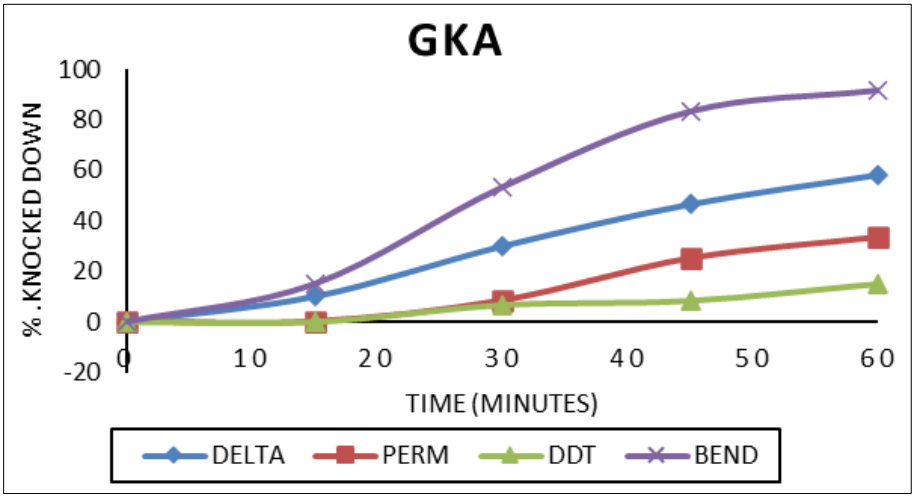

Fig 10: Percentage knockdown profile of *Anopheles* mosquitoes' WHO bioassay collected from Bunkure residential (BKA) site in dry season.

Table 1: Knockdown time of *Anopheles* mosquitoes' insecticide bioassay to DDT(4%), Permethrin (0.75%), Deltamethrin (0.05%), and Bendiocarb (0.1%) of mosquitoes collected for study locations.

Insecticides	WuDiL		GaRKo		BunKuRe	
	KT_{50}	95% Confidence Level (CL)	KT_{50}	95% Confidence Level (CL)	KT_{50}	95% Confidence Level (CL)
DDT	503.85$^{\#a}$	296.70 -711.00	145.02$^{\#b}$	92.13-197.91	194.57$^{\#b}$	84.38-304.75
PERM	154.64^{*b}	115.20 - 194.08	177.23$^{\$c}$	56.27-298.19	177.32$^{\#c}$	76.56-278.07
DELTA	90.14$^{\wedge a}$	61.31 -114.97	105.08$^{\$b}$	50.15-160.00	56.98$^{\wedge c}$	47.77-66.20
BEND	44.23$^{\$c}$	37.45 - 51.01	38.51^{*c}	32.11-44.91	49.13$^{\wedge b}$	41.13-57.13

Superscripts$^{*\#\wedge\$}$Values bearing the same superscripts of special characters down a column are not statistically different. abcde Values bearing the same superscripts of alphabets across a row are not statistically different ($p<0.05$).
C.I: Confidence Level

Table 2: Knockdown time of *Anopheles* mosquitoes' insecticide bioassay to DDT (4%), Permethrin (0.75%), Deltamethrin (0.05%), and Bendiocarb (0.1%) of mosquitoes collected for breeding sites

Insecticides	Agricultural		Residential	
	KT_{50}	95% Confidence Level (C.L)	KT_{50}	95% Confidence Level (CL)
DDT	296.86#a	160.54 - 433.18	278.23#a	131.00 - 425.45
PERM	177.33#a	114.09 -240.58	151.67#a	56.46 - 246.88
DELTA	83.40^b	55.17 -111.62	76.60#b	69.94 - 83.27
BEND	42.63*c	36.95 - 48.32	48.04*c	42.13 - 53.95

Table 3: Seasonal variation in knockdown time of *Anopheles* mosquitoes' insecticide bioassay to DDT (4%), Permethrin (0.75%), Deltamethrin (0.05%), and Bendiocarb (0.1%).

Insecticides	Rainy		Dry	
	KT_{50}	95% Confidence Level (CL)	KT_{50}	95% Confidence Level (CL)
DDT	371.85#a	226.58-517.12	145.10#b	101.75-188.45
PERM	199.84#b	126.66-223.01	112.01#b	72.71-151.31
DELTA	95.88^c	68.61-123.15	52.31*d	43.79-60.83
BEND	49.58*d	44.79-54.37	34.30^e	30.03-38.57

*.

Table 4 is the linear regression analysis showing the test and measure of association between median knockdown time with type of insecticide (TOI), seasons, breeding site (BS), and breeding locations (BL). There is a significant difference in the KT50s associated with the different types of insecticide. Using DDT as a reference category, switching from DDT to Permethrin or Deltamethrin or Bendiocarb is associated with reduction in the KT50 by 123.19 minutes (mins.) (p=0.002), 209.46 mins (p=0.000), and 245 mins (p =0.000) respectively if all other factors remain constant. An indication that the 3 insecticides have better knockdown profile than DDT. Transition from rainy to dry season also reduces the KT50 by 126.06 mins. Relatively, moving from agricultural site to residential is associated with a reduction in the KT_{50} by 76.05 mins. There is a significant difference between the KT50 of the insecticides in 3 different study locations. Relatively, transiting from Wudil to Garko or Bunkure is associated with reduced KT_{50} by a factor of 79.06 mins (p=0.019) and 83.02 mins (p=0.012).

Table 4: Association between median knockdown time with the type of insecticide (TOI), seasons, breeding sites (BS) and breeding locations (BL).

KT_{50}	Coefficient	S.E	P> value	95% Confidence Level
Insecticide type				
DDT	Ref			
PERM	-123.19	38.26	0.002	-199.05 to -47.33
DELTA	-209.46	36.92	0.000	-282.67 to -136.24
BEND	-245.16	37.20	0.000	-318.925 to -171.40
Season				
Rainy	Ref			
Dry	-126.06	31.80	0.000	-189.10 to -63.01
Site type				
AGRICULTURAL	Ref			
Residential	-76.05	32.75	0.022	-140.99 to -11.12
Site				
WUDIL	Ref			
GARKO	-79.06	33.12	0.019	-144.73 to -13.38
Bunkure	-83.02	31.92	0.011	-146.32 to -19.72

Table 5 shows DDT and permethrin recorded the lowest percent mortality in Wudil compared to Garko and Bunkure; deltamethrin recorded the highest percent mortality in Bunkure compared to Wudil and Garko; bendiocarb recorded the highest percent mortality in Garko, compared to Wudil and Bunkure. Table 6 showed that; DDT and permethrin recorded the lowest percent mortality in the agricultural site compared to the residential site, while deltamethrin and

bendiocarb recorded the highest percent mortality in the agricultural site compared to the residential site. In table 7, the dry season recorded the highest percent mortality for all the insecticides tested compared to the rainy season. From table 8 there was significant association observed (p=0.00) between percent mortality and insecticides [deltamethrin and bendiocarb, (35.69%) and (60.04%) increase in mortality respectively]; there was 18.64% increase in dry season compared to the rainy season. Moving from agricultural to residential site is associated with 8.40% p=(0.08) increase in mortality, but not statistically significant. Transiting from Wudil (reference) to Garko is associated with elevated insecticidal associated mortality by a factor of 7.11% (p=0.025) if all other variables remain constant. However, Wudil to Bunkure is associatd with no significant difference in mortality (p=0.119)**.** There was also significant association observed between percent mortality 7.11% (p=0.025) and Garko.

Table 5: Percentage mortality of *Anopheles* mosquitoes for breeding locations

Insecticide	WDL		GRK		BKR	
	Mortality ± S.E	95% Confidence Level (CL)	Mortality ± S.E	95% Confidence Level (CL)	Mortality ± S.E	95% Confidence Level (CL)
DDT	14.87±4.24#a	6.48-23.27	30.09±6.91#a	16.40-43.77	27.22±4.53#a	18.25-36.19
PERM	20.12±3.16#a	13.87-26.37	36.15±3.31#b	29.59-42.71	25.73±5.33#b	15.18-36.29
DELTA	60.13±6.45*a	47.35-72.90	50.77±6.31*a	43.27-58.27	65.41±2.92*b	62.63-68.18
BEND	83.83±3.38^a	77.13-90.53	90.82±3.55^a	83.79-97.84	78.70±4.97^b	68.85-88.54

Super scripts: $^{*\#\wedge\$}$Values bearing the same superscripts of special characters down a column are not statistically different. ^{abcde} Values bearing the same superscripts of alphabets across a row are not statistically different ($p<0.05$).

Table 6: Percentage mortality of *Anopheles* mosquitoes for breeding sites

Insecticides	Agricultural		Residential	
	Mortality ± S.E	95% Confidence Level (CL)	Mortality ± S.E	95% Confidence Level (CL)
DDT	22.50±3.32#a	15.92-29.09	26.85±7.32#a	12.36-41.34
PERM	25.34±3.34#a	18.72-31.97	32.06±3.44#a	25.25-38.87
DELTA	60.11±4.53^a	51.14-69.08	57.85±2.23*a	53.44-62.26
BEND	87.48±2.93*a	81.66-93.29	76.44-3.99^a	68.54-84.34

Table 7: Seasonal variation in percentage mortality of *Anopheles* mosquitoes

Insecticides	Rainy		Dry	
	Mortality ± S.E	95% Confidence Level (CL)	Mortality ± S.E	95% Confidence Level (CL)
DDT	19.80±4.14#a	11.60-28.00	31.55±3.94#a	23.75-39.36
PERM	23.68±3.13#a	17.48-29.89	34.63±3.77#a	27.16-42.10
DELTA	51.77±3.21*b	45.40-58.14	74.75±4.16^c	66.51-82.99
BEND	79.48±2.91^d	73.72-85.24	92.74±3.54*e	85.73-99.75

Table 8: Association between the insecticides mortality with TOI, Season, TOBS and BL.

Mortality	Coefficient	S.E	t	P> |t|	95% Confidence Level
Insecticides					
DDT	Ref				
PERM	3.70	3.57	1.04	0.302	-3.38 - 10.79
DELTA	35.69	3.48	10.25	0.000	28.78 - 42.60
BEND	60.04	3.51	17.10	0.000	53.08 - 66.99
Season					
Rainy	Ref				
Dry	18.64	2.99	6.23	0.000	12.71 - 24.57
Site type					
AGRICULTURAL	Ref				
Residential	8.40	3.08	2.72	0.008	2.29 - 14.51
Site					
WDL	Ref				
GRK	7.11	3.13	2.27	0.025	0.91 - 13.31
BKR	4.75	3.02	1.57	0.119	-1.24 - 10.74

Discussion

This study investigated the susceptibility of *Anopheles* mosquitoes to the main insecticides in Kano-south, Kano State, Nigeria based on WHO current criteria for characterising insecticides resistance/ susceptibility were susceptibility is defined by mortality rate greater than 98% 24 hours after exposure and resistance which also defined by mortality rate less than 98%. In both the rainy and dry seasons, larval collection was done from different *Anopheles* breeding sites from both agricultural and residential sites and

the larvae were reared into adult mosquitoes in the insectary. Insecticides bioassay was conducted using emerged female *An.* mosquitoes (F_0). In addition to being a useful tool for estimating an insecticide's insecticidal knockdown capability or capacity under conditions that have not been thoroughly studied, KT_{50} can also be used to gauge the strength of an insecticide's emerging resistance. The study reveals that bendiocarb has lower KT_{50} an indication of its greater knockdown potential compared to permethrin, deltamethrin and DDT. The higher KT_{50} in the rainy season could be due to high cropping practiced during the rainy season, and elements such as xenobiotic and agricultural pesticide concentration in breeding sites are dependent on rainfall levels, this could possibly result in a variation of the related insecticide resistance profile according to season (Tene-Fossog *et al*., 2022) [19]. In contrast Mahe *et al*., 2022 [11] reported that *Anopheles* populations from Hadejia town in Jigawa state observed that deltamethrin has a lower KT_{50} and KT_{90} values compared to permethrin. Mosquitoes from Wudil, Garko and Bunkure localities used in this research showed the highest resistance with DDT, followed by permethrin, then deltamethhrin and bendiocarb recorded the least resistance. Higher resistance recorded by DDT is most likely a result of the widespread use of DDT and pyrethroids (permethrin and deltamethrin) for crop protection (Hassan *et al*., 2018) [8]. The widespread use of coils and aerosols containing pyrethroids for the control of household pests and vectors may have created selection pressure on the *Anopheles* population leading to resistance (Hassan *et al*., 2018) [8]. Similarly, the same pattern was maintained for the insecticide susceptibility bioassays based on breeding sites (agricultural and residential) and seasons (rainy and dry). However, similar results were obtained from studies conducted by Ononamadu *et al.*, 2020 [15], WHO insecticides susceptibility bioassay revealed a high level of resistance to DDT and permethrin. A relatively low level of resistance was observed with bendiocarb. Similar, research work revealed high level of resistance to deltamethrin and possible resistance to bendiocarb in *An. gambiae* being the major malaria vector in Bichi, Nigeria collected from both residential and agricultural sites (Safiyanu *et al*., 2016) [17]. Extremely high DDT resistance was observed in Bayero University, Kano, *Aedes* mosquitoes was observed (Ibrahim and Muktar, 2022) [12]. It was also documented by Ibrahim and Muktar, 2022 [12] *Aedes aegypt*i were resistant to deltamethrin and lambda – cyalothrin, but susceptible to cyfluthrin as well as well as extremely high resistant to DDT, but susceptible to dieldrin, highlighted the complex nature of metabolic resistance.

Comparing this findings with previous works suggests that insecticide resistance in northern Nigerian *An. gambiae* complex has escalated in few years. It was previously reported by Ibrahim *et al*., 2014 [10] that *An. coluzzii* populations from two sites in the Sudan Savannah of northern Nigeria exhibiting mortalities with pyrethroids 50 – 78%.

Conclusion

The knockdown profile of *Anopheles* mosquitoes collected from the breeding locations, breeding sites in both rainy dry seasons using permethrin, deltamethrin, DDT and bendiocarb were determined. The results of median knockdown time (KT_{50}) of *Anopheles* mosquitoes' across the breeding locations (Wudil, Garko and Bunkue) shows that the KT_{50}; of DDT, permethrin, deltamethrin and bendiocarb were statistically different in Wudil; in Garko the KT_{50} of permthrin and deltamethrin were statistically different from that of DDT and bendiocarb and that DDT was also dfferent from that of bendiocarb. In Bunkure, the KT_{50} of DDT and permethrin were statistically significant to that of deltamethrin and bendiocarb. The KT_{50} of DDT and permethrin were statistically significant between Wudil and Garko; Wudil and Bunkure. For deltamethrin the KT_{50} was found to be statistically different across the three breeding locations. The KT_{50} for bendiocarb was statistically different between Wudil and Garko; Garko and Bunkure

The results of median knockdown time (KT_{50}) of *Anopheles* mosquitoes' collected from breeding sites (agricultural and residential) showed that the KT_{50}; of DDT and permethrin are statistically different from that deltamethrin and bendiocarb in both agricutural and residential sites; deltamethrin is also different from that of bendiocarb in the agricultural site; DDT, permethrin and deltamethrin are statistifically significant from bendiocarb in the residential site. The KT_{50}; of DDT and permethrin are statistically different from that deltamethrin and bendiocarb and that of deltamethrin is also different from that of bendiocarb, in both rainy and dry seasons. Comparing rainy to the dry season, all the insecticides tested were statistically significant across the two seasons except permethrin. Association between median knockdown time with type of insecticide (TOI), seasons, breeding sites (BS), and breeding locations (BL). DDT been as a reference category, permethrin, deltamethrin and bendiocarb were associated with reduction in the KT_{50} by 123.19 minutes (mins.), 209.46 mins, and 245 mins respectively, which were all statistically significant. An indication that all 3 insecticides have better knockdown profile than DDT. Moving from rainy to dry season also reduces the KT_{50} by 126.06 minutes which was also statistically different. Relatively, moving from agricultural site to residential is associated with a reduction in the KT_{50} by 76.05 mins. Relatively, transiting from Wudil to Garko or Bunkure is associated with reduction in KT_{50} by a factor of 79.06 mins ($p=0.019$) and 83.02 mins($p=0.012$) respectively. Furthermore, DDT and permethrin recorded the lowest percent mortality in Wudil compared to Garko and Bunkure; deltamethrin recorded the highest percent mortality in Bunkure compared to Wudil and Garko; bendiocarb recorded the highest percentage mortality in Garko, compared to Wudil and Bunkure. The results also showed that; DDT and permethrin recorded the lowest percentage mortality in the agricultural site compared to the residential site, while deltamethrin and bendiocarb recorded the highest percentage mortality in the agricultural site compared to the residential site. The dry season recorded the highest percentage mortality for all the insecticides tested compared to the rainy season. There was significant association observed between percentage mortality and insecticides (deltamethrin and bendiocarb), there was increase in dry season compared to the rainy season. There was also significant association observed between an increase in percentage mortality and Garko.

Authors' Contributions

The first author S.A Bichi carried out the research under the supervision of A.A. Imam, A. J. Alhassan was the Co-supervisor, Ononamadu did the statistics, A.Mahe assisted in the laboratory work.

Conflict of interest

The authors declare that there is no conflict of interest

regarding the publication of this paper.

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