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(RESEARCH ARTICLE)

Assessing female anopheles mosquito susceptibility to three insecticide classes in Ilorin metropolis Nigeria: Implications for environmental management and effective vector control

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## **Abstract**

This study investigates the environmental factors affecting the susceptibility of Female Anopheles mosquitoes to three classes of insecticides in Ilorin, Kwara State, Nigeria, following WHO standards. The analyzed breeding sites showed severe water pollution, with low dissolved oxygen, high conductivity, and heavy metal contamination. Anopheles mosquito populations at two of the five breeding sites, Akanlanbi and Aminlengbe, were resistant to all three insecticide types tested. The mosquitoes in Aminlengbe exhibited higher resistance to organochlorine (71% mortality), organophosphate (73%), pirimiphos methyl (73%), and carbamates (83%) and bendiocarb (88%) compared to those in Akanlanbi, which had lower resistance to pirimiphos methyl (94%) and carbamates (67%) and bendiocarb (86%). In contrast, mosquitoes from the other three sites (Edun, Niger, Shao-garage) were mostly susceptible (98%-100%) to the organophosphate insecticide pirimiphos methyl but showed varying susceptibility to the two carbamate insecticides tested (propoxur and bendiocarb). Mosquitoes at these sites were 98.4%-99.5% susceptible to bendiocarb, yet resistant to propoxur (88.7%-94.6%). The study attributes environmental pollution at these breeding sites to historical agricultural practices, industrial activities, and improper waste disposal, contributing to insecticide resistance among mosquito populations. This resistance poses significant challenges to malaria vector control efforts in Ilorin. The study emphasizes the urgent need for integrated vector management (IVM) strategies that incorporate environmental management into vector control plans. This study therefore recommends adopting pesticide and fertilizer degradation strategies, improving waste management practices, implementing comprehensive IVM approaches, developing supportive policies, and enhancing community awareness and participation to mitigate mosquito-borne diseases and ensure effective malaria control amidst rising insecticide resistance.

**Keywords:** Malaria; Vector; Anopheles; Insecticides; Environmental Management

## **1. Introduction**

In sub-Saharan Africa in particular, malaria remains a major source of illness and mortality (WHO, 2020). Five Plasmodium species—*P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi*—are responsible for the illness (CDC, 2021). *P. falciparum* is the most common and lethal of these species in Africa (WHO, 2020). The Anopheles mosquito, which contracts the disease by feeding on the blood of an infected person, is a key player in the malaria transmission cycle. After then, the parasite develops inside the mosquito and is transferred to a different human host by a bite (CDC, 2021). Despite notable advancements in the last 20 years in lowering malaria incidence and mortality rates, the illness continues to be a serious public health concern, according to the World Malaria Report (WHO, 2020).

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Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are two strategies that are critical to controlling malaria vectors (WHO, 2020). However, populations of mosquitoes have developed resistance as a result of the extensive usage of these insecticides (Ranson et al., 2011). Because it can decrease the effectiveness of vector control methods and raise transmission rates, insecticide resistance poses a serious challenge to attempts to combat malaria (Hemingway et al., 2016). Mosquitoes possess two types of resistance mechanisms: metabolic resistance, which occurs when the mosquitoes detoxify or sequester insecticides before they have a chance to work, and target-site resistance, which is caused by nervous system changes that hinder the insecticides' ability to bind to the mosquito (Hemingway et al., 2016).

An important part of the dynamics of malaria transmission is played by environmental factors. Anopheles mosquito distribution and life cycle are directly impacted by climatic factors as temperature, precipitation, and humidity (Paaijmans et al., 2009). For example, warmer temperatures may hasten the Plasmodium parasite's development within the mosquito, raising the risk of transmission (Paaijmans et al., 2009). Mosquito breeding sites are created by rainfall, and their survival and feeding habits are influenced by humidity (Paaijmans et al., 2009). The physicochemical characteristics of breeding environments, such as nutrient levels, pH, salinity, and water quality, have a major influence on mosquito larval growth and density in addition to climate (Fillinger and Lindsay, 2011). These environmental factors have been shown in studies carried out in several malaria-endemic regions of Africa to either enhance or impede mosquito. Environmental management must be incorporated into malaria control programs in order to solve the issues caused by pesticide resistance and guarantee sustainable vector control. In order to decrease mosquito breeding grounds and human-mosquito contact, environmental management entails altering or changing the surrounding environment. This can involve actions like enhanced habitat modification, better water management, and environmental sanitation. The routine monitoring of the physicochemical characteristics of mosquito breeding habitats is one of the most important parts of environmental management. Understanding the ways in which environmental factors impact mosquito populations can help with the development of focused interventions aimed at upsetting the mosquito life cycle and lowering mosquito abundance. This study looks at the long-term consequences of environmental management techniques while concentrating on the short-term effects of environmental variables on mosquito populations.

# **2. Literature Review**

The principal malaria vectors in sub-Saharan Africa, the *Anopheles gambiae species* complex, are remarkably adaptable, utilizing a variety of larval environments from ephemeral pools to permanent water bodies in rice fields and irrigation canals (WHO, 2013). Their adaptability allows them to thrive in diverse micro and macroenvironmental settings throughout tropical Africa. However, exposure to contaminated breeding grounds, especially in urban areas, may lead to insecticide resistance from the larval stage (Okogun et al., 2005). Environmental contaminants, including pesticides and physicochemical factors like dissolved oxygen, turbidity, conductivity, and heavy metal content, can greatly impact Anopheles mosquito ecology and contribute to resistance (Afrane et al., 2004). This indicates selective egg-laying behavior in Anopheline mosquitoes. Larvae and pupae growth rates vary significantly in different water samples; river and ocean water cultures show the best growth rates, while high alkalinity increases larval mortality in well water cultures (Geller et al., 2000). A complex interaction of physical, chemical, and biological water quality aspects determines suitability for *Anopheles gambiae s.l*. breeding (Robert et al., 1998). Urbanization and water salinity significantly affect *Anopheles melas* and *Anopheles gambiae s.s.* population density and spread (Akogbeto, 1995). Physicochemical characteristics like temperature, salinity, carbonate, and nitrate concentrations are linked to Anopheles larvae growth in pools (Robert et al., 1998). Additionally, *An*. *gambiae s.l*. habitats are associated with algae, water turbidity, and lack of emergent plants (Gimnig et al., 2001). Climate and human activities, such as livestock raising, impact mosquito populations, with water sources often drying up during the dry season, leaving sparse collections supporting limited larval development (Mala et al., 2011). Mosquito larvae obtain nutrients from decomposed plant material (Sattler et al., 2005), with larger breeding grounds like sunny pools along riverbanks fostering development due to abundant green algae (Sattler et al., 2005). Green algae presence may indicate mosquito larvae (Mala et al., 2011). Larval nutrient and algal contents are vital to the *Anopheles gambiae* complex's resource ecology (Mala et al., 2011). Optimal humidity, light, and temperature are necessary for larval development, which can be managed more effectively in an insectary setting than in rural fields (Dida et al., 2015). Indoor residual spraying (IRS) with stable pesticide formulations remains a critical mosquito control method (WHO, 2006). The WHO recommends specific pesticides for IRS to combat mosquito vectors.





**Source:** Karunaratne and Hemingway (2001)

Insecticide-treated nets' (ITNs') poor retreatment rates and quick efficacy loss after washing are their main limitations (Lines, 1996). By comparison, it has been demonstrated that long-lasting insecticidal nets (LLINs) decrease humanmosquito interaction. The biological activity of LLINs lasts for the same amount of time as the net itself, lasting 3–4 years for polyester nets and 4-5 years for polyethylene nets (WHO, 2005). A number of LLIN products, including DawaPlus 2.0, Duranet, Interceptor, Netprotect, PermaNet 2.5, and PermaNet 3.0, are recommended by the World Health Organization for use in public health (WHO, 2005). The WHO recommended insecticides for treatment of mosquito nets are detailed in Table 2

**Table 2** WHO recommended insecticides for the treatment of mosquito nets



**Source:** Karunaratne and Hemingway (2001)

Larval source management (LSM) is a malaria vector control strategy requiring careful consideration due to varying ecological and programmatic conditions. Effective LSM implementation demands robust infrastructure and capacity for entomological and epidemiological surveillance. A thorough evaluation of larvicide is essential to ensure the use of highquality vector control products and their optimal deployment in high-transmission areas. In situations where vector breeding sites are distinct, confined, and fixed, LSM can complement indoor residual spraying (IRS) programs, as per the World Health Organization's (WHO) position on larvicide (WHO, 2013). The primary malaria vector control strategies, long-lasting insecticidal nets (LLINs) and IRS, have achieved significant success in various countries. However, this progress is threatened by the emergence and spread of pesticide resistance in major African malaria vectors. The problem is exacerbated by the limited pesticide classes available for mosquito control: pyrethroids, carbamates, organophosphates, and organochlorines, with pyrethroids being the only class used solely for both LLINs and IRS. Hemingway and Ranson (2000) identify two main mechanisms of insecticide resistance: enhanced metabolic detoxification and reduced target site sensitivity. Metabolic detoxification involves enzymes like cytochrome P450s, glutathione S-transferases, and esterases, which degrade pesticides, preventing them from reaching their targets (Hemingway and Ranson, 2000). Reduced target site sensitivity decreases insecticide binding affinity.

The 'Knock-down resistance' (kdr) mutation in *Anopheles gambiae* causes cross-resistance to DDT and resistance to pyrethroids through two amino acid substitutions in the sodium channel: leucine to phenylalanine (Martinez-Torres et al., 1998) and leucine to serine (Ranson et al., 2000). Additionally, resistance to carbamates and organophosphates in Anopheles is due to a single amino acid change at position 119 in the ace-1 gene from glycine to serine (Weill et al., 2004).

## **3. Material and methods**

#### **3.1. Study Area**

In Kwara State, Nigeria, five (5) breeding sites for Anopheles mosquitoes were selected for the collection of larvae and pupae: Akanlanbi (N 08050.645' E 0040.55.128'), Aminlengbe (N 08049.606' E0040.56542'), Edun (N 08048.989' E0040.56078'), Niger (N 08048.931' E0040.55416'), and Shao garage (N 080 50.676' E0040.55527'). Residential villages, industrial mechanical workshops, and fishing are the main features of these places.



**Figure 1** Sampling map showing the sample locations

## **3.2. Methodology**

Using a standard dipper, a thorough survey of stagnant water bodies within the Ilorin metropolis was carried out during the dry season, which runs from January to March. Larvae of Anopheles mosquitoes were carefully collected from each identified breeding site. The larvae of Anopheles mosquitoes were gathered from five aquatic habitat breeding locations, which included ditches, puddles, river banks, and stream borders. A Global Positioning System (GPS) was used to precisely record each site's geographic coordinates, enabling future reference and accurate mapping. The larvae were recognized by utilizing a 35-mL dipper that was fixed to a 1.2-m pole, taking into account their spatial distribution on the water's surface. Using a pipette, all of the larvae that were collected were moved to a holding cup. The larvae were taken to the Zoological Insectary at the Department of Zoology, Kwara State University, Malete, Nigeria, once a sizable proportion had been collected. Morphological analysis involves the examining physical characteristics to distinguish between the two sexes. This was used to identify the larvae and distinguish between the male and female larvae. Female larvae were identified through its size that is larger than male larvae. Also, the female larvae possess a longer and more pointed abdomen with a distinctive tapering shape than male larvae (Gillies & Coetzee, 1987). Horsfall identified in (1955) that female larvae body shape is longer and slenderer in nature with more prominent and darker pigmentations on the thorax and abdomen than the male larvae possess. Ramsdale & Snow, (2000) identified that female larvae head and thorax is larger than male larvae while female larvae possess a small tubercle on the eighth abdominal segment, of which is absent in male larvae. The adult mosquito was raised from a female in a cage with carefully regulated surroundings. A 10% sugar solution was given till exposure, and the temperature range was 29°C to 39°C with a relative humidity of 33% to 79%.



**Table 3** Physicochemical properties of Anopheles mosquito breeding sites in Ilorin Metropolis

Source: Fieldwork 2024

Adult female Anopheles mosquitoes, 3–5 days old at the time of emergence, were chosen and sorted into replicates of 25–30 insects each to test for susceptibility. The World Health Organization's recommended protocol was followed for conducting susceptibility testing (WHO, 2013). Ten plastic tubes made up the testing package, five of which were labeled with a red dot to expose adult female mosquitoes to insecticides and five of which were labeled with a green dot for observation. A 16-mesh screen was installed at the top of each tube, and at the other end was a screw cap slide with a 20mm filling hole. This arrangement allowed for the controlled application of insecticides to mosquitoes and the monitoring of their reactions. To keep the insecticidal paper in place, a clean 12-by-15-cm sheet of paper was lined the inside of each tube. Ten silver clips were used for the holding tubes, and ten copper spring clips were used for the exposure tubes. Throughout the experiment, these clips made sure the paper was firmly attached to the tube walls and prevented any movement or detachment. Throughout the testing procedure, the consistent usage of copper clips for the exposure tubes and silver clips for the holding tubes was maintained. Standard WHO tests sheets with approved diagnostic dosages of 0.1% propoxur, 0.1% bendiocarb (carbamates), 0.1% pirimiphos methyl (organophosphate), and 4% DDT (organochlorine) were given to adult female mosquitoes.

Eight duplicates of 25 to 30 adult female mosquitoes that were not fed blood and were 3 to 5 days old were exposed to the insecticide-impregnated test papers in test tubes for one hour for each pesticide. The number of mosquitoes that were knocked down was methodically recorded at 10-minute intervals during the 1-hour exposure period. After being exposed, the mosquitoes were cautiously moved into holding tubes, given a 10% sugar solution, and their mortality was tracked for a whole day. In order to create a baseline for comparison with the treated groups, 20–25 female Anopheles mosquito samples were also subjected to untreated papers. This exacting procedure guaranteed reliable evaluation of mosquito reaction and pesticide efficacy.

Mosquitoes that were resistant to the insecticide and those that were vulnerable to it were kept under strict preservation in 1.5 ml Eppendorf tubes that were filled with dried silica gel. Following that, these mosquitoes were identified using a stereo microscope and the morphological criteria outlined by Gillies and Coetzee (1987) down to the species level. This precise identification process ensured accurate classification and analysis of the mosquito samples.



**Figure 2** Study Approach

# **4. Results**

**Table 4** Insecticide susceptibility of *Anopheles* mosquito populations from different sites





Anopheles mosquito populations at two (Akanlanbi and Aminlengbe) of the five breeding sites were resistant to all three types of pesticides tested. The Anopheles mosquito populations in Aminlengbe, however, were more resistant to organochlorine (71% mortality), organophosphate (73%), pirimiphos methyl (73%), and carbamates (83%) and bendiocarb (88%) than those in the Akanlanbi breeding site (76%), pirimiphos methyl (94%), and carbamates (67%) and bendiocarb (86%). Apart from the two previously mentioned sites, all other sites (Edun, Niger, Shao-garage) had populations of Anopheles mosquitoes that were susceptible (98% - 100%) to the organophosphate insecticide pirimiphos methyl, but displayed varying degrees of susceptibility to the two carbamate insecticides that were tested (propoxur and bendiocarb). All of the sites had mosquitoes that were between 98.4% and 99.5% susceptible to bendiocarb; yet, tests conducted on mosquitoes from these same sites revealed resistance to the pesticide propoxur (88.7% to 94.6%). Overall, one of the carbamate insecticides (propoxur) and the organochlorine DDT were ineffective against mosquitoes from all of the nesting areas. However, all mosquito populations—aside from Akanlanbi and Aminlengbe—were shown to be susceptible to the organophosphate insecticide (pirimiphos methyl) and the other carbamate insecticide (bendiocarb) (Tabe 4).

#### **4.1. Environmental implications**

The water quality analysis conducted in Anopheles mosquito breeding sites within the 5 sampled locations revealed alarming levels of traces of heavy metals in the collected larvae water samples. This arose as a result of various environmental factors contributing to this contamination. Spot checks and laboratory analyses were performed to understand the reasons behind this heavy metal occurrence in the breeding sites, which is believed to factor into the adult mosquitoes' resistance to the three classes of insecticides used. The following environmental factors were identified as responsible for this contamination:

#### *4.1.1. Habitat Modification and Heavy Metal Contamination*

The site under discussion was once used for large-scale agriculture but transitioned to urban settlements, leading to scattered settlements and deforestation, as shown in **Figure 1**. This change lacked proper drainage plans, causing significant environmental impacts. Key points highlight the link between habitat modification and heavy metal contamination in mosquito breeding sites:

The area's intensive agricultural use involved pesticides and fertilizers containing heavy metals like lead, arsenic, and cadmium. These chemicals can remain in the soil long after farming activities cease. Research confirms that pesticides and fertilizers are major sources of heavy metals in agricultural soils, which can leach into water bodies over time. Studies by Gupta & Gupta (1998) and Lehnhoff et al. (2023) highlight similar trace element toxicity related to crop production, livestock, and human health. With urbanization, natural vegetation was cleared, leading to deforestation. This increases soil erosion, causing heavy metals bound in the soil to wash into water bodies during rainstorms.

Vegetation clearance disrupts natural filtration processes that would otherwise mitigate contamination, as supported by Erinle et al. (2016) and Henke & Petropoulos (2013). The absence of a proper drainage plan results in water from rain and urban runoff accumulating in low-lying areas, forming stagnant pools ideal for mosquito breeding. These water bodies can become concentrated with heavy metals from the contaminated soil. Improper drainage worsens the problem by allowing pollutants to accumulate in breeding habitats. These factors combine to introduce and concentrate heavy metals in mosquito breeding sites, significantly contributing to the contamination observed in the collected larvae water sample.

#### *4.1.2. Industrial Waste*

The sites can be accessed via the Bode Sadu Jeba Road, which spans approximately 48 kilometers. Alongside this major road, numerous roadside mechanics and various industries were observed. These establishments are significant point sources of heavy metal pollutants. The activities carried out by these roadside mechanics and industries contribute to the release of heavy metals such as lead, cadmium, copper, and zinc into the environment. Automobile repair shops generate waste products, including used motor oil, batteries, brake pads, coolants, and metal parts, which often contain heavy metals that can result in soil and water contamination. Similarly, industrial facilities along the road may discharge effluents and waste containing heavy metals, further contributing to the environmental burden.

#### *4.1.3. Improper Waste*

Residents settling in the study area were observed to dispose of their waste improperly, resulting in a severe decline in hygiene standards. This unsanitary practice creates a conducive environment and breeding grounds for mosquitoes. The accumulation of improperly disposed waste not only contributes to environmental pollution but also facilitates the pooling of stagnant water, which is ideal for mosquito breeding. This cycle perpetuates the presence of mosquitoes and increases the risk of vector-borne diseases in the community



**Figure 3:** Improper Waste Disposal Practices Found on Study Area

#### **5. Discussion**

The study conducted in the Ilorin Metropolis identified significant pollution levels in Anopheles mosquito breeding sites through rigorous water quality assessments. These sites exhibited heightened conductivity, depleted dissolved oxygen levels, and elevated concentrations of heavy metals, creating an environment conducive to the proliferation of mosquito populations resistant to insecticides. This poses a substantial public health concern due to the potential exacerbation of malaria transmission in the area. The correlation observed between mosquito breeding sites and water pollution underscores the urgent necessity for integrated vector control strategies that comprehensively address environmental factors influencing disease transmission. The study's findings underscored the presence of pollutants in Anopheles mosquito breeding grounds, potentially contributing to the emergence of pesticide resistance among these populations. Alarmingly, the study revealed widespread resistance of Anopheles mosquitoes in these breeding sites to all three classes of insecticides investigated, thereby compromising the effectiveness of current malaria vector control measures.

This highlights the imperative of transitioning towards integrated vector management approaches, particularly emphasizing the control of mosquito larvae, to mitigate the spread of insecticide-resistant mosquito populations and uphold the efficacy of malaria prevention efforts in the region. Previous research conducted in similar contexts, such as Lagos (Oduola et al., 2012) and other regions in Africa (Aikpon et al., 2013; Yagoop et al., 2013), has documented varying susceptibilities of Anopheles mosquitoes to different carbamate insecticides. These variations underscore the importance of considering the chemical properties of pesticides in formulating tailored and effective vector control strategies.

The accumulation of pesticides from historical agricultural practices, industrial pollutants, inadequate drainage systems, and domestic waste at breeding sites likely exposed mosquito larvae to sub-lethal concentrations of insecticides, potentially facilitating the development of resistance mechanisms.

Therefore, adopting integrated strategies that concurrently address environmental pollution and pesticide resistance in mosquito populations is imperative for effective vector control and environmental stewardship

#### **6. Conclusion**

This study reveals alarming evidence of widespread insecticide resistance among Anopheles mosquitoes in Ilorin Metropolis, compromising the efficacy of recommended control measures. Furthermore, the findings suggest that pollution of breeding sites, primarily caused by the disposal of domestic waste into water bodies, contributes significantly to the development of insecticide-resistant mosquito populations.

### *Recommendation*

For effective vector control and environmental management, this study strongly recommends the following actions:

- Adoption of pesticide and fertilizer residue degradation strategies in soil and water: Implementing techniques to break down residual pesticides and fertilizers will reduce environmental contamination and minimize exposure of mosquito larvae to harmful chemicals.
- Improvement of waste management practices: Proper disposal and recycling of waste will help prevent pollutants from entering mosquito breeding sites, thereby reducing environmental pollution and its impact on vector populations.
- Implementation of Integrated Vector Management (IVM) for sustainability: Utilizing a holistic approach that integrates multiple strategies, such as larval source management, insecticide-treated nets, and community engagement, will enhance the effectiveness and sustainability of vector control efforts. Source management measures that target the periphery of water bodies that serve as mosquito breeding places is essential to addressing this issue, especially during the dry season when these regions are not flooded by rain. By taking a proactive position, the region's efforts to combat malaria can be sustained and insecticide resistance can be lessened
- Policy development: Establishing and enforcing policies that support environmentally sound practices and effective vector control measures will provide a regulatory framework for achieving long-term success in disease prevention.
- Implementation of public awareness and participation: Educating the community about vector-borne diseases, environmental stewardship, and the importance of participating in vector control initiatives will foster community engagement and support for sustainable environmental management practices.
- Expansion of operation of waste management companies in Ilorin: To ensure they effectively cover the states This will ultimately increase the efficacy of efforts to control malaria vectors.

### **Compliance with ethical standards**

#### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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