EVALUATION OF THE LIFE CYCLE OF *AEDES ALBOPICTUS* AND ITS CONTROL STRATEGY USING DELTAMETHRIN AND LAMBDA-CYHALOTHRIN AS LARVICIDES

Bioresearch Communications
Volume 11, Issue 2, July 2025

DOI:
doi.org/10.3329/brc.v11i2.82644

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ABSTRACT

Dengue fever, transmitted by *Aedes aegypti* and *Aedes albopictus*, has seen a global increase, with *A. albopictus* particularly prevalent in Dhaka, Bangladesh, during the study period, August 2023 to July 2024. This study focused on controlling dengue at the vector level, specifically targeting *A. albopictus*, which was identified in both larval and adult stages through morphological analysis. Results showed that *A. albopictus* larvae took about three times longer to develop into adults during winter than in summer. The effectiveness of two pyrethroid insecticides, lambda-cyhalothrin and deltamethrin, was assessed by determining lethal concentrations (LC_{50} and LC_{90}), emergence inhibition (IE_{50} and IE_{90}), and resistance ratios (RR) for late 3rd and early 4th instar larvae. Current LC_{50} and LC_{90} values for lambda-cyhalothrin were 0.29 ppm and 0.47 ppm, while deltamethrin values were 0.24 ppm and 0.69 ppm, showing a significant rise from previous levels and indicating increased resistance ($p<0.05$). Cytogenotoxicity tests of the determined doses revealed minimal DNA damage in human blood nucleoids and insignificant impact on mammalian cell viability. These findings suggest that lambda-cyhalothrin and deltamethrin remain effective against *A. albopictus* larvae without substantial genomic damage on non-target organisms; however, further study is needed to evaluate long-term effects and potential resistance mechanisms.

KEYWORDS: *Aedes albopictus*, lambda-cyhalothrin, deltamethrin, resistance, and cytogenotoxicity.

RECEIVED: 13 April 2025, ACCEPTED: 11 June 2025

TYPE: Original Research

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Introduction

Dengue fever, a significant global health concern, is spread by *Aedes* mosquitoes, particularly *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*. This viral infection has become increasingly prevalent in tropical and subtropical regions, with rising outbreaks and an expanding range of *Aedes* populations making dengue a major public health issue worldwide (Tabassum and Taylor-Robinson, 2019). Currently, the World Health Organization (WHO) reports endemic dengue cases in 129 countries. Bangladesh, for instance, experienced its deadliest outbreak in 2023 since its first documented epidemic in 2000 (Sarker *et al.*, 2024).

Chemical insecticides are widely used to control *Aedes* mosquitoes (Bisset Lazcano *et al.*, 2009). Pesticides is a chemical agent targeting pests which are essential in disease control but raise concerns due to potential adverse effects, particularly causing genetic damage. Over the past three decades, managing insect pests in agriculture, livestock, and public health has relied on insecticides like methyl-carbamates, organochlorines, and organophosphates. Despite their cost-effectiveness, concerns over their health, environmental, and ecological impacts have led to restrictions, prompting a search for safer alternatives. Pyrethroids have since emerged as a viable solution (El-Gerbed, 2014).

Pyrethroids act on the insect's nervous system, specifically targeting voltage-gated sodium channels. By keeping these channels open, pyrethroids cause continuous nerve impulses, muscle contractions, and ultimately paralysis and death of the insect (Field *et al.*, 2017). The WHO-endorsed pyrethroid insecticides, including lambda-cyhalothrin and deltamethrin are highly effective, for controlling mosquito, with a lower toxicity profile than previous options like organochlorines and organophosphates (Casida, 1980).

In recent years, however, the efficacy of established pyrethroid doses for larvicidal purposes has diminished in some areas. This reduced effectiveness is largely due to genetic mutations in mosquito populations, leading to resistance mechanisms such as increased detoxifying enzyme production. Consequently, some mosquito populations are surviving and reproducing despite exposure to standard insecticide doses.

Two key metrics are used to evaluate insecticide efficacy and resistance such as Emergence Inhibition (IE) and Resistance Ratio (RR). Emergence Inhibition, as defined by WHO, measures how effectively an insecticide prevents mosquito larvae from maturing into adults. This metric is critical for evaluating larvicidal and pupicidal efficacy. The IE values are specifically IE_{50} and IE_{90} that indicates the insecticide concentrations required to inhibit 50% and 90% of larval emergence into adults, respectively. A higher IE values denoting greater efficacy in preventing mosquito development. The Resistance Ratio (RR) measures how resistant a mosquito population has become to a given insecticide by comparing the lethal concentration required to kill a resistant mosquito population to that required for a susceptible reference population. A higher RR indicates greater resistance (Wei *et al.*, 2021). WHO (2016) guidelines also allow RR calculations from IE values, making these metrics essential in assessing insecticide efficacy and informing resistance management strategies.

Amid growing resistance to pyrethroids, it's crucial to monitor and minimize insecticide impacts on human health and the environment. The release of pesticides into the environment poses substantial health risks to both humans and animals. Studies indicate that insecticides interact with cellular macromolecules, disrupting essential functions and potentially causing long-term health problems (Kim *et al*., 2017). In recent years, the comet assay, also known as single-cell gel electrophoresis, has emerged as a sensitive, efficient method for detecting DNA damage and repair. Compared to traditional approaches, the comet assay is simple, rapid, and cost-effective, suitable for assessing genotoxicity in both in vivo and in vitro conditions (Muid *et al*., 2012). In addition to assessing genotoxicity, Vero cell line can be utilized to evaluate the cytotoxicity of the candidate pyrethroid insecticides (Kiesslich and Kamen, 2020).

Therefore, this study aimed to assess seasonal variation in life-cycle and to evaluate the efficacy of two commonly used pyrethroids named lambda-cyhalothrin and deltamethrin against *Aedes albopictus* larvae. The objectives include determining current lethal concentrations (LC_{50} and LC_{90}) of these insecticides, measuring IE_{50} and IE_{90} values, and calculating the resistance ratio (RR) of the mosquito population. In addition, this research investigated the genotoxic and cytotoxic potential of these insecticides. Current findings may provide critical insights into the current resistance levels of mosquito populations and the potential health risks associated with the use of these insecticides.

Materials and Methods

Aedes Culture and Identification

Aedes albopictus larvae were collected from various sites across Dhaka and reared in the insectary at the Department of Zoology, University of Dhaka, Bangladesh, from August 2023 to July 2024. Both natural sources and artificial ovitraps were used for collection. Larvae were fed budding yeast, and adults were sustained with a 10% sugar solution. Species identification was performed on the F_1 generation, following morphological criteria based on Anoopkumar et al. (2017).

Dose determination

To test the effectiveness of insecticides on late third- and early fourth-instar *Aedes albopictus* larvae (F_1 generation), bioassays were conducted at different concentrations following WHO (2005). The insecticides used were lambda-cyhalothrin 2.5 EC (Syngenta) and deltamethrin 2.5 EC (Bayer). A 1% stock solution of each was prepared in distilled water and diluted to create 6 serial concentrations. Bioassays were performed in a controlled environment with a 12-hour light/dark cycle.

For each test, batches of 20 healthy, lab-reared larvae were placed in 100 ml of tap water containing one of the insecticide concentrations in each glass beakers. Tests were repeated three times at room temperature, and larval mortality was assessed after 24 hours. Larvae exhibiting moribund characteristics were considered dead. Lethal concentrations (LC_{50} and LC_{90}) and Emergence Inhibition (IE_{50} and IE_{90}) were determined using log dosage-probit mortality regression analysis in R programming (WHO, 2016). Emergence inhibition (IE%) was calculated as:

$$\text{IE \%} = 100 - \frac{\text{\% adults emerged in treated batches}}{\text{\% adults emerged in control batches}} \text{ x } 100$$

Resistance Ratio (RR) Calculation

Resistance ratio (RR) was calculated by dividing the LC_{50} of the test population by the LC_{50} of a laboratory-susceptible strain (WHO, 2016). The susceptible strain (Lab-S) was obtained from the Shanghai Center for Disease Control and maintained in controlled conditions (27 ± 1°C, 70–80% relative humidity, 14-hour light/10-hour dark cycle) without insecticide exposure. For this strain (Lab-S), the LC_{50} for deltamethrin is 0.002 ppm (Deng *et al.*, 2021). According to WHO (2016), in bioassays, population with RR <5 considered as susceptible, 5–10 as moderately resistant, and >10 as highly resistant to candidate insecticide. RR was calculated as:

$$\text{The resistance ratio} = \frac{\text{LC50 determined during study period}}{\text{LC50 of laboratory susceptible strain}}$$

Comet Assay

Genotoxicity was assessed using a comet assay on human blood cells. Fresh blood was collected in EDTA tubes from Dhaka University Medical Centre, and DNA damage was analyzed on stained cells following method of Muid *et al*. (2012). A total of 100 cells per sample were observed at 400x magnification using a Nikon Eclipse 50i fluorescence microscope (Nikon, Japan).

Cytotoxicity Analysis

Cytotoxic effects were evaluated using the Vero cell line (from African green monkey's kidney epithelial cells) at the Centre for Advanced Research in Sciences (CARS), University of Dhaka. Cells were cultured in DMEM with 1% penicillin-streptomycin, 0.2% gentamicin, and 10% fetal bovine serum (FBS). Approximately 1.5 x 10^4 cells/100 µl were seeded in a 96-well plate and incubated at 37°C with 5% CO_2. On the following day, 25 µl of each sample was added. Positive and negative controls were also prepared, and each sample was tested in duplicate. Cytotoxicity was observed after 48 hours under an inverted light microscope. This test was repeated once.

Statistical Analysis

Statistical analyses were conducted in R programming (R 4.4.1). LC_{50}, LC_{90}, IE_{50}, and IE_{90} values were calculated, and a Likelihood Ratio Test (LRT) was used to compare current and previously determined LC_{50} and LC_{90} values in the study area. One-way ANOVA was applied to comet assay results to detect significant differences in DNA damage scores between treated and control groups **(Supplementary data set 1 and 2).**

Results and Discussion

Seasonal Variation in Life Cycle

In this study, *Aedes albopictus* was identified at the species level **(Figure 1),** and seasonal changes in its life cycle were recorded. During the summer months (March to August, with temperatures between 25°C and 35°C), the first instar larva develops into adult in an average of 8 days. In winter (September to February, 15°C–17°C), this development slowed to around 18–28 days. These results align with Marini et al. (2020), who observed *A. albopictus* requiring 8.8 to 10.4 days in warmer seasons (25°C–30°C) but about 35 days in cooler conditions (15°C). Similarly, Marinho et al. (2015) reported that *Aedes aegypti* development slowed significantly at lower temperatures, taking 9.33 to 13.08 days in warmer conditions (28°C–36°C) and 36.28 to 41.42 days at 16°C. This underscores the influence of temperature on mosquito development rates.

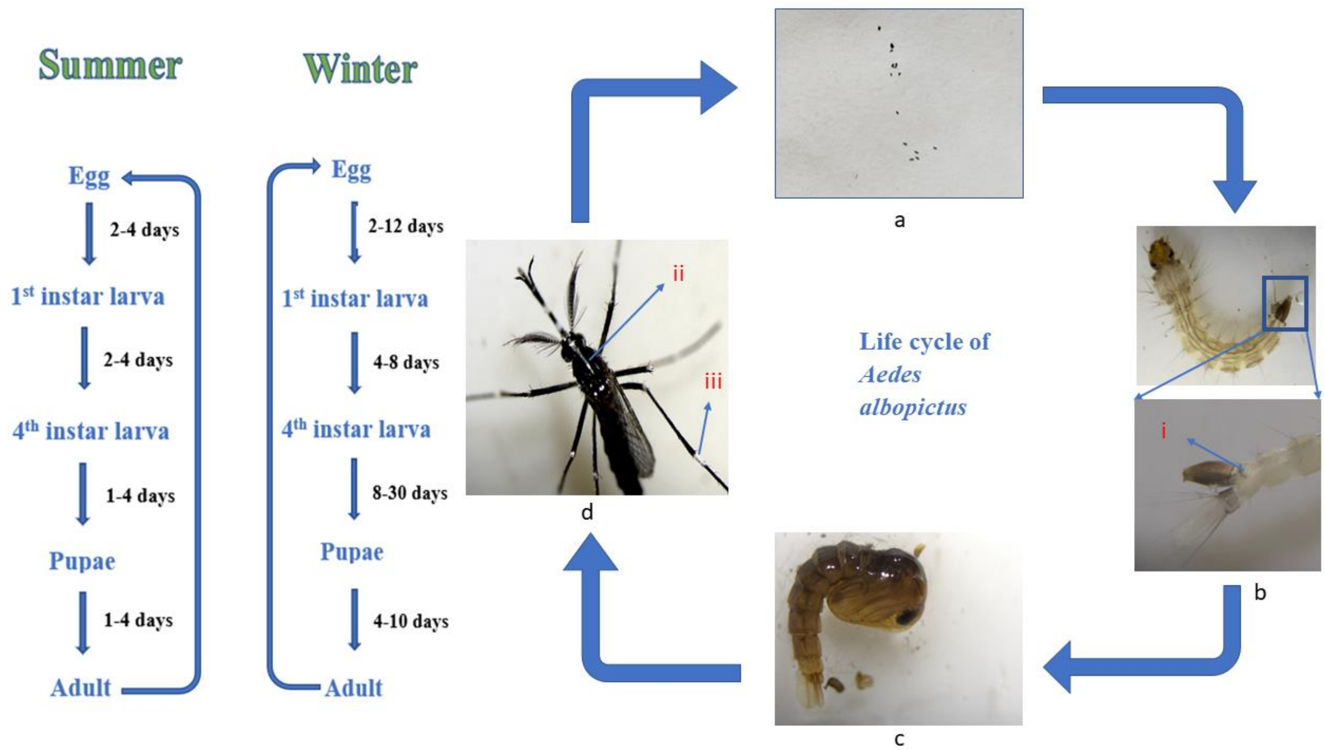


Figure 1. Life cycle of *A. albopictus*. (a) Eggs of *A. albopictus*. (b) Larvae of *A. albopictus*. i - represents a straight row of 7-12 comb scales without subapical spines indicating the larvae belongs to *A. albopictus*. (c) Pupae of *A. albopictus*. (d) Adult *A. albopictus*; ii - Median longitudinal white patches on scutum, iii - white stripe on leg.

In this study, each developmental stage showed clear temperature dependency. For instance, eggs hatched in an average of 2 to 4 days in summer and 2 to 12 days in winter. From the first instar larva to adulthood, development spanned 4 to 10 days in summer and 16 to 25 days in winter. Marini et al. (2020), found that eggs took 4.5 days to hatch at 25°C and 7.4 days at 15°C, with larval development extending from a week at 30°C to 35 days at 15°C.

Temperature also influenced body size, as demonstrated by Rueda et al. (1990), who reported that *Aedes aegypti* and other mosquito species developed into smaller adults at higher temperatures, likely due to accelerated developmental rates. Larger adults, which result from slower development in cooler months, may gain survival benefits in winter (De Majo *et al.*, 2019). Slower development in pre-diapause stages could allow larvae to build energy reserves, supporting larger adults with sufficient stores to sustain overwintering eggs (Costanzo *et al*., 2015; Diniz *et al*., 2017). This time delay for the development may be the strategy of a seasonal adaptation of mosquitoes.

Faster larval growth in summer could increase gonotrophic cycles, potentially raising bite rates in *A. albopictus*, which may lead to a higher basic reproductive number, R_0, and an elevated risk of disease transmission (Mordecai *et al.*, 2019). As summers lengthen with climate change, the threat of outbreaks may rise due to increased mosquito activity.

Understanding mosquito developmental timing is crucial for larvicide application, as most larvicides target specific stages and are effective for 3 to 4 weeks. Shorter life cycles may allow mosquitoes to bypass larvicidal effects, especially in temporary breeding sites (Marini *et al.*, 2020). Further research on these aspects may give the directions of explain recent pattern of outbreaks in Bangladesh.

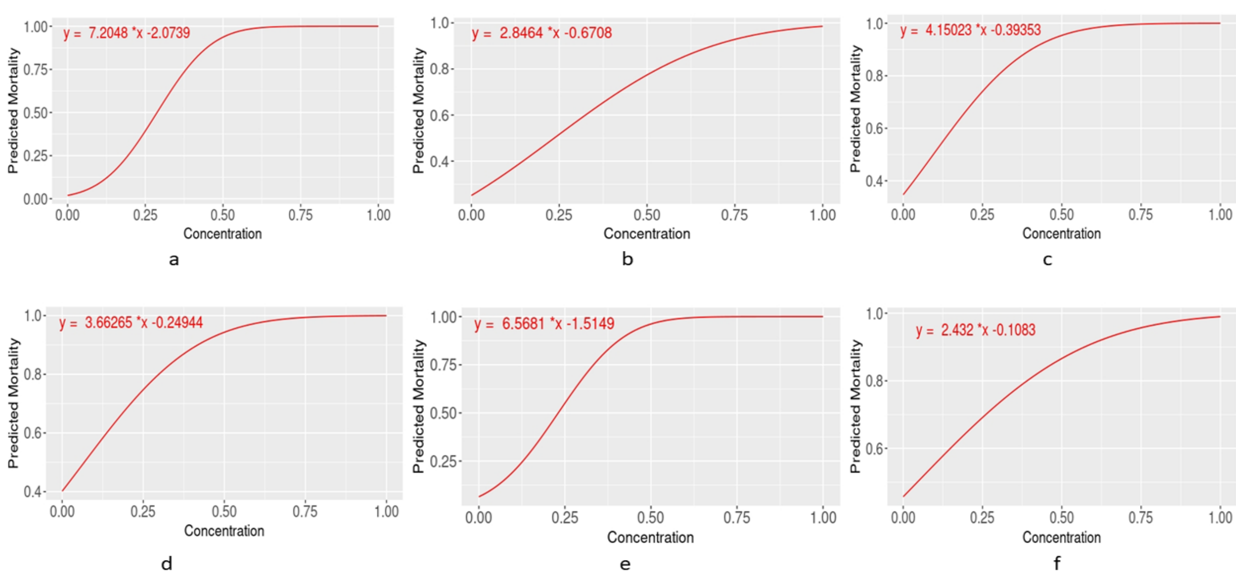
Current Doses of Lambda-Cyhalothrin and Deltamethrin

Controlling vector populations is a crucial method in reducing vector-borne diseases, especially those spread by mosquitoes. Among the methods used, chemical insecticides are highly effective for managing *Aedes* mosquitoes (Bisset Lazcano *et al.*, 2009). The WHO endorses lambda-cyhalothrin and deltamethrin are two pyrethroid insecticides those have high efficacy and relatively low toxicity compared to older insecticides like organochlorines and organophosphates (Casida, 1980). However, limited data is available on pyrethroid use specifically as larvicides, prompting us to conduct bioassays to establish lethal doses for these insecticides **(Table 1 and S. Table 1).**

Table 1. Mortality (%) of *A. albopictus* larva (late 3rd-early 4th instar) exposed 24hr to different concentrations of Lambda-cyhalothrin and Deltamethrin in the current study.

Concentration (ppm)	Number of Larvae	Larval mortality (%) after 24 hrs exposure of Lambda-cyhalothrin				Larval mortality (%) after 24 hrs exposure of Deltamethrin			
		Observation 1	Observation 2	Observation 3	Mean mortality (%)	Observation 1	Observation 2	Observation 3	Mean mortality (%)
0.15	20	5	5	10	6.67	35	35	40	36.67
0.2	20	50	40	25	38.33	30	65	55	50
0.25	20	50	35	45	43.33	60	55	45	53.3
0.3	20	35	70	45	50	55	50	70	58.33
0.35	20	65	80	50	65	45	65	65	58.33
0.4	20	85	90	65	80	55	95	60	70

Using a log-dose probit regression model in R (R 4.4.1), this study calculated LC_{50} and LC_{90} values for lambda-cyhalothrin at 0.29 ppm and 0.47 ppm, respectively **(Figure 2a),** and for deltamethrin at 0.24 ppm and 0.69 ppm **(Figure 2b).** A comparison of lethal concentrations is provided in **Figure 2g.** Available data on the LC values of deltamethrin for *Aedes* larvae is limited, and for lambda-cyhalothrin, it is scarce. Q. Liu *et al*. (2024) reported an LC_{50} of 0.058 ppm for deltamethrin against *A. albopictus* larvae in Zhejiang, China, whereas our findings in Dhaka, Bangladesh, reveal a concentration approximately four times higher.



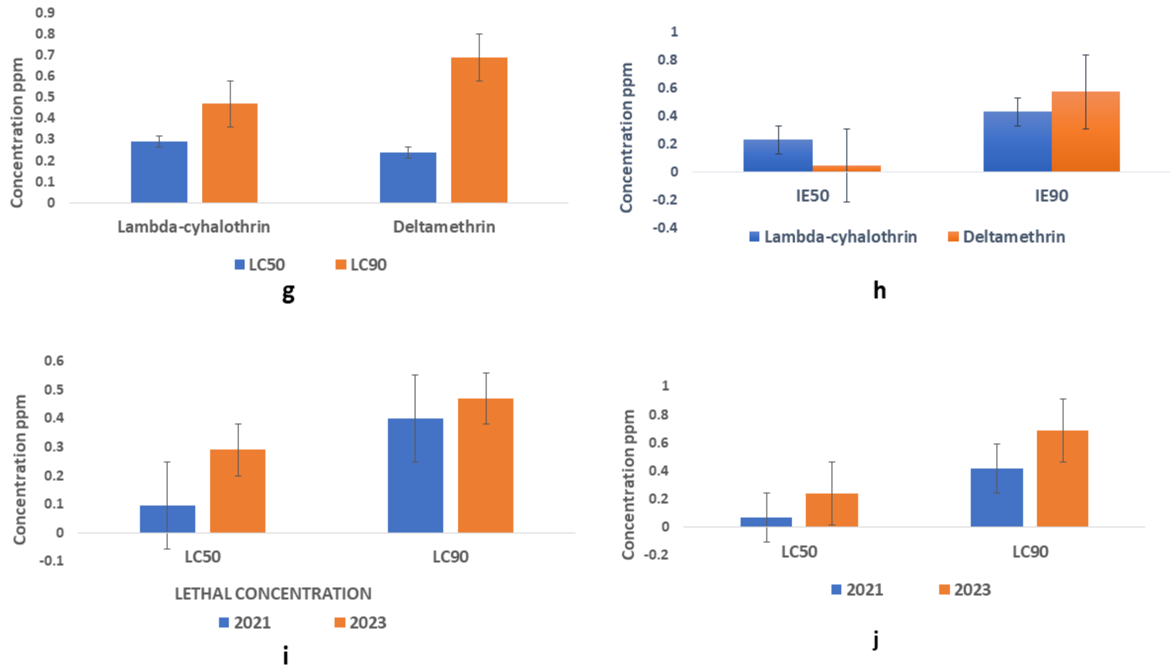


Figure 2. Graphical representation of insecticide susceptibility of *Aedes albopictus*. Log-Probit mortality analysis of (a) lambda-cyhalothrin and (b) deltamethrin in the current study period. Log-Probit mortality analysis of (c) lambda-cyhalothrin and (d) deltamethrin in 2021. Log-Probit analysis of IE (%) of (e) lambda-cyhalothrin and (f) deltamethrin in current study. Here X – axis represents insecticide doses (Concentration in ppm) and Y- axis represents mortality (%) in each graph. (g) Bar-graph represents currently determined LC_{50} and LC_{90} for lambda-cyhalothrin (0.29 and 0.47 ppm respectively) and deltamethrin (0.24 and 0.69 ppm respectively). (h) Bar-graph represents currently determined IE_{50} and IE_{90} for lambda-cyhalothrin (0.23 and 0.43 respectively) and deltamethrin (0.045 and 0.57 respectively). Comparison among LC_{50} and LC_{90} of 2021 and 2023 for (i) lambda-cyhalothrin and (j) deltamethrin. Significant differences were observed ($p < 0.05$) among the determined doses in two different periods.

Emergence Inhibition (IE) is another important parameter that quantifies the reduction in mosquito larvae successfully maturing into adults following insecticide exposure (WHO, 2016). IE_{50} and IE_{90} values represent concentrations that prevent 50% and 90% of larvae from reaching adulthood, respectively. The higher IE values signify better inhibition of mosquito development. Since no IE values were available for these insecticides in existing literature, we determined the IE_{50} and IE_{90} doses for both insecticides using larval bioassays adapted from Alsheikh *et al*. (2016) **(Table 2).**

Table 2. IE (%) of *A. albopictus* exposed to different concentrations of Lambda-cyhalothrin and Deltamethrin in a laboratory bioassay.

Concentration (ppm)	Number of Larvae	Emergence Inhibition, IE (%) for Lambda-cyhalothrin exposure				Emergence Inhibition, IE (%) for Deltamethrin exposure			
		Observation 1	Observation 2	Observation 3	Mean IE (%)	Observation 1	Observation 2	Observation 3	Mean IE (%)
0.15	20	15	20	35	23.33	55	60	60	58.33
0.2	20	55	55	55	55	70	75	75	73.33
0.25	20	55	40	60	51.67	65	60	70	65
0.3	20	40	75	75	63.33	65	70	70	68.33
0.35	20	80	90	70	80	75	80	80	78.33
0.4	20	85	95	85	86.67	80	85	85	83.33

The determined IE_{50} and IE_{90} for lambda-cyhalothrin at 0.23 ppm and 0.43 ppm **(Figure 2e)** and for deltamethrin at 0.045 ppm and 0.57 ppm **(Figure 2f).** A comparison of IE values is provided in **Figure 2h.**

Resistance Ratio (RR), which compares the IE_{50} of a field population to that of a susceptible strain, indicates resistance levels, with an RR of 10 or higher indicating high resistance (Mazzarri and Georghiou, 1995). WHO (2016) reported that IE values indicate resistance ratios which can serve as valuable metrics for tracking the development of insecticide resistance in field populations.

Resistance Status

The increasing resistance of *Aedes albopictus* to insecticides poses a major challenge to mosquito-borne disease control, driven largely by the extensive, repeated use of chemical agents. Globally, resistance to pyrethroids in *A. albopictus* has been documented, with studies in Cameroon showing resistance to deltamethrin and permethrin in 2017 (Yougang *et al.*, 2020). Other resistance cases have been noted in Thailand (Chuaycharoensuk *et al.*, 2011), India (Kushwah *et al.*, 2015), and the United States, particularly in Alabama and Florida (Liu *et al.*, 2004). In China's Zhejiang Province, *A. albopictus* populations exhibited varying resistance levels to beta-cypermethrin, deltamethrin, and permethrin. Relative to a susceptible strain, resistance ratios of *A. albopictus* larvae to beta-cypermethrin ranged from 8.17 to 36.06, to deltamethrin from 12.12 to 107.3, and to permethrin from 1.55 to 81.9, with a significant positive correlation observed between the three insecticides (Liu *et al.*, 2024).

In Bangladesh, CDC bottle bioassays revealed that adult *A. albopictus* from two districts were resistant to permethrin but remained susceptible to deltamethrin, malathion, and bendiocarb (Al-Amin *et al.*, 2022). Previous research on *Aedes aegypti* indicated high resistance to permethrin, moderate resistance to deltamethrin and malathion, and full susceptibility to bendiocarb (Al-Amin *et al.*, 2020). However, no previous studies have examined the larvicidal resistance status of *A. albopictus* in Bangladesh, making this research the first to evaluate larvicide susceptibility for this mosquito species in the region.

To evaluate the current susceptibility of *A. albopictus* to deltamethrin, the resistance ratio (RR) of the test population was determined. The LC_{50} dose for a laboratory-susceptible (Lab-S) strain of *A. albopictus* is 0.002 ppm (Deng *et al.*, 2021). Our test population indicated an LC_{50} of 0.24 ppm, suggesting a high resistance level to deltamethrin, with an RR exceeding 10.

The insecticides were investigated in 2021 **(S. Table 1)** in the same study area two years prior **(Figures 2c & 2d).** Comparisons of LC_{50} and LC_{90} values for lambda-cyhalothrin and deltamethrin determined in two different study periods, showed significant increases: LC_{50} for lambda-cyhalothrin tripled, and LC_{90} rose by 1.17 times, while LC_{50} for deltamethrin increased 3.4 times, and LC_{90} by 1.64 times **(Figures 2i & 2j).** Significant differences were observed (p <

0.05) among the determined doses in two different periods in Likelihood Ratio. Test indicating probable development of resistance.

Although this study did not conduct biochemical or molecular analyses, it provides crucial initial data on larvicide resistance in *A. albopictus* in Bangladesh. Similar studies in India have identified detoxifying enzymes and kdr mutations as primary factors in resistance (Bharati *et al.*, 2019; Chatterjee *et al*., 2018). These findings highlight the need for further research into genetic and molecular mechanisms underlying resistance in *A. albopictus.*

Genotoxicity and Cytotoxicity Assessments

Given the global reliance on LC_{50} values of insecticides to control mosquito populations, this study investigates the potential genotoxic and cytotoxic effects of the LC_{50} doses of lambda-cyhalothrin and deltamethrin to evaluate their environmental safety. Genotoxicity was assessed using the Comet DNA Assay, analyzing 100 cells per observation and totaling 200 cells for each lethal concentration. Undamaged nuclei appeared round, while damaged nuclei exhibited comet-like tails due to DNA strand breaks **(Figure 3).**

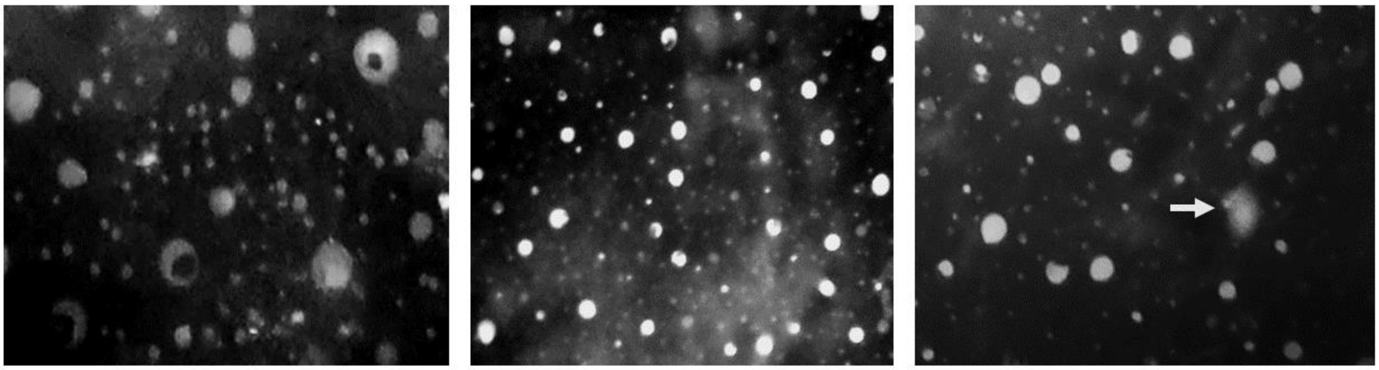


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Figure 3. Comet assay. Representative images: (a) Control group showed undamaged nucleoids, (b) Intact nucleoids after lambda-cyhalothrin exposure, (c) White arrow showed a damaged nucleoid after deltamethrin exposure. Comets were analyzed under Fluorescence microscope Nikon Eclipse 50i: 40X magnification. Significant comets were not found for both lambda-cyhalothrin and deltamethrin LC_{50} exposure.

Following Collins (2004), the average DNA damages were calculated. After 48-hour exposure to the LC_{50} doses, in the untreated control group, no comet structures were observed whereas, both lambda-cyhalothrin and deltamethrin groups showed slight comet formations **(Table 3)**. The mean damage score was 6 ± 1.41 for lambda-cyhalothrin and 3 ± 0.71 for deltamethrin. One-way ANOVA analysis revealed no significant differences ($p > 0.05$) between the control and treated groups, suggesting that the LC_{50} doses have minimal genotoxic effects on the human nuclear genome.

Table 3. Comet assay result analysis for LC_{50} of Lambda-cyhalothrin and Deltamethrin against *A. albopictus* larvae.

Treatment	**Dose**	**Nucleoid observed**	**Comet classes**				**DNA damage score**
			0 (no damage)	**1 (little)**	**2 (Med)**	**3 (High)**	
Control	0.00 ppm	200	200	0	0	0	0
Lambda-cyhalothrin	0.29 ppm	200	197 ± 0.71	1 ± 0.71	1 ± 0.71	1 ± 0.71	6 ± 1.41
Deltamethrin	0.235 ppm	200	198 ± 0	1 ± 0.71	1 ± 0.71	0 ± 0	3 ± 0.71

Additionally, cytotoxicity was evaluated using Vero cells (a mammalian cell line) following Konowalchuk *et al*. (1977). Results **(S. Table 2)** showed that cell viability remained above 95%, indicating that the LC_{50} doses of both lambda-cyhalothrin and deltamethrin have negligible cytotoxic effects on mammalian cells.

In summary, the LC_{50} concentrations of lambda-cyhalothrin and deltamethrin pose minimal genotoxic risks to the human nuclear genome and have an insignificant impact on mammalian cell viability. Nonetheless, further research on the genotoxic and cytotoxic effects of pyrethroids across various biological systems is necessary to draw definitive conclusions and understand the underlying mechanisms.

Conclusion

The extensive and repeated application of insecticides has led to an alarming rise in resistance within *Aedes* mosquito populations to pyrethroid-based insecticides. Despite this trend, lambda-cyhalothrin and deltamethrin, two prominent pyrethroids, still demonstrate effectiveness in controlling *Aedes albopictus* larvae. It is crucial, however, to exercise caution to avoid adverse impacts on non-target organisms. With resistance levels on the rise, further detailed assessments, including genome sequencing, are required to determine the current resistance status comprehensively and to guide future mosquito control strategies.

Acknowledgement

This work was funded by the UGC-Dhaka University grant 2022-23. The authors are very grateful to Professor Dr. Rakhahori Sarker of the Department of Botany, University of Dhaka for providing us fluorescence microscopic facilities. We are also very thankful to Mr. Shohel Mia, Institute of Statistical Research and Training, University of Dhaka for his technical support.

Author Declaration

The authors report there are no competing interests to declare.

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