Al Azhar Bulletin of Science Vol. 28, No. 1 (June), 2017, pp. 45-54

TAXONOMIC STUDIES ON TWO TEPHRITID SPECIES (ORDER: DIPTERA), *BACTROCERA OLEAE* AND *B. ZONATA*, USING THE CUTICULAR HYDROCARBONS PROFILE

Ahmad M. M. Galhoum

ahmadgalhoum@hotmail.com

Department of Zoology, Faculty of Science, Al–Azhar University, Cairo, Egypt

ABSTRACT

The outer surface of insects (cuticle) is sheltered by a complex mixture of cuticular hydrocarbons (CHCs) play an important role in avoiding desiccation and defend the insects against diseases infestation. Identification and chemical analyses of insect cuticular hydrocarbons are vital practice toward insect control. The obtained results indicated the two studied species obviously differ in CHCs components (35 and 29 components characterized *B. oleae* and *B. zonata* respectively) and shared twelve components. All these components can be used (quantitatively and qualitatively) to identify and taxonomically separate them.The objective of this paper is to evaluate the using of cuticular hydrocarbons as taxonomic tools in two dipteran species, *Bactrocera oleae* and *B. zonata*.

Keywords*: *Taxonomy, Diptera, Tephritidae, Bactrocera, Cuticular hydrocarbon.

INTRODUCTION

The integument of insects play vital role in protect insects from desiccation due to evaporation of different internal body liquids and infestation by pathogens (Gibbs and Rajpurohit, 2010), also it is help in communication as it contains complex mixture of hydrocarbons, ketones, aldehydes, fatty acids, methyl esters and aliphatic alcohols (Blomquist and Bagnères, 2010) and defense (Golebiowski *et al*; 2011).The number of hydrocarbons of the body of insects usually reach up to100 different types (Nelson *et al*., 1981). Insect species usually possess complex mixtures of hydrocarbons including n-alkanes, branched mono-, di-, ortrimethylalkanes, and others (Jackson and Blomquist, 1976).

Cuticular hydrocarbons are heritable and stable end products of genetically controlled metabolic pathways (Grunshawn *et al*., 1990 and Foley *et al.* 2007). Thomas and Dennis (1981) found no significant differences between male and female of the pupae of *Manduca sexta* (L.) as well as in different instars. Coby*et al.* (1998) confirmed the similarity of the cuticular and internal hydrocarbons. Applying CHCs as chemotaxonomic tool was investigated by different researchers (e.g. Carlson *et al. Glossina spp.*1993; Copren *et al.* 2005, termites; Calderón-Fernández, 2011, *Triatoma dimidiate*).

The present research focuses on analysis of cuticular hydrocarbons to be used as potential chemotaxonomic tool distinguished two fruit fly species, the olive fruit fly *Bactrocera oleae* (Rossi) and the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). The olive fruit fly *Bactrocera oleae* is a serious pest of olives in most countries around the Mediterranean Sea. The damage caused by this pest results in production losses that can exceed 80% (Rice *et al*., 2003). The peach fruit fly, *Bactrocera zonata* (Saunders), is one of the most harmful species of Tephritidae, it is attacking more than 40 species of fruit crops. The peach fruit fly is a serious pest of peach, guava and mango; secondary hosts include apricot, fig and citrus. This pest has established in Egypt since the late 1990s and is now widespread throughout the country (Delrio and Cocco, 2012).

MATERIALS AND METHODS

Insects

Specimens of a mixture of males and females adults of the two species of genus *Bactrocera* namely *B. oleae and B. zonata* were obtained from a culture rearing in Horticultural Insect Research Department, Plant Protection Research Institute, Cairo, Egypt. Before extraction of hydrocarbon, specimens were kept in refrigerator.

Hydrocarbon extraction and analysis.

Cuticular hydrocarbons were extracted from adult specimens using hexane as a solvent, separated from other lipid components and analyzed by gas chromatography-mass spectrometry (GC-MS) as described by Page *et al.,* (1990).

Gas Chromatography – Mass Spectrometry (GC/MS).

GC/MS analysis was conducted in "The Regional Center for Mycology and Biotechnology", Al-Azhar University. Samples were run on Thermo Scientific TRACE 1310 Gas Chromatograph, fitted with a silica capillary column DB-5, (Length 30 m. x Internal diameter 0.25 mm. x film thickness 0.25 µm), carrier gas of helium (flow rate 1 ml/min.). One microliter of sample was injected into the injector in pulsed splitless mode. The injector temperature was at 300 °C. The GC temperature program was started at 40 °C (5 min.) then raised to 275 °C (5 min.) at 5 °C/min. Mass spectrometric was operated in electron impact ionization mode with an ionizing energy of 70 ev. The ion source temperature was 300 °C. The electron multiplier voltage (EM voltage) was maintained 1650 v. above auto run. The instrument was manually turned using perfluorotributyle amine (PFTBA).

Compounds were identified by comparison of the spectra to the Wiley &NISTMASS SPECTRAL DATABASE and by comparison to literature relative retention indexes.

RESULTS

Seventy six cuticular hydrocarbons were identified by GC-MS from adults of two species belong to genus *Bacterocea* (Diptera: Tephritidae), *B. oleae* and *B. zonata* (No individual species contained all 76 components.) (Table 3). The classes of hydrocarbons found in both species are alkanes (28 components), alkenes (39 components), monocyclic hydrocarbons (4 components) alkyne (3) and polycyclic (2 components).The alkanes occurred as a continuous series of carbons ranged from C_5 toC_{12},alkensfrom C_{12} to C_{16} ,monocyclic C_7 and C_{16} and polycyclic C_{11}.

2- Cuticular Hydrocarbon Analysis of Bactrocera oleae:

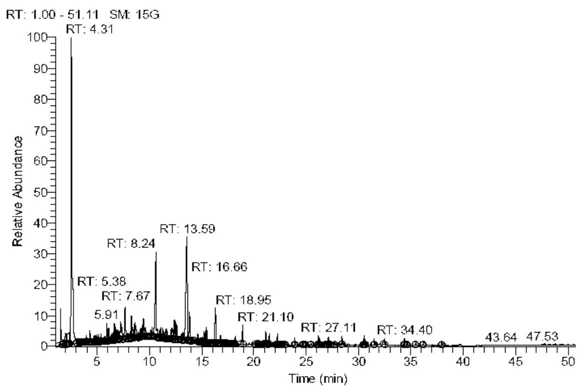

Figure (1): Chromatogram obtained by GC/MS: Cuticular hydrocarbons of *Bactrocera oleae.*

Bactrocera oleae had a mixture of forty seven hydrocarbons (Figure 1, Table 1) with chain lengths varying from C_6 to C_{16}. The hydrocarbon of *B. oleae* was classified within five categories namely, alkene (23), alkane (19), polycyclic hydrocarbons (2), alkyne (2) and monocyclic hydrocarbons (1). The most abundant hydrocarbons in *B. oleae* are cyclohexane (27.98%) followed by dodecane (10.59%), undecane (6.73%), decane (2.71%), tridecane (2.40), Heptane (CAS) (1.92%), Cyclohexane,1-methyl-2-propyl (1.43%), Undecane, 2,6-dimethyl- (1.37%), Decane, 4-methyl- (1.24%) and Hexane (CAS) (1.07%). Thirty six hydrocarbons represented as traces (i.e. less than 1%).

2- Cuticular Hydrocarbon Analysis of Bactrocera zonata:

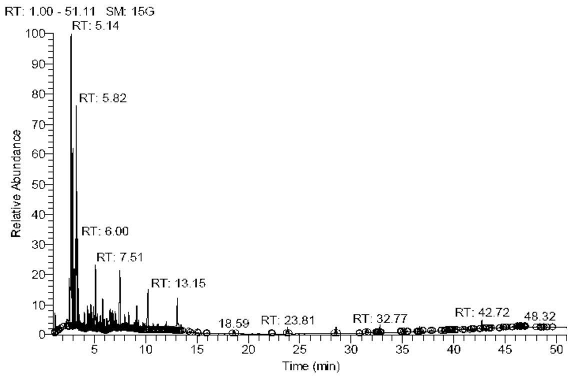

Figure (2): Chromatogram obtained by GC/MS: Cuticular hydrocarbons of *Bactrocera zonata*

Bactrocera zonata had a mixture of forty one hydrocarbons (Fig. 2, Table 2) with chain lengths varying from C_5 to C_{16}. The hydrocarbon of *B. zonata* are classified within four categories namely, alkene (22), alkane (14), monocyclic hydrocarbons (3) and alkyne (2). The most abundant hydrocarbons in *B. zonata* are Cyclohexane, methyl- (24.42%), Benzene, methyl- (CAS) (11.13%), Cyclopentane, ethyl- (6.09%),Benzene, (2-methyloctyl)- (4.88%), Undecane (2.88%), Nonane (CAS) (2.77%), Dodecane (2.71%),Cyclopentane, 1,2-dimethyl-, cis- (1.44%) and 1,1,2,3-tetramethylcyclohexane A (1.23%).Thirty three hydrocarbons represented as traces (i.e. less than 1%).

3- Comparing the cuticular hydrocarbons of *Bactrocera oleae* and *B. zonata*:

All of the major cuticular hydrocarbon components of the two species of genus ***Bactrocera*** were recorded (Table 3). All hydrocarbon components found in the two species were belonging to one of the following classes, alkane, alkene, alkyne, monocyclic hydrocarbons andpolycyclic hydrocarbons.

As shown in (Table 3) many components can be easily used to separate the two species of *Bactrocera*. Where 35 hydrocarbon compounds characterized *B. oleae*, 29 hydrocarbon compounds characterized *B. zonata*, they share 12 compounds. The alkene is the most dominant class of hydrocarbon among the peaks obtained by GC/MS in both species followed by Alkane (Alkene represents in *B. oleae* by 48.94% followed by Alkane 40.43% whileAlkene represents in *B. zonata* by 54.76% followed by Alkane 33.33%).

The alkene composition in *B. oleae* ranged from C6-C16 with C12, C9 and C10 predominating. The alkane is ranged from C6-C16and equally distributed. The alkyne C10 and C11, monocyclic hydrocarbon limited within C16, polycyclic limited within C11.

The alkene composition in *B. zonata* ranged from C7-C16equally distributed. The alkane ranged from C5-C15equally distributed. The alkyne limited within C11. The monocyclic compound C7, C8 and C15.

The major alkene compound in *B. oleae* is Cyclohexane (peak area 27.98%) and Cyclohexane, methyl- (24.42%) in *B. zonata*. The abundant alkane in *B. oleae* is dodecane (10.59%) and undecane (2.88%) in *B. zonata*. The major alkyne compound in *B. oleae* is Cyclooctene, 1, 2-dimethyl- (peak area 1.43%) and Naphthalene, decahydro-2-methyl- (0.26%) in *B. zonata*. The major monocyclic compound in *B. oleae* is Benzene, (1-butylhexyl)- (peak area 0.06%) and Benzene, methyl- (CAS) (11.13%) in *B. zonata*. The major polycyclic compound in *B. oleae* is Methylnaphthalene (peak area 0.28%) not represented in *B. zonata*.

Twelve hydrocarbon components are shared between the two *Bactrocera* spp. As shown in (Table 3), there is a quantitative difference among the shared components. In *B. oleae*,three hydrocarbon components are represented by a considerable quantities (Dodecane, 10.59%, Undecane, 6.73% and Tridecane, 2.40%) and the other nine components represented by traces. *Bactrocera zonata*, one component (Undecane) represented by 2.88% and the other components found as traces. The ratio between Dodecane present in *B. Olea* and that in *B. zonata* show a considerable difference (10.59% to 0.035 respectively), this is noticeable in undecane (6.73% to2.88% respectively) and tridecane

(2.40% to 0.03% respectively). Nonane relatively represented in *B. zonata* by higher ratio than *B. oleae* (2.77% to 0.28% respectively). The other shared components are represented by traces and the difference between each component is scant.

Table (1) Cuticular hydrocarbons of *Bactrocera oleae*

RT	Compound Name	Area %	Molecular Formula	Molecular Weight
2.49	Cyclohexane	27.98	C6H12	84
2.37	Pentane, 3-methyl- (CAS)	0.84	C6H14	86
2.27	Hexane (CAS)	1.07	C6H14	86
1.47	Heptane (CAS)	1.92	C7H16	100
4.83	Cyclohexane, 1,2,4-trimethyl	0.05	C9H18	126
4.03	Cyclohexane, 1,1,3-trimethyl	0.08	C9H18	126
5.05	Cyclohexane,1-ethyl-4-methyl-, trans	0.11	C9H18	126
4.75	Cyclohexane, 1,2,3-trimethyl	0.16	C9H18	126
3.93	Cyclohexane, 1,3,5-trimethyl-	0.18	C9H18	126
4.97	Cyclohexane, 1-ethyl-2-methyl	0.22	C9H18	126
3.68	Octane, 4-methyl-	0.09	C9H20	128
5.14	Nonane	0.28	C9H20	128
9.41	Cyclooctene, 1,2-dimethyl-	1.43	C10H18	138
6.47	1,2,3,5-tetramethylcyclohexane	0.32	C10H20	140
6.47	1,1,2,3-tetramethylcyclohexane	0.32	C10H20	140
6.12	Cyclohexane,2-ethyl-1,3-dimethyl	0.72	C10H20	140
7.26	Cyclohexane,1-methyl-2-propyl	1.75	C10H20	140
16.58	Naphthalene, 2-methyl	0.24	C11H10	142
17.01	Methylnaphthalene	0.28	C11H10	142
6.04	Heptane, 3-ethyl-2-methyl-	0.36	C10H22	142
7.67	Decane	2.71	C10H22	142
11.51	Naphthalene,decahydro-2-methyl	0.40	C11H20	152
8.97	Cyclopentane, 1,2-dipropyl-	0.40	C11H22	154
11.61	Cyclohexane, pentyl-	0.46	C11H22	154
8.24	Decane, 4-methyl-	1.24	C11H24	156
10.65	Undecane	6.73	C11H24	156
3.43	1-Heptene, 2-pentyl-	0.08	C12H24	168
4.39	1-Undecene, 7-methyl-	0.10	C12H24	168
5.79	Cyclooctane, 1-methyl-3-propyl	0.12	C12H24	168
14.85	1-Dodecene (CAS)	0.24	C12H24	168
3.16	2-Undecene, 6-methyl-, (Z)-	0.45	C12H24	168
13.09	Cyclohexane,1-methyl-4-(1-methylbutyl)-	0.57	C12H24	168
9.63	2,3-Dimethyldecane	0.78	C12H26	170
13.59	Dodecane	10.59	C12H26	170
15.22	Dodecane, 2-methyl-	0.47	C13H28	184
13.85	Undecane, 2,6-dimethyl-	1.37	C13H28	184
16.32	Tridecane	2.40	C13H28	184
10.24	Cyclotetradecane	0.72	C14H28	196
16.77	Decane, 2,3,5,8-tetramethyl	0.25	C14H30	198
18.95	Tetradecane	0.99	C14H30	198
6.39	1-Pentadecene	0.31	C15H30	210
22.25	Dodecane, 2,6,11-trimethyl	0.40	C15H32	212
21.47	Pentadecane	0.37	C15H32	212
22.34	Benzene, (1-butylhexyl)-	0.06	C16H26	218
20.19	Cyclopentane, undecyl	0.04	C16H32	224
10	1-Nonylcycloheptane	0.46	C16H32	224
16.66	Hexadecane	0.12	C16H34	226

Table (2): Cuticular hydrocarbons of *Bactrocera zonata*

RT	Compound Name	Area %	Molecular	Molecular
1.22	Butane, 2-methyl	0.47	C5H12	72
3.25	Benzene, methyl- (CAS)	11.13	C7H8	92
2.57	Cyclopentane, 1,2-dimethyl-,cis-	1.44	C7H14	98
2.79	Cyclohexane, methyl-	24.42	C7H14	98
2.95	Cyclopentane, ethyl-	6.09	C7H14	98
4.67	Benzene, 1,4-dimethyl-(CAS)	0.99	C8H10	106
3.58	Cyclohexane, 1,4-dimethyl-	0.26	C8H16	112
3.65	Cyclohexane, 1,3-dimethyl-,	0.12	C8H16	112
4.04	Cyclohexane, ethyl-	0.84	C8H16	112
3.51	Octane	0.95	C8H18	114
2.65	2,4-Dimethyl-1-heptene	0.60	C9H18	126
4.09	Cyclohexane, 1,1,3-	0.39	C9H18	126
4.34	Cyclohexane, 1,2,4-trimethyl-	0.79	C9H18	126
4.95	Cyclohexane, 1-ethyl-2-methyl-	0.64	C9H18	126
4.56	Heptane, 2,5-dimethyl-	0.45	C9H20	128
5.14	Nonane (CAS)	2.77	C9H20	128
6.00	Cyclohexane, 1,2-diethyl-	0.49	C10H20	140
6.33	1,1,2,3-tetramethylcyclohexane	0.16	C10H20	140
3.86	Octane, 2,6-dimethyl-	0.14	C10H22	142
5.93	Heptane, 3-ethyl-2-methyl-	0.32	C10H22	142
10.72	Naphthalene, decahydro-2-methyl-	0.26	C11H20	152
11.15	trans-Decalin, 2-methyl-	0.22	C11H20	152
4.22	Cyclodecane, methyl-	0.02	C11H22	154
9.93	Cyclopentane, 1,2-dipropyl-	0.35	C11H22	154
8.92	Decane, 5-methyl-	0.23	C11H24	156
10.24	Undecane	2.88	C11H24	156
6.25	1-sec-butyl-1-(2-methyl	0.49	C12H24	168
12.88	Cyclododecane	0.21	C12H24	168
4.44	2,6-Dimethyldecane	0.51	C12H26	170
12.03	Undecane, 2-methyl-	0.48	C12H26	170
13.15	Dodecane	2.71	C12H26	170
8.70	Cyclopentane, 1-pentyl-2-propyl-	0.12	C13H26	182
11.25	Heptylcyclohexane	0.27	C13H26	182
9.32	Nonane, 5-(1-methylpropyl)-	0.52	C13H28	184
13.45	Tridecane	0.39	C13H28	184
18.59	1-Tetradecene	0.22	C14H28	196
1.30	Tridecane, 4-methyl	0.40	C14H30	198
3.16	Benzene, (2-methyloctyl)-	4.88	C15H24	204
8.35	Decane, 2-cyclohexyl-	0.93	C16H32	224
9.54	1-Nonylcycloheptane	0.13	C16H32	224
23.81	1-Hexadecene (CAS)	0.33	C16H32	224

Table (3): Comparison of Cuticular hydrocarbons of *Bactrocera oleae* and *B. zonat*

No.	Hydrocarbons	Molecular formula	Molecular weight	*B. oleae*	*B. zonata*	HC class
1	Butane, 2-methyl	C5H12	72	−	+	Alkane
2	Cyclohexane	C6H12	84	+	−	Alkene
3	Hexane (CAS)	C6H14	86	+	−	Alkane
4	Pentane, 3-methyl- (CAS)	C6H14	86	+	−	Alkane
5	Benzene, methyl- (CAS)	C7H8	92	−	+	Monocyclic
6	Cyclohexane, methyl-	C7H14	98	−	+	Alkene
7	Cyclopentane, 1,2-dimethyl-, cis-	C7H14	98	−	+	Alkene
8	Cyclopentane, ethyl-	C7H14	98	−	+	Alkene
9	Heptane (CAS)	C7H16	100	+	−	Alkane
10	Benzene, 1,4-dimethyl- (CAS)	C8H10	106	−	+	Monocyclic
11	Cyclohexane, 1,3-dimethyl-	C8H16	112	−	+	Alkene
12	Cyclohexane, 1,4-dimethyl-	C8H16	112	−	+	Alkene
13	Cyclohexane, ethyl-	C8H16	112	−	+	Alkene
14	Octane	C8H18	114	−	+	Alkane
15	2,4-Dimethyl-1-heptene	C9H18	126	−	+	Alkene
16	Cyclohexane,1-ethyl-4-methyl-, trans	C9H18	126	+	−	Alkene
17	Cyclohexane, 1,1,3-trimethyl- (CAS)	C9H18	126	+	+	Alkene
18	Cyclohexane, 1,2,3-trimethyl	C9H18	126	+	−	Alkene
19	Cyclohexane, 1,2,4-trimethyl-	C9H18	126	+	+	Alkene
20	Cyclohexane, 1,3,5-trimethyl-	C9H18	126	+	−	Alkene
21	Cyclohexane, 1-ethyl-2-methyl-	C9H18	126	+	+	Alkene
22	Heptane, 2,5-dimethyl-	C9H20	128	−	+	Alkane
23	Nonane (CAS)	C9H20	128	+	+	Alkane
24	Octane, 4-methyl-	C9H20	128	+	−	Alkane
25	Cyclooctene, 1,2-dimethyl-	C10H18	138	+	−	Alkyne
26	1,2,3,5-tetramethylcyclohexane	C10H20	140	+	−	Alkene
27	Cyclohexane, 1,2-diethyl-	C10H20	140	−	+	Alkene
28	1,1,2,3-tetramethylcyclohexane	C10H20	140	+	+	Alkene
29	Cyclohexane,1-methyl-2-propyl	C10H20	140	+	−	Alkene
30	Cyclohexane,2-ethyl-1,3-dimethyl	C10H20	140	+	−	Alkene
31	Decane	C10H22	142	+	−	Alkane
32	Heptane, 3-ethyl-2-methyl-	C10H22	142	+	+	Alkane
33	Methylnaphthalene	C11H10	142	+	−	polycyclic
34	Naphthalene, 2-methyl	C11H10	142	+	−	polycyclic
35	Octane, 2,6-dimethyl-	C10H22	142	−	+	Alkane
36	Naphthalene, decahydro-2-methyl-	C11H20	152	+	+	Alkyne
37	trans-Decalin, 2-methyl-	C11H20	152	−	+	Alkyne
38	Cyclodecane, methyl-	C11H22	154	−	+	Alkene
39	Cyclohexane, pentyl-	C11H22	154	+	−	Alkene
40	Cyclopentane, 1,2-dipropyl-	C11H22	154	+	+	Alkene

Table (3): Continued:

No.	Hydrocarbons	Molecular formula	Molecular weight	*B. oleae*	*B. zonata*	*HC class*
41	Decane, 4-methyl-	C11H24	156	+	−	Alkane
42	Decane, 5-methyl-	C11H24	156	−	+	Alkane
43	Undecane	C11H24	156	+	+	Alkane
44	Cyclododecane	C12H24	168	−	+	Alkene
45	1-Dodecene	C12H24	168	+	−	Alkene
46	1-Heptene, 2-pentyl-	C12H24	168	+	−	Alkene
47	1-sec-butyl-1-(2-methyl butyl)cyclopropane	C12H24	168	−	+	Alkene
48	1-Undecene, 7-methyl-	C12H24	168	+	−	Alkene
49	2-Undecene, 6-methyl-, (Z)-	C12H24	168	+	−	Alkene
50	Cyclohexane,1-methyl-4-(1-methylbutyl)-	C12H24	168	+	−	Alkene
51	Cyclooctane, 1-methyl-3-propyl	C12H24	168	+	−	Alkene
52	2,3-Dimethyldecane	C12H26	170	+	−	Alkane
53	2,6-Dimethyldecane	C12H26	170	−	+	Alkane
54	Dodecane (CAS)	C12H26	170	+	+	Alkane
55	Undecane, 2-methyl-	C12H26	170	−	+	Alkane
56	Cyclopentane, 1-pentyl-2-propyl-	C13H26	182	−	+	Alkene
57	Heptylcyclohexane	C13H26	182	−	+	Alkene
58	Dodecane, 2-methyl-	C13H28	184	+	−	Alkane
59	Nonane, 5-(1-methylpropyl)-	C13H28	184	−	+	Alkane
60	Tridecane	C13H28	184	+	+	Alkane
61	Undecane, 2,6-dimethyl-	C13H28	184	+	−	Alkane
62	1-Tetradecene	C14H28	196	−	+	Alkene
63	Cyclotetradecane	C14H28	196	+	−	Alkene
64	Decane, 2,3,5,8-tetramethyl	C14H30	198	+	−	Alkane
65	Tetradecane	C14H30	198	+	−	Alkane
66	Tridecane, 4-methyl	C14H30	198	−	+	Alkane
67	Benzene, (2-methyloctyl)-	C15H24	204	−	+	monocyclic
68	1-Pentadecene	C15H30	210	+	−	Alkene
69	Dodecane, 2,6,11-trimethyl	C15H32	212	+	−	Alkane
70	Pentadecane	C15H32	212	+	−	Alkane
71	Benzene, (1-butylhexyl)-	C16H26	218	+	−	monocyclic
72	1-Hexadecene (CAS)	C16H32	224	−	+	Alkene
73	1-Nonylcycloheptane	C16H32	224	+	+	Alkene
74	Cyclopentane, undecyl	C16H32	224	+	−	Alkene
75	Decane, 2-cyclohexyl-	C16H32	224	−	+	Alkene
76	Hexadecane	C16H34	226	+	−	Alkane

Discussion

As taxonomy is in crisis due to inadequate funding, lack of taxonomists, the impact factor of taxonomicaljournals is very low, among other reasons, Guerra-García, *et al.*, (2008) concluded that taxonomy is in cross-roadsand suggested to apply the new approaches (i. e. biodiversity conservation, internet and web pages, molecular techniques, phylogeny…etc.)

Chemical analysis of cuticular hydrocarbons offers a non destructive and reliable chemotaxonomic method (De Renobales *et al.*, 1991). Also, the chemotaxonomic tools solve the different taxonomic problems, for example, the morphological similarity as the members of the *Anopheles gambiae* complex (Anyanwu, *et al*., 2000); differentiation of sibling species of sandflies (Ryan *et al*., 1986).

Using cuticular hydrocarbons as taxonomic tool, also, solvethe problem facing the taxonomists of finding a boundary or range beyond which a species canbe classed as independent (Sites and Marshall, 2004). Cuticular hydrocarbons are heritable and several genes have been implicated to play a role in CHC biosynthesis (Kather and Martin, 2012). This gave their characters its taxonomic value as they are stable and not easily changeable.

The present results showed many differences in cuticular hydrocarbon components of the two studied species. Five classes of hydrocarbons surveyed in this investigation were all represented in *Bactrocera oleae*, while four classes of them present in *B. zonata* (i.e. polycyclic class of hydrocarbons not pro in this species). Wagner *et al.* (1998) tested for differences in the relative abundance of classes of hydrocarbon ompounds among task groups of colonies of the harvester ant, *Pogonomyrmex barbatus*, they found differences in the proportions of the four major classes of hydrocarbons on the cuticle.

As shown in table (3), many cuticular hydrocarbon components distinguished each species and can be used to separate them taxonomically (35 and 30 CHs components for *B. oleae* and *B. zonata* respectively). GC/MS technique, is now established as a precise chemotaxonomic tool in different insect groups e.g. Sarcophagidae (Braga *et al*. 2013), blowfly (Moore *et al*. 2014 and Rodrigo *et al.* 2017).

Different studies obtained similar results in other species using GC/MS technique and, for example, the ant *Formica candida* stands outamongst other *Formica* species in the presence of alkadienes (Martin *et al*., 2008). The cricket *Gryllotalpa marismortui*, however, produces some of its alkanes in significantly higher amounts compared with its close relative *Gryllotalpa cossyrensis* (Broza*et al.*, 1998).

In conclusion, the present study aimed to investigate the qualitative and quantitative differences between cuticular hydrocarbon profiles of two Tephirtid species (*Bactrocera oleae* and *B. zonata*). The study stated the great differences between the CHs components of the two species and suggested to apply them as precise taxonomic tool side by side with classic taxonomy.

REFERENCES

Anyanwu, G. I.; Molyneux, D. H. and Phillips, A. 2000. Variation in Cuticular Hydrocarbons among Strains of the *Anopheles gambiae* sensustric to by Analysis of Cuticular Hydrocarbons Using Gas Liquid Chromatography of Larvae. MemInst Oswaldo Cruz, Rio de Janeiro, Vol. 95(3): 295-300.

Blomquist, G. C. and Bagnères, A. G. 2010. Insect hydrocarbons: Biology, biochemistry, and chemical ecology. Cambridge University Press, Cambridge.6:878-889

Braga, M. V.; Pinto, Z. T.;Queiroz, M. M. C.; Matsumoto, N.and Blomquist, G. J. 2013. Cuticular hydrocarbons as a tool for the identification of insect species: puparial cases from Sarcophagidae. Acta Trop., 128:479–485.

Broza, M.; Blondheim, Y. and Nevo, E. 1998. New species of more crickets of the *Gryllotalpagryllotalpa* group (Orthoptera: Gryllotalpidae) from Israel, based on morphology, song recordings, chromosomes and cuticular hydrocarbons, with comments on the distribution of the group in Europe and the Mediterranean region. Systematic Entomology, 23: 125–135.

Coby, S.; Veeresh , L. S; Hugh P. Young and Jane A. S. 1998. Sites of Synthesis and Transport Pathways of Insect Hydrocarbons : Cuticle and ovary as target tissues. Amer.Zool., 38:382-393.

De Renobales, M.; Nelson, D. R., and Blomquist, G. J. 1991.Cuticular Lipids, pp. 240-251,in K. Binnington and A. Retnakaran (eds.). Physiology of the Insect Epidermis. CSIRO,Melbourne, Australia.

Delrio, G. and Cocco, A. 2012. The Peach Fruit Fly, *Bactrocera zonata*: A Major Threat For Mediterranean Fruit Crops?. ISHS Acta Horticulturae 940: XXVIII International Horticultural Congress on Science and Horticulture for People: International Symposium on the Challenge for a Sustainable Production, Protection and Consumption of Mediterranean Fruits and Nuts

Foley, B.; Chenoweth, S.F.; Nuzhdin, S.V. and Blows, M.W. 2007. Natural geneticvariation in cuticular hydrocarbon expression in male and female *Drosophil amelanogaster*. Genetics. 175:1465–1477.

Gibbs, A. G. and Rajpurohit, S. 2010.Cuticular lipids and water balance. In Insect Hydrocarbons (ed. G. J. Blomquist and A. G. Bagneres), pp. 100-120. Cambridge: Cambridge University Press.

Gołębiowski, M; Boguś, M. I.; Paszkiewicz, M and Stepnowski, P. 2011.Cuticular lipids of insects as potential biofungicides: methods of lipid composition analysis. Anal. Bioanal. Chem., 399: 3177–3191.

Grunshawn, J. P.; Guermouche, H.; Guermouche, S.; Jago, N. D.; Jullien, R.; Knowles, E.; and Perez, F. 1990. Chemical taxonomic studies of cuticular hydrocarbons in locusts of the *Schistocerca americana* complex: Chemical relationships between New World and Old Worldspecies. J. Chem. Ecol., 16:2835–3858.

Guerra-García, J.M.; Espinosa, F. and García-Gómez, J.C. (2008). Trends in Taxonomy today: an overview about the main topics in Taxonomy. ZoologicaBaetica, 19, 15–49.

Jackson, L. L. and Blomquist, G. J. 1976. Insect waxes. Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, Holland.

Kather, R. and Martin, S. J. 2012. Cuticular hydrocarbon profiles as a taxonomic tool:advantages, limitations and technical aspects. Physiological Entomology (2012) 37: 25–32.

Martin, S. J.; Helantera, H. and Drijfhout, F. P. 2008. Evolution ofspecies-specific cuticular hydrocarbon patterns in *Formica* ants. Biological Journal of the Linnean Society, 95: 131–140.

Moore, H. E.; Adam, C. D. and Drijfhout, F. P. 2014. Identifying 1st. instar larvae for three forensically important blowfly species using “fingerprint” cuticular hydrocarbon analysis. Forensic Sci. Int., 240:48–53.

Nelson, D. R.; Dillwith, J. W. and Blomquist, G. J. 1981.Cuticular hydrocarbons of thehousefly, *Musca domestica*. Insect Biochem., 11:187–197.

Page, M.; Nelson, L. J.; Haverty, M. I. andBlomquist, G. J. 1990. Cuticular hydrocarbons of eight speciesof North American cone beetle, Conophthorus Hopkins. J. Chem. Ecol. 16:1 173-1198.

Rice, R. E.; Phillips, P. A.; Stewart-Leslie, J. andSibbett, G. S. 2003. Olive fruit fly populations measured in central and southern California.Calif. Agric.57: 122–127.

Rodrigo, R. B.; Marina, V. B.; Gary, J. B. and Margareth, M. 2017.Cuticular hydrocarbon profiles as a chemotaxonomic tool for three blowfly species (Diptera: Calliphoridae) of forensic interest. Journal of Natural History,Volume 51: Issue 25-26.

Ryan, L; Phillips, A.; Milligan, P.; Lainson, R., Molyneux, D. and Shaw, J. 1986. Separation of female *Psychodopygus wellcomei* and *P. complexus* (Diptera: Psychodidae) by cuticular hydrocarbonanalysis. Acta Trop, 43: 85-89.

Sites, J. W. and Marshall, J. C. 2004. Operational criteria for delimiting species. Annual Review of Ecology Evolution and Systematics, 35: 199–227.

Thomas, A. C. and Dennis, R. N. 1981. Characterization and distribution of the hydrocarbons found in diapausing pupae tissues of the tobacco hornworm, *Manducasexta* (L.). Journal of Lipid Research Volume 22:28-70.

Wagner, D.; Brown, M. F.; Brun, P.; Cuevas, W.; Moses, L. E.; Chao, D. L. and Gordon, D. M. 1998.Task-Related Differences In The Cuticular Hydrocarbon Composition Of Harvester Ants, *Pogonomyrmex* Barbatus. Journal Of Chemical Ecology, vol. 24, no.12.:2021-2042.

Calderón-Fernández, G. M., Girotti, J. R. and Patricia Juárez, M. 2011. Cuticular Hydrocarbons of *Triatoma dimidiata* (Hemiptera: Reduviidae): Intraspecific Variation and Chemotaxonomy. Journal of Medical Entomology 48(2):262-271.

Carlson, D. A., Milstrey, S. K. and Narang, S. K. 1993. Classification of tsetse *Glossina* spp. (Diptera: Glossinidae) by gas chromatographic analysis of cuticular components. *Bull. Entomol. Res.* 83: 507–515.

Copren, K. A., Nelson, L. J., Vargo, E. L. and Haverty, M. I. 2005. Phylogenetic analyses of mtDNA sequences corroborate taxonomic designations based on cuticular hydrocarbons in subterranean termites. *Mol. Phylogenet. Evol.* 35: 689–700.