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Original Article

Assessment of the Impact of Diflubenzuron on the Inhibition of Adult Emergence in *Aedes aegypti* (Diptera: Culicidae) from Hormozgan Province, Iran, 2025

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Abstract

Background: The presence of *Aedes aegypti* in Iran and the risk of transmitting viral diseases, such as dengue and Zika, underscore the importance of evaluating effective vector control methods.

Methods: This study investigated the effectiveness of diflubenzuron, an insect growth regulator that inhibits chitin synthesis, in preventing adult emergence of *Ae. aegypti* larvae from Hormozgan. The concentrations of 0.03, 0.06, 0.12 and 0.24 mg/L of 25% wettable powder formulations of diflubenzuron were used, according to WHO protocols.

Results: Diflubenzuron was highly effective at 0.24 mg/L, achieving 98% emergence inhibition (EI50=0.053 mg/L, EI_{90}=0.146 mg/L). At lower concentrations, e.g., 0.12 mg/L, emergence inhibition decreased to 83%, indicating a clear concentration-dependent reduction in efficacy. Larvae exposed to sublethal doses displayed abnormal movements and reduced responses to stimuli.

Conclusion: This experiment supports diflubenzuron's potential as an effective larvicide for vector control programs in Iran. However, field trials and resistance monitoring are recommended before widespread implementation.

Keywords: *Aedes aegypti*; Invasive mosquito; *Stegomyia aegypti*; Insect growth regulator; Emergence inhibition

Introduction

Mosquitoes are significant vectors of parasites, viruses, bacteria and nematodes (1, 2). *Aedes aegypti* is one of the most dangerous insects to humans because it is the primary vector of deadly diseases, such as dengue, chikungunya, Zika and yellow fever (3–4). The habitat of this vector is closely related to the conditions inside and outside homes where human populations reside (5–7). Chemical insecticides are the primary methods for vector control. However, many of these chemicals pose health risks to humans, harm the environment and contribute to the development of resistance in target insects (8, 9). It is essential to develop new chemical products that are less toxic, less persistent and safer for humans and the environment (10). Compounds that interfere with metabolic pathways, such as chitin synthesis, have garnered significant interest (11). Insect growth regulators (IGRs), which target specific physiological processes during normal development (12), are considered selective for target species and environmentally friendly (13, 14). Diflubenzuron (DFB) is an IGR that inhibits chitin synthesis and affects all larval and immature stages of the mosquito. It is an

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effective tool for controlling disease vectors and an encouraging alternative to traditional insecticides (15, 16). DFB kills larvae during molting because mosquitoes cannot shed their old cuticle; those that survive die at the pupal stage or during adult emergence (17, 18). The WHO has recommended DFB for controlling container-breeding mosquitoes (19). Dengue fever has emerged as a major public health concern in South Asia (20), with Pakistan currently experiencing one of its most significant outbreaks in recent years (21, 22). Given the extensive cross-border movement, trade, and tourism between Pakistan and Iran, there is a growing risk of disease spread in Iran, particularly in provinces such as Sistan and Baluchestan, where more than 1000 dengue cases have been reported. This province shares ecological and climatic characteristics with dengue-endemic regions of Pakistan. In addition, approximately 255 confirmed dengue cases have also been documented in Hormozgan Province, underscoring the expanding geographic distribution of the disease in southern Iran (23). This highlights the need for more effective vector control measures in Iran.

Therefore, identifying effective doses of chemical agents for this invasive species is crucial for vector control. This is the first study to determine the effective concentration of DFB needed to prevent the emergence of *Ae. aegypti* larvae from the Hormozgan Province strain through laboratory bioassays, providing region-specific EI_{50} and EI_{90} values to inform targeted vector control strategies in a high-risk area for dengue transmission.

Materials and Methods

This experimental study was conducted from March to May 2025 in Hormozgan Province, southern Iran, under controlled laboratory conditions. For mosquito rearing, a colony of adult *Ae. aegypti* was obtained from the insectarium of the Bandar Abbas Health Education and Research Station, which maintains a recently field-collected strain. This strain was maintained in a rearing cage (50 ×50 ×50 cm) with access to a 10% water-sugar solution via cotton wicks. Adult females were blood-fed on a rabbit twice weekly. To produce larvae, eggs from the colony were submerged in plastic trays with water after a dry period until they hatched and reached the 3rd and 4th instar larvae. The experiment was conducted at 27±2 °C, 65–75% RH and a 12:12 hour light: dark photoperiod in the insectary.

Testing of DFB was conducted following the World Health Organization's standard procedure for determining 50% and 90% adult emergence inhibition (EI_{50} and EI_{90}) (24). For the bioassays, formulations of DFB WP25% (Melli Shimi Keshavarz Company, Iran) were used. World Health Organization Pesticide Evaluation Scheme (WHOPES) recommends a dosage of 0.02–0.25 mg/L for WP formulations of this compound to control mosquito larvae (19, 25, 26). To determine the variation of EI_{90} concentration, late 3rd or early 4th instar larvae were exposed to DFB dilutions ranges 0.03, 0.06, 0.12 and 0.24 mg/L. For each concentration and the control, three replicates were conducted, with each replicate containing 20 *Ae. aegypti* larvae, resulting in a total of 60 larvae per concentration based on availability. For each replicate, 20 larvae were placed in 25 mL of dechlorinated water for one hour to acclimate. The larvae were then transferred to a 250 mL glass beaker containing 1 mL of each test solution and 74 mL of dechlorinated water. A small amount of larval food was added to each beaker every two days to prevent cannibalism. The controls were set up with dechlorinated water without DFB. Adult emergence inhibition (EI) was calculated based on the proportion of emerged adults in each treatment relative to the control group. As adult emergence in the control group exceeded 95%, no correction for control mortality was applied. The emergence inhibition percentage (EI %) for each concentration was cal-

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culated based on the number of moribund and dead larvae and pupae, which were considered "affected." Only mosquitoes that emerged completely free of exuviae were counted as alive (24). The experiment was checked every two to three days and stopped when all adults emerged in the controls. Adult emergence inhibition data were analyzed using probit analysis in SPSS software (version 26, IBM Corp.) to estimate the DFB concentrations causing 50% and 90% inhibition of adult emergence (EI_{50} and EI_{90}), along with their corresponding 95% confidence intervals. The significance of the dose–response relationship was assessed based on the probit regression model. A regression line was plotted to illustrate the relationship between diflubenzuron concentration and the percentage of emergence inhibition of *Ae. aegypti* larvae. The goodness-of-fit of the model and the presence of heterogeneity in the response data were evaluated using a chi-square goodness-of-fit test. A P-value< 0.05 was considered statistically significant.

Results

The inhibition of emergence concentrations of DFB in *Ae. aegypti* larvae were estimated using Probit analysis, with values shown in Table 1. In the control group, more than 95% of adults emerged. According to Table 1, increasing DFB doses significantly enhanced adult emergence inhibition as demonstrated by probit dose–response analysis (P< 0.001). Dose-response tests determined the emergence inhibitory concentrations for DFB in *Ae. aegypti* larvae. Probit analysis revealed that the EI_{50} and EI_{90} were 0.053 mg/L and 0.146 mg/L, respectively (Fig. 1). Concentrations between 0.03 and 0.24 mg/L DFB caused reduced movement, slow S-shaped movements, and unresponsiveness to light or touch in larvae. The goodness-of-fit chi-square test (P= 0.775) confirmed the suitability of the probit regression model. The P-value greater than 0.05 indicates the absence of significant heterogeneity in the dose–response data, validating the reliability of the estimated confidence limits.

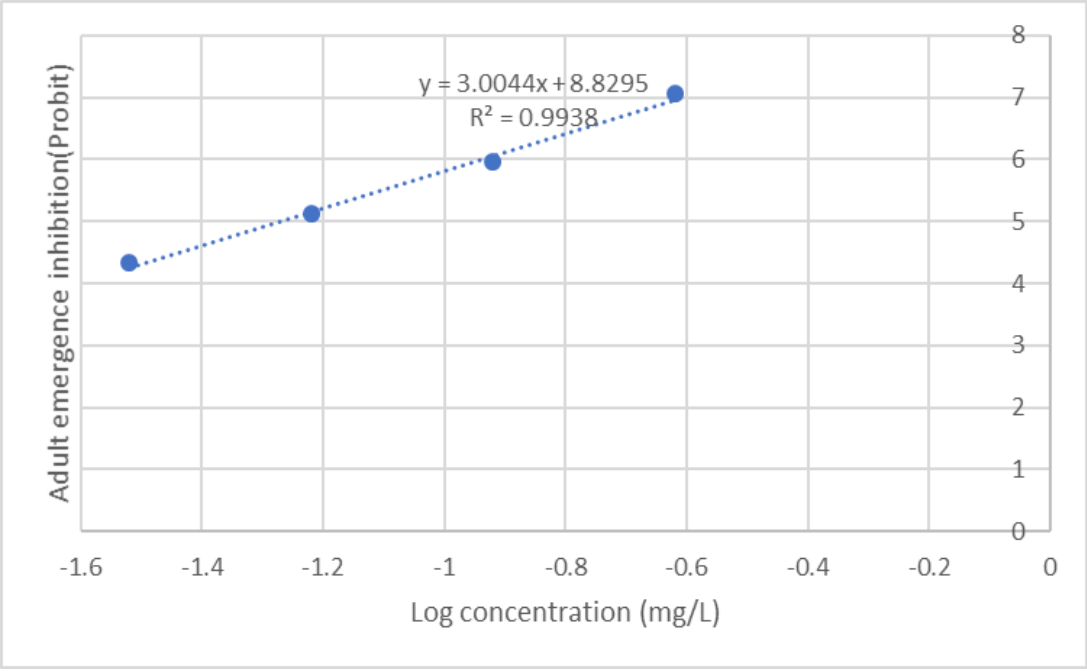

Fig. 1. Percentage emergence inhibition of *Aedes aegypti* larvae (late 3rd/early 4th instar) treated with various concentrations of diflubenzuron WP25%, at laboratory conditions in Bandar Abbas, Iran, 2025

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Table 1. Emergence inhibition of *Aedes aegypti* larvae exposed to various concentrations of diflubenzuron at laboratory conditions in Bandar Abbas, Iran, 2025

Concentration (mg/L)	Inhibition (%)	Statistical parameters			
		EI_{50} (95%CI) (mg/L)	EI_{90} (95%CI) (mg/L)	Slope±SE	X^2
0.24	98	0.053 (0.044–0.062)	0.146 (0.119–0.197)	2.91± 0.34	0.510 (p<0.001)
0.12	83				
0.06	55				
0.03	25				
Control	0				

Abbreviation: CI, 95% confidence interval; EI, emergence inhibition 50 and 90 (mg/L); SE, standard error. p< 0.05.

Discussion

Today, vector control strategies focus on using environmentally safe chemicals that are harmless to non-target organisms. IGRs, as toxic chemicals with a specific mode of action, have proven to be promising tools for controlling mosquitoes (27, 28). Monitoring the effective dose of DFB on *Ae. aegypti* is essential before implementing control measures. In this study, the chitin synthesis inhibitor DFB was tested against late instar larvae of *Ae. aegypti* mosquitoes of the Hormozgan strain. The findings show that DFB exhibited high emergence inhibition activity against late instar larvae of *Ae. aegypti*. The EI_{50} and EI_{90} values were estimated at 0.053 and 0.146 mg/L, respectively, demonstrating effective activity at relatively low concentrations. As DFB concentration increased, mortality significantly rose, reaching 98% at 0.24 mg/L, nearly matching the EI_{90} threshold, while the lowest mortality (25%) was observed at 0.03 mg/L. Larvae at sublethal concentrations displayed reduced swimming ability and no response to tactile or light stimuli, consistent with previous data on DFB-induced disruption of cuticular and epithelial development in mosquitoes (29). The EI values obtained in our study, while lower than the LC values reported by Montaño-Reyes et al. (30), were comparable to the LC values reported by Silva et al. (31) and the EI values reported by Fansiri et al. (32), Sankar et al. (33) and Seccacini et al. (34). The EI values obtained here were lower than the reported lethal concentrations (LC_{50}= 0.23 mg/L, LC_{90}= 0.47 mg/L) by Montaño-Reyes et al. (31), but higher than those in studies using optimized formulations or synergistic combinations. In Uberlândia, Minas Gerais, southeastern Brazil, the LC_{50} and LC_{95} of DFB were reported as 0.00519 and 0.01224 mg/L, respectively, causing high mortality across all larval stages of *Ae. aegypti* mosquitoes (31). An EI_{50} was reported as 0.00241 mg/L (32), while lower values (0.37–0.63 µg/L) were effective against both laboratory and field strains, with emergence inhibition ranging from 9.1% to 100% at a concentration of 16 µg/L (33). Variations in the purity of the active ingredient, formulation types, larval strain susceptibility and experimental conditions largely explain these differences. One study noted that formulation differences affected efficacy, with EC formulations showing lower EI_{50} values compared to technical DFB, with an EI_{50} of 1.59 µg/L, emphasizing the importance of formulation chemistry in determining biological effectiveness (34).

The World Health Organization recommends DFB at concentrations up to 0.25 mg/L for drinking water applications (19), making it acceptable for local communities as it causes no noticeable odor or turbidity. This study

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confirms that, within this range, mosquito emergence can be significantly reduced, supporting the suitability of this compound for operational programs. Previous research indicates that sublethal DFB concentrations reduce mosquito reproductive potential, including egg production and hatching rates (29). Combined with larval mortality, this can significantly decrease vector populations. Previous studies also note that adjuvants like verapamil may increase efficacy, offering promising options for dose optimization and cost savings in large-scale interventions (33). A study evaluating the toxicity of DFB on *Aedes* larvae from breeding sites at a university in Lagos, Nigeria, found the highest EI (≥90%) at a concentration of 0.0001 mg/L after 12 days, while similar inhibition was observed after 8 days at 0.005 mg/L. The DFB residue in water decreased over time, indicating its stability and lack of toxicity to non-target organisms, making it ideal for controlling mosquito larvae (35). When comparing the biological effects of two IGRs, DFB was more effective than pyriproxyfen by a factor of 11.4 (36).

The resistance status of a wild *Ae. aegypti* population from Martinique (Vauclin) to conventional larvicides (*Bacillus thuringiensis* var *israeliensis* [Bti] and temephos) and potential alternatives (spinosad, diflubenzuron and pyriproxyfen) was reported. Despite others, diflubenzuron and spinosad showed a residual efficacy of 16 weeks, suggesting that these chemicals may be promising alternatives to Bti and temephos for controlling insecticide-resistant *Ae. aegypti* populations (37). The EI_{50} (0.053 mg/L) and EI_{90} (0.146 mg/L) values obtained for the *Ae. aegypti* Hormozgan strain falls well within the WHO-recommended concentration range of 0.02–0.25 mg/L for diflubenzuron in mosquito control. This indicates that the Hormozgan strain is highly susceptible to diflubenzuron, supporting its potential as an effective larvicide for regional vector control programs. These findings provide a clear benchmark for public health officials to implement diflubenzuron at WHO-recommended doses, ensuring both efficacy and safety in community settings. Despite the proven effectiveness of DFB, reports of reduced susceptibility in *Ae. aegypti* populations (38, 39) highlight the need for ongoing resistance monitoring. Developing rotation strategies that combine DFB with other IGRs or insecticides can reduce selection pressure and improve operational efficiency.

This study was conducted under controlled laboratory conditions that do not fully simulate the natural habitats of *Ae. aegypti*, including environmental factors. Potential interactions between DFB and other control agents or natural predators were not investigated. The research focused on a single mosquito species within one geographic location, limiting its relevance to other species or regions. Future studies should assess the effectiveness of DFB in semi-field and field settings to better understand its practical application in integrated vector management. Due to the limited availability of larvae during the experimental period, the study used a total of 60 larvae per concentration. Although this sample size was sufficient for preliminary estimation of EI_{50} and EI_{90} values, it is lower than the number recommended by WHO guidelines, which may have reduced the statistical precision of the results. To increase the statistical precision and generalizability of emergence inhibition (EI) estimates, future bioassays should follow WHO recommendations by using at least four replicates of 25 larvae per concentration (100 larvae per concentration). Additional research is needed to explore long-term residual activity, resistance development and potential synergistic effects when these compounds are combined with other larvicides or biological agents. Moreover, evaluating ecological impacts on non-target organisms and conducting cost-effectiveness analyses would provide valuable insights for public health decision-makers.

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Conclusion

This study demonstrated that DFB is an effective IGR against *Ae. aegypti* larvae, significantly increasing mortality during molting. The results confirm the potential of DFB as a reliable larvicide at WHO-recommended concentrations for drinking water. The high effectiveness of DFB at low doses makes it a promising option for mosquito control programs. Based on these findings, integrating diflubenzuron into Iran's vector control programs, particularly in high-risk areas like Hormozgan Province, conducting regular resistance monitoring to ensure sustained efficacy, and implementing rotational strategies with other insect growth regulators or larvicides to mitigate resistance development are recommended. Future research should explore field application across different ecological settings, its persistence in natural environments and its compatibility with other vector control strategies.

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Ethical Considerations

This study was conducted as part of the student's thesis and was approved by the Ethics Committee of Tehran University of Medical Sciences under ethical approval number: IR.TUMS.SPH.REC.1402.247.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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