

Identification and Evaluation of Cotton and Tomato Plant Volatiles as Attractants for Certain Lepidopterous Insect Pests

Samah S. Ibrahim¹; A. G. El Gendy² and E. A. Omer²

¹ Plant Prot. Res. Inst., Agric. Res. Centre, Dokki, Giza, Egypt

² Medicinal and Aromatic Plants Dept., National Research Centre, Dokki (12622), Giza, Egypt

Email: samah_elasklany@yahoo.com , 7 Nadi El-Saied Street.



ABSTRACT

The objective of the current study was to identification and evaluate the essential oils “Eos.” of cotton and tomato plants to determine the responsive attraction of some pests; *Spodoptera littoralis* (Boisd.), *Pectinophora gossypiella* (Sound.) and *Earias insulana* (Boisd.) which caused a lot of damage to cotton and vegetable crops in Egypt. An experiment of two choice olfactometer systems was used in moths’ bioassay to study the effect of which part of cotton plant and three tomato varieties can attract or repellent of volatile oils EOs. Essential oils of cotton and tomato plants were extracted and chemically identified by Gas Chromatography/Mass Spectrometry (GC/MS). Eighty-six volatile compounds were identified from leaves, bolls and flowers of cotton representing (99.45, 99.57 and 99.02%) from total mass, respectively. The major constituent of their chemical composition was Caryophyllene (17.83, 22.01 and 24.63 % for cotton leaves, flowers and bolls, respectively), D-Limonene recorded the largest compound in tomato varieties (“Real Madrid” 20.35 %, “Bs” 10.49 % and “Alissa” 12.07 %). Laboratory bioassay of the target pest “female and male moths” of *P. gossypiella*, obtained that the highest total response were 83.09, 64.0 and 57.0 % for cotton leaves, flowers and bolls oil. *E. insulana* was the highest attracted moths to cotton bolls (64.91 %), while *S. littoralis* estimated a positive response to cotton leaves (76.6 %). The present results may provide a new strategy in the future to use plant essential oils as a mixture baited on pheromone traps to attract and kill those pests.

Keywords: Plant essential oils, *Spodoptera littoralis*, *Pectinophora gossypiella*, *Earias insulana*.

INTRODUCTION

The Egyptian cotton leaf worm (CLW) *Spodoptera littoralis* (Boisduval) (Lepidoptera :Noctuidae) is a major polyphagous key pest attacking several economically important Egyptian crops specially cotton (*Gossypium* sp.) causing a major loss in the crop (Ahmad, 1988 and Ellis, 2004). One % increase of infestation with the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) would reduce cotton yield about 2.5–6 %. Also, a unit infestation degree by the Spiny bollworm (SBW), *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) is particularly found in Asian and African countries causes a loss of 6–9 % in cotton yield, where it can damage up to three squares and one boll in 1-2 plants. All pests are major biotic constraint to achieve self-sufficiency in ensuring food security. Losses due to pest vary between 10-30% depending on the genetic constituent of crop. The annual crop losses due to these pests amounts to 260000 million per year all over the world (El-Sadaany *et al.* 2009 and Unlu, 2007). Essential oils (EOs) are defined as any volatile oil (s) that have strong aromatic components and give distinctive odor, flavor or scent to the products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. EOs called semiochemicals that found in glandular hairs or secretory cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants. The aromatic characteristics of essential oils provide various functions for the plants including (i) attracting or repelling insects, (ii) protecting themselves from heat or cold, and (iii) utilizing chemical constituents in the oil as defense materials. Many plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic fungi ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and anti-vector activities. (Pare and Tunlinson, 1999; Koul *et al.*, 2008; Rowan, 2011; Svensson, 2012 and Horas *et al.*, 2014).

Plants have evolved highly intriguing ways of defending themselves against insect attacks, including

through emission of defense volatiles. These volatiles serve the plant’s defense by directly repelling phytophagous insects and/or indirectly through attracting natural enemies antagonistic to the herbivores (Tamiru and Khan, 2017). Plant produce chemical cues, both volatile and non-volatile can be used as information for insects searching for a suitable host plant for feeding or oviposition (Hopkins *et al.*, 2009). For many insect herbivores olfactory cues are very important and are used by the insect to orientate towards and accept a specific host plants. (Bruce and Pickett, 2011; Muhammad, 2012). The aim of this study was to evaluate the relationship between the essential oils extracted from cotton different parts and three tomato varieties to attract *S. littoralis*, *P. gossypiella* and *E. insulana* pests. The responsive behavior of the major compounds found in those plants that may be attracted the studied pests.

MATERIALS AND METHODS

1. Sample of cotton and tomato plants

Samples of cotton *Gossypium barbadense* (L.) Malvaceae leaves were taken during different phenological stages of plant life. First and second samples were taken after 45 days of planting date, about 500 g of cotton leaves were cutting and kept in paper pages and transferred to laboratory, followed by flowers samples after the first two months at the beginning of the flowering period. About 500 g of fresh and green leaves from three tomato *Lycopersicon esculentum* Mill Solanaceae varieties “Elissa”, “Real Madrid” and “BS” was also taken from the open field and kept in the refrigerator until the extraction of volatile oils.

2. Extraction of essential oils (EOs)

The EOs from different samples of cotton (leaves, flower and bolls) and leaves of the mentioned tomato varieties were obtained from the entire plants by hydro-distillation using a Clevenger-type apparatus for 3 h according to (Guenther, 1961). The oily layer obtained was separated and dried with anhydrous sodium sulfate. All the EOs were kept in sealed air-tight glass vials covered with aluminum foil and maintained at 4 °C until further analysis.

3. Gas Chromatography/Mass Spectrometry (GC/MS)

The GC-MS analysis of the cotton and tomato samples were carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications, Instrument a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analysis was carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program 60 C for 1 min; rising at 4.0 C/min to 240 C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV using a spectral range of m/z 40-450.

4. Identification of essential oil constituents

The identification of chemical constituents of the essential oils were de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to *n*-alkanes C₈-C₂₂), mass spectrum matching to authentic standards (when available), and Wiley spectral library collection and NSIT library database).

5. Stock culture of *S. littoralis*, *P. gossypiella* and *E. insulana*

Newly hatched larvae of *S. littoralis*, *P. gossypiella* and *E. insulana* were obtained from a colony maintained in the laboratory for several generations at 27±1°C and 75±5% relative humidity (RH). Larvae were reared on a modified artificial diet as described by Abd El-Hafez et al. (1982). About 50 pupae were sexed and when emergence, moths were individually held in (3x2cm) plastic cups containers and fed with distilled water only until they were used in the olfactometer between 1 and 4 days age. Unmated moths were used as targeting females which produce the greatest reduction in oviposition. Moths were used once only in the bioassays. Then newly emerged moths were sexed and transferred immediately to plastic cups (3x2 cm). The adult feeding on water solution on a small piece of absorbent cotton wool.

6. Bioassay system

A two-choice olfactometer based on the design of Beerwinkle et al. (1996) was used in laboratory bioassays. In most cases, fifty moths were tested as a group (1, 2, 3 days for male and female moths of target pests, then put 50 µl of plant oil on filter paper then positioned horizontal at the center of y tube arm and in the other arm put water on filter paper as a control, the air flow was used 60–80 ml/min flower to the left and right of y tube arms. The moths which entered each chamber, and the time of attractiveness were counted and recorded.

7. Y-tube assay

Each adult of target insect pests was used in the Y-tube olfactometer on the same day that it emerged from the pupa to ensure uniformity of age among tested adults. In addition, a 1:1 sex ratio of adults was used for the assay. At the beginning of the assays, unmated moths were placed

individually at the bottom of the Y and were observed until a choice was recorded, or for a maximum of 15 min. A choice was recorded when the insect moved past the mid-point of one of the arms containing a volatile source. Non-responding individuals were marked as such and excluded from the subsequent statistical analysis.

We used two measures to determine attractiveness in the olfactometer.

% positive response (100*T/N), and % total response (100*(T + C)/N),

Where

T = number of moths entering the test chamber

C = number of moths entering control (blank) chamber

N = total number of moths in the olfactometer

We considered % positive response to be our primary criterion of attractiveness.

The extent to which moth activity, especially upwind movement, is stimulated by the presence of volatiles in the body of the olfactometer might influence % total response. However, % positive response is the best indicator (Del Socorro et al., 2010).

8. Statistical analysis:

For each plant tested, of the choice of moths to enter the test chamber, the numbers of moths entered the test and control chambers in the three replicate runs for each sex were compared with those in the 'blank' olfactometer using the number of moths caught in the test chamber as a percentage of the total moths placed in the olfactometer (% positive response), while the second one, with the number of moths caught in both the test and control chambers as a percentage of the total (% total response) (Gregg et al., 2010). Statistical analysis was carried out using analysis of variance (ANOVA) that conducted on all data (SAS Institute Inc., 1996).

RESULTS

1. Chemical Composition.

Chemical composition of the EOs of cotton leaves, flower and boll and tomato varieties as well were studied. The main constituents of each oil, their relative percentage of the total chromatogram area, and Kovats index are summarized in Table 1 and Fig 1. Insulated the chemical composition of cotton and tomato oil and some compounds may be induced attractive of target moths of studies. In the EOs of cotton leaves, sixty-six compounds were identified, representing 99.49% of the total oil, the major constituents being caryophyllene (17.83%), α-Pinene (16.65%), 2-β-Pinene (13.27%) and Humulene (9.20%). The EOs of cotton flower were analyzed, fifty-four compounds were identified, representing 99.02% of the total oil, the major constituent being caryophyllene (22.01%). Other compounds were Humulene (9.74%), Isobutyl phthalate (7.35%), Caryophyllene oxide (6.67%) and Farnesol (5.48%). On the other hand, the EO of cotton bolls were 45 compounds identified, representing 99.57% of the total oils, the main components being caryophyllene (24.63%), α-Pinene (23.45%), heptacosane (14.33%), 2-β-Pinene (7.59%) and Humulene (7.47%). Cotton (*Gossypium hirsutum* L.) plants store large amounts of monoterpenes and sesquiterpenes, several of those induced compounds are acyclic terpenoids [β-Pinene, Limonene and α-Humulene] (Loughrin et al., 1994; Essien et al., 2010).

Table 1. Relative percentage of volatile compounds of the essential oil of Leaves, bolls and flowers cotton plants

NO	Compound name	RT	Cotton		
			Leaves	Bolls	Flowers
1	1-Hexanol, 2-ethyl-	2.19	-	-	0.12
2	3-Hexanone	2.73	-	0.05	-
3	2-Hexanone	2.78	-	0.08	-
4	Hexanal	2.94	0.84	0.04	-
5	2-Hexenal	3.79	1.48	0.06	0.22
6	1-Hexanol	3.97	0.16	-	-
7	α -Thujene	5.23	0.13	0.06	-
8	α -Pinene	5.47	16.65	23.45	4.35
9	Camphene	5.95	0.73	0.27	-
10	Sabinene	6.59	0.3	-	-
11	2- β -Pinene	6.81	13.27	7.59	2.39
12	β -Myrcene	7.05	0.72	0.12	0.35
13	Furan, 2-pentyl	7.13	0.21	-	-
14	trans-2-(2-Pentenyl)furan	7.44	0.18	-	-
15	α -Phellandrene	7.7	0.21	0.12	0.4
16	4-Ethyl-2-hexynal	8.12	0.37	-	--
17	p-Cymene	8.39	0.28	0.14	0.51
18	dl-Limonene	8.5	3.63	1.96	4.04
19	β -Phellandrene	8.6	0.92	0.62	0.24
20	trans- β -Ocimene	8.69	0.24	0.38	0.12
21	cis- β -Ocimene	9.09	3.27	3.72	2.53
22	Y- Terpinene	9.59	0.94	0.4	1.7
23	Terpinolene	10.64	0.22	0.08	-
24	L-linalool	11.28	0.46	-	0.13
25	Nonanal	11.58	-	-	0.36
26	Citronella	13.61	0.26	-	0.2
27	2,6-Nonadienal, (E,Z)-	13.75	0.11	-	-
28	Terpinene-4-ol	14.89	0.29	-	0.33
29	Z-3-hexenyl butanoate	15	0.14	0.09	0.11
30	Dill Ether	15.14	-	0.05	-
31	α -Terpineol	15.6	0.89	0.13	0.63
32	Methyl chavicol	15.76	0.34	0.17	1.05
33	β -Cyclocitral	16.68	0.21	0.04	-
34	Z-3-hexenyl 2-methylbutanoate	16.98	0.11	-	-
35	Geraniol	17.94	0.12	-	-
36	Bornyl acetate	19.46	1.08	0.45	0.9
37	Myrtenyl acetate	21.25	-	0.08	0.45
38	α -Cubebene	22.05	-	0.15	-
39	6-Tridecen-4-yne, (E)-	22.16	0.27	-	-
40	α -Copaene	23.36	0.74	7.21	0.64
41	3,7-Octadiene-2,6-diol, 2,6-dimethyl	23.76	0.14	-	0.14
42	6-Tridecen-4-yne, (Z)-	24.38	0.25	-	-
43	Caryophyllene	25.38	17.83	24.63	22.01
44	Glycyl-L-proline	26.36	0.23	-	-
45	Nerylacetone	26.74	0.72	-	0.22
46	Humulene	26.96	9.2	7.47	9.74
47	1,3,5-Cycloheptatriene, 3,4-diethyl-7,7-dimethyl-	27.64	0.13	0.05	-
48	γ -Muuroolene	27.76	-	0.14	-
49	(E)- β -Ionone	28.07	0.41	-	0.13
50	Cubedol	28.7	0.14	0.05	0.5
51	α -Muuroolene	28.79	-	0.17	-
52	1-Adamantyl methyl ketone	28.85	0.33	-	-
53	δ -Cadinene	29.6	1.11	3.08	2.17
54	β -copaene	29.82	0.11	0.04	-
55	(\pm)-trans-Nerolidol	31.43	0.16	-	-
56	Epiglobulol	32.11	0.13	-	0.28
57	(-)-Caryophyllene oxide	32.37	2.27	0.82	6.67
58	Ledol	33.28	-	-	0.15
59	Humulene epoxide II	33.53	0.56	0.15	1.37
60	trans-Sesquisabinene hydrate	34.2	0.44	0.16	1.12
61	Longipinene epoxide	34.47	-	-	0.57
62	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	34.62	0.15	-	1.04
63	.tau.-Cadinol	34.81	0.31	0.09	0.47
64	tau.-Muurolol	34.91	0.12	-	0.2
65	Aromadendrene oxide-(2)	35.06	0.59	0.16	2.08
66	α -Cadinol	35.37	0.69	0.16	2.32
67	Lanceol, cis	35.97	-	-	0.7
68	Nootkatone (CAS)	37.15	0.13	-	0.88
69	2,6,10-Dodecatrienal, 3,7,11-trimethyl-	37.44	0.86	0.05	2.81
70	Farnesol	37.58	3.13	-	5.48
71	trans-Farnesal	38.5	1.98	0.11	4.95
72	Caryophyllene oxide	38.7	-	-	0.23
73	Trans,Trans-Farnesol 1tms	40.54	-	-	0.19
74	Isobutyl phthalate	43.04	-	-	7.35
75	Butyl phthalate	46.39	-	-	0.17
76	(+)-Nerolidol	48.41	-	0.18	0.18
77	trans-Phytol	51.18	5.25	-	0.93
78	2,4-Pentadien-1-ol, 3-pentyl-, (2Z)-	53.59	-	-	0.68
79	1-Iodo-2-methylundecane	55.08	-	0.22	-
80	9-Hexadecen-1-ol, (Z)-	55.98	0.23	-	-
81	10-Heneicosene (c,t)	56.14	0.27	-	-
82	Octadecane	56.93	1.33	-	1.28
83	Nonadecane	59.76	0.11	-	0.24
84	Heptacosane	61.48	-	14.33	-
85	(Z)6-Pentadecen-1-ol	61.67	0.17	-	-
86	n-1-Eicosanol	61.79	0.2	-	-
	Total		99.45	99.57	99.02

RT means retention time

The EOs. extracted from different varieties of tomato presented in Table 2, GC-MS analysis of tomato “Real Madrid” identified 40 constituents; representing 99.81 % of the total oil, the main components were D-Limonene (20.35%), γ -terpinene (16.94 %), estragol (13.53%) and o-cymene (7.99 %). When the EO of tomato variety “BS” was analyzed, forty compounds were identified, representing 99.84 % of the total oil, the major constituent being estragol (13.02%), γ -terpinene (10.83 %), D-Limonene (10.49%) and B-phellandrene (10.16%). In tomato variety “Alissa”, the EO were recorded and identified 40 components, representing 99.95 % of the total oil, the main constituents being; D-Limonene (12.07 %), γ -terpinene (11.24 %), estragol (7.06%), caryophyllene (5.79%) and o-cymene (4.60%). α -humulene, β -myrcene, ocimene, (D)- limonene, (Z) - 3-hexenylacetate and nonanal, constituted the main method used for recognition. Glomeruli were numbered according to a morphological atlas of *S.littoralis* males and females ALs (Saveer et al., 2012; Couton et al., 2009).

2. Bioassays of plant volatiles for moths

a. Tomato plant

On the % positive response of our experimental, responses of males compared with females attractiveness to males was strongly correlated with attractiveness to females specially “Alissa” variety. *S. littoralis* showed a significantly higher response to volatiles from different varieties of tomato leaves, as it recorded respectively 80, 56.6 and 50% (Fig 2).

Spodoptera littoralis (Boisd.), estimated a positive response to cotton leaves about 76.6 %, the highest number of moths attracted were 80, 56.6 and 50 % by the tomato varieties “Bs”, “Alissa” and “Real Madrid”. Data in Table 3 showed that the number of *S. littoralis* females attracted were respectively 0.56 ± 0.09 , 0.53 ± 0.14 and 0.44 ± 0.08 for “Real Madrid”, “Alissa” and “Bs” of tomato varieties.



Fig. 1. Isolation and evaluation of cotton and tomato volatile oils to major pests.

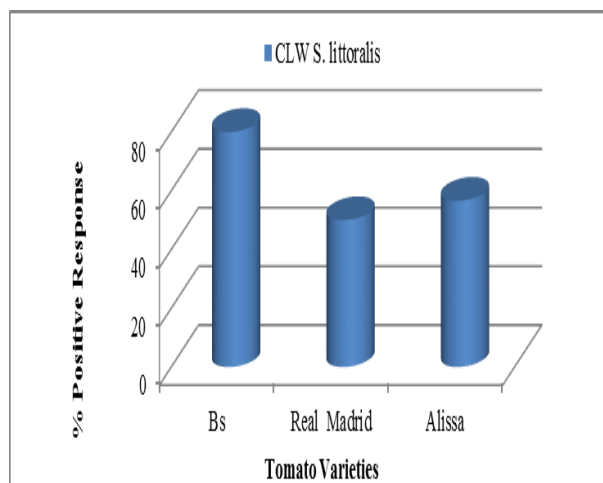


Fig. 2. % positive response of CIW *S. littoralis* to volatile compounds in tomato varieties.

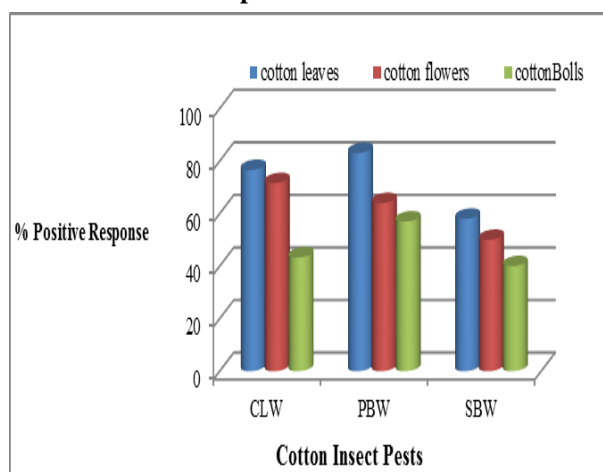


Fig. 3. % positive response of females cotton insect pests CIW, PBW, SBW to volatile compounds in cotton plant.

b. Cotton plant

The highest total responses were respectively 83.09, 64.0 and 57.0 % for cotton leaves, flowers and bolls oil. The results revealed that *E. insulana* was the highest attracted moths to cotton bolls (64.91 %). Data in Table 3, investigated that the highest number of attracted *S. littoralis* females were respectively 0.75 ± 0.08 , 0.74 ± 0.08 and 0.42 ± 0.10 for cotton leaves, flowers and bolls that may be due to 2- β -Pinene (13.27%). Also the time response showed significant differences between all cotton parts. *P. gossypiella* females showed highly significance to cotton leaves, flowers and bolls as well (0.71 ± 0.06); while *E. insulana* revealed that no significant differences between female moths attractive time, as it recorded respectively 0.12, 0.13 and 0.21 Sec. that may be due to the high amount of heptacosane 14.33 %. On cotton bolls (Table 4, Fig.3). In the present study we investigated the wind tunnel experiments to show that males were attracted towards the leaves, flowers and bolls, as it recorded a significance differences between leaves and bolls, but the female attraction are active at night and mate soon after emergence and started laying eggs from the third day onwards. The female lays small flat eggs singly, usually close to or upon the open fruit, it laying up to 456 eggs with an average of 125.

Table 2. Relative percentage of volatile compounds of the essential oil of different cultivars from tomato plants

	Compounds	RT	Alissa	Bs	Real Madrid
1	α-Pinene	5.44	2.18	0.46	
2	β-Pinene	6.77	0.79		
3	β-Myrcene	7.04	1.02	0.98	1.63
4	α-Terpinene	7.39		2.86	
5	α-Phellandrene	7.7	1.15	1.7	1.84
6	p-Mentha-1,4(8)-diene	8.04		0.46	0.56
7	o-Cymene	8.38	4.6	4.26	7.99
8	D-Limonene	8.49	12.07	10.49	20.35
9	β-Phellandrene	8.6		10.16	1.31
10	γ-Terpinene	9.59	11.24	10.83	16.94
11	p-Mentha-1,4(8)-diene	10.62		1.05	0.56
12	L-Linalool	11.28	2	3.26	2.42
13	Cis-Sabinene Hydrate	11.4			0.53
14	Nonanal	11.58		1.11	0.5
15	α-Phellandren-8-ol	14.17	0.9	0.47	
16	trans-p-Menth-2-en-1,8-diol	14.59	0.84	0.52	
17	Terpinene-4-ol	14.89	1.63	1.36	2.75
18	Dill ether	15.14	2.45	6.91	1.48
19	α-Terpineol	15.59	0.9	0.5	0.47
20	Estragol	15.76	7.06	13.02	13.53
21	Decanal	16.03		0.71	0.51
22	β-Cyclocitral	16.67		0.54	
23	Nerol	16.74		0.49	
24	Pulegone	17.55		0.45	
25	Carvone	17.91	3.92	4.62	2.19
26	Piperitone	18.31		0.88	
27	Anethole	19.81	1.19	4.28	1.12
28	Carvacrol	19.92	0.69		0.92
29	Thymol	20.27		0.52	
30	Myrtenyl acetate	21.24	0.68	1.05	0.51
31	δ-Elemene	21.52	1.46	0.7	
32	Geranyl acetate	23.68	0.67	0.93	0.58
33	Lanceol, cis	24	0.65		0.5
34	6-Tridecen-4-yne, (E)-	24.36		0.62	0.76
35	Nothosmyrnol	24.98	1.51	1.39	
36	β-Caryophyllene	25.32	5.79	3.9	2.17
37	3-Caren-10-al	26.35	0.63	0.64	
38	Neryl Acetone	26.73	1.08	0.73	1.03
39	Humulene	26.92	1.73	0.94	0.54
40	β-Ionone	28.06	4.17	2.18	3.84
41	(-)-Caryophyllene oxide	32.35	1.51	0.64	0.69
42	Dillapiole	34.02	3.24		
43	2,4-Dodecadienal, (E,E)-	34.09			0.53
44	(+) spathulenol	34.28	3.28		0.69
45	isospathulenol	34.67	0.87		
46	Cedren-13-ol, 8-	35.64	1.81		0.51
47	Tetradecanal	37.64	1.03	1.15	0.91
48	Hexahydrofarnesyl acetone	42.15	1.91		0.48
49	Isobutyl phthalate	43.01			0.61
50	9,17-Octadecadienal, (Z)-	43.92			0.57
51	9,12,15-Octadecatrienal	44.14	4.14	1.59	2.83
52	Farnesyl acetone C	44.64	0.7		0.55
53	Methyl palmitate	45.18	0.92		1.07
54	Linolelaidic acid, methyl ester	50.74			0.51
55	Linolenic acid, methyl ester	50.96	3.81	0.65	2.52
56	Phytol (CAS)	51.17	2.37		
57	Eicosane, 2-methyl-	58.09	1.44		
	Total		99.95	99.84	99.81

Table 3. Mean response of *S. littoralis* moths attracted to tomato and cotton plants under laboratory conditions.

Treatments	Tomato varieties volatile Leaves oil "100 µl"			cotton plant volatiles oil" 100 µl"				F.	LSD.
	B s	Real Madrid	Alissa	Leaves	Flowers	bolts			
Female moths	0.44 ± 0.08 bc	0.56 ± 0.09 abc	0.53 ± 0.14 abc	0.75 ± 0.09 a	0.74 ± 0.08 ab	0.41 ± 0.10 c	2.6	0.27	
Attracted Time "min."	0.25 ± 0.09 ab	0.09 ± 0.21b	0.27 ± 0.23 a	0.15 ± 0.02 ab	0.15 ± 0.02 ab	0.13 ± 0.01 ab	1.62	0.15	
Control " Water" 100 µl"	0.56 ± 0.08 a	0.44 ± 0.09 ab	0.61 ± 0.14 a	0.16 ± 0.08 b	0.22 ± 0.08 b	0.70 ± 0.09 a	5.4	0.26	
Attracted Time"min." control"	0.47 ± 0.09 a	0.09 ± 0.08 b	0.09 ± 0.03 b	0.04 ± 0.02 b	0.04 ± 0.01 b	0.10 ± 0.02 b	7.02	0.24	
Male moths	0.40 ± 0.13 b	0.57 ± 0.09 ab	0.63 ± 0.08 ab	0.75 ± 0.07 a	0.73 ± 0.08 a	0.42 ± 0.08 b	2.79	0.24	
Attracted Time "min."	0.12 ± 0.05 ab	0.08 ± 0.02 bc	0.16 ± 0.08 a	0.09 ± 0.01bc	0.10 ± 0.01bc	0.04 ± 0.01c	5.26	0.05	
Control " Water" 100 µl"	0.56 ± 0.13 a	0.40 ± 0.09 a	0.43 ± 0.08 a	0.31 ± 0.08 a	0.29 ± 0.08 a	0.54 ± 0.07 a	1.49	0.25	
Attracted Time"min." control"	0.24 ± 0.07 a	0.07 ± 0.02 b	0.09 ± 0.02 b	0.03 ± 0.02 b	0.01 ± 0.02 b	0.07 ± 0.01b	6.93	0.06	

Mean with the same letter within the same coelom is not significantly different (P<0.05).

Table 4. Mean response of Female *S. littoralis*, *P. gossypilla* and *E. insulana* moths attracted to cotton plant volatiles under laboratory conditions.

Cotton pests	Treatments	cotton plant volatiles oil				
		Leaves	Flowers	bolts	F.	LSD.
<i>S. littoralis</i>	Female moths	0.75 ± 0.09a	0.74 ± 0.08a	0.42 ± 0.10b	4.09	0.26
	Time	0.12 ± 0.02a	0.15 ± 0.02a	0.05 ± 0.01b	5.7	0.05
	Control	0.17 ± 0.08b	0.22 ± 0.08b	0.71 ± 0.09a	12.01	0.23
	Time	0.04 ± 0.02b	0.04 ± 0.01b	0.11 ± 0.03a	3.85	0.05
<i>P. gossypilla</i>	Female moths	0.49 ± 0.06b	0.71 ± 0.06a	0.60 ± 0.08ab	2.9	0.18
	Time	0.13 ± 0.03a	0.12 ± 0.01a	0.09 ± 0.01a	0.78	0.06
	Control	0.51 ± 0.06a	0.31 ± 0.63a	0.38 ± 0.08a	2.61	0.18
	Time	0.08 ± 0.01a	0.05 ± 0.01a	0.07 ± 0.02a	1.40	0.04
<i>E. insulana</i>	Female moths	0.44 ± 0.10a	0.57 ± 0.09a	0.61 ± 0.10a	1.24	0.22
	Time	0.13 ± 0.03ab	0.12 ± 0.02b	0.21 ± 0.05a	2.73	0.08
	Control	0.47 ± 0.10a	0.48 ± 0.09a	0.30 ± 0.10a	1.41	0.23
	Time	0.10 ± 0.02a	0.09 ± 0.02a	0.12 ± 0.05a	0.18	0.07

Mean with the same letter within the same coelom is not significantly different ($P \leq 0.05$).

Table 5. Mean response of Male *S. littoralis*, *P. gossypilla* and *E. insulana* moths attracted to cotton plant volatiles under laboratory conditions.

Cotton pests	Treatments	Cotton plant volatiles oil				
		Leaves	Flowers	bolts	F.	LSD
<i>S. littoralis</i>	Male moths	0.75 ± 0.07a	0.72 ± 0.08a	0.42 ± 0.08b	5.79	0.21
	Time	0.09 ± 0.01a	0.10 ± 0.01a	0.05 ± 0.01b	7.39	0.03
	Control	0.31 ± 0.08b	0.27 ± 0.08b	0.67 ± 0.07a	3.17	0.22
	Time	0.03 ± 0.01a	0.10 ± 0.02b	0.07 ± 0.01a	5.50	0.03
<i>P. gossypilla</i>	Male moths	0.61 ± 0.05a	0.55 ± 0.06a	0.52 ± 0.07a	0.69	0.16
	Time	0.11 ± 0.01a	0.08 ± 0.01a	0.10 ± 0.02a	1.36	0.04
	Control	0.38 ± 0.05a	0.46 ± 0.06a	0.45 ± 0.06a	0.91	0.16
	Time	0.06 ± 0.01a	0.08 ± 0.06a	0.09 ± 0.02a	1.24	0.04
<i>E. insulana</i>	Male moths	0.55 ± 0.08a	0.53 ± 0.08a	0.38 ± 0.10a	1.58	0.20
	Time	0.16 ± 0.04a	0.15 ± 0.03a	0.19 ± 0.04a	0.38	0.10
	Control	0.44 ± 0.08a	0.49 ± 0.08a	0.38 ± 0.10a	0.40	0.23
	Time	0.12 ± 0.03a	0.08 ± 0.01a	0.15 ± 0.05a	1.78	0.07

DISCUSSION AND CONCLUSION

That the essential oils extracted from cotton leaves are more attractive than that extracted from flowers and bolls as well as tomato varieties, these results are in harmony with that of Del Socorro *et al.* (2010) who recorded that potential sources of moth attractants might be used in managing *Helicoverpa armiger* (Hubner), include any plant which is attractive for adult nectar for aging and females for oviposition and feeding behaviors. (Sankara *et al.*, 2014, Morrison *et al.*, 2016) investigated that when females have a choice between pure air and the air emanating from their complex host of origin, they are attracted to the air tainted by the volatile compounds they have become accustomed to. They spent significantly more time in the branch of the tube leading to the odorous air than in the tube leading to the pure air. The current results along with the obtained data on laboratory assays to predict the behavioral effect of essential oils as attracted female moths of lepidopteran insect pests suggest their ability use as formulation in the field treatments in the future. Volatile attractants used in an attract-and-kill strategy could be useful in the integrated approach to control this pest on cotton. The next step is to determine the impact of the attract-and-kill approach on actual ovipositor and on non-target beneficial in the field. In addition, kairomone of plant volatiles as biological insect control methods produce rather subtle effects compared to conventional insecticides, which are lethal upon contact. Single biological control methods can therefore rarely replace insecticide treatments and the available biological tools should be developed as components of an integrated crop management program.

ACKNOWLEDGMENTS

Thanks to Prof. Dr. Yoosuf Eldeeb (Department of Cotton Pests, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt) for his help, encouragement and guidance during the course of this work.

REFERENCES

- Abd El-Hafez, Alia, Metwally, A.G., Saleh, M.R.A., 1982. Rearing pink bollworm *Pectinophora gossypiella* (Saund.) on kidney bean diet in Egypt. Res. Bull., Fac. Agric., Zagazig Univ., 576, 1-10.
- Ahmad, T.R., 1988. Field studies on sex pheromone trapping of cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae). Journal of Applied Entomology, 105(1/5), 212-215.
- Beerwinkle, K.R., Shaver, T.N., Lingren, P.D. and Raulston, J.R. 1996. Freechoice olfactometer bioassay system for evaluating the attractiveness of plant volatiles to adult *Helicoverpa zea*. *Southwestern Entomologist* 21, 395-405.
- Bruce, T.J.A., Pickett, J.A., 2011. Perception of plant volatile blends by herbivorous insects - Finding the right mix. *Phytochemistry*, 72(13), 1605-1611.
- Couton L., Minoli S., Kieu K., Anton S., Rospars, J.P., 2009. Constancy and variability of identified glomeruli in antennal lobes: computational approach in *Spodoptera littoralis*. *Cell Tissue Res.*, 337, 491-511.

- Del Socorro, A.P., Gregg, P.C., Alter, D., Moore, J.C., 2010. Development of a synthetic plant volatile-based attracticide for female noctuid moths. I. Potential sources of volatiles attractive to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Australian Journal of Entomology, 49, 10-20.
- Ellis, S.E. 2004. New Pest Response Guidelines: *Spodoptera*. US DA/APHIS/PPQ/PDMP. <http://www.aphis.usda.gov/ppq/manuals>
- El-Sadaany, G., El-Shaarawy, M.F. and El-Refaei, S.A. 2009. Determination of the loss in cotton yield as being affected by the pink bollworm *Pectinophora gossypiella* (Saund.) and the spiny bollworm, *Earias insulana* (Biosduval) Zeitschrift für Angewandte Entomologie, 79, 357-360.
- Essien, E.E., Aboaba, O.S. and Ogunwande, A. I. 2010. Constituents and antimicrobial properties of the leaf essential oil of *Gossypium barbadense* (Linn.). Journal of Medicinal Plants Research, 5(5) 702-705.
- Gregg, P.C., Socorro, A.P., Alter, D. and Moore, C.J. 2010. Development of a synthetic plant volatile-based attracticide for female noctuid moths. II. Bioassays of synthetic plant volatiles as attractants for the adults of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). 30. Australian Journal of Entomology, 49, 21-30.
- Guenther G., 1961. The Essential Oils. Nastrand Press, New York, USA
- Hopkins, R.J., van Dam, N.M., van Loon, J.J.A. 2009. Role of Glucosinolates in Insect-Plant Relationships and Multitrophic Interactions. Annual Review of Entomology 54, 57-83.
- Horas, R.V., Mario Antonio Navarro Da Silva, Vinicius Annes, Beatriz Helena N. Sales Maia, Francisco A. Marques, and Maria Fátima das Graças Fernandes da Silva. 2014. Noctuidae-Induced Plant Volatiles Current Situation And Prospects quim.nova, 37(10), 1663-1669.
- Koul, O., Sureshwalia and Dhaliwal, G.S. 2008. Essential Oils as Green Pesticides: Potential and Constraints, Biopestic. Int. 4(1): 63-84.
- Loughrin J.H., Manukian A., Heath R.R., Turlings T.C.J., Tumlinson J.H. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. Proc Natl Acad Sci, USA 91, 11836-11840.
- Morrison, William R., Adam Ingraio, Jared Ali & Zsofia Szendrei 2016. Identification of plant semiochemicals and evaluation of their interactions with early spring insect pests of asparagus, journal of plant interactions, 11(1): 11-19.
- Muhammad, Z. A. 2012. Influence of herbivore-induced changes in host plants on reproductive behaviors in *Spodoptera littoralis*, Swedish University of Agricultural Sciences, PHD, SLU Service/Repro, Alnarp 2012.
- Pare, P.W. and Tumlinson, J.H. 1999. Plant Volatiles as a Defense against Insect Herbivores Plant Physiology, October 121, 325-331.
- Rowan, D.D. (2011). Volatile Metabolites *Metabolites* 2011, 1, 41-63.
- Sankara, F., Dabiré, L.C.B., Ilboudo, Z., Dugravot, S., Cortesero, A.M., Sanon A. 2014. Influence of host origin on host choice of the parasitoid *Dinarmus basalis*: Does upbringing influence choices later in life? *Journal of Insect Science* 14:26. <http://www.insectscience.org>
- SAS Institute Inc. 1996. SAS/STAT Software. Changes and Enhancements through Release 6.12. SAS Institute Inc., Cary, North Carolina.
- Saveer, A.M., Kromann, S.H., Birgersson G., Bengtsson, M. Lindblom T and Balkenius, A. 2012. Floral to green: mating switches moth olfactory coding and preference. Proc R Soc B Biol Sci.; 279, 2314-22.
- Svensson, T. 2012. Induced plant volatiles and their effect on *Spodoptera littoralis* choice of host plant, Online publication: <http://stud.epsilon.slu.se>
- Tamiru, A. and Khan, Z. R. 2017. Volatile Semiochemical Mediated Plant Defense in Cereals: A Novel Strategy for Crop Protection, Agronomy, 7, 58.
- Unlu, L. and Ozturk, I. (2007). Relationships between the numbers of adult male pink bollworm *Pectinophora gossypiella* Saund., catches on pheromone traps and infestation ratio of cotton bolls. Journal of Entomology, 4(5): 397-400.

تعريف وتقييم الزيوت الطيارة لنباتات القطن والطماطم لجذب بعض آفات حشرية الأجنحة سماح سيد إبراهيم^١، عبد الناصر جابر الجندي^٢ و السيد ابو الفتوح عمر^٢ ^١ مركز البحوث الزراعية - معهد بحوث وقاية النباتات - قسم دودة ورق القطن ^٢ المركز القومي للبحوث - قسم النباتات الطبية و العطرية

تستهدف الدراسة تقييم العلاقة بين الزيوت الطيارة لنباتات القطن والطماطم لتحديد الاستجابة النسبية لبعض الآفات الهامة التي تسبب ضرر لنباتات القطن وبعض محاصيل الخضار بمصر مثل دودة ورق القطن، دودة اللوز القرنفلية و دودة اللوز الشوكية. تم استخدام جهاز ثنائي الاختيار Olfactometer 2 choice في تحديد مدى استجابة الزيوت المستخلصة من نباتات القطن و ثلاث هجن للطماطم و هل هي جاذبة ام طاردة. حيث تم استخلاص الزيوت الطيارة لنباتات القطن والطماطم و تعريف التركيب الكيماوي باستخدام جهاز GSMC و اظهرت النتائج وجود ٦٨ مركب باورق و ازهار و لوز القطن بنسبة ٩٩.٤٨ %، ٩٩.٥٧ % و ٩٩.٠٢ % علي التوالي. و اكثر المركبات كاربيلين يوجد بنسبة ١٧.٨٣، ٢٢.٠١ و ٢٤.٦٣ % في الاوراق و الازهار و لوز القطن علي الترتيب و مركب D Limone بنسبة ١٧.٨٣، ٢٢.٥ و ٢٤ % بهجن الطماطم ريال مدريد، BS و اليسا علي التوالي). و سجلت فرشاة الاناث و الذكور لدودة اللوز القرنفلية اعلي استجابة للزيت اوراق القطن، الازهار و اللوز بنسبة ٨٣.٥٩ %، ٦٤ % و ٧٥.٥ % علي التوالي. بينما اعلي استجابة لفرشاة دودة اللوز الشوكية تجاه الزيت المستخلص من لوز القطن و دودة ورق القطن تجاه زيت الورق و بالتالي تمدنا النتائج بأسلوب جديد و استراتيجي متطورة باستخدام خليط من الزيوت الطيارة لنباتات كوسيلة لجذب اناث الفرشاة بالمصائد الجاذبة.