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Toxicological and Biochemical Effects of Jojoba, *Simmondsia chinensis* Extract on Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

This study was conducted to evaluate the toxicity and biological effects of ethanolic leaf extract of *Simmondsia chinensis* on 2nd and 4th instar larvae of cotton leafworm, *Spodoptera littoralis, as* well as the effect on total protein content and the activity some enzymes of *S. littoralis* larvae after 48 and 72 hr of treatment. The obtained results indicated that the extract was more effective on the second instar larvae than the fourth one. The LC_{50} of the jojoba extract on the 2nd instar larvae was less than that on the 4th instar larvae , as well as it was decreased by increasing the period after treatment indicated that the larval mortality was higher on 2nd instars than 4th ones. Larval duration was shortened at all treatments of the 2nd instars compared with control. Pupae mortality was high on the 2nd instar treatments comparing with that of the 4th instars, also, the pupae duration was shortened at all treatments of the 2nd and 4th instars compared with control. The percentages of emerged moths were decreased by increasing the extract concentrations.There were significant differences in the egg laid per female between treatments and control. Total protein after 48 hr. of treating was decreased from 32 mg/ g b wt. in control to 31.8 mg/ g b wt. at treated larvae with – 0.36 % change, while it was 19.5 mg/g b wt. at control compared to 14 mg/ g b wt. after 72 hr. with –28.21 % change. As for the enzymes activity it was observed that nearly all enzyme activities were decreased in comparison with control except α-esterase activity which was decreased to 15.3µg-a-naphthal/min/mg protein after 48 hr. from treatment compared with control 27.7 µg-a-naphthal/min/mg protein and increased to 23.2 µg-a-naphthal/min/mg proteinafter72 hr. from treatment compared with control 19.5 µg-a-naphthal/min/mg protein. Also, AkP activity which increased to 20.5 U x 10^3/mg protein after 48hr. of treatment, compared with control7.9 U x 10^3/mg protein and decreased to 3.7U x 10^3/mg protein compared with control 5.8 U x 10^3/mg protein after72 hr. of treatment. Jojoba is suggested as a safe product with a potential for use as a bioinsecticide in integrated pest management especially in urban localities where use of chemical insecticides are discouraged.

Keywords: Jojoba, toxicity, biochemical, biological control and Lepidoptera

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered as one of the most serious insect pests to different Egyptian crops, where the pest attacks and cause heavy damages to different parts of host plants. Many control methods were carried out to suppress the pest population and to keep under the economic injury level. The recent intensive research is concerned mainly with avoiding the serious problems resulted from using harmful insecticides, that cause harmful residue in the chain of food and population of the surrounding environment, disruption of natural biological balance, destructive of enemies and pest resistance, because of the extracts of various plants possess distinct toxicity(Saleh *et al*., 1986) .Plant extracts show a broad spectrum of activity against a wide variety of pests and so they have been touted as attractive alternatives to synthetic chemical pesticides for pest management because they pose little threat to the environment or to human health. The deleterious effect of plant extracts or pure natural/ synthetic compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction in fecundity and fertility. Plants are well known producers of diverse kind of chemical compounds and many products that are used for defend plant against differentkinds of pests (Isman and Akhtar ,2007). Plant extracts and isolated metabolites have long been a subject of research due to the increased concern for adverse effects of conventional insecticides on human health and environment. The larvicidal activity of the neotropical "matico" *Piper tuberculatum* on the tobacco budworm, *Heliothis virescens* was evaluated by contact bioassays. The secondary compounds were extracted from mature spikes with fruits and seeds of wild plants and in vitro 12-months-old plants of *P. tuberculatum* (Lisset *et al*., 2015).

In Egypt, toxicity of two chemical insecticides (chlorpyrifos, es-fenvalerate); a bioinsecticide (protecto); an IGR (lufenuron) and jojoba oil against 2nd and 4th of *Spodoptera littoralis* and their effect on some biological characters and fecundity were studied on 4th instar larvae(Gaaboub *et al*., 2012) In addition, several laboratory studies were done on jojoba oil dealing with its pesticidal effect on various economic pests such as *Pectinophora gossypiella* , *Bemisia tabaci*, *Empoasca discipiens*, *Agrotis ipsilon*, *Sesamia cretica* , *Ostrinia nubilalis* and *Schistocerca gregaria* (Salem *et al*., 2003; Yacoub 2006 and Halawa *et al*., 2007) who stated that jojoba oil has different effects as toxic, antifeedant, growth and development inhibitors and ovipostion inhibitor. Seeds as well as foliar extracts of several plants have been reported to have toxic and potent growth reducing activity to insects (Rossi *et al*., 2012).

This work was conducted to determine the acute and delayed effects of Jojoba, *Simmondsia chinensis* extract on cotton leafworm, *Spodoptera littoralis*, as well as its effects on some detoxification enzymes, in addition to the effect of the plant extract on biochemical components.

MATERIALS AND METHODS

This experiment was conducted in the Toxicology laboratory of the Pesticides Department, Faculty of Agriculture, Menoufia University to study the toxicological, biological and biochemical effects of the ethanolic leaf extract of jojoba plant, *Simmondsia chinensis* on cotton leafworm, *Spodoptera littoralis.*

Insect culture:

Cotton leafworm, *Spodoptera littoralis* stock culture was obtained from Plant Protection Research Institute in

Dokki, Giza, Egypt which reared on castor bean leaves (El-Defrawi *et al*., 1964). The culture was maintained under laboratory conditions of 26±2 ºC and 55 % R.H.

Preparation of plant extracts:

Jojoba, *Simmondsia chinensis leaves* were left to dry at room temperature (26±2 ºC) for one month. The dried leaves were grounded to fine powder and extracted consecutively in a Soxhlet apparatus using ethanol solvent. Crude extracts were dried and filtered over anhydrous sodium sulfate and were subjected to remove the solvents used in the extraction. All solvents used, were previously redistilled using fraction column distillation. All the crude extracts obtained were kept in the freezer until bioassay.

Toxicity test:

The leaf dipping technique was adopted for toxicity bioassay of *S. chinensis*. Series concentrations at five replicates were prepared of fresh castor bean leaves dipped into these solutions for 20 seconds and air dried at room temperature. Leaves treated with water alone were used as control.

Ten larvae of 2^{nd} and 4^{th} of *S. littoralis* were introduced into glass jars (1L.) and were offered for 24 hr and then replaced by untreated leaves. Castor bean leaves were renewed daily for 72 hr. On the basis of preliminary experiments at least 6 concentrations of *S. chinensis* that caused mortality ranged between 20 to 90 % were used to determine LC_{50} values. Mortality was recorded at 24, 48 and 72 hr and was subjected to Abbott formula (Abbott 1925) for mortality correction. Probit analysis determined to calculate $LC_{50}$and LC_{90}values (Finney 1971).

Biological effects:

Freshly collected castor bean leaves were dipped for 20 seconds in different concentrations of *Simmondsia chinensis* (4, 2, 1, 0.5, 0.25 and 0.125 %), and left to dry. Second and fourth instar larvae for each concentration were introduced into glass jars covered with muslin cloth for 24 hours. Five replicates were tested for each concentration. Control larvae were feed on untreated leaves. Survived larvae were fed on untreated leaves for the rest of the experiment period. The larvae were examined and mortality percentages were recorded daily till pupation. Also, percentages of pupation and adult emergence were recorded. Adult biological data were counted. Also, larval, pupal and adult duration were recorded.

Biochemical effects:

To study the biochemical effects, fifty larvae of *S. littoralis* 2^{nd} and 4^{th} instar larvae were treated with LC_{50} of Jojoba leaves extract using castor bean leaves dipping technique, and replicated five times. After 48 and 72hrs from treatment the larvae were starved for about four hours before homogenization.

The biochemical bioassays has been carried out to determine total protein content, α and β esterase, ASAT (GOT), ALAT (GPT) and AkP activity.

Preparation insects for analysis:

Five larvae were homogenized in 2ml distilled water in a glass tissue grinder under an ice jacket. The homogenate was centrifuged at 8000 rpm for 20min at 4 ºC to remove cellular and mitochondrial debris. The supernatant was collected and stored in a deep freezer until use for determination detoxification enzymes, protein and enzymes metabolism.

Total protein content:

Total proteins were determined by the method of Bradford (1976).

Determination of non-specific esterases activities:

Detoxification enzymes such as alphaesterases (α-esterases) and Beta esterases (β- esterases) was determined according to the method of Van Asperen (1962)using α- and β–naphthyl acetate as substrates, respectively.

Determination of phosphatase enzyme:

Alkaline phosphatase (AkP) was determined according to the method described byPowell and Smith (1954).In this method, the phenol release by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium fnaseaerricyanide, the characteristic brown colour is produced.

Determination of transaminases:

Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were assayed according to the method of Reitman and Frankel (1957).

Data analysis:

The obtained data was statistically analyzed using analysis of variance (ANOVA) one way direction by F test at 5 % probability. The measurements were divided using Duncan's Multiple Range Test, in addition the standard errors of the means (SEM) were also calculated by CoSTAT Version 6.400 Copyright © 1998-2008.

RESULTS

Toxicity of *Simmondsia chinensis leaf* extract on 2^{nd} and 4^{th} instar larvae of cotton leafworm:

Mortality percentages of the 2^{nd} and 4^{th} instar larvae of cotton leafworm, *Spodoptera littoralis* after 24, 48 and 72 hrs of treatment with different concentrations of Jojoba leaf extract, *Simmondsia chinensis* was determined in Table (1). The obtained results show increase of larval mortality by increasing the concentration of jojoba leaf extract as well as by increasing time of observation where mortality percentages were increased at 72 hr more than 48 and 24 hr. It could be observed that the extract was more effective on the second instar larvae than the fourth one. The highest reduction percentages was observed with 4 % concentration on second stage larvae giving 98 % after 72 hr of treatment , while it was 88 on fourth stage larvae.

Table 1. Mortality % of *S. littoralis* larvae after treatment with *S. chinensis leaf* extract

Concentrations %	Mortality %		
	24 hr	48 hr	72 hr
2^{nd} instar larvae			
0.125	20	26	38
0.250	30	38	44
0.500	40	52	58
1.000	48	60	70
2.000	54	80	90
4.000	82	90	98
4^{th} instar larvae			
0.125	14	16	20
0.250	16	22	30
0.500	24	36	46
1.000	36	44	62
2.000	48	56	70
4.000	56	70	88

As for the toxicity of *S. chinensis* leaf extract on *S. littoralis* 2nd and 4th instar larvae after 24 , 48 and 72 hrs from treatment , results in Table (2) indicated that the LC_{50} of the jojoba extract on the 2nd instar larvae was less than that on the 4th instar larvae .

The same trend was observed on LC_{90} values where it decreased by increasing the period after treatment, and it was high for 4th instar larvae comparing with 2nd instar larvae.

Table 2.Toxicity of *S. chinensis* leaves on *S.littoralis*larvae after 24 , 48 and 72 hrs

Periods after treatment	LC_{50} (%)	LC_{90} (%)	Confidence limits		Slope Mean ±SE
			LC_{50}	LC_{90}	
			2nd instar larvae		
24 hr.	1.05	9.473	0.555-2.592	7.105-130.816	1.344±0.165
48 hr.	0.46	4.96	0.328-0.607	3.026-10.865	1.237±0.162
72 hr.	0.29	2.53	0.202-0.383	1.731-4.417	1.363±0.166
			4th instar larvae		
24 hr.	2.63	71.09	1.602-5.910	21.215-810.421	0.895±0.159
48 hr.	1.93	27.33	0.906-2.071	11.106-144.641	0.968±0.154
72 hr.	0.57	6.44	0.412-0.756	3.784-15.163	1.217±0.162

Effect of ethanolic *S. chinensis* leaf extract on some biological aspects of 2nd and 4th instar larvae of *S. littoralis*.

As for the effect of ethanolic *S. chinensis* leaf extract on some biological aspects of 2nd and 4th instar larvae of *S. littoralis* , results in Table (3) indicated that the larval mortality was higher on 2nd instars than 4th ones, where it was 82 % on 2nd instars at 4 % extract while it was only 30 % on 4th instars.

Larval duration was shortened at all treatment of the 2nd instars compared with control(16 days), where it was nearly 12 days with significant differences (LSD 5% = 0.99), on the other side, there were significant differences in larval duration of the 4th instar larvae and control nearly at all tested concentrations except at 0.125 and 0.250 % recording larval duration values 4.80, 4.66, 4.33 and 4 days at concentrations of 0.5, 1, 2 and 4 %, respectively (LSD 5% = 1.1), where it was 5.5 and 5 days at concentrations of 0.125 and 0.250, respectively.

Regarding to pupal mortality as affected by jojoba extract, results in Table (3) indicated that the mortalities was high on pupae of the 2nd instars comparing with that of the 4th instars. The highest mortality percentages of pupae were recorded with 1, 2 % of jojoba extract treated on 2nd instars recording 10 %, while it was ranged between 1-4 % for 4thinstar larvae at all tested concentrations.

It was observed that no mortality was recorded at control and the treatment of 0.125 % on 4th instar larvae.

Table 3. Effect of *S. chinensis* leaf extract on biological aspects of 4th larvae of *S. littoralis*

Concentrations %	Larval mortality%	Larval Duration (days) mean ±SE	Pupal mortality%	Pupal duration (days) mean ±SE	Emerged Moths %	no. of eggs/female mean ±SE (Fecundity)
			2nd instar larvae			
0.125	18	12.5b (± 0.84)	4	7.5 b (± 0.92)	86	500 b (± 41.23)
0.250	30	12.42 b (± 0.72)	6	7.34 b (± 0.88)	64	420 c (± 56.57)
0.500	40	12.31 b (± 0.66)	8	7. 39 b (± 0.86)	52	380 c (± 56.16)
1.000	48	12.27 b (± 0.35)	10	7.37 b (± 0.83)	42	220 d (± 28.22)
2.000	54	12.24 b (± 0.21)	10	7.25 b (± 0.82)	36	170 de (± 30.82)
4.000	82	12.21 b (± 0.11)	3	7.14 b (± 0.81)	15	140 e (± 37.42)
Control	0	16 a (± 0.21)	0	9.8 a (± 0..22)	99.8	2499.1 a (± 319.78)
LSD 5%		0.99		0.84		72.8
			4th instar larvae			
0.125	14	5.5 ab (± 4.5)	0	8.75 b (± 0.58)	86	1000 b (± 42.25)
0.250	16	5 abc (±3)	2	8.22 c (± 0.56)	82	925 bc (± 62.1)
0.500	16	4.8 bc (± 1.5)	4	7.61 d (± 0.47)	80	860 c (± 52.27)
1.000	18	4.66 bc (± 1.34)	3	7.5 d (± 0.45)	79	740 d (± 29.37)
2.000	20	4.33 c (± 3.67)	2	7.3 d (± 0.43)	78	620 e (± 8.17)
4.000	30	4 c (± 2)	1	7.11 d (± 0.42)	69	530 f (± 21.6)
Control	0	6 a (± 1)	0	9.8 a (± 0.88)	100	2254.2 a (± 319.78)
LSD 5%		1.1		0.59		81.7

Means in each column followed by the same letter (s) are not significantly different at 5% level.

As for pupal duration results in Table (3) revealed that it was shortened at all treatments of the 2nd and 4th instars compared with control (9.8 days) where it was nearly7 days with significant differences (LSD 5% =0.84) for 2nd instars and it was nearly 8 days with significant difference for 4th instar between all concentrations and control.

Regarding to the emerged moths, results in Table (3) indicated that the percentages of emerged moths were decreased by increasing the extract concentrations comparing with control which was 100 % for 2nd larvae and 99.8 % for 4th instars. The least percentages of emerged moths were recorded with the treatment of 4 % on 2nd instar larvae recording 15 %, while it was 69 % with the treatment of 4 % on 4th instar larvae.

As for the fecundity of females, results in Table (3) indicated that there were significant differences in the number of eggs per female between control and all other treatments, where it was 2499.1 and 2254.2 eggs for control of the 2nd and 4th instars comparing to 140, 530 eggs for 4% treatments of the 2nd and 4th instars, respectively with significant differences (LSD5%= 72.8 and 81.7, respectively.).

Effect of ethanolic *S. chinensis* leaf extract on total protein content and enzymes activity of 2nd and4th instar larvae of *S. littoralis*:

Total protein content:

Regarding to the effect of leaf extract of *S. chinensis* on total protein of *S. littoralis* larvae after 48and 72 hrs of treatment, results in Tables (4 and 5) indicated that the total protein content was decreased to31.8 mg/ g b. wt with – 0.36%change, while it was 14 mg/ g b.wt with –28.21 %change compared with 19.5 mg/ g b.wt in control, after 72 hrs.

Transaminase enzymes:

As for Aspartate amino transferase (ASAT),activity results in Tables (4 and 5) indicated that the ASAT activity was decreased to 187 (U x 10^3/mg protein) and– 10.95 % change less than control after 48 hrs of treating. On the other side, the enzyme activity was decreased to 143 (U x 10^3/mg protein) and –22.28 % change less than control after 72 hrs.

As for Alanine amino transferase (ALAT) enzyme, results in Tables (4 and 5) indicated that it was after 48hr less than after 72 hr of treatment. The activity of the enzyme was decreased to 20.5 (U x 10^3/mg protein) compared with control and % change was – 25.45 less than control after 48 hr from treatment, while the activity was decreased to 67.5 (U x 10^3/mg protein) compared with 72 (U x 10^3/mg protein) in control with –6.25 % change after 72 hr from treatment.

Non –specific esterase activity:

The data in Tables (4 and 5) showed that the activity of α-esterases was decreased to 15.3 (µg-a-naphthal/min/mg protein) and – 44.77% change less than control after 48 hr from treatment, where the α-esterases activity was increased to +18.97 % more than control after 72 hr from treatment.

Table 4. Effect of the leaf extract of *S. chinensis on* total protein and some enzymes of *S. littoralis* larvae after 48 hrs of treatment

Enzymes	larvae after 48 hrs of treatment			
	Control mean ±SE	Treated mean ±SE	% change	Activity ratio
Total protein (mg/g.b.wt)	32 ±1.5	31.8 ± 1.2	- 0.63	-
ASAT(U x 10^3/mg protein)	210 ±19	187 ± 1	- 10.95	0.89
ALAT(U x 10^3/mg protein)	27.5 ±2.3	20.5± 2.1	- 25.45	0.74
α-esterases (µg-a-naphthal/min/mg protein)	27.7 ±2.5	15.3 ± 1.1	- 44.77	0.55
β-esterases (µg-a-naphthal/min/mg protein)	11.5 ±1.5	8.3 ± 1	- 27.82	0.72
AkP (U x 10^3/mg protein)	7.9 ±0.2	20.5 ± 2.1	+ 159.49	2.59

U= unite of enzyme ability **AkP= Alkaline phosphatase**
ASAT = Aspartate amino transferase **ALAT = Alanine amino transferase**

Table 5. Effect of leaf extract of *S. chinensis* on total protein and important enzymes of *S. littoralis* larvae 72hrs of treatment

Enzymes	larvae after 72 hrs of treatment			
	Control mean ±SE	Treated mean±SE	% change	Activity ratio
Total protein (mg/g.b.wt)	19.5± 1	14± 0.5	- 28.21	-
ASAT(U x 10^3/mg protein)	184± 11	143± 9	- 22.28	0.78
ALAT(U x 10^3/mg protein)	72± 1.2	67.5± 13	- 6.25	0.94
α-esterases (µg-a-naphthal/min/mg protein)	19.5± 1	23.2± 1.1	+ 18.97	1.19
β-esterases (µg-a-naphthal/min/mg protein)	18.4± 1	12.2±1.3	- 33.96	0.66
AkP (U x 10^3/mg protein)	5.8± 0.5	3.7± 10.5	-36.21	0.64

U= unite of enzyme ability **AkP= Alkaline phosphatase**
ASAT = Aspartate amino transferase **ALAT = Alanine amino transferase.**

As for β-esterases, results in Tables (4 and 5) indicated that the β –esterase activity was decreased to 8.5 (µg-a-naphthal/min/mg protein) compared with control and % change – 27.82 % less than control after 48 hr from treatment, while the enzyme activity was decreased to 3.7 (µg-a-naphthal/min/mg protein)and % change was – 36.21%less than control after 72hr from treatment.

Phosphatase enzyme:

The data in Tables (4 and 5) show that alkaline phosphatase (AkP) was increased to + 20.5 (U x 10^3/mg protein) compared with control where the enzyme

activity was 7.9 (U x 10^3/mg protein) and+159.49 % change more than control after 48 hr from treatment, while the enzyme activity was decreased to 3.7 (U x 10^3/mg protein) compared with control and– 36.21 % change less than control after 72 hr of treatment.

Generally, the enzymes activity were decreased after 48 and 72 hrs from treatment of *S. littoralis* 4th instar larvae treatment except AkP enzyme activity was increased after 48 hr from treatment and α esterase activity was decreased after 48 hr and increased after 72 hr from treatment.

DISCUSSION

Gaaboub *et al*. (2012) studied the toxicity of two chemical insecticides (chlorpyrifos, es-fenvalerate); a bioinsecticide (protecto); an IGR (lufenuron) and jojoba oil against 2nd and 4th of instar larvae *Spodoptera littoralis* and their effect on some biological characters and fecundity, and found that Es-fenvalerate proved to be the most effective insecticide against 2nd and 4th instar larvae of *S. littoralis* after 24hrs, followed by chlorpyrifos, lufenuron, jojoba oil and protecto. As for the efficiency of the tested insecticides against the 2nd instar larvae of *S. littoralis*, LC_{50} values were 0.15, 0.17, 0.31, 4.61 ml/L and 16.64 g/L, while the larval durations were 16.9, 14.1,13,7,12,1 and 14,1 days for lufenuron, chlorpyrifos, esfenvalerate, protecto, and jojoba oil, respectively, in addition protecto, and jojoba oil, at lower concentrations showed an increase in pupation percentage, being 85.9% and 81.2%, respectively compared to 99.6% for control Gaaboub *et al*.(2012).

LC_{50} and LC_{30} values of the third instar larvae of the elm leaf beetle *Xanthogaleruca luteola* 48 h post treatment of neem, Achook® containing 0.03% Azadirachtin were 3.3 and 2.25 ppm respectively, Bita *et al*. (2013).. In addition, neem, Achook (0.03%) was decreased the nutritional physiology and gut enzyme activity of the lesser mulberry pyralid , *G. pyloalis*, and the LC_{25}, LC_{50}, and LC_{90} values on 4th instar larvae were estimated as 113.6, 256.84, and 1,210.02 ppm, respectively, Roya and Jalal (2013).

Furthermore, most plant extracts decreased larval weight 8 days after treatment (up to 33% with *C. erythraea*) and caused significant alterations on pupation (ranging from 5% to 85%) and adult emergence (below 2.5% with *R. officinalis*, *C. erythraea* and *A. iva*) Bouayad *et al*.(2013).

The insecticidal activities of emamectin benzoate, abamectin and spinosad were evaluated on the 4thinstar larvae of *Spodoptera littoralis* by leaf dipping technique as well as the biochemical changes in treated insects, which showed immediate effects with 24 hrs-LC_{50} values of 0.17, 0.23 and 38 ppm for the three pesticides, respectively, Megahed *et al*. (2013).

Methylene chloride and hexane extracts of *Azadirachta indica*, *Citrullus colocynthis*, *Ammi majus* and *Mentha microphylla* showed high toxicity on the 4th instar larvae of *Spodoptera littoralis* , Sayed *et al*. (2011). The toxicity of castor bean leaf extract against *Spodoptera frugiperda* larvae was high especially at 10 % w/v and the highest concentration evaluated as10% , Rossi *et al*.(2012). Methanol extracts of *Tagetes patula, Clerodentron phillomedis,* and *Catharanthus roseus* singly and in combination against the dengue vector, *Stegomyia agyptii* were effective, where the median LC showed a significant difference between the combined (2.25 mg/ml/3rd instar) and individual treatment (6.41 mg/ml/3rd instar for *T. patula*, 6.85 mg/ml/3rdinstar for *C. phillomedis* and 6.59 mg/ml/3rdinstar for *C. roseus*) Kokila *et al.* (2016). In addition, *A.indica* (Neem), *C.colocynthis* (Hanzal) and *T. vulgaris* (Zaatar) extracts exhibited insecticidal and antifeedant activities against *S.littoralis* , Hegab and Abdelatty (2013).

The larvae fed on treated leaves immersed in crude extracts of Damsissa, Camphor and Datura leaves for 24 hrs, increased larval mortality and antifeedant index, as well as pupal duration and pupal mortality were increased while pupal average weight and percent adult emergence was decreased, El-kholy *et al*. (2014).

Furthermore, the obtained results had been confirmed with Gaaboub *et al*. (2012) who used plant extracts: *Azadirachta indica*, *Citrullus colocynthis* and *Thymus vulgaris* which resulted (57.33, 50.67 %), (45.33, 66.66%) and (49.33, 57.33 %) mortality of larval and pupal stages of spiny bollworm, respectively, the tested extracts significantly decreased the numbers of laid eggs, as well as treating 2nd instar larvae of *S. littoralis* with botanical extracts inhibited adult emergences. Plant ethanolic extracts caused significant reduction in the number of laid eggs of *S. littoralis* females, due to physiological disturbance in hormonal systems of adults, Hegab and Abdelatty (2013).

Aqueous extract from *Koelreuteria paniculata* seeds had great physiological effects on larval development, fecundity and fertility of velvetbean, *Anticarsia gemmatalis* causing a significant increase in the larval mortality (68.3%), 52.4%reduction in weight of, as well in fertility (48.4%) and fecundity (49%) of adults. The proteinase activity of fecal extracts was significantly higher (56%) in adults fed with 0.15%treated leaves than that of the control Carlos *et al*. (2012).

Lipids and protein of haemolymph of *Plodia interpunctella* larvae treated with sublethal concentrations of two volatile oils and three fixed oils were increased but decreased their carbohydrate contents, Ibrahim *et al*. (2003). Furthermore, Biochemical analysis of the treated larvae, activity level of alanine aminotransferase, alkaline phosphatase, acid phosphatase and α-amylase as enzymatic components and urea and cholesterol as non-enzymatic ones changed significantly in LC_{50} and LC_{30} treatments, in addition Aspartate aminotransferase, lactate dehydrogenase, protein, glycogen, and glucose levels decreased in these treatments as well as the activity level of detoxifying enzymes such as esterase A, esterase B and glutathione S-transferase were significantly affected, Bita *et al*. (2013).

Cotton leafworm larvae fed on treated leaves with neem extract decreased protein, lipid, and glucose but the amount of uric acid was increased compared with the control , Roya and Jalal (2013). Marked

biochemical changes were recognized in treated *Spodoptera littoralis* larvae with Emamectin benzoate, abamectin and spinosad such as reduction of ALP and AchE activities, total protein, total lipids and glucose contents, increased GOT and GPT activities Megahed *et al*. (2013).

Treated larvae of *Plodia interpunctella* with Moroccan plant extracts decreased protein and carbon hydrate larval contents, as well as proteases and α-amylase activities, and the induction of glutathione S-transferase and esterase activities , Bouayad *et al*. (2013). As for α esterase and β esterase enzyme activities Coumarin and Azadirachtin caused significant increase in activity of α esterase of the treated 4thinstar larvae of *S. littoralis*, furthermore β esterase enzyme activity was significantly decreased in treated 4th instar larvae of *S. littoralis* by LC_{50} of Coumarin (2.59 mg /g b. wt.) and (1.88 mg/g b. wt.) of Azadirachtin, Saleh *et al*. (1986), and Gaaboub *et al*.(2012). As for alkaline phosphatase activity it was inhibited at treated 4th instar larvae of *S. littoralis* with LC_{50} of Coumarin or Azadirachtin, furthermore, plant extracts of Coumarin, Neemix, caused significant decrease in activity of GPT of the treated 4th instar larvae of *S. littoralis* Gaaboub *et al*. (2012).

Finally, the insecticidal effect of *A. indica, C. colocynthis* methylene chloride extracts on *S. littoralis* recorded marked decrease in total lipids, protein and glucose contents, also, the activity of ALAT and ASAT was highly affected Sayed *et al*. (2011).

CONCLUSION

The present research concluded that 2nd or 4th instar larvae of cotton leafworm, *S. littoralis* exposed to jojoba extract for 24 hrs were greatly suffered from toxic effects which give good evidence for using jojoba as an element for the integrated management of insects. Synthetic organic compounds and those of plant origin used in insect pest control are known to affect digestive enzymes and biochemical compounds therefore , jojoba extract is suggested as a safe product that may have the potential for use as a bio-insecticide in integrated pest management of urban localities where use of chemical insecticides are discouraged.

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التاثيرات التوكسيكولوجية والبيوكيماوية لمستخلص الجوجوبا على دودة ورق القطن

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أجريت هذة الدراسة لتقدير التاثيرات السامة والبيولوجية للمستخلص الايثانولى لاوراق الجوجوبا على العمر اليرقى الثانى و الرابع لدودة ورق القطن وكذلك التاثيرات على مكونات البروتين الكلى ونشاط بعض الانزيمات بعد 48 ، 72 ساعة من المعاملة. أظهرت النتائج ان مستخلص الجوجوبا كان اكثر تاثيرا على العمر اليرقى الثانى مقارنة بالعمر اليرقى الرابع ، التركيز النصفى المميت لنصف الافراد من مستخص الجوجوبا كان فى العمر اليرقى الثانى اقل من العمر اليرقى الرابع ووجد أنه يتناقص بزيادة الفترة بعد المعاملة. حدث نقص فى فترة العمر اليرقى فى معاملات العمر اليرقى الثانى مقارنة بالكنترول. حدث زيادة فى النسبة المئوية للموت فى العذارى فى معاملات العمر اليرقى الثانى مقارنة بمعاملات العمر اليرقى الرابع، أيضا حدث نقص فى فترة العمر للعذارى فى معاملات العمر اليرقى الثانى والرابع مقارنة بالكنترول. حدث نقص فى النسبة المئوية للفراشات التى ظهرت بعد المعاملة بزيادة تركيز المستخلص. تناقص المحتوى الكلى للبروتين من 32 ملليجرام/جرام من وزن الجسم فى الكنترول الى 31.4 ملليجرام/جرام من وزن الجسم فى اليرقات المعاملة بعد 48 ساعة من المعاملة وكانت النسبة المئوية للتغيير -0.63 ٪ بينما كانت 19.5 ملليجرام/جرام من وزن الجسم فى الكنترول مقارنة ب 14 ملليجرام/جرام من وزن الجسم فى المعاملة بعد 72 ساعة من المعاملة وكانت النسبة المئوية للتغيير -28.21 ٪ . أظهرت النتائج أن معظم نشاط الانزيمات إنخفض بعد المعاملة مقارنة بالكنترول ماعدا نشاط انزيم الالفا استيريز الذى تناقص الى 15.3 ميكروجرام/ا/نافثال/ق/ملليجرام بروتين مقارنة بالكنترول 27.7 ميكروجرام/ ا/نافثال/ق/ملليجرام بروتين بعد 48 ساعة من المعاملة وحدث زيادة فى نشاطه بعد 72 ساعة من المعاملة الى 23.2 ميكروجرام/ ا/نافثال/ق/ملليجرام بروتين مقارنة بالكنترول 19.5 ميكروجرام/ ا/نافثال/ق/ملليجرام بروتين ، وايضا حدث زيادة فى نشاط انزيم الالكالين فوسفاتيز مسجلا 20.5 وحدة * 10^3ملليجرام بروتين مقارنة بالكنترول 7.9 وحدة * 10^3 ملليجرام بروتين بعد 48 ساعة من المعاملة وتناقص الى 3.7 وحدة * 10^3 ملليجرام بروتين مقارنة بالكنترول 5.8 وحدة * 10^3 ملليجرام بروتين بعد 72 ساعة من المعاملة. ويوصى البحث باستخدام مستخلص الجوجوبا كمنتج حيوى آمن فى برامج الادارة المتكاملة للافات.