



IMPACT OF SOME CHITIN SYNTHESIS INHIBITORS ON THE FALL ARMYWORM, *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

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Maize, *Zea mays* L. is an economic cereal in Egypt. Numerous pests infested maize, but only a few of them caused high crop losses, such as FAW, *Spodoptera frugiperda*. Therefore, this study is urgent to determine: the effect of chitin synthesis inhibitors on FAW larvae, and evaluate LC<sub>90</sub> efficiency of CSIs on maize under the field. In this study, the mortality percentage increased with increasing exposure time to CSIs. Moreover, LC<sub>50</sub> for hexaflumuron was low (0.487 mg/L), followed by flufenoxuron (0.5712 mg/L) on the 2<sup>nd</sup> larva. On the 4<sup>th</sup> larva, hexaflumuron and diflubenzuron were more lethal than other CSIs. LC<sub>90</sub> of all CSIs ranged between 1.3815 5.0569; and 1.024514.6923 mg/L on the 2<sup>nd</sup> and 4<sup>th</sup> larvae, respectively. Results were elucidated that the toxicity index was low by novaluron and lufenuron treatments with high resistance ratios, respectively. However, it had a high value with diflubenzuron, followed by novaluron, with a few resistance ratios when compared with hexaflumuron, respectively. In field experiments, data showed that flufenoxuron, hexaflumuron and diflubenzuron could be used as recommended insecticides to FAW control, however, chlorfluazuron, lufenuron and novaluron were the lowest ones on maize. On the response of proteins, carbohydrates and lipids to CSIs, diflubenzuron, hexaflumuron and flufenoxuron caused the lowest significant reduction in protein. Wherever, the highest significant protein value was recorded with chlorfluazuron and lufenuron. The values of chitinase, phenoloxidase and carboxylesterase indicated a clear disturbance in physiological aspects for larvae. Now, CSIs are used for developing eco-friendly FAW management.

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INTRODUCTION

Maize, *Zea mays* L. (Family: Poaceae) is a main economic commodity of cereal crops in Egypt, and it could have a higher source of carbohydrates than other cereal crops. It has a third rank of cereals after wheat and rice (Purwanto *et al.*, 2015; Ali and Abdelaal, 2020). In Egypt, maize is mainly used as human food, livestock and poultry feed it as a raw material in numerous industrial products (Ali and Abdelaal, 2020).

Recently, the presence of insect pests was still opposed to the increase in maize production in Egypt. Numerous pests attack maize plants, but only a few of them cause highly economic crop losses, such as the fall armyworm, *Spodoptera*

*frugiperda* (Lepidoptera: Noctuidae) (Dahi *et al.*, 2020). In Africa, *S. frugiperda* was first found in both west and central Africa in 2016 (Goergen *et al.*, 2016) and then rapidly spread to North Africa in Egypt, where, FAW was first recorded on maize in 2019 in Upper Egypt (Dahi *et al.*, 2020), then rapidly dispersed to different countries in Egypt (Mohamed *et al.*, 2022). FAW caterpillars caused serious damage to more than 350 plant species, including many economic crops such as maize, rice, wheat, alfalfa, soybean, cotton, sugar beet, onion, potato and tomato (Montezano *et al.*, 2018; CABI, 2018). The fall armyworm problem in Africa and Egypt is exacerbated because of the widespread cultivation of maize, which is considered the preferred plant as a staple food in the region and is mainly the host plant of *S. frugiperda* (Harrison *et al.*, 2019; Ali and Abdelaal, 2020; Salem *et al.*, 2021). Besides, FAW is mainly an insect pest to maize (Sisay *et al.*, 2019), and reduces about 40% of maize yield in a monocropped system (Chabi-Olaye *et al.*, 2005). Recently on maize, the biological control by using plant extracts against the pink borer, *Sesamia cretica* was implemented by Ismail *et al.* (2023) and utilized silver nanoparticles from entomopathogenic fungi against the spiny bollworm, *Earias insulana* was conducted by Abdel-Raheem *et al.* (2023) on maize crop.

One of the management strategies for this invasive pest is the use of chitin synthesis inhibitor groups such as benzoylurea, including commonly named lufenuron, novaluron, diflubenzuron, chlorfluazuron, flufenoxuron, hexaflumuron, triflumuron, noviflumuron, bistrifluron and teflubenzuron as a persistent urea derivative insecticide against arthropods, including lepidopteran species (Liu *et al.*, 2019; IRAC, 2023). Diflubenzuron had activity as ovicide and larvicide, as reported by Merzendorfer (2013). This inhibited chitin biosynthesis, leading to defects in hatching and anti-molting. Many issues have resulted from the fact that diflubenzuron inhibits the incorporation of acetylglucosamine into insect chitin (Matsumura, 2010). Additionally, lufenuron is an effective insect growth regulator (IGR) against *S. frugiperda* (Lv *et al.*, 2023). They stated that lufenuron has highly insecticidal activity against *S. frugiperda* with an  $LC_{50}$  value of  $0.99 \text{ mg l}^{-1}$ . Shareef *et al.* (2022) found that novaluron acts as an IGR that causes abnormal endocuticle and abortive moulting in *S. frugiperda* larvae. Despite the larvicidal activity of novaluron, it had an ovicidal effect on *S. frugiperda* eggs. Moreover, the lethal concentration of hexaflumuron was determined against *Helicoverpa armigera* to reduce its infestation (Vojoudi *et al.*, 2017). The nine different concentrations of chitin synthesis inhibitors (CSIs) and noval chlorfluazuron contributed to the high toxicity in lepidopteran cells against the 5<sup>th</sup> instar of *S. frugiperda* in the laboratory (Huang *et al.*, 2015). Laboratory toxicity and field persistence studies of CSIs such as lufenuron and flufenoxuron were assessed on *S. littoralis* larvae (El-Sheikh and Aamir, 2011).

Uses of different chitin synthesis inhibitors in IPM, especially lepidopteran species, like diflubenzuron on *H. armigera*, chlorfluazuron on *S. litura* and *S. littoralis*, flufenoxuron on *S. litura*, hexaflumuron and lufenuron on *H. armigera*. Interestingly, the benzoylurea group has a unique biochemistry and mode of action when compared to other insect growth regulator pesticides in that they act as inhibitors of chitin biosynthesis, affecting CHS1 (Sun *et al.*, 2015). Abo-Elghar *et al.* (2004) hypothesized that the benzoylurea group may bind to membrane-bound sulfonylurea receptors (SURs). While, Choudhary *et al.* (2022) demonstrated that

benzoylphenyl urea products inhibited a post-catalytic step of chitin synthesis in an insect. Furthermore, IGRs are eco-friendly pesticides with high efficiency on lepidopteran species but low toxicity on mammals, birds, fishes and beneficial enemies and low environmental pollution (Lv *et al.*, 2023). In addition, chitinases, a type of chitinolytic enzyme, play a crucial role in breaking down chitin into chitooligosaccharides. It was responsible for breaking down chitin found in insects that do not have a natural chitin layer. It was shown to be completely lethal for lepidopteran species (Adrangi and Faramarzi, 2013). Different detoxication enzymes play an important role in studying insecticide resistance in *S. frugiperda* larvae (Yu, 1991). Furthermore, chitin synthesis inhibitors, including lufenuron, chlorfluazuron and hexaflumuron, showed a significant reduction in total protein with increased activity of GST,  $\beta$ -esterase, carboxylesterase, phenoloxidase, chitinase, and  $\alpha$ -esterase, which may be used as an indicator of insecticide resistance in the red palm weevil, *Rhynchophorus ferrugineus* (El-Sobki and Ali, 2020). The determination of lethal effects for chitin synthesis inhibitors may be important in maize management programs to suppress and defect the development of FAW. There is little information on the current status of these IGRs on FAW. Therefore, it is urgent to determine: (1) the lethal effects of chitin synthesis inhibitors (CSIs) on *S. frugiperda* larvae under laboratory conditions. (2) its impact of CSIs on some biochemical analyses such as total protein, total lipids, total carbohydrates and enzyme activities (3) Efficiency of the LC<sub>90</sub> dose of CSIs on *S. frugiperda* infestation under field conditions for understanding the current susceptibility status of FAW to various CSIs insecticides. This study will be designed to provide basic data to future experts for adopting the best management approach against this pest in Egypt.

## **MATERIALS AND METHODS**

### **Toxicity of chitin synthesis inhibitors on *S. frugiperda* larvae under laboratory conditions**

#### **Insect culture**

A laboratory colony of *S. frugiperda* was continuously reared in a plant protection research institute. Larvae fed on fresh castor leaves (*Ricinus communis* L.) till the pupation stage. Larvae were reared in rectangle-shaped plastic box (20 cm length X 10 cm width X 7 cm height) with covered by agrilic clothes, and contained a thin layer of fine saw-dust put on the bottom for moisture absorbers. Larvae were kept in an incubator at  $25\pm 2^{\circ}\text{C}$  and  $70\pm 5\%$  RH till pupation. Pupae were kept in glass jars (20 cm length X 8 cm diameter) contained a layer of fine saw-dust for moisture absorbers until the adult's emergence. The adults were fed on 50% honey bee solution in soaked a piece of cotton tissue. Each glass jar was provided with a paper bent in zigzag shape like an oviposition sheet. Tops of glass jar were covered with agrilic clothes and secured with rubber bands, each glass jar was examined at one day intervals to replace the oviposition sheet with new ones and renew the feeding solution of moths. The adult glass-jar was maintained at the same conditions of temperature and RH%. The deposited egg- masses were kept in the same previous plastic box until the hatching. The insect culture of FAW was reared for at least six generations to become a sensitive strain in an incubator at  $25\pm 2^{\circ}\text{C}$  and  $70\pm 5\%$  RH.

### Toxicity of selected chitin synthesis inhibitor (CSIs) compounds

The dipping castor-leaf technique method was used as a method of application against the 2<sup>nd</sup> and 4<sup>th</sup> larval of *S. frugiperda*. Plastic boxes of 9 cm length X 8 cm width X 4 cm height were used for the tested of six concentrations (0.2, 0.3, 0.4, 0.5, 0.75 & 1 ml/L) for the selected chitin synthesis inhibitors Table (1). All larvae treatments were individually applied in each previously plastic box. Three replicates for each concentration were used. Each replicate contained 10 larvae. Fresh castor leaves were dipped in each concentration of each tested compound for 30 second. The control (untreated application) was only treated by the distilled water as check treatment. Both numbers of alive and dead of the 2<sup>nd</sup> and 4<sup>th</sup> larvae of *S. frugiperda* were counted and recorded after 3 and 5 days after treatment. Then alive larvae in each treatment were transferred singly to previous plastic box contained fresh castor leaves as diet and maintained under laboratory conditions (25±2°C and 70±5% RH). The corrected mortality % was calculated by Abbott's formula (1925). Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>), toxicity index and resistance ratio values were detected by the using of Ldp-line software computer program (Bakr, 2007) to calculate probit analyses according to Finney (1971) to illustrate toxicological studies and dose-response regression line. The toxicity index of selected compounds was measured by using Sun's equation (1950) as:

$$\text{Toxicity index} = \frac{\text{LC}_{50} \text{ /or LC}_{90} \text{ of the "A" compound}}{\text{LC}_{50} \text{ /or LC}_{90} \text{ of the "B" compound}} \times 100$$

Where "A" is the most effective compound and "B" is the other compared selected compound.

### Biochemical studies on the fall armyworm, *S. frugiperda* larvae

To comparison between the efficiency of the tested chitin synthesis inhibitors, the biochemical analysis of *S. frugiperda* larvae were determined at Insect physiology department, Plant protection research institute, Agricultural research center. For preparation of insect samples for biochemical analysis, the 2<sup>nd</sup> and 4<sup>th</sup> larval samples after treatments were collected and prepared as described by Abd El-Kareem *et al.* (1998). Total proteins were determined by the method of Bradford (1976). Total lipids were estimated by the method of Knight *et al.* (1972). Total carbohydrates were extracted and prepared for estimating by the phenol-sulphuric acid reaction according to Dubois *et al.* (1956) and Crompton and Birt (1967). For enzyme activity assays, carboxylesterase level was measured according to Simpson *et al.* (1964). Also, phenoloxidase level was determined according to the method illustrated by Ishaaya (1971). In addition, the colloidal chitin was prepared as described by Bade and Stinson (1981), then the chitinase activity was estimated according to the methods of Ishaaya and Casida (1974) and Waterhouse *et al.* (1961).

### Efficiency of LC<sub>90</sub> dose of CSIs on *S. frugiperda* infestation under field conditions

A field experiment was conducted in a private maize (Bioneer 444 variety) farm in Kafer-Singerg village, Menoufia governorate, Egypt, to determine the efficacy of LC<sub>90</sub> dose of the selected CSIs under field conditions for controlling of *S. frugiperda* infestations at 12<sup>th</sup> July 2023 during season 2023 Table (1). The experiment area was divided into 21 plots, each plot size was 100 m<sup>2</sup>. Each treatment

was replicated three times. Plots were separated from approximately 1 m of bare ground for reducing spray drift. The foliar applications of the tested insecticides were implemented in completely randomized block design using the calibrated Hand-Held compression sprayer “Kwazar” before the spraying, while the control (untreated application) was only treated by the distilled water as check treatment. Randomly samples of 10 maize plants per replicate were directly inspected in the field at 3, 5, 7 and 10 days after treatment to count and record *S. frugiperda* larvae and their infestation percentage. In addition, both pre-counts and infestation % were estimated for all replicates. The reduction percentage of the density of *S. frugiperda* larvae and the infestation % were calculated according to Henderson and Tilton (1955).

Table (1): list of the selected chitin synthesis inhibitors against *S. frugiperda* larvae.

No.	Trade name	Conc. & Form.	Active ingredient	The used dose/liter in field	Local Company
1	Daflox	48% SC	Diflubenzuron	2.2 ml	Kanza Group
2	Topron-S	5% EC	Chlorfluazuron	2.4 ml	Agrochem
3	Klifron	5% EC	Lufenuron	2.2 ml	Agrochem
4	Novo	10% DC	Flufenoxuron	5.1 ml	Soltaire Development and Investment
5	Demeron-S	10% EC	Hexaflumuron	1.4 ml	Agrochem
6	Akio	10% EC	Novaluron	2.2 ml	Lotus Agricultural Development

Conc. & Form. = Concentration & Formulation, DC= Dispersible Concentrate, SC= Suspension concentrates, EC= Emulsifiable Concentrate

### Statistical analysis

The obtained data were statistically analyzed by using SAS computer software program including f-test (SAS, 2004). Comparison between the obtained means by using the calculated least significant differences (LSD) at 0.05 level of probability. Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>), slope, and chi-square ( $\chi^2$ ) were calculated by the using of Ldp-line software computer program (Bakr, 2007) to calculate probit analysis according to Finney (1971).

## RESULTS AND DISCUSSION

### Toxicity of chitin synthesis inhibitors on *S. frugiperda* larvae under laboratory conditions

A laboratory test was performed to evaluate the impact of six tested chitin synthesis inhibitors against *S. frugiperda* larvae with different concentrations Table (2 and 3). In the case of 4<sup>th</sup> larval instar of *S. frugiperda*, the obtained data was showed that the mortality % increased with the concentration of all tested CSIs. After 3 and 5 days after treatment (DAT), the first concentration (0.2 ml/l) was not significantly effect on all CSIs treatments. In which, mortality % in 4<sup>th</sup> instar was not exceed up 10.0 and 13.33 % at 3 and 5 DAT, respectively Table (2). Also, insignificantly effects were reported at 3 and 5 DAT with the concentration of 0.3 ml/l in all treatments. With 0.4 ml/l concentration, no significant effect was also found between the all investigated CSIs with mortality % ranging from 16.7- 26.7 %, but a significant mortality % was stated at 5 DAT with 0.4ml/l concentration between all CSIs treatments Table (2). The highest mortality % was 56.67% with diflubenzuron,

followed by hexaflumuron application (50 % mortality). At 0.5ml/l concentration after 3 DAT, the highest mortality % of 4<sup>th</sup> instar of *S. frugiperda* larvae was 86.7 and 66.7 % with hexaflumuron and diflubenzuron, but the lowest value of mortality percentage was stated with chlorfluazuron and lufenuron in 4<sup>th</sup> of FAW larval populations (26.7 and 33.3%), respectively. Moreover, at 0.5ml/l concentration after 5DAT, the increase in the mortality % was observed up to reach to maximum with diflubenzuron (100 %), followed with each of hexaflumuron (93.33%) and flufenoxuron (86.67 %). However, chlorfluazuron, lufenuron and novaluron caused low effect on in 4<sup>th</sup> of FAW larval populations ranging from 53.33-66.67 % mortality Table (2). The present data in Table (2) indicated that the mortality % was more increased at 5 DAT than 3 DAT in both of 0.75 and 1.0 ml/l in 4<sup>th</sup> of FAW larval populations. With 0.75 ml/l, the treatment of diflubenzuron (80.0 & 100% mortality) and hexaflumuron (73.3 & 100% mortality) had the highest significant effects on 4<sup>th</sup> of FAW larvae at 3 and 5DAT, respectively, but flufenoxuron had a high significant effect only at 5 DAT (93.33% mortality). With 1.0 ml/l, the mortality in 4<sup>th</sup> of FAW larvae was extended from 26.7-80.0 % and 46.67-100 % at 3 and 5 DAT, respectively Table (2).

Data in Table (3) illustrated that the CSIs effective level was more a slight higher on 2<sup>nd</sup> instar than 4<sup>th</sup> instar of FAW larvae with all concentrations of all treatments at the two tested dates. At 3 DAT, the mortality percentage ranged from 3.33-26.7%, 10.0-36.7%, 16.7-46.7%, 36.7-50.0%, 33.3-70.0% and 60.0-86.7% with 0.2, 0.3, 0.4, 0.5, 0.75 and 1.0 ml/l for all CSIs treatments on 2<sup>nd</sup> instar of FAW larvae Table (3). There were significant differences between treatments except with each of 0.4, 0.5 and 1.0 ml/l showing non-significant effect. At 5 DAT, the mortality effect had been the same trend as at 3 DAT. The lethal effect ranged from 6.67-36.67%, 23.33-56.67%, 33.33-60.0%, 63.33-86.67%, 50.0-86.67% and 66.67-90.0% with 0.2, 0.3, 0.4, 0.5, 0.75 and 1.0 ml/l for all CSIs treatments on 2<sup>nd</sup> instar of FAW larvae Table (3). There were significant differences between treatments except with 0.4 and 1.0 ml/l recording non-significant effect on the 2<sup>nd</sup> instar of *S. frugiperda* larvae. The obtained results showed that the increasing mortality percentage of FAW larvae occurred along with the increasing concentration of the applied CSIs.

Table (2): Mortality of FAW larvae when treated in 4<sup>th</sup> instar by some CSIs under laboratory conditions

Treatments	Corrected Mortality % Mean $\pm$ SE) at DAT											
	0.2 cm/l		0.3 cm/l		0.4 cm/l		0.5 cm/l		0.75 cm/l		1.0 cm/l	
	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT
Diflubenzuron 48% SC	3.33a $\pm 3.3$	6.67a $\pm 3.3$	6.67a $\pm 3.3$	13.33a $\pm 3.3$	26.7a $\pm 8.8$	56.67a $\pm 8.8$	66.7ab $\pm 6.7$	100a $\pm 0$	80.0a $\pm 5.8$	100 a $\pm 0$	80.0a $\pm 5.8$	86.67ab $\pm 3.3$
Chlorfluazuron 5% EC	3.33a $\pm 3.3$	6.67a $\pm 3.3$	10.0a $\pm 5.8$	23.33a $\pm 3.3$	23.3a $\pm 3.3$	36.67ab $\pm 3.3$	26.7c $\pm 3.3$	60.0b $\pm 0$	26.7b $\pm 3.3$	80.0bc $\pm 5.8$	33.3c $\pm 8.8$	80.0b $\pm 5.8$
Lufenuron 5% EC	3.33a $\pm 3.3$	6.67a $\pm 3.3$	13.3a $\pm 3.3$	20.0a $\pm 5.8$	26.7a $\pm 3.3$	46.67ab $\pm 8.8$	33.3c $\pm 6.7$	53.33b $\pm 3.3$	33.3b $\pm 6.7$	60.0d $\pm 5.8$	26.7c $\pm 8.8$	46.67c $\pm 3.3$
Flufenoxuron 10% DC	10.00a $\pm 0$	13.33a $\pm 3.3$	10.0a $\pm 5.8$	13.33a $\pm 6.7$	16.7a $\pm 3.3$	40.0ab $\pm 5.8$	40.0c $\pm 11.5$	86.67a $\pm 3.3$	60.0a $\pm 5.8$	93.33ab $\pm 3.3$	60.0b $\pm 5.8$	86.67ab $\pm 3.3$
Hexaflumuron 10% EC	6.67a $\pm 3.3$	13.33a $\pm 3.3$	16.7a $\pm 3.3$	26.67a $\pm 3.3$	23.3a $\pm 8.8$	50.0a $\pm 5.8$	86.7a $\pm 6.7$	93.33a $\pm 3.3$	73.3a $\pm 3.3$	100 a $\pm 0$	73.3ab $\pm 3.3$	100a $\pm 0$
Novaluron 10% EC	3.33a $\pm 3.3$	6.67a $\pm 3.3$	13.3a $\pm 3.3$	16.67a $\pm 3.3$	23.3a $\pm 8.8$	26.67b $\pm 6.7$	46.7bc $\pm 12$	66.67b $\pm 12.0$	60.0a $\pm 11.5$	73.33cd $\pm 8.8$	73.3ab $\pm 3.3$	86.67ab $\pm 3.3$
F value	0.84	0.71	0.66	1.45	0.30	2.44	7.26	12.45	10.27	10.06	12.51	25.26
LSD	9.38	12.579	55.56	13.91	20.54	20.97	25.85	16.77	20.54	15.69	19.67	11.09
Prob.> F	0.55	0.63	0.66	0.27	0.90	0.05	0.002	0.0002	0.001	0.001	0.0002	0.0001

FAW = Fall Army worm DAT =Day after Treatment LSD =Least Significant Difference SE =Standard Error Prob. =Probability

Means signed by the same letter in the same column is not significant difference between them.

Table (3): Mortality of FAW larvae when treated in 2<sup>nd</sup> instar by CSIs under laboratory conditions.

Treatments	Corrected Mortality % Mean $\pm$ SE) at DAT											
	0.2 cm/l		0.3 cm/l		0.4 cm/l		0.5 cm/l		0.75 cm/l		1.0 cm/l	
	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT	3DAT	5DAT
Diflubenzuron 48% SC	13.3ab $\pm 3.3$	20.0ab $\pm 5.8$	26.7ab $\pm 8.8$	43.33ab $\pm 3.3$	40.0a $\pm 5.8$	60.0a $\pm 10$	40.0a $\pm 10$	63.33b $\pm 6.7$	43.3ab $\pm 3.3$	83.33a $\pm 3.3$	80.0a $\pm 5.8$	86.67a $\pm 3.3$
Chlorfluazuron 5% EC	6.67b $\pm 3.3$	16.67ab $\pm 6.7$	13.3b $\pm 3.3$	30.0bc $\pm 5.8$	23.3a $\pm 3.3$	36.67a $\pm 8.8$	36.7a $\pm 6.7$	86.67a $\pm 3.3$	43.3ab $\pm 3.3$	86.67a $\pm 3.3$	63.3a $\pm 3.3$	86.67a $\pm 3.3$
Lufenuron 5% EC	6.67b $\pm 6.7$	20.0ab $\pm 10$	20.0ab $\pm 5.8$	30.0bc $\pm 5.8$	23.3a $\pm 6.7$	33.33a $\pm 12.0$	46.7a $\pm 16.7$	70.0ab $\pm 10$	53.3ab $\pm 16.7$	83.33a $\pm 3.3$	63.3a $\pm 6.7$	83.33a $\pm 6.7$
Flufenoxuron 10% DC	26.7a $\pm 3.3$	36.67a $\pm 8.8$	36.7a $\pm 3.3$	56.67a $\pm 3.3$	36.7a $\pm 8.8$	56.67a $\pm 3.3$	50.0a $\pm 10$	80.0ab $\pm 0$	60.0ab $\pm 5.8$	73.33ab $\pm 12.0$	60.0a $\pm 20.8$	66.67a $\pm 18.6$
Hexaflumuron 10% EC	16.7ab $\pm 8.8$	36.67a $\pm 3.3$	26.7ab $\pm 8.8$	56.67a $\pm 8.8$	46.7a $\pm 18.6$	60.0a $\pm 11.6$	36.7a $\pm 16.7$	70.0ab $\pm 5.8$	70.0a $\pm 15.3$	73.33ab $\pm 12.0$	86.7a $\pm 8.8$	90.0a $\pm 5.8$
Novaluron 10% EC	3.33b $\pm 3.3$	6.67b $\pm 3.3$	10.0b $\pm 5.8$	23.33c $\pm 3.3$	16.7a $\pm 8.8$	43.33a $\pm 8.8$	40.0a $\pm 10$	83.33a $\pm 3.3$	33.3b $\pm 8.8$	50.0b $\pm 5.8$	66.7a $\pm 12$	90.0a $\pm 5.8$
F value	2.67	3.02	2.36	7.05	1.39	1.60	0.20	2.49	1.63	3.06	0.93	0.97
LSD	16.24	20.97	19.67	16.77	30.53	29.35	37.74	17.79	31.934	23.72	34.32	27.496
Prob.> F	0.05	0.05	0.03	0.003	0.296	0.233	0.956	0.05	0.05	0.05	0.495	0.475

FAW = Fall Armyworm DAT =Day after Treatment LSD =Least Significant Difference SE =Standard Error Prob. =Probability

Means signed by the same letter in the same column is not significant difference between them.

Analysis by probit regression line was tabulated in Table (4 & 5) and illustrated in Figures (1 &2) determined the lethal effect of diflubenzuron,

chlorfluazuron, lufenuron, flufenoxuron, hexaflumuron and novaluron on the exposed 2<sup>nd</sup> and 4<sup>th</sup> larval instar of FAW. The lethal concentration of LC<sub>50</sub> and LC<sub>90</sub> of the six tested CSIs calculated based on the mortality ratios was recorded in Table (4) on the exposed 2<sup>nd</sup> larval instar of FAW. The calculated LC<sub>50</sub> for hexaflumuron was low concentration (0.487 mg/l with slope value of 2.8306±0.463), followed by flufenoxuron (0.5712 mg/L with slope value of 1.3532±0.414) on 2<sup>nd</sup> larval instar. Both novaluron and chlorfluazuron treatments resulted a high value of LC<sub>50</sub> on the 2<sup>nd</sup> larval instar of FAW (0.7958 & 0.77 mg/l with slope values of 2.9239±0.516 & 2.5563±0.482), respectively, Table (4). The lethal concentration of LC<sub>90</sub> of the six tested CSIs ranged between 1.3815 and 5.0569 mg/l on the 2<sup>nd</sup> larval instar of *S. frugiperda* Table (4). The obtained results were conducted that the calculated toxicity index was low value by novaluron and lufenuron treatments (61.181 & 63.247, respectively) with a high resistance ratio (1.634 & 1.581, respectively) when compared with hexaflumuron treatment Table (4). As shown in the 2<sup>nd</sup> instar of *S. frugiperda* larvae, data in Table (5) showed that the calculated LC<sub>50</sub> for hexaflumuron and diflubenzuron were low value (0.4972 & 0.5216 mg/l with slope value of 3.4051±0.4950 & 4.3711±0.5694, respectively) on 4<sup>th</sup> instar of *S. frugiperda* larvae. However, the highest value of LC<sub>50</sub> was found with the exposed 4<sup>th</sup> larval instar with chlorfluazuron and lufenuron pesticides (1.5802 & 1.7071 mg/l with slope value of 1.6389 ±0.4864 & 1.371±0.4658, respectively). Accordingly, both of hexaflumuron and diflubenzuron were more lethal effect than other CSIs pesticides in case of the 4<sup>th</sup> instar of *S. frugiperda* larvae.

Table (4): Toxicity of some CSIs against 2<sup>nd</sup> larval instar of FAW at 3 days after treatments.

Treatments	Lethal effects						
	LC <sub>50</sub>	LC <sub>90</sub>	Slope	Slope+/-	$\chi^2$	toxicity Index***	RR***
Diflubenzuron 48% SC	0.6013* (0.49-0.79)**	2.2226 (1.40-6.01)	2.2574	0.445	5.122	81.032	1.234
Chlorfluazuron 5% EC	0.77 (0.63-1.06)	2.4427 (1.56-6.24)	2.5563	0.482	0.818	71.723	1.394
Lufenuron 5% EC	0.6793 (0.56-0.90)	2.1731 (1.43-5.13)	2.5378	0.467	1.968	63.247	1.581
Flufenoxuron 10% DC	0.5712 (0.41-1.00)	5.0569 (1.99-188.38)	1.3532	0.414	0.728	85.289	1.172
Hexaflumuron 10% EC	0.487 (0.41-0.58)	1.3815 (1.02-2.39)	2.8306	0.463	3.887	100	1
Novaluron 10% EC	0.7958 (0.66-1.06)	2.1834 (1.48-4.70)	2.9239	0.516	5.015	61.181	1.634

LC = Lethal Concentration \*Concentration (ml/l) \*\*Confidence Limits 95% (Lower-Upper limits) \*\*\*Toxicity Index and Resistance Ratio (RR) compared with Hexaflumuron treatment

The lethal value of LC<sub>90</sub> of CSIs was extended between 1.0245 and 14.6923 mg/l on 4<sup>th</sup> instar of *S. frugiperda* larvae. The toxicity index was high value with diflubenzuron followed by novaluron treatments (95.211 & 80.161, respectively) with a few resistance ratios (1.05 & 1.247, respectively) when compared with hexaflumuron Table (5).



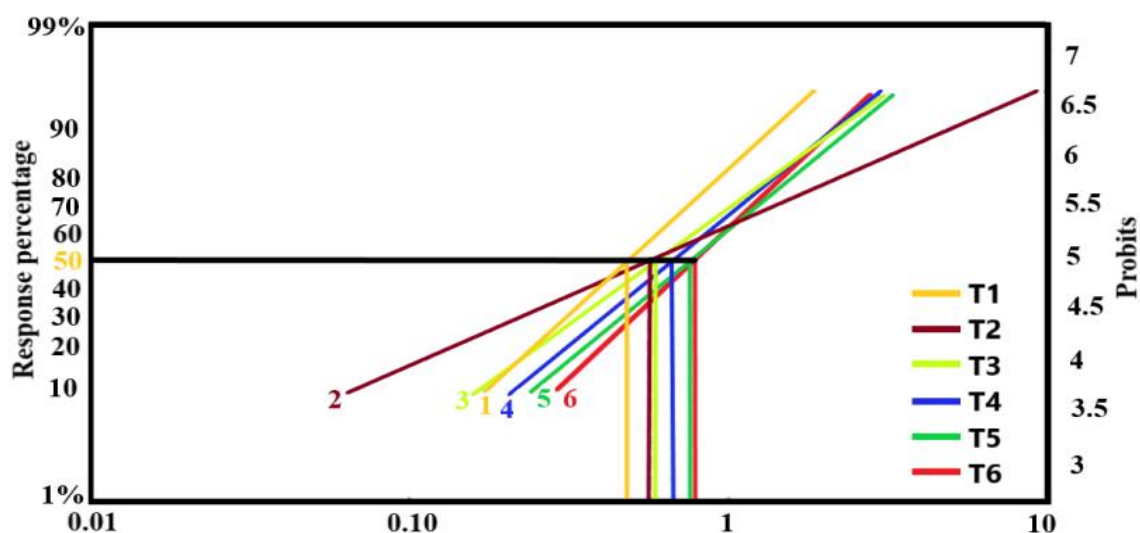


Figure (1): Toxicity of chitin synthesis inhibitors against 2<sup>nd</sup> instar of FAW larvae: T1= Diflubenzuron, T2= Chlorfluazuron, T3= Lufenuron, T4= Flufenoxuron, T5= Hexaflumuron and T6= Novaluron.

Table (5): Toxicity of different chitin synthesis inhibitors against 4<sup>th</sup> larval instar of FAW at 3 days after treatments.

Treatments	Lethal effects						
	LC <sub>50</sub>	LC <sub>90</sub>	Slope	Slope+/-	$\chi^2$	Toxicity Index***	RR***
Diflubenzuron 48% SC	0.5216* (0.46-0.59)**	1.0245 (0.8545-1.36)	4.3711	0.5694	9.4986	95.211	1.05
Chlorfluazuron 5% EC	1.5802 (0.98-7.96)	9.5657 (3.19-566.39)	1.6389	0.4864	2.8507	31.456	3.179
Lufenuron 5% EC	1.7071 (-----)	14.6923 (-----)	1.371	0.4658	5.8963	29.115	3.435
Flufenoxuron 10% DC	0.718 (0.60-0.94)	2.1157 (1.43-4.63)	2.7308	0.4854	3.9039	69.22	1.445
Hexaflumuron 10% EC	0.4972 (-----)	1.1829 (-----)	3.4051	0.4950	22.3711	100	1
Novaluron 10% EC	0.6197 (0.54-0.74)	1.4671 (1.12-2.34)	3.4243	0.5151	1.4501	80.161	1.247

LC = Lethal Concentration \*Concentration (ml/L) \*\*Confidence Limits 95% (Lower-Upper limits) \*\*\*Toxicity Index and Resistance Ratio (RR) compared with Hexaflumuron treatment

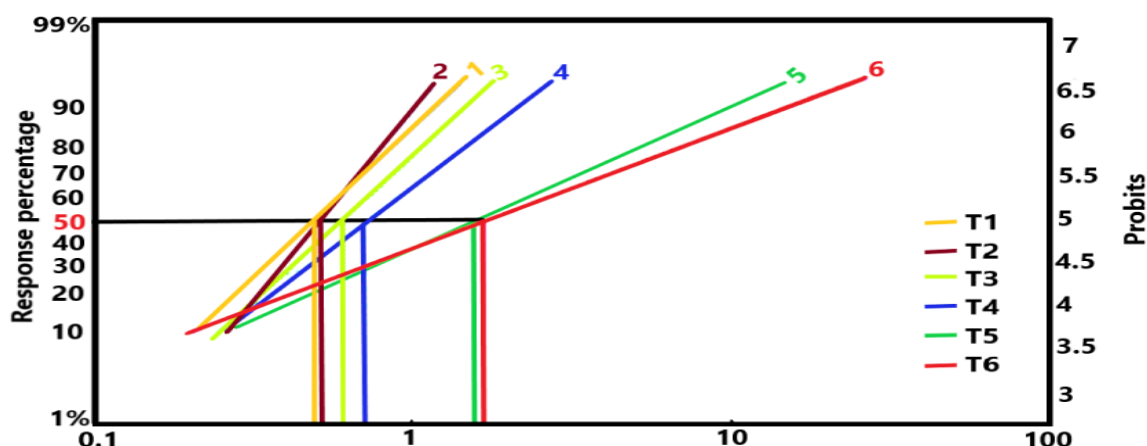


Figure (2): Toxicity of chitin synthesis inhibitors against 4<sup>th</sup> instar of FAW larvae: T1= Diflubenzuron, T2= Chlorfluazuron, T3= Lufenuron, T4= Flufenoxuron, T5= Hexaflumuron and T6= Novaluron.

### Efficacy of six different chitin synthesis inhibitors against FAW larvae under field conditions.

To ascertain the efficiency of different chitin synthesis inhibitors CSIs in suppressing of *S. frugiperda* larvae population infesting maize plants, six CSIs namely: diflubenzuron, chlorfluazuron, lufenuron, flufenoxuron, hexaflumuron and novaluron were used in the present work with the used LC<sub>90</sub> dose being 2.2, 2.4, 2.2, 5.1, 1.4 and 2.2 ml/l, respectively at 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 10<sup>th</sup> day after treatment (DAT) Table (6). After 3 DAT, flufenoxuron and chlorfluazuron were recorded the highest reduction value of 96.52 and 87.36 % against *S. frugiperda* larvae on maize plants, respectively. A moderately effect was elicited decrease in FAW larvae by diflubenzuron and hexaflumuron with 84.64% and 82.17% at 3 DAT, respectively. At the time that a low effect was stated by lufenuron and novaluron (74.23 and 78.12 reduction %, respectively). Significant differences were found between all treatments with LSD is 12.744 at Prob.>|F| equal 0.03 Table (6). On the other direction, lufenuron excited decrease in FAW larvae populations on maize plants with the highest reduction value of 90.59 %, and followed by hexaflumuron (88.18 %) and diflubenzuron (86.86 %) against *S. frugiperda* larvae on maize plants at 5 DAT. at the time that there were non-significant difference was reported between at applied treatments (LSD equal 11.483 at Prob.>|F| equal 0.59). Similarly, no significant difference were found between all treatments at 7 and 10 DAT. The reduction percentage in *S. frugiperda* larvae on maize plants was extended between 87.10 – 77.28% and 90.20 – 82.31% at 7 and 10 DAT, respectively Table (6).

Generally, Novo (flufenoxuron 10% DC), Demeron-S (hexaflumuron 10% EC) and Daflox (diflubenzuron 48% SC) compounds could be used as recommended reference insecticides to control of *S. frugiperda* larvae infestations. With using of LC<sub>90</sub> doses on the maize variety “Bioneer 444” after 10 days after treatment, chlorfluazuron gave a low effect against *S. frugiperda* larvae (82.04 reduction %). However, a moderate effect was obtained with both of lufenuron and novaluron (83.59 and 83.18 reduction %, respectively).

Table (6): Reduction percentages of FAW larvae when treated by different CSIs under field conditions

Treatments	Mean numbers of FAW larvae/ plant before treatment	Mean numbers and reduction % of FAW larvae after treatments								
		3 DAT		5 DAT		7 DAT		10 DAT		Overall R%
		Mean $\pm$ SE	R%	Mean $\pm$ SE	R%	Mean $\pm$ SE	R%	Mean $\pm$ SE	R%	
Diflubenzuron 48% SC	1.8 $\pm$ 0.2	0.2 $\pm$ 0.1	84.64abc $\pm$ 5.4	0.33 $\pm$ 0.0	86.86a $\pm$ 1.5	0.27 $\pm$ 0.0	87.10a $\pm$ 1.4	0.23 $\pm$ 0.0	90.20a $\pm$ 1.8	87.20ab
Chlorfluazuron 5% EC	1.6 $\pm$ 0.2	0.17 $\pm$ 0.0	87.36ab $\pm$ 1.8	0.4 $\pm$ 0.1	81.20 a $\pm$ 5.7	0.4 $\pm$ 0.0	77.28 a $\pm$ 4.8	0.37 $\pm$ 0.0	82.31 a $\pm$ 3.6	82.04b
Lufenuron 5% EC	1.77 $\pm$ 0.0	0.37 $\pm$ 0.1	74.23c $\pm$ 4.6	0.23 $\pm$ 0.1	90.59 a $\pm$ 4.1	0.3 $\pm$ 0.1	84.56 a $\pm$ 4.4	0.33 $\pm$ 0.0	84.97 a $\pm$ 4.8	83.59ab
Flufenoxuron 10% DC	1.8 $\pm$ 0.2	0.07 $\pm$ 0.0	96.52a $\pm$ 1.8	0.4 $\pm$ 0.1	84.55 a $\pm$ 0.6	0.27 $\pm$ 0.1	86.93 a $\pm$ 3.2	0.3 $\pm$ 0.0	86.38 a $\pm$ 4.4	88.60 a
Hexaflumuron 10% EC	1.63 $\pm$ 0.1	0.23 $\pm$ 0.0	82.17bc $\pm$ 3.0	0.27 $\pm$ 0.0	88.18 a $\pm$ 2.5	0.3 $\pm$ 0.0	84.00 a $\pm$ 1.3	0.23 $\pm$ 0.0	89.37 a $\pm$ 1.9	85.93ab
Novaluron 10% EC	1.6 $\pm$ 0.1	0.27 $\pm$ 0.0	78.12bc $\pm$ 6.1	0.33 $\pm$ 0.1	84.57 a $\pm$ 5.0	0.27 $\pm$ 0.1	84.31 a $\pm$ 6.2	0.3 $\pm$ 0.0	85.71 a $\pm$ 2.8	83.18ab
Control (without treatment)	2.1 $\pm$ 0.2	1.73 $\pm$ 0.3	-----	3.00 $\pm$ 0.1	-----	2.43 $\pm$ 0.2	-----	2.90 $\pm$ 0.4	-----	-----
F value			3.53	-----	0.77	-----	0.81	-----	0.73	1.62
LSD			12.744		11.483		12.189		10.526	5.6669
Prob.> F			0.03		0.59		0.56		0.62	0.05

FAW = Fall Army worm DAT =Days after Treatment LSD =Least Significant Difference SE =Standard Error Prob. =Probability

Means signed by the same letter in the same column is not significant difference between them.

### Biochemical analysis for FAW larvae after treated by chitin synthesis inhibitors

Results in Table (7) showed 6 parameters in larval treated by the tested CSIs. These parameters were main body components like total protein, total carbohydrates and total lipids, and enzymes as chitinases, phenoloxidases and carboxylesterases in FAW larvae. On the response of total protein value to exposure to CSIs, diflubenzuron, hexaflumuron and flufenoxuron caused the lowest significant reduction in total protein (6.43, 6.7 & 7.74 mg /g.b.wt in the 2<sup>nd</sup> instar and 37.77, 42.8 & 45.47 mg /g.b.wt in the 4<sup>th</sup> instar of *S. frugiperda* larvae , respectively), wherever, the highest significant of protein value was recorded with chlorfluazuron (15.3 & 58.43 mg /g.b.wt in the 2<sup>nd</sup> and 4<sup>th</sup> larval instar, respectively), followed by lufenuron (11.1 & 45.47 mg /g.b.wt in the 2<sup>nd</sup> and 4<sup>th</sup> larval instars, respectively), but there were more significantly decreased than untreated larvae (19.13 & 60.57 mg /g.b.wt in the 2<sup>nd</sup> and 4<sup>th</sup> larval instars, respectively). Similarly, the nearly alignment was detected in the total of carbohydrates and lipids after larvae exposed to the tested CSIs. The total carbohydrates were ranged from 2.73-5.47 and 5.07- 10.43 mg /g.b.wt, while, the total lipids were extended between 2.73-5.47 and 5.07- 10.43 mg /g.b.wt in the 2<sup>nd</sup> and 4<sup>th</sup> larval instars, respectively) Table (7).

Regarding to the enzyme analyses of treated *S. frugiperda* larvae, data illustrated that a reverse relationship between the effect of the tested CSIs and the levels of chitinases, means that, the increase of pesticide effect will decrease this enzyme activity in both the 2<sup>nd</sup> and 4<sup>th</sup> larval instars. The highest value of chitinases was found with larvae treated with chlorfluazuron (103.0  $\mu$ g NAGA/min /g.b.wt), while, the lowest once

with diflubenzuron and hexaflumuron (50.33 & 45.67  $\mu\text{g}$  NAGA/min /g.b.wt, respectively). Contrariwise, a positive relationship was recorded between the effect of the tested CSIs and the levels of phenoloxidas and carboxylesterases, means that, the increase of pesticide effect will decrease these enzymes activities in both the 2<sup>nd</sup> and 4<sup>th</sup> larval instars. The highest value of phenoloxidas and carboxylesterases was stated with larvae treated with diflubenzuron and hexaflumuron, while, the lowest once was reported with chlorfluazuron and novaluron Table (7). Values of the measured parameters indicated a clear disturbance in the metabolism and the physiological aspects for *S. frugiperda* larvae.

Table (7): Amounts of total proteins, carbohydrates and lipids and certain selected enzymes activities (Mean  $\pm$ SE) in FAW larvae treated by chitin synthesis inhibitors.

Treatments	Biochemical selected parameters											
	Main body components						Enzyme activities					
	Total proteins (mg /g.b.wt)		Total carbohydrates (mg /g.b.wt)		Total lipids (mg /g.b.wt)		Chitinases ( $\mu\text{g}$ NAGA/min /g.b.wt)		Phenoloxidas (O.D. units/min /g.b.wt)		Carboxylesterases ( $\mu\text{g}$ Meb /min /g.b.wt)	
	2 <sup>nd</sup> instar	4 <sup>th</sup> instar	2 <sup>nd</sup> instar	4 <sup>th</sup> instar	2 <sup>nd</sup> instar	4 <sup>th</sup> instar	2 <sup>nd</sup> instar	4 <sup>th</sup> instar	2 <sup>nd</sup> instar	4 <sup>th</sup> instar	2 <sup>nd</sup> instar	4 <sup>th</sup> instar
Diflubenzuron 48% SC	6.43 $\pm 0.23\text{d}$	37.77 $\pm 0.96\text{d}$	2.73 $\pm 0.09\text{f}$	5.07 $\pm 0.18\text{e}$	1.8 $\pm 0.15\text{cd}$	2.6 $\pm 0.15\text{c}$	50.33 $\pm 1.86\text{e}$	121.33 $\pm 2.33\text{d}$	5.15 $\pm 0.10\text{a}$	52.03 $\pm 1.70\text{a}$	258 $\pm 1.53\text{b}$	276 $\pm 3.51\text{c}$
Chlorfluazuron 5% EC	15.3 $\pm 0.40\text{b}$	58.43 $\pm 0.64\text{a}$	5.17 $\pm 0.09\text{ab}$	10.43 $\pm 0.23\text{a}$	3.16 $\pm 0.12\text{a}$	4.2 $\pm 0.06\text{a}$	103.0 $\pm 8.14\text{a}$	169.0 $\pm 3.51\text{b}$	2.72 $\pm 0.07\text{e}$	34.1 $\pm 0.32\text{cd}$	80.33 $\pm 8.17\text{e}$	208.67 $\pm 5.21\text{e}$
Lufenuron 5% EC	11.1 $\pm 0.49\text{c}$	45.47 $\pm 1.32\text{c}$	4.87 $\pm 0.22\text{bc}$	7.83 $\pm 0.18\text{bc}$	2.03 $\pm 0.12\text{c}$	4.57 $\pm 0.15\text{a}$	69.67 $\pm 1.45\text{cd}$	189.0 $\pm 5.51\text{a}$	3.03 $\pm 0.09\text{d}$	34.57 $\pm 1.06\text{cd}$	134.67 $\pm 3.18\text{d}$	265.33 $\pm 3.93\text{cd}$
Flufenoxuron 10% DC	7.74 $\pm 0.34\text{d}$	42.8 $\pm 1.11\text{c}$	4.41 $\pm 0.05\text{d}$	7.37 $\pm 0.12\text{c}$	2.5 $\pm 0.06\text{b}$	3.5 $\pm 0.15\text{b}$	62.33 $\pm 0.88\text{d}$	117.67 $\pm 1.45\text{d}$	3.37 $\pm 0.19\text{bc}$	36.47 $\pm 0.55\text{c}$	169.0 $\pm 5.86\text{c}$	373.33 $\pm 8.82\text{a}$
Hexaflumuron 10% EC	6.7 $\pm 0.23\text{d}$	42.67 $\pm 0.77\text{c}$	3.7 $\pm 0.12\text{e}$	6.6 $\pm 0.26\text{d}$	1.63 $\pm 0.09\text{d}$	1.97 $\pm 0.12\text{d}$	45.67 $\pm 2.33\text{e}$	145.67 $\pm 3.38\text{c}$	3.39 $\pm 0.06\text{bc}$	41.83 $\pm 0.93\text{b}$	275.0 $\pm 3.21\text{a}$	314.0 $\pm 2.52\text{b}$
Novaluron 10% EC	10.4 $\pm 0.25\text{c}$	54.77 $\pm 1.07\text{b}$	4.7 $\pm 0.15\text{cd}$	8.13 $\pm 0.19\text{b}$	2.97 $\pm 0.12\text{a}$	4.53 $\pm 0.09\text{a}$	83.0 $\pm 1.73\text{b}$	149.0 $\pm 3.06\text{c}$	3.61 $\pm 0.05\text{b}$	37.0 $\pm 0.58\text{c}$	122.0 $\pm 2.31\text{d}$	267.0 $\pm 2.31\text{cd}$
Control (without treatment)	19.13 $\pm 1.18\text{a}$	60.57 $\pm 1.56\text{a}$	5.47 $\pm 0.15\text{a}$	10.33 $\pm 0.35\text{a}$	3.13 $\pm 0.15\text{a}$	4.43 $\pm 0.12\text{a}$	74.33 $\pm 3.18\text{bc}$	154.0 $\pm 7.81\text{c}$	3.22 $\pm 0.06\text{cd}$	32.83 $\pm 1.92\text{d}$	127.0 $\pm 4.73\text{d}$	255.33 $\pm 6.77\text{d}$
F value	75.14	65.21	49.87	72.49	29.38	70.21	29.60	33.43	62.98	33.50	247.33	97.97
LSD	1.6597	3.3358	0.4034	0.6879	0.3614	0.3764	10.95	13.172	0.2991	3.4991	14.114	15.807
Prob.	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

FAW = Fall Armyworm LSD =Least Significant Difference Prob. =Probability

Means signed by the same letter in the same column is not significant difference between them.

One of the management strategies of the invasive fall armyworm, *S. frugiperda*, is the use of chitin synthesis inhibitor groups such as benzoylurea as a derivative urea insecticide. It is likely that this was reported by Liu *et al.* (2019), who found that many chemicals have been effected on chitin biosynthesis in arthropod pest management, including benzoylphenyl ureas, resulting in deformation, anti-molting and lethality. They also stated that benzoylphenyl ureas is a commonly used CSI in the suppression of arthropod pests, including hexaflumuron, flufenoxuron, lufenuron, novaluron, noviflumuron, chlorfluazuron, teflubenzuron, triflumuron, bistrifluron and diflubenzuron. Moreover, benzoylurea may block the action of a postcatalytic step of chitin synthesis (Nauen and Smagghe, 2006). In the present study, the mortality percentage was higher at 5DAT than 3DAT in 4<sup>th</sup> of FAW larval populations with all tested CSIs. Also, CSIs effective level was slightly higher on 2<sup>nd</sup> instar than 4<sup>th</sup> instar of FAW larvae with all concentrations of all treatments at the

two tested dates. Moreover, the calculated LC<sub>50</sub> for hexaflumuron was low (0.487 mg/l, followed by flufenoxuron (0.5712 mg/L) on 2<sup>nd</sup> larval instar. On 4<sup>th</sup> instar of *S. frugiperda* larvae, hexaflumuron and diflubenzuron were more lethal effective than other CSIs pesticides. The lethal concentration of LC<sub>90</sub> of the six tested CSIs ranged between 1.3815 and 5.0569 mg/l & 1.0245 and 14.6923 mg/l on 2<sup>nd</sup> & 4<sup>th</sup> larval instars of *S. frugiperda*. The obtained results showed that the calculated toxicity index was low by novaluron and lufenuron treatments (61.181 & 63.247, respectively) with a highly resistant ratio (1.634 & 1.581, respectively) when compared with hexaflumuron treatment. However, the toxicity index was high with diflubenzuron, followed by novaluron treatments (95.211 & 80.161, respectively) with a few resistance ratios (1.05 & 1.247, respectively), when compared with hexaflumuron application. Similarly, the obtained findings are in agreement with the result of Shareef *et al.* (2022) found novaluron to have moderate toxicity to *S. frugiperda*. They determined the LC<sub>50</sub> value in a mixture with emamectin benzoate and indoxacarb to be 7.7 ppm and (31.7 ppm) respectively. It was also used alone, presenting 18 ppm on *S. frugiperda* larvae. Also, benzoylurea involving lufenuron binds chitin synthase in terrestrial arthropods, causing chitin biosynthesis inhibition (Douris *et al.*, 2016). In addition, Idrees *et al.* (2023) found that the three insect growth regulators, diflubenzuron, lufenuron and buprofezin showed significant larval mortality against 2<sup>nd</sup> instar of *S. frugiperda* by using the leaf-dip technique.

In field experiments, the obtained data in this study showed that flufenoxuron, hexaflumuron and diflubenzuron pesticides could be used as a recommended reference insecticide to control *S. frugiperda* larvae infestations; however, chlorfluazuron, lufenuron and novaluron had the lowest efficiency against *S. frugiperda* larvae infestations on maize crops. Similar to the present results in this study, MacBean (2012) stated that IGRs were anti-molting agents through ingestion, leading to larvae death. Chlorfluazuron and lufenuron act by contact action, while hexaflumuron is a systemic pesticide. Not only, they were target for lepidopteran larvae but they also had an effect on coleopteran larvae. Behind the anti-molting activity of lufenuron on lepidopteran larvae, but also anti-feeding and sterility effects (MacBean, 2012). Starting in about 2000, IGRs (CSIs and ecdysteroid agonists) have been used in condensate in the Americas (Nascimento *et al.* 2021) against FAW. In numerous studies, the benzoylurea group ceases the chitin synthesis on a large scale in lepidopteran pests, as found by Ishaaya *et al.* (2003). Not only, were novaluron and lufenuron active against lepidopteran species, but they were also powerful against whiteflies and leafminers. However, diflubenzuron was highly effective against pests, including moths, beetles, flies, scale-insects, sucking bugs and mites on different crops (Ganguly *et al.*, 2020). Subsequently, FAW is a migratory lepidopteran species in which resistance alleles can spread when it invades new areas (Nguyen *et al.*, 2021), so the present studies gave the current status of the benzoylurea group of CSIs against FAW in Egypt to decrease the acquired resistance of FAW to conventional insecticides.

On the response of total protein, carbohydrates and lipids values to exposure to CSIs, diflubenzuron, hexaflumuron and flufenoxuron caused the lowest significant reduction in total protein in *S. frugiperda* larvae, wherever, the highest significant protein value was recorded with chlorfluazuron, followed by lufenuron, but there

were more significantly decreased than untreated larvae in larval instar. Subsequently, proteins are the most important component in all living organisms because they're necessary for the main functions of the organisms (Fagan *et al.*, 2002). Similarly, Amein *et al.* (2021) who reported that lufenuron reduced the total protein (mg/larva), total carbohydrate (mg glucose/larva) and total lipid (mg oleic/larva) with reduction percentages of 13.7%, 12.9 % and 38.9% in *S. littoralis* larva, respectively. Also, the total protein decreased by 13.702%, lipid by 12.934%, and carbohydrate by 38.846% in *Agrotis ipsilon* larvae (Abo Elela, 2023). Additionally, our results were proved by Kandil *et al.* (2012), who recorded inhibition of the total protein of the pink bollworm, *Pectinophora gossypiella* with hexaflumuron and chlorfluazuron at LC<sub>50</sub> values. But, hexaflumuron was more efficient in reducing the total protein than chlorfluazuron in *P. gossypiella*. Subsequently, El-Sobki and Ali (2020) reported the highly significant effect of CSIs compounds on total proteins in the red palm weevil, *Rhynchophorus ferrugineus*. They found the highest significant reduction in total protein (45.96%) with hexaflumuron treatment compared to chlorfluazuron (23.54%) and lufenuron (7.29%) in *R. ferrugineus*.

A reverse relationship between the effect of the tested CSIs and the chitinase activities, means that, the increase in pesticide effectiveness will decrease this enzyme activity in both 2<sup>nd</sup> and 4<sup>th</sup> larval instars of FAW in the present study. The highest chitinase activities were detected in larvae treated with chlorfluazuron, while the lowest were detected with diflubenzuron and hexaflumuron. Contrariwise, a positive relationship was recorded between the effect of the tested CSIs and the levels of phenoloxidase and carboxylesterase, meaning that the increase in pesticide effect will decrease these enzyme activities in both 2<sup>nd</sup> and 4<sup>th</sup> larval instars. The values of the measured parameters indicated a clear disturbance in the metabolism and physiological aspects of *S. frugiperda* larvae. Similarly, Hussain *et al.* (2010) suggested that any change in chitinolytic enzyme activity may be related to insecticide activities. Similarly, as in the present study, Assar *et al.* (2016) stated that the activity of chitinase and phenoloxidase enzyme increased with the application of IGRs and bio-insecticides for controlling *S. littoralis*. Moreover, El-Sobki and Ali (2020) found that any changes in CSIs increased the chitinase activity in the 6<sup>th</sup> larval instar of *S. littoralis*. Also, after exposure of larvae to hexaflumuron, lufenuron and chlorfluazuron treatments, chitinase recorded 2.28 % increasing in activity compared to untreated treatment in *R. ferrugineus*.

In the present study, both phenoloxidase and carboxylesterase enzymes may play an important role in defense mechanisms in insect pests, as reported by Jiang *et al.*, (1997). In arthropods, phenoloxidase enters into melanin synthesis and clotting, generating quinones (Eleftherianos and Revenis, 2011). The obtained data elucidated a strong relationship between phenoloxidase and carboxylesterase enzymes and the CSIs activities, similarly, a high activity of phenoloxidase was observed in hexaflumuron, which recorded a 46.72% reduction, while a low phenoloxidase activity was observed in lufenuron applications, with a reduction of 22.56% (El-Sobki and Ali, 2020). Also, Feng *et al.* (2018) found that carboxylesterase provided resistance mechanisms to insecticides in insect, especially organophosphates. It was responsible for cross-resistance to a wide range of insecticides. The esterases were

divided into arylesterases, acetylerases, carboxylesterases and acetylcholinesterase (Dahan-Moss and Koekemoer, 2016). Hilliou *et al.* (2021) elucidated that insecticide resistance increases levels of detoxification enzymes, including carboxylesterases in many insect pests.

## CONCLUSIONS

The present study has improved the current status of CSIs to cater to FAW management programs. CSIs have a devastating effect on *S. frugiperda* in Egypt. The results suggest that diflubenzuron, hexaflumuron and flufenoxuron work better in these conditions. Due to its appearance and rapid spread, *S. frugiperda* represents a serious threat to maize cultivation in Egypt, and it has a high resistance to numerous chemical pesticides. So, the study helps in better decision making in CSIs choice to establish the most suitable IGRs insecticidal schedule against FAW on maize crops.

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## CONFLICT OF INTEREST

The work's authors declare that there are no conflicts of interest associated with its publication.

## تأثير بعض مثبطات تخليق الكيتين على دودة الحشد الخريفية *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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### الخلاصة

تعتبر الذرة من الحبوب الهامة في مصر ويصاب بالعديد من الآفات الحشرية وتسبب دودة الحشد الخريفية *Spodoptera frugiperda* خسائر كبيرة. ولذا تناولت الدراسة دراسة تأثير مثبطات تخليق الكيتين على يرقات دودة الحشد على الذرة. وأظهرت النتائج أن LC50 لمبيد Hexaflumuron كانت منخفضة، يليه Diflubenzuron و Flufenoxuron على العمر اليرقي الثاني. بينما كانت منخفضة مع Hexaflumuron و Diflubenzuron على العمر الرابع عن المركبات الأخرى. أوضحت النتائج أن معامل السمية كان منخفضاً مع Novaluron و Lufenuron. بينما كانت أعلى مع Diflubenzuron و Novaluron مقارنةً بمعاملة Hexaflumuron. وقد بينت التجربة الحقلية أنه يمكن استخدام Flufenoxuron و Hexaflumuron و Diflubenzuron لمكافحة دودة الحشد. وأظهرت النتائج أن محتوى البروتين باليرقات منخفض مع Diflubenzuron

و Hexaflumuron و Flufenoxuron. بينما سُجلت أعلى قيم للبروتين مع Chlorfluazuron و Lufenuron. وقد أشارت النتائج الى تغير في انزيمات الحشرة مما يؤثر في العمليات الفسيولوجية في اليرقات. وبناءً عليه فيمكن استخدام مثبطات الكيتين في تطوير برنامج الإدارة المتكاملة لدودة الحشد على الذرة. الكلمات المفتاحية: الخصائص البيوكيميائية، مثبطات تخليق الكيتين، دودة الحشد الخريفية، التركيز المميت، *Spodoptera frugiperda*.

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