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Biochemical Studies in Larvae of *Agrotis ipsilon* (Hüfnagel) Affected by Recent Insecticides

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ABSTRACT

As field populations of black cutworms grow more resistant to conventional insecticides, the need for new and effective chemical means to control this insect is more important than ever. In this study, we examined the biochemical mechanisms underlying the toxicity of the sublethal effect of the new insecticides (emamectin benzoate, indoxicarb, chloranitraniliprole, and pyridalyl) against two strains of *Agrotis ipsilon* comprising the laboratory-susceptible (L-S) and the field-resistant (BK-R). Activity measurements of the main detoxification enzymes showed that new insecticides inhibited the activities of both glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT), whereas the significant activity of glutathione S-transferase (GST) was observed, suggesting that the inhibition of detoxification contributes to the enhancement toxicity against *A. ipsilon* larvae. A significant decrease in the effect of sublethal dose was also observed between the control larvae in the content of protein, lipid, and glycogen, and treated larvae in two strains. According to these results, the treated larvae were negatively affected in both two tested strains compared with untreated larvae in control.

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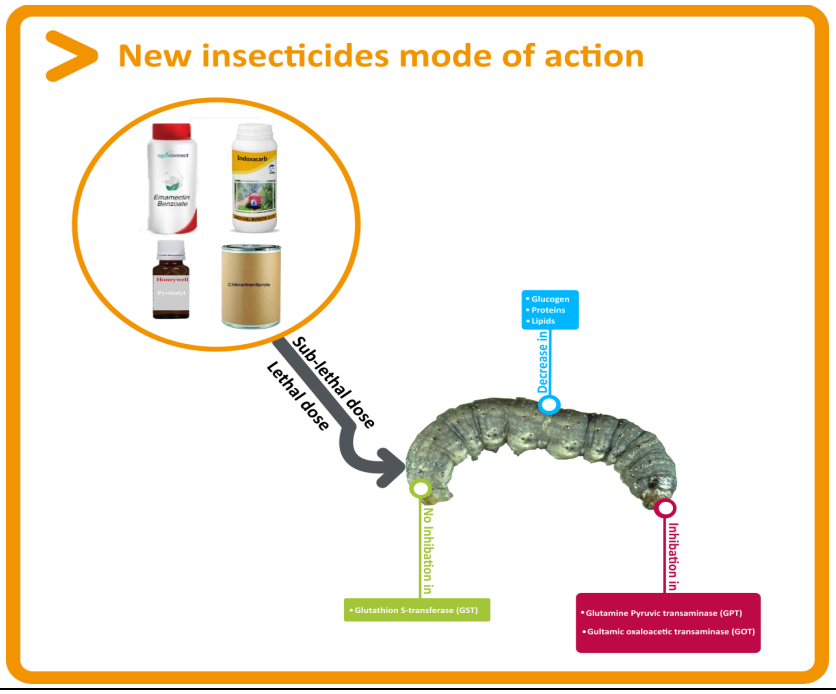
GRAPHICAL ABSTRACT



1. Introduction

The black cutworm, *Agrotis ipsilon* Hüfnagel (Lepidoptera: Noctüidae), is an important global pest. It is one of the most dangerous underground pest species and can feed on more than 100 species of host plants; it is highly widespread in many regions of the world, including Egypt throughout the year. Once larvae reach the fourth instar, they attack various field crops, causing serious damage and shortages of crops. They especially prefer to attack the young plant in the growing stages by cutting seedlings or digging a tunnel in old plant bases and the destruction of their growth points, which leads to huge economic losses in the crop, especially in the seedling stage, ranging from 20 to 37% and may reach 80% depending on the severity of infestation [1,2]. Infestation caused by larvae and their habits requires special efforts to combat this pest. Therefore, most uses of insecticides to control black cutworms have specific timing to control the larval stages, which are the most destructive stage for this insect [3, 4]. As a result, injudicious use of insecticides exclusively in the management of *A. ipsilon* may have a detrimental impact on human health, untargeted organisms, and the natural environment. In addition to this, frequent use of the same chemical pesticides may cause insect resistance to particular insecticides; therefore, the traditional control methods for this species were not considered promising, and this represents a great challenge to control this pest. Therefore, there is a greater need to develop alternative strategies that included investigating several new insecticides from different chemical groups with different modes of action and studying the efficacy, sublethal impact as an essential part of integrated management of black cutworm [5, 6].

Resistance in insects to a different group of insecticides was due to insensitivity of the target site and detoxification of insecticides by metabolic enzymes, e.g., Glutathione S-transferase (GST), glutamic oxaloacetic transaminase (GOT), and glutamine pyruvic transaminase (GPT) [7,8]. Glutathione S-transferases (GST) are the mainly cytosolic enzyme that catalyzes the conjugation of

electrophile molecules with reduced glutathione (GSH), potentially toxic substances become more water-soluble and generally less toxic [9]. GST plays an important role in insecticide resistance and is involved in the metabolism of organophosphorus and organochlorine compounds [10]. Transaminases, glutamic oxaloacetic transaminase (GOT), and glutamine pyruvic transaminase (GPT) are critical enzymes in the biological processes. They play a role in amino acid catabolism and biosynthesis. The disruption of transaminases from the normal value denotes biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification processes and metabolism [11]. Pesticides have other effects besides their acute and chronic toxicity. Some of the non-responses are related to hepatic changes including induction of transaminases. These hepatic changes indirectly affect the levels of hormones and various biogenic amines which act at vital biological sites.

These enzymes are biomarkers to measure the level of resistance, tolerance, or susceptibility in an organism during insecticide detoxification mechanisms [12]. These enzymes also take part in the chemical equilibrium, which is essential for insects in different physiological processes. Insecticides are known to cause a disturbance in enzymatic equilibrium needed to perform different physiological processes [13]. Energy reserves such as proteins, lipids, and glycogen in the hemolymph are also an important indicator of the level of metabolism in insects, and a dynamic balance of the absorption and metabolism [14-16]. These energy reserves are closely related to physiological processes such the molting and reproduction [17, 18].

Insecticides are known to cause a disturbance in enzymatic equilibrium and energy reserves needed to perform different physiological processes [13]. Accordingly, fourth instar larvae of *A. ipsilon* were exposed to sublethal doses of new insecticides (emamectin benzoate, indoxicarb, chloranitraniliprol, and pyridalyl) to assess the detoxification enzymes activities; glutathion S-transferase (GST), glutamic oxaloacetic transaminase (GOT), and glutamine pyruvic transaminase (GPT) as well as energy reserves; proteins, lipids, and glycogen content of *A. ipsilon*. This study will provide more information about the effects of new insecticides exposure on the physiological response of *A. ipsilon*.

2. Experimental

2.1. Black cutworm test population

The two black cutworm populations used in this study were susceptible and resistant strains of *Agrotis ipsilon*, comprising laboratory-susceptible (L-S) and Biala Kafr El-Sheikh-resistant (BK-R), which were collected from field larvae at various heavily sprayed sites in Biala district at Kafr El-Sheikh Governorate (Egypt), on June 2020. These sites were previously known to be exposed to insecticides from different groups during the cotton growing seasons. All of the black cutworm larvae were maintained continually from their date of collection with the susceptible-laboratory strain and reared for several years without exposure to any pesticides, in controlled laboratory conditions at in the laboratory at a temperature of 25 °C ± 2 °C and 65 ± 5%, relative humidity with a 12-h light: dark photoperiod at the Department of Insect Population Toxicology, Central Agricultural Pesticides Laboratory, Agriculture Research Center, Dokki, Giza, Egypt. The black cutworm larvae were reared in a big glass container with sawdust in the bottom and fed on castor bean leaves (*Ricinus communis* L.). Early 4th stage *A. ipsilon* larvae were available for biochemical assays.

2.2. Toxicity study

The leaf dipping method was used to determine the median lethal concentration (LC_{50}) and sublethal concentration (LC_{25}) of insecticides on

two strains of *A. ipsilon*. Ten newly hatched 4th instar larvae were placed in glass jars (14 × 5 × 12 cm) containing castor bean leaves (3.5 cm in diameter) dipped for 10 s in five aqueous concentrations for each the emamectin benzoate (5% SG), indoxicarb (15% EC), chloranitraniliprole (20% SC) and pyridalyl (50% EC), whereas distilled water was used as a control. Bioassays were carried out under controlled conditions at 25 °C ± 2 °C, 65 ± 5% relative humidity and the light-dark period was 12 h with four replicates. The LC_{50} and LC_{25} for larvae were calculated by probit-analysis method of [19] After recording the percentage of their mortality at time-interval 24 h.

2.3. Sample preparation

After larvae of L-S strain were fed on leaves treated with the corresponding sublethal concentrations (LC_{25}) 0.05, 0.18, 3.3, and 14.5 mg. L^{-1} for insecticides, emamectin benzoat, indoxicarb, chloranitraniliprole, and pyridalyl, respectively, and LC_{25} of BK-R strain were 1.6, 2.1, 7.0, and 28.1 mg. L^{-1}, respectively, as estimated in this study, these larvae were homogenized in 10 volumes (w/v) of 0.1 M phosphate buffer pH 7.4 for one minute using a Teflon tissue homogenizer surrounded by crushed ice. The homogenate was divided into two parts: The first part was taken to determine the biochemical constitutes (total proteins, total lipids, and glycogen content), while the second was centrifuged at 8000 rpm for 20 min using a cooling centrifuge at 4 °C and the clear supernatants were used immediately for the determination of enzyme activity.

2.3.1. Sample assay

Glutathion S-transferase (GST) activity was determined according to the method of [20] using 4-chloro-1,3-dinitrobenzene (CDNB) as a substrate. Glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) were measured by the method of [21]. Proteins content was assayed using the Lowry *et al*. method [22] using bovine serum albumin (BSA) as standard protein, glycogen content and total lipids were measured by [23,24], respectively.

2.4. Statistical analysis

The data obtained from the experiments were analyzed by ANOVA test. The means were compared by Tukey's range test, in which statistically significant differences were accepted at $P < 0.05$.

3. Results

3.1. Relative toxicity of four new insecticides

Toxicity of four new insecticides against the fourth larval instar of *A. ipsilon* showed that emamectin benzoate was the most toxic, followed by indoxicarb, chloranitraniliprole, and then pyridalyl. Based on LC_{50} values obtained from a larvicidal bioassay, it is estimated that the susceptibility levels toward emamectin benzoate, indoxicarb, chlorantraniliprole and pyridalyl observed in BK-R strain was lower than those of L-S strain by 2.5-, 10.8-, 24.1-, and 51.1-fold, respectively (Table 1).

3.2. The effect of four new insecticides on content of proteins, lipids and glycogen

The results showed that there was a significant reduction in total proteins, total lipids, and glycogen content in resistant BK-R larvae lower than those in larvae for susceptible L-S or untreated (Table 2 and Fig. 1). The highest reduction was noticed in emamectin benzoate or indoxicarb treated *Agrotis* larvae, while pyridalyl or chloranitraniliprol treatment exhibited the lowest one.

Table 1. Determination of median lethal concentration LC_{50} of four new insecticides in the two strains of *A. ipsilon* larvae. The error bars represent the standard errors (±SE) of four replicates.

Treatment	LC_{50} (mg. L^{-1}) at 95 % Confidence limit	Slope (±SE)	Relative toxicity
Susceptible-Strain (L-S)			
Pyridalyl	39.32 (18.1-53.34)	1.52 ± 0.39	2.82
Indoxicarb	0.862 (0.65-1.30)	0.98 ± 0.43	0.129
Emamectin benzoate	0.111 (0.08-0.16)	2.03 ± 0.19	1.000
Chloranitraniliprole	7.20 (6.82-8.26)	1.9 ± 0.32	0.015
Resistant-Strain (BK-R)			
Pyridalyl	76.92 (53.43-94.28)	1.59 ± 0.42	0.058
Indoxicarb	7.98(3.08-13.54)	1.52 ± 0.19	0.558
Emamectin benzoate	4.45(2.51-6.42)	1.67 ± 0.22	1.000
Chloranitraniliprole	29.83 (17.57-44.31)	1.52 ± 0.68	0.149

Table 2. Proteins, lipids; and glycogen content in 4th instar larvae of the laboratory susceptible (L-S) and field resistant (BK-R) strains of *A. ipsilon* after treatment with (LC_{25}) of four new insecticides.

Treatment	Total protein[1]		Total lipids[1]		Glycogen content[1]	
	Activity	% of Control	Activity	% of Control	Activity	% of Control
Susceptible-Strain (L-S)						
Pyridalyl	23.12^{a}±1.73	92.44	18.44^{b}±2.02	94.52	21.12^{a}±2.43	89.08
Indoxicarb	15.71^{b}±2.54	62.81	15.38^{b}±2.78	78.83	19.27^{b}±2.33	81.27
Emamectin benzoate	14.34^{b}±3.37	57.34	12.21^{b}±3.15	62.58	16.05^{b}±1.16	67.69
Chloranitraniliprole	20.00^{a}±3.08	79.97	17.11^{b}±1.11	87.70	20.33^{a}±2.27	85.74
Control	25.01^{a}±2.44		19.51^{a}±1.81		23.71^{a}±4.64	
Resistant-Strain (BK-R)						
Pyridalyl	28.30^{b}±3.90	79.36	27.24^{a}±4.08	95.34	28.51^{b}±3.63	93.48
Indoxicarb	20.22^{b}±4.89	56.70	19.11^{b}±2.05	66.89	24.92^{b}±3.56	81.70
Emamectin benzoate	17.17^{c}±1.63	48.15	15.30^{b}±3.36	53.55	22.06^{b}±2.16	72.33
Chloranitraniliprole	25.31^{b}±2.45	70.98	25.77^{a}±4.59	90.20	27.12^{b}±1.48	88.92
Control	35.66^{a}±3.26		28.57^{a}±2.68		30.50^{a}±5.11	

[1.] Protein, lipids and glycogen contents are expressed as mg/g tissue.

Means ± SE followed in the same column by the same letter are not significantly different at ($P< 0.05$; Tukey's HSD test).

3.3. The effect of four new insecticides on the activity of detoxifying

Results showed that the activity levels of all evaluated enzymes, including glutathion S-transferase (GST), glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) in the treated groups of L-S strain, were significantly different from those of the treated groups of BK-R strain (Table 3 and Fig. 2). The activity level of GST was increased as well as decreased activities GOT, and GPT with significant differences were observed in the treated *A. ipsilon* larvae of BK-R strain compared with treated *A. ipsilon* larvae of L-S strain. Indoxicarb was the most inhibitory for GST while treatment of larvae with emamectin benzoate and pyridalyl resulted in pronounced inhibition of GOT and GPT, respectively.

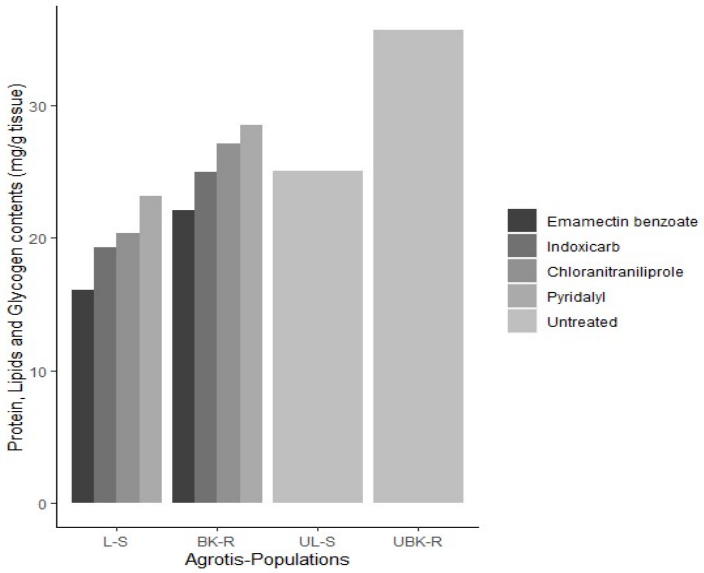


Fig. 1. Protein; lipids; and glycogen content in 4th instar larvae of the laboratory susceptible (L-S) and field resistant (BK-R) *A. ipsilon* after treatment with four new insecticides (LC_{25}).

Table 3. Activity levels of glutathion S-transferase (GST), glutamic oxaloacetic transaminase (GOT), and glutamine pyruvic transaminase (GPT) in 4th instar larvae of the laboratory susceptible (L-S) and field resistant (BK-R) *A. ipsilon* after treatment with four new insecticides (LC_{25}).

Treatment	GST[1]		GOT[2]		GPT[2]	
	Activity	% of Control	Activity	% of Control	Activity	% of Control
Susceptible-Strain (L-S)						
Pyridalyl	53.86^{c}±1.72	197.00	509.83^{c}±2.11	62.58	181.25^{d}±3.23	41.17
Indoxicarb	39.73^{b}±5.59	145.32	639.48^{b}±3.37	78.49	328.29^{b}±3.71	74.57
Emamectin benzoate	49.44^{b}±3.39	180.83	426.51^{d}±1.13	52.35	300.12^{b}±2.34	68.17
Chloranitraniliprole	43.50^{b}±1.46	159.11	620.03^{b}±2.20	76.11	251.17^{c}±4.22	57.05
Control	27.34^{a}±1.95	11	814.70^{a}±5.87		440.24^{a}±2.66	
Resistant-Strain (BK-R)						
Pyridalyl	62.30^{d}±1.65	156.93	527.65^{c}±1.95	53.37	172.15^{c}±1.48	34.17
Indoxicarb	40.02^{b}±2.11	100.81	690.33^{b}±7.75	69.82	266.27^{b}±3.56	52.85
Emamectin benzoate	53.17^{c}±4.79	133.93	458.43^{d}±1.34	46.37	228.00^{b}±2.16	45.25
Chloranitraniliprole	45.31^{b}±2.33	114.13	607.24^{b}±3.83	61.42	185.41^{c}±3.63	36.80
Control	39.70^{a}±3.41		988.67^{a}±5.61		503.83^{a}±1.32	

[1.] Activity of glutathion S-transferase (GST) is expressed as nmole of CDNB conjugated formed/min. mg protein.

[2.] Activity of glutamic oxaloacetic transaminase (GOT) or glutamine pyruvic transaminase (GPT) is expressed as unit/mg protein.

Means ± SE followed in the same column by the same letter are not significantly different at ($P< 0.05$; Tukey's HSD test).

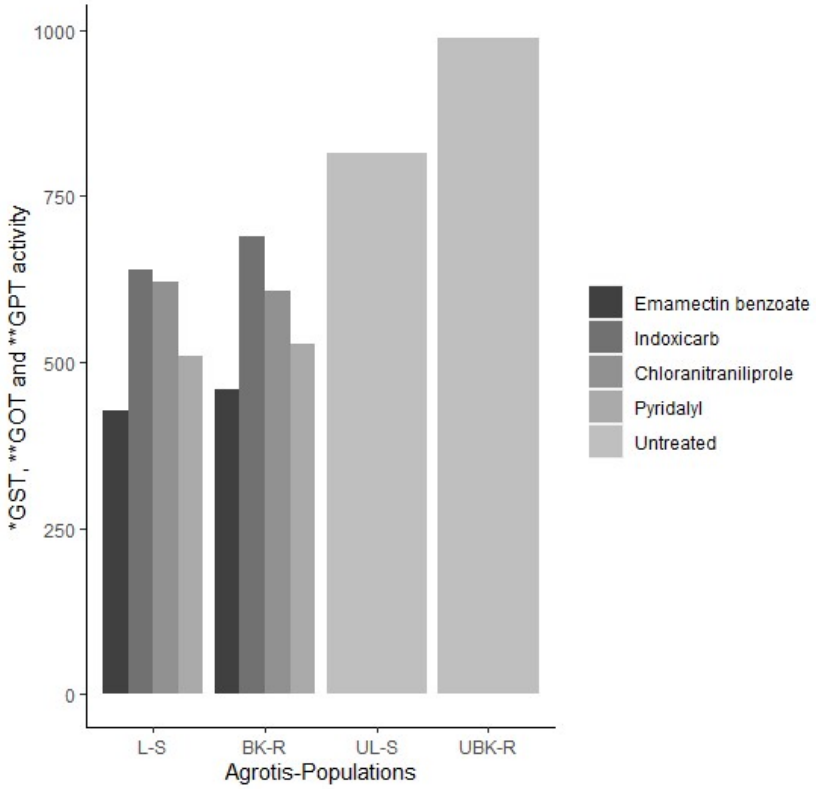


Fig. 2. Activity levels of glutathion S-transferase (GST), glutamic oxaloacetic transaminase (GOT), and glutamine pyruvic transaminase (GPT) in 4th instar larvae of the laboratory susceptible (L-S) and field resistant (BK-R) *A. ipsilon* after treatment with four new insecticides (LC_{25}).

*Activity of GST is expressed as nmole of CDNB conjugated formed/min. mg protein.

**Activity of GOT or GPT is expressed as unit/mg protein.

4. Discussion

All types of insecticides have a negative effect on the reproduction and development of the insect by cause disturbance in enzymatic equilibrium and energy reserves needed to perform different physiological processes [13]. Insects have a range of detoxification mechanisms to cope with a large diversity of toxic compounds introduced into the body for their survival. Among these mechanisms, detoxification enzymes play an important role in detoxifying these toxic compounds and cause insect of resistance [25]. This study revealed the toxicity of new insecticides; emamectin benzoate, indoxicarb, chloranitraniliprole, and pyridalyl at a sublethal concentration (LC_{25}) against both laboratory-susceptible (L-S) and field-resistant (BK-R) strains of *Agrotis ipsilon*. Therefore, in this study, we focused on the detoxification enzymes in two populations of black cutworm to assess whether this toxicity was caused by inhibits these enzymes.

The results obtained from this study showed an increase in activity of the defensive enzyme, glutathion S-transferase (GST) in insecticide-treated larvae, which may be attributed to their overproduction. [26] Çağatay et al. reported that GST plays an important role in the detoxification of insecticides by act as catalyzing the conjugation of glutathione with electrophilic compounds, and ligand-binding proteins that lead to the sequestration of xenobiotics and rendering them non-toxic to insects. [27] Le Gall

et al., reported that GST enzyme activity also plays an important role in resistance development in an insect. Ismail; Pavlidi, et al.; Hu et al. [28,29,30], found that there is an increase in GST activity in strains resistant to many Lepidopteran species such as *Spodoptera exigua*, *Spodoptera littoralis*, and *Agrotis ipsilon* compared to the susceptible strain.

Glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) are essential, critical enzymes in biological processes that play a role in the catabolism of amino acids and biosynthesis. The disturbance of transaminases from the normal value due to the toxic stress of pesticides refers to a biochemical imbalance in the tissues and cellular function because they participate in detoxification and metabolism [31]. From the data in this study, it was clear that there decreased activity of GOT and GPT in all of the insecticide-treated larvae strains compared with untreated larvae may have caused liver changes, including transaminase induction, and these hepatic changes indirectly affect the levels of hormones and various biogenic amines that act in the vital sites [32]. It may also lead to tissue damage, decreased synthesis, or catabolism of GOT and GPT, suggesting a negative effect on the physiological mechanisms of the insect [31,32].

Proteins, lipids, and glycogen are necessary substances for energy in many physiological processes of insects. The current study showed decreased proteins, lipids, and glycogen levels in all of the insecticide-treated larvae strain as a result of the insecticide-induced stress [33,34]. Lohar and Wright [35] reported that *Tenebrio molitor* suffered from the depletion of fats in the blood, lipid bodies, and eggs when exposed to malathion as a result of those lipids being converted into proteins in the detoxification mechanism against toxic substances entering the insect. Ismail [28] reported that decrease protein content might be attributed to the destruction or necrosis of cells or impaired incorporation of amino acids into polypeptide chains, consequent impairment in protein synthesis machinery by its tool (amino acids) of the detoxification mechanism, where the protein helps to synthesize microsomal detoxifying enzyme, contributing to detoxify the toxicants that entered into the insect body or through imbalance between the rate of protein synthesis and the rate of biodegradation [36]. Sak et al. [37] demonstrated the glycogen depletion in *Pimpla turionellae* appears as a result of cypermethrin-induced effects on the glycolysis pathway. Based on our results, it was observed that the effect on GST, GOT, and GPT, as well as contents of protein, lipids, and glycogen, were higher in the resistant strain than susceptible strain (Figures 1 and 2) which could be a reason for adverse effects of insect. According to these results, new insecticides have potential applications for the integrated management of *A. ipsilon*.

Conclusions

Biochemical parameters in the susceptible and resistant strains of treated *Agrotis ipsilon* larvae showed alterations of key enzymes responsible for detoxification, including glutathion S-transferase (GST) Glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) that paralleled with relative changes of total proteins, total lipids, and glycogen content. The deviation of the results from normal values and the unbalance of biochemical targets which could be a reason for adverse effects of insect may indicate a possible role for new insecticides (emamectin benzoate, indoxicarb, chloranitraniliprole, and pyridalyl) as a novel chemical application and alternative to conventional insecticides for managing black cutworm resistance in control programs.

Declarations

Ethics approval and consent to participate

The manuscript does not contain any studies involving human participants, human

data or human tissue.

Consent for publication
Not applicable.

Availability of data and materials
All data generated during this study are included in this published article.

Competing interests
The author declares that there are no competing interests.

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Author's contributions
The author contributed to the production and writing of the manuscript.
The author(s) read and approved the final manuscript.

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References

[1] FL Fernandes, JFS Diniz, PR Silva, E Mosca, Damage of *Agrotis ipsilon* (Lepidoptera: Noctüidae) on Coffea Arabica in Brazil. Revista Colombiana de Entomología, 39 (2013) 49-50. ID: 83306108.

[2] F He, S Sun, H Tan, X Sun, C Qin, S Ji, X Li, J Zhang, X Jiang, Chlorantraniliprole against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctüidae): From biochemical/physiological to demographic responses. Scientific Reports, 9 (2019) 10328.
https://doi.10.1038/s41598-019-46915-0

[3] SM Ismail, Field persistence of certain new insecticides and their efficacy against black cutworm, *Agrotis ipsilon* (Hüfnagel). Bulletin of the National Research Centre, 45 (2020) 17–24.
https://doi.org/10.1186/s42269-020-00481-y

[4] MJ Joshi, A Rana, PV Raj, S Kaushal, AG Inamdar, KS Verma, RS Chandel, The potency of chemical insecticides in management of cutworm, *Agrotis ipsilon* Hüfnagel (Noctüidae: Lepidoptera): A review, Journal of Entomology and Zoology Studies 8 (2020) 307-311.

[5] C Xu, Z Zhang, K Cui, Y Zhao, J Han, F Liu, W Mu, Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctüidae). PLoS One 1 (2016) e0156555.
https://doi.org/10.1371/journal.pone.0156555

[6] Rimpy, SK Verma, Efficacy of novel insecticides against cutworms (Lepidoptera: Noctüidae) infesting cabbage. International Journal of Chemical Studies, 6 (2018) 824-827.

[7] Van den Bosch TJM, Welte CU, Detoxifying symbionts in agriculturally important pest insects. Microbial Biotechnology, 10 (2017) 531-540.
https://doi.org/10.1111/1751-7915.12483

[8] MAH Kandil, EA Sammour, NF Abdel-Aziz, EA Agamy, AM El-Bakry, NM Abdelmaksoud, Comparative toxicity of new insecticides generations against tomato leafminer *Tuta absoluta* and their biochemical effects on tomato plants. Bulletin of the National Research Centre, 44 (2020) 126.
https://doi.org/10.1186/s42269-020-00382-0

[9] DF Grant, F Matsumura, Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti*. Pesticide Biochemistry and Physiology, 33 (1989) 132-143.
https://doi.org/10.1016/0048-575(89)90004-7

[10] J Wu, J Li, C Zhang, X Yu, AGS Cuthbertson, S Ali, Biological impact and enzyme activities of *Spodoptera litura* (Lepidoptera: Noctüidae) in response to synergistic action of Matrine and *Beauveria brongniartii*, Invertebrate Physiology, 11 (2020) 584405.
https://doi.10.3389/fphys.2020.584405

[11] WF Tordior, EAH VanHeemstra-Leqin, Field studies monitoring exposure and effect in the development of pesticides. Elsevier: Amsterdam, Oxford, New York, (1980) eBook ISBN: 9780080874777

[12] R Buès, JC Bouvier, L Boudinhon, Insecticide resistance and mechanisms of resistance to selected strains of *Helicoverpa armigera* (Lepidoptera: Noctüidae) in the south of France, Crop Protection, 24 (2005) 814–820. https://doi.org/10.1016/j.cropro.2005.01.006

[13] MM Khan, RM Kaleem-Ullah, JA Siddiqui, S Ali, Insecticide resistance and detoxification enzymes activity in *Nilaparvata lugens* Stål against neonicotinoids. Journal of Agricultural Science, 12 (2020) 24. https://doi.org/10.5539/jas.v12n5p24

[14] S Chowanski, J Lubawy, M Spochacz, P Ewelina, S Grzegorz, G Rosinski, M Slocinska, Cold induced change in lipid, protein and carbohydrate levels in the tropical insect *Gromphadorhina coquereliana*, Comparative Biochemistry and Physiology, Part A, 183 (2015) 57-63. https://doi.10.1016/j.cbpa.2015.01.007

[15] NGC Ferreira, R Morgado, MJG Santos, AMVM Soares, S Loureiro, Biomarkers and energy reserves in the isopod *Porcellionides pruinosus*: the effects of long-term exposure to dimethoate. Science of the Total Environment, 502 (2015) 91-102. https://doi.10.1016/j.scitotenv.2014.08.062

[16] SM Ismail, ShH Sameh, MA El-Malla, MM Abdel-Sattar, Influences of sublethal dose of certain insecticides on biological and biochemical against *Spodoptera littoralis* (Boisdüval). Egyptian Journal of Plant Protection Research, 6 (2018) 1-16.

[17] AL Lutz, I Bertolaccini, RR Scotta, MC Curis, MA Favaro, LN Fernandez, DE Sánchez, Lethal and sublethal effects of chlorantraniliprole on and on *Spodoptera cosmioides* (Lepidoptera: Noctüidae). Pest Management Science, 74 (2018) 2817–2821. https://doi.10.1002/ps.5070

[18] M Nawaz, W Cai, Z Jing, X Zhou, JI Mabubu, H Hua, Toxicity and sublethal effects of chlorantraniliprole on the development and fecundity of a non- specific predator, the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas). Chemosphere, 178 (2017) 496-503. https://doi.10.1016/j.chemosphere.2017.03.082

[19] DJ Finney, Probit analysis, 3rd edn. Cambridge Univ. Press, Cambridge, England, (1971) p 318.

[20] DA Vessey, TD Boyer, Differential activation and inhibition of different forms of rat liver glutathione S-transferase by the herbicides 2,4-dichlorophenoxy acetate (2,4-D) and 2,4,5-trichlorophenoxy acetate (2,4,5-T). Toxicology and Applied Pharmacology, l73 (1984) 492–499. https://doi.10.1016/0041-008x(84)90101-7

[21] S Reitman, SA Frankel, colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. American Journal of Clinical Pathology, 28 (1957) 56-36. https://doi.10.1093/ajcp/28.1.56

[22] OH Lowry, JN Rosebrough, LAL Farry, JR Randall, Protein measurements with folin phenol reagent. Journal of Biological Chemistry, 193 (1951) 265-271. PMID: 14907713

[23] E Van Handel, Microseparation of glycogen, sugars, and lipids. Analytical Biochemistry, 11 (1965) 266-271. https://doi.org/10.1016/0003-2697(65)90014-X

[24] JA Knight, S Anderson, JM Rawle, Chemical basis of the sulphophosphat-vanilin reaction for estimating total serum lipids. Clinical Chemistry, 18 (1972) 199-202. PMID: 5020813

[25] X Wang, SK Khakame, C Ye, Y Yang, Y Wu, Characterisation of field-evolved resistance to chlorantraniliprole in the diamondback moth, *Plutella xylostella*, from China. Pest Management Science, 69 (2013) 661–665. https://doi.org/10.1002/ps.3422

[26] NS Çağatay, P Menault, M Riga, J Vontas, R Ay, Identification and characterization of abamectin resistance in *Tetranychus urticae* Koch populations from greenhouses in Turkey. Crop Protection, 112 (2018) 112–117. https://doi.org/10.1016/j.cropro.2018.05.016

[27] VL Le Gall, GM Klafke, TT Torres, Detoxification mechanisms involved in ivermectin resistance in the cattle tick, *Rhipicephalus* (Boophilus) *microplus*. Scientific Reports, 8 (2018)1–10.
https://doi.org/10.1038/s41598-018-30907-7

[28] SM Ismail, Effect of sublethal doses of some insecticides and their role on detoxication enzymes and protein-content of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctüidae). Bulletin of the National Research Centre, 44 (2020) 35-41.
https://doi.org/10.1186/s42269-020-00294-z

[29] N Pavlidi, J Vontas, TV Leeuwe, The role of glutathione S-transferases (GSTs) in insecticide resistance in crop pests and disease vectors. Current Opinion in Insect Science, 27 (2018) 97-102.
https://doi.10.1016/j.cois.2018.04.007

[30] B Hu, S Hu, H Huang, Q Wei, M Ren, S Huang, X Tian, J Su, Insecticides induce the co-expression of glutathione S-transferases through ROS/CncC pathway in *Spodoptera exigua*. Pesticide Biochemistry and Physiology, 155 (2019) 58-71.
https://doi.org/10.1016/j.pestbp.2019.01.008

[31] EE. Enan, IG. Berberian, Interaction of pesticide exposure level with some biochemical enzymes among field workers. Journal of the Egyptian Society of Parasitology, 3 (1986) 76-90.

[32] KS. Hamadah, Disturbance of phosphatase and transaminase activities in of the red palm weevil. *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) by certain insecticidal compounds. JoBAZ 80 (2019) 52.
https://doi.org/10.1186/s41936-019-0123-1

[33] EL Arrese, JL Soulages, Insect fat body: Energy, metabolism and regulation. Annual Review of Entomology, 55 (2010) 207-225.
https://doi.org/10.1146/annurev-ento-112408-085356

[34] S Suchail, AL Navenant, Y Capowiez, A Thiéry, M Rault, An exploratory study of energy reserves and biometry as potential tools for assessing the effects of pest management strategies on the earwig, *Forficula auricularia* L. Environmental Science and Pollution Research, 25 (2018) 22766-22774.
https://doi.10.1007/s11356-018-2371-x

[35] MK Lohar, DJ Wright, Changes in the lipid content in heamolymph, fat body and oocytes of malathion treated *Tenebrio molitor* L. adult females. Pakistan Journal of Zoology, 25 (1993) 57-60.

[36] RR Binning, J Coats, X Kong, RL Hellmich, Susceptibility to Bt protein is not required for *Agrotis ipsilon* aversion to Bt maize. Pest Management Science, 71 (2015) 601-606.
https://doi.10.1002/ps.3901

[37] O Sak, F Uckan, E Eegin, Effects of cypermethrin on total body weight, glycogen, protein, and lipid contents of *Pimpla turionellaei* (L.) (Hymenoptera: Ichneumonidae). Belgian Journal of Zoology, 136 (2006) 53-58.

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