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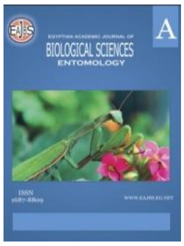

Chemotaxonomic Study of Cuticular Chemical Compounds on Male and Female of *Anthidium amabile* Alfken, 1932 (Hymenoptera: Megachilidae)

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ABSTRACT

Bees within the family Megachilidae are economically important members of natural and agroecosystems as play an important role in the pollination of many plant species. Tribe Anthidiini contains a diverse, cosmopolitan group of solitary bees. Cuticular chemical components analysis is a precise tool for chemotaxonomy, and perhaps they can be used as a complement to morphology and genetic characters in phylogenetic studies. The aims of this study were to describe the cuticular chemical compounds of males and females of *Anthidium amabile* Alfken, 1932, to evaluate the obtained components to use some chemical compounds as characters that differentiate between male and female, and others as a chemotaxonomic tool. The investigation used gas chromatography coupled to mass spectrometry (GC–MS). The results indicated that the male and female of this species has significant differences in cuticular chemical components, the male cuticle is characterized by three compounds not found in female while the female cuticle obtains twenty-two chemical compounds not found within the male cuticle.

INTRODUCTION

The main functions of insect cuticle are protection against desiccation, xenobiotic penetration of harmful environmental elements and serving in chemical communication (Hadley, 1984, Ayasse *et al*., 2001; Simmons *et al.*, 2003; Mant *et al*., 2005).

The chemotaxonomic significance of cuticular chemical compounds was reported on several insect groups, for example, Lepidoptera (Arsene *et al*., 2002), Hymenoptera (Abdalla *et al.*, 2003; Nunes *et al.*, 2009), Dictyoptera and Orthoptera (Imen *et al.*, 2005), Diptera (Galhoum,2017 and 2018), Coleoptera (Elshaier, *et al* 2017). The content and composition of cuticular hydrocarbons in insects can vary with age, diet, development stage, sex, temperature regimes, and geographic origin of species or populations (Bagnères and Blomquist 2010).

Determining the factors associated with CHC variation in insects is an important general step toward understanding their evolution, and their potential utility in enhancing available pest monitoring and management strategies (Li *et al.* 2021). Ngumbi *et al.* (2020) find that identifying sex-related variation in CHCs in *A. transitella* (Lepidoptera: Pyralidae) could be useful in developing new or enhancing existing pest monitoring and management strategies.

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Females and males independently evolve some traits that enhance survival and reproduction under the pressure of divergent selection forces, thus leading to sexual dimorphism of traits such as body size (Bear and Antónia, 2013). Because males and females of the same species share the majority of their genomes, the genetic basis of sex-specific traits that evolve under sexual selection is poorly understood. It is widely assumed that sexually dimorphic regulation of gene expression facilitates sex-specific adaptations (Pei *et al.*, 2021).

Sexually dimorphic cuticular hydrocarbon (SDCHC) profiles are widespread in insects (Ingleby *et al.*, 2014). The cuticular lipids of the female mainly consist of hydrocarbons (Galhoum, 2018).

Anthidium Fabricius, 1804, is among the most diverse genera of Megachilidae, containing more than 160 species worldwide (Ascher and Pickering, 2018). *Anthidium* are solitary bees, commonly known as wool-carder bees (Michener, 2007), they are known as the most useful pollinators of plants as they have specialized apparatus (Scopa) for collecting pollen grains (Müller, 2012). They also play a major role in ecosystems (Michener, 2000).

MATERIALS AND METHODS

Insects:

The present investigation is carried out on preserved insects of the specimen of male and female *Anthidium amabile* Alfken, 1932. The specimens were collected using sweeping nets during different field trips in Egypt, then, killed by placing them in a poison bottle. A stereo light microscope was used to examine the specimens. Taxonomic keys used for identification and confirmed in the entomological collection of the Plant Protection Research Institute, Giza, Egypt.

Hydrocarbon Extraction and Analysis*:*

Cuticular chemical compounds were extracted using hexane as a solvent from adult male and female specimens, 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 the 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 ionizing energy of 70 ev. The ion source temperature was 300 °C. The electron multiplier voltage (EM voltage) was maintained 1650 v. above autorun. The instrument was manually turned using perfluorotributylamine (PFTBA).

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

RESULTS

The total number of chemical compounds included in the cuticle of both sexes of this species is 35 compounds (Table 1 and Fig. 1). Overall (in both sexes), including 10 common compounds in both sexes, 22 compounds that distinguish female from the male; and only 3 compounds that distinguish male from female.

Both sexes share 10 compounds, (Table, 1) these compounds are:

Benzene, (1-butyloctyl); Benzene, (1-methyldodecyl) 1.25; Benzene, (1-Ethyloctadecyl); Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid (Z, Z); Oleic Acid; Octadecanoic acid; 2,3-Dihydroxypropyl elaidate 9- Octadecenoic acid (Z)-,2-hydroxy-1- (hydroxymethyl) ethyl ester; 2- Hydroxy- 3- [(9e)- 9-Octadecenoyloxy] Propyl (9e)- 9- Octadecenoate and Benzene, (1-Pentylhexyl).

Table 1: Cuticular chemical compounds of male and female *Anthidium amabile* analyzed by GC/ Mas.

Compound names	M.F.	Female		Male	
		Area%	M. W.	Area%	M. W.
Benzene, (1-butylhexyl)-	C16H26	0.68	218	-	-
Benzene, (1-Pentylhexyl)	C17H28	0.72	232	2.03	232
1-butyloctylbenzene,	C18H30	0.70	246	1.22	246
Benzene, (1-methyldodecyl)- 1.25	C19H32	1.25	260	2.06	260
1-Ethyloctadecylbenzene	C26H46	1.77	358	1.46	358
Heneicosane, 11-phenyl-	C27H48	1.45	372	-	-
2-Propoxybenzaldehyde	C10H12O2	0.61	164	-	-
Tetradecanoic acid	C14H28O2	0.82	228	-	-
Hexadecen-1-ol, trans-9-	C16H32O	1.60	240	-	-
n-Hexadecanoic acid	C16H32O2	-	-	13.30	256
1-Hexadecanol	C16H32O	-	-	2.11	242
Hexadecanoic acid, methyl ester	C17H34O2	0.96	270	1.04	270
Oxiraneoctanoic acid, 3-octyl-, cis	C18H34O3	0.43	298	-	-
9,12-Octadecadienoic acid (Z, Z)-	C18H32O2	2.89	280	3.73	280
Oleic Acid	C18H34O2	24.96	282	1.84	282
Octadecanoic acid	C18H36O2	4.53	284	5.82	284
8-Octadecenoic acid, methyl ester	C19H36O2	1.64	296	-	-
Heptadecanoic acid, 9-methyl-, methyl ester	C19H38O2	0.85	298	-	-
Octadecanoic acid, 4-hydroxy-, methyl ester	C19H38O3	0.58	314	-	-
Eicosanoic acid	C20H40O2	0.82	312	-	-
1-Docosene	C22H44	-	-	2.60	308
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)	C21H34O2	3.09	318	-	-
9,12-Octadecadienoic acid (Z, Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H38O4	0.73	354	-	-
2,3-Dihydroxypropyl elaidate 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl Ester	C21H40O4	2.83	356	1.90	356
Docosanoic acid	C22H44O2	1.91	340	-	-
Ethyl iso-allocholate	C26H44O5	0.67	436	-	-
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandro st-8-en-17-yl)-	C27H42O4	1.01	430	-	-
Cholesta-8,24-dien-3-ol,4-methyl-, (3á,4à)-	C28H46O	0.64	398	-	-
Stigmast-5-en-3-ol, (3á,24s)-	C29H50O	1.23	414	-	-
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	C35H68O5	0.49	568	-	-
2-Hydroxy-3-[(9e)-9-Octadecenoyloxy] Propyl (9e)-9-Octadecenoate	C39H72O5	1.97	620	2.46	620
Silicone oil	C6H18OSi2	1.19	162	-	-
Cyclooctasiloxane, hexadecamethyl-	C16H48O8Si8	0.61	592	-	-
Cyclononasiloxane, octadecamethyl-	C18H54O9Si9	0.42	666	-	-
Cyclodecasiloxane, eicosamethyl-	C20H60O10Si10	1.04	740	-	-

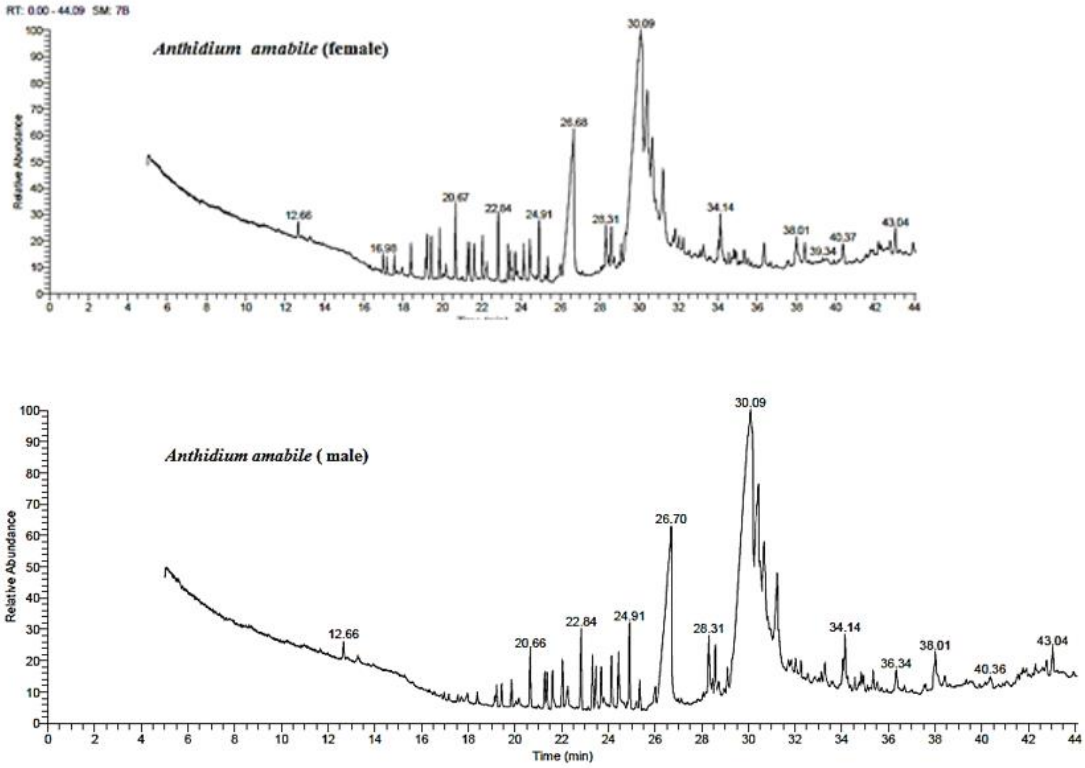

Fig..1: Chromatogram of GC-MS analysis for the cuticular chemical profile of male of *Anthidium amabile.*

In the male the total number identified by GC-MS is (13) chemical compounds only three compounds not found in females; namely n- Hexadecanoic acid, 1-Hexadecanol and 1-Docosene.

The most abundant compounds in males are, n- Hexadecanoic acid (13.30**%**); Octadecanoic acid (5.82**%**) and 9,12-Octadecadienoic acid (Z, Z) (3.73**%**) while the following compounds represented by scarce amounts, Hexadecanoic acid, methyl ester (1.04**%**), 1-butyloctylbenzene, (1.22**%**) and 1-Ethyloctadecylbenzene (1.46**%**).

Female *A. amabile* distinguished from male *A. amabile* in having twenty-two compounds, as follows:

One Alcohol (Ethyl iso-allocholate); One Keton (2-Propoxybenzaldehyde); Two hydrocarbons (Benzene, (1-butylhexyl) and (Heneicosane, 11-phenyl), Two glycerides (9,12-Octadecadienoic acid (Z, Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester) and (Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester); Two sterol (Cholesta-8,24-dien-3-ol,4-methyl-, (3á,4à)) and (Stigmast-5-en-3-ol, (3á,24s)) Four Organosiloxane (Silicone oil; Cyclooctasiloxane, hexadecamethyl; Cyclononasiloxane, octadecamethyl and Cyclodecasiloxane, eicosamethyl); finally Ten fatty acid (Tetradecanoic acid; Hexadecen-1-ol, trans-9; Oxiraneoctanoic acid, 3-octyl-, cis; 8-Octadecenoic acid, methyl ester; Heptadecanoic acid, 9-methyl-, methyl ester; Octadecanoic acid, 4-hydroxy-,methyl ester; Eicosanoic acid; 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z); Docosanoic acid and Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandro st-8-en-17-yl)).The most abundant compound in the cuticle of the female is Oleic Acid (24.96%) followed by Octadecanoic Acid (4.53%) and 5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (All-Z) (3.09 %), while Oxiraneoctanoic acid, 3-octyl-, cis and Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester, represented by traces (0.43% and 0.49% respectively).

DISCUSSION

Although the chemical composition of insect cuticles is known in some species (Lockey 1988) knowledge about this composition in many Egyptian species is still rare and requires sufficient study. The investigation of chemical classes obtained by GC/MS to the cuticle of female *Anthidium amabile,* somewhat agree with that previously recorded by many authors investigated other insects e.g. Al-Dawsary (2014) in red palm weevil *Rhynchophorus ferrugineus* recorded hydrocarbons, Carboxylic acid, Alcohols, Ketones, Aldehydes, Esters and Amines), Alnajim *et al.* (2019) in *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (Fabricius) classified fatty acids, hydrocarbon waxes, sterols, Lockey (1988 & 1991) (normal and branched saturated and unsaturated hydrocarbons, free fatty acids, free alcohols, alkyl esters, glycerides, sterols and aldehydes). Although the small number of cuticular hydrocarbon (5 compounds) that was recorded in the current study is questionable, it was documented in some studies, Drijfout *et al.* (2010) estimated the number of cuticular hydrocarbon compounds in insects as ranging between five and fifty. Fatty acids are important constituents of cuticle and are commonly engaged in forming more complex lipids, such as triglycerides, which store energy (Desbois and Smith 2010). Fatty acids are most diverse and abundant in the cuticle of collected females, representing 45.24% of all obtained compounds). This result match with Alnajim *et al.* (2019) in the investigation of cuticular lipids of *Rhyzopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst). Although these species have been studied by traditional taxonomy, the addition of supplementary characters through biochemical, molecular, ecological and other biological sciences, makes the identification of species more accurate and confident, which is what the integrative taxonomy aims at (Yeates *et al.* 2010). The present study is an attempt to complement the previous traditional taxonomic investigation and biochemical approach, toward integrative taxonomy.

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ARABIC SUMMARY

التركيب الكيميائي لجليد كل من ذكر وأنثى *Anthidium amabile* Alfken, 1932
(Hymenoptera: Megachilidae) كأداة تصنيفية كيميائية

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يعتبر النحل من فصيلة ميجاكيليدي من الحشرات الهامة اقتصاديًا حيث يلعب دور هام في النظم البيئية الطبيعية والزراعية حيث انه يلعب دورًا مهمًا في تلقيح العديد من الأنواع النباتية. تحتوي قبيلة أنثيديني على مجموعة متنوعة وعالمية من النحل انفرادي المعيشة. وتعتبر المكونات الكيميائية للجليد أداة دقيقة للتصنيف الكيميائي ، و يمكن استخدامها كمكمل للصفات المورفولوجية و الوراثية. استهدفت الدراسة الحالية وصف المركبات الكيميائية الجلدية لذكر وأنثى النوع *أنثيديوم أمابيل* وذلك لاستخدامها كأداة تصنيفية كيميائية للتفريق بين الذكور و الاناث.. وقد تم استخدم كروماتوغرافيا الغاز مقرونة بمقياس الطيف الكتلي (GC-MS) لفصل و تعريف المركبات الكيميائية الموجودة في جليد هذا النوع. حيث أشارت النتائج إلى أن الذكور والإناث من هذا النوع لديهم فروق معنوية في المكونات الكيميائية للجليد، حيث يتميز جليد الذكور بثلاثة مركبات غير موجودة في الإناث بينما يحتوي جليد الإناث على اثنين وعشرون مركبًا كيميائيًا غير موجودة داخل جليد الذكور.