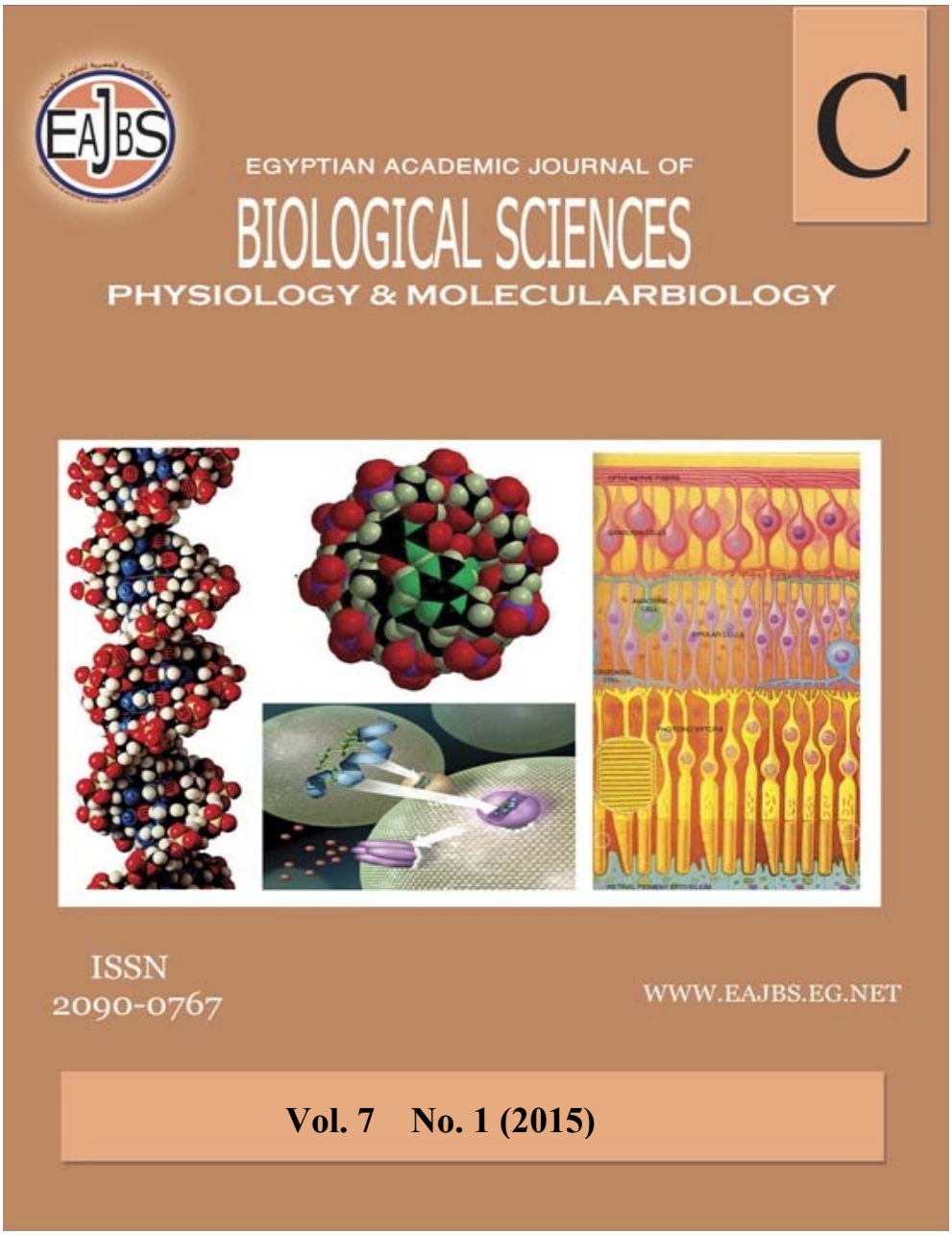

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Toxicological effect of the botanical extract castor oil seeds *Ricinus communis* and their biochemical activity on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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ABSTRACT

The present work aimed to studying the effect of ethanol extract of *Ricinus commnuis* L. seeds on biological and physiological for cotton leafworm *Spodoptera littoralis* 2nd and 4th instar larvae. The results cleared that the larval duration were 17.08 &15.58 days and 10.11 & 9.36 days for treated and untreated of the 2nd & 4th instar larvae, respectively. The pupation percent was 60.0 and 96.0 % for treated and untreated of the 2nd instar larvae and 61.0 and 93.0 for treated and untreated of the 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 of the 2nd instar larvae and it was 11.96 and 12.51 days for treated and untreated of the 4th instar larvae. The emergence % was 82.0 compare to 97.0 % and 86.0 compare to 98.0 % for the 2nd & 4th instar larvae, respectively. On the other hand, the malformed adult was 28.0 and 2.0 % for the 2nd instar larvae (treated and untreated) and 16.0 & 5.0 % for 4th instar larvae (treated and untreated), respectively. There are decreasing in fecundity for treatment by botanical extract to *S. littoralis* 2nd and 4th instar larvae. 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 and total protein activities post treatment were considered throughout the present study.

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 *S. 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 helped in increasing agriculture 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 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 effort has 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 researchers, Meisner *et al*., (1983); Ley *et al.,* (1988); Malakov *et al.,* (1994); El-Khayat, *et al.,* (1998); Nugroho *et al.,* (1999); Hamed, (2000); Abdel Galeil and Nakatani (2003); Abd El-Mageed *et al.,* (2007); Reda *et al.* (2012) and Hanan, *et al.* (2013).

To complete the picture detailed study was planned to find out the effect of these compounds on some biochemical components of the 4th instar larvae such as total soluble protein, Acetyl choline-esterase, Non-specific α and β esterase, Acid and alkaline phosphatase and Transaminases enzymes activities Also the biochemical changing were extensively studied by several researchers, Ahmed (1990); Schmidt *et al.,* (1998); McKeon *et al.,* (2000); Abdel-Aal, (2003); Desuky *et al.,* (2005); Rashad *et al.,* (2006); Zahia *et al*. (2009); Bakr *et al*. (2010); Ramos-Lopez *et al.,* (2010); Sayed *et al.,* (2011) and Mervat *et al* (2012). These attempts were elucidate to rationalize the using of insecticides via IPM program on cotton crop.

MATERIALS AND METHODS

Rearing technique:

The tested insect was obtained from the Department of the cotton leafworm, Plant Protection Research Institute (PPRI), Agriculture Research Centre (CRI), Ministry of Agriculture, Dokki, Giza.

The stock culture of susceptible *S. littoralis* was reared on castor 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 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 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 in to the rearing jars and then it ready to start the experiments.

Tested plant:

Order: Malpighiales

FAMILY	SPECIES	USED PART
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 absolute ethanol.

Laboratory Assay:

The leaf dipping technique used to test larvicidal action of plant extracts against the 2nd and 4th instars *S. littoralis* larvae of 1-day old were used. Fresh castor bean leaves were dipped in serial concentration 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 same solvent used in extraction test.

Mortality percentages of all treatments were rated at 3days 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).

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

*Toxicity index =

$$\frac{LC_{50} \text{ of the most effective compound (has the lowest } LC_{50})}{LC_{50} \text{ of other tested compound}} \times 100$$

*Relative potency =

$$\frac{LC_{50} \text{ of the least effective compound (has the highest } LC_{50})}{LC_{50} \text{ of other tested compound}}$$

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 castor bean oil treated with ethanol extract of *R. communis* at its determined LC_{50} for 72h. ,after which time larvae were offered 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 castor 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.

Biochemical responses:

After 72 hours following the feeding of 4th instar *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°C and the supernatants were used directly for enzyme assays Raies (1992).

M**ain contents**

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

Enzymes assay

The following enzymes activity was determined:

i- Acetyl choline-esterase activity was determined using acetylcholine

bromide (AChBr) as substrate according to the method described by Simpson *et al*. (1964).

ii- Non-specific α and β esterase activity was measured as described by Van Asperen (1962) using α naphthyl acetate and β naphthyl acetate, respectively, as substrates.

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

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

Statistical analysis

Mortality percentages of all treatments were rated at one day 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

Toxicological activity of certain solvent extracts for *R. communis* against *S. littoralis* 2nd and 4th larval instar.

The efficiency of some solvent extracts for *R. communis* were evaluated against *S. littoralis* 2nd and 4th larval instar, 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 tested insecticides varied tremendously according to the concentration used, the solvent, and the treated instar. As a general trend the higher the concentration was the 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 10031, 1415, 623.26 and 10320 ppm, respectively. In case of LC_{50}, it exerted 65685, 8829, 2239 and 49089 ppm, respectively. However LC_{90} values revealed 2334386, 286383, 25414 and 950392 ppm, respectively.

Table 1: Toxicity values (ppm.) of the tested solvent extract for *R. communis* to the 2nd instar larvae of *S. littoralis*.

Treatments	LC_{25}	LC_{50}	LC_{90}	Slope	Toxicity index	Relative potency
Hexane extract	10031	65685	2334386	0.82	3.4 %	1
Acetone extract	1415	8829	286383	0.84	25.4 %	7.4
Ethanol extract	623.26	2239	25414	1.2	100 %	29.3
Water extract	10320	49089	950392	0.99	4.6 %	1.3

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

Treatments	LC_{25}	LC_{50}	LC_{90}	Slope	Toxicity index	Relative potency
Hexane extract	14735	72938	1523041	0.97	2.4 %	1
Acetone extract	4296	19213	330816	1.04	8.9 %	3.8
Ethanol extract	122.4	1724	262750	0.59	100%	42.3
Water extract	10460	49262	935894	1.0	3.5 %	1.5

The relative potency based on the least effective extract (hexane), showed that ethanol extract was the most toxic extract since it had the highest relative potency of 29.3 folds of hexane followed by acetone with relative potency of 7.4 folds and water with relative potency of 1.3 folds as presented in Table (1).

Data in Table 2 and Fig. 2 showed that the LC_{25} of hexane, acetone, ethanol and water against 4th instar larvae of *S. littoralis* were 14735, 4296, 122.4 and 10460 ppm, respectively. In case of LC_{50}, it exerted 72938, 19213, 1724 and 49262

ppm, respectively. However LC_{90} values revealed 1523041, 330816, 262750 and 935894ppm, respectively.

The relative potency based on the least effective extract (hexane), showed that ethanol extract was the most toxic extract since it had the highest relative potency of 42.3 folds of hexane followed by acetone with relative potency of 3.8 folds and water with relative potency of 1.5 folds as presented in Table (2).

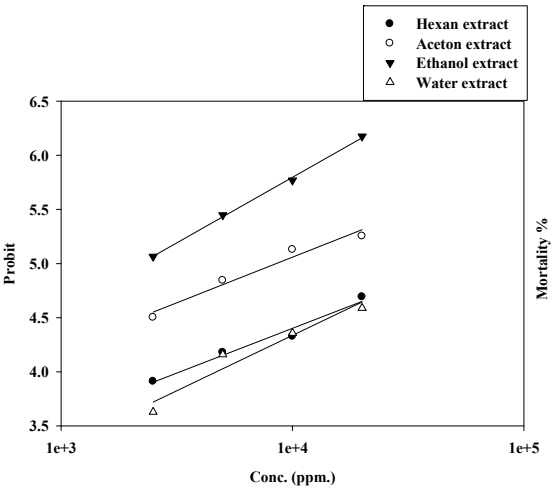

Fig 1 : Toxicity regression lines of some botanical extract castor oil plant *Ricinus communis* against 2nd instar *Spodoptera littoralis* larvae.

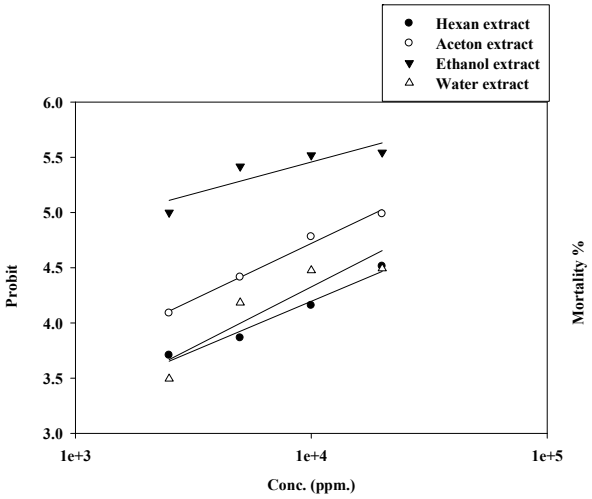

Fig 2 : Toxicity regression lines of some botanical extract castor oil plant *Ricinus communis* against 4th instar *Spodoptera littoralis* larvae.

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

The present study of *R. communis* 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).

Data concerning the effect of LC_{50} value of the ethanol extract of tested plant, *R. communis* on the biological aspects of the 2nd and 4th larval instar of *S. littoralis*.

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

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

On the other hand, the larval mortality percentages were 56.0 and 4.0 % for treatment and control, respectively. The pupation percentage was 60.0 and 96.0 % for treated and untreated larvae of *S. littoralis*, respectively.

The same table indicted 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 & 0.3400 gm for treatment, and 0.3488 & 0.3711 gm for untreated, 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 treatment with LC_{50} which was (11.80 and11.00 days) for both ♂ and ♀, compared with their controls (12.09 and 11.33 days), respectively. The pupal mortality % in *R. communis* treatment reached to 27.4

%, while it does not exceed than 6.0 % in untreated.

Table 3: Effect of LC_{50} of *R. communis* seeds ethanol extract on 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 %		60.0	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 %		27.4	6.0

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

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

Table 4: Effect of LC_{50} 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

The percentage of male and 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 adult longevity, male longevity were 11.01

and 12.00 days for the treatment and the untreated, respectively, while female longevity was 11.73 and 11.88 days for the treatment and untreated, respectively.

The same Table (4) showed the affected by treatment by *R. communis* extract , the average total number of eggs laid by female throughout its life span was 853.0 eggs/female, while it reach to 1208.5 eggs/female in the untreated, these agree with such finds of Abdel-Aziz and Omer (1995).

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

Data in Table (5) indicate that the treatment of the 4th instar larvae of *S. littoralis* by LC_{50} concentration of *R. communis* led to the prolongation and directly increased 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).

On the other hand, the larval mortality percentages were 48.0 and 7.0 % for treatment and untreated, respectively. The pupation percentage was 61.0 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 and female pupal weight were 0.2695 & 0.3123 gm for treatment, and 0.2973 & 0.3334 gm for untreated, respectively.

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

Table 5: Effect of LC_{50} of *R. communis* 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 %	61.0	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

Influence of *R. communis* ethanol extract on *S. littoralis* adult stage when treated as 4th instar larvae:

Regarding the effect of *R. communis* extract on adult stage, data in Table (6) indicate that the total

emergence percent for 4th instar larvae treated with *R. communis* were 86.00 and 98.00 % for treatment and untreated, respectively. Reducing of adult emergence by plant extract had mentioned before by Sharaby and Ammar (1997).

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

Table 6: Effect of LC_{50} 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	86.0	98.0
	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

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

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

Data in the same Table (6) showed the affected by treatment by *R. communis* extract , the average total number of eggs laid by female throughout its life span was 789.0 eggs/female while it reach to 805.0 eggs/female for untreated, these agree with such finds of Abdel- Aziz and Omer (1995).

Biochemical effects

This part of study deal with the effect of sublethal concentration of *R. communis* 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 and some enzymatic systems (Esterases, Acetyl choline-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 previous parts.

Effect on total soluble protein

As seen in Table (7), treatment of 4th instars *S. littoralis* larvae for 24h. with LC_{50} of *R. communis* 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% after 72h., as its value was increase from 38.3 in the control to 47.51 mg/ml in treated 2nd instar larvae. The obtained results are in contrary with that obtained by Abdel-Hafez *et al.,* (1988), Ahmed and Mostafa (1989), Ahmed *et al.,*

(1993), EL-Kordy *et al.,* (1995), and Desuky *et al.*, (2005) they found that the IGRs and Nemmazal compounds caused a significant reduction in the level of the total soluble protein of the 4th instar larvae of *S. littoralis.*

Enzyme assay:

Effect on acetyl choline esterase, **alpha and beta esterase activities:**

The esterases constitutes 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 enzymes is including the specific and non-specific esterases. Acetylcholine esterase 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 acetylcholine esterase. The non-specific esterases, the esterases that hydrolyze α- and β- naphthyl acetate (α- and β- esterases), which are considered as aromatic ester hydrolases. The catalysis of hydrolysis reaction by non-specific esterases consider 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* 4th instar larvae 24 and 72 hours following treatment with the calculated LC_{50} of *R. communis* ethanol extract are shown in Table (7). The activity of alpha esterase in treated 4th 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 4th instar larvae for 24h.than their control; this percentage was much lower than the increase of alpha esterase. Similarly, treatment of 4th instar larvae for 72h. caused a marked decrease in alpha esterase activity than that recorded in untreated larvae by 34.2 %.

Table 7: Enzyme activities in 4th instar *S. littoralis* larvae 24 hours following treatment with LC_{50} concentrations of indoxacarb 15% EC.

Biochemical aspects	After 24h.			After 72h.		
	Control	Treated	% Increase or decrease than control	Control	Treated	% Increase or decrease than control
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) (µg oxaloacetate/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 = treated – control ÷ control X 100

According to such influence it could be emphasized that increase of *R. communis* 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* ethanol extract to either 4th instars after

Effect on acid and alkaline phosphatase activities:

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 4th instar larvae following their treatment for 24h. with LC_{50} of *R. communis* ethanol extract making a 12.6 % decrease. Following treatment of 4th 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 4th instar larvae for 24h. by 55.6%, and it decreased in treated 4th instars for 72h. by 79.1%. (Table 7). These results on the contrary of those obtained by Mostafa (1998) showed that the activity of alkaline and acid phosphatase increased in IGR's treated larvae than control ones. El-Guindy *et al.,* (1985) stated that the higher acid phosphatase activity in the laboratory strain of the larvae of *S. littoralis* might explain why that strain was more tolerant than other strains for the tested compounds.

Effect on transaminases activities:

The amino transferases, especially alanine amino transferase (GPT) is one of

24 and 72h., the activity of acetyl choline esterase was reduced by 17.9 and 35.3 % for the respective mentioned treatments, than that of their equivalent control. These results are in disagreement with those obtained by Mostafa (1998) who found that the activity of α- and β-esterases increased in larvae of *Agrotis ipsilon* treated with IGRs than control ones.

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* 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 4th 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 4th 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 disagreement with Rashad *et al.,* (2006) who found increase in ALT and AST enzyme activities in a newly emerged adults of Pink bollworm, *Pectinophora gossypiella* (Saund.) feeding on LC_{50} level of Neemazal solutions for 24 hrs than control ones. 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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