*Tropical Biomedicine* 43(2): 231-237 (2026)
https://doi.org/10.47665/tb.43.2.014


RESEARCH ARTICLE

# Resistance profiling of *Aedes aegypti* using intensity concentrations enhances the evaluation of insecticide effectiveness for vector control

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**ARTICLE HISTORY**

Received 11 February 2026
Revised 29 May 2026
Accepted 30 May 2026
Published: 30 June 2026

**ABSTRACT**

The detection of insecticide resistance is pivotal for characterising the phenotypic resistance of field-collected *Aedes aegypti* populations, enabling the early identification of resistance trends and safeguarding the efficacy of chemical interventions in vector control programmes. Although the World Health Organization (WHO) adult tube test remains the standard screening method, its reliance on binary classifications of susceptibility and resistance limits the ability to compare the magnitude of resistance among different insecticides. To address this limitation, probit analysis was performed on adult mortality data obtained from a field-collected strain exposed to WHO-recommended discriminating and intensity concentrations. $LC_{50}$ values were subsequently normalised using a concentration ratio (CR) framework, allowing resistance levels to be quantified and compared across insecticides. The relationship between the CR and slope parameters derived from probit analysis enabled the inference of resistance magnitude within field populations. The results demonstrated that pirimiphos-methyl retained high efficacy against the tested populations, whereas substantial intra-population variation in resistance intensity was observed among pyrethroids and malathion. Lambda-cyhalothrin exhibited the highest level of resistance, followed by permethrin and alpha-cypermethrin. These findings highlight the value of data-driven approaches for enhancing the interpretation of insecticide resistance profiles and providing a more comprehensive assessment of resistance status. By utilising mortality data generated from WHO-recommended discriminating and intensity bioassays, resistance can be evaluated beyond the binary outcomes of standard susceptibility tests, thereby supporting more informed decisions on insecticide rotation and resistance management in vector control programmes.

**Keywords:** Dengue; pyrethroids; intensity resistance; WHO adult assay; *Aedes aegypti*.

## INTRODUCTION

Dengue poses a significant global health threat, with over five million cases and more than 5,000 deaths reported in 2023 alone, marking one of the highest incidence levels in recent history and affecting over 80 countries across all Africa, Americas, South-East Asia, Western Pacific and Eastern Mediterranean Regions globally (WHO, 2023). This growing burden is further compounded by the global reliance on a limited arsenal of insecticides for adult mosquito control, particularly pyrethroids, which remain the most widely used and effective class despite the concerns about resistance and the absence of newly approved adulticides over the past 15 years (WHO, 2011).

In Malaysia, the dengue burden continues to intensify, driven by factors such as the expanding distribution of *Aedes* mosquitoes and climate change. The warm temperatures, high humidity and annual rainfall ranging from 2,000 to 4,000 mm (Jamaludin & Abdul Aziz, 2007) creates ideal environment for *Aedes* mosquito breeding and accelerating mosquito life cycles and enhances viral replication, thereby increasing the likelihood of dengue transmission (Hii *et al*., 2016). The heightened risk is evident in the recent rise in dengue cases, with 122,423 reported cases in 2024 (MOH, 2024). This marks a notable increase from 2023, during which 117 dengue-related deaths were recorded (MOH, 2023, 2024). Among Malaysia's 13 states and 3 federal territories, Selangor has consistently recorded the highest number of dengue cases, accounting for approximately half of the national total over the past three years (MOH, 2022a, 2023, 2024).

Due to rapid urban development and high population density, Selangor continues to experience a disproportionately high dengue burden, particularly the Klang Valley, which encompasses Kuala Lumpur and surrounding districts of Petaling Jaya, Shah Alam, Subang Jaya, Klang, Gombak, and Hulu Langat (Raihan *et al*., 2023). This region has also experienced water supply disruptions, often prompting residents to store water in containers. Such practices create ideal breeding grounds for mosquitoes, leading to increased

mosquito density (Hemme *et al*., 2009). In densely populated urban settings, especially in high-rise buildings, the risk of mosquito-human contact is further heightened. Collectively, these factors contribute to the rising incidence of dengue in residential communities (Gubler, 2011).

In the absence of specific antiviral treatments and a fully effective dengue vaccine, vector control remains the primary strategy to curb dengue transmission. Current interventions target both larval and adult mosquito populations through chemical and biological methods (Tham, 1993). Chemical interventions, such as larviciding and adulticiding, remain the mainstream strategies in vector control. More recently, the release of *Wolbachia*-infected mosquitoes has emerged as an innovative tool in dengue control programs (Hoffmann *et al*., 2024; Nazni *et al*., 2019; Cheong *et al*., 2023). Other interventions include the use of *Bacillus thuringiensis israelensis* (*Bti*) for larviciding (Lee *et al*., 1997; Yap *et al*., 2002), community engagement in reducing vector habitats (Azmawati *et al*., 2013), and the deployment of mosquito traps to suppress mosquito populations and interrupt transmission (Shahar *et al*., 2022). Ultimately, the success of dengue prevention relies on an integrated vector control strategy, where all interventions are directed toward reducing mosquito populations (WHO, 2004). Although various methods have been implemented, chemical intervention remains a primary approach in dengue control (Wilson *et al*., 2020). Nevertheless, the effectiveness of this strategy may be jeopardised by the development of insecticide resistance in mosquito vectors (Ranson *et al*., 2010). As resistance increases, the effectiveness of chemical control may be compromised, potentially leading to operational failure and increased disease transmission.

Primarily, the use of pyrethroids has increased in many dengue-endemic countries, particularly for adult mosquito control targeting *Aedes* species (van den Berg *et al*., 2021; WHO, 2011). Pyrethroid resistance among *Aedes* species has become widespread across many South-East Asian regions (WHO, 2025). Malaysian *Ae. aegypti* strains were reported exhibiting higher levels of resistance to insecticides pyrethroids (Loke *et al*., 2012; Ishak *et al*., 2015; Rosilawati *et al*., 2017; Siti-Futri *et al*., 2020; Rasli *et al*., 2018, 2021). Notably, the majority of resistant *Aedes* populations have been identified in dengue hotspot areas, highlighting the potential challenges in controlling cases using insecticide pyrethroids for space spray interventions.

Resistance profiling via the World Health Organization (WHO) bioassay at the discriminating concentration (DC) constitutes a standard methodology for evaluating insecticide resistance within wild-caught *Aedes* populations across endemic regions (WHO, 2022). Although this approach offers a pragmatic and standardised framework for reporting resistance, the resulting adult mortality data are inherently binary, categorising mosquito populations as either resistant or susceptible based on a singular diagnostic concentration. Consequently, this methodology may fail to encompass the nuanced variations in susceptibility, particularly in populations demonstrating cross-resistance to various pyrethroid insecticides. Furthermore, the identification of resistance through adult assays at the DC level primarily serves as an indicator of resistance management preparedness rather than as evidence of operational insecticide failure. This is attributable to the fact that the DC is typically calibrated at twice the lethal concentration required to achieve total mortality in a susceptible laboratory strain. Accordingly, an exclusive reliance on WHO adult assays at the DC level may obscure the true complexity and magnitude of resistance within field populations. Integrating phenotypic data from adult DC assays with intensity assays facilitates a more precise estimation of resistance levels and provides comprehensive insights into the operational implications of resistance within vector populations (Bagi *et al*., 2015; Dusfour *et al*., 2019).

To enhance the interpretation of insecticide resistance across pyrethroid and organophosphate compounds with varying discriminating concentrations (DCs), this study establishes a standardised analytical framework incorporating phenotypic responses of field strains derived from WHO susceptibility and intensity bioassays via probit regression analysis. The ratio of $LC_{50}$ estimates relative to their respective WHO DCs enables a quantitative assessment of resistance magnitude across diverse insecticides. For instance, pyrethroids exhibit substantial variation in intrinsic potency; earlier-generation compounds, such as permethrin, require higher DCs than later-generation pyrethroids like deltamethrin. Subsequently, slope analysis of the log-probit response curves was correlated with the concentration ratio (CR) comprehensive insights into sample response variations, effectively characterising the uniformity or heterogeneity of resistance profiles within *Aedes* populations.

## MATERIALS AND METHODS

### Mosquito strains

Two different strains of *Ae. aegypti* were used in this study, which were the laboratory-susceptible strain maintained at filial generation F1106 and the field-collected strain reared to F2 generation. Both strains were maintained in the Arthropod-Containment Laboratory (ACL) of the Medical Entomology Unit at the National Institute of Health, Malaysia with the laboratory strain serving as the control group and the field-collected strain being used for insecticide resistance profiling.

### *Aedes* sampling

Field-collected *Aedes* mosquitoes were sampled in a dengue outbreak locality (2.96706° N, 101.66904° E), a pre-selected site with a recorded history of persistent dengue transmission and recognised as an endemic area. Ovitraps were deployed for mosquito sampling, a method proven effective in capturing *Aedes* mosquitoes even at low population densities, making it preferable over larval survey for field sampling (Lee, 1992). After 5 days in the field, during which the ovitraps were expected to attract female mosquitoes to lay eggs, the ovitraps were retrieved and brought to the laboratory for species identification. Mosquito species were identified based on established taxonomic characteristics of *Aedes* mosquitoes (Christophers, 1960). Identified *Ae. aegypti* samples were reared in ACL following the Standard Operating Guidelines of the Medical Entomology Unit. The colony was maintained until the $F_2$ and $F_3$ generations for subsequent bioassays.

### Insecticides

Insecticides-impregnated papers were obtained from the Vector Control Research Unit (VCRU), Universiti Sains Malaysia (USM), Penang. The selected insecticides include pyrethroids (permethrin, deltamethrin, alpha-cypermethrin, and lambda-cyhalothrin), and organophosphates (malathion and pirimiphos-methyl). The concentrations of the respective insecticides are based on the DC described by the WHO (2022), with the IC of the respective insecticides at five- and ten-fold the DC (Table 1).

**Table 1.** Concentrations of pyrethroid and organophosphate insecticides

| | Insecticides | DC | Five-fold the DC | Ten-fold the DC |
|---|---|---|---|---|
| Pyretroid | Alpha-cypermetrin | 0.05% | 0.25% | 0.5% |
| | Deltametrin | 0.03% | 0.15% | 0.3% |
| | Lambda-cyhalotrin | 0.05% | 0.25% | 0.5% |
| | Permetrin | 0.4% | 2.0% | 4.0% |
| Organophosphate | Pirimiphos-methyl | 0.17% | 0.85% | 1.7% |
| | Malathion | 1.5% | 7.5% | 15% |

**Insecticide resistance profiling via WHO adult assay**

Female *Ae. aegypti* mosquitoes aged 5 to 7 days and maintained on a sugar diet were used for the bioassay, following the WHO guidelines (WHO, 2022). A total of six tubes marked with a green dot were prepared, with the inner surfaces lined with blank paper, referred to as holding tubes. Additionally, four tubes marked with a red dot, with the inner surfaces lined with insecticide-treated paper, were designated as treatment tubes, while two tubes marked with a yellow dot, with the inner surfaces lined with control paper, served as control tubes. This setup was replicated according to the number of insecticides being tested. All papers affixed to the inner surfaces of the tubes were secured using copper or silver rings to ensure the papers remained firmly in place. For each tube marked with a green dot, 25 *Ae. aegypti* mosquitoes were introduced, resulting in a total of 150 mosquitoes per experimental set. After the mosquitoes were introduced into the green dot-marked tubes, they were left to acclimatise for one hour to allow them to adjust to the environment within the tubes. Subsequently, the mosquitoes were gently blown into the red and yellow tubes. The mosquitoes were exposed to the insecticide paper in the tubes marked with a red dot, while those in the yellow dot-marked tubes were exposed to the control paper. The exposure period lasted for one hour. Following the exposure, the mosquitoes were gently blown back into the tubes marked with a green dot for a 24-hour recovery period, post-exposure to the insecticide. Mortality was then monitored, recorded, and analysed to determine the percentage of mortality. If mortality in the yellow dot-marked control tubes exceeded 20%, the experiment was repeated. If mortality in the control tubes ranged from 5% to 20%, a correction to the mortality percentage observed in the insecticide-treated tubes was applied. This corrected mortality was perform using Abbott's formula (Abbott, 1925).

**Data analysis**

Mortality data for both DC and IC were recorded 24 hours after exposure to the tested insecticides. Mortality for each insecticide was determined using four replicate tubes per assay. The binomial outcomes of resistance and susceptibility were interpreted according to the WHO guidelines (WHO, 2022), where mortality greater than 98% indicated susceptibility, while mortality less than 90% signified resistance.

Probit analysis was employed to determine $LC_{50}$ based on the relationship between log-transformed insecticide concentration and observed mosquito mortality. Mortality data were converted into probit units prior to analysis to linearise the concentration–response relationship. The goodness-of-fit of each probit was assessed using the chi-square ($\chi^2$), which compares observed and expected mortality values. A non-significant $\chi^2$ value ($p > 0.05$) indicates an adequate model fit to the data.

The CR was then calculated as the ratio of $LC_{50}$ to the corresponding DC for each insecticide. The CR served as a comparative metric, calculated by dividing the $LC_{50}$ value by the corresponding DC for each insecticide, to standardised the resistance magnitude prior to compare resistance across compounds. Additionally, correlation analysis between CR values and slope parameters was performed to evaluate the relationship between resistance intensity and the heterogeneity of population response.

**Phenotypic response matric classification**

To evaluate the resistance characteristics of field mosquito populations across all tested insecticides, a two-dimensional phenotypic response matrix was constructed by integrating the CR and the Probit regression slope (β). The integration of these two parameters enabled simultaneous assessment of resistance intensity and population response structure. In general, higher slope values indicate a more homogeneous response to insecticide exposure, whereas lower slope values reflect greater heterogeneity in individual tolerance levels within the population. Rather than applying fixed numerical thresholds, the CR–β matrix was interpreted comparatively across insecticides based on their relative positioning within the four quadrants of the two-dimensional distribution. Within this framework, insecticides in the upper-left quadrant (low CR, high β) indicate low resistance and a uniform susceptible response. Those in the lower-right quadrant (high CR, low β) show high resistance and significant phenotypic variation, suggesting strong selection pressure and diverse tolerance levels. The upper-right quadrant (high CR, high β) identifies populations with high resistance and uniform responses, indicating a more established resistance. Finally, the lower-left quadrant (low CR, low β) shows low resistance but high heterogeneity, potentially marking an early stage of resistance where both tolerant and susceptible individuals are present.

## RESULTS

**Phenotypic susceptible profile across diagnostic and intensity concentrations**

Ideally, a susceptible population would exhibit over 98% mortality in response to the insecticide at DC. In this study, the field-collected *Ae. aegypti* exhibit high phenotypic resistance to all tested pyrethroid and organophosphate insecticides. At the standard WHO bioassay at DC, absence of adult mortality was recorded for alpha cypermethrin (0.05%), deltamethrin (0.03%), lambda-cyhalothrin (0.05%), permethrin (0.4%), pirimiphos-methyl (0.17%) and malathion (1.5%) (Figure 1A). Exposure to higher intensity concentrations (IC) resulted in a concentration-dependent increase in mortality rates (Figure 1B). However, at at five- and ten-fold the DCs, the majority of the insecticides failed to restore full susceptibility, with mortality rates remaining below 98%, except for pyrimiphos-methyl which achieved 100% at both the five- and ten-fold the DC (Figure 1A). The validity of the field strain susceptibility data was assessed by comparison with a laboratory strain used as the control group, which was subjected to the same insecticide treatments. No mortality correction was performed, as the laboratory strain exposed to the tested insecticides exhibited complete mortality (Figure 1B).

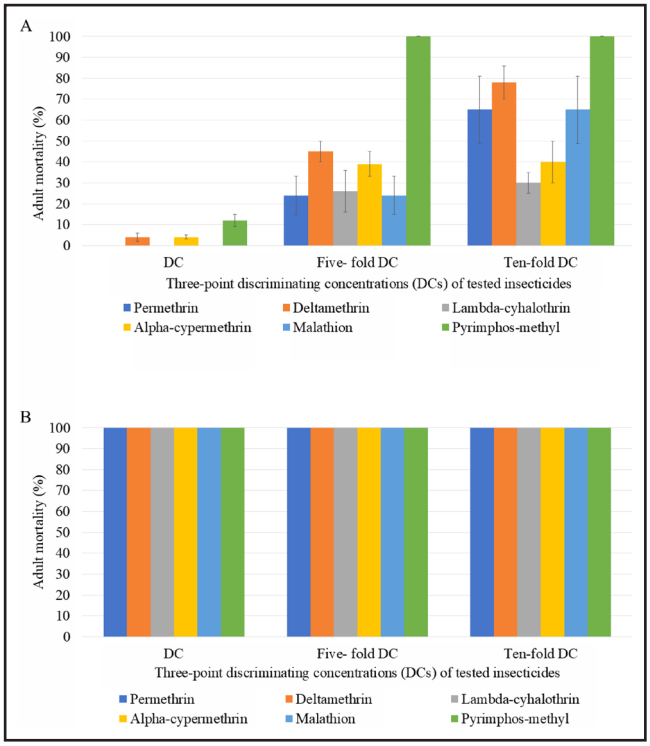


**Figure 1.** Mortality rates of *Aedes aegypti* strains exposed to pyrethroids and organophosphates at diagnostic and intensity concentrations. (A) Field strain; (B) Laboratory strain.

**Concentration-response relationships and estimation of $LC_{50}$**

The response dynamics of the field strain across the three concentration points were further characterised using probit regression analysis of the phenotypic mortality data, allowing evaluation beyond binary susceptibility classifications (Figure 2). The log transformation of insecticide concentrations linearised the sigmoidal concentration-response relationships, providing reliable estimations of the lethal concentration that required to cause 50% mortality ($LC_{50}$). Chi-square ($\chi^2$) goodness-of-fit demonstrated that all regression models adequately described the concentration-mortality relationships ($p < 0.0001$). Based on $LC_{50}$ values, the insecticides were ranked from the highest concentration required to the lowest start with permethrin (3.2%), lambda-cyhalothrin (1.9%), alpha-cypermethrin (0.66%), malathion (0.40%), deltamethrin (0.20%), and pirimiphos-methyl (0.11%) (Figure 2). However, because the baseline diagnostic doses vary substantially between compounds, these absolute $LC_{50}$ values reflect specific toxicological thresholds rather than a standardised comparison of resistance severity across the different chemical classes.

**Interaction matrix of concentration ratio (CR) and probit slope**

The range of CR values demonstrated varying magnitudes of susceptibility loss among the tested compounds, with higher CR values indicating greater resistance intensity relative to the corresponding WHO diagnostic concentrations, resulting in the highest CR being observed in lambda-cyhalothrin among pyrethroids, while malathion showed a higher CR compared to pirimiphos-methyl for organophosphates (Figure 2). Standardising the metrics altered the resistance ranking among the pyrethroids. Although permethrin displayed a higher $LC_{50}$ than lambda-cyhalothrin, lambda-cyhalothrin exhibited the highest resistance intensity using CR framework (Figure 2 and Figure 3).

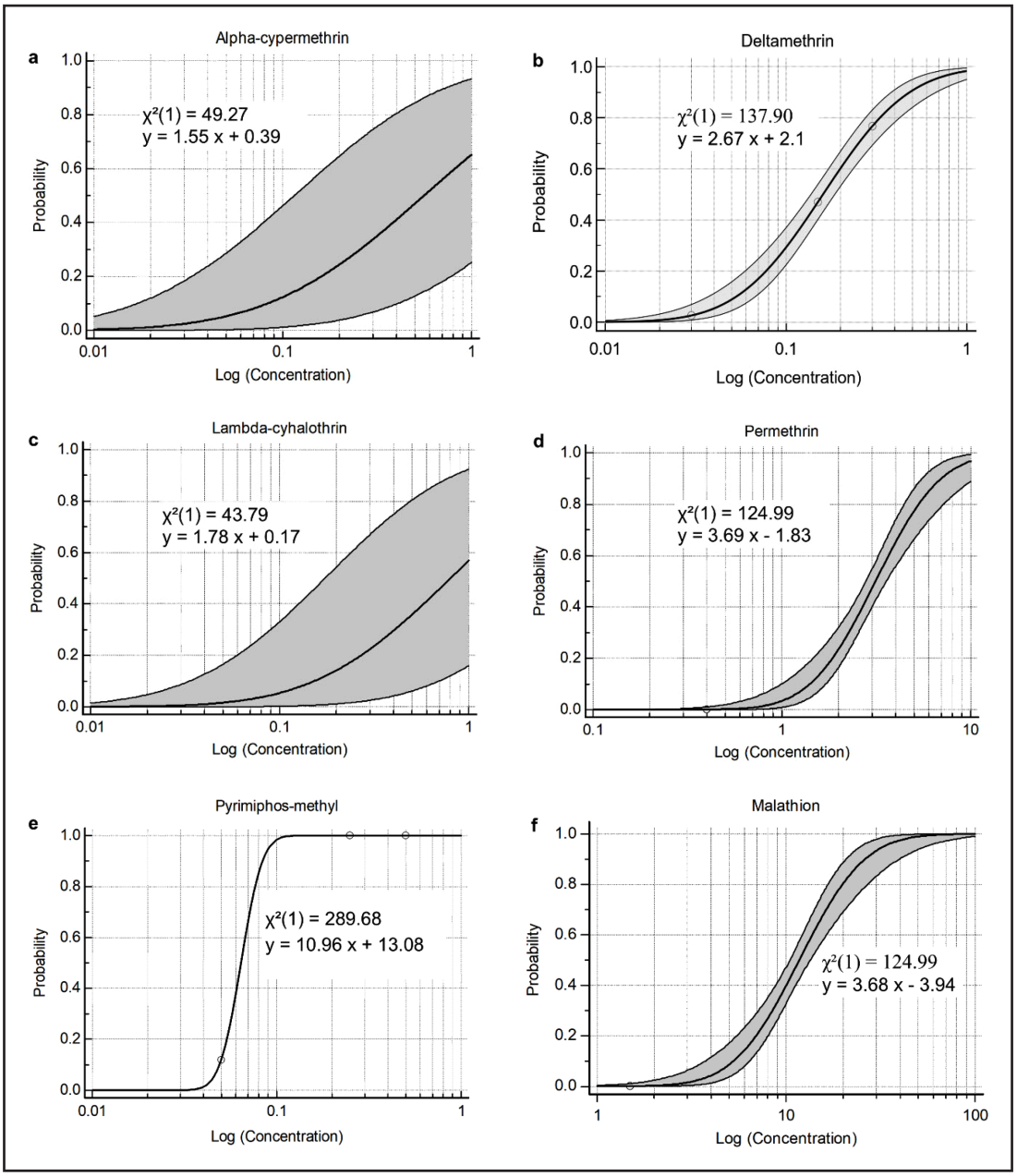


**Figure 2.** The predicted mortality from probit transformation of phenotypic response of field-collected *Aedes aegypti* against tested insecticides and log-concentration of probit analysis of alpha-cypermethrin (a) deltamethrin (b) lambda-cyhalothrin (c) permethrin (d) pirimiphos-methyl (e) malathion (f), with shaded area indicating 95% confidence interval, CI, minimum and maximum probit regression estimates.

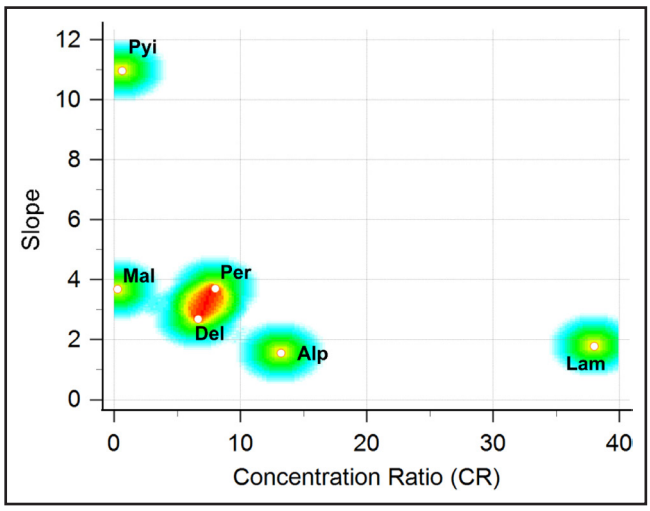


**Figure 3.** Correlation coefficient between the concentration ratio (CR) and the slope of alpha-cypermethrin (Alp), deltamethrin (Del), lambda-cyhalothrin (Lam), permethrin (Per), pirimiphos-methyl (Pyi), malathion (Mal) against the field-collected *Aedes aegypti* strain. Colours represent the density of data points with red and yellow areas indicate high-density regions where multiple data points are closely clustered together, green areas represent lower-density regions with fewer data points, light blue areas indicate the least density or areas where data points are sparse.

The regression slope values provided additional insights into the distribution of heterogeneous phenotypic response within the mosquito population (Chilcuit & Tabashnik, 1995). The overall correlation between the CR and slope matrix indicated a negative correlation ($r = -0.5182$, $p < 0.5$). The two-dimensional matrix as shown in Figure 3 showed pirimiphos-methyl represents the highest efficacy with the plot located in the upper-left quadrant, which also indicates a highly homogeneous susceptible phenotypic response of the field strain. The lower-left quadrant of the graph matrix showed an early warning of resistance in the insecticide malathion, revealing incipient heterogeneous response; even though its resistance number is still safely low as the CR is < 1, the uneven response of the test population indicates an early warning. The pyrethroid insecticides collectively clustered in the lower-right quadrant, with deltamethrin recording better efficacy than permethrin and alpha-cypermethrin, as deltamethrin recorded a lower CR than other pyrethroid insecticides required to kill 50% of the test samples. For lambda-cyhalothrin, the plot moved deeper into the quadrant with the highest CR among the pyrethroid insecticides, indicating the most highly heterogeneous resistant phenotype. None was detected in the upper-right quadrant, showing that no insecticide tested fell into the failure of insecticide intervention.

## DISCUSSION

Dengue control in high-rise buildings presents challenges due to the vertical dispersal of *Aedes* mosquitoes, which are predominantly found at lower levels but can also sustain populations on upper floors through oviposition in available breeding sites (Hamid *et al.*, 2020). In the present study, *Ae. aegypti* specimens were collected from a high-rise residential site with persistent dengue transmission for more than 60 weeks. This site is notably associated with densely populated residential areas, spans 7.6 hectares and comprises 14 blocks, each with five levels. If dengue cases are reported, chemical interventions such as fogging must be initiated within one week of notification, with repeated insecticide spraying if transmission persist (MOH, 2022b). The implementation of insecticide interventions aims to halt the transmission, leading in significant insecticides exposure in mosquito population.

Current control efforts continue to rely heavily on chemical interventions, including fogging and ultra-low volume space spraying to target adult mosquitoes, as well as larviciding to eliminate immature stages (MOH, 2022b). The reliance on chemical insecticides necessitates continuous monitoring of field mosquito populations to track resistance development and maintain intervention efficacy. Determining phenotypic resistance is often the preferred method in most countries. In Malaysia, the management of insecticide resistance involves detecting phenotypic resistance through insecticide susceptibility testing using the WHO adult bioassay, as outlined in the Ministry of Health (MOH, 2022b). These assays are conducted at designated concern sites by district health authorities. Results obtained at DC, along with recommendations for further intensity bioassays at sites with continued reports of resistance, serve as key reference information for implementing appropriate mitigation strategies such as insecticide rotation and integrated vector management (IVM), to effectively manage resistance and maintain intervention efficacy (WHO, 2004, WHO, 2022). While metabolic detoxification and target site mutations offer insights into the genetic and biochemical basis of resistance, phenotypic resistance relies on observational skills to assess insecticide effectiveness in field populations, without the need for advanced expertise in enzyme assays or molecular techniques.

However, quantifying resistance through phenotypic assays is challenging. Resistance detected via bioassay at DC reflects survival at twice the lethal dose for susceptible laboratory strains but may not confirm the presence of resistance alleles in field populations, as resistance develops gradually over time. The outcome serves as an early indicator of resistance emergence and a prompt for initiating resistance management strategies before they reach a critical level. The option to rotate insecticides within the same mode of action is not viable if the results from a single-dose test against a field strain show resistance to all tested insecticides, as observed in these findings. In this case, the field-collected strain exhibited resistance to all insecticides tested at DC (Figure 1). To address this, we extended the screening concentrations to five- and ten-fold the DC to obtain a broader resistance profile. Hence, we introduce quantitative approaches in interpreting resistance magnitude beyond the conventional analysis of susceptibility data that yields a binary outcome of either susceptible or resistant.

The present study utilises two continuous metrics which are resistance magnitude, as quantified by the CR, and population response variability, represented by the probit regression slope ($\beta$). Through the integration of these parameters into a two-dimensional phenotypic response matrix, this framework facilitates the concurrent assessment of resistance intensity and response variance within wild mosquito population subjected to insecticide selection pressure. Furthermore, the relative distribution of insecticides across the matrix quadrants offers a systematic framework for the interpretation of resistance profiles, thereby differentiating populations based on phenotypic homogeneity or heterogeneity while simultaneously considering resistance magnitude. The proposed framework provides complementary layers of information for resistance interpretation.

Firstly, concentration-response mortality curves were used to assess the mortality patterns of field strains across different insecticides. Concentration-response mortality curves demonstrated distinct mortality patterns among field-collected *Ae. aegypti* exposed to different insecticides. Variations in curve shape and slope suggested differences in phenotypic susceptibility and response dynamics across compounds (Figure 2). In this study, the probit regression analysis enabled estimation of $LC_{50}$ and slope parameters of each tested insecticides, while chi-square ($\chi^2$) goodness-of-fit statistics indicated that the regression models

adequately described the concentration–mortality relationships (Figure 1). The concentration–response patterns were sigmoidal, transformation of mortality percentages into probit units linearised the relationship, allowing more reliable estimation of toxicity parameters and facilitating comparison among insecticides (Finney, 1971). This modelling approach enhances the interpretability and statistical robustness of the data, allowing for accurate estimation of $LC_{50}$.

Secondly, $LC_{50}$ values were used to identify which insecticides showed the highest concentration require to kill the 50% of the test population. In theory, a higher $LC_{50}$ value within the same population suggests a greater degree of resistance to that insecticide. However, because each insecticide has its own unique DC, standardising resistance levels using the CR provides a more comparable assessment across tested insecticides. The magnitude of resistance was contextualised by comparing the $LC_{50}$ of field populations with the DC set by WHO (2022) for each insecticide, which serves as a reference point for distinguishing susceptibility in fully sensitive populations. The CR implemented in this study quantifies resistance by dividing the $LC_{50}$ of the field strain by the corresponding DC value, instead of comparing the $LC_{50}$ of the field strain with the $LC_{50}$ value of the laboratory strain (Kont *et al*., 2023), enabling direct comparison across insecticides. This approach provides a common reference framework based on the WHO DC, which is designed to discriminate against fully susceptible populations. In addition, the proposed framework utilises $LC_{50}$ derived from concentration–response modelling as a measure of central tendency for phenotypic resistance, while also allowing extrapolation towards concentrations associated with near-complete mortality, thereby supporting broader interpretation of resistance within the standard WHO bioassay framework.

Thirdly, we explored the relationship between CR and the slope of the concentration-response curve for each insecticide. In general, a concentration-response curve with a lower $LC_{50}$ tends to shift to the left and displays a steeper slope, indicating lower variability among susceptible individuals and suggesting that the insecticide induces faster mortality at lower concentrations. Conversely, a higher $LC_{50}$ value results in a rightward-shifted curve with a shallower slope, implying greater heterogeneity and uniform resistance in the test population and the need for higher concentrations to achieve effective mortality (Carroll & Ruppert, 1988). By correlating these two parameters, the distribution of phenotypic resistance responses can be visualised, highlighting how variations in slope may reflect differences in population heterogeneous resistance response, which correspond to varying levels of resistance intensity (Figure 3). Previous study had implemented probit regression combining $LC_{50}$ with other parameters like knockdown time, which has been reported to provide insight in resistance level (Grossman *et al*., 2018). However, in this study we correlated the CR, instead of $LC_{50}$ alone, with the slope values ($\beta$) to better characterise insecticide-specific resistance profiles within the phenotypic response matrix framework. This approach allows each insecticide to be categorised based on its combined resistance magnitude and population response variability, rather than being interpreted solely through comparative $LC_{50}$ values. Increasing resistance magnitude is generally associated with greater heterogeneity in phenotypic response. This reflects a transition from uniform susceptibility towards increasingly variable tolerance within the population across different insecticides. Field-collected *Ae. aegypti* strain exhibited uniformly high resistance to lambda-cyhalothrin, permethrin, and alpha-cypermethrin. For deltamethrin, a moderate level of resistance was observed, accompanied by a more heterogeneous response profile, similar to that observed for malathion (Figure 3). In contrast, pirimiphos-methyl demonstrated a relatively homogeneous susceptibility pattern in the tested population, indicating a distinct insecticide-specific response profile within the overall resistance landscape (Figure 3).

This study was based solely on phenotypic resistance data and did not incorporate information on the underlying resistance mechanisms, highlighting the need for further investigation into the roles of allelic expression and metabolic enzyme activity in driving resistance. Future work should priorities establishing insecticides resistance thresholds that are evidence-based and validated using real-time field sample representative of the target population. Furthermore, the coexistence of uniform and heterogeneous resistance patterns may contribute to the differential survival response to various insecticides, underscoring the complex dynamics underlying mosquito responses to pyrethroid exposure across varying resistance levels.

Employing broader screening in resistance assessment is crucial for capturing the full spectrum of resistance across pyrethroid insecticides, many of which remain widely use in current vector control program. Previous studies in Malaysia have largely focused on only one or two pyrethroids (Rasli *et al*., 2025). The findings of this study demonstrate that broader evaluation provides greater insight into the differential toxicity and resistance responses among compounds within the same insecticide class. The inclusion of multiple pyrethroids also enables the identification and comparative ranking of insecticides with relatively higher toxicity among the tested compounds.

This study further improves the sensitivity of resistance interpretation by emphasising the relationship between insecticide concentration and mosquito mortality through the incorporation of phenotypic response data derived from IC testing. Evaluating resistance phenotypes across varying IC levels offers a clearer understanding of resistance magnitude, providing more meaningful insights than assessments based solely on a single diagnostic concentration (Khoa & Kont, 2017; Burgess *et al*., 2020; Kont *et al*., 2023). In contrast, standard DC assays, which rely on a single predetermined concentration, yield only binary outcomes and may overlook variations in resistance intensity. Consequently, robust monitoring the magnitude of insecticide resistance requires long-term surveillance and repeated sampling to accurately track resistance development over time (Penilla-Navarro *et al*., 2024). In this context, probit regression provides a useful quantitative framework for predicting resistance magnitude and assessing variations in susceptibility within mosquitoe populations across different insecticides.

## ACKNOWLEDGEMENT

We would like to express our gratitude to the Director General of Health Malaysia for granting permission to publish this paper, and to the Director of the Institute for Medical Research, National Institute of Health, Malaysia, for his continued support.

**Conflict of Interest**

The authors declare there is no conflict of interest.

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