Tropical Biomedicine 43(1): 90-102 (2026)
https://doi.org/10.47665/tb.43.1.012

RESEARCH ARTICLE

Evaluation of pyriproxyfen against *Aedes aegypti* and *Aedes albopictus* from Penang, Malaysia

Mohamad, N.A.[1], Ishak, I.H.[1,2], Hasan, H.A.[1,2*]

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
[2]Vector Control Research Unit, Universiti Sains Malaysia, 11800 Penang, Malaysia
*Corresponding author: hadura@usm.my

ARTICLE HISTORY

Received: 17 August 2025
Revised: 9 January 2026
Accepted: 13 February 2026
Published: 31 March 2026

ABSTRACT

Aedes aegypti and *Aedes albopictus* mosquitoes are the primary vectors of tropical diseases, including dengue fever (DHF), chikungunya, and Zika, particularly in tropical regions such as Malaysia. Vector control efforts are now increasingly focused on biological and environmentally friendly approaches, one of which is using insect growth regulator (IGR)-based insecticides such as pyriproxyfen. Pyriproxyfen works by disrupting the metamorphosis process of mosquito larvae into adults without causing direct death, but it is effective in inhibiting the emergence of adult mosquitoes by more than 90%, depending on the dose and environmental conditions. This study evaluated the concentration-dependent effects of pyriproxyfen on laboratory and field populations of *Aedes* spp. Laboratory strains exhibited 100% adult mortality at 1.04 ppm (*Ae. aegypti*) and 1.08 ppm (*Ae. albopictus*), whereas field populations required 1.15 – 1.71 ppm depending on locality. LC_{50} values were lowest in laboratory strains (0.267 ppm for *Ae. aegypti* and 0.236 ppm for *Ae. albopictus*), while field strains ranged from 0.326 to 0.571 ppm. LC_{95} values followed a similar trend, with 1.026 ppm (*Ae. aegypti*) and 1.107 ppm (*Ae. albopictus*) in laboratory strains. Inhibition of emergence (IE%) was high in laboratory strains, reaching 97.89% at 0.50 ppm in *Ae. aegypti* and 63.54% at 0.50 ppm in *Ae. albopictus*. Field populations required higher concentrations for similar suppression, with IE% ranging from 17.68 – 98.23% in *Ae. aegypti* and 13.14-98.24% in *Ae. albopictus*, peaking at 1.50 ppm. From this finding, it is suggested that pyriproxyfen effectively inhibits adult emergence in both laboratory and field populations, with laboratory strains more sensitive at lower concentrations and field strains responding at higher doses. These results provide strong evidence for pyriproxyfen as an effective, adaptive, and sustainable component of integrated vector management in Malaysia.

Keywords: *Aedes*; insect growth regulator; pyriproxyfen.

INTRODUCTION

The *Aedes* genus comprises of several medically significant mosquito species involved in disease transmission to humans, with *Aedes aegypti* and *Aedes albopictus* being the most well-known examples. *Aedes aegypti* transmits dengue, chikungunya, and Zika viruses, which are its primary vectors. It can thrive and spread widely across tropical and subtropical regions, especially in cities and densely populated areas. Its anthropophilic behaviour patterns and ability to reproduce in artificial environments make it one of the most significant species for arboviral disease transmission (Moura *et al*., 2023). On the other hand, *Ae. albopictus*, or the 'Asian tiger mosquito', is also a major vector for the same diseases but generally has a wider distribution, being present in rural and highly vegetated regions. With distinctive morphological characteristics, such as the black and white stripes on its legs, it is easy to recognise in the field (Campos *et al*., 2020).

In Malaysia, both *Ae. aegypti* and *Ae. albopictus* possess complementary but different ecological distributions. *Aedes aegypti* are commonly found in densely populated urban areas, while *Ae. albopictus* has a broader range in suburban and rural areas (Mahmud *et al*., 2025). Several studies indicate that in areas such as Selangor, a shift in dominance from *Ae. aegypti* to *Ae. albopictus* is occurring, attributed to the high ecological adaptability of the latter species to environmental changes (Abdullah *et al*., 2025). Both species are also active in several states, including the Federal Territories of Kuala Lumpur and Putrajaya, Sabah, Penang, and Kedah, which perennially experience high dengue incidence (Cheong *et al*., 2023).

Malaysia's constant humid and hot tropical environment supports the continuous life cycle of *Aedes* mosquitoes. Urbanisation, as well as poor water and waste infrastructure, has created many potential breeding sites, such as abandoned containers, gutters, and water standing in housing areas. This promotes the breeding

of *Aedes* mosquitoes, which can produce eggs in standing water as small as a bottle cap (Sabar *et al*., 2023). *Aedes* eggs are also highly resistant to desiccation, lasting for up to eight months in dry conditions, and easily hatching once re-exposed to water. These are all characteristics that make *Aedes* mosquitoes highly resistant to environmental stress and seasonality and are largely responsible for dengue transmission in Malaysia. Presently, ongoing vector surveillance and control measures are required to prevent dengue outbreaks from spreading (Nazni *et al*., 2025).

Current vector control measures primarily rely on the use of insecticides and community clean-up campaigns, which have had limited impact on dengue control. Several shortcomings, including cost, delivery challenges, insecticide resistance, sustainability issues, and environmental safety concerns, impede the effectiveness of these interventions (Buhler *et al*., 2019). While insecticides remain a primary tool for controlling vector populations, their use is associated with high costs and potential adverse effects on human health and the environment. Moreover, extensive insecticide application often leads to resistance development in vector species, diminishing the efficacy of these chemicals (Gan *et al*., 2021). Given these challenges, there is a pressing need for cost-effective, environmentally friendly, and safe alternatives for vector control. The World Health Organisation emphasises the importance of integrated vector management strategies, integrating chemical, biological, and environmental interventions to achieve sustainable control of mosquito populations (WHO, 2020).

Innovative approaches, such as the release of genetically modified mosquitoes and the use of biological control agents, are under exploration to address the limitations of traditional methods and enhance the effectiveness of dengue prevention efforts (Achee *et al*., 2015). The lack of a universally effective dengue vaccine necessitates alternative strategies to control the disease's transmission. While two vaccines, Dengvaxia® (CYD-TDV) by Sanofi Pasteur and Qdenga® (TAK-003) by Takeda, have been licensed, their availability and efficacy are limited (Thomas, 2023). This situation highlights the need for alternative methods and cost-effective, environmentally friendly chemicals that are safer for non-target organisms to enhance vector control and management.

Insect growth regulator (IGR), such as pyriproxyfen, is a promising tool for controlling *Aedes* mosquitoes. Pyriproxyfen belongs to a group of insecticides, used specifically to prevent the growth and development of *Aedes* mosquitoes, including *Ae. aegypti* and *Ae. albopictus* (Moura & Corbi, 2024). Pyriproxyfen is a juvenile hormone analogue which inhibits the natural metamorphosis to pupae and adult mosquitoes. Pyriproxyfen induces developmental defects, prolongation of life stages, and death before adults (Juarez *et al*., 2021). Additionally, surviving individual mosquitoes to adulthood often display morphological abnormalities, such as wing, leg, and proboscis deformities, that affect their fertility and motility.

The effectiveness of pyriproxyfen in inhibiting the emergence of adult mosquitoes is very high, with Inhibition of Emergence (IE) of more than 70% to almost 100%, depending on the dose and environmental conditions of the area of treatment. Its effective dose ranges from 0.005 µg/L to 1 mg/L, with a common recommended concentration of 0.01 mg/L or 10 ppb, especially for third instar larvae targeting (Moura *et al*., 2023). Field evaluations also indicate that its performance remains stable under varying environmental conditions, making it suitable for routine public health operations in diverse settings (Carvalho *et al*., 2020). The two major advantages of pyriproxyfen over conventional chemical insecticides are its target insect specificity and lower resistance potential.

As this pesticide operates at the developmental level of insects, it has less impact on non-target insects and is thus more environmentally friendly (Campos *et al*., 2020). Its formulation range also allows application in a wide range of breeding habitats, including natural and artificial containers typically associated with urban mosquito proliferation. In real life, pyriproxyfen is widely used as a larvicide against water containers that bear the possibility of being the breeding ground for mosquitoes, such as flowerpots, tires, or watercourses. In addition, the technology of auto-dissemination techniques offers the potential to extend the control radius without any additional direct intervention, since adult mosquitoes infested with pyriproxyfen can spread the compound to other regions as they oviposit. Therefore, pyriproxyfen is a very valuable integrated vector management tool, especially in dengue fever endemic areas such as tropical and subtropical areas (Leles *et al*., 2024).

This study aims to investigate the effect of pyriproxyfen on *Aedes* mosquito larvae. It focuses on treatment concentrations appropriate to domestic and peri-domestic breeding sites commonly encountered in tropical regions. Through an experimental approach, this study evaluated the level of inhibition of emergence (IE), morphological changes, and potential mortality of the larvae at various ecologically relevant concentrations of pyriproxyfen. These results provide strong evidence for the optimisation of application protocols within integrated vector management frameworks. The data further demonstrate that pyriproxyfen performs consistently under ecologically relevant conditions, confirming its operational suitability in field-representative environments. The study findings can also serve as a basis for policy decisions on the use of IGR-based larvicides in the tropics.

MATERIALS AND METHODS

Mosquito culture

In this research, both laboratory and field strains of *Ae. aegypti* and *Ae. albopictus*, were utilised. The laboratory strains for these species were obtained from the Vector Control Research Unit (VCRU) at Universiti Sains Malaysia, located in Penang. Eggs on Whatman No. 1 filter paper were placed in a separate enamel tray filled with 500 ml of chlorine-free water to initiate hatching and larval development. Under controlled laboratory settings, the larvae were raised at a temperature of 28 ± 30 °C, with a relative humidity of 70 ± 10%, following a 12-hour light and 12-hour dark cycle. To promote growth, the larvae were provided with a finely blended mixture of dog biscuits, beef liver, milk powder, and yeast powder in a weight ratio of 2:1:1:1 until they reached the 3rd or early 4th instar stage for larval bioassay purposes.

This study employed the ovitrap technique, where field populations of both species were gathered from three different sites located in residential areas: Taman Free School (5°24'13.8954"N, 100°18'34.9554"E), Tingkat Teluk Kumbar (5°17'16.4178"N, 100°13'55.2576"E), and Tingkat Paya Terubong (5°22'22"N, 100°16'47"E). Taman Free School and Tingkat Paya Terubong are situated in the Northeast region, while Tingkat Teluk Kumbar is positioned in the Southwest region of Penang. Each of these selected sites was identified as a hotspot for dengue (http://idengue.arsm.gov.my/), associated with a significant population of *Aedes* mosquitoes and a high incidence of dengue cases.

The ovitraps utilised were essentially mosquito home traps composed of black plastic containers filled with 300 ml of dechlorinated water. A hardboard paddle was inserted diagonally within each ovitrap to facilitate the attachment of eggs during the laying process. In all three sites, a total of 10 ovitraps were deployed to gather wild strains. Each week, the wooden paddles were retrieved and substituted with fresh ones for a one-month duration, aiding in the collection of sufficient wild strains for both *Ae. aegypti* and *Ae. albopictus*. The retrieved paddles were air-dried for 48 hours before being placed in dechlorinated water, allowing the eggs to hatch. The hatching process for the eggs required approximately 24 to 48 hours.

Following the identical conditions as previously mentioned for laboratory strains, larvae from field strains were raised until they reached adulthood. Once in the adult phase, *Ae. aegypti* and *Ae. albopictus* were distinguished and placed into separate cages sized 30 x 30 x 30 cm according to species, with access to a 10% sucrose solution through a cotton swab (Masters *et al*., 2020). After three days post-emergence, a laboratory rat (*Rattus norvegicus*) was introduced into the cage to provide a blood meal for female lab mosquitoes. The rat was maintained according to animal care guidelines, including housing in a sanitized, temperature and humidity-controlled environment with unrestricted rodent pellets and water. All procedures were conducted in accordance with approved ethical standards throughout the blood-feeding process for continuous monitoring to minimize stress and discomfort (animal ethics: USM/IACUC/2020/ (126) (1113)). Moist Whatman No. 1 filter papers were shaped into cones and positioned in the cage for egg-laying. The eggs that were produced were allowed to dry, and after three days, the collected eggs were immersed in dechlorinated water to generate the F_1 generation, which was subsequently utilised for the bioassay experiment.

Serial dilutions

A concentrated solution containing 110000 ppm PPF was diluted with distilled water through a series of dilutions to obtain the required concentration. An initial assessment was conducted to identify the concentration levels that resulted in 0% to 100% mortality rates among the larval populations of both the laboratory and wild strains of *Ae. aegypti* and *Ae. albopictus*. From this data, the necessary concentration range was computed, leading to the selection of eight concentrations to be employed in this study. The concentrations were determined based on the differences noted in the initial assessment findings (Juarez *et al*., 2021; Su *et al*., 2019). For testing, the lab strain larvae of *Ae. aegypti* and *Ae. albopictus* were exposed to concentrations of 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 ppm of PPF, whereas the larvae from all three locations of *Ae. aegypti* and *Ae. albopictus* were subjected to concentrations of 0.15, 0.3, 0.45, 0.6, 0.75, 0.9, 1.05, 1.2, 1.35, and 1.5 ppm of PPF during testing. The volume of distilled water that needed to be added to get the desired concentrations was calculated using this formula:

$$C_1V_1 = C_2V_2$$

where C_1 = concentration of stock solution before dilution
V_1 = volume of stock solution before dilution
C_2 = desired concentration after dilution
V_2 = volume of solution (desired concentration)

Larval bioassay

Late-stage larvae, specifically the late third and early fourth instars of *Ae. aegypti* and *Ae. albopictus*, were selected for larval bioassay trials according to established WHO guidelines (WHO, 2017). Each trial involved placing 25 larvae into paper cups of 250 ml volume that contained 200 ml of various specified concentrations of PPF. Each concentration was tested across three additional replicates. The experiment was conducted on three separate occasions to guarantee reliable, accurate, and consistent results by reducing potential errors and confirming the outcomes. A negative control was established for the bioassay, where pyriproxyfen was excluded and only distilled water was utilised, allowing for comparisons against the various PPF concentrations. Larvae from both the control and treatment groups were provided with food every other day until they transformed into pupae or perished. After five days of exposure, any surviving larvae and pupae were washed with water and placed into a plastic cup filled with dechlorinated water. Daily observations were made to record larval and pupal deaths, as well as the emergence of adults, until all subjects either died or became adults. Pupal mortality data were analysed using probit analysis to determine LC_{50} and LC_{95}, as outlined in the statistical analysis section. Moribund larvae and pupae, along with adult mosquitoes that were unable to separate from their pupal cases, were categorised as affected and counted as dead (WHO, 2005).

Percentage of Adult Emergence Inhibition (IE%)

Using a method similar to larval bioassays, the percentage inhibition of adult emergence (IE%) was determined to assess the impact of pyriproxyfen on the transition of mosquitoes from larvae to adulthood. Four distinct strains of *Ae. aegypti* and *Ae. albopictus* were collected for this purpose: the laboratory strain, Taman Free School strain, Tingkat Teluk Kumbar strain, and Tingkat Paya Terubong strain, which were reared until they reached the late third and early fourth instar larval stages. The concentration of PPF mentioned in the serial dilutions section was applied. The emergence rate in the control group was maintained at around 90% before evaluating the percentage of adult emergence inhibition (IE%) in the treated group. Experiments were conducted in triplicate to guarantee the consistency of the data obtained. Larvae and pupae in moribund states, along with adult mosquitoes that were unable to separate from their pupal cases, were classified as impacted and counted as deceased. The tally of adults that emerged successfully from both treated and control groups was recorded, and the IE% was calculated using the formula provided by WHO (2016).

$$\text{IE\%} = 100 - \left(\frac{\%\text{ adult emergence in treated group}}{\%\text{ adult emergence in control group}}\right) \times 100$$

Statistical analysis

Values for LC_{50} and LC_{95} were derived through Probit analysis, and analysis of variance (ANOVA) was performed with SPSS version 26. Independent factors comprised the species of *Aedes* vector and the level of PPF concentration employed to assess the mortality rates in *Ae. aegypti* and *Ae. albopictus*. The threshold for statistical significance was established at $p < 0.05$. Independent variables such as (i) PPF concentration, (ii) *Aedes* vector species, and (iii) strains of *Aedes* vector were analysed to explore their correlation and impact on mortality rates (%).

Probit analysis and ANOVA were utilised to evaluate the influence of different factors on the mortality rates of *Ae. aegypti* and *Ae. albopictus* larvae. The percentage of larval mortality (%) served as the dependent variable, while the fixed elements consisted of the genus and the quantity of target mosquito vectors.

Probit analysis was used to determine lethal concentration values, LC_{50} and LC_{95}, representing the levels of pyriproxyfen that lead to 50% and 95% mortality of the mosquito larvae. The figures provided a numerical estimation of PPF's toxicity against the evaluated *Aedes* species. ANOVA was applied to examine the hypothesis regarding the effects of PPF concentration, *Aedes* vector species, and strains of *Aedes* vector on larval mortality rates.

RESULTS

Larval and pupal mortality

No larval mortality was recorded throughout the experiment, but pyriproxyfen causes pupal mortality in both *Ae. aegypti* and *Ae. albopictus* in all localities as well as lab strains. The pupal mortality increased with the increase of pyriproxyfen concentration. Mosquitoes from lab strains, Taman Free School and Tingkat Paya Terubong showed a similar pattern where a higher dose is needed for larvae of *Ae. albopictus* treated to show complete mortality compared to *Ae. aegypti* from the same area. However, for Tingkat Teluk Kumbar, *Ae. aegypti* require a higher dose for complete mortality as compared to *Ae. albopictus.*

Concentration-dependent effects of pyriproxyfen

For laboratory strains, *Ae. aegypti* shows 100% mortality at 1.04 ppm while *Ae. albopictus* shows 100% mortality at 1.08 ppm (Figure 1). In the Taman Free School, 100% mortality was recorded for *Ae. aegypti* and *Ae. albopictus* at 1.21 ppm and 1.71 ppm, while in Tingkat Paya Terubong, 100% mortality for *Ae. aegypti* and *Ae. albopictus* was recorded at 1.15 ppm and 1.44 ppm (Figure 1). Only in Tingkat Teluk Kumbar, *Ae. aegypti* showed 100% mortality at a higher dose of 1.37 ppm compared to 1.21 ppm recorded for *Ae. albopictus* (Figure 2).

Lethal concentrations (LC_{50} and LC_{95})

For both species, laboratory strains showed the lowest LC_{50} and LC_{95} compared to the wild strains from all three localities, with 0.267 ppm and 1.026 ppm recorded for *Ae. aegypti* and 0.236 ppm and 1.107 ppm recorded for *Ae. albopictus,* respectively (Table 1). Among the three localities, the lowest LC_{50} was recorded in *Ae. aegypti* was 0.332 ppm in Tingkat Paya Terubong, while the highest was 0.399 ppm recorded in Tingkat Teluk Kumbar (Table 1). For *Ae. albopictus*, the lowest LC_{50} recorded was 0.326 ppm in Tingkat Teluk Kumbar, while the highest was 0.571 ppm in Taman Free School (Table 2).

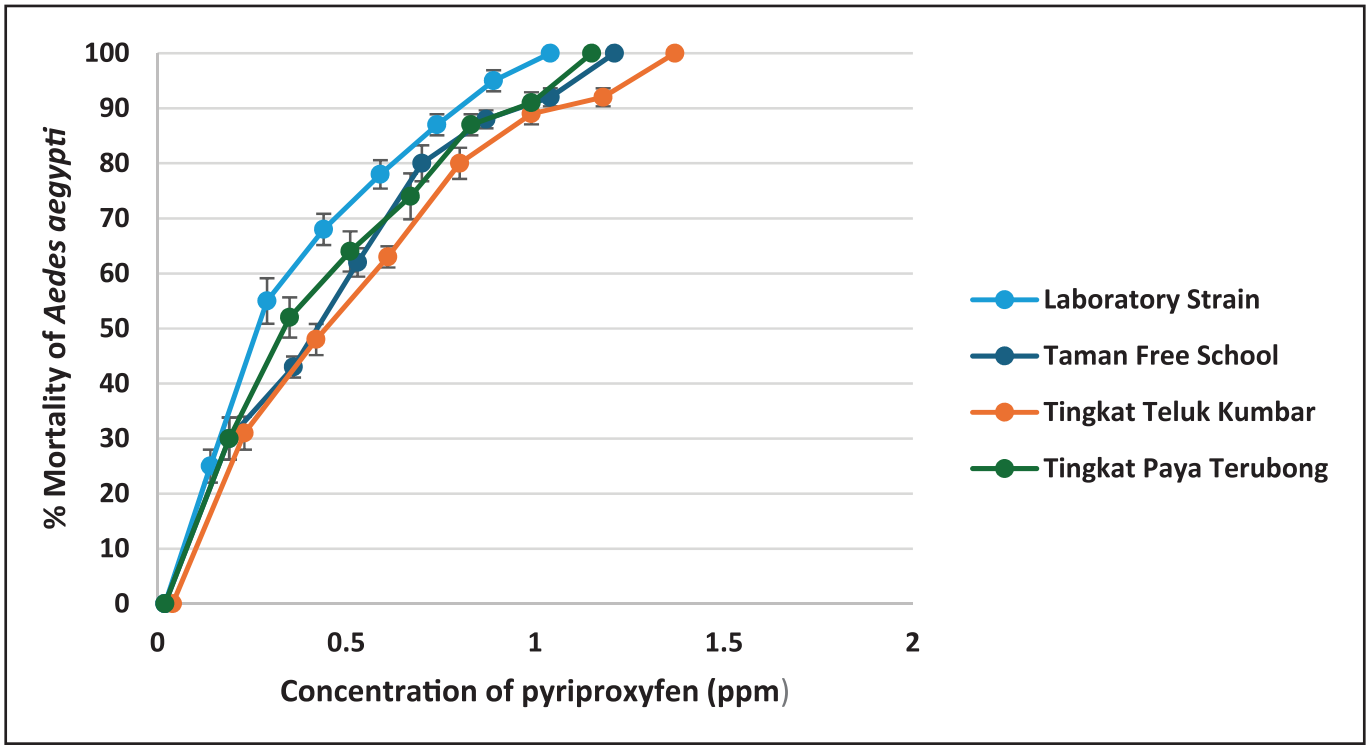

Figure 1. Mortality of *Aedes aegypti* at different concentrations of pyriproxyfen and different localities.

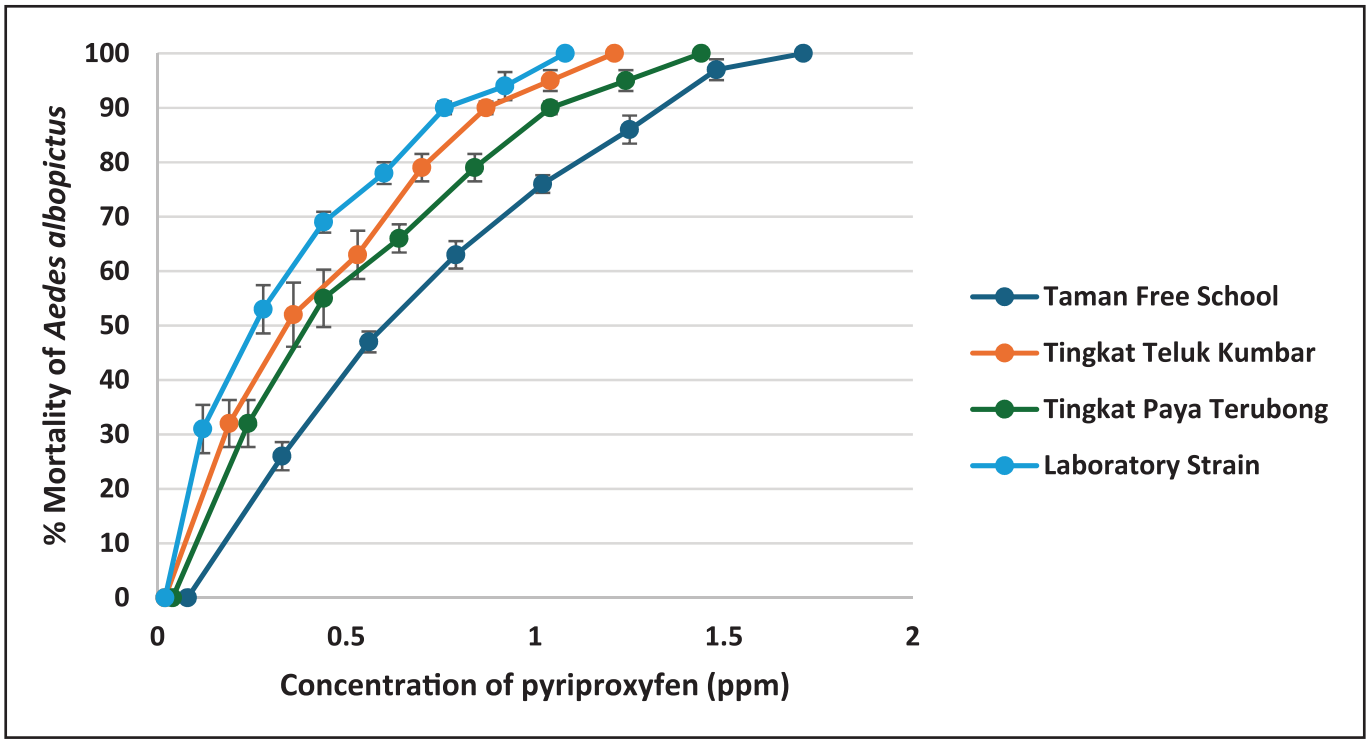

Figure 2. Mortality of *Aedes albopictus* at different concentrations of pyriproxyfen and different localities.

Table 1. Mean LC_{50} and LC_{95} (ppm) values with 95% confidence limit on *Ae. aegypti* from different localities against pyriproxyfen after 24 hours of exposure

Localities	N	LC_{50} (ppm)	LC_{95} (ppm)	Regression equation
Laboratory strain	400	0.267 (0.237–0.296)	1.026 (0.889–1.231)	Y= 1.54+2.68X
Taman Free School	400	0.352 (0.272–0.424)	1.270 (0.977–1.978)	Y= 1+2.5X
Tingkat Teluk Kumbar	400	0.399 (0.319–0.471)	1.398 (1.108–2.017)	Y= 1.13+2.78X
Tingkat Paya Terubong	400	0.332 (0.260–0.395)	1.274 (0.990–1.922)	Y= 1.24+2.57X

Values in the bracket indicate the 95% confidence interval for LC_{50} and LC_{95} (ppm) of *Ae. aegypti* respectively.

Table 2. Mean LC_{50} and LC_{95} (ppm) values with 95% confidence limit on *Ae. albopictus* from different localities against pyriproxyfen after 24 hours of exposure

Localities	N	LC_{50} (ppm)	LC_{95} (ppm)	Regression equation
Laboratory strain	400	0.236 (0.176–0.293)	1.107 (0.830–1.732)	Y= 2+2.5X
Taman Free School	400	0.571 (0.472–0.661)	1.648 (1.341–2.279)	Y= 0.88+3.53X
Tingkat Teluk Kumbar	400	0.326 (0.248–0.395)	1.202 (0.929–1.853)	Y= 1.5+2.5X
Tingkat Paya Terubong	400	0.393 (0.350–0.433)	1.385 (1.211–1.644)	Y= 1.18+2.87X

Values in the bracket indicate the 95% confidence interval for LC_{50} and LC_{95} (ppm) of *Ae. albopictus* respectively.

Morphological abnormalities

In this study, several morphological abnormalities were observed after exposure to the LC_{50} of PPF, where larvae and pupae failed to darken and harden their cuticle and died as white pupae in Figure 3(a) and 3(b) or became partially melanised and died (Figure 4). Melanisation is described as the darkened colour pigmentation typically found on the larvae or pupae of mosquitoes, as shown in Figure 3(c). The degree of melanisation was qualitatively observed and compared to untreated control larvae or pupae. Pupae also showed redness in the anterior part and eventually died (Figure 5). At 0.15 ppm, the legs of adult individuals adhered to the exoskeletal remnants of pupae, resulting in an inability to complete the process of emergence (Figure 6).

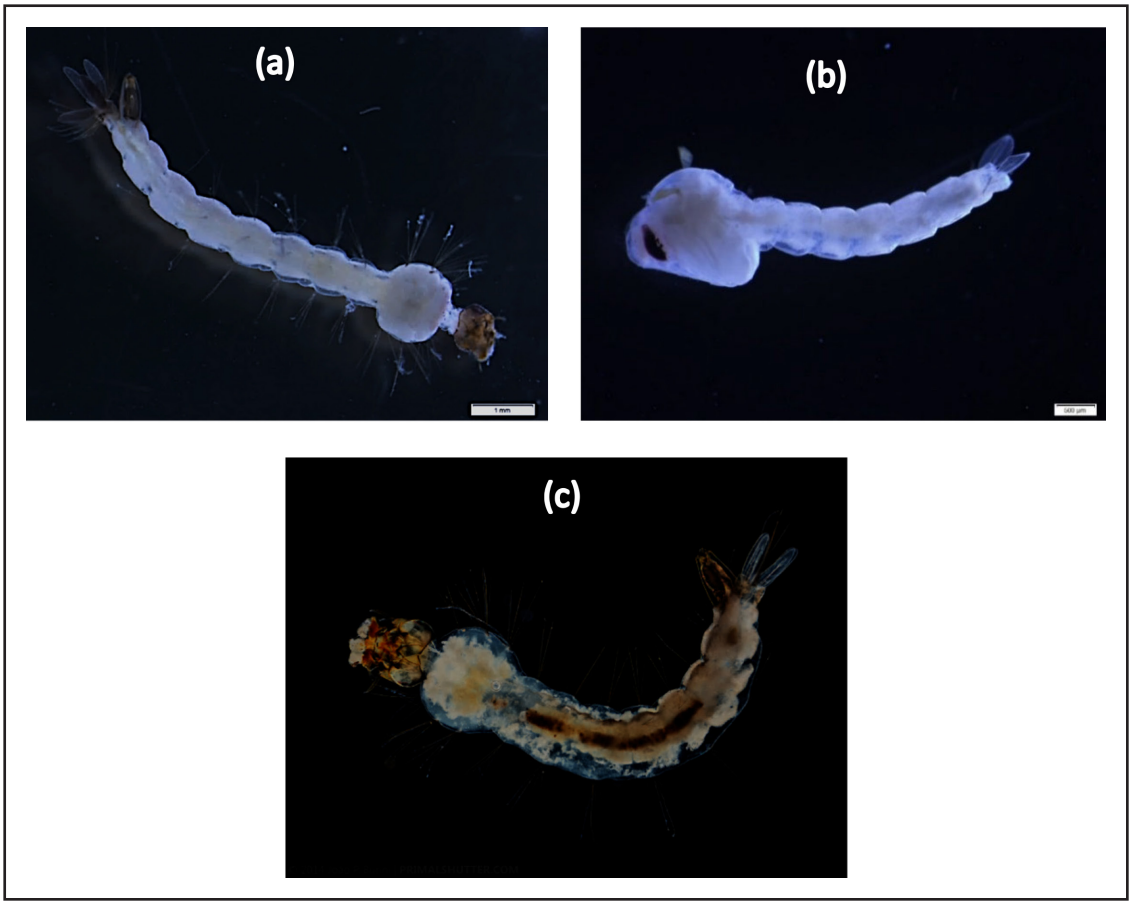

Figure 3. Larvae (a) and pupae (b) of *Ae. aegypti* lacks melanisation after exposure to pyriproxyfen in comparison to a normal *Ae. aegypti* larvae (c).

Figure 4. Partially melanized pupae of *Ae. aegypti* after exposure to pyriproxyfen.

Figure 5. Pupae of *Ae. aegypti* exhibits red anterior after exposure to pyriproxyfen.

Figure 6. Adherence of exoskeletal remnants of pupae in *Ae. aegypti* which failed to emerge as an adult after PPF exposure.

Inhibition of emergence (IE) in *Ae. aegypti* and *Ae. albopictus*

The inhibition of emergence (IE) was assessed across different strains and localities under treatment and control conditions for *Ae. aegypti* (Table 3) and correlation analysis (Table 4) were recorded. The laboratory strain and mosquito populations from all three localities of *Ae. aegypti* and *Ae. albopictus* showed a significant reduction in emergence under the treatment conditions, indicating effective inhibition by the pyriproxyfen application compared to the control group. The laboratory strain showed no inhibition, with emergence proceeding as expected, confirming the baseline of untreated conditions.

In the laboratory strain, IE in *Ae. aegypti* began at 14.72% with 0.05 ppm and gradually increased, reaching 97.89% at 0.50 ppm (Table 3). In contrast, IE in *Ae. aegypti* field strains from Taman Free School, Tingkat Teluk Kumbar and Tingkat Paya Terubong exhibited lower inhibition at 0.15 ppm, recording 17.68%, 28.79%, and 21.78% respectively, compared to 44.65% in the laboratory strain. As concentrations increased, the field populations experienced greater suppression in adult emergence. Inhibition of emergence increased at 0.30 ppm to 34.67% in Taman Free School, 31.63% in Tingkat Teluk Kumbar, and 32.86% in Tingkat Paya Terubong. A further increase in concentration to 0.45 ppm resulted in 54.82% inhibition at Taman Free School, 49.75% at Tingkat Teluk Kumbar, and 51.79% at Tingkat Paya Terubong. The highest inhibition was recorded at 1.50 ppm, with 98.23% in Taman Free School, 97.04% in Tingkat Teluk Kumbar, and 97.01% in Tingkat Paya Terubong (Table 3). These results indicated that while the laboratory strain exhibited higher susceptibility at lower concentrations, field populations responded with marked inhibition at higher concentrations, suggesting effective suppression of emergence across laboratory and field populations of *Ae. aegypti*.

In Table 4, significant correlations ($p < 0.01$) were observed between several locality pairs, particularly in relation to the inhibition of adult emergence. The lab strain showed strong, significant correlations in inhibition of emergence with Taman Free School ($p = 0.008$), Tingkat Teluk Kumbar ($p = 0.006$), and Tingkat Paya Terubong ($p = 0.007$), indicating consistent effects across these locations. Similarly, Taman Free School's survivorship/emergence was significantly correlated with Tingkat Teluk Kumbar ($p = 0.008$) and Tingkat Paya Terubong ($p = 0.007$), suggesting similar response patterns among these field populations.

Moreover, strong correlations in IE were found between Taman Free School and both Tingkat Teluk Kumbar ($p = 0.000$) and Tingkat Paya Terubong ($p = 0.000$), reinforcing the uniform effectiveness of the treatment across sites. Comparable trends were seen between Tingkat Teluk Kumbar and Tingkat Paya Terubong ($p = 0.000$). These significant correlations highlight a consistent impact of pyriproxyfen across different strains and localities, especially in inhibiting adult emergence of *Ae. aegypti* (Table 4).

In Table 5, IE in *Ae. albopictus* increased progressively with higher concentrations of pyriproxyfen across all strains. The laboratory strain showed a gradual increase from 10.63% IE at 0.05 ppm to 63.54% at 0.50 ppm. Among field strains, IE at 0.15 ppm remained relatively low, with 13.14% in Taman Free School, 23.05% in Tingkat Teluk Kumbar, and 19.13% in Tingkat Paya Terubong. Marked increases were observed at 0.30 ppm, where IE reached 26.33%, 46.32%, and 38.23% in Taman Free School, Tingkat Teluk Kumbar, and Tingkat Paya Terubong, respectively. Tingkat Teluk Kumbar recorded 64.12% at 0.60 ppm, the highest among the three locations. The highest IE values were recorded at 1.50 ppm, with 88.83% in Taman Free School, 98.24% in Tingkat Teluk Kumbar, and 95.87% in Tingkat Paya Terubong (Table 5), indicating increased susceptibility at higher concentrations.

The IE of the laboratory strain showed significant correlations ($p < 0.01$) with all field populations, namely Taman Free School ($p = 0.001$), Tingkat Teluk Kumbar ($p = 0.001$), and Tingkat Paya Terubong ($p = 0.001$), as presented in Table 6. Similarly, the

Table 3. Inhibition of Emergence (IE) of different strains of *Aedes aegypti* at different concentrations of pyriproxyfen

Concentration (ppm)	Lab strain		Taman Free School		Tingkat Teluk Kumbar		Tingkat Paya Terubong	
	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)
0.05	85.28	14.72	–	–	–	–	–	–
0.10	70.13	29.87	–	–	–	–	–	–
0.15	55.35	44.65	82.32	17.68	71.21	28.79	78.22	21.78
0.20	40.33	59.67	–	–	–	–	–	–
0.25	30.17	69.83	–	–	–	–	–	–
0.30	20.41	79.59	65.33	34.67	68.37	31.63	67.14	32.86
0.35	15.24	84.76	–	–	–	–	–	–
0.40	10.37	89.63	–	–	–	–	–	–
0.45	5.30	94.70	45.18	54.82	50.25	49.75	48.21	51.79
0.50	2.11	97.89	–	–	–	–	–	–
0.60	–	–	30.11	69.89	32.93	67.07	31.31	68.69
0.75	–	–	18.64	81.36	20.48	79.52	19.43	80.55
0.90	–	–	12.07	87.93	12.98	87.02	12.27	87.73
1.05	–	–	8.03	91.97	9.21	90.79	8.32	91.68
1.20	–	–	5.44	94.56	6.21	93.79	6.29	93.71
1.35	–	–	2.98	97.02	4.17	95.83	4.02	95.98
1.50	–	–	1.77	98.23	2.96	97.04	2.99	97.01

Table 4. Correlation Analysis (*Aedes aegypti*)

P-Value	Lab strain		Taman Free School		Tingkat Teluk Kumbar		Tingkat Paya Terubong	
	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)
Lab strain Survivorship/ Emergence (%)		.798	.796	.002**	.281	.003**	.294	.003**
Lab strain Inhibition of Emergence (%)	.798		.392	.008**	.387	.006**	.371	.007**
Taman Free School Survivorship/ Emergence (%)	.796	.392		.960	.008**	.909	.007**	.932
Taman Free School Inhibition of Emergence (%)	.002**	.008**	.960		.307	.000**	.323	.000**
Tingkat Teluk Kumbar Survivorship/ Emergence (%)	.281	.387	.008**	.307		.358	.000**	.335
Tingkat Teluk Kumbar Inhibition of Emergence (%)	.003**	.006**	.909	.000**	.358		.375	.000**
Tingkat Paya Terubong Survivorship/ Emergence (%)	.294	.371	.007**	.323	.000**	.375		.352
Tingkat Paya Terubong Inhibition of Emergence (%)	.003**	.007**	.932	.000**	.335	.000**		

**. Correlation was significant at the 0.01 level (2-tailed).

Table 5. Inhibition of Emergence (IE) of different strains of *Aedes albopictus* at different concentrations of pyriproxyfen

Concentration (ppm)	Lab strain		Taman Free School		Tingkat Teluk Kumbar		Tingkat Paya Terubong	
	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)
0.05	89.37	10.63	–	–	–	–	–	–
0.10	78.75	21.25	–	–	–	–	–	–
0.15	68.18	31.82	86.86	13.14	76.95	23.05	80.87	19.13
0.20	57.63	42.37	–	–	–	–	–	–
0.25	49.27	50.73	–	–	–	–	–	–
0.30	47.12	52.88	73.67	26.33	53.68	46.32	61.77	38.23
0.35	44.10	55.90	–	–	–	–	–	–
0.40	42.31	57.69	–	–	–	–	–	–
0.45	39.12	60.88	60.58	39.42	43.56	56.44	47.39	52.61
0.50	36.46	63.54	–	–	–	–	–	–
0.60	–	–	48.71	51.29	35.88	64.12	40.58	59.42
0.75	–	–	42.47	57.53	28.16	71.84	33.77	66.23
0.90	–	–	36.62	63.38	20.49	79.51	26.95	73.05
1.05	–	–	29.97	70.03	12.78	87.22	20.17	79.83
1.20	–	–	23.69	76.31	5.07	94.93	13.40	86.60
1.35	–	–	17.49	82.51	3.76	96.24	6.56	93.44
1.50	–	–	11.17	88.83	1.76	98.24	4.13	95.87

Table 6. Correlation Analysis (*Aedes albopictus*)

P-Value	Lab strain		Taman Free School		Tingkat Teluk Kumbar		Tingkat Paya Terubong	
	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)	Survivorship/ Emergence (%)	Inhibition of Emergence (%)
Lab strain Survivorship/ Emergence (%)		0.22	.550	.000**	.934	.000**	.847	.000**
Lab strain Inhibition of Emergence (%)	.022		.760	.001**	.852	.001**	.965	.001**
Taman Free School Survivorship/ Emergence (%)	.550	.760		.253	.000**	.121	.000**	.159
Taman Free School Inhibition of Emergence (%)	.000**	.001**	.253		.697	.000**	.505	.000**
Tingkat Teluk Kumbar Survivorship/ Emergence (%)	.934	.852	.000**	.697		.450	.000**	.527
Tingkat Teluk Kumbar Inhibition of Emergence (%)	.000**	.001**	.121	.000**	.450		.292	.000**
Tingkat Paya Terubong Survivorship/ Emergence (%)	.847	.965	.000**	.505	.000**	.292		.360
Tingkat Paya Terubong Inhibition of Emergence (%)	.000**	.001**	.159	.000**	.527	.000**	.360	

**. Correlation was significant at the 0.01 level (2-tailed).

survivorship of the lab strain was significantly correlated with Tingkat Teluk Kumbar (p = 0.000) and Tingkat Paya Terubong (p = 0.000).

Taman Free School's IE was also significantly correlated with IE in Tingkat Teluk Kumbar (p = 0.000) and Tingkat Paya Terubong (p = 0.000). Meanwhile, survivorship/ emergence in Tingkat Teluk Kumbar showed significant correlation with Tingkat Paya Terubong (p = 0.000), and their IE values were also correlated (p = 0.000). These consistent patterns of significant correlation suggest that pyriproxyfen similarly affects *Ae. albopictus* populations across all tested locations.

DISCUSSION

The results of the study indicate that exposure to pyriproxyfen strongly inhibits *Aedes* larvae from developing into adult mosquitoes. This outcome is consistent with a variety of previous results that pyriproxyfen acts effectively at low dosages and possesses a comparatively long residual activity, therefore being the most promising candidate for environment-oriented vector control (Fansiri *et al*., 2022; Rahman *et al*., 2024; Rhyne & Richards, 2020). Importantly, the present findings provide the first quantitative assessment of pyriproxyfen efficacy against *Aedes* populations collected from Penang, extending existing knowledge by generating locality-specific data under Malaysian field conditions. This dataset offers critical evidence on larval response patterns in an area with persistent dengue transmission and intensive vector control activities.

Evidence from autodissemination studies and larval bioassays in Malaysia demonstrates that pyriproxyfen primarily suppresses pupal or adult emergence rather than inducing immediate larval mortality (Nabila *et al*., 2025). Previous study in Bandar Baru Bangi, Selangor, Malaysia, using the Mosquito Home System with pyriproxyfen, found 10-35% larval mortalities in WHO larval bioassays, but the main effect was a significant reduction in egg, larval, and ovitrap indices, not direct larval death. This suggests that while some larval mortality may be observed in bioassays (likely due to high concentrations or specific conditions), the primary action is pupal or adult emergence inhibition (Mohd Ngesom *et al*., 2021). In comparison with these earlier studies, the Penang populations examined in the present work exhibited variation in concentration-dependent emergence inhibition, indicating potential differences in local susceptibility. Such variation highlights the importance of regional evaluations to support evidence-based larvicide application strategies and optimise integrated vector management programmes at the sub-national level.

In the present study, the limited immediate larval mortality observed across treatments indicates that population suppression was driven primarily by disruption of developmental progression rather than acute toxicity, consistent with the developmental outcomes reported by Yadav *et al*. (2019). This pattern supports the suitability of pyriproxyfen for control strategies that target emergence suppression and long-term population reduction. These findings are further supported by studies showing that even non-lethal doses of pyriproxyfen can disrupt reproductive fitness and development in *Aedes* mosquitoes, reinforcing its utility in integrated vector management strategies (Kancharlapalli & Brelsfoard, 2024).

For lab strains, both *Ae. aegypti* and *Ae. albopictus* showed complete mortality at the lowest tested concentrations. In Taman Free School and Tingkat Paya Terubong, complete mortality for both species was also achieved, but at higher concentrations. However, in Tingkat Teluk Kumbar, *Ae. aegypti* required a higher dose than *Ae. albopictus* to reach 100% mortality. A study assessing the susceptibility of *Ae. aegypti* populations, including the Rockefeller laboratory reference strain, reported complete inhibition of adult emergence (100% mortality) in this susceptible lab strain when exposed to pyriproxyfen at diagnostic doses (Darriet & Corbel, 2006). Another comparison of insecticide susceptibilities in laboratory strains of *Ae. aegypti* confirmed that pyriproxyfen was the most effective insecticide tested, with 100% inhibition of adult emergence in susceptible lab strains (Campos *et al*., 2020; Carvalho *et al*., 2020). These reference values provide an essential comparative standard for interpreting the responses of Penang field populations examined in this study. The requirement for higher concentrations in specific locations suggests heterogeneity in susceptibility, potentially reflecting local environmental conditions or prior insecticide exposure, and highlights the importance of incorporating field-based data into larvicide evaluation and resistance-monitoring frameworks.

This study shows higher LC_{50} and LC_{95} values for pyriproxyfen in both laboratory and field strains compared to the 0.02 ppm reported for *Ae. aegypti* and *Ae. albopictus* by WHO (2001). The values currently reported by Darriet and Corbel (2006) are also notably lower than those in the present study, with LC_{50} and LC_{95} as low as 0.00011 mg/L and 0.00032 mg/L, respectively, against *Ae. aegypti* larvae, indicating reduced susceptibility in the current field populations. Some field strains of other species, such as *Anopheles stephensi*, remain fully susceptible to low concentrations of pyriproxyfen, with LC_{50} values around 0.000169 mg/L, suggesting variability depending on the mosquito species and geographical origin (Azizi *et al*., 2019).

The differences between the low LC_{50} and LC_{95} values reported in earlier studies and the higher values observed in the current study may be attributed to several factors. Environmental conditions such as temperature, water quality, and breeding habitats can affect the mosquitoes' response to the insecticide. Additionally, variations in larval age and bioassay methods, including differences in exposure duration or larval density, may influence the results. The formulation or purity of pyriproxyfen used may also differ between studies. Furthermore, mosquitoes in the current study areas may have been exposed to other insecticides, leading to selection pressure that reduces their susceptibility to pyriproxyfen, thus requiring higher concentrations for effective control.

Most field and laboratory studies confirm that the main effect of pyriproxyfen is to disrupt metamorphosis, leading to pupal mortality or failure to emerge as adults, rather than causing immediate larval death. This is consistent with the mode of action of juvenile hormone analogues (Hustedt *et al*., 2020). The morphological abnormalities observed, such as incomplete melanisation and failure to emerge from pupal cases, are consistent with the interference of pyriproxyfen in cuticle formation and moulting processes. These sub-lethal effects can contribute to population suppression by reducing the number of viable adults (Sihuincha *et al*., 2005), and melanisation changes are part of these morphological abnormalities observed in exposed immature mosquitoes (Sihuincha *et al*., 2005). The altered melanisation is linked to the interference with juvenile hormone pathways that regulate cuticle sclerotisation and pigmentation, processes essential for normal pupal development (Devillers & Devillers, 2020). This can manifest as darkened or precociously pigmented areas on the pupae. Such sub-lethal effects in Penang field populations may contribute to sustained suppression of adult emergence, reinforcing the operational value of pyriproxyfen in local vector management strategies. While direct studies on pyriproxyfen-induced melanisation in mosquitoes are limited, research in other insects, such as honeybees, shows that pyriproxyfen can cause anomalous pigmentation in pupae by affecting phenoloxidase activity, an enzyme involved in melanin synthesis (Devillers & Devillers, 2020).

The previous findings demonstrate that pyriproxyfen effectively inhibits adult emergence in both *Ae. aegypti* and *Ae. albopictus* across various strains and localities. Systematic reviews and experimental studies have consistently shown that pyriproxyfen prevents juvenile *Aedes* mosquitoes from becoming adults, with granule formulations achieving 90-100% inhibition of adult emergence for up to 90 days in multiple settings and strains (Hustedt *et al*., 2020; Mohd Ngesom *et al*., 2021). Laboratory and field studies confirm that pyriproxyfen

is highly effective as a larvicide, with low concentrations sufficient to inhibit the emergence of both *Ae. aegypti* and *Ae. albopictus*.

In both *Ae. aegypti* and *Ae. albopictus*, inhibition of emergence (IE) increased progressively with higher concentrations of pyriproxyfen. Laboratory strains of both species showed greater susceptibility at lower concentrations. All field strains of both species showed strong suppression of adult emergence at the highest concentration tested, demonstrating the effective performance of pyriproxyfen. In Taman Free School, Tingkat Teluk Kumbar, and Tingkat Paya Terubong, IE consistently increased with higher doses. These findings are supported by previous research, which reported emergence inhibition rates of 80% or higher at the two highest concentrations (0.02 and 0.04 mg/L) in both *Ae. aegypti* and *Ae. albopictus*, confirming a dose-dependent increase in emergence inhibition across populations (Moura & Corbi, 2024).

Correlation analyses further support the dose-dependent relationship between pyriproxyfen concentration and emergence inhibition. Such findings are crucial for determining optimal dosages for field applications, ensuring effective vector control while minimising environmental impact. Factors such as genetic diversity, environmental conditions, and prior exposure to insecticides can influence pyriproxyfen efficacy (Campos *et al*., 2022). The efficacy of pyriproxyfen is highly dependent on environmental conditions such as water temperature, light exposure, and the content of organic matter that can interfere with the stability of the chemical compound (Campos *et al*., 2023).

CONCLUSIONS

This study shows that pyriproxyfen is efficient as a larvicide to inhibit the development of *Aedes* larvae from becoming adult mosquitoes. This effectiveness is due to the action of pyriproxyfen as an insect juvenile hormone analogue that interferes with the process of metamorphosis. Pyriproxyfen treatment successfully reduced the adult mosquito emergence rate, even at low concentrations, and showed promise as a long-term and environmentally safe vector control agent. Semi-field and large-scale studies need to be conducted to assess the longevity of pyriproxyfen when exposed to varying environmental conditions and to monitor closely the long-term resistance development that may result from repeated use. Field application, therefore, requires adjustment of dose and appropriate method of delivery, for example, the use of auto-dissemination technology through the exploitation of the egg-laying behaviour of female mosquitoes for the dissemination of the active ingredient to other breeding sites. These findings constitute a usable scientific basis for the application of pyriproxyfen as an important element in an environmentally friendly, sustainable mosquito control strategy with great potential for integration into national dengue control programs in endemic regions such as Malaysia and other tropical countries.

ACKNOWLEDGEMENTS

The authors thank the School of Biological Sciences and the Vector Control Research Unit (VCRU), Universiti Sains Malaysia, Penang, for the facilities used in this study. We also thank One Team Network Sdn Bhd for providing the research materials. The first author, Noor Adila Mohamad, gratefully acknowledges the Ministry of Higher Education Malaysia for the scholarship (Hadiah Latihan Persekutuan) support throughout her study.

Conflict of interests

The author declares that they have no conflicts of interests.

REFERENCES

Abdullah, N.A.M.H., Dom, N.C., Salleh, S.A., Salim, H., Precha, N. & Dapari, R. (2025). Spatiotemporal dynamics of dengue hotspots in an urbanizing landscape: a five-year analysis in Selangor, Malaysia. *Clinical Epidemiology and Global Health* **32**: 101966. https://doi.org/10.1016/j.cegh.2025.101966

Achee, N.L., Gould, F., Perkins, T.A., Reiner, R.C., Morrison, A.C., Ritchie, S.A., Gubler, D.J., Teyssou, R. & Scott, T.W. (2015). A critical assessment of vector control for dengue prevention. *PLOS Neglected Tropical Diseases* **9**: e0003655. https://doi.org/10.1371/journal.pntd.0003655

Azizi, K., Soltani, A., Ali Amiri, S., Fakoor Ziba, M.R. & Moemenbellah-Fard, M.D. (2019). Laboratory and semi-field evaluations on lethal and residual effects of temephos and pyriproxyfen insecticides to control malaria mosquito larvae, *Anopheles stephensi* Liston. *Journal of Health Sciences & Surveillance System* **7**: 40-47. https://doi.org/10.30476/jhsss.2020.85116.1059

Buhler, C., Winkler, V., Runge Ranzinger, S., Boyce, R. & Horstick, O. (2019). Environmental methods for dengue vector control – a systematic review and meta analysis. *PLOS Neglected Tropical Diseases* **13**: e0007420. https://doi.org/10.1371/journal.pntd.0007420

Campos, K.B., Alomar, A.A., Eastmond, B.H., Obara, M.T. & Alto, B.W. (2022). Brazilian populations of *Aedes aegypti* resistant to pyriproxyfen exhibit lower susceptibility to infection with Zika virus. *Viruses* **14**: 2198. https://doi.org/10.3390/v14102198

Campos, K.B., Alomar, A.A., Eastmond, B.H., Obara, M.T., Dias, L.D.S., Rahman, R.U. & Alto, B.W. (2023). Assessment of insecticide resistance of *Aedes aegypti* (Diptera: Culicidae) populations to insect growth regulator pyriproxyfen, in the northeast region of Brazil. *Journal of Vector Ecology* **48**: 12-18. https://doi.org/10.52707/1081-1710-48.1.12

Campos, K.B., Martins, A.J., Rodovalho, C.M., Bellinato, D.F., Dias, L.S., Macoris, M.L.G., Andrighetti, M.T.M., Lima, J.B.P. & Obara, M.T. (2020). Assessment of the susceptibility status of *Aedes aegypti* (Diptera: Culicidae) populations to pyriproxyfen and malathion in a nation-wide monitoring of insecticide resistance performed in Brazil from 2017 to 2018. *Parasites & Vectors* **13**: 531. https://doi.org/10.1186/s13071-020-04406-6

Carvalho, B.L., Germano, R.N.L., Braga, K.M.L., Araújo, E.R.F., Rocha, D.A. & Obara, M.T. (2020). Susceptibility of *Aedes aegypti* populations to pyriproxyfen in the Federal District of Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* **53**: e20190489. https://doi.org/10.1590/0037-8682-0489-2019

Cheong, Y.L., Nazni, W.A., Lee, H.L., NoorAfizah, A., MohdKhairuddin, I.C., Kamarul, G.M., Nizam, N.M.N., Arif, M.A.K., NurZatilAqmar, Z.M., Irwan, S.M. *et al.* (2023). Spatial distribution and long-term persistence of *Wolbachia*-infected *Aedes aegypti* in the Mentari Court, Malaysia. *Insects* **14**: 373. https://doi.org/10.3390/insects14040373

Darriet, F. & Corbel, V. (2006). Laboratory evaluation of pyriproxyfen and spinosad, alone and in combination, against *Aedes aegypti* larvae. *Journal of Medical Entomology* **43**: 1190-1194. https://doi.org/10.1093/jmedent/43.6.1190

Devillers, J. & Devillers, H. (2020). Lethal and sublethal effects of pyriproxyfen on *Apis* and Non-*Apis* Bees. *Toxics* **8**: 104. https://doi.org/10.3390/toxics8040104

Fansiri, T., Pongsiri, A., Khongtak, P., Nitatsukprasert, C., Chittham, W., Jaichapor, B., Pathawong, N., Kijchalao, U., Tiangtrong, S., Singkhaimuk, P. *et al.* (2022). The impact of insect growth regulators on adult emergence inhibition and the fitness of *Aedes aegypti* field populations in Thailand. *Acta Tropica* **236**: 106695. https://doi.org/10.1016/j.actatropica.2022.106695

Gan, S.J., Leong, Y.Q., Barhanuddin, M.F.H., Wong, S.T., Wong, S.F., Mak, J.W. & Ahmad, R.B. (2021). Dengue fever and insecticide resistance in *Aedes* mosquitoes in Southeast Asia: a review. *Parasites & Vectors* **14**: 315. https://doi.org/10.1186/s13071-021-04785-4

Hustedt, J.C., Boyce, R., Bradley, J., Hii, J. & Alexander, N. (2020). Use of pyriproxyfen in control of *Aedes* mosquitoes: a systematic review. *PLOS Neglected Tropical Diseases* **14**: e0008205. https://doi.org/10.1371/journal.pntd.0008205

Juarez, J.G., Garcia-Luna, S.M., Roundy, C.M., Branca, A., Banfield, M.G. & Hamer, G.L. (2021). Susceptibility of South Texas *Aedes aegypti* to pyriproxyfen. *Insects* **12**: 460. https://doi.org/10.3390/insects12050460

Kancharlapalli, S.J. & Brelsfoard, C.L. (2024). The impact of non-lethal doses of pyriproxyfen on male and female *Aedes albopictus* reproductive fitness. *Frontiers in Insect Science* **4**: 1430422. https://doi.org/10.3389/finsc.2024.1430422

Leles, L.F.D.O., Alvarez, M.V.N., Cortes, J.J.C., Alonso, D.P., Ribolla, P.E.M. & Luz, S.L.B. (2024). Impact of long term pyriproxyfen exposure on the genetic structure and diversity of *Aedes aegypti* and *Aedes albopictus* in Manaus, Amazonas, Brazil. *Genes* **15**: 1046. https://doi.org/10.3390/genes15081046

Mahmud, M.A.F., Faizul Akmal, A., Eida Nurhadzira, M., Mohd Hazrin, H., Hanipah, S. & Izfa Riza, H. (2025). Spatial and breeding site analysis of *Aedes* spp. at dengue-prone areas in Kuala Lumpur, Malaysia. *International Journal of Environmental Health Research* **36**: 93-112. https://doi.org/10.1080/09603123.2025.2488484

Mohd Ngesom, A.M., Ahmad, N.W., Lee, H.L., Md Lasim, A., Greenhalgh, D., Sahani, M., Hod, R. & Othman, H. (2021). Evaluating the potential of pyriproxyfen dissemination using mosquito home system against *Aedes albopictus* at dengue hotspot area. *Sains Malaysiana* **50**: 2379-2393. http://doi.org/10.17576/jsm-2021-5008-20

Moura, L. & Corbi, J.J. (2024). Regionality in vector control: effect of fluctuating temperature in the susceptibility of *Aedes aegypti* (Diptera: Culicidae) larvae to Pyriproxyfen. *Parasitology Research* **123**: 23. https://doi.org/10.1007/s00436-023-08065-1

Moura, L., de Nadai, B.L. & Corbi, J.J. (2023). One does not simply apply larvicides: *Aedes aegypti* from Araraquara (Brazil) has reduced susceptibility to pyriproxyfen. *Veterinary Parasitology: Regional Studies and Reports* **41**: 100875. https://doi.org/10.1016/j.vprsr.2023.100875

Nabila, F., Hwa, L.C., Ngesom, A.M.M. & Zuharah, W.F. (2025). Evaluating Aedes Tech Mosquito Home System (AMHS) effectiveness on *Aedes* mosquitoes. *Pertanika Journal of Tropical Agricultural Science* **48**: 451-481. https://doi.org/10.47836/pjtas.48.2.08

Nazni, W.A., Teoh, G.N., Nuradila, M.A., Hakimi, S.I.S.N., Tanusshni, M., Arif, M.A.M., Hanini, A.N., Shazia,I.A., Tan, A.M., Rabizah, H. *et al.* (2025). Impact of sterile *Aedes aegypti* males releases on vector dynamics: insights from Malaysian field trials. *Infectious Diseases of Poverty* **14**: 33. https://doi.org/10.1186/s40249-025-01303-x

Rahman, A.U., Khan, I., Usman, A. & Khan, H. (2024). Evaluation of insect growth regulators (IGRs) as biological pesticides for control of *Aedes aegypti* mosquitoes. *Journal of Vector Borne Diseases* **61**: 129-135. https://doi.org/10.4103/0972-9062.392257

Rhyne, M.N. & Richards, S.L. (2020). Impact of the insect growth regulator pyriproxyfen on immature development, fecundity, and fertility of *Aedes albopictus*. *Journal of the American Mosquito Control Association* **36**: 11-15. https://doi.org/10.2987/19-6893.1

Sabar, N.N., Yu, K.X., Ahmad, R., Zurainee, M.N., Tengku-Idris, T.I.N., Dianita, R., Sabrina, R.M. & Wan Najdah, W.M.A. (2023). Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Malaysia (2010 to 2022): a review. *Asian Pacific Journal of Tropical Medicine* **16**: 434-445. https://doi.org/10.4103/1995-7645.378561

Sihuincha, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J.D., López-Sifuentes, V., Vidal-Ore, C. & Devine, G.J. (2005). Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *Journal of Medical Entomology* **42**: 620-630. https://doi.org/10.1093/jmedent/42.4.620

Su, T., Thieme, J., Lura, T., Cheng, M.L. & Brown, M.Q. (2019). Susceptibility profile of *Aedes aegypti* L. (Diptera: Culicidae) from Montclair, California, to commonly used pesticides, with note on resistance to pyriproxyfen. *Journal of Medical Entomology* **56**: 1047-1054. https://doi.org/10.1093/jme/tjz019

Thomas, S.J. (2023). Is new dengue vaccine efficacy data a relief or cause for concern? *NPJ/ Vaccines* **8**: 55. https://doi.org/10.1038/s41541-023-00658-2

World Health Organisation (WHO). (2001). Report of the Fourth WHOPES Working Group Meeting. WHO/HQ Geneva. https://www.sumivector.com/wp-content/uploads/sumilarv-05g-paper-1h.pdf Accessed 25 February 2025

World Health Organisation (WHO). (2005). Guidelines for laboratory and field testing of mosquito larvicides. https://iris.who.int/bitstream/handle/10665/69101/WHO_CDS_WHOPES_GCDPP_2005.13.pdf Accessed 26 January 2025

World Health Organisation (WHO). (2016). Monitoring and managing insecticide resistance in *Aedes* mosquito populations. Interim guidance for entomologists. https://iris.who.int/bitstream/handle/10665/204588/WHO_ZIKV_VC_16.1_eng.pdf Accessed 26 January 2025

World Health Organisation (WHO). (2017). WHO specifications and evaluations for public health pesticides–Pyriproxyfen. https://www.who.int/pq-vectorcontrol/prequalified-lists/PYRIPROXYFEN.pdf Accessed 26 January 2025

World Health Organisation (WHO). (2020). Global vector control response: Progress in planning and implementation. https://iris.who.int/bitstream/handle/10665/336658/9789240007987-eng.pdf?sequence=1 Accessed 25 February 2025

Yadav, K., Dhiman, S., Acharya, B.N., Ghorpade, R.R. & Sukumaran, D. (2019). Pyriproxyfen treated surface exposure exhibits reproductive disruption in dengue vector *Aedes aegypti*. *PLOS Neglected Tropical Diseases* **13**: e0007842. https://doi.org/10.1371/journal.pntd.0007842