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Toxicological, biochemical, and histological impacts of bio-insecticides on *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) along with their field effectiveness compared to chemical insecticides

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Abstract
The present work aimed to evaluate the larvicidal, toxicological, biochemical and histological effects of bio-insecticides against the black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) larvae in comparison chemical insecticides under laboratory conditions, in addition to evaluating their effects as larval population reduction under field conditions. Based on the toxicity index, cypermethrin was the most potent against the 2nd instar larvae and the rest tested insecticides arranged descendingly to be protecto, acetamiprid, Biosiana, metaflumizone, and azadirachtin, while, on the 4th instar larvae, protecto was superior, followed by Biosiana, cypermethrin, metaflumizone, acetamiprid and azadirachtin, respectively. The effects of bio-insecticides (Azadirchtin, Metaflumizone, Biosiana and Protecto) on the activities of some key enzymes of *A. ipsilon* 4th instar larvae varied between inhibition and activation according to the estimated insecticide. The abnormal structure of the 4th instar larvae midgut caused by the tested insecticides descendingly arranged the effects of insecticides as protecto, metaflumizone, Biosiana, cypermethrin, acetamiprid and azadirachtin, respectively. Under field conditions, cypermethrin recorded the highest reduction percentages of *A. ipsilon* population, followed by protecto, acetamiprid, metaflumizone, Biosiana and azadirachtin, respectively.

Keywords: *Agrotis ipsilon*, black cutworm, larvicidal effect, toxicity, histology, enzymes, reduction percentages

Introduction
The black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) is one of destructive insect pests in many regions worldwide feeding on wide a range of plant species (Mrowezynski *et al.*, 2003, Napiorkowska & Gawowska 2004 [30], Harrison & Lynn, 2008 and Abdou & Abdel-Hakim, 2017) [24, 6]. In Egypt, *A. ipsilon* is an economic pest not only to cotton but also to many of other crops and vegetables (Abo El-Ghar *et al.*, 1996 and Abdou & Abdel-Hakim, 2017) [8, 6]. This species has a special way of life and behavior; with a nocturnal activity, where the newly hatched larvae feed on the leave's epidermis of the young seedlings; then, when larvae become older (as fourth instar) it feed by cutting plant stems at the soil surface, causing considerable damage to several plants in a night (Abo El-Ghar *et al.*, 1996; Showers, 1997 and Shakur *et al.*, 2007) [8, 45, 42]. Also, Bhattacharyya *et al.* (2014) [12] stated that larvae of *A. ipsilon* eat only the tender parts of young plants at ground level at night, while during daytime it hides inside soil cracks or under debris around the plants. *A. ipsilon* has a substantial adaptation with the help of the wide range of host plants in addition to the fast-evolving insecticide resistance (Showers, 1997 and Binning *et al.*, 2015) [45, 13].

When following integrated pest management, highly effective insecticides to control *A. ipsilon* are needed to overcome this harmful problem (Falin *et al.*, 2019) [20]. The commonly used insecticides for controlling *A. ipsilon* include pyrethroids, organophosphates, and carbamates (Eldaly, 2022) [19]. Overuse or misuse of insecticides may develop insect resistance and may also harm the non-target organisms (Haq *et al.*, 2004 and Abd El-Mageed & Shalaby, 2011) [23, 3,]. Accordingly, evaluation of other insecticides' efficiencies continuously for controlling this insect pest became urgent; which gives the chance to the effective alternatives for replacing the failed controlling agents (Denholm *et al.*, 1999 and

Mohan & Gujar, 2003) [16, 29], in addition to conserve natural enemies in the agro-ecosystems using compounds safe to environment (Haq *et al.*, 2004 and Abd El-Mageed & Shalaby, 2011) [23, 3]. Therefore, delaying the insect resistance problem may be achieved by using different groups of insecticides in a rotation program to provide crop protection (Razaq *et al.*, 2007 and Abd El-Mageed & Shalaby, 2011) [37, 3].

Therefore, the present work aimed to evaluate the toxicological, histological and biochemical effects of different insecticide groups [Azadirachtin 2.3% EC (secondary metabolite present in neem seeds), Metaflumizone 22.6% SC (a semicarbazone broad-spectrum insecticide), Biosiana 2.5% WP (based on entomopathogenic fungi *Beauveria bassiana* $1x10^8$ CFU's/gm), Protecto 9.4% WP (bacterial insecticides of *Bacillus thuringensis* var. *kurstaki*, (32000 I.U. /mg)), Acetamiprid (neonicotinoid insecticide) and Cypermethrin 20% EC (pyrethroidal insecticide)] against *A. ipsilon* larvae (2^{nd} and 4^{th} instars) under both laboratory and field conditions.

Materials and Methods

1. Tested insecticides

Six commercial formulations belonging to different groups (according to their mode of action) were selected and tested in the present study, Okios (Azadirachtin 3.2% EC, Sipcam Inagra S.A), Protecto (Protecto 9.4% WP, (*Bacillus thuringensis* var. kurstaki, (32000 I.U. /mg), Bioinsecticide Production Unit, Plant Protection Research Institute, Agricultural Research Center), Biosiana (Biosiana 2.5% WP, (*Beauveria bassiana*, (1×108 CFU/g), Bioinsecticide Production Unit, Plant Protection Research Institute, Agricultural Research Center), Zonemita (Metaflumizone 22.6% SC, Kafr El Zayat Pesticides and Chemicals), Acetagro (Acetamiprid 20% SP, Jiangsu Inter - China Group Corporation), Sparkle (Cypermethrin 25% EC, Elhelb for Pesticides and Chemicals).

2. Insect cultures

A stock colony of *A. ipsilon* was reared in the laboratories of the Plant Protection Research Institute at 27±2°C, 65±5% R.H and a 16-h light: 8-h dark photoperiod. Larvae were fed on fresh leaves of castor bean (*Ricinus communis* L.), while the adults were fed on a 10% sucrose solution.

Approximately 1500 individuals of *A. ipsilon* 2^{nd} instar larvae were transferred to the laboratory at the Plant Protection Research Institute, Mansoura Branch, under the same conditions. Larvae were reared on pepper leaves (*Capsicum annuum* L.) inside plastic containers (measuring 40 x 30 x 25 cm^3). Generous amounts of shredded paper were put inside containers to minimize larval cannibalism. The formed pupae were introduced in glass jars (measuring 20 x 20 x 30 cm^3) covered with muslin till adult emergence, which were provided with 10% of honey solution, and pieces of oleander (*Nerium oleander* L.) branches with their leaves to receive egg masses deposited by adults.

3. Bioassay

3.1 Laboratory studies

3.1.1 Toxicity of insecticides

The six commercial formulations were assessed using the leaf dipping technique. The pepper plant leaves were dipped for 20 seconds prior to air drying. Six diluted aqueous concentrations were prepared for each toxicant: Acetamiprid 20% SP (100, 50, 25, 12.5, 6.25, and 3.13 ppm), Azadirachtin 2.3% EC (4000, 2000, 1000, 500, 250, and 125 ppm), Cypermethrin 20% EC (75, 37.5, 18.75, 9.38, 4.69, and 2.34 ppm), Metaflumizone 22.6% SC (160, 80, 40, 20, 10, and 5 ppm), Biosiana 2.5% (50, 25, 12.5, 6.25, 3.13, and ppm), and Protecto 9.4% WP (100, 50, 25, 12.5, 6.25, and ppm). The effectiveness was evaluated against 2^{nd} and 4^{th} instar larvae by measuring mortality percentages after 24 h for chemical insecticides and after 72 h for bio-insecticides. Each treatment was replicated four times with 10 larvae per replication, alongside a control group of 10 untreated larvae with four replicates Abou-Taleb *et al.* (2010) [10].

3.1.2 Histological and biochemical effects of insecticides

The calculated LC_{50} of each evaluated insecticide was prepared, and the 4^{th} instar larvae of *A. ipsilon* were treated using the dipping method as previously mentioned. After 24 hours, the live larvae in each treatment and control were collected in Eppendorf tubes and divided into two groups. The first group was used for biochemical evaluation, while the other was used for histological evaluation.

Biochemical effects: The Eppendorf tubes for this estimation were frozen at -20°C. Colorimetrically, by using UV visible spectrophotometer (model V1200, China), the activities of certain five detoxifying enzymes were estimated at Mansoura Branch of the Plant Protection Research Institute. According to Reitman & Frankel (1957) [38], GPT (the glutamate pyruvate transaminase) and GOT (glutamate oxaloacetic transaminase) activities were estimated at 505 nm. According to Pan *et al.* (2016) [32], GST (Glutathione S-transferase) activity was estimated at 340 nm; while, ALP (alkaline phosphatase) and ACP (acid phosphatase) activities were estimated at 510 nm according to Powell & Smith (1954) [34].

Histological effects: According to Abouelghar *et al.* (2013) [9], from the middle of the midgut, larval-gut was dissected and fixed in Bouin's solution. Transverse sections (5 mm each) were stained with Hematoxylin-Eosin (HE), and compared with the tissues taken from the control, the morphological alterations of the midgut cell structures in addition to organization of each specimen were analyzed by microscopic examination. Also, Malpighian tubules were examined comparing to the control.

3.2. Field studies

3.2.1 Area of application

Field trials were conducted during the 2023 cotton season at the Aga district, Dakahlia governorate. Giza 94 cotton variety was sown at the 15^{th} of April 2023. An area of about 2800 m^2 infested with *A. ipsilon* was selected for the present study. The normal agricultural practices were carried out. The selected area was divided into seven blocks (six insecticide treatments in addition to a control treatment), and each block was divided into four replicates (the area of each replicate was about 100 m^2). In a randomized complete block design, all treatments as well as the control were assigned to plots.

3.2.2 Preparation of baits

Baits were prepared as described by Salama *et al.* (1995) [41]. For each treatment, 100 ml of each insecticide at the

recommended concentration was thoroughly mixed with 2.5 kg of bran and 0.4 kg of molasses. Water was added gradually until the desired consistency was achieved. Shortly before field application, baits were prepared with ensuring all ingredients were fully mixed for a homogeneous distribution.

3.2.3 Field application of baits

The pretreatment counts of *A. ipsilon* larvae/ 25 plants in each replicate were estimated one day before the treatment (on the 13th of May 2024). On the 14th of May 2024, the prepared baits were manually distributed on the soil surface behind hills. Then, the number of *A. ipsilon* larvae/ 25 plants (recorded as number of larvae, damage, and/or cut stems) in each replicate was counted at 3 days post-treatment.

4. Statistical analysis

In the laboratory experiment, Abbott's formula (Abbot, 1925) [1] was used for correction of the mortality percentage of each treatment. The lethal concentrations of 50 and 90% of insect population (LC_{50} and LC_{90}) were estimated by the Finney method Finney (1971) by using LDP-line software. By using Sun equation (Sun, 1950) [47], the toxicity index was calculated. Reduction percentages of *A. ipsilon* larvae in each replicate of the field experiment were calculated by the formula of Henderson and Tilton (1955) [26]. Analysis of variance (ANOVA) and the standard error (SE) were used for the results of the bioassay test, enzyme activity, and reduction percentages in the field experiment for analyzing by using CoHort software (CoHort, 2004) [15].

Results

1. Toxicity studies

The effects of the evaluated insecticides against *A. ipsilon* larvae (2nd and 4th instars) were estimated. The mortality percentages after one day for the chemical synthetic insecticides and after 3 days for bio-insecticides were calculated post-treatment (Table 1). The highest concentrations caused the highest mortality percentages, where the initial kill of the chemical insecticides for the 2nd instar larvae reached 100% for both Acetamiprid and Cypermethrin, while the initial kill of these treatments on the 4th instar larvae reached 52.5 and 95.0%, respectively. Concerning the bio-insecticides, the initial kill of Azadirchtin, Metaflumizone, Biosiana and Protecto for the 2nd instar larvae reached 72.5, 85.0, 100, and 100%, respectively, while, on the 4th instar larvae, the percentages reached 87.5, 75.0, 85.0 and 87.5%, respectively.

Data illustrated in Table (1) showed that mortality percentages declined as treatment concentrations decreased. Additionally, mortality rates were generally higher in 2nd instar larvae compared to 4th instar larvae, indicating that 2nd instar of *A. ipsilon* larvae is more susceptible to the evaluated insecticides than the 4th instar.

Table 1: Mortality percentages caused by the certain insecticides against the 2nd and 4th-instar larvae of Black cutworm, *Agrotis ipsilon*

Tested compounds	Concentration (ppm)	Mortality after days post-treatments	
		2nd instar larvae	4th instar larvae
After 3 days			
Azadirchtin	4000	72.5	87.5
	2000	52.5	45.0
	1000	32.5	12.5
	500	15.0	2.5
	250	5.0	0
	125	0	0
Metaflumizone	160.0	85.0	75.0
	80.0	75.0	57.5
	40.0	55.0	25.5
	20.0	50.0	15.0
	10.0	35.0	2.5
	5.0	22.5	0
Biosiana	50	100.0	85.0
	25	82.5	67.5
	12.5	50.0	52.5
	6.25	17.5	30.0
	3.13	5.0	15.0
Protecto	100	100.0	87.5
	50	92.5	67.5
	25	82.5	60.0
	12.5	70.0	40.0
	6.25	50.0	25.0
After 1 day			
Acetamiprid	100	100.0	52.5
	50	90.0	37.5
	25	80.0	20.0
	12.5	72.5	10.0
	6.25	45.0	5.0
	3.13	27.5	0
Cypermethrin	75	100.0	95.0
	37.5	92.5	60.0
	18.75	80.0	32.5
	9.38	70.0	17.5
	4.69	62.5	2.5
	2.34	45.0	0

Results in Table (2) displayed the toxicity of the tested insecticides on *A. ipsilon* 2nd instar larvae. The tested insecticides showed larvicidal activity with LC_{50} values ranging from 2.96 to 1894.72 ppm and LC_{90} values from 31.39 to 9857.32 ppm. According to the results, the LC_{50} of Cypermethrin was the least concentrated, followed by Protecto, Acetamiprid, Biosiana, Metaflumizone and Azadirachtin (which showed the obviously highest LC_{50}), respectively. Biosiana and Cypermethrin exhibited the highest larvicidal activity, with LC_{90} values of 31.39 and 31.40 ppm, followed by Protecto, Acetamiprid, Metaflumizone and Azadirachtin, respectively. The toxicity index at LC_{50} on *A. ipsilon* 2nd instar larvae arranged the treatments in descending order as follows: Cypermethrin, Protecto, Acetamiprid, Biosiana, Metaflumizone and Azadirachtin.

Table 2: Toxicity of certain insecticides against 2nd-instar of Black cutworm *Agrotis ipsilon*

Compounds	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope ±SE	Toxicity index
		Lower	Upper		Lower	Upper		
Azadirachtin	1894.72	1260.34	4941.83	9857.32	4114.04	123895.77	1.789±0.458	0.16
Metaflumizone	21.92	9.37	33.25	252.76	117.96	2794.81	1.207±0.336	13.50
Biosiana	12.26	9.08	15.68	31.39	22.32	74.57	3.14±0.777	24.12
Protecto	5.93	1.31	9.59	37.31	25.92	37.85	1.605±0.457	49.86
Acetamiprid	7.24	4.65	10.05	45.12	28.25	107.79	1.613±0.290	40.85
Cypermethrin	2.96	0.46	5.57	31.40	19.21	107.03	1.249±0.345	100.00

Concerning the toxicity of tested insecticides on *A. ipsilon* 4th instar larvae (Table, 3), the calculated LC_{50} values ranged from 12.02 to 2192.57 ppm, while, LC_{90} values ranged from 66.59 to 5157.63 ppm. The LC_{50} value of Protecto was the least, followed by Biosiana, Cypermethrin, Metaflumizone, Acetamiprid and Azadirachtin, respectively. The LC_{90} value of Protecto was also the least, followed by Cypermethrin, Biosiana, Metaflumizone, Acetamiprid and Azadirachtin, respectively. The toxicity index at LC_{50} arranged the treatments in descending order as follows: Protecto, Biosiana, Cypermethrin, Metaflumizone, Acetamiprid, and Azadirachtin.

Table 3: Toxicity of certain insecticides against 4th-instar of Black cutworm *Agrotis ipsilon*

Compounds	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope ±SE	Toxicity index
		Lower	Upper		Lower	Upper		
Azadirachtin	2192.57	1786.53	2734.04	5157.63	3821.71	9358.24	3.45±0.660	0.55
Metaflumizone	74.04	55.74	105.00	323.38	191.85	975.25	2.002±0.394	16.24
Biosiana	17.91	5.15	28.09	142.97	90.55	509.52	1.421±0.383	67.15
Protecto	12.02	3.68	18.01	66.59	43.18	250.54	1.724±0.504	100.00
Acetamiprid	94.84	60.57	291.79	746.65	258.09	22946.21	1.43±0.393	12.68
Cypermethrin	25.52	20.00	32.40	80.25	57.04	147.01	2.576±0.426	47.12

2. Biochemical studies

The effects of bio-insecticides on the activities of some key enzymes inside the bodies of *A. ipsilon* 4th instar larvae were evaluated compared to the normal activities of these enzymes in control treatment (Table 4). Azadirachtin significantly inhibited ALT activity, while it significantly activated AST, ALP, ACP, AChE and GST. Metaflumizone significantly inhibited the activities of AST, ACP and AChE, while, this insecticide significantly activated ALP and GST, but it did not significantly affect the activity of ALT. The insecticide Biosiana significantly inhibited the activities of ALT, ALP and AChE, while, this insecticide significantly activated AST and GST, but it did not significantly affect the activity of ACP. With respect to Protecto, it significantly inhibited the activities of ACP and AChE, while it significantly activated AST, ALP and GST, but this insecticide did not significantly affect the activity of ALT.

Table 4: Effect of certain insecticides on the activities of some detoxifying enzymes inside the bodies of the 4th-instar larvae of black cutworm *Agrotis ipsilon*

Treatments	AST U/ml ± SE	ALT U/ml ± SE	Alkaline phosphatase U/ml ±SE	Acid phosphatase U/ml ± SE	AChE (ug AchBr /min/gm bwt.)	GST (Mmol sub. conjugated/min. mg protein)
Azadirachtin	0.206 ± 0.00006^{a}	0.117 ± 0.00009^{bc}	211.0 ± 1.15^{b}	50.33 ± 0.88^{a}	282.67 ± 0.88^{a}	2.466 ± 0.002^{b}
Metaflumizone	0.159 ± 0.0015^{c}	0.121 ± 0.002^{ab}	252.0 ± 1.73^{a}	19.0 ± 1.15^{d}	54.0 ± 1.53^{e}	2.475 ± 0.002^{a}
Biosiana	0.203 ± 0.0012^{a}	0.112 ± 0.001^{c}	87.33 ± 1.20^{d}	37.67 ± 0.88^{b}	104.67 ± 1.45^{c}	1.207 ± 0.001^{c}
Protecto	0.167 ± 0.00008^{b}	0.119 ± 0.00003^{ab}	$206.0 \pm 1.73b^{b}$	25.67 ± 1.45^{c}	79.0 ± 1.00^{d}	1.075 ± 0.001^{d}
Control	0.053 ± 0.00009^{d}	0.123 ± 0.003^{a}	179.0 ± 3.46^{c}	39.0 ± 0.58^{b}	124.33 ± 2.03^{b}	0.881 ± 0.001^{e}
LSD (0.05)	0.0033	0.0058	6.423	3.254	4.529	0.005

LSD: Least Significant Difference

3. Histological studies

Figure (1) showed cross sections of *A. ipsilon* 4th instar larvae midgut in normal (untreated) larvae. This figure shows that larvae showed normal midgut structures with normally arranged columnar epithelial cells rest on the intact basement membrane, with normal malpighian tubes, and peritrophic membrane surrounding food debris (lumen).

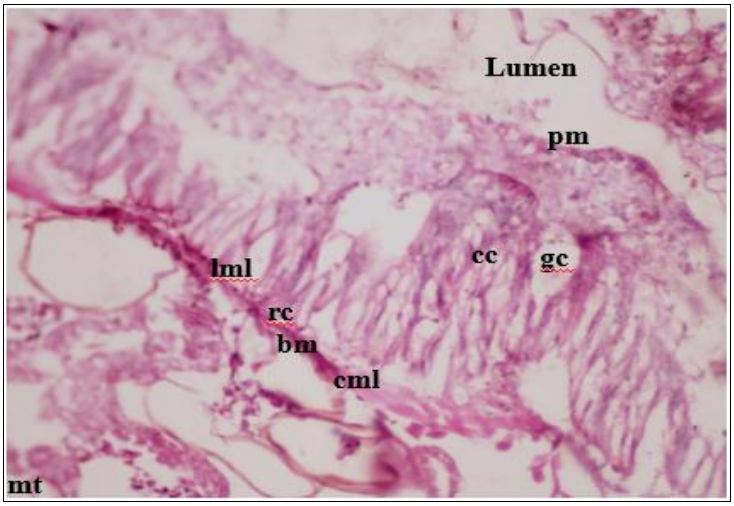

Fig 1: The cross section of the normal midgut of *A. ipsilon* 4th instar larvae (X400) in control treatment [Note: bm) basement membrane, rc) regenerative cells, lml) longitudinal muscle layer, cml) circular muscle layer, cc) columnar cells, gc) goblet cells, pm) peritrophic membrane, and mt) malpighian tubes].

When *A. ipsilon* 4th instar larvae were treated with Azadirachtin, cross section of midgut showed a separation of the basement membrane and a mild degeneration of its epithelial lining associated with desquamation of some cells in the lumen, in addition to shrinkage and atrophied of the peritrophic membrane (Fig. 2). Protecto caused separation, fragmentation of the basement membrane associated with sever degeneration, and thinning of the epithelial lining of the midgut. Fragmentation of the peritrophic membrane was noticed with an increase in the space between it and the epithelial layer of the midgut.

Treatment with Biosiana showed partial separation of basement membrane of the larval midgut and vacuolar degeneration of its epithelial lining, in addition to degeneration of the epithelial lining malpighian tubes (Fig. 2). There was severe damage caused in the basement membrane represented as a thickening and separation of with sever degeneration, necrosis of epithelial lining midgut and malpighian tubes when 4th instar larvae were treated with Metaflumizone, also, the basement membrane separated, and epithelial lining showed sever vacuolar degeneration.

Acetamiprid caused changes in the midgut of black cutworm, which showed as a separation of the basement membrane associated with degeneration, and necrosis of its epithelial lining; in addition, atrophied peritrophic membrane was observed (Fig., 2). Thickening and separation of basement membrane associated with vacuolar degeneration, necrosis and desquamation of its epithelial lining when *A. ipsilon* larvae were treated with Cypermethrin.

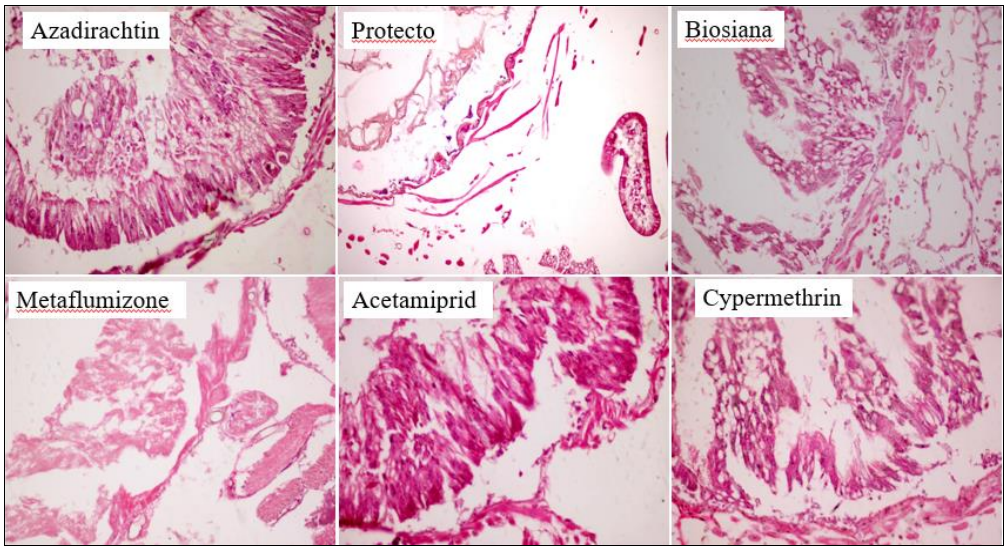

Fig 2: The cross section of the midgut of *A. ipsilon* 4th instar larvae (X400) treated with certain insecticides.

4. Field performance

The effects of the tested insecticides on *A. ipsilon* larval population after 3 days of bait application are presented in Tables (5). The population of *A. ipsilon* larvae was higher significantly in the control treatment than the populations in the areas treated with the evaluated insecticides. According to reduction percentages, the highest effective treatment on *A. ipsilon* population under field conditions was Cypermethrin, followed by Protecto, Acetamiprid, Metaflumizone, Biosiana and Azadirachtin, respectively with a reduction percentage of 98.21, 84.72, 76.23, 64.58, 55.63 and 43.82%, respectively.

Table 5: Efficiency of baits treated with certain insecticides against black cutworm *Agrotis ipsilon* population in the field

Insecticide	Field recommended rate	Pre-Spray	Total number of larvae (or its symptoms) after 3 days	
			Mean No.	% Reduce.
Azadirachtin	100cm^3/100L	11.00^{e}	6.50^{b}	43.82
Metaflumizone	70 cm^3/100L	12.75^{bcd}	4.75^{c}	64.58
Biosiana	250g/100L	11.25^{de}	5.25^{c}	55.63
Protecto	300 gm/feddan	14.00^{ab}	2.25^{d}	84.72
Acetamiprid	25 gm/100L	12.00^{cde}	3.00^{d}	76.23
Cypermethrin	250cm^3/feddan	13.25^{abc}	0.25^{e}	98.21
Control		14.50^{a}	15.25^{a}	------
LSD $_{0.05}$		1.675	0.949	------

Discussion

The black cutworm, *A. ipsilon* is a dangerous pest in Egypt and many countries. This pest is usually controlled by using chemical insecticides, especially organophosphorus compounds (Abou-Taleb *et al.*, 2010) [10]. Frank *et al.* (1990) [22] and Khan *et al.* (2010) [2]7 mentioned that environmental contamination resulted from the intensive use of pesticides. The present study evaluated various effects (larvicidal, toxicological, histological and biochemical impacts in addition to field performance) of certain bio-insecticides, which are safe to the environment, on *A. ipsilon* compared with chemical insecticides. According to the obtained data, the larvicidal efficiency in addition to the toxicity index at LC_{50} and LC_{90} revealed that the two bio-insecticides, Protecto and Biosiana are among the most superior insecticides against *A. ipsilon* larvae. On contrary, Metaflumizone and Azadirachtin were amongst the least effective treatments against the tested pest larval stage. These findings are partially similar to Salama *et al.* (1995) [41] and Abou-Taleb *et al.* (2010) [10]; they reported that the bio-insecticide (which containing *B. huringiensis* var. *kurstaki*) is a possible substitute to chemical insecticides for controlling *A. ipsilon* and *Spodoptera littoralis* (Boisd.). Also, the present study showed that Cypermethrin was of the highest effective treatments on the 2nd instar larvae of *A. ipsilon*, and was of the lowest effective treatments on the 4th instar larvae under laboratory conditions. Abdel Aziz *et al.* (2024) [2] reported that Cypermethrin showed highest values of LC_{50} and LC_{25} on the 4th instar larvae of the same insect pest. According to Schmidt *et al.* (1997) [44] and Shaurub *et al.* (2022) [43], the plant extract of *Melia azedarach* exhibited significantly adverse effects on the biological parameters of *A. ipsilon* larvae including hemolymph nutrient.

The disturbances of enzyme activities inside the bodies of *A. ipsilon* larvae might affect larval growth (Abo El-Ghar *et al.*, 1996) [8]. Alanine aminotransferase (ALT) is considered as a marker enzyme for evaluating organ damage and usually it is released in serum as a result of the damage in the hepatic membrane by a chemical attack (Saki *et al.*, 2011) [40]. The defensive enzyme, glutathion S-transferase (GST) is important for the detoxification of insecticides in insecticide-treated larvae (Çağatay *et al.*, 2018) [14] and plays an important role in insect resistance development (Le Gall *et al.*, 2018 and Pavlidi *et al.*, 2018) [28, 33]. In the present study, the effects of bio-insecticides (Azadirchtin, Metaflumizone, Biosiana and Protecto) on the activities of certain enzymes inside the bodies of *A. ipsilon* 4th instar larvae were evaluated. As shown in this table (4), the activities of AST, ALT, ALP, ACP, AChE and GST varied between inhibition and activation according to the estimated insecticide. The present results are supported by Çağatay *et al.* (2018) [14]; they reported that GST increased in insecticide-treated larvae. Also, Nasiruddin & Mordue (1993) [31] and Bezzar-Bendjazia *et al.* (2017) [11] hypothesized that azadirachtin disrupts enzymes secretion. Derbalah *et al.* (2012) [17] and Abdelkhalek *et al.* (2022) [5] found that a normal ALP activity after plant extracts treatments, which indicated that there was no lysosomal disruption after the plant extract applications. Derbalah *et al.* (2012) and Abdelkhalek *et al.* (2022) reported that there were no significant effects on ALT levels in *A. ipsilon* larvae treated with botanical extracts. Also, the present results agreed with the results of Abdullah *et al.* (2024); reported that most of the estimated enzymes (GPT, GOT, GS-T, ALP and ACP) were inhibited inside the bodies of aphid (*Aphis gossypii* (Glover)) and thrips (*Gynaikothrips ficorum* Marchal) when treated with garlic and camphor essential oils as well as mineral oil. According to Abo El-Ghar *et al.* (1996), the plant extract of *M. azedarach* exhibited significantly adverse effects on the enzyme activities *A. ipsilon* larvae.

Histologically, the present results recorded that the most effective sub-lethal treatments on *A. ipsilon* 4th instar larvae were Protecto and Metaflumizone followed by Biosiana, Cypermethrin, Acetamiprid and Azadirachtin, respectively. The effects appeared in the histological disturbances of the mid-gut as separation, fragmentation of basement membrane, thinning of the epithelial lining of the mid gut and fragmentation of the peritrophic membrane with increase the space between it and the epithelial layer of the midgut; which led to loss of the permeability and mixing lysis cells with lumen content. These abnormal structures of midgut and malpighian tubes (in treatments of Biosiana and Metaflumizone insecticides) may show the functional differences between treated larvae and untreated ones. The obtained results of the present study agreed with the results of Abdou & Abdel-Hakim (2017) [6]; they found that certain insecticides resulted histopathological damages and destroyed of epithelial cells in addition to a separation of epithelial cells from the basement membrane of *A. ipsilon* midgut which led to the loss of the midgut functions as absorption or digestion. According to the study of Abdel Aziz *et al.* (2024) [2] on *A. ipsilon* larvae, deltamethrin, cypermethrin, lambda-cyhalothrin and gamma-cyhalothrin treatments caused detachment and lysis of numerous membranes and cells of the midgut structures and damage in the muscular layer with breaks in many places. Also, the present results are supported by the studies of Nasiruddin & Mordue (1993), Roel *et al.* (2010) and Shu *et al.* (2018) [31, 39, 46]; where they hypothesized that there were adverse effects on the epithelial cells of the midgut of lepidopteran larvae when treated with azadirachtin. In the study of Abouelghar *et al.* (2013), *S. littoralis* larvae were fed on spinosad-treated leaves; which showed that regenerative

cells of the larval midgut seemed to be sensitive to the treatment; so, these cells could not replace the damaged cells, also, the epithelial cells of midgut showed apoptosis signs; which manifested as cell shrinkage and presence of condensed chromatin, associated with some vacuolization. This process is described as a cell mechanism against the pathogenic and toxic agents (Dougherty *et al.*, 2006) [18]. These findings explain the obtained effects of Protecto on *A. ipsilon* larvae in the present study. The obtained results were also similar to those obtained in the midgut of *S. littoralus* larvae which treated with biopesticides of *Bacillus thuringiensis* (Bt) (Quesada-Morga & Santiago-Alvarez, 2001 and Abdelkefi-Mesrati *et al.*, 2011) and exotoxin protein which extracted from *Metarhizum anisopliae* (Quesada-Morga *et al.*, 2006). Roel *et al.* (2010) [39, 36, 4] found histo-physiological alterations in the midgut of *S. frugiperda* (such as degeneration of the epithelial lining and in the peritrophic matrix) when treated with the sub-lethal doses of neem oil (*Azadirachta indica* (Meliaceae)).

After 3 days of bait application in the cotton field, the population of *A. ipsilon* larvae was higher significantly in the control treatment in comparison with the treated areas with the evaluated insecticides. According to reduction percentages, the highest effective treatment on *A. ipsilon* population under field conditions was that of Cypermethrin followed by Protecto, Acetamiprid, Metaflumizone, Biosiana and Azadirachtin, respectively. These findings are in agreement with Abou-Taleb *et al.* (2010) [10]; they found that chemical insecticide exhibited relatively higher reduction percentages of *A. ipsilon* under field conditions after 3 days of bait application, while, after 6, 9 and 12 days, the bio-insecticide (depending on the *B. thuringiensis* var. *kurstaki*) exhibited higher reduction percentages. Also, Salama *et al.* (1995) [41] found that baits treated with *B. thuringiensis* var. *kurstaki* revealed a considerable reduction of *A. ipsilon* population in soybean fields (more than lambda-cyhalothrin); avoiding the chemical insecticides residues in the seeds which used to produce oil, and it was less harmful to the natural enemies. Also, Abdel Aziz *et al.* (2024) [2] found that Cypermethrin showed lower effects on *A. ipsilon* population under field conditions in comparison with other chemical insecticides. Heibatian *et al.* (2018) [25] mentioned that deltamethrin showed the highest larvicidal effect on *Agrotis segetum* (Denis & Schiffermüller) infesting sugar beet field (in Iran) followed by Bt treatment, while azadirachtin showed a significant oviposition deterrent for female moths of this pest.

Conclusion: The bio-insecticides, Protecto and Biosiana could be used successfully for controlling of *A. ipsilon*; these bio-insecticides showed highly toxic effects against the pest larvae, severe disturbances in enzyme activities, obvious histological disturbances in the larval mid-gut, and acceptable reduction in pest population in the field, especially Protecto.

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