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Research Article

Methoxyfenozide as a potent insect growth regulator: Disruption of growth, development and chitin synthesis in *Aedes aegypti* for sustainable vector control

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Abstract
Aedes aegypti is a major vector of arboviral diseases such as dengue, chikungunya, Zika, and yellow fever. Traditional chemical insecticides have led to widespread insecticide resistance and posed risks to non-target organisms and the environment. Insect growth regulators (IGRs) offer a promising alternative by disrupting mosquito development rather than inducing immediate lethality. The present study evaluated the efficacy of methoxyfenozide, a non-steroidal ecdysteroid agonist, in disrupting the development and chitin synthesis of *Ae. aegypti* larvae to explore its potential as a mosquito control agent. Larvicidal activity, inhibition of adult emergence, and effects on chitin synthesis were assessed using standard bioassays and biochemical quantification techniques. Lethal concentrations and emergence inhibition dosages were determined using probit analysis. Methoxyfenozide exhibited dose-dependent larvicidal activity, with respective LC_{50} and LC_{90} values of 6.326 mg/L and 115.615 mg/L after 24 hours, and 4.180 mg/L and 119.100 mg/L after 48 hours, indicating a delayed action. The compound significantly inhibited adult emergence ($p < 0.05$), exhibiting an IE_{90} value of 1.209 mg/L. Morphological abnormalities, such as arrested moulting and structural deformities, were also observed. Biochemical analysis revealed a substantial reduction in chitin content in methoxyfenozide-treated larvae, with a greater decrease observed after longer treatment durations and at higher dosages. The study revealed that methoxyfenozide effectively disrupted *Ae. aegypti* development, inhibited both larval survival and adult emergence, as well as impeded chitin content deposition. The effective mode of action and minimal toxicity to non-target species highlight its potential for inclusion in integrated vector management strategies.

Keywords: *Aedes aegypti*, Chitin synthesis inhibition, Larvicidal activity, Insect growth regulator, Methoxyfenozide, Vector control

INTRODUCTION

The prevalence of dengue fever mosquitoes, *Aedes aegypti* (Linnaeus) and *Aedes albopictus*, worldwide, particularly in tropical and subtropical regions, has caused widespread transmission of several arboviruses, including dengue (DENV), chikungunya (CHIKV), Zika (ZIKV), and yellow fever (YFV), inflicting a large number of diseases and mortalities (Benelli and Mehlhorn, 2016). In addition, various other species, such as *Aedes vittatus* and *Aedes polynesiensis*, have been reported in a few countries in the Southeast Asia Region. The absence of effective therapy and vaccines for these arboviruses, except for YFV, has exacerbated

the global arboviral disease burden (WHO, 2024a). Besides, emergence and re-emergence of these arboviruses has heightened disease prevalence.

Among various *Aedes*-borne diseases, dengue has emerged as one of the fastest-spreading diseases, resulting in approximately 100–400 million annual infections. Remarkably, more than 100 countries across the five WHO Regions have displayed dengue endemism, putting 3.5 billion people at disease risk (WHO, 2024b). In India, *Ae. aegypti* is the major vector of dengue and Chikungunya. Despite numerous control efforts, the Directorate of National Vector-Borne Disease Control Programme (NVBDCP) recorded a total of 289,235 dengue cases and 485 deaths in 2023, which declined slightly to 233,519 cases and 297 deaths in 2024. As of March 2025, 12,043 cases and 6 fatalities have been recorded (NVBDCP, 2025). It is apparent that several dengue cases go unrecorded due to being asymptomatic, mild, and self-managed; hence, it is difficult to accurately assess the actual scenario (Waggoner *et al*., 2016).

Due to the lack of effective medication and vaccines, *Aedes*-borne diseases are currently managed through vector control. Among the various interventions, chemical-based interventions remain predominant for controlling *Aedes* populations. Intensive efforts have been made to reduce mosquito density at various life stages. Yet, larvicidal measures are considered ideal and most effective due to their restricted mobility and captivity in small aquatic ecosystems (Killeen *et al*., 2013).

Nonetheless, the prolonged use of these chemicals has led to the elimination of susceptible individuals and, consequently, the selection of resistant ones, resulting in the evolution of insecticide resistance in *Ae. aegypti* (Hazarika *et al*., 2025). Ironically, mosquitoes have developed resistance to the applied chemicals and other compounds with similar mode of action, impeding effective disease control. Apart from this, the application of these residual and non-degradable chemicals in households, as well as in surrounding fields and other outdoor areas, has harmed the health of individuals, non-target organisms, and the environment (Şengül Demirak and Canpolat, 2022). For a few years, the focus has been diverted to using Insect Growth Regulators/Disruptors (IGRs/IGDs) as innocuous alternatives for lethal chemicals. This has been attributed to the efficacy of IGRs at sub-lethal dosages, their quick biodegradation, comparatively less residual nature, and low toxicity to non-target organisms, particularly vertebrates, making their use beneficial in Integrated Pest Management (IPM) and resistance programs (Zibaee *et al*., 2011; Punniyakotti *et al*., 2024). These compounds affect insect growth and development by inhibiting the post-moulting chitin synthesis (Chitin synthesis inhibitors; CSI) or disrupting the endocrine regulatory mechanism of insects (Juvenile Hormone Analogues and Ecdysteroids), thereby reducing the successive generations and finally bringing the insect below the threshold limits (Tunaz and Uygun 2004; Tanani *et al*., 2022; Ma *et al*., 2024).

Methoxyfenozide (RH-2485) is an endocrine disruptor which influences the development of certain insect pests, specifically displaying selectivity for lepidopteran pests (Ahmed *et al*., 2022). It binds to the ecdysteroid receptor complex, competing with ecdysteroids and mimicking the steroid insect moulting hormone 20-hydroxyecdysone (20E), causing a premature and incomplete lethal moult (Smagghe *et al*., 2012). Methoxyfenozide has been found effective against *Spodoptera exigua* (Chen *et al.,* 2019), *Helicoverpa armigera* (Saber *et al*., 2013) and *Plutella xylostella* (Arruda *et al.,* 2020) among several other lepidopterans. Hamaidia and Soltani (2021) reported that methoxyfenozide exhibited an LC_{50} value of 24.54 µg/L and an LC_{90} value of 70.79 µg/L against fourth-instar larvae of *Culex pipiens.* They also found that larval treatment with LC_{50} dosage of methoxyfenozide negatively altered their autogeny capacity and oviposition.

Despite the growth regulatory efficacy of methoxyfenozide against several insect pests, limited studies have been conducted regarding its efficacy against the dengue vector *Ae. aegypti* (Fansiri *et al*., 2022; Sankar and Kumar, 2023). Considering the management potential of methoxyfenozide, its plausible safety in the environment, and the low possibility of resistance development, the present study aimed to determine its efficacy against *Ae. aegypti* larvae. In addition, since it is a known moulting hormone mimic, its effect on chitin synthesis in *Ae. aegypti* larvae was also assessed, hypothesizing that the probable dual effect of methoxyfenozide may help in the formulation of a new strategy for its use in IPM.

MATERIALS AND METHODS

Procurement of chemical

Methoxyfenozide, with a purity of > 98.0%, and the analytical grade chemicals used in biochemical assays were procured from M/S Sigma-Aldrich, India.

Strain of *Aedes aegypti*

The present study involved the larvae of *Ae. aegypti* (AND-*Ae. aegypti*) maintained without any insecticide selection pressure for the past twelve years at the Insect Pest and Vector Laboratory (IPAV), Acharya Narendra Dev College, University of Delhi, India. The strain has been reared according to the methodology adopted by Kumar *et al*. (2009) under controlled environmental conditions.

Larvicidal assay with methoxyfenozide against *Aedes aegypti*

Methoxyfenozide was tested for larvicidal efficacy against early fourth instars of *Ae. aegypti*. The larvicidal assay was conducted at a controlled temperature of

28±1 °C, following the standard World Health Organisation (WHO) protocol (WHO, 2016) with minor modifications (Samal *et al*., 2022a, b). Early fourth instars of *Ae. aegypti* were exposed for 24 hours to a range of methoxyfenozide concentrations (0.5 mg/L to 10 mg/L) prepared using ethanol as a solvent. Care was taken not to use small, lethargic, or damaged larvae. During the treatment period, the larvae were not given any food. The dead and moribund larvae were scored after 24 hours, and the percentage mortality was calculated.

The assay with each concentration was run in 4 replicates with an equal number of concurrent controls using ethanol instead of methoxyfenozide. Since growth regulators are slow-acting insecticides, the delayed effects were also noted by recording observations after 48 h of treatment. The percent larval mortality in each bioassay was calculated using the formula given in Equation 1:

Larval mortality (%) = (Total number of dead larvae / Total number of exposed larvae) X 100 …… Eq. 1

Any assay with a pupation rate of greater than 10% was discarded and repeated. In cases where mortality in the control set ranged from 5% to 20%, the mortality of the treated groups was corrected using Abbott's formula (Abbott, 1925) (Equation 2). Conversely, the test was rejected when the mortality rate exceeded 20% in the larval stage.

% Corrected mortality = (% Test mortality - % Control mortality) / (100-% Control mortality X 100) ..... Eq. 2

During the assay, behavioural alterations were also recorded in the larvae using Olympus Magnus MIPS Camera (40X). Changes were observed after every hour until no aggressive moments were observed.

Effect of methoxyfenozide on the adult emergence of *Aedes aegypti*

The efficacy of methoxyfenozide in inhibiting adult emergence from treated larvae of *Ae. aegypti* was assessed according to the WHO protocol (WHO, 2016). A range of methoxyfenozide concentrations (0.1 mg/L to 0.5 mg/L) was prepared using ethanol as the solvent. Thirty early fourth instars were added to the homogeneous solution of 1 mL methoxyfenozide and 199 mL dechlorinated water. Each concentration was replicated three times along with simultaneous controls. After 24 h, the treated larvae were washed thoroughly with dechlorinated water and reared until they reached adulthood. Larval mortality and adult emergence were recorded daily, and the percentage inhibition of adult emergence (IE) was determined using the formula in Equation 3.

IE% = 100 − {T X 100/C} ……..Eq. 3

Where T = percentage of adult emergence in methoxyfenozide-treated sets, and C = percentage of adult emergence in the control set.

During the assay, any morphological aberrations in the larvae and intermediates formed were observed and photographed using a Magnus MIPS Camera, Olympus India Pvt. Limited at 40X.

Statistical analysis of the data

Using the computer software program SPSS 22.0, the lethal concentrations (LC) resulting in 30%, 50%, 70% and 90% larval mortality and inhibition of adult emergence values (IE) resulting in 30%, 50%, 70% and 90% inhibition, were calculated from a log dosage-probit mortality regression line. Furthermore, the 95% confidence intervals (CI) for each lethal concentration and adult emergence inhibition, as well as the slope, standard error (SE) and chi-square values were calculated. When the 95% CI of the LC_{50} values did not overlap, they were considered significantly different (Sharma *et al*., 2016).

Chitin content analysis in *Aedes aegypti*

Chitin content in fourth instar *Ae. aegypti* larvae was quantified using the method of Lehmann and White (1975), with modifications described by Zhang and Zhu (2006). Twenty early fourth-instar larvae were homogenised in 1 mL of distilled water, followed by 15 minutes of centrifugation at 1800 × *g*. The obtained pellets were resuspended in 0.4 mL of SDS and incubated at 100°C for 15 min. Pellets were then cooled and centrifuged again, followed by washing and resuspension in 0.3 mL of potassium hydroxide (KOH). Subsequently, these were incubated at 130 °C for 1 h, resulting in chitin deacetylation. The samples were then cooled to ice temperature, mixed with 0.8 mL of ice-cold 75% ethanol, incubated for 15 minutes, and treated with 30 µL of Celite 545. The treated samples were centrifuged (1800 × *g*, 5 min, 4 °C), and the pellets were washed with 40% ethanol and distilled water. They were then resuspended in 0.5 mL of water.

Now, 100 µL of chitosan suspension was mixed with 50 µL of 10% sodium nitrite ($NaNO_2$) and potassium hydrogen sulphate ($KHSO_4$) each. The mixture was incubated for 15 min at ambient temperature and subsequently centrifuged. A 60 µL aliquot of supernatant was taken and reacted with 20 µL of 12.5% ammonium sulfamate, followed by 20 µL of MBTH (3-methyl-2-benzothiazolinone hydrazine). The mixture was incubated at 100 °C for 5 min. After cooling, 20 µL of 0.83% iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was added. Absorbance was read at 650 nm using a microplate reader (NanoQuant Infinite® M200 PRO). Chitin content was calculated as glucosamine equivalents using a standard curve (Sigma-Aldrich).

Impact of methoxyfenozide on chitin content in *Aedes aegypti*

A total of five hundred newly hatched *Ae. aegypti* fourth instar larvae were selected and divided into five groups of 100 larvae each. Four groups were separately treated with the IE_{30}, IE_{50}, IE_{70} and IE_{90} concentration of

methoxyfenozide, while the fifth group of 100 larvae was exposed to ethanol as a control, following the WHO standard protocol (WHO, 2016) with minor modifications (Kumar *et al*., 2009) at a controlled temperature of 28 ± 1°C. After 24 h and 48 h of methoxyfenozide exposure, 20 surviving larvae were collected from each experimental and control group. The experiment was repeated five times. The chitin content of the larvae at each time point was measured using the previously described protocol (Lehmann and White, 1975; Zhang and Zhu, 2006).

RESULTS

The present study evaluated the effects of methoxyfenozide on the survival and growth of early fourth instar larvae of *Ae. aegypti*. The effect of emergence inhibition values on the chitin content of larvae was also estimated to assess their growth regulatory effects.

Larvicidal efficacy of methoxyfenozide against *Aedes aegypti*

The larvicidal potentialities of methoxyfenozide observed against the early fourth instar of *Ae. aegypti* after 24 h of exposure are presented in Table 1. The results showed moderate LC_{50} and LC_{70} values of 6.326 mg/L and 20.772 mg/L, respectively (Table 1, Fig. 1a). The r-value of 0.957 indicated a strong correlation between the dosage and the percentage of larval mortality. The increased duration of treatment, up to 48 h, however, increased the efficacy of methoxyfenozide, resulting in reduced LC_{50} and LC_{70} values of 4.180 mg/L and 13.380 mg/L, respectively (Table 1, Fig. 1b), indicating latent effects. Remarkably, a slight increase in the LC_{90} value was observed with an extended treatment duration.

The assay with methoxyfenozide, nonetheless, did not affect the movement and behaviour of *Ae. aegypti* larvae instantly, yet abnormal agitation, restlessness, and sluggishness was observed just after a few minutes. Reduced larval motility prevented the larvae from rising to the surface, resulting in asphyxiation. Subsequently, the larvae exhibited tremors and convulsions, followed by paralysis, stiffness, and death.

Effect of methoxyfenozide on the adult emergence of *Aedes aegypti*

Larval exposure to methoxyfenozide at concentrations of 0.1 mg/L to 0.5 mg/L showed a dose-dependent effect, inducing an adult emergence inhibition of 70-90% (Fig. 2). It was interesting to note that at such a low dosage, methoxyfenozide could substantially inhibit adult emergence, indicating its growth-regulatory potential.

The significant adult emergence suppression in *Ae. aegypti* mosquitoes treated with larval methoxyfenozide resulted in an IE_{30} value of 0.002 mg/L, compared to an IE_{90} value of 1.209 mg/L (Table 2), which contrasts with the respective LC_{50} values of 1.927 and 115.615 mg/L.

Morphological aberrations in treated larvae

The treatment of *Ae. aegypti* larvae treated with methoxyfenozide were arrested in their life cycle and exhibited several morphological changes (Fig. 3). In comparison to the control larva (Fig. 3a), a pigmented deposition on the cuticle and disintegration of the gut viscera were observed in the treated larvae (Fig. 3b), which increased with the treatment duration. The treated larvae also displayed a deformed thorax (Fig. 3c) and shrinkage in the anal papillae (Fig. 3d). A few pupal-adult intermediates were obtained during the assay, signifying arrested growth (Fig. 3e).

Effect of methoxyfenozide on the chitin content of *Aedes aegypti*

Exposure to methoxyfenozide caused a dose-dependent reduction in the chitin content of the *Ae. aegypti* larvae (Table 3, Fig. 4). After 24 h, chitin levels decreased from 64.33 µg/larva (control) to 34.13 µg/larva (IE_{70}; 1.88-fold reduction; $p < 0.05$), and further to 23.58 µg/larva (IE_{90}; 2.73-fold reduction). Similar trends were observed after 48 h, with the chitin content decreasing from 91.33 µg/larva (control) to 41.33 µg/larva (IE_{90}; a 2.21-fold reduction; $p < 0.05$). These findings align with methoxyfenozide's role as an inhibitor of chitin synthesis.

Table 1. Larvicidal efficacy of methoxyfenozide against *Aedes aegypti* after 24 h and 48 h of treatment

Parameters	LC values in mg/mL (95% Fiducial Limits)	
	After 24 h	After 48 h
LC_{30}	1.927 (1.029-3.608)	0.147 (0.024-0.891)
LC_{50}	6.326 (3.378-11.847)	4.180 (0.688-25.395)
LC_{70}	20.772 (11.092-38.901)	13.380 (3.597-35.259)
LC_{90}	115.615 (61.7.35-216.519)	119.100 (91.605-273.537)
Slope	1.211	0.406
SE	0.139	0.400
χ^2 (df)	0.410 (4)	0.428 (4)

LC_{30} - Lethal Concentration at which 30% larvae are killed, LC_{50} - Lethal Concentration at which 50% larvae are killed, LC_{70} - Lethal Concentration at which 70% larvae are killed; LC_{90} - Lethal Concentration at which 90% larvae are killed; SE= Standard error. χ^2= chi-square. df = degree of freedom.

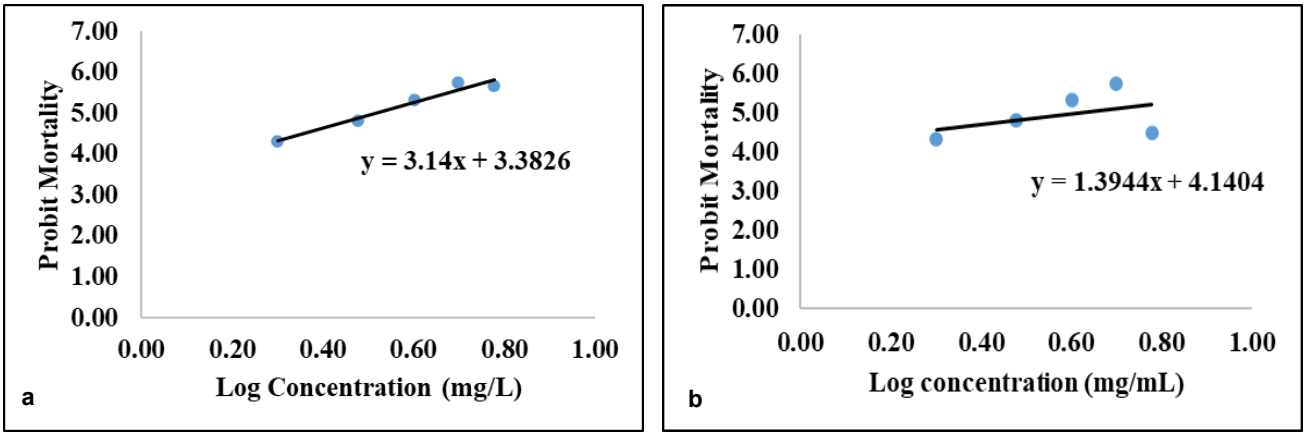

Fig. 1. *Percent mortality of Aedes aegypti early fourth instars after (a) 24 h and (b) 48 h exposure to different concentrations of methoxyfenozide*

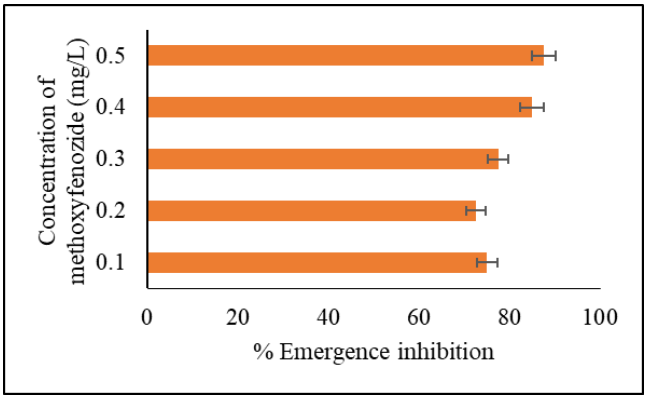

Fig. 2. *Percent adult emergence inhibition from the Aedes aegypti larvae treated with methoxyfenozide for 24 h*

DISCUSSION

The increasing prevalence of *Ae. aegypti* at the global level is largely attributed to its adaptability to changing climatic conditions and growing resistance to conventional insecticides. The challenges associated with mosquito management have prompted a shift in research toward alternative control interventions. One such measure is the application of Insect Growth Regulators (IGRs) (Şengül Demirak and Canpolat, 2022). There is a renewed interest in the use of these compounds as potential substitutes for traditional insecticides. This study investigates the larvicidal and growth-inhibiting effects of methoxyfenozide, against *Ae. aegypti*, demonstrating its potential as an effective agent for controlling this mosquito species, which transmits dengue, Zika, and Chikungunya viruses (Da Costa *et al.*, 2022).

The present findings indicated that methoxyfenozide exhibited significant ($p < 0.05$) larvicidal activity against *Ae. aegypti* larvae in their early fourth instar. After 24 h of exposure, the lethal concentrations, LC_{50} and LC_{70}, were determined to be 6.326 mg/L and 20.772 mg/L, respectively. In comparison, Hamaidia and Soltani (2021) reported much lower LC_{50} and LC_{90} values (24.54 µg/L and 70.79 µg/L, respectively) of methoxyfenozide against fourth-instar larvae of *Cx. pipiens.* Present studies also showed that when the exposure period was extended to 48 hours, the efficacy of methoxyfenozide increased, reducing the LC_{50} and LC_{70} values to 4.180 mg/L and 13.380 mg/L, respectively. The decline in LC values with increased treatment duration suggests the latent effects exerted by the compound, likely due to progressive physiological or biochemical alterations in the larvae. Similar delayed-action effects of IGRs have been documented in a few previous researches, reinforcing their viability as a tool for *Aedes* management (Kamal and Khater, 2010; Fansiri *et al.*, 2022; Sankar *et al.*, 2024). The assays with three IGRs (Diflubenzuron, nikkomycin Z, and polyoxin D) against the larvae of *An. quadrimaculatus* Say exhibited 86.7% larval mortality at 12.5 µg/L DFB, whereas the other two IGRs (Zhu *et al.*, 2007) had insignificant effects.

Despite its effectiveness, methoxyfenozide exhibited moderate potency compared to other IGRs. Herath *et*

Table 2. Inhibition of adult emergence (IE) in *Aedes aegypti* on exposure of fourth instar larvae with methoxyfenozide

Parameters	IE values (mg/mL)	95% Fiducial Limits
IE_{30}	0.002	0.0001 - 0.018
IE_{50}	0.015	0.0003 – 0.051
IE_{70}	0.089	0.004 – 0.155
IE_{90}	1.209	0.566 – 15.528
Slope	1.226	
SE	0.169	
χ^2 (df)	3.837 (3)	

IE_{30} - concentration that inhibits 30% of adult emergence; IE_{50} - oncentration that inhibits 50% of adult emergence; IE_{70} - concentration that inhibits 70% of adult emergence; IE_{90} - concentration that inhibits 90% of adult emergence; SE= Standard error; χ^2= chi-square; df = degree of freedom

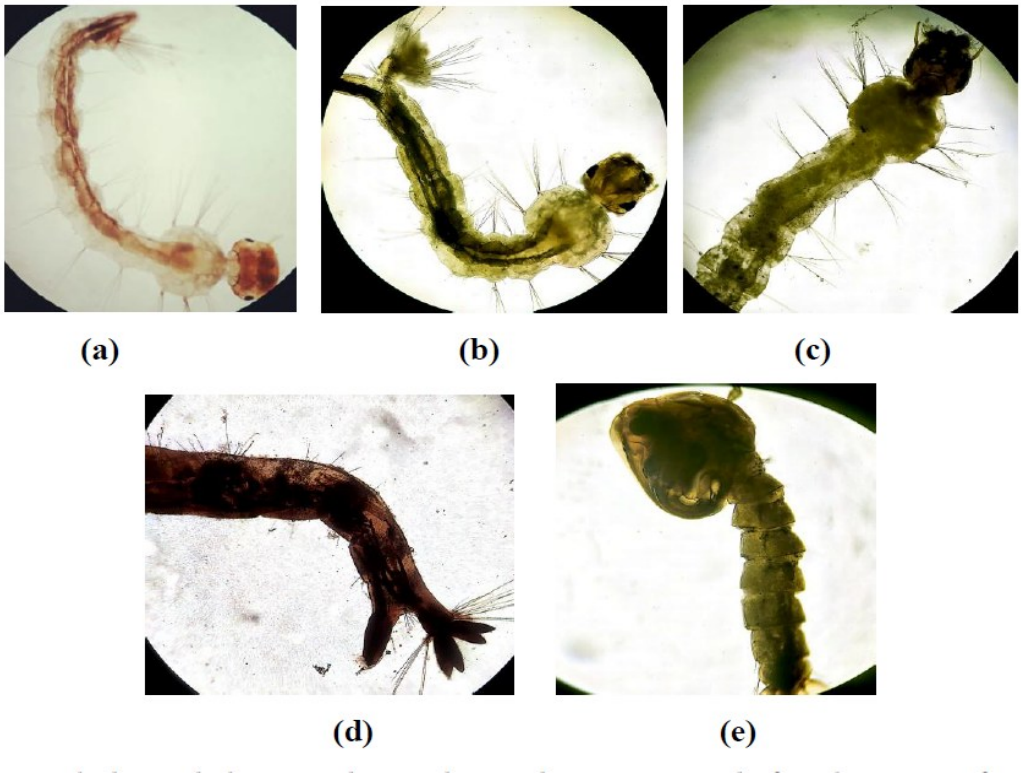

Fig. 3. *Morphological changes observed in Aedes aegypti early fourth instars after 24 h of exposure with methoxyfenozide; (a) Larva under control conditions; (b) treated larva showing disruption and disintegration of gut; (c) treated larva showing deformation near thorax region; (d) treated larva witj disintegrated anal papillae and deposition near siphon region; (e) a pupal-Adult intermediate*

al. (2024) treated *Ae. aegypti* with novaluron and documented LC_{50} values in the range of 0.047-0.049 ppm after 7 days of exposure and 0.02-0.05 ppm after 14 days of exposure. Application of 1.0 ppm pyriproxyfen and novaluron could cause 98-100% mortality of *Ae. aegypti* larvae (Rahman *et al*., 2024). Likewise, the treatment of *Cx. pipiens* with pyriproxyfen has resulted in LC_{50} values as low as 0.00111 ppm and 0.00013 ppm at 20 ºC and 32 ºC, respectively, which are significantly lower than those of methoxyfenozide (El-Shazly and Refaie, 2002). These findings suggest that although methoxyfenozide is effective, higher doses may be necessary to achieve comparable results.

The present study also showed that methoxyfenozide treatment caused notable behavioural changes in *Ae. aegypti* larvae, including increased restlessness, sluggish movement, and tremors, which were followed by paralysis and stiffness. Similar results have been reported by Fiaz *et al.* (2021), who observed significant changes in the behavioural response of *Ae. aegypti* larvae when subjected to the novaluron treatment. They documented significantly reduced activity in the treated larvae, who spent more time resting compared to the control group. These symptoms likely resulted from damage to the breathing siphon, which may have impaired respiratory function, making the larvae more susceptible to environmental stressors (Salem *et al*., 2024)

Besides its lethal effects on larvae, methoxyfenozide significantly reduced the emergence of adult mosquitoes. The inhibition of adult emergence was dose-dependent, ranging from 70% to 90% at methoxyfenozide concentrations ranging from 0.1 mg/L to 0.5 mg/L. The estimated IE_{30} and IE_{90} values were 0.002 mg/L and 1.209 mg/L, respectively, indicating strong efficacy in disrupting developmental processes essential for adult mosquito development. Comparable results have been reported for other IGRs, such as novaluron, which also demonstrated effective suppression of *Ae. aegypti* adult emergence at low concentrations, resulting in an IE_{99} value of 0.001 ppb (Herath *et al*., 2024). The present results are also in congruence with those of Hamaidia *et al.* (2018), who observed reduced adult emergence in *Cx. pipiens* treated with methoxyfenozide at LC_{50} levels. Likewise, Fansiri *et al.* (2022) reported IE efficacy of diflubenzuron (DFB) against *Ae. aegypti* revealing an IE_{50} value of 2.41 µg/L comparable to the present study. Seccacini *et al.* (2008) obtained the respective IE_{50} values of pyriproxyfen and DFB emulsifiable concentrates as 0.01 and 0.02 ppb against *Ae. aegypti*. In comparison, Sankar *et al.* (2024) reported an IE_{50} of 0.37 µg/L when *Ae. aegypti* fourth instars were treated with diflubenzuron.

However, despite its potential, methoxyfenozide's relatively lower efficacy compared to other well-established larvicides highlights the need for optimisation in its ap-

Table 3. Mean chitin content in the fourth instar larvae of *Aedes aegypti* when exposed to IE_{30}, IE_{50}, IE_{70} and IE_{90} concentrations of methoxyfenozide for 24 h and 48 h

Concentration of exposure	Chitin content (µg/larva) ± SEM After 24h of exposure	Fold change	Chitin content (µg/larva) ± SEM After 48h of exposure	Fold change
Control	64.33 ± 8.25a	1	91.33 ± 3.75a	1
IE_{30}	58.93 ± 6.95a	1.09	79.53 ± 8.25b	1.15
IE_{50}	44.23 ± 3.75b	1.45	73.08 ± 1.60b	1.25
IE_{70}	34.13 ± 4.15c	1.88	60.18 ± 3.90c	1.52
IE_{90}	23.58 ± 5.50d	2.73	41.33 ± 5.05d	2.21

Each assay consisted of 100 larvae and was conducted in 5 replicates (n=500); IE values and 95% CI for methoxyfenozide are expressed in µg/L, IE_{30} - concentration that inhibits 30% of adult emergence, IE_{50} - concentration that inhibits 50% of adult emergence, IE_{70} - concentration that inhibits 70% of adult emergence, IE_{90} - concentration that inhibits 90% of adult emergence; SEM= Standard error of mean; Figures in each column followed by different letters are significantly different ($p < 0.05$), one-way ANOVA followed by Tukey's all pair wise multiple comparison test

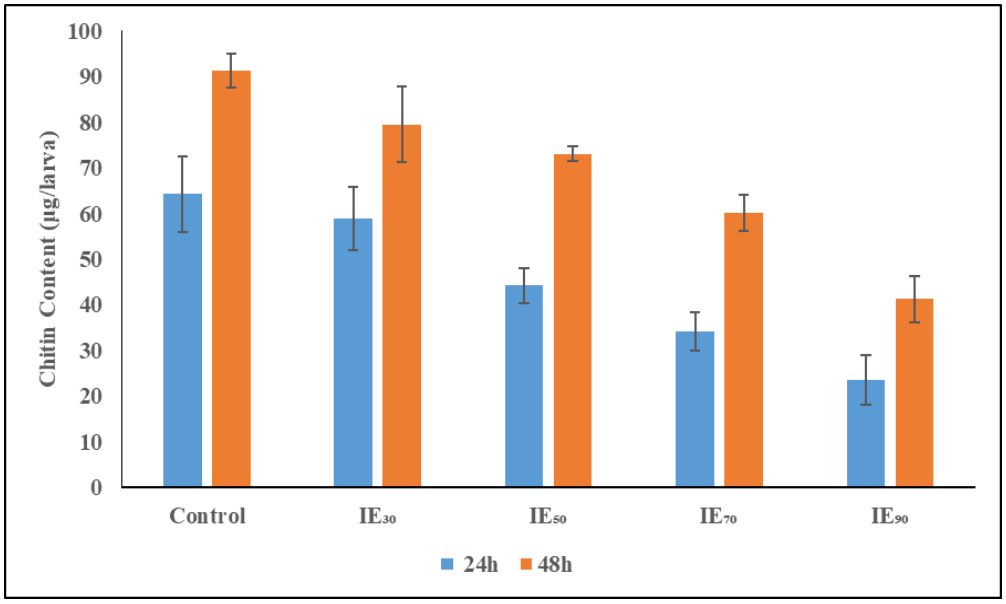

Fig. 4. *Mean chitin content in the fourth instar larvae of Aedes aegypti when exposed to IE_{30}, IE_{50}, IE_{70} and IE_{90} concentrations of methoxyfenozide for 24 h and 48 h*

plication. Its unique dual-action mechanism, affecting both larval survival and adult emergence, suggests that it could be used in combination with other agents to enhance overall mosquito control strategies. For instance, pairing methoxyfenozide with traditional larvicides, such as temephos, or biological control agents, such as *Bacillus thuringiensis israelensis* (Bti), could improve effectiveness while mitigating the risk of resistance development.

Methoxyfenozide has been recognized for its hormonal disruption action (El-Shewy *et al.*, 2024). Nevertheless, the present studies also investigated the possibility of interfering with chitin biosynthesis and disrupting mosquito development. The results demonstrated a significant, concentration-dependent decrease in chitin levels in *Ae. aegypti* fourth instar larvae following methoxyfenozide exposure indicating disruption in the chitin synthesis process. After 24 h, larvae exposed to increasing concentrations (IE_{30} to IE_{90}) exhibited progressive chitin depletion, with the highest inhibition (a 2.73-fold reduction) observed at IE_{90}. These findings indicate that methoxyfenozide could effectively inhibit chitin synthesis, preventing normal cuticle formation and moulting, ultimately leading to developmental arrest and mortality. This trend continued after 48 hours, with chitin levels declining across all tested concentrations, reaching a 2.21-fold reduction at IE_{90}. However, the reduction in chitin content was lower compared to the 24-hour period. The increase in chitin content with increased exposure duration of methoxyfenozide appears to be due to prolonged hormonal misregulation and activation of their immune system leading to sustained or excess chitin synthesis.

Earlier studies have shown that mosquitoes resist the action of any toxicant by increasing their cuticle thickness due to deposition of chitin content (Samal and Kumar, 2021). Lucchesi *et al.* (2022) estimated the chitin content in susceptible (Cp-S) and DFB-resistant (Cp

-R-1043M) strains of *Cx. pipiens* and revealed respective values of 30.89 ± 1.02 µg (1.54 µg/larva) and 47.77 ± 2.02 µg (2.39 µg/larva). They suggested that this is probably due to the activation of the mosquitoes' defence mechanism, leading to reduced insecticide penetration by increasing the thickness of their cuticle.

The present study supports on the efficient use of chitin synthesis inhibitors and their role in mosquito control. Effective declines in chitin levels and larval mortality have been observed in *Anopheles* spp. and *S. littorallis* with other insect growth regulators (IGRs), such as diflubenzuron and novaluron, both of which act by inhibiting chitin deposition in the insect exoskeleton (Zhang and Zhu, 2006). Salokhe *et al.* (2013) discovered reduced chitin levels in *Ae. aegypti* larvae after exposure to LC_{20} and LC_{40} values of lufenuron; the decrease level was negatively correlated with the lufenuron concentration. The chitin content decreased significantly in the midgut as well as the epidermis of the larvae of *Lymantria dispar* treated with fenoxycarb or RH-2485 (Zhang *et al.,* 2020). However, methoxyfenozide appears to act more gradually than IGRs, such as pyriproxyfen, which functions effectively at much lower concentrations. While pyriproxyfen primarily targets adult emergence inhibition, methoxyfenozide impacts both larval development and adult emergence, making it a useful tool in integrated vector management programs.

In addition to chitin inhibition, methoxyfenozide exposure resulted in noticeable physiological abnormalities in larvae, including lethargy, impaired mobility, and structural deformities. These effects suggest that methoxyfenozide may interfere with broader metabolic processes beyond chitin synthesis, which could further contribute to its efficacy in mosquito control. Comparable physiological impairments have been observed with other IGRs, reinforcing their potential in reducing mosquito populations (Khan *et al*., 2016; Lisi *et al*., 2024).

The present findings demonstrated methoxyfenozide as a valuable candidate for mosquito management, particularly for *Ae. aegypti* population control. While methoxyfenozide shows promise in mosquito control, its potency relative to other insect growth regulators (IGRs) requires further evaluation. Further research is necessary to optimize its dosage by assessing its long-term field performance, persistence in aquatic environments, and potential non-target effects. Exploring its compatibility with other vector control strategies could enhance its role in sustainable mosquito management programs, ultimately contributing to reducing vector-borne diseases.

Conclusion

The findings highlight methoxyfenozide as a promising insect growth regulator with strong larvicidal potential against *Ae. aegypti*. The studies demonstrated the effective potential of methoxyfenozide to induce morphological deformities in the larvae, cause behavioural changes and restlessness, and significantly inhibit adult emergence. Although it is known as a hormonal disruptor, it also inhibits chitin synthesis in larvae, suggesting its potential use as an effective agent for mosquito management. Future research requires a comprehensive field-based evaluation of methoxyfenozide, including its impact on non-target organisms and its integration into existing vector management strategies, to assess its long-term efficacy and environmental safety.

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Conflict of interest

The authors declare that they have no conflict of interest.

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