Development and enzyme activity of the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) treated with Dual-action synthetic insecticides

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Abstract

Background: The uncontrolled excessive usage of insecticides in agriculture to manage pests leads to considerable side effects such as resistance, regeneration, and residue. The present study aimed to evaluate the toxicity of environmentally-safe mixtures pesticides belonging to different groups (Extreme 36% SC (methoxyfenozide 30% + Spinetoram 6%), Atifos-super 55% EC (chlorpyrifos 50% + cypermethrin 5%), and Tempo-xl 30% EC (chlorpyrifos 25% + lufenuron 5%) against the 4th instar larvae of *Agrotis ipsilon* using leaf-dip method. The current data indicated that Tempo-xl recorded the highest efficacy against treated larvae with LC_{50}, followed by Extreme and Atifos-super (4.09, 05.03, and 9.78 ppm, respectively). Moreover, the percentage of pupation, adult emergence, growth index, fecundity, and egg hatching exhibited a significant decrease; also, the larval and pupal weights detected a significant reduction. The biochemical analysis showed that the tested compounds significantly enhanced the total protein, α- and β- esterase, acetylcholinesterase, protease, and chitinase. Therefore, Tempo-xl could be recommended as an eco-friendly alternative to synthetic insecticides for the appropriate integrated pest management (IPM) program of *A. ipsilon*.

Keywords: mixture biocides, Extreme, Atifos-super, Tempo-xl, *Agrotis ipsilon*, biological, biochemical.

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Introduction

Insects are known to be sensitive to the climate changes. Over the years, the uncontrolled and intensive application of conventional pesticides has commanded to diverse problems such as climate change, contamination of fish, birds, beneficial insects, non-target plants, water, air, soil, and crops, also biodiversity loss of natural enemies and insect impedance to various insecticides (Tirado et al, 2010; Tudi et al, 2021). These pesticides residues influence human health risks via the environment and food contamination (Shahzad et al, 2020).

The black cutworm, *A. ipsilon* (Hufnagel, 1766; Lepidoptera: Noctuidae), is a polyphagous, severe, and destructive insect pest in the world. It is a nocturnal pest, widely distributed in temperate regions (Abdou & Abdel-Hakim, 2017; Rodingpuia & Lalthanzara, 2021). Adults feed on plant nectar and corn; however, larvae attack the young plants during the growing stage by cutting seedlings or excavating an underpass in old plant roots leading to destruction and considerable economic losses in the yield reach to 100% in some cases (Abd-El-Aziz et al, 2019; Wang et al, 2021). Many farmers use organophosphorus, pyrethroids, and carbamate methyl to control this severe pest, which developed high impedance to them because of high-frequency

applications and exposure to high concentrations (Binning et al, 2014). Investigators and manufacturers should be examined the efficient and secure alternative combinations for this pest and eco-friendly for human health according to the IPM protocols (Veres et al, 2020). So, using a mixture of insecticides induced a higher larval decrease than the identical insecticide alone (Xu et al, 2016).

Methoxyfenozide is considered a Reduced Risk Pesticide (RR) and organophosphates alternative (OPs) according to Environmental Protection Agency (EPA) because it is an eco-friendly compound with fewer toxic effects on mammals, natural enemies, and beneficial insects (Shah et al, 2015). Spinetoram is a mixture of two spinosyns which are bio-insecticides based on the fermentation product of the soil actinomycete bacterium *Saccharopolyspora spinosa* (Sparks et al, 2008). Spinetoram is considered RR according to EPA (Bacci et al, 2016). Chlorpyrifos plays a vital role; in pest control throughout variable crops, fruits, and vegetables (Kopjar et al, 2018). Cypermethrin is globally utilized for controlling cotton, fruit, and vegetable pests; because of its moderately residual impacts, efficacy at low rates, and lower cost, this pesticide is frequently overused and induces environmental problems (Mohammadi et al, 2019). Lufenuron (Match 5%) is diverse in combination between chitin synthesis inhibitors (CSIs) or Insect Growth regulator (IGR) that interferes with chitin synthesis, disrupts the hormonal balance in the molting process, and inhibits the insect's growth (Gelbic et al, 2011).

The current study aimed to investigate the effectiveness of three mixtures of pesticides Extreme 36% SC (methoxyfenozide 30% + spinetoram 6%), Atifos-super 55% EC (chlorpyrifos 50% + cypermethrin 5%), and Tempo-xl 30% EC (chlorpyrifos 25% + lufenuron 5%) on the toxicity, biology, and biochemistry of the black cutworm, *A. ipsilin* as a step towards a better understanding of the nature of their action aiming at extending their useful life in controlling many harmful pests.

MATERIALS AND METHODS

Insects rearing

Eggs of *A. ipsilon* were obtained from the colony of the Plant Protection Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt, without exposure to any insecticides. The hatched larvae were kept in glass jars (1 L) with fresh castor oil leaves (*Ricinus communis* L.) for feeding. The third-instar larvae were reared individually to avoid larval cannibalism by putting them in plastic cell trays. Each tray contained 18 separated cells, each cell having five tiny pores on its outer lateral walls for aeration, and its bottom was covered with glass sheets to prevent larval escape and with sawdust to reduce moisture until pupation (He et al, 2019). Five couples of female and male moths were kept in a glass jar (2 L) with a hanging piece of cotton wet with honey solution (10%) for feeding. Strips of dark or white cloth were fixed in the muslin as hanging sites for egg deposition, which were collected daily. All the rearing procedures were performed under laboratory conditions at 25 ± 2 °C and 65 ± 5% relative humidity (RH) with a photoperiod of 12:12 h (L:D), according to (Ahmed et al, 2013; Abdou & Abdel-Hakim, 2017).

Tested Materials

Table 1. The tested mixture pesticides according to the Agricultural Pesticides Committee (APC, 2020) and their classification used to monitor mortality rates.

Reg. No., Trade name, Conc. & production company	Common name	Main group& Molecular Formula	IRAC group	Sub-group or Active Ingredient	Primary site of action/ MoA	WHO Toxicity classification	IUPAC name
Extreme (36% SC) from DOW AgroSciences-UK	Methoxyfenozide 30% + Spinetoram 6%	Methoxyfenozide C22H28N2O3 Spinetoram C42H69NO10	Growth and Development Targets 18	Diacylhydrazines	EcR agonists IGR (Mimic the moulting hormone, ecdysone, inducing a precocious moult) (Arruda et al, 2020;	U (Warning toxic to mammals)	**Methoxyfenozide** N'-tert-butyl-N'-(3,5-dimethylbenzoyl)-3-methoxy-2 methylbenzohydrazide **Spinetoram** (2R,5R,9R,10S,14R,15S,19S)-15-[(2R,5S,6R)-5-(dimethylamino)-6-methyloxan-2-yl]oxy-7 [(2R,3R,4R,5S,6S)-4-ethoxy-3,5 dimethoxy-6-methyloxan-2-yl]oxy-19-ethyl-14-methyl-20

			Nerve and Muscle Targets 5	Spinosyns	Hamaidia & Soltani, 2021) nAChR allosteric modulators – Site I (Nerve action) (Causing hyper excitation of the nervous system. Acetylcholine is the major excitatory neurotransmitter in the insect central nervous system) (Salgado & Sparks, 2010)		oxatetracyclo[10.10.0.02,10.05,9]docos-11-ene-13,21-dione
2660 **Atifos -super** (55% EC) from Starchem Industrial Chemicals-Egypt	Chlorpyrifos 50%+ Cypermethrin 5%	Chlorpyrifos C9H11Cl3NO3PS Cypermethrin C22H19Cl2NO3	Nerve and Muscle Targets 1 Nerve and Muscle Targets 3	1B Organophosphate 3A Pyrethroid	AChE inhibitors (Nerve action) (Mehta et al, 2009) VGSC modulators (Nerve action) (Raszewski et al, 2015; Meslin et al, 2021)	Mod II (Harmful toxic to mammals) Moderately hazardous	**Chlorpyrifos** diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ5-phosphane **Cypermethrin** [cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate
2460 **Tempo- xl** (30% EC) from Starchem Industrial Chemicals-Egypt	Chlorpyrifos 25% + Lufenuron 5%	Chlorpyrifos C9H11Cl3NO3PS Lufenuron C17H8Cl2F8N2O3	Nerve and Muscle Targets 1 Growth and Development Targets 15	1B Organophosphate Benzoylureas	AChE inhibitors (Nerve action) (Mehta et al, 2009) CSI, Growth regulation (Inhibit the enzyme that catalyzes the polymerization of Chitin.) (Gelbic et al, 2011)	Mod II (Harmful toxic to mammals) Moderately hazardous	**Chlorpyrifos** diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ5-phosphane **Lufenuron** N-[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]carbamoyl]-2,6-difluorobenzamide

Reg.No., registration number; Conc., concentration; SC, suspension concentrate = (flowable concentrate); EC, emulsifiable concentrate; IRAC group, insecticide resistance action committee group; MoA : Mode of action classification; EcR, ecdysone receptor; IGR, Insect Growth regulator; nAChR, nicotinic acetylcholine receptor; AChE, Acetylcholinesterase; VGSC, voltage gated sodium channel; CSI, chitin synthesis inhibitor; WHO, world health organization; IUPAC name according to PubChem (2021) https://pubchem.ncbi.nlm.nih.gov, International Union of Pure and Applied Chemistry name.**Toxicity study**

Bioassay

The leaf dipping method was used to determine the LC_{25}, LC_{50}, and LC_{90} of the three tested pesticides on *A. ipsilon*, according to (Abo El-Ghar et al, 1994). Castor plant leaves (3.5 cm in diameter) were immersed in seven concentrations (diluted with distilled water) of Extreme, Tempo-xl, and Atifos-super for 10 s and then allowed to dry at room temperature (29 ± 2 °C) for one h. Sixty of the newly hatched 4th instar larvae were placed in glass jars covered with a clean muslin cloth and divided into four replicates (15 larvae / replicate) for each concentration of each tested pesticide. The larvae were starved for four hrs. then were allowed to feed on the treated leaves for 24 hrs. then the survived larvae were transferred to clean jars with new untreated castor leaves. Moreover, analogous replicates of the similar larvae number fed on untreated castor oil leaves dipped in distilled water were used as a control. The mortality was recorded daily and then corrected the mortality percentage after 24, 48, and 72 h.

Biological Studies

Newly moulted fourth instar larvae were treated with the LC_{50} concentration of Extreme 36% SC, Atifos-super 55% EC, and Tempo-xl 30% EC. Treated larvae were checked daily to determine the effect of post-treatment on surviving insects (larval, pupal, and adult longevity durations, larval and pupal weights, larval mortality, pupation, adult emergence, sterility, growth index, hatchability, and fertility percentages). These parameters were compared with untreated control larvae.

Biochemical Studies

Preparation of Samples for Biochemical Studies

The fourth instar larvae of *A. ipsilon* were fed on castor oil leaves immersed in LC_{50} of Extreme, Atifos-super, and Tempo-xl for 24 hr. The survival of treated larvae was transformed into plastic jars and fed on clean leaves until reaching the 6th instar larvae. Homogenates were collected from treated as well as untreated 6th instar larvae by homogenizing in insect-appropriate buffer (100 mM phosphate buffer, pH 7.0 containing one mM EDTA, one mM PTU, one mM PMSF, and 20% glycerol) using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. The samples were centrifuged at 4500 rpm for 5 minutes under cooling (4°C) (Biofuge 28RS Heraeus, Sepatech centrifuge) to remove the remnant of tissues. The resulting supernatants were collected and divided into small aliquots (0.5 ml) and stored at -20 °C until analysis. All biochemical tests were estimated in the Physiology Department, Plant Protection Research Institute, Dokki, Egypt.

Determination of Protein Content and Enzyme Activities

The total protein content in larvae treated with LC_{50} homogenate was determined after six days post-treatment according to the method estimated by (Bradford, 1976). The activity of both α and β (EC 3.1.1.43 & EC 3.1.1.1) non-specific esterases was determined according to (Van Asperen, 1962) by using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively. Also, the activity of the enzyme acetylcholine esterase (AChE, EC 3.1.1.7) was measured using acetylcholine bromide (AChBr) as substrate at a level of 6 x 10-3 M according to (Simpson et al, 1964). Moreover, the protease enzyme (EC 3.4.21.112) activity was determined by the casein digestion method as described by (Ishaaya et al, 1971). Furthermore, Chitinase (EC 3.2.1.14) was examined by using (3, 5-dinitrosalicylic acid reagent) to determine the free aldehydic groups of hexosamine liberated on chitin digestion (Ishaaya & Casida, 1974).

Statistical Analysis

Abbott's formula was used to correct the percent mortality rate of treated *A. ipsilon* (Abbott, 1925) and subjected to Finney probit analysis (Finney, 1971) to estimate the LC_{25}, LC_{50}, and LC_{90} values. All data were subjected to one-way analysis of variance (ANOVA), followed by the Duncan multiple range F-test to determine the significant differences among the treatment means at probit $P < 0.05$ (Duncan, 1955). The analyses were performed by SPSS 11 computer program (Snedecor & Cochran, 1980).

RESULTS

Mortality rates of *A. ipsilon* to different pesticides

The data obtained indicated that the highest corrected mortality percent of the 4th instar larvae of *A. ipsilon* was caused by the highest concentration of pesticides and vice versa after 24, 48, and 72 h. for Extreme, Atifos-super, and Tempo-xl pesticides, respectively (Table 2).

Table 2. Susceptibility of the newly hatched 4th instars larvae of *A. ipsilon* after 24, 48 & 72 h. post treatment with different concentrations of Extreme, Tempo-xl, and Atifos-super.

		Corrected mortality %		
Conc. (ppm)	**pesticides**	**24 hours**	**48 hours**	**72 hours**
40	Extreme	82.76	91.07	98.21
	Tempo-xl	86.21	96.43	98.18

20	Extreme	81.04	89.29	96.43
	Tempo-xl	84.48	94.64	98.18
10	Extreme	75.86	92.86	94.65
	Tempo-xl	81.05	92.86	98.18
5	Extreme	70.69	89.29	91.07
	Tempo-xl	79.31	91.07	96.36
2.5	Extreme	67.24	82.14	89.29
	Tempo-xl	75.86	89.29	94.55
1.25	Extreme	55.17	82.14	92.86
	Tempo-xl	74.14	87.5	92.73
0.625	Extreme	53.45	80.36	89.29
	Tempo-xl	72.41	85.71	90.91
60.5	Atifos-super	91.38	94.64	98.18
30.25	Atifos-super	87.93	92.86	96.36
15.12	Atifos-super	86.21	91.07	94.55
7.56	Atifos-super	84.48	89.29	92.73
3.7	Atifos-super	82.76	87.50	90.91
1.84	Atifos-super	81.03	85.71	89.09
0.945	Atifos-super	75.86	85.71	87.27

Conc.: Concentration level (ppm), ppm: parts per million

Relative toxicity of different pesticides against *A. ipsilon*

The least toxicity values of the sub-lethal concentrations (LC_{25}, LC_{50} & LC_{90}) were displayed for Atifos-super (3.05, 9.78 & 89.82, respectively) (Fig. 2). Meanwhile, the highest toxicity values were recorded for Tempo-xl (1.81, 4.09 & 19.28 ppm, respectively) (Fig. 3). The current results also showed that Tempo-xl was the most toxic in the various values followed by Extreme (Fig. 1) and Atifos -super.

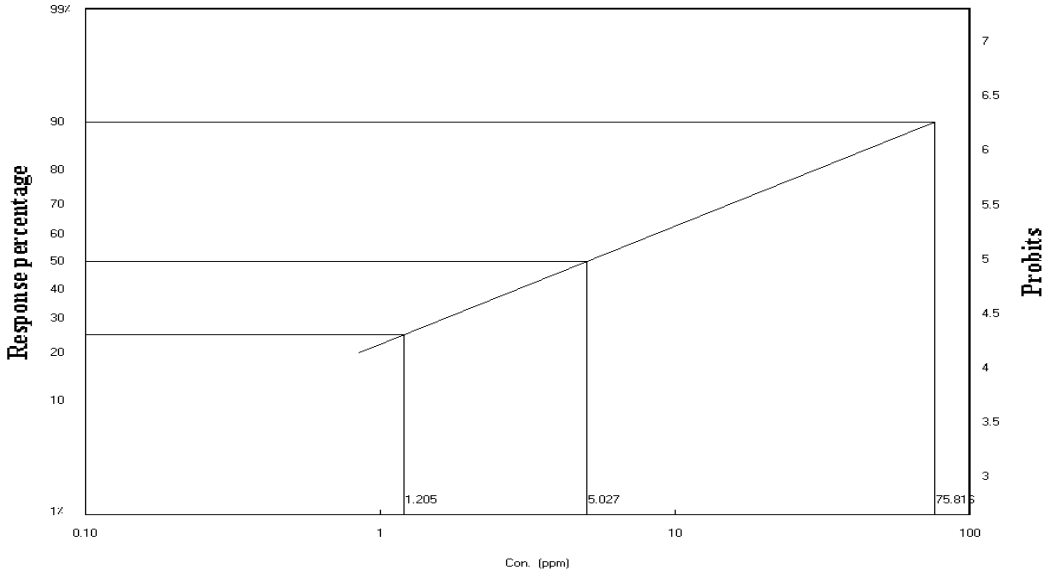

Fig. 1. Toxicity regression line of Extreme against 4th instar larvae of *A. ipsilon* that calculated LC_{25}, LC_{50}, and LC_{90} values.

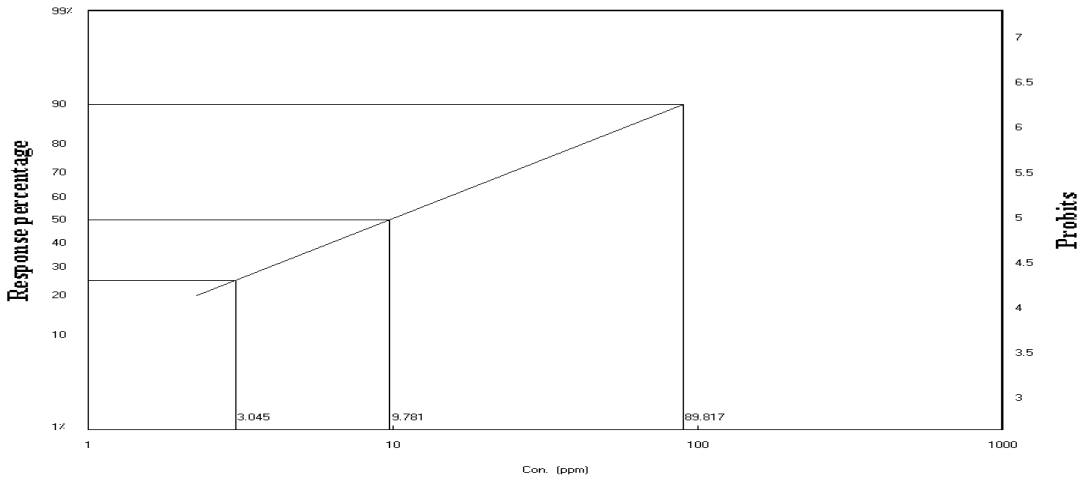

Fig. 2. Toxicity regression line of Atifos -super against 4th instar larvae of *A. ipsilon* that calculated LC_{25}, LC_{50}, and LC_{90} values.

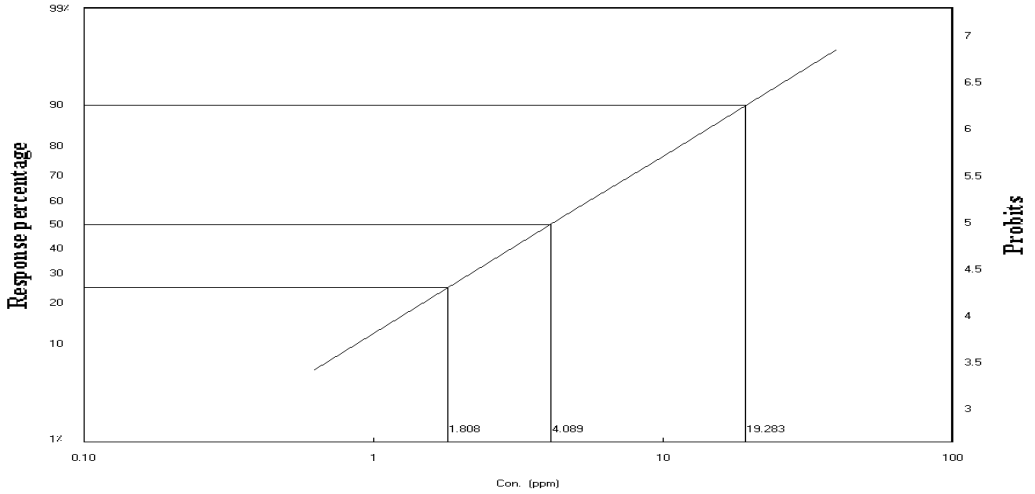

Fig. 3. Toxicity regression line of Tempo-xl against 4th instar larvae of *A. ipsilon* that calculated LC_{25}, LC_{50}, and LC_{90} values.

Biological studies

Growth and development of treated *A. ipsilon* with LC_{50} of the tested pesticides as revealed in (Table 3)

Data obtained showed a non-significant prolongation at $P \geq 0.05$ in the mean larval duration of the 4th instar larvae treated with LC_{50} for Atifos-super and Extreme compared to the control; meanwhile, a non-significant shortage was observed for Tempo-xl. Moreover, a non-significant increase was observed in the mean pupal durations for Tempo-xl followed by Extreme and Atifos-super. Furthermore, the pupation percentage exhibited a significant reduction at $P < 0.05$ for Tempo-xl, Atifos-super, and Extreme. Also, a significant decrease was detected in the adult emergence percentage for Extreme, Tempo-xl, and Atifos-super. In addition, adult longevity showed a significant reduction for Extreme; meanwhile, a significant increase was observed for Atifos-super. Moreover, data obtained showed a significant decline in the adult growth index after treatment with Atifos-super followed by Tempo-xl and Extreme, respectively. The obtained data indicated a significant decrease in the larval weights treated with Tempo-xl and a non-significant reduction for Extreme and Atifos-super. Moreover, a non-significant decrease in the pupal weights was detected for Extreme followed by Tempo-xl and Atifos-super, respectively.

Table 3. Growth and development of the 4th instar larvae *A. ipsilon* treated with LC_{50} of the three tested pesticides.

Pesticides	Larval duration (Days) Mean ± SE	Pupal duration (Days) Mean ± SE	Pupation % Mean ± SE	Adult emergence % Mean ± SE	Adult longevity (Days) Mean ± SE	Growth index % Mean ± SE	Larval weight (Gm.) Mean ± SE	Pupal weight (Gm.) Mean ± SE
Control	14.75± 1.49^{a}	11.75±1 .49^{a}	96.32± 0.48^{a}	92.52± 1.39^{a}	13.00± 1.08^{ab}	7.28± 0.67^{a}	0.51± 0.02^{a}	0.31± 0.04^{a}
Extreme	17.00± 1.73^{a}	13.25±1 .89^{a}	76.25± 1.65^{b}	24.5± 1.32^{b}	8.75± 2.52^{b}	4.13± 1.77^{b}	0.37± 0.03^{ab}	0.28± 0.01^{a}
Atifos-super	18.50± 1.44^{a}	12.00±1 .78^{a}	41.34± 1.43^{c}	8.66± 0.62^{c}	16.25± 1.32^{a}	0.54± 0.05^{c}	0.41± 0.02^{ab}	0.31± 0.03^{a}
Tempo-XL	13.75± 1.49^{a}	14.25±2 .46^{a}	35.15± 1.20^{d}	24.29± 2.11^{b}	12.00± 1.47^{ab}	2.12± 0.29^{bc}	0.27± 0.09^{b}	0.29± 0.02^{a}

Each value represents mean of four replicates; SE = Standard error; Gm. = Grams; within a column, means possessing the same letter differ non-significantly at $P \geq 0.05$

Activity of the tested pesticides at LC_{50} on the reproductive potency of *A. ipsilon*.

The treated 4th instar larvae showed a significant reduction in the mean number of eggs laid by females and the percentage of egg hatching for Tempo-xl, Extreme, and Atifos-super as compared to control (Table 4). However, a significant rise was revealed in the percentage of sterility index for Tempo-xl, Extreme, and Atifos-super, respectively as compared to control which recorded zero (Table 4).

Table 4. Reproductive potency of the 4th instar larvae *A. ipsilon* treated with LC_{50} of the three tested pesticides.

Pesticides	Fecundity Mean ± SE	Egg hatching% Mean ± SE	Non-hatching eggs% Mean ± SE	Sterility index % Mean ± SE
Control	1693.75±106.16^{a}	89.13±5.26^{a}	10.87±5.26^{b}	0.0±0.0^{b}
Extreme	182.00±41.09^{b}	21.39±1.79b^{c}	78.61±1.79^{ab}	97.42±0.42^{a}
Atifos-super	222.25±65.23^{b}	31.18±13.36^{b}	68.82±13.36^{a}	95.41±2.12^{a}
Tempo-XL	173.00±73.39^{b}	5.85±3.91^{c}	94.15±3.91^{a}	99.33±1.03^{a}

Each value represents mean of four replicates; SE = (Standard error); within a column, means possessing the same letter differ non-significantly at $P \geq 0.05$

Biochemical studies

The obtained data showed that Tempo-xl followed by Extreme and Atifos-super caused a significant accumulation ($P < 0.05$) in total protein content 25.37±0.01, 24.75±0.025, and 24.48±0.01 mg/gm in larvae of *A. ipsilon* as compared to control (22.19±0.05 mg/gm) (Fig. 4). Moreover, a significant increase was exhibited in the activity of α-esterase in Atifos-super followed by Tempo-xl and Extreme (436.45±4.32, 302.14±4.88, and 190.34±4.64 µg α-Naphthol/min/ml, respectively) as compared to control (87.72±2.48 µg α-Naphthol/min/ml) (Fig. 5). Additionally, the current results revealed a significant augmentation in the activity of β-esterase (384.17±2.50, 309.14 ± 1.30, 199.32± 1.88 µg β-Naphthol/min/ml for Atifos-super, Tempo-xl, and Extreme, respectively) as compared to control (73.89±2.52 µg β-Naphthol/min/ml) (Fig. 6). Also, data obtained exhibited that the highest activity of acetyl-cholinesterase were observed in larvae treated

with Atifos-super (966.34±1.49 µg Acetyl-cholinebromide/min/ml) followed by Tempo-xl and Extreme (718.48±12.02 and 504.88±0.5 µg Acetyl-cholinebromide/min/ml, respectively) as compared to the control (324.77 ± 3.55 µg Acetyl-cholinebromide/min/ml) (Fig. 7). Moreover, the activities of proteases significantly raised in larvae treated with Atifos-super and Extreme (610.9± 22.42 and 580.4 0± 9.65 O.D. units x 103 /min. / ml); meanwhile, it was non-significantly increased at $P \geq 0.05$ in larvae treated with Tempo-xl (531.7 0± 12.32 O.D. units x 103 /min. / ml) as compared to control (511.30 ± 7.97 O.D. units x 103 /min. / ml) (Fig. 8). Furthermore, a significant increase in the chitinase activity was revealed due to the effect of Tempo-xl followed by Extreme and Atifos-super (29.78±1.17, 20.75 ±1.01 and 14.52±0.22 µg NAGA/ min /ml, respectively) as compared to untreated larvae (12.58±0.09 µg NAGA/ min /ml) (Fig. 9).

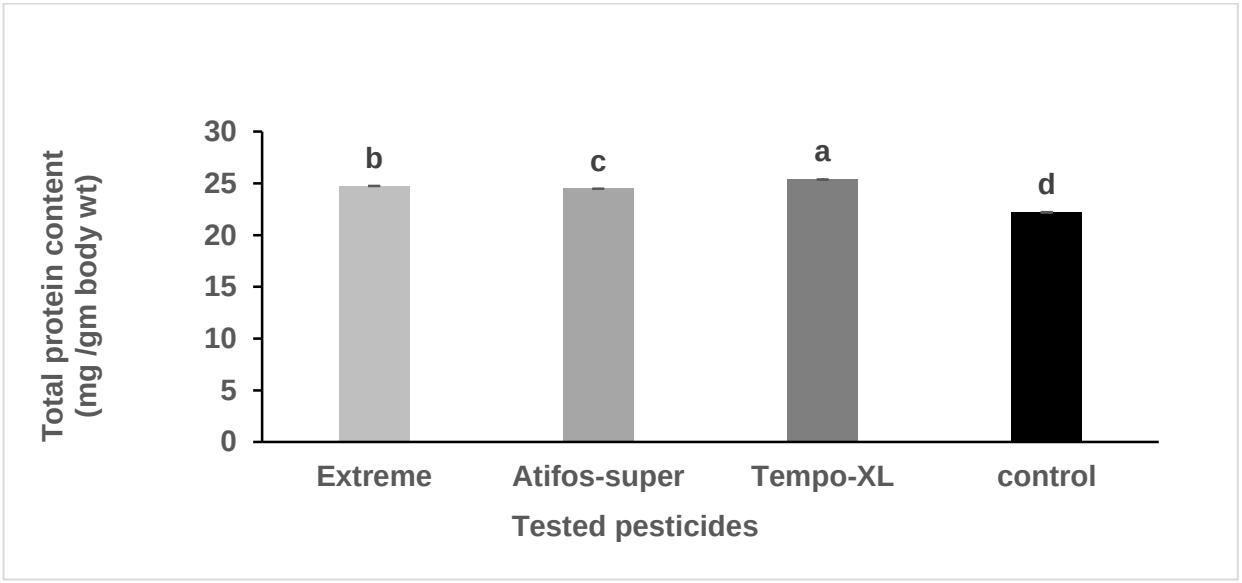

Fig. 4. Effects of LC_{50} tested pesticides on total protein content of 4th instar larvae of *A. ipsilon*.

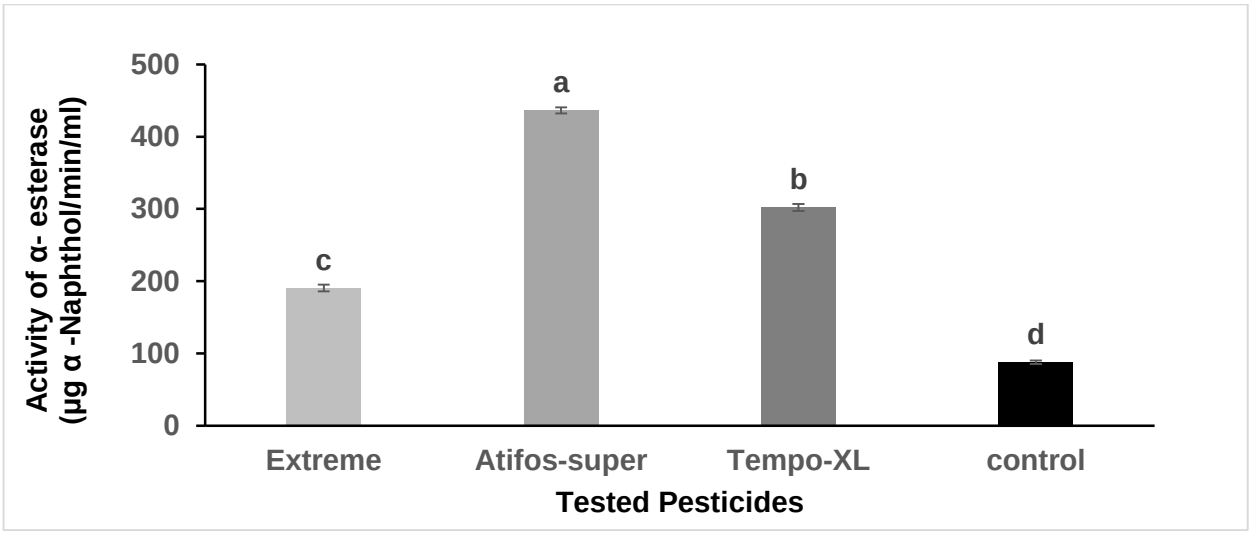

Fig. 5. Effect of LC_{50} of the tested pesticides on the activity of α-esterase enzyme of 4th instar larvae of *A. ipsilon*.

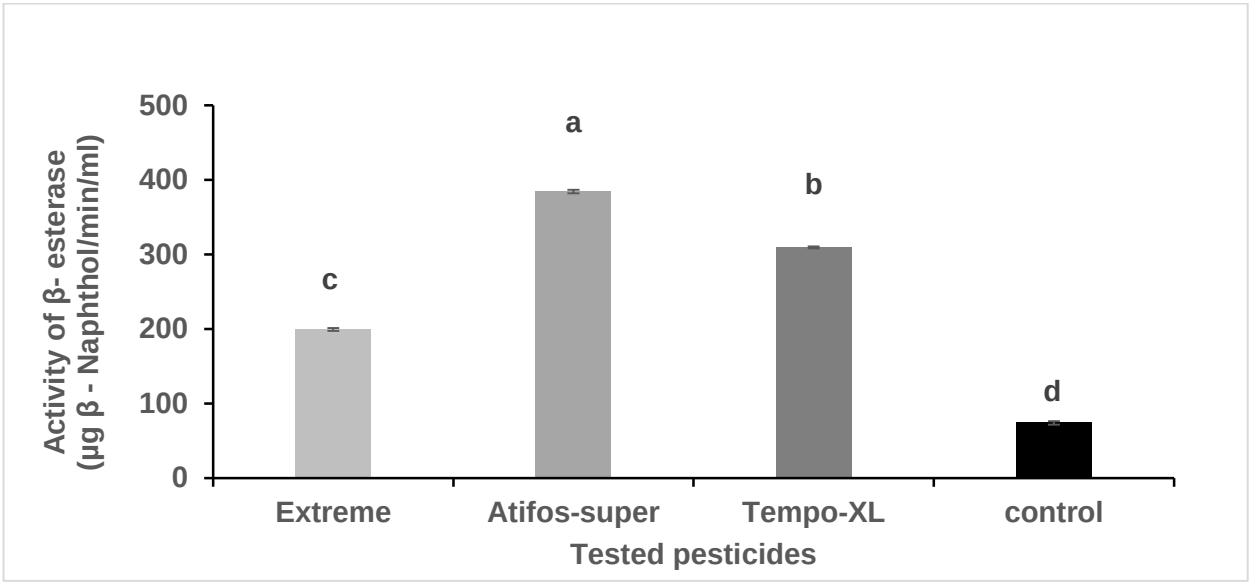

Fig. 6. Effect of LC_{50} of the tested pesticides on the activity of β-esterase enzyme of 4th instar larvae of *A. ipsilon*.

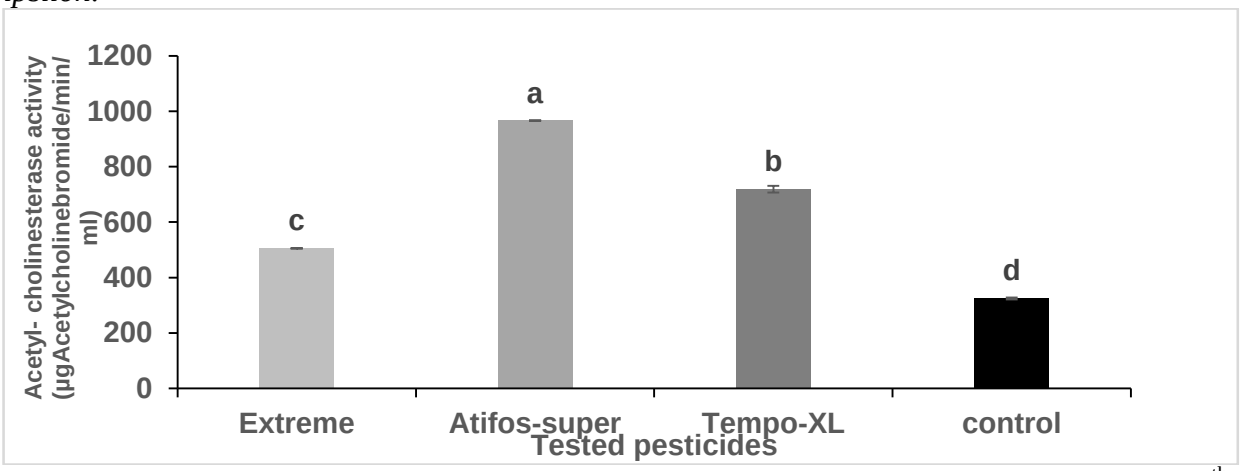

Fig. 7. Effect of LC_{50} of the tested pesticides on the activity of acetylcholinesterase enzyme of 4th instar larvae of *A. ipsilon.*

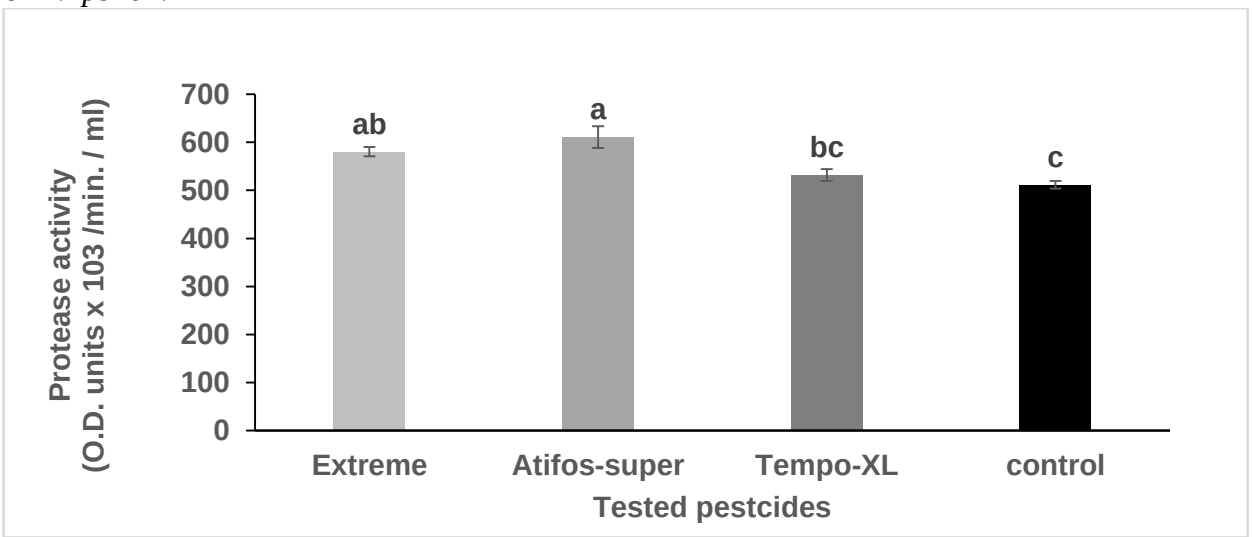

Fig. 8. Effect of LC_{50} of the tested pesticides on the activity of protease activity enzyme of 4th instar larvae of *A. ipsilon*.

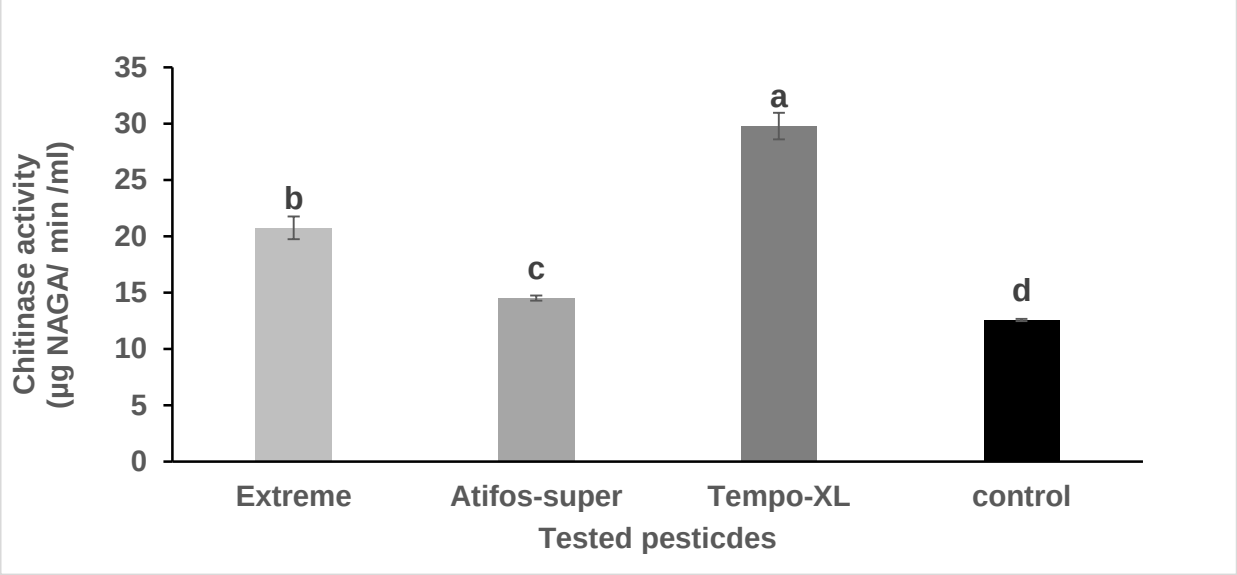

Fig. 9. Effect of LC_{50} of the tested pesticides on the activity of chitinase activity enzyme of 4th instar larvae of *A. ipsilon*.

DISCUSSION

The black cutworm is one of the most important insect pests of vegetables and several field crops in Egypt and the world (Abdou & Abdel-Hakim, 2017); also, it induces significant economic damage to a broad range of crops via root damage, as it consumes cotton, corn, wheat, and numerous vegetables (Wang et al, 2021). One of the most critical problems of this pest is its resistance to almost all chemical groups used against it. Mixing pesticides (synergists) with different modes of action may delay the development of resistance within pest populations; this is making the insecticides more toxic to the pest, and reducing the amount of pesticide usage quantity (Yu, 2008).

The present data indicated that the highest mortality percent of the 4th instar larvae of *A. ipsilon* was caused by the highest concentration of Extreme, Atifos-super, and Tempo-xl pesticides and vice versa after 24, 48, and 72 hrs. Also, the larval duration was prolonged non-significantly for Atifos-super and Extreme; meanwhile, Tempo-xl shortened it. Moreover, the mean pupal duration showed a non-significant increase for Tempo-xl followed by Extreme and Atifos-super. This prolongation of larval and pupal durations in *A. ipsilon* reflects metamorphic disruption. These results coincided with Hussein & Eldesouky (2019), who exhibited a significant prolongation in the larval and pupal durations of the 4th instars larvae of *Spodoptera littoralis* exposed to sublethal-concentrations of chlorfluazuron (IGR).

The current results revealed a significant reduction in the pupation percentage for Tempo-xl, Atifos-super, and Extreme. These results agreed with Fahmy (2014), who noted that only one-third of treated *A. ipsilon* larvae with two CSIs were pupating successfully. Moreover, the current data showed a significant decrease in the larval weights treated with Tempo-xl and a non-significant reduction for Extreme and Atifos-super. Furthermore, a non-significant decrease in the pupal weights was detected for Extreme, Tempo-xl, and Atifos-super; these pupae are derived from small-sized and weaker larvae that will be less in weight than untreated insects; due to the lack of proper sclerotization of the newly formed puparium and evaporation of body fluids leading to decreased pupal weight. These results concurrence with Hussein & Eldesouky (2019), who exhibited a significant reduction in the larval and pupal weights, and pupation percentage of the 4th instars larvae of *S. littoralis* exposed to sublethal-concentrations of chlorfluazuron. Shaurub et al (2018) stated that the reduction in the pupal weight was due to decreased food consumption and food utilization efficiency after larval treatment.

The obtained results cleared a prolongation in the longevity of *A. ipsilon* moths treated with Atifos-super and Tempo-xl than larvae treated with Extreme; this prolongation may be due to the adverse effects of any foreign chemicals lead to the accumulation of toxic xenobiotics, where longevity is an intricate balance of absorption,

excretion, intoxication, and detoxification. These results corresponded with Hussein & Eldesouky (2019), who showed a significant increase in the adult longevity of *S. littoralis* exposed to chlorfluazuron. Moreover, the current study revealed that Tempo-xl and Atifos-super showed severe failure in adult emergence percentage due to their more extended latent effect; this reduction may be due to the block of the maturation of the initial insect integument (imaginal discs) by the toxin (Suh et al, 2000). These results agreed with Abdel-Rahim et al (2008) on *A. ipsilon* treated with spinosad; Hussein & Eldesouky (2019) on *S. littoralis* treated with chlorfluazuron. Furthermore, data obtained showed a significant reduction in the adult growth index treated with Atifos-super followed by Tempo-xl and Extreme due to their chemical reaction.

The current results showed a suppression of egg production in females treated with Tempo-xl followed by Extreme and Atifos-super; this may be due to interference of the tested mixture pesticides with the ecdysteroid hormone that may lead to abnormal oocyte growth and egg formation (oogenesis), vitellogenesis, and embryogenesis processes (Lafont et al, 2005; Dhadialla et al, 2009). Moreover, the obtained results exhibited a reduction in the egg hatchability for Tempo-xl followed by Extreme and Atifos-super because of the improper chitin formation during the embryonic development or the sterilization of either egg and or/sperms (Abdel-Aal, 2012). So, very high degrees of sterility were detected in the present study for Tempo-xl followed by Extreme and Atifos-super. The current results conceded with Abdel-Rahim et al (2008), who found that fecundity and fertility were inhibited when treating the 4th instar larvae of *A. ipsilon* with spinosad.

Proteins help synthesize microsomal detoxifying enzymes that assist in the removal of toxins that enter the insect body (Wilkinson, 1976). Proteins are essential in many characters as body size, growth rate, and fecundity (Aguila et al, 2013). The obtained data showed that Tempo-xl followed by Extreme and Atifos-super caused a significant accumulation in total protein content in larvae of *A. ipsilon*. This elevation can be explained by the natural increase of protective hydrolytic and detoxifying enzymes after exposure to xenobiotics leads to dissociation into amino acids, which reduce insect energy storage (Fahmy, 2014; Assar et al, 2016). These results disagreed with Elbarky et al (2008), who indicated a significant decrease in the total proteins of treated *S. littoralis* larvae with spinetoram.

Eesterases are critical detoxifying enzymes that trap or hydrolyze the ester bond in synthetic chemicals before they reach their target sites in the nervous system (Darvishzadeh & Sharifian, 2015). The current study revealed that the Atifos-super followed by Tempo-xl and Extreme elevated the activity of α & β-esterases; these results agreed with Abd El-Mageed & Shalaby (2011), who revealed that the esterase enzymes play a vital role in insecticidal poisoning according to the type of component of the tested insecticide mixtures. Furthermore, the previous studies indicated that IGRs cause different levels of significant changes in α & β-esterases on *S. littoralis* (Bakr et al, 2013), spinetoram slightly increased the α-esterase (Rashwan, 2013); also, the elevated esterase activity due to pyrethroids (Yang et al, 2004). Moreover, Zibaee et al (2016) demonstrated that the 4th instar larvae of *Tuta absoluta* exposed to chlorpyrifos increased the α-esterase activity.

Acetylcholinesterase plays a vital role in hydrolyzing acetylcholine into acetic acid and choline at cholinergic synapses of the nervous system (McHardy et al, 2017). The changes in AChE activity disrupt cholinergic transmission in the head ganglia and ventral nerve cord, resulting in uncoordinated leg movement (Lang et al, 2012). The current study indicated that Atifos-super followed by Tempo-xl and Extreme elevated the activity of AChE; this could be due to: reducing AChE metabolism, rising AChE expression, or increasing AChE activity itself (Shawer et al, 2022). This elevation due to Atifos-super treatment could be attributed to the repetitive firing of nerves induced by organophosphate and pyrethroids that stimulated an excessive acetylcholine release at cholinergic nerve terminals, causing the block of cholinergic synaptic transmission (Abd El-Mageed & Shalaby, 2011). These results disagree with Pang (2014), who observed that organophosphates and pyrethroids create neurotoxicity by deterring the AChE activity. Assar et al (2016) proved that AChE in *S. littoralis* was significantly inhibited with spinetoram; however, spinetoram induced a moderate increase in the AChE activity (Elbarky et al, 2008; Rashwan, 2013).

The metabolic enzyme protease is a highly essential enzyme in the alimentary canal of insects and plays a vital role in insect growth, development and reproduction, enzyme activation, toxin activation/detoxification, and inflammation processes; also, they act as a target for insect pest management and cleave the peptide

bonds in the proteinous insect foods to release the amino acids that are absorbed by the epithelial cells of the insect midgut (Terra et al, 1996). Proteolysis plays a conspicuous role in insect physiology and food digestion facilitated by serine, cysteine, aspartic proteinases or endopeptidases, and metal-loproteinases (Mahdavi et al, 2013). The protease activity in the current study significantly increased in 4th instar larvae of *A. ipsilon* treated with Atifos-super and Extreme. Moreover, some insecticide synergists cause various protease changes in the resistance of *Tribolium castaneum* and *Musca domestica* (Saleem et al, 2000; Ahmed et al, 2002).
Chitinase is an enzyme that helps the breakdown of the β-1,4-glycosidic bonds in chitin and Chito-oligo-saccharides. Moreover, it plays an essential role during chitin ecdysis (Shi et al, 2017). The reduction in an insect's chitinase enzymatic activity causes extreme exoskeleton faults and lethality at all developmental stages (Chen &Yang, 2020). The present study declared a significant elevation in the chitinase activity by the impact of Tempo-xl, Extreme, and Atifos-super compared to untreated larvae. These results conformed to Rashwan, 2013; Assar et al, 2016, who stated a pronounced increase in the activity of chitinase of *S. littoralis* treated with spinetoram.

CONCLUSION

The current study concluded that the highest mortality percent caused by the highest concentration of Extreme, Atifos-super, and Tempo-xl pesticides, respectively (after 24, 48, and 72 h.) and vice versa for the 4th instar larvae of *A. ipsilon*. Tempo-xl exhibited a significant toxic effect on different development stages of *A. ipsilon*, causing a severe reduction of the larval weight and damaging inhibition of growth. Likewise, it remarkably reduced the pupation and disturbed the development. Therefore, Tempo-xl could be recommended as an eco-friendly alternative to synthetic insecticides to control this hazardous insect. The results of this study will supply a scientific foundation for the reasonable utilization of these new types of insecticides in strategies to manage *A. ipsilon* outbreaks.

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