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Cuticular Hydrocarbons as Chemotaxonomic Character for Two Species of Genus *Alphitobius* Stephens (Coleoptera: Tenebrionidae) in Egypt

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Abstract: ***Genus Alphitobius Stephens, 1829 (Alphitobiini Reitter, 1917, subfamily Tenebrioninae Latreille, 1802) is represented in Egypt by two species, A. diaperinus (Panzer) and A. laevigatus (Fabricius, 1781). Species of Alphitobius have been identified based on adult morphological characters. Species identification is often difficult because they are closely similar and are not so easy to be distinguished morphologically. This study constitutes an attempt to apply cuticular hydrocarbons to distinguish the two species in Egypt. The data of GC/MS analysis showed that the two species shared eleven of sixty hydrocarbon compounds, A. diaprinus was characterized by having twenty nine compounds not found in A. laevigatus while A. laevigatus had twenty compounds not represented in A. diaprinus. These results suggest that the application of cuticular hydrocarbons as chemotaxonomic character can represent a contribution toward developing modern, precise and confirmed research tool in taxonomy.***

Keywords: *Cuticular hydrocarbons, Alphitobius, GC/MS, Chemotaxonomy.*

1. Introduction

The lesser mealworm, *A. diaperinus* is a serious pest of poultry facilities, causing structural damage to poultry houses and is associated with the transmission of fatal pathogens (e.g., nematodes and bacteria) to poultry and humans (Crippen and Poole, 2012). Black fungus beetle, *A. laevigatus*, is endemic to Sub-Saharan Africa but currently has a cosmopolitan synanthropic distribution (Schawaller and Grimm, 2014). It is mycetophagous and known as a pest of various stored products (such as bread, flour products, rice, cocoa etc.) (Maitip *et al.*, 2017).

Hydrocarbons represent universal constituents of the insect cuticle (Chapman, 1998). Cuticular hydrocarbons (CH) are considered to be stable end products of genetically controlled metabolic pathways (Grunshawn *et al.*, 1990). The common function of (CH) is to protect against desiccation. In social insects, CH are regarded as the main signals responsible for nestmate recognition (Howard and Blomquist, 2005).

Hydrocarbons are highly reliable tool in insect taxonomy and play an important role in chemotaxonomy, as they are permanent and abundant components of their cuticle which can be determined even from dead individuals (Kather and Martin, 2012). Cuticular hydrocarbons have been most successful as taxonomic tool in sibling species identification of *Anopheles* mosquitoes (Carlson and Service, 1979), sandflies (Phillips *et al.*, 1990), *Drosophila* (Cobb and Jallon, 1990), population genetic variation (Dapporto *et al.*, 2009) and Aphid species identification (Raboudi *et al.*, 2005).
Due to many reasons (i.e. inadequate funding, lack of taxonomists, the extremely low recruitment of young scientists into taxonomy and systematics and very low impact factor of taxonomical journals) taxonomy is in crisis (Guerra-garcía *et al.*, 2008). However, the advances in molecular and biochemical techniques are given some light to taxonomy. Biochemical techniques can act as useful supportive tool to morphological approach. Many authors suggested creative ideas for modernizing the taxonomic work. Bisby *et al.* (2002) suggested that all data about taxonomic work concerning species (i.e. nomenclature, descriptions, images, publications and debate) should be available on the web. Wheeler *et al.* (2004) recommended applying the new technologies in taxonomy to approach taxonomy as large-scale international science.

The present study reports the identification of the hydrocarbon components in the cuticle of two species of genus *Alphitobius*, as a preliminary, relatively precise approach to determine the affinity of cuticular hydrocarbon patterns between the species of this genus.

2. Materials and Methods

Insects

The present study is carried out on preserved insects of two species which belong to genus *Alphitobius*, obtained from the side collection of the Plant Protection Research Institute, Dokki, Giza governorate, Egypt.

Hydrocarbon extraction and analysis.

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

Gas Chromatography – Mass Spectrometry (GC/MS).

GC/MS analysis was conducted in Central Agricultural Pesticides Laboratory. Samples were run on a Aglient 6890 GC-MS, fitted with a silica capillary column PAS-5 ms. (Length 30 m. x Internal diameter 0.32 mm. x film thickness 0.25 µm), carrier gas of helium. One microliter of sample was injected into the injector in pulsed splitless mode. The injector temperature was at 280 °C. The GC temperature program was started at 60 °C (2 min.) then raised to 280 °C if 5 °C/min. Mass spectrometric was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230 °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 and Wily mass spectral database and by comparison to literature relative retention indexes. Only one component was chosen, among those suggested by different database; for each peak and some components were neglected as they aren't belonging to hydrocarbon classes.

1- Cuticular hydrocarbons of *Alphitobius diaprinus*:
Of sixty-eight compounds obtained by GC/MS analysis (Fig; 1), only fifty-six are hydrocarbons. As shown in Table 1, *Alphitobius diaprinus* had a mixture of hydrocarbons with chain lengths varying from C_9 to C_{35}. The hydrocarbon of *A. diaprinus* was classified within ten categories namely, alkane (28), n- alkane (8), cycloalkane (6), monocyclic hydrocarbons (5), bicyclic hydrocarbons (3), alkene (2) and one compound was classified within each of alkyne, oil, tricyclic and heterocyclic hydrocarbons. The most abundant hydrocarbon in *A.diaprinus* was azulene (6.2%) followed by dodecane (5%), undecane (4.5%), docosane (4.3%), pentatriacontane (3.5), naphthalene, decahydro (3.31%), docosane (2.94%), n –docosane (2.6), n – tetracosane (2.44). Twenty hydrocarbons represented as traces (i.e. less than 1%).

3. Results

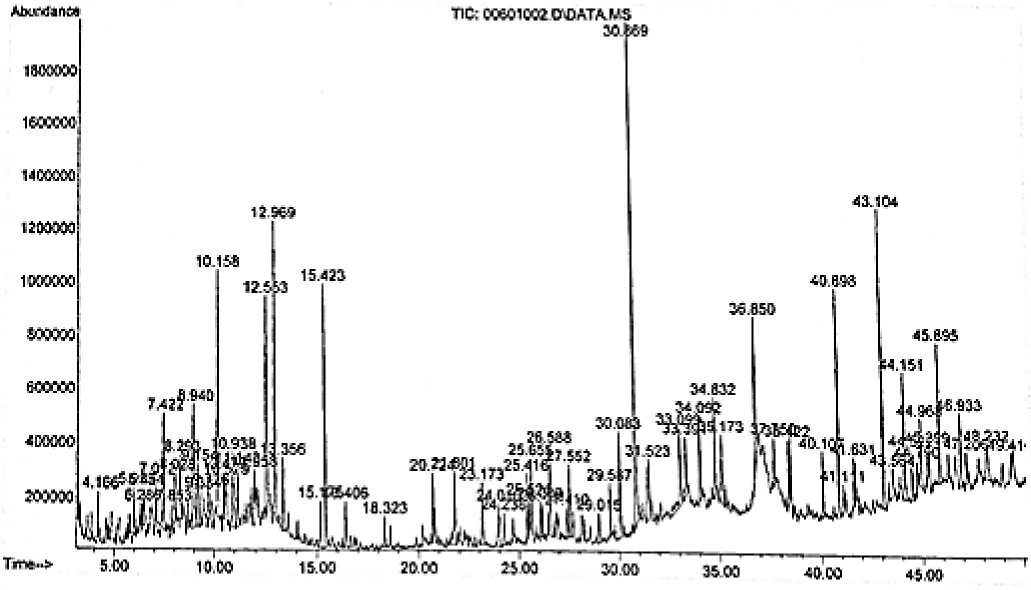
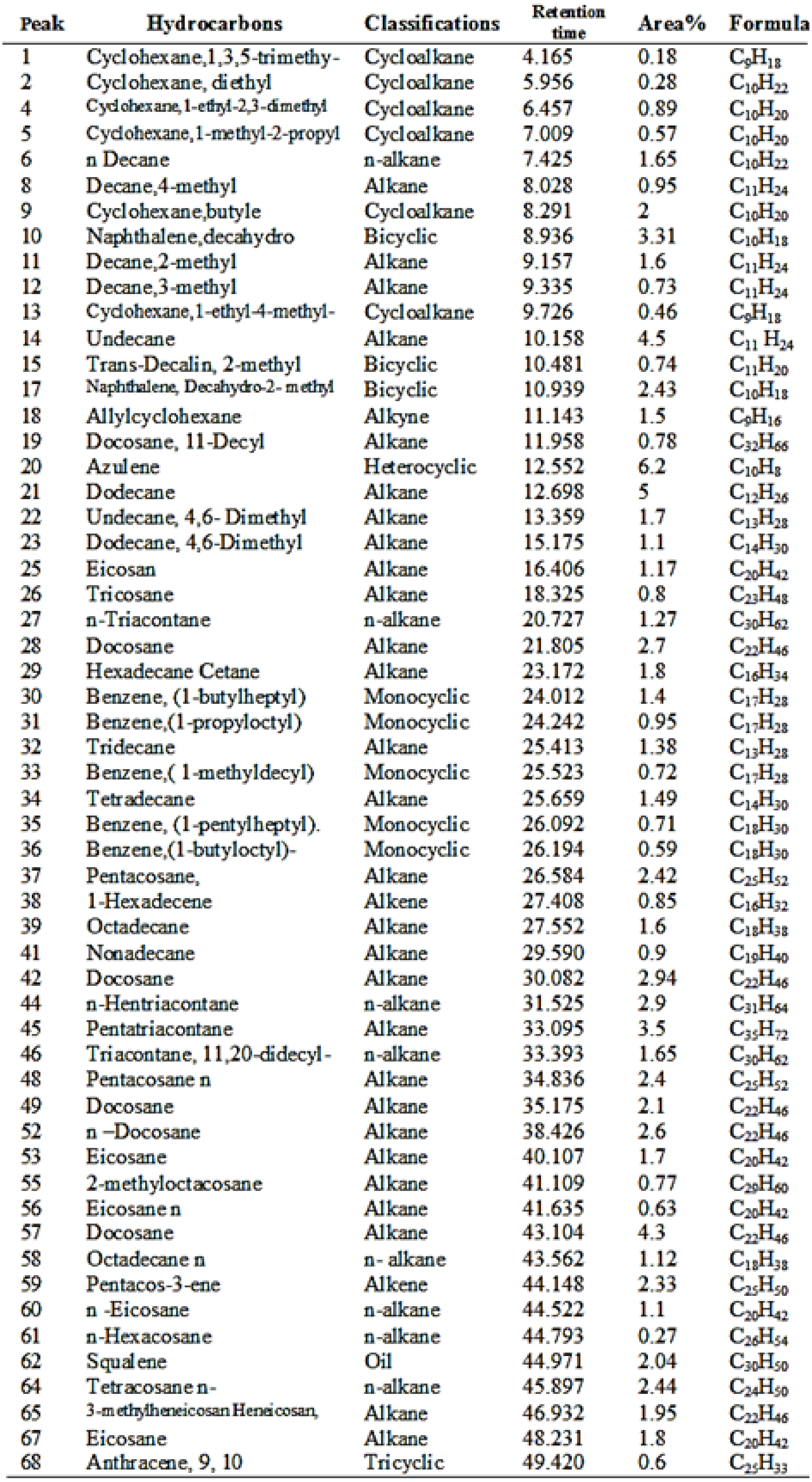


Fig. 1 Chromatogram obtained by GC/MS: cuticular hydrocarbons on *Alphitobius diaprinus*.

A. diaprinus possess 29 hydrocarbons not found in *A. laevigtus* representing 39.2% of the total peak areas. Three compounds of these were predominant namely, Pentatriacontane (3.5%), n- Hentriacontane (2.9%), n-Pentacosane (2.4%).

Table (1): Cuticular hydrocarbons of *Iphitobius diaprinus*



Peak	Hydrocarbons	Classifications	Retention time	Area%	Formula
1	Cyclohexane,1,3,5-trimethy-	Cycloalkane	4.165	0.18	C_9H_{18}
2	Cyclohexane, diethyl	Cycloalkane	5.956	0.28	$C_{10}H_{22}$
4	Cyclohexane,1-ethyl-2,3-dimethyl	Cycloalkane	6.457	0.89	$C_{10}H_{20}$
5	Cyclohexane,1-methyl-2-propyl	Cycloalkane	7.009	0.57	$C_{10}H_{20}$
6	n Decane	n-alkane	7.425	1.65	$C_{10}H_{22}$
8	Decane,4-methyl	Alkane	8.028	0.95	$C_{11}H_{24}$
9	Cyclohexane,butyle	Cycloalkane	8.291	2	$C_{10}H_{20}$
10	Naphthalene,decahydro	Bicyclic	8.936	3.31	$C_{10}H_{18}$
11	Decane,2-methyl	Alkane	9.157	1.6	$C_{11}H_{24}$
12	Decane,3-methyl	Alkane	9.335	0.73	$C_{11}H_{24}$
13	Cyclohexane,1-ethyl-4-methyl-	Cycloalkane	9.726	0.46	C_9H_{18}
14	Undecane	Alkane	10.158	4.5	$C_{11}\ H_{24}$
15	Trans-Decalin, 2-methyl	Bicyclic	10.481	0.74	$C_{11}H_{20}$
17	Naphthalene, Decahydro-2- methyl	Bicyclic	10.939	2.43	$C_{10}H_{18}$
18	Allylcyclohexane	Alkyne	11.143	1.5	C_9H_{16}
19	Docosane, 11-Decyl	Alkane	11.958	0.78	$C_{32}H_{66}$
20	Azulene	Heterocyclic	12.552	6.2	$C_{10}H_8$
21	Dodecane	Alkane	12.698	5	$C_{12}H_{26}$
22	Undecane, 4,6- Dimethyl	Alkane	13.359	1.7	$C_{13}H_{28}$
23	Dodecane, 4,6-Dimethyl	Alkane	15.175	1.1	$C_{14}H_{30}$
25	Eicosan	Alkane	16.406	1.17	$C_{20}H_{42}$
26	Tricosane	Alkane	18.325	0.8	$C_{23}H_{48}$
27	n-Triacontane	n-alkane	20.727	1.27	$C_{30}H_{62}$
28	Docosane	Alkane	21.805	2.7	$C_{22}H_{46}$
29	Hexadecane Cetane	Alkane	23.172	1.8	$C_{16}H_{34}$
30	Benzene, (1-butylheptyl)	Monocyclic	24.012	1.4	$C_{17}H_{28}$
31	Benzene,(1-propyloctyl)	Monocyclic	24.242	0.95	$C_{17}H_{28}$
32	Tridecane	Alkane	25.413	1.38	$C_{13}H_{28}$
33	Benzene,(1-methyldecyl)	Monocyclic	25.523	0.72	$C_{17}H_{28}$
34	Tetradecane	Alkane	25.659	1.49	$C_{14}H_{30}$
35	Benzene, (1-pentylheptyl).	Monocyclic	26.092	0.71	$C_{18}H_{30}$
36	Benzene,(1-butyloctyl)-	Monocyclic	26.194	0.59	$C_{18}H_{30}$
37	Pentacosane,	Alkane	26.584	2.42	$C_{25}H_{52}$
38	1-Hexadecene	Alkene	27.408	0.85	$C_{16}H_{32}$
39	Octadecane	Alkane	27.552	1.6	$C_{18}H_{38}$
41	Nonadecane	Alkane	29.590	0.9	$C_{19}H_{40}$
42	Docosane	Alkane	30.082	2.94	$C_{22}H_{46}$
44	n-Hentriacontane	n-alkane	31.525	2.9	$C_{31}H_{64}$
45	Pentatriacontane	Alkane	33.095	3.5	$C_{35}H_{72}$
46	Triacontane, 11,20-didecyl-	n-alkane	33.393	1.65	$C_{30}H_{62}$
48	Pentacosane n	Alkane	34.836	2.4	$C_{25}H_{52}$
49	Docosane	Alkane	35.175	2.1	$C_{22}H_{46}$
52	n –Docosane	Alkane	38.426	2.6	$C_{22}H_{46}$
53	Eicosane	Alkane	40.107	1.7	$C_{20}H_{42}$
55	2-methyloctacosane	Alkane	41.109	0.77	$C_{29}H_{60}$
56	Eicosane n	Alkane	41.635	0.63	$C_{20}H_{42}$
57	Docosane	Alkane	43.104	4.3	$C_{22}H_{46}$
58	Octadecane n	n- alkane	43.562	1.12	$C_{18}H_{38}$
59	Pentacos-3-ene	Alkene	44.148	2.33	$C_{25}H_{50}$
60	n -Eicosane	n-alkane	44.522	1.1	$C_{20}H_{42}$
61	n-Hexacosane	n-alkane	44.793	0.27	$C_{26}H_{54}$
62	Squalene	Oil	44.971	2.04	$C_{30}H_{50}$
64	Tetracosane n-	n-alkane	45.897	2.44	$C_{24}H_{50}$
65	3-methylheneicosan Heneicosan,	Alkane	46.932	1.95	$C_{22}H_{46}$
67	Eicosane	Alkane	48.231	1.8	$C_{20}H_{42}$
68	Anthracene, 9, 10	Tricyclic	49.420	0.6	$C_{25}H_{33}$

2- Cuticular hydrocarbons of *Alphitobius laevigatus*:
GC/MS analysis results indicated the presence of fifty compounds in the cuticle of *Alphitobius laevigatus* (Fig2), only forty-two were hydrocarbons. As shown in Table 2, the cuticular hydrocarbons of *Alphitobius laevigatus* chain lengths varying from C_8 to C_{35}.They were classified within nine classes, alkane (23), alkene (5), n- alkane (4), cycloalkane (4), bicyclic hydrocarbons (2) and one compound classified within each of alkyne, oil, n- alkene and heterocyclic hydrocarbons. The major hydrocarbon in *A.*

*laevigatus*is azulene (13%) followed by undecane (12.94%), dodecane (9.6%), 2-octene, 2, 3, 7-trimethyl (3.84%), n decane (3.50), naphthalenedecahydro (3.24%), nonadecane (3%), 1-nonadecene (2.88), cyclodecene, 1-methyl (2.78%), longifolene (2.48), n–decene (2.36). Ten hydrocarbons were represented as traces (i.e. less than 1%).

Lockey (1979) studied a mixture of species of genus *Alphitobius* and stated that it lacked n-alkenes; this disagree with the results obtained during the present study.

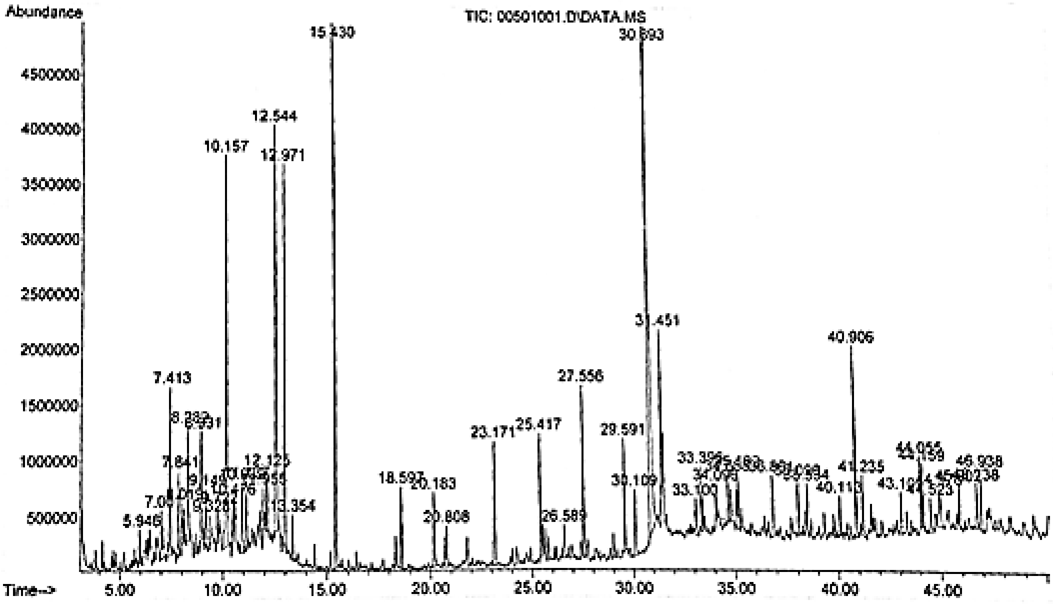


Fig. 2 Chromatogram obtained by GC/MS: cuticular hydrocarbons on *Alphitobius laevigatus*

A. laevigtus possessed 20 hydrocarbons which were not found in *A. diaprinus* representing 22.44% of the total peak areas. Two compounds of these were predominant namely, n-decane (2.25%) and hexadecane (2.23%).

Table (2): Cuticular hydrocarbons of *Alphitobius laevigatus*.

Peak	Hydrocarbons	classifications	Retention time	Area %	Formula
1	Trans-1,4-diethylcyclohexane	Cycloalkane	5.948	0.48	C_8H_{16}
2	Cyclohexane,1-methyl-2-pentyl	Cycloalkane	7.001	2.16	$C_{12}H_{24}$
3	n Decane	n-alkane	7.417	3.50	$C_{10}H_{22}$
5	Nonane,2,6-dimethyl	Alkane	8.019	0.84	$C_{11}H_{24}$
6	2-Octene,2,3,7-trimethyl	Alkene	8.283	3.84	$C_{11}H_{22}$
7	Naphthalene,decahydro	Bicyclic	8.928	3.24	$C_{10}H_{18}$
8	Heptadecane, 7-methyl	Alkane	9.148	1.46	$C_{18}H_{38}$
9	Nonane,3,7-Dimethyl	Alkane	9.327	0.94	$C_{11}H_{24}$
10	1-Ethyl-4-methyl Cyclohexane	Cycloalkane	9.726	0.72	C_9H_{18}
11	Undecane	Alkane	10.159	12.94	$C_{11}H_{24}$
12	Naphthalene, decahydro	Bicyclic	10.473	0.72	$C_{10}H_{18}$
13	Cyclodecene, 1-methyl	Alkyne	10.931	2.78	$C_{10}H_{18}$
14	Cyclohexane, Octyl	Cycloalkane	11.135	1.96	$C_{14}H_{28}$
15	Undecane,2-methyl	Alkane	11.958	0.82	$C_{12}H_{26}$
16	Octadecane, 5,14- dibutyl-	Alkane	12.128	1.08	$C_{26}H_{54}$
17	Azuline	Heterocyclic	12.544	13	$C_{10}H_8$
18	Dodecane	Alkane	12.968	9.6	$C_{12}H_{26}$
19	Undecane, 4,6- Dimethyl	Alkane	13.35	1.24	$C_{13}H_{28}$
21	Longifolene	Oil	18.597	2.48	$C_{15}H_{24}$
22	n -Decene	n-alkene	20.184	2.36	$C_{10}H_{20}$
23	Pentadecane	Alkane	20.804	1.02	$C_{15}H_{32}$
24	Hexadecane	Alkane	23.172	1.46	$C_{16}H_{34}$
25	Heptadecane	Alkane	25.413	1.86	$C_{17}H_{36}$
26	Tricosane	Alkane	26.565	1.30	$C_{23}H_{48}$
28	Nonadecane	Alkane	29.95	3	$C_{19}H_{40}$
33	Heneicosan	Alkane	33.393	1.6	$C_{21}H_{44}$
34	Eicosane	Alkane	34.097	0.78	$C_{20}H_{42}$
35	Eicosane	Alkane	34.692	0.14	$C_{20}H_{42}$
36	Docosane	Alkane	35.184	1.44	$C_{22}H_{46}$
37	1-nonadecene	Alkene	36.865	2.88	$C_{19}H_{38}$
38	Docosane	Alkane	38.087	1.38	$C_{22}H_{46}$
39	Octadecane	Alkane	38.537	0.72	$C_{18}H_{38}$
40	Eicosane	Alkane	40.116	1.04	$C_{20}H_{42}$
42	Heneicosan	Alkane	41.237	1.94	$C_{21}H_{44}$
43	n-Eicosane	n-alkane	43.113	1.06	$C_{20}H_{42}$
44	n-Pentacos-3-ene	Alkene	44.055	1.08	$C_{25}H_{50}$
45	n-Pentacos-3-ene	Alkene	44.157	1.66	C_{25}H50
46	Eicosane	Alkane	44.522	0.72	$C_{20}H_{42}$
47	n Eicosane	n-alkane	44.98	2.10	$C_{20}H_{42}$
48	Nonadecane	Alkane	45.905	1.36	$C_{19}H_{40}$
49	Mediaglycole 1	Alkene	46.737	1.46	$C_{10}H_{20}$
50	n-octylicosane	n-alkane	46.941	1.04	$C_{35}H_{72}$

3- Comparing the cuticular hydrocarbons of *Alphitobius diaprinus* and *Alphitobius laevigatus*

All of the major cuticular hydrocarbon components of the two species of genus *Alphitobius* were recorded (Table 3). All hydrocarbon components found in the two species were belong to one of the following classes, alkane, alkene, alkyne, cycloalkane, n- alkane, bicyclic hydrocarbons, tricyclic hydrocarbons, heterocyclic hydrocarbons and oils.

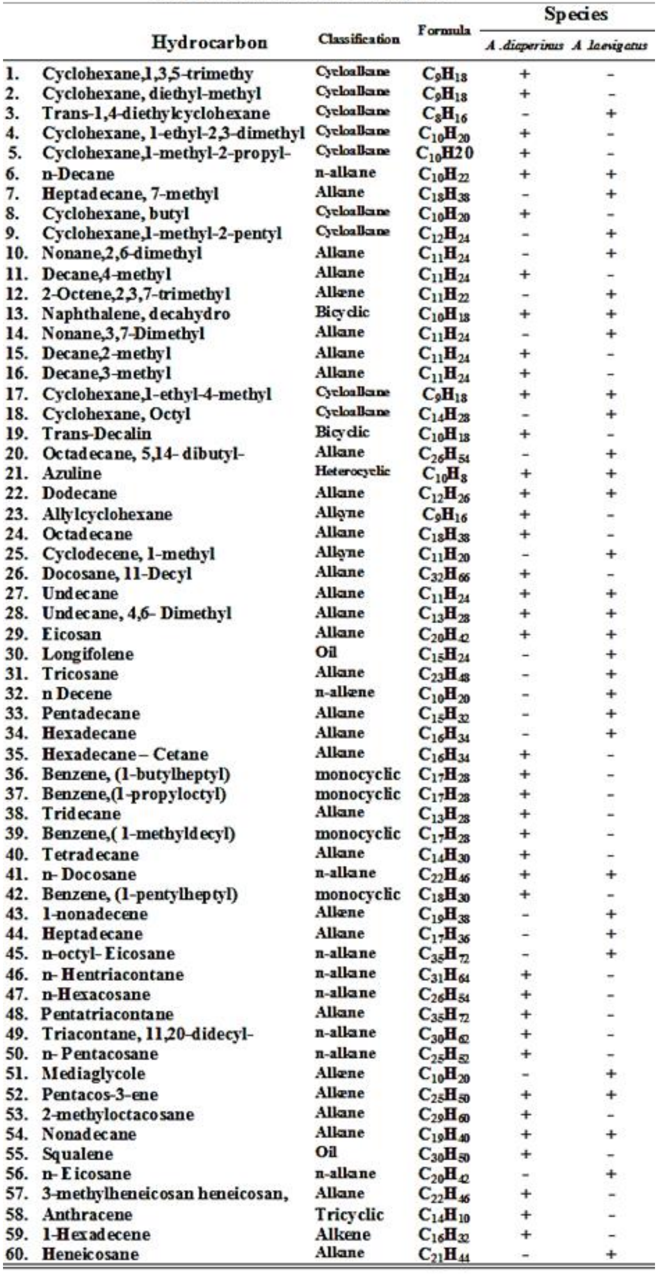
As shown in (Table 3) many components can be easily used to separate the two species of *Alphitobius*. Where 29 hydrocarbon compounds characterized *A.diaprinus*, 20 hydrocarbon compounds characterized *A. laevigatus*, they share 11 compounds. The alkane is the most dominant class of hydrocarbon among the peaks obtained by GC/MS, it represents 54.8% and 41.01% of all classes in *A. diaprinus* and *A. laevigatus* respectively.

The alkane composition in *A. diaprinus* was ranged from C_{11}-C_{35} with C_{11}, C_{20} and C_{22} predominating. The n-alkane is ranged from C_{10}- C_{31} with C_{30} predominating. The cycloalkane was limited within C_9 and C_{10} with C_{10} predominating.

The alkane composition in *A. laevigatus* was ranged from C_{11}-C_{35} with C_{11}, C_{20} and C_{22} predominating. The n-alkane was represented by four compounds two of them were C_{10} and one for C_{20} and C_{35}. The cycloalkane was four compounds, C_8, C_9, C_{12} and C_{14}.

The major alkane compound in *A. diaprinus* was dodecane (peak area 5%) and undecane (12.94%) in *A. laevigatus*. The abundant cycloalkane in *A. diaprinus* was cyclohexane, butyle (2%) and cyclohexane, 1-methyl-2-pentyl (2.16%) in *A. laevigatus* .n-hentriacontane (2.9%) was the major n-alkane in *A. diaprinus*, while n-decane (3.50%) was the abundant compound in *A. laevigatus*.

Table(3) Comparison of Cuticular hydrocarbons of *A. diaprinus* and *A. laevigatus*



	Hydrocarbon	Classification	Formula	Species	
				A .diaperinus	*A laevigatus*
1.	Cyclohexane,1,3,5-trimethy	Cycloalkane	C_9H_{18}	+	-
2.	Cyclohexane, diethyl-methyl	Cycloalkane	C_9H_{18}	+	-
3.	Trans-1,4-diethylcyclohexane	Cycloalkane	C_8H_{16}	-	+
4.	Cyclohexane, 1-ethyl-2,3-dimethyl	Cycloalkane	$C_{10}H_{20}$	+	-
5.	Cyclohexane,1-methyl-2-propyl-	Cycloalkane	$C_{10}H20$	+	-
6.	n-Decane	n-alkane	$C_{10}H_{22}$	+	+
7.	Heptadecane, 7-methyl	Alkane	$C_{18}H_{38}$	-	+
8.	Cyclohexane, butyl	Cycloalkane	$C_{10}H_{20}$	+	-
9.	Cyclohexane,1-methyl-2-pentyl	Cycloalkane	$C_{12}H_{24}$	-	+
10.	Nonane,2,6-dimethyl	Alkane	$C_{11}H_{24}$	-	+
11.	Decane,4-methyl	Alkane	$C_{11}H_{24}$	+	-
12.	2-Octene,2,3,7-trimethyl	Alkene	$C_{11}H_{22}$	-	+
13.	Naphthalene, decahydro	Bicyclic	$C_{10}H_{18}$	+	+
14.	Nonane,3,7-Dimethyl	Alkane	$C_{11}H_{24}$	-	+
15.	Decane,2-methyl	Alkane	$C_{11}H_{24}$	+	-
16.	Decane,3-methyl	Alkane	$C_{11}H_{24}$	+	-
17.	Cyclohexane,1-ethyl-4-methyl	Cycloalkane	C_9H_{18}	+	+
18.	Cyclohexane, Octyl	Cycloalkane	$C_{14}H_{28}$	-	+
19.	Trans-Decalin	Bicyclic	$C_{10}H_{18}$	+	-
20.	Octadecane, 5,14- dibutyl-	Alkane	$C_{26}H_{54}$	-	+
21.	Azuline	Heterocyclic	$C_{10}H_8$	+	+
22.	Dodecane	Alkane	$C_{12}H_{26}$	+	+
23.	Allylcyclohexane	Alkyne	C_9H_{16}	+	-
24.	Octadecane	Alkane	$C_{18}H_{38}$	+	-
25.	Cyclodecene, 1-methyl	Alkyne	$C_{11}H_{20}$	-	+
26.	Docosane, 11-Decyl	Alkane	$C_{32}H_{66}$	+	-
27.	Undecane	Alkane	$C_{11}H_{24}$	+	+
28.	Undecane, 4,6- Dimethyl	Alkane	$C_{13}H_{28}$	+	+
29.	Eicosan	Alkane	$C_{20}H_{42}$	+	+
30.	Longifolene	Oil	$C_{15}H_{24}$	-	+
31.	Tricosane	Alkane	$C_{23}H_{48}$	-	+
32.	n Decene	n-alkene	$C_{10}H_{20}$	-	+
33.	Pentadecane	Alkane	$C_{15}H_{32}$	-	+
34.	Hexadecane	Alkane	$C_{16}H_{34}$	-	+
35.	Hexadecane – Cetane	Alkane	$C_{16}H_{34}$	+	-
36.	Benzene, (1-butylheptyl)	monocyclic	$C_{17}H_{28}$	+	-
37.	Benzene,(1-propyloctyl)	monocyclic	$C_{17}H_{28}$	+	-
38.	Tridecane	Alkane	$C_{13}H_{28}$	+	-
39.	Benzene,(1-methyldecyl)	monocyclic	$C_{17}H_{28}$	+	-
40.	Tetradecane	Alkane	$C_{14}H_{30}$	+	-
41.	n- Docosane	n-alkane	$C_{22}H_{46}$	+	+
42.	Benzene, (1-pentylheptyl)	monocyclic	$C_{18}H_{30}$	+	-
43.	1-nonadecene	Alkene	$C_{19}H_{38}$	-	+
44.	Heptadecane	Alkane	$C_{17}H_{36}$	-	+
45.	n-octyl- Eicosane	n-alkane	$C_{35}H_{72}$	-	+
46.	n- Hentriacontane	n-alkane	$C_{31}H_{64}$	+	-
47.	n-Hexacosane	n-alkane	$C_{26}H_{54}$	+	-
48.	Pentatriacontane	Alkane	$C_{35}H_{72}$	+	-
49.	Triacontane, 11,20-didecyl-	n-alkane	$C_{30}H_{62}$	+	-
50.	n- Pentacosane	n-alkane	$C_{25}H_{52}$	+	-
51.	Mediaglycole	Alkene	$C_{10}H_{20}$	-	+
52.	Pentacos-3-ene	Alkene	$C_{25}H_{50}$	+	+
53.	2-methyloctacosane	Alkane	$C_{29}H_{60}$	+	-
54.	Nonadecane	Alkane	$C_{19}H_{40}$	+	+
55.	Squalene	Oil	$C_{30}H_{50}$	+	-
56.	n- Eicosane	n-alkane	$C_{20}H_{42}$	-	+
57.	3-methylheneicosan heneicosan,	Alkane	$C_{22}H_{46}$	+	-
58.	Anthracene	Tricyclic	$C_{14}H_{10}$	+	-
59.	1-Hexadecene	Alkene	$C_{16}H_{32}$	+	-
60.	Heneicosane	Alkane	$C_{21}H_{44}$	-	+

4. Discussion

Morphological taxonomic researches in insects are becoming rare. Rather, molecular and biochemical methods are proving useful as taxonomic tools (Verdyck *et al.*, 1998).

Cvacka *et al.* (2006) stated that hydrocarbon profiles may serve as fingerprints defining particular species, the results obtained by the present study confirmed this conclusion. Hydrocarbon components are genetically fixed and represent a unique, species-specific phenotype (Coyne *et al.*, 1994). (Drijfhout, 2009) stated that across 78 ant species, almost 1000 cuticular hydrocarbons have been identified, with no two-species having the same combination of compounds.

As shown in table (3), a number of cuticular hydrocarbons are absent from *A. diaprinus* but are found in *A. laevigatus*, others were found in both species. This provides numerous characters to test species differences. In contrast to morphological traits and genetic markers, which are based on a large number of small discrete changes, cuticular hydrocarbons are often discrete, being either present or absent and allowing species recognition cues to be measured unambiguously (Kather and Martin, 2012).

The total number of cuticular hydrocarbons identified during the present study was 56 and 42 compounds in *A. diaprinus* and *A. laevigatus* respectively. Kather and Martin (2012) concluded that, the cuticular hydrocarbon profile of a species could consist of a few to more than 100 compounds, which could vary in size and structure.

The carbon numbers of the cuticular hydrocarbons analyzed by GC/MS in the two species under investigation were ranged from C_8 to $C_{35.}$ These chain lengths indicate that they even distributed, to some extent, among short, medium and long carbon chains. Lockey (1988) concluded that species living in dry conditions generally contains longer hydrocarbon chains in comparison to their relatives living in wet conditions.

Alkanes is the predominant constituent of cuticular hydrocarbon of the two species. The predominance of alkanes is obtained for other species of the order Coleoptera (e.g. *Agabus* sp., Alarie, 1998;).

In contrast to Haverty *et al.* (2000) who suggested that, insects cannot synthesize n-alkanes longer than thirty-four carbons, one hydrocarbon identified in *A. laevigatus* namely n- octylicosane contains thirty-five carbons.

In conclusion, hydrocarbons are very useful tools for the identification of insects since even parts of insects may be used for the analysis; these highly stable molecules can be used also for older specimens. This method is a simple and feasible technique for taxonomical purposes. More research is needed concerning insect taxonomy applying new trends to enrich this important field of basic science and construct a huge electronic database for all insect categories.

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