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Chemotaxonomic Study of Males of Two Wool Carder Bee Species (Hymenoptera: Megachilidae: Megacklinae: Anthdinii: Anthidium)

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ABSTRACT

Cuticular hydrocarbons are present on the surface of all insects and play an important role in the life of insects. Although primarily anti-desiccation agents, cuticular hydrocarbons are emerging as important chemicals in insect communication. Using Cuticular hydrocarbons of insect cuticles is important in taxonomy because they have a wide variety of chemical compounds e.g.; hydrocarbons, monoester waxes, triglycerides, and free fatty acids. So cuticular chemical components are a precise tool for chemotaxonomy, and they can be used as an accompaniment to morphology and genetic characters in phylogenetic studies. Solitary bees (leaf-cutting) of *Anthidium* Fabricius, 1804 are economically important in natural and agroecosystems and play an important role in pollinating many domesticated and wild plant species. This study aimed to describe the cuticular chemical profile of males of two species of the genus *Anthidium* to be applied as a chemotaxonomic tool. The investigation used gas chromatography coupled to mass spectrometry (GC–MS). Investigated specie revealed 40 compounds in total, with nine compounds in common. The chemical diversity was higher in, *A. tessellatum* (30 compounds) while, in *A. pulchellum* (19 compounds). Males of *A. tessellatum* were distinguished by twenty-one exclusive compounds, and males of *A. pulchellum* were characterized by ten compounds. Most abundant compounds and that represented by scarce quantity were recorded for each species.

Keywords: *Chemical Profile*, Chemot*axonomy, GC-MS, Solitary Bees, Leaf-cutting, Anthidium tessellatum,* and *A. Pulchellum*

1. Introduction

Anthidium is a worldwide genus of Megachilidae, compressing about 160 species worldwide (Ascher and Pickering, 2012). *Anthidium* (wool-carder bees) are solitary bees, (Michener, 2007), and the most useful pollinators of plants as they have specialized apparatus (Scopa) for collecting pollen grains (Müller and Bansac, 2004). They also play a major role in ecosystems (Michener, 2000). The main functions of insect cuticle are protection against, desiccation and xenobiotic penetration of harmful environmental elements and serving in chemical communication (Nicolas *et al.,* 2013). As a supplementary tool for classification, the cuticle chemical composition has been used as a taxonomic marker in several insect groups over the last 30 years, for example, Lepidoptera (Arsène *et al.,* 2002), Hymenoptera (Nunes *et al.,* 2010). These studies concluded the suitability and significance of using the chemical composition of insect cuticle (especially cuticular hydrocarbons) as chemotaxonomic parameters for species distinction. Dos' Santos, and do Nascimento (2015), identified and statistically analyzed the cuticular hydrocarbons of males of 17 species of orchid bees (Hymenoptera, Apidae) and revealed 108 compounds, which allowed for the taxonomic classification. The cuticular hydrocarbons of insects can vary with temperature regimes, sex, development stage, age, diet, and geographic origin of species or populations (Blomquist and Bagnères, 2010). Defining the factors related to Cuticle Hydrocarbons (CHCs) differences in insects is vital to understanding their evolution and potential utility in enhancing pest monitoring and management strategies (Li *et al.,* 2021). The present study aims to evaluate using the chemical structure of an insect cuticle as a taxonomic tool that helps in identifying males of insects.

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Materials and Methods

Insects

The present investigation was carried out using preserved males of two species of the genus *Anthidium.* The specimens were collected using sweeping nets throughout different field trips in Egypt, killed in poison bottles. A stereo light microscope was used to examine the specimens. Taxonomic keys (Michener, 2007) were used for the identification of specimens and the identification was confirmed in the entomological collection of the Plant Protection Research Institute, Giza, Egypt.

Hydrocarbon extraction and analysis

Cuticular chemical compounds were extracted by immersing one specimen from each species in n-hexane as a solvent for two minutes; then filtered and kept in dark bottles at 4◦**C**. Extracts were kept in a refrigerator at 4◦**C** until analysis, as described by El Shaier, (2022).

Gas Chromatography-Mass Spectrometry (GC/MS).

Extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) as described by Nelson and Buckner (1995). GC/MS analysis has been conducted in "The Regional Center for Mycology and Biotechnology (RCMB), Cairo, Egypt. the two extracts were analyzed on a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph, the column used was DB-5, (Length 30 m. x Internal diameter 0.25 mm. x film thickness 0.25 µm), with helium as carrier gas (flow rate 1 ml/min.). One microliter of each extract was injected into the injector in pulsed splitless mode, as described by Wurdack *et al*., (2015).

The initial GC temperature was 40 °C (held for 5 min.) then raised to 275 °C (5 min.) at a rate of 5 °C/min. ass spectrometric detection was performed in electron impact 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). All compounds were identified by comparison with Computer Library (WILEY & NISTMASS SPECTRAL DATABASE) attached to the GC–MS instrument and by comparison to literature relative retention indexes.

Results

Analysis of male's cuticle of the two investigated species revealed 40 compounds (Figure 1 & Table 1) in total, including hydrocarbons, monoester waxes, triglycerides, and free fatty acids to be represented. The compounds that were common in both species were nine compounds,

1) Benzene, (1-Butylhexyl)-
2) Benzene, (1-Pentylhexyl
3) Benzene, (1-Butyloctyl)-
4) Benzene,(1-Pentyloctyl)-
5) Benzene, (1-Propylheptadecyl)-
6) 1-Eicosanol
7) 9-Octadecenoic Acid, (2-Phenyl-1,3-Dioxolan-4-Y L) Methyl Ester, Cis.
8) Cyclodecasiloxane, Eicosamethyl-
9) [(2-Fluorophenyl)Methyl]-

The chemical diversity was higher in, *A. tessellatum* (30 compounds) than *A. pulchellum* (19 compounds). (Table 1).

Male of ***Anthidium pulchellum*** was characterized by ten compounds, not found in the cuticle of another male,

1) Heptadecanoic Acid, 9-Methyl-, Methyl Ester
2) Octadecanoic Acid Methyl Ester
3) Tributyl Acetylcitrate;
4) Linoleic acid Ethyl Ester
5) Octadecanoic Acid,2,3-Dihydroxypropyl Ester.
6) Ursodeoxycholic Acid
7) Stigmast-5-En-3-Ol, (3á,24s)-
8) 1,1-bis(dodecyloxy)hexadecane 1,1-didodecoxy hexadecane
9) Cyclooctasiloxane, Hexadecamethyl-
10) Cyclononasiloxane, Octadecamethyl-

Male of ***Anthidium tessellatum*** was characterized by twenty-one compounds:

1) Benzene, Pentadecyl-
2) Heneicosane, 11-Phenyl-
3) Tetradecanoic Acid
4) Butanoic Acid, 2-Methyl-, 2-Methoxy-4-(2-Propenyl) Phenyl Ester
5) Hexadecanoic Acid, Methyl Ester
6) Oleic Acid
7) Octadecanoic Acid
8) E,E,Z-1,3,12-Nonadecatriene-5,14-Diol
9) 8-Octadecenoic Acid, Methyl Ester
10) Octadecanoic Acid, 4-Hydroxy-, Methyl Ester
11) Hexadecanoic Acid, 2,3-Dihydroxypropyl Ester
12) Retinal
13) Androstan-17-One.3-Ethyl-3-Hydroxy-, (5à)-
14) Docosanol.
15) Ethyl Iso-Alcoholate.
16) Propanoic Acid,2-(3-Acetoxy-4,4,14-Trimethylandro St-8-En-17- Yl)-.
17) Palmitic Acid,2-tetradecyloxy)Ethyl ester
18) Hexadecenoic Acid,1-(Hydroxymethyl)-1,2-Ethanediyl Ester.
19) 2-Hydroxy-3-[(9e)-9-Octadecenoyloxy] Propyl (9e)-
20) 9-Octadecenoic Acid,1,2,3-Propanetriyl Ester, (E,E,E).
21) 2,3-Dihydroxypropyl elaidate.

The relative contribution of cuticular chemical compounds (Table 1):

Anthidium pulchellum

The most abundant compounds in males of this species are, **Octadecanoic Acid,2,3-Dihydroxypropyl Ester** (4.19%), **Benzene, (1-Pentyloctyl)-** (3.05%)) and **Cyclodecasiloxane, Eicosamethyl-** (3.01%)); while the following compounds represented by scarce amounts, **Benzene, (1-Butylhexyl)- and** 9-Octadecenoic acid, (2-Phenyl-1,3-Dioxolan-4-Y L) methyl ester, Cis- (0.68%). (Table 1)

Anthidium testellatum

The most abundant compounds in males of this species are, Oleic Acid (16.08%) followed by Octadecanoic acid (5.72%); while the following compounds are represented by scarce amounts, Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Eth Anediyl Ester and 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)- (0.55% and 0.56%) respectively. (Table 1)

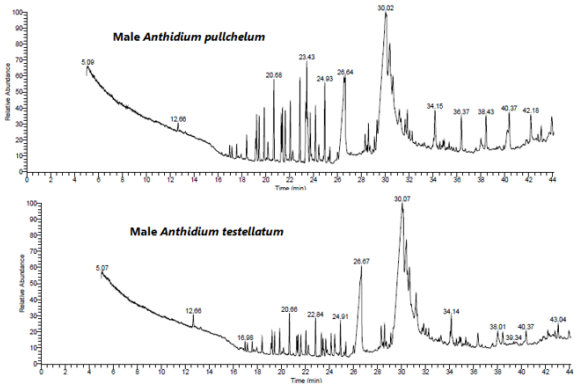

Figure 1. Chromatogram of GC-MS analysis for a cuticular chemical profile of males of two species of genus *Anthidium*.

Table 1. Chemical composition of the cuticle in males of two *Anthidium*, species and relative contribution (%)

	Compound Name	Chemical formula	Classification	Area (%)	
				A.pulchellum	*A.testellatum*
	Benzene, (1-Butylhexyl)-	C16H26	Hydrocarbon	0.68	0.65
	Benzene, (1-Pentylhexyl	C17H28		1.03	0.70
	Benzene, (1-Butyloctyl)-	C18H30		2.22	1.34
	Benzene, (1-Pentyloctyl)-	C19H32		3.05	1.70
	Benzene, Pentadecyl-	C21H36		-	0.65
	Benzene, (1-Propylheptadecyl)-	C26H46		2.60	1.72
	Heneicosane, 11-Phenyl-	C27H48		-	1.59
	Tetradecanoic Acid	C14H28O2	Fatty acid	-	0.88
	Butanoic Acid, 2-Methyl-, 2-Methoxy-4-(2-Propenyl)Phenyl Ester	C15H20O3		-	0.93
	Hexadecanoic Acid, Methyl Ester	C17H34O2		-	1.02
	Oleic Acid	C18H34O2		-	16.08
	Octadecanoic Acid	C18H36O2		-	5.72
	E,E,Z-1,3,12-Nonadecatriene-5,14-Diol	C19H34O2		-	2.83
	8-Octadecenoic Acid, Methyl Ester	C19H36O2		-	1.74
	Heptadecanoic Acid, 9-Methyl-, Methyl Ester	C19H38O2		0.78	-
	Octadecanoic Acid, 4-Hydroxy-, Methyl Ester	C19H38O3		-	0.70
	Octadecanoic Acid Methyl Ester	C20H34O4		0.93	-
	Androstan-17-One, 3-Ethyl-3-Hydroxy-, (5à)-	C21H34O2		-	3.25
	Propanoic Acid,2-(3-Acetoxy-4,4,14-Trimethylandro St-8-En-17-Yl)-	C27H42O4		-	1.03
	9-Octadecenoic Acid, (2-Phenyl-1,3-Dioxolan-4-Y L) Methyl Ester, Cis	C28H44O4		0.68	0.75
	Linoleic Acid Ethyl Ester	C20H36O2	Long-chain fatty acid	1.69	-
	1-Eicosanol	C20H42O	Fatty alcohol	0.90	1.88

	Docosanol	C22H46O	Aliphatic alcohol	-	1.80
	1,1-bis(dodecyloxy)hexadecane1,1-didodecoxyhexadecane	C40H82O2	Alcohol	1.01	-
	Ethyl Iso-Alcoholate	C26H44O5	Alcohol	-	1.35
	Hexadecanoic Acid, 2,3-Dihydroxypropyl Ester	C19H38O4	Glycerides	-	0.75
	2,3-Dihydroxypropyl elaidate	C21H40O4		-	3.13
	Octadecanoic Acid,2,3-Dihydroxypropyl Ester	C21H42O4		4.19	-
	Hexadecenoic Acid,1-(Hydroxymethyl)-1,2-Ethanediyl Ester	C35H68O5		-	0.55
	2-Hydroxy-3-[(9e)-9-Octadecenoyloxy] Propyl (9e)-	C39H72O5		-	0.73
	Retinal	C20H28O	Terpenes	-	0.95
	Tributyl Acetylcitrate	C20H34O8	Organooxygen	0.93	-
	Ursodeoxycholic Acid	C24H40O4	Other (Bile acid)	1.78	-
	Stigmast-5-En-3-Ol, (3á,24s)-	C29H50O	Sterol	0.98	-
	Palmitic Acid,2-(tetradecyloxy)Ethyl ester	C32H64O3	Fatty acid ester	-	0.59
	9-Octadecenoic Acid,1,2,3-Propanetriyl Ester, (E,E,E)	C57H104O6	triglyceride	-	0.56
	Cyclooctasiloxane, Hexadecamethyl-	C16H48O8Si8	Organosiloxane	0.95	-
	Cyclononasiloxane, Octadecamethyl-	C18H54O9Si9		1.58	-
	Cyclodecasiloxane, Eicosamethyl-	C20H60O10Si10		3.01	1.19
	[(2-Fluorophenyl)Methyl]-	C12H10FN5	Amino purines	1.25	1.26

DISCUSSION

The data obtained from the studied males of the two species of genus *Anthidium* by GC-MS technique offer a precise characterization of species via analyzing their cuticular chemical compounds, this conclusion is supported by many authors (Martin, *et al.,* 2013, Ngumbi *et al.,*2020). Pokorny *et al.,* (2014**)** proved that the CHCs of the cuticle are possibly useful for chemotaxonomy because they had shown distinguished cuticular chemical profiles that separate two cryptic species, also numerous publications have confirmed the benefits of chemotaxonomy for the distinction of various insect species (e.g. Ye et al., 2007, Martin et al., 2008).

Hydrocarbons are biologically very stable, and it secreted by a range of glands on the cuticle of the insect (Kaib et al., 1991). For example, the species-specific cuticular hydrocarbon profiles (*Vespa spp*) remain unchanged even after 20 years of being stored at ambient temperatures (Martin et al., 2009).

The cuticular chemical profile is genetically controlled, which confirms its accuracy and to some extent not affected by environmental factors. Ingleby *et al.,* (2010) reported scientific progress in understanding the hereditary basis of hydrocarbon variation in insects. Bosorang *et al.,* (2014) suggested that studying specimens of known taxa from different regions could help confirm the significance of CHCs as a taxonomic tool.

Furthermore, Dallerac *et al*. (2000) identified the gene responsible for producing several cuticular hydrocarbons (dienes) in Drosophila melanogaster. Mutation in these desaturase genes can cause sexual isolation in flies (Fang et al., 2002) and species isolation in moths (Roelofs *et al*., 2002).

The chemical composition of cuticle in males of the investigated species of the genus Anthidium agrees with those commonly recorded in insects (e.g. Kosaki and Yamaoka, 1996). The prevailing compounds in the males are oleic acid (16.08 % in *A. testellatum*) and Octadecanoic Acid,2,3-Dihydroxypropyl Ester (4.19 % in *A. pullshellum*), Châline *et al.,* (2005) concluded that alkanes and the longer-chain alkenes are the most abundant compounds on the bee cuticle, Poiani *et al.,* (2017) stated that hydrocarbons are the most dominant compounds in *Apis mellifera*. The other 42 compounds are represented in the cuticle by relatively small quantities that do not exceed 10% of the entire amount of the compounds (i.e. ranging from 0.55% - to 5.82%).

Vaničkova *et al.,* (2017) stated that the chemical constituents of *Bactrocera dorsalis* epicuticle

comprising of fatty acid esters, aliphatic hydrocarbons, aldehydes, and fatty acids were qualitatively and quantitatively different in both sexes. The present investigation adds aromatic hydrocarbons to the recorded compounds in the cuticle.

For more confirmation and accuracy, it is preferable to use an integrated taxonomic tool (e.g. biochemical, molecular, morphological, behavioral, or ecological). Therefore, researchers are combining several taxonomic tools to obtain greater power (Copren *et al.*, 2005; Schlick-Steiner, 2006).

Investigations carried out on cuticular chemical profiles of members of the genus *Anthidium*, mainly targeting the study of their role in scent recognition (Nadine *et al.*, 2005) and pollen recognition (Beryl and Neff, 1981), there are many studies concerning applying chemical profiles as a taxonomic tool, e.g.; (Elshaier, *et al.,* 2017; Elshaier,2022; Galhoum, 2017 & 2018).

The present study provides a detailed description of the cuticular chemistry of males of members of the genus *Anthidium* and finds some clear qualitative and quantitative variations. Our findings mainly provide a foundation for chemotaxonomic studies and investigate the roles of cuticular chemistry in functions such as desiccation resistance, protection from pathogens and injury, and chemical communication.

CONCLUSION

Using cuticular hydrocarbons as a taxonomic tool in insect classification is beneficial because they have some advantages like, they exhibit high natural variation that is under direct selection; stability over decades, and easy identification of cryptic species.

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