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Susceptibility of *Culex quinquefasciatus* to selected lambda-cyhalothrin, DDT and pirimphos-methyl in Isoko North Local Government, Delta State

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Abstract

Lymphatic filariasis (LF) and malaria are two major health concerns in sub-Saharan Africa, with Nigeria inclusive. The use of insecticides averting the significant mortality and morbidities caused by these disease vectors has led to the upregulation of insecticide resistance. This study determined the susceptibility of *Culex* mosquito to select lambda-cyhalothrin, Dichloro-Diphenyl-Trichloroethane (DDT) and pirimphos-methyl in Isoko North Local Government, Delta State. Larvae of mosquito were obtained from potential breeding sites, reared to adult and exposed to the selected insecticides as well as synergist (piperonyl butoxide) using WHO bioassay protocol. Knockdowns for 50% and 95% were estimated using probit analysis. Mortality in *Culex* exposed to lambda-cyhalothrin was resistant (36%). Complete mortality was recorded in *Culex* mosquitoes exposed to Piperonyl Butoxide (PBO)+ lambda-cyhalothrin. Similarly, complete mortality was recorded in *Culex* mosquitoes exposed to pirimiphos-methyl. *Culex* mosquitoes exposed to DDT were susceptible (99%). The difference between the mortality of mosquitoes was significant ($p<0.0001$). Percentage knockdown increased with time in all the selected insecticides exposed to the mosquitoes from 10 to 60 minutes. The difference between the knockdowns was significant ($p<0.05$). Knockdown Time (KDT) for 50% of lambdacyhalothrin, PBO+lambdacyhalothrin, DDT and pirimiphos methyl range between 6.1 minutes and 178.6 minutes, and KDT for 95% between 11.6 and 339.3 minutes. Lamda-cyhathorin should be synergized with PBO especially for the control of *Culex* mosquitoes while adopting pirimiphos-methyl. Vector surveillance is crucial to elucidate pyrethroid resistance.

Keywords: Culex mosquitoes, Lambda-cyhalothrin, Pirimiphos-methyl, Susceptibility

Introduction

Lymphatic filariasis (LF) remains one of the most widespread and debilitating vector-borne diseases, particularly in sub-Saharan Africa. LF is caused primarily by the parasitic worm *Wuchereria bancrofti*, which leads to chronic conditions including elephantiasis and hydrocele, resulting in significant morbidity, disability, and social stigma (Almeida-Souza *et al*., 2023). In Nigeria, the disease remains endemic in many states and continues to pose a major public health burden (Muhammad *et al*., 2017). *Culex* mosquitoes are the primary vector responsible for the transmission of *W. bancrofti* in many urban and peri-urban settings. The control of *Culex* mosquito populations has historically relied on the use of insecticides incorporated into insecticide-treated nets (ITNs) and indoor residual spraying (IRS) (Alto and Lord, 2016; Andersen, 2020). Continuous reliance on chemical intervention is the major problem in disease endemic areas where mosquitoes have emerging resistance, this significantly undermining the efficacy of recommended control strategies (Balthazard-Accou *et al*., 2021; Bain and Dobermann, 2022).

Resistance of *Culex* species to different insecticides recommended for vector control is driven by several mechanisms (Hernandez and Pietrantonio, 2025). Mosquito gene mutations responsible for knockdown resistance (kdr) in the voltage-gated sodium channels (VGSCs), confers resistance to pyrethroids and some organochlorines like DDT (Becker *et al*., 2020). Pyrethroids like permethrin, lambda-cyhalothrin, alphacypermethrin, and several other related insecticides are widely used for vector control because of their rapid knockdown effect, high potency, and relatively low toxicity to mammals (Ahamad *et al*., 2023). Metabolic resistance is another mechanism adopted by mosquito to detoxifying enzymes to break down or sequester insecticides before they reach their target sites (Bradbury, 2020; Barik, 2021). Such enzymes include cytochrome P450 monooxygenases, glutathione-S-transferases (GSTs), and esterases. There are a number of studies in Nigeria reporting resistance of mosquitoes to several insecticides (Dambach *et al*., 2018; Cataldo, 2019; Bun, 2019; Ojianwuna *et al.,* 2021). Several studies have investigated mosquito resistance to pyrethroids, organochlorides, and organophosphates across various regions. However, despite the growing evidence of widespread resistance, there remains limited information on the resistance status of mosquitoes in Isoko North Local Government Area (LGA) of Delta State—an area where lymphatic filariasis (LF) is endemic and recommended vector control interventions are routinely deployed (Enwemiwe *et al*., 2025).

The problems of relying on insecticides as the sole intervention measures and the emergence of resistance has triggered the search for alternative interventions in mosquitoes (Şengül Demirak and Canpolat, 2022). Many substances with insecticidal potentials such as naphthalene, petroleum products, botanical ashes, and many essential oils have been tried on *Culex* with the potentials to avert the problem of resistance

Nigerian Journal of Science and Environment 2025 Volume 23 (3) 174 – 189 ISSN: 3043 – 4440
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(Ojianwuna *et al.,* 2021a; Ojianwuna *et al.,* 2021b, Ojianwuna *et al.,* 2022; Ojianwuna *et al.,* 2024; Ojianwuna *et al.,* 2024a; Ojianwuna *et al.,* 2024b; Ojianwuna *et al.,* 2024c).

Insecticide resistance is the major setbacks to planned intervention, and thriving disease spread in many areas (Deming *et al*., 2016). The role of *Culex* mosquitoes as efficient vectors for the transmission of LF cannot be undermined considering the growing concern of insecticide resistance. Therefore, this study assessed the susceptibility of *Culex* mosquito populations in Isoko North LGA to lambda-cyhalothrin, DDT, and pirimiphos-methyl.

Methodology

Study area

This study was conducted in Isoko North Local Government Area (LGA), Delta State, Nigeria (approximately 5.525° N, 6.234° E). Isoko North LGA has diverse landscapes such as swamps, rivers, wetlands and many temporary stagnant waters that contribute to the breeding of mosquitoes. Isoko has a tropical climate characterized by wet and dry seasons, and high level of humidity and rainfall. Isoko North has several temporary breeding sites including stagnant pools, ponds, and puddles (Asare *et al*., 2016). During the raining period water gathers in several depressions. The seasonal flooding of the area further increases the availability of temporary mosquito breeding areas in the area. Four communities were sampled for this study namely Ozoro, Owhelogbo, Oghara-Iyede and Otor-Owhe. Selection of collection sites was guided by ecological suitability, areas with shallow pools and stagnant water, ideal for *Culex* breeding. Farming is the major human activities. Increasing agricultural production through the application of pesticides can lead to resistance in associating vectors.

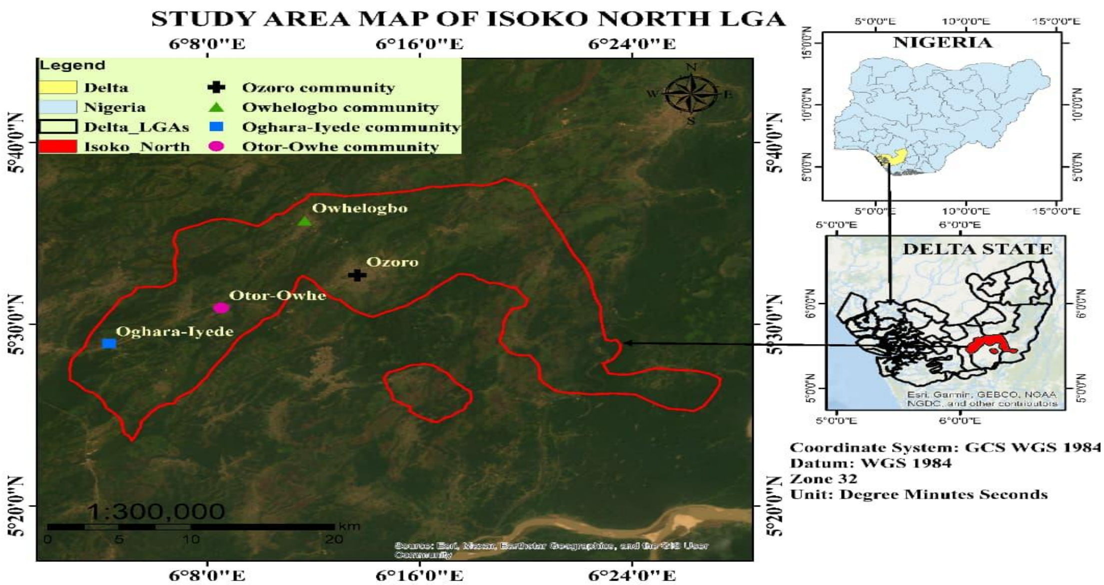

Figure 1. Map of Nigeria showing Delta state and the sampling communities

Study Design

Mosquito collection and rearing

Mosquito collection was done in the early hours of the morning using scooping spoons and ladles in Isoko North LGA, Delta State. The *Culex* mosquito collection was done from multiple breeding sites including stagnant water bodies, puddles, ponds, and swamps, which are known breeding grounds (Rasheed and Zaidi, 2024). Larvae and pupae were transferred into transparent buckets and transported to the Entomology Laboratory for sorting and rearing (Mamai, *et al*., 2020). The collection was carefully pipetted to remove debris and predators, isolating only mosquito larvae (L'Ambert *et al*., 2023). The morphological presentation such as siphon shape and body structure were used to confirm the identification of *Culex* larvae. The larval trays were netted and fed low-yeast biscuit and yeast. Upon emergence, mosquitoes were sucked into the adult holding cages and sorted males from females by aspiration (Houri-Zeevi *et al*., 2025). The mosquitoes were then fed 10% sugar solution and kept for 48 hours to acclimatize (Costanzo and Occhino, 2023).

Insecticide and synergist testing

The mosquitoes were then exposed to grades of insecticides including 0.05% lambdacyhalothrin, 4% DDT, and 0.25% pirimiphos-methyl. Twenty-five (25) female mosquitoes in four replicated were used for the exposure while one replicate unexposed served as control. Readings of knockdown were taken for 10, 15, 20, 30, 40, 50 and 60

Nigerian Journal of Science and Environment 2025 Volume 23 (3) 174 – 189 ISSN: 3043 – 4440
https://doi.org/10.61448/njse2332513

minutes. Thereafter, readings after 24 hours were taken. The exposure was done adopting the WHO protocols of bioassays (WHO, 2016). Additionally, synergist assays using PBO (piperonyl butoxide) were conducted to detect metabolic enzymes responsible for resistance in mosquitoes. Mortality of < 90% was termed confirmed resistance, 90-97% was termed suspected resistance, 98-100% susceptible.

Preservation

The dead mosquitoes were then preserved in 1.5ml tube containing silica gel for molecular analysis for resistance genes.

Data Analysis

The differences between mortality of mosquitoes with time was tested using one-way analysis of variance (ANOVA) test. Probit analysis model for knockdown for 50% and 95% was done (Dutta*et al*., 2023).

Result

Susceptibility of mosquitoes

Culex mosquitoes exposed to lambda-cyhalothrin were 36% resistant. The same population pre-exposed to piperonyl butoxide (PBO) and to the insecticides resistant to mosquitoes showed an increase in mortality (Table 1). Mortality increased from 36 to 100% in *Culex* mosquitoes exposed to lambda-cyhalothrin. The increase in mortality of *Culex* exposed to lambda-cyhalothrin was 64%. There was complete mortality in *Culex* mosquitoes exposed to pirimiphos-methyl respectively. *Culex* mosquitoes exposed to DDT were susceptible (99%). The difference between the mortality of mosquitoes was significant (F= 13.75, p<0.0001).

Table 1. Susceptibility of *Culex quinquefasciatus* mosquitoes to selected insecticides and synergists in Isoko North LGA, Delta State.

Insecticide	Conc. (%)	Mortality (%)	Status
Lambdacyhalothrin	0.05	36	Confirmed Resistance
PBO+Lambdacyhalothrin	4+0.05	100	Susceptible
Pirimiphos-methyl	0.25	100	Susceptible
DDT	4	99	Susceptible

Nigerian Journal of Science and Environment 2025 Volume 23 (3) 174 – 189 ISSN: 3043 – 4440
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Percentage knockdown increased with time in all the selected insecticides exposed to the mosquitoes from 10 to 60 minutes (Figure 1). In mosquitoes exposed to lambdacyhalothrin knockdown was below 20%. From 20 minutes of exposure, knockdown increased in mosquitoes exposed to DDT and pirimiphos methyl. Mosquitoes exposed to lambda-cyhalothrin synergist with PBO caused significant increase in knockdown ($p<0.05$) of mosquitoes. Mean knockdown was complete from 30 minutes to 60 minutes of exposure. The difference between the knockdowns was significant ($p<0.05$).

Nigerian Journal of Science and Environment 2025 Volume 23 (3) 174 – 189 ISSN: 3043 – 4440
https://doi.org/10.61448/njse2332513

Table 4.2. Knockdown time of *Culex quinquefasciatus* mosquitoes exposed to selected insecticides in Isoko North, Delta State

		Knockdown time (minutes)						
Insecticides	**Conc. (%)**	**10**	**15**	**20**	**30**	**40**	**50**	**60**
Lambdacyhalothrin	0.05	0.25 ± 0.25	1.50 ± 0.50	1.75 ± 0.48	2.75 ± 1.03	3.25 ± 1.03	3.50 ± 0.87	3.50 ± 0.87
PBO+Lambdacyhalothrin	4+0.05	20.50 ± 1.56	23.00 ± 0.58	24.75 ± 0.25	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
Pirimiphos-methyl	0.25	0.50 ± 0.50	0.75 ± 0.48	1.75 ± 0.48	15.00 ± 2.97	18.25 ± 2.69	18.75 ± 2.32	22.50 ± 0.87
DDT	4	0.75 ± 0.48	1.00 ± 0.41	1.50 ± 0.29	4.25 ± 0.85	13.00 ± 0.91	15.50 ± 0.65	22.00 ± 0.71

Note: PBO signifies piperonyl butoxide, values are knockdown time of mosquitoes.

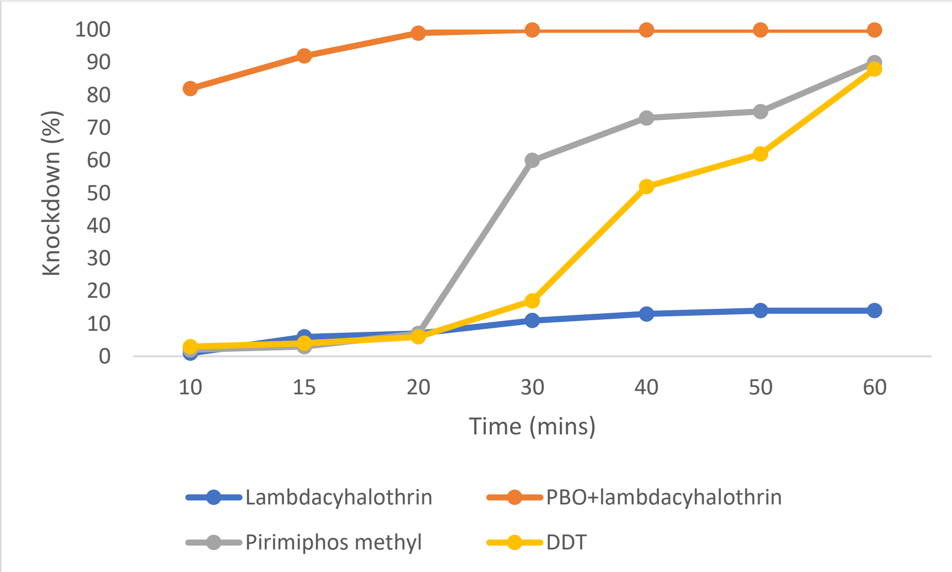

Figure 2. Percentage knockdown time of *Culex quinquefasciatus* mosquitoes exposed to selected insecticides in Isoko North LGA, Delta State. (F= 13.75; p<0.0001).

Probit model

The knockdown resistant time (KDT) for 50% and 95% of the mosquitoes exposed to lambdacyhalothrin and DDT was high. KDT for 50% of lambdacyhalothrin, PBO+lambdacyhalothrin, DDT and pirimiphos methyl range between 6.1 minutes and 178.6 minutes, and KDT for 95% between 11.6 and 339.3 minutes.

Table 3. Probit model of *Culex quinquefasciatus* mosquitoes exposed to several insecticide class in Isoko North, Delta State.

Chemical insecticide	Conc. (%)	Regression equation	Pearson $\chi 2$ goodness of fit (p-value)	KDT_{50}	KDT_{95}
Lambdacyhalothrin	0.05	2.18+0.71x	7.20 (0.0008)	178.6	339.3
PBO+Lambdacyhalothrin	4+0.05	2.54+86.0x	2.93 (0.033)	6.1	11.6
Pirimiphos-methyl	0.25	16.93-23.43x	6.45 (0.0013)	33.3	63.3

Nigerian Journal of Science and Environment 2025 Volume 23 (3) 174 – 189 ISSN: 3043 – 4440
https://doi.org/10.61448/njse2332513

DDT	4	14.89- 26.43x	6.47 (0.0013)	34.1	64.8

Discussion

This study determined the susceptibility of *Culex* mosquitoes to selected insecticide class in Isoko North LGA, Delta State. *Culex* mosquitoes are very known vectors for the transmission of lymphatic filariasis and other mosquito-borne diseases in Nigeria and across sub-Saharan Africa (Kweka *et al*., 2019; Barik, 2021). Previous studies by Renault *et al*. (2023) and Miranda *et al*. (2024) have highlighted the ecological adaptability of *Culex* mosquitoes to a wide range of environments, including both natural and artificial habitats in urban and semi-urban areas. Their preponderance outweighs those of other mosquitoes transmitting diseases. An understanding of their composition, distribution and resistance to recommended insecticides is vital in monitoring local transmission dynamics of diseases, informed strategics of planned vector control, as well as implementation of interventions (Bain and Dobermann, 2022; Almeida-Souza *et al*., 2023).

The mosquitoes in this study showed variation in susceptibility to the selected insecticides including lambda-cyhalothrin (a pyrethroid), DDT (an organochlorine), and pirimiphos-methyl (an organophosphate) recommended for vector control. The findings revealed that *Culex* mosquitoes were resistant to lambda-cyhalothrin and showed reduced susceptibility to DDT, while being largely susceptible to pirimiphos-methyl. This pattern of resistance suggests that *Culex* mosquitoes in the study area could have been exposed to multiple classes of insecticides, resulting in the development of resistance. This observation is in agreement with the work of Deming *et al*. (2016) and Cataldo (2019), who emphasized that mosquito populations often exhibit high variability in their susceptibility to insecticides, complicating uniform application of vector control measures. Furthermore, Fernandez (2022) and Poulton (2021) noted that resistance to pyrethroids and organophosphates is becoming increasingly common due to widespread use in both public health and agricultural settings. A study by Ojianwuna *et al.* (2021) conducted in Isoko South showed resistance to mosquitoes to the selected pyrethroids. It is pertinent to routinely monitor resistance as infrequent and unmonitored application of insecticides exerts strong selection pressure on mosquito

Nigerian Journal of Science and Environment 2025 Volume 23 (3) 174 – 189 ISSN: 3043 – 4440
https://doi.org/10.61448/njse2332513

populations, encouraging the survival of resistant strains (Cuervo-Parra, 2016; Lalah *et al*., 2022).

Resistance of mosquitoes is guided by underlining mechanisms. In this present study utilizing *Culex* mosquitoes, knockdown resistance (kdr) time mutations were low, and metabolic detoxification is evidenced through the synergist assays; exposure to PBO. The presence of kdr mutations in *Culex* populations suggest target-site insensitivity to pyrethroids caused by mutations in the voltage-gated sodium channels. This result is consistent with earlier findings by Fernandez (2022) and Hutton *et al*. (2023), who described how these mutations reduce the effectiveness of pyrethroid insecticides. Increase in mortality was observed in mosquitoes pre-exposed to piperonyl butoxide before insecticide exposure. This suggests that detoxifying enzymes, particularly cytochrome P450 monooxygenases, may be responsible for breaking down insecticides before they can exert their effects. Similar conclusions were drawn in the study of Issa and Smith (2014), whereby overexpression of detoxifying enzymes was observed as a major factor in the development of insecticide resistance. These findings demonstrate that resistance in *Culex* mosquitoes is driven by a combination of genetic and metabolic mechanisms, both of which diminish the efficacy of standard insecticide-based control methods.

Culex mosquito populations in Isoko North LGA are not only widespread but also highly resistant to at least two major classes of insecticides. This pattern mirrors what has been observed in other regions of Africa (Deming *et al*., 2016; Rakotoson *et al*., 2017), emphasizing the urgency of adapting current vector control strategies. The study underscores the importance of implementing integrated vector management (IVM) approaches that combine chemical, biological, and community-based interventions for effective control (Hakizimana, 2019; Gowelo, 2020). Moreover, it highlights the critical need for routine local monitoring of resistance patterns to inform evidence-based decisions about insecticide use. As Kim *et al*. (2016) and Sippy *et al*. (2020) suggested, incorporating surveillance data into decision-making can greatly enhance the success and sustainability of vector control programs. By linking mosquito species identification, insecticide susceptibility status, and resistance mechanisms, this study provides a holistic view of the current challenges in controlling *Culex* mosquitoes in Isoko North. The findings offer practical insights for designing more effective and sustainable interventions that could significantly reduce the burden of lymphatic filariasis and improve public health outcomes in the region.

Conclusion

Culex quinquefasciatus mosquitoes in this study exhibit widespread resistance to pyrethroids, organophosphates, and organochlorines in Isoko North LGA. The high levels of resistance, especially to DDT and lambda-cyhalothrin, were linked to both kdr mutations and metabolic detoxification mechanisms, such as cytochrome P450 overexpression. There is urgent need to divert the attention of relying on single dose of insecticides with reported resistance into ecofriendly and sustainable vector control approach.

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