EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

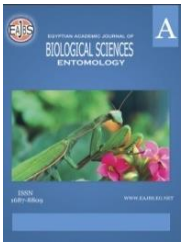
Vol. 17 No. 2 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 17(2) pp.69-77 (2024)*
DOI: 10.21608/EAJBSA.2024.359314

Egypt. Acad. J. Biolog. Sci., 17(2):69-77 (2024)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Latent Effects of Chitin Synthesis Inhibitor; Chlorfluazuron on The Fall Armyworm; *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Lobna R. Amin and Tarek R. Amin
Plant Protection Research Institute, Agricultural Research Center
***E-mail:** odahss1433@gmail.com

ARTICLE INFO
Article History
Received:22/4/2024
Accepted:10/6/2024
Available:14/6/2024

Keywords:
latent effect, fall armyworm, chitin synthesis inhibitors, chlorfluazuron, main metabolites, digestive enzymes.

ABSTRACT

The fourth larval instar of *Spodoptera frugiperda* was treated, for 24 h, with LC_{50} of chlorfluazuron as a chitin synthesis inhibitor (0.5027 ppm) to examine its latent effects after counting acute mortality and ecdysis to the 5th instar. Percentage mortality after 24, 48 and 72 h postecdysis was 2, 12 and 18% from the whole number of treated larvae. Larval weight and main metabolites were significantly decreased as compared to control larvae. This reduction increased over time, especially for lipids. The early depression of lipids suggested that the larvae suffered from malnutrition probably due to starvation and that digestion did not go properly. Treatment reduced proteases and amylase activity even after 72 h postecdysis but had no significant changes for β-glucosidase and lipase. Starvation and the observed effect on digestive enzymes may lead to a decrease in body metabolites and consequently body weight, so it is suggested that larvae are no longer able to complete their development well and will be adversely affected. It could be concluded that treatment of the fall armyworm larvae with chlorfluazuron led to lethal and sub-lethal latent effects more or less like that induced by the acute effects of the insecticide. Latent effects, from the practical point of view, must be taken into consideration in pest management programs that aim to decrease insect population to decrease cost and toxic hazards.

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), is a lepidopteran pest that feeds in enormous numbers on the leaves and stems of over 80 plant species, inflicting significant damage to maize, rice, sorghum, sugarcane, and other vegetable crops as well as cotton.

Latent effects are those in which exposure to toxicants is followed by some period of time after the specific response is developed (Vandivert and Eaton, 2014). Besides acute effects, different chemical insecticides could induce significant changes in subsequent developmental stages. In general, latent effects contributed most to the overall insecticide effect (Matthias and Groning, 2024).

Some insecticides showed latent effects on the fall armyworm. Methomyl caused latent larval mortality, reduction of pupation percentage and adult reproductive potentials (Salem *et al*., 2023), while spinetoram caused slowing in larval and pupal growth, adult malformation, and disruption of proteins, carbohydrates and digestive enzymes (Salem *et al*., 2024).

Chitin synthesis inhibitors (CSI´s) are insect growth regulators, interfering with chitin synthesis and deposition causing death during moulting. Insects treated with CSI´s and succeeded in moulting were unable to feed and probably died of starvation (Mulder and Gijswijt, 1973; Neuman and Guyer, 1987). Interference with feeding is an important delayed effect of diflubenzuron and chlorfluazuron and can be considered as a secondary mode of action of these compounds (Amin, 1998). Starvation stress caused by treatment of *Agrotis ipsilon* with CSI´s affected main metabolites of the larvae (Afifi, 2002).

There is little information about the latent effects of CSI´s on *S. frugiperda*, so the fourth larval instar of *S. frugiperda* was treated, for 24 h, with LC_{50} of chlorfluazuron as chitin synthesis inhibitor, and its latent effects were examined after ecdysis to 5th instar. The purpose of the research was to evaluate the latent effects of chlorfluazuron, represented by the assumption of its ability to cause death rates and affect weight, metabolites and digestive enzymes of the larvae and to discuss this through the ability of the pesticide to affect feeding and nutrition.

MATERIALS AND METHODS

Insect Rearing Technique:

The culture of fall armyworm was initiated with larvae gathered from maize cultivation at Qena province existed in Upper Egypt. Where they were kept at the laboratory for several generations and reared in laboratory environments under controlled conditions, 25 ± 2 °C, 70 ± 10 % RH), and fed on castor leaves according to Dahi (1997). Larvae have daily provided and fed on castor bean leaves. The resultant pupae were placed in clean moist sawdust jars to supply the pupation site. However, adults were supplied 10 % sugar solution.

Pesticide Used:

Common name: Chlorfluazuron 5%, E.C.

Trade name: Caprice, Atabron, Fertabron (Caprice 5% EC).

Chemical name: [N-(4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)-3,5-dichlorophenyl)-N(1)- (2,6-difluorobenzoyl)urea], and it was obtained from El-Helb for pesticides and chemicals company, Egypt.

Determination of LC_{50}:

A small pilot trial was carried out to determine the LC_{50} of chlorfluazuron on *S. frugiperda* 4th instar larvae. Fresh castor bean leaves (*Ricinus communis*) were dipped for 60 seconds in several concentrations of chlorfluazuron (0.01, 0.1, 0.5, 1and 10 ppm), left to dry and introduced to larvae for 24 h. Treated leaves were then removed and fresh untreated ones were provided. Mortality counts were recorded after 72 h. The data were corrected using Abbott's formula (1925). The obtained results were expressed graphically and LC_{50} values were calculated using a computerizing LDP line software program according to Finney (1971).

Preparation of Insects for Biochemical Analysis:

The fifth larval instar insects were made according to Amin's instructions (1998). The larvae were homogenized in distilled water (50 mg/mL). The homogenates were centrifuged at 8000 r.p.m. for 15 minutes at 4ºC in a chilled centrifuge. The deposits were

discarded, and the supernatant was divided into small amounts before being stored in a deep freezer (-20ºC) until needed for the tests.

The digestive tracts of *S. frugiperda* fifth larval instars were removed to provide crude gut samples for digestive enzyme assays, as described by Osuna-Amarillas *et al.* (2012). Midguts were homogenized in assay solution (0.IM phosphate buffer, pH 8) and centrifuged at 14,000 g for 10 minutes at 4ºC. The supernatants were collected and utilized as an enzyme source.

Chemicals Used:

The bovine albumin standard was bought from Stanbio Laboratory (Texas, USA). Commasie brilliant blue G-250 was from Sigma (Sigma Chemicals Co.). The remainder of the chemicals were of good quality and obtained from commercial local businesses.

Apparatus:

Insects were homogenized for biochemical examination using a cooled glass (coated with an ice jacket) Teflon tissue homogenizer (ST-2 Mechanic, Preczyina, Poland). A double-beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to assess the absorbance of colored substances.

Biochemical Assays:

Bradford's (1976) method was used to measure total proteins, with Coomassie Brilliant Blue G250 and bovine serum albumin serving as the reference. The total carbohydrates in the sample acid extract were determined using Dubois *et al*.'s (1956) phenol-sulphuric acid reaction. Total carbohydrates were extracted and prepared for testing in accordance with Crompton and Birt (1967). The total lipids were determined using the Knight *et al.* (1972) technique with phosphor vanillin reagent. Lipase was tested colorimetrically using the spectrum diagnostic kit (www.spectrumdiagnostics.com). Lipase splits a synthetic substrate (DGMRE) to produce the colorful end product, methylresorufin. The increasing absorbance of red methylresorufin was measured photometrically at 578 versus air. Tatchell *et al.* (1972) described assessing total proteolytic activity (protease) in gastrointestinal extracts as an increase in free amino acids split from substrate protein (bovine serum albumin). The generated amino acids were colorimetrically tested using ninhydrin reagent, as described by Lee and Takabashi (1966). Amylase was determined using Amin's (1998) improvements to the procedures reported by Ishaaya and Swirski (1976), with soluble starch as the substrate. Lindorth's (1988) method was used to assess β-glucosidase activity by measuring the amount of glucose released during the enzymatic hydrolysis of salicin.

Statistical Analysis:

The collected values were pooled from three replicates. Means and standard deviations were obtained, and the data were analyzed using ANOVA with Costat statistical software (Cohort Software, Berkeley). Duncan's multiple range tests confirmed the relevance of variable treatments ($p<0.01$). %weight loss was calculated as follows: $\%\text{weight loss}=\frac{control\ weight-treated\ weight}{control\ weight}\times 100$.

RESULTS

The fourth larval instar of *S. frugiperda* was treated with LC_{50} of chlorfluazuron to show its effects on the subsequent instar (5^{th} instar). Probit analysis as shown by the concentration-mortality regression line (Fig. 1) illustrated that LC_{50} of chlorfluazuron was 0.5027 ppm (slope = 0.899±0.09, α= 0.051).

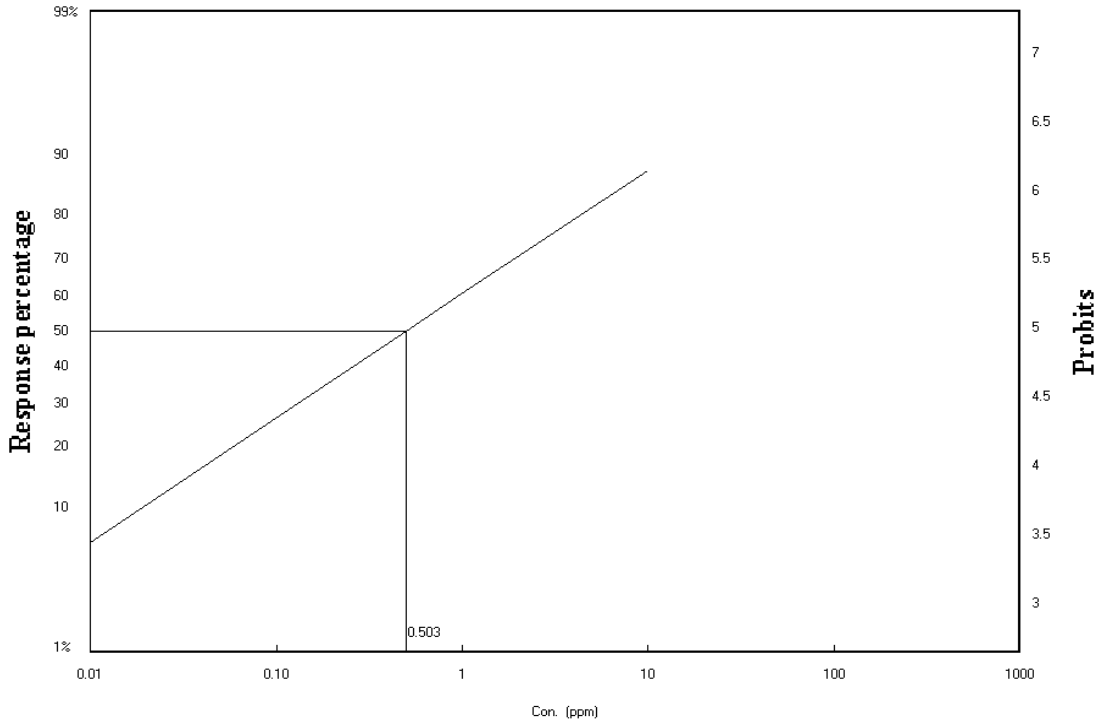


Fig.1: concentration-mortality regression line for the 4th larval instar of *Spodoptera frugiperda* treated with variable concentrations of chlorfluazuron.

Latent Biological Effects:

After ecdysis to the 5th instar, i.e. after acute mortality was calculated and 50% of the larvae were killed, the insecticide was able to induce mortality (Table 1).

Mortality after 24, 48 and 72 h postecdysis was 2, 12 and 18% from the whole number of treated larvae, while 1.2% of control larvae normally died during this period. Control mortality percentage is considered small in comparison to treated larvae (Fig. 2).

Table 1: latent biological effects on *Spodoptera frugiperda* treated as 4th larval instar for 24 h with LC_{50} of chlorfluazuron

Hours post ecdysis to 5th instar	**Body weight (mg)**		**% weight loss**	**%mortality**	
	control	**treated**		**control**	**treated**
24	55 ± 3^{cd}	40 ± 4.1^{e}	27.2%±2.5	0^{b}	2 ± 1^{b}
48	86 ± 5^{b}	48 ± 3.8^{de}	44.2%±4.1	1 ± 0.2^{b}	12 ± 4.2^{a}
72	120 ± 11^{a}	68 ± 3.3^{c}	45.8%±3.6	1.2 ± 0.3^{b}	18 ± 5^{a}

Data is presented as mean±SD.

Means with distinct superscripts for each parameter are significantly different (P<0.01, ANOVA, LSD test).

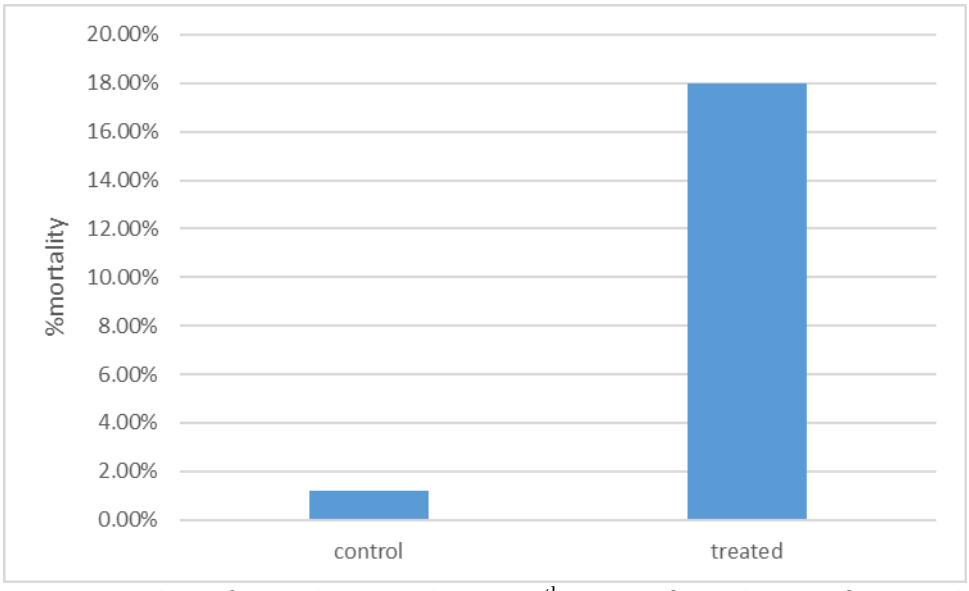


Fig. 2: Mortality after 72 h postecdysis to 5th instar of *Spodoptera frugiperda.*

Another important observation was that the weight of the treated larvae was significantly decreased than that of control larvae (Table, 1). Percentage weight loss increased over time (Fig. 3). It was 27.2, 44.2 and 45.8% after 24, 48 and 72 h post ecdysis, respectively.

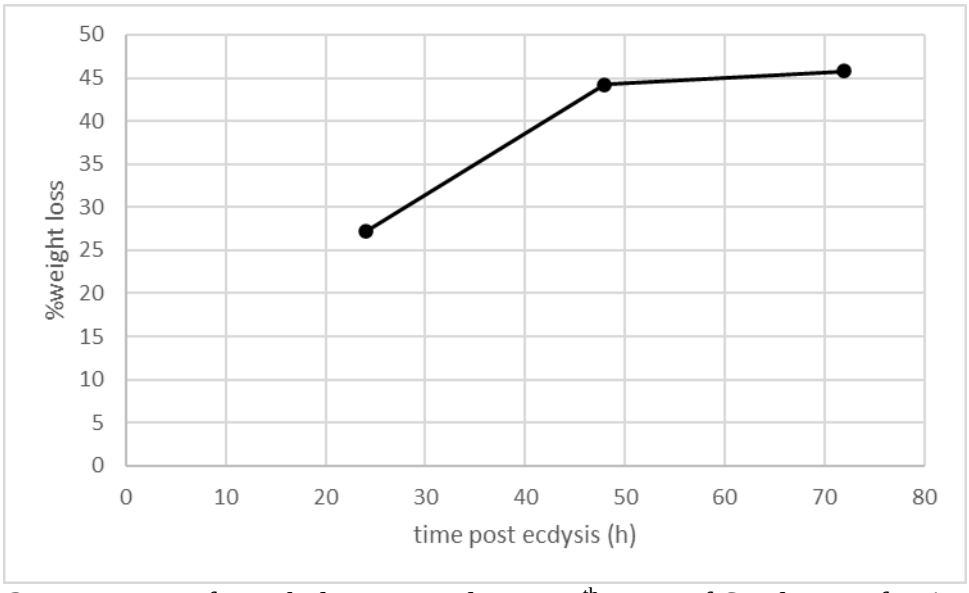


Fig. 3: Percentage of weight loss postecdysis to 5th instar of *Spodoptera frugiperda.*

Latent Effect on Larval Metabolites:

Treatment of the 4th larval instar with the LC_{50} of chitin synthesis inhibitor; chlorfluazuron affected the main metabolites of the next larval instar **(**Table, 2). The insecticide caused a significant decrease in all of the tested insect metabolites. This reduction increased over time and was severe for lipids and proteins. Proteins and lipids were adversely affected after 24 h, while carbohydrates showed significant changes after 48 h. The percentage amount of carbohydrates, proteins, and lipids, 72h postecdysis to 5th instar, was 70.6, 63.4 and 30% as compared to control, respectively (Fig. 4).

Table 2: Delayed effects on the main metabolites after ecdysis to fifth instar of *Sposoptera frugiperda* larvae treated by LC_{50} of chlorfluazuron

Hours post ecdysis to 5th instar	Total proteins (mg/g.b.wt)		Total carbohydrates (mg/g.b.wt)		Total lipids (mg/g.b.wt)	
	Control	Treated	Control	Treated	Control	Treated
24	47.7±1.5[b]	35±4[c]	30.7±3.8[c]	27.7±2.1[c]	5.3±0.8[c]	3.6±0.3[d]
48	58.7±3.2[a]	45±4[b]	39.3±3.1[b]	31±2[c]	8.4±0.7[b]	3.6±0.3[d]
72	56.3±2.1[a]	35.7±2.5[c]	46±2[a]	32.5±2.5[c]	10.3±0.9[a]	3.1±0.4[d]

Data is presented as mean±SD.
Means with distinct superscripts for each parameter are significantly different (P<0.01, ANOVA, LSD test).

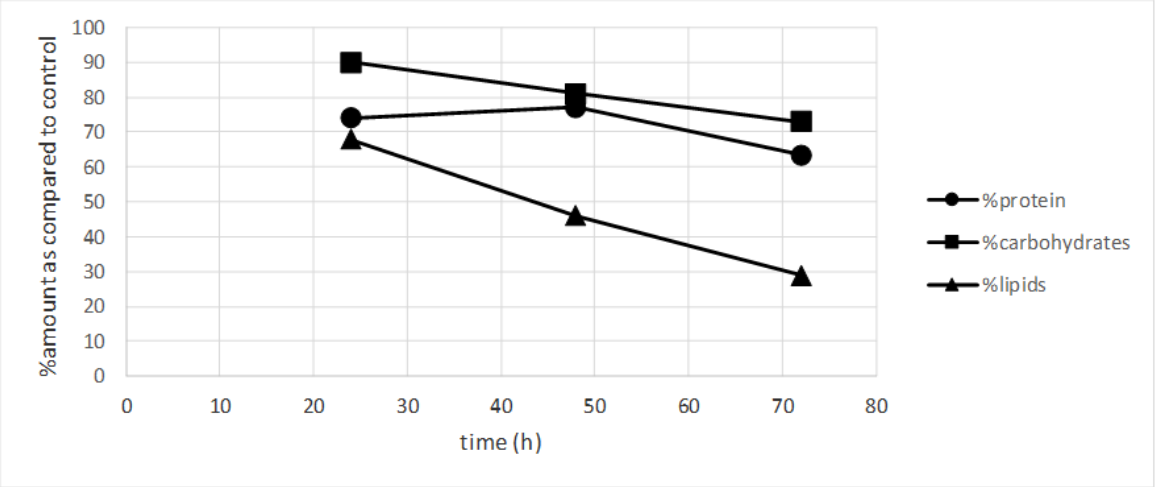


Fig.4: percentage amount of main metabolites as compared to control post ecdysis to 5th instar.

Latent Effects on Digestive Enzymes:

Treatment during the 4th larval instar was able to reduce protease and amylase activity even after 72 h post ecdysis, but had a non-significant effect on β-glucosidases and lipase activity. Proteases activity after 72 h post ecdysis to the 5th instar of *S. frugiperda* (**Table, 3**) was 21.9 and 37.8 µg glucose/min/ mg protein for treated and control, respectively. Amylase activity was less affected and equaled 6.7 and 9.9 µg glucose/min/ mg protein for treated and control, respectively.

Table 3: Delayed effects on digestive enzymes after 72 h post ecdysis to fifth instar of *Spodoptera frugiperda* larvae treated by LC_{50} of chlorfluazuron

Insects	*B-glucosidase (µg glucose/min/ mg protein)*	*Protesases (µg alanine/min/mg protein)*	*Amylase (µg glucose/min/mg protein)*	*Lipase (µU/mg protein)*
Treated	**1.7±0.1[a]**	**21.9±3.3[b]**	**6.7±0.5[b]**	**232±21[a]**
Control	**1.8±0.05[a]**	**37.8±4.6[a]**	**9.9±2.1[a]**	**241±9[a]**

Data is presented as mean±SD.
Means with distinct superscripts for each parameter are significantly different (P< 0.01, ANOVA, LSD test).

DISCUSSION

Although acute mortality was counted after 3 days from treatment of the 4th larval instar of fall armyworm with LC_{50} of chlorfluazuron, it is still capable of causing sub-lethal and lethal effects in the following days. Larvae might suffer abnormal physiological situations such as desiccation and starvation after treatment by chitin synthesis inhibitors (DJeghader *et al*., 2014). The present results showed that a number of larvae of *S. frugiperda* that entered to 5th instar died slowly. Amin (1998) proved that starvation itself, within a few days, can induce larval mortality besides that occurs during molting due to treatment, i.e. can be added to the toxicological evaluation of CSI´s in *S. littoralis* larvae.

Latent effect of diflubenzuron represented by the reduction of food intake capacity and growth of larvae and pupae of *Pericallia ricini* Fabr. (Lepidoptera: Arctiidae), was explained by Nathan and Nathan (2011). They suggested that the mouth parts may become weak and the larvae not able to claw food properly by weak jaws thus the food intake capacity was ultimately reduced.

Starvation and the observed effect on digestive enzymes in the present study may lead to a decrease in body metabolites and consequently body weight. The early depression of lipids as indicated by the results suggested that the larvae have malnutrition probably due to starvation and that digestion did not go properly. Carbohydrates, proteins and lipids are very efficiently utilized by insects. The utilization of these nutrients depends on the digestive enzymes. Previously, Amin (1998) referred to the reduced efficiency of conversion of ingested food to body substances (E.C.I), partially, due to the effect of CSI´s on some carbohydrases. The date palm weevil; *Rhynchophorus ferrugineus* (Olivier) (Coleoptera) larvae were more sensitive to starvation than adults, and their main metabolites were more reduced (Abdel- Hamid, 2018).

Some of the digestive enzymes of *S. frugiperda* larvae were reduced by the treatment of chlorfluazuron might be due to the effect of the insecticide on the perotrophic membrane. Previously, Culter *et al.* (2005) stated that novaluron (CSI) may affect the integrity and/or growth of the petritrophic membrane of *Leptinotarsa decemlineata*. This membrane protects the midgut from digestive enzymes, pathogenic organisms and harmful substances. Treatment reduced chitin production, which is constantly occurring in the petritrophic membrane. Gut cells secrete digestive enzymes, and if these cells are affected in any way, it may affect enzyme secretion. Forty-eight hours of starvation led to the complete breakdown of the gut epithelial cells of *S. litura* (Rosaiah and Mukkerjee, 1988).

Due to the importance of insecticides' latent effects in pest control, Matthias and Groning (2024) introduced an analytical framework for mechanistically predicting latent effects. This enables to reduce the recommended amount of insecticides in field use, since the concentration causing 15% mortality of the may fly *Cloeon dipterum*, as reported by the same authors, 29days post-exposure was 1000 times lower than the concentration that caused the same mortality 4 days later, underscoring the time-dependent nature of this latent impact amplification (LEA).

From the present work, it is suggested that *S. frugiperda* larvae which suffered from a high reduction in body metabolites, a decrease of some digestive enzyme activity and died slowly due to treatment by LC_{50} of chlorfluazuron as chitin synthesis inhibitor, were not able to complete their development well, and will be severely affected.

It could be concluded that treatment of the fall armyworm larvae with chlorfluazuron led to lethal and sub-lethal latent effects more or less like that induced by the acute effects of the insecticide. Latent effects, from the practical point of view, must be taken into consideration in pest management programs that aim to decrease insect population to decrease cost and toxic hazards.

Declarations:
Ethical Approval: Not applicable
Authors Contributions: All authors participated in the design of the study, material preparation, data collection, and analysis.
Competing interests: The authors declare no conflict of interest.
Availability of Data and Materials: The data that support the findings of this study are available from all author upon reasonable request.
Source of Funding: The current research was not funded.
Acknowledgements: Not applicable

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18 (2), 256-267.

Abdel-Hameid, N.F. (2018). Starvation effect on bioactive components of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) ; (Coleoptera: Curculionidae). *Middle East Journal of Applied Sciences,* 8:337- 344.

Afifi, M.A. (2002). Starvation stress of two chitin synthesis inhibitors on the cutworm, *Agrotis ipsilon* (HUFN) larvae. *Egyptian Journal of Applied Sciences,* 17:356-362.

Amin, T.R. (1998). Biochemical and Physiological Studies of Some Insect Growth Regulators on the Cotton Leafworm, *Spodoptera littoralis* (Boisd.). p. 28. Ph.D. Thesis, Fac. Agric. Cairo Univ., Egypt.

Bradford, M.M., I976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical biochemistry,*72:248-254.

Crompton, M. and L.M. Birt, 1967. Changes in the amounts of carbohydrates, phosphagen , and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina. Journal of Insect Physiology,* 13: 1575 - 1595.

Cutler GC, Scott-Dupree CD, Tolman JH, Harris CR. Acute and sublethal toxicity of novaluron, a novel chitin synthesis inhibitor, to *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Pest Management Science,* 2005 Nov;61(11):1060-8. doi: 10.1002/ps.1091. PMID: 16015578.

Dahi, H. F. (1997). New approach for management the population of cotton leafworm *Spodoptera littoralis* (Boisd.) and pink bollworm *Pectinophora gossypiella* (Saund.) in Egypt. M. Sc. Thesis, Fac. Agric., Cairo Univ., 142 pp.

Djeghader , N.E.H., L. Aïssaoui, K. Amira and H. Boudjelida (2014). Impact of a Chitin Synthesis Inhibitor, Novaluron, on the Development and the Reproductive Performance of Mosquito *Culex pipiens. World Applied Sciences Journal,* 29 (7): 954-960.

Dubios, M., K.A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28:350 - 358.

Finney, D. J. (1971). Probit Analysis. 3rd Edition, Cambridge University Press, Cam

Ishaaya, I. and E.Swirski(1976). Trehalase,invertase and amylase activities in the black scale, *Saissetia oleae*, and their relation to host adaptability. *Journal of Insect Physiology,*16:1025-1029.

Knight, J.A., S. Anderson, and J.M. Rawle, 1972. Chemical basis of the ulfo-phospho-vanillin reaction for estimating total serum lipids. *Clinical Chemistry*, 18:199-202.

Lee, Y.P. and T. Takabshi. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. *Analytical biochemistry,* 14: 71-77.

Lindroth , R.L. (1988) . Hydrolysis of phenolic glycosides by mid gut β-glucosidases in *Papilo glaucus* subspecies. *Insect biochemistry*, V 18: 789-792.

Matthias, L. and J. Groning (2024). Latent pesticide effects and their mechanisms. *Science of the total environment,* 909 (2024) 168368.

Mulder, R. and M. J. Gijswijt (1973). The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pesticide Science,* 4:737-745.

Nathan, L.S. and R.J. Nathan (2011). Effect of chitin biosynthesis inhibitor- diflubenzuron on fifth instar larvae of p. ricin Fabr. *The Asian Journal of animal science,* 6:66-70.

Neuman, R. and W. Guyer (1987). Biochemical and toxicological differences in the modes of action of the benzoylureas. *Pesticide Science,* 20:147-156.

Osuna-Amarillas, P.S.; F.J. Cinco-Moroyoqui; J.L. Cardenas-Lopez; J.M.Ezquerra-Brauer; R.Stelo-Mundo; M.O. Certez-Rocha; J.M. Barrom-Hoyos; O. Rouzaud-Sandez and J. Borboa-Flores. 2012. Biochemical and kinetic characterization of the digestive trypsin-like activity of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera : Bostrichidae). *Journal of Stored Products Research,* 51:41-48.

Rosaiah, B. and S.P. Mukkerjee (1988). Histopathological changes in the alimentary canal of starved larvae of *Spodoptera litura. Indian Journal of Entomology,* 47:249-254.

Salem, S., Dahi, H., Abdel-Galil, F., & Mahmoud, M. (2023). Efficacy of Common Synthetic Insecticides for Management of Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Egypt. *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control,* 15(1), 157-170. doi: 10.21608/eajbsf.2023.303457.

Salem, S.A.R., Alhousini, E.M.E., Al-Amgad, Z. et al. Efficiency of spinetoram on biological, biochemical, and histological parameters in the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Egypt. *Journal of Plant Diseases and Protection, 131*, 489–499 (2024). https://doi.org/10.1007/s41348-023-00835-4.

Tatchell, R.J.; S.F. Araman and F.N. Boctor. 1972. Biochemical and physiological studies of certain ticks (Ixodoidae). *The journal Parasitology Research,* 39: 3454-350.

Vandivort, T.C. and D.L. Eaton (2014). Principles of toxicology. Elsevier online reference medal in biomedical sciences.