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Effect of certain plant oils on some biological and biochemical aspects on the cotton leaf worm *Spodoptera littoralis.*

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ABSTRACT

The latent effects of three plant oils; namely thyme , bitter and neem on certain biological and biochemical parameters of the 6th instar larvae of *Spodoptera littoralis* treated as 2nd and 4th instars with the LC_{50} values of these oils. The results showed that the 2nd instars were more susceptible to all the tested oils than the 4th instar larvae. All treatments recorded significantly difference in all durations for both 2nd and 4th instars. Also, highly significant prolongation in pupal duration and the most prolonged oil was occurred by thyme on the 2nd instars, while bitter was more effective on the 4th instar larvae. Longevity of both sexes was reduced specially for male moths. No significant reduction was noticed on female longevity as a result of pretreated 2nd but significant reduction was recorded for pretreated 4th instars especially with thyme. Highly significant reduction in pupal weight produced from the treated 2nd and 4th larval instars with the three products. All oils caused deformations with various degrees for larvae, pupae and adults resulted from the treated 2nd and 4th instar larvae. The highest deformations exist in larval-pupa intermediate by thyme as pretreated 2nd and 4th of the larval instars. Bitter gave the highest pupal deformation as the pretreated 4th instars followed by neem for the 2nd and 4th instars. Generally, all oils exhibited higher effect on the males. Total malformation during all generation recorded its maximum value with neem treatment as 2nd instars then thyme was the most effective in both 4th and 2nd of the larval instars followed by bitter. Highly significant stimulation in chitinase and α- & β -esterases activity was recorded with all treatments and the most effective one caused by thyme followed by bitter. Highly significant inhibition in protease activity and acetylcholine esterase (AChE) was attained by (bitter & thyme) and (neem & thyme) oils, respectively. While high significant stimulation was recorded in protease and non significant stimulation of AChE was noticed by neem and bitter, respectively.

Keywords: *S. littoralis*, plant oils thyme, bitter and neem, chitinase, protease, α - esterase, β-esterase, AchE.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boised) is swarming polyphagous, foliage feeding insect that is distributed throughout the world. The insect is one of the major cotton pests that has at least 7 generations during the cotton season as well as infesting more than 120 other crops and vegetables of economic importance (Khawas and Abd El-Gawad, 2002). In Egypt, insecticidal activity of natural plant extracts may play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms. Few botanicals such as neem oil and some essential oils which characterized by a strong odour and formed by aromatic plants as secondary

metabolites can used as biological control agents (Bakkali *et al*., 2008). Thyme (*Thymus vulgaris* L.) which grows in several regions in the world and is used by the Greeks and Romans in cooking as a source of honey. Lectin was extracted from seeds of *Citrullus colocynthis* and act as α-amylase inhibitors, lectin-like (Ramzi and Sahragard 2013), Botanical pesticides may control the pest population through a different action activity acting primarily as an oral poison, acute toxicity, feeding deterrent, repellent , growth regulator or inhibition of reproduction. The mechanisms of toxicity of essential oils have not been fully identified and display symptoms similar to toxins with a neurotoxic mode of action. They are very effective against more than 200 species of insect-pests some of whom are resistant to chemical pesticides or are otherwise difficult to control.

The aim of the present investigation is to determine insecticidal effects of the three different plant extract oils; thyme, bitter and neem against the 2nd and 4th larval instars of *S. littoralis*. Also, it extended to evaluate the most potent promising plant extracts on some biological and physiological aspects in the tested pest.

MATERIALS AND METHODS

Tested pest

A colony of cotton leaf worm, *S. littoralis*, was maintained in the laboratory for many generations without contamination with insecticides according to Ghoneim, (1985). The tests were carried out on the 2nd and 4th instars larvae.

Plant extracts used:-

The crude oils which used in this study was obtained from Recent Pesticides Company and formulated as 40% EC.

1-Thyme: *Thymus vulgaris* (Labiatae) (Family: Lamiaceae).

2-Bitter: *Citrullus colocynthis* (Handal) (Family: Cucurbitaceae)

3- Neem: *Azadirachta indica* (Family: Meliaceae),

Toxicity tests

Four concentrations of each extract were prepared in water ranged from 0.062% to 0.5 % as required for the bioassay tests against the 2nd and 4th instars of *S. littoralis.* The castor bean leaves were dipped in each concentration of the plant extracts for 20 second and left to dry then five replicates of 10 larvae of each concentration were fed on the treated leaves for 48 hrs. The surviving larvae were transferred to clean cups and supplied daily with untreated leaves until pupation. For control, plant leaves were dipped in fresh water. Mortality was recorded daily after treatment and the LC_{50}s were determined for each tested oil.

Biological experiments:

The effect of LC_{50}s on some biological aspects of the treated instars and its subsequent developmental stages were studied on the 2nd and 4th instars larvae of *S. littoralis* which fed on castor bean leaves treated with LC_{50}'s of thyme, bitter and neem oils for 48 hrs. In control, leaves were treated with distilled water only. Larval and pupal duration, pupal weight, pupation%, adult emergence %, adult longevity, malformation of different stages and sex ratio, were recorded.

Biochemical studies:

All the biochemical analysis was carried out in Physiology Department, Plant Protection Research Institute.

Tissue preparation:

Total body tissue samples were collected from late 6th larval instars treated as 4th instars fed on treated leaves with LC_{50} values of the used compounds. Insect bodies of

treated or untreated were homogenized in distilled water (one gm. insect bodies / 5 ml) using a chilled glass teflon tissue grinder for 3 min. Homogenates were centrifuged at 8000 r.p.m for 15 min at -2^{o}C in a refrigerated centrifuge. The supernatant used stored at -5^{o}C until the use (Max-2 week) for determination of some enzyme activities included chitinase, protease, AchE, α- and β- non specific esterases.

Enzyme activity

Chitinase: Colloidal chitin was determined according to Bade and Stinson (1981).

Protease: was determined according to Tatchell *et al.,* (1972)

Acetylcholine esterase (AchE): was determined according to Simpson *et al*. (1964) using acetylcholine bromide (AchBr) as substrate.

α- and β- non specific esterases were determined according to Van Asperen (1962) method using α- naphthyl acetate or β- naphthyl acetate as substrates, respectively.

Statistical analysis:

Mortality were recorded daily after treatment until the end of experiment and corrected according to Abbott (1925). Also, mortality values were analyzed by probit analysis (LPD line) to obtain LC_{50} and slope for each extract according to a method adopted by Finney (1971). LC_{50} values, were computed and used for calculating the toxicity index (Sun, 1950) which was used for comparing the relative toxicity of insecticides used.

Sun's toxicity index= LC_{50} or LC_{90} of the most toxic compound/ LC_{50} or LC_{90} of the tested compounds x 100.

Relative Potency = LC_{50} of the least toxic compound/ LC_{50} of the tested compounds Activity ratio=enzymatic activity of the tested strain/ enzymatic activity of the control**.**

RESULTS AND DISSCUSSION

1-Toxicity tests:-

Based on the LC_{50} values of the tested products, the present results indicated that all the tested insecticides have larvicidal activities against both 2nd and 4th instar larvae. As shown in Table (1), thyme oil proved to be the most toxic plant extraction to both 2nd and 4th instars larvae; the corresponding LC_{50} values were 0.078 & 0.113%, respectively followed by bitter, where the corresponding LC_{50} values were 0.116 & 0.184%, respectively and of neem these values were 0.153& 0.21%, respectively. By comparison, the other two oils produced showed low activity recording toxicity index values of 67.24&61.41 and 50.98 & 53.81 for bitter and neem oils, respectively as effective as thyme on 2nd and 4th instars. The relative potency level can be used as a convenient method in comparing the degree of toxicity of the different compounds in this study. The potency levels of the tested insecticides are expressed as number of folds, at the required toxicity level, compared with the least efficient toxicant included in the evaluation in the study.

Concerning the relative potency levels based on the LC_{50} values as represented in Table (1), the larvacidal activity values of thyme and bitter oils were 1.96 & 1.86 and 1.32&1.14 times as toxic as larvacidal action of neem oil against the 2nd and 4th instars of *S. littoralis*, respectively.

Table 1: Susceptibility of the 2nd and 4th instars larvae of the cotton leaf worm *S. littoralis* to three plant extract oils.

Treatments	2nd instars				
	LC_{50}%	Slope ±S.E	95%Fiduciual limits lower–upper	Toxicity index	Relative potency levels
Thyme	0.078	1.36 ± 0.32	0.038 - 0.111	100	1.96
Bitter	0.116	1.44±0.28	0.076- 0.155	67.24	1.32
Neem	0.153	0.782±0.29	0.076- 0.33	50.98	1
Treatments	4th instars				
	LC_{50}%	Slope +S.E	95%Fiduciual limits	Toxicity index	Relative potency levels
			lower–upper		
Thyme	0.113	1.055±0.27	0.055- 0.166	100	1.86
Bitter	0.184	1.653±0.31	0.134- 0.245	61.41	1.14
Neem	0.21	0.878±0.27	0.122- 0.387	53.81	1

According to the estimated LC_{50} values, the 2nd instar larvae reflected higher level of susceptibility towards all the tested oil than the 4th instars. These results are in harmony with Shabnum and Wagay (2011) who concluded that three of the essential oils, thyme (*Thymus vulgaris*) were highly toxic to the cutworms *Agrotis ipsilon.* Similary, Abd El-Mageed and Shalaby (2011) reported higher susceptibility of some new insecticides to the 2nd instars than 4th instars of *S. littoralis* and this may be contributed to the tolerance levels which were generally less than those of old ones.

Effect of the LC_{50} values of the tested products on larval duration, pupal duration and adult longevity.

The results of the larval duration starting from initial instars treated up to pupation recorded from the treated 2nd and 4th instar larvae with the LC_{50}'s values of the tested products are presented in (Table 2). The obtained results showed in significant decreased in case of pretreated 2nd instars which ranged 11.0, 10.8 &10.5 days for thyme, bitter and neem, compared with 12.2 days for control. While there were significant prolongation in larval duration for the pretreated 4th instars ranged between 10.48 & 11.58 days as compared with 8.25 days for control.

Table 2: Effect of LC_{50} values of three plant extract oils on larval duration, pupal duration and adult longevity of 2nd and 4th instar larvae of cotton leaf worm *S. littoralis*.

Compounds	Larval duration (mean days±S.E)		Pupal duration (mean days±S.E)		Adult longevity(mean days±S.E) 2nd		4th	
	2nd	4th	2nd	4th	♂	♀	♂	♀
Thyme	11.0± 0.58	10.48± 0.85 [a]	19.93± 0.65 [a]	19.0± 1.68 [a]	4.33± 1.2	3.5± 0.65	4.0± 0.58 [ab]	3.25± 0.75 [b]
Bitter	10.8± 0.85	10.79± 0.65 [a]	19.5±2.1 [a]	21.2± 0.86 [a]	3.67± 0.67	4.2± 0.49	2.8± 0.37[b]	3.25± 0.25 [b]
Neem	10.5± 0.65	11.58± 0.44 [a]	18.6± 1.029 [a]	19.2± 0.05 [a]	3.75± 0.48	4.0± 0.58	2.67± 0.06[b]	3.33± 0.012 [b]
Control	12.2±0.3	8.25± 0.3 [b]	13.57± 0.53 [b]	13.57± 0.53 [b]	4.7± 0.33	5.5± 1.21	4.7± 0.33 [a]	5.5± 1.21 [a]
F value	1.376[ns]	5.2*	27.744***	15.38***	0.32[ns]	1.525[ns]	4.476*	3.921**
L.S.$D_{0.05}$	-	2.1096	2.5754	2.4775			1.4381	1.7321

All the tested oils showed highly significant prolongation in pupal duration in both 2nd and 4th instars. The most prolonged pupae resulted from thyme treatment on the 2nd instars (19.93 days) followed by bitter and then neem 19.5&18.6 days,

respectively. Whereas the 4th instars showed highly significant prolongation reached its maximum by bitter 21.2 days followed by neem and thyme 19.2 &19.0 days, respectively compare with 13.57 days for control. Both males and females moths longevity were reduced with all the tested oils specially the males which were more affected as a results of both pretreated 2nd and 4th instars to record 3.75 & 2.67 days and significant reduction of 3.67 & 2.8 days, by neem and bitter, respectively. While insignificant reduction was recorded on females longevity as a results of pretreated 2nd instars but significant reduction for pretreated 4th instars ranged 3.5 &4.2 and 3.25& 3.33 days, respectively especially with thyme .

All treatments recorded significantly difference in all durations for both 2nd and 4th instars. The decrease in the larval, pupal and adults longevity here may reflect metamorphosis disruption these results are agreed with Hatem *et al.* (2008) they showed significantly prolonged larval and pupation periods with azadirachtin superior than Spinosad. Similarly Riba *et al.,* (2003) azadirachtin (neem oil) has growth regulatory effect causing greatly extended instar lengths, delayed molts, mortality at ecdysis of *N.viridula.*

2-Effect of LC_{50} values of three plant extract oils on pupation, pupal weight, adult emergence and sex ratio.

The data presented in Table (3) showed highly significant reduction in pupal weight resulted from both 2nd and 4th instars treated with LC_{50} values of thyme, bitter and neem oils where ranged between 0.315 & 0.32 and 0.312 & 0.346 gm compared with 0.413 gm of control.

Table 3: Effect of LC_{50} values of three plant extract oils on pupation, pupal weight, adult emergence and sex ratio of 2nd and 4th instar larvae of cotton leaf worm *S. littoralis*.

Compounds	Pupation %		Pupal weight (gm ±S.E)		Adult emergence %		Sex ratio 2nd	Sex ratio 4th
	2nd	4th	2nd	4th	2nd	4th	♂ ♀	♂ ♀
Thyme	36.51	46.77	0.315±0.01 b	0.334±0.01 b	23.81	17.74	1.5 : 1	1.75:1
Bitter	42.19	40	0.32±0.012 b	0.346±0.01 b	20.31	25	1.17: 1	2 : 1
Neem	49.15	45.24	0.315±0.11 b	0.312±0.02 b	25.42	12.29	1.14: 1	1 : 1
Control	92	92.00	0.413±0.01 a	0.413±0.01 a	84	84	0.91: 1	0.91: 1
F value	--	-	33.6629***	20.7946***				
$L.S.D_{0.05}$	----	-	0.02986	0.0343				

The treated 2nd instars with LC_{50} values of the plant oils markedly reduced metamorphosis to pupae to approximately by the third compared with control. Pupation percentage was the lowest 36.51% when the 2nd instars treated with thyme and was 46.77% with the 4th instars respectively, Pupation percentage was slightly higher 40% and 42.19% by bitter against both 2nd and 4th instars and reached to 45.24% and 49.15% for neem in case of treated 2nd and 4th instars, respectively. Table (3)showed that the percentages of adult emergence were reduced to 12.29 and 17.74% for neem and thyme on pretreated 4th instars while bitter having the highest reduction in the adult emergence percentages (20.31%) in case of pretreated 2nd instars compared to 84% for control. Also, all the tested oil generally affected on the sex ratio by producing of males more than females (about 2 folds), the most effective oil was thyme which affected on both pretreated 2nd and 4th instars (1.5:1) and (1.75:1), respectively followed by bitter (2 : 1) on the pretreated 4th instars compare with control (0.91:1). *i. e.* high proportion of males production. This is interesting to decrease in population build up. These results are occurance with these found by Hummelbrunner and Isman (2001) they recorded that thymol prolonged both larval

and pupal periods as well as reduced the pupal weight, and significantly more male adults were produced than females and pupation (35%) of *S. litura* was by thymol.

The failure in adult emergence was observed by Bakr *et al.,* (2012) when tested three newly insecticides against larvae of *S. littoralis,* also, the neem oil reduced the pupation to 52.33% compared with control (69.67%).

3-Effect of LC_{50} values of three plant extract oils on deformations of larvae, pupae and adult.

Results illustrated in Table (4) showed that the tested plant extract oils caused deformations with various degree for larvae, pupae and adults resulted from either 2nd or 4th instar larvae of *S. littoralis* fed on castor oil bean leaves treated with the LC_{50} values of each oil. Deformations and malformations were recorded based on the external morphology.

The highest percentage of deformation exist in larval - pupa intermediate and recorded (28.57 &14.52 %) for thyme in pretreated 2nd and 4th instars, respectively followed by neem in case of 2nd instars treatment (27.12%) and recorded the lowest value in pretreated 4th instars with neem (2.38%) as compared with control which didn't show any larval deformations.

Table 4: Effect of LC_{50} values of three plant extract oils on deformations of larvae, pupae and adult malformations of 2nd and 4th instar larvae of cotton leaf worm *S. littoralis*.

Compounds	Larva- pupa%		m.p &p.ad %		Dead pupae %		Total deformation %		Adult malformation %				Total malformation	
									2nd		4th			
	2nd	4th	2nd	4th	2nd	4th	2nd	4th	♂	♀	♂	♀	2nd	4th
Thyme	28.57	14.52	9.52	20.97	3.17	8.06	41.269	43.55	7.94	7.94	9.68	4.84	57.14	58.06
Bitter	12.5	6.67	7.81	6.67	14.06	8.33	34.37	21.67	9.38	6.25	15	6.67	50	43.34
Neem	27.12	2.38	13.56	14.29	8.47	16.67	49.15	33.34	10.17	6.78	2.38	4.76	66.1	40.48
Control	-	-	2	2	4	4	6	6	-	2	-	2	8	8

The pupal deformations and pupa- adult intermediate manifested in Table (4)showed that thyme gave the highest deformation (20.97%) as pretreated 4th instars followed by neem treatment to the 2nd and 4th instars(13.56 &14.29 %), respectively. Some pupae failed to emerge to moths and dead during pupal stage (16.67%) in pretreated 4th instar with neem followed by bitter (14.06 %) in pretreated 2nd instar compared with control (4%).

The total deformation during larval and pupal stages were recorded in Table (4). Neem was the most effective on by causing highest total deformation (49.15%) in pretreated 4th instars whereas thyme caused highest deformation for both pretreated 2nd and 4th instars (41.27 & 43.55%), respectively.

All tested plant oils caused various degrees of adult malformations when *S. littoralis* treated in the 2nd and 4th instars and the adult males showed higher effect than females. Pretreated 4th instars with bitter recorded the highest malformation (15%) followed by neem (10.17%) on the pretreated 2nd instars while the lowest value of males malformation was by neem on the pretreated 4th instars. The females moth recoded malformation in range of (4.76 & 7.94%) for neem and bitter for 4th and 2nd instars, respectively.

The total malformation during generation recorded its maximum value with neem treatment (66.1%) with 2nd instars then thyme had higher effect in both 4th and 2nd instars (58.06 & 57.17%), while bitter recorded (50 & 43.34%), respectively compared with (8%) for control.

Several abnormality, possibly related to defective molting were observed as affected by plant extract oils. Larvae had problem in discarding the old cuticle or precocious molt lead to production of abnormal chitin deposition. Our results agreed

with (Riba *et al.,* (2003) they recorded that azadirachtin exhibiting classic symptoms of abnormal molts, pupa-adult intermediate and growth inhibition, Increasing doses of azadirachtin in larval stages resulted several abnormalities and deformities on *S. exigua* and could be a result from the following kinds of growth disruption, which some of them involved negatively in vital activities such as feeding, walking or flying (Elumalai *et al.,* 2010; Zarate *et al.,* 2011 and Korrat ,*et al.*, 2012) whose explained that the metamorphosis of the larvae of *S. littoralis* treated with three newly insecticides were failed to pupate, deformed prepupae and pupae can't complete the molting process and died. As well, larvae fed on high dose of two IGRs reduced phosphorous liberation for energy metabolism, decreased rate of metabolism, as well as decreased rate of transport of metabolites so, the dead larvae showed the symptom of improper metamorphosis from one instar to another instar.

Enzyme activity:

1-Chitinase activity:

Table (5) demonstrated the effect of thyme, bitter and neem oils on chitinase and protease activities in total homogenate of 6th instar larvae of *S. littoralis* resulted from the treated when treated as 4th instar for 48 hrs with LC_{50} values of the three tested oils. The tested oils caused highly significant stimulation in chitinase activity and the most effective ones was thyme with remarkably significant stimulation (+ 39.27%) followed by bitter (12.74%) and neem (5.32%) compared with control. Depending on the activity ratios, the tested oils enhanced chitinase by 1.05, 1.39 and 1.13 folds (Table, 5) when treated by thyme, bitter and neem oils, respectively than control. Our results in agreement with these finding by Abd El-Mageed and Shalaby (2011) they found that chitinase activity was increased when *S. littoralis* larvae was treated with different insecticides**.** In addition, the increase in chitinase activity could be attributed to the secondary effect of chitin synthesis inhibitor, or may be a secondary effect for the reduced activity of β-ecdysone metabolizing enzymes, followed by β-ecdysone accumulation which result in hyperchitinase activity. (Yu and Terriere, 1977).

Table 5: Changes in chitinase and protease activities of 6th instar larvae when treated in the 4th instar with LC_{50} values of three plant extract oils.

Treatment	Chitinase *Mean±SE	Change %	Activity ratio	Protease *Mean±SE	Change %	Activity ratio
Thyme	700±5.78^{a}	5.32	1.05	42.93±1.36^{c}	-37.9	0.62
Bitter	925.67±39.4^{b}	39.27	1.39	26.9±1.23^{d}	-61.1	0.39
Neem	749.33±18.8^{b}	12.74	1.13	82.8±2.61^{a}	19.77	1.198
Control	664.67±24.6^{b}		1	69.13±2.02^{b}		1
F value	26.162***			178.533***		
L.S.D$_{0.05}$	82.201			6.1537		

*Mean with the same letter are not significant different.

*Mean: µg NAGA/min/g.b.wt , SE: Stander error

2-Protease activity:

Results represented in Table (5) revealed that highly significant effect of the tested products on the protease activity which fluctuated between increase and decrease as a result of treatment. It was obvious that both treatments with bitter and thyme exhibited remarkable inhibition in protease activity which was significantly high -61.1% in bitter treatment and moderate -37.9% in thyme treatment. While high significant stimulation was recorded 19.77% by neem treatment compare with control.

The activity ratios of protease based on control proved less value by 0.62 and 0.39 time for thyme and bitter, respectively but neem was enhanced by 1.196 times

than control. Our result are agreed with El- Sheikh *et al.,* (2009) and Abdel-Aal and El- Sheikh(2012) who recorded significant increase in protease of *S. littoralis* larvae with treated with spinosad , tebufenozide, Diple 2x and its mixture with two insecticides and change in the activity of chitinase .

3-Non specific esterases(α- esterases and β – esterases)activity:

Data shown in Table (6) illustrated that all treatments caused remarkable stimulation in α- -esterase activity which was significantly high 61.16 and 60.75% in bitter and neem treatment and low 26.86% in thyme treatment compared with control.

The activity ratio, of α- esterase was enhanced by 1.27, 1.61 and 1.61 times as a result of treated by thyme, bitter and neem oils than control, respectively. Similarly data in the same Table (6) revealed high significant stimulation in all treatments in β - esterases activity and the most effective was bitter (120.7%) followed by neem (97.27%), while thyme showed the least stimulation (52.14%). In the same trend the activity ratio, of β - esterase as represented in Table (6), was enhanced by 1.52, 2.21 and 1.97 times as a result of treated by thyme, bitter and neem oils than control, respectively.

Table 6: changes in α- and β- esterase activities of 6th instar larvae when treated in the 4th instar with LC_{50} values of three plant extract oils.

treatment	α- esterase *Mean±SE	Change %	Activity ratio	β- esterase *Mean±SE	Change %	Activity ratio
Thyme	204.67± 3.7[b]	26.86	1.27	166.33+2.85[c]	52.14	1.52
Bitter	260±8.08[a]	61.16	1.61	241.33+4.37[a]	120.7	2.21
Neem	259.33±5.21[a]	60.75	1.61	215.67+4.9[b]	97.27	1.97
Control	161.33±4.49[c]		1	109.33+4.7[d]		1
F value	71.946***			184.708***		
L.S.$D_{0.05}$	18.328			13.974		

*Mean with the same letter are not significant different.

*Mean: Ug α naphthol/min/g.b.wt.

SE: Stander error.

Our results are agreed with El- Sheikh, *et al.,* (2009) who found that the tested compounds caused a disturbance in the activities of enzymes either with increase α- and β- esterase activities or with decrease. The non significant alternation in esterases under this study may be due to the absence of affinity between the chemical structure of plant oils and the tissue esterases but such phenomenon my be able to change with the metabolite of oils.

Acetylcholine esterase (AchE) activity:

The general fluctuation in the activity of the studied enzymes (α- , β-esterases and AchE) in the present work may indicate that general esterases are involved in the detoxification process of the tested oils. Highly significant inhibition in AchE was recorded as a results of treatment with neem and thyme oils by (-56.5 and 34.86%), respectively as represented in Table (7). It was of interest to note that bitter exhibited non significant stimulation of AchE (6.56%) compared with control and significant with neem and thyme.

According to activity ratios, of AchE represented in Table (7), the obtained values -56.5 and -34.86 times less for neem and thyme treated, while +6.54 times more for bitter treatment compared with the control. AchE has a key role in neurotransmitter by hydrolyzing the neurotransmitter achetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides.

Our results are agreed with that recorded by Abd-el-Aziz and El-Gohary(2013) who mentained that all treated insecticides inhibited acetylcholine esterase in the 4th instar larvae of *S. littoralis*. El- Sheikh, *et al.,* (2009) found the change of response to spinosad could be associated with the decrease in AchE activity.

Table 7: Changes in AchE activities of 6th instar larvae when treated in the 4th instar with LC_{50} values of Thyme , Bitter and Neem oils.

Treatment	AchE *Mean±SE	Change %	Activity ratio
Thyme	26.7+1.21[b]	-34.86	0.65
Bitter	43.67+1.26[a]	+6.54	1.065
Neem	17.83+0.83[c]	-56.5	0.43
control	40.99+1.13[a]		1
F value	120.365***		
$L.S.D_{0.05}$	**3.625**		

This activity is apparently strongest during pupation; pupae were very susceptible after larval exposure (SenthilNathan, *et al.,* 2005). The inhibitory effects of the extracted lectin on digestive α-amylase of *E. ceratoniae* larvae. Proteins have been discovered and characterized, including lectins, ribosome - inactivating proteins, and protease and α-amylase inhibitors, which have shown insecticidal effects on different insect pests Ramzi and Sahragard (2013). Elumalai *et al.,* (2010) reported that neem oil showed significant insecticidal activity. It may due to reduction in the total protein content which is a major component for the metamorphosis of the larval instars, Our data support this hypothesis decreased levels of digestive enzyme may be due to the direct effect of botanical oil and enzyme regulation. Neem derivatives may interfere with the production of certain types of proteins.It is evident that exposure to botanical insecticides (Neem oil) in larval diet has significant effects on activities of several enzymes found in the late instar larvae and adult *S. litura*.

In addition, BiF, *et al*., (2008) stated that different commercial neem formulations probably have several modes of action. Primary of which is an interference with the neuroendocrine system in insects which controls the synthesis of ecdysone and juvenile hormone, and others are a more direct role in the inhibition of molting, the antifeedant and growth regulating effects. Other secondary effects was repellency, loss of flying ability and disrupting sexual communication (Jacobson, 1989) The reason behind deformities and malformations may be a result of changes in, chitinase ecdysis is initiated by apolysis, the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysal membrane formation. Molting fluid contains protease and chitinase enzymes that digest the main constituents of the old endocuticle. Thus, the disruption in chitinase enzymes postulated the reason of deformations that agree with Nasr *et al.,* (2010). The results obtained here consider promising for controlling *S. littoralis*. Sublethal effects of plant extracts may be of great importance in regulating population of a target pest by affecting physiological process. 48 hours feeding period was enough to promote morphological abnormalities, feeding suppression, inhibiting molting, preventing the formation of new integument and mortality of this pest. This suggests that these pests would complete fewer generations, multiply less quickly and would remain exposed to natural enemies for a longer period. This would supply natural control. High proportion of male's production interesting to decrease in population build up. These factors will cause less damage and will help increase crop yield. It can be concluded from the present study that larvicidal and growth inhibitory activity by the plant oil compounds could be

beneficial as possible control of the *S. littoralis*. This is true specifically of thymol which has shown potential in both larval and adult.

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ARABIC SUMMARY

تأثير بعض الزيوت النباتية علي بعض النظم البيولوجية والبيوكيميائية فى دودة ورق القطن

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تمت دراسة التأثير المتأخر لثلاث زيوت نباتية تشمل زيت الزعتر والحنظل والنيم على بعض النظم البيولوجية والبيوكيميائية على العمر اليرقى السادس لدودة ورق القطن والتى سبق معاملتها فى العمراليرقى الثانى والرابع بالجرعة النصف مميتة لهذه الزيوت حيث اظهرت النتائج ان العمر اليرقى الثانى كان اكثر حساسية من العمرالرابع لكل الزيوت المستخدمة حيث ان كل المعاملات سجلت فروقا معنوية مختلفة فى كل الفترات العمرية لكل من العمراليرقى الثانى والرابع . كما سجلت ايضا اطالة عالية المعنوية فى فترة التعذير، وقد كان زيت الزعتر الاكثر اطالة فى معاملات العمر الثانى بينما زيت الحنظل كان الاكثر تأثيرا على العمر الرابع .اظهرت النتائج انخفضا فى عمر الفراشات وخاصة الذكور بينما لم يكن هناك انخفاضا معنويا فى فترة عمر الاناث الناتجة من معاملة العمر الثانى ولكن لوحظ انخفاضا معنويا فى الاناث السابق معاملاتها فى العمر الرابع وخاصة زيت الزعتر، كما سجلت كل الزيوت انخفاضا عالى المعنوية فى وزن العذارى فى كل من معاملات العمرالثانى والرابع وقد سببت كل الزيوت تشوهات بدرجات متفاوتة فى كل من اليرقات والعذارى وكذلك الفراشات سواء الناتجة من معاملات العمر اليرقى الثانى اوالرابع حيث وجد ان اعلى نسبة تشوهات وجدت فى الطور الوسطى بين اليرقة والعذراء عند المعاملة بزيت الزعتر فى كل من العمرين الثانى والرابع.اما بالنسبة للتشوهات التى حدثت فى كل الجيل فقد كانت اعلاها فى معاملات العمراليرقى الثانى بزيت النيم ثم الزعتر على العمرالثانى والرابع ثم تبعه الحنظل .عموما وجد ان كل الزيوت كان لها تاثير مثبط خاصة الذكور .كما وجد زيادة عالية المعنوية فى كل من النشاط الانزيمى للكيتين والفا وبيتا استيرازس وقد كان الاكثر تأثيرا مستخلص الزعتر والحنظل على التوالى .ادت المعاملات بزيت الحنظل والزعتر الى تثبيط عالى المعنوية فى نشاط انزيم البروتييز وكذلك اظهر كل من زيت النيم والزعتر تثبيطا فى الاسيتيل كولين استيريز على التوالى. بينما سجل النيم والحنظل نشاطا عالى المعنوية لانزيم البروتييز وغير معنوى للاسيتيل كولين استيريز على التوالى.