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ANALYTICAL STUDY OF TWO COMMERCIAL SEX PHEROMONE LURES FOR MONITORING MALES OF COTTON LEAFWORM (*CLW*) AND SPINY BOLLWORM (*SBW*) IN EGYPT

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ABSTRACT

A simple and reliable GC-FID method was applied for the determination of the of the synthetic sex pheromone components of the Egyptian armyworm *Spodoptera littoralis* (Cotton leafworm, *CLW*) (I) pheromone isomers *cis*-9, *trans*-11-tetradecadienyl acetate (major) is the active pheromone while *cis*-9, *trans*-12-tetradecadienyl acetate (minor) is the synergist in ratio 9:1, *w/w*, and *Earias insulana* (Spiny bollworm, *SBW*) (II) pheromone isomers *(10E, 12E)-10, 12-hexadecadienal* (major) is the active pheromone while *(Z)-11-hexadecenal* (minor) is the synergist in ratio 99:1, *w/w* in their pure form. The proposed method showed high sensitivity with good linearity over the concentration range of 12.5-400 µg / ml of major, 5-80µg / ml of minor in case of I and 25-400 µg / ml of major and 1-32µg / ml of minor in case of II. The method is successfully applied to the analysis of commercial lures containing (I) and (II) with excellent recovery. The kinetics investigation are carried out in Egyptian environmental condition by monitoring the ratio of the pheromone isomers in order to achieve optimum biological activity and follow up longetivity of the lure in the trap under field conditions. GC-FID is considered to be the most promising method for this purpose because of its sensitivity, specificity and versatility. Moreover uncertainty of measurement for the pheromone components of *CLW* and *SBW* was calculated.

Keywords: pheromone, cotton leafworm (*CLW*, *Spodoptera littoralis)*, spiny bollworm (*SBW*, *Earias insulana*), GC analysis.

INTRODUCTION

The word pheromone comes from the Greek words *Pheran* (to transfer) and *Horman* (to excite). Pheromones are unique and highly specific chemical signals produced by an organism that triggers a natural behavioral response in another member of the same species [1]. In insects, the use of pheromones to control phases of the lives of pest species is one method of pest management. Sex-attractant pheromones are used in certain products (Lures) to lure and trap unwanted or harmful insects [2].

Cotton is considered by far the main cash crop in Egypt with ~ 90 % of the long fibers produced being exported [3, 4], where cotton presents ≥ 40% of the whole Egyptian export quota [5]. Among Egypt's principle areas of cotton cultivation, Sharqiya, Gharbiya, Menoufiya and Daqahliya provinces are the hardest hit by cotton worms [4]. Cotton is Cultivated in March and harvest in September [6]. Cotton plantations are dying due to a combination of severe infestation with boll and cotton worms as well as piercing insects which thrive in high temperature and humidity of July and August during their growth stage. Pests are such serious threat to cotton production and the cost of cotton pests control is ≈ 68, 000, 000 L.E every year [7].

The most destructive pests of these are Cotton leafworm (*CLW*), *Spodoptera littoralis*, and Spiny bollworm (*SBW*), *Earias insulana* [8]. *Cis*-9, *trans*-11-tetradecadienyl acetate (*Z*9, *E*11-14: AC) and *cis*-9, *trans*-12-tetradecadienyl acetate (*Z*9, *E*12-14: AC) [9], were identified as major components of the female sex pheromone of *Spodoptera littoralis, CLW* while The isomers (10*E*, 12*E*)-10, 12-hexadecadienal (*E*10, *E*12-16:Ald) and (*Z*)-11-hexadecenal (*Z*11-16:Ald) are the main components of female sex pheromone used for monitoring and mass trapping of *Earias insulana, SBW* [10, 11]. Chemical control is currently used in the management of these two pests in Egypt as the major control program [12], although recently, some alternatives to chemical control have begun to be explored [13].

Sex pheromones have been successfully used for monitoring, mass trapping and mating disruption of a diversity of lepidopteran insect pests including *CLW* and *SBW* [14, 15]. pheromone lure dispensers of *CLW* filled with sex pheromone blend formulation of the 2 isomers, namely *Z*9, *E*11-14: AC and *Z*9, *E*12-14: AC in ratio 9: 1 *w/w*, while *SBW* lures filled with sex pheromone blend formulation of the 2 isomers *E*10, *E*12-16: Ald and *Z*11-16: Ald in ratio 99: 1, *w/w*. Pheromone trap data gives early warning of the infestation and also exhibits the density of insect population. For either of these applications, measuring the amount collected and that left in pheromone formulation is important and critical to success [16, 17]. These sex pheromones, unfortunately, some of these pheromone lures worked efficiently in other countries but did not give the same results under Egyptian climate.

The aim of this work is to develop and validate a simple, sensitive GC-FID method to evaluate two commercial pheromone lures under Egyptian environmental conditions as an important step for developing an effective system for monitoring these two destructive pests in Egyptian cotton field. As well as the use of this method is to monitor the ratio of the pheromone

isomers in order to achieve optimum biological activity and predict the longetivity of the lures.

MATERIALS AND METHODS

A. Chemicals

a) Pure standards of *CLW* pheromone isomers

Cis-9, *trans*-11-tetradecadienyl acetate (*Z*9, *E*11-14: AC) and *Cis*-9, *trans*-12-tetradecadienyl acetate (*Z*9, *E*12-14: AC) manufactured by Trifolio-M GmbH-Germany were kindly supplied from the Ministry of Agriculture (MARC), located at Dokki, Giza. Their purity were checked in MARC laboratories relative to the reference standards obtained from the manufacturing company and were found to be 99.65 + 0.03% and 98.89 + 0.11%, respectively.

b) Pure standards of *SBW* pheromone isomers

(*Z*)-11-hexadecenal (*Z*11-16: Ald) and (10*E*, 12E)-10, 12-hexadecadienal (*E*10, *E12*-16: Ald) manufactured by Trifolio-M GmbH-Germany were kindly supplied from the Ministry of Agriculture (MARC), located at Dokki, Giza. Their purity was checked in MARC laboratories relative to the reference standards obtained from the manufacturing company and were found to be 98.90 + 0.23% and 99.80 + 0.40%, respectively.

B. Pheromone lure specifications

Pheromone lures (controlled lures) for monitoring *CLW* manufactured by Production Unit of Pesticide Alternatives in Sendion were evaluated in this study. They were kindly supplied from the Ministry of Agriculture (*MARC*). The lures are red body, red Cap polyethylene vial filled with sex pheromone blend formulation. Each vial claimed to contain 1 ± 0.2 mg of the 2 isomers *Z*9, *E*11-14: AC and *Z*9, *E*12-14: AC in ratio 9: 1 *w/w* (Any changes in the reported ratio 9:1, *w/w* affect its biological activity [18]).

Pheromone lures for monitoring *SBW* manufactured by Trifolio-M GmbH-Germany were kindly supplied from the *MARC*. The lures are white body, white Cap prepared from Polyethylene filled with sex pheromone blend formulation. Each vial claimed to contain 2 ± 0.25 mg of the 2 isomers, namely; (10*E*, 12E)-10, 12-hexadecadienal (*E*10, *E12*-16: Ald) and (*Z*)-11-hexadecenal (*Z*11-16: Ald) in ratio 99: 1, *w/w*.

C. Gas chromatography

Analysis were performed on GC-system, Hewlett-Packard HP-6890 series, computerized equipped with two columns in the same injection unit, were packed with 5%-pheny l-95% dimethylpolysiloxane as the stationary phase, with different film thickness,

Column (1): ZB-5: [(0.25 µm, 30 m × 0.25 mm, id.)]
Column (2): ZB-5: [(0.50 µm, 30 m × 0.25 mm, id.)]

for optimum performance, flame ionization detector, and a split/splitless injector.

***Chromatographic dual-column conditions**

- ***CLW***

Nitrogen was used as the carrier gas with a flow rate 1 ml /min. The column was kept at 70^{o}C for 1 min. and then programmed at a rate of 10^{o}C /min to 270^{o}C (10 min.). The injector and detector temp were maintained at 280^{o}C and 300^{o}C, respectively.

- ***SBW***

Nitrogen was used as the carrier gas with a flow rate 0.4 ml /min. The column was kept at 120^{o}C for 1 min. and then programmed at a rate of 20^{o}C /min to 180^{o}C (1 min.), 20^{o}C /min to 235^{o}C (1 min) and finally programmed at a rate of 20^{o}C /min to 270^{o}C (10 min). The injector and detector temp were maintained at 280^{o}C and 300^{o}C, respectively.

D. Procedure

a) Linearity

i. Linearity of CLW pheromone isomers

- **L*inearity of cis-9, trans-11-tetradecadienyl acetate***

Aliquots equivalent to 125-4000 µg of Z9, E11-14: AC isomer were accurately transferred from its stock solution (1000 µg mL^{-1}) into a series of 10-mL calibrated volumetric flasks, and then completed to volume with acetone. One microliter of each prepared solution was injected separately and chromatographed under the specified chromatographic conditions described above. The peak area for each injected solution was recorded. The calibration curve was constructed representing the relationship between the relative peak areas (peak area of the pheromone relative to that of external standard pheromone 50 µg mL^{-1}) versus the corresponding concentration of Z9, E11-14: AC and the regression equation were computed.

- **Linearity of cis-9, trans-12-tetradecadienyl acetate**

Aliquots equivalent to 50-800 µg of Z9, E12-14: AC isomer from its working standards solution (100 µg mL^{-1}) were transferred accurately into series of 10-ml calibrated volumetric flasks, and then completed to volume with acetone. One micro liter of each prepared solution was injected separately into the gas chromatograph under the specified chromatographic conditions described. The peak area for each injected solution was recorded. The calibration curve was constructed representing the relationship between the relative peak areas (peak area of the pheromone relative to that of external standard pheromone 10 µg mL^{-1}) versus the corresponding concentration of Z9, E12-14: AC and the regression equation were computed.

ii. Linearity of SBW pheromone isomers

▪ **Linearity of (10E, 12E)-10, 12-hexadecadienal isomer**

Aliquots equivalent to 250 - 4000 µg of E10, E12-16: Ald isomer were accurately transferred from its stock solution (2000 µg mL^{-1}) into a series of 10-mL calibrated volumetric flasks, then were completed to volume with acetone. One microlitre of each prepared solution was injected separately and chromatographed under the specified chromatographic conditions described above. The peak area for each injected solution was recorded. The calibration curve was constructed representing the relationship between the relative peak areas (peak area of the pheromone relative to external standard pheromone 100 µg mL^{-1}) versus the corresponding concentration of E10, E12-16: Ald and the regression equation was computed.

▪ **Linearity of (Z)-11-hexadecenal isomer**

Aliquots equivalent to 10-320 µg mL^{-1} of Z11-16: Ald isomer were accurately transferred from its working solution (20 µg mL^{-1}) into a series of 10-mL calibrated volumetric flasks. Complete to volume with acetone. One microlitre of each prepared solution was injected and chromatographed under the specified conditions described above. Peak area for each injected solution was recorded. The calibration curve was constructed representing the relationship between the relative peak areas (peak area of the pheromone relative to external standard pheromone 2 µg mL^{-1}) versus the corresponding concentration of Z11-16: Ald was constructed and the regression equation was computed.

Assay parameters and method validation were summarized in Table-1.

Table-1. Validation parameters of the proposed GC method for determination of Z9, E11-14: AC, Z9, E12-14: AC, E10, E12-16: Ald and Z11-16: Ald in pure sample.

Pheromone isomer / Validation parameter	Z9, E11-14: AC	Z9, E12-14: AC	E10, E12-16:Ald	Z11-16:Ald
Linearity range (µg mL^{-1})	12.5-400	5-80	25-400	1-32
Regression equation				
- Correlation coefficient (r^2)	0.9998	0.9997	0.9998	0.9999
- Intercept	+ 0.0179	+ 0.094	+ 0.0255	+ 0.0301
- Slope	0.0199	0.1012	0.0098	0.4884
- Mean ± SD	99.56 ± 1.65	100.34 ± 1.67	99.67 ± 1.46	99.85 ± 0.88
Accuracy			100.55 ± 1.69	95.96 ± 1.94
Repeatability (Mean ± RSD [a])	101.46 ± 0.641	102.54 ± 1.394	99.39 ± 1.743	97.15 ± 1.864
Intermediate precision (Mean ± RSD [b])	102.41 ± 0.959	103.52 ± 1.515	97.83 ± 1.935	96.85 ± 2.080
(LOD) * (µg mL^{-1})	1.43×10^{-1}	2.096×10^{-2}	0.139	0.085
(LOQ) ** (µg mL^{-1})	4.33×10^{-1}	6.351×10^{-2}	0.308	0.235

a. The intraday average (n = 8) of 100 µg ml^{-1} for Z9, E11-14: AC, 10 µg ml^{-1} for Z9, E12-14: AC, 200 µg mL^{-1} for E10, E12-16:Ald and 2 µg mL^{-1} for Z11-16:Ald, concentrations repeated 8 times within the same day.
b. The interday (n = 8) average of 100 µg ml^{-1} for Z9, E11-14: AC, 10 µg ml^{-1} for Z9, E12-14: AC, 200 µg mL^{-1} for E10, E12-16:Ald and 2 µg mL^{-1} for Z11-16:Ald concentrations repeated 8 times in three successive days.
* Limit of detection
** Limit of quantitation

b) Analysis of lure dispensers

The pheromone lure was divided quantitatively into small pieces using a metallic scissor and collected quantitatively into a dry 25-mL calibrated volumetric flask. Ten milliliter of acetone was accurately added by one mark pipette, and the flask was firmly closed by the stopper. The solution was kept for ~ 18-24 hours at room temperature (20 ±5°C) for complete extraction.

The extract was quantitatively filtered into another dry 25-mL calibrated volumetric flask and chromatographed by applying the proposed method using the specified chromatographic condition. Recovery was calculated by comparing peak area of the sample with its corresponding external standard.

E. Experimental specifications (field evaluation)

For determination the Release Rates of two Pheromone Isomers from their Lures under Field Conditions, field experiment was planned and done on Giza-80 cotton plantation during cotton season 2008 which is cultivated in field located at the Ministry of Agricultural Research Center (MARC), Dokki, Giza -

Egypt. The experiment was conducted and sex pheromone lures were evaluated between May 15 and July 5, 2008 (cotton season) under Egyptian climate conditions; temperature 22^{o}C $_{(min.)}$ -34^{o}C $_{(max.)}$ and relative humidity (RH %) 58 ± 5% (in shadow) under the proposed trap specification {Funnel pheromone traps baited with CLW and SBW-lures were hung on wooden stakes at height ~ 20 cm above plant surface and increasing traps level gradually according to the increase in plant growth. The traps were placed at 100 m intervals along planted rows.

The experimental trial period under field conditions was 50 days using the specified experimental design? Pheromone lures were collected from traps every five days after application; transferred to the laboratory using an ice-box for immediate analysis as detailed under analysis of commercial lure. The extract chromatographed immediately by the proposed method or kept refrigerated (0 ± 5^{o}C) till analysis. An initial reading (zero time) was obtained from freshly prepared lure content. Three different GC injections were made from each sample collection. Recovery was calculated by comparing peak area of sample with that of its corresponding external standard.

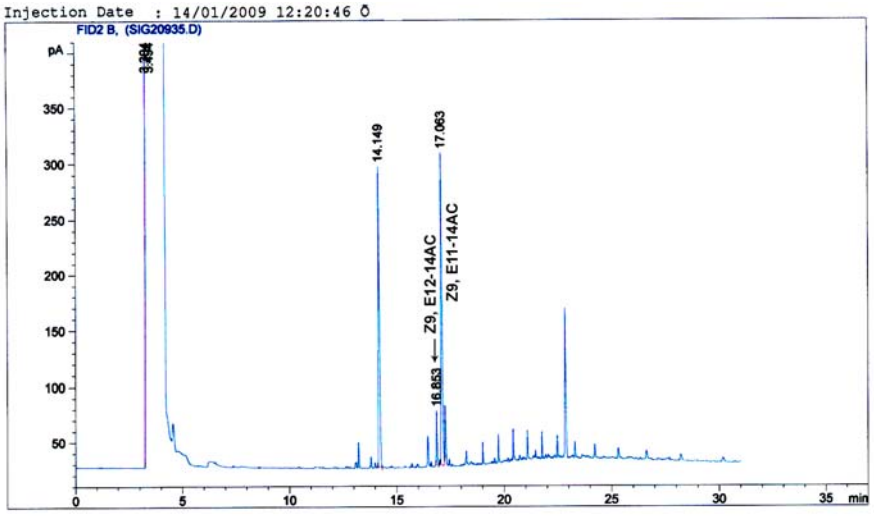
RESULTS AND DISCUSSIONS

The use of pheromone lures in pest management requires quantitative determination of the content in order to measure the release rate of the pheromone and longetivity of the lures. Capillary gas chromatography was widely used for determination of many insect pheromones [19]. GC-FID analysis is the most accurate method, provided that peaks are sharp and the analysis is consistent [19].

Pheromone lure dispensers for monitoring *CLW* claimed to contain 1 ± 0.2 mg of the 2 isomers *Z*9, *E*11-14: AC and *Z*9, *E*12-14: AC in ratio 9: 1, *w/w*. The optimum condition of analysis was obtained by using optimized different temperature programming and flow rate. A satisfactory separation of *CLW* pheromone peak *Z*9, *E*11-14: AC and the synergist *Z*9, *E*12-14: AC peak with best peak resolution was performed using GC analysis in column ZB-5: [0.25 µm, 30 m × 0.25 mm, *id.*], carrier gas flow: 1 mL min^{-}1, injector temp.: 280^{o}C, detector (FID) temp.: 300^{o}C and temperature programming mentioned under the chromatographic conditions of *CLW*.

While *SBW* pheromone lures claimed to contain 2 ± 0.25 mg of the 2 isomers, namely; *E*10, *E12*-16: Ald and *Z*11-16: Ald in ratio 99: 1, *w/w*. The GC-method was established from the satisfactory separation of *SBW* pheromone peak of *E*10, *E*12-16: Ald from the synergist *Z*11-16: Ald peak using column ZB-5: [0.25 µm, 30 m × 0.25 mm, *id.*], carrier gas flow: 0.4 mL min^{-1}, injector temp.: 280^{o}C, detector (FID) temp.: 300^{o}C and temperature programming mentioned under the chromatographic conditions of *SBW*.

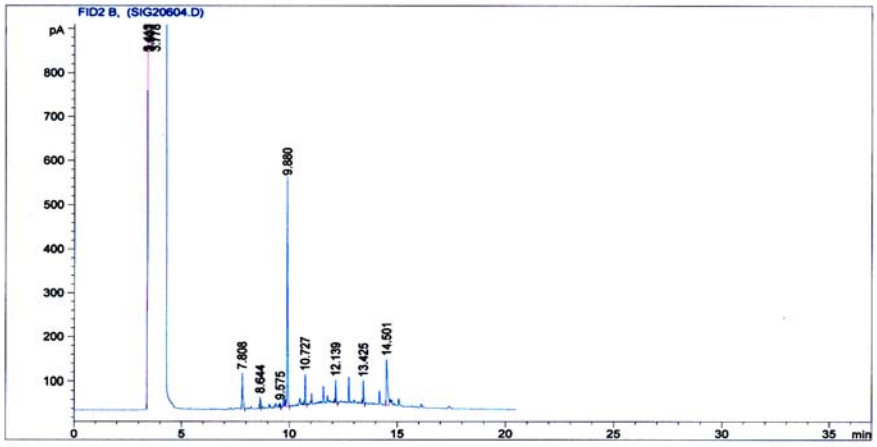
The chromatograms obtained following the assay lures samples are shown in Figures 1 and 2.



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	3.304	PV S	0.0768	7.06607e5	1.09887e5	52.53823
2	3.494	VB S	0.1515	6.35995e5	5.54068e4	47.28798
3	14.149	VB	0.0509	1076.60303	270.42670	0.08005
4	16.853	BB	0.0386	131.87981	49.49746	0.00981
5	17.063	VV	0.0523	1128.97546	279.88507	0.08394
Totals :				1.34494e6	1.65893e5	

Figure-1. GChromatogram containing cis-9, trans-11-tetradecadienyl acetate and cis-9, Trans -12 - tetradecadieny l acetate (9: 1, w/w) in its pheromones lure (using the specified chromatographic conditions).



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	3.413	PV S	0.0426	4.31475e5	1.30437e5	23.15129
2	3.507	VV S	0.1872	1.15388e6	1.02737e5	61.91248
3	3.778	VB S	0.1084	2.76007e5	3.21617e4	14.80947
4	7.808	PB	0.0386	221.54196	79.22459	0.01189
5	8.644	PV	0.0382	70.54768	26.35418	0.00379
6	9.575	VV	0.0293	16.68583	8.73180	0.00090
7	9.880	VP	0.0347	1223.37219	514.81854	0.06564
8	10.727	VV	0.0361	156.70830	67.49857	0.00841
9	12.139	VP	0.0299	97.80854	49.89868	0.00525
10	13.425	VV	0.0376	126.26949	51.47520	0.00678
11	14.501	BV	0.0597	449.56296	101.49915	0.02412
Totals :				1.86372e6	2.66235e5	

Figure-2. GChromatogram containing (10E, 12E)-10 12-hexadecadienal (E10, E12-16 Ald) and (Z)-11-hexadecenal (Z11-16: Ald) (99: 1, w/w) in its pheromone lure [using the specified chromatographic conditions].

a) Release study of cotton leafworm and spiny bollworm lures

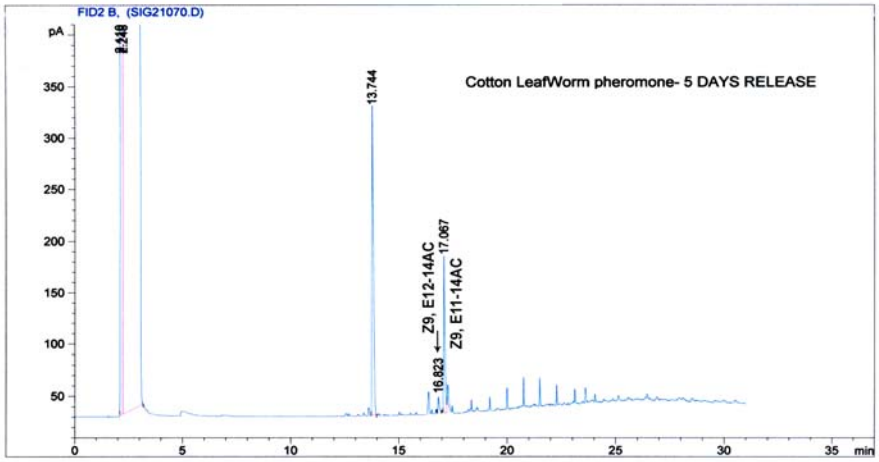
▪ **Cotton leafworm (CLW), spodoptera littoralis**

Each *CLW*-lure was claimed to contain 1 mg lure^{-1} pheromone isomers. Cotton Leaf Worm (*CLW*) pheromone isomers *cis*-9, *trans*-11-tetradecadienyl acetate (major) is the active pheromone while *cis*-9, *trans*-12-tetradecadienyl acetate (minor) is the synergist in ratio 9:1, *w/w* which responsible for optimum efficiency of the *CLW* pheromone in the lure. By monitoring the concentration of the *CLW* pheromone isomers in their lures over 50 days, it was found that the two *CLW* pheromone isomers gradually decrease keeping the same ratio between them (9: 1, *w/w*) to reach their minimum value (~35% of their claimed concentration in the lure after ~30 days release study) which was accompanied by the loss of the biological activity of the trap as shown in Table-2 and Figures 3-5.

Table-2. Quantity and percentage remaining of CLW pheromone components during the experimental period (GC-analysis).

Day	***Z*9, *E*11-14: AC**		***Z*9, *E*12–14: AC**		**Ratio** ***Z*9, *E*11-14: AC** / ***Z*9, *E*12-14: AC**
	Concentration (mg lure $^{-1}$)*	**% Remaining**	**Concentration** (mg lure $^{-1}$)*	**% Remaining**	
0	0.909	101.00	0.101	101.00	9.00 : 1
5	0.829	91.20	0.096	95.05	8.64 : 1
10	0.739	81.30	0.087	86.14	8.49 : 1
15	0.614	67.55	0.072	71.29	8.53 : 1
20	0.488	53.69	0.056	55.45	8.71 : 1
25	0.427	46.97	0.048	47.52	8.90 : 1
30	0.317	34.87	0.039	38.61	8.13 : 1
35	0.294	32.34	0.0299	29.60	9.83 : 1
45	0.235	25.85	0.028	27.72	8.39 : 1

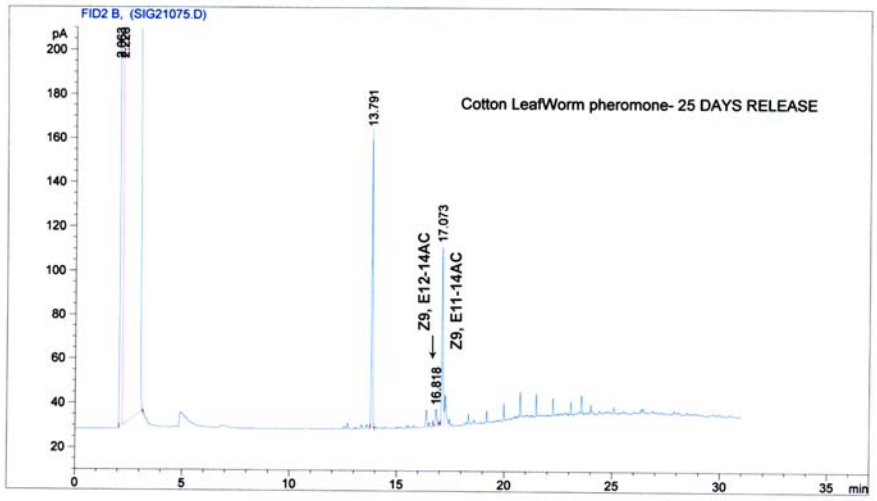
*Labeled concentration of the lure = 1 mg containing *Z*9, *E*11-14: AC (major) and *Z*9, *E*12-14: AC (minor) with ratio
(9: 1, *w/w*)



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.110	BV S	0.0912	1.51219e6	2.31513e5	31.25250
2	2.248	VB S	0.3703	3.32420e6	1.49607e5	68.70133
3	13.744	PB	0.0653	1509.85876	297.01114	0.03120
4	16.823	VB	0.0642	66.39487	15.11924	0.00137
5	17.067	PV	0.0603	657.63239	149.50717	0.01359
Totals :				4.83863e6	3.81581e5	

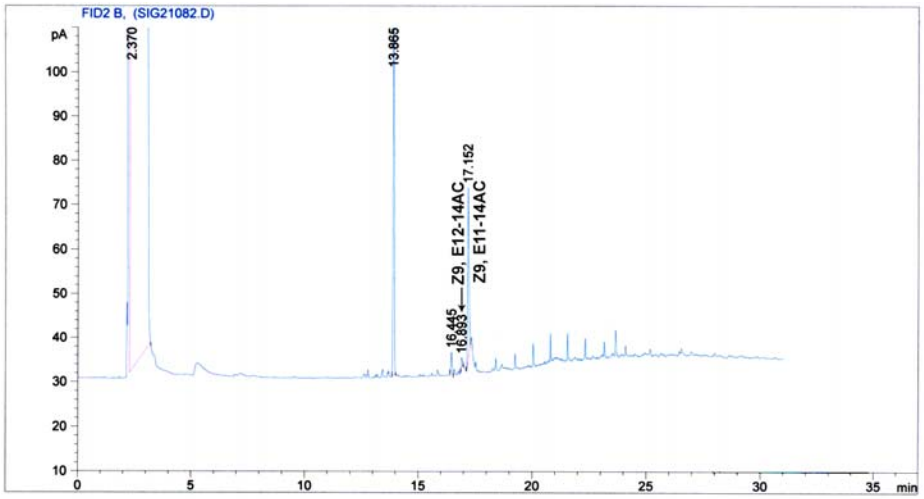
Figure-3. GChromatogram for cis-9, trans-11-tetradecadienyl acetate and cis-9, trans-12 -tetradecadienyl acetate on 5 days release study [using the specified chromatographic conditions].



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.063	PV S	0.0933	1.93974e6	2.65398e5	36.76632
2	2.226	VB S	0.3555	3.33524e6	1.56381e5	63.21716
3	13.791	BB	0.0484	489.19955	134.67451	0.00927
4	16.818	BB	0.0630	30.30594	6.93694	0.00057
5	17.073	PV	0.0608	352.03156	80.80959	0.00667
Totals :				5.27585e6	4.22001e5	

Figure-4. GChromatogram fo r cis-9, trans-11-tetradecadienyl acetate and cis-9, trans -12 –tetradecadienyl acetate on 25 days release study [using the specified chromatographic conditions].



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.370	VB S	0.2885	3.37729e6	1.37258e5	99.98461
2	13.865	PB	0.0421	326.01889	101.88120	0.00965
3	16.445	BP	0.0442	18.69004	5.10755	0.00055
4	16.893	PB	0.0437	9.12398	2.59683	0.00027
5	17.152	PB	0.0502	165.88965	39.95441	0.00491
Totals :				3.37781e6	1.37407e5	

Figure-5. GChromatogram for cis-9, trans-11-tetradecadienyl acetate and cis-9, trans-12-tetradecadienyl acetate on 45 days release study [using the specified chromatographic conditions].

- **Spiny bollworm (SBW), earias insulana**

Each SBW-lure was claimed to contain 2 mg lure^{-1} pheromone isomers. SBW pheromone isomers (10E, 12E)-10, 12-hexadecadienal (major) is the active pheromone while (Z)-11-hexadecenal (minor) is the synergist in ratio 99:1, w/w, which is responsible for optimum efficiency of the SBW pheromone in the lure. By monitoring the concentration of the SBW pheromone isomers in their lures over 50 days under the Egyptian climate conditions (temperature 22^{o}C $_{(min.)}$–34^{o}C $_{(max.)}$ and relative humidity (RH %) 58 ± 5%) using the proposed GC-method, it was found that the concentration of (Z)-11-hexadecenal (synergist) gradually decrease to reach its minimum value (~26% of its claimed concentration in the lure) which was accompanied by the loss of the biological activity of the trap and was not detected after 25 days as shown in Table-3 and Figures 6 and 7.

It was concluded that the trap will lose its validity after 25 days. This result was assessed by biological activity study of the SBW pheromone trap over three successive months (June-August, 2002) reported by AL-Elimi *et al*., [2], where it was found that moths' activity was detected June only while nearly failed to detect the other 2 documented activity peaks (July and August peaks). (Z)-11-hexadecenal is essential for significant catch and satisfactory activity.

b) Uncertainty of analytical determinations

The term uncertainty "of measurement" is defined as a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand [20]. It may arises from many possible sources; such as sampling, matrix effects and interferences, environmental conditions, uncertainties of masses and volumetric equipment, reference values, approximations and assumptions in methods and procedures.

- For a certain measurement result y, the total uncertainty termed as *combined standard uncertainty* and denoted by $U_C(y)$, is an estimated standard deviation equal to positive square root of total variance obtained by combining all uncertainty components as follows:

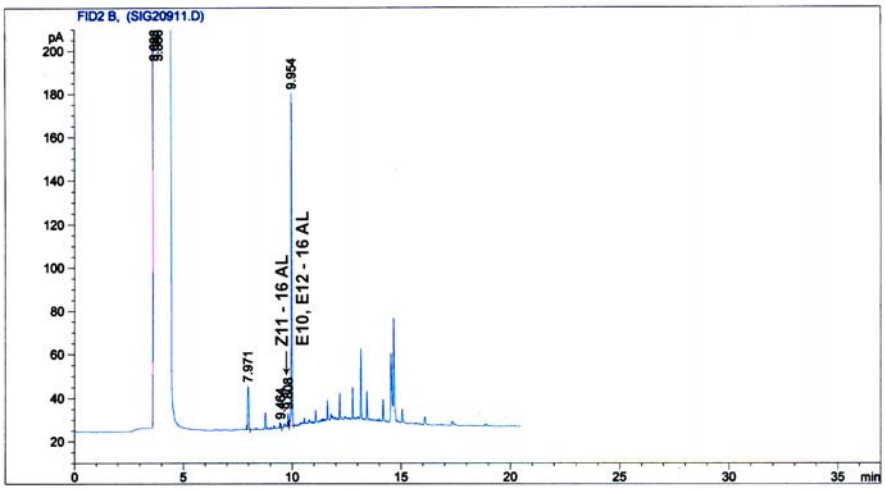
$$U_C(y(x_1, x2,...)) = \sqrt{\sum_{i=1,\,n} c_i^2\, u(x_i)^2}$$

Where $y(x_1, x2...)$ is a function of several parameters $x_1, x2...$, c_i is a sensitivity coefficient evaluated as $c_i=\partial y/\partial x_i$, rate of change in final result with changes in parameter. When the uncertainty on a parameter is expressed directly in terms of its effect on y, c_i is equal to 1.

- For most purposes in analytical chemistry, an *expanded uncertainty* denoted by U should be used. It provides an

interval within which the value of measurand is believed to lie with a higher level of confidence. U is obtained by multiplying $U_C(y)$, combined standard uncertainty by a coverage factor K as: $U = K \times U_c(y)$

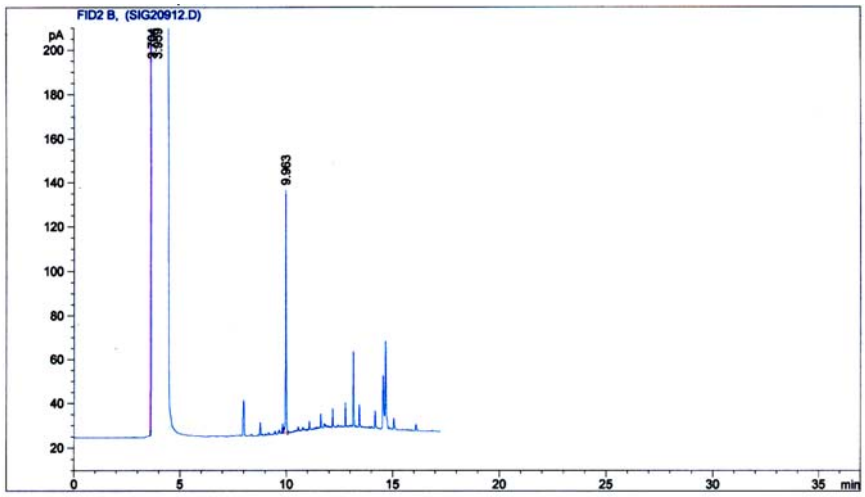
The choice of the factor k is based on the level of confidence desired. For an approximate level of confidence of 95%, K = 2. Tables 4 and 5 illustrate uncertainty of measurement for *Z*9, *E*11-14: AC (major) and *Z*9, *E*12-14: AC (minor) pheromone components of *CLW*. Tables 6 and 7 illustrate uncertainty of measurement for *E*10, *E*12-16: Ald (major) and *Z*11-16: Ald (minor) pheromone components of *SBW*.



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	3.698	PV S	0.1399	3.84137e5	4.57493e4	48.91367
2	3.866	VB S	0.1661	4.00777e5	2.85984e4	51.03256
3	7.971	PB	0.0525	73.28043	19.86351	0.00933
4	9.464	BP	0.0343	4.50123	1.99856	0.00057
5	9.808	PP	0.0259	10.53277	6.17627	0.00134
6	9.954	BB	0.0325	333.98563	152.84958	0.04253
Totals :				7.85337e5	7.45286e4	

Figure-6. GChromatogram for both (10E, 12E)-10, 12-hexadecadienal and (Z)-11-hexadecenal on 25 days release study of SBW lure [using the specified chromatographic conditions].



Area Percent Report

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	3.704	PP S	0.0706	1.03703e5	2.21598e4	61.09319
2	3.959	VB S	0.3811	6.58024e4	2877.41992	38.76511
3	9.963	BB	0.0315	240.52823	110.22313	0.14170
Totals :				1.69746e5	2.51475e4	

Figure-7. GChromatogram for (10E, 12E) -10, 12-hexadecadienal only after 25 days release study of SBW lure [using the specified chromatographic conditions].

Table-3. Quantity and percentage remaining of SBW pheromone components during the experimental period (GC- analysis).

Day	*E*10, *E*12-16:Ald		*Z*11-16:Ald	
	Concentration (mg lure $^{-1}$) *	**% Remaining**	**Concentration** (mg lure $^{-1}$) *	**% Remaining**
0	1.753	88.55	17.00×10^{-3}	85.00
5	1.644	83.03	12.17×10^{-3}	60.85
10	1.416	71.51	10.34×10^{-3}	51.70
15	1.266	63.93	9.39×10^{-3}	46.95
20	0.615	31.06	6.15×10^{-3}	30.75
25	0.542	27.37	5.20×10^{-3}	26.00
30	0.390	19.69	ND^{+}	ND^{+}
35	0.349	17.62	ND^{+}	ND^{+}
40	0.291	14.69	ND^{+}	ND^{+}
45	0.198	10.00	ND^{+}	ND^{+}
50	0.192	9.69	ND^{+}	ND^{+}

Labeled concentration of the lure: 2 mg of both *E*10, *E12*-16: Ald (major) (1.98 mg) and *Z*11-16: Ald (minor) (0.02 mg) with ratio (99: 1, w/w) $^{+}$ ND: Not detected.

Table-4. Uncertainty of measurement of Z9.E11-14AC (major) pheromone component of CLW.

No.	Concentration (mg lure $^{-1}$)	SD
1	0.9093477	0.005314
2	0.906429	
3	0.9176438	
4	0.9059183	**RSD**
5	0.9163393	
6	0.9159371	0.005828
Average*	0.9119359	

(y) Measurand	0.9119359 ± 0.005
True value	Measured value ± U_{Exp}
	0.9119359 ± 0.0514

Parameter	Symbol	Method of determination	Unit of quantity	Value of quantity (x)	Uncertainty of the quantity u (x)	Distribution	Sensitivity coefficient (c_i)	Relative standard uncertainty u (x) / x
Uncertainty due to repeatability	U_{Rep}	الإنحراف المعيارى الناتج من التكرارية	mg	0.9119359	0.005314	Normal 1	1	0.005828
Uncertainty of balance	U_M	ينتج من شهادة معايرة الجهاز العيارى	mg	210	0.032	Normal 2	1	0.000076
Uncertainty of temperature effect	U_T	تنتج من اللايقين لدرجات الحرارة	^oC	5	0.25	Normal 2	1	0.025000
Uncertainty of pipette	U_{Cal}	ينتج من شهادة معايرة الماصة	mL	10	0.025	Rectangular 1.7	1	0.001445
Uncertainty of syringe	U_Y	ينتج من شهادة معايرة السرنجة	uL	4	0.003	Normal 2	1	0.000375
$U_c(y)$	Combined standard uncertainty of $y = \sqrt{(c_1 . U_{Rep})^2 + (c_2 . U_M)^2 + (c_3 . U_T)^2 + (c_4 . U_{Cal})^2 + (c_5 . U_Y)^2}$							0.0257
U_{Exp}	Expanded Uncertainty (K = 2 C.L 95%) = $U_c(y) \times 2$							0.0514

c_i: c is the sensitivity coefficient of the parameter, i = 1, n where n is the no. of the parameter
U: Relative standard uncertainty of the parameter = u (x) / x
* Measured value

Table-5. Uncertainty of measurement of Z9.E12-14AC (minor) pheromone component of CLW.

No.	Concentration (mg lure $^{-1}$)	SD
1	0.1019643	0.00051
2	0.1020848	
3	0.1029734	
4	0.1030299	**RSD**
5	0.1030269	
6	0.1029734	0.00493
Average*	0.1026755	

(y) Measurand	0.10267545 ± 0.0005
True value	Measured value ± U_{Exp}
	0.10267545 ± 0.051

Parameter	Symbol	Method of determination	Unit of quantity	Value of quantity (x)	Uncertainty of the quantity u (x)	Distribution	Sensitivity coefficient (c_i)	Relative standard uncertainty u (x) / x
Uncertainty due to repeatability	U_{Rep}	الإنحراف المعيارى الناتج من التكرارية	mg	0.1026755	0.000506	Normal 1	1	0.004930
Uncertainty of standard balance	U_M	ينتج من شهادة معايرة الجهاز العيارى	mg	210	0.032	Normal 2	1	0.000076
Uncertainty of temperature effect	U_T	تنتج من اللايقين لدرجات الحرارة	^oC	5	0.25	Normal 2	1	0.025000
Uncertainty of pipette	U_{Cal}	ينتج من شهادة معايرة الماصة	mL	10	0.025	Rectangular 1.7	1	0.001445
Uncertainty of syringe	U_Y	ينتج من شهادة معايرة السرنجة	uL	4	0.003	Normal 2	1	0.000375
$U_c(y)$	Combined standard uncertainty of $y = \sqrt{(c_1 . U_{Rep})^2+(c_2 . U_M)^2+(c_3 . U_T)^2+(c_4 . U_{Cal})^2+(c_5 . U_Y)^2}$							0.0255
U_{Exp}	Expanded uncertainty (K = 2 C.L 95%) = $U_c(y) \times 2$							0.051

c_i: c is the sensitivity coefficient of the parameter, i = 1,n where n is the no. of the parameter
U: Relative standard uncertainty of the parameter = u (x) / x
*Measured value

Table-6. Uncertainty of Measurement of E10, E12-16: Ald (major) pheromone component of SBW.

No.	Concentration (mg lure $^{-1}$)	SD
1	1.705	0.0644
2	1.717	
3	1.751	
4	1.849	RSD
5	1.751	
6	1.852	0.03635
Average*	1.770833	

(y) Measurand	1.770833 ± 0.0644
True value	Measured value ± U_{Exp}
	1.770833 ± 0.0883

Parameter	Symbol	Method of determination	Unit of quantity	Value of quantity (x)	Uncertainty of the quantity u (x)	Distribution	Sensitivity coefficient (c_i)	Relative standard uncertainty u (x) / x
Uncertainty due to repeatability	U_{Rep}	الإنحراف المعيارى الناتج من التكرارية	mg	1.770833	0.0644	Normal 1	1	0.036350
Uncertainty of standard balance	U_M	ينتج من شهادة معايرة الجهاز العيارى	mg	210	0.032	Normal 2	1	0.000076
Uncertainty of temperature effect	U_T	تنتج من اللايقين لدرجات الحرارة	^oC	5	0.25	Normal 2	1	0.025000
Uncertainty of pipette	U_{Cal}	ينتج من شهادة معايرة الماصة	mL	10	0.025	Rectangular 1.7	1	0.001445
Uncertainty of syringe	U_Y	ينتج من شهادة معايرة السرنجة	uL	4	0.003	Normal 2	1	0.000375
$U_c(y)$	Combined standard uncertainty of $y = \sqrt{(c_1 . U_{Rep})^2 + (c_2 . U_M)^2 + (c_3 . U_T)^2 + (c_4 . U_{Cal})^2 + (c_5 . U_Y)^2}$							0.0441
U_{Exp}	Expanded Uncertainty (K = 2 C.L 95%) = $U_c(y) \times 2$							0.0883

c_i: c is the sensitivity coefficient of the parameter, i = 1, n where n is the no. of the parameter
U: Relative standard uncertainty of the parameter = u (x) / x
*Measured value

Table-7. Uncertainty of Measurement of Z11-16: Ald (minor) pheromone component of SBW.

No.	Concentration (mg lure $^{-1}$)	SD
1	0.0157	0.00066
2	0.0151	
3	0.0167	
4	0.0169	RSD
5	0.0159	
6	0.0160	0.041282
Average*	0.01605	

(y) Measurand	0.01605 ± 0.001
True value	Measured value ± U_{Exp}
	0.01605 ± 0.0965

Parameter	Symbol	Method of Determination	Unit of quantity	Value of Quantity (x)	Uncertainty of the quantity u (x)	Distribution	Sensitivity coefficient (c_i)	Relative standard uncertainty u (x) / x
Uncertainty due to repeatability	U_{Rep}	الإنحراف المعيارى الناتج من التكرارية	mg	0.01605	0.00066	Normal 1	1	0.041282
Uncertainty of standard balance	U_M	ينتج من شهادة معايرة الجهاز العيارى	mg	210	0.032	Normal 2	1	0.000076
Uncertainty of temperature effect	U_T	تنتج من اللايقين لدرجات الحرارة	^oC	5	0.25	Normal 2	1	0.025000
Uncertainty of pipette	U_{Cal}	ينتج من شهادة معايرة الماصة	mL	10	0.025	Rectangular 1.7	1	0.001445
Uncertainty of syringe	U_Y	ينتج من شهادة معايرة السرنجة	uL	4	0.003	Normal 2	1	0.000375
$U_c(y)$	Combined Standard Uncertainty of $y = \sqrt{(c_1 . U_{Rep})^2 + (c_2 . U_M)^2 + (c_3 . U_T)^2 + (c_4 . U_{Cal})^2 + (c_5 . U_Y)^2}$							0.0482
U_{Exp}	Expanded Uncertainty (K = 2 C.L 95%) = $U_c(y) \times 2$							0.0965

c_i: c is the sensitivity coefficient of the parameter, i = 1, n where n is the no. of the parameter
U: Relative standard uncertainty of the parameter = u (x) / x
*Measured value

CONCLUSIONS

a) It is clear that *CLW* pheromone in its lure is more stable than that of the *SBW* pheromone.
b) The *CLW* Funnel trap should be replaced every 30 days under the Egyptian climate conditions (Temperature $22^{o}C_{(min.)}$ - $34^{o}C_{(max.)}$ and the relative humidity (RH %) 58 ± 5% in shadow).
c) Optimum activity for *SBW* catch was achieved with (10*E*, 12E)-10, 12-hexadecadienal and (*Z*)-11-hexadecenal in ratio 99:1, *w/w*.
d) The concentration of the synergist is essential for the lure activity, which sustained up to ≥ 26% from its claimed concentration in the lure, showing significant catch and satisfactory activity for *SBW* pheromone trap.
e) The *SBW* Funnel trap was efficiently used up to 25 days under the Egyptian climate conditions.
f) GC-FID is considered to be the most promising method for this purpose because of its sensitivity, specificity and versatility. The advantages of this gas chromatographic method were the ease of performance as only one solvent is used and there was no need for complicated pre-treatment. Inspite of being an expensive tool, it was capable of separating the two SBW pheromone isomers from the additives in the lure within a short time (< 10 minutes).

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