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SUSCEPTIBILITY OF ANOPHELES FUNESTUS TO PIRIMIPHOS-METHYL: EVIDENCE FOR ITS CONTINUED ROLE IN MALARIA VECTOR CONTROL IN NORTHERN NIGERIA

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Abstract

Insecticide resistance in malaria vectors threatens vector control programs in Nigeria. The study assessed the insecticide susceptibility status and molecular identification of An. funestus populations in Gajerar Giwa village, Katsina State, Nigeria. Bioassays were conducted using WHO test protocols with DDT (4%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%). Molecular identification was performed using cocktail PCR assays. Results showed possible resistance to DDT (95% mortality) and bendiocarb (90% mortality), while full susceptibility was observed to pirimiphos-methyl (100% mortality). Knockdown analysis indicated a gradual increase in mortality with exposure duration, suggesting partial resistance mechanisms at play. PCR confirmed the presence of An. funestus s.s. These findings provide crucial insights into insecticide resistance profiles of An. funestus in northern

Introduction

Malaria remains a significant public health problem in Nigeria, contributing to high morbidity and mortality rates, particularly in the Sahelian northern regions (World Health Organization [WHO], 2023). The primary vectors in these areas include members of the *An. gambiae* complex and the *An. funestus* group, both of which are efficient malaria transmitters (Awolola *et al.*, 2014). Insecticide-based interventions such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS) remain the cornerstones

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Nigeria and emphasize the importance of routine monitoring to guide malaria vector control. The study further highlights the potential operational benefits of organophosphates in indoor residual spraying, given their sustained efficacy compared to carbamates and organochlorines. Considering the widespread emergence of pyrethroid resistance in northern Nigeria, the continued susceptibility of An. funestus to pirimiphos-methyl offers a valuable opportunity for integrated resistance management and policy decisions in malaria control programs.

Keywords: An. funestus s.s, malaria, pirimiphos-methyl

Of malaria control. However, the widespread emergence of insecticide resistance poses a substantial challenge (Ranson & Lissenden, 2020).

Previous studies in northern Nigeria have demonstrated multiple resistance mechanisms in both An. gambiae s.l. and *An. funestus* populations, driven by both metabolic detoxification and target-site mutations (Ibrahim *et al*., 2020; Awolola *et al*., 2018). The persistence of *An. funestus* in areas with permanent water bodies and irrigated agriculture, such as those surrounding Gajerar Giwa village, underscores its role as a major malaria vector. Therefore, there is a need to continually monitor the resistance status of this species to guide vector control.

Methods

Study site: Mosquito collections were carried out in Gajerar Giwa village, Rimi LGA, Katsina State, within the Sahel savanna ecological zone. 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

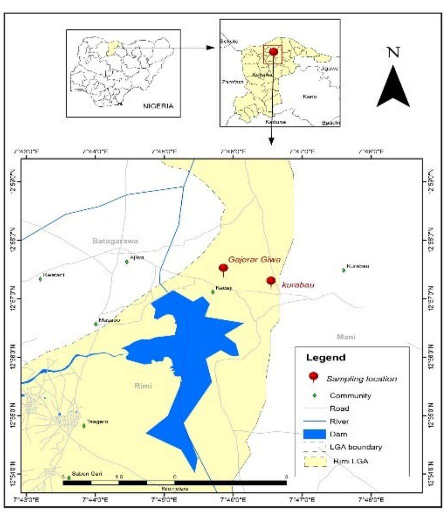

FIG.1. Constructed by Onyekachi Aziujali, Department of Geography and Regional Planning, Faculty of Earth and Environment. Federal University Dutsin-Ma (2025)

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 $25^{o}C \pm 2$ 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™ 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 pirimiphos-methyl (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 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.

Results
Bioassays revealed 95% mortality with DDT, indicating suspected resistance; 90% mortality with bendiocarb, suggesting possible resistance; and 100% mortality with pirimiphos-methyl, indicating full susceptibility.

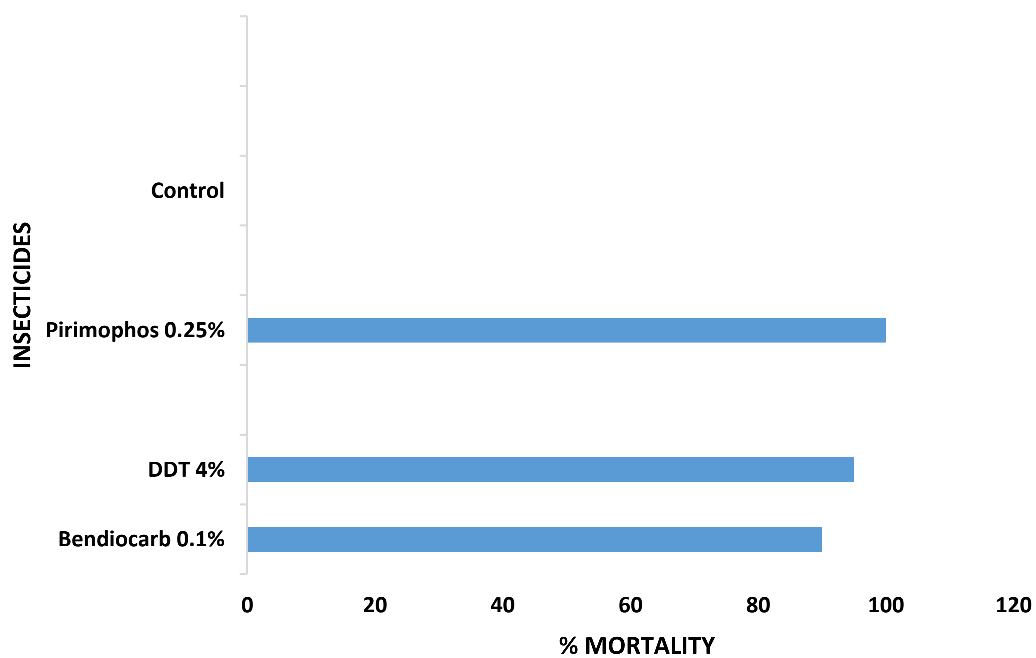

FIG. 2. 24 hour percentage mortality rate after exposure to insecticides. Error bars represent variability in the data. Dichlorodiphenyltrichloroethane (DDT)

There was steady increase in knockdown with increase in time by the *An. funestus* upon exposure to 4% DDT, 0.1% bendiocarb, and the 0.25% pirimiphos-methyl, notably from 30 minutes of exposure.

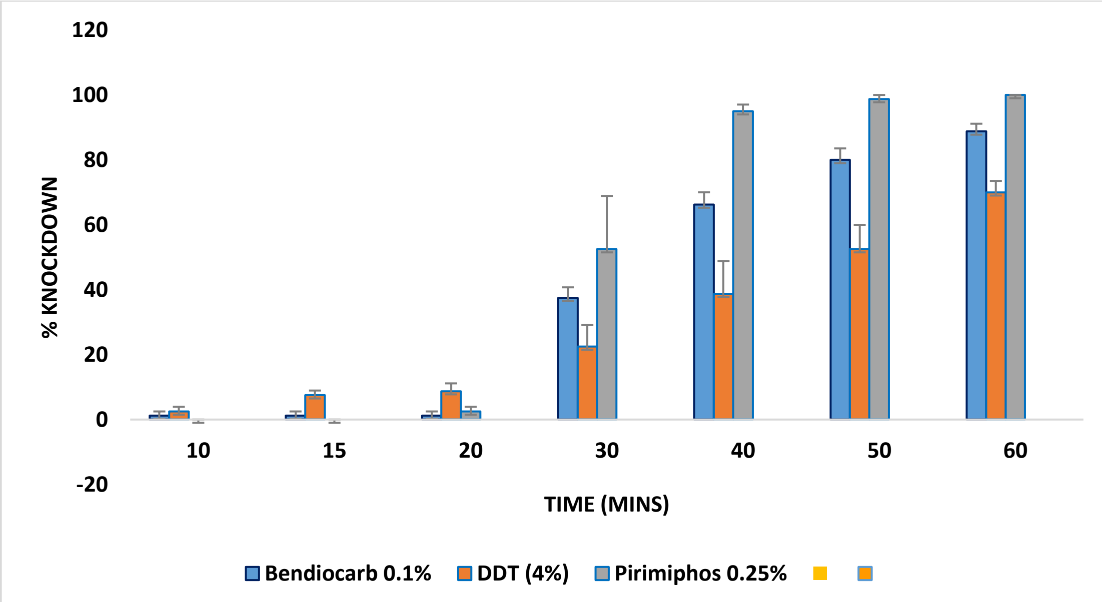

FIG. 3. Knockdown rate over time upon exposure to insecticides for *An. funestus.* Error bars represent variability in the data.

Specie identification

The cocktail PCR assay targeting members of the *An. funestus* group produced bands at ~560 bp, corresponding to *An. funestus s.s*. No amplification was detected at fragment sizes corresponding to *An. rivulorum, An. vaneedeni*, or other sibling species, confirming the exclusive presence of *An. funestus s.s*. in the samples analyzed.

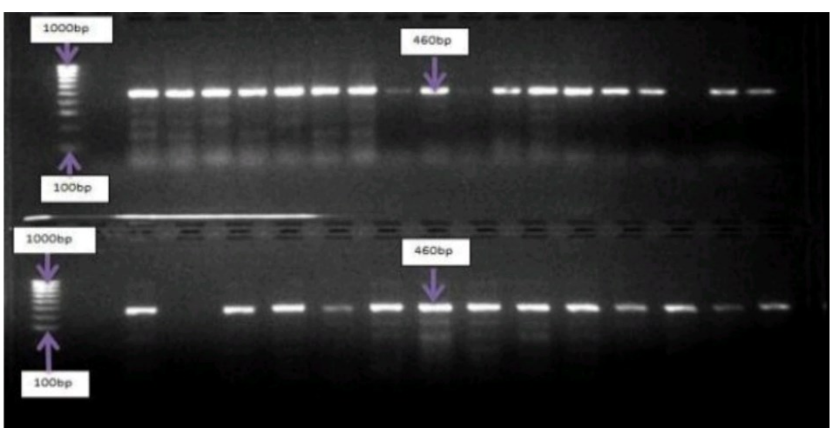

FIG.4: Gel electrophoregram for *An. funestus s.l* specie identification. Lane M =100bp molecular weight marker, Lane 1-34 at 560bps= *An. funestus s.s*

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Discussion

The results from Gajerar Giwa demonstrate varying resistance patterns across insecticide classes. Resistance to DDT is consistent with previous reports from Kano, Kaduna, and Bauchi States, where *An. funestus* populations displayed moderate DDT resistance due to historical organochlorine used (Ibrahim *et al*., 2020; Awolola *et al*., 2018). This trend reflects the long-standing use of DDT for vector control in Nigeria during the 1950s–1970s, which likely exerted strong selective pressure (Ranson & Lissenden, 2020). The observed 95% mortality in this study suggests that resistance is not yet fully fixed in the population, and residual susceptibility may be linked to heterogeneous resistance allele frequencies.

Possible resistance to bendiocarb aligns with findings in northeastern Nigeria, where *ace-1* mutations and metabolic resistance mechanisms have been implicated in carbamate resistance (Oduola *et al*., 2016). The 90% mortality threshold suggests an emerging resistance pattern that could compromise the long-term utility of carbamates in indoor residual spraying (IRS). Similar patterns of carbamate resistance have been documented across West Africa, indicating that the spread of resistance alleles may be facilitated by gene flow among *An. funestus* populations (Riveron *et al*., 2016).

The susceptibility to pirimiphos-methyl suggests organophosphates remain effective, supporting their continued use in IRS campaigns as observed in Maiduguri and Zaria (Tchouakui *et al*., 2020). This is particularly important given the widespread failure of pyrethroid-based interventions, which dominate long-lasting insecticidal nets (LLINs) (Ibrahim *et al*., 2020). The full susceptibility of *An. funestus* to pirimiphos-methyl indicates that IRS with organophosphates could complement LLIN distribution in resistance management strategies. Furthermore, organophosphates target different enzymatic pathways compared to carbamates and pyrethroids, reducing the likelihood of cross-resistance (Njoroge *et al*., 2019).

The PCR confirmation of *An. funestus* s.s. highlights the dominance of this species in semi-permanent aquatic habitats typical of Sahelian regions. Its strong anthropophilic and endophilic behaviors make it a key malaria vector in this region (Awolola *et al*., 2014; Koekemoer *et al*., 2002). Importantly, previous studies have shown that *An. funestus* can sustain year-round malaria transmission due to its ecological adaptability, making resistance monitoring critical (Coetzee & Koekemoer, 2013).

Overall, these findings emphasize the necessity of integrating molecular monitoring with phenotypic resistance assays to inform evidence-based vector control strategies. The emergence of resistance in *An. funestus* populations underscores the importance of insecticide rotation and the introduction of next-generation LLINs treated with synergists such as piperonyl butoxide (PBO), which can mitigate metabolic resistance

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(WHO, 2023). Without sustained surveillance and adaptive management, the gains made in malaria control risk being undermined by escalating resistance.

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Conclusion

This study establishes that *An. funestus* s.s. is the predominant malaria vector in Gajerar Giwa village, exhibiting suspected resistance to DDT and bendiocarb but full susceptibility to pirimiphos-methyl. Routine resistance monitoring and the incorporation of organophosphates in IRS programs remain vital to sustaining malaria control in northern Nigeria.

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