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Research Article

Local Variability in Permethrin Susceptibility of *Anopheles* Mosquitoes in Batagarawa, Katsina State, Nigeria

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ABSTRACT
Malaria has been one of the major public health challenges in Nigeria, with vector control heavily reliant on pyrethroid-based interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Widespread insecticide resistance in *Anopheles* mosquitoes threatens these gains, yet locality-specific resistance patterns remain poorly understood. This study assessed locality-based variations in the susceptibility of *Anopheles* mosquitoes to permethrin in Batagarawa Local Government Area (LGA), Katsina State, Northwestern Nigeria. The study hypothesized that no significant difference existed between localities in mosquito resistance or susceptibility to permethrin. *Anopheles* larvae were collected from five localities, reared under controlled insectary conditions, and tested using Word Health Organization (WHO) standard tube bioassays with 0.75% permethrin. Knockdown times (KDT_{50}, KDT_{95}) and 24-hour mortality were recorded and analyzed. Breeding habitats showed broadly similar physicochemical characteristics, with minor differences in pH and temperature. Knockdown dynamics varied significantly across sites ($p < 0.05$), but 24-hour mortality ranged from 7% to 39%, far below the WHO threshold for susceptibility (≥98%). All populations exhibited confirmed resistance, with no operationally meaningful differences between localities. The findings confirm widespread permethrin resistance in *Anopheles* populations across Batagarawa Local Government Area, undermining the effectiveness of pyrethroid-based vector control tools. It is therefore recommended that there is need for transitioning to next-generation LLINs, diversifying IRS insecticides, expanding resistance surveillance with molecular diagnostics, and integrating ecological management strategies to sustain malaria control efforts in Batagarawa.

Keywords: *Anopheles* Mosquitoes; Insecticide resistance; Malaria vector control; Permethrin; Pest Control

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INTRODUCTION

Malaria remains a critical public health concern in sub-Saharan Africa, with Nigeria contributing nearly 27% of global malaria cases and deaths (World Health Organization, WHO, 2023). In 2021, Nigeria recorded approximately 68 million malaria cases and 194,000 deaths, around 80% of which occurred among children under five (World Health Organization, 2023). The disease accounts for approximately 60% of outpatient visits, 11% of maternal mortality, and 30% of child mortality in the country (Dawaki *et al.*, 2016). Vector control through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) remains central to malaria control efforts (Kleinschmidt *et al.*, 2018). Permethrin, a synthetic pyrethroid, is among the most widely used chemical insecticides in Nigeria's malaria vector intervention programs. However, its continued effectiveness is jeopardized by rising levels of insecticide resistance in *Anopheles* mosquito populations (Ranson & Lissenden, 2016).

Across Africa, permethrin has been extensively deployed for malaria vector control, primarily

through its incorporation into LLINs and, to a lesser extent, IRS. As a World Health Organization–approved pyrethroid, permethrin has been widely adopted due to its rapid knockdown effect, relative safety for human use, and cost-effectiveness, making it suitable for large-scale public health interventions in resource-limited settings (WHO, 2018). Between 2000 and 2020, pyrethroids—including permethrin—accounted for over 80% of insecticides used in LLIN distributions across sub-Saharan Africa and contributed substantially to reductions in malaria morbidity and mortality (Bhatt et al., 2015; WHO, 2022). However, the prolonged and widespread reliance on permethrin has imposed strong selection pressure on *Anopheles* mosquito populations, leading to the emergence and spread of pyrethroid resistance across multiple African regions and threatening the long-term sustainability of current vector control strategies (Ranson et al., 2016; Hancock et al., 2018).

In Nigeria, permethrin, a synthetic pyrethroid, is among the most widely used chemical insecticides in malaria vector intervention programs. However, its continued effectiveness is increasingly jeopardized by rising levels of insecticide resistance in *Anopheles* mosquito populations (Ranson & Lissenden, 2016). Evidence from various regions of Nigeria indicates significant variation in *Anopheles* susceptibility to permethrin. In Lagos State, mortality rates to permethrin (0.75%) ranged from 4.25% to 22.0%, indicating high resistance (Idowu *et al.,* 2020). In Ibadan, mortality rates varied between 83.5% and 87.7%, suggesting moderate susceptibility but still below full effectiveness (Ibrahim *et al.,* 2013). Abba *et al.* (2023) also reported that *Anopheles* populations were susceptible to permethrin and alphacypermethrin across communities in Southern Gombe, highlighting pronounced regional heterogeneity.

Further, in the Sahel region of Nigeria, *Anopheles funestus* populations exhibited pronounced resistance to permethrin, with mortality rates of 48.3% and evidence implicating metabolic resistance mechanisms such as cytochrome P450 monooxygenases and glutathione S-transferases; notably, the addition of piperonyl butoxide (PBO) partially restored susceptibility, increasing mortality to approximately 78.7% (Atoyebi *et al.,* 2020). Recent analyses of generational impacts of metabolic resistance development further underscore how resistance can influence mosquito lifespan and reproductive traits, with potential epidemiological implications (Adesoye *et al.,* 2024). In addition, spatial distribution modeling of the *Anopheles gambiae* complex in Nigeria reveals substantial ecological and geographic variability that may further shape insecticide susceptibility patterns (Adeogun *et al.,* 2023).

Collectively, these findings emphasize that permethrin effectiveness varies considerably across ecological zones and localities. Yet, studies specifically examining such locality-based variations in Katsina State, particularly in Batagarawa, remain scarce. Addressing this knowledge gap is critical, given permethrin's central role in Nigeria's vector control strategy. This study therefore aims to assess locality-based variation in susceptibility of *Anopheles* mosquito populations to permethrin across different localities in Batagarawa, Katsina State, northwestern Nigeria. It is hypothesized that susceptibility levels vary significantly among localities, reflecting underlying ecological, genetic, or operational differences.

This study is limited in scope to Batagarawa LGA of Katsina State, focuses exclusively on *Anopheles* mosquitoes, evaluates only permethrin as the test insecticide, and employs the WHO tube bioassay as the sole susceptibility assessment method. Understanding these local susceptibility patterns will support targeted decision-making on vector control measures, including insecticide rotating or incorporating synergists such as PBO into LLINs, and ultimately strengthen malaria control programs in the region.

MATERIAL AND METHODS

Study Design

This study employed a cross-sectional experimental design to evaluate locality-based variation in the susceptibility of *Anopheles* mosquito populations to permethrin within Batagarawa Local Government Area (LGA), Katsina State, northwestern Nigeria. The design integrated field-based larval sampling with laboratory-controlled insecticide susceptibility bioassays, following World Health Organization (WHO) guidelines.

Mosquito larvae and pupae were collected from multiple breeding sites across five geographically distinct localities—Batagarawa town, Dabaibayawa, Barawa, Babbar Ruga, and Ajiwa—during the peak malaria transmission season (July–August 2025). Field-derived specimens were reared in the Insectary of Umaru Musa Yar'adua University Katsina, under standardized insectary conditions to minimize environmental and physiological variability prior to testing.

Susceptibility to permethrin was assessed using WHO standard tube bioassays, with mortality and knockdown responses measured as primary outcome variables. The study design enabled comparative analysis of permethrin efficacy across localities, allowing the detection of spatial heterogeneity in insecticide susceptibility potentially driven by ecological, genetic, or operational factors. By maintaining uniform rearing conditions, insecticide concentrations, and exposure protocols across all experimental units, the design ensured that observed differences in susceptibility could be attributed primarily to population-level variation rather than methodological bias.

Study Area

This study was conducted in Batagarawa Local Government Area (LGA) of Katsina State, Northwestern Nigeria (Figure 1). Batagarawa LGA lies approximately between latitude 12°55'N and longitude 7°37'E. It shares boundaries with Katsina LGA to the south, Rimi LGA to the north, and Charanchi LGA to the east. The area has an estimated population of over 180,000 people, predominantly engaged in farming and trading. The climate is characterized by a tropical savannah type, with a distinct wet season (May–September) and dry season (October–April), and an average annual rainfall of about 850 mm. Malaria remains hyperendemic in the region, with *Anopheles* mosquitoes serving as the primary vectors, contributing significantly to the persistent malaria burden (WHO, 2023).

Figure 1. **Map of Katsina State showing the study area, Batagarawa Local Government Area**

Larval Collection

Mosquito larvae and pupae were collected in the months of July and August 2025, from five selected localities within Batagarawa LGA: Batagarawa Town, Dabaibayawa, Barawa, Babbar Ruga, and Ajiwa villages. Each locality was surveyed for potential mosquito breeding sites, including stagnant pools, irrigation channels, and temporary rain-filled depressions. At least four well-established breeding sites were identified and sampled per locality. Standard dipping techniques were employed, using larval collection sets to collect larvae and pupae into labeled plastic containers.

Larval sampling was conducted between 09:00 AM and 1:00 PM, a period corresponding to minimal larval vertical movement and reduced disturbance of breeding habitats. At each breeding site, sampling was carried out for approximately 15–30 minutes, depending on habitat size and larval density. Sampling effort was standardized spatially by covering an estimated surface area of approximately 1 m^2 per breeding site, using repeated dips distributed evenly across the habitat to ensure representative coverage. This standardized temporal and spatial sampling approach was adopted to allow comparability of larval abundance and species composition across localities.

For each breeding site, in situ physico-chemical parameters of the water were measured immediately prior to larval collection to minimize disturbance-related bias. Water temperature (°C) and pH were measured using a portable digital pH/temperature meter (HI98107, Hanna Instruments, Romania), while dissolved oxygen (ppm) was measured using a portable dissolved oxygen meter (HI9147, Hanna Instruments, Romania). Measurements were taken at a depth of approximately 5–10 cm below the water surface and at three randomly selected points per breeding site, with mean values used for subsequent analyses.

Approximately 500 mL of water was collected from each breeding site in sterile, labeled polyethylene bottles and transported in insulated containers to the laboratory within 6 hours of collection. In the laboratory, electrical conductivity (µS cm^{-1}) was measured using a digital conductivity meter (HI-2300, Hanna Instruments, Romania), and turbidity (NTU) was measured using a turbidity tube (Lovibond, USA), following manufacturer-recommended analytical protocols. All meters were calibrated daily using standard reference solutions supplied by the manufacturers. Collected mosquito larvae were then transported to the insectary for rearing under controlled conditions.

Mosquito Rearing

All collected *Anopheles* larvae and pupae were carefully transported to the insectary at Umaru Musa Yar'adua University, Katsina, where they were reared under strict laboratory controls. Specimens were housed in shallow plastic trays filled with dechlorinated tap water, maintained at a temperature of 25 ± 2 °C and relative humidity of 80 ± 10%. Larval feeding followed the protocol of Leite *et al.* (2024), using a daily diet of powdered yeast mixed with finely ground cabin biscuits.

Upon pupation, no more than 200–300 individuals per cage, individuals were gently transferred into small clean-water cups placed within screened emergence cages, to minimize overcrowding and stress. The cage is constructed of aluminum frames frames (30 × 30 × 30 cm) with fine polyester mesh netting (approximately 1.2 mm aperture). Upon emergence, adult mosquitoes were maintained in these cages and supplied *ad libitum* with a 10% (w/v) sucrose solution delivered via cotton pads, in accordance with Leite *et al.* (2024). Only three-day-old, non–blood-fed adult female *Anopheles* mosquitoes were selected for subsequent insecticide susceptibility bioassays.

To preserve experimental integrity and avoid cross-contamination, all trays, cages, and feeding apparatus were thoroughly sterilized prior to use. Each batch of mosquitoes was tagged with its locality of origin, ensuring that geographic provenance was preserved for later analysis of variability in susceptibility.

Insecticide Preparation

For susceptibility testing, standard WHO insecticide-impregnated papers coated with 0.75% permethrin were sourced from an accredited WHO collaborating centre (the Vector Control Research Unit at Universiti Sains Malaysia). These unexpired, quality-assured papers adhere to WHO recommendations for insecticide bioassays and were handled following WHO guidelines to maintain efficacy and validity of the results.

Bioassay Procedures

Insecticide susceptibility tests were carried out using the standard WHO tube bioassay protocol (WHO, 2016). For each of the five study localities—Batagarawa Town, Dabaibayawa, Barawa, Babbar Ruga, and Ajiwa—three-day-old, non-blood-fed, sugar-fed adult female *Anopheles* mosquitoes were tested. In each assay, four replicates of 25 mosquitoes were exposed to permethrin-impregnated filter papers, while an equal number of mosquitoes served as controls, exposed to papers treated only with the carrier and solvent.

Mosquitoes were introduced into exposure tubes lined with the treated papers and observed at 10, 15, 20, 30, 40, 50, and 60 minutes to record knockdown effects. After 60 minutes of exposure, mosquitoes were gently transferred to clean holding tubes lined with untreated papers and provided with 10% sucrose solution on cotton pads. Mortality was assessed after a 24-hour recovery period.

All bioassays were conducted in an insectary maintained at a temperature of 25 ± 2 °C and relative humidity of 80 ± 10%, following WHO-recommended conditions. Each insecticide-impregnated paper was used only once to preserve potency, and all materials (cages, tubes, and feeders) were sterilized before use to prevent contamination. Mosquito susceptibility was interpreted using WHO classification criteria: mortality rates of 98–100% indicating full susceptibility, 90–97% suggesting possible resistance (requiring confirmation), and <90% confirming resistance.

Ethical Considerations

This study was conducted in compliance with institutional, national, and international guidelines for research involving live mosquito vectors. All experimental procedures were reviewed and approved by the Departmental Research Ethics Committee of Biological Sciences, Umaru Musa Yar'adua University, Katsina, Nigeria. Permissions for larval collection were obtained from relevant community leaders and local authorities in each study locality.

No human or vertebrate hosts were used at any stage of the research. All mosquitoes were reared and handled under controlled laboratory conditions to minimize environmental impact and ensure biosafety. Insecticide handling followed WHO-recommended safety protocols, including the use of personal protective equipment and proper waste disposal measures. All materials contaminated with insecticides were disposed of according to approved chemical safety regulations to prevent accidental environmental contamination.

Data Collection and Analysis

Knockdown and mortality data were recorded for each replicate across all five study localities. Progressive knockdown was documented at 10, 15, 20, 30-, 40-, 50-, and 60-minutes post-exposure, and cumulative mortality was assessed after a 24-hour recovery period.

Key endpoints analyzed included:

- Percentage mortality at 24 hours post-exposure.
- Knockdown times for 50% (KDT_{50}) and 95% (KDT_{95}) of the tested populations, estimated through probit regression analysis.

$$\textbf{Percentage mortality} = \frac{\textbf{Total number of dead mosquitoes}}{\textbf{Total tested}} \times \mathbf{100}$$

Comparisons of insecticidal efficacy among mosquito populations from different localities were carried out using appropriate inferential statistical tests. One-way analysis of variance (ANOVA) or Kruskal–Wallis tests were applied for multiple-group comparisons depending on the distribution of the data. Descriptive statistics (means, standard deviations, and percentage absolute meanic deviations (Abdullahi, 2024a) were computed to summarize key parameters. All analyses were conducted using standard statistical software (GraphPad Instat 3, and Stat_mirroring-B1.3 (Abdullahi, 2024b)), and statistical significance was defined as $p < 0.05$.

RESULTS

Physicochemical Characteristics of the Breeding Sites

The physicochemical characteristics of *Anopheles* mosquito breeding sites across the five sampled localities in Batagarawa LGA are summarized in Table 1. The larval habitats were primarily temporary to semi-permanent water bodies, such as roadside ditches and shallow rain-fed ponds. Most sites contained water with an earthy to greenish hue and an earthy or grassy odor, with variable sun exposure ranging from shaded to partially shaded and fully sunlit conditions. Aquatic vegetation was diverse across all localities, typically comprising floating, submerged, and emergent plant species.

Among the measured parameters, pH differed significantly among localities ($p = 0.0039$). The highest average pH was recorded in Locality C (8.40 ± 0.42), indicating slightly alkaline conditions, whereas Localities D (7.18 ± 0.65) and E (7.32 ± 0.42) exhibited values closer to neutral. Temperature also varied significantly ($p = 0.0004$), with Localities B (29.16 ± 0.86 °C) and E (29.02 ± 0.83 °C) being marginally warmer than the other sites.

Other measured parameters—dissolved oxygen (DO), conductivity, turbidity, and water depth—did not differ significantly among the localities ($p > 0.05$). Dissolved oxygen ranged from 6.16 ± 1.62 ppm in Locality E to 8.22 ± 1.33 ppm in Locality A. Conductivity values fluctuated widely between sites, though no clear spatial pattern was evident ($p = 0.9775$). Turbidity and water depth showed overlapping ranges across all localities, consistent with broadly similar habitat types.

Overall, the breeding sites sampled in Batagarawa LGA exhibited ecologically comparable conditions conducive to *Anopheles* mosquito development. Apart from modest but statistically significant differences in pH and temperature, the physicochemical environment of larval habitats appeared broadly consistent across localities, providing a relatively uniform ecological background for subsequent susceptibility analyses.

Adulticidal Efficacy of Permethrin Against *Anopheles* Mosquitoes

The knockdown times (KDT_{50} and KDT_{95}) and 24-hour mortality of adult female *Anopheles* mosquitoes exposed to 0.75% permethrin varied across the five study localities in Batagarawa LGA (Table 2).

Knockdown analysis revealed significant differences among localities. The median knockdown time (KDT_{50}) ranged from 94.30 ± 13.45 min in Locality D to 215.04 ± 79.49 min in Locality C ($p = 0.0211$). Similarly, the time required to achieve 95% knockdown (KDT_{95}) followed a

comparable trend, ranging from 168.73 ± 27.43 min in Locality D to 393.28 ± 147.32 min in Locality C (p = 0.0253). In both cases, mosquitoes from Locality C exhibited markedly delayed knockdown responses, suggesting reduced susceptibility, while those from Locality D responded faster to permethrin exposure.

Mortality rates after 24 hours of recovery also differed significantly among the populations (p = 0.0016). The lowest mortality was recorded in Locality C (7.00 ± 5.92%), followed by Localities B (20.00 ± 12.65%), E (19.00 ± 5.92%), and A (24.00 ± 4.90%). The highest mortality was observed in Locality D (39.00 ± 3.32%). None of the mosquito populations achieved mortality levels approaching the WHO-defined susceptibility threshold (≥98%), indicating confirmed resistance across all the localities.

Locality-Based Susceptibility Classification

Across all five localities, mortality rates ranged from 7.00 ± 5.92% in Locality C to 39.00 ± 3.32% in Locality D (Table 2). These values fall well below the WHO-defined susceptibility threshold, confirming that all sampled mosquito populations exhibited confirmed resistance to permethrin at the tested concentration.

Although variation in knockdown dynamics was observed, this did not correspond to effective mortality outcomes. For example, Locality D exhibited the fastest knockdown (KDT_{50} = 94.30 ± 13.45 min; KDT_{95} = 168.73 ± 27.43 min) yet achieved only 39% mortality after 24 hours. Conversely, mosquitoes from Locality C not only showed markedly delayed knockdown (KDT_{50} = 215.04 ± 79.49 min; KDT_{95} = 393.28 ± 147.32 min) but also produced the lowest mortality (7%).

Table 1. Physicochemical characteristics of *Anopheles* mosquito breeding sites across five localities in Batagarawa LGA, Katsina State, Nigeria.

Parameter	Locality A	Locality B	Locality C	Locality D	Locality E	P-value
pH	7.60 ± 0.44 (11.76)^{ab}	7.58 ± 0.35 (15.31)^{ab}	8.40 ± 0.42 (14.08)^{a}	7.18 ± 0.65 (15.91)^{b}	7.32 ± 0.42 (21.92)^{b}	0.0039
Temperature (℃)	27.15 ± 0.56 (21.96)^{b}	29.16 ± 0.86 (12.32)^{a}	27.92 ± 0.38 (12.0)^{ab}	27.74 ± 0.72 (23.69)^{b}	29.02 ± 0.83 (21.51)^{a}	0.0004
DO (ppm)	8.22 ± 1.33 (13.09)	6.32 ± 1.13 (12.18)	6.95 ± 0.85 (21.35)	6.57 ± 1.14 (13.84)	6.16 ± 1.62 (9.28)	0.0942
Conductivity (µS/cm)	229.83 ± 124.82 (12.73)	190.17 ± 49.93 (3.37)	245.83 ± 103.41 (15.56)	275.89 ± 196.08 (17.56)	209.80 ± 98.93 (14.07)	0.9775
Turbidity (NTU)	74.17 ± 21.10 (12.95)	65.00 ± 24.49 (18.18)	78.33 ± 19.08 (16.12)	64.44 ± 32.78 (17.12)	55.00 ± 23.07 (12.84)	0.6652
Water depth (meters)	0.18 ± 0.10 (19.61)	0.18 ± 0.15 (8.14)	0.56 ± 0.80 (17.28)	0.25 ± 0.16 (11.8)	0.23 ± 0.09 (22.99)	0.8140
Breeding site longevity	Temporary, Semi-permanent	Temporary, Semi-permanent	Temporary, Semi-permanent	Temporary, Semi-permanent	Temporary, Semi-permanent	
Breeding site nature	Street Side Ditches, Shallow Pond	Street Side Ditches, Shallow Pond	Street Side Ditches, Shallow Pond	Street Side Ditches, Shallow Pond	Street Side Ditches, Shallow Pond	
Water smell	Earthy, Grassy	Earthy, Grassy	Earthy, Grassy	Earthy, Grassy	Earthy, Grassy	
Water color	Earthy, Greenish	Earthy, Greenish	Earthy, Greenish	Earthy, Greenish	Earthy, Greenish	
Water origin	Rainfall	Rainfall	Rainfall	Rainfall	Rainfall	
Exposure to sunlight	Shaded, Partially Shaded, Sunlight	Shaded, Partially Shaded, Sunlight	Shaded, Partially Shaded, Sunlight	Shaded, Partially Shaded, Sunlight	Shaded, Partially Shaded, Sunlight	
Vegetation presence	Floating, Submerse, Emergent	Floating, Submerse, Emergent	Floating, Submerse, Emergent	Floating, Submerse, Emergent	Floating, Submerse, Emergent	

Note: Values represent mean ± standard deviation (% absolute meanic deviation shown in brackets). Means sharing the same superscript letter(s) in a row are not significantly different ($p > 0.05$).

Table 2. Knockdown time and mortality of *Anopheles* mosquitoes exposed to Permethrin (0.75%) across five localities in Batagarawa LGA, Katsina State, Nigeria.

Estimators	Locality A	Locality B	Locality C	Locality D	Locality E	P-value
KDT_{50} (min)	125.13 ± 15.96 (13.42)^{ab}	119.68 ± 22.65 (17.85)^{ab}	215.04 ± 79.49 (13.25)^{a}	94.30 ± 13.45 (18.77)^{b}	129.28 ± 23.34 (16.45)^{ab}	0.0211
KDT_{95} (min)	223.94 ± 30.15 (12.66)^{ab}	212.90 ± 43.76 (17.37)^{ab}	393.28 ± 147.32 (12.99)^{a}	168.73 ± 27.43 (18.85)^{b}	232.57 ± 45.52 (16.93)^{ab}	0.0253
Mortality (%)	24.00 ± 4.90 (17.14)^{ab}	20.00 ± 12.65 (8.00)^{b}	7.00 ± 5.92 (16.09)^{b}	39.00 ± 3.32 (11.76)^{a}	19.0 ± 5.92 (16.09)^{b}	0.0016

***Keys*:** KDT_{50} = knockdown time for 50% of mosquitoes; KDT_{95} = knockdown time for 95% of mosquitoes.

***Note*:** Values represent mean ± standard deviation (% absolute meanic deviation shown in brackets). Means sharing the same superscript letter(s) in a row are not significantly different ($p > 0.05$).

DISCUSSION

Analysis of physicochemical parameters in larval habitats across the five localities in Batagarawa LGA revealed generally comparable ecological conditions, with two notable exceptions: pH and water temperature. Temporary to semi-permanent, rain-fed water bodies—such as roadside ditches and shallow ponds—comprised the majority of breeding sites. These were characterized by earthy to greenish coloration, a grassy or earthy smell, and varied exposure to sunlight (shaded, partially shaded, or fully exposed). Aquatic vegetation across all sites included floating, emerging, and submerged plants, highlighting habitat heterogeneity.

Statistical analysis showed significant inter-locality variation in pH, with Locality C registering a more alkaline average, while Localities D and E remained closer to neutral. Temperature also differed significantly, with relatively higher mean values in Localities B and E. Other measured parameters—dissolved oxygen (DO), conductivity, turbidity, and water depth—did not vary significantly across localities. Conductivity, turbidity, and depth displayed overlapping ranges among sites.

Although modest, the observed differences in pH and temperature have important ecological implications. Even small deviations in pH can influence microbial communities and chemical speciation—such as levels of unionized ammonia—and thus affect larval growth, survival, and competitive dynamics (Multini *et al.*, 2021). The slightly alkaline water in Locality C may alter microbial feeding environments or impose physiological stress that influences larval performance. This could contribute to developmental delays or adult phenotypic changes that later affect insecticide susceptibility.

Likewise, temperature exerts a strong influence on larval development and adult physiology. Warmer breeding sites (Localities B and E) may yield faster-developing larvae, but these adults are often smaller and may display altered metabolism, including enhanced activity of detoxification enzymes (Rueda *et al.*, 1990; Bayoh & Lindsay, 2003). Several studies demonstrate that higher rearing or ambient temperatures can modulate pyrethroid resistance by upregulating detoxification gene expression or enzyme activity, which can reduce insecticide efficacy (Oliver & Brooke, 2017).

Dissolved oxygen levels and vegetation structure also play indirect but significant roles in larval ecology and eventual adult susceptibility. *Anopheles* larvae rely more directly on water-column oxygen than do culicines; suboptimal oxygen conditions can influence microbial composition and larval stress responses (Service, 1993). Vegetation contributes to habitat complexity, food availability, and microclimate, which in turn can affect developmental rates and larval resilience. Organic matter from decaying vegetation may promote microbial communities that interact with larval physiology and detoxification systems (Evans *et al.*, 2022). In agricultural settings, organic inputs or agrochemical runoff can further shape microbial and chemical environments, potentially selecting for larvae with pre-adapted detoxification mechanisms that confer cross-resistance in adults (Oliver & Brooke, 2018; Nkya *et al.*, 2013). Although transmissible evidence was not collected in this study, the presence of farming and trading in Batagarawa suggests that breeding sites may be influenced by local agrochemicals or other contaminants.

Overall, the ecological variation in larval habitats—namely, higher pH in Locality C, elevated temperatures in Localities B and E, and consistent suitability in other physicochemical parameters—likely contributes to spatial variability in larval development, adult physiology, and ultimately insecticide susceptibility. These habitat-driven differences reinforce the value of integrating environmental monitoring with entomological surveillance to better understand local drivers of resistance.

Patterns of knockdown and mortality across localities in Batagarawa LGA revealed substantial heterogeneity in permethrin performance. Locality D demonstrated the fastest KDT_{50}/KDT_{95} values and the highest 24-hour mortality, whereas Locality C exhibited the slowest knockdown and the lowest mortality—an indicator of high-intensity resistance. Localities A, B, and E produced intermediate knockdown rates but uniformly low mortality. Across all populations, mortality remained substantially below the WHO ≥98% susceptibility threshold (WHO, 2016), confirming widespread operational resistance. The dissociation between fast knockdown and poor mortality—particularly in Locality D—suggests transient physiological incapacitation without lethal effect, a signature of tolerance and rapid recovery (Brogdon & McAllister, 1998). Such transient knockdown is consistent with temporary sodium-channel disruption followed by detoxification-driven physiological restoration. Evidence from African *Anopheles* indicates that metabolic enzyme induction (P450s, GSTs) supports rapid recovery after initial neural shock (Awolola *et al.*, 2009; Atoyebi *et al.*, 2020; Bariami *et al.*, 2012). Cuticular thickening and

reduced penetration may further delay permethrin accumulation, contributing to prolonged KDT values (Rajatileka *et al.*, 2011).

The severe resistance observed in Locality C—slow knockdown and minimal mortality—suggests a combination of target-site (kdr) mutations and high detoxification efficiency acting synergistically. This aligns with documented patterns of pyrethroid resistance intensification in northern Nigeria, where agricultural selection pressures amplify metabolic resistance pathways (Nkya *et al.*, 2013; Ibrahim *et al.*, 2023; Muturi *et al.*, 2019).

Operationally, these findings indicate that mosquitoes in Locality C are highly resilient to permethrin, whereas Locality D—despite faster knockdown—still fails to achieve lethal susceptibility, underscoring uniformly compromised LLIN performance across the LGA.

The detection of confirmed permethrin resistance across all surveyed *Anopheles* populations in Batagarawa LGA has critical operational implications for malaria control in Katsina State and beyond. Nigeria's malaria control strategy relies heavily on pyrethroid-based LLINs and, in some regions, indoor residual spraying (IRS) (Federal Ministry of Health, FMoH, 2022). When local vector populations exhibit high levels of resistance, the protective efficacy of LLINs may be compromised: nets that once provided both a physical barrier and a lethal chemical effect may now act primarily as a barrier, allowing some resistant mosquitoes to survive long enough to seek blood meals later (WHO, 2018).

Locality-specific differences in knockdown speed and mortality indicate that resistance is not homogeneous. The relatively "less resistant" phenotype in Locality D (faster knockdown, highest but still insufficient mortality) suggests that interventions such as LLINs supplemented with synergists (e.g., PBO nets) or IRS using non-pyrethroid chemistries may temporarily restore some control in areas with similar resistance intensity (Protopopoff *et al.*, 2018). Conversely, in Locality C, with delayed knockdown and extremely low mortality, more aggressive rotation or combination strategies are likely required, possibly deploying next-generation LLINs containing pyrethroid–pyriproxyfen or pyrethroid–chlorfenapyr blends (WHO, 2022).

The widespread agricultural activity in northern Nigeria, including Katsina State, also provides a context for interpreting these findings. Agrochemicals, especially pyrethroid-based pesticides used in crop protection, can exert off-target selection pressure on mosquito populations breeding in irrigation channels and transient rain pools (Nkya *et al.*, 2013; Oliver & Brooke, 2018). Such ecological overlap can accelerate the selection and spread of resistance alleles, intensify metabolic detoxification capacity, and ultimately reduce the operational lifespan of pyrethroid-based vector control tools.

These local variations underscore the importance of moving beyond "one-size-fits-all" intervention strategies. Incorporating routine insecticide resistance monitoring at sub-LGA scales, integrating physicochemical and land-use mapping, and aligning insecticide selection with local resistance profiles will be essential for sustaining vector control impact (Ranson & Lissenden, 2016). Moreover, as WHO guidelines recommend, integrated vector management (IVM) — including larval source management, environmental modification, community-based interventions, and rotation of insecticide classes — should be prioritized to slow resistance evolution and maintain malaria gains (WHO, 2018, 2022).

From an epidemiological standpoint, failure to address heterogeneity in resistance may allow focal malaria transmission hotspots to persist despite high LLIN coverage. This scenario risks undermining national malaria elimination goals and increasing the cost and complexity of control programs over time (Sherrard-Smith *et al.*, 2022). The findings from Batagarawa thus provide both a warning and an opportunity: a warning that resistance is entrenched even in less intensively urbanized LGAs, and an opportunity to pilot adaptive, evidence-based control strategies that can be scaled across the Sahelian zone of Nigeria.

CONCLUSION

This study investigated locality-based variations in the susceptibility of *Anopheles* mosquitoes to permethrin across five communities in Batagarawa LGA, Katsina State, Northwestern Nigeria. Contrary to our initial hypothesis that no significant differences would exist among localities, we observed measurable heterogeneity in knockdown dynamics (KDT_{50} and KDT_{95}). However, this variability did not translate into operationally meaningful differences in mortality outcomes. Across all sites, 24-hour mortality rates remained well below the WHO threshold for susceptibility, confirming widespread resistance to permethrin.

The findings highlight that although local ecological conditions may influence the speed of knockdown, they do not currently overcome the high levels of

resistance present in these vector populations. This persistent pyrethroid resistance has serious implications for malaria control, particularly for LLIN and IRS interventions that rely on permethrin or related compounds. Continuous monitoring, integration of molecular diagnostics, and exploration of next-generation insecticide formulations or combination strategies will be critical for restoring and maintaining vector control efficacy in Katsina State and beyond.

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