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Widespread Insecticide Resistance in Outdoor-Resting Anopheles Mosquitoes in Gombe: Implications for Malaria Control

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Abstract
Malaria, a life-threatening disease transmitted by Anopheles mosquitoes, remains a significant public health concern in many parts of the world, including Nigeria. Understanding the susceptibility of these mosquitoes to insecticides is crucial for effective vector control strategies. This study aimed to characterize the habitats favoured by Anopheles mosquitoes and assess their susceptibility to commonly used insecticides in Billiri and Kaltungo Local Government Areas of Nigeria. A comprehensive survey was conducted across various ecological zones within the selected areas to identify and characterize potential mosquito breeding sites. Susceptibility tests were carried out using standard WHO protocols to evaluate the resistance status of Anopheles mosquitoes to insecticides commonly used in vector control programs. Insecticide susceptibility tests indicated varying levels of resistance among anopheles populations to commonly used insecticides, highlighting the importance of regular monitoring and rotation of insecticide classes, the findings underscore the urgency of implementing integrated vector management strategies that consider both environmental factors and insecticide resistance profiles to effectively combat malaria transmission in the region. Further investigations incorporating molecular techniques and long-term surveillance are recommended to enhance our understanding and management of Anopheles mosquitoes in these areas. Two-way ANOVA was used to determine the significant differences in the means of insecticides using SPSS 20.

Keywords: Anopheles Gambiae, Insecticide Resistance, DDT, Bendiocarb, Deltamethrin

Introduction

Malaria continues to be one of the most significant public health concerns globally, particularly in sub-Saharan Africa, where the disease is endemic. It is transmitted primarily by Anopheles mosquitoes, which act as vectors for the Plasmodium parasites responsible for malaria. According to the World Health Organization (WHO), malaria caused approximately 627,000 deaths worldwide in 2020, with the majority occurring in sub-Saharan Africa (WHO, 2021). Despite various control efforts, the disease remains a major burden, especially in rural and peri-urban communities where healthcare infrastructure is often inadequate. The primary strategy for controlling malaria involves vector control, which includes the use of insecticide-treated nets (ITNs), indoor residual spraying (IRS), and larviciding. These methods have significantly reduced malaria transmission in many regions. However, the effectiveness of these interventions is increasingly threatened by the development of insecticide resistance in mosquito populations. Resistance can render these control measures ineffective, leading to a resurgence of malaria cases (Hemingway et al., 2016). Insecticide resistance in Anopheles mosquitoes has emerged as a critical obstacle in the global fight against malaria. This resistance occurs when mosquito populations develop the ability to withstand insecticides that would normally be lethal, allowing them to survive treatments aimed at reducing their numbers. The ability of mosquitoes to develop resistance stems from a combination of genetic adaptations, behavioural shifts, and enhanced detoxification processes within their systems (Hemingway et al., 2016; Ranson & Lissenden, 2016). These adaptations not only make controlling mosquito populations more difficult but also threaten to undo the progress made in reducing malaria transmission.

The rise of insecticide resistance is driven by several key factors. The most prominent among them is the widespread and often indiscriminate use of insecticides in both agriculture and public health. In many regions, the same classes of chemicals used to protect crops are also employed in mosquito control programs. This dual exposure accelerates the development of resistance as mosquitoes are continuously subjected to low doses of

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insecticides, giving those with resistant traits a survival advantage (Edi et al., 2012). Additionally, the improper use of insecticides, such as incorrect dosing or incomplete coverage during spraying campaigns, can contribute to the selection pressure on mosquito populations, further promoting resistance. Mosquitoes can develop resistance through several mechanisms, each of which undermines the effectiveness of insecticides in different ways. One of the primary mechanisms is **target site resistance**, which involves changes to the insecticide's target sites within the mosquito's body. For instance, mutations in the voltage-gated sodium channels can lead to what is known as knockdown resistance (kdr). These mutations alter the structure of the sodium channels, which are critical for nerve function, making it difficult for pyrethroids and other insecticides to bind effectively, thereby allowing mosquitoes to survive exposure (Martinez-Torres et al.,1998). Another key mechanism is metabolic resistance, where mosquitoes develop the ability to break down insecticides before they can reach their target sites. This is achieved through the increased activity of detoxifying enzymes, such as cytochrome P450 monooxygenases, esterases, and glutathione S-transferases (Poupardin et al., 2010). These enzymes can neutralize a wide range of insecticides, rendering them ineffective even at doses that would normally be fatal. Cuticle resistance is another form of adaptation, where changes in the mosquito's cuticle, or outer layer, reduce the penetration of insecticides into their bodies. This physical barrier prevents sufficient amounts of the chemical from reaching the internal target sites, thereby reducing its effectiveness (Wood et al.,2010). Lastly, behavioral resistance involves changes in mosquito behaviour that allow them to avoid contact with insecticides altogethr. For example, mosquitoes might shift their feeding times to periods when insecticide-treated nets are less effective or change their resting locations to places that are less likely to be treated with insecticides. These behavioural adaptations can significantly diminish the impact of vector control measures (Gatton et al.,2013).

The consequences of insecticide resistance are far-reaching, particularly for malaria control efforts. One of the most concerning developments is the resistance to pyrethroids, the primary class of insecticides used in insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Pyrethroids are favoured in public health due to their effectiveness, low cost, and relative safety for humans. However, as resistance to these chemicals spreads, the protective effect of ITNs and IRS diminishes, leading to increased malaria transmission (Ranson et al.,2011). The spread of pyrethroid resistance has been documented across many African countries, posing a significant challenge to malaria control programs. In regions where resistance is high, the effectiveness of ITNs and IRS in reducing malaria cases has been compromised, leading to concerns about the resurgence of the disease (N'Guessan et al., 2007; Yadouleton et al., 2011). This situation underscores the urgent need for new strategies in vector control, as well as the development of novel insecticides with different modes of action that mosquitoes have not yet developed resistance to. In response to the growing threat of insecticide resistance, public health organizations and governments are advocating for integrated vector management (IVM) strategies. IVM involves the coordinated use of multiple control methods to manage mosquito populations and reduce the risk of resistance. One approach within IVM is the rotation of insecticides with different modes of action, which can help prevent mosquitoes from becoming resistant to any single class of chemicals. Additionally, there is ongoing research into the development of new insecticides that target mosquitoes through different mechanisms, providing more tools in the fight against malaria (WHO, 2012).

Malaria remains a significant global health issue, with the World Health Organization (WHO) estimating that in 2021, there were 241 million cases and 627,000 deaths worldwide, primarily in sub-Saharan Africa (WHO, 2022). Controlling the vectors of malaria, predominantly mosquitoes from the genus *Anopheles*, is critical for reducing the transmission of this disease. Over the years, various vector control strategies have been developed and implemented, ranging from chemical interventions to environmental management and the introduction of biological control agents. This literature review discusses the current state of malaria vector control, focusing on the efficacy and challenges associated with different control measures, including insecticide-treated nets (ITNs), indoor residual spraying (IRS), larval source management, and novel strategies like genetic modification of mosquitoes. ITNs have been one of the most effective tools in reducing malaria transmission. ITNs work by providing a physical barrier to mosquitoes, while the insecticide (usually a pyrethroid) on the nets kills or repels mosquitoes that come into contact with it. Multiple studies have documented the effectiveness of ITNs in reducing malaria morbidity and mortality. For example, (Lengeler 2004) conducted a comprehensive review of randomized control trials and found that ITNs reduced malaria cases by about 50% and child mortality by 17%. However, the efficacy of ITNs is increasingly threatened by the development of insecticide resistance among *Anopheles* mosquitoes. Resistance to pyrethroids, the only class of insecticides approved for use on ITNs, has been widely reported in many parts of Africa (Ranson & Lissenden, 2016). In regions with high levels of resistance, the protective effect of ITNs is significantly diminished, leading to concerns about the sustainability of this intervention.

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Indoor residual spraying (IRS) is another cornerstone of malaria vector control. IRS involves the application of insecticides to the interior walls of homes, where it remains effective for several months, killing mosquitoes that land on treated surfaces. The effectiveness of the IRS has been demonstrated in various settings, particularly when used in conjunction with ITNs. A study in Mozambique showed that combining IRS with ITNs provided a synergistic effect, significantly reducing malaria transmission compared to using either intervention alone (Casimiro et al.,2006). However, similar to ITNs, the efficacy of IRS is compromised by the development of insecticide resistance. Mosquito populations in many malaria-endemic areas have developed resistance to the insecticides commonly used in IRS, such as DDT and pyrethroids (Hemingway & Ranson, 2000). The emergence of resistance necessitates the rotation of different insecticides and the development of new compounds with novel modes of action to prevent the further spread of resistance. For instance, the use of non-pyrethroid insecticides like organophosphates or carbamates in IRS programs has been explored as an alternative to pyrethroids, showing promise in areas with high pyrethroid resistance (Oxborough et al.,2014). Larval source management (LSM) involves the targeting of mosquito larvae at their breeding sites, thereby reducing the number of adult mosquitoes capable of transmitting malaria. LSM strategies include environmental management, such as draining stagnant water bodies, and the application of larvicides. LSM has been particularly effective in urban areas where breeding sites are well-defined and can be systematically targeted. A study in Dar es Salaam, Tanzania, demonstrated that larval control through regular application of larvicides significantly reduced malaria transmission (Geissbühler et al.,2009).

Despite its potential, the implementation of LSM is challenging in rural and peri-urban areas where mosquito breeding sites are dispersed and difficult to manage. Additionally, LSM requires sustained community engagement and resources, which can be difficult to maintain over time. The effectiveness of LSM is also contingent on the ecology of local mosquito populations, which can vary significantly across different regions, making it a less universally applicable strategy than ITNs or IRS. Biological control involves the use of natural predators, pathogens, or genetically modified organisms to reduce mosquito populations. The introduction of larvivorous fish, such as *Gambusia affinis* (mosquitofish), into water bodies has been used as a biological control method to target mosquito larvae. Studies have shown that these fish can effectively reduce mosquito larvae populations, particularly in small, contained water bodies (Chandra et al.,2008). Another promising area of research is the genetic modification of mosquitoes. One approach involves the introduction of genetically modified mosquitoes that are either sterile or carry genes that reduce their ability to transmit malaria. For instance, the release of sterile males has been explored as a method to reduce mosquito populations by preventing reproduction. Additionally, the use of gene-driven genetic systems that spread a particular trait rapidly through a population has shown the potential to drive genes that confer resistance to malaria parasites into mosquito populations (Burt, 2014). However, the release of genetically modified mosquitoes into the environment raises ethical, ecological, and regulatory concerns, which need to be carefully considered (Alphey et al.,2010).

While significant progress has been made in malaria vector control, several challenges remain. The widespread development of insecticide resistance is perhaps the most pressing issue, threatening the effectiveness of both ITNs and the IRS. Additionally, the sustainability of vector control programs is often compromised by a lack of resources, infrastructure, and community engagement, particularly in low-income settings (White et al.,2011). Future efforts in malaria vector control must focus on overcoming these challenges through the development of new tools and strategies. The integration of multiple control measures—such as combining ITNs, IRS, and LSM offers a promising approach to mitigating the limitations of individual interventions. Furthermore, continued research into new insecticides, biological control methods, and genetic technologies is essential for maintaining the effectiveness of vector control programs in the face of evolving challenges. Malaria vector control is a complex and evolving field that requires the integration of multiple strategies to effectively reduce the transmission of the disease. While ITNs, IRS, and LSM have proven effective, the emergence of insecticide resistance poses a significant threat to their continued success. The exploration of novel approaches, such as genetic modification of mosquitoes, along with sustained investment in research and community engagement, will be crucial for the future of malaria control. By addressing these challenges, it is possible to sustain and build on the gains made in the global fight against malaria.

Statement of the Problem

Malaria remains a significant public health concern in Gombe State, Nigeria, with high transmission rates and a substantial burden on the local population. However, limited research has been conducted to assess the susceptibility status of local mosquito populations to insecticides in specific areas such as Billiri and Kaltungo Local Government Areas (LGAs). The absence of region-specific data hampers evidence-based decision-making and resource allocation for malaria control programs. This study aims to address the aforementioned challenges

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by conducting a comprehensive assessment of the susceptibility status of Anopheles mosquitoes in Billiri and Kaltungo LGAs of Gombe State. Gombe State has a significant burden of malaria, with high transmission rates and a substantial impact on public health. Understanding the diverse species abundance and insecticide resistance patterns is crucial for designing effective malaria control strategies tailored to the local context. Anopheles mosquitoes are the primary vectors of malaria in Nigeria, including Gombe State. The emergence of insecticide resistance among mosquito populations poses a significant threat to malaria control efforts. Assessing the susceptibility status of Anopheles mosquitoes to commonly used insecticides in Billiri and Kaltungo LGAs helps monitor resistance patterns and guides the selection of appropriate insecticides for vector control interventions

Aim and Objectives

The aim of the study is to investigate the diversity of Anopheles mosquito species and their resistance status against commonly used insecticides in Billiri and Kaltungo local government areas of Gombe state. **The objectives of the study are to:**

1. Determine the susceptibility status of local Anopheles mosquito populations to commonly used insecticides, such as pyrethroids and organophosphates.
2. Determine how the findings of species diversity and susceptibility status inform targeted malaria control interventions in Billiri and Kaltungo LGAs

Research questions

1. What is the susceptibility status of local Anopheles mosquito populations to commonly used insecticides, such as pyrethroids and organophosphates?
2. How can the findings of species diversity and susceptibility status inform targeted malaria control interventions in Billiri and Kaltungo LGAs?

Materials and Methods

The study was conducted in four communities in Gombe State: Lakolin, Unguwar Sarkin, Bare, and Awai. These communities were selected based on their high malaria transmission rates and distinct ecological characteristics.

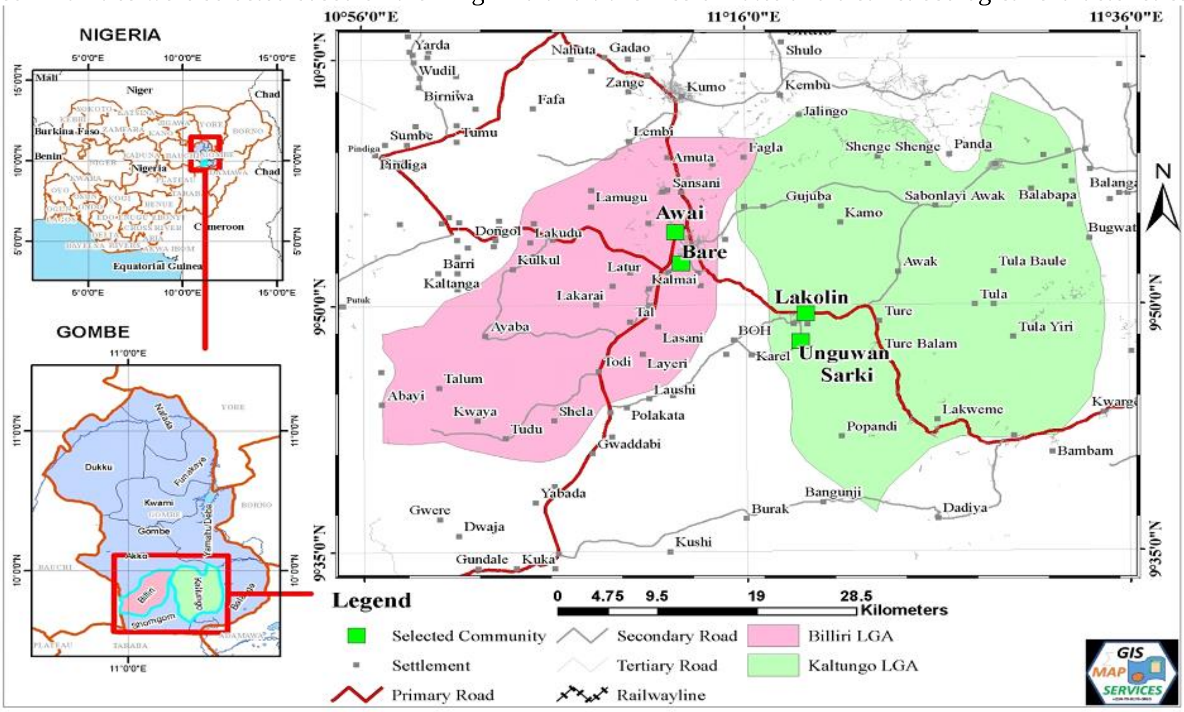

Figure 3.1: Map of Study Area Showing Selected Community

Mosquito Collection and Identification: Mosquitoes were collected using standard entomological techniques, including CDC light traps and pyrethrum spray collections. The species composition was determined by morphological identification using taxonomic keys, and the results are presented as percentages of the total Anopheles mosquitoes collected in each location (Gillies & De Meillon, 1968).

Insecticide Susceptibility Testing: Susceptibility tests were conducted using standard WHO protocols (WHO, 2016). Mosquitoes were exposed to insecticide-impregnated papers for one hour, and mortality rates were recorded 24 hours post-exposure. The insecticides tested included DDT, Bendiocarb, Deltamethrin, and

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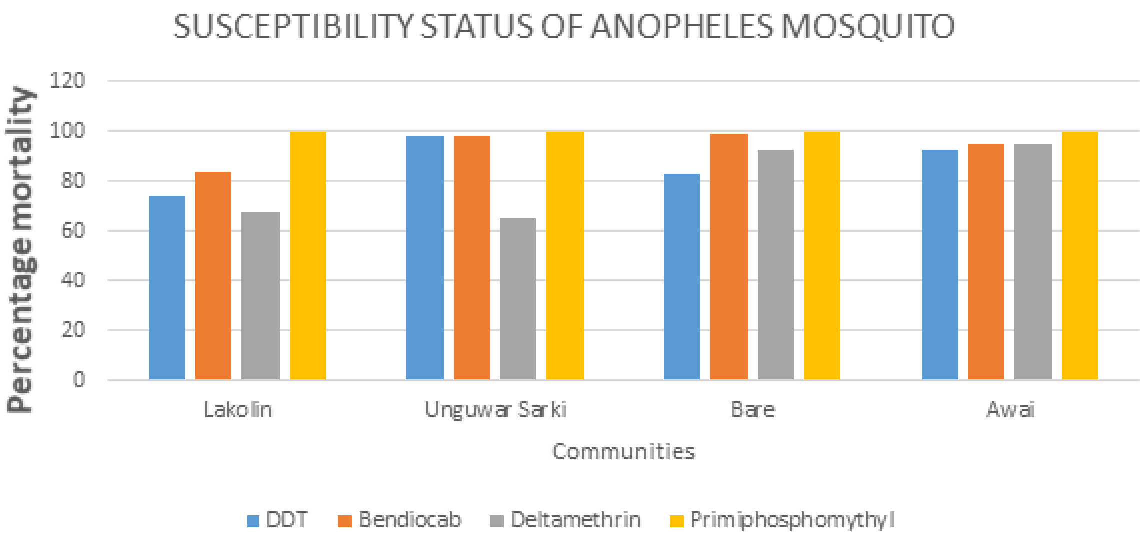
Primiphosphomethyl. Mortality rates were categorized as resistant (mortality <90%), suspected resistance (mortality 90-98%), and susceptible (mortality >98%) (WHO, 2016).

Results



The susceptibility status of Anopheles mosquitoes to different insecticides is summarized in Fig 1

Insecticide Susceptibility: The susceptibility status reveals that two communities one each in Billiri and Kaltungo Local Government Areas that is Bare and Lakolin showed resistance to DDT with percentage mortality of 83 and74% respectively while Awai and Unguwar sarkin indicate suspected resistance with percentage mortality of 93 and 98% respectively. There was an indication of resistance in Bendiocab in Lakolin where the mortality rate was at 84%. Unguwar Sarki and Awai suggest suspected resistance with 98 and 95% mortality respectively. Only Bare in Billiri showed resistance to Bendiocab with a percentage mortality at 99%. None of the four communities was susceptible to Deltamethrin as there was an indication of suspected resistance in Bare and Awai all in the Billiri local government Area with 93 and 95% mortality rates respectively. All communities tested in the Kaltungo local government area have shown complete resistance with 65 and 68% mortality, while all communities showed 100% susceptibility to Primiphosphomythyl. %). Comparatively, the percentage of mortalities of *Anopheles gambiae* across the four insecticides differed significantly (p<0.05). The results demonstrate significant variations in the susceptibility of Anopheles mosquitoes across the four insecticides (p<0.05).

Table 1 The species composition of Anopheles mosquitoes in the four communities

Species composition					
Location	***Anopheles gambiae* (%)**	***Anopheles maculipalpis* (%)**	***Anopheles pretoriensis* (%)**	***Anopheles rufipes***	**Unidentified**
Lakolin	257(50.0%)	89(39.2%)	25(4.5%)	0(0%)	0(0%)
Unguwar Sarkin	177(34.4%)	108(47.6%)	78(14.0%)	0(0%)	0(0%)
Bare	54(10.5%)	5(2.2%)	203(36.6%)	34(55.7%)	27(34.1%)
Awai	26(5.0%0)	25(11.0%)	249(44.9%)	27(44.3%)	52(65.8%)
Total	**514(100%)**	**227(100%)**	**555(100%)**	**61(100%)**	**79(100%)**

Susceptibility Status of Anopheles Species in the Study Area

From the 1436 mosquitoes used in this study (Table 2), *Anopheles pretoriensis* was the significantly dominant Anopheles species with 555(100%) and was preceded by *Anopheles gambiae. Anopheles maculipalpis* and *Anopheles rufipes* were 227(100) and 61(100) respectively. Contrary to the earlier reports made by (Olatunbosun-Oduola et al., 2019) they reported the dominance of *Anopheles gambiae* in their studies but didn't relate the

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abundance of the *Anopheles gambiae* sister species to their role in malaria transmission (Asmare et al.,2022). The data analysis has shown these species composition not significantly different. The remaining 79(100) were not identified due to damaged or missing body parts.

Discussion

Susceptibility Status of Anopheles Species in the Study Area: The findings of this study regarding insecticide resistance in Anopheles mosquitoes in Gombe State align with similar studies conducted in northern Nigeria, offering significant insights into the regional patterns of resistance. Previous research by (Abdulmalik, 2023) and (Abba et al., 2024) revealed varying levels of resistance to multiple insecticides, including DDT, deltamethrin, and permethrin, among Anopheles mosquitoes in both rural and peri-urban areas of Gombe State. These findings are consistent with the observations in the present study, where significant resistance to these insecticides was also documented. The consistency of these results highlights the widespread nature of insecticide resistance among Anopheles mosquitoes in Gombe State, as well as other northern regions of Nigeria. Various factors contribute to this emerging resistance, including the extensive use of insecticides in agriculture, the implementation of vector control interventions, and inherent genetic predispositions in mosquito populations. For instance, the misuse of insecticides in agricultural practices, where the same compounds used in vector control are applied to crops, may create selection pressure that accelerates the development of resistance in mosquito populations (Hemingway et al., 2016).

Moreover, the resistance patterns observed in Anopheles mosquitoes in Gombe State are not isolated but are part of a broader trend seen across Nigeria and the African continent. For example, research conducted in southwestern Nigeria reported high resistance levels to DDT and pyrethroids, particularly deltamethrin, in Anopheles gambiae populations (Oduola et al.,2012). Similar resistance trends have been documented in other West African countries, such as Ghana and Benin, where widespread resistance to multiple insecticides has been reported. These findings suggest that insecticide resistance in malaria vectors is a significant regional challenge, with potential implications for the effectiveness of current malaria control programs. Globally, the issue of insecticide resistance in malaria vectors is not confined to Africa. Countries like India have also reported resistance to DDT, pyrethroids, and organophosphates in Anopheles species, further emphasizing the global scale of this problem (Kaur et al., 2019). The persistence and spread of resistance worldwide highlight the need for coordinated international efforts to monitor and manage this threat effectively. Without such efforts, the progress made in reducing malaria transmission could be at risk, especially in regions where resistance levels are high and alternative vector control strategies are limited. The high levels of resistance observed in this study, particularly to DDT, bendiocarb, and deltamethrin, pose significant challenges to malaria control programs in Gombe State. The findings indicate that the efficacy of these commonly used insecticides is diminishing, which could undermine efforts to control mosquito populations and reduce malaria transmission. However, the complete susceptibility of Anopheles mosquitoes to primiphosphomethyl, as observed in this study, suggests that this insecticide could be a viable alternative in regions where resistance to other insecticides is prevalent. Nevertheless, the emergence of resistance to multiple insecticides necessitates a reevaluation of current vector control strategies. The World Health Organization (WHO) has long advocated for integrated vector management (IVM) as a sustainable approach to combating insecticide resistance. IVM strategies include rotating insecticides with different modes of action, employing non-chemical control measures, and enhancing community participation in vector control efforts (WHO, 2012). Implementing these strategies in Gombe State and other affected regions could help mitigate the impact of resistance and sustain the effectiveness of malaria control programs.

Species Composition and Diversity: The analysis of species composition in the current study indicates that the distribution and abundance of Anopheles mosquito species have remained consistent with findings from previous research in Nigeria. This consistency suggests that the ecological conditions and environmental factors influencing mosquito populations in Gombe State have not changed significantly over time. In this study, Anopheles pretoriensis emerged as the dominant species, which is noteworthy given the traditionally recognized role of Anopheles gambiae as the primary malaria vector in Nigeria.The dominance of Anopheles pretoriensis in this study area aligns with findings from other studies conducted in northern Nigeria. For example, research by (Abiodun et al.,2018) and (Usman et al.,2020) documented variations in the predominant Anopheles species across different regions and periods in northern Nigeria. These variations are likely influenced by local environmental factors, such as climate, vegetation, and human activities, which affect mosquito breeding sites and population dynamics. The presence of *Anopheles pretoriensis* as the dominant species in this study area raises important questions about its role in malaria transmission. While Anopheles gambiae has long been identified as the primary vector of malaria in Nigeria, the increasing prevalence of other Anopheles species, such as *Anopheles pretoriensis,* suggests that alternative vector species may also contribute significantly to malaria transmission.

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This finding underscores the need for further research to determine the vector competence of *Anopheles pretoriensis* and its potential impact on malaria epidemiology in Gombe State.

Conclusion

This study highlights the widespread insecticide resistance in outdoor resting Anopheles mosquitoes in Gombe State. The findings call for urgent action to strengthen resistance monitoring and management efforts to sustain the effectiveness of malaria control interventions. By comparing these results with other studies globally, this research underscores the need for coordinated efforts to combat insecticide resistance and protect the gains made in malaria control.

Recommendations

1. Considering the susceptibility status results indicating the emergence of insecticide resistance, establishing routine surveillance programs is imperative. These programs should regularly monitor mosquito populations for changes in resistance patterns and assess the effectiveness of control interventions. Early detection of resistance allows for timely adjustments to control strategies, such as switching insecticide classes or implementing alternative vector control measures.
2. Rotating insecticide classes and using synergistic combinations can delay the development of resistance and preserve the efficacy of insecticides. Research into alternative vector control tools, such as biological control agents and spatial repellents, can provide additional options for managing insecticide resistance.
3. Community engagement and education programs should be tailored to address the specific findings of the susceptibility status and species composition. Communicating the importance of malaria control, the emergence of insecticide resistance, and the role of alternate vector species, such as *Anopheles pretoriensis*, can empower communities to take proactive measures to protect themselves from malaria. Emphasizing the importance of proper insecticide use and adherence to control measures is essential for community participation and support.
4. Building on the results presented, investment in research and innovation is necessary to address knowledge gaps and develop context-specific control strategies. Research into the ecology and behaviour of *Anopheles pretoriensis* and other vector species can inform targeted interventions. Innovations in insecticide formulations, vector control tools, and surveillance methods can enhance the effectiveness of malaria control efforts in Gombe State.

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