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Ahmad Galhoum

Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt,
ahmadgalhoum@hotmail.com

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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 to 100 different types (Nelson *et al.*, 1981). Insect species usually possess complex mixtures of hydrocarbons including n-alkanes, branched mono-, di-, or trimethylalkanes, 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 dimidiata*).

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 & NIST MASS 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 *Bactroceea* (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 C₅ to C₁₂, alkenes from C₁₂ to C₁₆, monocyclic C₇ and C₁₆ and polycyclic C₁₁.

2- Cuticular Hydrocarbon Analysis of Bactrocera oleae:

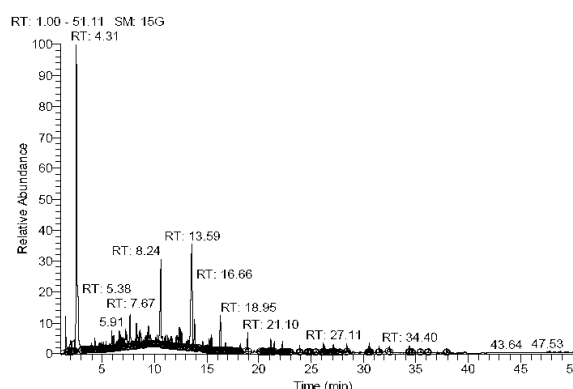


Figure (1): Chromatogram obtained by GC/MS: Cuticular hydrocarbons of *Bactrocera oleae*.

Bactrocera oleae had a mixture of forty seven hydrocarbons (Figure 1, Table 1) with chain lengths varying from C₆ to C₁₆. 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*:

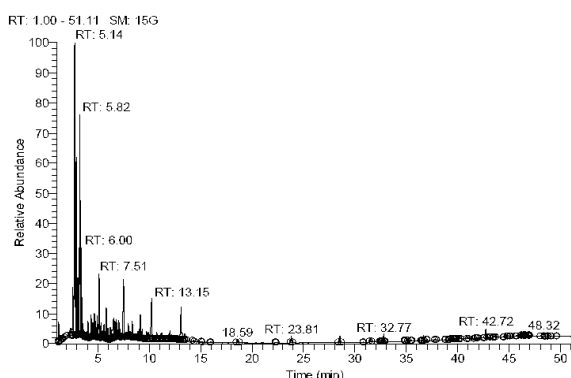


Figure (2): Chromatogram obtained by GC/MS: Cuticular hydrocarbons of *Bactrocera zonata*

Bactrocera zonata had a mixture of forty one hydrocarbons (Fig. 2, Table 2) with chain lengths varying from C₅ to C₁₆. 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 and polycyclic 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% while Alkene 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-C₁₆ and 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-C₁₆ equally distributed. The alkane ranged from C₅-C₁₅ equally 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 Methyl naphthalene (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% to 2.88% 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	C ₆ H ₁₂	84
2.37	Pentane, 3-methyl- (CAS)	0.84	C ₆ H ₁₄	86
2.27	Hexane (CAS)	1.07	C ₆ H ₁₄	86
1.47	Heptane (CAS)	1.92	C ₇ H ₁₆	100
4.83	Cyclohexane, 1,2,4-trimethyl	0.05	C ₉ H ₁₈	126
4.03	Cyclohexane, 1,1,3-trimethyl	0.08	C ₉ H ₁₈	126
5.05	Cyclohexane,1-ethyl-4-methyl-, trans	0.11	C ₉ H ₁₈	126
4.75	Cyclohexane, 1,2,3-trimethyl	0.16	C ₉ H ₁₈	126
3.93	Cyclohexane, 1,3,5-trimethyl-	0.18	C ₉ H ₁₈	126
4.97	Cyclohexane, 1-ethyl-2-methyl	0.22	C ₉ H ₁₈	126
3.68	Octane, 4-methyl-	0.09	C ₉ H ₂₀	128
5.14	Nonane	0.28	C ₉ H ₂₀	128
9.41	Cyclooctene, 1,2-dimethyl-	1.43	C ₁₀ H ₁₈	138
6.47	1,2,3,5-tetramethylcyclohexane	0.32	C ₁₀ H ₂₀	140
6.47	1,1,2,3-tetramethylcyclohexane	0.32	C ₁₀ H ₂₀	140
6.12	Cyclohexane,2-ethyl-1,3-dimethyl	0.72	C ₁₀ H ₂₀	140
7.26	Cyclohexane,1-methyl-2-propyl	1.75	C ₁₀ H ₂₀	140
16.58	Naphthalene, 2-methyl	0.24	C ₁₁ H ₁₀	142
17.01	Methylnaphthalene	0.28	C ₁₁ H ₁₀	142
6.04	Heptane, 3-ethyl-2-methyl-	0.36	C ₁₀ H ₂₂	142
7.67	Decane	2.71	C ₁₀ H ₂₂	142
11.51	Naphthalene,decahydro-2-methyl	0.40	C ₁₁ H ₂₀	152
8.97	Cyclopentane, 1,2-dipropyl-	0.40	C ₁₁ H ₂₂	154
11.61	Cyclohexane, pentyl-	0.46	C ₁₁ H ₂₂	154
8.24	Decane, 4-methyl-	1.24	C ₁₁ H ₂₄	156
10.65	Undecane	6.73	C ₁₁ H ₂₄	156
3.43	1-Heptene, 2-pentyl-	0.08	C ₁₂ H ₂₄	168
4.39	1-Undecene, 7-methyl-	0.10	C ₁₂ H ₂₄	168
5.79	Cyclooctane, 1-methyl-3-propyl	0.12	C ₁₂ H ₂₄	168
14.85	1-Dodecene (CAS)	0.24	C ₁₂ H ₂₄	168
3.16	2-Undecene, 6-methyl-, (Z)-	0.45	C ₁₂ H ₂₄	168
13.09	Cyclohexane,1-methyl-4-(1-methylbutyl)-	0.57	C ₁₂ H ₂₄	168
9.63	2,3-Dimethyldecane	0.78	C ₁₂ H ₂₆	170
13.59	Dodecane	10.59	C ₁₂ H ₂₆	170
15.22	Dodecane, 2-methyl-	0.47	C ₁₃ H ₂₈	184
13.85	Undecane, 2,6-dimethyl-	1.37	C ₁₃ H ₂₈	184
16.32	Tridecane	2.40	C ₁₃ H ₂₈	184
10.24	Cyclotetradecane	0.72	C ₁₄ H ₂₈	196
16.77	Decane, 2,3,5,8-tetramethyl	0.25	C ₁₄ H ₃₀	198
18.95	Tetradecane	0.99	C ₁₄ H ₃₀	198
6.39	1-Pentadecene	0.31	C ₁₅ H ₃₀	210
22.25	Dodecane, 2,6,11-trimethyl	0.40	C ₁₅ H ₃₂	212
21.47	Pentadecane	0.37	C ₁₅ H ₃₂	212
22.34	Benzene, (1-butylhexyl)-	0.06	C ₁₆ H ₂₆	218
20.19	Cyclopentane, undecyl	0.04	C ₁₆ H ₃₂	224
10	1-Nonylcycloheptane	0.46	C ₁₆ H ₃₂	224
16.66	Hexadecane	0.12	C ₁₆ H ₃₄	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	Cyclododecane, 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. zonata*

No.	Hydrocarbons	Molecular formula	Molecular weight	<i>B. oleae</i>	<i>B. zonata</i>	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	<i>B. oleae</i>	<i>B. zonata</i>	HC class
41	Decane, 4-methyl-	C ₁₁ H ₂₄	156	+	-	Alkane
42	Decane, 5-methyl-	C ₁₁ H ₂₄	156	-	+	Alkane
43	Undecane	C ₁₁ H ₂₄	156	+	+	Alkane
44	Cyclododecane	C ₁₂ H ₂₄	168	-	+	Alkene
45	1-Dodecene	C ₁₂ H ₂₄	168	+	-	Alkene
46	1-Heptene, 2-pentyl-	C ₁₂ H ₂₄	168	+	-	Alkene
47	1-sec-butyl-1-(2-methyl butyl)cyclopropane	C ₁₂ H ₂₄	168	-	+	Alkene
48	1-Undecene, 7-methyl-	C ₁₂ H ₂₄	168	+	-	Alkene
49	2-Undecene, 6-methyl-, (Z)-	C ₁₂ H ₂₄	168	+	-	Alkene
50	Cyclohexane,1-methyl-4-(1-methylbutyl)-	C ₁₂ H ₂₄	168	+	-	Alkene
51	Cyclooctane, 1-methyl-3-propyl	C ₁₂ H ₂₄	168	+	-	Alkene
52	2,3-Dimethyldecane	C ₁₂ H ₂₆	170	+	-	Alkane
53	2,6-Dimethyldecane	C ₁₂ H ₂₆	170	-	+	Alkane
54	Dodecane (CAS)	C ₁₂ H ₂₆	170	+	+	Alkane
55	Undecane, 2-methyl-	C ₁₂ H ₂₆	170	-	+	Alkane
56	Cyclopentane, 1-pentyl-2-propyl-	C ₁₃ H ₂₆	182	-	+	Alkene
57	Heptylcyclohexane	C ₁₃ H ₂₆	182	-	+	Alkene
58	Dodecane, 2-methyl-	C ₁₃ H ₂₈	184	+	-	Alkane
59	Nonane, 5-(1-methylpropyl)-	C ₁₃ H ₂₈	184	-	+	Alkane
60	Tridecane	C ₁₃ H ₂₈	184	+	+	Alkane
61	Undecane, 2,6-dimethyl-	C ₁₃ H ₂₈	184	+	-	Alkane
62	1-Tetradecene	C ₁₄ H ₂₈	196	-	+	Alkene
63	Cyclotetradecane	C ₁₄ H ₂₈	196	+	-	Alkene
64	Decane, 2,3,5,8-tetramethyl	C ₁₄ H ₃₀	198	+	-	Alkane
65	Tetradecane	C ₁₄ H ₃₀	198	+	-	Alkane
66	Tridecane, 4-methyl	C ₁₄ H ₃₀	198	-	+	Alkane
67	Benzene, (2-methyloctyl)-	C ₁₅ H ₂₄	204	-	+	monocyclic
68	1-Pentadecene	C ₁₅ H ₃₀	210	+	-	Alkene
69	Dodecane, 2,6,11-trimethyl	C ₁₅ H ₃₂	212	+	-	Alkane
70	Pentadecane	C ₁₅ H ₃₂	212	+	-	Alkane
71	Benzene, (1-butylhexyl)-	C ₁₆ H ₂₆	218	+	-	monocyclic
72	1-Hexadecene (CAS)	C ₁₆ H ₃₂	224	-	+	Alkene
73	1-Nonylcycloheptane	C ₁₆ H ₃₂	224	+	+	Alkene
74	Cyclopentane, undecyl	C ₁₆ H ₃₂	224	+	-	Alkene
75	Decane, 2-cyclohexyl-	C ₁₆ H ₃₂	224	-	+	Alkene
76	Hexadecane	C ₁₆ H ₃₄	226	+	-	Alkane

Discussion

As taxonomy is in crisis due to inadequate funding, lack of taxonomists, the impact factor of taxonomical journals is very low, among other reasons, Guerra-García, *et al.*, (2008) concluded that taxonomy is in cross-roads and 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, solve the problem facing the taxonomists of finding a boundary or range beyond which a species can be 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 present in this species). Wagner *et al.* (1998) tested for differences in the relative abundance of classes of hydrocarbon compounds 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 out amongst other *Formica* species in the presence of alkenes (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 Tephritid 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 a precise taxonomic tool side by side with classic taxonomy.

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