GAS CHROMATOGRAPHY COMBINED TO MASS SPECTROMETRY ANALYSES OF SOME CITRUS PEELS BIOACTIVE COMPOUNDS AGAINST THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

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(Manuscript received 1 November 2017)

Abstract

Hexane (Hex) extracts of fresh navel orange (*citrus sinensis*) and lemon (*citrus limon*) peel were prepared to isolate and identify toxic bioactive secondary metabolites for potential use as natural ecofriendly biopesticides against the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Hex extracts were fractionated by column chromatography. The fractions that showed activity against the larvae, were identified and quantified by gas chromatography combined to mass spectrometry(GC/MS), after checking component profile by thin layer chromatography (TLC). The results revealed that F_2 and F_3 fractions were the toxic active fractions of both lemon and orange peel Hex extracts, but lemon fractions were more toxic than orange ones. F_2 and F_3 fractions of orange extract causd 60 and 20% larval mortality, respectively. Both lemon extract fractions caused 100% morality. Orange fractions had approximately equal d-limonene content (≈86%) as illustrated by mass spectral fragmentation pattern. However, F_2 fraction contained slightly higher amounts of linalool, α-terpinol and citral than F_3 fraction. The active fraction of both lemon and orange peel extracts contained d-limonene, α-pinene, linalool, citronllal and citral. Limonene oxide, geranial and terpineol were only detected in orange peel extracts. The results indicated that secondary metabolites quality and quantity differ according to the *citrus* spp., and the quantity of d-limonene is much lower (≈15%) in lemon than orange extracts in spite of lemon peel was more toxic than the orange. However, d-limonene was a major compound in both extracts. The results suggest that citrus extract contains many toxic compounds, other than limonene, and /or they could potentiate each other to give the observed toxicity. It could be concluded that the quantity of d-limonene is not a determinant factor, but it is probable that other metabolites confer toxicity allover citrus extract depending on the species.

Keywords: natural biopesticides, *citrus*, cotton leafworm, toxicity, fractions, GC/MS analysis.

INTRODUCTION

Numerous investigations have been performed at identifying chemical composition of extracts from different *Citrus* species. Citrus fruits are sources of

essential oils (EOs) due to their aromatic secondary metabolites which usually obtained from the peels. Peels of citrus fruits comprise of two layers, orange outer layer as flavedo and inner white layer as albedo. The flavedo layer contains EOs in the range of 0.5 to 3 Kg/ton of fruit (Sattar and Mahmud,1992). Eos are vegetable products whose constituents are basically complex mixture of terpenic hydrocarbons and oxygenated derivatives such as aldhydes, alcohols and esters (Murugan *et al*.,2012). Unlike most major plant species, citrus plants contain a large volume of Eos and mostly composed of monoterpene compounds (Weiss, 1997). However, the composition and flavor quality of citrus fruits considerably depend on their cultivar, maturity, genotype, origin, climate, season and ripening stage (Parastar *et al.* , 2012).

Citrus species have been reported as a source of botanical insecticides. Peel and seed solvent extracts from a variety of citrus plants contain secondary metabolites that show insecticidal activity against several insect species (Salvatore *et al*. ,2004 ; Siskos *et al*. , 2007 ; Loh *et al.* , 2011). Identification of toxic secondary metabolites constituents could be the first step in the investigation of natural insecticides based on peel waste. Among different methods, gas chromatography combined to mass spectrometry (GC-MS) is the primary choice for the analysis of citrus extracts by many investigators (e.g Parastar *et al*. , 2012).

Oxygenated monoterpene aldhydes like citral, are reported to be responsible for the chemical resistance of lemon to attack by Ceratitis *Capitata* (da Silva Branco *et al*. ,2000). It is northworthy that limonene was the major component in all EOs analysed (Dutra *et al*., 2016). However, there are toxic bioactive compounds like pinene (Michaelakis *et al*., 2009) and linalool (Yamaski *et al*., 2007).

Among various species of agricultural pests, the cotton leafworm *Spodoptera littoralis* (Boisd.) is one of major polyphagous pest which attacks economically important crops in Egypt such as cotton, cabbage and vegetables causing extensive dmage. Studies concerned with bioactivity of citrus peels against this pest are relatively few. Amin *et al.* (2017) studied bioactivity of fresh navel orange and lemon peels crude extracts against *S.littoralis* larvae. They found that the bioactivity depends on solvent of extraction, *Citrus* species and method of treatment. Hexane (Hex)extract was more efficient than methanol extract specially for contact treatment. So Hex extracts were selected for further studies in this topic.

The aim of this paper was to extract, isolate and identify toxic bioactive secondary metabolites of fresh navel orange (*Citrus sinensis*) and lemon (*citrus limon*) peel Hex extracts for potential use against the cotton leafworm, *S. littoralis*. Chemical composition of lemon and orange extracts were compared to detect the differences and significance between the two species with respect to their toxic constituents. Thin layer chromatography (TLC) and advanced gas chromatography techniques were used.

MATERIALS AND METHODS

- **Collection of citrus fruits :**

Navel orange and lemon fruits were fresh and of eating quality, and purchased from local market during citrus season (october-january). They were brought to the laboratory, and the fruits were cleaned thoroughly by washing with tap water in order to clean dust or any particles. Fruits were inspected carefully to find any kind of diseases or pest infestation, and the infested ones were discarded. Fruits were kept in a refrigerator (2-8°C) for few days till be used for extraction.

- **Chemicals :**

Silica gel used for fractionation on column chromatography was Kiesel gel 60 (70-230 and 230-400 mesh, Merck). TLC strips 20×20 cm was purchased from Merck. Ethyl acetate (EtOAc), Hex and methanol (MeOH) were provided by the company sigma Aldrich, while dichloromethane (CH_2Cl_2) was from Analar. All solvents were HPLC grade.

- **Extraction :**

About 10 kg of navel orange or 5 kg of lemon fruits were dissected to get peels. The peels were weighed and cut into small pieces. Each 1 kg of peels were homogenized with 1 liter of absolute Hex for 5 min in electric blinder. The resultant homogenate was macerated for 72 hr, at room temperature, in a double amount of the used solvent to ensure efficient extraction. The homogenate was filtered using filter paper (Whatman No. 1). The filterates were concentrated to dryness under vaccum pressure using rotary evaporator (Labconco, Germany) at 35°C. The resultant plant residue was considered as crude extract. It collected in a glass stoppered tubes and strored at -10°C in a deep freezer till use.

- **Fractionation procedure of the Hex peel extracts:**

Hex extracts of orange and lemon peels were subjected to fractionation by column chromatography to isolate active metabolites in the extracts. Half gm of extract was mixed with 1 gm of silica gel then transefered onto the column (75 x 11 mm, silica gel 70-230 mesh, Merck Kieselgel 60), as described by Kirchner, (1978). Extract was eluted initially with *n*-hexane then introducing *n*-hexane: ethyl acetate (90:10,80:20, 70:30, 60:40, 50:50, 40:60, 30:70. 20:80, 10:90, each of 10 ml v/v, respectively). Then MeOH was employed to remove components not removed by the other mobile phases. As soon as column chromatography was completed, the obtained fractions were concentrated under a stream of nitrogen for complexity analysis using thin layer chromatographic plates (TLC) and visualized by spraying with vanillin reagent according to the method mentioned by Kirchner (1978).

Fractions which showed bioactivity against the experimental insect were re-chromatographed using similar silica column but eluted with *n*-hexane-CH_2Cl_2 as a solvent system. For each fraction, the column was eluted successively with 100% *n*-hexane, and *n*-hexane-CH_2Cl_2 mixtures, initially at 1% CH_2Cl_2 in *n*-hexane and increasing CH_2Cl_2 by 1% to 10% then by 10% to 100% CH_2Cl_2. Then MeOH was employed to remove components not removed by the other mobile phases. Then all fractions were identified by GC/MS system.

- **TLC analysis:**

TLC analysis was carried out according to Kirchner (1978) in order to check the component profile obtained from fractionation. TLC was performed on 20×20cm TLC plates silica gel 60 F_{254}precoated. Lemon and navel orange Hex fractions (100µg/5ml Hex) were spotted on the middle of the starting line (1cm away from one end of the plate). The plate was put into covered beaker and its inner surface was lined with filter paper to aid in saturating the atmosphere with solvent vapor. Fifty milliliters of solvent mixture (60% Hex and 40% EtOAc.) were poured into the beaker, whereas the level of the fraction spots on TLC plate was above the solvent level. After development the plates were air dried for 10 min at room temperature . Photographs of the TLC plates were obtained by a sony digital camera (4x optical zoom)under UV light (254 nm) provided by a spectroline lamp or after staining by vanillin reagent . Similar fractions were collected together and kept in a deep freezer tell use for bioassay and GGc/Ms analysis.

- **GC/MS analysis :**

Secondary metabolites of the reported fractions that showed activity against larvae, were identified and quantified by chromatographic analyses. GC/MS analyses were performed on Gas Chromatograph-Mass spectrometry Agilent technologies GC 7890B system,5977A MSD detector (USA). Gas Chromatography/mass spectrometer system under computer control at 70 eV. The mobile phase is Helium high purity (99.9999% pure) used at a flow rate of 1ml/min. The instrument equipped with a capillary column HB-5MS (30 m length, 0.25 mm thickness, 0.25m diameter). 1 µl sample was injected into the split/splitless inlet in split mode ratio 50 at 250 °C using a micro syringe. The temperature of the GC/MS interface was 250 °C and temperature of ion source 200°C. The oven temperature program started at 50°C hold 0.5 min increase of 10 °C/ min to 190°C, hold 1 min and increasing by 10°C/min to reach 220°Cm hold 1 min and increasing by 10°C/min to reach 300°Cm hold 2 min. the range of scan mode (50–550 amu) used for data acquisition. The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule.

Identification of constituents present was based on comparing with computer matching of their retention indices and mass spectra fragmentation patterns against the library spectra Wiley330.000 and NIST08.LIB, (Adams, 2007). built up using pure substances and components of known constituents. The percentage of composition was computed from gas chromatography peak areas.

The chemical composition of peel extracts was assessed at analytical laboratories of central agricultural pesticides laboratory (CAPL), by the aid of Dr. Rasha Mohamed Abd El Rasoul

- **Insect colony :**

Bioassays were conducted using 4 *th* larval instar of the cotton leafworm, *S. littoralis* obtained as egg masses from an established laboratory colony in pest physiology laboratory, plant protection research institute, Sharkia branch (Egypt). Larvae were reared on castor bean leaves. The colony was maintained at 25±2°C, 60-70%RH, and photoperiod 12:12 (L:D)h.

- **Bioassay of active fractions:**

After fractionation of *C. sinensis* and *C. limon* peels Hex extracts, the separated fractions were tested against the newly moulted 4th larval instar to detect the active fraction that, later on, subjected to TLC and Gc/MS analyses. The petri dish residual exposure bioassay (Siskos *et al.*,2007) was used to evaluate the insecticidal activity of different citrus peel crude extracts against the larvae. In glass petri dishes (bottom, internal dimeter 9 cm; hight, 1.5 cm). serial concentrations of each fraction were prepared, and 1 ml of each was spread on the bottom, and the petri dishes were rotated manually until solvent evaporation to achieve an even distribution of the sample. Three replicates of 10 randomly selected larvae were used for each extract. The larvae were introduced into petri dishes, and exposed to the extract for 30 min. The insects were exposed to the extract through contact with the cuticle and probably also with prologs. Then, the larvae were transferred to clean dishes supplied with their food (castor bean leaves). Mortality was recorded after 72 hr post-treatment. Control dishes were treated by the same manner with solvent only; the same number of larvae were introduced into these dishes, and controls were run simultaneously with the treatments.

The experiments were conducted in the laboratories of pest physiology department, plant protection Research Institute.

- **Statistics :**

Data obtained from bioassays were corrected for control mortality using Abbott`s formula (1925). Fractionation and bioassay test were done in triplicates. Data obtained from Gc/Ms analyses were computed from Gc peak areas.

RESULTS AND DISCUSSION

Peels from plants of the genus *Citrus* are obtained as waste by-products of the citrus processing industry. The citrus peels contain many secondary metabolites that are physiologically active to insects and could be used as natural botanical pesticides as ecofriendly products. So identification of such metabolites is of a valuable interest.

Amin *et al*. (2017) indicated that a potent contact larvicide can be obtained from Hex extracts of fresh lemon or orange peel. Fractionation of Hex crude extracts obtained 12 fractions for each citrus species, (L_1-L_{12}) for lemon and (O_1-O_{12}) for orange peel extracts. According to TLC analysis, similar fractions were added together, so lemon crude extract fractions was reduced to be 6 fractions, while that of orange crude extract was reduced to 4 fractions (Table, 1).

Bioassay test using contact method was performed on different fractions to detect the bioactive fractions toxic to 4th larval instar of *S. littoralis*. The results (Table, 2) showed that lemon fractions were more toxic than those of orange.

Table 1. Fractions before and after TLC analysis.

Fraction number	Reduced fractions	
	C. limon	*C. sinensis*
F_1	L_1	O_1
F_2	L_{2+3+4}	$O_{2+3+4+5}$
F_3	L_5	O_{6+7+8}
F_4	L_{6+7}	$O_{9+10+11+12}$
F_5	L_{8+9+10}	---
F_6	L_{11+12}	---

F_{1-6} :Fractions after TLC analysis ; L_{1-12} :Lemon fractions before TLC analysis ; O_{1-12}: orange fractions before TLC analysis.

Table 2. percentage mortality counts of 4th larval instar of *S. littoralis* treated by contact method with different fractions of lemon and orange peel extracts.

	% corrected mortality	
	C. limon	*C. sinensis*
F_1	0.00	0.00
F_2	100	60 (55-65)
F_3	100	20.5 (19-22)
F_4	0.00	0.00
F_5	0.00	----
F_6	0.00	----

- Data were corrected according to Abbott`s formula (1925).

F_2 and F_3 fractions of lemon peel extract showed 100% mortality after 72 hr post treatment , while the highest mortality recorded for orange extract was 60% (F_2 fraction). F_3 caused low activity (20% mortality). The remaining factions i.e F_1,F_4,F_5 and F_6 of lemon extract, and $F_1$and F_4 of orange extract did not show any toxicity against the larvae.

The most potent fractions of lemon and orange extracts were selected for further analysis to identify and compare the bioactive secondary metabolites present in such fractions. F_2 and F_3 fractions of both lemon and orange Hex peel extracts were subjected to GC/MS analysis, as they recorded the highest toxicity against the cotton leafworm.

The identification of individual components was based on comparison of mass spectral fragmentation patterns with those stored in the mass spectral library built up using pure substances and the mass spectra from the literature. The percentage of composition was computed from gas chromatography peak areas.

Many compounds were identified (Table 3) in the semi-purified F_2 and F_3 factions of orange extract. Although the two fractions differed in their bioactivity, they had approximately equal value of D-Limonene (≈86%). However F_2 contained higher amounts of linalool, α-terpinol and citral than F_3 fraction.

F_2 and F_3 fractions of Hex extracts of lemon peel caused 100% mortality, however there are variable metabolites eluted in the different fractions differ in their structure and physical properties, and might share in toxicity (table 4). F_2 fraction contained d-limonene(15.16%), 3-carene (13.42%), γ-terpinene(5.2%), α-pinene (2.24%) and α-fernesene (4.32%). On the other hand, F_3fraction contained the overall highest amount of citral (42.03%), followed by d-limonene (14.07%), linalool (5.11%), β-pinene (4.34%) , β-myrcene (3.37%), γ-terpinene (1.62%) and terpinen-4-ol (1.51%).

Table 3. The main constituents of fraction2 and fraction3 from navel orange Hex peel extract identified by GC-MS.

No.	Orange fraction2			Orange fraction3		
	Components	RT (min.)	Ratio (%)	Components	RT (min.)	Ratio (%)
1	α-pinene	4.229	0.18	α-pinene	4.379	0.25
2	Bicyclo (3.1.0)-hexane	4.957	0.22	β-Myrcene	5.163	1.70
3	β-Myrcene	5.151	1.31	α- Phelladerene	5.391	0.06
4	Octanal	5.329	0.54	3-Carene	5.483	0.16
5	D-limonene	5.707	85.57	D-limonene	5.786	86.94
6	2-pyrrolidinone	5.975	0.42	γ-Terpinene	6.204	0.07
7	Linalool	6.645	1.76	Linalool	6.799	0.17
8	Cis- Verbenal	6.777	0.08	Limonene oxide	7.411	0.04
9	limonene oxide	7.446	0.22	Geranial	7.604	0.05
10	Citronllal	7.623	0.09	Naphthalene	8.121	0.20
11	Terpineol	7.995	0.89	α- Terpineol	8.201	0.08
12	Hexadecane	8.379	0.38	Decanal	8.361	0.10
13	Geranial	8.487	0.57	Citral	9.817	0.10
14	2,6,10-dodecatrien-1-ol	8.739	0.38	Caryophyllene	11.44	0.08
15	Carvone	8.997	0.06	α -Famesene	11.56	0.04
16	Citral	9.100	0.26	n-Hexadecanoic acid	17.945	0.26
17	1,6-hexanediol dimethacrylate	9.151	0.21	Linoelaidic acid	20.263	0.29
18	2,6-octadienal	9.323	0.23	Heptamethoxy flavon	25.865	4.17
19	2(3H)-Naphthalenone	16.252	0.22	Hexamethoxy flavone	26.105	2.36
20	n-Hexadecanoic acid	17.969	2.11	9H-Fluorene-2-carboxylic acid	26.408	0.21
21	Linoelaidic acid	20.229	0.24	Methyltris(trimethyl siloxy)silane	26.551	0.25
22	9,12,15-octadecatrien-1-ol	20.303	1.35	-	-	-
23	4`,5,6,7,8pentamethoxy flavone	29.407	0.89	-	-	-
	Total		98.18			97.58

RT :retention time.

Table 4. The main constituents of fraction2 and fraction3 from lemon Hex peel extract identified by GC-MS.

No.	Lemon fraction2			Lemon fraction3		
	Components	RT (min.)	Ratio (%)	Components	RT (min.)	Ratio (%)
1	Bicyclo(3.1.0)hexene	4.281	0.22	Bicyclo(3.1.0)hexane	4.940	0.49
2	α -Pinene	4.390	2.24	β-pinene	4.997	4.34
3	3-Carene	5.037	13.42	β -myrcene	5.169	3.37
4	D-Limonene	5.809	15.16	D-Limonene	5.769	14.07
5	β -Ocimene	6.072	0.72	Tricyclo(2.2.1.0[2,6])heptane	5.849	1.28
6	γ-Terpinene	6.295	5.20	β -Ocimene	6.021	2.08
7	Decane	6.370	0.28	ɣ -Terpinene	6.204	1.62
8	4-Carene	6.684	2.35	2-Carene	6.673	0.41
9	Citronllal	7.603	0.10	Linalool	6.834	5.11
10	Decanal	8.367	0.29	2,4,6-octatriene	7.246	0.86
11	Citral	8.916	1.55	Terpinen-4-ol	8.024	1.51
12	Geranyl acetate	10.839	0.31	Decanal	8.367	0.70
13	Cyclohexane	11.056	2.11	oxiranecarboxaldehyde	8.653	0.62
14	Caryophyllene	11.485	3.75	Citral	8.985	42.03
15	Trans-α-Bergamatene	11.623	4.80	4-methyl-1,5-Heptadiene	10.582	1.08
16	trans- α -Bergamotene	11.348	0.25	Bicyclo(2.1.0)pentane	10.759	0.24
17	(E)- β -Famesene	11.806	0.48	Geranyl acetate	10.833	0.37
18	Humulene	11.909	0.78	Dodecanal	11.171	0.38
19	8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	12.241	1.01	3,5-heptadienal	11.360	0.24
20	Naphthalene	12.315	0.21	Caryophyllene	11.446	0.53
21	cis- α -Bisabolene	12.424	0.68	trans- α -Bergamotene	11.583	0.66
22	α -Farnesene	12.515	4.32	α -Farnesene	12.447	0.54
23	β -Bisabolene	12.561	6.89	β -Bisabolene	12.498	1.24
24	Benzene 1- butyllhexyl	12.841	3.61	Benzene -1- pentylhexyl	13.935	0.93
25	ɣ-Elemene	13.208	3.33	1,3,6,10-Dodecatetraene	14.003	0.42
26	Benzene-1- butylheptyl	14.020	4.37	Benzene -1- ethylnonyl	14.329	1.15
27	Benzene -1- pentylheptyl	15.119	2.82	4-(2,2-Dimethyl-6-methylenecyclohexyl)butanal	14.478	0.77
28	Benzene -1- propylnonyl	15.342	2.01	alpha-Bisabolol	14.627	2.01
29	Hexadecanal	16.200	12.65	n-Hexadecanoic acid	18.020	2.59
30	Octadecane	28.457	8.60	Linoelaidic acid	20.229	0.69
31		-	-	cis-Vaccenic acid	20.292	0.69
32		-	-	7H-Furo(3,2-g) (1) benzopyran-1-one	21.505	2.61
33		-	-	1.3-Butanedione	28.400	0.83
Total			96.5			96.4

The GC-MS pattern of the toxic fractions of lemon and orange peel extracts illustrated in fig. (1,2,3,4).

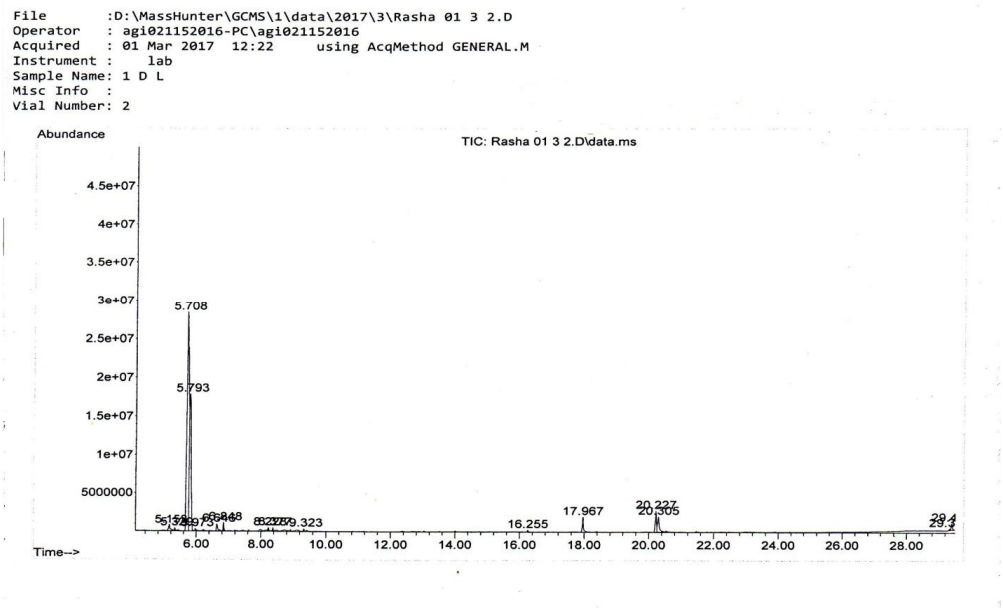

Fig. 1. Chromatogram (GC-MS) of fraction2 from navel orange Hex peel extract.

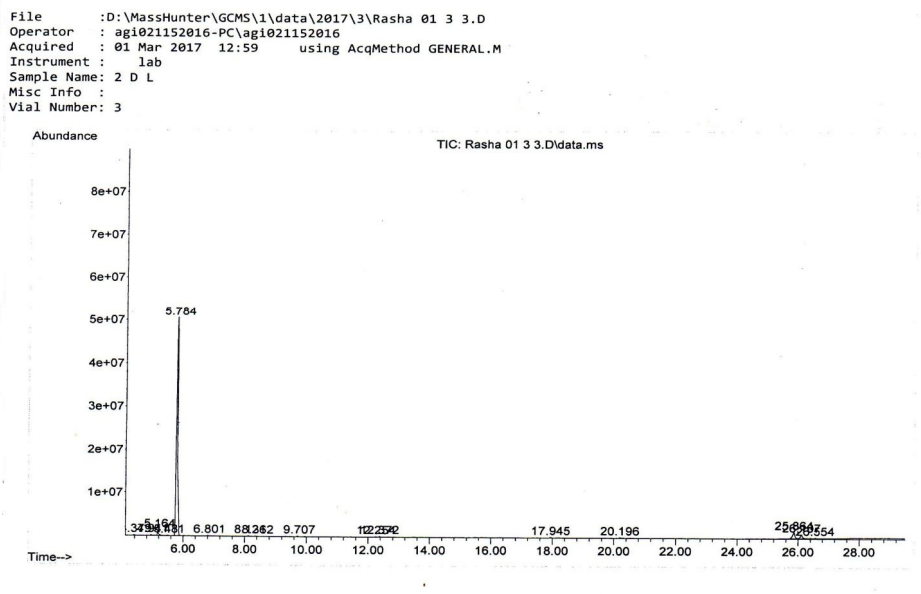

Fig. 2. Chromatogram (GC-MS) of fraction 3 from navel orange Hex peel extract**.**

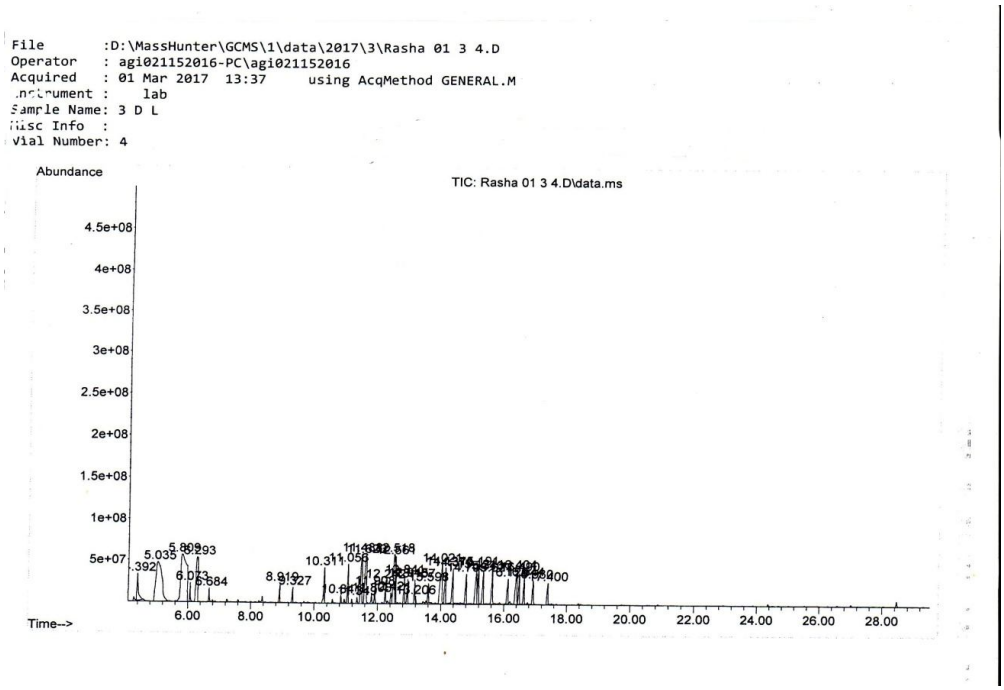

Fig. 3. Chromatogram (GC-MS) of fraction 2 from lemon Hex peel extract.

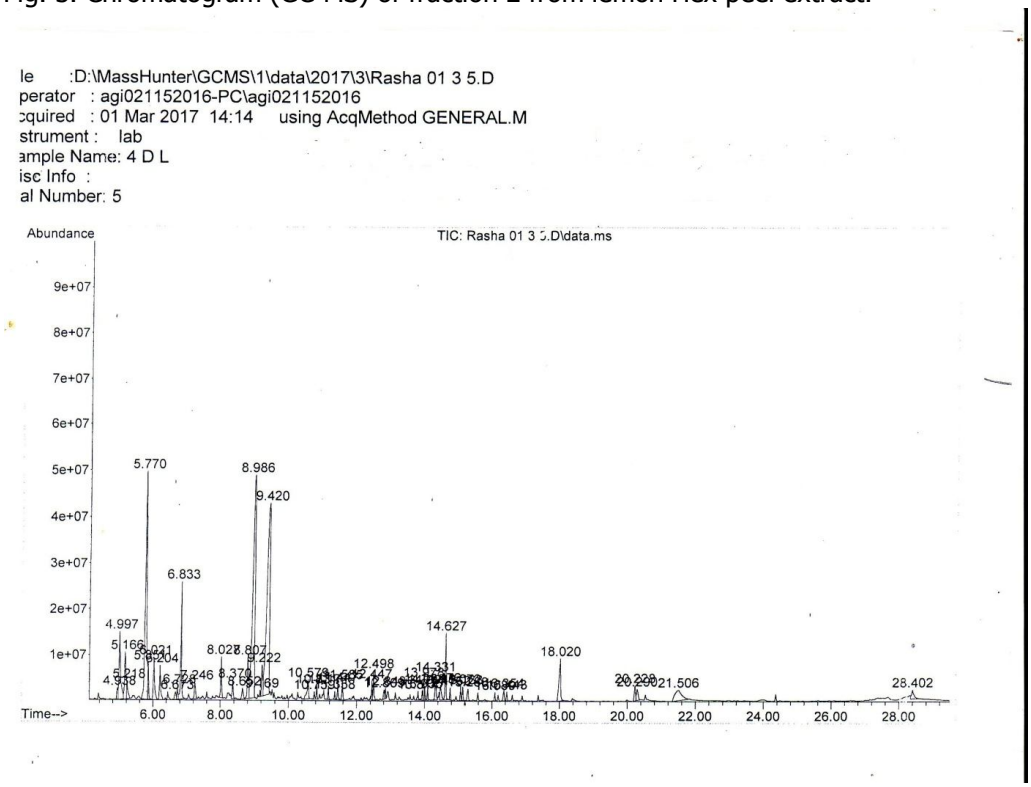

Fig. 4. Chromatogram (GC-MS) of fraction 3 from lemon Hex peel extract.

The active fractions of lemon and orange peel extracts contained D-limonene, α-pinene, linalool, citronllal and citral. Limonene oxide, Geranial and terpineol were only detected in orange peel extract. The results indicated that secondary metabolites

quality and quantity differ according to the *Citrus* spp., and quantity of D-limonene is much lower in lemon extract, while it is a major compound in both extracts.

The variation of the composition of secondary metabolites of citrus peel as shown between lemon and orange peel extracts, might be depend on the *Citrus* spp. . However, there are some factors that affect the citrus composition such as collection time, temperature, humidity, sun exposure and extraction method (Dutra *et al*., 2016). Here a through comparative study between lemon and orange, the extraction procedure, type of solvent and GC-MS conditions were identical for all samples and therefore, the influence of technical parameters on the chemical composition of peel extracts was negligible.

There are much reviews about comparison between orange and lemon constituents (Campolo *et al*., 2014 ; Mickaelkis *et al.* , 2009 ; Parastar *et al*. , 2012). They agree that orange contains higher content of d-limonene than lemon. It seems that there are other active compounds in citrus extract besides d-limonene that add to toxicity of lemon or orange peel extracts. Although d-limonene has insecticidal capacity, it showed lower toxicity than oils when used alone, since insecticidal activity may also affected by minor compounds (Martins *et al*., 2017).

In addition to the monoterpenoids; d-limonene [(R)-4-isopropenyl-1-methylcyclo-hexane], there are many active compounds have insecticidal activity like citral (mixture of neral and gernial) (Salvatore *et al*., 2004), α-terpineol , β-myrcene , linalool and pulegone (Coats *et al.* , 1991).Some authors found that there are secondary metabolites in citrus, and more active than limonene. Citral was the most efficient monoterpenoid, followed by limonene, then α-pinene and α-terpineol, against larvae of the Caribbean fruit fly (Styer and Greany, 1983). β-pinene revealed stronger toxicity than many already known p-menthane type molecules such as S-(+)-limonene, carvone, menthane and menthol (Mickealkis *et al*., 2008). A cyclic type compounds such as nerol, gerniol and neryl acetate were also recorded in citrus essential oils (Mickealkis *et al*., 2009).

It is difficult in the present work, to implicate certain compound(s) in toxicity of the studied extracts. We found th at d-limonene content was ≈15% in lemon extract fractions, which showed very high toxicity as compared to orange extract fractions (≈85% d-limonene). Also, both fractions (F_2 andF_3) had different mixtures of metabolites with different quantities. If it is supposed that citral in F_3 (42.03%) might responsible for toxicity, the situation become not valid, because F_2 which showed the same toxicity had relatively low value of citral (1.55%). The toxic factors seem not only depend on citrus spp., but also on fraction level in citrus extracts.

Lemon essential oil is a mixture of many p-menthane-type molecules, which are known for their larvicidal toxicity, and subsequently, it may be concluded why LC_{50} value revealed stronger toxicity compared to other *Citrus* species (Mickealkis *et al*., 2009). This might explains why lemon extracts fractions were more toxic than orange

extracts fraction, regardless of the d-limonene content observed in the present work. Martins *et al.* (2017) found that sweet orange (*C. sinensis*) and Silcian lemon (*C. limon*) contained 83.33 and 59.78% d-limonene, respectively. The sweet orange oil presented lethal concentration against *Dymicoccusbrevips* at 2.21% (LC_{50}) and the Silcilian lemon oil at 0.72%(LC_{50}) , indicating that lemon was more toxic than orange oil.

The results suggest that citrus extract contains many compounds, other than limonene, are toxic and/or potentiate each other to give the observed toxicity. Jiang *et al*. (2009) mentioned that insecticidal activity may also affected by minor compounds which promote a synergism with the major constituents, thus increasing mortality.

It could be concluded that the quantity of d-limonene is not the determinant factor, but it is probable that other metabolites confer toxicity allover citrus extract depending on the species.

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"التحليل الكروماتوجرافي للمكونات الطبيعية لقشور بعض الموالح الفعالة ضد دودة ورق القطن"

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تم في هذه الدراسة استخدام مذيب الهكسان لاستخلاص مكونات قشور البرتقال و الليمون الطازجة وذلك لفصل و تعريف المكونات الفعالة ضد العمر اليرقي الرابع لدودة ورق القطن٠

و قد استخدم لفصل و تعريف المركبات جهاز عمود الفصل الكروماتوجرافى و كروماتوجراف الطبقةالرقيقة (TLC) و جهاز الكروماتوجراف الغازي –الكتلي ٠ و قد اثبت استخدام هذه التقنيات كفاءة العمل على قشور الموالح٠

وقد قسم المستخلص النباتي بعد الفصل الى ٦ أجزاء (ف١-ف٦)٠ وكان جزئي المستخلص ف٢ ،ف٣ من قشور الليمون والبرتقال هما الفاعلين ضد الآفة٠ وقد اشترك كلا من قشور الليمون والبرتقال في احتوائها على .d- limonene, α-pinene, linalool, citral, citronllal.و لكن قشور البرتقال فقط احتوت على limonene oxide, geranial, terpineol٠ وقد اوضحت النتائج ان مكونات الليمون والبرتقال تختلف كميا ونوعيا في المكونات الكيميائية وان مركب d-limonene كان كميا هو الاكبر من المكونات الاخرى و خاصة في البرتقال و لكن كان اقل في الليمون (حوالي ١٥%) عن البرتقال (حالي ٨٦%) و مع ذلك كان مستخلص قشور الليمون هو الأكثر سمية (١٠٠% نسبة الموت) عن قشور البرتقال (٦٠% نسبة الموت)٠

و قد رجحت الدراسة ان مستخلصات الموالح تحتوي على مركبات أخرى سامة بخلاف d-limonene قد تكون في نفس سميته وان هذه المركبات قد تنشط فعل بعضها البعض لتنتج السمية الملاحظة في التجارب٠ والخلاصة ان مركب d-limonene ليس هوالعامل المحدد للسمية في الليمون والبرتقال وان العوامل المحددة للفاعلية قد تختلف حسب صنف الموالح٠