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RESEARCH

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Potential of insect growth regulators for the control of *Musca domestica* (Diptera: Muscidae) with respect to their biochemical and histological effects

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Abstract

Background Housefly causes a variety of health problems to humans and animals. Therefore, it is crucial to find out effective methods for the control of housefly larvae to avoid health problems associated with the presence this disease vector insect. Efficacy of insect growth regulators (IGRs), chlorfluazuron, lufenuron, methoxyfenozide and pyriproxyfen, against larvae of *Musca domestica* (Diptera: Muscidae), was assessed. The IGRs were mixed with food media at concentrations of 2.5, 5.0, 10.0, 25.0, 50.0, 75.0 and 100.0 mg/kg (ppm).

Results The highest larvicidal activity was obtained by chlorfluazuron and pyriproxyfen with LC_{50} values of 6.79 and 7.10 ppm, respectively, 72 h post-treatment. Also, 25.0 ppm of chlorfluazuron and 75.0 ppm of methoxyfenozide as well as lufenuron were shown to fully suppress adult emergence and survival percentages (0.0%). Moreover, the activity of three digestive enzymes suppressed in the treated larvae with pyriproxyfen and chlorfluazuron: amylase (enzyme ratio (ER) = 0.71 and 0.78), lipase (ER = 0.54 and 0.63) and proteases (ER = 0.62 and 0.66), respectively. Also, methoxyfenozide and pyriproxyfen inhibited acetylcholinesterase (AChE) and general-esterase (GE) activity with ER (0.28 and 0.59) and (0.61 and 0.72), respectively. The histological examination of *M. domestica* larvae treated with IGRs showed changes in midgut; for example, the epithelial cells were broken, deformed and loss their columnar structure. Also, the peritrophic membrane disappeared completely.

Conclusions The findings of the current study indicate that the tested IGRs have a potential to be applied in IPM programs of *M. domestica*.

Keywords Chlorfluazuron, Pyriproxyfen, Methoxyfenozid, House fly, Enzymes, Histological changes

Background

Houses, ranches, cattle stables and poultry farms are among the locations where the housefly, *Musca domestica* L. (Diptera: Muscidae), is a major insect pest that poses a health risk to the populations as described in several studies (Kumar et al., 2014; Malik et al., 2007; Salamatian et al., 2020; Sasaki et al., 2000). This insect pest is a vector for over 100 human and animal pathogens (Issa, 2019). Traditional chemical insecticides are a primary method for the managing of *M. domestica*. However,

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mammals and ecosystem are negatively impacted when chemical insecticides are used for public health insect control without wisdom (Kumar et al., 2012). Additionally, the emergence of insect resistance is a big threat associated with the ongoing use of chemical insecticides (Li et al., 2012; Shono et al., 2004; Wang et al., 2019). Recently, safe and effective control methods have been applied for the control of *M. domestica*, such as entomopathogenic fungi, ozone fumigation, essential oils and monoterpenes (Acharya et al., 2015; Metwally et al., 2023; Yoon & Tak, 2023; Zhang et al., 2017).

Due to their various advantages over traditional insecticides, including reduced environmental risk and relative safety for ecosystem and livestock, insect growth regulators (IGRs) are considered among the safe control methods for managing insects with public health importance (Gupta & Jindal, 2014; Mulla, 1995; Tunaz & Uygun, 2004). IGRs disrupt some physiological processes in insects, such as cuticle formation, metamorphosis, and the development of immature stages (Oberlander & Silhacek, 2000). IGRs are classified into three categories: chitin synthesis inhibitors, juvenile hormone agonists and ecdysteroid agonists (Mondal & Parween, 2000; Oberlander et al., 1997). Several investigations reported the insecticidal efficacy of IGRs against *M. domestica*, particularly chitin synthesis inhibitors, such as diflubenzuron, hexaflumuron, lufenuron and buprofezin (Kočišová et al., 2004; Assar et al., 2010; Khalil et al., 2010; Albayyar & Abdel Qader, 2021; Tanani et al., 2022; Sankar & Kumar, 2023). However, little information is available on efficacy of the other two types of IGRs, such as juvenile hormone mimic and molting hormone agonist on *M. domestica*. Likewise, there is a lack of knowledge on the biochemical and histological effects of these IGRs. Therefore, we focus in the current investigation on examining the susceptibility of *M. domestica* third instar larvae to four IGRs, including chlorfluazuron and lufenuron (chitin synthesis inhibitor), methoxyfenozide (molting hormone agonist) and pyriproxyfen (juvenile hormone mimic) using a food media technique as well as their biological, biochemical and histological effects.

Methods

House fly colony

Culture of *M. domestica* was provided by Medical Entomology Research Institute, Dokki, Giza, Egypt. Male and female adults were reared in plastic cages with 20 L capacity and fed on dry diet milk powder and sucrose solution (cotton pads soaked in 10% sucrose solution). Larvae fed on an artificial diet (200 mg of wheat bran, 100 mg of milk powder, 5 mg of yeast and 200 ml distilled water) under laboratory conditions (25 °C, 65% RH and 12-h light and 12-h dark photoperiod). The third instar larvae were used in all bioassays (El-Geneady et al., 2023).

Tested IGRs

Chlorfluazuron (95%) was obtained by Simonis BV, Doetinchem, The Netherlands, and lufenuron (94%) was supplied by and Syngenta Crop Protection, Switzerland. Methoxyfenozide (95%) and pyriproxyfen (98%) were obtained from Kafr El-Zayat Pesticides and Chemicals Co., Egypt.

Larvicidal activity bioassay

The tested IGRs were assessed on larvae of *M. domestica,* using a food media technique (Wright, 1971). IGRs stock solutions were prepared in acetone. IGRs were applied at different concentrations, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0 and 100.0 mg/kg (ppm). A series of ten grams of artificial diet (200 mg of wheat bran, 100 mg of milk powder, 5 mg of yeast and 200 ml distilled water) was treated with stock solutions of IGRs separately in plastic jars (400 ml). Each jar was treated with 1 ml of acetone solution and mixed thoroughly with diet. The treated diet in the jars kept for other 30 min before introducing house fly larvae. In case of control treatment, the artificial diet treated with 1 ml of acetone. Then, ten third instar larvae of *M. domestica* were introduced into each jar. All treated jars were kept under laboratory conditions of 25 °C and 65% RH. The above procedure was replicated four times. Larval mortality was counted 24, 48 and 72 h post-treatment. On the other hand, the treated larvae with IGRs were observed daily until complete pupation and adult emergence, and percentages of pupation, adult emergence and total survival were calculated using the following equations:

$$\text{Pupation (\%)} = (\text{Number of pupae} / \text{Total number of larvae}) \times 100$$

$$\text{Adult emergence (\%)} = (\text{Number of adults} / \text{Total number of pupae}) \times 100$$

$$\text{Survival (\%)} = (\text{Number of adults} / \text{Total number of larvae}) \times 100$$

Biochemical tests

The effect of tested IGRs at 25 ppm (~ 0.5 LC_{50} after 24 h) on the activity of three digestive enzymes (amylase, lipase and proteases), acetylcholinesterase (AChE), general-esterase (GE) and adenosinetriphosphatase (ATPase)

of third instar larvae of *M. domestica* was tested. After treatment, 1 g of larvae was taken 24 h post-treatment for enzyme activity measurements.

Preparation of enzyme sources

The enzyme sources from treated larvae were prepared according to methods provided by Amin (1998).

Determination of enzyme activities

Digestive enzymes were determined as follows: amylase activity is measured as µg glucose released/min/g body weight in accordance with Amin's (1998) modifications using the technique given by Ishaaya and Swirski (1976). With a few minor adjustments, the method of Tahoun and Abdel-Ghaffar (1986) was used to measure lipase activity. The technique is predicated on measuring the triolein substrate's ester content decline. The activity was measured in µmoles of liberated oleic acid/min/g of body weight. With minor adjustments, the amount of free amino acids that were separated from the substrate protein (albumin) over a one-hour incubation period at 30 °C was used to calculate the proteolytic (or proteases) activity (Tatchell et al., 1972). The amino acids were expressed as µg alanine/min/g.b.wt. Acetylcholinesterase (AChE) activity was measured by using acetylcholine bromide (AchBr) as substrate (Simpson et al., 1964). α-Esterases and β-esterases, namely general-esterase (GE), were measured by using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively (Van Asperen, 1962). Adenosinetriphosphatase (ATPase) was determined according to Amaral et al. (2001). ATPase activity was expressed in µmoles of Pi released per minute per milligram protein.

Histological tests

The effect of the four IGRs at 25 ppm on histology of *M. domestica* third instar larvae midgut was assessed. Ten larvae were taken 24 h post-treatment and placed into 10% formalin as fixative for dehydration. After dehydration larvae were passed through a graded series of ethanol for two h. Then, the larvae were placed in soft paraffin wax for 24 h at 50 °C. Series sections at 6 µ were made by microtome and mounted on clean slides using Mayer's albumin (Bancroft et al., 1990). Sections were prepared and stained with hematoxylene and examined microscopically.

Data analysis

To adjust mortality percentages, Abbott's formula (1925) was used. The corrected mortality percentages were subjected to probit analysis (Finney, 1971) in order to estimate the LC_{50} value. ANOVA was used to analyze the percentages of pupation, adult emergence, survival and tested enzymes. Tukey's HSD test was used to calculate mean separations at a significance level of < 0.05 by SPSS 21.0 software (SPSS, Chicago, IL, USA).

Table 1 Toxicity of four insect growth regulators against third larval instar of *Musca domestica* after 24, 48 and 72 h of treatment

Treatments	Exposure time (hours)	LC_{50}[a] (mg/kg)	95% confidence limits (ppm)		Slope[b] ± SE	(χ2)[c]	*P*[d]	Toxicity index
			Lower	Upper				
Chlorfluazuron	24	11.39	9.20	13.67	1.62 ± 0.15	7.42	0.059	100
	48	8.54	6.72	10.35	1.69 ± 0.19	1.59	0.452	
	72	6.79	4.69	8.79	1.36 ± 0.21	2.92	0.232	
Lufenuron	24	51.4	44.29	60.59	1.79 ± 0.15	9.11	0.059	22.17
	48	40.65	34.01	49.47	1.38 ± 0.11	10.04	0.074	
	72	27.72	23.23	33.66	1.49 ± 0.12	8.05	0.089	
Methoxyfenozide	24	109.59	86.92	150.92	1.52 ± 0.17	8.58	0.072	10..39
	48	40.77	34.84	48.14	1.63 ± 0.14	8.59	0.072	
	72	25.86	22.29	30.26	1.85 ± 0.13	0.72	0.949	
Pyriproxyfen	24	47.44	36.38	68.42	1.48 ± 0.16	1.59	0.661	24.03
	48	10.12	8.05	12.65	1.21 ± 0.13	3.04	0.385	
	72	7.1	5.56	8.81	1.26 ± 0.14	2.61	0.455	

[a] The concentration causing 50% mortality

[b] Slope of the concentration–mortality regression line ± standard error

[c] Chi-square value

[d] Probability value

Results

Larvicidal activity of IGRs

Table 1 shows the LC_{50} values of four IGRs against *M. domestica* third instar larvae after 24, 48 and 72 h of treatment. The tested IGRs had varying levels of insecticidal action, and their toxicity increased as exposure time and concentration increased. After 72 h of exposure, the highest larvicidal activity was obtained by chlorfluazuron and pyriproxyfen with LC_{50} values of 6.79 and 7.10 ppm, respectively, while the two other IGRs, methoxyfenozide and lufenuron, had considerably higher levels of effectiveness after 72 h of treatment, with LC_{50} values of 25.86 and 27.72 ppm, respectively.

Latent effects of IGRs

The delayed effect of four IGRs on the percentages of pupation, adult emergence and survival is presented in Tables 2, 3 and 4. The percentages of pupation, adult emergence and survival of treated larvae decreased significantly with increasing concentrations of tested IGRs compared to untreated third instar larvae (100, 90.0 and 90.0%), respectively. Our results demonstrated that a complete suppression of pupation percentage (0.0%) was achieved by the following treatments: 50.0 ppm of chlorfluazuron, 75.0 ppm of methoxyfenozide and 100 ppm of lufenuron. On the other hand, 25.0 ppm of chlorfluazuron and 75.0 ppm of methoxyfenozide and lufenuron were shown to full suppress adult emergence and survival percentages (0.0%).

Table 2 Effect of four insect growth regulators on pupation percentage of *Musca domestica* treated as third instar larvae

Treatments	Pupation (% ± SE)			
	Chlorfluazuron	Lufenuron	Methoxyfenozide	Pyriproxyfen
0.0	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
2.5	65.6 ± 6.0ab	90.6 ± 3.1a	93.8 ± 3.6ab	71.9 ± 3.3ab
5.0	56.2 ± 6.2bc	87.5 ± 4.5a	90.6 ± 3.0abc	53.1 ± 4.0bc
10.0	37.5 ± 4.5bcd	81.2 ± 8.2ab	87.5 ± 5.1abc	46.8 ± 9.0bc
25.0	9.4 ± 3.1cd	71.9 ± 6.1ab	78.1 ± 6.1bc	37.5 ± 5.9bc
50.0	0.0 ± 0.0d	40.6 ± 1.6bc	65.6 ± 3.3c	15.6 ± 1.0c
75.0	–	9.4 ± 3.0cd	0.0 ± 0.0d	–
100	–	0.0 ± 0.0d	0.0 ± 0.0d	–
F	13.1	19.4	45.7	8.6
df	5, 18	7.24	7, 24	5, 18
P	< 0.01	< 0.01	< 0.01	< 0.01

Mean values within each column followed by the same letter are not significantly different ($P < 0.05$)

Table 3 Effect of four insect growth regulators on adult emergence percentage of *Musca domestica* treated as third instar larvae

Treatments	Adult emergence (% ± SE)			
	Chlorfluazuron	Lufenuron	Methoxyfenozide	Pyriproxyfen
0.0	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
2.5	56.5 ± 5.6ab	83.1 ± 6.4ab	72.8 ± 6.2ab	62.1 ± 5.3ab
5.0	30.8 ± 5.8bc	67.2 ± 3.6abc	68.0 ± 4.7b	52.1 ± 7.9abc
10.0	15.5 ± 2.4cd	50.7 ± 6.3bc	65.2 ± 6.2bc	45.8 ± 1.2abc
25.0	0.0 ± 0.0d	41.4 ± 7.4cd	58.9 ± 6.4bc	24.9 ± 5.8bc
50.0	0.0 ± 0.0d	18.3 ± 5.5de	45.1 ± 5.3c	6.3 ± 2.8c
75.0	–	0.0 ± 0.0e	0.0 ± 0.0d	–
100	–	0.0 ± 0.0e	0.0 ± 0.0d	–
F	20.7	31.2	89.1	7.1
df	5, 18	7, 24	7, 24	5, 18
P	< 0.01	< 0.01	< 0.01	< 0.01

Mean values within each column followed by the same letter are not significantly different ($P < 0.05$)

Table 4 Effect of four insect growth regulators on larval surviving to adulthood percentage of *Musca domestica*

Treatments	Larval surviving to adulthood (% ± SE)			
	Chlorfluazuron	Lufenuron	Methoxyfenozide	Pyriproxyfen
0.0	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
2.5	37.5 ± 5.2b	75.0 ± 5.1ab	68.8 ± 6.3ab	46.9 ± 8.1ab
5.0	21.8 ± 1.9bc	59.4 ± 3.0abc	59.4 ± 5.9b	31.2 ± 6.0bc
10.0	9.4 ± 3.1bc	43.8 ± 6.1bc	59.4 ± 6.0b	25.0 ± 2.6bc
25.0	0.0 ± 0.0c	31.3 ± 2.3cd	46.9 ± 7.8bc	12.5 ± 5.1bc
50.0	0.0 ± 0.0c	12.5 ± 2.5de	31.3 ± 8.1c	3.1 ± 1.0c
75.0	-	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0c
100	-	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0c
F	25.1	31.8c	52.9	12.1
df	5, 18	7, 24	7, 24	5, 18
P	< 0.01	< 0.01	< 0.01	< 0.01

Mean values within each column followed by the same letter are not significantly different ($P < 0.05$)

Table 5 Activity of digestive enzymes in larvae of *M. domestica* treated with 25.0 mg/kg of four insect growth regulators

Treatments (25 mg/kg)	Amylase[a]		Lipase[c]		Proteases[d]	
	Mean ± SE	ER[b]	Mean ± SE	ER	Mean ± SE	ER
Control	21.8 ± 0.6b		193.3 ± 2.4a		168.0 ± 2.6c	
Chlorfluazuron	17.1 ± 0.4c	0.78	122 .0 ± 1.1d	0.63	111.3 ± 1.7d	0.66
Lufenuron	23.9 ± 0.5ab	1.09	180.0 ± 1.8b	0.93	272.6 ± 9.4b	1.62
Methoxyfenozide	26.1 ± 0.3a	1.19	162.3 ± 0.9c	0.84	339.7 ± 8.6a	2.02
Pyriproxyfen	14.9 ± 0.3c	0.71	103.7 ± 1.9e	0.54	104.0 ± 1.6d	0.62
F	53.1		251.5		152.1	
P	< 0.01		< 0.01		< 0.01	

Mean values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 4, 10)

[a] Amylase (ug glucose/min/mg protein)

[b] Enzyme ratio (ER) = mean activity of enzyme in different treatments/mean activity of enzyme in control group)

[c] Lipase (nU/min/mg protein)

[d] Proteases (ng alanine/min/mg protein)

Table 6 Activity of acetylcholinesterase (AChE), general-esterase (GE) and Adenosinetriphosphatase (ATPase) in larvae of *M. domestica* treated with 25.0 mg/kg of four insect growth regulators

Treatments (25 mg/kg)	AChE[a]		GE[c]		ATPase[d]	
	Mean ± SE	ER[b]	Mean ± SE	ER	Mean ± SE	ER
Control	504.0 ± 6.4ab		7.2 ± 0.2a		293.0 ± 4.7b	
Chlorfluazuron	527.0 ± 2.9a	1.05	6.3 ± 0.1b	0.88	219.0 ± 4.8c	0.74
Lufenuron	482.0 ± 3.3b	0.96	6.7 ± 0.1ab	0.93	223.0 ± 2.3c	0.76
Methoxyfenozide	139.3 ± 3.4d	0.28	4.4 ± 0.1d	0.61	325.0 ± 3.6a	1.11
Pyriproxyfen	300.3 ± 3.5c	0.59	5.2 ± 0.1c	0.72	208.0 ± 1.8c	0.71
F	832.2		58.2		101.6	
P	< 0.01		< 0.01		< 0.01	

Mean values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 4 10)

[a] AChE (ng AchBr/min/mg protein)

[b] Enzyme ratio (ER) = mean activity of enzyme in different treatments/mean activity of enzyme in control group)

[c] GE (ug α- naphthol/min/mg protein)

[d] ATPase (nmol Pi/min/mg protein)

Biochemical effects of IGRs

Data in Tables 5 and 6 show the impact of a 25 ppm concentration of tested IGRs on three digestive enzymes (amylase, lipase, and proteases), AChE, GE and ATPase of *M. domestica* larvae. The activity of three digestive enzymes was suppressed in larvae exposed to pyriproxyfen and chlorfluazuron: amylase (ER = 0.71 and 0.78), lipase (ER = 0.54 and 0.63) and proteases (ER = 0.62 and 0.66), respectively. However, the two other IGRs, methoxyfenozide and lufenuron, caused different responses as they induced enzyme activities with ER (1.19 and 1.09) on amylase and ER (2.02 and 1.62) on proteases, respectively. In contrast, lipase activity was inhibited by the methoxyfenozide and lufenuron with ER (0.84 and 0.93), respectively, but it was on par with control (Table 5). Also, methoxyfenozide and pyriproxyfen inhibited the activity of AChE and GE with ER (0.28 and 0.59) and (0.61 and 0.72), respectively. However, chlorfluazuron increased the activity of AChE with ER (1.05). In the case of ATPase, pyriproxyfen, chlorfluazuron and lufenuron inhibited enzyme activity with ER (0.71, 0.74 and 0.76), respectively, while methoxyfenozide increased enzyme activity with ER (1.11), but it was on par with control (Table 6).

Histological effects of IGRs

The effect of a 25 ppm of tested IGRs on midgut of *M. domestica* larvae is presented in Fig. 1. Across section in the midgut of untreated larvae of *M. domestica* appeared a single layer of columnar epithelial cells surrounded by the basement membrane. The lumen of midgut contains peritrophic membrane enclosing food particles. The wall of gut contains two distinct layer of muscles layer under basement membrane, longitudinal muscles fibers to the outside and circular muscles fibers to the inside indicated good feeding on protein diet. In the case of larvae treated with tested IGRs, the epithelial cells in the midgut were broken, deformed and loss their columnar structure, although the peritrophic membrane is still intact in many areas in treatment of chlorfluazuron and methoxyfenozide, while in the larvae treated with lufenuron and pyriproxyfen, the epithelial cells lost their association with columnar structure in some point and appeared destroyed beside, and become elongate in size than

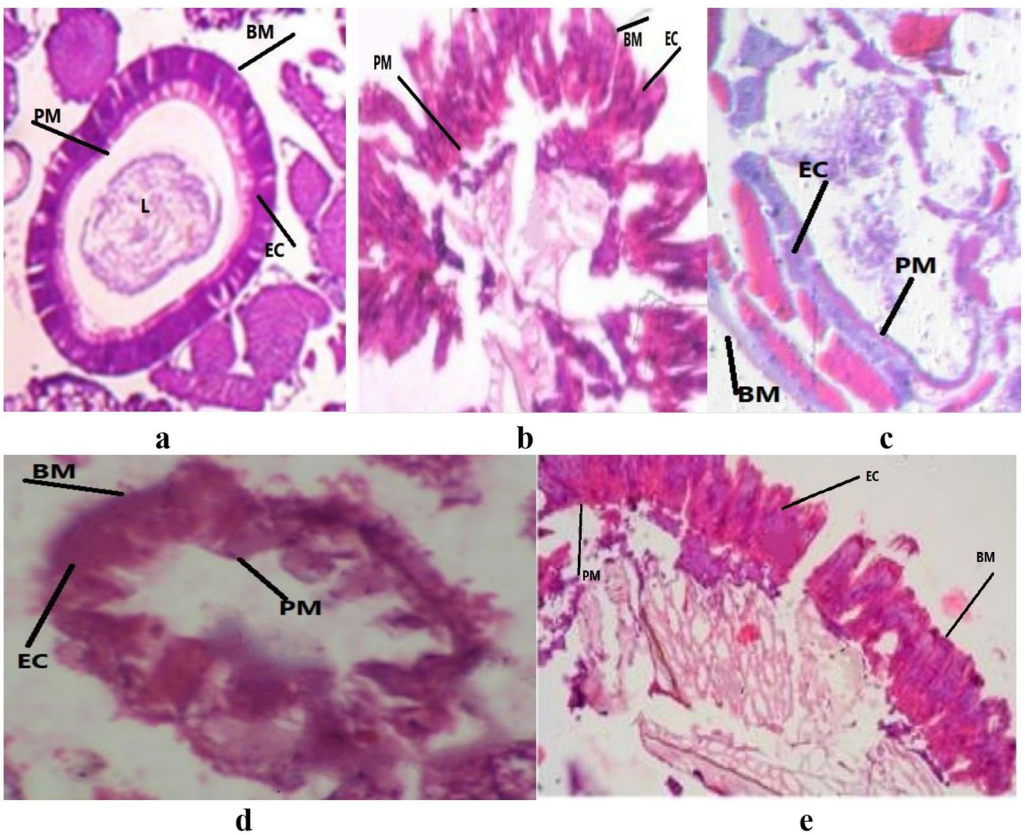

Fig. 1 Transverse sections on midgut of third instar larvae of *M. domestica* treated with IGRs (25.0 mg/kg) (**a**, untreated, **b**, chlorfluazuron, **c**, lufenuron, **d**, methoxyfenozide and **e**, pyriproxyfen), BM: basement membrane, EC: epithelium cell, PM: peritrophic membrane, L: lumen

control. Also, the peritrophic membrane disappeared completely (Fig. 1).

Discussion

Efficacy of IGRs has been reported against *M. domestica* in several studies (Alzahrani, 2021; Khan, 2021; Shah et al., 2015; Tanani et al., 2022). Yet, few studies have described a larvicidal activity of tested IGRs against *M. domestica*. Furthermore, no pervious information is available on biochemical and histological effects of tested IGRs on *M. domestica.* The results of the current study revealed that the toxicity of tested IGRs increased as exposure time and concentration increased. The larvicidal activity chlorfluazuron and pyriproxyfen was more than methoxyfenozide and lufenuron. The higher toxicity of IGRs shown in this current investigation was matched with previous reports indicated that IGRs were very effective against *M. domestica*. Kočišová et al. (2004) showed that diflubenzuron induced a complete mortality of first instar larvae of *M. domestica*. Abo El-Mahasen et al. (2010) found that tebufenozide and pyriproxyfen displayed a potent larvicidal activity against *M. domestica* with full larval mortality at 2000 ppm. Albayyar & Abdel Qader (2021) demonstrated that lufenuron caused a complete larval mortality of *M. domestica* at concentration of 1000 mg/l. Beside their effects on larval mortality, the IGRs induced a significant reduction in the resulting pupae and adults as well as reduced adult emergence and a complete suppression of pupation percentage was achieved by chlorfluazuron (50.0 ppm), methoxyfenozide (75.0 ppm) and lufenuron (100 ppm). A full inhibition of adult emergence and survival percentages of *M. domestica* were obtained by chlorfluazuron (25.0 ppm), methoxyfenozide (75.0 ppm) and lufenuron (75.0 ppm). Our results supported by previous studies indicated the inhibition of pupation and adult emergence of *M. domestica* by IGRs, such as Kočišová et al. (2004) who mentioned that cyromazine and diflubenzuron had a high adverse effect on the development of earliest stages of housefly larvae. Abo El-Mahasen et al. (2010) showed that tebufenozide and pyriproxyfen reduced the pupation and adult emergence of *M. domestica* and a complete suppression was obtained at 1000 and 2000 ppm. The suppression of pupation, adult emergence and total survival of *M. domestica* by tested IGRs observed here are due to their adverse effects on metamorphosis and development of immature stages (Biale et al., 2017; Cetin et al., 2006; Khalil et al., 2010).

The results also show that the activity of three digestive enzymes suppressed in larvae exposed to pyriproxyfen and chlorfluazuron. However, methoxyfenozide and lufenuron increased the activity of amylase and proteases and decreased the activity lipase. There was no published research on the impact of tested IGRs on *M. domestica* larvae's digestive enzymes. However, many studies indicated that the IGRs caused changes of nutrition components of *M. domestica*, such as Assar et al. (2010) who indicated that the IGRs (tebufenozide and pyriproxyfen) increased the glucose, total protein and amino acids contents of *M. domestica* larvae, while total protein and amino acids contents decreased in larvae treated with chitin inhibitors (hexaflumuron and lufenuron). Tanani et al. (2022) demonstrated that lufenuron, fufenoxuron and hexafumuron reduced total carbohydrate, protein and lipid contents in treated larvae. Our results relived that IGRs affect the activity of digestive enzymes of house fly. This findings agree with several studies indicating that IGRs disturb the activity of digestive enzymes of other insects, such as *Pseudaletia separata* (Huang et al., 2008) and *Callosobruchus maculatus* larvae (Khatter & Abuldahb, 2011). Also, methoxyfenozide and pyriproxyfen inhibited the activity of AChE and GE of *M. domestica* larvae. However, chlorfluazuron increased the activity of AChE. Pyriproxyfen, chlorfluazuron and lufenuron inhibited the activity of ATPase, while methoxyfenozide induced the activity of ATPase. These results are supported by El-Bermawy (1994) who found that pyriproxyfen inhibited phosphatase enzymes activity in larval, pupal and adult stage of *M. domestica*. Assar et al. (2010) indicated that lufenuron and pyriproxyfen increased the activity of acid and alkaline phosphatases; in contrast, tebufenozide exhibited a severe reduction in the activity of these enzymes. Chen et al. (2019) showed that methoxyfenozide and lufenuron inhibited the chitinase, AChE and carboxylesterase enzymes activities in *Spodoptera exigua* Hübner larvae. Valbon et al. (2021) found that pyriproxyfen reduced GE enzymes of *Aedes aegypti* larvae. Increasing of enzyme activity after exposure to insecticides may be due to the induction of the enzyme genes which are tissue specific and depending on the insecticide doses and the time after exposure (Feng et al., 2018).

Larvae of *M. domestica* treated with tested IGRs showed histological changes; for example, the epithelial cells in the midgut were broken, deformed and loss their columnar structure. Also, the peritrophic membrane disappeared completely. There were no published researches on the impact of tested IGRs on histology of midgut of *M. domestica* larvae. However, similar effects were reported on other insects treated with IGRs. For example, Costa et al. (2017) indicated that lufenuron caused disorganization, vacuolization and desquamation of the midgut epithelium of *Anthonomus grandis* Boheman. Fiaz et al. (2019) demonstrated that pyriproxyfen induced significant cytotoxic and histological changes in *A. aegypti* larvae's midgut, such as presence of cytoplasmic

vacuolization and damage to brush border of the digestive cells. Our findings suggest that IGRs caused changes in the epithelial cells and the peritrophic membrane. These histopathological changes may cause the insect to die or may have an impact on reproduction by inhibiting the insect's ability to digest and absorb nutrients (Mordue and Nisbet 2000). Catae et al. (2014) stated that the midgut has become an interesting target site to the toxicity of insecticides on certain insects and their ability to cause morphological disorders in cells and tissues. These effects can interfere with cellular immunology and compromise nutritional reserves.

Conclusions

Based on the present study, the tested IGRs displayed remarkable acute and latent toxicities on *M. domestica* larvae. Our finding also indicted that some IGRs may induce their insecticidal activity via suppression of vital enzymes or adverse changes in midgut histology. Among the tested IGRs, chlorfluazuron and pyriproxyfen were the most potent toxicants and should be applied in IPM strategies of *M. domestica*. The use of insect growth inhibitors is highly important to delay the development of insect resistance as well as to reduce the risk on mammals and the environment.

Abbreviations

IGRs	Insect growth regulators
IPM	Integrated pest management
ER	Enzyme ratio
AChE	Acetylcholinesterase
GE	General-esterase
ATPase	Adenosinetriphosphatase

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Author contributions

AME involved in conceptualization, methodology, investigation, writing. SSAH and AMG took part in conceptualization; data curation; methodology; investigation. HAG involved in conceptualization; methodology; investigation; writing. SAMA took part in conceptualization; validation; visualization; writing—review and editing.

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Consent for publication

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Competing interests

The authors declare no competing interests.

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