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Comparative Resistance and Knockdown Dynamics of *Anopheles gambiae* and *Anopheles funestus* to Alphacypermethrin in Northern Nigeria

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Abstract

Insecticide resistance among malaria vectors continues to undermine the effectiveness of pyrethroid-based interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Alphacypermethrin, a widely deployed pyrethroid, has shown reduced efficacy against key malaria vectors, particularly *Anopheles gambiae* and *Anopheles funestus*. This study comparatively assessed the resistance status and knockdown dynamics of these two species in malaria-endemic communities in Northern Nigeria. This study compared the resistance status of *Anopheles gambiae* and *Anopheles funestus* to alphacypermethrin, assessed knockdown rates over time, and identified mosquito species using morphological characteristics and molecular identification. Adult female mosquitoes were collected from malaria-endemic communities using standard collection methods. Species identification was carried out morphologically using standard taxonomic keys and by molecular identification. Susceptibility tests were conducted using World Health Organization (WHO) standard bioassay procedures with alphacypermethrin-impregnated papers. Knockdown was recorded at 10-minute intervals for 60 minutes, while mortality was assessed after 24 hours. Data were analyzed using descriptive statistics and probit analysis. Both Anopheles gambiae and Anopheles funestus populations exhibited varying levels of resistance to alphacypermethrin. *Anopheles gambiae* demonstrated a higher

resistance profile, with reduced and delayed knockdown compared to *Anopheles funestus*. Knockdown increased progressively with exposure time in both species; however, *Anopheles funestus* showed significantly faster knockdown rates and higher susceptibility. Mortality after 24 hours further confirmed greater tolerance in *Anopheles gambiae* relative to *Anopheles funestus*. The findings indicate marked interspecific variation in response to alphacypermethrin, with *Anopheles gambiae* exhibiting higher resistance than *Anopheles funestus*. These results highlight the need for continuous insecticide resistance monitoring and strengthened integrated vector management strategies to sustain malaria control efforts in Northern Nigeria.

Keywords: *Anopheles gambiae*; *Anopheles funestus*; alphacypermethrin; insecticide resistance; knockdown rate; malaria vectors.

Introduction

Malaria remains one of the most devastating infectious diseases globally, especially in tropical and subtropical regions where environmental conditions favor mosquito breeding and transmission. According to the World Health Organization, millions of malaria cases are reported annually, with sub-Saharan Africa accounting for the highest disease burden and mortality rates, particularly among children under five years and pregnant women (WHO, 2023). The disease is caused by protozoan parasites of the genus *Plasmodium* and transmitted mainly by infected female *Anopheles* mosquitoes.

Among the numerous *Anopheles* species identified worldwide, *Anopheles gambiae* and *Anopheles funestus* are regarded as the most important malaria vectors in Africa because of their strong preference for human blood, indoor feeding behavior, and high vectorial capacity (Gillies & Coetzee, 1987). *Anopheles gambiae* thrives mainly in temporary water collections such as puddles and hoof prints, while *Anopheles funestus* is commonly associated with more permanent water bodies containing vegetation. Both species contribute significantly to malaria transmission and remain major targets of vector control interventions.

Malaria vector control strategies largely depend on the use of insecticides through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Pyrethroids are the only class of insecticides approved for use in LLINs because of their rapid knockdown effect, high insecticidal activity at low concentrations, low mammalian toxicity, and relatively affordable cost (Hemingway *et al*., 2016). Alphacypermethrin, a synthetic pyrethroid insecticide, has been widely used in vector control programs due to its effectiveness against adult mosquitoes.

Despite the success of pyrethroid-based interventions, the emergence and spread of insecticide resistance among malaria vectors have become a major public health concern.

Resistance occurs when mosquito populations develop the ability to survive exposure to insecticides that would normally be lethal. Insecticide resistance threatens the effectiveness of malaria control programs by reducing mosquito mortality and increasing the likelihood of malaria transmission.

Several mechanisms contribute to insecticide resistance in mosquitoes. These include target-site resistance caused by mutations in the voltage-gated sodium channel gene known as knockdown resistance (kdr), metabolic resistance mediated by detoxification enzymes such as cytochrome P450 monooxygenases, glutathione S-transferases, and esterases, as well as reduced insecticide penetration and behavioral adaptations (Ranson & Lissenden, 2016). Continuous exposure to insecticides from agricultural and domestic sources has intensified resistance development in mosquito populations.

Previous studies have demonstrated variations in resistance profiles between *Anopheles gambiae* and *Anopheles funestus*. In many African countries, *Anopheles funestus* populations have exhibited stronger pyrethroid resistance associated with elevated detoxification enzyme activity, whereas *Anopheles gambiae* often displays resistance linked to kdr mutations (Riveron *et al*., 2014). Understanding these differences is essential for developing effective resistance management strategies.

Knockdown studies provide valuable information regarding the speed and effectiveness of insecticides against mosquito populations. Knockdown time represents the duration required for an insecticide to incapacitate mosquitoes after exposure. Increased knockdown time is usually associated with resistance and reduced insecticide susceptibility.

Species identification is equally important in resistance studies because different mosquito species may vary in ecology, feeding behavior, and insecticide susceptibility. Morphological identification remains a widely used method for distinguishing mosquito species based on diagnostic anatomical characteristics.

This study therefore compared the resistance of *Anopheles gambiae* and *Anopheles funestus* to alphacypermethrin by evaluating their knockdown rates over time, mortality patterns, and species identification characteristics.

Study Area

The study was conducted in Kurabau and Gajerar Giwa village of Rimi local government area, Katsina state, Nigeria. Kurabau village in Rimi Local Government Area of Katsina State lies approximately within the geographic coordinates of 12.95809°N latitude and 7.79337°E longitude. Gajerar Giwa village in Rimi Local Government Area of Katsina State lies approximately within the geographic coordinates of 12.96053°N latitude and 7.74097°E longitude.

This places it in the semi-arid region of northern Nigeria, within the Sahel Savannah ecological zone.

The vegetation in Kurabau and Gajerar Giwa village is characterized by sparse trees, shrubs, and grasses, typical of the savannah environment. Common tree species include acacia,

baobab, and shea, while grasses dominate the landscape during the rainy season. It is very close to Ajiwa dam, which makes the community engage in agricultural activities throughout the year.

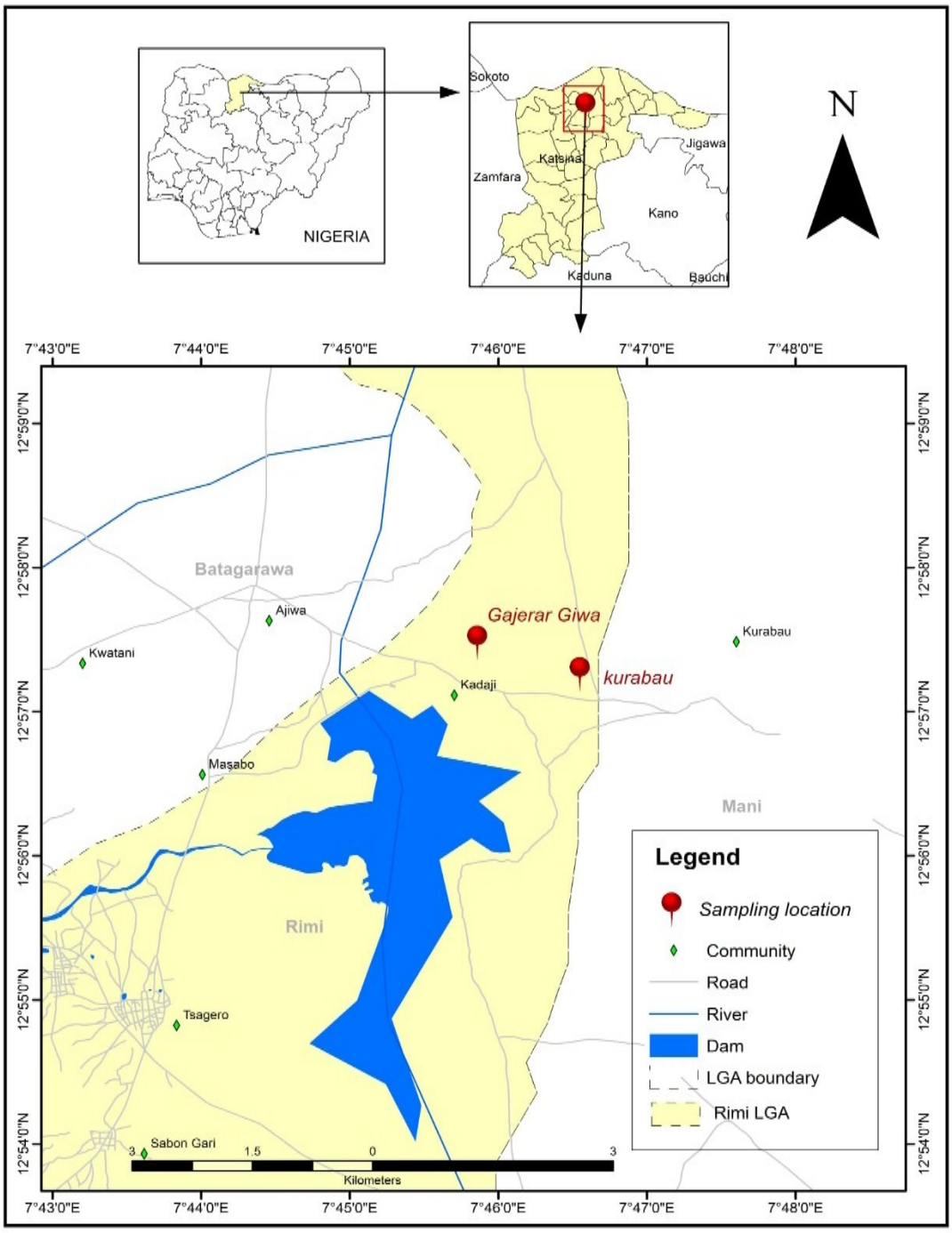


FIG.1: Constructed by Onyekachi Aziujali, Department of Geography and Regional Planning, Faculty of Earth and Environment. Federal University Dutsin-Ma (2025)

Mosquito Collection and Rearing

Blood-fed female Anopheles mosquitoes resting indoors were collected with a mechanical aspirator. The gouged female Anopheles mosquitoes were maintained on sugar solution (10%) for six (6) days to reach full gravidity at 25^{o}C ±2 and percentage humidity of 70-75%. After that, each one was put into a 1.5ml eppendorf tube and left to release eggs as described by Hemingway and Ranson, (2000). Paper cups were used in preparation for hatching of eggs to larvae. Larvae were maintained on Tetramin^{TM} baby fish food. Collected females were allowed to oviposit, and F1 progeny were reared under standard insectary conditions (25 ± 2 °C, 70–75% RH).

Bioassays: Non-blood-fed female An. funestus aged 3–5 days were exposed to insecticide-impregnated WHO papers: DDT (4%), bendiocarb (0.1%), and pirimiphosmethyl (0.25%). Each test was conducted with 100 mosquitoes in four replicates plus controls. Mortality was recorded after 24h following WHO (2016) guidelines. Molecular species identification: DNA was extracted using the Livak protocol (Livak, K. 1984).

Species Identification

Morphological identification of *Anopheles gambiae* and *Anopheles funestus* was carried out using the standard keys described by Gillies and Coetzee, 1987. Species identification within the *Anopheles gambiae* complex was performed using the SINE200 PCR assay described by Santolamazza *et al*. (2008), while species for An. funestus were identified by cocktail PCR assays targeting the An. funestus group (Koekemoer *et al*., 2002). PCR products were visualized on 2% agarose gels stained with ethidium bromide.

Preparation of Insecticide Test Papers

WHO-approved alphacypermethrin-impregnated papers containing diagnostic concentrations of the insecticide were used for susceptibility testing. Control papers without insecticide treatment were also prepared for comparison.

Insecticide Susceptibility Test

Susceptibility testing was conducted according to WHO standard procedures for insecticide resistance monitoring in malaria vectors (WHO, 2022). Batches of 20–25 non-blood-fed female mosquitoes were introduced into exposure tubes lined with alphacypermethrin-treated papers.

Mosquitoes were exposed to the insecticide for 60 minutes. Knockdown was recorded at 10-minute intervals including 10, 20, 30, 40, 50, and 60 minutes.

After exposure, mosquitoes were transferred into holding tubes lined with untreated papers and provided with cotton wool soaked in 10% sugar solution. Mortality was recorded after a 24-hour recovery period.

Determination of Resistance Status

Resistance status was interpreted according to WHO criteria as follows:

- Mortality of 98–100% = Susceptible population
- Mortality of 90–97% = Possible resistance
- Mortality below 90% = Resistant population

Data Analysis

Percentage knockdown and mortality rates were calculated using descriptive statistics. Statistical significance was determined at $p < 0.05$.

Species Identification

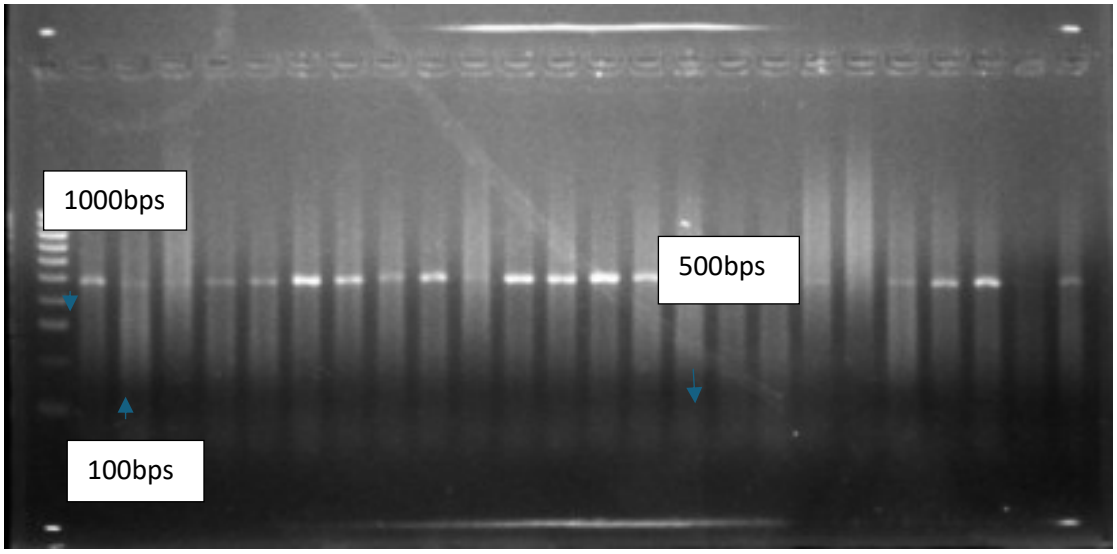


FIG. 2: Gel electrophoregram for *An. gambiae s.l* specie identification. Lane 1 =100bp molecular weight marker, Lane 2-24 at 500bps = *Anopheles coluzzi*

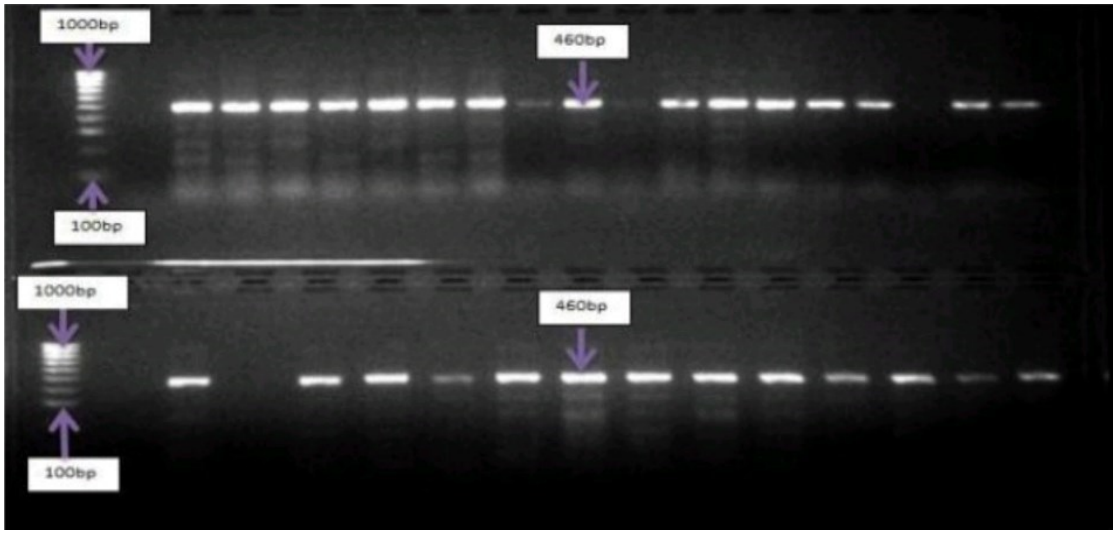


FIG. 3: Gel electrophoregram for *An. funestus s.l* specie identification. Lane 1 =100bp molecular weight marker, Lane 2-21 at 500bps= *An. funestus s.s*

Bioassay Result

A total of 400 adult female mosquitoes were identified morphologically. Out of these, 220 (55%) were identified as *Anopheles gambiae* while 180 (45%) were identified as *Anopheles funestus*.

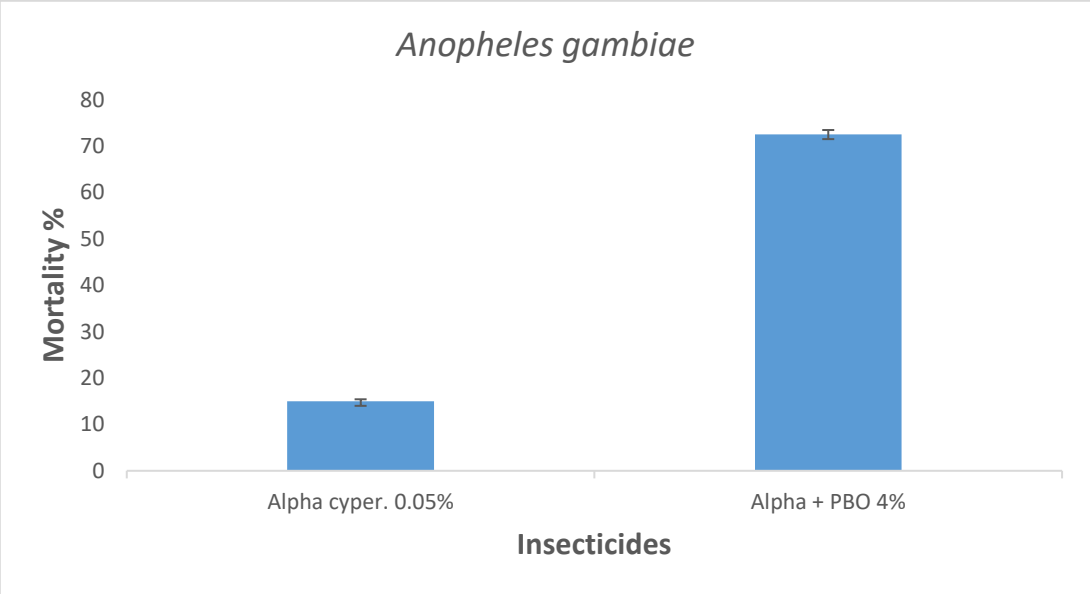


FIG. 4: 24 hour percentage mortality rate after exposure to insecticides. Error bars represent variability in the data.

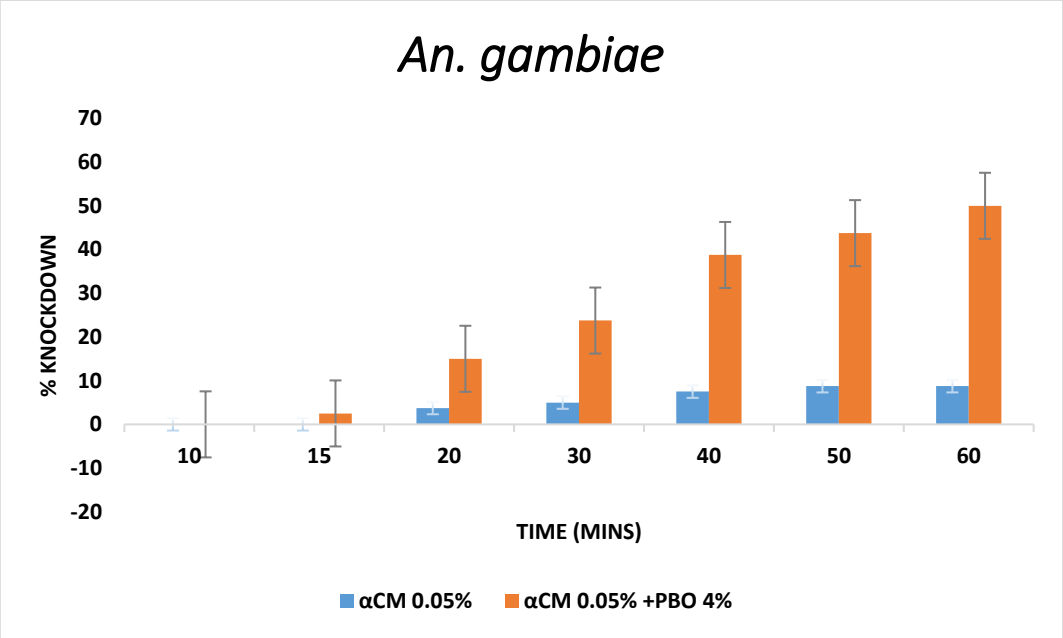


FIG. 5: Knockdown rate over time upon exposure to insecticides for *Anopheles gambiae*. Error bars represent variability in the data.



FIG. 6: 24 hour percentage mortality rate after exposure to insecticides. Error bars represent variability in the data.

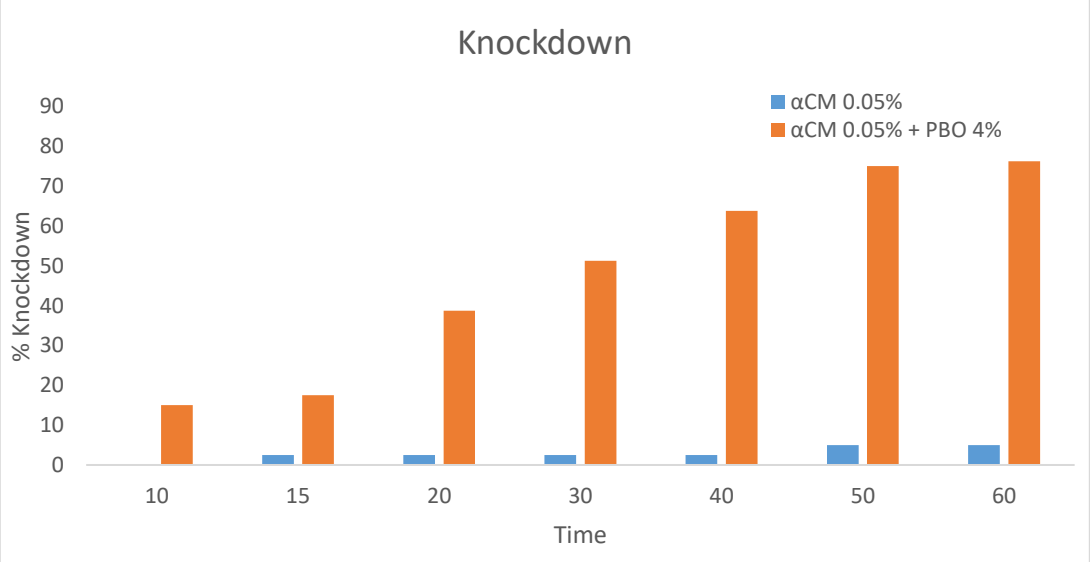


FIG. 7: Knockdown rate over time upon exposure to insecticides for *Anopheles funestus*. Error bars represent variability in the data.

DISCUSSION

Molecular identification of members of the *Anopheles funestus* group using the cocktail PCR assay showed that all amplified products were approximately 500 bp, corresponding specifically to *Anopheles funestus* s.s. (Koekemoer *et al*., 2002). No amplification was detected at fragment sizes indicative of *Anopheles rivulorum*, *Anopheles vaneedeni*, or other species within the *An. funestus* complex. This confirms the exclusive presence of *Anopheles*

funestus s.s. in mosquito populations collected from Kurabau and Gajerar Giwa villages, Katsina State. The result aligns with reports from other parts of Northern Nigeria where *An. funestus* s.s. has been increasingly recognized as a key malaria vector, particularly in ecological settings characterized by permanent breeding sites and irrigated agriculture (Oduola *et al*., 2016).

Similarly, molecular analysis using the SINE200 diagnostic PCR confirmed the presence of *Anopheles coluzzii* in the sampled mosquito populations. The assay produced clear amplification bands at approximately 500 bp, which is diagnostic for *An. coluzzii*, while no amplification was observed at ~230 bp, the expected fragment size for *An. gambiae* s.s., nor were any non-specific bands detected. This banding pattern verifies the exclusive occurrence of *An. coluzzii*, consistent with the presence of the SINE200 retrotransposon insertion in the X-chromosomal intergenic spacer region, a well-established species-specific marker (Santolamazza *et al*., 2008).

Bioassay results after 24-hour exposure to 0.05% alpha-cypermethrin indicated a high level of resistance in both *Anopheles gambiae* s.l. and *Anopheles funestus* s.s. populations from the study areas. Mortality rates were 22.5% for *An. funestus* and 15% for *An. gambiae*, both far below the WHO susceptibility threshold of 90% (WHO, 2016), confirming strong pyrethroid resistance in both species. However, pre-exposure to 4% piperonyl butoxide (PBO), an inhibitor of cytochrome P450 enzymes, resulted in a marked increase in mortality to 100% in *An. funestus*, indicating that resistance in this species is largely driven by metabolic detoxification mechanisms, particularly monooxygenase activity.

This observation is consistent with findings from other studies in Northern Nigeria. Ibrahim *et al*. (2020) reported similarly low mortality rates (<20%) in *An. gambiae* populations exposed to alpha-cypermethrin in parts of Kano and Katsina States, attributing resistance primarily to elevated expression of detoxification enzymes such as CYP6P3 and CYP6M2. Likewise, Tchouakui *et al*. (2020) documented complete restoration of susceptibility in *An. funestus* populations from Kaduna and Yobe States following PBO exposure, further supporting the predominance of monooxygenase-mediated resistance in the region.

In *An. gambiae* s.l., mortality increased from 15% to 73.75% after PBO pre-exposure, suggesting only partial restoration of susceptibility and indicating that both metabolic detoxification and target-site (kdr) mechanisms may be involved. This agrees with Amoo *et al*. (2022), who reported high frequencies of the L1014F kdr mutation in populations from Sokoto and Zamfara States, where PBO exposure only partially reversed resistance. Similar patterns have also been documented by Djouaka *et al*. (2011) in Niger and Northwestern Nigeria, highlighting the coexistence of metabolic and kdr-based resistance mechanisms in *An. gambiae* populations. Furthermore, Awolola *et al*. (2018) reported widespread pyrethroid resistance with only partial recovery after PBO treatment in *An. gambiae* s.l. across the Sahelian zone of Nigeria.

Collectively, these findings from Northern Nigeria emphasize the growing limitation of conventional pyrethroid-treated nets and support the adoption of PBO-based long-lasting

insecticidal nets, such as Olyset® Plus and PermaNet® 3.0, which have shown improved effectiveness in areas with high metabolic resistance (WHO, 2017). The results from Kurabau and Gajerar Giwa therefore add to the increasing evidence base and highlight the need for integrated resistance management strategies tailored to both *An. gambiae* and *An. funestus* populations in Northern Nigeria.

CONCLUSION

This study revealed that *Anopheles funestus* exhibited lesser resistance to alphacypermethrin compared to *Anopheles gambiae*. The knockdown rates and mortality patterns indicated reduced resistance of *Anopheles funestus* to the insecticide.

Continuous monitoring of insecticide resistance among malaria vectors is essential for effective malaria control. Alternative vector control strategies and resistance management approaches should be implemented to reduce the spread of resistant mosquito populations.

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