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Original Article

Cuticular Hydrocarbon (CHCs) in *Cataglyphis savignyi* (Hymenoptera: Formicidae) in Damietta Province, Egypt

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Abstract

Cuticular hydrocarbon is one of the most important fingerprints in insects. However, few studies have been hitherto carried out in Egypt. *Cataglyphis savignyi* is the common *Cataglyphis* species in Egypt and widely distributed in most habitats. This study analyzes the profile of cuticular hydrocarbon (CHCs) in this species. Sampling was carried out from many different habitats in Damietta Province, Egypt. Gas Chromatography-Mass Spectrometry (GC-MS) was the suitable instrument for the separation and identification of compounds. Many peaks corresponding to CHCs compounds were clarified with extremely significant variation but not in a significant amount. Pentadecane, nonadecane; alkanes, β-elemene and aromadenrene; alkenes, butylated hydroxytoluene; alkylaldehde & anethole, phenol were the main identified components. According to the relative abundance, alkylaldehdic & phenol component "butylated hydroxytoluene & anethole, respectively" recorded the major ones. This study is the informative study about the common structural components (CHCs) in *Cataglyphis savignyi*.

1. Introduction

The study of cuticular hydrocarbon is one of the most recent branches in science that grows rapidly having an importance in many research fields. As defined, cuticular hydrocarbons (CHCs) cues are endogenous compounds (genotype) that may be further modulated by exogenous, environmental, sources (Vander Meer and Morel, 1998; Liang and Silverman, 2000). Typical structural and functional materials of cuticular hydrocarbons have been affirmed their importance (Lucas *et al.,* 2005b). Cuticular hydrocarbons play essential and multifaceted roles in life of insects. Protection from adverse environments such as dehydration (Gibbs, 1998; Gibbs *et al.,* 2003; Gibbs and Rajpurohit, 2010), entomopathogenic microorganism penetration (Blomquist *et al.,* 1987), and recognition in various social insects such as

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polistine wasps, honeybees, termites, and ants are the essential functions (Howard and Blomquist, 1982; Lockey, 1988; Singer, 1998; Martin and Drijfhout, 2009). Aside these function, CHCs are useful for some social species to communicate with nestmates using a special chemical signal as sociochemicals' compounds (Blomquist *et al.,* 1987; Bonavita-Cougourdan *et al.,* 1987; Yamaoka, 1990; Meskali *et al.,* 1995; Lahav *et al.,* 1999; Liebig *et al.,* 2000; Akino *et al.,* 2004; Gamboa, 2004; Lorenzi *et al.,* 2004; Howard and Blomquist, 2005; Lucas *et al.,* 2005b; Ozaki *et al.,* 2005; Greene and Gordon, 2007; Hefetz, 2007; Martin *et al.,* 2008; Guerrieri *et al.,* 2009; d'Ettorre and Lenoir, 2010; Bos and d'Ettorre, 2012; Chernenko *et al.,* 2013; Costanzi *et al.,* 2013; Emery and Tsutsui, 2013), Kin, patriline (Boomsma *et al.,* 2003). They are also useful for identifying gender, recognizing mates, conspecifics and colony members (Howard and Blomquist, 1982; Vander Meer and Morel, 1998; Lacey *et al.,* 2008; Oppelt et al., 2008), as caste-specific cue (Depickère *et al.,* 2004; Antonialli-Junior *et al.,* 2007; Oettler *et al.,* 2008), for predator avoidance, parasitism, and as fertility and dominance signals (Elmes *et al.*, 2002; Howard and Blomquist, 2005; Richard *et al.,* 2007; Blomquist and Bagnères, 2010).

Surprisingly, the hydrocarbon pattern, related to biological functions, has been designated by different names, including the following: signature, fingerprint, bar code, template, visa and label. An intriguing aspect of this pattern is "super positioning", which allows insects to display specific, colonial and individual signals on the same channel, also called multipurpose signals (Denis *et al.,* 2006; d'Ettorre and Moore, 2008).

Cataglyphis ants are of the most characteristic and conspicuous insects of arid regions around the Mediterranean basin and can be seen running very rapidly. They are called "the thief of the cooking pot" in Arabic, and "thief" in Greek (due to their rapid stealing of food particles) or "Englishmen" (due to their activity in the hot midday sun at siesta time) (Harkness and Wehner, 1977). *Cataglyphis* species was the common species in the variation of cuticular hydrocarbon profiles (Hefetz and Orion, 1982; Ali, 1988; Hefetz and Lenoir, 1992; Keegans *et al.,* 1992; Agosti *et al.,* 1996; Dahbi *et al.,* 1996; Oldham *et al.,* 1999; Gokcen *et al.,* 2002). *Cataglyphis savignyi* (Dufour, 1862) is one of the most common Egyptian species of ants, which is widely distributed in almost habitat in Damietta Province. Therefore, the main aim of this paper is to use the technique of gas chromatography coupled to mass spectrometry (GC–MS) to study the hydrocarbon composition of this species and the variation of its CHCs components.

2. Materials and Methods

2.1. Study area

Damietta (or Dumyat) is a Province in the North part of Egypt. It lies between longitudes 31 ° 28`& 32 ° 04`N and latitude 31 ° 10` & 31 ° 30`E, covering about 1029 km^2 on the extreme of Damietta branch of the Nile River, about 5% of the Delta's area and about 1% of the Egypt area (SEAM, 2005). This Province is bordered by E1-Dakahlia Governorate from southwest, by the Mediterranean Sea from north and Lake Manzala from east. Damietta Governorate's Mediterranean coastline extends from El-Deeba village westward to Gamasa along Mediterranean Sea for about 42 Km (SEAM, 2005).The sampling of *Cataglyphis savignyi* was carried out in two main regions (coastal and Delta regions) with twenty sites. As shown in Fig (1), the coordinates of each site were recorded using a hand-held Global Positioning System (GPS) by using Garmin® GPS III plus.

2.2. Extraction and isolation

Protocols for the extraction of cuticular hydrocarbons and Gas Chromatography-Mass Spectrometry (GC-MS) analyses are as described in (Fournier *et al.,* 2009). Briefly, the elution of the hydrocarbons from the cuticle was carried by suspending the sampled ants in hexane (organic solvent). The amount of hexane depends on the size of the insect, as it should be fully immersed in the solvent, and the amount of sample needed for the analytical technique. By a Haep Labor Consult HTML-133 shaker (Bovenden, Germany), shaking the suspending samples for 10-20 min at 50 C was performed for quick elution of the cuticular hydrocarbons. After that, ants were removed and the extracted solution was allowed to evaporate and then was resuspended in 20 ml of hexane.

Gas Chromatography-Mass Spectrometry (GC-MS) is a selective and sensitive analytical technique for the separation, identification, and quantification of components of complex organic mixtures (Townshend, 1995).

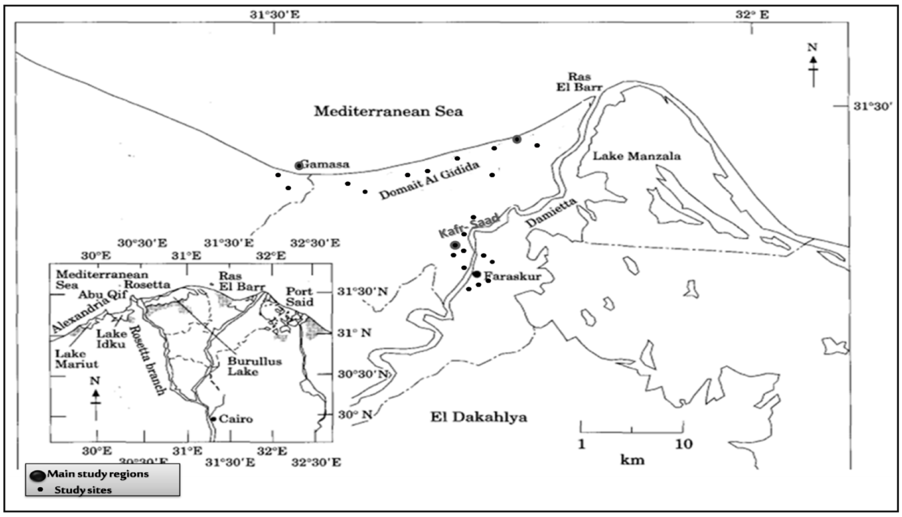

Fig. 1. A map showing thefour main study regions and correlating study sites in Damietta Province.

2.3. Data analysis

Analyses of variance (ANOVA) were used to test for differences by using SPSS (Statistical Package for Social Sciences, Inc.).

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3. Results

3.1. Cuticular hydrocarbons profile of *Cataglyphis savignyi*

The cuticular hydrocarbon of *Cataglyphis savignyi* was analyzed using GC-MS for the different samples. The chromatograms of these cuticular hydrocarbons revealed the existence of numerous peaks, expressing different components. The identification of these components based on their spectra in relation to their retention time was confidently assigned with the standards of mass spectra. The structural profile of these cuticular hydrocarbons consists of n- alkanes, monomethylalkanes, dimethylalkanes, alkenes, fatty acids and ester.

The common compounds have been identified in this study like: alkanes (e.g pentadecane, nonadecane, hentriacontane, dotriacontane, and tritetracontane), alkenes (e.g cis 1-chloro-9 Octadecene, β-elemene, aromadenrene), phenols (e.g anethole), alkylaldehde (e.g butylat d

4. Discussion

The ability of ants to produce such a large diversity of CHCs, having a range of biophysical properties, has helped them adapt to environments that range from tropical to arctic, as well as to develop a complex chemical communication system (Morrison and Witte, 2011; Berville *et al.,* 2013). Hydrocarbon profiles may serve as fingerprints defining particular species (Ali *et al.,* 1988). As previously reported by many authors (Lockey, 1988; Dietemann *et al.,* 2003; Lucas *et al.,* 2005a; Martin *et al.,* 2008), the main components of CHCs in ant species were alkanes, alkenes, and methyl-branched alkanes.

Martin *et al.* (2013) clarified the importance of n-alkanes as significant segregations on a larger spatial scale. Their study suggests that, rather than isolation due to dispersal barriers, divergence may be due to more gradual changes in environment. Additionally, a positive correlation was between spatial and chemical distance for n-alkanes, which are not only nestmate cues but also a genetic underpinning.

Pentadecane, nonadecane, hentriacontane, and dotriacontane were the most identified alkanes among the

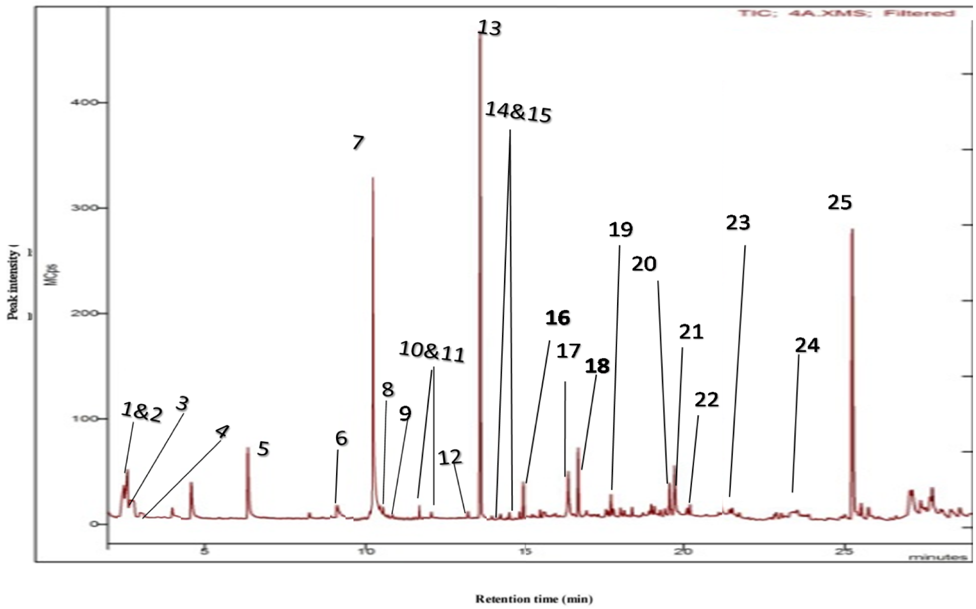

Fig. 3. Representative GC-MS profiles of Cataglyphis savignyi collected from different study sites. Numbered peaks arecompounds unique to each. Peaks numbered indicated the identified compounds (Comp., cf. Table 1).

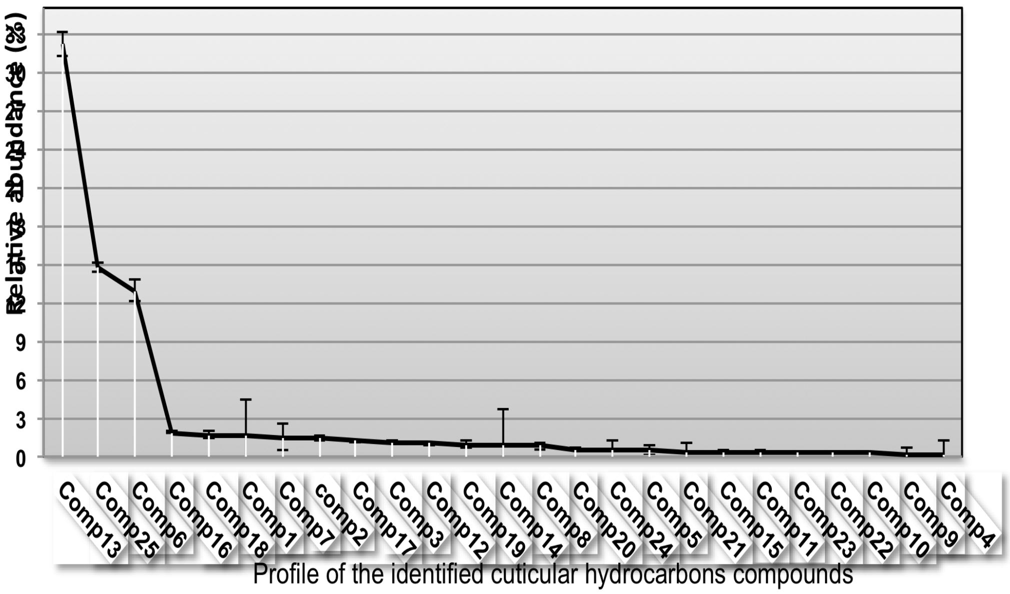

Fig. 4. Compound importance curve of the identified hydrocarbon compounds in the cuticle of Cataglyphis savignyi.Comp. abbreviation, cf. Table 1.

Table 1. Compounds identified in cuticular hydrocarbons with the average of peak area and average of relative abundance (%) of *Cataglyphis savignyiby* GC-mass spectrography.

No.	RT.	Profile compounds of the extracted cuticular hydrocarbons	Compound code	Average of peak area	Mini.	Maxi.	Average %	Mean± SE
1	2.399	1- methyl- Cis-2- (N,N- Dimthylaminomethyl- D2) Isopropylidenecyclopentane	**Comp.1**	**2.42E+08**	**0**	**18.44**	**1.69**	**1.69±0.92**
2	2.441	2-Methyl heptanoic acid	Comp.2	6.03E+08	0	4.91	1.38	1.38±0.34***
3	2.501	Butanoic acid, 2 methyl-(CAS)	Comp.3	7.44E+08	0	16	1.17	1.7±084
4	2.545	Methyl heptanoic acid	Comp.4	3.69E+07	0	1.15	0.06	0.6±0.05
5	6.328	4-methyl-Phenol (CAS)	Comp.5	1.88E+08	0	9.01	0.68	0.68±0.44
6	9.69	Decenal	Comp.6	4.60E+08	0	2.23	0.15	0.15±0.11
7	10.08	Anethole	Comp.7	7.02E+09	0	34.29	12.97	12.97±2.8***
8	10.38	Pentadecane	Comp.8	6.88E+08	0	3.15	0.86	0.86±0.18
9	11.78	β-elemene	Comp.9	1.85E+08	0	1.14	0.18	0.18±0.07***
10	11.88	Cis 1-chloro-9 Octadecene	Comp.10	2.63E+08	0	1	0.24	0.24±0.8***
11	11.94	Aromadenrene	Comp.11	3.83E+08	0	1.78	0.29	0.29±0.12***
12	13.17	3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsilloxy)tetrasiloxane	Comp.12	1.57E+09	0	4.67	1	1±0.27*
13	13.55	Butylated hydroxytoluene	Comp.13	4.35E+10	0.54	50.54	32.13	32.13±2.81
14	13.61	Phenol, 2,4- bis (1,1 dimethylethyl)-(CAS)	Comp.14	3.21E+09	0	4.41	0.91	0.91±0.31**
15	14.91	1-tetradecanol (CAS)	Comp.15	5.84E+08	0	0.92	0.29	0.29±0.06***
16	15.03	Nonadecane	Comp.16	1.76E+09	0	17.34	1.91	1.91±0.79
17	15.54	Cyclooctasiloxanehexadecamethyl-	Comp.17	2.28E+09	0	6.56	1.2	1.2±0.39**
18	16.726	5(1H)-Azulenone, 2,4,6,7,8,8a- hexahydro-3,8-dimethyl-4-(1- methylethylidene)	Comp18	2.39E+09	0	16.49	1.73	1.73±0.83**
19	17.598	OctadecamethylcyclononasilOxane	Comp19	1.84E+09	0	4.94	0.91	0.91±0.3***
20	19.4	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasil oxane	Comp20	1.32E+09	0	3.21	0.58	0.58±0.20***
21	19.557	n-Hexadecanoic acid	Comp21	5.83E+08	0	0.99	0.31	0.31±0.08***
22	21.124	TetracosamethylcyclododecasilOxane	Comp22	6.21E+08	0	2.14	0.25	0.25±0.13**
23	22.43	4,8,13-Cyclotetradecatriene-1,3-diol.1,5,9-trimethyl-12-(1-methylethyl)-	Comp23	2.24E+08	.00	2.81	.26	0.26±0.16*
24	24.113	Butyl 9,12,15- octadecatrienoate	Comp24	2.98E+09	0	36.11	1.75	1.75±0.0.172
25	25.715	Bis(2ethylhexyl) phthalate&1,2-Benzenedicarboxylic acid, 3-nitro-(CAS)	Comp25	2.07E+10	6.96	25.85	14.75	14.75±1.16

Note: Min. = Minimum, Max. = Maximum, RT= Rentation time. The given values are the means ± standard error of relative abundance % of cuticular components. * Significant value at the 0.05 level, ** Significant value at the 0.001 level, and *** Significant value at the 0.0001 level.

identified component of CHCs in *Cataglyphis savignyi*. Hentriacontane, dotriacontane, and tritetracontane were found in little amounts and in two or three sites only, so we ignored their existence among the structural profile of CHCs on contrary to other mono alkanes. As previously reported by Ali *et al*. (1988), pentadecane and nonadecane were identified as components of CHCs in *Cataglyphis savigny*, and pentadecane was the most abundant substance in the dufour glands of this species. The previous study also explained the presence of pentadecane as a less volatile substance for the foraging behavior of *Cataglyphis savignyi* at daytime in a very hot environment (Delye, 1968).

n- alkenes, cis 1-chloro-9 Octadecene, β− elemene and aromadenrene were detected in the current study. The importance of these unsaturated compounds was related to be excellent discriminatory compounds or nest-mate discrimination (Martin *et al.,* 2008). Other cuticular components alcohol, alkylphenol, aldehyde, fatty acids and esters were for protection (Dani *et al.,* 1995).

Finally, not all compounds within a profile may function independently as recognition cues (van Wilgenburg *et al.,* 2010). A recent study of *Formica* ants and *Vespa* hornets has shown that CHCs profiles are a mixture of correlated and non-correlated compounds. Importantly that the relative amounts of compounds within a homologous series often are constant, both within colonies and species (Martin *et al.,* 2008; Martin and Drijfhout, 2009). Consequently, a single compound may provide the same information as its homologs (van Wilgenburg *et al.,* 2010). Meanwhile, fatty acid or unsaturated acids act as ant repellent against predatory (Dani *et al.,* 1995). As detected in *P. annularis, P. do-*

minulus, and *P. sulcifer*, there are different fatty acids that present in van der vecht glands, nest pedicle and cuticular gland secretion (Espelie and Hermann, 1990; Dani *et al.,* 1995&1996).

Among the identified CHCs, the maximum mean of relative abundance recorded in butylated hydroxytoluene and anethole. Alkylaldehde, butylated hydroxytoluene, is widely known as an antioxidant component for decreasing the rate of lipid peroxidation and scavenges free radical (Sharma and Wadhwa, 1983; Blum and Roitberg, 1999). Meanwhile, the phenol compound, anethole, was also stated as an efficient antioxidant, antimicrobial, acaricide, strong bioactive property, repellent (Koul *et al.,* 2007; Anwar *et al.,* 2009; Shahat *et al.,* 2011; Senra *et al.,* 2013).

Cataglyphis savignyi as the common species that widely distributed in all habitats, we need to know many about its adaptation and habitation. Based upon the present study, we hope to complete our future studies using data in spatial ecology.

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الملخص العربى

الهيدروكربونات الجليدية فى حرامى الحلة (فورميسيدى) فى المحافظة الساحلية، دمياط، مصر

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نظرا لاهمية الهيدروكربونات داخل حياة الحشرات وقلة تواجد معلومات عن تلك المكونات الرئيسية فى الحياة المجتمعية لحرامى الحلة. اختار عدد من الأماكن المختلفة البيئات فى محافظة دمياط. ولقد استخدام جهاز GC-MS للتعرف على مكونات الهيدروكربونات الجليدية فى حرامى الحلة ووجد اعداد كبيرة من المركبات منها أليفاتية مثل الألكانات و الألكينات ومن بينهما ما يجمع به عدد من مجموعة الميثيل أو اروماتية مثل مركبات فينولية وأحماض دهنية مشبعة وغير مشبعة واسترات. لم تستخدم كل المركبات فى تحليل البيانات ولكن استخدمت فقط خمسة وعشرين مركبا لتواجدهم بنسب جيدة، ووجد أن butylated hydroxyto-luene and anethole هم أكثر المركبات تواجد. تعتبر تلك الدراسة دراسة تعريفية لمكونات تلك المركبات والتعرف على طبيعتها التركيبية.

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Cuticular Hydrocarbon (CHCs) in *Cataglyphis savignyi* (Hymenoptera: Formicidae) in Damietta Province, Egypt

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