



## BREEDING HABITATS AND INSECTICIDE SUSCEPTIBILITY OF *Anopheles gambiae* AND *Culex quinquefasciatus* IN ILORIN, NIGERIA

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### ABSTRACT

Urban mosquito breeding habitats and insecticide resistance pose major public health challenges in Nigeria. This study characterised larval habitats and insecticide susceptibility of *Anopheles gambiae* and *Culex quinquefasciatus* in Ilorin. Larvae were collected from ten sites during the 2011 rainy season, and habitats were profiled by water physicochemical properties (temperature, pH, conductivity, total dissolved solids, heavy metals) and vegetation. Adult susceptibility was tested against five WHO-recommended insecticides. *An. gambiae* predominated in sunlit, turbid, polluted waters with submerged vegetation; *Cx. quinquefasciatus* occurred in both sunlit and shaded habitats. Propoxur gave the highest mortality: 80.3% in *An. gambiae* and 94.1% in *Cx. quinquefasciatus*. Primiphos methyl was least effective, killing only 4.9% of *An. gambiae*. Pyrethroids showed moderate efficacy (60–75%). Principal component analysis revealed strong correlations of iron and manganese with polluted sites, indicating heavy metal contamination. These pre-long-lasting insecticidal net baseline data expose alarming resistance to DDT and organophosphates and underline the urgent need for integrated vector management, insecticide rotation, larval habitat modification, and continuous resistance monitoring.

**Keywords:** Malaria, insecticide resistance, *Anopheles gambiae*, *Culex quinquefasciatus*, urban breeding habitats, Nigeria

### INTRODUCTION

Female mosquitoes transmit malaria, lymphatic filariasis, yellow fever, and arboviruses (Manyi *et al.*, 2017). In Nigeria, *Anopheles gambiae* and *Culex quinquefasciatus* are the primary vectors of malaria and lymphatic filariasis, respectively (Fagbohun *et al.*, 2019; Matowo *et al.*, 2019). *An. gambiae* alone accounts for the vast majority of malaria cases in sub-Saharan Africa, and Nigeria recorded 27% of global cases in 2019, the highest national burden (WMR, 2020). By 2023, 96% of malaria deaths were concentrated in just 29 countries, including Nigeria (WMR, 2023).

Urbanisation continuously creates mosquito breeding sites in blocked drains, earthen pots, polluted pools, and stagnant water (Oduola *et al.*, 2016; Mattah *et al.*, 2017; Agbor *et al.*, 2020). Larval distribution and fitness depend on physical (temperature, pH, conductivity), chemical (heavy metals, total dissolved solids), and biological parameters, which in turn are modulated by climatic factors (Kabula *et al.*, 2011; Fouque & Reeder, 2019; Ande *et al.*, 2022).

Insecticide resistance increasingly undermines control efforts. Pyrethroid resistance is widespread in Ilorin (Oduola *et al.*,

2016), driven by agricultural insecticide use (Fagbohun *et al.*, 2019), and *kdr* mutations (Matowo *et al.*, 2019), and resistance to multiple classes has been reported nationally (Chukwuekezie *et al.*, 2020; Yusuf *et al.*, 2021). Despite these challenges, detailed characterisation of urban larval habitats and contemporary susceptibility data for Ilorin are lacking. Furthermore, pre-intervention baseline data are extremely rare in West Africa, yet they are essential for detecting temporal resistance trends. This study aimed to: (1) characterise the physicochemical properties of mosquito breeding habitats in Ilorin, (2) determine the insecticide susceptibility status of *An. gambiae* and *Cx. quinquefasciatus*, and (3) provide evidence-based recommendations for vector control.

### MATERIALS AND METHODS

#### Study Area

Ilorin metropolis, Kwara State, Nigeria (4°28'10"E, 8°34'28"N to 4°35'35"E, 8°24'09"N). Area ~105 km<sup>2</sup>, population 364,666 (2006 census). Ten sampling points were located near the Post Office (8°29'16"N, 4°33'52"E) (Figure 1).

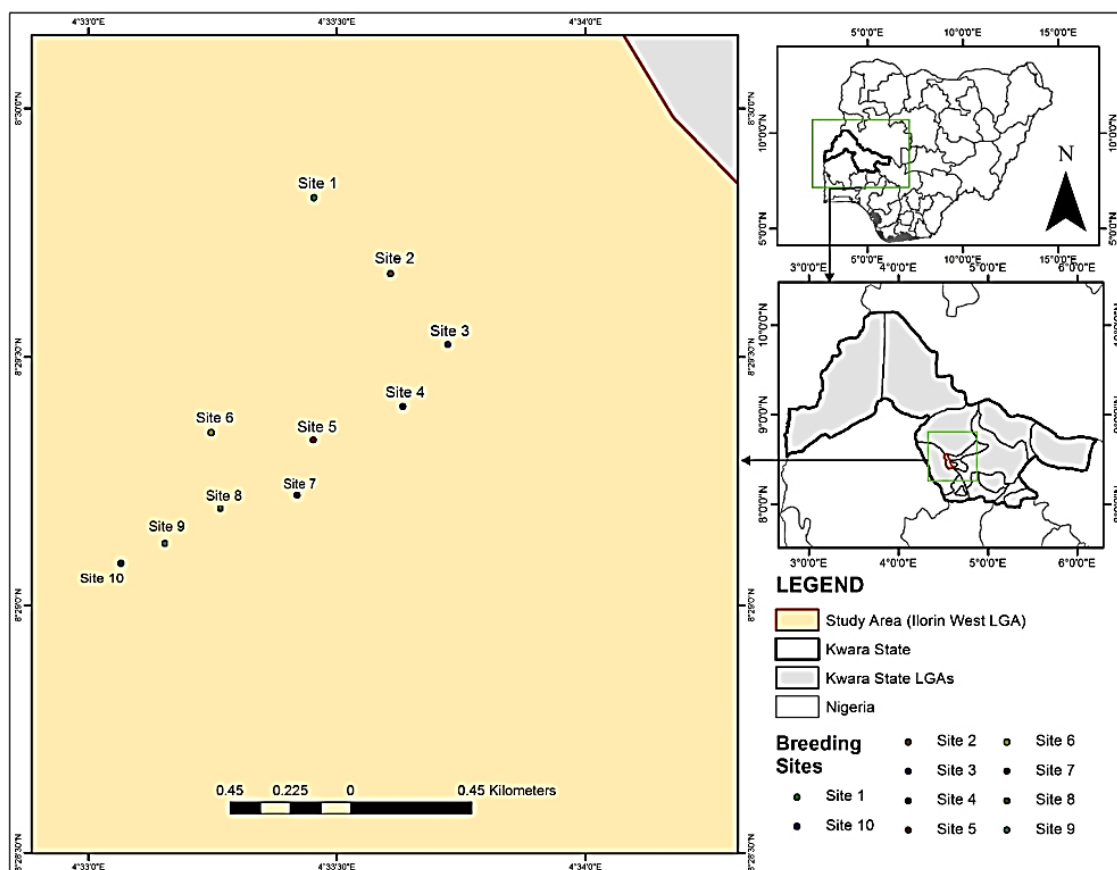


Figure 1: Mosquito Breeding Sites Sampling Points

### Larval Habitat Characterisation

At each site, we recorded water origin, nature (puddle/ditch), characteristics (clear/turbid/polluted/dark), sunlight exposure (shaded/partial/sunlit), vegetation type (emergent/submerged/floating), and larval presence. Temperature, conductivity, total dissolved solids (TDS), and pH were measured on-site with a Consort C561 multi-parameter analyser and a pH meter. Heavy metals (Cd, Pb, Mn, Fe, Zn) were determined by atomic absorption spectrophotometry (AAS PG990FG) following APHA (1998).

### Mosquito Collection and Identification

Larvae were sampled during the rainy season (May–June 2011) using a 350 ml dipper (MR4, 2007). Across the 10 sites, 1,200 larvae were collected (30 dips per site on average), transported to the laboratory in 5,000 ml containers filled with site water, and reared to adults. These samples represent a pre-scale-up baseline, obtained before widespread distribution of long-lasting insecticidal nets (LLINs) and intensified urbanisation. Such historical benchmarks are rare in West Africa and remain valuable for detecting temporal trends in resistance. A limitation is that data from a single rainy season may not capture the full seasonal variation. Larvae and emerged adults were identified morphologically as *An. gambiae* s.l. and *Cx. quinquefasciatus* (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987).

### Physicochemical Analyses

Ten parameters: temperature, pH, conductivity, TDS, dissolved oxygen (DO), Cd, Pb, Mn, Fe, Zn, were measured following APHA (1998) standards.

### Insecticide Susceptibility Tests

Adult females (2–5 days old, non-blood-fed) were exposed to WHO discriminating dosages of five insecticides: alphacypermethrin 0.5%, DDT 4%, pirimiphos-methyl 0.25%, propoxur 0.1%, and deltamethrin 0.1%. For each insecticide and species, four replicates of 25 mosquitoes were tested, with control papers run in parallel. Knockdown was recorded at 1 h, and mortality at 24 h (WHO, 1998). Control mortality was <5% in all assays.

### Data Analysis

Larval density (larvae/L) = total larvae / (total dips × 0.35 L). Principal component analysis (PCA) examined correlations among physicochemical parameters and sites (PAST software). Two-way ANOVA compared species and site effects ( $p < 0.05$ ). Probit analysis (GraphPad Prism 6) was used to calculate  $KDT_{50}$  and  $KDT_{90}$ .

## RESULTS AND DISCUSSION

### Breeding Habitat Characteristics

The ten sites (Table 1) showed distinct species-habitat associations. *An. gambiae* dominated sunlit, turbid or polluted waters with submerged vegetation (Sites 1–5). *Cx. quinquefasciatus* occurred in both sunlit and shaded habitats, often with floating or emergent vegetation (Sites 6–10). Larval density ranged from 5 larvae/L (Site 1) to 20 larvae/L (Site 8).

**Table 1: Characterisation of Mosquito Breeding Habitats in Ilorin**

Site	GPS Coordinates	Water Characteristics	Vegetation	Sunlight	Mosquito Species	Dips	Density (larvae/L)
1	N08°29.395, E004°33.585	Turbid	Submerged	Sunlit	<i>An. gambiae</i>	30	5
2	N08°29.395, E004°33.582	Polluted, turbid	Submerged	Sunlit	<i>An. gambiae</i>	15	7
3	N08°29.397, E004°33.594	Dark, polluted, turbid	Submerged	Sunlit	<i>An. gambiae</i>	15	8
4	N08°29.403, E004°33.568	Dark, polluted, turbid	Submerged	Sunlit	Mixed	30	15
5	N08°29.397, E004°33.637	Turbid	Submerged	Sunlit	<i>An. gambiae</i>	30	7
6	N08°29.298, E004°33.710	Dark, polluted, turbid	Floating/emergent	Partial	Mixed	30	10
7	N08°29.305, E004°33.722	Dark, polluted, turbid	Floating/emergent	Partial	<i>Cx. quinquefasciatus</i>	30	15
8	N08°29.329, E004°33.733	Dark, turbid	Floating/emergent	Partial	<i>Cx. quinquefasciatus</i>	20	20
9	N08°29.085, E004°37.066	Polluted, turbid	Emergent	Partial	<i>Cx. quinquefasciatus</i>	30	15
10	N08°29.085, E004°37.065	Turbid	Emergent	Shaded	<i>Cx. quinquefasciatus</i>	20	10

#### Physicochemical Parameters

Temperature ranged from 26.8°C to 33.4°C, and pH from 6.28 to 8.34. Conductivity (236–1382  $\mu\text{S}/\text{cm}$ ) and TDS (236–691 ppm) peaked at Sites 1–4 and were lowest at Site 10. DO was relatively constant (4.7–5.2 mg/L). Heavy metal concentrations varied: Pb was highest (0.28 ppm) at Sites 1, 2, 4, and 6; Fe and Mn peaked at Site 4 (0.778 and 0.796 ppm, respectively); Cd and Zn were low or undetectable.

#### Principal Component Analysis

PCA of physical parameters (Figure 2) showed that PC1 (48% of variance) was driven by conductivity and TDS, which strongly correlated with Sites 1–4, identifying them as

polluted, high-salt habitats. PC2 (22%) separated Site 10 (high TDS but low conductivity). Temperature, pH, and DO showed weak loadings, indicating they were less discriminating among sites. The chemical parameter PCA (Figure 3) gave PC1 (55% of variance) dominated by Fe and Mn, with high loadings at Sites 2, 4, 6, and 9—areas near drainage outlets and markets, consistent with industrial runoff. PC2 (18%) captured Pb and Cd, but no site clustered exclusively with these metals, reflecting their sporadic distribution. Elevated Fe and Mn in polluted sites may impose oxidative stress on larvae, potentially selecting for cross-resistance to insecticides via up-regulation of detoxification enzymes.

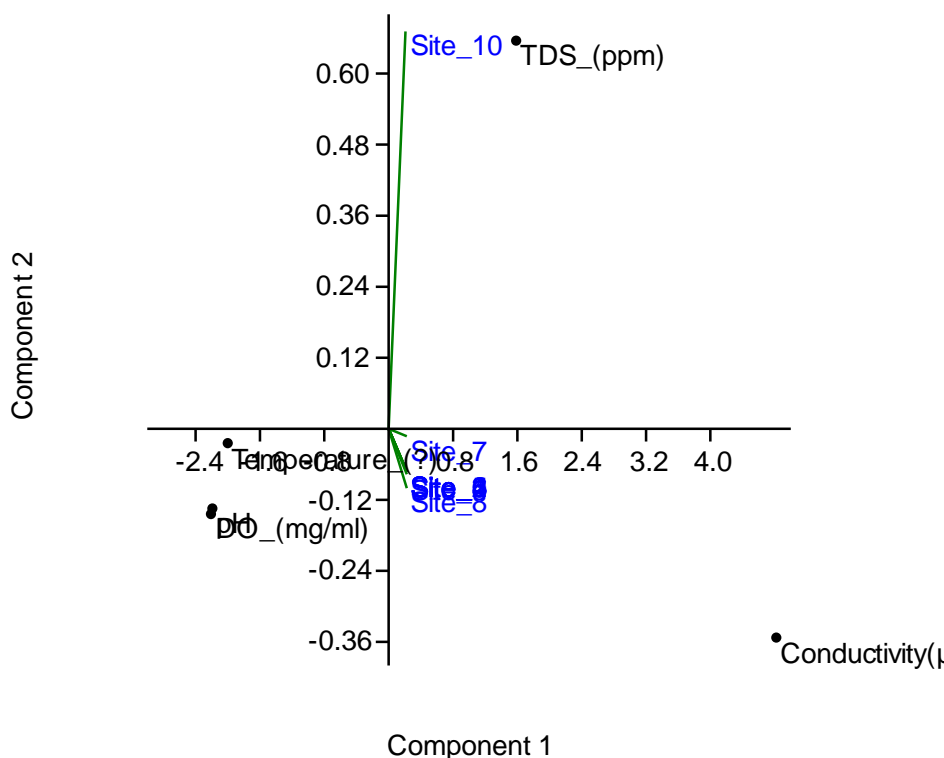


Figure 2: Principal Component Analysis Correlating Physical Parameters with Mosquito Breeding Habitats

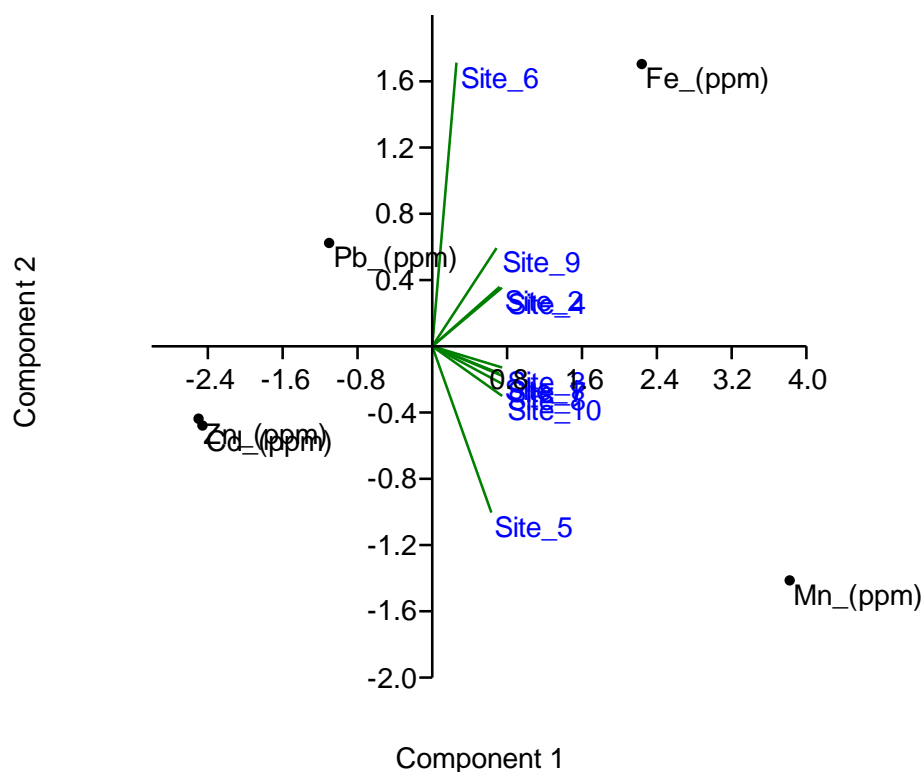


Figure 3: Principal Component Analysis Correlating Chemical Parameters with Mosquito Breeding Habitats

#### Insecticide Susceptibility

Propoxur was the most effective insecticide against both species, whereas pirimiphos-methyl showed near-complete failure against *An. gambiae* (Table 2). For *An. gambiae*, mortality was highest with propoxur (80.3%), followed by alphacypermethrin (69.4%) and deltamethrin (60.2%); DDT and pirimiphos-methyl killed only 16.1% and 4.9%, respectively. Knockdown was fastest for alphacypermethrin

(KDT<sub>50</sub> = 9.3 min) and slowest for DDT (KDT<sub>50</sub> = 41 min). In *Cx. quinquefasciatus*, propoxur (94.1%), DDT (88.1%), and alphacypermethrin (75%) achieved high mortality, while pirimiphos-methyl caused only 30.9% mortality. KDT<sub>50</sub> ranged from 10.9 min (alphacypermethrin) to 32.8 min (propoxur). Pyrethroid resistance appears moderate but is increasing.

Table 2: Percentage Mortality and Knockdown Times

Insecticide	<i>Anopheles Gambiae</i> s.l.				<i>Culex Quinquefasciatus</i>			
	Mortality (%)	KDT <sub>50</sub> (min)	KDT <sub>90</sub> (min)	R <sup>2</sup>	Mortality (%)	KDT <sub>50</sub> (min)	KDT <sub>90</sub> (min)	R <sup>2</sup>
Alphacypermethrin	69.4	9.3	26.2	0.86	75.0	10.9	31.1	0.62
DDT	16.1	41	47	0.49	88.1	28.2	73.5	0.79
Primiphos methyl	4.9	18.1	25.7	0.11	30.9	26.4	43.7	0.45
Propoxur	80.3	25.3	107.7	0.71	94.1	32.8	81	0.68
Deltamethrin	60.2	12.3	42.2	0.78	69.1	13.4	32.9	0.49

Notes: n=100 mosquitoes per insecticide (4 replicates of 25). Control mortality <5% in all assays.

#### Discussion

This study reveals clear species-specific habitat preferences: *An. gambiae* favoured sunlit, turbid, polluted waters with submerged vegetation, whereas *Cx. quinquefasciatus* exploited a broader range, including shaded, organically enriched sites. These patterns align with earlier reports (Paaijmans et al., 2009; Mwangangi et al., 2010) and highlight the ecological plasticity of *Culex* vectors (Minakawa et al., 2002). The high conductivity and TDS values at Sites 1–4 reflect organic pollution from urban

runoff, which provides microbial food for larvae while simultaneously exposing them to heavy metals.

Elevated Fe and Mn concentrations in sites near drainage outlets and markets may impose oxidative stress, potentially co-selecting for insecticide resistance through enhanced detoxification enzyme activity (Fossog et al., 2012; Tongo & Ezemonye, 2015). This finding underscores the need to consider water quality in resistance management.

Propoxur (a carbamate) remains the most effective adulticide, corroborating earlier studies (Chandre et al., 1998). However, its high KDT<sub>90</sub> in *An. gambiae* (107.7 min) hints at emerging

metabolic resistance. DDT resistance is extreme in *An. gambiae* (16.1% mortality), consistent with widespread *kdr* mutations in West Africa (N'Guessan et al., 2007). The near-complete failure of pirimiphos-methyl against *An. gambiae* (4.9% mortality) is alarming and likely reflects agricultural selection. Pyrethroid efficacy (60–75%) has declined relative to earlier Nigerian surveys (Oduola et al., 2016), signalling progressive resistance. The 2011 data provide a crucial baseline; resistance is expected to have intensified, making repeated assessments urgent.

## CONCLUSION

This study provides the first integrated characterisation of larval habitats and insecticide susceptibility in Ilorin, exposing severe resistance to DDT and organophosphates and moderate resistance to pyrethroids. To sustain vector control, we recommend:

- i. Insecticide Rotation: Use propoxur for indoor residual spraying in rotation with pyrethroids and avoid pirimiphos-methyl in *An. gambiae*-endemic areas.
- ii. Larval Source Management: Prioritise sites with high conductivity and TDS (Sites 1–4) for drainage clearing, vegetation removal, and larviciding, coupled with community education on stagnant water elimination.
- iii. Routine Resistance Monitoring: Establish biannual WHO tube tests and molecular screening for *kdr* and metabolic resistance markers (CYP450, esterases) across Ilorin.

Integrated vector management that combines these strategies is urgently required. Continuous surveillance and research into resistance mechanisms remain priorities.

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