

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

PART (1)

First of all, it should admit that the comparison between insecticides and plant extracts were somewhat have a bios for plant extracts from the side of concentrations used.

1. Effect of different tested compound as antifeedants on 4th instar larvae of *S. littoralis*:

In this trial only 4th instar larvae of *S. littoralis* were used to establish the presence of antifeeding properties in these tested compounds. The obtained results are given in Table (1).

Data in Table (1) indicated that coumarin gave the lowest consumed leaf area (cm^2) treated 24 hrs followed by azadirachtin extract therefore, the two plant extracts were the best for lowering the consumed area of leaves. While lannate and protecto gave the highest consumed area in cm^2 at 24 hrs treatment. All tested compounds gave significantly lower consumed area than control. Also, the same trend was obtained for percentages of eaten areas.

The mean percentages of eaten area were 6.06, 8.23, 10.19 and 15.79 obtained after coumarin, azadirachtin, lannate and protecto treatments, respectively. In addition, data in Table (1) clearly indicate the important role of tested compounds used in determining antifeeding activities. Coumarin showed the highest antifeeding activity mean (69.07) followed by azadirachtin (58.17). On the other hand, lannate and protecto gave the lowest antifeeding activity means (48.15 and 19.50, respectively). Data in Table (1) also showed increasing

Table (1): Effect of different concentrations of coumarin, azadirachtin, protecto and lannate as antifeedants on 4th instar larvae of *S. littoralis* (Boisd.).

| Compound | Concentration | Consumed area in (Cm) ² treated 24 his | % of eaten area | Antifeedant activity |
|----------------|---------------|---|-----------------|----------------------|
| Lannate ppm | 100 | 1.50±0.14 | 3.98 | 79.72 |
| | 50 | 2.76±0.13 | 7.32 | 62.69 |
| | 25 | 4.14±0.12 | 10.98 | 44.04 |
| | 12.5 | 4.32±0.16 | 11.52 | 41.29 |
| | 6.25 | 6.48±0.11 | 17.19 | 12.39 |
| Means | | 3.84 | 10.19 | 48.02 |
| Protecto | 2.0 | 5.03±0.17 | 13.34 | 32.01 |
| | 1.0 | 5.48±0.33 | 14.67 | 25.23 |
| | 0.5 | 5.73±0.11 | 15.2 | 22.53 |
| | 0.25 | 6.25±0.66 | 16.58 | 15.49 |
| | 0.125 | 7.23±0.08 | 19.18 | 2.24 |
| Means | | 5.94 | 15.79 | 19.50 |
| Coumarin | 10 | 0.80±0.04 | 2.12 | 89.19 |
| | 5 | 0.98±0.13 | 2.60 | 86.75 |
| | 2.5 | 1.61±0.24 | 4.27 | 78.24 |
| | 1.25 | 2.85±0.22 | 7.56 | 61.47 |
| | 0.625 | 5.20±0.16 | 13.79 | 29.72 |
| Means | | 2.28 | 6.06 | 69.07 |
| Azadirachtin | 10 | 1.40±0.14 | 3.71 | 81.7 |
| | 5 | 1.68±0.12 | 4.46 | 77.27 |
| | 2.5 | 3.13±0.19 | 8.30 | 57.69 |
| | 1.25 | 3.64±0.16 | 9.66 | 50.77 |
| | 0.625 | 5.66±0.08 | 15.02 | 23.45 |
| Means | | 3.10 | 8.23 | 58.17 |
| Control | | 7.30±0.04 | 19.62 | - |

consumed area in cm² by decreasing the concentration of each tested compounds. The obtained results indicate a positive relationship between the concentration of tested compounds and antifeeding activities. The highest used concentration (10%) of coumarin & azadirachtin gave the greatest antifeeding activity (89.19) and (81.7), respectively. On the other hand, the lowest used one of lannate (6.25%) and protecto (0.125%) gave the least antifeeding activity values 12.39 and 2.25, respectively. These results agree with the results of **Mansour (1981)** who found that coumarin at higher concentration reduced the food consumption, acted as an antifeedant, completely inhibiting larval feeding, while lower concentrations of the compound decreased the amount of food digested and ingested.

All furano coumarins a substituent in the furan ring decrease antifeeding activity at higher concentrations. (**Luthria et al., 1989**).

Methanol extract of *Azadirachta indica* and hexane extract of *Thevetia nerifolia* leaves showed antifeedant effects against *Agrotis janata*. (**Babu et al., 1997**).

The new *B. thuringiensis* products containing crystals and endospores were more toxic towards the Egyptian cotton leafworm than those with crystals alone. In this respect, crystal endospore complex was most effective in reducing the consumption of fed leaves per day by the larvae. (**Zaiad, 2001**).

The antifeedant activity of Neemix 4.5% EC, a commercial formulation of azadirachtin from the neem tree (*A. indica*) indicated that feeding by adults *Nezara viridula* was significantly reduced in treated pods compared with controls.

The antifeedant effect of azadirachtin was significantly greater on pods treated with 5% aqueous solution than on those treated with 0.5%, indicating that the antifeedant activity was related to concentration. (**Abudulai *et al.*, 2003**).

PART (2)

Insecticidal activities of the tested compounds against 4th instar larvae of the cotton leafworm, *S. littoralis* in laboratory:

Data of the larvicidal activities of the two tested insecticides, namely lannate and protecto and the plant extracts coumarin and azadirachtin against the 4th instar larvae of the cotton leafworm, *S. littoralis* are tabulated in Tables (2 to 5). The comparative responses of insect larvae to the tested compounds expressed as LC₅₀ are summarized in Table (6) and illustrated as LD-p lines in Fig. (1). It could be noticed from the examination of the obtained results the important role of insecticide source and type and tested concentration on the insecticidal activities. The activities of the tested compounds differed upon the period after treatment. To facilitate the presentation of data, each compound will be discussed separately as follow:

1. Lannate (chemical insecticide):

Data in Table (2) showed the higher potency of lannate against the 4th instar larvae of the cotton leafworm, *S. littoralis*. Lannate showed considerable initial activity within 24 hrs from the treatment, these were proportionally related to the tested concentrations. The percent initial kill reached, 26.66, 36.66, 36.66, 40.0 and 43.33 % with 6.25, 12.5, 25, 50 and 100 ppm, respectively. The LC₅₀ was 13.72 ppm after 72 hrs from the treatment. It is clearly evident that the longer time after treatment has the higher residual mortality on tested larvae and vice versa. This was true with the all tested concentrations. The corresponding mortality percentages after 48 hrs reached 30.0,

Table (2): Accumulative mortality of 4th instar larvae of *S. littoralis* (Boisd.) to lannate in the laboratory at 26 ± 2°C and 60 ± 5% RH.

| Concentration | % Accumulative mortality | | |
|---------------|--------------------------|-----------|-----------|
| | 24 hrs | 48 hrs | 72 hrs |
| 100 ppm | 43.33±0.5 | 73.33±0.8 | 90.0±0.0 |
| 50 ppm | 40.0±0.0 | 66.66±0.5 | 80.0±0.0 |
| 25 ppm | 36.66±0.4 | 53.33±0.3 | 73.33±0.5 |
| 12.5 ppm | 36.66±0.3 | 46.66±0.5 | 63.33±0.5 |
| 6.25 ppm | 26.66±0.0 | 30.0±0.5 | 46.66±0.5 |
| Control | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

46.66, 53.33, 66.66 and 73.33 % with the same mentioned concentrations, respectively. The prolongation of time to 72 hrs recorded more larvicidal activity, reached 46.66, 63.33, 73.33, 80.0 and 90.0 % kill at 6.25, 12.5, 25, 50 and 100 ppm, respectively.

2. Protecto (biocide):

Data in Table (3) indicated insecticidal activity of the biocide protecto against 4th instar larvae of the cotton leafworm. Protecto showed very poor initial efficacy. It showed no kill after 24 hrs from treatment with all tested concentrations (0.125-2.0 g /ml) and the same performance was recorded after 48 hrs with (0.125-0.5 g /ml). Unsatisfactory kill was recorded after 72 hrs, reaching 10.0, 13.33, 16.66, 26.66 and 36.66 % with the concentrations of 0.125, 0.25, 0.5, 1.0 and 2.0 g /ml, respectively. The prolongation of post — treatment interval resulted in gradual increase in larval mortality, especially with the highest concentration, showing 53.33 and 80.0 % kill after 5 and 7 days, respectively. The LC50 of protecto was 0.391 g /ml after 7 days from the treatment.

3- Coumarin (plant extract):

Data in Table (4) indicated the poor insecticidal activity of coumarin against the 4th instar larvae of the cotton leafwoun in laboratory. The product showed slight initial activity when used at higher concentrations (5 and 10%). Within 48 hrs after treatment, the larval mortality reached 10.0, 10.0, 16.66, 20.0 and 30.0 % with 0.625, 1.25, 2.5, 5 and 10 %, respectively. The prolongation of post- application intervals to 72 hrs and 5 days showed slight increase in the mortality. The percent kill reached

Table (3): Accumulative mortality of 4th instar larvae of *S. littoralis* (Boisd.) to protecto in the laboratory at 26 ± 2°C and 60 ± 5% RH.

| Concentration | % Accumulative mortality | | | | |
|---------------|--------------------------|-----------|-----------|-----------|-----------|
| | 24 hrs | 48 hrs | 72 hrs | 5 days | 7 days |
| 2.0 % | 0.0±0.0 | 13.33±0.2 | 36.66±0.5 | 53.33±0.3 | 80.0±0.3 |
| 1.0 % | 0.0±0.0 | 10.0±0.0 | 26.66±0.0 | 43.33±0.3 | 66.66±0.5 |
| 0.5 % | 0.0±0.0 | 0.0±0.0 | 16.66±0.3 | 33.33±0.5 | 53.33±0.5 |
| 0.25 % | 0.0±0.0 | 0.0±0.0 | 13.33±0.3 | 30.0±0.3 | 43.33±0.8 |
| 0.125 % | 0.0±0.0 | 0.0±0.0 | 10.0±0.5 | 20.0±0.8 | 30.0±0.8 |
| Control | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

Table (4): Accumulative mortality of 4th instar larvae of *S. littoralis* (Boisd.) to coumarin in the laboratory at $26 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH.

| Concentration | % Accumulative mortality | | | |
|---------------|--------------------------|-----------|-----------|-----------|
| | 24 hrs | 48 hrs | 72 hrs | 5 days |
| 10 % | 10.0±0.3 | 30.0±0.3 | 53.33±0.0 | 86.33±0.5 |
| 5 % | 10.0±0.0 | 20.0±0.5 | 40.0±0.5 | 80.0±0.5 |
| 2.5 % | 6.66±0.0 | 16.66±0.3 | 40.0±0.0 | 66.66±0.0 |
| 1.25 % | 3.33±0.3 | 10.0±0.0 | 23.33±0.5 | 60.0±0.0 |
| 0.625 % | 0.0±0.0 | 10.0±0.0 | 13.33±0.3 | 43.33±0.3 |
| Control | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

13.33, 23.33, 40.0, 40.0 and 53.33 % after 72 hrs and 43.33, 60.0, 66.66, 80.0 and 86.33 % after 5 days at the same mentioned concentrations, respectively.

4- Azadirachtin (plant extract):

Data in Table (5) recorded that the tested azadirachtin extract showed very poor activity against the 4th instar larvae of the cotton leafworm within 24 and 48 hrs from feeding on treated leaves. This was pronounced with the five tested concentrations. In terms of figures, the percent mortality after 24 hrs reached 0.0, 0.0, 3.33, 6.66 and 16.66 %, while after 48 hrs reached 0.0, 13.33, 13.33, 16.66 and 26.66 % at 0.625, 1.25, 2.5, 5 and 10 %, respectively. The larval mortality reached 13.33, 23.33, 36.66, 40.0 and 43.3 % after 72 hrs, respectively.

The prolongation of post application intervals to 5 and 7days showed slight increase in the mortality. The percent mortality reached 20.0, 30.0, 43.33, 50.0 and 73.33 % after 5 days and 30.0, 43.33, 53.33, 66.66 and 83.33 % after 7 days at the same mentioned concentrations, respectively. The LC₅₀ reached (1.854 nil /100 ml) after 7 days from the treatment.

Reviewing the aforementioned results as shown in Table (6) could be concluded the lethal values of different tested compounds against 4th instar larvae of *S. littoralis*. The LC₅₀ frequently used to measure of tested compounds toxicity is the 50 percent lethal concentration. It is the concentration of poison that kill half of the population.

Table (6) and Fig. (1) show the results of probit analysis obtained by the exposure of 4th instar larvae of *S. littoralis* to different concentrations of tested compounds. There was a group

Table (5): Accumulative mortality of 4th instar larvae of *S. littoralis* (Boisd.) to azadirachtin in the laboratory at 26 ± 2°C and 60 ± 5% RH.

| Concentration | (/)0 Accumulative mortality | | | | |
|---------------|-----------------------------|-----------|-----------|-----------|-----------|
| | 24 hrs | 48 hrs | 72 hrs | 5 days | 7 days |
| 10 % | 16.66±0.0 | 26.66±0.2 | 43.33±0.5 | 73.33±0.3 | 83.33±0.3 |
| 5 % | 6.66±0.3 | 16.66±0.3 | 40.0±0.5 | 50.0±0.0 | 66.66±0.8 |
| 2.5 % | 3.33±0.0 | 13.33±0.3 | 36.66±0.5 | 43.33±0.0 | 53.33±0.8 |
| 1.25 % | 0.0±0.0 | 13.33±0.3 | 23.33±0.0 | 30.0±0.5 | 43.33±0.0 |
| 0.625 % | 0.0±0.0 | 0.0±0.0 | 13.33±0.3 | 20.0±0.0 | 30.0±0.3 |
| Control | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

Table (6): Lethal concentrations of the tested compounds against the fourth instar larvae of *S. littoralis*, after 3&7 clays after treatment.

| Compounds | Lethal values and their 95% confidence limits % | | | | |
|-------------------------------|---|---------------------------------|---------------------------------|-------------|-------|
| | LC50 | LC90 | LC ₉₅ | Slope ±SD | r |
| Lannate (ppm , | 13.729 (3.71-23.83) | 217.527 (109.73- 1343.07) | 476.045 (191.88- 6291.09) | 1.068±0.275 | 0.933 |
| Protecto (g /100 ml) | 0.391 (0.21-0.634) | 6.873 (2.65- 92.61) | 15.491 (4.67- 443.15) | 1.029±0.256 | 0.966 |
| Coumarin (g /100 ml | 0.843 (0.26-1.41) | 14.188 (6.72- 107.47) | 31.589 (11.83- 520.88) | 1.045±0.268 | 0.961 |
| Azadirachtin (ml /100 ml) | 1.854 (1.08-2.82) | 22.939 (10.59- 143.97) | 46.802 (17.75- 500.13) | 1.173±0.261 | 0.959 |

SD: Standard deviation of mortality regression line

r: Correlation coefficient

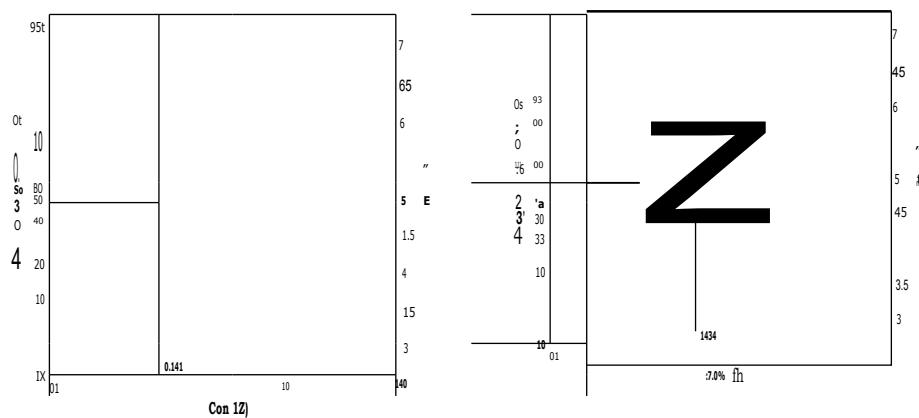
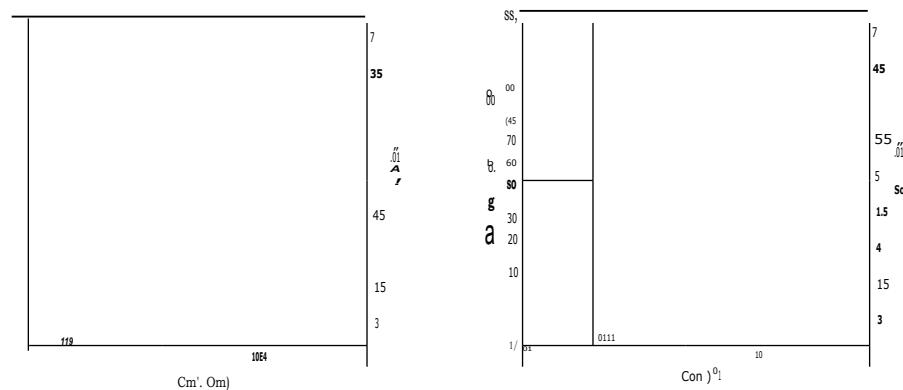


Fig (1): LC₅₀'s of the tested compounds on the 4th instar larvae of *S. littoralis*

of effective tested compounds having LC₅₀ lower than one. The chemical insecticide lannate recording the value of LC₅₀ (13.729 ppm) & plant extract azadirachtin having the value of LC₅₀ (1.854 ml /100 ml) and then coumarin having the value of LC₅₀ (0.843 g /100 ml). On the other hand, bio-insecticide protecto recording LC₅₀ (0.391 g /100 ml) the values of slope varied between 1.029 to 1.173. There was a considerable difference between the highest slope and the lowest one. The same trend was obtained at LC₉₀ and LC₉₅. Significant positive correlation coefficient values between concentrations and mortality % were obtained for all tested compounds, indicating that the increasing concentrations increased the mortality percentages. In summary, data indicated clearly that chemical insecticide (lannate) was the most effective one followed by plant extracts (coumarin and azadirachtin), respectively. While the bio-insecticide (protecto) was the least effective against 4th instar larvae of *S. littoralis*.

Such findings are in agreement with those obtained by several researchers in Egypt and other countries. The toxicity and sublethal effects of profenofos, emamectinbenzoate, spinosad and azadirachtin were studied on 2n^d and 4th instar larvae of *S. littoralis* under laboratory conditions. Based on the LC₅₀ values, profenofos was most toxic, the toxic action extended for 2 days, whereas the toxicity of emamectinbenzoate, spinosad, hexaflumuron, azadirachtin persisted for 5 days based on LC₅₀ values. A negative relationship was found between the time elapsed after treatment (24, 48, 72, 96 and 120 h) and the LC₅₀ values of the insecticides. (El-Aw, 2003).

The efficiency of three plant extracts, i.e. Neemazal, soyabean and garlic (Biorepel), and five insect growth regulators (IGRs), i.e. benzoylphenylurea, chlorfluazuron, flufenoxuron, lufenuron and tebufenozide against *Spodoptera littoralis* were studied. Among plant extracts, Neemazal recorded the highest initial and residual reduction percentages in both seasons in cotton fields, with the highest initial reduction reaching 30.33% and the highest residual reduction reaching 39.88%. However, in clover fields, Biorepel recorded the highest initial reduction in the 1999 season (32.11%) and Neemazal recorded the highest residual reduction percentage in the same season (36.03%). Among IGRs, the initial reduction percentages were at least 92.00% in both crops for all the tested compounds, except for Mimic which recorded an initial reduction percentage less than 77.0% in the cotton field in 1999. Residual reduction percentages did not exceed 87.00% in both crops for all the tested compounds in 2000. (Desuky *et al.*, 2005).

PART (3)

Effect of different tested compounds on some biological parameters of *S. littoralis* (Boisd.)

The latent effect of treating 4th larval instar of *S. littoralis* by different tested compounds on the biology and fecundity of stages that survived after larval treatment was studied. Accordingly, mortality percentages, pupation percentage, adult emergence percentage, sex ratio, fecundity of adult, larval and pupal duration and adult longevity were determined.

1- Biological effects of lannate after treatment of 4th instar larvae of *S. littoralis*:

The obtained results are illustrated in Table (7) and Fig. (2). Data indicated that increasing lannate concentrations increased mortality percentages of larvae. The highest percentage mortality (70.0 %) was recorded by concentration 100 ppm and the lowest was (48.3 %) at concentration 6.25 ppm, while at concentrations 50, 25, and 12.5 ppm, mortality percentages were (66.6, 61.6, and 53.3 %), respectively. The sub-lethal concentrations of lannate prolongs the larval period to reach maximum of 13.2 day at the highest concentration of 100 ppm compared with untreated larvae 9.5 days. The pupal duration seemed to be relatively longer for treated larvae than the check group and the averages were 8.4 and 11.5 days of the lower and the higher concentrations 6.25 and 100 ppm, respectively. On the other hand, the concentrations 12.5, 25 and 50 ppm showed slight difference in the pupal duration between each other where they recorded (9.5, 10.3 and 10.8) days, respectively. While it was 7.0 days at the check groups.

Z : Biological effects of lannate on 4th instar larvae of *S. littoralis* Boisd.) fed for 24 hrs. on treated leaves.

| S. littoralis | Larval stage | Pupal stage | Adult stage | | | | Deposit | Mortality (%) | Carcasses | Alive |
|----------------|--------------|-------------|-------------|--------------|------------|--------------|--------------|---------------|--------------|-------------|
| | | | % Malformed | Emergence | Malformed | Longevity | | | | |
| 001 | FEI | SI | 0.0 | 1717 | | W1 | WE | 10.1 | 9.3 | 0.0 |
| 50 | 66.9 | 77.1 | 10.8 | SS.0 | RN | W2 | 1.0 | 6.0 | W0 | W0 |
| 52 | 91.9 | 81.1 | COI | WS | 29 | IT.1 | 0.6 | 7W | 6.0 | 11.0 |
| 52.1 | 83.3 | 81.6 | II | WS | 017 | WS | SE | 0117 | OSI | -- |
| 6.2S | 38.17 | 10.0 | 1713 | PL | C8 | WS | 6.0 | 609.0 | 300 | 69.99 |
| Control | 0.0 | 1.0 | 00 | FO.16 | W0 | S.0 | 6.0 | W011 | 0001 | 89.3 |
| 15.11 | | | | TS.13 | 691 | 1.1.2 | 2.2.2 | OZ.2 | N.2.1 | 67.9 |

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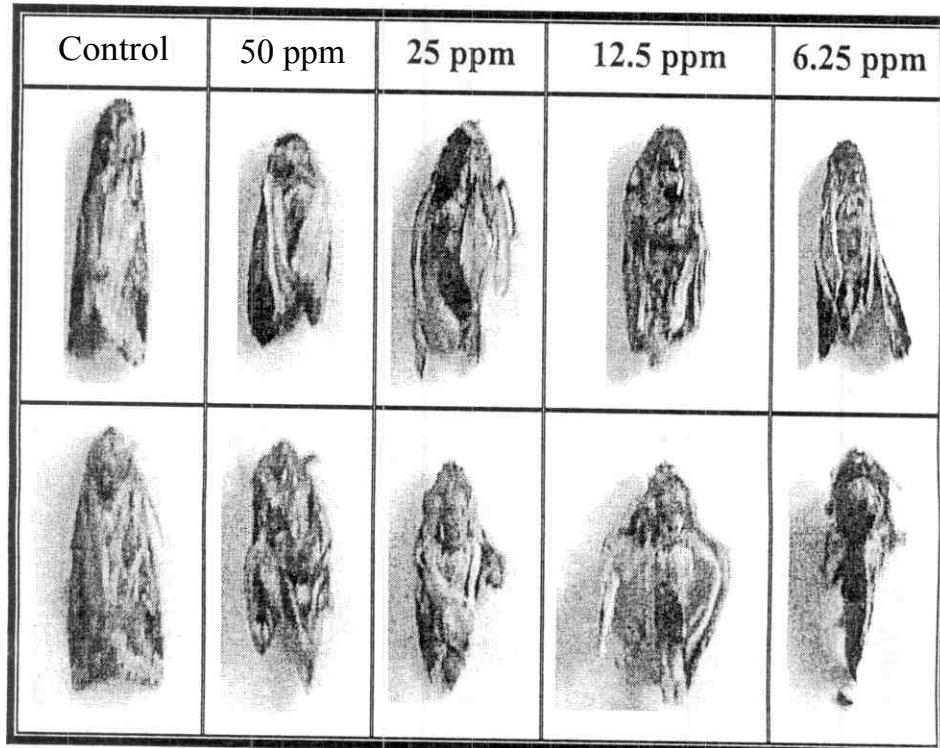


Fig (2): Malformed adults resulted from treatment of *S. littoralis* larvae in sublethal concentration of lannate.

The data presented in the same Table (7) indicated that there was a reduction in emergence percentage with all used concentrations compared with the emergence percentages resulted from untreated larvae. The emergence percentage recorded (44.4 %) at the highest concentration 100 ppm and (77.4 %) at the lowest concentration 6.25 ppm, while of untreated larvae reached (90.0 %).

Data in Table (7) indicated significant increase in adult defoimation percentage and this rate increased with increasing lannate concentrations. In addition, the untreated (control) did not show any adult malformation. The results indicated that longevity of adult females was shortened with lannate concentration from 6.25 to 100 ppm. Also, concentrations of lannate gave the higher female / male sex ratio. Results in Table (7) indicated significant decreases in number of eggs / female and hatchability % with increasing concentrations of lannate (6.25 to 100 ppm). The highest number of eggs/female and hatchability percentage were obtained by control. Also, female sterility percentage increased by increasing lannate concentration.

The fourth-instar larvae of *S. littoralis* (Boisd.) was dipped in solutions of phosfolan (colane), chlorpyrifos (Dursban) or methomyl (lannate) at the LC₂₅ or LC₅₀ and rearing until the F4 generation, all 3 compounds significantly reduced the pupation rate and pupal weight, as well as prolonging the pupal and larval period and reduced the percentage of adult emergence, lengthened the pre-oviposition period and also reduced the number of eggs laid by the females. (Hosny *et al.*, 1978).

The sub lethal doses of cypermethrin and deltamethrin on 4th instar larvae of *S. littoralis* recorded in both insecticides resulted in a gradual decrease in larval survival, larval weight gain, rate of pupation, pupal weight, adult emergence and adult weight. Significant increases in pupal period and proportion of deformed pupae and de formed adults were observed. (**Abdul-Ghaffar et al, 1997**).

The treatment of the 4th instar larvae of *S. littoralis* with fenvalerate reduced the percentage of pupation, adult emergence and reduced egg production and egg hatchability in the resulting female moths. Also, larval and pupal durations became, longer while the adult life span was shortened than the check. (**Mohamady, 2000**).

The effects of various selected insecticides on some main biological aspects of the cotton leafworm, *S. littoralis* were studied. Results indicated that proclaim and consult, significantly, decreased male and pupal weights, whereas all tested insecticides except telion decreased female pupal weight. Proclam, consult and Dipel 2X- significantly decreased the percentage of pupal survivorship. Except, Teliton, all tested insecticides significantly, decreased fecundity. Significant difference in the percentages of egg hatchability was found with all tested insecticides. Among all treatments, slight and insignificant increases in the pupal duration were observed. (**El- Aw, 2003**).

2- Effect of protecto on some biological parameters of *S. littoralis* (Boisd.), when 4th instar larvae were fed for 24 hrs on treated leaves:

The obtained data are presented in Table (8) and Fig. (3). Results indicated that increasing protecto concentration increased mortality percentage of larvae from minimum 20.0 % at 0.125 g /100 ml to maximum of 50.0 % at 2.0 g /100 ml. The highest tested concentration of protecto exhibited significant high mortality percentage compared by untreated. All protecto concentrations exhibited longer larval duration when compared with untreated to reach maximum of (11.0 days) at the highest concentration 2.0 g /100 ml. compared with untreated larvae (8.6 days). Also, significant increase in the pupal duration was observed after exposure of *S. littoralis* larvae to protecto concentrations compared with untreated larvae.

All concentrations of protecto showed significant effect on emergence rates of adults, longevity and sex ratio. On the other hand, results in the same table indicated significant decrease in number of eggs /female with increasing concentrations of portecto (0.125 to 2.0 g /100 ml). The highest number of eggs /female was obtained by control (1611.0). The minimum of eggs /female 774.0 was recorded at the tested protecto concentration (1.0 g /ml).

Data in Table (8) indicate significant increased in pupal deformation percentage and this rate increased with increasing protecto concentrations.

Also, significant decrease in eggs hatchability was observed at various protecto concentrations. Adult female

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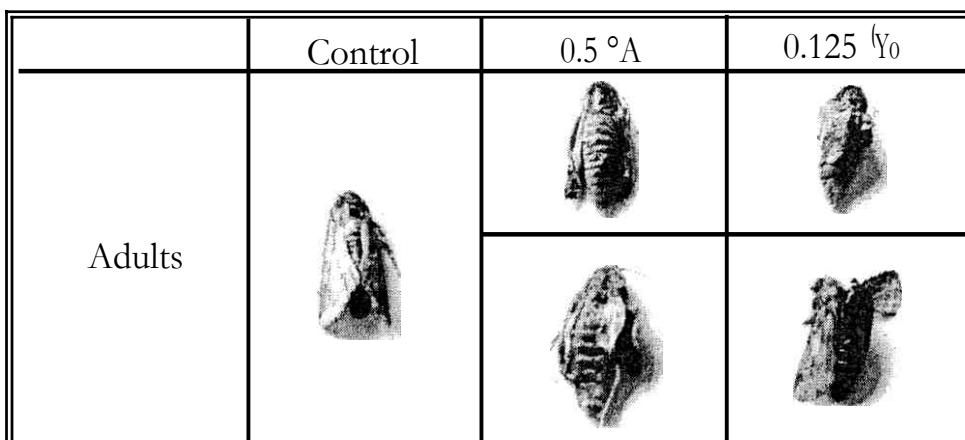
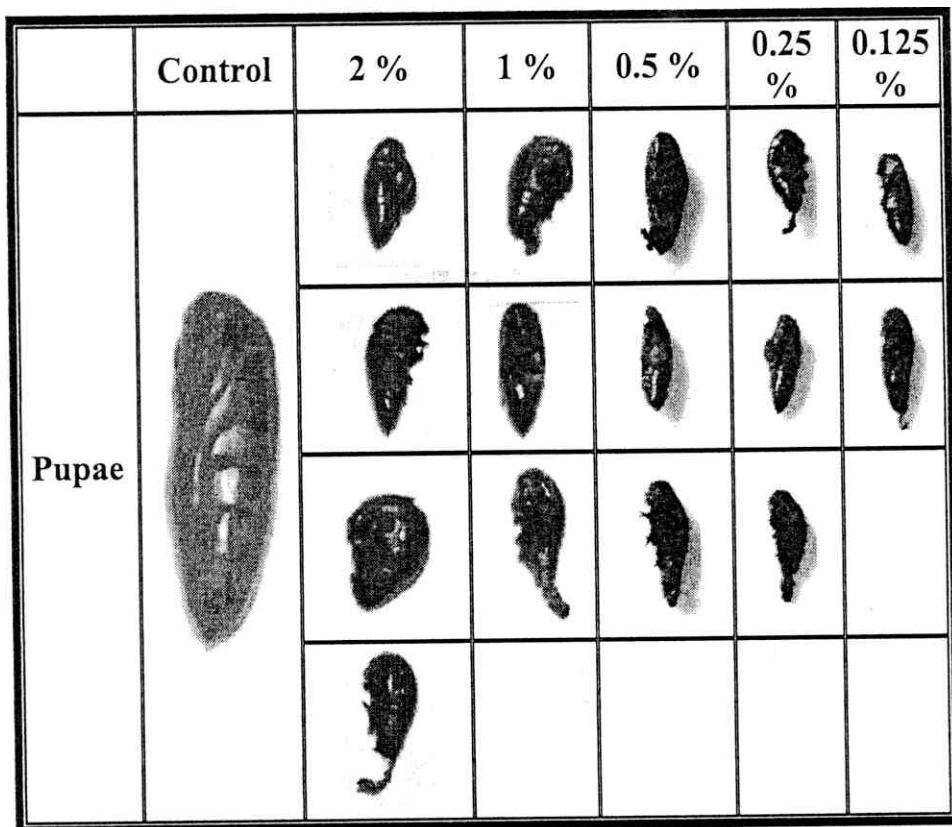


Fig (3): Malformed pupae and adults resulted when the 4th instar larvae of *S. littoralis* were fed on castor bean leaves treated with Protecto

sterility increased with rising protecto concentrations. The sterility percentages ranged from 100.0 % at 2.0 g /100 ml to 31.48 at 0.125 g /100 ml protecto.

These results agreed with **Hou and Chou (1993)** who found that the sublethal doses of *B. thuringiensis* decreased the pupation rate, pupal weight and adult emergence and prolonged the pupal duration of the diamond back moth. The pupation and adult emergence were around 60% compared with over 90% in the check groups. Also the larval mortality was only 28%.

Atallah et al., (2001) tested biocide agerin (based on *B. thuringiensis*) against the 2nd and 4th instars of *S. littoralis*, *A. ipsilon* and *S. cretica* under laboratory conditions. Results indicated that *S. cretica* 2nd instar larvae were the most susceptible to agerin followed by *S. littoralis*, while *A. ipsilon* larvae were the least sensitive to agerin which, also, affected the resulted pupae and adults of *S. littoralis*.

Koja et al., (2006) studied two *B. thuringiensis* commercial formulations, Dipel-2X and protecto against eggs and 1st instar larvae of *Earias insulana* (Lepidoptera: Arctiidae). These treatments caused 73.3% larval mortality after 10 days. Larval period was extended to 21.3 and 19.1 days when Dipel-2X or protecto was used, compared with 17.5 days in the control. Pupal stage duration was not significantly affected; however, the weight of pupae was significantly reduced when larvae were treated with either *B. t* product longevity of adults was shortened in treated insects. Several morphological malformations were observed in pupae and also emerged moths.

3. Effect of coumarin on some biological parameters of *S. littoralis* (Boisd.), when 4th instar larvae were fed for 24 hrs on treated leaves:

Data in Table (9) and Fig.(4) indicated that increasing coumarin concentration increased larval mortality percentage from 25.0% at 0.625% to 60.0% at 10 % concentration. The concentrations of coumarin caused elongations in the larval period to reach maximum of (17.2 days) at the highest concentration (10 %) compared with untreated larvae (10.9) days.

The pupal duration seemed to be relatively longer at treated larvae than the cheek group and the averages were (10.7) and (12.7) days for the lower and the higher concentrations 0.625 and 10 %, respectively. While it was (9.7 days) at the check group. The data presented in the same Table (9) indicated that there was a reduction in emergence percentages with all used concentrations, compared with the emergence percentage resulted from untreated larvae. The emergence percentage recorded (50.0 %) at the highest concentration 10% and (88.8 %) at the lowest concentration 0.625 %, while with untreated larvae reached (91.6 %). Results showed increase percentages of pupae and adult deformation and this rate increased with increasing coumarin concentrations. The higher malformed percentage was obtained at 10 % that recorded (12.5 %) at pupae and (16.6 %) among adults, followed by 2.5 % that recorded (5.8 %) at pupae and (7.6 %) in adults. On the other side, the lowest value was obtained by 1.25 % that recorded (7.5 %) among pupae and (6.2 %) in adult. No malformation was recorded in case of the control

Results and Discussion

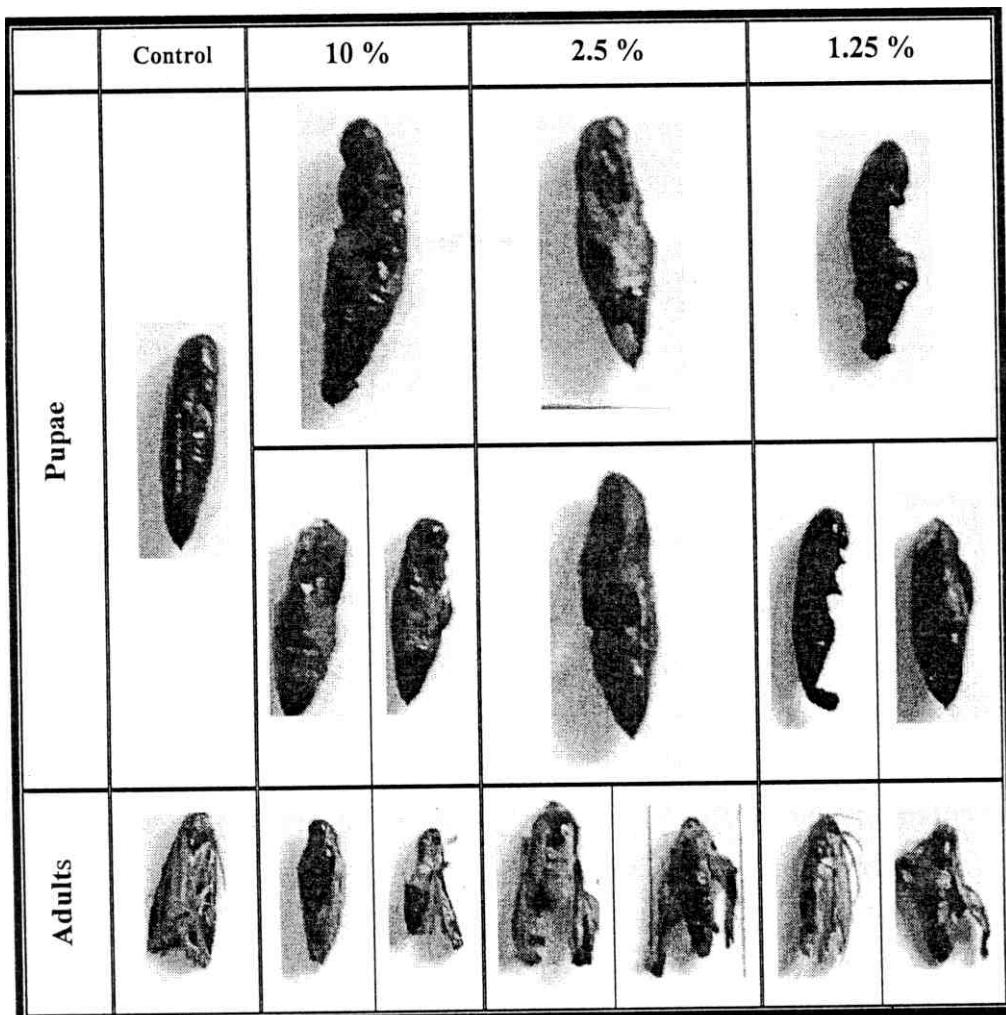


Fig (4): Malformed pupae and adults resulted when the 4th instar larvae of *S. littoralis* were fed on castor bean leaves treated with Coumarin

pupae and adults. In addition, the lowest concentration (0.625) did not show any malformation being similar to control. The results indicated that longevity of adult males was shortened with coumarin concentration from 0.625 to 10 %. Also, concentrations of coumarin gave the higher male/ female sex ratio.

Results in Table (9) showed decreased number of eggs /female and hatchability % with increasing concentrations of coumarin (0.625 to 10 %). The highest number of eggs / female and hatchability percentage were obtained by control. Also, female sterility percentage increased by increasing coumarin concentration.

These results agree with the results of **Sammour and Abdalla (1988)**. They found that larvae of *Heliothis armigera* (*Helicoverpa armigera*) were reared in laboratory and treated with teflubenzuron 0.5-21149 (propanol 0- (2-(4-phenoxyphenoxy)ethyl) oxime) (a juvenile hormone analogue) and coumarin. These compounds, at 20, 10 and 1000 micro g, caused 45, 0, 23.3 and 16.0% larval mortality, resp. And further 15.0, 30.1 and 32.3 % pupal mortality sub lethal doses of the compounds resulted in 91.2, 90.4 and 77.1% sterility, resp. treatments with insect growth regulators reduced the number of egg chambers.

The phagodepress ion activity of five coumarins benzopyran-2-ones), 6-hydroxy-7-isoprenyloxy- coumarin (1), 6-methoxy-7-isoprenyloxy coumarin (2), 6, 7-methyl lenedioxycoumarin (3), 5 -methoxy-6, 7methylene dioxy coumarin (4) and 6-methoxy-7-(2-hydroxy ethoxy) coumarin (5),

from the Argentine native herb *pterocaulon polystachyum*, was tested by **Vera et al., (2006)** against *S. frugiperda* (Lepidoptera: Noctuidae) larvae. The compounds were added to an artificial diet at doses ranging from 50 to 200 g per g of diet. The results indicating that 50 micro g/g of compound 1 and 3 (non-methoxylated coumarins) incorporated to the larval diet caused 80 and 50% of pupal mortality, respectively, while, 100 micro g /g dose of compounds 2, 4, 6 and 7 produced 60, 50, 10 and 80% pupal mortality, respectively. Larval growing rate during the early larval instars was significantly reduced by treatments with the methylenedioxy coumarins 3 and 4 coincidentally, the larval period duration was significantly increased by the latter compounds.

4- Effect of azadirachtin on some biological parameters of *S. littoralis* (Boisd.), when 4th instar larvae were fed for 24 hrs on treated leaves:

Data in Table (10) and Fig. (5) indicated that increasing azadirachtin concentrations increased mortality percentage from 21.6 at 0.625 % to 55.0 at 10 %, all azadirachtin concentrations exhibited high mortality percentages compared with control. All azadirachtin concentrations exhibited longer larval duration when compared with untreated larvae. Also, at 10 % the longest larval duration was recorded (15.2 days) followed by 5 % (14.0 days) when compared with untreated larvae (10.3 days). Also, insignificant effects of azadirachtin occurred on duration of pupae compared to untreated pupae.

Results showed higher malformed pupal rates at various azadirachtin treatments, the higher malformed percentage was

S. littoralis

| Conc. % | Larval stage | | | Pupal stage | | | Adult stage | | | | | | % Hatching | % Sterility | |
|---------------|----------------|--------------------|--------------------|----------------|----------------|----------------|------------------|---------------------|------|---------|------------|---------|---------------|----------------|--|
| | % Mortality | Duration (days) | Duration (days) | % Malformed | % Emergence | % Malformed | Sex ratio M:F | Longevity (days) | | | Egg/female | Deposit | | | |
| | | | | | | | | M | F | Deposit | | Hatch | | | |
| 10 | 55.0 | 15.2 | 10.7 | 11.1 | 66.6 | 0.0 | 5.0 | 4.0 | 7.0 | 5.3 | 852.0 | 385.0 | 45.1 | 78.66 | |
| 5 | 43.3 | 14.0 | 10.5 | 0.0 | 73.5 | 12.0 | 5.0 | 6.0 | 8.7 | 10.7 | 1128.0 | 600 | 53.1 | 66.73 | |
| 2.5 | 35.0 | 13.5 | 10.0 | 7.6 | 79.4 | 9.6 | 4.0 | 3.0 | 11.0 | 8.0 | 1150.0 | 693.0 | 60.2 | 61.54 | |
| 1.25 | 28.3 | 12.3 | 9.8 | 9.3 | 83.7 | 5.5 | 1.0 | 1.0 | 7.0 | 6.0 | 1200.0 | 800 | 66.6 | 55.60 | |
| 0.625 | 21.6 | 11.7 | 9.3 | 6.3 | 85.1 | 0.0 | 3.0 | 2.0 | 7.2 | 5.8 | 1227.0 | 900 | 73.3 | 50.01 | |
| Control | 0.0 | 10.3 | 9.0 | 0.0 | 91.6 | 0.0 | 7.0 | 4.0 | 6.0 | 6.3 | 2000.0 | 1800 | 90.0 | -- | |
| I.S.D (5%) | 12.30 | 0.86 | 0.74 | 2.21 | 18.7 | 2.63 | 1.19 | 1.12 | 1.23 | 2.13 | 136.0 | 107.0 | 13.39 | -- | |

Results and Discussion

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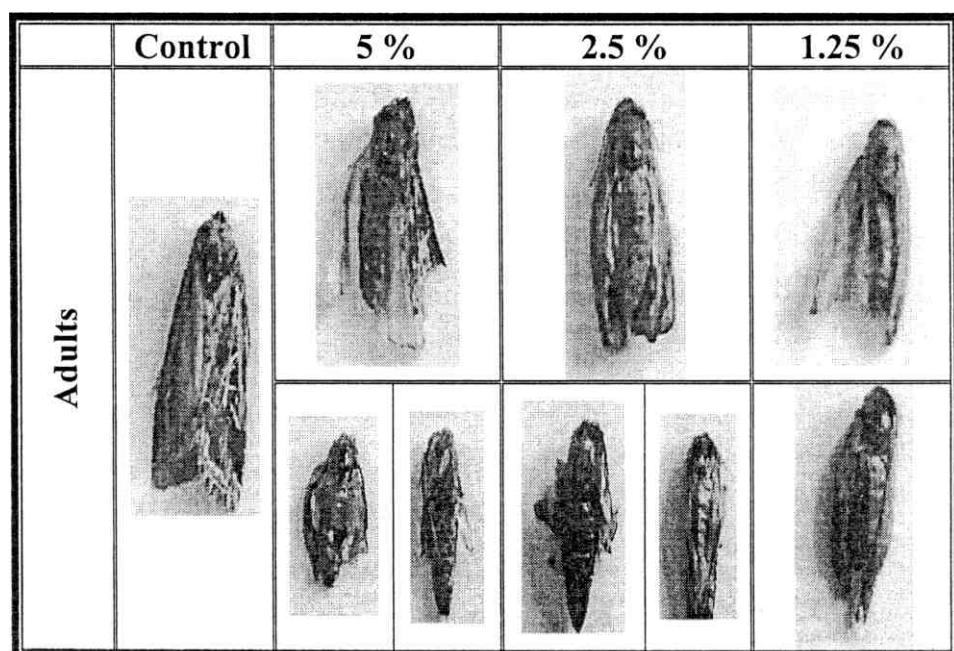
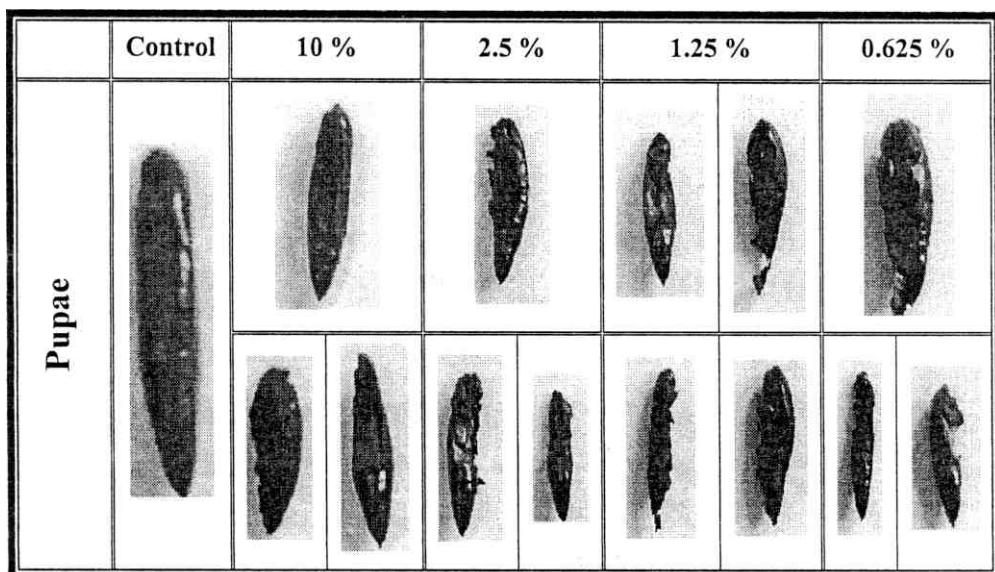


Fig (5): Malformed pupae and adults resulted when the 4th instar larvae of *S. littoralis* were fed on castor bean leaves treated with azadirachtin

obtained at 10 % followed by 1.25 %. On the other side, the higher value was obtained by 0.625 %. Also, highest malformed adults rate was detected at 5 % followed by 2.5 % and then by 1.25 %.

The data presented in the same Table (10) indicated that there was Significant decrease in adult emergence percentage occurred with increasing concentrations of azadirachtin.

The lowest value of emergence percentage was detected at 10 % followed by 5 % and then by 2.5 % compared with control adult emergence that recorded (91.6 %). The results indicated that longevity of adult females increased with rising azadirachtin concentration from (0.625 to 5 %) while in higher concentration (10 %) the longevity of adult female was shortened. Also, the male ratio was generally higher than female.

Results indicated significant reductions in number of eggs and hatchability % of eggs laid per female. The deposited eggs was 2000.0 in control females and was found to be reduced to 852.0, 1128.0, 1150.0, 1200.0 and 1227.0 eggs /female at 10, 5, 2.5, 1.25 and 0.625 % of azadirachtin, respectively.

Also, higher concentrations of azadirachtin gave the highest sterility percentage (78.66 %), while, sterility percentage decreased by decreasing azadirachtin concentrations.

These results agreed with **Shalaby et al. (1997)** used the leaf crude extract of *M azedarach* L. seeds against many pests as *S. littoralis* (Boisd.) larvae, *E. insulana* (Boisd.) and *P. gossypiella* (Saund). The results indicated that the crude extract caused mortality of newly hatched larvae of these species, prolonged larval duration, pupal duration and reduced moth

emergence. Also, **El-Meniawi *et al.* (1999)** studied the effect of sub lethal conc. of the ethanolic neem seed extract (*Azadirachta indica*) on the reproduction, activities of the cotton leafworm *S. littoralis* (Boisd.), by continuous mating. The results revealed that treatment of adults as well as 4th instar larvae with the sub lethal concentrations, caused significant reduction of each of the female oviposition activity, total deposited eggs per female, hatchability percentages and life-span of moth. .

Abd El-Rady and Osman (2005) found that (Neemix 4.5% azadirachtin) neem and (Nat—I 96%) jojoba oil on 4th instar *A. ipsilon* larvae caused an increase in larval and pupal durations and decrease in pupal weight. Also, Neemix was more effective than Nat-1, decreased pupation, pupal weight, adult emergence, fecundity and fertility. Malformed pupae were increased with the Neemix, while Nat-1 was high in malformed adults.

PART (4)

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

A cross 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 fact, the microvilli of columnar cell extending from their free ends. The wall of gut contains two distinct layers of muscle fibers, longitudinal muscle fibers to the outside and circular muscle fibers to the inside. The spaces between the different gut wall layers are almost filled with connective tissue.

1. Effect of lannate:

The present histological study on the effect of lannate on the mid-gut of treated larvae of *S. littoralis* after 1, 2 and 3 days of treatments revealed certain changes appeared within Plate (1) that showed epithelium cells detached from the basement membrane in many areas and thickness of epithelial cells in the mid-gut of 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.

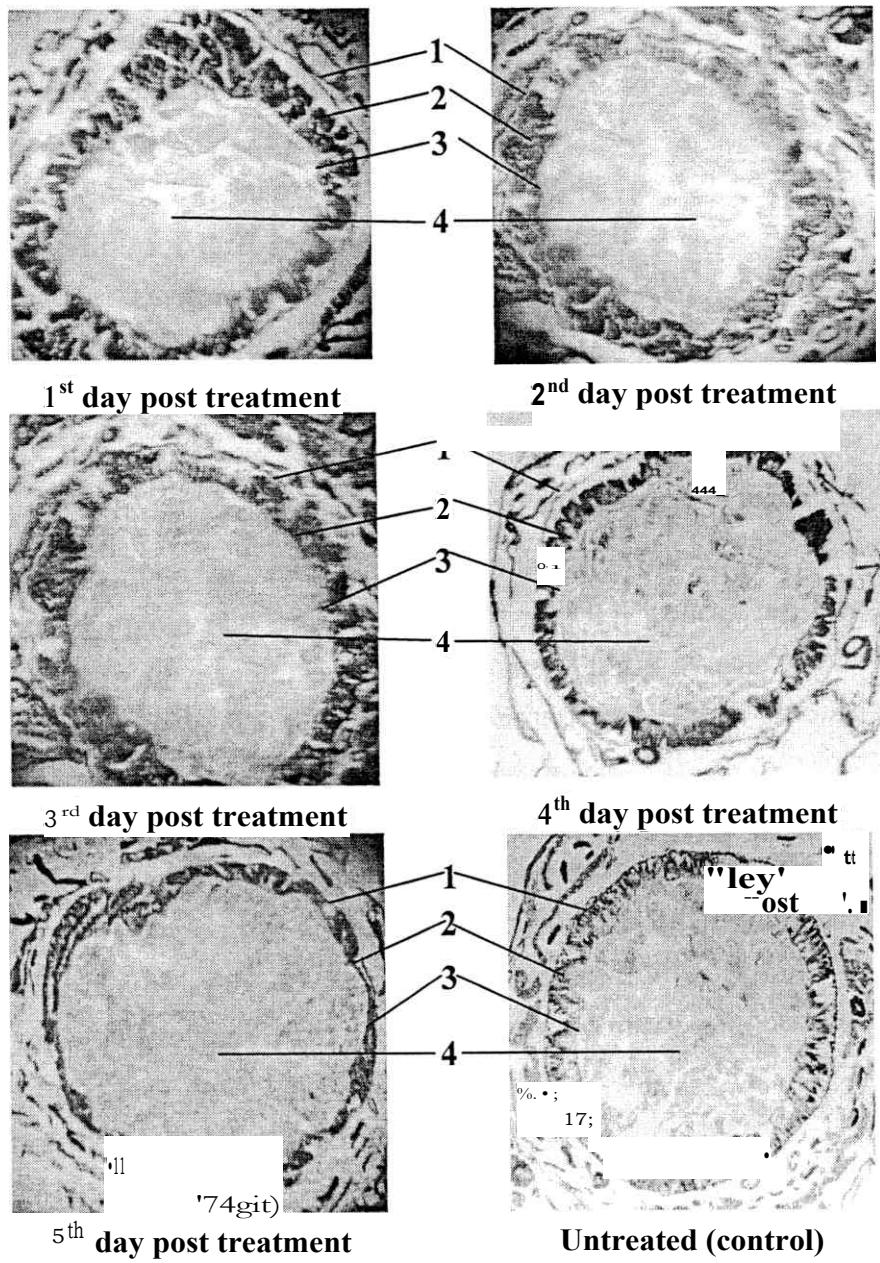


Plate (1): Effect of lannate on the mid gut of the 4th instar larvae of *S. littoralis*.

Results and Discussion

2. Effect of protecto:

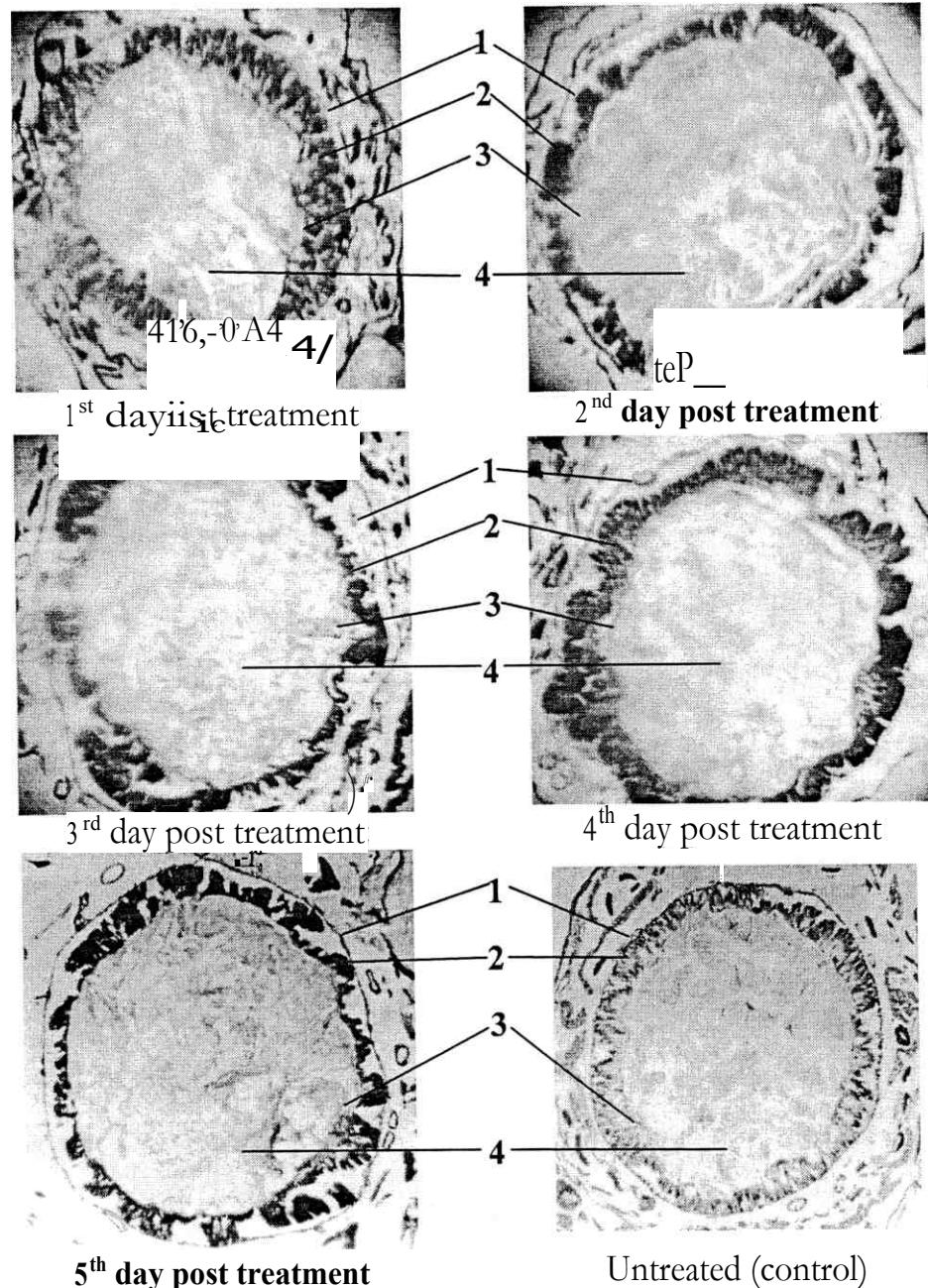
Plate (2) indicated that, the epithelial cells lost their close association with the basement membrane and with each other. The epithelial cells were destroyed and lost their columnar structure in some point and caused disorganization of peritrophic membrane and in some cases disappeared after 1,2 and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

3. Effect of coumarin:

Platezz(3) indicated that, the effect of coumarin on the mid-gut tissues of 4th larvae of *S. littoralis* was most pronounced and extensive after 1 and 2 days of treatment, where some cells become thick 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 separated 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 were treated by coumarin and the control in concern of epithelium and basement membrane also, peritrophic membrane not affected and still intact.

4. Effect of azadirachtin extract:

Plate (4) indicated that, there is no effect of azadirachtin extract on the 4th larval instar of *S. littoralis* after 1 and 2 days of treatment, but after 3 and 4 days of treatments many



(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*.

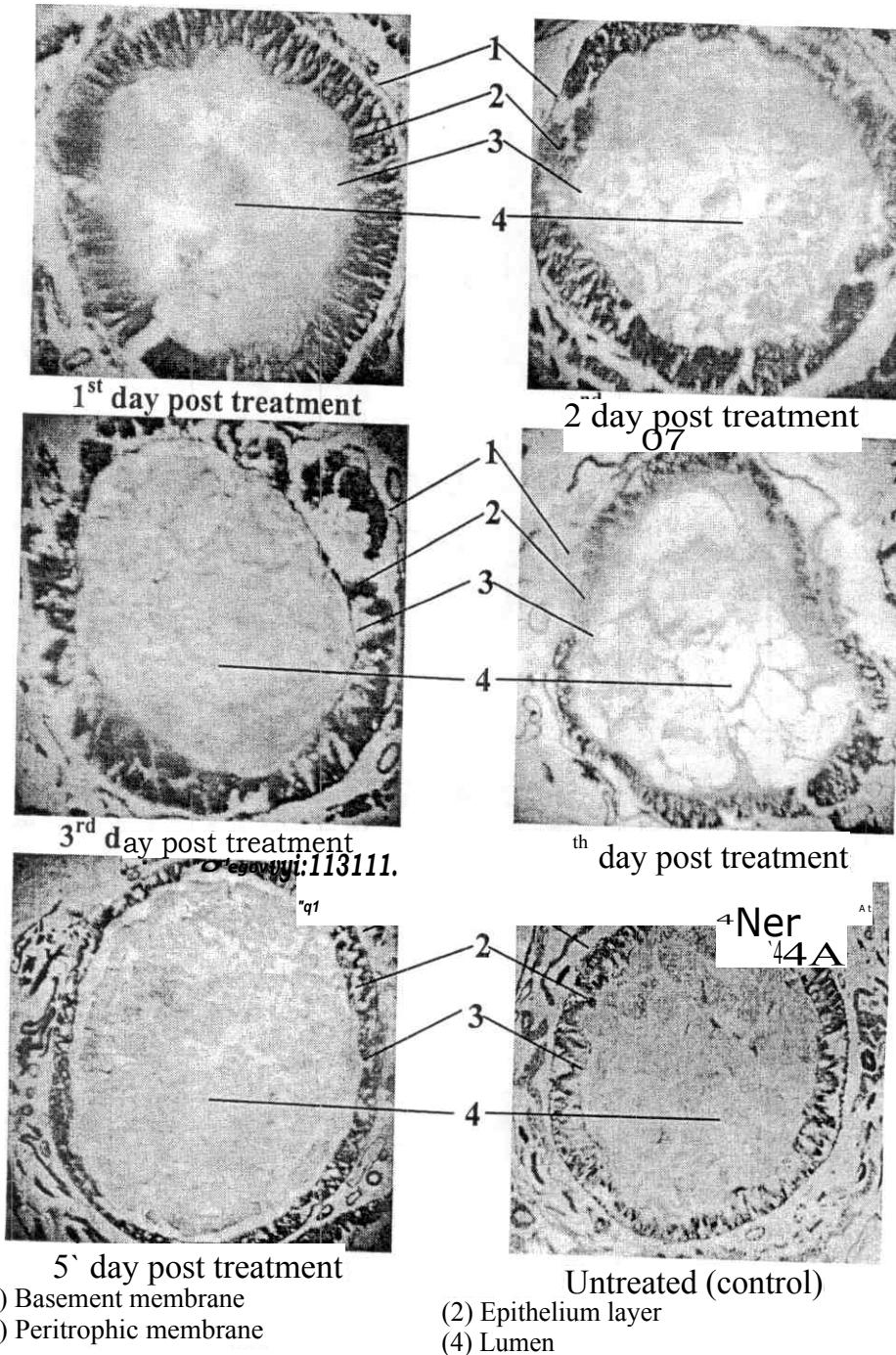


Plate (3): Effect of coumarin on the mid gut of the 4th instar larvae of *S. littoralis*.

Results and Discussion

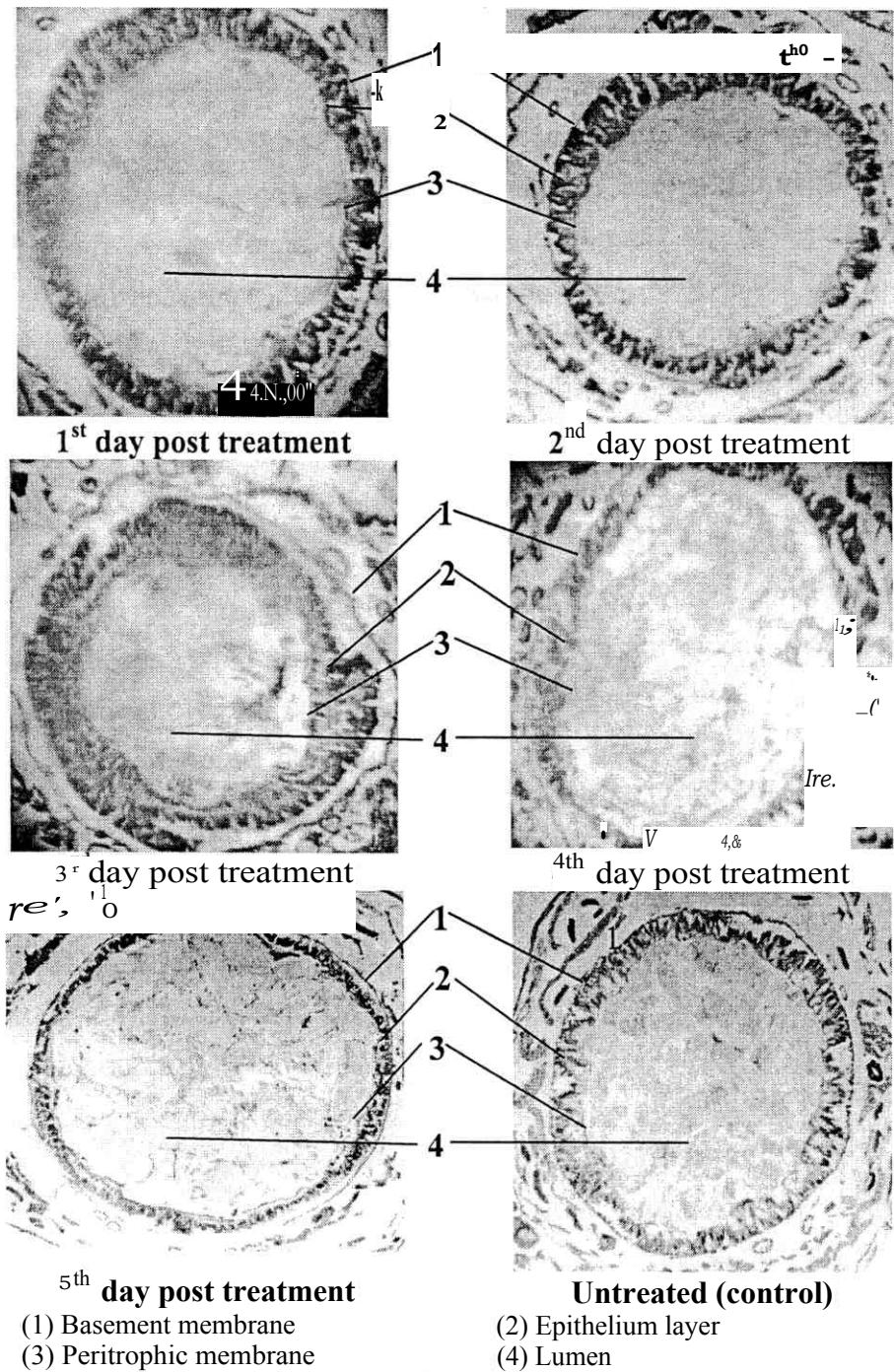


Plate (4): Effect of azadirachtin on the mid gut of the 4th instar larvae of *S. littoralis*.

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 epithelium 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 microvilli of some columnar cells are still intact, also peritrophic membrane and basement membrane not affected.

These results partially agree with findings of **Saad *et al.*, (1985)** who studied the effect of Fenvalerate on spiny bollworm larvae in the 4th instars. They reported that damage caused by the treatment was found to be characterized by elongation and vacuolization of epithelial cells detachment of membranes and lysis. **Tawfik (1998)** found that histological studies revealed that Vertimec and Neemaza 1 compounds caused distraction for the mid-gut epithelium of the pink and spiny bollworms and affected on the uniform of the epithelial cells. **Zidan *et al.*, (1998)** indicated that *B. thuringiensis* (MVP II) caused morphological changes to mid-gut epithelium of treated larvae of pink and spiny bollworms.

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

El-Lebody (1998) found that feeding the 4th instar larvae of *E. insulana* on Xentari-contaminated diet, using the highest concentration (1.25 ppm) at the rate of 5 ml/100g diet, for 24 hrs

caused many pathological effects in the mid-gut of the treated larvae. These effects, as shown in cross sections of the mid-gut, were separated of the epithelial cells from the basement membrane as well as elongation, vacuolization and breakdown of the larval mid-gut epithelium. Also, Xentari caused disorganization and disintegration of peritrophic membrane.

Gamil (2004) stated that histological changes were observed in *S. littoralis* mid-gut. Although, damage in mid-gut tissue by the two tested bacteria was relatively similar. 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.

PART (5)

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

In these tests, only 4th instar larvae of *S. littoralis* was treated with the LC₅₀ concentration for each compound for biochemical assays to evaluate total soluble protein, the activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and the carbohydrate hydrolyzing enzymes (trehalase, invertase, amylase), a-f3 esterases, phosphatases (acid and alkaline) and acetylcholinesterase (AChE).

The obtained results are tabulated in Tables (11-21) and illustrated in Figures (6-16).

1. Carbohydrate hydrolyzing enzymes:

1.1. Invertase:

Data in Table (11) and Fig. (6) indicated that, in general, the tested compounds showed reduction in the activity of invertase of the treated 4th instar larvae of *S. littoralis* than the untreated ones. 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 were treated with (LC₅₀) by protecto, lannate and azadirachtin compared with 1.636 mg/g b.w. while, there were no effects on the activity of invertase when larvae treated (at LC₅₀) by coumarin (1.636 mg/g b.w.). 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, lannate and azadirachtin, respectively.

Table (11): Effect of the tested compounds on the activities of invertase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | Invertase activity (mg/g body weight) | | | | | |
|---------------------|---------------------------------------|-------|-------|-----------------|-------|-------|
| | 1 st | 3rd | 5th | 7 th | 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 |
| Lannate | 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

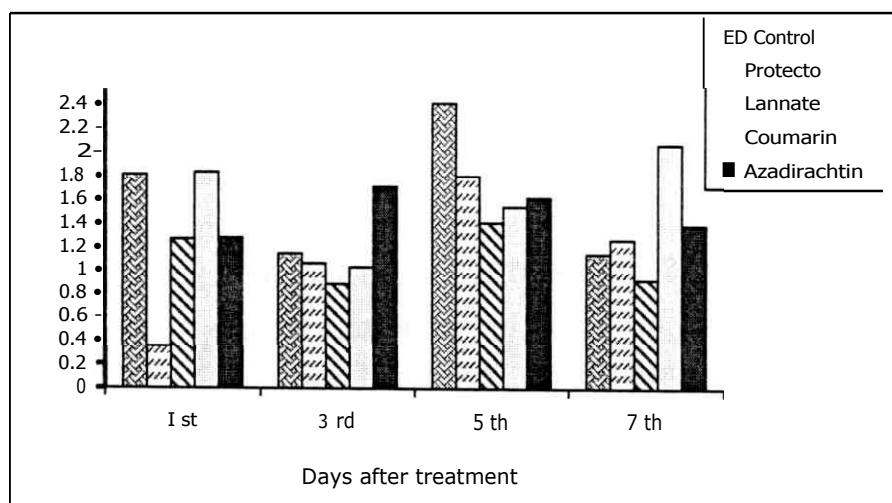


Fig. (6): Effect of the tested compounds on the activities of invertase enzyme of the fourth instar larvae of *S. littoralis*

1.2. Trehalase:

Data in Table (12) and Fig. (7) indicated that coumarin was the only treatment caused significant increase in the activity of trehalase of 4th instar larvae of *S. littoralis* than untreated ones and other tested compounds. 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 were treated (at LC₅₀) by protecto, lannate, coumarin and azadirachtin, respectively, compared with 0.697 mg/g b.w. for the control. On the contrary, protecto and lannate showed reduction in enzyme activity, reaching 82.06 and 77.33 % less than the check. The percent differences in the activity of this enzyme between treatments irrespective to the time after treatment reached 106.74 % increase in case of coumarin than the check.

1.3. Amylase:

Data in Table (13) and Fig. (8) indicated that coumarin and azadirachtin caused significant increase 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 b.w. when larvae were treated at LC50 by protecto, lannate, coumarin and azadirachtin, respectively, compared with 0.261 mg/g b. w for the untreated control. The differences between treatments in the activity of this enzyme reached 125.28 and 132.56 % increase than the control in case of coumarin and azadirachtin, respectively. The other products variously decreased this enzyme activity, i.e. 72.41 and 78.92 % less than the untreated control in

Table (12): Effect of the tested compounds on the activities of trehalase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | Trehalase activity (mg/g body weight) | | | | | |
|---------------------|---------------------------------------|-----------------|-------|-------|-------|--------|
| | 1 st | 3 rd | 5th | 7th | Mean | % |
| 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 |
| Lannate | 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

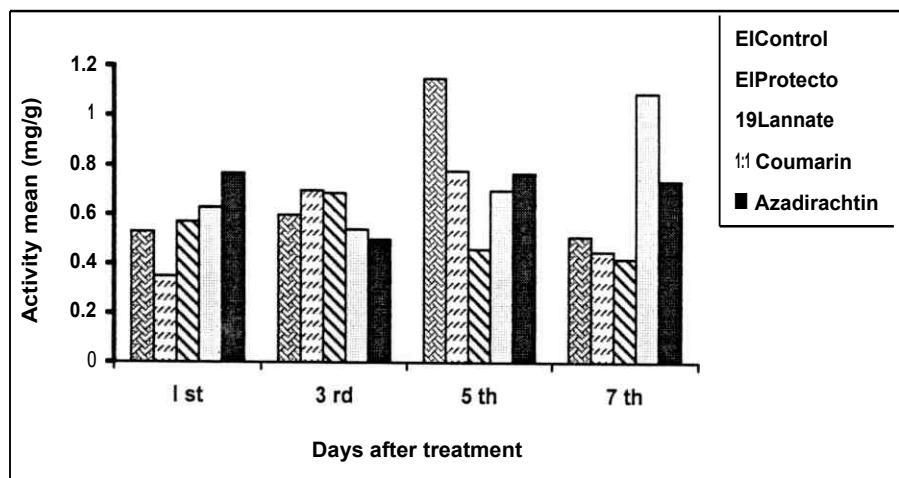


Fig. (7): Effect of the tested compounds on the activities of trehalase enzyme of the fourth instar larvae of *S. littoralis*.

Table (13): Effect of the tested compounds on the activities of amylase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | Amylase activity (mg/g body weight) | | | | | |
|---------------------|-------------------------------------|-----------------|-------|-----------------|-------|--------|
| | 1 st | 3 rd | 5th | 7 th | Mean | % |
| 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 |
| Lannate | 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

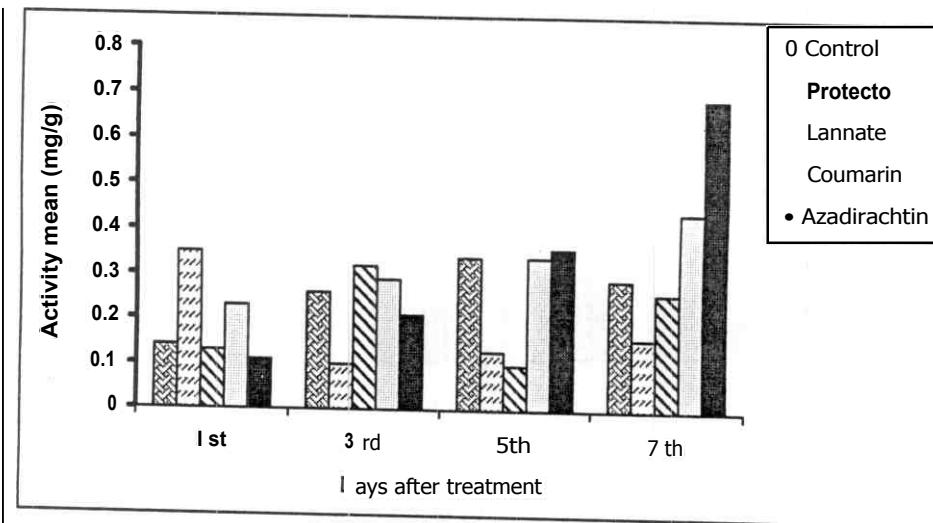


Fig. (8): Effect of the tested compounds on the activities of amylase enzyme of the fourth instar larvae of *S. littoralis*

Results and Discussion

case of protecto and lannate. Such results are in accordance with those obtained by **Mohamady (2000)**, who investigated the effect of treatment of the 4th instars larvae of *S. littoralis* with the LC₂₅ and LC₅₀ of fenvalerate on the activity of amylase enzyme at different time intervals (24, 48 and 72 hrs). The results indicated that, there was great reduction in the activity of amylase after treatment. On the other hand, **Khedr (2002)** found an increase in the activity of trehalase enzyme of *S. littoralis* (2nd & 4th instar larvae) after treatment with Biorepel.

2. Transaminase enzymes:

2.1. Glutamic pyruvic transaminase (GPT):

Data in Table (14) and Fig. (9) showed that, the tested compounds caused significant decrease 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 1.07, 0.58, 0.93 and 1.02 mg/g b.w. When larvae were treated at LC₅₀ 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, lannate, coumarin and azadirachtin, respectively.

2.2. Glutamic oxaloacetic transaminase (GOT)

Data in Table (15) and Fig. (10) 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

Table (14): Effect of the tested compounds on the activities of GPT enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | G.P.T activity (mg/g body weight) | | | | | |
|---------------------|-----------------------------------|-----------------|-----------------|-----------------|------|-------|
| | Days after treatment | | | | | Mean |
| | 1 st | 3 rd | 5 th | 7 th | | |
| 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 |
| Lannate | 0.46 | 0.70 | 0.45 | 0.73 | 0.58 | 53.21 |
| Coumarin | 0.63 | 0.94 | 0.88 | 1.28 | 0.93 | 85.32 |
| Azadirachtin | 0.44 | 1.95 | | 0.58 | 0.58 | 93.57 |

... at (0.05) for treatment = 0.13

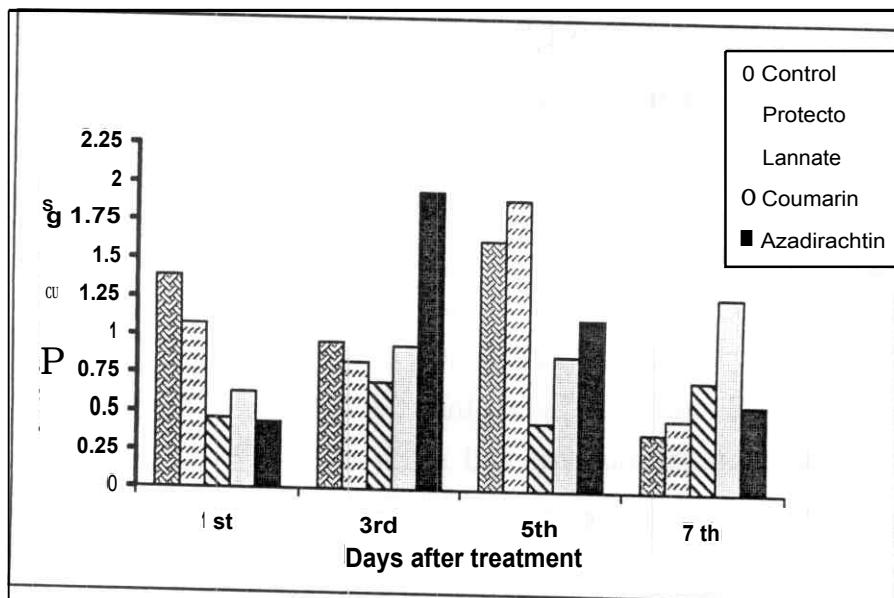


Fig. (9): Effect of the tested insecticides on the activates of GPT enzyme of the fourth instar larvae of *S. littoralis*.

Table (15): Effect of the tested compounds on the activities of GOT enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | G.O.T activity (mg/g body weight) | | | | | |
|---------------------|-----------------------------------|-------|-------|------|------|--------|
| | 1st | 3rd | 5th | 7th | Mean | % |
| 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 |
| Lannate | 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

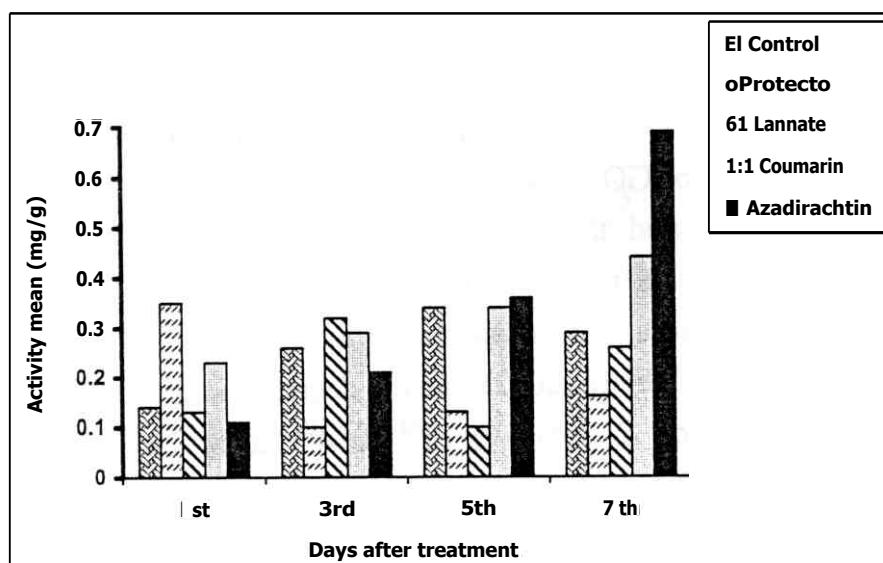


Fig. (10): Effect of the tested compounds on the activities of GOT enzyme of the fourth instar larvae of *S. littoralis*

changed from 7.32 mg/g b. w of larvae (normal) to 7.49, 8.40 and 9.97 mg/g b.w., respectively. On the contrary, lannate gave (5.94 mg/g b.w.) showed 81.15 decrease 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 reached 136.20, 114.75 and 102.32 % more than the check in case of azadirachtin, coumarin and protecto respectively. Such findings are in agreement with those obtained by **El-Sheakh et al., (1990)** who found that the treatment of 4th larval instar of *S. littoralis* with sumithion and glyceollin (plant extract) increased GOT activity. **Aly (1999)** showed that the treatment of 4th larval :instar of *S. littoralis* with LC₅₀ of plant extract of river gum, *Eucalyptus camaldulensis* Dehn and lemon scented spotted gum, *E. citriodora* Hook, in hexane and ethanol caused an increase in GOT activities. In the same time, **Mohamady (2000)** investigated the effect of treatment of the 4th instar larvae of *S. littoralis* with the LC₂₅ and LC₅₀ 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. **Khedr (2002)** found a decrease in the activity of GOT enzyme of *S. littoralis* (2nd & 4th instar larvae) after treatment with Biorepel.

3. Alpha and beta esterases (a-E and

3.1. Alpha esterase (a-E):

Data in Table (16) and Fig. (11) indicated that, the tested compounds coumarin and azadirachtin caused significant

Table (16): Effect of the tested compounds on the activities of a - esterase enzyme of the 4 instar larvae of *S. littoralis*

| Treatments | a - esterase activity (mg/g body weight) | | | | | |
|---------------------|--|-----------------|-----------------|-----------------|-------|--------|
| | 1 st | 3 rd | 5 th | 7 th | 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 |
| Lannate | 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

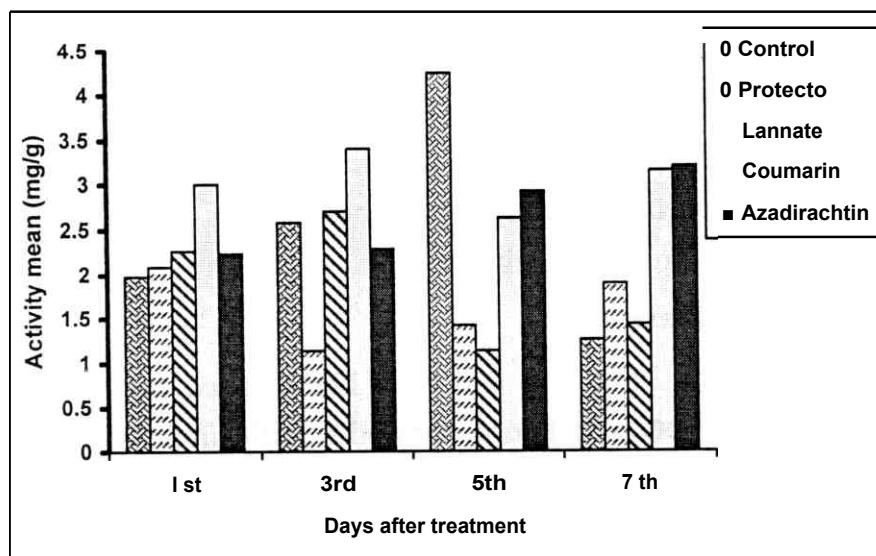


Fig. (11): Effect of the tested compounds on the activities of a - esterase enzyme of the fourth instar larvae of *S. littoralis*

increase in the activity of a-esterase of the treated 4th instar larvae of *S. littoralis* than the untreated larvae. The mean values of a-esterase enzyme activities in the supernatant of the homogenate larvae reached 1.643, 1.890, 3.047 and 2.665 mg/g b.w. when larvae were treated at LC₅₀ by the tested protecto, lannate, coumarin and azadirachtin, respectively, compared with 2.510 mg/g b.w of the untreated control. 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 lannate showed slight decrease in a - esterase activity, reached to 65.45 and 75.29 % less than the control, respectively.

Abdel Hafez et al. (1993a) measured aliphatic esterase and non-specific esterase activity during the poisoning of *S. littoralis* larvae with dioradin and dennate under laboratory. Data indicated that all tested compounds caused a variable reduction in the activity of esterases than that in control. While, **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.

3.2. Beta esterase O -E):

Data in Table (17) and Fig. (12) revealed that, 4th instar larvae of the cotton leafivorm showed significant decrease in f3 - esterase enzyme activities in the supernatant of the homogenated larvae which were treated at LC₅₀ by protecto (1.855 mg/g b.w.), lannate (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

Results and Discussion

Table (17): Effect of the tested compounds on the activities of - esterase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | 13 - esterase activity (mg/g body weight) | | | | | |
|--------------|---|-----------------|-----------------|-----------------|-------|-------|
| | Days after treatment | | | | | Mean |
| | 1 st | 3 rd | 5 th | 7 th | | |
| 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 |
| Lannate | 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 |
| Azadirachtin | 0.931 | 2.077 | 2.073 | 2.447 | 1.882 | 69.73 |

L.S.D. at (0.05) for treatment = 0.21

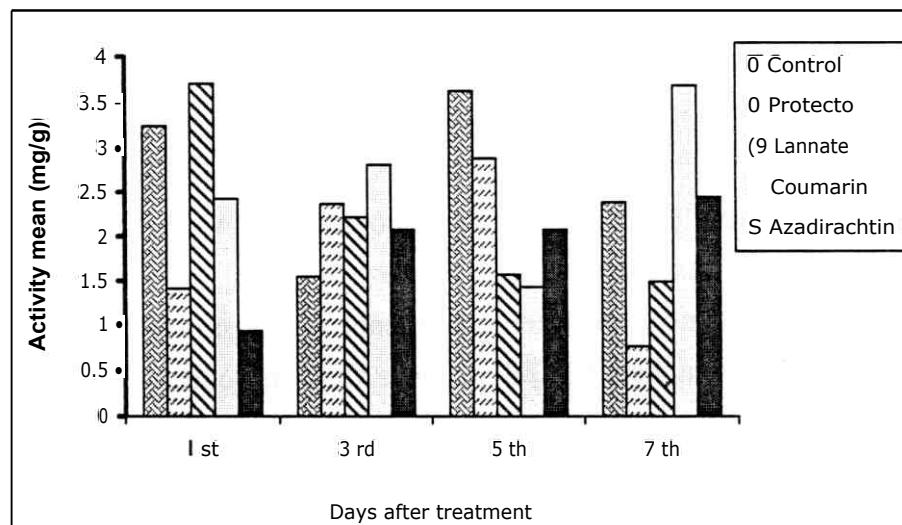


Fig. (12):Effect of the tested compounds on the activities of (3- esterase enzyme of the fourth instar larvae of *S. littoralis*

decrease 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.

Abdel Hafez et al., (1993a) measured aliphatic esterase and non-specific esterase activity during the poisoning of *S. littoralis* larvae with di oradin, dennate and sumuline (IGR's/insecticides mixtures) or each of their components alone under laboratory and field conditions. Data indicated that all tested compounds caused a variable reduction in the activity of esterases than that in control. The highest level of reduction was obtained for the IGR's diflubenzuron alone or in its mixtures.

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

Data in Table (18) and Fig. (13) showed that, only protecto caused significant increase in the concentration of total protein of the treated 4th instar larvae of *S. littoralis* than the untreated ones. The other tested compounds caused significant reduce of total protein. The values of total protein in the supernatant of the homogenated larvae reached 3.120, 1.291, 0.635 and 0.475 mg/g b.w. when larvae were treated with LC₅₀ of the tested protecto, lannate, 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.

On the contrary, **El-Sheakh et al. (1990)** found that the treatment of 4th larval instar of *S. littoralis* with sumithion and glyceollin (plant extract) increased the total soluble protein. It was increased after 2 days of sumithion treatment, while after

Table (18):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) | | | | | |
|--------------|--|-------|-------|-------|-------|--------|
| | 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 |
| Lannate | 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

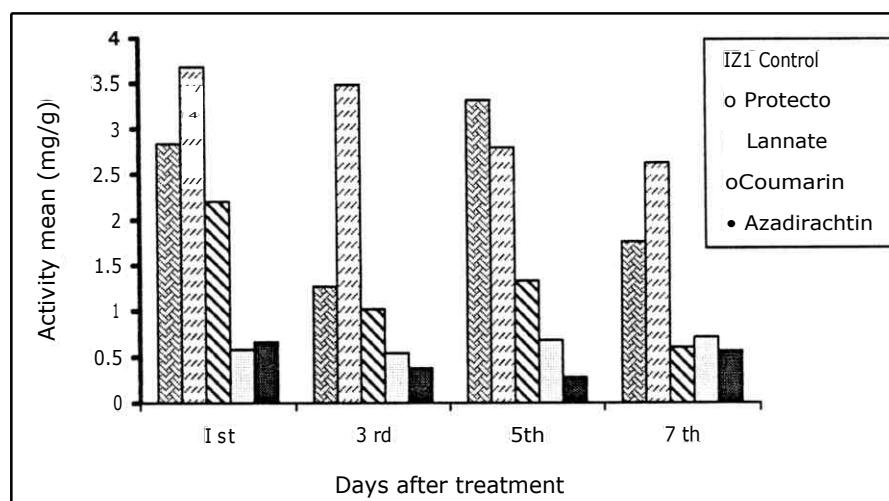


Fig. (13): Effect of the tested compounds on the concentration of total soluble protein of the fourth instar larvae of *S. littoralis*

one day of Glyceollin treatment, the total soluble protein content decreased than the control. Mohamady (2000) investigated the effect of treatment of the 4th instar larvae of *S. littoralis* with the LC₂₅ and LC₅₀ 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.

5. Acetylcholinesterase (AChE):

Data in Table (19) and Fig. (14) 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 homogenated larvae reached to 0.175., 0.266 and 0.249 mg/g b.w when larvae were treated with LC₅₀ of the tested protecto, coumarin and azadirachtin compared with the check (0.148 mg/g b.w). On the contrary, lannate gave (0.118 mg/g b.w) and showed 79.72 % decrease in (AChE) activity than the check. 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.

These results agree with Farag (1981) who reported that the level of AChE was higher in the cypermethrin resistant laboratory strain of *S. littoralis* than the susceptible one.

Zidan *et al.* (1996) studied the effects of pyriproxyfen (juvenile hormone analogue), *B. thuringiensis* and KZ mineral oil on the larval enzymes of *S. littoralis*. Pyriproxyfen resulted in the best immediate inhibitory effect on AChE

Table (19): Effect of the tested compounds on the activities of acetylcholinesterase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | Acetylcholinesterase activity (mg/g body weight) | | | | | |
|---------------------|--|-----------------|-----------------|-----------------|-------|--------|
| | Days after treatment | | | | | Mean |
| | 1 st | 3 rd | 5 th | 7 th | | % |
| 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 |
| Lannate | 0.134 | 0.285 | 0.033 | 0.020 | 0.118 | 79.72 |
| Coumarin | 0.532 | 0.051 | 0.192 | 0.288 | 0.266 | 179.72 |
| Azadirachtin | 0.327 | 0.060 | 0.255 | 0.356 | 0.249 | 168.24 |

L.S.D. at (0.05) for treatment = 0.56

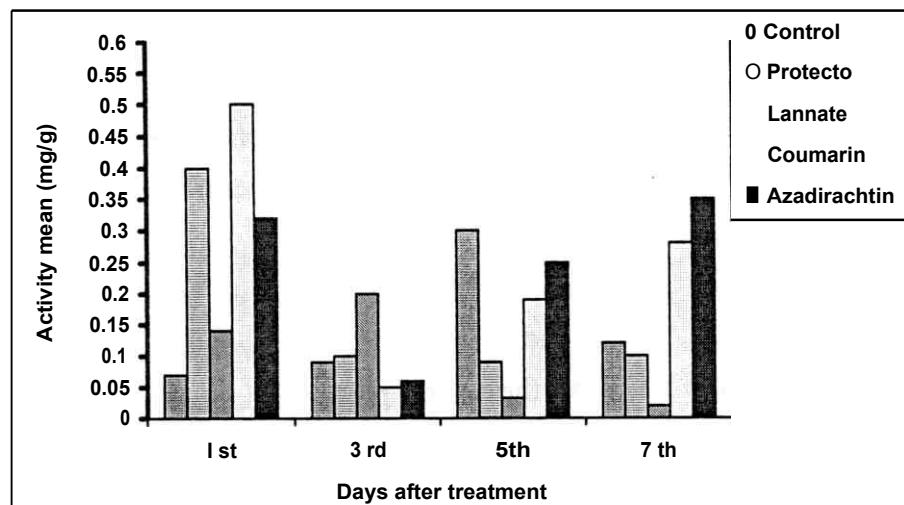


Fig. (14): Effect of the tested compounds on the activities of acetylcholinesterase enzyme of the fourth instar larvae of *S. littoralis*

(acetylcholinesterase) whereas *B. thuringiensis* and KZ oil caused better latent effects.

Byrne et al.(1999), on the field populations of the beet armyworm, *S. exigua*, indicated the presence of two forms of acetylcholinesterase (AChE), the target site of organophosphorus (OP) and carbamate insecticides, using a diagnostic concentration of the carbamate, methomyl. In toxicological bioassays, the response of populations to topical applications of technical methomyl and the OP, chlorpyrifos-oxon, were related to the relative frequencies of the AChE alleles present. All populations, including a laboratory reference strain, showed a high degree of tolerance to methomyl; however, chlorpyrifos-oxon proved to be extremely potent, even against populations in which the resistant AChE variant predominated.

6. Alkaline and Acid phosphatase:

6.1. Alkaline phosphatase:

Data in Table (20) and Fig. (15) revealed that, the tested compounds reduced the activity of 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 homogenated larvae reached to 0.058, 0.077, 0.054 and 0.073 mg/g b.w. when larvae were treated with the LC₅₀ 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 (20): Effect of the tested compounds on the activities of alkaline phosphatase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | alkaline phosphatase activity (mg/g body weight) | | | | | |
|---------------------|--|-------|-------|-------|-------|-------|
| | 1st | 3rd | 5th | 7th | Mean | 0/0 |
| 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 |
| Lannate | 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 |
| Azadirachtin | 0.049 | 0.090 | 0.067 | 0.087 | 0.073 | 85.88 |

L.S.D. at (0.05) for treatment = 0.02

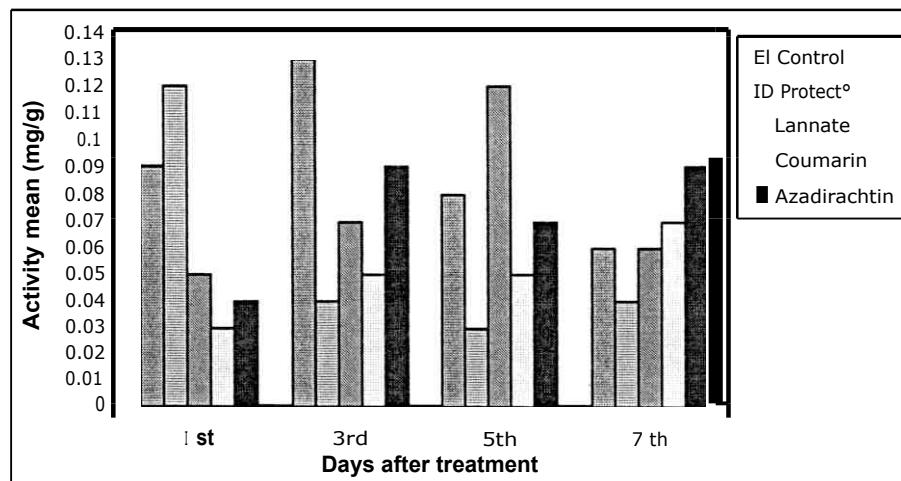


Fig. (15): Effect of the tested compounds on the activities of alkaline phosphatase enzyme of the fourth instar larvae of *S. littoralis*

6.2. Acid phosphatase:

Data in Table (21) and Fig. (16) indicated that, the tested compounds increased the activity of acid phosphatase enzyme of the 4th instar larvae of *S. littoralis* than the untreated larvae. The mean values of acid phosphatase enzyme activities in the supernatant of the homogenated larvae reached to 0.037, 0.035, 0.049 and 0.035 mg/g b.w. when larvae were treated with LC₅₀ by protecto, lannate, coumarin and azadirachtin, respectively compared with 0.034 mg/g b.w. of the untreated control. The percent differences between treatments were 108.82, 102.94, 144.11 and 102.94 % more than control when larvae were treated by protecto, lannate, coumarin and azadirachtin, respectively.

Abdel-Hafez *et al.* (1993b) studied the efficacy of two OP insecticides, two IGR's and their combined mixtures on laboratory strain of *S. littoralis*. Data showed that the larvae treated with the LC₅₀ of the tested compounds showed variable reduction (much lower than control) in Alla

Table (21): Effect of the tested compounds on the activities of acid-phosphatase enzyme of the 4th instar larvae of *S. littoralis*

| Treatments | Acid phosphatase activity (mg/g body weight) | | | | | |
|---------------------|--|-----------------|-----------------|-----------------|-------|--------|
| | 1 st | 3 rd | 5 th | 7 th | Mean | % |
| 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 |
| Lannate | 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

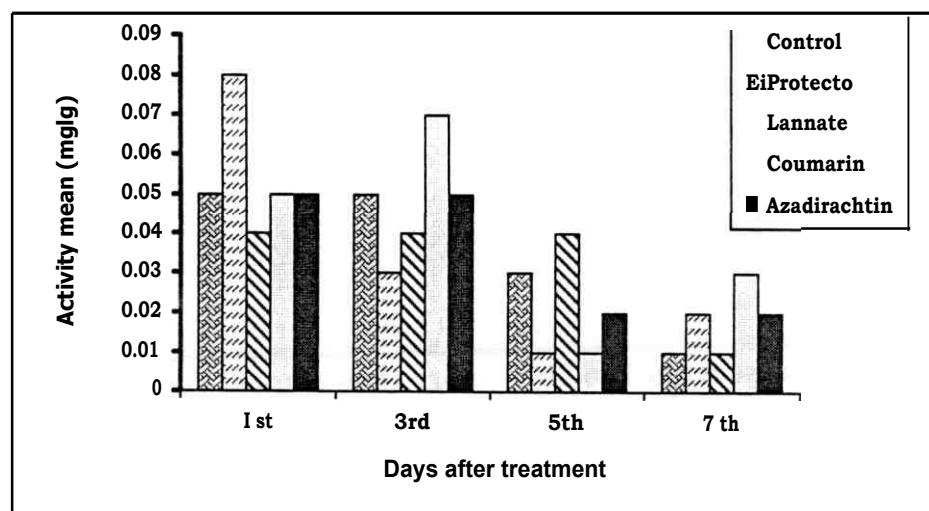


Fig. (16): Effect of the tested compounds on the activities of acid phosphatase enzyme of the fourth instar larvae of *S. littoralis*

Table (22): Effect of the tested compounds on the changes (+, -) of the studied tested biochemical aspects of 4th instar larvae of *S. littoralis*.

| Tested biochemical aspects | Compounds | | | |
|----------------------------|-----------|----------|----------|--------------|
| | Protecto | Lannate | Coumarin | Azadirachtin |
| Invertase | (-31.05) | (-30.86) | (0.0) | (-8.19) |
| Trehalase | (-17.93) | (-22.66) | (6.74) | (0.0) |
| Amylase | (-27.59) | (-21.07) | (25.28) | (32.56) |
| G.P.T | (- 1.83) | (-46.78) | (-14.67) | (-6A2) |
| G.O.T | + | (2.32) | (-18.85) | (36.20) |
| α - esterase | (-34.54) | (-24.70) | (21.38) | (6.17) |
| β - esterase | (-31.27) | (-16.70) | (-4.00) | (-30.17) |
| Total soluble protein | + | (35.88) | (-43.77) | (-72.34) |
| Acetylcholinesterase | + | (18.24) | (79.05) | (79.72) |
| Alkaline phosphatase | (-31.76) | (-9.71) | (-39.47) | (-14.11) |
| Acid phosphatase | + | (8.82) | (2.94) | (44.11) |

to % more than control

++ = increase from 31 to 60 % more than control

+++ = increase from 61 to 100 % more than control

0.0= no effect

- = decrease from 1 to 30 % less than control

= decrease from 31 to 60 % less than control

= decrease from 61 % less than control