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# ACTIVITY OF CASTOR OIL SEEDS *Ricinus communis* ETHANOL EXTRACT AGAINST COTTON LEAFWORM *Spodoptera littoralis* (BOISD.)



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## ABSTRACT

The present work aimed to study the effect of ethanol extract of *Ricinus commnuis* L. seeds on biological and physiological aspects for the cotton leafworm *Spodoptera littoralis*, 2nd and 4th instar larvae. The results cleared that the larval duration was 17.08 and 15.58 days & 10.11 and 9.36 days for treated and the untreated 2nd & 4th instar larvae respectively. The pupation percentage was 60.0 and 96.0 % for treated and untreated 2nd instar larvae and 61.0 and 93.0 for treated and untreated 4th instar larvae. The pupal weight was affected by the botanical extract. The pupal duration was 11.40 and 11.71 days for treated and untreated 2nd instar larvae and it was 11.96 and 12.51 days for treated and untreated 4th instar larvae. The emergence percentages resulted from treated 2nd and 4th instar larvae were 82.0 and 85.2 % compared with 97.0 % and 92.0 % for that resulted from the untreated larvae, respectively. On the other hand, the malformed adult was 28.0 and 2.0 % for 2nd instar larvae (treated and untreated) and 16.0 and 5.0 % for 4th instar larvae (treated and untreated) respectively. There are decreasing in fecundity of *S. littoralis* adults resulted from the treated 2nd and 4th instar larvae by the botanical extract used for this study. Effect of $LC_{50}$ value of the tested plant extract on the biochemical aspects of the 4th larval instar of *S. littoralis* was detected. The biochemical aspects AST, ALT, alkaline and acid phosphatase activities, the as well as the total protein content post treatment of the 4th instar larvae were considered throughout the present study.

**Keywords:** Botanical extract **-** Castor oil seeds - Cotton leafworm **-** *Spodoptera littoralis*

## INTRODUCTION

The Egyptian cotton, *Gossypium barbadense* L., is one of the most economic agricultural cash and industrial crops. It has been attacked by sever from many pest insects. The most of yield and quality losses are caused by insect pests, specially the Egyptian cotton leafworm *Spodoptera littoralis*.

*S. littoralis* is one of the most injurious insect pests to cotton in the Middle East. *S. littoralis* larvae feed mainly on leaves, stems and flowers of cotton plants .The control of this pest is based mainly on foliage treatments with chemical synthetic insecticides.

The widespread of synthetic pesticides since 1945 was utilized helped in increasing agricultural production and decreasing the incidence of endemic and epidemic diseases .However, the massive application of pesticides,

resulted in building up pest resistance to these poisons, and also resulted in adverse effects on the environment.

The present work is an attempt to implement a new promising approach to suppress the population of *S. littoralis* by using new types of the pest control agents, plant extracts. Plant extracts can be used in programs of integrated pest management, cheap, used safely, economically and environmentally acceptable. Over the last ten years, much efforts have been directed towards plants as a source of biologically active compounds. Today over thousands species of plants are known that possess dome insecticidal activities. Many plants have a history of use as folk remedies and are still in local use by different societies throughout the world to kill or repel insects. The pesticidal and biological activities of plant extracts were extensively studied by several investigators, Meisner *et al*., (1983); Ley *et al*., (1988); El-Khayat, *et al*., (1998); Nugroho *et al*., (1999); Hamed, (2000) ;Abd El-Mageed and EL Gohary (2007) and Abdel Aziz and El-Din (2007).

To complete the picture, detailed study was planned to find out the effect of these compounds on some biochemical components of the 4$^{th}$ instar larvae such as total soluble protein content, as well as Acetyl choline-esterase, non-specific α and β esterase, acid and alkaline phosphatases and Transaminases enzymes activities Also the biochemical changing were extensively studied by several investigators, Ahmed *et al.,*(1990); Schmidt *et al*., (1998); McKeon *et al*., (2000); Abdel-Aal, (2003); Desuky *et al.,* (2005); Rashad *et al*., (2006); Ramos-Lopez *et al.,* (2010) and Sayed *et al*., (2011).These attempts were elucidate to rationalize the using of insecticides via IPM program on cotton crop.

## MATERIALS AND METHODS

**1. Rearing technique:**

The tested insect was obtained from the Department of the cotton leafworm, Plant Protection Research Institute (PPRI), Agricultural Research Centre (ARC).

The stock culture of susceptible *Spodoptera littoralis* was reared on castor bean oil leaves *Ricinus communis* L. for several generations at laboratory conditions of 25 ± 1 °C and 65 – 70 ± 5% R.H. Egg masses were placed on castor bean 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 rearing jars bottomed with saw dust to absorb excess humidity. Castor bean oil 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 10% sugar solution and branches of *Nerium oleander* as suitable site for oviposition (El-Defrawi *et al.,* 1964). Newly laid egg masses were collected daily and transferred into the rearing jars and then it ready to start the experiments.

**2. Plant tested:**

**Order:** Malpighiales

| FAMILY | SPECIES | PART USED |
|---|---|---|
| Euphorbiaceae | *Ricinus commnuis* L. | Seeds |

**Plant preparation:**

Plant was collected from the field, cleaned from debris and was dried under room temperature at least one week, dried grounded in electric mill and were sieved in the rough 0.5 mm sieve.

**Extraction:**

Extraction was carried out according to the method adopted by Freedman, *et al.,* (1979), ground plants were soaked in hexane, acetone, ethanol and water extract.

**3. Laboratory Assay:**

The leaf dipping technique was used to test larvicidal action of the plant extracts against the 2nd and 4th instars of *S. littoralis* larvae. Fresh castor bean leaves were dipped in serial concentrations of the plant extract which was 20000, 10000, 5000 and 2500 ppm. for 10 seconds to define ($LC_{50}$) of hexane, acetone, ethanol and water extract. The treated leaves were left to dry before being offered to larvae. The larvae were allowed to feed on treated leaves for 72 hours. Five replicates contained 10 larvae/jar for each concentration and also for the control experiments which carried out using the same technique but leaves dipped only in the same solvent used in the extraction test.

Mortality percentages of all treatments were rated at 3days after treatment and corrected according to Abbott Formula (Abbott 1925). Results were illustrated graphically as log/probit regression lines using Sigma Plots software for Windows (version 11) depending on method described by (Finney1972). Mortality data were subjected to probit analysis using the Statistical Analysis System Version 9.1 program PROC PROBIT (SAS Institute 2003).

The efficiency of tested insecticides was measured according to Sun's equation (1950).

$$\text{*Toxicity index} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of other tested compound}} \times 100$$

$$\text{*Relative potency} = \frac{LC_{50} \text{ of the least effective compound}}{LC_{50} \text{ of other tested compound}}$$

**4. Biological responses:**

In order to study the biological response of *S. littoralis* to ethanol extract of *R. communis* the 2nd and 4th instar larvae were offered the castor bean oil treated with ethanol extract of *R. communis* at its determined $LC_{50}$ for 72h. after which time larvae were offered to untreated leaves. Treatment comprised 10 larvae and was replicated 10 times. The same numbers of larvae were considered as a control, these larvae were offered to castor bean oil leaves immersed only in same solvent used in extraction test. The

following parameters were recorded; larval duration, pupal weight, pupal duration, male longevity, female longevity, fecundity and fertility.

**5. Biochemical responses:**

After 72 hours following the feeding the 4$^{th}$ instar of *S. littoralis* larvae on castor bean oil treated with ethanol extract of *R. communis* at its determined $LC_{50}$ any survival larvae exhibiting toxic symptoms and healthy larvae were selected after 24 hrs and 72 hrs post treatment. The larvae of each treatment and control were placed in clean jars then starved for 4 hrs. The starved larvae were homogenized in distilled water (5 larva/5 ml distilled water) using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 3500 r.p.m for 10 minutes at 5$^{o}$C and the supernatants were used directly for enzyme assays as described by Raies (1992).

**Main contents**

A - Total soluble protein as described by Bradford (1976).

**Enzymes assay**

**The following enzymes activity were determined as follows:**

B - Acetylcholine-esterase activity was determined using acetylcholine bromide (Ach Br) as substrate according to the method described by Simpson *et al*. (1964).

C - Non-specific α and β esterases activity were measured as described by Van Asperen (1962) using α naphthyl acetate and β naphthyl acetate, respectively, as substrates.

D - Acid and alkaline phosphatase activity were measured from the larval haemolymph as described by Laufer and Schin (1971).

E - Transaminases activity were measured from the larval haemolymph as described by Reitman and Frankel (1957).

**6. Statistical analysis**

Mortality percentages of all treatments were rated at 3 days after treatment and corrected according to Abbot Formula (Abbott 1925). Results were illustrated graphically as log/probit regression lines using Sigma Plots software for Windows (version 11) depending on (Finney1972). Mortality data were subjected to probit analysis using the Statistical Analysis System Version 9.1 program PROC PROBIT (SAS Institute 2003)

## RESULTS AND DISCUSSION

**1. Toxicological activity of certain solvent extracts for *R. communis* against *Spodoptera littoralis* 2$^{nd}$ and 4$^{th}$ larval instars.**

The efficiency of some solvent extracts for *Ricinus communis* were evaluated against *S. littoralis,* 2$^{nd}$ and 4$^{th}$ larval instars, which were hexane, acetone, ethanol and water extract are tabulated in Tables (1&2) and graphically illustrated as toxicity lines in Figs. (1&2).

Examination of the tabulated data indicated that the toxicity of the tested insecticides varied tremendously according to the concentration used, the solvent, and the treated instar. As a general trend, the higher concentration caused higher mortality rate and vice versa.

Data in Table 1 and Fig. 1 showed that the $LC_{25}$ of hexane, acetone, ethanol and water against 2nd instar larvae of *S. littoralis* were 3174, 3779, 1792 and 7040 ppm, respectively. In case of $LC_{50}$, it these values were 7142, 9667, 4163 and 16565ppm, respectively. However, the $LC_{90}$ values revealed 33353, 57603, 20655 and 84206ppm, respectively.

**Table 1: Toxicity values (ppm.) of the tested solvent extracts for *R. communis* seeds against the 2nd instar larvae of *S. littoralis* using dipping technique.**

| Treatments | $LC_{25}$ | $LC_{50}$ | $LC_{90}$ | Slope | Toxicity index | Relative potency |
|---|---|---|---|---|---|---|
| Hexane extract | 3174 | 7142 | 33353 | 1.9 | 58.3 | 2.3 |
| Acetone extract | 3779 | 9667 | 57603 | 0.8 | 43.1 | 1.7 |
| Ethanol extract | 1792 | 4163 | 20655 | 1.8 | 100 | 4.0 |
| Water extract | 7040 | 16565 | 84206 | 1.7 | 25.1 | 1 |

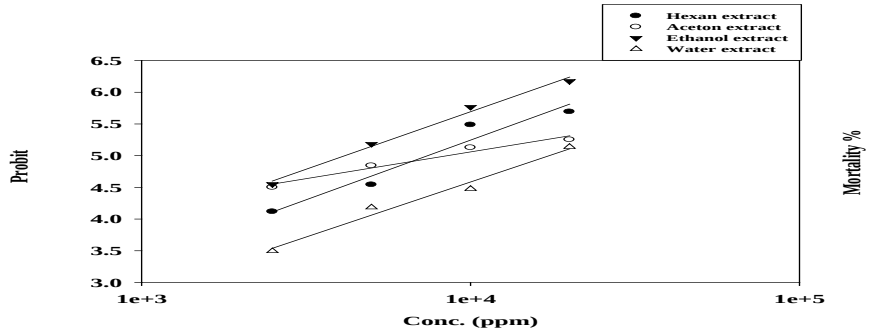


**Fig (1): Toxicity Regression lines of some botanical extract for castor oil seeds *Ricinus communis* against 2nd instar *S. littoralis* larvae.**

The relative potency based on the least effective extract (water), showed that ethanol extract was the most toxic extract since it had the highest relative potency of 3.9 folds of water followed by hexane extract with relative potency of 2.2 folds and then acetone with relative potency of 1.86 folds as presented in Table (1).

Data illustrated in Table 2 and Fig. 2 showed that the $LC_{25}$ of hexane, acetone, ethanol and water against the 4th instar larvae of *S. littoralis* were 4937, 6144, 3288 and 9056ppm, respectively. In case of $LC_{50}$, these values exerted 11614, 15814, 8165 and 19659 ppm, respectively. However $LC_{90}$ values revealed 59009, 95327, 45963 and 100391ppm, respectively.

**Table 2: Toxicity values (ppm) of the tested solvent extract for *R. communis* seeds against the 4th instar larvae of *S. littoralis* using dipping technique.**

| Treatments | $LC_{25}$ | $LC_{50}$ | $LC_{90}$ | Slope | Toxicity index | Relative potency |
|---|---|---|---|---|---|---|
| Hexane extract | 4937 | 11614 | 59009 | 1.8 | 70.3 | 1.7 |
| Acetone extract | 6144 | 15814 | 95327 | 1.1 | 51.6 | 1.2 |
| Ethanol extract | 3288 | 8165 | 45963 | 1.4 | 100 | 2.4 |
| Water extract | 9056 | 19659 | 100391 | 1.9 | 41.5 | 1 |

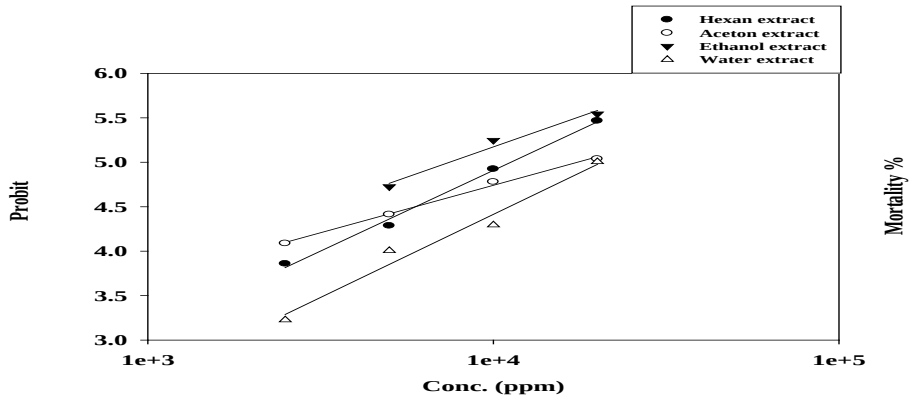


**Fig (2): Toxicity Regression lines of some botanical extracts for castor bean oil leaves *Ricinus communis* against 4th instar *S. littoralis* larvae.**

The relative potency based on the least effective extract (water), showed that ethanol extract was the most toxic extract since it had the highest relative potency of 2.8 folds of water followed by hexane extract with relative potency of 1.8 folds and then acetone with relative potency of 1.5 folds as presented in Table (2).

**2. Influence of *R. communis* seeds ethanol extract on the biological aspects for *S. littoralis*.**

The present study of *R. communis* seeds extracts on *S. littoralis* larvae gave promising results. Thus, it was selected for further studies on biological aspects for immature and adult stage of *S. littoralis* (i.e. larval duration, larval mortality, pupation percent, pupal weight, pupal duration for both ♂ and ♀, adult emergence percent, malformation percent, adult longevity for ♂ and ♀, sex ratio and fertility).

**Influence of *R. communis* seeds ethanol extract on some biological aspects of S*. littoralis* immature stages when treated as 2nd instar larvae:**

Data in Table (3) indicated that the treatment of the 2nd instar larvae of *S. littoralis* by $LC_{50}$ concentration of *R. communis* seeds extract led to the prolongation and directly increase in the larval duration, it was 17.08 days compared with the untreated one which being 15.58 days. This result is in full agreement with those found by Dimetry *et al.,* (1998) and Ismail *et al.,* (2002).

**Table 3: Effect of $LC_{50}$ of *R. communis* seeds ethanol extract on biological aspects of the immature stages of *S. littoralis* when treated as 2nd instar larvae.**

| Biological aspects | | *R. communis* | Untreated |
|---|---|---|---|
| Larval duration (days ± S.E) | | 17.08 ± 0.44 | 15.58 ± 0.23 |
| Larval mortality % | | 56.0 | 4.0 |
| Pupation % | | 44.0 | 96 |
| Pupal weight (gm) | Mean Pupal weight | 0.3104 ± 0.015 | 0.3599 ± 0.044 |
| | ♂ pupal weight | 0.2809 ± 0.010 | 0.3488 ± 0.043 |
| | ♀ pupal weight | 0.3400 ± 0.024 | 0.3711 ± 0.031 |
| Pupal duration (days ± S.E) | Mean Pupal duration | 11.40 ± 0.359 | 11.71 ± 0.19 |
| | ♂ pupal duration | 11.8 ± 0.16 | 12.09 ± 0.27 |
| | ♀ pupal duration | 11.00 ± 0.20 | 11.33 ± 0.17 |
| Pupal mortality % | | 18.0 | 3.0 |

On the other hand, the larval mortality percentages were 56.0 and 4.0 % for treated and untreated larvae, respectively. The pupation percentage were 44.0 and 96.0 % for treated and untreated larvae of *S. littoralis*, respectively.

As shown in Table (3) results indicated that the mean pupal weight were 0.3104 and 0.3599 gm for treated and untreated, respectively. While, the male and female pupal weight were 0.2809 and 0.3400 gm for larval treatment, and 0.3488 and 0.3711 gm for untreated larval, respectively.

The mean pupal duration was 11.40 and 11.71 days for treated and untreated one, respectively. Data in Table (3), revealed that pupal duration decrease in both male and female by larval treatment with $LC_{50}$ which (11.80 and11.00 days) for both ♂ and ♀, compared with their untreated larval (12.09 and 11.33 days), respectively. The pupal mortality percentage in *R. communis* seeds extract treatment reached to 18.0 %, while it did not exceed than 3.0 % in untreated larvae.

**Influence of *R. communis* seeds ethanol extract on some biological aspects of *S. littoralis* adult stage when treated as 2nd instar larvae:**

Regarding the effect of *R. communis* seeds ethanol extract on adult stage, data in Table (4) indicated that the total adult emergence percentage for 2nd instar larvae treated with *R. communis* extract were 82.0 compared with 97.0 % for untreated larvae. The treatment by *R. communis* caused adult malformation of 28.0% compared with 2.0% for the untreated larvae. On the other hand, the adult stage was affected by *R. communis* seeds extract treatment; the normal adult was 72.0% for treatment compared with 98.0 % for untreated.

The percentage of male & female sex ratio % were 0.55: 0.45 for *R. communis* extract and 0.46: 0.54 for untreated, respectively. Also, *R. communis* extract decrease the adult longevity, where male longevity recorded 11.01 and 12.00 days resulted from larval treatment and the untreated larvae, respectively, while female longevity was 11.73 and 11.88 days produced from larval the treatment and untreated, respectively.

The same Table (4) showed the effect of the larvae treatment by *R. communis* extract on the resulted adult fecundity, the average total number of eggs laid by treated female throughout its life span was 853.0 eggs/female, while it reach to 1208.5 eggs/female in the untreated female. The results are accordance with those findings by Abdel- Aziz *et al.,* (1995).

**Table 4: Effect of $LC_{50}$ value of *R. communis* seeds ethanol extract on the adult stage of *S.littoralis* when treated as 2nd instar larvae**

| Biological aspects | | *R. communis* | Untreated |
|---|---|---|---|
| Emergence % | Total emergence % | 82.0 | 97.0 |
| | Normal adult % | 72.0 | 98.0 |
| | Malformed adult % | 28.0 | 2.0 |
| Sex ratio % | ♂ | 0.55 | 0.46 |
| | ♀ | 0.45 | 0.54 |
| Longevity (days ± S.E) | ♂ | 11.01 ± 0.66 | 12.00 ± 0.48 |
| | ♀ | 11.73 ± 0.71 | 11.88 ± 0.42 |
| Fecundity (No. of egg / female) | | 853.0 ± 32.3 | 1208.5 ± 22.8 |

**Influence of *R. communis* seeds ethanol extract on *S. littoralis* immature stages when treated as 4th instar larvae:**

Data summarized in Table (5) indicate that the treatment of the 4th instar larvae of *S. littoralis* by $LC_{50}$ concentration of *R. communis* seeds ethanol extract led to slight increase in the larval duration, it was 10.11 days compared with the untreated one 9.36 days. This result is in full agreement with those found by Dimetry *et al.,* (1998) and Ismail *et al.,* (2002).

**Table 5: Effect of $LC_{50}$ value of *R. communis* seeds ethanol extract on the immature stages of *S.littoralis* when treated as 4th instar larvae.**

| Biological aspects | | *R. communis* | Untreated |
|---|---|---|---|
| Larval duration (days ± S.E) | | 10.11 ± 0.31 | 9.36 ± 0.09 |
| Larval mortality % | | 48.0 | 7.0 |
| Pupation % | | 52.0 | 93.0 |
| Pupal weight (gm) | Mean Pupal weight | 0.2909 ± 0.073 | 0.3153 ± 0.067 |
| | ♂ pupal weight | 0.2695 ± 0.015 | 0.2973 ± 0.065 |
| | ♀ pupal weight | 0.3123 ± 0.085 | 0.3334 ± 0.035 |
| Pupal duration (days ± S.E) | Mean Pupal duration | 11.96 ± 0.18 | 12.51 ± 0.21 |
| | ♂ pupal duration | 11.23 ± 0.23 | 12.00± 0.27 |
| | ♀ pupal duration | 12.69 ± 0.09 | 13.02 ± 0.32 |
| Pupal mortality % | | 14.8 | 8.0 |

On the other hand, the larval mortality percentages were 48.0 and 7.0 % for treatment and untreated larvae, respectively. The pupation percentages

were 52.0 and 93.0 % for treated and untreated larvae of *S. littoralis*, respectively.

The mean pupal weight was 0.2909 and 0.3153 gm for treated and untreated, respectively. While, the male & female pupal weight were 0.2695 & 0.3123 gm for treatment, and 0.2973 & 0.3334 gm for untreated, respectively.

Data shown in Table (5), revealed that pupal duration was increased in both male and female by treatment with $LC_{50}$ value which being 11.23 and 12.69 days for both ♂ and ♀, compared with their untreated one 12.00 and 13.02 days, respectively. The pupal mortality % reached to 14.8 % in treatment, while it did not exceed than 8.0 % in untreated one.

**Influence of $LC_{50}$ value of *R. communis* ethanol extract on *S. littoralis* adult stage when treated as 4th instar larvae:**

Regarding the effect of *R. communis* extract on the adult stage, data in Table (6) indicated that the total emergence percentages for the 4th instar larvae treated with *R. communis* were 85.2 and 92.00 % for treatment and untreatment, respectively. Reducing the adult emergence by plant extract had mentioned before by Sharaby and Ammar (1997).

The treatment with *R. communis* seeds ethanol extract indicated adult malformation with 16.6 % compared with 5.0 % for the untreated adult. On the other hand, the adult stage was affected by *R. communis* treatment; the normal adult emergence was 83.4 % for treatment compared with 95.0 % for the untreatment.

The male and female sex ratio were 0.58 and 0.42 for *R. communis* extract treatment & 0.53 and 0.47 for untreatment, respectively (Table, 6).

For adult longevity, *R. communis* extract decrease the adult longevity, in this respect the male longevity was 13.00 and 10.04 days for the treatment and the untreatment, respectively, while female longevity was 13.19 and 9.99 days for the treatment and untreatment respectively.

Data represented in Table (6) showed the average total number of eggs laid by treated female throughout its life span was 789.0 eggs/female while it reached to 805.0 eggs/female for untreated female, these results are agree with such findings by Abdel- Aziz *et al.,* (1995).

**Table 6: Effect of $LC_{50}$ value of *R. communis* seeds ethanol extract on the adult stage of *S.littoralis* when treated as 4th instar larvae.**

| Biological aspects | | *R. communis* | Untreated |
|---|---|---|---|
| Emergence % | Total emergence % | 85.2 | 92.0 |
| | Normal adult % | 83.4 | 95.0 |
| | Malformed adult % | 16.6 | 5.0 |
| Sex ratio % | ♂ | 0.58 | 0.53 |
| | ♀ | 0.42 | 0.47 |
| Longevity (days ±S.E) | ♂ | 13.00 ± 0.72 | 10.04 ± 0.76 |
| | ♀ | 13.19 ± 0.89 | 9.99 ± 0.59 |
| Fecundity (No. of egg / female) | | 789.0 ± 43.0 | 805.0 ± 38.0 |

**3. Biochemical aspects**

This part of study deal with the effect of sublethal concentration of *R. communis* seeds ethanol extract on certain biochemical homogenate

constituents of the 4th instar larvae of *S. littoralis* under laboratory conditions. This investigation included the following homogenate constituents; total soluble protein content and some enzymatic activities, including (non-specific esterases, acetylcholine-esterase, phosphatases and transaminases). Such examinations were undertaken as an attempt to interpret the primary mode of actions of the tested insecticides as well as illustrate the biological disturbance which was observed in the treated larvae in the previous study.

**Effect on total soluble protein**

As shown in Table (7), treatment of 4th instars *S. littoralis* larvae for 24h. with $LC_{50}$ value of *R. communis* seeds ethanol extract caused an increase in the total soluble protein from 30.8 to 36.11 mg/ ml, giving a 17.2 % increase than their value in the control. Also, a marked increase of 24% was noticed after 72h.; where the value was increase from 38.3 in the control to 47.51 mg/ml in treated 4th instar larvae. The obtained results are in agreement with some investigators such as Aly 1999; Desuky *et al.*, (2005) and Taksira 2007.

**Table 7: Enzyme activities in the *S. littoralis* 4th instar larvae following treatment with $LC_{50}$ concentrations of *R. communis* seeds ethanol extract.**

| Biochemical aspects | After 24h. | | | After 72h. | | |
|---|---|---|---|---|---|---|
| | Control | Treated | *Increase or decrease than control (% Changes) | Control | Treated | *Increase or decrease than control (% Changes) |
| Total soluble Proteins | 30.8 ±0.53 | 36.11 ±1.48 | +17.2 | 38.3 ±1.18 | 47.51 ±1.82 | + 24 |
| α - Esterase (µg α-naphthol released/ml./min.) | 502.7 ± 0.06 | 380.9 ± 0.09 | -24.2 | 584.9 ± 0.02 | 250 ± 0.08 | -57.3 |
| β - Esterase (µg β-naphthol released/ml./min.) | 650.7 ±0.5 | 530.8 ± 0.3 | -18.4 | 730.5 ± 0.1 | 480.4 ± 0.3 | -34.2 |
| Acetyl Choline-esterase (µg AchBr/ml/min ) | 790.02 ±0.3 | 648.7 ±0.2 | -17.9 | 849.03 ±0.1 | 549.4 ±0.1 | -35.3 |
| Acid phosphatase (µg phenol/ml/min ) | 95.88 ± 0.05 | 83.79 ± 0.01 | -12.6 | 128.9 ± 0.01 | 45.78 ± 0.03 | -64.5 |
| Alkaline phosphatase (µg phenol/ml/min ) | 9.1 ± 0.02 | 4.04 ± 0.03 | -55.6 | 10.4 ± 0.1 | 2.17 ± 0.2 | -79.1 |
| ALT (GPT) (µg pyruvate/ml/min) | 21.96 ± 0.3 | 18.6 ± 0.1 | -15.3 | 30.46 ± 0.4 | 12.7 ± 0.1 | -58.3 |
| AST (GOT) ( µgoxaloacetate/ml/min) | 37.86 ± 0.01 | 26.93 ± 0.02 | -28.9 | 45.8 ± 0.02 | 18.1 ± 0.01 | -60.5 |

*** Mean significant difference between treated and control.**

**% Increase or decrease than control % Changes = treated – control ÷ control X 100**

**Enzyme assay**

**Effect on acetylcholine esterase, alpha and beta esterases activity:**

The esterases constitute a large group of enzymes of generally broad specific which occur in multiple forms in both animals and insects (Cook and Forgash, 1965). This group of enzyme is including the specific and non-specific esterases. Acetylcholineesterase is one of the most important enzyme belong to the specific esterases, the great majority of traditional insecticides are more poisons and the main target for most of them is the acetylcholineesterase. The non-specific esterases, that hydrolyze α- and β-naphthyl acetate, which are considered as aromatic ester hydrolases. The catalysis of hydrolysis reaction by non-specific esterases considers one of the main reactions responsible for detoxification mechanism of toxic compounds in insects (Ahmed and Forgash, 1976).

The activity of alpha and beta esterase in *S. littoralis* 4$^{th}$ instar larvae 24 and 72 hours following treatment with the calculated $LC_{50}$ of *R. communis* seeds ethanol extract are shown in Table (7). The activity of alpha esterase in treated 4$^{th}$ instar larvae for 24h. was 380.9µg α-naphthol /ml /min/ g larval weight as compared to 502.7 µg α-naphthol /ml /min/g larval weight in the control, being a reduction by 24.2 %. Moreover; 57.3 % reduction was found in treated larvae for 72h. Beta esterase activity was also decreased by 18.4 % in larvae treatment as 4$^{th}$ instar larvae for 24h.than their control; this percentage was much lower than the increase of alpha esterase. Similarly, treatments of 4$^{th}$ instar larvae for 72h. caused a marked decrease in alpha esterase activity than that recorded in untreated larvae by 34.2 %. According to such influence it could be emphasized that increase of *R. communis* seeds ethanol extract toxicity on larval stage of *S. littoralis* with time elapsed is due to the inhibition power on non-specific esterases which are responsible to detoxification process.

As seen in Table (7), As a result of treatment with $LC_{50}$ of *R. communis* seeds ethanol extract to either 4$^{th}$ instars after 24 and 72h., the activity of acetylcholine-esterase was reduced by 17.9 and 35.3 % for the respective mentioned treatments, than that of their equivalent control. These results are in agreement with those obtained by Aly (1999) and Taksira (2007).

**Effect on acid and alkaline phosphatase activity:**

The term of phosphatases are defined as enzymes that hydrolyze any phosphorus ester or anhydride bond, including P-O-C, P-F and others. One generalization can be made safely, all the OP compounds can be hydrolyzed, in mammals, insects and plants by phosphatases, commonly the major metabolic route (O' Brien, 1967).

Acid phosphatase activity was significantly reduced from 95.88 to 83.79 µg phenol/ml/min in 4$^{th}$ instar larvae following their treatment for 24h. with $LC_{50}$ of *R. communis* seeds ethanol extract making a 12.6 % decrease. Following treatment of 4$^{th}$ instar larvae for 72h. this enzyme's activity was highly significantly reduced from 128.9 to 45.78 µg phenol/ml/min, (i.e. a 64.5 % reduction). Likewise; alkaline phosphatase activity decreased in treated 4$^{th}$ instar larvae for 24h. by 55.6%, and it decreased in treated 4$^{th}$ instars for 72h. by 79.1%. (Table 7). These results are in agreement with those obtained by Saeed and Nagvi (1987) and Aly (1999).

**Effect on transaminases activity:**

The amino transferases, especially alanine amino transferase (GPT) is one of the component of oxidative metabolism of proline, which in certain insects is utilized during the initial periods of lights (Bursell, 1963), it also acts as a catalytic agent in the metabolism of carbohydrate (Katunuma *et al.,* 1968). The inhibitory effect of *R. communis* seeds ethanol extract at $LC_{50}$ level on transaminases is shown in Table (7) and for Glutamic oxaloacetic transaminase (GOT) [also known as Aspartate transaminase (AST)], and for Glutamic pyruvic transaminase (GPT) [also known as Alanine transaminase (ALT)]. From the obtained results it could be seemed that ALT and AST activities in $4^{th}$ instar larvae after 24h. of treatment were reduced than their control by 15.3 and 28.9 % for the respective mentioned enzymes, meanwhile; treatment the $4^{th}$ instar larvae for 72h. were reduced the enzymes activities by 58.3 and 60.5 % than their control for the respective mentioned enzymes. These result is agreement with Desuky *et al.,* (2005). From the above results it could be concluded that the chronic effect of the tested non-traditional insecticides on transaminases activities may led to the disturbance of protein metabolism and synthesis of certain specific compounds according to (Bursell, 1963).

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## نشاط المستخلص الإيثانولى لبذور الخروع ضد دودة ورق القطن

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تهدف الدراسة الحالية الي دراسة تأثير المستخلص الايثانولي لبذور نبات الخروع علي النظم البيولوجية والفسيولوجية ليرقات العمر الثاني والرابع لدودة ورق القطن ، حيث اوضحت الدراسة ان مدة الطور اليرقي كانت ١٧.٠٨ و ١٥.٥٨ يوم وكذلك ١٠.١١ و ٩.٣٦ يوم لكل من المعاملة والمقارنة لكل من العمر الثاني والرابع ، بالترتيب . كانت النسبة المؤية للتعذير ٦٠ ، ٩٦ % لكل من المعاملة والمقارنة للعمر اليرقي الثاني ، وبلغت ٦١ ، ٩٣ % للمعاملة والمقارنة ليرقات العمر الرابع ، واوضحت النتائج ان هناك تاثير للمستخلص الايثانولي علي اوزان العذاري. بلغت مدة الطور العذري ١١.٤ و ١١.٧١ يوم لكل من المعاملة والمقارنة للعمر اليرقي الثاني وبلغت ١١.٩٦ و ١٢.٥١ يوم لكل من المعاملة والمقارنة للعمر اليرقي الرابع. بلغت النسبة المئوية لخروج الفراشات ٨٢.٠ مقابل ٩٧.٠ % للمقارنة وكذلك ٨٦.٠ للمعاملة مقابل ٩٨.٠ % مقابل المقارنة لكل من العمر اليرقي الثاني والرابع بالترتيب ، وعلي الجانب الاخر ، بلغت النسبة المئوية للتشوهات في الفراشات الناتجة ٢٨.٠ و ٢.٠ % لكل من المعاملة والمقارنة في العمر اليرقي الثاني وبلغت ١٦.٠ و ٥.٠ % لكل من المعاملة والمقارنة للعمر اليرقي الرابع. اوضحت النتائج وجود انخفاض في عدد البيض الموضوع لكل انثي عند المعاملة بالمستخلص الايثانولي من بذور الخروع ليرقات العمر الثاني والرابع لدودة ورق القطن. شملت الدراسة ايضا تأثير الجرعة نصف السامة النصفية من المستخلص الايثانولي تحت الدراسة علي المظاهر الكيموحيوية ليرقات العمر الرابع لدودة ورق القطن وركزت الدراسة علي تقدير كل من الـــ AST والـــ ALT والانزيمات التي لها علاقة بالتحليل المائي في جسم الحشرة والفا وبيتا استريز والاستيل كولين استيريز وكذلك البروتينات الكلية.