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Investigating multi-resistance mechanism of Anopheles gambiae s.l to insecticides in Osun State, Nigeria

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Abstract

The increasing resistance to pyrethroids and other insecticide classes by Anopheles mosquitoes continues to threaten the effectiveness of core vector-control interventions and risks reversing hard-won gains. This study investigated resistance mechanisms in Anopheles gambiae s.l. populations in two urban areas (Olorunsogo and Freedom Park) in Osogbo, Osun State. Larvae were collected from the field and reared to adults for WHO bioassay insecticide susceptibility testing using different doses of pyrethroids and an organophosphate. Synergist assays following WHO procedures assessed the involvement of monooxygenases in resistance. Sibling species were identified by Polymerase Chain Reaction (PCR) - Intentional Mismatch Primer- and allelespecific PCR was used to detect the L1014F Knockdown resistance (Kdr) mutation. Metabolic enzyme activities were measured by biochemical assays. High levels of pyrethroid resistance were observed at both locations, with variation between insecticides. Preexposure to PBO produced differing recoveries in mortality: PBO+permethrin yielded 96% mortality in Olorunsogo and 75% in Freedom Park, while PBO+deltamethrin produced 99% and 87%, respectively. Kdr allele frequencies were low, at 0.84 and 0.86 in Olorunsogo and Freedom Park. Monooxygenase activity was very low in resistant populations (P = 0.000), whereas glutathione Stransferases were significantly overexpressed (59.52 ± 1.44; P = 0.001). βesterase activity was also elevated (18.86; P = 0.000). The findings reveal multiple resistance mechanisms in urban Osun State populations of An. coluzzii, including metabolic detoxification and targetsite mutation. Therefore, proactive, evidencebased resistance management strategies are urgently needed to restore and sustain the efficacy of current vectorcontrol tools and strategies in the state.

Introduction

Mosquito-borne infections continue to represent a significant vector-borne health issue worldwide (WHO, 2024). *Anopheles, Aedes, and Culex* mosquitoes comprise species that may serve as vectors for several mosquito-borne diseases affecting people (Busari *et al.,* 2025). Consequently, vector control is a crucial component in the eradication of malaria.

In the twenty-first century, indoor residual spraying (IRS), pyrethroid-treated bed nets (ITNs) with residual insecticides, and other insecticide-based approaches were widely used as front-line tools against malaria vectors and other vector-borne diseases (Karunamoorthi and Sabesan, 2013), within an Integrated Vector Management framework (WHO, 2013). *Anopheles* mosquitoes are resistant to deltamethrin, permethrin, and lambda-cyhalothrin, and show varying levels of resistance to bendiocarb and pirimiphos-methyl, which is concerning as it could significantly affect the effectiveness of current vector control methods. During knockdown resistance, sensitivity declines due to mutations in the target site and metabolic pathways of the mosquito Adeogun *et al*., (2025). *Anopheles* mosquitoes have developed two-point mutations in the voltage-gated sodium channel that confer resistance to pyrethroids and DDT: one in Burkina Faso (Soma *et al*., 2021) and Côte d'Ivoire, where the leucine-phenylalanine substitution occurs at residue 1014 (L1014F) Ranson *et al*., (2011). The second mutation was observed in Kenya, where leucine is replaced by serine at the same codon position (L1014S) Okoko

et al., (2023). In Nigeria, both mechanisms have been shown to confer resistance in *An. gambiae s.l.* Haruna *et al.,* (2025). Target site resistance involves mutations in the voltage-gated sodium channel (Vgsc) gene, mediating resistance to DDT and pyrethroids Adesoye *et al*., (2024), as well as mutations in acetylcholinesterase (ACE), which is linked to resistance to carbamates and organophosphates Haruna *et al.,* (2025). Increased activity of detoxifying enzyme families—including non-specific esterases, glutathione-S-transferases (GSTs), and monooxygenases (cytochrome P450s)—are associated with resistance to various malaria control insecticides Omotayo *et al.,* (2021).

In Nigeria, the efficacy of chemical-based strategies through the use of pyrethroid insecticides has drastically reduced across the states (Adeogun *et al.,* 2025; Busari *et al.,* 2025; Adeleke *et al*., 2018; Awolola *et al.,* 2003), demanding urgent need for proactive and effective control strategies. This could be due to multiple resistance of mosquito, leading to phenotypic and genotypic variations. as well as increasing the disease burden transmitted by the vector globally.

In Osun State, vector control is significantly challenged by agricultural practices that involve the frequent and intensive application of pesticides (Adeleke *et al.,* 2018; Busari *et al.,* 2025). While some studies have indicated resistance in *Anopheles gambiae s.l*., which may impede the effectiveness of insecticides used for vector control and contribute to the knockdown resistance observed in adult mosquitoes, there is a dearth of baseline data regarding both targeted and mutational resistance in *An. gambiae s.l.* Hence, Constant monitoring is necessary to comprehend the levels and resistance mechanisms of vectors in combating mosquito-borne diseases. This study aims to examine the multi-resistance mechanisms of *An. gambiae s.l.* in selected metropolitan districts of Osogbo, Osun State, Nigeria.

Materials and Methods

The Study Area

This study was carried out in Osogbo (Lat. 7◦ 33' N) and Olorunda Long. (4◦ 31' E) Local Government Areas (LGA), Osun State, Nigeria (Fig 1). These were selected based on a previous report by Adeleke *et al*. (2025) on the widespread suitability of larval habitats for Anopheles mosquito breeding. Three communities were further purposively selected from each of the two LGAs. The communities from Osogbo LG are Olorunsogo, Owode-Ilesha garage and Ijetu while in Olohunda LG are Freedom park, Oke-fia, and Ajegunle.

Osun State is a southwestern state sharing boarders with Kwara and Oyo. It is a lowland forest zone comprising of both wet and dry seasons. Residents are predominantly preoccupied by trading and farming. In addition, is the rich cultural heritage and natural resources present.

Ethical approval

Ethical approval was sought and obtained from the Health Planning, Research and Statistics Department of the Ministry of Health, Osogbo, Osun State, with reference number: OSHREC/PRS/569T/1264.

Community entry and mobilization

Before the commencement of the study, mobilization visits were made to the study communities. These was led by community health workers and heads. This was to sensitize residents on the rationale and benefits of the study.

Collection Of Mosquito Larvae

A thorough larval prospection was conducted throughout the study communities to identify productive and potential *Anopheles* larval breeding habitats. Larval collection was conducted between May and August 2024. All types of breeding habitat were surveyed, comprising of gutters, puddles and pools of water in the selected communities of prospection. The four-instar larva stage were collected via scoop and sieve with 0.55mm mesh into a well-labelled transparent container. The larvae sample was transported to the Department of Animal and Environmental Biology Laboratory, Osun State University, Osogbo, Nigeria, reared till emergence into adult. Emerged adult mosquitoes were then transferred with the aid of an aspirator into a clean and transparent container. The mosquitoes were fed with 10% sugar solution, maintained under laboratory condition at 72±1% relative humidity and 25- 27 ^{0}C Temperature.

Morphological Identification of Adult Mosquitoes

The adult mosquitoes were observed and identified under Stereo microscope morphologically. All female Anopheles gambiae s.l used for insecticide bioassay were separated from the male due to their epidemiological irrelevance, using the dichotomous key described by Gilles and Coetzeez (1987).

Insecticide Susceptibility Bioassay

The insecticide susceptibility test was carried out following the protocol provided by WHO, with the use of impregnated papers (WHO,2016) in the Molecular Epidemiology and Vector Biology unit (MOVEB), Department of Animal and Environmental Biology, Osun State University. The recommended impregnated paper used were 0.05% deltamethrin, 0.75% permethrin and 0.05% of alphacypermethrin. Twenty-five newly emerged mosquitoes were introduced into the WHO kits with untreated paper in duplicates, followed by the ones with impregnated paper in four replicates. The bioassay was done for 60mins at 27±2 0C and 72±1% relative humidity, the knockdown was then recorded at 10min interval. After an hour of exposure, the adult mosquitoes were then transferred into a holding bottle for 24hrs with 10% sugar solution. Susceptibility status was determined after the holding hours. The susceptible were sorted from the resistance and individual sample were preserved in an eppendorf tubes containing desiccant (silica beads) for further analysis.

$$\text{mortality} = \frac{\text{Total number of dead mosquitoes after 24 h}}{\text{Total number of exposed mosquitoes}} \times 100\%.$$

Synergy Bioassay

This procedure was carried out following the WHO susceptibility bioassay (WHO, 2016). Adult mosquitoes were introduced to piperonyl butoxide (PBO) for an hour and then transferred to WHO bottle with 0.05% deltamethrin, 0.75% permethrin paper, readings and mortality were recorded

Biochemical Assay

This test was conducted to evaluate the mechanism of action of the enzymes and some cellular antioxidants (monooxygenases, beta-esterase, Acetylcholinesterase, Glutathione-S-Transferases) implicated for metabolic resistance in mosquitoes. An adult female mosquito, 2-5 days old, was exposed to the insecticide prior to the assay. The determination of these biochemical activities was performed according to the protocol provided by Omedes et al., (2023). P450 monooxygenase was assessed by the total content of the heme at 620nm in the end point mode. The mixture reaction contained 20µl of homogenate, 60µl of 90mM phosphate buffer (pH= 7.2), 200µl of working solution 0.2%. TMBZ with 250mM of sodium acetate buffer (pH= 5.0), and 25 µl of 3% hydrogen peroxide. For beta-esterase, β-napthyl acetate was dissolved in acetone to create a 10mM stock solution. The stock solution was then diluted to 0.5-1mM in phosphate buffer (pH =7.4). the reaction was added up with 200µl of phosphate buffer (pH =7.4), 20µl of the enzyme source and 20µl of the substrate solution (β- napthyl acetate). The absorbance was measured at 600nm. In determining AChE, 10µl of homogenate was used, then 90µl of 50mM potassium phosphate buffer (pH =7.0) and 100µl of Eliman reagent (2Mm of acetythiocholine iodide and 0.23 mM of DTNB mixed before the measurement). The optical density was measured at 405nmin the kinetic mode for 30min at 30℃. GST reaction contained 15µl of homogenate and 195µl of 100mM potassium phosphate buffer (pH= 6.5) with 9mM of GSH in 1mM of CDNB (1-chloro- 2,4-dinitrobenzene). The GST was accessed towards CDNB at 340nm in the kinetic mode for 20min at 25℃.

Molecular identification of sibling species and knock-down resistance gene

The DNA of hundred adult Anopheles mosquito samples was extracted using DNA extraction kit by Nigerian Institute for Medical Research (NIMR) in the MOVEB laboratory, Osun State University. Further analysis was carried out using the polymerase chain Reaction to identifying the sub-species of the complex using protocol by Wilkin *et al.,* (2006). IMP-PCR was used for the detection of mutational genes associated with pyrethroids and organophosphate according to the method described by Huynh *et al.,* (2007); Weil *et al.,* (2004).

Data Analysis

Correlation between the insecticide mortalities were analyzed using pearson correlation. The kdr (KDT50 and KDT95) was analyzed using chi-square while a one-sample t-test was employed to compare mean activities of enzymes used in the enzymatic resistance mechanism of the mosquitoes. Insecticide susceptibility assay was analyzed using WHO standard formula (WHO, 2022). All data were significant at $p \leq 0.05$ (2-tailed). Data from the Insecticide susceptibility test was analyzed and calculated using a standardized formula by WHO data was analyzed Percentage.

The knock down rate (KDT50 and KDT95) was determined using SPSS software, version 21.0 (probit model). Correlation between mortality to deltamethrin, permethrin, alphacypermethrin and synergy was analysed using pearson correlation. Mutational frequency were calculated using F(R) = (2 x RR + RS)/2 N, where RR = Total number of homozygote resistant, RS = Total number. The KDR frequency was analyzed using chi-square ($\chi 2$). One sampleT-test was employed to compare mean activities of enzymes of the exposed mosquitoes (both resistance and susceptible).

Result

Molecular identification of *Anopheles gambiae s.l* sibling species

A total number of six hundred mosquitoes were identified morphologically belonging to the complex of *Anopheles gambiae s.l*. DNA extraction was performed by randomly selecting of one hundred and fifty mosquitoes for molecular identification of the sibling species of *An. gambiae s.l* encountered during the study. Molecular analysis revealed only 1% of *An. gambiae s.s.* were present while the remaining 99% were *An. coluzzii* from the sample collected.

Insecticide Bioassay

Pyrethroids and Organophosphates

All *Anopheles* mosquitoes collected during the study showed strong resistance to permethrin and alphacypermethrin, followed by deltamethrin, except in the case of pirimiphosmethyl. Resistance levels varied across discriminating doses of permethrin, deltamethrin, alphacypermethrin, and pirimiphosmethyl. Mortality rates at Olorunsogo were 39%, 58.6%, 82%, and 100%, while at Freedompark they were 52.78%, 49.11%, 59%, and 100% respectively (Table 1). A high level of permethrin resistance was observed in Olorunsogo, indicated by knockdown times (KDT_{50} and KDT_{95}). Pirimiphos-methyl was the most effective insecticide tested, with no resistance detected at either site. Both locations achieved 100% mortality within 24 hours. KDT_{50} and KDT_{95} were 39.72 and 79.14 minutes in Olorunsogo and 45.45 and 88.98 minutes in Freedompark, respectively (Fig 2). The mortality rates across sites were significantly correlated (R2= 0.030, p = 0.01).

Table 1: Knockdown time of different doses of insecticides and the percentage mortalities in the study areas.

Insecticide	Parameters	Study site	
		Olorunsogo	Freedompark
Permethrin	% Mortality	39	52.78
	(24 h)	>120	>120
	KDT50(min)	>120	>120
	KDT95(min)	Resistant	Resistant
	Susceptibility status		
Deltamethrin	% Mortality	82	49.11
	(24 h)	108.05	97.00
	KDT50(min)	>120	>120
	KDT95(min)	Resistance suspected	Resistance suspected
	Susceptibility status		
Alphacypermethrin	% Mortality	58.6	59
	(24 h)	>120	87.76
	KDT50(min)	>120	>120
	KDT95(min)	Resistant	Resistance suspected
	Susceptibility status		
Pirimiphos	% Mortality	100	100
	(24 h)	39.72	45.45
	KDT50(min)	79.14	88.98
	KDT95(min)	Susceptible	Susceptible
	Susceptibility status		

All Knockdown time values greater than 120 min is reported as >120

Synergy Bioassay

The results from the synergy assay for both sites, involving pre-exposure to PBO, revealed mortality in the *An. coluzzii* subjected to the bioassay. However, PBO+Permethrin improved efficacy but did not fully restore susceptibility (Table 2). In Olorunsogo, the mortality rate increased significantly to 96%, with a KDT_{50} of 70.83 minutes and a KDT_{95} of 110.50 minutes. In Freedompark, the mortality rate was 75%, with a KDT_{50} of 95.96 minutes and a KDT_{95} still exceeding 120 minutes (Fig 4). PBO+ Deltamethrin showed a

mortality rate reached 99%, with KDT_{50} of 49.28 minutes and a KDT_{95} of 106.20 minutes, indicating a high level of susceptibility, in Olorunsogo while resistance was suspected in Freedompark, with a mortality rate of 82.85%, and a KDT_{50} of 60.20 minutes and a KDT_{95} of 102.86 minutes (Fig 5).

Table 2: Knockdown time of synergy and the percentage mortalities from the both sites.

Insecticide	Parameters	Study site	
		Olorunsogo	Freedompark
PBO+Permethrin	% Mortality	96	75
	(24 h)	70.83	95.96
	KDT50(min)	110.50	>120
	KDT95(min)	Resistance suspected	Resistance suspected
	Susceptibility status		
PBO+Deltamethrin	% Mortality	99	82.85
	(24 h)	49.28	60.20
	KDT50(min)	106.20	102.86
	KDT95(min)	Susceptible	Resistance suspected
	Susceptibility status		

Mutant alleles frequency

The kdr genotyping mutation (L1014F) was high in both study areas. In Olorunsogo, 84% were homozygous for the resistant allele (RR), 4 individuals (16%) were homozygous for the susceptible allele (rr), and no heterozygotes (Rr) were detected (Table 3). In Freedom Park, 88% were homozygous resistant (RR), 3 individuals (12%) were homozygous susceptible (rr), and no heterozygotes were observed. Using Chi-square, P value for Hardy-Weinberg expectations for kdr gene ($x2 = 25$, $df = 1$ $P < 0.05$).

Table 3: KDR Frequency and Alleles Distribution of *An. coluzzii* from the two sites

Locations	**No screened**	**Kdr Mutation**			**Kdr alleles Frequency (%)**
		rr	Rr	RR	
Olorunsogo	25	4	0	21	0.84
Freedom park	25	3	0	22	0.88

Metabolic Enzymes Assay

Metabolic activities based on the insecticide susceptibility of mosquitoes are presented as mean values (Table 4). The results indicated that monooxygenase activity was lower in resistant mosquitoes compared to susceptible and control groups. Conversely, GST activity was significantly higher in the resistant population (P ≤ 0.05; 0.001). β-esterase activity showed a gradual increase from the control to the susceptible and then to the resistant group, with the highest mean observed in resistant mosquitoes. A one-sample T-test revealed significant differences in enzyme activities among the susceptibility groups (P ≤ 0.05; 0.00). Acetylcholinesterase activity was slightly reduced in the susceptible group relative to the control (Table 5) (P ≤ 0.05; 0.03).

Table 4: Mean Activities of Metabolic Enzymes control and exposed An. colluzi to pyrethroids

Susceptibility Status (N=11)		**Enzymes**	
	Monooxygenase (ng/L)	GST (U/L)	β-esterase (nmole/mg protein)
Control	55.70± 0.33	53.43± 2.32	18.16± 0.72
Susceptible	55.35± 0.51	55.71± 1.39	18.54± 0.72
Resistance	53.60± 1.02	59.52± 1.44	18.86± 0.93

Table 5: Mean Activities of Metabolic Enzymes in control and exposed *An. coluzzii* to pyrethroids

Susceptibility Status	**Acetylcholinesterase**
Control	17.21± 1.07
Susceptible	15.65± 0.52

Discussion

This study found that, among 100 *An. gambiae s.l.* analysed, 99% were identified as *An. coluzzii* and 1% as An. gambiae s.s. The predominance of *An. coluzzii* over An. gambiae s.s. corroborates earlier reports from Osun State (Iwalewa *et al.,* 2025; Busari *et al.,* 2024). Afolabi *et a*l. (2019) similarly reported a higher prevalence of *An. coluzzii* than An. gambiae in Ondo, Southwestern Nigeria. Studies from Nigeria (Oduola *et al.,* 2012), Ghana (De Souza *et al.,* 2010), and Benin (Yaouleton *et al.,* 2010) also document dominance of the M form over the S form. This pattern may be related to greater larval tolerance of physico-chemical variation in *An. coluzzii*, which can restrict gene flow and promote shifts in species composition.

The field populations examined were highly resistant to pyrethroids, with no mortality observed in response to the organophosphate tested. Resistance to permethrin was particularly pronounced in

Olorunsogo compared with Freedom Park, although mortality rates varied by site. These observations align with numerous reports of widespread pyrethroid resistance in Nigeria, including studies by Obembe *et al.,* 2025 (Kwara State), Obembe *et al.,* 2024 (national), Muhammad *et al.,* 2021, Omotayo *et al.,* 2022, Awolola *et al.,* 2009 (Lagos), and Adeleke *et al.,* 2018 (Osun State). Mosquito dispersal facilitates spread of resistance haplotypes, including the kdr-W mutation that confers knockdown resistance to pyrethroids.

Analysis of kdr genotypes indicated homozygous resistant (RR) frequencies of 0.84 and 0.88 at the study sites. Homozygous susceptible (rr) frequencies were 0.16 at Olorunsogo and 0.12 at Freedom Park. No heterozygotes were detected at either site. Consistent with previous reports (Djouka *et al.,* 2008; Cukwuekezie *et al.,* 2020; Omotayo *et al.,* 2021; Ekedo *et al.,* 2023), heterozygote frequencies were low in the sampled populations. The observed kdr allele distribution deviated from Hardy–Weinberg expectations, suggesting selection, nonrandom mating, or population structure affecting the L1014 locus. The prominence of kdr mutations in West Africa has been driven by prolonged use of pyrethroid-based control measures, reducing pyrethroid efficacy in many settings.

Piperonyl butoxide (PBO) is a synthetic synergist with minimal intrinsic insecticidal activity. Pre-exposure to PBO increased mortality at both sites, although mortality in Freedom Park was slightly lower than in Olorunsogo. This contrasts with some studies reporting near-complete restoration of susceptibility by PBO (Awolola *et al.,* 2018; Martins *et al.,* 2021) and likely reflects local differences in resistance profiles. PBO does not affect target-site mutations (e.g., kdr L1014F) and may not fully restore susceptibility when multiple mechanisms such as elevated GSTs, esterases, or alternative pathways contribute to resistance.

Biochemical assays showed lower monooxygenase activity in the resistant groups; monooxygenase activity was significantly higher in the susceptible and control ($P = 0.000$). This observation aligns with Hamid-Adiamoh *et al.,* 2020, but contradicts studies reporting elevated monooxygenases in pyrethroid-resistant populations (Cuamba *et al.,* 2010; Awolola *et al.,* 2018). Reduced monooxygenase activity in resistant specimens could reflect the involvement of cuticular resistance or antioxidant defenses that limit insecticide uptake or mitigate oxidative damage.

In contrast, GST activity was significantly overexpressed in the resistant group ($P = 0.001$). Earlier studies document GST involvement in Anopheles resistance (Ifeoluwa *et al.,* 2020; Koumo *et al.,* 2025; Adeogun *et al.,* 2025). GST-mediated detoxification likely contributes to the high pyrethroid resistance observed at both sites. Additionally, β-esterase activity was significantly elevated in resistant populations ($P = 0.000$), consistent with reports linking increased esterases to pyrethroid resistance (Hemingway *et al.,* 2004; Zhong *et al.,* 2024; Olaniran *et al.,* 2020). Some studies, however, have found no significant difference in β-esterase activity (Omotayo *et al.,* 2021), indicating geographic and temporal variation in resistance mechanisms.

Overall, biochemical results indicate limited involvement of monooxygenases in the resistant *An. coluzzii* examined, while GSTs and β-esterases are implicated as major contributors to resistance at the study

sites. The significant GST overexpression and the variable effect of PBO at one site highlight a serious challenge for malaria control. Local differences in PBO efficacy imply that reliance on PBO-based interventions may not uniformly restore pyrethroid susceptibility and could pose a risk to effective vector control.

Pyrethroid resistance in Anopheles has become a major public-health challenge in Nigeria because malaria control depends heavily on pyrethroid-based interventions. Growing resistance demands urgent attention because it undermines control efforts and risks increasing the burden of malaria and other vector-borne diseases.

Conclusion

Anopheles coluzzii remains the dominant vector in Osun State and exhibits strong resistance to pyrethroids. Although *kdr* mutations are present, their low frequency indicates they are not the primary resistance mechanism. Instead, metabolic enzymes, particularly GSTs and β-esterases, play a major role, with evidence of additional complex mechanisms. The limited effectiveness of PBO highlights the crucial need for locally tailored, evidence-based vector control strategies. Therefore, there is an urgent need to manage resistance and prevent the continuous transmission of malaria through proactive vector surveillance and environmental management.

List of Abbreviations

PCR -Polymerase Chain Reaction

KDR -Knock-down Resistance

IRS- Indoor Residual Spray

ITN- Insecticide Treated Net

DDT- Dichlorodiphenyltrichloroethane

PBO -Piperonyl butoxide

RR -Homozygous Resistant

RS- Heterozygous Susceptible

LGA -Local Government Area

MOVEB -Molecular Epidemiology and Vector Biology

WHO- World Health Organization

IMP - Intentional Mismatch Primer

pH- Potential Hydrogen

TMBZ - 3,3’,5, 5’ Tetramethylbenzidine

GSH- Reduced Glutathione

OSMoH–REC - Osun State Ministry of Health Research and Ethics Committee

Declarations

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Authors’ contributions

Conceptualization–Z. O, M.A

Data curation–Z.O., Y.O., Q.O., M.M., D.I., L.O.,

H.K., G.O., G.B., C.T., T.A., I.O.

Supervision–A.M., K.A., A.O., M.A.

Data analysis–Z.O.

Manuscript draft—Z.O.

Manuscript editing– M. A., L.O.

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Data availability

All data were generated and analyzed during the study are present in the article.

Consent to participate

Verbal consent of resident was obtained durig the community mobilization visit

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest regarding the article

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Figures

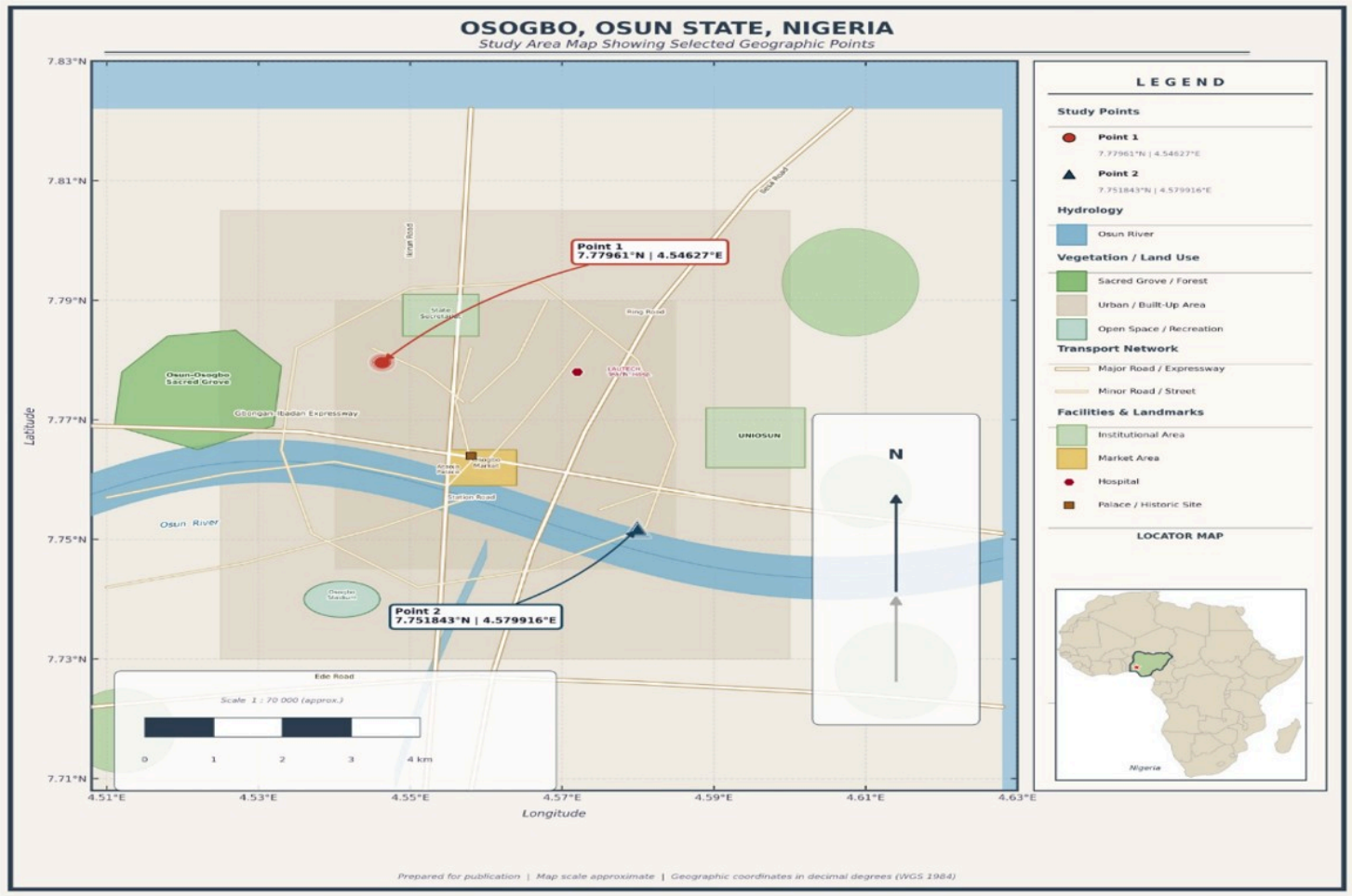


Figure 1

Showing the study location sites

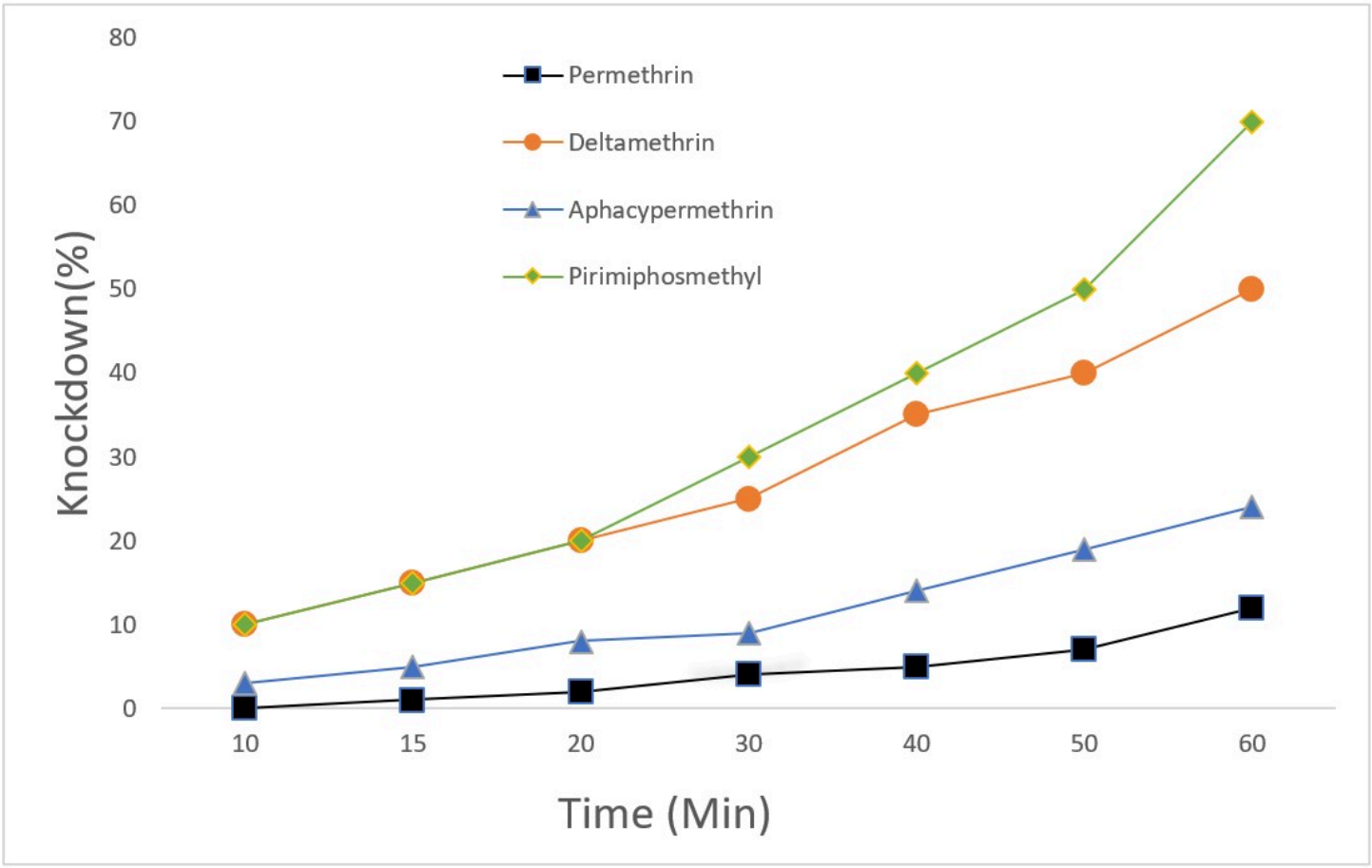


Figure 2

Percentage Knockdown of *An. coluzzii* exposed to the Permethrin, Deltamethrin, Alphacypermethrin and Pirimiphosmethyl from Olorunsogo

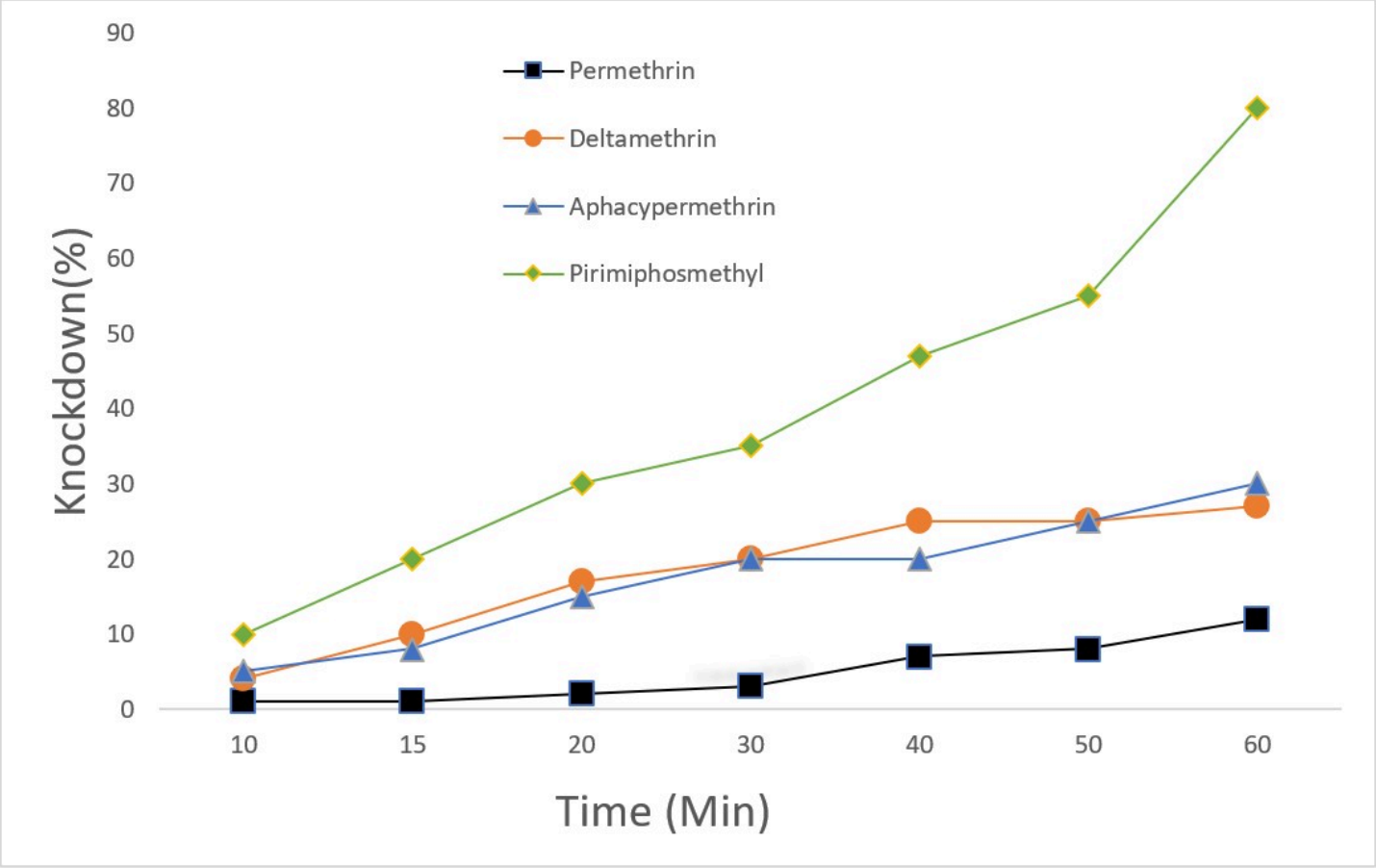


Figure 3

Percentage Knockdown of *An. coluzzii* exposed to the Permethrin, Deltamethrin, Alphacypermethrin and Pirimiphosmethyl from Freedompark

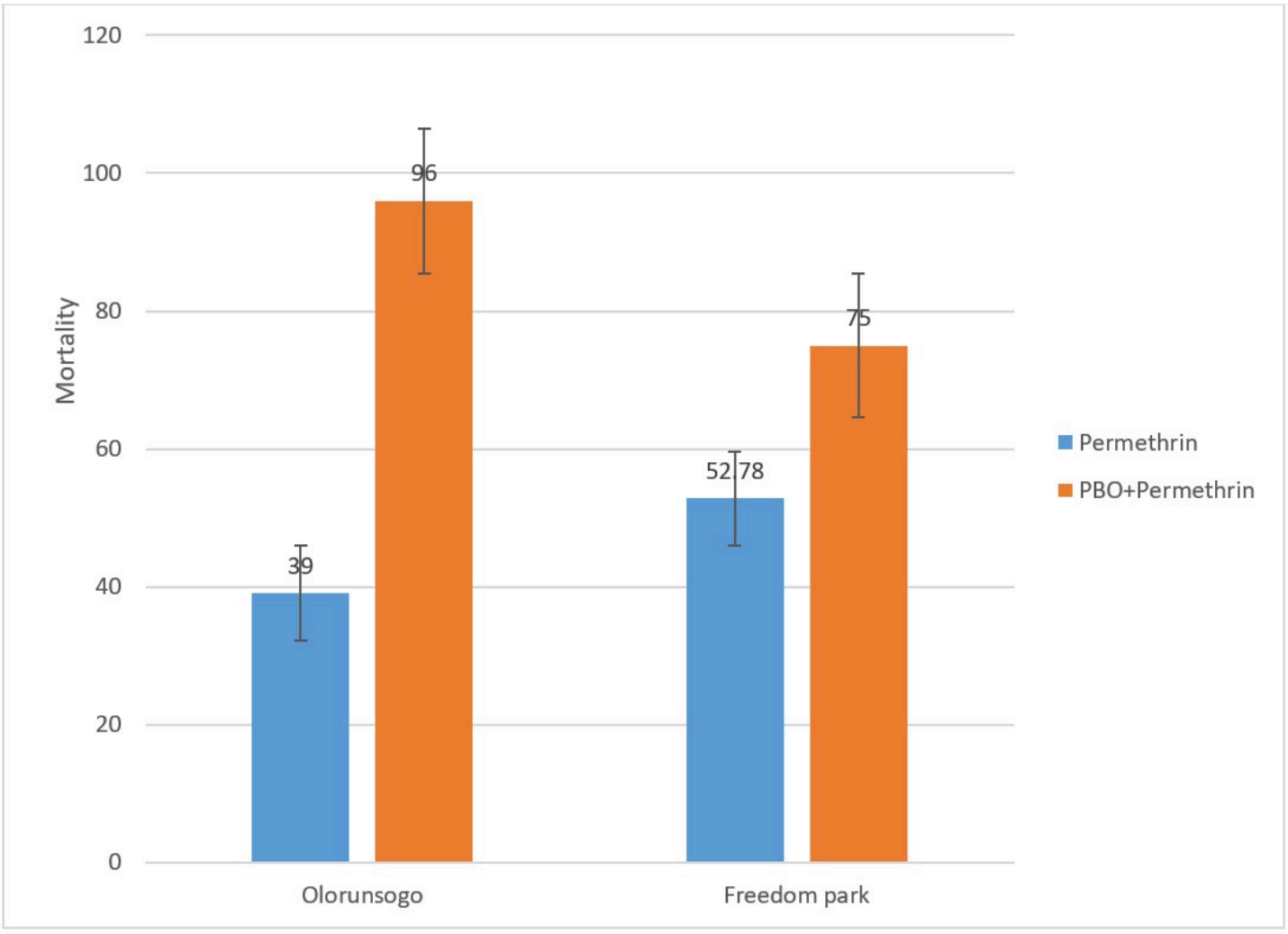


Figure 4

Percentage Knockdown (60mins) of *An. coluzzii*exposed to Permethrin and PBO+ Permethrin from Freedompark

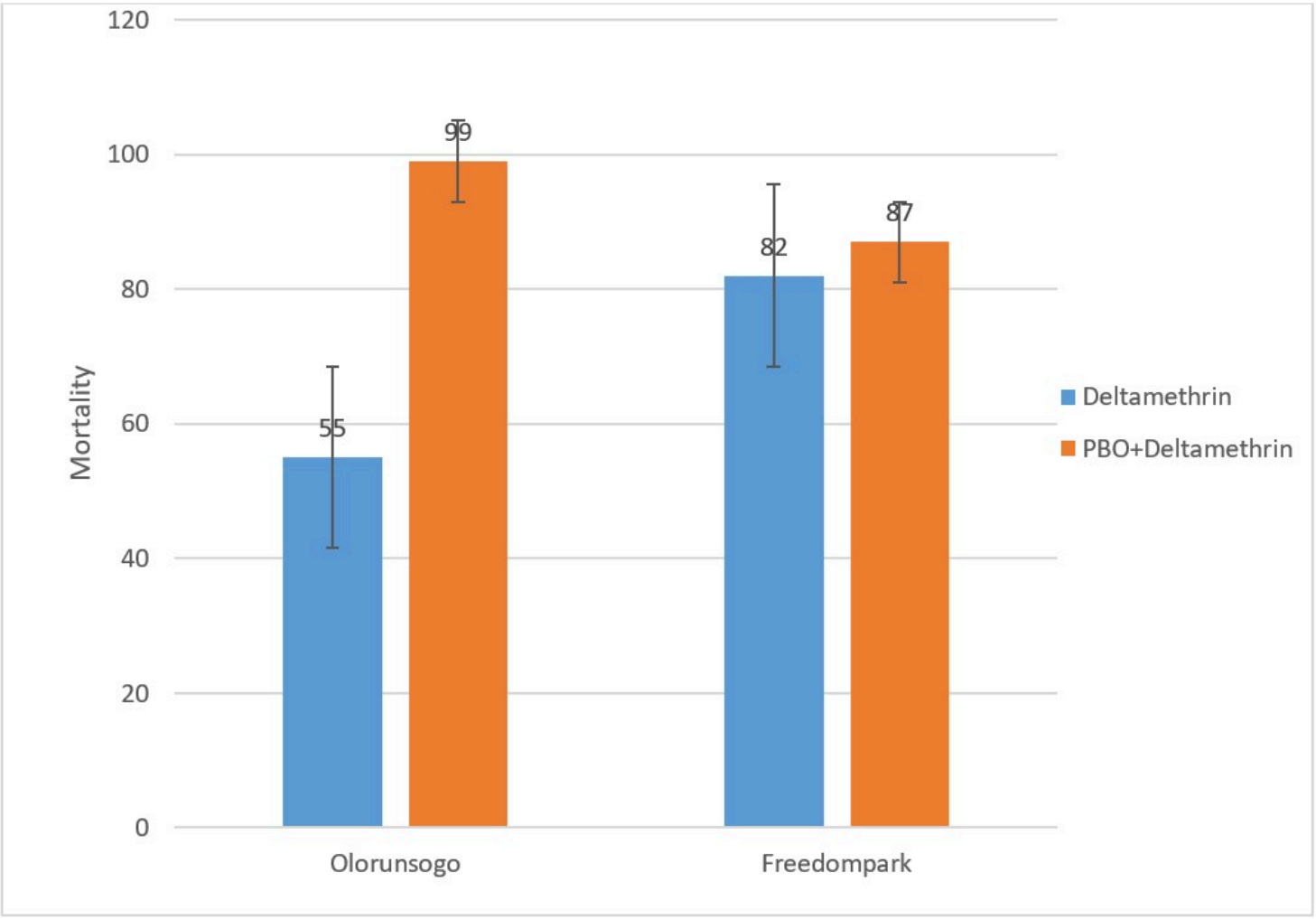


Figure 5

Percentage Knockdown (60mins) of *An. coluzzii* exposed to Permethrin and PBO+ Deltamethrin from the two study sites