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การยับยั้งการเกิดตัวเต็มวัยและอัตราการตายจำแนกตามระยะของสารยับยั้ง การสังเคราะห์ไคติน novaluron, triflumuron และ lufenuron ที่มีต่อยุงลายบ้าน (*Aedes aegypti*) พาหะนำโรคไข้เลือดออก

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บทคัดย่อ

สารควบคุมการเจริญเติบโตของแมลง (insect growth regulators: IGRs) กลุ่มยับยั้งการสังเคราะห์ไคติน (chitin-synthesis inhibitors: CSIs) ได้รับความสนใจเพิ่มขึ้นในการควบคุมยุง เนื่องจากมีคุณสมบัติในการรบกวนกระบวนการเจริญเติบโตของแมลงมากกว่าการออกฤทธิ์ที่ทำให้ตายอย่างรวดเร็ว การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพในการกำจัดลูกน้ำ โดยยับยั้งการเป็นตัวเต็มวัย และเปรียบเทียบประสิทธิภาพในการควบคุมลูกน้ำยุงลายบ้าน (*Aedes aegypti*) ของสาร novaluron, triflumuron และ lufenuron ภายใต้สภาวะห้องปฏิบัติการ โดยใช้ลูกน้ำระยะที่ 3 สัมผัสสารในช่วงความเข้มข้น 0.05 - 6.25 µg/L และบันทึกอัตราการตายของลูกน้ำที่เวลา 24, 48 และ 72 ชั่วโมง และประเมินการยับยั้งการเกิดตัวเต็มวัยเพื่อคำนวณค่า EL_{50} และ EL_{90} ผลการศึกษาพบว่าไม่พบการตายของลูกน้ำในช่วง 24 ชั่วโมงแรกในทุกกลุ่มทดลอง และอัตราการตายยังคงอยู่ในระดับต่ำที่ 72 ชั่วโมง โดยมีค่าสูงสุดที่ $11.67 \pm 3.51\%$ สำหรับ novaluron, $8.33 \pm 1.53\%$ สำหรับ lufenuron และ $6.33 \pm 2.08\%$ สำหรับ triflumuron ที่ความเข้มข้นสูงสุด อย่างไรก็ตาม สารทั้งสามชนิดสามารถยับยั้งการเกิดตัวเต็มวัยได้ 100% ที่ความเข้มข้น 6.25 µg/L โดย lufenuron มีประสิทธิภาพสูงสุด ให้ค่า EL_{50} ต่ำที่สุด (0.122 µg/L) และ EL_{90} เท่ากับ 1.638 µg/L รองลงมาคือ novaluron ($EL_{50} = 0.156$ µg/L; $EL_{90} = 1.903$ µg/L) และ triflumuron ($EL_{50} = 0.228$ µg/L; $EL_{90} = 2.040$ µg/L) และจากการวิเคราะห์อัตราการตายจำแนกตามระยะการเจริญเติบโตแสดงให้เห็นถึงความแตกต่างของสารแต่ละชนิดต่อระยะการตายของแมลง โดย lufenuron ส่งผลให้เกิดอัตราการตายในระยะลูกน้ำสูงที่สุด ขณะที่ novaluron เห็นผลเด่นชัดในระยะตัวโม่ง และ triflumuron พบอัตราการตายแบบกระจายทั้งระยะลูกน้ำและตัวโม่ง ผลการศึกษานี้ชี้ให้เห็นว่าการประเมินประสิทธิภาพโดยอาศัยอัตราการตายของลูกน้ำในระยะสั้นเพียงอย่างเดียว อาจนำไปสู่การประเมินค่าประสิทธิภาพของสารกลุ่มยับยั้งการสังเคราะห์ไคตินต่ำกว่าความเป็นจริง โดยจากผล



การศึกษานี้สรุปว่า lufenuron แสดงประสิทธิภาพสูงที่สุด ซึ่งสนับสนุนศักยภาพในการนำไปประยุกต์ใช้ในโปรแกรมควบคุมยุงต่อไป

คำสำคัญ: ยุงลายบ้าน, การยับยั้งการเกิดตัวเต็มวัย, สารยับยั้งการสังเคราะห์ไคติน, การตายจำแนกตามระยะ, สารควบคุมการเจริญเติบโตของแมลง

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Emergence inhibition and stage-specific mortality of chitin-synthesis inhibitors (novaluron, triflumuron, and lufenuron) against *Aedes aegypti*, a dengue vector

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ABSTRACT

Chitin-synthesis inhibitor (CSI) insect growth regulators (IGRs) are increasingly used in mosquito control due to their ability to disrupt insect development rather than induce rapid mortality. This study assessed the larvicidal activity, emergence inhibition, and the relative efficacy and stage-specific activity of three CSI compounds (novaluron, triflumuron, and lufenuron) against *Aedes aegypti* under laboratory conditions. Third-instar larvae were exposed to concentrations ranging from 0.05 to 6.25 µg/L, and larval mortality was recorded at 24, 48, and 72 hours, while adult emergence inhibition was used to estimate El_{50} and El_{90} values. No larval mortality was observed at 24 hours across all treatments, and mortality remained low at 72 hours, reaching a maximum of $11.67 \pm 3.51\%$ for novaluron, $8.33 \pm 1.53\%$ for lufenuron, and $6.33 \pm 2.08\%$ for triflumuron at the highest concentration. In contrast, all compounds demonstrated strong, concentration-dependent emergence inhibition, achieving 100% inhibition at 6.25 µg/L. Lufenuron exhibited the highest potency with the lowest El_{50} (0.122 µg/L) and El_{90} (1.638 µg/L), followed by novaluron ($El_{50} = 0.156$ µg/L; $El_{90} = 1.903$ µg/L) and triflumuron ($El_{50} = 0.228$ µg/L; $El_{90} = 2.040$ µg/L). Stage-specific mortality analysis further demonstrated compound-specific differences in the timing and developmental stage at which mortality occurred. Lufenuron predominantly affected earlier larval stages, whereas novaluron showed greater effects during the pupal stage, and triflumuron exhibited a more distributed pattern across larval and pupal stages. These findings highlight that reliance on short-term larval



mortality alone may underestimate the efficacy of chitin-synthesis inhibitor IGRs. In contrast, emergence inhibition and stage-specific mortality provide a more biologically relevant and informative assessment of their activity. Among the tested compounds, lufenuron exhibited the highest potency, supporting its potential application in mosquito control programs.

Keywords: *Aedes aegypti*, emergence inhibition, chitin-synthesis inhibitors, stage-specific mortality, insect growth regulators



Introduction

Vector-borne diseases remain a major global public health challenge, accounting for more than 17% of all infectious diseases and causing over 700,000 deaths annually.¹ Among these, dengue is the most widespread arboviral disease transmitted by mosquitoes, with *Aedes aegypti* serving as the primary vector responsible for transmitting dengue, Zika, and chikungunya viruses.^{1,2} The global incidence of dengue has increased dramatically in recent decades, with approximately 3.9 billion people at risk of infection and an estimated 96 million symptomatic cases annually, particularly in tropical and subtropical regions.^{1,3} In Thailand, dengue continues to be a significant public health concern, with over 100,000 reported cases in recent years.¹

Effective vector control remains the cornerstone of disease prevention due to the lack of specific antiviral treatments and limitations in vaccine coverage.⁴ However, conventional mosquito control strategies rely heavily on chemical insecticides, particularly organophosphates and pyrethroids, which act on the nervous system and induce rapid mortality. The extensive and prolonged use of these insecticides has led to widespread

resistance in *Ae. aegypti* populations, reducing their effectiveness and compromising control programs.⁵ In addition, concerns regarding environmental toxicity and non-target effects further limit their long-term sustainability.⁶

Insect growth regulators (IGRs) have emerged as an alternative approach for mosquito control due to their specific mode of action targeting insect development rather than causing immediate mortality. Among IGRs, chitin-synthesis inhibitors (CSIs), including novaluron, triflumuron, and lufenuron, belong to the benzoylphenyl urea group and interfere with the formation of chitin, an essential component of the insect cuticle.⁷ By disrupting molting and cuticle formation, CSIs prevent successful transition between developmental stages, ultimately leading to death during larval-pupal or pupal-adult transformation.⁸ Unlike conventional insecticides, CSIs typically exhibit delayed action, allowing larvae to survive initial exposure but causing them to fail at subsequent molts.⁹

Due to this delayed mode of action, the efficacy of CSIs cannot be accurately assessed using short-term larval mortality alone. Previous studies have demonstrated that larval mortality within 24-72 hours is often minimal, while substantial effects occur



during later developmental stages, particularly at pupation or adult emergence.^{9,10} Therefore, emergence inhibition (EI), expressed as EI₅₀ and EI₉₀, has been recommended as a more appropriate indicator of IGR efficacy, as it reflects the compound's ability to suppress adult mosquito production.¹¹

In addition to overall emergence inhibition, evaluating stage-specific mortality provides further insight into the developmental windows at which CSIs exert their effects. Differences among compounds in targeting larval or pupal stages may influence their operational performance and suitability for vector control programs.^{8,10} However, comparative data on stage-specific effects of different CSIs against *Ae. aegypti* remain limited.

Therefore, this study aimed to evaluate larval mortality, emergence inhibition, and stage-specific mortality of three chitin-synthesis inhibitors (novaluron, triflumuron, and lufenuron) against *Aedes aegypti* under laboratory conditions. The findings provide a more comprehensive assessment of CSI efficacy while highlighting their practical relevance for community-based mosquito control. Despite low acute larval mortality, the strong emergence

inhibition observed suggests that these compounds can effectively suppress adult mosquito populations under operational conditions. Their delayed mode of action and stage-specific effects support their integration into vector control programs, particularly in settings with insecticide resistance or where sustainable, long-term control strategies are needed.

Objectives

This study aimed to evaluate larval mortality, emergence inhibition (EI₅₀ and EI₉₀) of novaluron, triflumuron, and lufenuron against *Ae. aegypti*, and to compare their relative efficacy and developmental stage of action.

Materials and methods

Study design

This laboratory-based experimental study was conducted to evaluate the larvicidal activity and emergence inhibition of three chitin-synthesis inhibitors (CSIs), including novaluron, triflumuron, and lufenuron, against *Ae. aegypti*. The study followed standard procedures adapted from the World Health Organization (WHO) guidelines for laboratory testing of mosquito larvicides (2005).



Mosquitoes

A laboratory-susceptible strain of *Ae. aegypti* was obtained from the National Institute of Health, Thailand. Larvae were reared under controlled environmental conditions at $26 \pm 2^\circ\text{C}$ and 70 - 80% relative humidity with a 12:12 h light-dark photoperiod. Third-instar larvae (L3) were used for all experiments.

Compounds and preparation

Novaluron PESTANAL[®] (analytical standard), triflumuron PESTANAL[®] (analytical standard), and lufenuron PESTANAL[®] (analytical standard) were used in this study. Silicone oil (Dow Corning 200) and all insect growth regulators were purchased from Sigma-Aldrich Pte Ltd. Acetone used in all preparations was of analytical grade. Stock solutions of each compound were prepared using appropriate solvents and then diluted to obtain the desired test concentrations of 0 (control), 0.05, 0.25, 1.25, and 6.25 $\mu\text{g/L}$.

Larval bioassay

Larval bioassays were conducted following WHO guidelines. Briefly, 25 third-instar (L3) *Ae. aegypti* larvae were introduced into 500 mL plastic containers containing 150 mL of dechlorinated water and 100 mg of

larval food. Four concentrations of each chitin-synthesis inhibitor (0.05 - 6.25 $\mu\text{g/L}$) were tested, with dechlorinated water serving as the control. Each concentration was performed in four replicates.

All test units were maintained at $25 \pm 2^\circ\text{C}$, 60 - 70% relative humidity, and a 12:12 h light-dark photoperiod. Larval mortality was assessed at 24, 48, and 72 hours post-exposure. Each experiment was repeated three times on separate days. Emergence inhibition data were analyzed using probit regression to estimate EI_{50} and EI_{90} values.

Emergence inhibition assay

Following initial exposure, all test units were observed daily until all individuals either emerged as adults or died. The number of dead and alive larvae, pupae, and adults was recorded daily to determine stage-specific mortality and adult emergence outcomes. Emergence inhibition was calculated based on the proportion of individuals that failed to emerge as viable adults. EI_{50} and EI_{90} values were estimated using probit regression analysis.

Data analysis

Larval mortality and emergence inhibition were expressed as mean $\pm$ standard



deviation (SD), while stage-specific mortality was expressed as mean $\pm$ standard error (SE). Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons when assumptions of normality and homogeneity of variance were met. When these assumptions were not satisfied, the non-parametric Kruskal-Wallis test followed by Dunn's post hoc test was applied. Statistical significance was set at $p < 0.05$. Dose-response relationships for emergence inhibition were analyzed using probit regression to estimate El_{50} and El_{90} values with 95% confidence intervals.

Results

Larvicidal effect of novaluron, triflumuron, and lufenuron

At 24 hours, no larval mortality was observed in any treatment group for all three compounds ($0.00 \pm 0.00\%$), indicating the absence of immediate larvicidal activity.

At 48 hours, larval mortality remained low for all compounds. Novaluron showed slightly higher mortality at the highest concentration ($5.00 \pm 2.65\%$ at $6.25 \mu\text{g/L}$), with a statistically significant difference compared to the control ($p = 0.046$). In contrast, triflumuron

and lufenuron exhibited negligible mortality ($<1\%$ and $\leq 1.67\%$, respectively), with no statistically significant difference observed between treatment groups ($p > 0.05$).

At 72 hours, a concentration-dependent increase in larval mortality was observed for all three compounds, although overall mortality remained low. Novaluron produced the highest mortality ($11.67 \pm 3.51\%$ at $6.25 \mu\text{g/L}$), followed by lufenuron ($8.33 \pm 1.53\%$) and triflumuron ($6.33 \pm 2.08\%$). Statistically significant differences compared to the control emerged at concentrations $\geq 0.25 \mu\text{g/L}$ for novaluron and lufenuron ($p < 0.05$), whereas triflumuron showed significant effects only at higher concentrations ($\geq 1.25 \mu\text{g/L}$) (Table 1).

Novaluron demonstrated the strongest early larvicidal effect among the tested compounds, followed by lufenuron, while triflumuron exhibited the weakest activity. However, despite these differences, larval mortality remained low ($<12\%$) across all treatments, indicating that the three chitin-synthesis inhibitors did not act as fast-acting larvicides but instead exhibited delayed toxic effects.

**Table 1.** Larval mortality of *Aedes aegypti* exposed to novaluron, triflumuron, and lufenuron.

Concentration ($\mu\text{g/L}$)	48 h			72 h		
	Novaluron	Triflumuron	Lufenuron	Novaluron	Triflumuron	Lufenuron
0 (Control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.05	1.67 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	1.67 ± 0.58	0.67 ± 0.58	0.67 ± 0.58
0.25	2.00 ± 1.00	0.33 ± 0.58	1.00 ± 1.00	3.67 ± 1.53	1.33 ± 1.15	1.67 ± 1.15
1.25	2.00 ± 1.00	0.33 ± 0.58	0.33 ± 0.58	6.67 ± 1.53	3.67 ± 1.15	3.67 ± 0.58
6.25	5.00 ± 2.65	0.67 ± 0.58	1.67 ± 1.15	11.67 ± 3.51	6.33 ± 2.08	8.33 ± 1.53

Emergence inhibition (EI_{50} and EI_{90})

In contrast to the low larval mortality observed within 72 hours, all three chitin-synthesis inhibitors demonstrated strong, concentration-dependent inhibition of adult emergence.

Emergence inhibition increased markedly with concentration for all compounds, reaching complete inhibition (100%) at 6.25 $\mu\text{g/L}$. Novaluron exhibited a progressive increase from $26.67 \pm 8.33\%$ at 0.05 $\mu\text{g/L}$ to $78.67 \pm 4.62\%$ at 1.25 $\mu\text{g/L}$, achieving 100% inhibition at the highest concentration. Triflumuron showed a similar trend but with a lower initial

effect ($10.67 \pm 4.62\%$ at 0.05 $\mu\text{g/L}$), followed by a sharp increase at higher concentrations, reaching complete inhibition at 6.25 $\mu\text{g/L}$.

Dose-response analysis further confirmed significant differences in potency among the compounds. Lufenuron exhibited the highest activity, with the lowest EI_{50} (0.122 $\mu\text{g/L}$) and EI_{90} (1.638 $\mu\text{g/L}$), followed by novaluron ($\text{EI}_{50} = 0.156 \mu\text{g/L}$; $\text{EI}_{90} = 1.903 \mu\text{g/L}$) and triflumuron ($\text{EI}_{50} = 0.228 \mu\text{g/L}$; $\text{EI}_{90} = 2.040 \mu\text{g/L}$). These results indicate that lufenuron required the lowest concentration to achieve both 50% and 90% inhibition of adult emergence (Table 2).

Table 2. EI_{50} and EI_{90} values of novaluron, triflumuron, and lufenuron against *Aedes aegypti*.

Compound	EI_{50} ($\mu\text{g/L}$)	95% CI	EI_{90} ($\mu\text{g/L}$)	95% CI
Novaluron	0.156	0.130 - 0.187	1.903	1.462 - 2.477
Triflumuron	0.228	0.196 - 0.265	2.040	1.610 - 2.586
Lufenuron	0.122	0.101 - 0.149	1.638	1.251 - 2.145



Stage-specific mortality

Stage-specific mortality analysis demonstrated distinct developmental patterns among novaluron, triflumuron, and lufenuron across all tested concentrations. Mortality was distributed across larval, pupal, and adult stages, with significant differences observed depending on both compound and concentration.

At low concentrations (0.05 - 0.25 µg/L), lufenuron consistently produced higher larval mortality compared to novaluron and triflumuron. At 0.05 µg/L, larval mortality reached $34.0 \pm 4.2\%$ for lufenuron, significantly higher than novaluron ($15.0 \pm 2.5\%$) and triflumuron ($6.0 \pm 2.6\%$) ($p = 0.0005$). Pupal mortality at this concentration remained low and comparable among compounds (11.0 ± 1.9 - 3.0%), while adult mortality was minimal. At 0.25 µg/L, larval mortality increased for all compounds, with lufenuron remaining the highest ($47.0 \pm 3.8\%$), followed by triflumuron ($31.0 \pm 6.4\%$) and novaluron ($21.0 \pm 1.9\%$) ($p = 0.0078$), whereas pupal and adult mortality showed no significant differences.

At intermediate concentration (1.25 µg/L), clear divergence in stage-specific effects was observed. Lufenuron produced the highest larval mortality ($56.0 \pm 2.8\%$), while novaluron exhibited the highest pupal mortality ($45.0 \pm 1.0\%$), significantly greater than lufenuron ($28.0 \pm 4.3\%$) ($p = 0.0218$). In contrast, triflumuron showed significantly higher adult mortality ($23.0 \pm 1.9\%$) compared to both novaluron and lufenuron ($p = 0.0045$), indicating a broader distribution of mortality across developmental stages.

At the highest concentration (6.25 µg/L), larval mortality predominated for all compounds, with lufenuron achieving the highest level ($94.0 \pm 2.6\%$), followed by triflumuron ($88.0 \pm 1.6\%$) and novaluron ($83.0 \pm 1.0\%$) ($p = 0.0076$). Despite this, novaluron maintained significantly higher pupal mortality ($17.0 \pm 1.0\%$) compared to triflumuron ($12.0 \pm 1.6\%$) and lufenuron ($6.0 \pm 2.6\%$) ($p = 0.0076$). No adult mortality was observed at this concentration, as all individuals either died at earlier developmental stages or failed to progress beyond pupation (Table 3).



Table 3. Stage-specific mortality (%) of *Aedes aegypti* following exposure to novaluron, triflumuron, and lufenuron across different concentrations.

Concentration ($\mu\text{g/L}$)	Stage	Novaluron (mean $\pm$ SE %)	Triflumuron (mean $\pm$ SE %)	Lufenuron (mean $\pm$ SE %)
Control	Larvae	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	Pupae	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	Adult	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
0.05	Larvae	15.0 $\pm$ 2.5	6.0 $\pm$ 2.6	34.0 $\pm$ 4.2
	Pupae	11.0 $\pm$ 1.9	11.0 $\pm$ 3.0	11.0 $\pm$ 1.9
	Adult	3.0 $\pm$ 1.9	8.0 $\pm$ 1.6	1.0 $\pm$ 1.0
0.25	Larvae	21.0 $\pm$ 1.9	31.0 $\pm$ 6.4	47.0 $\pm$ 3.8
	Pupae	29.0 $\pm$ 4.4	26.0 $\pm$ 2.6	26.0 $\pm$ 2.6
	Adult	6.0 $\pm$ 1.2	5.0 $\pm$ 1.9	2.0 $\pm$ 1.2
1.25	Larvae	31.0 $\pm$ 3.4	18.0 $\pm$ 5.3	56.0 $\pm$ 2.8
	Pupae	45.0 $\pm$ 1.0	41.0 $\pm$ 4.4	28.0 $\pm$ 4.3
	Adult	6.0 $\pm$ 3.5	23.0 $\pm$ 1.9	6.0 $\pm$ 3.5
6.25	Larvae	83.0 $\pm$ 1.0	88.0 $\pm$ 1.6	94.0 $\pm$ 2.6
	Pupae	17.0 $\pm$ 1.0	12.0 $\pm$ 1.6	6.0 $\pm$ 2.6
	Adult	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

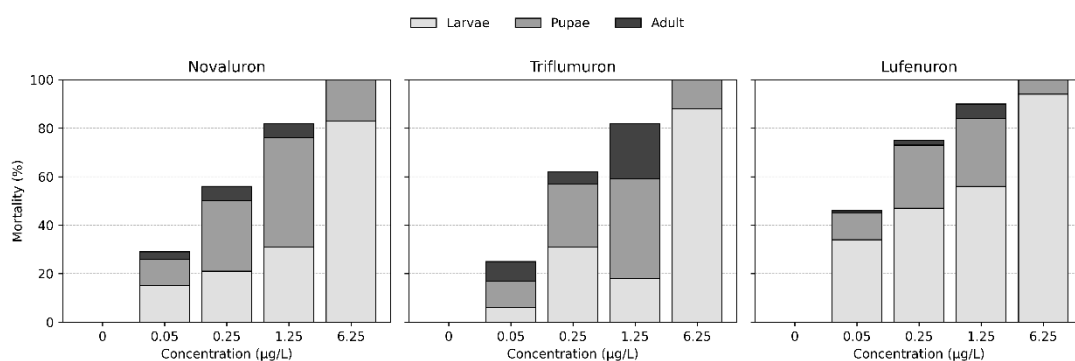


Figure 1. Stage-specific mortality of *Aedes aegypti* exposed to chitin-synthesis inhibitor IGRs.



The figure illustrates the distribution of larval, pupal, and adult mortality of *Ae. aegypti* following exposure to increasing concentrations of novaluron, triflumuron, and lufenuron. Mortality is expressed as the percentage of individuals that failed to reach the subsequent developmental stage, partitioned by life stage.

For novaluron, mortality began at low concentrations (0.05 - 0.25 µg/L), with a gradual increase in larval and pupal mortality, while adult mortality remained relatively low. At higher doses (≥ 1.25 µg/L), pupal mortality predominated, followed by near-complete larval mortality at the highest concentration (6.25 µg/L).

For triflumuron, mortality was observed across all stages, with pupal and adult mortality contributing substantially at intermediate concentrations (0.25 - 1.25 µg/L). At the maximum tested concentration (6.25 µg/L), larval mortality accounted for nearly all observed deaths, indicating a stage shift in susceptibility from later to earlier instars as the dose increased.

For lufenuron, mortality was most pronounced at the larval stage across concentrations, with relatively minor pupal and adult contributions. Mortality increased rapidly from 0.05 µg/L upward, reaching

>90% larval mortality at 6.25 µg/L, indicating that this compound strongly disrupts development at earlier stages compared to novaluron and triflumuron.

Discussion

This study demonstrated that novaluron, triflumuron, and lufenuron produced minimal acute larval mortality within 72 h but strong, concentration-dependent inhibition of adult emergence in *Ae. aegypti*. These findings indicate that the primary mode of action of chitin-synthesis inhibitors (CSIs) is developmental disruption rather than rapid larvicidal activity. This pattern is consistent with previous studies showing that CSI-treated larvae often survive initial exposure but fail during subsequent molts or adult emergence.^{12,13}

The low acute mortality observed across all compounds is biologically consistent with the mechanism of CSIs, which interfere with chitin formation and molting processes rather than acting on the nervous system. In the present study, no mortality was observed at 24 h and only low mortality was recorded at 72 h, even at the highest concentration. This delayed effect has been reported in *Ae. aegypti*, where novaluron exposure resulted in altered development and mortality during later stages rather than immediate larval



death.¹ Similarly, triflumuron has been shown to impair development, survival, and reproductive capacity in *Ae. aegypti*, with mortality distributed across developmental stages rather than concentrated at early larval stages.^{13,14}

In contrast to larval mortality, emergence inhibition (EI) showed a strong dose-response relationship for all compounds and reached complete inhibition at the highest concentration. These findings support the use of EL_{50} and EL_{90} as primary indicators of efficacy for CSIs. Previous studies have consistently demonstrated that EI is a more appropriate endpoint for insect growth regulators, as it reflects cumulative developmental disruption rather than immediate toxicity. For example, lufenuron has been shown to exhibit high potency against *Ae. aegypti*, with low EL_{50} values and strong suppression of adult emergence.⁶ Similar findings have been reported for novaluron and other IGRs, where emergence inhibition was the most sensitive and reliable measure of activity.^{18,19}

The comparative potency observed in this study (lufenuron > novaluron > triflumuron) aligns with previous research, although differences in magnitude may occur depending on experimental conditions and mosquito populations. Lufenuron has been reported to

exhibit strong activity against *Ae. aegypti*, including effects on adult emergence and reproductive parameters.^{17,18} Novaluron has also demonstrated effective emergence inhibition in laboratory and field populations of dengue vectors,¹⁹ while triflumuron, although effective, generally shows slower or less pronounced activity relative to other CSIs.^{13,14}

Stage-specific mortality analysis provided further insight into the mode of action of these compounds. In this study, lufenuron predominantly induced mortality during the larval stage, whereas novaluron showed the greatest effect at the pupal stage, and triflumuron exhibited a more distributed effect across larval, pupal, and adult stages. These findings suggest that, despite belonging to the same chemical class, the compounds differ in their primary developmental targets. Previous studies support the concept that CSI-induced mortality is stage-dependent and associated with molting processes; however, direct evidence confirming specific stage dominance for each compound in *Ae. aegypti* remains limited in the literature. Therefore, the stage-specific patterns observed in this study should be considered as experimental findings that extend current knowledge rather than fully established characteristics.¹²⁻¹⁴



The observed shift in mortality from later to earlier stages with increasing concentration further supports the mechanism of action of CSIs. At lower concentrations, larvae may survive initial molts and die during pupation or adult emergence, whereas at higher concentrations, disruption occurs earlier in development. This concentration-dependent shift has been reported in studies of chitin-synthesis inhibitors and reflects the progressive inhibition of cuticle formation across developmental stages.^{12,15}

These results highlight a key limitation of relying solely on short-term larval mortality to assess CSI efficacy. If only 24 - 72 h mortality had been considered, all compounds would appear weakly active. However, emergence inhibition and stage-specific mortality revealed substantial biological effects. This supports the use of developmental endpoints in mosquito bioassays and aligns with previous studies that emphasize EI as the primary metric for insect growth regulators.¹⁷⁻¹⁹

The high emergence inhibition observed for all compounds suggests strong potential for reducing adult mosquito populations. The differences in stage-specific activity may also have practical implications. Lufenuron, with its strong larval-stage activity,

may be more effective in rapidly developing populations, whereas novaluron may provide enhanced control during later developmental stages. Triflumuron, with its broader stage distribution, may offer more gradual but sustained suppression. These characteristics could be considered when selecting compounds for integrated vector management programs, particularly in areas with insecticide resistance.

The present study demonstrates that novaluron, triflumuron, and lufenuron act primarily as developmental inhibitors rather than acute larvicides. Emergence inhibition and stage-specific mortality provide more accurate measures of efficacy than short-term larval mortality. Among the tested compounds, lufenuron exhibited the highest potency, while novaluron and triflumuron showed distinct stage-specific patterns. These findings support the use of chitin-synthesis inhibitors as effective tools for mosquito control and highlight the importance of using appropriate biological endpoints in their evaluation.

The delayed mode of action and high potency observed for chitin-synthesis inhibitors may provide operational advantages in mosquito control strategies beyond conventional larviciding. Because exposed



individuals can survive initial contact and continue normal behaviors, including oviposition, these compounds are particularly well-suited for transfer-based approaches such as autodissemination. In such systems, contaminated adult females can disseminate active ingredients to multiple breeding sites, where they subsequently affect immature stages. Previous studies have demonstrated that lufenuron can be transferred by gravid *Ae. aegypti* females to oviposition sites, resulting in reduced fertility, fecundity, and successful adult emergence.¹⁷ In addition, autodissemination strategies using insect growth regulators have been shown to effectively suppress mosquito populations by exploiting natural oviposition behavior.²⁰ Therefore, the combination of delayed action and strong emergence inhibition observed in this study supports the potential application of these compounds in autodissemination-based vector control strategies.

Conclusions

This study demonstrates that novaluron, triflumuron, and lufenuron exhibit low acute larvicidal activity but strong, concentration-dependent emergence inhibition against *Ae. aegypti*, confirming that emergence inhibition is a more sensitive efficacy endpoint

than short-term larval mortality for chitin-synthesis inhibitors. Lufenuron showed the highest potency (EL_{50}/EL_{90}), followed by novaluron and triflumuron. Distinct stage-specific mortality patterns were observed, indicating differences in developmental targets among compounds. Overall, the results emphasize that chitin-synthesis inhibitors act primarily as developmental disruptors rather than fast-acting larvicides. The use of emergence inhibition and stage-specific mortality endpoints provides a more comprehensive evaluation of their efficacy and should be considered in laboratory testing protocols. These findings support the potential application of these compounds, particularly lufenuron, in mosquito control strategies and integrated vector management programs. Further studies are recommended to evaluate their efficacy in field populations, including insecticide-resistant strains, and under real-world environmental conditions.

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