Biochemical and histological effect of some plant extracts, insecticide (methomyl) and bio insecticide (protecto) against cotton leafworm, *Spodoptera littoralis* (Boisd.)

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Abstract

The toxic effect of four different compounds, namely protecto (bioinsecticide based on *Bacillus thuringienses*), coumarin (plant extract of Chicory flower), Neemix (plant extract of *Azadirachta indica*) and lannate (chemical insecticide) against the fourth instars larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) by LC_{50} concentrations were evaluated under laboratory conditions. Methomyl led to basement and peritrophic membrane detachment and destruction, appearance of numerous vacuoles, destruction of epithelial cells that emptied their cytoplasmic contents in the lumen, while protecto caused detachment and destruction of the basement and peritrophic membrane, vacuolization and destruction of the epithelial cells. Plant extracts (coumarin and azadirachtin) caused basement membrane detachment followed by destruction, destruction of epithelial cells which showed thickness in some points, in some cases the epithelial cells appeared deformed and become elongate in size than those of the control. The tested compounds caused significant decrease in the total soluble protein content of the 4th instar larvae. All compounds (except methomyl) caused significant increase in the activity of acetylcholinesterase enzyme and caused significant increase in the activity of acid phosphatase and amylase enzyme (except protecto and methomyl decrease the activity of amylase enzyme), on contrary, tested compounds showed significant decrease in the activity of alkaline phosphatase enzyme, coumarin and azadirachtin significantly increased α-esterase, while the other compounds caused significant decrease in the activity of this enzyme and β-esterase enzyme. Also tested compounds expect methomyl caused significant inhibition in the activity of GOT, on contrary, all tested compounds except coumarin caused significant decrease in the activity of GPT and invertase activity. Trehalase showed the same decreasing effects (except coumarin), while azadirachtin similar to control and had no effect.

Key word: Biochemical, histological, plant extracts, methomyl, bio-insecticide, cotton leafworm, *Spodoptera littoralis* (Boisd.)

Introduction

The cotton leaf worm, *S. littorals* (Boisd.) is considered as one of the most severe destructive cotton pests in Egypt and many other parts of the world infesting over 112 plant species belonging to 44 families. The larval stage of *S. littoralis* is known as a notoriously leaf eater accepting almost all herbaceous plant (Hill., 1975). Cotton leaf worm control program was based mainly on use of insecticides, which created some problems such as insecticides- resistance, environmental pollution and hazard to natural enemies and beneficial insects (Toscano *et al.*, 1974 and Abbas *et al*., 1996**).** Hence, it recent approaches now focus upon the use of environmentally safe compounds as pest control agents, the entomopathogenic bacteria, *B. thuringensis* and plant extracts as pesticide alternatives possess distinct toxicity and also lead to antifeeding activity and inhibition growth of some pests (Sharaby and Ammar, 1997; Badr *et al*., 2000 and D'Andrea *et al*; 2001). Latent effect of larval exposure to various antifeedants was reported to disturb growth and development in the subsequent pupal and adult stage, i.e., *S. littoralis* (Meisner *et al*., 1982); *Ostrinia nubilalis* (Arnason *et al*.,1987), *S. littoralis* Adel and Abd El-Hakim (2007) and *Heliothis virescens* (Barnaby and Klocke, 1987) The histological studies are very important to do on the mid gut of the treated larvae because it show and explain the changes resulting from treating larvae with different tested compounds**.** The changes in the biochemical content especially the transaminase enzymes activities such as GOT, GPT, trehalase, invertase, amylase and soluble protein content have an important role in biological and physiological activities of insects. (Mead-Hala, 2000 and Khedr, 2002). Esterase in insects have been implicated in reproductive behavior, pheromone and hormone metabolism, digestion, neurotransmission, the action of end resistance to insecticide, particularly organophosphates. Esterases may contribute to resistance by hydrolyzing the pesticide (Parkes, *et. al*. 1993, Gaaboub, 2004 Hanafy, *et. al.* 2005).

The aim of the present work to evaluate histological effects of tested compounds against the mid gut of 4th instar larvae of *S. littoralis* and biochemical study was conducted on the treated larvae, i.e. total protein and enzyme activities, (acid phosphatase, alkaline phosphatase, alpha and beta esterases, GOT and GPT transaminase enzymes, trehalase, amylase and invertase carbohydrate hydrolyzing enzymes and acetyl cholinesterase enzymes).

Material and methods

The tested compounds

Plant extracts

Coumarin: - Isolated from the flower of *chichorium intybus* (chicory), Azadirachtin (Neemix): from the neem tree, *A. indica* (Meliaceae), 6 (2, 4-dinitrophenylamino hexanyl) - 22, 23-dihydroazadirachtin

Bio insecticide (protecto)

W.P. based on *B. thuringiensis subsp. Kurstaki* ($32x10^3$ I. U/mg). Active ingredient 9.4%

Insecticides (methomyl):

Lannate 90% W.P. (a synthetic carbamate compound) Carbamate oxime

Chemical name: S. methyl- N-[(methyl carbomoyl) oxy] thioecetimidate.

Insect maintenance

The stock culture of susceptible Egyptian cotton leaf worm, *S. littoralis* was reared on castor leaves (*Ricinus communish*) for several generations at laboratory conditions 27 ± 1°C and 70 ± 5% RH. Egg masses were placed on castor oil leaves in cylindrical glass jars. The jars were covered with muslin cloth and fastened with rubber band; first instar larvae hatched within 2-3 days, the newly hatched larvae were transferred into reading jars bottomed with sheeted of towel paper to absorb excess humidity. Castor bean leaves were provided daily to the larvae in sufficient amounts, the accumulated faces and debris were cleaned out daily. After pupation, pupae were collected and placed in wide clean jars until adult emergence. Then, the emerged adults were supplied with a piece of cotton wetted with 5-10% sugar solution and branches of tafla (*Nerium oleander*) as a suitable site for oviposition. Newly laid egg masses were collected daily and transferred into the rearing jars.

Histopathological studies

All tested compounds at their LC_{50} concentration were applied to the fourth instar larvae using the leaf dipping technique method at the LC_{50} concentration of each compound. The larvae were collected after 1, 2, 3, 4 and 5 days post treatment then, transferred into Bouin's solution. It was used as a fixative, for dehydration and removal of the yellow colour of Bouin's solution the larvae were rinsed in a series of ethanol solutions. They were transferred first into 50% ethyl alcohol for 2 hrs. (Two changes) then left for 24 hours. Then the larvae passed through a series of alcoholic treatment each for two hours at room temperature starting with 80% followed by 90%, 96% and ending with 100% alcohol. After dehydration the larvae were placed in solution of amylacetate solution and soft paraffin wax and leaving them for 24 hours at 50°C. The larvae were replaced by soft paraffin wax three times at 24 hours intervals at 50°C. A mixture of one part of hard paraffin wax was added to the larvae. The larvae were imbedded in wax mixture used in the last step. Serial sections at 6 microns were made by microtome and mounted on clean slides using Mayer's albumin. Sections were mounted on glass slides and stained with haematoxylene and counterstained in alcoholic solution and prepared for examination and photo microscopy.

Biochemical studies

Samples preparation

Larval samples used for biochemical assays were collected at 1, 2, 3, 4, 5 and 7 days post treatment of the 4^{th} instar larvae with the LC_{50} concentration for each compound. Untreated larvae were used as control. Samples were homogenized in distilled water using a Teflon homogenizer. The homogenates were centrifuged at 500 r.p.m for 10 minutes at 5°C. The supernatants were immediately assayed to determine the total soluble protein, the activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and the carbohydrate hydrolyzing enzymes (Trehalase, invertase, amylase),α-β esterases, phosphatesas (acid and alkalin).

Determination of total soluble protein

Colorimetric determination of total soluble protein in total homogenate *S. littoralis* larvae was carried based out as described by (Gornall *et al.*, 1949). A volume of 0.2 ml of larval homogenate was added to 5 ml of Biuret reagent and incubated for 30 min at 20-25°C. The absorbance of the sample against a blank Biuret reagent was measured at wave length of 546 nm.

Determination of enzymes activities

Transminase enzymes (GOT and GPT)

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities were determined colorimetrically according to the method of Reitman and Frankle (1957). GOT transfer the amino group from L-aspartate to α-ketyo acid (α-ketoglutaric acid) producing a new amino acid (L-glutamate) and a new keto acid (oxaloacetic acid). GPT transfer the amino group from D,L alanine to α-keto acid (α-keto glutaric acid), resulting in a new amino acid (L-glutamate) and a new keto acid (pyruvic acid). Oxaloacetate or pyruvate reacts with 2, 4-dinitrophyenylhedrazine forming oxaloacetate or pyruvate hydrazone which in alkaline medium form a brown colour which can measure spectrophotometerically. The reaction mixture consisted of 1ml of a mixture of phosphate buffer (pH 7.4) 0.2 mM α-ketoglutaric and 200 mM D-L alanine or L-aspartate, 0.2 ml of larval homogenate was then added to the reaction mixture. The mixture was incubated for 30 min. then after, 10 ml of 0.4 N

NaOH was added. The optical density of the produced brown color is measured after 5 min using spectrophotometer at 520 nm. The enzyme activity is expressed as M Pyruvate/gm body weight/min.

Carbohydrate hydrolyzing enzymes

The methods used to determine the digestion of trehalose, starch and sucrose by trehalase, amylase and invesrtase enzymes respectively, were similar to those described by Ishaaya and Swiriski (1976). The free aldehydic group of glucose were determined using 3, 5 dinitrosalicylic acid reagent. The optical density (OD) of the produced colour is measured at 550 nm using spectrophotometer. The enzymatic activity was expressed as mg glucose released/gm body weight/min.

Determination of non-specific esterases activity

Alpha- and Beta- esterases (α-E, β-E) were determined according to the method of Van Asperen (1962). Using α-naphthyl acetate and β- naphthyl acetate as substrates, respectively. Napthol produced as a result of substrate hydrolysis can be measured by the addition of diazoblue sodium lauryl sulphate solution which produces a strong blue colour in case of α-naphthol or strong red colour in the case of β-naphthol. The colour was measured spectrophotometrically. The developed colour was read at 600 and 555nm for α- and β-naphthol, respectively. The activity was expressed as mg α- or β- naphthol released/min/ larva.

Acid and alkaline phosphatases enzymes (AcP & AlkP)

Acid phosphatase (AcP) and alkaline phosphatase (AlkP) were determined according to the method described by Powell and Smith (1954).

Acetylcholine esterase (AChE):

Acetylcholine esterase (AChE) was measured according to the method described by Simpson *et al.,* (1964), measured at 515 nm.

Results and discussion

Histological changes in the mid-gut of the 4th instar larvae of *S. littoralis* as affected by the tested compounds

Across section in the mid-gut of untreated larvae of *S. littoralis* appeared the epithelium of mid-gut surrounded by the basement membrane. They possess oval, conspicuous nuclei nearly central in position. Scattered between them are small goblet cells with reduced granular cytoplasm and spherical nuclei. The epithelial shows a striated (brush border) which is in the fact, the microvilli of columnar cell extending from their free ends. The wall of the gut contains two distinct layers of muscle fibers longitudinal muscles fibers to the outside and circular muscles fibers to the inside. The spaces between the different gut wall layers are almost filled with connective tissue.

Effect of methomyl

The present histological study on the effect of methomyl on the mid-gut of treated larvae of *S. littoralis* after 1, 2 and 3 days of treatments revealed certain changes appeared within Plates (1) that showed epithelium cells detached from the basement membrane in many areas and thickness of epithelial cells in the mid-gut larvae and some cells were broken and emptied their cytoplasmic contents in the space between the epithelial and peritrophic membrane. However, after 4 and 5 days of treatments complete degeneration of the mid-gut epithelial cells becomes more deformed and losses the columnar structure while the peritrophic membrane still intact in many areas.

Effect of protecto

Plates (2) indicated that, the epithelial cells lost their close association with the basement membrane and with each other. The epithelial cells were destroyed and lose their columnar structure in some point and caused disorganization of peritrophic membrane and in some cases disappeared after 1, 2 and 3 days of treatments. While after 4 and 5days of treatments the mid-gut epithelial cells were filled with scattered vacuoles and the basement membrane appear and still intact and the epithelium cells destruction in some point.

Effect of coumarin

Plates (3) indicated that, the effect of coumarin on the mid-gut tissues of 4th larvae of *S. littoralis* were most pronounced and extensive after 1 and 2 days of the treatments, where some cells become thickness and deformed also, emptied their cytoplasmic contents in the space between the epithelial and peritrophic membrane. After 3 and 4 days of treatment, the epithelium cells are destroyed, broken and separation completely from the broken basement membrane. The cellular debris from degenerating cells filled the gut lumen. While after 5 days of treatments microscopic examination showed no differences between the larvae which treated by coumarin and the control in concern of epithelium and basement membrane also, peritrophic membrane not affected and still intact.

Effect of azadirachtin

Plates (4) indicated that, there is no effect of azadirachtin extract on the 4th larvae instar of *S. littoralis* after 1 and 2 days of treatments, while after 3 and 4 days of treatments many abnormalities appeared in internal components of the mid-gut compared with the untreated larvae, the epithelial cells appeared deformed, destroyed in some points and in some cases the epithelial become elongate in size than control, detachment of the epithelium cells from the basement membrane in the same areas, but it was still intact in other. Also, azadirachtin caused

disintegration of peritrophic membrane compared with control. while after 5 days of treatment the epithelial cells become more apparent and the microvili of some columnar cells are still intact, also peritrophic membrane and basement membrane not affected.

Mohamed (2002) found that plant seed oil extracts, i.e., sunflowers, soybean, castor and cotton caused abnormalities in the tissue of mid-gut larvae of pink bollworms.

Gamil (2004) studded that histological changes were observed in *S. littroalis* mid-gut. Although, damage in mid-gut tissue by the two tested bacteria was relatively similar, Ahmed *et. al.* (2007) studied the effect of different neem products on the mid gut tissues of the black cut worm, *Agrotis ipsilon*. Its occurrence was much more evident and severe when *S. marcescens* was tested compared to the effect of HD 129 and protecto. Heba (2005) found that *B. thuringiensis* var. kurstaki caused detachment and destruction of the basement and peritrophic membranes, vacuolization and destruction of the epithelial cells.

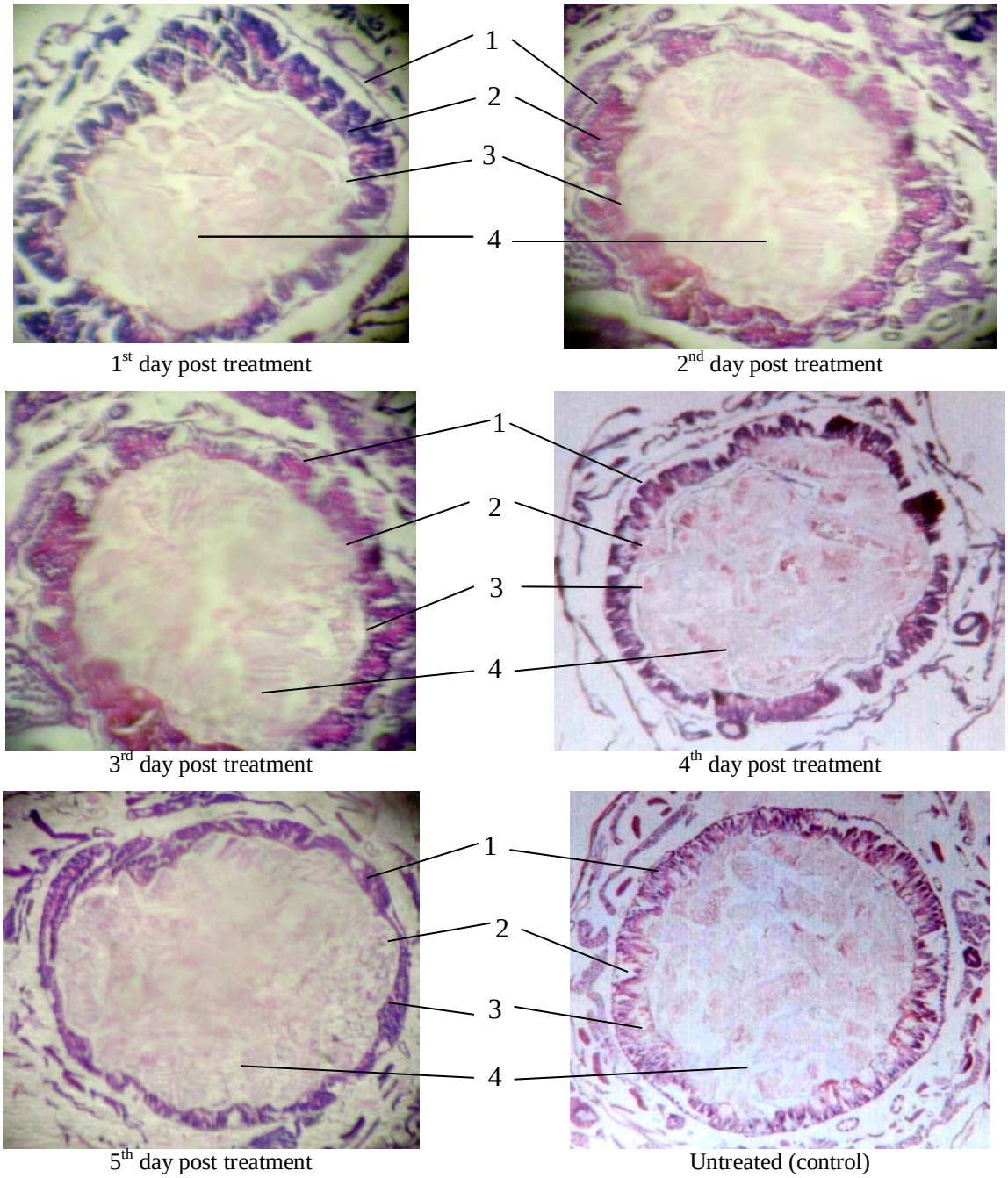


Plate 1. Effect of lannate on the mid gut of the 4th instar larvae of *S. littoralis*.
(1) Basement membrane (2) Epithelium layer
(3) Peritrophic membrane (4) Lumen

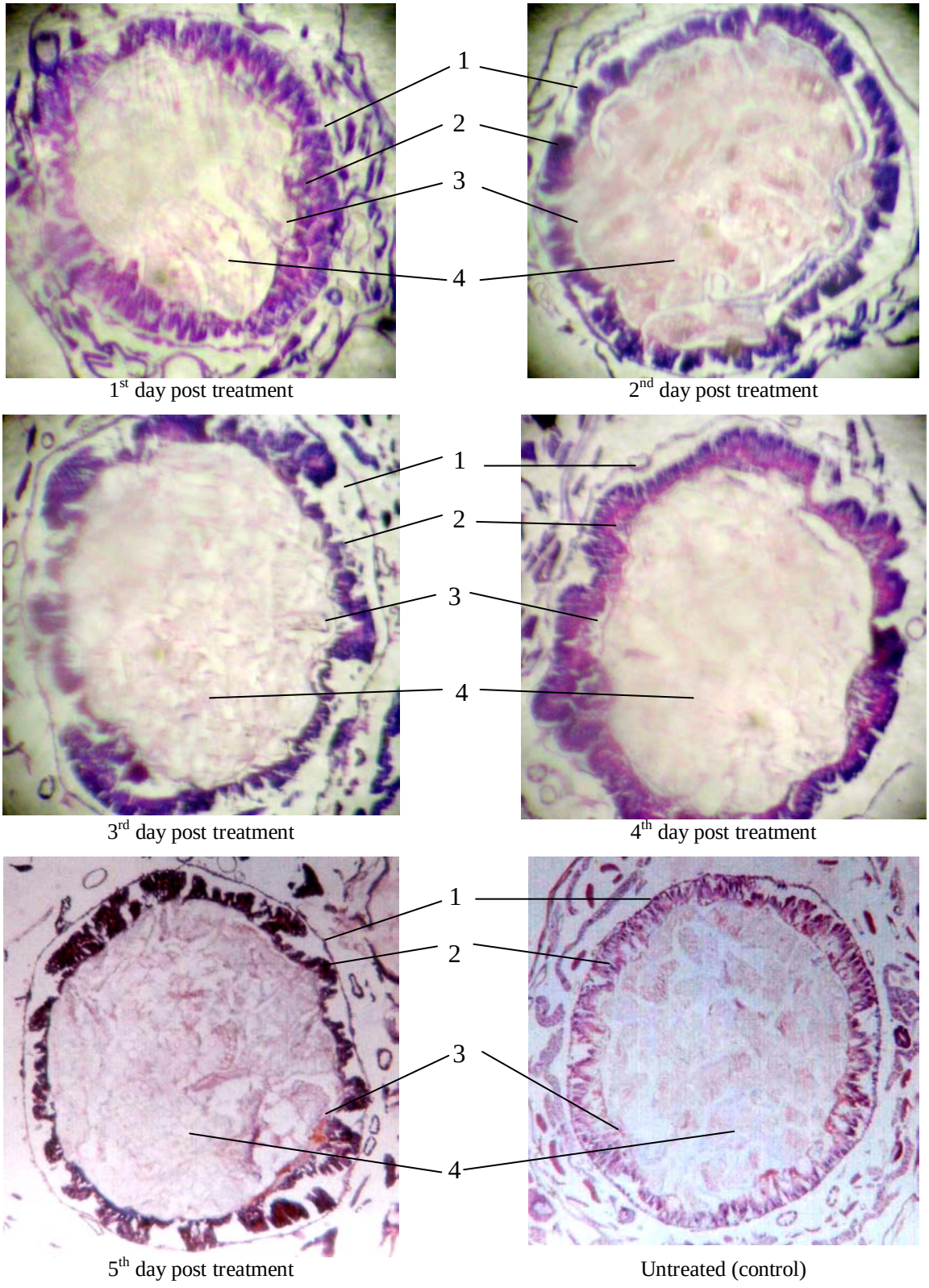
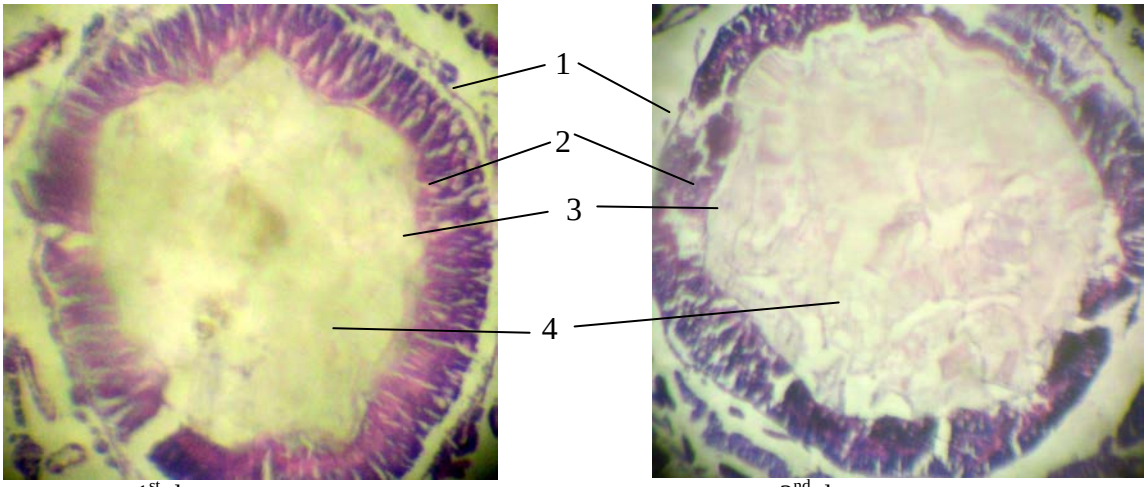


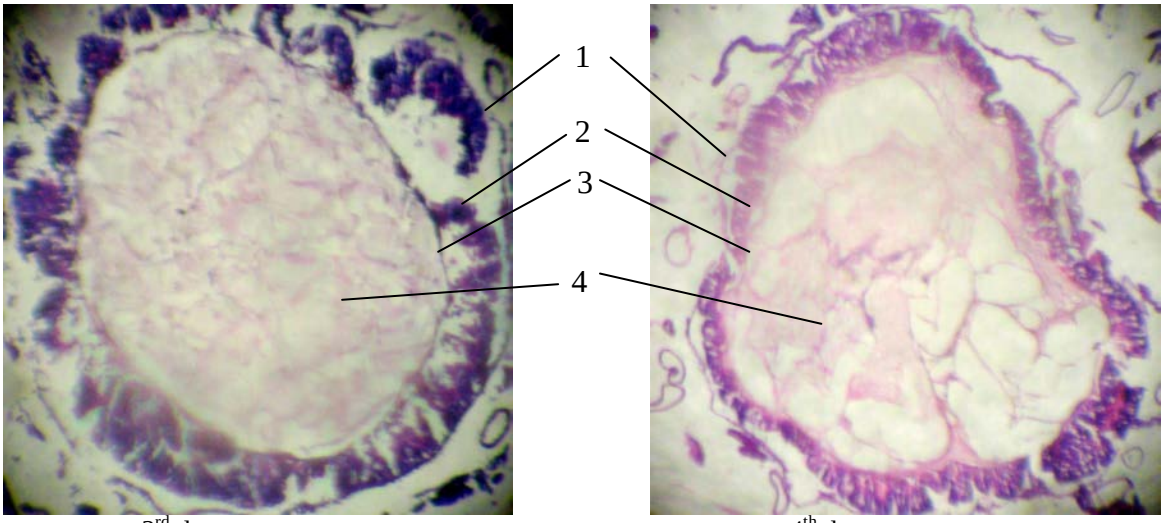
Plate 2. Effect of protecto on the mid gut of the 4th instar larvae of *S. littoralis.*

(1) Basement membrane (2) Epithelium layer
(3) Peritrophic membrane (4) Lumen



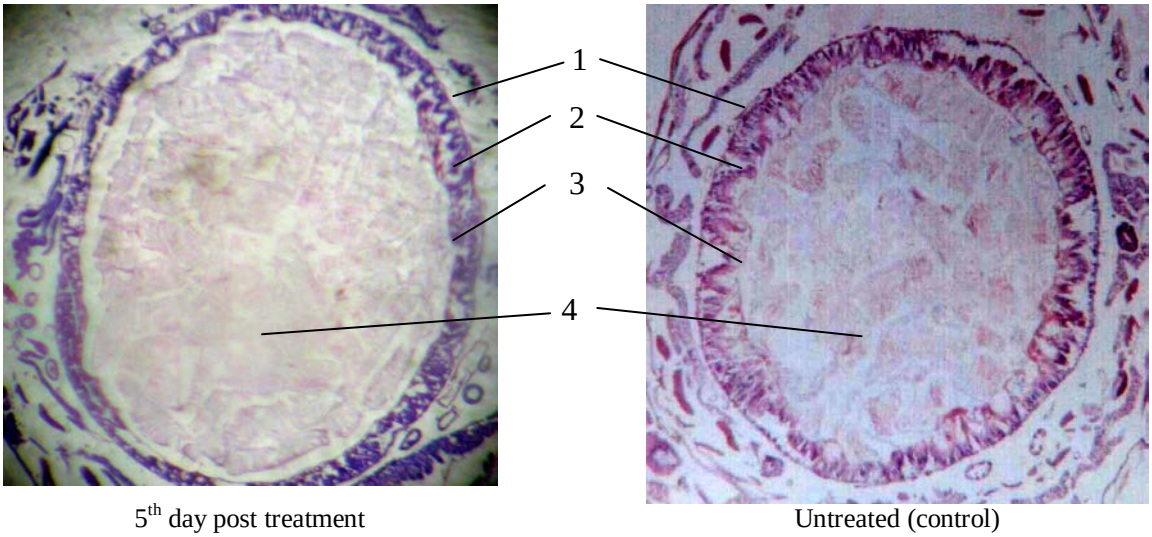
1st day post treatment

2nd day post treatment



3rd day post treatment

4th day post treatment



5th day post treatment

Untreated (control)

Plate 3 Effect of coumarin on the mid gut of the 4th instar larvae of *S. littoralis*.
(1) Basement membrane (2) Epithelium layer
(3) peritrophic membrane (4) Lumen

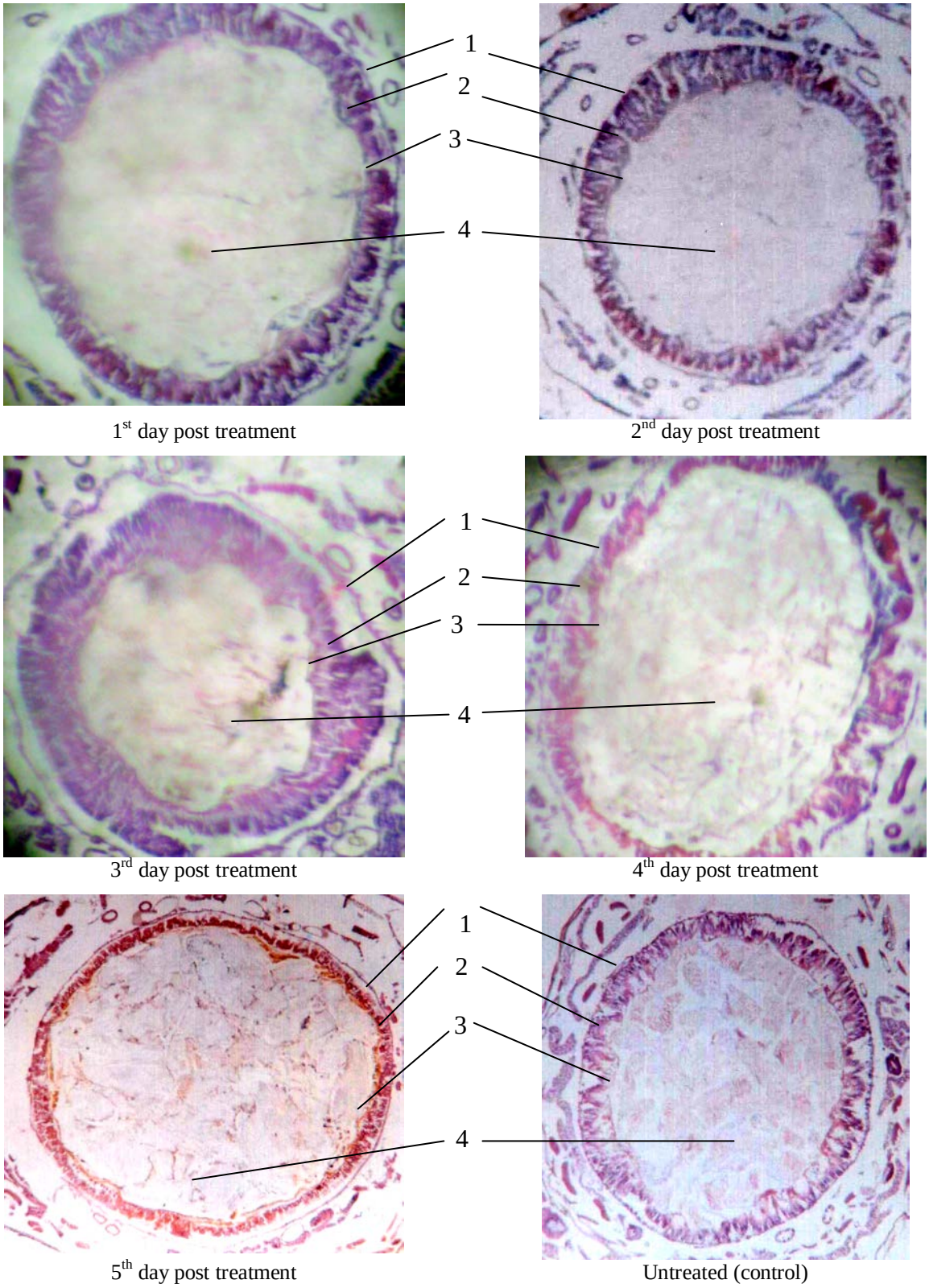


Plate 4. Effect of azadirachtin on the mid gut of the 4th instar larvae of *S. littoralis*.

(1) Basement membrane (2) Epithelium layer
(3) peritrophic membrane (4) Lumen

Effect of the tested compounds on some biochemical aspects of the cotton leafworm larvae in the laboratory

Fourth instar larvae of *S. littoralis* treating with the LC_{50} concentration for each compound was used for biochemical assay to evaluate total soluble protein, the activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and the carbohydrate hydrolyzing enzymes (trehalase, invertase, amylase), α-β esterases, phosphatesas (acid and alkaline) and acetylcholinesterase (AChE).

Concentration of total soluble protein (T.S.P)

Data in Table (1) showed that, all tested compounds significantly reduce the total protein except protecto which significantly increase in the concentration of total protein. The other tested compounds caused significant reduce of total protein. The values of total protein in the supernatant of the homogenate larvae reached to 3.120, 1.291, 0.635 and 0.475 mg/g b.w. when larvae treated with LC_{50} of the tested protecto, methomyl, coumarin and azadirachtin, respectively, compared with 2.296 mg/g b.w. of the control. The percent differences between treatments in the concentration of total protein reached 135.88 % more than the check in case of protecto Mohamed-Sondos, 2002 and Abo-El-Ftooh (2004). Mohamady (2000) investigated the effect of treatment of the 4th instar larvae of *S. littoralis* with the LC_{25} and LC_{50} of fenvalerate on the total protein at different time intervals (24, 48 and 72 hrs). The results indicated that there was high reduction in the level of total protein due to the treatment.

Table (1): Effect of the tested compounds on the concentration of total soluble protein of the 4th instar larvae of *S. littoralis*

Treatments	**Total soluble protein (mg/g body weight) Days after treatment**					
	1st	**3rd**	**5th**	**7th**	**Mean**	**%**
Control	2.844	1.265	3.312	1.763	2.296	100.0
Protecto	3.673	3.475	2.796	2.626	3.120	135.88
Methomyl	2.203	1.026	1.330	0.604	1.291	56.22
Coumarin	0.587	0.548	0.687	0.718	0.635	27.66
Azadirachtin	0.662	0.387	0.288	0.561	0.475	20.68

L.S.D. at (0.05) for treatment = 0.32

Carbohydrate hydrolyzing enzymes:

Invertase

Data in Table 2 indicated that, the tested compounds showed reduction in the activity of invertase of the treated 4th instar larval of *S. littoralis*. The mean values of invertase activities in the supernatant of the homogenate larvae reached to 1.128, 1.131 and 1.502 mg/g b.w when larvae treated with (LC_{50}) by protecto, methomyl and azadirachtin compared with 1.636 mg/g. while, there were no effects in the activity of invertase when larvae treated by coumarin (1.636 mg/g). The percent differences between treatments in the activity of this enzyme irrespective to the time after treatment reached 68.94, 69.13 and 91.80 % less than the check in case of protecto, methomyl and azadirachtin, respectively. In general, protecto and methomyl were high effective on invertase activity 68.94 and 69.13, respectively.

Table (2). Effect of the tested compounds on the invertase, trehalase and amylase activty of the 4th instar larvae of *S. littoralis*

Treatments	Invertase activity (µg Glu/g body weight/min) Days after treatment					
	1st	3rd	5th	7th	Mean	%
Control	1.816	1.153	2.418	1.156	1.636	100.0
Protecto	0.353	1.076	1.808	1.278	1.128	68.94
Methomyl	1.274	0.899	1.416	0.935	1.131	69.13
Coumarin	1.827	1.046	1.542	2.077	1.636	100.0
Azadirachtin	1.284	1.710	1.623	1.393	1.502	91.80
L.S.D. at (0.05) for treatment = 0.34						
	Trehalase activity (µg Glu/g body weight/min) Days after treatment					
Control	0.530	0.600	1.150	0.506	0.697	100.0
Protecto	0.353	0.704	0.775	0.455	0.572	82.06
Methomyl	0.574	0.697	0.466	0.419	0.539	77.33
Coumarin	0.639	0.540	0.702	1.097	0.744	106.74
Azadirachtin	0.771	0.500	0.772	0.747	0.697	100.0
L.S.D. at (0.05) for treatment = 0.13						
	Amylase activity (µg Glu/g body weight/min) Days after treatment					
Control	0.143	0.265	0.344	0.291	0.261	100.0
Protecto	0.353	0.100	0.133	0.169	0.189	72.41
Methomyl	0.131	0.329	0.100	0.265	0.206	78.92
Coumarin	0.230	0.292	0.347	0.442	0.327	125.28
Azadirachtin	0.113	0.215	0.364	0.692	0.346	132.56
L.S.D. at (0.05) for treatment = 0.08						

Trehalase

Data in Table 2 indicated that coumarin and azadirachtin are almost the same with control for the the activity of trehalase. The mean values of trehalase activities in the supernatant of the homogenated larvae reached 0.572, 0.539, 0.744 and 0.697 mg /g b.w. when larvae treated (at LC_{50}) by protecto, methomyl, coumarin and azadirachtin, respectively. Compared with 0.697 mg/g. for the control on the contrary, protecto and lannate showed reduction in enzyme activity, reaching 82.06 and 77.33 % less than the check. About 22.77 and 17.94 % inhibition were obtained by methomyl and protecto, respectively.

Amylase

Data in Table 2 indicated that coumarin and azadirachtin caused significant increase while protecto and methomyl caused significant decrease in the activity of amylase of 4th instar larvae of *S. littoralis* compared to the untreated ones and other tested compounds. The mean values of amylase activities in the supernatant of the homogenated larvae reached to 0.189, 0.206, 0.327 and 0.346 mg/g. when larvae were treated at LC_{50} by protecto, methomyl, coumarin and azadirachtin, respectively, compared with 0.261 mg/g for the untreated control. The other products variously decreased this enzyme activity, i.e. 72.41 and 78.92 % less than the

untreated control in case of protecto and methomyl. Khedr (2002) found an increase in the activity of trehalase enzyme of *S. littoralis* (2nd & 4th instar larvae) after treatment with Biorepel.

Transaminase enzymes:

Glutamic pyruvic transaminase (GPT)

Data in Table 3 showed that the tested compounds caused significant decreased in activity of GPT of the treated 4th instar larvae of *S. littoralis* than the untreated control. While, protecto showed no significantly increased the activity of GPT. The mean values of GPT enzyme activities in the supernatant of the homogenated larvae reached to 1.07, 0.58, 0.93 and 1.02 mg/g. When larvae were treated at LC_{50} by protecto, lannate, coumarin and azadirachtin, respectively, compared with 1.09 mg/g b. w for the untreated larvae. The tested compounds decreased this enzyme activity, i.e. 98.17, 53.21, 85.32 and 93.57 % less than the untreated check in case of protecto, methomyl, coumarin and azadirachtin. It was found that the most inhibitor compound was methomyl followed by coumarine this result agree the studied by Vera *et. al.* (2006).

Glutamic oxaloacetic transaminase (GOT)

Data in Table 3 revealed the significant increasing effect of protecto, coumarin and azadirachtin on GOT activity of the treated 4th instar larvae of the cotton leafworm compared with the untreated larvae. The enzyme activity changed from 7.32 mg/g of larvae (normal) to 7.49, 8.40 and 9.97 mg/g , respectively. On the contrary, methomyl gave (5.94 mg/g b.w.) showed 81.15 decreased in GOT activity than the check. In general, the percent differences between treatments in the activity of this enzyme irrespective of the time after treatment reached136.20, 114.75 and 102.32 % more than the check in case of azadirachtin, coumarin and protecto respectively. Mohamady (2000) investigated the effect of treatment of the 4th instar larvae of *S. littoralis* with the LC_{25} and LC_{50} of fenvalerate on the activity of GOT at different time intervals (24, 48 and 72 hrs). Data showed that, there was irregular effect on GOT activity at the different time intervals where it fluctuated between increase and decrease throughout the 72 hrs period of the experiment.

Alpha and beta esterases (α-E and β-E)

Alpha esterase (α-E)

Data in Table 4 were caused indicated that, the tested compounds coumarin and azadirachtin significant increase in the activity of α–esterase of the treated 4th instar larvae of *S. littoralis* than the untreated larvae. The mean values of α–esterase enzyme activities in the supernatant of the homogenate larvae reached to 1.643, 1.890, 3.047 and 2.665 mg/g b.w when larvae were treated at LC_{50} by the tested protecto, lannate, coumarin and azadirachtin, respectively, compared with 2.510 mg/g b.w the untreated control.

Table 3. Effect of the tested compounds on the activities of GPT and GOT enzymes of the 4th instar larvae of *S. littoralis*

Treatments	GPT activity (mg/g pyrovate/ body weight/min) Days after treatment					
	1st	3rd	5th	7th	Mean	%
Control	1.39	0.96	1.63	0.39	1.09	100.0
Protecto	1.08	0.83	1.90	0.48	1.07	98.17
Methom-yl	0.46	0.70	0.45	0.73	0.58	53.21
Coumari-n	0.63	0.94	0.88	1.28	0.93	85.32
Azadirac-htin	0.44	1.95	1.13	0.58	1.02	93.57
L.S.D. at (0.05) for treatment = 0.13						
	GOT activity (mg/g pyrovate/ body weight/min) Days after treatment					
Control	11.18	9.37	4.28	4.44	7.32	100.0
Protecto	9.68	6.47	9.78	4.02	7.49	102.32
Methomyl	3.49	7.23	5.43	7.59	5.94	81.15
Coumarin	6.29	8.32	9.36	9.64	8.40	114.75
Azadirachtin	12.14	11.16	12.09	4.49	9.97	136.20
L.S.D. at (0.05) for treatment = 1.11						

The differences in the activity of this enzyme between treatments reached 121.39 and 106.18 % more than the check in case of coumarin and azadirachtin, respectively. Protecto and methomyl showed slight decrease in α – esterase activity, reached to 65.45 and 75.29 % less than the control, respectively. Mohamed and Azab (2002) found that pyrethroids caused a remarkable increase in alpha-esterase of pink bollworm compared to that recorded in untreated check larvae.

Beta esterase (β -E)

Data in Table 4 revealed that, 4th instar larvae of the cotton leafworm showed significant decreased in β -esterase enzymes activities in the supernatant of the homogenated larvae which treated at LC_{50} by protecto (1.855 mg/g b.w.), methomyl (2.248 mg/g b.w.), coumarin (2.591 mg/g b.w.) and azadirachtin (1.882 mg/g b.w.), respectively. Compared with 2.699 mg/g b.w. of the control. The tested compounds showed decreased in the activity of this enzyme after treatment reached 68.72, 83.29, 95.99 and 69.73 % less than the check in case of protecto, lannate, coumarin and azadirachtin, respectively.

Acetyl cholinesterase (ACHE)

Data in Table 4 indicated that, the tested compounds increased the activity of (AChE) of the 4th instar larvae of *S. littoralis* compared with the untreated larvae. The mean values of (AChE) in the supernatant of the homogenate larvae reached to 0.175, 0.265, 0.266 and 0.249 mg/g b.w when larvae were treated with LC_{50} of the tested protecto,

lannate, coumarin and azadirachtin compared with the check (0.148 mg/g b.w). The percent differences between treatments in the activity of this enzyme reached 118.24, 179.05, 179.72 and 168.24 % more than the check in case of protecto, lannate, coumarin and azadirachtin, respectively.

Alkaline and Acid phosphatase

Alkaline phosphatase

Data in Table 4 revealed that, the tested compounds inhibited the alkaline phosphatase of the treated 4th instar larvae of *S. littoralis* than the untreated ones. The mean values of alkaline phosphatase enzymes activities in the supernatant of the homogenate larvae reached to 0.058, 0.077, 0.054 and 0.073 mg/g b.w. when larvae were treated with the LC_{50} by the tested protecto, lannate, coumarin and azadirachtin, respectively, compared with 0.085 mg/g b.w. of the untreated control. The tested compounds caused decrease in Alkaline phosphatase activity reached to 68.24, 90.58, 63.52 and 85.88 % less than control. In case of protecto, lannate, coumarin and azadirachtin, respectively.

Table 4. Effect of the tested compounds on the activities of α - esterase, β - esterase, acetyl-cholinesterase, alkaline phosphates, acid-phosphates enzymes of the 4th instar larvae of *S. littoralis*

Treatments	α - esterase activity (µg a-naphth/g body weight/min) Days after treatment					
	1st	3rd	5th	7th	Mean	%
Control	1.989	2.581	4.242	1.260	2.510	100.0
Protecto	2.090	1.148	1.429	1.905	1.643	65.45
Methomyl	2.272	2.701	1.144	1.437	1.890	75.29
Coumarin	3.004	3.401	2.632	3.153	3.047	121.39
Azadirachtin	2.244	2.295	2.922	3.200	2.665	106.18
L.S.D. at (0.05) for treatment = 0.23						
	β - esterase activity (µg a-naphth/g body weight/min) Days after treatment					
Control	3.236	1.552	3.625	2.386	2.699	100.0
Protecto	1.418	2.361	2.880	0.760	1.855	68.72
Methomyl	3.706	2.213	1.578	1.496	2.248	83.29
Coumarin	2.423	2.815	1.437	3.687	2.591	95.99
Azadirach-tin	0.931	2.077	2.073	2.447	1.882	69.73
L.S.D. at (0.05) for treatment = 0.21						
	Acetyl cholinesterase activity (µg AchBr/g body weight/min) Days after treatment					
Control	0.071	0.099	0.302	0.120	0.148	100.0
Protecto	0.401	0.105	0.090	0.104	0.175	118.24
Methomyl	0.03	0.045	0.094	0.1	0.0672	29.05
Coumarin	0.532	0.051	0.192	0.288	0.266	179.72
Azadirach-tin	0.327	0.060	0.255	0.356	0.249	168.24
L.S.D. at (0.05) for treatment = 0.56						
	Alkaline phosphates activity (µg phenol/g body weight/min) Days after treatment					
Control	0.092	0.103	0.082	0.061	0.085	100.0
Protecto	0.119	0.041	0.031	0.042	0.058	68.24
Methomyl	0.057	0.075	0.122	0.055	0.077	90.58
Coumarin	0.035	0.059	0.052	0.070	0.054	63.52
Azadirach-tin	0.049	0.090	0.067	0.087	0.073	85.88

Table 4. cont.

L.S.D. at (0.05) for treatment = 0.02						
	Acid-phosphates activity (µg phenol/g body weight/min) Days after treatment					
Control	0.045	0.049	0.031	0.013	0.034	100.0
Protecto	0.085	0.025	0.017	0.020	0.037	108.82
Methomyl	0.042	0.046	0.035	0.018	0.035	102.94
Coumarin	0.053	0.107	0.011	0.026	0.049	144.11
Azadirachtin	0.045	0.052	0.020	0.024	0.035	102.94
L.S.D. at (0.05) for treatment = 0.002						

Acid phosphatase

Data in Table 4 indicated that, the tested compounds were increased the activity of acid phosphatase enzymes compared to untreated larvae. The mean values of acid phosphatase enzymes activities in the supernatant of the homogenate larvae reached to 0.037, 0.035, 0.049 and 0.035 mg/g b.w. when larvae were treated with LC_{50} by protecto, lannate, coumarin and azadirachtin, respectively compared with 0.034 mg/g b.w. of the untreated control. The percent differences between treatments 108.82, 102.94, 144.11 and 102.94 % more than control when larvae treated by protecto, lannate, coumarin and azadirachtin, respectively. Abdel-Hafez *et al.* (1993**)** studied the efficacy of two OP insecticides, two IGR's and their combined mixtures on laboratory of strain *S. littoralis*. Data showed that the larvae with the LC_{50} of the tested compounds caused variable reduction (much lower than control) in alkaline phosphatase.

In general, all tested compounds decreased the total soluble protein of the 4th larvae cotton leaf worm except the bio-insecticide, protecto. The carbohydrate enzymes were inhibited by protecto and methomyl. All tested compound reduced the activity of GPT, α - esterase and β - esterase**.** Alkaline phosphate was inhibited by all tested compounds while the activity of acid phosphates was increased. It was interested that the effect of tested compounds on the different enzymes was agreement with their effect on the mid gut membrane. The disturbances of the enzyme activities in the treated larvae due to the damage of mid gut membrane, particularly epithelial tissues.

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