TOXIC AND LATENT EFFECT OF THREE ISOLATED BOTANICAL CHEMICALS ON THE EGYPTION COTTON LEAFWORM, SPODOPTERA LITTORALIS (BOISD).NOCTUIDAE :LEPIDOPTERA

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Abstract

The toxic effect of three chemicals ,were isolated from plants , Anaphalis margaritacea , Chenopodium ambrosioides and Ambrosia maritime against the second and fourth instar larvae of the cotton leafworm, Spodoptera littoralis was bioassayed in the laboratory tests. The molecular formula of these chemicals were C15H12O4, $C_{27}H_{30}O_{14}$ and $C_{18}H_{16}O_7$,respectively .The 2^{nd} and 4^{th} instar larvae were left to contact for 24h with the residual film of each chemical at different concentrations. The first chemical ($C_{15}H_{12}O_4$) was the most toxic against the two instar larvae with LC_{50} values of 6 and 28 P.P.M. for both instars , while the third chemical was the least toxic one with LC50 values of 60and 84 P.P.M. for the two instar larvae, respectively. The biological parameters of the treated larval instars were obviously affected specially with the first chemical. Therefore, the larval and pupal duration were prolonged, the pupation and adult emergence percentages were decreased, the pupal weight was reduced. Also, the fecundity and fertility were retard ,and the longevity was shorted ,also the sex ratio of the emerged adults was shifited in respect to control .The Pupal and adult malformation percentages were recorded as a result of the larvae treated of 4^{th} instar with these chemicals at the LC50 values. Most of the tested biological activities of the treated larvae with the three botanical chemicals were more affected with the third chemical (CC18 H16 O7) treatments than the second chemical $(C_{27}H_{30}\ O_{14}\)$ ones.

INTRODUCTION

The cotton leafworm , Spodoptera littoralis (Boisd) is one of the major pests that cause a considerable damage to many of the important vegetables and field crops in Egypt. The massive application of pesticides has created serious problems such as the build - up of pest resistance, the upsetting of natural balance , acute and chronic hazards to man and animals. It is therefore necessary to complement our reliance on synthetic pesticides with less hazardous ,safe ,and biodegradable substitutes. The use of natural products of plant origin is a new trend that preserve the environment from contamination with harmful toxicants. The most familiar one of these chemicals in the

recent years is azadirachtin that isolated from the fruits of *Melia azedarach* and seeds of *Azadirachta indica* (Meliaceae) .Azadirachtin exhibits extremely low acute mammalian toxicity, yet it is very effective as control agent for many insect groups (Sharma *et.al.*,1992). Besides azadirachtin, other plant derived extracts and phytochemicals have been shown to possess insecticidal activity and some morphogenetic effects. The insecticidal activity of several isolated phytochemicals was evaluated against several Lepidoptous insects particularly *S. littoralis* (Simmonds *et al.*, 1990; Amr *et al.*,1995).Also some morphogenetic effects of some plant derived chemicals against several Lepidoptera species were recorded(Urinova *et al.*,1989; Abo- El -Ghar *et al.*,1994)

The present study aimed to evaluate the insecticidal and morphogentic activity of some plant derived chemicals against *S. littoralis* .

MATERIALS AND METHODS

1- Insect rearing

The cotton leaf worm, *S. littoralis* was reared in the laboratory for several generations at room temp. ranged between 25 - 28 C° and 60 -65% R.H. The instar larvae were fed on castor bean leaves, *Ricinus communis* (L.) in a wide glass jars until pupation period and adults emergence. The newly emerged adults were mated inside glass jars supplied with a piece of cotton wetted with 10% sugar solution as feeding source for the emerged moths and branches of Tafla leaf (*Nerium oleander* L.) or castor bean leaves as an oviposition site (El- Defrawi *et al.*,1964). Egg masses were kept in plastic jars until hatching.

2- Material used

Three chemicals were isolated from three plants :The first plant, Anaphalis margaritacea (Asteraceae). It was a stoloniferous plant , the common name is Pearly everlasting, the samples of this plant collected from North American . The second plant, Chenopodium ambrosioides (Chenopodiacea) , and the third plant , Ambrosia maritime (Compositae) , the samples of the latter plants were collected from North Sinai .All samples were collected and separated by Prof. Dr. Ahmed A. Ahmed in Biochemical Division, Faculty of Science ,El- Minia University. The molecular formula for these botanical chemicals were. $C_{15}H_{12}O_4$, $C_{27}H_{30}O_{14}$ and $C_{18}H_{16}O_7$, respectively.

- The chemical structure of the compound as following:
- The first chemical:

Molecular formula: C₁₅H₁₂O₄

Molecular weight: 256 mg

Systematic name: dihydroxy dibenzoic acid (phenolis)

Structure formula:

•The second chemical:

Molecular formula: C27H30O14

Molecular weight:578 mg

Systematic name: chrysophan (glucoside)

Chemical name: K-3,7-dirho- Oside

The third chemical

Molecular formula: C₁₈H₁₆O₇

Systematic name: 5,6 dihydroxy-3,4,7 trimethoxy flavone

Molecular weight: 344mg

Structure formula:

3- Test procedures

A series of different concentrations were prepared on the active ingredient basis (p.p.m) by diluting the formulated chemical in the solvent. About 5-8 concentrations, i.e. 25 -1600 ppm for each chemical of the three tested isolated chemicals were prepared. Thin film technique was used as a method of application ,where the tested concentrations were applied through ethanol to the surface of a 0.9 cm diameter Petri - dish. One ml of each concentration of the tested plant chemicals was spread on the inner surface of the Petri dish, by moving the dish gently in circles. The Petri -dish used as control was treated with 1 ml ethanol only. The ethanol was evaporated under room temperature. Ten larvae of 4th or 2nd instar larvae of cotton leafworm were exposed about 24 hours in each perti-dish , then transferred to clean glass containers and fed on fresh castor leaves until the pupation period according to Ahmed(1985). Five replicates of each concentration were prepared. The larval mortality percentages of both 2nd and 4th instar were recorded and corrected according to the check by using Abbott s' formula. (1925) .The data were then analyzed by using probit analysis (Finney,1971) and LC50 values were estimated for each tested chemical .The biological parameters of treated larval instars were evaluated as larval and pupal period, the pupation and adult emergence percentages, pupal weight. The fecundity, fertility, longevity and sex ratio of the adult stage treated as 4th instar larvae with LC₅₀ values each of the phytochemicals were evaluated . Also, the observed malformations were recorded and photographed.

4- - Separation technique.

The fresh plant(root and flower) was dried with out sunlight, after that was ground to fine powder. The ground material was transferred to a suitable container and soaked in methanol-methylene chloride (MeOH-CH $_2$ Cl $_2$) (1:1) for 24 hrs at room temperature. The solvent (MeOH- CH $_2$ Cl $_2$) was evaporated under reduced pressure to give a green oily residue. This oily material was quantitatively transferred by leading the material dissolved in the minimum amount of CH $_2$ Cl $_2$

On a small quantity of normal phase silica gel, then dried by evaporation the solvent under vacuum to the top of column chromatography, which packed with normal phase Silica gel (60, E- Merck) and eluted with hexane (3 L), followed by a gradient of hexane–CH $_2$ Cl $_2$ up to 100% CH $_2$ Cl $_2$ and CH $_2$ Cl $_2$ –MeOH up to 15% MeOH (2L. of each solvent mixture). These fractions were further re-fractionated by column Sephadex LH-20 with eluted solvent of the following system: n-hexane, methylene chloride, methanol (7:4:0.5 or 7:4.1) and also by using HPLC (High Performance

Liquid Chromatography) with eluted solvent of the following system: water , methanol (45:55 or 40:60) to give pure compounds in the end compounds in the end.

RESULTS AND DISCUSSION

1-Toxic effect

Data presented in Table(1) demonstrated that the three chemicals that isolated from three various plants, *Anaphalis margaritacea L., Chenopodium ambrosioides*, and *Ambrosia maritima* (L.) were effective against 2^{nd} and 4^{th} instar larvae of *S. littoralis*. The first chemical ($C_{15}H_{12}O_4$) was the most toxic against 2^{nd} and 4^{th} instar larvae. The LC_{50} values were 6 and 28 P.P.M., respectively. While the third chemical was the least toxic one as their LC_{50} values were 60 and 84 P.P.M., respectively.

Table 1 . Insecticidal activity of dibenzoic acid ,chrysophan, and trimethoxy flavone against $Spodoptera\ littoralis\ 2^{nd}$ and 4^{th} instar larvae.

Compounds	2 nd instar				4 th instar				
	LC ₅₀	Slope function		nfidence nit	LC _{s0} values ppm	Slope	95%confidence		
	ppm		Upper	Lower			Upper	Lower	
C ₁₅ H ₁₂ O ₄	6	6.1	5.6	6.8	28	5.2	24	32	
C ₂₇ H ₃₀ O ₁₄	56	2.9	48	68	72	2.86	60	88	
C ₁₈ H ₁₆ O ₇	60	5	56	68	84	4.6	76	92	

These results agreement with those obtained by Mahmoud (2002)who demonstrated a toxic activity of four ethanolic extracts of *Cassia fistula , , imbrosia maritima, Tipuana tipu* and *lantana camara* by using a contact method against 4th instar of *A. ipsilon* larvae . Also, Osman (1999) found that 40% of a chloroformic extract of *A .maritima* caused 100% mortality to *A. ipsilon* larvae. after 7-days of the treatment ,using a contact method .Pizzale *et al.*(1997)proved that some isolated plant essential oils belonged to the family Asteraceae had insecticidal activity. These oils were analyzed by capillary gas chromatography- mass spectrometry(GC-MS) using two columns with different polarities(SE 52 and Carbowax).The antioxidant activity of these oils was evaluated with two methods: Crocin and Rancimat tests. Swidan (1994) showed a toxic activity of 25% mortality of *Ambrosia maritima*, against the 4th instar larvae of *S. littoralis*. Also, Abdallah and Kandil (1986) isolated and indicated the toxic activity of eleven fractions obtained from *Chenopodium ambrosioides*, *Barnoof, Conya dioscoridis* and *Ulliq, Convolvulus arvensis*.

2. Latent effect

2.1. Larval and pupal periods

Data in Tables (2 and 3) showed that the second and fourth *Spodoptera littoralis* larvae treated at the LC₅₀ values with the three tested chemicals increased the larval period . The effect was more pronounced with the first chemical($C_{15}H_{12}O_4$), where the larval period highly significant (p<0.01) increased to average 23 and 21 days for the larvae treated as 2^{nd} and 4^{th} instars, respectively, as compared to 14.5 and 11days larval period of the check (of untreated 2^{nd} and 4^{th} instar larvae ,respectively). While the larval treatment of 2^{nd} and 4^{th} instars with the third chemical (C_{15} H_{16} O_7) significanty (p<0.05) increased the larval period to average 20 and 16days for the two instars, respectively. The second chemical ($C_{27}H_{30}O_{14}$) caused none significant increase in the larval period to average19.3 and 15 days for the two instars, respectively .

The larvae treated in the second instar larvae with the three tested chemicals at the LC $_{50}$, highly significant (p<0.01) increased the pupal period of the resulting pupae to average 11.5, 9.4 and 9.5days for the three chemicals ,respectively (Table 2),as compared to 8.1days for control. Whereas, the larvae treated in the fourth instar with the first chemical (C $_{15}H_{12}O_4$) only highly significant (p<0.01)increased the pupal period to average 10.9 days ,as compared to 7.1days of the check.(Table 3). While the larvae treated in the same instar with the second and third chemicals induced none significant increase for the pupal period to average 8 and 9.2days for the two chemicals , respectively ,as compared to 7.1days of the check .

These results are in agreement to those obtained by Mahmoud (2002), who recorded an increase in the larval and pupal period of *A. ipsilon* as a result of the larval treatment of 4th instar with both *C. fistula* and *A. maritima* extracts by a conact method. Also , Osman (1999) found an increase in the larval and pupal period of *A ipsilon* after the larval treatment with a chloroformic extract of *A. maritima* using a conact method . Antonious *et al.*(1992) recorded that the longest pupal period was obtained when the larvae of *S. littoralis* treated with phytochemicals extracted by methanol , benzene or hexane of *Dieffenbachia maculata* and *Adhatada vasica*.

Table 2. Effect of some phytochemicals on the larval and pupal development of $Spodoptera\ littoralis\ treated$ as 2^{nd} instar larvae with LC_{50} values.

Compounds	Larval periods (days) ±	% Pupation ± SD		Pupal period (days)	Pupal weight (gm)	% Moths emergence ± SD	
	SD	Normal	Malfo.	± SD	<u>+</u> S.E	Normal	Malfo.
C ₁₅ H ₁₂ O ₄	23_+3.0**	51.8 <u>+</u> 1.6**	4.2	11.5 <u>+</u> 0.5**	0.216 <u>+</u> 0.06**	30.6 <u>+</u> 1.2**	3.7
C ₂₇ H ₃₀ O ₁₄	19.3 ± 0.2°.s	55.0 <u>+</u> 3.0**	11	9.4 <u>+</u> 0.31**	0.303 <u>+</u> 0.04**	66.6 <u>+</u> 1.3**	3.4
C ₁₈ H ₁₆ O ₇	20.0 ± 1.5*	59.7 <u>+</u> 1.4**	9	9.5 <u>+</u> 0.2**	0.266 <u>+</u> 0.01**	50.5 <u>+</u> 2.0**	3.5
Check	14.5 ± 0.6	100 <u>+</u> 0.0	0	8.1 + 0.4	0.365+ 0.05	97.4 <u>+</u> 1.2**	0.0
F value	8.9	776.1		39.5	47.9	1198.6	
P- value	0.01	0.0003		0.0002	0.0001	0.0002	
L.S.D at 5 %	5.2	2.8		0.77	0.03	2.87	
%	7.9	4.2		1.17	0.05	4.3	

^{** =} Highly Significant (p<0.01) S.D.=Standard deviation

Table 3. Effect of some phytochemicals on the larval and Pupal development of Spodoptera littoralis treated as 4^{th} instar larvae with LC_{50} values.

Compounds	Larval		tion	Pupal period (days)	Pupal weight (gm)	% Moths emergence	
	SD	Normal	Malfo.	± SD	<u>+</u> S.E.	Normal	Malfo.
C ₁₅ H ₁₂ O ₄	21_+4.6**	55.0 <u>+</u> 5.0**	2.1	10.9 <u>+</u> 0.5**	0.244 <u>+</u> 0.05**	55.7 <u>+</u> 0.8**	4.1
C ₂₇ H ₃₀ O ₁₄	15.0 ± 1.0*	58.8 ± 1.04**	7.2	8.0 <u>+</u> 1.8 ^{ns}	0.375 <u>+</u> 0.01*	71.0 <u>+</u> 4.0	2.9
C ₁₈ H ₁₆ O ₇	16 ± 1.0*	65.6 <u>+</u> 2.1**	8	9.2 <u>+</u> 1.1 ^{ns}	0.329 <u>+</u> 0.01**	63.5 <u>+</u> 1.9**	3.6
Check	11.0 ± 2.0	100.0 <u>+</u> 0.0	0	7.1 + 0.8	0.440 <u>+</u> 0.05	98.8 <u>+</u> 1.2	0.0
F value	16.3	267.3		5.8	14.5	313.7	
P- value	0.003	0.00003		0. 03	0.003	0.00005	
L.S.D at 5 %	4.3	4.4		2.4	0.06	3.7	
%	6.1	6.5		3.6	0.1	5.6	

^{** =} Highly Significant (p<0.01)

^{*} Significant (p<0.05)

Malfo.= Malformatiom%

L.S.D.= Least significant difference

S.D.=Standard deviation

^{*} Significant (p<0.05) Malfo.= Malformatiom%

L.S.D.= Least significant difference

2.2. Pupation and Pupal weight

Tables(2and 3) demonstrated that the larvae treated in 2^{nd} and 4^{th} instar with the three tested chemicals at the LC50, highly significantly (p<0.01) reduced the pupation period percent. The first chemical ($C_{15}H_{12}O_4$) had the highest effect on pupation , it averaged 51.8 and 55 % for pupae treated as 2^{nd} and 4^{th} instar larvae , respectively ,as compared with that of the check (100%) .Whereas, the second ($C_{27}H_{30}O_{14}$) and third ($C_{18}H_{16}O_7$) chemicals had the next effect on the pupation, they averaged 55, 59.7 and 58.8, 65.6 % for pupae treated as 2^{nd} and 4^{th} instar larvae , respectively.

Likewise, the larval treatment of 2^{nd} and 4^{th} instar with the three tested chemicals at the LC₅₀, highly significantly (p<0.01) reduced the pupal weight of the resulting pupae (Table2 & 3). The first chemical was the most suppressive one on the pupal weight, it averaged 0.0216 and 0.0244 gm for pupae treated as 2^{nd} and and 4^{th} instar larvae, respectively, as compared to 0.365 and 0.440 gm pupal weight of the check (pupae produced from untreated 2^{nd} and 4^{th} instar larvae, respectively) . Whereas, the third ($C_{18}H_{16}O_7$) and second ($C_{27}H_{30}O_{14}$) chemicals induce the next decrease of the pupal weight, they averaged 0.266, 0.303 and 0.329, 0.375 gm for pupae treated as 2^{nd} and 4^{th} instar larvae, respectively.

These results were similar to those obtained by Mahmoud(2002) who mentioned that the larval treatment of *A.ipsilon* with *C. fistula*, *A. maritima* and *T. tipu* extracts highly significantly (p<0.01) reduced the pupation percent to about 50% of the control, and also decreased the weight of the resulting pupae. Also, Osman (1999) demonstrated a decrease in the pupal weight of *A. ipsilon* following treatment of larvae with a chloformic extract of *A. maritima* using a contact method Amr *et al.* (1995) indicated a reduced pupal weight of *S. littoralis* treated as larvae with a chloroformic and ethanolic extacts of *Nerium oleander* leaves. There are similar results were obtained by Abo-El-Ghar *et al.*(1994) who showed a significant reduction in the pupal weight of *A. ipsilon* treated as larvae with acetone and ethanolic extracts of *Vinca rosea* and *Melia azedarach* and also they added that the larval feeding on the ethanolic extracts at concentrations of 0.02 and 0.01% completely failed to pupate. Also, Antonious (1992) recorded that the castor oil leaves treated with *Dieffenbachia maculate* extracts proved to be more effective than those treated with *Adhatoda vasica* extracts in reducing the pupation of *S. littoralis*.

2.3. Moths emergence

Data in Tables (2 &3)showed that second and fourth instar larvae of *S. littoralis* treated with the three tested chemicals at the LC_{50} values induce highly significant (p<0.01) reduction in the moths emergence. The first chemical ($C_{15}H_{12}O_4$) had the highest effect on the moths emergence, where it averaged 30.6 and 55.7 % for adults treated as 2^{nd} and 4^{th} instar larvae respectively ,as compared 97.4 and 98.9 % adult

emergence of the check (adults produced from untreated 2^{nd} and 4^{th} instar larvae, respectively) . While the larval treatment of the two instars with the third chemical ($C_{18}H_{16}O_{7}$) had the next effect on the adult emergence to average 51 and 64 % for

adults treated as 2^{nd} and 4^{th} instar larvae, respectively. Whereas, the second $(C_{27}H_{30}O_{14})$ chemical had the least effect on the adults emergence to average 67 and 71% for adults treated as 2^{nd} and 4^{th} instar larvae, respectively.

These results are agreement with those obtained by Mahmoud (2002) who demonstrated that the larval treatment of 4th instar of *A. ipsilon* with *A. maritima* extract induced the highest reduction in the adult emergence by a contact method . Also, Abo -El-Ghar *et al.*(1994) recorded a decrease in the adult emergence of *A. ipsilon* treated as larvae instar with petroleum ether extacts of *L.cylindrica*, *A. majus*, *C. elegans* and *V.rosea*, as compared to control. Antonious *et al.*(1992) found that a decrease in the adult emergence of *S. littoralis* after the larval treatment with ethanolic or methanolic extracts of *D. maculata* and *A. vasica* plants.

Table 4. Effect of some phytochemicals on the adults of *Spodoptera littoralis* treated as 4^{th} instar larvae with LC₅₀ values.

Compounds	Fecundity eggs/ f	Fertility eggs/ f	Longevity (days)	Adult sex ratio(%)	
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Male	Female
C ₁₅ H ₁₂ O ₄	93.3 <u>+</u> 1.5**	89.2 <u>+</u> 1.3**	8.0 ± 1.3**	44.4	55.6
C ₂₇ H ₃₀ O ₁₄	110.8 ± 5.0**	104.6 ± 1.95**	12.6 ± 1.5*	40.0	60.0
C ₁₈ H ₁₆ O ₇	115.8 <u>+</u> 0.8**	109.0 ± 2.7**	8.5 ± 1.0 **	43.3	56.7
Check F value P- value L.S.D at 5 %	558.7 ± 1.65 43441.6 0.000004 3.7 5.7	509.6 ± 8.6 4462.6 0.00001 10.5 16.3	15.2 ± 0.7 21.7 0.001 2.6 3.9	39.5	60.5

^{** =} Highly Significant (p<0.01)

^{*} Significant (p<0.05)

2.4. Adult fecundity and fertility

Data presented in Table(4) showed that the treatment of 4^{th} instar larvae of S. *littoralis* with the three tested chemicals at the LC₅₀ values highly significant (p<0.01) retard the fecundity of the emerged adults .The first chemical (C₁₅H₁₂O₄) had the strongest effect on the fecundity , where it reduced the fecundity to average 93.3 eggs/f, as compared to 558.7eggs/f of the check. While the third (C₁₈H₁₆O₇) and second(C₂₇ H₃₀ O₁₄) chemicals had the next effect on the adult fecundity ,where they reduced the adult fecundity to average 115.8 and 110.8 eggs /f for adults treated as 4^{th} instar with the two chemicals ,respectively.

Likewise, the larval treatment of 4^{th} instar of S. littoralis with the three tested chemicals at the LC_{5o} values highly significant (p<0.01) inhibited the eggs fertility (Table,4). The first chemical($C_{15}H_{12}O_4$) had the highest effect on eggs fertility ,where it reduced the eggs fertility to 89.2 eggs/ f. ,as compared to 509.6 eggs /f of the check. Whereas, the third ($C_{18}H_{16}O_7$) and second (C_{27} H_{30} O_{14}) chemicals had the following effect on the eggs fertility, it averaged 109 and 104.6 eggs/f for adults treated as 4^{th} instar larvae with the two compounds, respectively.

These results were similar to those obtained by Mahmoud (2002) who recorded that a reduction in the adult fecundity and fertility of *A. ipsilon* treated as 4th instar larvae with both *A. maritima* and *C. fistula* extracts by a contact method. Also, Osman (1999) cleared that a decrease in the percent of eggs hatching of *A. ipsilon* treated with chloroform extracts of *A. maritima*, via oral administration. Whereas, Abo-El – Ghar *et al.* (1994) showed fecundity deterrent effects of plant extracts against *A. ipsilon* were found in the polar (acetone ,ethanol and water) extracts of *M. azedarach, V. rosea ,L.cylindrica, P.vera, C.elegans ,A.majus and A.graveolens* rather than non_polar (petroleum ether).

2.5. Adult longevity

Table(4) indicated that the 4th instar larvae of *S. littoralis* treated with the three tested chemicals at the LC₅₀ values caused a highly significant (p<0.01) decrease in the longevity of the emerged adults, as compared to control. The first chemical ($C_{15}H_{12}O_4$) had the highest effect in reducing of adult longevity to average 8 days ,as compared to 15days of control. Whereas the third chemical had the next effect on the adult longevity ,where it averaged 8.5days .While the least shorten of the longevity was obtained by the larval treatment of 4th instar larvae with the second chemical (C_{27} $H_{30}O_{14}$), it averaged 12.6days.

These results agreement with that obtained by Mahmoud (2002) who recorded a significant decrease in the adult longevity of *A. ipsilon* by the larval treatment of 4th instar with *A. maritime* and *T. tipu* extracts by a contact method .Osman(1999) mentioned that chloroform extract of *A. maritima* decreased the adult longevity of *A. ipsilon* treated as larvae with LC₅₀ values , using a contact method.

2.6. Adult sex ratio

Data obtained in Table (4) showed that the larval treatment of 4^{th} instar of S. *littoralis* with the three tested chemicals at the LC_{50} values shifted the sex ratio ,it increased the males and decreased the females than that of the check .The effect was more pronounced with the first and third chemicals ,where it reached 44.4,43.3 and 55.6, 56.7% for both adult males and females respectively, as compared to 39.5 and 60.5 % of adult males and females respectively, of the check. While the sex ratio of both males and females did 'nt differ than that of the check at the larval treatment of 4^{th} instar with the second compound (C_{27} H_{30} O_{14}), it reached to 40 and 60% for both adult males and females respectively, treated as 4^{th} instar .

2.7. Morphogenetic effects

Data presented in Tables(2&3)showed that the larval treatment of 2nd and 4th

instars of *S. littoralis* with the three tested chemicals at the LC₅₀ values induced a noticeable increase in the pupal malformations , as compared to the check The second ($C_{27}H_{30}O_{14}$) and third ($C_{18}H_{16}O_7$)chemicals induce the highest percent ,it reached 11, 9 and 7.2 ,8 % for pupae treated as 2^{nd} and 4^{th} instar larvae with the two chemicals , respectively ,as compared to 0% pupae malformations of the check. Whereas, the first chemical ($C_{15}H_{12}O_4$) recorded the lowest percent ,it reached 4.2 and 2.1% for pupae treated as 2^{nd} and 4^{th} instar larvae ,respectively.

With regarded to the adult malformations(Tables 2 & 3) ,it was found that the larval treatment of 2^{nd} and 4^{th} instar of S .//ittoralis with the first $(C_{15}H_{12}O_4)$ and third($C_{18}H_{16}O_7$) chemicals at the LC₅₀ values caused the highest percent of adult malformations, it reached 3.7, 3.5 and 4.1,3.6% for adults treated as 2^{nd} and 4^{th} instar larvae with the two chemicals, respectively, as compared to 0 % of the check. Whereas, the second chemical $(C_{22}H_{30}O_{14})$ induce the next percent of adult malformations, it reached 3.4 and 2.9 % for adults treated as 2^{nd} and 4^{th} instars ,respectively, as compared to control.(0%) .

These results are similar to that obtained by Mahmoud(2002) ,who demonstrated that the pupal and adult malformation percent as result of the larval treatment of 4^{th} instar larvae of A. ipsilon with the A. maritima ,T.tipu, L. camara

and *C.fistula* extracts ,the effect was more pronounced with both *A. maritima* and *T. tipu* extracts ,where the highest pupal and adult malformations were recorded by the larval treatment with the two extracts, while the other *C.fistula* and *L. camara*. extracts had the least effect one. Also, the same results obtained by Abo- El_Ghar *et.al.*(1994) on *A. ipsilon* and Antionious *et al.*(1992) on *S. littoralis* .

Malformations of *S. littoralis* pupae resulting from the larval treatment of 2nd and 4th instars in the present work mostly appeared as malformed prepupa failed to cast the old cuticle with complete blackening of the body leading to death (Fig.1),or abnormal pupae showing body shrinkage or undersized pupae (Fig..2) or malformed pupa failed to cast the larval skin (Fig.3). Adult malformations often appeared as poorly developed moths failed to emerge from the pupal cast (Fig.4) or a moth showing poorly developed and deformed twisted wings(Fig5 and 6), as compared to the pupa and adult of the control (Fig.7and 8).





Fig 1. malformation prepupa failed to cast the old cuticle with complete blackening of the body leading to death



Fig 2 . abnormal pupa showing body shrinkage or undersized pupa



Fig 3 . malformation pupa failed to cast the larval skin $\,$



Fig 4 .Adult malformations often appeared as poorly moth failed to emerge from the pupal cast





Fig 5. and 6. Moth showing poorly developed and deformed twisted wings





Fig 7. and 8. normal pupa and adults

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التأثير السمى والمتأخر لثلاث مركبات كميائية نباتية معزولة على يرقات دودة ورق القطن الكبرى

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اجريت هذه الدراسة بغرض نقيم التاثير السمى لثلاث مركبات معزولة من نباتات الانفليس والزربيح والديمسيسة اختبرت معمليا ضد برقات العمر الثاني والرابع لدودة ورق القطين الكبيري. والزربيح والديمسيسة اختبرت معمليا ضد برقات العمر الثاني والرابع في دريق القطين الكبيري المركب المركب المركب المركب المركب المائي والرابع في تلامس لمدة ٢٤ ساعة مع فيلم المتبقيات الخاصة بكل مركب كميائي عنيد التركيزات مجال الدراسة. وقد بينت الدراسة ان المركب الاول كان له التأثير الأقوى والغالب حييث بغت قيمة التركيز النصفي له (٢٨ ، ٢ ، ٢٨) لكلاً العمرين الثاني والرابع على النتائي ، بينما لعمرين على الناثير الأقل حيث بلغت قيمة التركيز النصفي له (60,84 ppm) على كلا العمرين على الترتيب . تأثرت المعايير البيولوجية لليرقات المعاملة . وقد كان التأثير كان اكثر وضوحا مع معاملة يرقات العمر الثاني والرابع بالمركب الأول حيث أدى ذلك الى استطالة في ودرجة الخصوبة مما أدى الى قصر في عمر الفراش المخترق وتغيير في النسبة الجنسية ودرجة الخصوبة مما أدى الى قصر في عمر الفراش المخترق وتغيير في النسبة الجنسية بالمركبات الثلاثة نسب من التشوهات في طور العذراء والحشرة الكاملة. ولقد لموحظ في اغلب بالمركبات الثلاث أن المعاملة بالمركب الثالث أحدثت المعاملة بالمركبات الثلاث المعاملة بالمركب الثالث أحدثت تأثيرا لافتا النظر تالي للمركب الأول مقارنة بمعاملات المركب الثاني.