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ASSESSING THE EFFICACY OF CHITIN SYNTHESIS INHIBITORS AGAINST MALAYSIAN *Aedes albopictus* SKUSE LARVAE

Noor Izuddin Nureen-Afrina[1], Navindra Kumari Palanisamy[1,2],
Mohd Nasir Nadzimah[1,2], Mohamad Rodi Isa[1],
Asri Lailatul-Nadhirah[1] & Othman Wan-Norafikah[1,2]*
[1]Faculty of Medicine,
Universiti Teknologi MARA (UiTM),
Selangor Branch, Sungai Buloh Campus,
Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia.
[2]HW ReNeU,
Universiti Teknologi MARA (UiTM),
40450 Shah Alam, Selangor, Malaysia.
*Corresponding author e-mail: *norafikah@uitm.edu.my*

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ABSTRACT

Insect growth regulators (IGRs) are insecticides that hinder the growth and reproduction of insects including mosquitoes by imitating hormones in their immature stages. IGRs from the type of chitin synthesis inhibitors (CSIs) impede the moulting and growing of insect larvae by disrupting their chitin synthesis. The effectiveness of three CSIs namely diflubenzuron, novaluron and cyromazine in causing the mortality among several populations of *Aedes albopictus* larvae were evaluated via the larval bioassays. Emergence inhibition at 50% of the population (EI_{50}) values of these three CSIs for *Ae. albopictus* reference strain larvae were initially obtained through the larval bioassays conducted using late third instar larvae at several concentrations that caused mortalities between 5% and 95%. These EI_{50} values of the reference strain were then applied on the field populations of the same larval species. Emergence inhibition percentage (EI%) was then calculated for each *Ae. albopictus* population tested. All *Ae. albopictus* populations showed EI% of more than 50% upon exposure to diflubenzuron, novaluron and cyromazine, indicating the effectiveness of these CSIs in controlling *Ae. albopictus* larvae at the study sites. These findings validate the potential use of these CSIs as alternative insecticides for mosquito control programmes in the future. However, the usage of CSIs and other types of IGRs by the public, needs to be monitored in order to avoid any resistance development among local *Ae. albopictus* larvae against IGRs.

Keywords: Insect growth regulators; chitin synthesis inhibitors; *Aedes albopictus*; Malaysia

ABSTRAK

Pengatur pertumbuhan serangga (PPS) adalah racun serangga yang membantutkan pertumbuhan dan pembiakan serangga termasuk nyamuk dengan cara menyerupai hormon pada peringkat pramatang mereka. PPS daripada jenis penghalang sintesis kitin (PSK) menghalang penyalinan kulit dan pertumbuhan larva serangga dengan cara mengganggu sintesis kitin mereka. Keberkesanan tiga PSK iaitu diflubenzuron, novaluron dan kiromazin dalam menyebabkan kematian di kalangan beberapa populasi larva *Aedes albopictus* telah dinilai melalui bioasai larva. Pada mulanya, nilai perencatan kemunculan pada tahap 50% populasi (EI_{50}) bagi ketiga-tiga PSK ini bagi strain rujukan larva *Ae. albopictus* telah diperolehi melalui bioasai larva yang dijalankan menggunakan larva peringkat hujung ketiga pada beberapa kepekatan yang menyebabkan kematian di antara 5% dan 95%. Nilai EI_{50} bagi strain rujukan ini kemudiannya diguna pakai ke atas populasi lapangan bagi spesies larva yang sama. Peratus perencatan kemunculan (EI%) bagi setiap populasi *Ae. albopictus* yang diuji kemudiannya dikira. Kesemua populasi *Ae. albopictus* menunjukkan EI% melebihi 50% selepas pendedahan kepada diflubenzuron, novaluron dan kiromazin, yang mana ianya menunjukkan keberkesanan kesemua PSK ini dalam mengawal larva *Ae. albopictus* di kawasan kajian. Hasil penemuan ini mengesahkan potensi penggunaan kesemua PSK ini sebagai racun serangga alternatif bagi program kawalan nyamuk pada masa hadapan. Walau bagaimanapun, penggunaan PSK dan jenis-jenis PPS yang lain oleh orang ramai perlu dipantau untuk mengelakkan sebarang pembangunan kerintangan di kalangan larva *Ae. albopictus* tempatan terhadap PPS.

Kata kunci: Pengatur pertumbuhan serangga; penghalang sintesis kitin; *Aedes albopictus*; Malaysia

INTRODUCTION

Dengue is one of many arboviruses that are transmitted to humans by mosquitoes, particularly *Aedes aegypti* and *Aedes albopictus* (Ogunlade et al. 2021). Dengue is endemic in tropical and subtropical climates (Uno & Ross 2018). Dengue remains a major public health concern for Malaysians and local health authorities for many years (Abidemi & Aziz 2020). *Aedes aegypti* commonly breeds in domestic water storage containers (Kumar et al. 2016) while *Ae. albopictus* is easily found in natural settings such as tree holes (Paul et al. 2018) as well as artificial breeding sites like plastic and rubber containers placed outdoors (Mohd et al. 2026). Although *Ae. aegypti* is the primary dengue vector, the adaptability of the secondary dengue vector; *Ae. albopictus* to survive in various breeding habitats should not be disregarded (Wan-Norafikah et al. 2018). *Aedes aegypti* is widely distributed in urban and transitional settings while *Ae. albopictus* dominates rural areas (Basari et al. 2016).

Without any specific antiviral drugs for dengue infection and also the ongoing development of dengue vaccine, the preventive measures of this infection rely mainly on the vector control programmes. Control methods using chemical substances in the form of larvicides and adulticides to combat the larval and adult mosquito stages, respectively, have become the most employed techniques in Malaysia and other countries. Regrettably, the uncontrolled application of insecticides in the mosquito control activities and also the agricultural pest management have driven to the occurrence of insecticide resistance among mosquitoes and other insects (Bukar et al. 2025; Shettima et al. 2023). Failures in the vector control programmes conducted due to insecticide resistance detected in mosquitoes have been reported in some countries (Arham et al. 2021) and this is where the substitute vector control approaches are needed in order to serve the purpose of eliminating the mosquito populations.

Insect growth regulators (IGRs) are chemicals with active ingredients that imitate the hormones of immature insects. IGRs interfere the mosquito reproduction, egg hatching, and moulting of mosquito larvae from one phase to the next (Rahman et al. 2024). The toxicity impact of IGRs on mammals and other non-target organisms is minimal (Scheff et al. 2024). Chitin synthesis inhibitors (CSIs) are one group of IGRs that impede the moulting and growing processes of mosquito larvae by disrupting their chitin synthesis (Belinato et al. 2009). IGRs have been used for mosquito larval control in certain countries. For example, the usage of pyriproxyfen and novaluron in Pakistan caused more than 90% *Ae. aegypti* larval mortality (Rahman et al. 2024), whereas novaluron has been used to control the field populations of *Ae. aegypti* and *Ae. albopictus* in India (Herath et al. 2024). However, the ability of IGRs especially CSIs in controlling *Aedes* immatures are still underreported. Therefore, the purpose of this research was to evaluate the efficacy of CSIs including diflubenzuron, novaluron and cyromazine in controlling *Ae. albopictus* larval populations from Malaysian residential and agricultural areas.

MATERIALS AND METHODS

Study Localities

This study covered both residential and agricultural areas in Peninsular Malaysia (Table 1). Residential sites included both dengue-risk and dengue-free areas. Dengue-free areas refer to residential areas with no reported dengue cases within the last five years before the conduct of this study, while dengue-risk areas are residential areas with reported dengue cases within the same period. Agricultural sites including oil palm estates, paddy fields, and rubber plantations were chosen as they represent major agricultural crops in Malaysia and no cases of mosquito-borne infections have been reported from these areas to date. Three sites were selected for each residential and agricultural setting, respectively, with a total of fifteen field populations of *Aedes albopictus* for this study. A laboratory-reared *Ae. albopictus* strain, which was originally collected from Selangor, Malaysia, and has been maintained for over fifty generations without insecticide exposure, was used as a reference strain in this study.

Table 1. Lists of selected mosquito populations in Peninsular Malaysia

Classification of Populations	Type of Areas	Populations	Locality Coordinate
Residential	Dengue-free	Selangor	3°6′52.39″ N, 101°28′9.20″ E
		Kedah	5°31′21.35″ N, 100°32′35.67″ E
		Pahang	3°26′59.45″ N, 102°26′39.25″ E
	Dengue-risk	Johor	1°40′39.55″ N, 104°1′18.64″ E
		Selangor	3°2′56.91″ N, 101°29′17.21″ E
		Kuala Lumpur	3°6′38.84″ N, 101°44′58.57″ E
Agricultural	Paddy fields	Selangor	3°30′1.27″ N, 101°9′31.88″ E
		Kedah	5°32′45.23″ N, 100°32′27.88″ E
		Negeri Sembilan	2°44′30.20″ N, 102°7′49.52″ E
	Rubber estate	Selangor	3°12′28.68″ N, 101°32′25.81″ E
		Pahang	3°27′21.60″ N, 102°27′31.21″ E
		Johor	1°33′52.19″ N, 104°14′9.72″ E
	Oil palm plantations	Johor	2°0′13.01″ N, 103°51′47.50″ E
		Selangor	3°9′16.44″ N, 101°27′37.08″ E
		Pahang	3°27′37.07″ N, 102°28′9.62″ E

Larval Surveillance and Sample Colonization

A larval survey was conducted at each locality to collect *Ae. albopictus* larvae. All natural and artificial containers with stagnant water found at each locality were checked for any presence of mosquito larvae. Mosquito larvae discovered from these mosquito breeding points were collected using the 3 ml plastic pipettes and placed in labelled containers. Collected larvae were brought back to the laboratory, fed with a half-cooked beef liver diet and reared to adult stage. Identification of adult mosquitoes was performed using taxonomic keys (Jeffery et al. 2012) and only *Ae. albopictus* adults (F0) were further bred to produce F1 eggs. Eggs of *Ae. albopictus* (F1) were hatched and reared in the same manner as the F0 generation until they reached the late third instar larvae which were utilized in this study. Simultaneously, *Ae. albopictus* reference strain (F90) was maintained in the laboratory in a similar manner as *Ae. albopictus* field populations (F0 and F1).

Insect Growth Regulators (IGRs)

Three insect growth regulators (IGRs) from the group of chitin synthesis inhibitors (CSIs) utilized in this study were diflubenzuron (Dimilin) 25% w/w wettable powder (Uniroyal Chemical Company Inc., Crompton Corporation, Connecticut, USA), novaluron (Rimon) 10% w/w emulsifiable concentrate (Zeenex (Malaysia) Sdn. Bhd., Kuala Lumpur, Malaysia), and cyromazine (Trigard) 75% w/w wettable powder (Syngenta Crop Protection Sdn. Bhd., Selangor, Malaysia).

Larval Bioassays using CSIs

Larval bioassays using CSIs were conducted according to the standard method by World Health Organization (2016). For each chitin synthesis inhibitor (CSI), several test concentrations were prepared using the stock solutions diluted in 200 ml of dechlorinated tap water per cup, aiming to produce 5–95% emergence inhibition (EI) in *Ae. albopictus* reference strain. Each test involved five replicates of twenty late third instar larvae placed in individual cups, with continuous exposure to the CSI mixture. These test cups were run concurrently with two control sets that were unexposed to any CSI and contained similar volume of dechlorinated tap water and number of third instar larvae. Larvae in all cups were nourished with half-cooked beef liver chunk, and the cups were covered with mesh to avoid any escapee. Observations were made every two days to record the mortality and successful emergence until all individuals had either emerged or died. Experiments were carried out in the laboratory under maintained environment of 27±2°C room temperature (RT), 75±10% relative humidity (RH) and a 12-hour light/dark cycle.

Subsequently, larval bioassays using the same CSIs were carried out on all *Ae. albopictus* field populations using the EI_{50} value of each CSI acquired from the larval bioassays using CSIs of *Ae. albopictus* reference strain. Larval bioassays using CSIs for all *Ae. albopictus* field larval populations were run in the same manner as described earlier.

Statistical Analysis

Emergence inhibition (EI) reflects the failure of larvae to mature into adults due to IGR exposure. This includes moribund and mortality at the larval and pupal stage as well as incomplete adult emergence. For each CSI, several concentrations inducing mortality rates between 5% and 95% in *Ae. albopictus* larvae of the reference strain by the end of the larval bioassays were chosen for probit analysis to develop a regression line. From this regression line, the emergence inhibition value for 50.00% of individuals in the reference strain colony (EI_{50}) was obtained for each CSI. The EI_{50} value of all CSIs acquired for *Ae. albopictus* reference strain were later applied in the larval bioassays involving all field populations of the same species to calculate the emergence inhibition percentage (EI%) for each field population exposed to each CSI. The emergence inhibition percentage (EI%) was determined using the following formula:

$$\text{Emergence Inhibition (EI\%)} = 100 - \frac{\text{\% emerged adult mosquitoes in treated batches x 100}}{\text{\% emerged adult mosquitoes in control batches}}$$

The test was considered invalid and repeated if adult mosquito emergence in the control group was below 90.00%. However, if mosquito emergence in control set ranged between 91.00% and 99.00%, the results were corrected using the Abbott's formula (Abbott 1925):

$$\frac{\text{EI \% of treated -EI \% of control}}{\text{100-EI \% of control}} \text{ x 100}$$

The Normality Test using the Shapiro-Wilk test was conducted on the EI% data for all *Ae. albopictus* larval populations to determine their distribution pattern. An EI% for each *Ae. albopictus* field population that was more than 50.00% indicated the effectiveness of the CSIs tested (Lau et al. 2015a).

Statistical analyses were carried out using the SPSS software at a significance level of $P=0.05$ to determine any significant differences in EI% ($P\leq0.05$) among assorted *Ae. albopictus* populations and the association between EI% of different CSIs. The Kruskal-Wallis Test and the Mann-Whitney U Test were performed for these analyses.

RESULTS

The emergence inhibition (EI) value for 50% of individuals (IE_{50}) of *Ae. albopictus* reference strain exposed to diflubenzuron, novaluron and cyromazine that were attained from the regression lines of probit analyses were 0.000355 mg/L (0.00023 – 0.000559 mg/L), 0.000175 mg/L (0.000134 – 0.000175 mg/L) and 0.0931 mg/L (0.0870 – 0.1018 mg/L), respectively. These EI_{50} values of *Ae. albopictus* reference strain were then used in the larval bioassays of *Ae. albopictus* field populations using similar CSIs. The emergence inhibition percentage (EI%) calculated for all *Ae. albopictus* larval populations exposed to diflubenzuron, novaluron and cyromazine were all beyond 50.00% (Table 2).

Table 2. The emergence inhibition percentage (EI%) for chitin synthesis inhibitors (CSIs) of diflubenzuron, novaluron and cyromazine across various *Aedes albopictus* larval populations

Classification of Populations	**Type of Areas**	**Populations**	**EI% Diflubenzuron**	**EI% Novaluron**	**EI% Cyromazine**
Reference	Laboratory	Laboratory	99.00	98.00	99.00
Residential	Dengue-free	Selangor	100.00	99.00	98.95
		Kedah	100.00	95.79	98.95
		Pahang	100.00	91.58	100.00
	Dengue-risk	Johor	100.00	99.00	100.00
		Selangor	100.00	95.79	100.00
		Kuala Lumpur	100.00	97.95	100.00
Agricultural	Paddy fields	Selangor	97.84	91.35	96.76
		Kedah	100.00	93.51	93.51
		Negeri Sembilan	98.89	92.22	100.00
	Rubber estates	Selangor	97.89	91.58	97.89
		Pahang	100.00	98.95	100.00
		Johor	100.00	91.35	97.84
	Oil palm plantations	Johor	100.00	91.35	100.00
		Selangor	100.00	93.51	100.00
		Pahang	100.00	94.87	100.00

Table 3. Median and interquartile range (IQR) of EI% for chitin synthesis inhibitors (CSIs) of diflubenzuron, novaluron and cyromazine across various *Ae. albopictus* larval populations, obtained from the Kruskal-Wallis Test (*P=0.05*)

Classification of Populations	**Type of Areas**	**Chitin Synthesis Inhibitors (CSIs)**											
		Diflubenzuron				**Novaluron**				**Cyromazine**			
		No. of Populations	**Median EI% (IQR)**	**H Statistic**	***P Value***	**No. of Populations**	**Median EI% (IQR)**	**H Statistic**	***P Value***	**No. of Populations**	**Median EI% (IQR)**	**H Statistic**	***P Value***
Reference	Laboratory	1	-	7.51	0.19	1	-	6.19	0.29	1	-	6.91	0.23
Residential	Dengue-free	3	100.00 (0.00)			3	95.79 (0.00)			3	98.95 (0.00)		
	Dengue-risk	3	100.00 (0.00)			3	97.95 (0.00)			3	100.00 (0.00)		
Agricultural	Paddy fields	3	98.89 (0.00)			3	92.22 (0.00)			3	96.76 (0.00)		
	Rubber estates	3	100.00 (0.00)			3	91.58 (0.00)			3	97.89 (0.00)		
	Oil palm plantations	3	100.00 (0.00)			3	93.51 (0.00)			3	100.00 (0.00)		

IQR = Interquartile range

Table 4. The Mann-Whitney U Test analyses (*P=0.05*) for comparison between the EI% of diflubenzuron, novaluron and cyromazine, respectively, across different classifications of *Ae. albopictus* larval populations

Classification of Populations	**No. of populations**	**Chitin Synthesis Inhibitors (CSIs)**											
		Diflubenzuron				**Novaluron**				**Cyromazine**			
		U Statistic	**Z Value**	***P Value***	**Median EI% (IQR)**	**U Statistic**	**Z Value**	***P Value***	**Median EI% (IQR)**	**U Statistic**	**Z Value**	***P Value***	**Median EI% (IQR)**
Residential vs Agricultural	6 vs 9	18.00	-1.52	0.33	100.00 (0.75)	8.50	-2.20	0.08	94.19 (6.41)	20.00	-0.93	0.46	100.00 (1.84)
Residential vs Reference	6 vs 1	0.00	-2.45	0.29		2.00	-0.51	0.86		2.00	-0.56	0.86	
Agricultural vs Reference	9 vs 1	3.00	-0.59	0.80		1.00	-1.24	0.40		4.00	-0.19	1.00	

IQR = Interquartile range

From the Normality Test performed, the results of emergence inhibition percentage (EI%) for all *Ae. albopictus* populations were not normally distributed. Following this, descriptive statistical analyses were presented in the form of median and interquartile range (IQR) (Table 3). The Kruskal-Wallis Test carried out revealed no significant difference in EI% of diflubenzuron, novaluron and cyromazine, respectively, across different type of areas (*P>0.05*) (Table 3). Therefore, no Post Hoc analysis was performed. Furthermore, the Mann-Whitney U Test performed showed no significant difference between the EI% of diflubenzuron, novaluron and cyromazine exposures, respectively, across different classifications of *Ae. albopictus* larval populations (*P>0.05*) (Table 4). In contrast, the Mann-Whitney U Test analyses demonstrated a significant difference between the EI% of all *Ae. albopictus* larval populations exposed to diflubenzuron and novaluron, and between the EI% of similar *Ae. albopictus* larval populations exposed to novaluron and cyromazine (*P≤0.05*) (Table 5).

Table 5. The Mann-Whitney U Test analyses (*P=0.05*) for comparison between the EI% of all *Ae. albopictus* larval populations exposed to diflubenzuron, novaluron and cyromazine

Chitin Synthesis Inhibitors (CSIs)	**U Statistic**	**Z Value**	***P Value***	**Median EI% (IQR) for Diflubenzuron**	**Median EI% (IQR) for Novaluron**	**Median EI% (IQR) for Cyromazine**
Diflubenzuron vs Novaluron	14.00	-4.42	<0.001	100.00 (0.75)	94.19 (6.41)	100.00 (1.84)
Diflubenzuron vs Cyromazine	102.00	-1.16	0.25			
Novaluron vs Cyromazine	30.00	-3.74	<0.001			

IQR = Interquartile range

DISCUSSION

The development of alternative insecticides, known as insect growth regulators (IGRs), has been driven by the increasing resistance observed in insect populations, particularly in mosquitoes, resulting from the massive and prolonged use of conventional insecticides. This resistance has ultimately undermined vector control efforts. IGRs are either synthetic or natural substances that interfere with key physiological, biochemical, or molecular processes essential to an insect's development, growth, and reproductive cycle (Pener & Dhadialla 2012). There are several types of IGRs, including chitin synthesis inhibitors (CSIs). Notable examples of CSIs include diflubenzuron, novaluron and cyromazine. These CSIs disrupt the normal development of insects by hindering ecdysis and the formation of a new chitin-based exoskeleton. They also interfere with embryonic development which usually results in mortality (Hamilton et al. 2021). Diflubenzuron, novaluron and cyromazine generally trigger the early stage of moulting process in insect larvae before the exoskeleton is fully developed (Farnesi et al. 2012). However, CSIs only target developmental pathways without affecting synaptic communication, making them arthropod-specific (Merzendorfer 2013). CSIs also exhibit low toxicity to vertebrates (Asgarian et al. 2023; Farnesi et al. 2012), making them suitable alternative larvicides in controlling *Aedes* population.

In this study, three CSIs tested namely diflubenzuron, novaluron and cyromazine, displayed high efficacy against *Ae. albopictus* larvae as indicated by emergence inhibition percentage (EI%) of each population that was more than 50.00% across the *Ae. albopictus* laboratory reference strain and field populations from different residential and agricultural sites. Diflubenzuron was the most effective, with recorded EI% for all larval populations of *Ae. albopictus* were beyond 97.00%. Cyromazine was the second most effective CSI against *Ae. albopictus* larval populations from all residential and agricultural sites as indicated by EI% of more than 93.00%. On the other hand, novaluron was more effective against *Ae. albopictus* larval populations from residential sites (EI% = 91.35% - 99.00%) than larval populations of the same species from agricultural sites (EI% = 91.35% - 98.95%). Moreover, results obtained showed that the efficacy of diflubenzuron, novaluron and cyromazine utilized in this study were not affected by diverse past exposures to typical public health insecticides and agricultural pesticides used by local health authorities and plantation owners, respectively. Furthermore, the efficacies of all these CSIs tested were also not affected by the differences in the classification of localities. In other words, these CSIs were effective on the *Ae. albopictus* laboratory strain as well as on *Ae. albopictus* from all types of residential and agricultural sites. However, exposures to novaluron among all *Ae. albopictus* larval populations may significantly affect their susceptibility towards diflubenzuron and cyromazine. Therefore, careful consideration should be given in implementing a combination of novaluron application with diflubenzuron or cyromazine application if needed at these residential and agricultural sites.

Diflubenzuron specifically inhibits adult emergence by preventing chitin formation, which results in imperfect moulting in exposed larvae (Sankar et al. 2024). Previous studies have shown that diflubenzuron effectively inhibited adult emergence and reduced egg hatching rates in *Ae. aegypti* field populations from Thailand (Fansiri et al. 2022). The mixture of diflubenzuron and *Bacillus thuringiensis israelensis* (BTI) bacteria applied in road sewers in Italy was reported to be effective against *Ae. albopictus* larvae for up to 14 days (Giatropoulos et al. 2022). Moreover, diflubenzuron has showed prolonged residual efficacy for up to four months in *Ae. aegypti* larvae from Lao PDR, suggesting its potential as a replacement for conventional insecticides to which *Aedes* mosquitoes are resistant to (Marcombe et al. 2018). Diflubenzuron was also found to be effective against *Ae. aegypti* larval populations from several areas in Brazil (Garcia et al. 2018). Besides its broad-spectrum efficacy and relatively low toxicity to mammals at recommended dosages, diflubenzuron is also widely accepted by the regulatory authorities, including the World Health Organization (Araujo et al. 2025). However, resistance emergence to diflubenzuron in certain arthropods has been reported, which reduces their susceptibility and encouraging the needs for regular resistance monitoring and insecticide rotation plans (Mastrantonio et al. 2025). This is important to prevent heavy reliance on a single insecticide and to curb future resistance development, especially in *Aedes* mosquito populations.

Novaluron operates through a similar mode of action as diflubenzuron, preventing the emergence of adult insects. When applied in aqueous form, novaluron has been shown to induce apoptosis in the digestive cells of *Ae. aegypti* larvae, leading to mortality (Fiaz et al. 2021). Its efficacy has been observed for up to three months against *Ae. aegypti* and *Ae. albopictus*, with adult emergence inhibited by up to 95.00% in laboratory, field and semi-field evaluations (Gunathilaka et al. 2020; Herath et al. 2024). In Columbia, fourteen weeks of exposure at highest concentration of 0.585 mg/L of novaluron have resulted in almost 100.00% inhibition of adult emergence among *Ae. aegypti* collected from urban environment (Quimbayo et al. 2022). Novaluron offers similar benefits to diflubenzuron, being highly effective against *Aedes* larvae, posing minimal risk to non-target organisms at operational dosages, and being practical for usage in small insect breeding habitats (Farnesi et al. 2012). As with diflubenzuron, long-term resistance monitoring in field populations, especially among endemic vectors such as *Aedes* is recommended following novaluron application (Herath et al. 2024).

Cyromazine, originally developed to target dipteran agricultural pests, is also known as triazine-based moulting disruptors (Khan 2024). It disrupts normal cuticle formation by causing wounds through tissue damage (Binnington et al. 1987), and pupation processes, and has demonstrated larvicidal activity against mosquito larvae in laboratory studies (Khan 2024). Unlike benzoylphenyl ureas such as diflubenzuron and novaluron, cyromazine has a slightly different chemical structure, which may reduce the risk of immediate cross-resistance. However, field trials evaluating the effectiveness of cyromazine against *Aedes* mosquitoes are relatively limited in comparison to the other CSIs (Khan 2024). Only few studies have assessed *Aedes* susceptibility to cyromazine. For example, Lau et al. (2015b) and Elia-Amira et al. (2022) reported on high susceptibility to cyromazine in Malaysian *Ae. albopictus* field populations. Another study had combined the cyromazine with an agricultural fertilizer to simultaneously nurture plants and eliminate crop pest insects and *Ae. albopictus* larvae which was effective for up to 15 days (Darriet 2022). Recently, Yuksel et al. (2025) has reported on an effective dosage of 65.846 g a.i/ha for cyromazine to be applied against *Ae. aegypti* larvae in Turkey. Nonetheless, these findings remain insufficient to draw definitive conclusions on the overall efficacy of cyromazine. Further research is necessary to evaluate its effectiveness in different types of aquatic habitats and to assess the possibility of resistance development in immature mosquito stages (Asgarian et al. 2023; Khan 2024).

CSIs are reliable and safe to most untargeted organisms and remain effective for a long period (Mulla 1995). A longer period of efficacy would allow for fewer applications of these IGRs, which would also lower labour and purchase costs. However, the slow-acting effects of IGRs including CSIs may limit their use in only long-term vector control strategies, making them unsuitable to be utilised during dengue outbreaks (Elia-Amira et al. 2022). Hence, the use of CSIs and other IGRs are recommended as continuous alternative vector control activities to be carried out by communities with supports and guidance from local health authorities.

CONCLUSION

In conclusion, CSIs of diflubenzuron, novaluron and cyromazine showed effectiveness against all *Ae. albopictus* larval populations tested in this study, thus, signifying their promising application in diverse residential and agricultural localities in Malaysia. However, further laboratory experiments and field evaluations on CSIs and other IGRs, are still needed to better comprehend their action mechanisms and the susceptibility level of mosquito immatures towards these IGRs before they can be executed on the ground. Larval surveillance and monitoring of mosquito susceptibility towards IGRs are essential to be constantly conducted to assess the efficacy of IGRs in controlling mosquito juveniles and avoid any misapplication of IGRs, which could later lead to the occurrence of resistance to IGRs in mosquito larvae and finally failures in vector control programmes.

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AUTHORS DECLARATION

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The authors agree that this research was conducted in the absence of any self-benefits, commercial, financial, or interest conflicts.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statements
Not applicable.

Authors' Contributions
Conceptualization: OWN, MNN; Methodology: OWN; Experiments: NINA, ALN; Data curation: NINA, OWN; Data analysis: NINA, MRI, OWN; Data validation: MRI, OWN, NKP; Writing – Original draft preparation: NINA; Writing – Reviewing & Editing: OWN, NKP, MRI; Supervision: OWN, NKP; Funding acquisition: OWN, MNN; Project administration: OWN, MNN.

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