Toxicity and Antifeedant Effects of Apricot Kernel Extract and Its Main Components against Cotton Leaf Worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) Larvae With Reference To Some Physiological Effects

Wael M. Khamis[1]; Sahar E. El-Desouky[1] and Abir A. Gad[2]

ABSTRACT

Toxicity and antifeedant effect of hexane apricot kernel extract (HEAK) and its gas chromatography-mass spectroscopy (GC-MS) identified fatty acid (FA) components palmitic acid (PA), Oleic acid (OL) and FA derivatives (Linoleic acid methyl ester (LAME)) were assessed against 2nd and 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. Methoxyfenozide (Runner 24% SC®) was used as reference insecticide to (HEAK) and its components. The results were statistically analyzed by probit analysis to calculate (LC_{50}) of mortality and the concentrations caused 50% of antifeedant index (AI_{50}) after 24, 48 and 72 hrs of exposure. The results of toxic and antifeedant effects showed the definite prevalence of HEAK versus its FA components against the two instar larvae of *S. littoralis* throughout 72 hrs of exposure. However the toxic initiation of the extract and its components paced methoxyfenozide by the first 24 hrs of exposure, methoxyfenozide still owned a superior toxicity and antifeedant response on the treated larvae. This work has been associated with some physiological tests including haemocytes count and corpora allata (CA) activity.

Key words: apricot kernel extract, *Spodoptera littoralis*, methoxyfenozide, haemocytes, corpora allata, fatty acids.

INTRODUCTION

Larvae of cotton leaf worm (CLW), *Spodoptera littoralis* (Lepidoptera: Noctuidae) is so far one of the major destructive agricultural lepidopterous pests within subtropical and tropical zones. The pest causes a variety of damage as a leaf feeder and sometimes as a cutworm on seedlings. It can attack various economically important crops throughout the year (EPPO, 1997).

Even thought, conventional insecticides have become an indispensable tool in controlling some pest economically, rapidly and effectively, the extensive use of insecticides can have adverse impacts on non-target organisms and general environmental contamination (Georgiou, 1987 and Ditrich, 1962). According to Nas (2004) recent orientations towards botanical pesticides application in crop protection programs is on the rise. Many researchers have been recourse to develop an alternative plant extracts as pesticides to be used against insect pests.

Natural plant products are comparatively less toxic, easily biodegradable and have made them to be the best alternate to the synthetic pesticides. Effective insecticidal properties were investigated in several plant species of various families (Koshiya and Ghelani, 1990). Apricot kernels, depending on the variety, contain the toxic cyanogenic glycoside amygdalin (Gomez *et al*., 1998). Amygdalin can be hydrolyzed to form glucose, benzaldehyde and hydrocyanic acid. Enzymatic release of cyanide occurs in the presence of β-glucoronidase, an enzyme found in the human intestine (Shragg *et al*., 1982). Previous studies had been carried out on the extraction methods and chemical profiles of apricot kernel. A typical extracted oil fraction makes out around 45 % of the weight of the seed. The triglyceride fatty acids (FAs) are mostly Oleic (OL), linoleic (LA) and Palmitic acid (PA). (Gupta *et al.,* 2012)

Recent experimental attempt had been achieved on the insecticidal activity of some FAs like those originated in hexane extract of apricot kernel (HEAK) and many other plant extracts. Insectistatic and insecticidal activities of LA were performed against *Spodoptera littoralis* larvae (Youssef *et al*., 2013) and *Spodoptera frugiperda* larvae (Ramos-López *et al*., 2012). However, there was rarity of publications on the toxicity and antifeedant responds of OL and PA on insects, some recent studies have been revealed on the insecticidal activity of OL against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae) (Rahuman *et al*., 2008; Kannathansan *et al*., 2008).

Methoxyfenozide have been chosen as a reference insecticide in this study as it is considered as the newest and most potent synthetic ecdysone agonist and it binds to ecdysteroid receptors, inducing a premature and lethal molt in many insects, primarily lepidopteron pests (Smagghe *et al*. 2003) also its sub lethal effects include delayed developmental rates (Adel and Sehnal, 2000, Biddinger *et al*. 2006). Therefore, suggesting of apricot

[1] Dept. of Cotton Pesticides Bioassay Research, Plant Protection Research Institute, Agriculture Research Center, Al Sabhia, Alexandria, Egypt.
[2]Dept. of Applied Entomology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt.
Received October 11, 2016, Accepted December 4, 2016

kernel extract in this study was attributed to its main constituents that possess toxic effect.

The aim of this study is to determine the lethal effect and antifeedant activity of methoxyfenozide on larvae of *S. littoralis* as a reference to HEAK. This work has been associated with some physiological tests that might elucidate approaches of insecticidal activity of HEAK's main FA constituents.

MATERIALS AND METHODS

Plant material and extraction:

Samples were collected from mature apricot fruits (*Prunus armeniaca*). After the shell removal, the kernels were left for drying at room temperature for about 10 days. The dried kernels were grinded in blender. Kernel oil was extracted with n-hexane (under heat temperature 45 ± 5 ºC, 12 hr.) in Soxhelt extractor. Hexane solvent was removed under reduced pressure by rotary evaporator. The crude extract was stored in a sealed glass bottle at 0 ºC.

Gas chromatography–mass spectroscopy (GC-MS) analysis

The chemical composition of HEAK was performed in Nuclear Research Center (NRC), Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt by using Trace_ GC Ultra-ISQ mass spectrometer (Thermo-Scientific, Waltham, MA) with a direct capillary column TG–5MS (30 m_0.25 mm_0.25 mm film thickness). The column oven temperature was initially held at 60 ºC hold 3 to 150 with 10 ºC min^{-1} ramp and then increased to 260 ºC withhold time 5min with 5 ºC min^{-1} ramp and split mode. The injector and detector (MS transfer line) temperatures were kept at 250 ºC. Helium was used as a carrier gas at a constant flow rate of 1 ml min^{-1}. Extract derivatization was done using BSTFA/TMCS (80:20, v: v) for 1 h at 70 ºC, after evaporation to dryness of dichloromethane/methanol mixture. The resulting solution was dried and then dissolved in hexane. The solvent delay was 2 min and diluted samples of 1 ml were injected automatically using Auto sampler AS3000 (Thermo-Scientific, Waltham, MA) coupled with GC in the splitless mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and quadruple temperatures were set at 200 ºC and 150 ºC, respectively. The components were identified by comparison of their retention times (Rt) and mass spectra with those of WILEY 09 (Flavor & Fragrance Natural & Synthetic Compounds) and NIST 11 (National Institute of Standards and Technology, Gaithersburg, MD) Mass Spectral databases.

Tested synthetic compounds:

The following synthetic compounds represent the main FA components found in HEAK previously identified by GC-MS analysis.

1. Oleic acid (OL) 98% and Palmitic acid (PA) were purchased from (Lobachemie Co.).
2. Linoleic acid methyl ester 99% (LAME) was purchased from (Sigma Aldrich).
3. Methoxyfenozide (Runner 24% SC® from Dow Agro Science co.) was used as a reference insecticide.

Strain of cotton leaf worm *S. littoralis*

The *S. littoralis* strain was reared in Integrated Protection Laboratory; Plant Protection Research Institute; Agriculture Research Center according to (El-Defrawi *et al*., 1964).

Toxicity tests:

Leaf of *Ricinus communis* were dipped for twenty seconds in sub lethal concentrations for HEAK and the tested synthetic fatty acid compounds with the concentrations range of (100-10000 mg L^{-1}) and (0.01-5 mg L^{-1}) for methoxyfenozide (Runner 24% SC®). The treated leaves were left to dry at room temperature. One or two treated leaves were placed in each cup (7cm dia x 9cm L) for starved (24 hr.) 2nd and 4th instar larvae of *S. littoralis*, respectively. The larvae were allowed to feed on the treated leaves for 48 hr. then the leaves were replaced by new untreated one at 72 hr. For each concentration, 4 replicates were maintained. Each replicate has five larvae. Larval mortality was recorded after 24, 48 and 72 hr. of exposure periods. Mortality percent was calculated using the Abbott formula (Abbott, 1925) and subjected to probit analysis according to (Finney, 1971) by LDP line.

Antifeedant effect:

Leaf discs (3cm dia.=706.81mm^2) were dipped for twenty seconds in sub lethal concentrations, not exceed the upper limit of LC_{50} values of the same previous tested treatments against 2nd and 4th instar larvae of *S. littoralis*. Likewise, the experiment was design and allowed to run as well as in toxicity assay. The uneaten area of each leaf disc was weighted. The percent antifeedant activity was calculated based on the formula of (Saleh *et al.,* 1986). Then all the data undergo concentration-responds probit analysis by LDP line to calculate the concentration that cause 50% of antifeedant index (AI_{50}).

$$\text{Antifeedant activity \%} = \left(1 - \frac{\text{\% of treated leaf eaten}}{\text{\%of untreated leaf eaten}}\right) X100$$

Haemolymph preparation:

The haemolymph samples from 4th instars larvae were taken after 72 hrs of treatment with apricot kernel extract and its main components. To calculate the differential haemocytes count (DHC), 100 cells were identified to their typical haemocytes type after staining a smear of haemolymph with Wright's stain (Essawy, 1990).

Corpora allata activity:

Corpora allata (CA) volume was used as an indicator of the juvenile hormone (JH) level (Pflugefelder, 1948). Larvae were dissected after 24, 48 and 72 hrs of treatment. The method of Armstrong and Carr (1964) was used to calculate the CA surface area.

RESULTS AND DISCUSSION

Fatty acids and other components in Hexane extract of apricot kernel analysis by (GC-MS):

GC-MS analysis of HEAK identified mainly 3 FAs (Table 1). The total FAs were approximately 77.90% of the crude extract. The FA components were named, using official libraries, by PA (7.68%), OL (63.78%) and LAME (6.44%). Occurrence of other unidentified compounds in tested extract may be associated with chemical processes during heating of extraction system under condition of n-hexane used.

The obtained Gas Liquid Chromatography (GLC) analysis data of HEAK's FAs composition came nearly equivalent to other previous analyses that possessed OL (74.59%), LA (19.57%), Stearic acid (0.96%), PA (4.11%), Palmitoleic acid (0.59%) and Arachidonic acid (0.18%). (Abdel Rahman, 2011)

In this respect, HEAK and some of its main fatty acid components were studied for its toxicity and antifeedant effect against CLW larvae in corresponded to methoxyfenozide (Runner 24% SC®).

A. Toxicity tests:

Toxic effect studies were conducted on HEAK and its main identified FA components with the concentrations range of (100 - 10000 mg L^{-1}) and (1250 - 10000 mg L^{-1}) against 2nd and 4th instar larvae of *S. littoralis* respectively during 72 hours of exposure. Meanwhile, the concentrations range of methoxyfenozide was (0.01-5 mg L^{-1}). The toxic effect of the tested compounds 2nd instar larvae of CLW were demonstrated by LC_{50} values calculated from probit analysis (Table 2).

The recorded data of LC_{50} values after 24 hours of exposure against 2nd instar larvae of CLW showed the highest toxic compound was OL with LC_{50} value (3799.71 mg L^{-1}) followed by HEAK (LC_{50} value 3995.77 mg L^{-1}) then LAME (LC_{50} value 5406.96 mg L^{-1}). In the mean mean time no apparent toxic effect was observed for methoxyfenozide after 24 hours of exposure. On contrary, after 48 and 72 hours of exposure the data of LC_{50} values show a broad shift in toxic effect rises of HEAK (3035.615 and 2034.14 mg L^{-1}, respectively) followed by LAME (3260.12 and 2105.734 mg L^{-1}, respectively) versus to a clear drop in OL toxicity (3447.39 and 2157.43 mg L^{-1}, respectively). The initiation of methoxyfenozide toxic effect began after 48 hours at LC_{50} value 8.77 mg L^{-1} and then increased after 72 hours at LC_{50} value 0.152 mg L^{-1} to exceed the toxic effects of the crude extract and its tested components.

Likewise, the data illustrated in (Table 3) of LC_{50} values of the tested treatments against 4th instar larvae of *S. littoralis* showed that HEAK had the highest toxic effects and descending followed by LAME and then lasted by OL all over the indicated interval hours of exposure. The final LC_{50} values after 72 hours were 3358.65 4220.92 6028.77 mg L^{-1} for HEAK, LAME and OL, respectively. While the toxic effect of methoxyfenozide (LC_{50} value 7.145 mg L^{-1}) delayed and not appeared before 72 hours of exposure. On the other hand, there was no evidence for lethal effect for palmitic acid within the concentrations range of (100-10000 mg L^{-1}) against the treated instar larvae of *S. littoralis.*

Finally, it was conclusively recognized the prevail of HEAK toxicity comparing to its main fatty acid components against the two instar larvae of *S. littoralis* during 72 hours of exposure. The toxic action in the treated larvae was significantly occurred in 2nd more than 4th instar larvae.

Table 1. Identified fatty acids profile of hexane extract of apricot kernel *Prunus armeniaca*

No	Identified compounds	Unsaturated bonds	Retention times	V/W	Area%	Molecular weight
1	Palmitic acid	C16:0	31.56	0.20	7.68	C16H32O2
2	Oleic acid	C18:1	35.46	1.66	63.78	C18H34O2
3	Linoleic acid methyl ester	C18:2	35.82	0.17	6.44	C19H34O2

Table 2. Toxicity of apricot kernels extract, its main fatty acids components and methoxyfenozide against 2nd instar larvae of *S. littoralis* after different periods of exposure

Treatments	Exposure time (hrs)	LC_{50} (mg L^{-1})	Confident limits	Slope ±S.E.
Apricot kernel Extract	24	3995.77	(3174.77-5027.81)	2.57±0.41
	48	3035.615	(2342.478-385.82)	2.392±0.37
	72	2034.14	(1492.57-2735.27)	1.90±0.29
Linoleic acid methyl ester	24	5406.96	(4055.09-9269.90)	2.14±0.57
	48	3260.12	(6557.439-4356.11)	2.22±0.48
	72	2105.734	(1535.51-2863.55)	1.83±0.29
Oleic acid	24	3799.71	(2711.73-5105.79)	1.95±0.43
	48	3447.39	(2596.90-4546.71)	2.02±0.34
	72	2157.43	(1307.21-3225.69)	1.39±0.318
Methoxyfenozide	24	-	-	-
	48	8.77	(2.90-1300.47)	0.673±0.237
	72	0.152	(0.128-0.183)	1.66±0.13

[a] seven different concentrations of tested crude extract and its main components (within the range of 100-10000 mg L^{-1}) and methoxyfenozide (within the range of 0.01-5 mg L^{-1}), N=120 for each treatment.
(-) no toxic effects occurred after 24hrs exposure.

Table 3. Toxicity of apricot kernels extract, its main fatty acids components and methoxyfenozide against 4th instar larvae of *S. littoralis* after different periods of exposure

Treatments [a]	Exposure time (hrs)	LC_{50} (mg L^{-1})	Confident limits (mg L^{-1})	Slope ±S.E.
Apricot kernel Extract	24	4355.44	(3650.418-5272.66)	3.189±0.49
	48	3875.874	(3213.88-4850.28)	3.11±0.57
	72	3358.65	(2759.00-4134.31)	3.03±0.55
Linoleic acid methyl ester	24	5480.93	(4647.39-6663.26)	3.63±0.62
	48	4887.22	(4192.372-5756.71)	4.051±0.65
	72	4220.92	(3400.99-5358.39)	2.49±0.43
Oleic acid	24	9422.25	(6897.90-25542.23)	2.193±0.70
	48	7736.38	(5651.52-14490.32)	1.855±0.43
	72	6028.77	(4445.49-10285.47)	1.70±0.41
Methoxyfenozide	24	-	-	-
	48	-	-	-
	72	7.145	(4.66-11.68)	1.25±0.22

[a] seven different concentrations of tested crude extract and its main components (within the range of 1250-10000 mg L^{-1}) and methoxyfenozide (within the range of 0.05-15 mg L^{-1}), N=120 for each treatment.
(-) no toxic effects occurred after 24 and 48 hrs exposure.

The data of toxic action of hexane HEAK and some of its main FA components against 2nd and 4th instar larvae of CLW were agreed with the insecticidal activity of some active agents naturally occurred in essential oil from ripe fruits of *Melia azedarach* and some free individual FAs like LA against *S. littoralis* larvae. (Farag *et al.* 2011; Yousef*, et al.,* 2013)

However, there were no a definite justifications for the high significant toxicity of HEAK comparing individually by its main FAs OL and PA and their esters compound of LAME, this phenomenon might be attributed to the naturally occurrence of these active agents and other components in the crude extract bulk, may give an indication of some of their synergistic action. This thought meets the report of (Hummelbrunner and Isman, 2001) concerning synergistic effects of complex mixtures (crude extract) of phytochemicals. In addition, apricot kernels contain the toxic cyanogenic glycoside amygdalin depending on their variety (Gomez *et al*., 1998). Amygdalin can be hydrolyzed by β-glucoronidase in the human intestine to form glucose, benzaldehyde and released cyanide in the form of hydrocyanic acid (Shragg *et al*., 1982).

However, PA (C16:0) concentrations have no toxic effects against the treated larvae of CLW, unsaturated FAs of LAME (C18:2) and OL (C18:1) showed relative significant effects. This finding was discussed by several authors such as (Wallace *et al*., 2006; Margarida

et al., 2010). Detection of double bond numbers could not explain why 18:3 FAs were more toxic to growth than 18:2 FAs, and especially why 18:1was not toxic for ruminal bacterium *Butyrivibrio fibrisolvens*. The free carboxyl group disrupted the cell integrity and consequently help growth inhibition to take place. This might give a prospect that the form of free carboxylic FA has more toxicity as originated in LA than their methyl esters form of LAME as study case in this research.

Toxicity effect of the crude extract was rivaled by a common IGR insecticide (Runner 24% SC®; Methoxyfenozide) meet a previous study carried out on lepidopteron species treated with lethal concentrations of ecdysone agonists, the larvae died in a double cuticle without successful ecdysis, meanwhile at lower or sublethal concentrations many survived larvae underwent abnormal and lethal pupation. (Sa'enz de Cabezo´n *et al*., 2005)

B. Antifeedant effect:

Comparative studies for all treatments according to concentration-antifeedant responds were illustrated by probit line analysis to find out concentrations that fulfilled with 50% antifeedant activity (AI_{50}). AI_{50} values of all tested treatments on second instar larvae of *S. littoralis* after different times of exposure were shown in (Table 4). AI_{50} values of HEAK were 1475.47 and 2111.787 mg L^{-1} after 24 and 72 hours, respectively while after 48 hours its antifeedant effect was below 50% at its highest concentration. Methoxyfenozide had a high significant antifeedant activity as the AI_{50} values were 0.938, 3.11 and 1.30 mg L^{-1} after 24, 48 and 72 hours, respectively. Besides, the highest concentrations of LAME and OL had an antifeedant activity values lower than 25%. The presented data in (Table 5) showed AI_{50} values of all treatments on 4th instar larvae after different times of exposure. HEAK had relative antifeedant activity presented in AI_{50} values of 3814.74, 3079.88 and 3207.53 mg L^{-1} after 24, 48 and 72 hours, respectively. AI_{50} values of methoxyfenozide indicated as 1.43, 1.54 and 1.61 mg L^{-1} after 24, 48 and 72 hours, respectively ravel out its high antifeedant activity. On contrary, the antifeedant activity values were lower than 25% at the highest concentrations of LAME and OL.

The previous data showed that the antifeedant responds of HEAK was definite prevalence over its FA components but methoxyfenozide was so far owned superior antifeedant responses due to his extreme low AI_{50} values on the treated larvae.

Antifeedant data against *S. littoralis* larvae emphasized that HEAK clearly revealed significant high effect comparing to its tested FAs of Oleic, PA and LAME. The rate of feeding significantly varied depending on the concentration of the crude extract and its tested active components. This indicates that some active agents in the plants inhibit larval feeding activity or make the food unpalatable or the substances directly act on stimulating the deterrent receptor on chemosensilla of the larva and some antifeedant are thought to block. Also, sugars and amino acids could interfere with the perception of feeding stimulant receptors, while others may cause erratic bursts of electrical impulses in the nervous system resulting in feeding deterrence. (Jeyarajan *et al*., 1990; Jeyasankar *et al*., 2012; Jeyasankar *et al*., 2014)

Table 4. AI_{50} values of HAEK, its main components and methoxyfenozide to on 2nd instar larvae of *S. littoralis*

Treatments [a]	Exposure time (hrs)	AI_{50}[b] (mg L^{-1})	Confident limits (mg L^{-1})	Slope ± SD	X^2
Apricot kernel extract	24	1475.47	(2108.70-1670.78)	3.02±0.32	2.15
	48	>50% antifeedant activity at highest concentration.			
	72	2111.787	(1755.58-2738.48)	1.99±0.24	6.67
Linoleic acid methyl ester	24	>25% antifeedant activity at highest concentration.			
	48				
	72				
Oleic acid	24				
	48				
	72				
Methoxyfenozide	24	0.938	(0.62-1.72)	0.85±0.12	0.90
	48	3.11	(1.39-17.38)	0.60±0.12	2.12
	72	1.30	(0.88-2.28)	1.04±0.14	3.81

[a] Different concentrations of tested crude extract and its main components (within the range of 100-2500 mg L^{-1}) and methoxyfenozide (within the range of 0.05-2.5 mg L^{-1}), N=120 for each treatment.
[b] Concentrations of treatments fulfill 50% antifeedant activity

Table 5. AI_{50} values of HAEK, its main components and methoxyfenozide to on 4th instar larvae of *S. littoralis*

Treatments [a]	Exposure time (hrs)	AI_{50} [b] (mg L^{-1})	Confident limits (mg L^{-1})	Slope ± SD	X^2
Apricot kernel extract	24	3814.74	(3373.58-4512.61)	2.13±0.29	1.15
	48	3079.88	(2648.57-3698.38)	1.56±0.27	0.61
	72	3207.53	(2564.62-4479.19)	1.087±0.27	3.03
Linoleic acid methyl ester	24	>25% antifeedant activity at highest concentrations.			
	48				
	72				
Oleic acid	24				
	48				
	72				
Methoxyfenozide	24	1.43	(0.82-2.86)	0.47±0.10	1.03
	48	1.54	(0.87-4.26)	0.52±0.13	1.25
	72	1.61	(0.97-3.12)	0.51±0.10	5.11

[a] Different concentrations of tested crude extract and its main components (within the range of 1250-5000 mg L^{-1}) and methoxyfenozide (within the range of 0.1-5 mg L^{-1}), N=120 for each treatment.
[b] Concentrations of treatments fulfill 50% antifeedant activity

The main FA components represented as OL (C18) and LAME (C19) of the crude extract had a significant very low antifeedant effect only in high concentrations and particularly seemed to be attractive in very low concentrations. This findings were agree with (Ganesan *et al*., 2006) observations that Longer-chain-length FAs (C12–C18) were attractive at lower levels but repellent at higher levels for *Aedes aegypti* L. oviposition. While, Straight-chain FAs in the C6–C10 range possessed antifeedant and repellent actions against pine weevils, *Hylobius abietis* L. and activity dropped with increasing chain length (Mansson *et al*., 2006).

Disturbance in feeding behavior in CLW larvae treated with methoxyfenozide attributed to its mode of action mimics natural ecdysone, where the reduction of weight could be due to a decrease in feeding activity as a consequence of the activity of the insecticide and the presence of the double cephalic capsule, which interferes with larval feeding. Inhibitions of methoxyfenozide and other IGRs on S. littoralis, and Mythimna unipuncta were detected (Gobbi *et al*. 2000; Pineda *et al*. 2007; Federico *et al.,* 2011).

C-Haemocytes count:

Five haemocyte types that have been identified as Prohaemocytes (Pr), Granulocytes (Gr), Plasmatocytes (Pl), Oenocytoides (Oe) and Spherulocytes (Sp) were monitored after treatment. The effects of apricot kernel extract and its main components after 72 hrs from treatment were evaluated on the haemolymph parameters. All tested compounds clearly affected on the different haemocytes counts of 4th instar larvae of S. littoralis.

(Table 6) shows the fluctuation of the calculated mean number of different haemocytes in the haemolymph of the 4th instar larvae of S. littoralis as a result of apricot kernel extract and its main components after 72 hrs of exposure.

With regard to apricot kernel extract treatment, a reduction in the percentage of the prohaemocyte was observed amounted 9.2 and 8.3 cell , when LC_{25} and LC_{50} were used, respectively (Table 6). A significant reduction was observed in the number of granulocyte after treatment with LC_{25} and LC_{50} values of apricot kernel extract 26.3 and 23 cell. The same trend was observed in the number of plasmatocytes of the LC_{25} and LC_{50} values where the number of plasmatocytes were 24.3 and 23.1 cell, respectively. While the number of Oenocytoides increased being 17.6 and 19.3 cell, respectively. The same trend was observed in spherule cells number being 10.1 and 12.3 cell, respectively.

In the linoleic acid treatment, reduction in the number of the prohaemocyte count was estimated by 8 and 6.5 cell, respectively when LC_{25} and LC_{50} were used compared to control, respectively, A significant reduction appeared in the number of granulocyte after treatment with LC_{25} and LC_{50} values of linoleic acid. 32.2 and 31.7 cell. The same trend was observed in the number of plasmatocyte in the LC_{25} and LC_{50} values were 35 and 33.2 cell, respectively, while the number of Oenocytoides increased being 20.4 and 21.3 cell, respectively. The same trend was observed in spherule cells number being 10.4 and 11.3 cell, respectively.

Table 6. Effect of HAEK crude extract, its main components on number of haemocytes in the 4th instar larvae of *S. littoralis* after 72hrs hours of exposure

Treatments		Haemocytes *number (cells)*/100 cell				
		Pr	Gr	Pl	Oe	Sp
Apricot kernel extract	Lc_{25}	9.2±0.2^{ab}	26.3±1.2^{c}	24.3±1.6^{c}	17.6±1.2^{ab}	10.1±1.2^{a}
	Lc_{50}	8.3±0.3^{b}	23±1.3^{c}	23.1±1.2^{c}	19.3±1.3^{a}	12.3±1.2^{a}
Linoleic acid methyl ester	Lc_{25}	8±0.5^{b}	32.2±1.2^{ab}	35±1.4^{a}	20.4±0.6^{a}	10.4±0.5^{a}
	Lc_{50}	6.5±0.3^{b}	31.7±1.3^{b}	33.2±1.0^{a}	21.3±0.7^{a}	11.3±0.7^{a}
Oleic acid	Lc_{25}	7.4±0.3^{b}	35.6±1.0^{a}	34.8±1.2^{a}	20.2±0.8^{a}	10.2±0.5^{a}
	Lc_{50}	6.1±0.2^{b}	34.3±0.4^{a}	32.1±0.8^{a}	22.8±0.8^{a}	11.8±0.7^{a}
Methoxyfenozide	Lc_{25}	7.3±0.3^{b}	24.8±0.3^{a}	22.2±0.6^{b}	16.2±1.0^{a}	10.2±0.5^{a}
	Lc_{50}	5.3±0.3^{b}	22.8±0.3^{b}	20.7±0.6^{b}	17 ±1.0^{a}	9.7 ±0.4^{a}
Control		12±0.6^{a}	30±1.2^{b}	30.1±1.5^{b}	15±0.2^{b}	6.5±0.1^{b}

- Each value represents the mean ± *SE.*
- Mean in same column followed by the same letters are not significant.
- Probability level at 0.01.

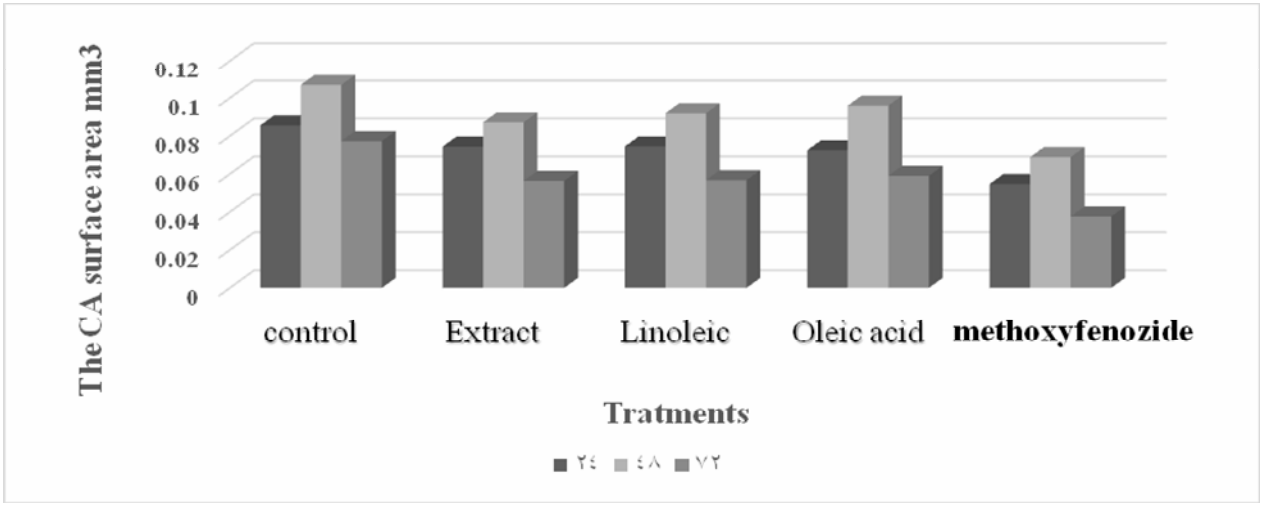

Figure 1. Effect of HAEK crude extract, its main components on the CA surface area in 4th instar larvae of *S. littoralis*

The Oleic acid treatment was characterized its increase in the number of prohaemocyte by 7.4 and *6.1* cell, respectively at LC_{25} and LC_{50}. A significant increase was observed in the number of granulocytes after treatment with LC_{25} and LC_{50} values *35.6* and *34.3* cell, respectively. The same trend was observed in plasmatocyte number being *34.8* and *32.1*cell, respectively. Moreover, oenocytoide cells number increased to *20.2* and *22.8 cell,* respectively .The number of spherule cells increased compared with control *10.2* and *11.8* cell (Table 6).

On contrast, methoxyfenozide treatment significantly decreased the number of prohaemocyte to be *7.3* and *5.3* cell, respectively at LC_{25} and LC_{50} values. Also, the number of granulocyte significant decreased being *24.8* and *22.8* cell, respectively .The same trend was observed in the number of plasmatocyte being *22.2* and *20.7* cell, respectively at LC_{25} and LC_{50} values. Furthermore, the number of oenocytoide increased being *16.2* and *17* cell, respectively. The same trend was observed in spherule cells number being *10.2* and *9.7* cell, respectively at LC_{25} and LC_{50} values (Table 6). The present result shows that the effect of apricot kernel extract on the different haemocyte counts in 4th instar larvae was similar to the effect of tested insecticide (methoxyfenozide).

These results were similar to those obtained in previous studies (Arnold and Hinks, 1976) when they observed a high mitotic and rapid turnover of spherule cells, possibly as a mechanism of releasing products of their metabolism into the haemolymph. In addition, spherule cells were played an important role in

recreation of some haemolymph proteins (Akai and Sato, 1973). Also, agreed to the results of (Gad and Abdel-Megeed 2006), they observed that spinosad and proclaim decreased the total and differential haemocyte counts also effect and damage on the larval DNA in the Spodoptera littoralis.

(Zibaee *et al*. 2011) mentioned that estimation of Aspartate (AST) and Alanine aminotransferases (ALT) activity significantly increased in Eurygaster integriceps after exposure to pyriproxyfen. They concluded that possible damages of this insecticide to haemocytes and fat bodies are the reason in elevation of their activity.

D- Corpora allata activity:

Results reported in Fig., (1) depict that treatment of S. littoralis 4th larval instar with crude extract and its main components caused markedly decreases in the CA surface area after 24, 48, 72 hrs of exposure.

Apricot kernel extract at LC_{50} markedly decreased the CA surface area, it was 0.0563 mm^3 in the treated larvae while control was 0.0776 mm^3, the percentage of decrease was 27.44 % after 72 hrs of exposure. Furthermore, linoleic and Oleic acid at LC_{50} decreased the CA surface area, it were 0.0573 and 0.0586 mm^3 in the treated larvae with linoleic and Oleic acid, respectively. The percentage of decrease were 26.2 and 24.4 % after 72 hrs of exposure, respectively. Also, treatment with methoxyfenozide at LC_{50} caused a sharp decrease in the CA surface area. The CA surface area was 0.0378 mm^3 in the treated larvae about 51.3 % 72 hrs of exposure.

These results are in agreement with Abou-Taleb *et al*. (2013), they reported a decrease in the CA activity in 4th larval instar of S. littoralis after treatment with insecticides lufenuron and chlorfluazuron.

CONCLUSION

It is noticeably showed that toxic action of the HAEK crude extract and its FA constitutes initiated much earlier than methoxyfenozide particularly within the period of 24 hours against 2nd instar and the first 48 hours against 4th instar larvae of *S. littoralis*. We can apparently depend on the toxic and antifeedant responds of HEAK and its main FA constituents in 2nd more than 4th instar larvae.

Prospective studies would be oriented to words the active agents of HEAK and its synergistic effects with other conventional insecticides against CLW and other insect pests.

Abbreviations:

- Cotton leaf worm (CLW)
- Hexane extract of apricot kernel (HEAK)
- Palmitic acid (PA)
- Linoleic acid (LA)
- Corpora allata (CA)
- Linoleic acid methyl ester (LAME)
- Oleic acid (OL)
- Insect growth regulator (IGR)
- Fatty acid (FA)

REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Abdel-Rahman, M. K. 2011. Can apricot kernels fatty acids delay the atrophied hepatocytes from progression to fibrosis in dimethyl nitrosamine (DMN)-induced liver injury in rats? Lipids in Health and Disease. 10: 114.

Abou-Taleb, H. K., H. M. Zahran and A. A. Gad 2015. Biochemical and physiological effects of lufenuron and chlorfluazuron on *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). J. Econ. Entomol. 12(2): 77-86.

Adel, M. M. and F. Sehnal 2000. Azadirachtin potentiates the action of ecdysteroid agonist RH-2485 in *Spodoptera littoralis*. J. Insect Physiol. 46: 267-274.

Akai, H. S. and S. Sato 1973. Ultrastructure of the larval haemocytes of the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae). Int. J. Insect. Morphol. Embryol. 2: 207-231.

Amish, P. V., L. V. Jaswant and N. Subrahmanyam 2010. A review on FAME production processes fuel. 89 (1): 1-9.

Armstrong, W. D. and C. W. Carr 1964. Physiological chemistry laboratory directions (3rd ED).Burges publishing Co., Minneapolis, Minnesota

Arnold, J. W. and C. F. Hinks 1976. Haemopoiesis in Lepidoptera. The multiplication of circulating haemocytes. Can. J. Zoo. 54: 1003-1012.

Biddinger, D., L. Hull, H. Huang, B. McPheron and M. Layer. 2006. Sub lethal effects of chronic exposure to tebufenozide on the development, survival, and reproduction of the tufted apple bud moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 99: 834-842.

Ditrich, V. 1962. "A comparative study of toxicological test methods on a population of the two spotted spider mite (*Tetranychus urticae*)". J. Econ. Entomol. 55: 644–648.

El-Defrawi, M. E., A. Toppozada, N. Mansour and M. Zaid. 1964. Toxicological studies on Egyptian cotton leaf worm *Prodenia litura* (F.). I: Susceptibility of different larval instars to insecticides. J. Econ. Entomol. 57: 591 – 593.

EPPO, 1997. *Spodoptera littoralis* and *Spodoptera litura*. In: Smith IM, McNamara DG, Scott PR, Holderness M (eds). Quarantine pests for Europe. 2nd edition. Wallingford, UK: CAB International. 518-525.

Essawy, M. M. 1990. Changes in different haemocytes counts of the last larval instar of *Spodoptera littoralis* (Boisd.) during wound healing. Alex. Sci. Exch. 11(4): 151-176.

Farag, M., M. H. Ahmed, H. Yousef and A. A. Abdel-Rahman. 2011. Repellent and insecticidal activities of *Melia azedarach* L. against cotton leaf worm *Spodoptera littoralis* (Boisd.). Z. Naturforsch. 66: 129-135.

Federico, R., N. F. Marilina, I. S. Marcela and E. R. Alicia 2011. Lethal and sublethal effects of cypermethrin and methoxyfenozide on the larvae of *Rachiplusia nu* (Guenee) (Lepidoptera: Noctuidae). Invertebrate Reproduction & Development. 1–9: I First.

Finney, D. J. 1971. Probit analysis. 3 rd Edn. Cambridge University Press, Cambridge. XV, 333S. 41 Rechenbeispiele, 20 Diagr., 8 Tab., 231 Lit., L 5.80.

Gad, A. A. and A. Abdel-Megeed 2006. Effects of spinosad and emamectin benzoate on the blood picture and DNA structure of the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) .The Egyption. Sci. Magazine. 4: 76-80.

Ganesan, K., M. J. Mendki, M. V. Suryanarayana, S. Prakash and R. C. Malhotra 2006. Studies of *Aedes aegypti* (Diptera: Culicidae) ovipositional responses to newly identified semiochemicals from conspecific eggs. Aust. J. Entomol. 45:75–80.

Georgiou, G. P. 1987. Insecticides and Pest Resistance: The Consequences of Abuse, Faculty Research Lecture, Academic Senate, University of California, Riverside, Calif, USA.

Gobbi, A., F. Budia, M. I. Schneider, P. Del Estal, S. Pineda and E. Vin˜ uela 2000. Accio´ n del tebufenocida sobre *Spodoptera littoralis* (Boisduval), *Mythimna unipuncta* (Haworth) *Spodoptera exigua* (Hu¨ bner). Boletı´n de Sanidad Vegetal-Plagas. 26: 119–127.

Gomez, E., L. Burgos, C. Soriano and J. Marrin 1998. Amygdalin content in the seeds of several apricot cultivars. J. Sci. Food Agric. 77: 184-186.

Gupta, A., P. C. Sharma, B. Tilakratne and A. K. Verma. 2012. Studies on physic-chemical characteristics and fatty acid composition of wild apricot (*Prunus armeniaca* Linn.) kernel oil. Indian J. of natural products and resources. 3(3): 366-370.

Heidi, A. A., A. M. Kordy, A. E. Khaled, N. A. Hassan and N. R. Abdelsalam 2015. Efficiency of using some new insecticides against cotton leaf worm (*Spodoptera littoralis*) based on biochemical and molecular marker. Alex. Sc. Exch. J. 36(4): 303-313.

Hummelbrunner, L. A. and M. B. Isman 2001. Acute, Sub lethal, Antifeedant and Synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm *Spodoptera litura* (Lepidoptera: Noctuidae). Journal of Agricultural and food chemistry. 49: 715-720.

Jeyarajan, S., P. C. Babu, G. Srimannarayana and Y. Geethanjali 1990. Antifeedant and morphogenetic effects of azadirachtin rich fractions on *Spodoptera litura* F. Botanical Pesticides in Integrated Pest Management. 13: 376-380.

Jeyasankar, A., P. Selvaraj and E. Kuppusamy 2014. Antifeedant and insecticidal activities of selected plant extracts against Epilating beetle *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae). Advances in Entomology. 2 (1): 14-19.

Jeyasankar, A., S. Premalatha and K. Elumalai. 2012. Biological activities of *Solanum pseudocapsicum* (Solanaceae) against cotton bollworm, *Helicoverpa armigera* Hübner and armyworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Asian Pacific J. of Biomedicine. 2: 981-986.

Kannathansan, K., A. Senthilkumar, V. Venkatesalu and M. Chandrasekaram. 2008. Larvicidal activity of fatty acid methyl esters of *Vitex* species against *Culex quinquefasciatus*. Parasitol. Res. 103(4): 999-1001.

Koshiya, D. J. and A. B. Ghelani. 1990. Antifeedant activity of different plant derivatives against *Spodoptera litura* (Fab) on groundnut. Proceedings of the Symposium on Botanical Pesticides in Integrated Pest Management. 270-275.

Mansson, P. E., F. Schlyter, C. Erikkson and K. Sjodin 2006. Noanoic acid, other alkanoic acids, and related compounds as antifeedant in *Hylobius abietis* pine weevils. Entomol. Exp. Appl. 121: 191-201.

Margarida, R. G., C. C. Maia, S. B. Charles, J. R. Anthony, M. Nest, R. L. Tony, A. G. Ian and J. W. Robert 2010. Toxicity of unsaturated fatty acids to the iohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. BMC Microbiology. 10: 52-56.

Nas, M. N. 2004. "In vitro studies on some natural beverages as botanical pesticides against *Erwinia amylovora* and *Curobacterium flaccumfaciensis* subsp. Poinsettia". Turkish J. of Agriculture and Forestry. 28(1): 57–61.

Pflugefelder, O. (1948). Volumetrissche untersuchugen van den corpora allata der Honigbiene *Apis mellifera* Biol. Zbl. 66: 211-235.

Pineda, S., M. I. Schneider, G. Smagghe, A. M. Martinez, P. Del Estal, E. Vin˜ uela, J. Valle and F. Budia 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. of Econ. Entomol. 100: 773–780.

Rahuman A. A., P. Venkatesan, G. Gopalakrishnan. 2008. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus coloncynthis* (Linn.). Parasitol. Res. 103(6): 1383-1390.

Ramos-López, M. A., M. M. González-Chávez, N. C. Cárdenas-Ortega, M. A. Zavala-Sánchez and G. S. Pérez. 2012. Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*. African J. of Biotechnology. 11(18): 4274-4278.

Sa´enz de Cabezo´ n, M., V. F. Zalom and I. Pe´rez-Moreno. 2005. Effects of methoxyfenozide on *Lobesia botrana* (Den y Schiff) (Lepidoptera: Tortricidae) egg, larval and adult stages. Pest Manag. Sci. 11: 1133-1137.

Saleh, M. A., M. M. El-Bolok, K. A. Abdel Salam and N. A. Ibrahim 1986. Plant extracts affecting insect feeding growth and metamorphosis. Bull. Fac. Agric. Cairo. Uni. 37(1): 526-539.

Shragg, T. A., T. E. Albertson and C. J. Fisher. 1982. Cyanide poisoning after bitter almond ingestion. West J. Med. 136: 65-69.

Smagghe G, Pineda S, Carton B, Del Estal P, Budia F, Vin˜ uela E. 2003. Toxicity and kinetics of methoxyfenozide in greenhouse-selected *Spodoptera exigua* (Lepidoptera: Noctuidae). Pest Manag. Sci. 59, 1203-1209.

Wallace, R. J., L. C. Chaudhary, N. McKain, N. R. McEwan, A. J. Richardson, P. E. Vercoe, N. D. Walker and D. Paillard 2006. *Clostridium proteoclasticum*: a ruminal bacterium that forms stearic acid from linoleic acid. FEMS Microbiol Lett. 265: 195-201.

Youssef, H., F. Saheir, Y. El-Lakwah and A. El Sayed 2013. Insecticidal activity of linoleic acid against *Spodoptera Littoralis* (Boisd.). Egypt. J. Agric. Res. 91 (2):573-580.

Zibaee, A., I. Zibaee and J. J. Sendi (2011). A juvenile hormone analog, pyriproxifen, affects some biochemical components in the haemolymph and fat bodies of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Pestic. Biochem. Physiol. 100: 289–298.

الملخص العربي

دراسة السمية والتأثير المانع عن التغذية لمستخلص نبات المشمش ومكوناته الفعالة ضد دودة ورق القطن بمرجعية بعض التأثيرات الفسيولوجية

وائل محمود خميس، سحر السيد الدسوقى، عبير عبد المجيد جاد

تم دراسة تأثير السمية والسلوك المانع عن التغذية لمستخلص الهكسان لنواة المشمش ومكوناته من الاحماض الدهنية التى تم تعريفها بواسطة التحليل بجهاز كروماتوجرافى غاز- مطياف الكتلة والتى تشمل على الأحامض الدهنية(البالمتيك والأوليك) ومشتق الأحماض الدهنية (الأستر ميثيل لحامض اللينوليك) ضد العمر اليرقى الثانى والرابع لدودة ورق القطن تحت الظروف المعملية. وتم الأستعانه بمبيد الميثوكسي فينوزيد (رنر٢٤% مركز معلق) كمبيد مرجعى لمستخلص نواة المشمش ومكوناته. خضعت جميع النتائج لبرنامج تحليل خط السمية لحساب التركيزات النصف قاتلة والتركيزات المسببة للأمتناع بنسبة ٥٠% عن التغذية بعد ٢٤، ٤٨ و٧٢ ساعة من التعرض للمعاملات. وقد تبين من نتائج تأثيرات السمية والمانع للتغذية سيادة مستخلص نواة المشمش مقارنة بمكوناته من الأحماض الدهنية ضد العمرين اليرقيين لدودة ورق القطن خلال ٧٢ ساعة من المعاملة. وعلى الرغم من أن بداية فعل تأثير السمية للمستخلص ومكوناته سبقت الميثوكسي فينوزيد بـ ٢٤ ساعة بعد التعرض للمعاملة، فان مبيد الميثوكسى فينوزيد مازال يمتلك التفوق فى أستجابة اليرقات المعاملة للسمية والتأثير المانع عن التغذية. هذا وقد تم تدعيم خطوات البحث بإجراء بعض الدراسات الفيسيولوجية متضمن العد الكلى لأنواع خلايا الدم وتقدير نشاط غدة الجسم الكروى.