Toxicity and Lethal Effects of Selected Insect Growth Regulators (IGRs) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae)[1]

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Abstract *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a polyphagous insect pest that is responsible for significant financial losses in important crops. Insect growth regulators (IGRs), such as lufenuron, diflubenzuron, and methoxyfenozide, may function as selective pesticides in an integrated pest management (IPM) program for *S. frugiperda* and other lepidopteran pests. Thus, this study examined the toxicity and biochemical effectiveness of 3 IGR insecticides against second-instar *S. frugiperda* larvae in laboratory testing. Furthermore, molecular docking analysis was used to assess each compound's binding to the enzymes glutathione-S-transferase (GST) (PDB ID: 1jlv), α-esterase (PDB ID: 4fng), and acetylcholine esterase (AChE) (PDB ID: 6tt0) to identify the molecular mode of action of the IGRs. Toxicity results indicated that lufenuron and diflubenzuron were the most toxic of the 3 IGRs tested against *S. frugiperda*, followed by methoxyfenozide, based on the median lethal concentration (LC_{50}) values and 95% confidence interval limits. Furthermore, findings showed that treatment with lethal concentrations (LC_{50}) of methoxyfenozide and lufenuron drastically shortened the duration of the larval and pupal stages of *S. frugiperda*. Compared to the control group, all tested pesticides had an inhibitory impact on AChE, α-esterase, and GST at 24 and 96 h after treatment. The molecular docking analysis revealed that each IGR tested presented a noteworthy propensity for binding to AchE, α-esterase, and GST, with energy scores varying between −4.927 and −7.115 kcal/mol. In addition to offering more effective pest control options for *S. frugiperda*, these findings should aid in the advancement of knowledge regarding the biochemical and molecular mechanisms of action of the evaluated IGR insecticides.

Key Words lethal effects, fall armyworm, enzyme activity, molecular docking, IGRs

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), also known as the fall armyworm, is a very destructive migratory pest (FAO 2018). Its damage results in massive financial losses for several important crops, including maize and cotton, in American and Caribbean countries (Kenis et al. 2023, Zhu et al. 2020). Asia and Africa have experienced a recent increase in the occurrence of this pest and its damage to crops (Goergen et al. 2016, Li et al. 2020). It has a strong detoxifying mechanism against pesticides and plant toxins, a rapid life cycle, a high degree of adaptability, and a high incidence of infestation (Wang et al. 2022a, Wu et al. 2021). Consequently, it represents a risk to several agricultural ecosystems worldwide (Kenis et al. 2023, Zhu et al. 2020).

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Integrated pest management (IPM) incorporated several environmentally-friendly methods of pest control that have been the subject of research efforts worldwide (Idrees et al. 2021, 2017; Qadir et al. 2021). Insecticides remain as one of these methods of management; however, concern remains on how to use pesticides to manage target insect pests in an environmentally conducive manner. Unfortunately, their use can be ineffective and, since these pesticides are indiscriminately overused, insect resistance to many types of insecticides has developed (Goergen et al. 2016). Selection of new conventional insecticides, insect growth regulators, and botanical pesticides should be continually considered for the management of *S. frugiperda* (Idrees et al. 2023).

Chemical pesticides have been the most popular method of managing pests on plants since 1940, because of their efficiency, simplicity of use, and positive results (Paredes-Sánchez et al. 2021). The last 40 yr have seen the development of insecticides that are different from conventional insecticides in that they operate selectively on certain biochemical sites found in certain insect species (Ishaaya et al. 2005). Using this method has produced insect growth regulators (IGRs) as insecticides (Dhadialla et al. 1998) with a unique mode of action, high biological activity, low vertebrate toxicity compared to conventional insecticides, and low environmental hazards (Haixiang et al. 2023, Tunaz and Uygun 2004).

Among the chitin synthesis inhibitors that are effective in controlling young insects are lufenuron and diflubenzuron. These compounds prevent the production of chitin, which is the primary building block of the insect exoskeleton (Desneux et al. 2007, Douris et al. 2016). Methoxyfenozide (20-hydroxyedysone) is another highly active insect ecdysteroid hormone mimic that acts to control molting and metamorphosis throughout the insect's life cycle. Because of the critical role ecdysterone in insect development, agonists or antagonists of this hormone are thought to interfere with the target pest's physiological functions (Oberlander and Smagghe 2001, Yanagi et al. 2006). Insects that are exposed to lethal or sublethal concentrations of IGR pesticides may have abnormalities in their biological and physiological characteristics, including altered diapause, weight loss in both larvae and pupae, malformations in pupae, and delayed or accelerated development (Biddinger et al. 2006, Eizaguirre 2007, Pineda et al. 2007, Seth et al. 2004, Smagghe et al. 2004, Sundaram et al. 2002). When compared with immediate lethality, these impacts have received less research attention despite their existence. Consequently, the present study (1) examines the toxicity of lufenuron, diflubenzuron, and methoxyfenozide against second-instar *S. frugiperda*, (2) evaluates the impact of these IGRs on the development of the *S. frugiperda*, and (3) determines the activation of detoxification enzymes, for example, cytochrome P450, α-esterase, and glutathione-S-transferase (GST), by the IGRs (Pineda et al. 2009). Consequently, the interactions between the amino acids, the 20 hydrogen bond lengths (A), the affinity (Kcal/mol), and the docking energy score of the IGRs against the GST enzyme's active site were identified through molecular docking analysis in comparison to the co-crystallized ligand, diethyl hydrogen phosphate (DHP).

Materials and Methods

Insects. Insects used in these assays were from a laboratory colony of *S. frugiperda* maintained at 25 $\pm$ 2°C and 65–70% relative humidity (RH) without exposure to any insecticides for 10 generations. The adult moths were given a 10% (w/v)

sugar solution as a food source, while the larvae were fed the leaves of castor beans, *Ricinus communis* L. Eggs were gathered and transferred to glass jars where they were held until larvae emerged after which they were supplied with castor bean foliage. All test and assays were conducted using second-instar larvae as determined by molting incidence.

IGRs. Commercial formulations of IGRs were used in the study. These were lufenuron (Lofine 10% SC, Tianjin Hanbang Plant Protection Agent Co., Ltd., Qichacha, China), diflubenzuron (Bestmilyn 48% SC, Agrobest Group Tarim Elklary Tom Kolwk Co., Izmir, Turkey), and methoxyfenozide (Met No 24%SC, Shawzing Shangozen Biochemical Co., Ltd. India).

Bioassays. The leaf dipping technique using second-instar *S. frugiperda* larvae was employed to determine the toxicity of the tested IGRs (Awad et al. 2024). Five concentrations (ranging from 0.00125 to 200 mg/L) of each IGR were prepared, while the distilled water was used for the control. After immersing fresh castor bean leaves for 15 s in each IGR concentration and the water for the control, 20 second-instar larvae were placed on each leaflet in jars. These treatments were replicated 5 times. Larval mortality was checked and recorded daily for 4 d. Probit analysis was conducted to determine the concentration-mortality response of each IGR (Finney 1971).

IGR impact on *S. frugiperda* development. Once the median lethal concentration (LC_{50}) of each IGR on *S. frugiperda* was established, a total of 120 second-instar larvae (3 replicates of 40 larvae each) were placed on caster bean leaves treated with the LC_{50} of each IGR as well as the untreated control. After 4 d, 60 larvae were randomly selected from each treatment and housed separately in a 30-ml cup containing untreated castor leaves. Larval duration, percentage pupation, pupal duration, pupal weight, percentage adult emergence, and sex ratio were recorded.

Biochemical sample preparation. Second-instar larvae were exposed to the LC_{50} of each IGR as previously described. Activity of AChE and detoxifying enzymes, for example, α-esterase, cytochrome P450, and GST, were measured at 24 and 96 h using 100 mg fresh body weight of the surviving larvae. Each analysis was conducted with 5 replicates. An enzyme assay was used to determine the pH at which the larvae should be homogenized in 100 mM phosphate buffer. As per Bradford (1976), the homogenates were centrifuged for 15 min at 12,000 rpm, and the supernatants were used to measure how well the enzyme activity correlated with the protein level.

Acetylcholine esterase (AChE) activity. AChE activity evaluation followed the protocol of Ellman et al. (1961). Enzyme solution, 75 mM acetylthiocholine iodide (ATChI), and 100 mM dithio-bis-nitro benzoic acid (DTNB) made up the reaction solution. Absorbance was monitored at 405 nm for 5 min.

α-esterase activity. Van Aspern (1962) described the method of measuring α-esterase enzyme activity using α-naphthyl acetate (α-NA) as a substrate. After adding the enzyme solution to 30 mM substrate, the mixture was incubated at 30°C for 15 min. To halt the process, a mixture solution containing 1% fast blue B and 5% sodium dodecyl sulfate was added. The optical densities of α-NA were determined at 600 nm.

Cytochrome P450 activity. The method of Hansen and Hodgson (1971) measured cytochrome P450 activity. After incubating at 27°C for 3 min with 2 mM p-nitro anisole, 9.6 mM NADPH was added to the enzyme solution. Absorbance was measured 405 nm.

Glutathione S-transferase (GST) activity. The method outlined by Habing et al. (1974) was used to measure the GST enzyme activity in the samples. 30 mM 1-chloro,2,4-dinitrobenzene (CDNB), 50 mM GSH, and an enzyme solution were all included in the reaction mixture. Absorbance at 340 nm was measured for 5 min.

Docking analysis. Molecular docking was conducted using Gaussian 09 to evaluate a ligand's possible energetic and geometric fit between the tested IGRs and the protein's active site. The IGRs crystal structures against the enzymes GST (PDB ID: 1jlv), α-esterase (PDB ID: 4fng), and AChE (PDB ID: 6tt0) were retrieved from the protein data bank (http://www.rcsb.org.pdb). Using the MOE 2015 program, the co-crystallized ligand was re-docked in the original enzyme structure for molecular docking investigations. The chemical structures of the selected ligands were drawn using ChemDraw 18.0, converted into 3-dimensional conformations, and saved as MDL mol files. These ligands were energy-minimized and docked into the active site of the enzyme. The docking process involved generating multiple binding poses, which were evaluated using MOE's scoring functions to estimate binding affinity. Compounds with more negative binding scores were considered to have stronger interactions with the enzyme. The ligand exhibiting the lowest binding affinity was given the highest numerical docking score for comparison purposes.

Data analysis. The statistical software SPSS V.22 was used to analyze the data. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to verify the normality of continuous variables, and data were examined for parametric test assumptions. The data were normalized using the arcsine square rRoot method. One-way ANOVA was utilized to determine the mean and standard deviation of the biological and biochemical data, with a minimum of 3 repetitions for each group. Tukey's pairwise comparison was employed for post-hoc analysis. Utilizing MiniTab (V. 14), Chi ($\chi 2$) was employed to compare the actual and predicted frequencies of the sex ratio. Significance was defined as a *P*-value of less than 0.05. Data visualization (V. 2022.02.4) was performed using R studio.

Results

IGR toxicity. The LC_{50} values of the 3 IGRs against second-instar *S. frugiperda* were 0.08 mg/L (0.02–0.22 confidence limits [CLs]) for lufenuron, 0.27 mg/L (0.17–0.55 CL) for diflubenzuron, and 63.01 mg/L (47.58–81.80 CL) for methoxyfenozide (Table 1). Based on these LC_{50} values and their respective 95% CLs, lufenuron and diflubenzuron were the most toxic of the 3 IGRs to *S. frugiperda*; however, toxicity of the two were not statistically different based on their overlapping CLs. Both were statistically more toxic to the *S. frugiperda* larvae than methoxyfenozide.

IGR exposure impact on development of *S. frugiperda*. When exposed to the LC_{50} of the 3 IGRs as second-instar larvae, durations of the larval stage for treatments with lufenuron (14.7 ± 1.72 d) and methoxyfenozide (14.52 ± 1.14 d) (Table 2) were statistically shorter than the untreated control (16.23 ± 1.47 d) and diflubenzuron (16.22 ± 1.62 d) treatments (Table 2). Pupal duration was significantly shorter in larvae treated with either lufenuron (12.6 ± 1.1 d), diflubenzuron (11.93 ± 1.1 d), or methoxyfenozide (12.07 ± 2.31 d) than larvae fed on untreated leaves (10.39 ± 2.32 d) (Table 2). The percentage of larvae successfully pupating did not differ among the treatments. Male pupal weight did not differ among the treatments, while only slight differences were observed in female pupal weights among the treatments

Table 1. Toxicity of three IGRs insecticides against second-instar *Spodoptera frugiperda.*

IGRs	LC_{50} (mg/L) (95% CL)	LC_{90} (mg/L) (95% CL)	Slope ± SE	*P*-value	χ^2
Lufenuron	0.08 (0.02–0.22)	33.03 (5.0–3586.9)	0.48 ± 0.11	0.186	4.80
Diflubenzuron	0.27 (0.166–0.548)	3.44 (1.26–39.57)	1.16 ± 0.26	0.101	4.75
Methoxyfenozide	63.01 (47.58–81.80)	231.48 (156.97–482.30)	2.26 ± 0.40	0.766	0.53

Table 2. Effect of exposing second-instar *S. frugiperda* to LC_{50}s of three IGRs on the development of *S. frugiperda*.

Treatments	Larval duration (d)	Pupal duration (d)	Pupation %	Mean pupal weight (g)		Adult emergence (%)
				Males	Females	
Control	16.23 ± 1.47a	10.39 ± 2.32b	95 ± 8.66a	0.23 ± 0.04a	0.21 ± 0.05b	100 ± 0a
Lufenuron	14.7 ± 1.72b	12.6 ± 1.1a	98.41 ± 2.75a	0.25 ± 0.031a	0.23 ± 0.02ab	71.67 ± 10.41b
Diflubenzuron	16.22 ± 1.62a	11.93 ± 1.1a	83.03 ± 9.05a	0.24 ± 0.04a	0.24 ± 0.02ab	80.17 ± 9.53ab
Methoxyfenozide	14.52 ± 1.14b	12.07 ± 2.31a	98.24 ± 3.04a	0.25 ± 0.02a	0.26 ± 0.02a	92.59 ± 12.83ab
F-value	20.75	12.55	3.64	1.27	6.40	5.26
P-value	0.0001	0.0001	0.064	0.289	0.001	0.027

Means (± SE) that do not share the same lowercase letter in the column are significantly different ($P = 0.05$).

Table 3. Effect of exposing second-instar *S. frugiperda* to LC_{50}s of three IGRs on the sex ratio of emerging *S. frugiperda* adults.

Treatments	Male ratio %	Female ratio %	χ^2	*P*-value
Control	50.98 ± 1.69	49.02 ± 1.7	0.1156	0.734
Lufenuron	50 ± 0	50 ± 0	0	1
Diflubenzuron	47.44 ± 12.85	52.55 ± 12.85	0.7813	0.377
Methoxyfenozide	49.47 ± 6.36	50.53 ± 6.36	0.3371	0.854

(Table 2). Adult emergence rate in the lufenuron treatment was significantly lower than the untreated control; however, the emergence rates in the diflubenzuron and methoxyfenozide treatments did not differ significantly and were not significantly different from that of lufenuron (Table 2). The sex ratios of the emerging adults were not significantly affected by the tested IGRs (Table 3).

IGR impact on detoxifying enzymes. The assays to assess the effects of the tested IGRs on *S. frugiperda* detoxifying enzyme activity revealed that, when compared to the control group, exposure to the lufenuron LC_{50} significantly lowered AChE activity at 24 h (Table 4). At 96 h of exposure, AChE activity did not differ significantly from that of the control. Diflubenzuron and methoxyfenozide displayed AChE activity levels that also were significantly decreased in comparison to the control and to lufenuron at 24 h, while only methoxyfenozide exhibited AChE activity that was significantly lower than the control at 96 h. Therefore, the impact of the IGRs on AChE activity was as follows, from highest to lowest: Control $>$ Lufenuron $>$ Diflubenzuron $=$ Methoxyfenozide at 24 h and Control $\geq$ Lufenuron $\geq$ Diflubenzuron $\geq$ Methoxyfenozide at 96 h.

In comparison to the control, α-esterase activity was significantly inhibited by all 3 IGRs at 24 and 96 h (Table 4). The α-esterase activity observed after 24 h of exposure to lufenuron, diflubenzuron, and methoxyfenozide did not differ among the 3 IGRs. At 96 h, α-esterase activity of diflubenzuron and methoxyfenozide did not differ significantly; however, the activities of both were significantly lower than that of lufenuron and of the control. Relative activity, from highest to lowest, of the enzyme was as follows: Control $>$ Lufenuron $=$ Methoxyfenozide $=$ Diflubenzuron at 24 h and Control $>$ Lufenuron $>$ Methoxyfenozide $=$ Diflubenzuron at 96 h.

Cytochrome P450 activity was significantly decreased by all 3 IGRs at 96 h of exposure ($F = 16.70$, $P = 0.001$] when contrasted with the control group (Table 4). At 24 h, enzyme activity in the methoxyfenozide treatment was significantly lower than that of the control. Likewise, GST activity was significantly lower than that of the control at 24 and 96 h after exposure to all 3 IGRs (Table 4).

Molecular docking. In an effort to better understand the potential molecular mechanisms associated with the modes of action of the 3 IGRs, data were collected on the amino acid interactions, hydrogen bond lengths in Ao, affinity by bond strength in Kcal/mol, and docking energy score between the tested IGRs and GST, α-esterase, and AChE (Table 5). The validation of the docking approach was conducted by comparing the active site of the pocket with the co-crystallized ligand of diflubenzuron, lufenuron, and methoxyfenozide. The tested compounds yielded good

Table 4. The activity of detoxification enzymes (AChE, α-esterase, cytochrome P-450, and GST) after 24 and 96 h of exposure to second-instar *S. frugiperda* with the LC_{50}s of the respective IGRs.

	AChE (mmole/mg protein)		α-esterase (mmole/mg protein)		Cytochrome P450 (μmole/min/mg of protein)		GST (μmol/mg of protein)	
Treatments	**24 h**	**96 h**	**24 h**	**96 h**	**24 h**	**96 h**	**24 h**	**96 h**
Control	7.57 ± 0.19a	4.68 ± 0.52a	0.88 ± 0.09a	0.62 ± 0.02a	0.51 ± 0.01ab	0.31 ± 0.037a	53.24 ± 6.15a	36.6 ± 2.08a
Lufenuron	6.51 ± 0.29b	3.74 ± 0.7ab	0.48 ± 0.06b	0.4 ± 0.03b	0.59 ± 0.11a	0.55 ± 0.042b	33.73 ± 1.94b	26.91 ± 2.8b
Diflubenzuron	2.66 ± 0.27c	2.98 ± 0.23bc	0.39 ± 0.021b	0.29 ± 0.02c	0.42 ± 0.03bc	0.39 ± 0.07b	23.65 ± 2.05c	22.12 ± 2.01b
Methoxyfenozide	2.93 ± 0.08c	2.12 ± 0.28c	0.43 ± 0.024b	0.31 ± 0.04c	0.31 ± 0.025c	0.32 ± 0.03b	31.23 ± 1.7bc	24.17 ± 3.34b

Means (± SE) that do not share the same lowercase letter in the column are significantly different ($P = 0.05$).

Table 5. Docking interaction data calculations of diflubenzuron, lufenuron, and methoxyfenozide against AChE (PDB ID: 6tt0), α-esterase enzyme (PDB ID: 4fng), and GST (PDB ID: 1jlv) active spots.

Enzyme	Compounds	Ligand	Amino acid	Interaction	Affinity Bond strength (Kcal/mol)	Affinity Bond length (in Ao from the main residue)	Interaction	Energy score (S) (Kcal/mol)
AChE	Diflubenzuron	C 6	TRP 84	3.81	−0.6	3.81	H-pi	−5.9675
		6-ring	PHE 330	3.72	0	3.72	pi-pi	
	Lufenuron	6-ring	PHE 331	3.96	0	3.96	pi-pi	−7.1157
	Methoxyfenozide	6-ring	TRP 84	3.99	0	3.99	pi-pi	−7.6565
α-esterase	Diflubenzuron	N 12	GLU 379	2.95	−3.6	2.95	H-donor	−6.1869
		N 12	GLU 379	3.1	−4.7	3.1	H-donor	
	Lufenuron	CL 39	GLN 255	3.79	−0.6	3.79	H-donor	−6.9594
	Methoxyfenozide	O 34	SER 378	3.37	−0.8	3.37	H-acceptor	−6.5986
GST	Diflubenzuron	CL 30	GLU 64	3.5	−0.4	3.5	H-donor	−4.927
		O 19	TYR 105	2.71	−2.3	2.71	H-acceptor	
	Lufenuron	O 19	TYR 105	2.93	−2.8	2.93	H-acceptor	−5.4364
	Methoxyfenozide	N 12	TYR 113	4.56	−0.7	4.56	H-pi	−6.0004

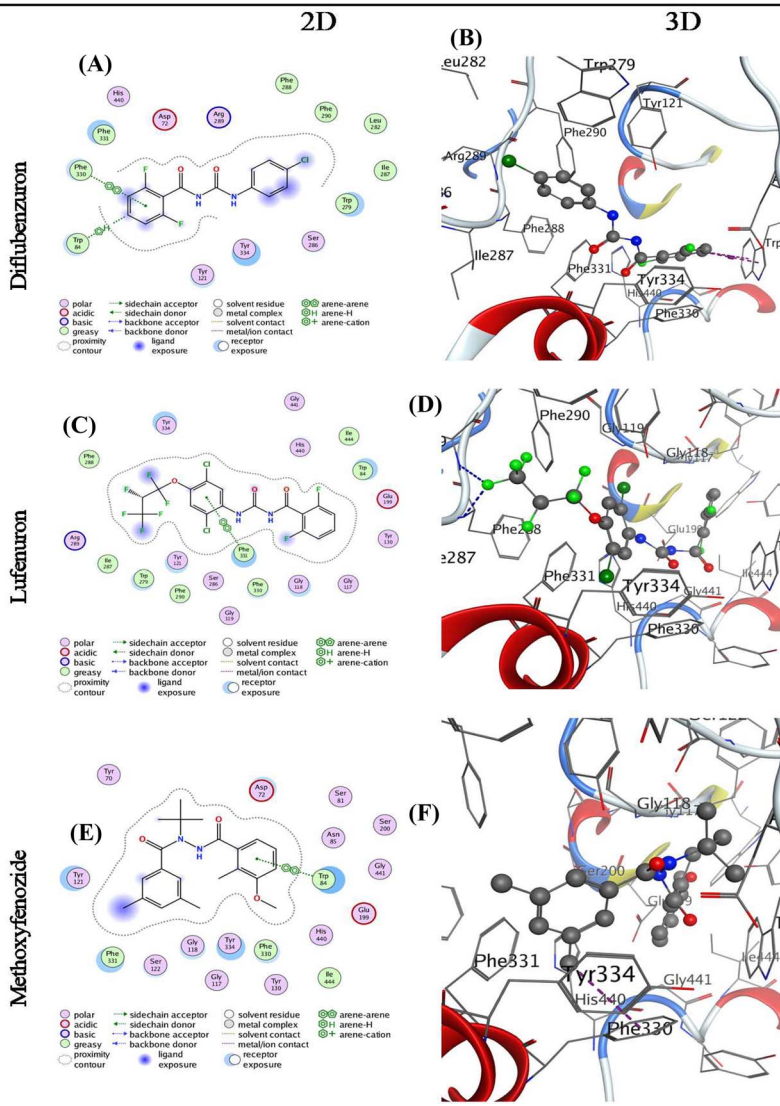

Fig. 1. Two-dimensional and three-dimensional interactions of diflubenzuron (A, B), lufenuron (C, D), and methoxyfenozide (E, F) against acetylcholine esterase (AChE) enzyme (PDB ID: 6tt0) active spots.

energy scores (S) = −5.9675, −7.1157, −7.6565 kcal/mol, respectively, against the AChE enzyme (PDB ID: 6tt0) and (S) −6.1869, −6.9594, −6.5986 kcal/mol, respectively, against α-esterase enzyme (PDB ID: 4fng), and lastly (S) = −4.927, −5.4364, −6.0004 kcal/mol, respectively, against GST (PDB ID: 1jlv) (Table 5; Figs. 1, 2, 3). The greater the engagement, the lower the energy score. Consequently, for our docked molecules, the interaction occurred in the following order: methoxyfenozide >

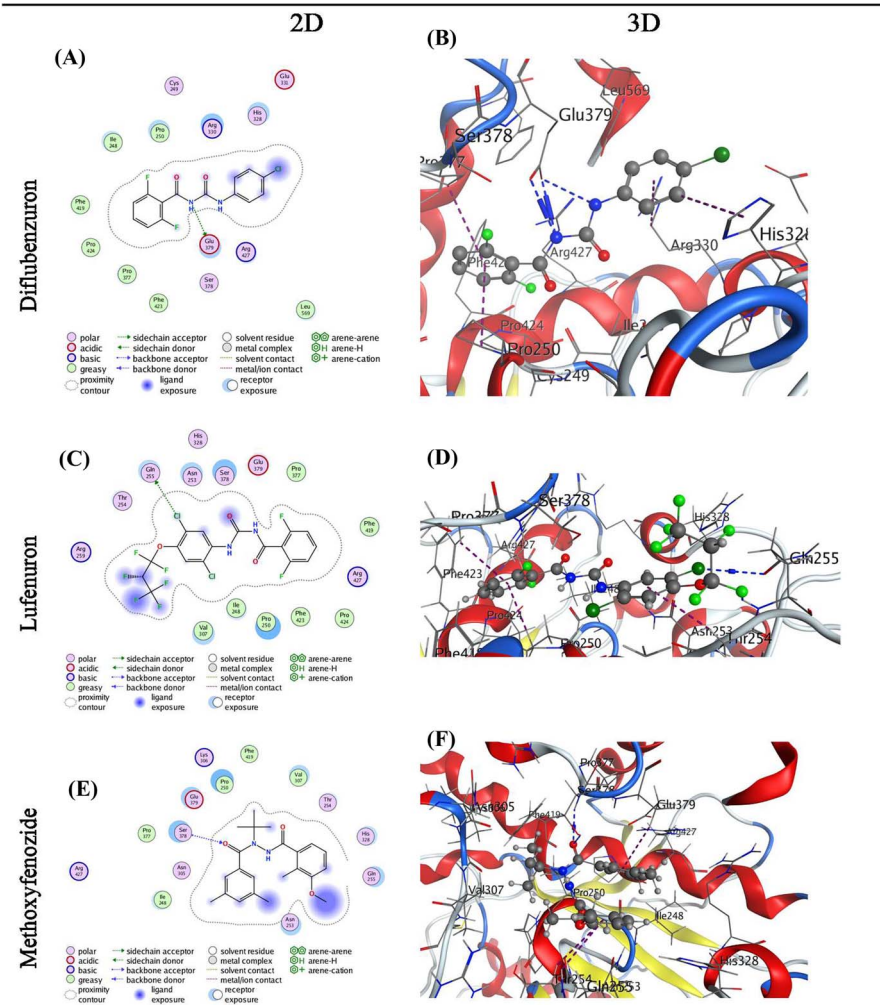

Fig. 2. Two-dimensional and three-dimensional interactions of diflubenzuron (A, B), lufenuron (C, D), and methoxyfenozide (E, F) against α-esterase enzyme (PDB ID: 4fng) active spots.

lufenuron > diflubenzuron for AChE enzyme (PDB ID: 6tt0) and GST (PDB ID: 1jlv) (Table 5; Figs. 1, 3). While, for α-esterase enzyme (PDB ID: 4fng), the interaction occurred in the following order: lufenuron > methoxyfenozide > diflubenzuron (Table 5; Fig. 2).

Discussion

The ultimate objective of this research was to assess the activity of 3 selected IGRs as management tactics for the fall armyworm, *S. frugiperda*, which has invaded

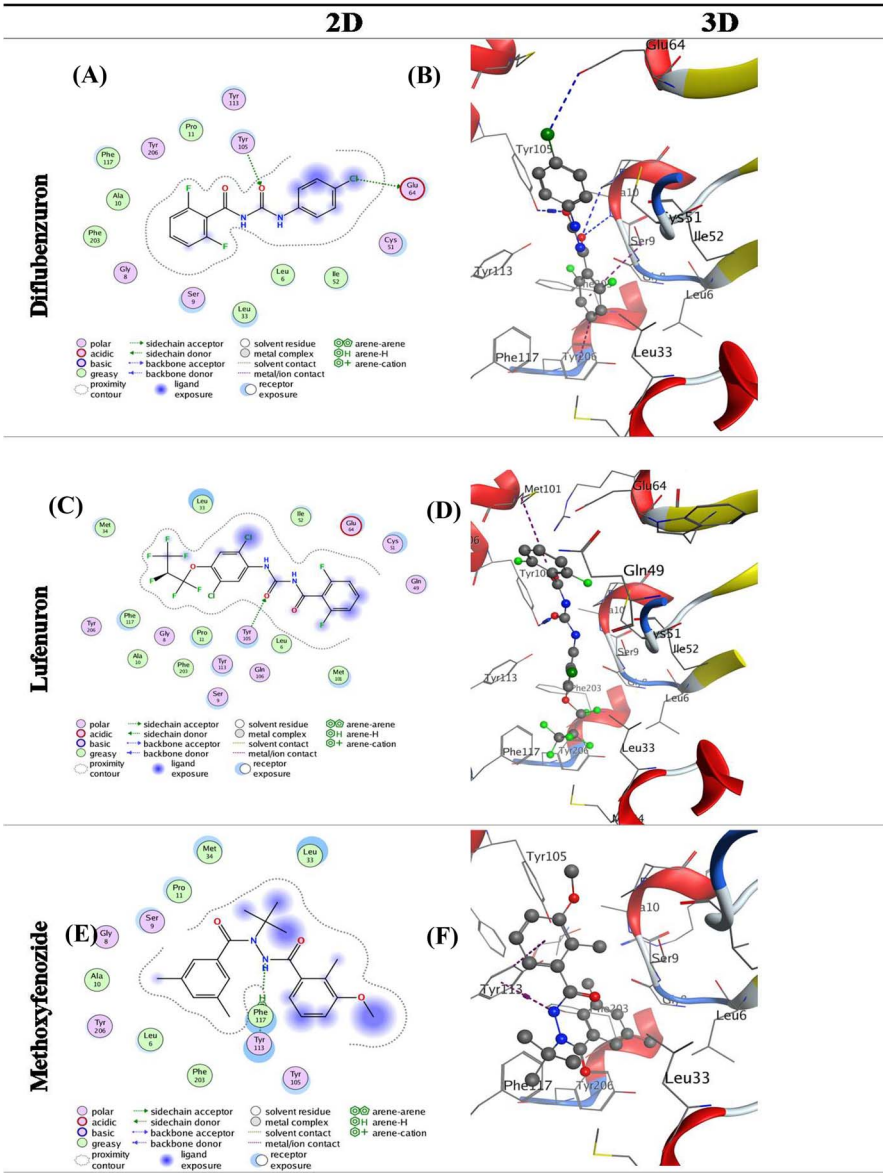

Fig. 3. Two-dimensional and three-dimensional interactions of diflubenzuron (A, B), lufenuron (C, D), and methoxyfenozide (E, F) against GST (PDB ID: 1jlv) active spots.

areas of Asia, Africa, and the Middle East from its native range in the Americas. This insect is a serious pest to a variety of crops, is difficult to manage (Chormule et al. 2019), and endangers food security and the livelihoods of smallholder farmers globally (Mohamed et al. 2022). IGRs are environmentally friendly pesticides that effectively

suppress the growth of pests by interfering with their regular growth and development processes (Lou and Wu 2024). Compared with chemical insecticides, IGRs offer several advantages, such as relative non-toxicity to mammals, low rate of development of resistance, high selectivity, and safe ecotoxicity profiles (Jiang et al. 2020, Guo et al. 2023). Several investigations have verified the biological activity of these agents against insect pests (Joseph 2017, Martínez et al. 2021).

Our toxicity bioassays revealed that lufenuron and diflubenzuron were the most toxic of the 3 IGRs tested herein against *S. frugiperda*. In comparison, methoxyfenozide was far less toxic. The greater toxicity of diflubenzuron and lufenuron is likely due to their mode of action as chitin biosynthesis inhibitors that impair insect growth and development by preventing the deposition of the new endocuticle during the molting process (Ono et al. 2017, Schneider et al. 2004). Toxicity of these IGRs appears to respond to species and larval age. Abou-Taleb et al. (2015) reported an LC_{50} of 1.7 mg/L for lufenuron against fourth-instar *S. littoralis* (Boisduval). Laecke and Degheele (1991) also reported that the LC_{50}s of diflubenzuron against fourth- and fifth-instar *S. exigua* (Hübner) were 295 and 16 mg/l, respectively. Moreover, Metayi and Attia (2023) determined the LC_{50} of 0.211 mg/L methoxyfenozide against second-instar *S. littoralis* after 72 h. of exposure.

Insecticides at both lethal and sublethal dosages may have direct or indirect nonlethal effects on insects (Cruz et al. 2021). Siviter et al. (2020) also noted that several chemicals are known to limit the growth of insect larvae. Hence, the impact of exposure of second-instar *S. frugiperda* to the LC_{50}s of the 3 IGRs was studied on selected developmental parameters of the insect. These findings showed that feeding *S. frugiperda* with LC_{50}s of lufenuron and methoxyfenozide shortened duration of the larval period, while the duration of the pupal stage was shortened by all 3 IGRs. Percentage pupation, sex ratio, and pupal weight were not significantly affected by the tested pesticides. Adult emergence rate was significantly lower in the lufenuron treatment in comparison to the untreated control, while percentage emergence rates in the methoxyfenozide and diflubenzuron treatments were not significantly different from that of the untreated control.

In insects, detoxification enzymes are often the enzymatic defense against foreign substances and are essential for preserving normal physiological processes (Li and Liu 2007). Insects may swiftly adjust to environmental challenges, such as pesticides, since a variety of exogenous or endogenous substances trigger detoxification enzyme activity (Zhang et al. 2017). The IGRs tested herein included two chitin production inhibitors (lufenuron and diflubenzuron) and one molting hormone agonist (methoxyfenozide). These findings imply that the 3 IGRs acted similarly to some extent on the activity of the detoxification enzymes assessed. An inhibitory effect was observed for all the tested insecticides on AChE, α-esterase, and GST in comparison to the control group. This would be an accurate representation of an insect's protective mechanism and much lower resistance (El-Sayed et al. 2023). Likewise, these results are in line with the *in vitro* assembly experiment results. The similarity of experimental data with theoretical data is relatively great when compared to data produced through theoretical calculations. The most important factor affecting the biological activity of IGRs against enzymes is interaction. As far as the author is aware, this is the first molecular docking study to investigate insecticides, such as lufenuron, diflubenzuron, and methoxyfenozide, against the AChE, α-esterase, and GST enzyme active sites.

Therefore, further molecular study is required to fully understand the relationship between the harmful effects of IGRs and their potential enzyme-inhibiting activities.

In conclusion, *S. frugiperda* has developed resistance to a wide range of insecticides due to long-term usage of chemical pesticides. To address this critical issue, chemical pesticide alternatives must be found to minimize such resistance, save the environment from pesticide pollution, and enhance agricultural productivity. These results demonstrated that *S. frugiperda* were affected by the IGRs, for example, lufenuron, diflubenzuron, and methoxyfenozide. Moreover, the evaluated IGR insecticides exhibited strong binding affinities and high energy scores with the evaluated enzymes. However, to verify our findings, further field-based IGR investigations are still required.

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