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Susceptibility And Emerging Resistance of Culex Mosquitoes to Lambda-Cyhalothrin and Dichlorvos in Minna, Niger State, Nigeria

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Abstract- ***The persistent challenge of controlling Culex mosquitoes, which are critical vectors for debilitating diseases such as lymphatic filariasis and various arboviruses, continues to hinge on the application of chemical insecticides. A formidable obstacle to these control efforts on a global scale is the escalating development of resistance to these chemicals within mosquito populations. This investigation was therefore designed to evaluate the current susceptibility profile of wild Culex mosquitoes sourced from Minna, Nigeria, when exposed to two widely utilized insecticides: lambda-cyhalothrin and dichlorvos. Field-collected immature stages from breeding sites in the Bosso Local Government Area were reared under controlled laboratory conditions to adulthood. Subsequent susceptibility testing was performed using the standard World Health Organization (WHO) tube bioassay protocol. Adult mosquitoes were exposed to filter papers treated with two distinct concentrations of each insecticide: a lower concentration (0.4% for lambda-cyhalothrin and 3.0% for dichlorvos) and a higher, diagnostic concentration (0.7% for lambda-cyhalothrin and 7.0% for dichlorvos). Knockdown was monitored at 10-minute intervals for one hour, with final mortality assessed after a 24-hour recovery period. The results revealed a stark, concentration-dependent response. The lower concentrations induced mortalities of only 52.63% ± 5.42 for lambda-cyhalothrin and 66.66% ± 3.83 for dichlorvos after one hour, figures that fall substantially below the WHO's 98% susceptibility benchmark, thereby confirming resistance. Conversely, the higher diagnostic doses achieved mortalities of 98.48% ± 1.51 and 98.67% ± 1.33, technically classifying the population as susceptible. However, the critical finding was that after 24 hours, mortality for the low concentrations remained incomplete at 87.54% and 84.06%, indicating a resilient sub-population. This study concludes that while the high diagnostic doses remain effective for now, the clear evidence of resistance at lower concentrations signals an urgent need for proactive insecticide resistance management strategies in the region to preserve the utility of these vital public health tools.***

Index Terms—Culex Mosquitoes, Insecticide Resistance, Lambda-Cyhalothrin, Dichlorvos, WHO Bioassay, Minna, Nigeria

I. INTRODUCTION

Culex mosquitoes represent a ubiquitous and significant threat to public health across the globe, functioning as primary vectors for pathogens that cause lymphatic filariasis and several forms of viral encephalitis, including the West Nile virus [1]. The impact of these diseases is profoundly felt across the African continent, where lymphatic filariasis, for instance, imposes a severe burden on more than 40 million individuals in sub-Saharan Africa [2]. For decades, the cornerstone strategy for reducing the transmission of these diseases has involved the chemical suppression of mosquito populations, employing a range of insecticides as larvicides to target breeding sites and as adulticides to kill mature mosquitoes [3].

The long-term viability of this chemical-based approach, however, is critically threatened by the rapid and adaptive evolution of insecticide resistance within mosquito populations [4]. The extensive and often intensive application of a limited repertoire of insecticide classes, not only in public health initiatives but also, and quite pivotally, within the agricultural sector, creates a powerful and persistent selection pressure. This environment selectively favors the survival and reproduction of mosquito strains possessing heritable traits that confer resistance [5]. The consequence of this selection is a major operational impediment that undermines the success of vector control programs worldwide [6]. Notably, the development of multi-insecticide resistance in Culex quinquefasciatus, a key species within the Culex pipiens complex, has been well-

documented in numerous scientific reports [7], [8]. The physiological mechanisms that mosquitoes employ to survive insecticide exposure are complex and multifaceted. They primarily involve two major strategies: enhanced metabolic detoxification and target-site insensitivity. Metabolic resistance entails the upregulation or modification of enzyme systems such as cytochrome P450 monooxygenases, glutathione S-transferases, and carboxylesterases that efficiently break down or sequester the insecticide before it can reach its target [9], [10]. Target-site insensitivity, on the other hand, arises from genetic mutations in the insecticide's binding site within the nervous system, such as the voltage-gated sodium channel or acetylcholinesterase enzyme, rendering the toxin ineffective [9]. In the specific context of Nigeria, the historical legacy of DDT use in past malaria campaigns, combined with the ongoing and intensive application of synthetic pyrethroids in agriculture particularly in the cultivation of cotton and vegetables is widely believed to be a significant driver of cross-resistance in local mosquito populations [11], [12].

Given this concerning backdrop, the continuous and vigilant monitoring of the susceptibility status of mosquito vectors is not merely beneficial but essential for implementing evidence-based vector management. It is against this imperative that the present study was conceived, with the specific objective of determining the contemporary susceptibility and resistance status of field populations of Culex mosquitoes in Minna, Niger State, Nigeria, to two insecticides currently in use: lambda-cyhalothrin, a pyrethroid, and dichlorvos, an organophosphate.

II. MATERIALS AND METHODS

A. Study Area and Mosquito Collection

The study was conducted within the Bosso Local Government Area of Minna, Niger State, Nigeria. Immature stages of Culex mosquitoes, comprising larvae and pupae, were systematically collected from a variety of natural and peri-domestic breeding habitats. These sites included stagnant ground pools, open drainage gutters, and water-filled depressions such as discarded vehicle tyre tracks. Collection was performed using standard plastic dippers or scoops. The collected samples were carefully transferred into transparent plastic containers partially filled with water from the source to minimize stress during transport to the insectary.

B. Mosquito Rearing and Colony Establishment

In the laboratory, third and fourth instar larvae were separated from debris and pooled together to promote synchronized development into adulthood. The larval cohorts were maintained in controlled environmental conditions, with temperature regulated at 26 ± 2 °C and relative humidity at 74 ± 3%. The larvae were fed a diet of finely ground commercial fish food. Upon pupation, the pupae were transferred into small containers placed inside larger screened adult mosquito cages to facilitate the emergence of adults. The newly emerged adult mosquitoes were provided with a 10% (weight/volume) glucose solution, made with distilled water, which was offered ad libitum on cotton wool pads to sustain them until testing [13]. All bioassays were conducted using 2 to 5-day-old, non-blood-fed adult female mosquitoes to ensure standardization.

C. Insecticides and Test Chemical Preparation

The insecticides used in this study were technical-grade compounds, ensuring high purity. Lambda-cyhalothrin, representing the pyrethroid class, and dichlorvos, representing the organophosphate class, were selected for testing. Analytical-grade acetone was used as the universal solvent for preparing all insecticide concentrations. The 10% glucose solution used for maintaining adult mosquitoes was prepared using distilled water.

D. Preparation of Insecticide-Impregnated Papers

The impregnation of filter papers was carried out following meticulously established methodologies [14]. In brief, standard Whatman No. 1 filter papers were trimmed to the dimensions of 12 x 15 cm, the size specified for WHO tube bioassays. Each paper was then placed on a supportive bed of pins to ensure it remained flat and allowed for even application. Pre-calculated volumes of the technical-grade insecticides were diluted in acetone to achieve the target concentrations: 0.4% and 0.7% for lambda-cyhalothrin, and 3.0% and 7.0% for dichlorvos. A precise volume of 2 ml of each insecticide-acetone solution was applied dropwise and distributed evenly across the surface of each paper using a graduated dropper, taking care to avoid pooling. Control papers were treated in an identical manner using 2 ml of pure acetone alone. All impregnated papers were then air-dried for a full 24 hours in a dark, well-ventilated

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space to allow for the complete evaporation of the solvent before being used in bioassays.

E. WHO Susceptibility Bioassay Procedure

The insecticide susceptibility bioassays were performed in strict adherence to the standard WHO tube test protocol [15]. For each insecticide concentration, four replicate tests were conducted. Each replicate involved exposing 20 to 25 adult female mosquitoes, aged 2-5 days and not having taken a blood meal, to the treated papers. Mosquitoes were gently transferred from their holding containers into the exposure tubes, which were lined with the insecticide-impregnated papers. The exposure period was set at 60 minutes, during which the number of knocked-down mosquitoes was recorded at 10-minute intervals. The assays were conducted under subdued lighting conditions. Following the one-hour exposure, all mosquitoes, including those that were knocked down and those still active, were carefully transferred back to their clean holding tubes. They were then provided with a 10% glucose solution on a cotton wool pad and held for a 24-hour recovery period, after which the final mortality was scored. Mosquitoes were considered dead if they were unable to stand or coordinate movement.

F. Data Analysis

The percentage mortality was calculated for each of the four replicates per concentration. In instances where mortality in the control group fell between 5% and 20%, the test mortality rates were adjusted using Abbott's formula to correct for natural death unrelated to the insecticide [15]. Any assay where control mortality exceeded 20% was considered invalid and the data were discarded. The corrected mortality figures were then interpreted based on WHO guidelines: a population is classified as susceptible if mortality is 98% or higher, suspected of having resistance if mortality is between 90% and 97% (requiring further verification), and confirmed as resistant if mortality is below 90% [15]. The data were subjected to statistical analysis using one-way analysis of variance (ANOVA), and where significant differences were found, a post-hoc test was applied for multiple comparisons. All results are presented as the Mean ± Standard Error of the Mean (SEM), and a p-value of less than 0.05 was deemed statistically significant.

III. RESULTS

A. Susceptibility Profile at Low Insecticide Concentrations

The bioassay results for the low concentrations of the insecticides revealed a clear pattern of reduced efficacy against the field-collected Culex population. As presented in Table I, the control group exhibited minimal mortality throughout the one-hour exposure period, culminating at 4.00 ± 0.98%, thereby validating the experimental conditions. Exposure to the 0.4% concentration of lambda-cyhalothrin produced a gradual, time-dependent increase in mortality. The knockdown began at 5.12% after 10 minutes and progressively rose to a final mortality of 52.63 ± 5.42% at the 60-minute mark. In contrast, the 3.0% concentration of dichlorvos induced a more rapid and pronounced initial knockdown, achieving 23.18% mortality within the first 10 minutes. However, this rate of increase slowed over time, culminating in a final one-hour mortality of 66.66 ± 3.83%. Crucially, the mortality rates for both insecticides at these lower concentrations were significantly lower than the 98% threshold established by the WHO for defining susceptibility, providing clear initial evidence of resistance in the tested mosquito population.

TABLE I: PERCENTAGE MORTALITY OF CULEX MOSQUITOES EXPOSED TO LOW CONCENTRATIONS OF INSECTICIDES OVER 1 HOUR

Parameters	Time (minutes)					
	10	20	30	40	50	60
Control dead	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.00±0.98	4.00±0.98
Control alive	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	96.00±2.32	96.00±2.32
0.4 % dead LC	5.12 ± 2.75[a]	14.20±3.30[a]	21.77±2.34[a]	29.42±2.26[a]	41.69±5.35[a]	52.63±5.42[a]
0.4 % alive LC	94.88±2.75[a]	85.80±3.30[a]	77.90±2.00[a]	67.42±3.64[a]	58.31±5.35[a]	47.37±5.42[a]
3.0 % dead Ds	23.18±3.83[b]	31.88±1.45[b]	43.48±2.51[b]	56.52±2.51[b]	64.35±3.29[b]	66.66±3.83[b]
3.0 % alive Ds	76.82±3.83[b]	68.12±1.45[b]	56.52±2.51[b]	43.48±2.51[b]	33.33±3.83[b]	28.99±3.83[b]

Values are Mean ± SEM of three replicates. Means with different superscript letters within a column are significantly different ($p<0.05$). LC = lambda-cyhalothrin; Ds = Dichlorvos.

B. Efficacy at High Diagnostic Concentrations

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When the same mosquito population was exposed to the higher, recommended diagnostic concentrations, a markedly different susceptibility profile emerged. The results, detailed in Table II, demonstrate that both 0.7% lambda-cyhalothrin and 7.0% dichlorvos were highly effective. The knockdown was rapid and sustained, leading to final mortality rates of 98.48 ± 1.51% and 98.67 ± 1.33%, respectively, after the one-hour exposure. These values meet and marginally exceed the WHO's 98% susceptibility benchmark. Statistically, the mortality at these high concentrations was significantly greater than that observed at the corresponding low concentrations, underscoring the dose-dependent nature of the insecticidal effect. According to the standard WHO classification criteria, the Culex population from Minna would therefore be classified as susceptible to these insecticides when tested at these specific diagnostic doses.

TABLE II: PERCENTAGE MORTALITY OF CULEX MOSQUITOES EXPOSED TO HIGH CONCENTRATIONS OF INSECTICIDES OVER 1 HOUR

Insecticides	Time (minutes)					
	10	20	30	40	50	60
Control dead	0.00±0.00	0.00±0.00	0.00±0.00	4.16±1.09	8.33± 1.45	8.33±1.45
Control alive	100.00±0.00	100.00±0.00	100.00±0.00	95.83±2.33	91.66±3.47	91.66±3.47
0.7 % dead LC	18.03±2.4^{b}	31.81±2.6^{b}	46.97±1.5^{c}	59.09±2.6^{b}	77.27±2.62^{bc}	98.48±1.51^{c}
0.7 % alive LC	81.97±4.01^{a}	68.19±2.62^{a}	53.03±1.52^{b}	40.91±2.62^{a}	22.73±2.62^{a}	1.52±1.51^{a}
7.0 % dead Ds	30.67±3.5^{b}	42.67±3.53^{c}	53.33±5.8^{c}	69.33±3.5^{c}	80.00±2.30^{c}	98.67±1.33^{c}
7.0 % alive Ds	69.33±3.53^{b}	57.33±6.11^{b}	46.67±5.81^{b}	30.67±3.52^{b}	20.00±2.30^{a}	1.33±1.33^{a}

Values are Mean ± SEM of three replicates. Means with different superscript letters within a column are significantly different ($p<0.05$). LC = lambda-cyhalothrin; Ds = Dichlorvos

C. Delayed Mortality Assessment after 24-Hour Recovery

The final mortality assessment, conducted after a 24-hour recovery period with access to glucose, provided deeper insight into the resilience of the mosquito population. As illustrated in Figure 1, the high concentrations of both insecticides ultimately achieved complete (100%) mortality, confirming the eventual lethality of the diagnostic doses. However, the situation was different for the low concentrations. The 0.4% lambda-cyhalothrin treatment resulted in a 24-hour mortality of 87.54 ± 2.65%, while the 3.0% dichlorvos treatment resulted in 84.06 ± 3.85% mortality. This failure to achieve complete lethality after a full day, despite the initial exposure, indicates that a substantial proportion of the mosquitoes possessed a sufficient level of tolerance to not only survive the initial knockdown but also to recover from its effects. This finding strongly suggests the presence of a developing resistance mechanism within a segment of the population

IV. DISCUSSION

This study provides a critical and nuanced assessment of the insecticide susceptibility status of Culex mosquitoes in Minna, Nigeria. The central finding is one of concentration-dependent susceptibility, where the population remains nominally susceptible to high diagnostic doses but exhibits significant and concerning resistance to lower concentrations. This dichotomy presents a complex scenario for public health officials and vector control programs.

The superior initial knockdown and one-hour mortality observed with the low concentration of dichlorvos (an organophosphate) compared to lambda-cyhalothrin (a pyrethroid) is a noteworthy observation. This could be partially attributed to the distinct mode of action of organophosphates, which act as acetylcholinesterase inhibitors and can cause a faster paralytic effect [16]. However, a more plausible and significant explanation lies in the well-documented and widespread development of pyrethroid resistance in mosquito populations across West Africa [8], [12]. The historical use of DDT and the massive, ongoing application of pyrethroids in the agricultural sector, particularly for protecting crops like cotton, have created an intense selection pressure. This has favored the survival and proliferation of mosquitoes with genetic mutations and metabolic adaptations that confer cross-resistance to pyrethroids like lambda-cyhalothrin [11], [17]. Our results are consistent with findings from other regions, such as the work by Yadouléton et al. [18] in Benin, who reported robust pyrethroid resistance in Culex

quinquefasciatus. The fact that the high diagnostic concentrations of both insecticides achieved the WHO susceptibility threshold is operationally reassuring. It indicates that, for the time being, these chemicals can still be effective in vector control operations within the study area when applied at the correct, recommended concentrations. This finding classifies the population as "susceptible" in a strict, binary interpretation of the WHO guidelines [15]. However, the most critical finding of this study is the clear evidence of resistance at the lower concentrations, a signal that is amplified by the 24-hour mortality data. The failure of these concentrations to achieve 100% mortality after the recovery period is a definitive warning sign. It indicates the presence of a sub-population with a significantly higher tolerance. While a degree of natural variation in susceptibility, known as vigor tolerance, can exist in any population [19], the consistent survival of a notable fraction of mosquitoes under insecticide pressure in a field-collected sample strongly points toward the emergence of heritable, genetic resistance. The ability of these individuals to withstand a one-hour exposure and subsequently recover suggests the activation of robust physiological resistance mechanisms. These likely involve enhanced metabolic detoxification, where enzyme systems such as cytochrome P450 monooxygenases or carboxylesterases are overexpressed to break down the insecticide before it can act on its target [9], [20]. The possibility of target-site insensitivity, such as the kdr mutations, cannot be ruled out and warrants further molecular investigation.

V. CONCLUSION

In summary, this study concludes that the Culex mosquito population in Minna, Nigeria, currently demonstrates susceptibility to the high diagnostic concentrations of lambda-cyhalothrin and dichlorvos. However, the unambiguous evidence of resistance at lower concentrations, confirmed by the incomplete 24-hour mortality, signals an urgent and pressing need for proactive insecticide resistance management strategies. Over-reliance on these particular insecticide classes, especially pyrethroids, is a risky strategy that could accelerate the development of full-blown, operational resistance. To safeguard the future efficacy of vector control in the region, we strongly recommend the immediate implementation of a robust and continuous resistance monitoring program. Furthermore, public health authorities should strategically plan for the rotation of insecticide classes with divergent modes of action and seriously pursue the integration of effective non-chemical control methods. Such a multi-pronged approach is essential to sustain the gains made in disease prevention and to avert a potential resurgence of mosquito-borne illnesses.

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AUTHORS' CONTRIBUTIONS
JTV conceived the study. JTV and TJO participated in the design and quality assessment of the study. EK and TJO took part in the selection and extraction of the plant samples used. AOO and TJO reconstituted the chemicals/reagents used, as well as carried out the experiment, with the assistance of the Laboratory Technologists. HAA carried out the statistical analysis and drafted the manuscript, with significant input from TJO. All authors proofread the manuscript and made inputs. All authors approved the final manuscript for publication.

COMPETING INTERESTS
The authors declare that they have no competing interests.

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