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Research Article

Pyrethroid, Pyrroles and Neonicotinoids Insecticides Resistance on *Anopheles gambiae* in Keffi and Nasarawa Communities of Nasarawa State, Nigeria

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ABSTRACT

Development and spread of insecticide resistance in malaria vectors is a threat to vector control and malaria prevention efforts. This study the resistance status of pyrethroids and new insecticide classes on the survival of *An. gambiae* complex and their knockdown gene frequencies were determined. WHO tube tests and CDC bottle bioassays were conducted on two to five days old adult females. *An. gambiae* s.l. survivors were morphologically identified and species-specific identification and detection of *An. gambiae* s.s. Overall, total of 3,608 *An. gambiae* s.l. were exposed to alphacypermethrin, deltamethrin, Chlorfenapyr and clothianidin. A total of 1,204 *An. gambiae s.l*. were exposed to alphacypermethrin and deltamethrin. The average mortality rate of 89.9 % (n=1134) was recorded with 10.1% (n=61) survivors in alphacypermethrin exposure and 90.7% (n=1148) with 9.3% (n=56) survivors recorded in deltamethrin exposure. A total of 600 *An. gambiae* s.l. were also exposed to Chlorfenapyr and clothianidin. Mortality rate due to Chlorfenapyr was 98.7 (n=596) with 1.3% (n=4) survivors while with clothianidin gave exposure mortality rates of 97.7% (n=593) with 2.3% (n=7) survivors. Analysis of survivors indicated *An. gambiae* s.s ranged from 80%(n=44) in Nasarawa to 86.1% (n=62) in Keffi. Significantly, deltamethrin exposed *An. gambiae* (0.83 vs 0.50), P≥0.004217 (0.50 vs 0.25, P ≥ 0.003892) in both Nasarawa and Keffi. Only alpha-cypermethrin exposed *An. gambiae* s.l. Although pyrethroid resistance was recorded in *An. gambiae* s.s though, malaria vectors were susceptible to both chlorfenapyr and clothianidin. Findings indicate need for deployment of new generation insecticides resistance management and malaria control programs.

Keywords: *Anopheles gambiae*; Nasarawa Communities; Neonicotinoids; New Generation Insecticides; Pyrethroid; Pyrroles; Resistance

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INTRODUCTION

Malaria control faces various biological obstacles, including the resistance of parasites to anti-malarial drugs (WHO, 2016) and of mosquito vectors to insecticides (Ranson *et al.*, 2011). So far, the fight against this disease is essentially based on chemotherapy targeting the parasite in humans using anti-malarial drugs, as well as actions aiming at simultaneously reducing the human-vector contact, the density and the longevity of Anopheles vectors (Ibrahim *et al.,* 2019). Insecticide-based vector control is a cornerstone in the fight against malaria. Yet insecticide resistance in malaria vectors threatens to undermine its effectiveness. In 2012, WHO released the *Global plan for insecticide resistance management in malaria vectors* (GPIRM). Among other priorities, GPIRM highlighted the need for strengthened resistance monitoring (WHO, 2018).

Among the different methods that are commonly used are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) (Kelly-Hope *et al.,* 2008). With the continued increase in climate change,

case reports of these diseases have skyrocketed especially in Africa where insecticide resistance has become a major challenge to vector control (Obame-Nkoghe *et al.,* 2024).

Studies carried out in Benin (Yadouléton *et al.,* 2010) and Côte d'Ivoire (Tia *et al.,* 2017) have shown that the frequency of the L1014F resistance allele of the kdr gene is higher in *Anopheles gambiae* sensu lato (s.l.) in agricultural areas usually treated with insecticides, as compared to rural areas where farmers grow only food or local consumption products. The hypothesis of the contribution of certain agricultural practices to the selection and extension of resistance in vectors cannot be excluded. In the current context where pyrethroids resistance is widespread in Africa (Ibrahim *et al.,*2019)

The resistance of Anopheles to insecticides has been blamed on several factors such as Agricultural activities by farmers, environmental factors such as weather and climate change etc. These insecticides, herbicides, and pesticides are used to protect agricultural crops. Obviously, these pesticides are marketed under different trade names belonging to all the chemical classes of insecticides such as Organophosphates, Organochlorine, Pyrethroids and Carbamates. (Coetzee *et al.,* 2000). The indiscriminate use of these insecticides, herbicides and pesticides in agriculture and the washing of these chemicals to mosquito breeding sites have resulted into the production of emergent resistant adult mosquitoes consequently increasing mosquitoes' population.

The justified the widespread use of a broad spectrum of insecticides in agriculture was considered here as a major source of selection pressure and possibly the main driver of insecticide resistance in major malaria vectors (members of *An. gambiae* complex) in Nigeria. Not much is known about types of insecticides nor the kind of degree of resistance they confer on different members of *the An. gambiae* complex.

Nigeria's National Malaria Elimination Programme (NMEP 2022) recommends investigation on the level, spread and mechanism of Pyrethroid use in ITN and Indoor residual Spraying IRS, as a major research priority.

Investigation on the efficacy of alternative new generation insecticides on pyrethroid resistance *An. gambiae* s.l. is a research priority for Nigeria.

In the guinea savannah which Nasarawa state belongs, mass distribution of treated net was deployed in the state, there is still records of resistance to the major pyrethroids commonly used in Insecticide treated nets (ITNs) and Indoor residual Spraying (IRS) and the efficacy of alternative new generation insecticides on pyrethroid resistant An. *gambiae* s.l. According to the World Malaria Report 2023 Although the WHO has received some resistance monitoring data for the new generation insecticides chlorfenapyr and clothianidin, the recommendation considers that, compared with pyrethroid-only nets or pyrethroid-piperonyl butoxide (PBO) nets, pyrethroid-chlorfenapyr ITNs had an increased killing effect against pyrethroid-resistant malaria vectors and should have a greater impact against malaria. (WMR 2023).

In Nigeria in general, and Nasarawa state in particular, the extent of pyrethroid resistance in predominant malaria vectors *An. coluzzii*, *An. gambiae* s.s and *An. arabiensis* in the guinea savannah is also unknown and in-depth investigation is needed. Newer classes of pyrroles and the neonicotinoids insecticides with no history of resistance and different mechanisms of action were recently introduced for resistance management and mitigation purposes Susceptibility of major malaria vectors to these newer classes of insecticides are also unknown. Recent investigations have shown that in n less than a decade, pyrethroid resistance in *An*. *coluzzii* from northern Nigeria has escalated, posing a serious setback to the effort to reduce malaria burden by 90%, in line with WHO projection by the year 2030 (Ibrahim *et al.,*2019). It is therefore of utmost importance to continue surveillance of this resistance and its underlying mechanisms in these areas to inform the malaria control program on its progress. This will help in the implementation of effective evidence-based control measures hence the need for this investigation.

The aim of the study is to determine resistance status to pyrethroid and new-generation insecticides in *An. gambiae* s. l from two agricultural communities each in Keffi and Nasarawa local government area of Nasarawa state.

MATERIALS AND METHODS

Study Area

This study was carried out in Keffi and Nasarawa Local Government Areas of Nasarawa State in the Guinea Savanah of north central Nigeria with an area of 138km^2. Keffi local government population is 92,664 by 2006 census (NPC, 2006), It is located on longitude 8.952 and latitude 7.891 (Akwa *et al*. 2007). Keffi has a tropical climate with mean annual rainfall of about 1,357 mm/a (NMA, 2012). The rainfall starts in March and lasts till October while the dry season starts from November and lasts till early March. The relief of the study area shows undulating highlands to average

height of about 850m above sea level (Kana *et al.,* 2014).

Nasarawa local government area has a population of about 189,835 made up of 95,168 male and 94,667 female, (NPC 2006). This is located on Longitude 8.23828 and Latitude 8.43879, Nasarawa has a common mass of 370.7 $km^{2.}$ The area lies within the Guinea Savannah and has tropical climate with mean annual rainfall of about 1,357 mm/a (Nigerian Meteorological Agency, Lafia 2012). The rainfall starts in March and lasts till October while the dry season starts from November and lasts till February. The rainy season on average lasts for 215 days while the dry season lasts for 150 days. Though, the state lies between latitude 7° 45′ and 9° 25′ N of the equator and between longitude 7° and 9° 37′ E of the Greenwich meridian. It shares a boundary with Kaduna state in the North, Plateau State in the East, Taraba and Benue states in the south while Kogi and the Federal Capital Territory flanks it in the West. The state has a total land area of 26,875.59 square kilometers and a population of about 1,826,883, according to the 2006 population Census estimate with a density of about 67 persons per square kilometers (NPC 2006, Nasarawa state website 2021).

Sample Collection and Mosquito Rearing

Larval stages of *Anopheles* mosquitoes were sampled from pockets of water beside the stream site from both (Sabon Gari) Keffi and (Kurudu) Nasarawa local government area. Larval sampling was done continuously for the period of three months from Dec. 2021 to Feb. 2022. All the tests are carried out each month from the two local governments. Mosquito larvae were scooped using a scooping ladle and emptied into a clean well labelled transparent plastic container. These was transported to the Insectary Laboratory of the Department of Zoology, Nasarawa State University, Keffi Nigeria for rearing to adult stage before susceptibility test was carried out on Keffi and Nasarawa LGAs.

The collection was done during dry seasons. Larvae of An. gambiae were collected from the positive breeding sites encountered using the dipping method for the two LGAs. The latter consists in collecting mosquito larvae from the surface of the breeding sites with a ladle. The collected larvae were filtered and kept in well labelled jars according to the surveyed sites and then transported to the insectary Nasarawa State University Keffi, Zoology Department. These larvae were raised under standard conditions of temperature (27 °C ± 2) and humidity (65% ± 10) until adult stage.

Procedures for Susceptibility Test.

The *Anopheles* mosquitoes were reared to adult stage in a bowl in the laboratory, the larva were fed with flake and yeast while the adults were fed with 10% glucose solution (Umar *et al.*, 2008, WHO, 2016). This was carried out at the PMI-Evolve insectary laboratory in Nasarawa State University Keffi, Department of Zoology.

WHO Method of Susceptibility Test

Susceptibility test was carried out using WHO standard procedures. Two insecticides from Pyrethroid Class of Insecticides (Alpha-Cypermethrin and Deltamethrin Impregnated Paper) were used. There were twelve (12) tubes with three different colors, six tubes with a green dot were used as holding period this is done during pre-test of 60mins for acclimatization period and post-test for 24hrs, while the remaining six tubes, four tubes with red dots were used for exposure of the insecticides for the period of 60mins on the two insecticides, then the remaining two tubes with colour yellow dots were used as control. During this exposure period, time was taken with respect to designated time of one hour to record the number of mosquito's knockdown at interval of 10 minutes for the next 60 minutes. After the exposure of 60minutes, mosquitoes were transferred to the green tube, and they were fed with a pad of cotton wool soaked with 10% glucose solution. The holding tubes were kept in a secluded and sterilized place for 24 hours, adult mosquitos' mortality was assessed after 24 hours of post-exposure**.** (WHO 2016).

Tests for Insecticides Susceptibility

Insecticide susceptibility bioassays were performed according to the WHO protocol (WHO, 2013) with the insecticides from four major public health classes. Four replicates of Fi (20–25, 2–4 days old females) per tube were used for each insecticide, alongside 25 unexposed females (control). To confirm the efficacy of the papers, the fully susceptible *An. gambiae* (Kisumu eggs was transported from Kenya, raised to adult in the PMI-Evolve insectary laboratory in Keffi, Nasarawa State University Keffi department of Zoology colony) (Mitchell *et al.,* 2012) was tested first or simultaneously with the experimental populations. Four (4) insecticides were tested, including: (i) the type I pyrethroid: deltamethrin (0.05%) and alpha-cypermethrin (0.05%); (ii) the type I Pyrrole: chlorfenapyr and type I neonicotinoid: clothianidin. Knockdown rates were recorded for both types during the exposure, at intervals of 15 min, 30 min, 45 min and 60 min. After 1hr. exposure mosquitoes were transferred to holding tubes and supplied with 10% sugar solution. Mortality was recorded 24 h after

exposure. Populations were considered susceptible to insecticide where mortality was > 98%, suspected to be resistant (moderately resistant) where mortality is between 90 and 97%, and resistant where mortality was found to be ≤ 90% (WHO 2016). Morphological Identification was done after the test exposure using morphological keys by Coetzee 2020.

CDC Bottle Bioassay Washing and Coating for Susceptibility Test

The bottles were washed with clean soapy water and rinse thoroughly, dry in the sun with the caps off. Masking tape was used to label both the bottles and the caps. Then 1 ml of acetone was added to the control bottle and the caps were put back tightly. 1 ml of stock solution of insecticides Alpha-Cypermethrin, Deltamethrin (Pyrethroids) and Chlorfenapyr and Clothianidin (Pyrrole and Neonicotinoid) were added to all the four bottles for each of the insecticide to be used and the caps were covered back tightly (CDC 2015, Brogdon, & Chan (2010).). The contents inside the bottle were swirling so that the bottom is coated then inverted and swirled to coat the inside of the cap. The bottle was placed on their sides for a moment to let the contents pool and gently rotate the bottle so that the sides were coated.

The coated bottles were kept in dark for 24hrs of coating the bottles with the insecticides of different choice, then susceptibility test was carried out using four bottles for each of the insecticides used (Alpha-Cypermethrin and Deltamethrin (Pyrethroids) and Chlorfenapyr and Clothianidin (Pyrrole and Neonicotinoid) and one control bottle each with 25 female anopheles' mosquitos were aspirated and introduced into the coated bottles. A timer was used to record the number of dead or alive mosquitoes after 15 minutes to 30 minutes for pyrethroids while 60mins and days were observed on the new generation insecticides. The mortality in the control bottle was recorded along with temperature and relative humidity as well.

CDC Bottle Assay Tests for Chlorfenapyr and Clothianidin

For chlorfenapyr, a serial dilution from the technical grade insecticide was made to 100 microgram per ml. Five (5) CDC bottles were coated with 1ml each of this concentration. These bottles were covered and kept in the dark for 24hrs. A total of 20 of 3–5-day old female *Anopheles* mosquitoes each were introduced into five coated bottles and one uncoated bottle (control). These were monitored for a period of 60 minutes and later 24hours for the two LGAs to establish resistant status.

For clothianidin a solution of 800 ppm of acetone/mero® mixture was first made by pipetting 178µL mero® and mixed with 200mL of acetone. 40µg/ml of stuck solution of clothianidin was prepared by mixing 4mg (4,000µg) of technical grade clothianidin with 100ml of acetone/MERO solution (CDC 2015). 10ml of the stock solution (40µg/ml) was diluted with 90 ml of 800ppm of acetone/Mero® solution to make a working solution of 4µg/ml. 1ml each of the clothianidin working solution (4µg/ml) was used to coat four bottles of 250ml volume according to the standard operating procedure bottle assay. The coated bottles underwent dark period of 24hrs after which 25 each of the female *Anopheles* mosquito's pp (2-4 day old) were exposed to four replicates of the coated bottles and one uncoated (control). These were monitored for a period of 30minute, 60 minutes and later 24hrs holding period for the two LGAs to establish resistant status.

Procedures for Molecular Analysis

All survivors (pyrethroid resistant) Anopheles *gambiae* s.l. mosquito samples from all pyrethroid susceptibility were morphologically identified with the (Coetzee 2020) keys and sent for Molecular analysis in Nigerian Institute of Medical Research Lagos for PCR analysis. The aim is to identify and determine the proportion of *An. coluzzii* and *An. gambiae* from survivors (resistant samples) from pyrethroid exposed *An. gambiae* s.l. from Deltamethrin and Alphacypermethrin and to estimate the frequency of the knock down resistance (Kdr) gene in the pyrethroid resistant population. The specimens analyzed were selected from (i) mosquitoes that survived insecticide exposure during routine insecticide susceptibility tests. The identification of *An. coluzzii* and *An. gambiae* and Kdr PCR assays were preceded by a Priori Polymerase Chain Reaction (PCR) assay for identification of members of the *Anopheles gambiae* complex (Scott *et al.,* 1993). This includes DNA extraction using essential extraction kits followed by PCR analysis.

i. Species Identification by PCR

All *Anopheles* mosquito from the two sites were separately analyzed. DNA extracted from each specimen using standard method were amplified with the *Anopheles gambiae* species specific multiplex PCR[1]. PCR products were separated in agarose gel, stained with ethidium bromide and visualized under UV Tran illuminator. The PCR diagnosis bands for this assay include: a 464-base pair (bp) band for *Anopheles* melas, 390bp for An. gambiae s.s and 315bp for *An. arabiensis.*

An. coluzzii and An. gambiae Molecular Identification.

Based on the outcome of the species specific-PCR assay, aliquot of DNA from each sample was processed for subsequent test to identify the molecular M and S using established protocols (Favia *et al.,* 1994; Della *et al*., 2001).

ii. Kdr Genotyping

The presence of the knock down resistance alleles were tested as earlier described. The presence of both the west (*kdr-w*) and East (*Kdr-e*) African kdr mutations were determined using specific primers and protocols designed for these assays (Martinez - Torres *et al*., 1998; Ranson *et al.,* 2000). Details procedures of laboratory protocols are contained in the references provided. Specifically, the West African kdr genotype is characterized by three different PCR bands: 293bp common to both susceptible and resistant specimens; 137bp susceptible band and 195bp kdr band.

The presence of the three bands in a single specimen indicates heterozygote that's the presence of both dominant and the recessive gene. The frequency of the kdr gene was calculated using established genotype formula:

f (R) = (2RR + Rr) / 2n. Where f = frequency, n = number of samples analysed, RR = number of homozygote resistant, Rr = number of heterozygote resistant

Data Analyses

Data were analysed using Analysis of Variance (ANOVA) developed by (Statistics Kingdom, 2017) Melbourne Australia http://www.statskingdom.com and quickcalcs for Chi Square test (x^2) using GraphPad Software, https://www.graphpad.com 2022 2365 Northsides Dr Suite 560 San Diego CA 92108 U.S.A.

RESULTS

Resistance Status of Pyrethroids (WHO Method)

Alpha-cypermethrin and Deltamethrin

Pyrethroids insecticides were tested on *An. gambiae* s.l. in the two agricultural communities of Nasarawa state (i.e., Keffi and Nasarawa communities.). Resistance to alphacypermethrin varied across the months in both sites. In Keffi mortality of *Anopheles gambiae* s.l. varied from 88-96% while in Nasarawa resistance to alpha-cypermethrin varied from 92-95%. Susceptibility to Alpha-cypermethrin 98-100% mortality was recorded in the month of February 2022 in both sites. A significant difference in Alpha-cypermethrin resistance was recorded across both sites $F_{2, 3} = 11.76$, P = 0.04. Fig. 5. In Keffi mortality of *Anopheles gambiae* s.l. Due to deltamethrin varied from 87-92% while in Nasarawa resistance to deltamethrin varied from 95-97%. Susceptibility to deltamethrin 100% mortality was recorded in Keffi in the month of February 2022. No significant difference in deltamethrin resistance was recorded across both sites. $F_{2, 3}= 1.34$ P = 0.38 (Fig. 2, 3).

Chlorfenapyr and Clothianidin

Mortality rates in chlorfenapyr *An. gambiae* from keffi local government area were ranged from 97 (CI: 93.7 – 100.3) to 100 while Nasarawa local government area recorded 100% mortality. No significant difference in mortality rate was recorded between keffi and Nasarawa in December, January and February (F2, 3 =0.7, P = 0.562). While for Clothianidin exposed An. gambiae from keffi, mortality rates ranged from 98% (CI: 95.3 – 100.7) while Nasarawa mortality rate ranged from 97% (CI: 93.7 – 100.3) to 100%. No significant difference in mortality rate was recorded within the month Dec, Jan and Feb. ($F_{2, 3} = 1.50$, P = 0.35) (Figure 4 and 5).

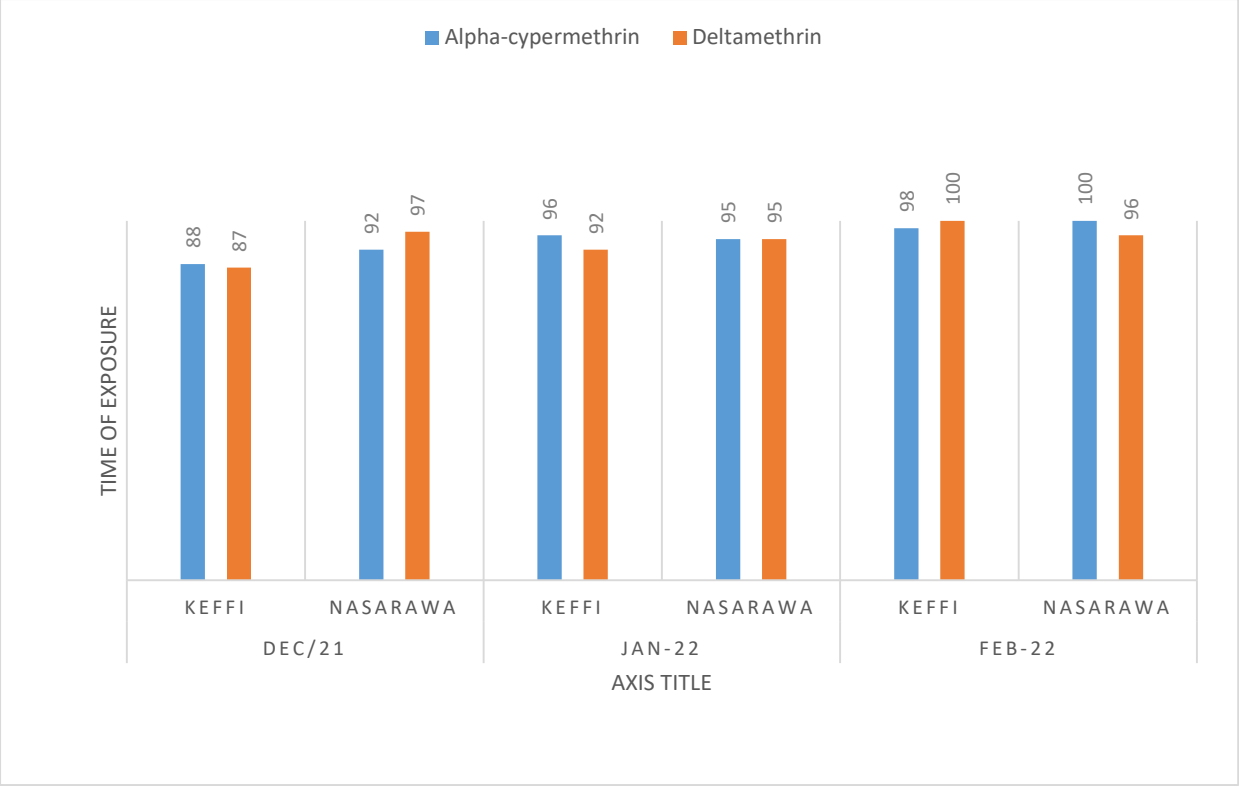

Fig. 2: Susceptibility Test using WHO Assay Between Alpha-cypermethrin and Deltamethrin Insecticide from the Keffi and Nasarawa LGAs.

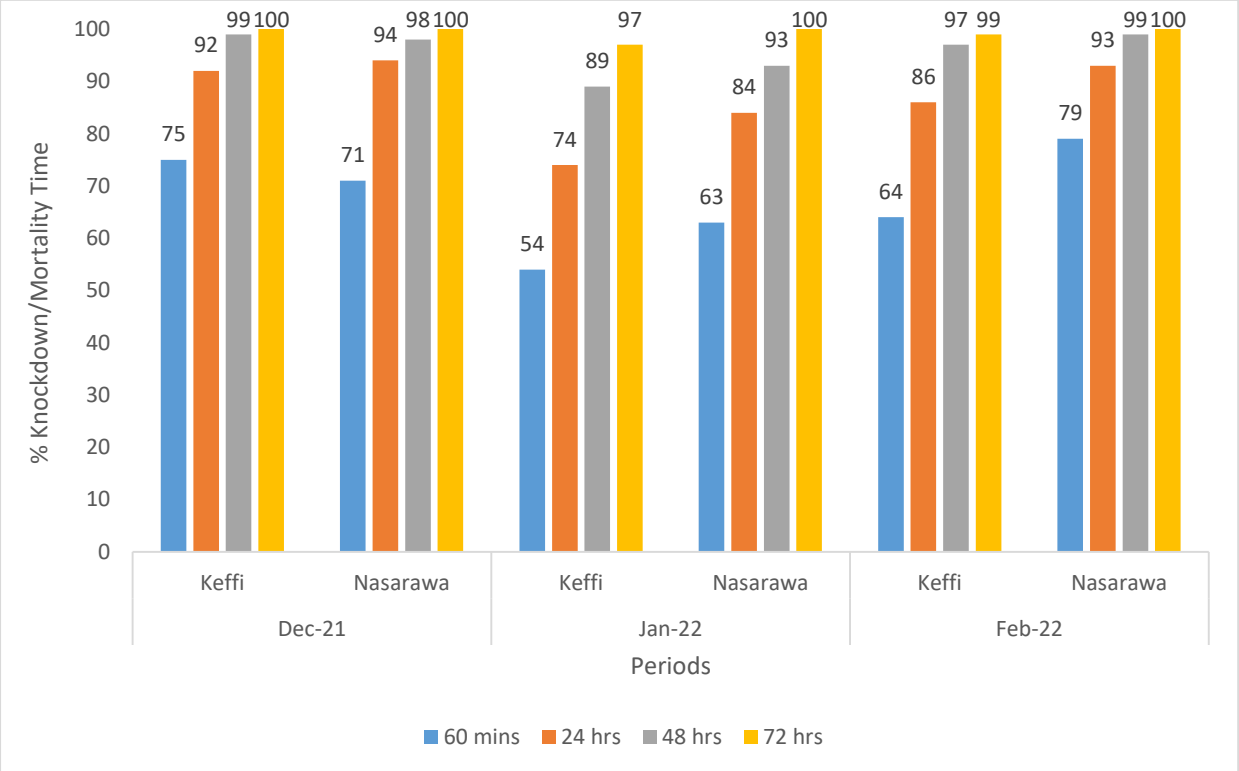

Fig. 3: Susceptibility Test using WHO Assay Between Alpha-cypermethrin and Deltamethrin Insecticide from the Keffi and Nasarawa LGAs.

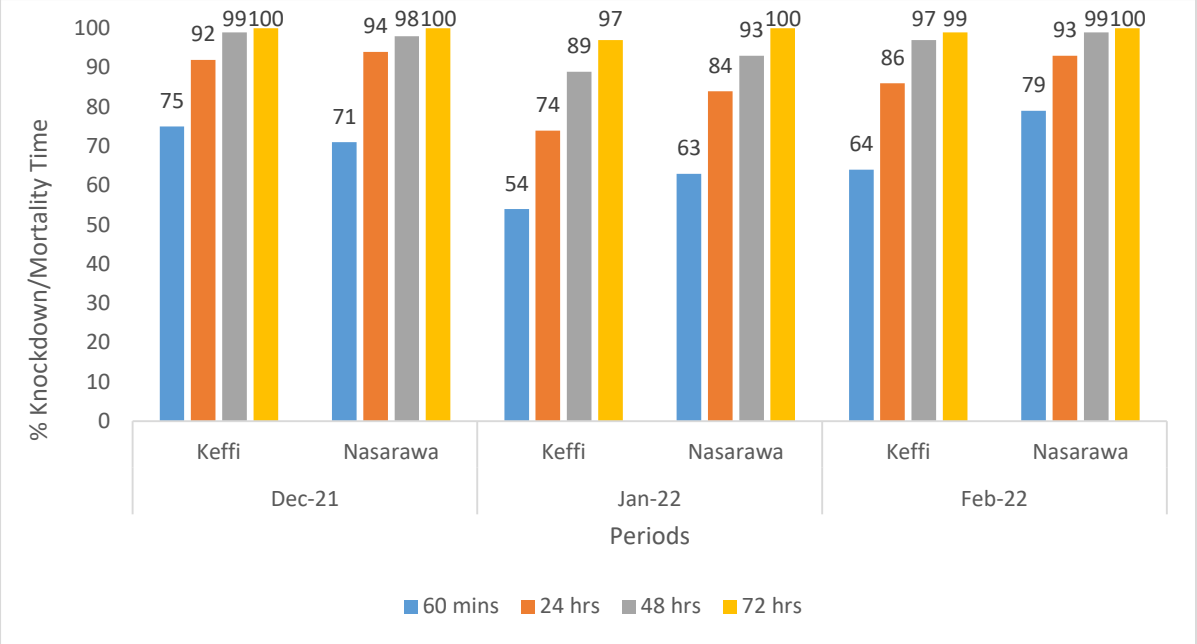

Fig. 4: Susceptibility Test using CDC Bottle Bioassay Chlorfenapyr Insecticide for Keffi and Nasarawa LGAs.

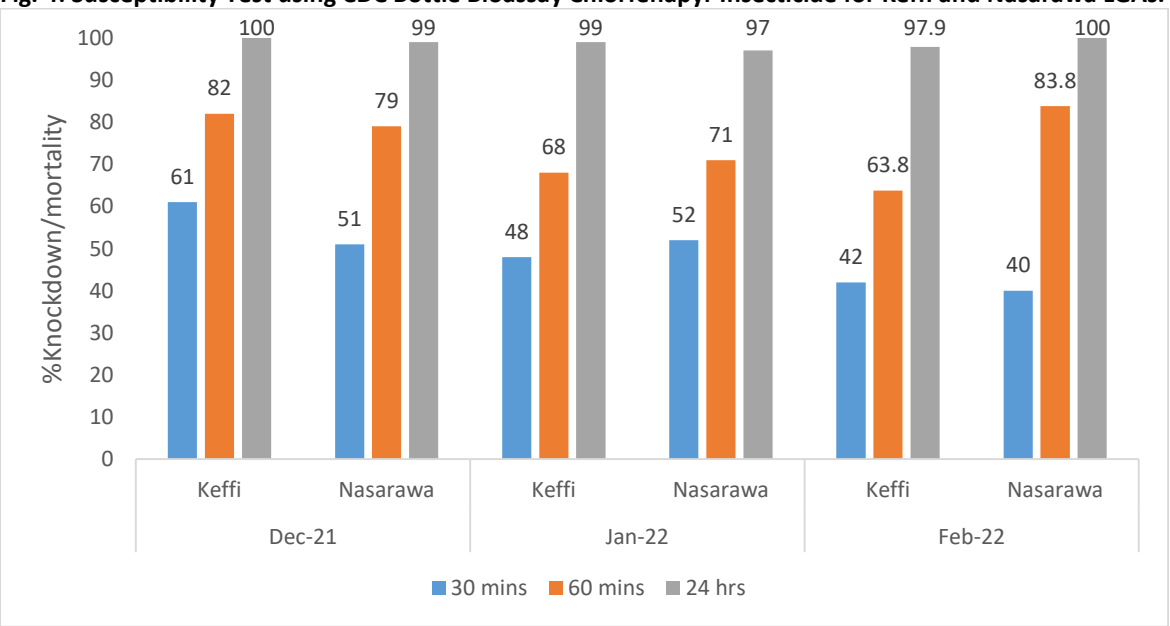

Fig. 5: Susceptibility Test using CDC Bottle Bioassay Clothianidin Insecticide from Keffi and Nasarawa LGAs.

Resistant Samples (Survivals) An. gambiae s.l. from Alpha-cypermethrin, Deltamethrin, Chlorfenapyr and Clothianidin tests.

Overall, a total of 3,608 *An. gambiae* s.l. were exposed to alpha-cypermethrin, deltamethrin, Chlorfenapyr and clothianidin. Of this number 1204 *An. gambiae* s.l. Exposed to alpha-cypermethrin and 1204 An. gambiae s.l. exposed to deltamethrin. Mortality rate of 89.9 %(n=1143) was recorded with 10.1% (n=61) survivals after alphacypermethrin exposure while mortality rates of 90.7% (n=1148) with 9.3% (n=56 survivors) were exposed in deltamethrin exposure. No significant difference between the number of alphacypermethrin and deltamethrin survivors χ^2 =0.05, P=0.82.

Pyrroles and Neonicotinoids (Chlorfenapyr and Clothianidin)

A total of 600 *An. gambiae* s.l. were exposed to Chlorfenapyr and another set of 600 An. *gambiae* s.l. exposed to clothianidin. Mortality rate due to Chlorfenapyr exposure was 99.3 (n=596) with 0.7% (n=4) survivals recorded while mortality rates recorded with clothianidin exposure were 97.7%

(n=593) with 2.3% (n=7 survivals). No significant difference between the number of chlorfenapyr and clothianidin survivors χ^2 =0.33, P=0.563. Comparing the number of pyrethroid survivors and those of pyrroles and neonicotinoids as a measure of resistance indicated that overall, significantly higher survivors were recorded with both pyrethroids than the newer insecticides pyrroles and clothianidin (χ^2 =5.33, P=0.02).

Table 1 Summary of Susceptible Tests Carried out on Insecticides from Different Communities.

Insecticide	Class of Insecticides	Sample Source	Total Tested	No. Dead	No Alive
Alpha-cypermethrin	**Pyrethroids**	Resistant mosquito (Sabon Gari)	602	567(94.2)	35 (5.8)
		Resistant mosquito (Kurudu)	602	576(95.7)	26(4.3)
		Total	**1204**	**1143(89.9)**	**61(10.1)**
Deltamethrin	**Pyrethroids**	Resistant mosquito (Sabon Gari)	601	571(95)	30(5)
		Resistant mosquito (Kurudu)	603	577(95.7)	26(4.3)
		Total	**1204**	**1148(90.7)**	**56(9.3)**
Chlorfenapyr	**Pyrrole**	Resistant mosquito (Sabon Gari)	300	296(98.7)	4(1.3)
		Resistant mosquito (Kurudu)	300	300 (100)	0 (0)
		Total	**600**	**596(99.3)**	**4 (0.7)**
Clothianidin	**Neonicotinoid**	Resistant mosquito (Sabon Gari)	300	297(99)	3(1)
		Resistant mosquito (Kurudu)	300	296(98.7)	4(1.3)
		Total	**600**	**593(98.8)**	**7 (1.2)**

Table 2: Data Analysis between Insecticides

Class of Insecticides	Insecticides	P-Value
Pyrethroids	Alpha-cypermethrin Deltamethrin	≥0.05, P=0.82.
Pyrrole/ Neonicotinoid	Chlorfenapyr Clothianidin	≥0.33, P=0.563

(χ2≥5.33, P=0.02)

There is significant difference in pyrethroid resistance and those of pyrroles and neonicotinoids recorded

DISCUSSION

Insecticide resistance remains the greatest threat to the future of malaria control and to the sustainability of the achievements of recent years. This investigation indicated the presence of both resistance and susceptibility to both deltamethrin and Alphacypermethrin. In both Keffi and Nasarawa the presence of two major malaria vectors *An. gambiae and An. coluzzii* were recorded in deltamethrin resistant samples while for Alphacypermethrin the presence of all 3 major malaria vectors *An. gambiae, An. coluzzii and An. arabiensis* were recorded. This suggests the possibility of higher alphacypermethrin resistance across both sites than deltamethrin. This finding is supported by the higher numbers of alphacypermethrin survivors recorded compared to deltamethrin and accords with the findings across many countries that resistance to pyrethroids continues to be widespread (WHO, 2017).

Novel alternative insecticides suitable that can complement the pyrethroids and improve the control of pyrethroid resistant malaria vectors are urgently required for sustaining LLIN as a means of malaria control. No resistance was recorded to either of the two new generation insecticides Chlorfenapyr or clothianidin tested in this work. This finding may be due to the different mode of action of these insecticides although all. *An. gambiae* s.l. mosquitoes were from pyrethroid resistant areas. This suggests the relevance of chlorfenapyr as a potential compound to manage the pyrethroid resistance observed at the monitoring sites and is consistent with several studies carried out elsewhere with chlorfenapyr. In addition, chlorfenapyr is considered a pro-insecticide that is activated by oxidase enzymes suggesting a potential for negative cross-resistance (Raghavendra *et al*., 2011). Several trials and studies have been conducted on the Interceptor® G2 net as described by Bayili *et al.,* (2017) working in Burkina Faso, Camara *et al*. (2018) working in Côte d'Ivoire, and Ngufor *et al*. (2011) working in Benin. These studies indicate that chlorfenapyr-treated nets evaluated in several areas with documented pyrethroid resistance have been proven to be effective for controlling pyrethroid-resistant malaria

vectors and could contribute to insecticide resistance management in Nigeria.

A high degree of susceptibility of field populations of pyrethroid resistant An. gambiae s.l. to clothianidin was recorded in this study. This may indicate a lack of cross-resistance with pyrethroid insecticides (Agumba *et al*., 2019). The target sites of clothianidin are different from that of pyrethroids, and the lack of cross-resistance indicates that enzymes involved in the metabolic detoxification of pyrethroids do not affect clothianidin. Recent studies have indicated full susceptibility to clothianidin with all susceptible insectary strains and > 98% mortality for wild *Anopheles* at all sites in 11 out of 16 countries. However, tests in at least 1 site in 5 countries produced mortality < 98%, which could potentially be a sign of existing clothianidin resistance (Oxborough *et al.,* 2019).

Adoption of the policies outlined in the Global Plan for Insecticide Resistance Management (GPIRM) produced by the WHO which encourages insecticide rotation to be undertaken in areas of insecticide resistance using insecticides with different modes of action such as the ones tested in this study Chlorfenapyr and clothianidin was strictly adhered. Further research work on resistance mechanisms should be determined as knowing them is important in managing resistance. The local government workers should work alongside the national malaria and elimination Programme team by sensitizing communities on mosquito insecticide treated net usage to combat and reduce drastically the population of these dreadful vectors of diseases.

Efforts at mosquito vector control and selected measures including larviciding of breeding sites should be implemented annually particularly a month before the onset of rainfall in the communities in order to reduce the menace of mosquito vector borne diseases.

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