

## Impact of some insect growth regulators on biochemical parameters of a fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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### Abstract

The current study aimed to assess the effect of three distinct insect growth regulators (IGRs): chitin synthesis inhibitors (lufenuron), juvenile hormone analogs (pyriproxyfen), and ecdysone receptor agonists (methoxyfenozide) on the biochemical parameters of *Spodoptera frugiperda* larvae at the second and fourth instar stages, which were fed leaves of castor bean treated with these compounds. According to the results, lufenuron was the most effective compound against the *S. frugiperda*, followed by pyriproxyfen, and methoxyfenozide was the least effective compound. To assess the impact of IGRs on protein, carbohydrate, and lipid levels, as well as the activity of chitinase, acetylcholinesterase, and carbohydrate-hydrolyzing enzymes (amylase, invertase, and trehalase) at LC<sub>50</sub> concentrations. In the fourth instar larvae of *Spodoptera frugiperda*, all tested IGRs dramatically reduced total proteins, lipids, and carbohydrate content, with the exception of methoxyfenozide which increased total (proteins, carbs, and lipids) while pyriproxyfen and lufenuron which decreased total (protein, carbs, and lipids). Lufenuron increased chitinase activity significantly. Acetylcholinesterase activity was markedly elevated by the tested IGRs. The tested IGRs caused a significant decrease in amylase activity, with the exception of methoxyfenozide for fourth-instar larvae, and an increase with all treatments for fourth-instar larvae of invertase. In contrast, all tested IGRs treatments for fourth-instar larvae increased trehalase activity except lufenuron decreased the enzyme activity.

**Keywords:** *Spodoptera frugiperda*, insect growth regulators (IGRs), lufenuron, pyriproxyfen, methoxyfenozide, biochemical effects

### Introduction

The devastating insect pest known as fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is currently posing a serious threat to agricultural output worldwide. Various crops, including corn, rice, sorghum, sugarcane, cabbage, beetroot, groundnut, soybean, alfalfa, onion, pasture grasses, millet, tomato, potato and cotton, are harmed by fall armyworm (Day *et al.* 2017) [12]. *Spodoptera frugiperda* reduce yields by feeding on the host plant's reproductive and vegetative components. Larvae instars eat near the ground, causing holes in leaves and consuming them from the edge inward. Despite this, the majority of farmers favor the use of chemical pesticides, which harm pollinators, natural enemies, and all other non-target insects. Insect growth regulators (IGRs) are currently seen to be a viable substitute for synthetic insecticides in the management of this pest (El-Lateef *et al.*, 2025) [17]. Because of the way they work, they have a bigger impact on insect species' early stages than on their adults. The majority of IGRs have a distinct method of action that interferes with the hormonal balance of insects or disturbs their cuticle production or moulting process (Khedr *et al.*, 2005) [25]. They have detrimental effects on target

pests and are known for their slow action against a limited range of sensitive phases of the insect life cycle (Siddall, 1976) [34]. IGRs have recently been categorised as chitin synthesis inhibitors (CSIs) and compounds that block the action of insect hormones, such as juvenile hormone analogues and ecdysteroids, based on their mode of action (Tunaz and Uygun, 2004) [38]. The study aimed to evaluate the efficiency of the previous categories of IGRs through selection the lufenuron, pyriproxyfen, and methoxyfenozide against 2<sup>nd</sup> and 4<sup>th</sup> of *S. frugiperda*. Also, impact of the tested IGRs' latent effects on some biochemical characteristics of fall armyworms was the goal of the continuing study.

### Materials and methods

#### Insect

Agricultural Research Centre, Dokki, Giza, Egypt's Plant Protection Research Institute provided the strain of fall armyworm (FAW), *S. frugiperda* larvae, used in this investigation. According to El-Defrawi *et al.* (1964) [16], larvae were raised in a laboratory with regulated conditions (25±2°C, 65±5% R.H., photoperiod 12 h L: 12 h D) and the rearing process was modified by Bakr *et al.* (2010) [6].

### The Tested Insect Growth Regulators

**Table 1:** lists of the common names, trade names, manufacturer, mode of action, and recommended dose for the chosen IGRs

Trade name	Common Name	Recommended dose	Mode of action	Obtained from
Hoori moon 10% SC	Lufenuron	80cm/fdan	Chitin Synthesis Inhibitor (CSIs)	Egypt Agricultural Development
Admafin 10%EC	Pyriproxyfen	37.5 cm/100 Liter water	Juvenile Hormone Analogues and Mimics (JHAs)	Kanza group for Pesticide and chemicals
Banzr 24% SC	Methoxyfenozide	37.5 cm/100 Liter water	Ecdysone Agonists	Dow Agro Sciences, Egypt

### Bioassays Test

The tested IGRs were diluted with distilled water to obtain the concentrations from  $10^{-1}$  to  $10^{-7}$  ppm for using in bioassay studies against 2<sup>nd</sup> and 4<sup>th</sup> larval instar of *S. frugiperda*. The (LC<sub>50</sub>) for the investigated IGRs was determined using a leaf-dipping bioassay. We prepared series concentrations (in water) as mentioned above of the examined substances. Castor bean leaves were immersed for ten seconds in each concentration and dried. Newly moulted 2<sup>nd</sup> and 4<sup>th</sup> instar larvae were fed treated leaves for 24 hours before switching to untreated leaves for 24 hours. After three days, each IGR's LC<sub>50</sub> was noted and determined using probit analysis (Finney, 1971) [20].

### Biochemical tests

For each treatment, three grams of fresh larvae were stored in plastic tube in a deep freezer set at  $-17\pm 1^\circ\text{C}$  until using in biochemical tests. Also, the same procedure was occurred in untreated sample. Larval tissue homogenized in phosphate buffer (PH 7) in a cold condition. 50mg of larval tissue mixed with 1ml of distilled water in an eppendorf tube followed by chilled centrifuge at 8000 rpm for 10 minutes at  $5^\circ\text{C}$ . The supernatant release for the following tests

#### 1. Total protein content determination

Using standard bovine serum albumin, the Bradford (1976) [9] method was used to estimate total proteins.

#### 2. Total carbohydrate content determination

Using an anthron reagent, the method outlined by Singh and Sinha (1977) [36] was used to determine the total carbs.

#### 3. Total lipid content determination

The phosphovanillin reagent and standard curve were used to quantify total lipid in accordance with the procedure outlined by Knight *et al.* (1972) [27].

#### 4. Chitinase activity

In accordance with the procedure outlined by Ishaaya and Casida (1974), chitinase was evaluated using 3,5-dinitrosalicylic acid reagent to ascertain the free aldehydic groups of hexosamine released upon chitin digestion.

#### 5. Acetylcholinesterase enzyme activity

Acetylcholine bromide (AChBr) was used as a substrate to evaluate the activity of the acetylcholine esterase enzyme (AChE) in accordance with Simpson *et al.* (1964) [35].

#### 6. Carbohydrate hydrolyzing enzyme activities

According to the procedure outlined by Ishaaya and Swirski (1976) [22], the technique was predicated on the digestion of trehalose, starch, and sucrose by trehalase, amylase, and invertase, respectively.

### Statistical analysis

Abbott's Formula (1925) [1] was used to correct mortality data, and probit analysis was used to determine the LC<sub>50</sub> value, which was then expressed as ppm with fiducial limits (FL) and slope (Finney, 1971) [20]. According to Duncan (1955) [13], significant differences between the treatments were portioned using the LSD test at probability levels of (P=0.05) after the data were subjected to an ANOVA using the "Costat" program (1988).

### Results and discussion

#### Toxicological effects

The data in table 2 demonstrated the effectiveness of the tested IGRs (methoxyfenozide, lufenuron, and pyriproxyfen) against 2<sup>nd</sup> and 4<sup>th</sup> larval instar of *S. frugiperda*. The LC<sub>50</sub> values for the second instar were 0.090, 0.025, and 0.050 ppm, respectively, and the LC<sub>50</sub> values for the fourth instar were 0.150, 0.075, and 0.095 ppm, respectively.

**Table 2:** Toxicity data of tested IGRs against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. frugiperda* after 72 hr. of treatment

Tested compounds	2 <sup>nd</sup> instar larvae		4 <sup>th</sup> instar larvae	
	LC <sub>50</sub> (ppm) its limits at 95%	Slope	LC <sub>50</sub> (ppm) its limits at 95%	Slope
Methoxyfenozide	0.090 (0.05–0.10)	1.2 ± 2.0	0.150 (0.1–0.2)	1.2±2.0
Lufenuron	0.025 (0.01–0.1)	1.0 ± 1.8	0.075 (0.01–0.1)	1.5±2.5
Pyriproxyfen	0.050 (0.01–0.20)	1.3 ± 2.2	0.095 (0.01–0.1)	1.8±2.8

### Biochemical studies

Data in table 3 indicate some biochemical analysis for the 4<sup>th</sup> larval instar as total protein, carbohydrates, and lipids after the treatment by the tested IGRs.

#### 1. Total protein content (μU/mg protein)

Total protein content is probable involvement in growth, development, morphogenesis, and several mediators of the insect metabolic route, protein has long been an intriguing biochemical tool for insect biochemists (Kar *et al.*, 1994) [23].

Data in table 3 showed that lufenuron and pyriproxyfen reduced the protein content of the 4<sup>th</sup> larval instar of *S. frugiperda* except methoxyfenozide. Duncan analysis categorized the tested IGRs into three groups. The following ascending order to lufenuron, pyriproxyfen, and methoxyfenozide in the values 19.61, 21.17, and 22.91 μU/mg protein, respectively.

These findings are consistent with those of Awadalla *et al.* (2017) [5], who observed that the fourth larval instar of *S. littoralis* treated with lufenuron from the IGRs group had lower total protein content. According to El-Gabaly (2015) [15] in Egypt, the total protein content of the fourth larval instar of *S. littoralis* decreased when exposed to lufenuron at their LC<sub>50</sub> values in comparison to the control. The suppression of DNA and RNA production could be the cause of this decrease in protein concentration.

The reduction in the enzymatic activity of several enzymes may be the cause of the decrease in total protein in the treated fourth larval instar. These are the findings that Abd El-Aziz *et al.* (2007) [2] presented. Assar *et al.* (2016) [4] found that treating the fourth larval instar of *S. littoralis* with teflubenzuron and hexaflumuron as insect growth regulators reduced the overall protein content. The primary biochemical elements required for an organism to grow,

develop, and carry out its essential functions are total proteins. The suppression of DNA and RNA synthesis could be the cause of the decrease in protein content (Elbarky *et al.*, 2008) [14].

## 2. Total carbohydrate content (mg/g body weight)

Duncan analysis grouped the tested IGRs into two significant groups. The first group indicate lufenuron and pyriproxyfen, where cause decreasing in total carbohydrates with values 3.31, 3.34 (mg/g.b.wt.). the second group represent methoxyfenozide, that caused increasing value to 5.61(mg/g.b.wt.). note that, the value of the control recorded 4.53(mg/g.b.wt.). Insect metabolism, metamorphosis, flying muscle development, reproduction, and embryonic development are all significantly influenced by carbohydrates (Chapman, 1998) [10]. These findings are consistent with those of Awadalla *et al.* (2017) [5], who noted that following lufenuron treatment, *S. littoralis* carbohydrate content significantly decreased in the fourth larval instar. Abdel-Aal (2012) [3] investigated the biological and insecticidal effects of three insect growth regulators (pyriproxyfen, tebofenozid, and chlorfluazuron) on *S. littoralis* larvae in their fourth instar. Additionally, LC<sub>50</sub> of the used IGRs was recorded for varying degrees of significant changes in the total protein and carbohydrate contents of the female ovaries pre-treated as fourth instar larvae. Additionally, some aberrant ovarian histology structures were identified.

## 3. Total lipid content (mg/gram)

The data in Table (3) showed that treatment with lufenuron, pyriproxyfen, and methoxyfenozide revealed

a significant reduction to the total lipids compared to control. Duncan anylisis showed that the tested IGRs had the same group. In the fourth instar larvae, the total lipid value was 6.61 (mg/g.b.wt.) with control, whereas it was 4.53, 4.65, and 5.79 (mg/g.b.wt.) with lufenuron, pyriproxyfen, and methylfenozide, respectively. According to Assar *et al.* (2016) [4], using teflubenzuron and hexaflumuron as insect growth regulators reduced the total lipid content of *S. littoralis* larvae in their fourth instar. El-Sheikh *et al.* (2013) [19] found that teflubenzuron was an effective insect growth regulator. According to Kiran *et al.* (1998) [26], during the active larval stage, the fat body produces a variety of proteins and releases them into the hemolymph. In order to boost food consumption, Hochachka and Somero (1973) [21] found that total lipids, free fatty acids, and phospholipids were all increased. Lipids and stored carbohydrates are the primary energy sources for the orders Lepidoptera and Orthoptera, according to studies by Ellis *et al.* (2002) [18], Costamagna and Landis (2004) [11]. According to Rawi *et al.* (1995) [31], some hazardous substances have been shown to alter the amount of fat in various body organs in both vertebrates and invertebrates. The most appropriate reserves for energy storage are lipids. Lipids can provide up to eight times as much energy per unit weight as carbs (Beenackers *et al.*, 1985) [8]. They proposed that the longer larval phase of the treated insects and their inability to consume could be the cause of the decreased fat content in the larvae, and that the fat reserves may have been used for maintenance during the longer larval period.

**Table 3:** Impact of the three tested IGRs LC<sub>50</sub> on the total proteins ( $\mu$ U/mg protein), total carbohydrates (mg/g body weight) and total lipids (mg/g body weight) of *S. frugiperda* larvae

Treatments	Total proteins $\pm$ SE	Total carbohydrates $\pm$ SE	Total lipids $\pm$ SE
Control	22.53 $\pm$ 0.76a	4.53 $\pm$ 0.24b	6.61 $\pm$ 2.19a
Lufenuron	19.61 $\pm$ 0.19c	3.31 $\pm$ 0.20 c	4.53 $\pm$ 0.44b
Pyriproxyfen	21.17 $\pm$ 2.18b	3.34 $\pm$ 0.35c	4.65 $\pm$ 1.88b
Methoxyfenozide	22.91 $\pm$ 0.39a	5.610 $\pm$ 0.56a	5.79 $\pm$ 2.526b

Means with the same letter are not significantly different, SE = Standard Error

## 4. Chitinase activity ( $\mu$ g NAGA/min/mg protein)

Data in Table (4) demonstrated that the tested compounds methoxyfenozide caused a significant decrease in the chitinase activity for the larvae in their fourth instar; the value was 10.43 ( $\mu$ g NAGA/min/mg protein). While lufenuron and pyriproxyfen caused an increase in the chitinase activity for the larvae in their fourth instar; the values were 50.99 and 19.67 ( $\mu$ g NAGA/min/mg protein), respectively, in comparison to the control of 15.05 ( $\mu$ g NAGA/min/mg protein). According to Kassem *et al.* (1986) [24], lufenuron inhibited the formation of chitin, which prevented the larvae from effectively moulting into the following stage. According to Locke (1964) [29], chitinase is compartmentalised in the moulting fluid during a typical

moulting process to aid in the digestion of the old cuticle, and the byproducts of this digestion are then used to synthesise the new cuticle.

## 5. Acetylcholinesterase activity ( $\mu$ U/mg protein)

Data in table 1 indicated that lufenuron, which recorded the lowest value of LC<sub>50</sub> was 0.075 ppm, in the same line data in table 4 recorded the highest value of acetylcholinesterase (AChE) 3.420 ( $\mu$ U/mg protein), this may be explain hydrolysis the neurotransmitter acetylcholine (AChE) in the nervous system's cholinergic synapses, AChE plays a crucial function in neurotransmission. It is also the target of a number of neurotoxic insecticides (Salgado *et al.*, 1998 and Rashwan, 2013) [30, 32].

**Table 4:** Impact of the three tested IGRs LC<sub>50</sub> on the activity of chitinase ( $\mu$ g NAGA/min/mg protein) and the activity of acetylcholinesterase ( $\mu$ U/mg protein) of *S. frugiperda* larvae

Treatments	Activity of chitinase $\pm$ SE	Activity of acetylcholinesterase $\pm$ SE
Control	15.05 $\pm$ 4.22b	1,953 $\pm$ 37a
Methoxyfenozide	10.43 $\pm$ 1.90c	2,249 $\pm$ 15b
Pyriproxyfen	19.67 $\pm$ 5.38b	2,451 $\pm$ 22b
Lufenuron	50.99 $\pm$ 9.33a	3,420 $\pm$ 62b

Means with the same letter are not significantly different, SE = Standard Error

### 6. Amylase activity ( $\mu\text{g glucose/min /g body weight}$ )

Data in table (5) indicated quantity analysis of digestive enzymes (amylase, invertase, and trehalase). The following are the descending order arrangement for amylase activity: lufenuron, pyriprofen, and methoxyfenozide values of, 1.149, 626.40, and 439.21 ( $\mu\text{g glucose/g.b.wt}$ ), respectively, when compared to control 861.34 ( $\mu\text{g glucose/g.b.wt}$ ). Diflubenzuron and chlorfluazuron, two insect growth regulators, increased the activity of amylase in the larvae of the pink ballworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), according to Kandil *et al.* (2005).

### 7. Invertase activity ( $\mu\text{g glucose/min /g body weight}$ )

In descending order arrangement for invertase activity: lufenuron, pyriproxifen and methoxyfenozide values of, 5500, 4819.7, and 4706.14 ( $\mu\text{g glucose /g.b.wt}$ ), respectively, in comparison to control 3967 ( $\mu\text{g glucose /g.b.wt}$ ).

### 8. Trehalase activity ( $\mu\text{g glucose/min /g body weight}$ )

Finally, the descending order arrangement for trehalase activity: lufenuron, pyriproxifen and methoxyfenozide values of, 4,577.34, 4,555.89, and 2,614.68 ( $\mu\text{g glucose /g.b.wt}$ ), respectively, in comparison to control 2,672.94 ( $\mu\text{g$

$\text{glucose /g.b.wt}$ ). Only when food is present in the gut does the gut produce and release enzymes (Barnard, 1973) [7]. After digestion is complete, the enzymes are mostly reabsorbed and sent to the pancreas, where they are released once more at the subsequent meal (Liebow and Rothman, 1975) [28]. It was discovered that the most significant enzyme involved in the digestion and metabolism of carbohydrates in insects is trehalase (Wigglesworth, 1972 and Wyatt, 1967) [39, 41]. According to Terra and Ferreira (1981) [37], trehalase activity drops during hunger and increases after feeding, indicating that it is dependent on food rather than haemolymph (Trehalase tires). According to Shukla *et al.* (2015) [33], trehalose is the primary blood sugar in insects and serves as a rapid source of energy and resistance to abiotic stress. Trehalose hydrolysis is controlled by Trehalase, an enzyme. Trehalase, an enzyme that catalyses trehalose, plays an important role in energy metabolism and glucose production in insects. Insect Trehalase (Tre-1 and Tre-2) plays crucial roles in energy supply, growth, metamorphosis, stress recovery, chitin synthesis, and flying.

From the previous, we can conclude that lufenuron recorded the highest value in amylase, invertase, and trehalase. On the other hand, methoxyfenozide recorded the lowest value in both amylases, and invertase.

**Table 5:** Impact of the three tested IGRs LC<sub>50</sub> on Amylase activity ( $\mu\text{g glucose/min /g body weight}$ ), Invertase activity ( $\mu\text{g glucose/min /g body weight}$ ) and Trehalase activity ( $\mu\text{g glucose/min /g body weight}$ ) of *S. frugiperda* larvae

Treatments	Amylase activity $\pm$ SE	Invertase $\pm$ SE	Trehalase activity $\pm$ SE
Control	861.34 $\pm$ 206.91b	3967 $\pm$ 150 a	2,672.94 $\pm$ 547.41c
Methoxyfenozide	439.21 $\pm$ 49.96 d	4706.14 $\pm$ 510c	2,614.68 $\pm$ 341.06d
Pyriproxifen	626.39 $\pm$ 59.38 c	4819.7 $\pm$ 419b	4,555.89 $\pm$ 1,030.0 b
Lufenuron	1,149.64 $\pm$ 43.10 a	5500 $\pm$ 306 d	4,577.34 $\pm$ 475.41a

Means with the same letter are not significantly different, SE = Standard Error

### Conclusion

This study refers to the significant biochemical effects of the insect growth regulators methoxyfenozide, pyriproxifen, and lufenuron on *Spodoptera frugiperda* larvae. Lufenuron exhibited the strongest impact, notably enhancing chitinase and AChE activities while reducing protein, carbohydrate, and lipid levels. Conversely, methoxyfenozide inhibited certain digestive enzymes. These findings support the use of IGRs as safer alternatives to conventional pesticides in pest management.

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