Cuticular chemical composition as a tool for the identification of puparial cases of some forensically important Egyptian blow flies

Eman E. Zaher
Zagazig University Faculty of Science
Salwa S. Rashed
Zagazig University Faculty of Science
Fatma A. Abdel-Halim
Zagazig University Faculty of Science
Samar M. Mohammed
Zagazig University Faculty of Science
Abeer Mohsen Salem (mabeer@sci.cu.edu.eg)
CU Faculty of Science: Cairo University Faculty of Science https://orcid.org/0000-0001-8552-5372

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Abstract

Cuticular chemical compounds for many insect species were proven to be unique and species specific. Because of their uniqueness, analysis of such chemical profiles, especially cuticular hydrocarbons was used for many purposes including identification of insects. Blow flies are one of the first flies that reach corpses so play a significant role in estimating the minimum post mortem interval. Accurate estimation depends on precise identification of the collected specimens. When only damaged empty puparial cases were left behind, morphological and even molecular identification methods of blow flies is so problematic. The aim of this study was to analyze the chemical compositions of the puparial exuviae of *Lucilia sericata, Chrysomya albiceps* and *Chrysomya marginalis*using gas chromatography/mass spectrometry (GC–MS) to evaluate their accuracy in Dipteran identification. Adults were collected from Giza and Cairo Governorates and reared under laboratory conditions until emergence of first-generation adults to obtain their empty puparial cases. GC–MS was used to analyze the chemical composition of these exuviae. Twelve classes of chemical compounds were identified from the three species at retention times 18.78 to 35.03. Alcohol represented the highest percentage (28.6%) of compounds in *Lucilia sericata* profile. Meanwhile, alkanes (*n*-alkanes, branched alkanes and cycloalkanes) constitute the major cuticular components of the three fly species with the highest percentage in *Chrysomya marginalis* cuticle. These findings could be considered as a preliminary step toward using hydrocarbon composition as a feasible tool for differentiation between forensic species in Egypt.

Introduction

Flies of family calliphoridae (blow flies) are strong flying insects, highly mobile and typically one of the first flies reaching corpses within minutes after death (Goff et al., 1993; Kabadaia, 2015). Accordingly, these flies may provide a useful solution in determining the minimum time since death which is known as the minimum post mortem interval (PMI_{MIN}). Usually, forensic entomologists precisely calculate PMI_{MIN} depending on species identification, duration of different stages at different temperatures, and other certain abiotic factors as geographic location, climate, latitude and so on (Turchetto and Vanin, 2004).

Usually, in death investigations, forensic professionals collect all insect specimens (adults, eggs, maggots and pupae) on corpses or around them. Sometimes, fresh insect samples are absent and only puparial cases are typically found. The exuvial identification of forensically important flies is so problematic, as they are often destroyed by the mechanical activity of adult emergence. Accordingly, traditional taxonomical identification of these deteriorated exuviae is very difficult. Also, natural degradation of DNA, enzymes and proteins during aging process deeply compromise molecular analysis of forensic samples (Gibbs and Crockettj, 1998; Ye et al., 2007). One alternative method to identify and age insect species is cuticular hydrocarbon analysis (CHC). The external layer of insect exoskeleton is the cuticle which acts as a mechanical support, prevents desiccation, protects against different microorganisms and serves as a contact or close-range pheromone. It is composed of a mixture of esters, alcohols, ketones, aldehydes, fatty acids and hydrocarbons (Blomquist et al., 1987; Suarez et al., 2011). In many insect species; hydrocarbons predominate the cuticle (Gibbs and Crockettj, 1998) and proven to be very stable (Drijfhout, 2010; Braga et al., 2016). Cuticular hydrocarbons are composed mainly of *n*-alkanes, branched methyl-alkanes, and alkenes (Blomquist et al., 1987; Blomquist and Bagnères, 2010; Drijfhout, 2010). The constituents of CHCs profile differ among various insect taxa; in the number of compounds, their proportions, chemical compositions, and chain lengths (Lockey, 1988; Howard and Blomquist, 2005; Sprenger and Menzel, 2020). These differences, along with certain other properties allowed the cuticular hydrocarbon content to be utilized for precise calculations of the weathering time of exuviae as well as the post mortem interval (Zhu et al., 2007).

Many authors reported the uniqueness of CHCs and successfully identified many species (Carlson, 1988; Anyanwu et al., 2000 & 2001; Horne and Priestmann, 2002; Bejarano et al., 2003; Shaalan et al., 2019; Moore et al., 2022). Also, studies confirmed that several CHCs exhibit changes in their relative abundance with chronological age of insect samples in different life stages (Urech et al., 2005; Zhu et al., 2006; Braga et al., 2016; Moore et al., 2021). In many insect species; CHCs function as sexual attractant pheromones or clues for species discrimination hence, very useful in speciation (Rundle et al., 2005). Changes in the composition of these clues alter mating preferences and pre-mating isolation and can be produced by changing the diet (Stennett & Etges, 1997) or temperature (Buckley et al., 2003). This explains the divergence in the cuticular hydrocarbons of geographically isolated populations due to differences in food and/or temperature which may lead to reproductive isolation. Hence, CHCs represent better indicators of recent speciation events and reproductive isolation than other genetic and morphological characters, that require more time to be expressed after speciation events.

The aim of this preliminary study is to use the technique of gas chromatography/mass spectrometry (GC–MS) to analyze the chemical composition of the puparial exuviae of three widely distributed Egyptian blow flies of forensic relevance. As far as we know this is the first study on Egyptian calliphorids investigating their cuticular hydrocarbon composition. Only few studies were done on other insect taxa as Hymenoptera (El Surtasi et al., 2016; Elshaier, 2021), Mantodea (Mohammad et al., 2009), or other dipteran species (Galhoum, 2017; 2018; Shaalan et al., 2019).

Material and methods

Flies collection and identification

Stock colonies of *Lucilia sericata, Chrysomya albiceps* and *Chrysomya marginalis* were established from flies initially collected during May, June & July 2019 from El-Mansuryia, Giza Governorate and Cairo Governorate, Egypt. Collected adults were transferred to be reared in the Entomology laboratory, Zoology department, Zagazig University where they were maintained in rearing cages under laboratory conditions at (27˚C ± 2) and (55–70%) relative humidity.

Adults were provided with water, sugar and meat as oviposition media. Meat was supplied in a clear plastic cup with damp cotton piece to prevent drying out of meat and checked daily for oviposition. After that, each deposited egg batch was transferred to a new plastic jar containing fresh meat. Newly hatching larvae were transferred to new jars containing fresh meat, covered with muslin and fastened with rubber bands. Dry autoclaved sieved sawdust was used as a medium for pupation. The pupae were sieved from the sawdust and transferred in petri dishes to the rearing cages for adult emergence. After adult emergence, puparial exuviae (cases) were collected for cuticular hydrocarbon analysis.

Morphological identifications were done using the identification key of adult Calliphoridae (Lutz et al., 2018) at Entomology Department, Faculty of Science, Ein Shams University, Egypt.

Cuticular hydrocarbon analysis

The extraction procedures of Ye et al., (2007) were slightly modified. Three replicates were analyzed for each species. Eight puparial cases of *L. sericata* and *Ch. albiceps* and only six puparial cases of *Ch. marginalis* (as large size) were used for each replicate. Puparial exuviae were washed with distilled water, cleaned by tip of fine paint brush and then dried at filter papers. Puparial exuviae of each replicate were immersed in 5mL n-hexane in glass vial and gentle swirl for 10 to 15 minutes at room temperature. After that, puparial cases were removed from the extracts. The extracts were then filtrated and collected in clean glass vials and stored at -20 ˚C till GC-MS analysis.

Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a splitless, injection volume of 1 µl and the following temperature program: 45°C for 2 min; rising at 10°C /min to 300°C and held for 10 min. The injector and detector were held at 280°C and 300°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 25–700. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Statistical analysis

In the present study, the data were analyzed using SPSS version 22. The peaks were selected for analysis based on their retention time (RT). Peaks with RT > 18 was chosen and annotated according to their retention times. The relative abundance of each peak was calculated based on their computed area under curve for each hydrocarbon. Discriminant analysis was executed, and discriminant functions were conducted using Wilk's lambda method. One-way ANOVA was applied to study the statistical effect of fly species on the percent composition of hydrocarbons. Least significant differences (LSD) test was used to illustrate the statistical differences in the studied variables among the different species.

Results

Twelve classes of chemical compounds were identified from the empty puparial cases of the three fly species at retention time 18.78 to 35.03. The percentages and the types of the extracted compounds were listed in Table 1. The profiles for *Chrysomya albiceps* and *Lucilia sericata* were very similar. Alcohol represented the highest percentage of compounds with 28.6% in *L. sericata*. However, in the three fly species *L. sericata*, *Ch. albiceps* and *Chrysomya marginalis*, alkanes (*n*-alkanes, branched alkanes and cycloalkanes) constitute the major component of cuticular hydrocarbons with 28.5, 50 and 89.4%, respectively. The chromatographs in Fig. 1 showed the characteristic peaks for each fly. Among studied species, the CHCs abundance in *Ch. albiceps* was lower than that in the others (18 compounds) as shown in Table 2a and 2b.

The retention times, names, and the frequency of each hydrocarbon in the three flies were listed (Table 2a & 2b). Forty-two compounds were identified with chain lengths ranging from C12 to C45. Heptacosane (7.6%) is the n-alkane dominated the chemical profile of *Ch. marginalis*, while Dodecane is the major one found in *L. sericata* (2.36%) and *Ch. albiceps* (1.88%). The predominant methyl branched alkane is 2-Methyltetracosane in *L. sericata* and *C. albiceps* and accounted for 5.53 and 4.92%, respectively. While the most abundant methyl branched alkane in *C. marginalis* is 2,6,10,14-Tetramethylhexadecane, (2.65%). The three species shared one compound in common which is the cycloalkane, 1-(2-Octyldecyl)octahydropentalene. Halogen branched hydrocarbons were detected in the chemical profiles of *L. sericata* and *C. albiceps*, but none was found in *C. marginalis* profile. Also, alkenes with different function groups as acid anhydride, alcohol and ester were detected in *L. sericata* and/or *C. albiceps* as illustrated in Table 2a & 2b peaks number 29, (26, 18) and (11, 7), respectively.

Alkadienes were represented in *L. sericata* with peak 30 (aldehyde) and in *L. sericata* and *C. albiceps* profiles with peak 35 (alcohol). The only cycloalkadienes observed in the chromatogram is the ketone compound, 3-(Dodecenyl)dihydro-2,5-furandione (peak 36) in the profiles of *L. sericata* and *C. marginalis*. The later species revealed several specific

compounds demonstrated by peaks 34, 33, 31, 28, 27, 25, 23, 20, 17, 15, 13, 9, 8, 6, 5, 4 and 2. The chromatogram of *C. marginalis* shows more alkanes than those found in other species. According to test equality of group means, most peaks differed significantly ($P < 0.01$) among all species.

Discriminant analysis

According to multiple regression analyses using Fisher discriminant method, twelve spectral peaks identified as characteristic variables among the three species of flies. They include peak 1 (Hydroxymethylcyclododecane), peak 2 (Heneicosane), peak 3 (Dodecane), peak 4 (Triacontane), peak 5 (Tetracosamethyl-cyclododecasiloxane), peak 6 (Tricosane), peak 7 (Oxalic acid, allyl pentadecyl ester), peak 10 (9-*t*-Butyl-4-iodo-2,2-dimethyladamantane), peak 11 (Oxalic acid, allyl octadecyl ester), peak 12 (2-Ethyl-1-decanol), peak 14 (2-Butyl-1-octanol), and peak 18 (Phytol).

Peaks 1, 3, 11, 12, 14 and 18 were found in *Lucilia sericata*. Peaks 1, 3, 7, 10, 14, and 18 were identified in *Chrysomya albiceps*. However, in *Chrysomya marginalis*, peaks 2, 4, 5 and 6 were only presented. According to one-way ANOVA, all the peaks showed significant differences among the studied species.

Two canonical standardized functions were obtained by discriminant analysis (Table 3). Function 1 explained 83.8% of the variations in the dependent variables (Fly species) and function 2 interpreted 16.2% of variable rate. To validate this result, species were plotted according to their scores on these two functions. Each individual was correctly assigned to its species as observed in Fig. 2 permitting establishment of a confident identification.

In Table 4, the unstandardized coefficients of the canonical discriminant function are displayed. The higher the value of the coefficient, the higher the ability to predict the change in the dependent variable. The canonical discriminant function for the three species is discernible.

Table 1
The frequency and percentage of each hydrocarbon classes in the three flies

Peak No.	Hydrocarbon class	Frequency (% of total abundance)		
		Lucilia sericata	*Chrysomya albiceps*	*Chrysomya marginalis*
1	Cyclic Alkane	2 (9.5%)	2 (11.1%)	2 (10.5%)
2	Alkane	2 (9.5%)	2 (11.1%)	15 (78.9%)
3	Alcohol	6 (28.6%)	3 (16.7%)	—
4	Ester	2 (9.5%)	2 (11.1%)	—
5	Ketone	3 (14.3%)	2 (11.1%)	1 (5.3%)
6	Halogenated alkane	2 (9.5%)	2 (11.1%)	—
7	Ether	1 (4.8%)	2 (11.1%)	—
8	Halogenated Cycloalkane	—	1 (5.6%)	—
9	Acid	—	—	1 (5.3%)
10	Acid anhydride	1 (4.8%)	1 (5.6%)	—
11	Aldehyde	1 (4.8%)	—	—
12	Epoxide	1 (4.8%)	1 (5.6%)	—

Table 2

a. Classes and percent composition of the compounds isolated from the cuticle of the three flies

Peak No.	Hydrocarbon class	Compound	RT	Percent composition			p-Value
				Lucilia sericata	*Chrysomya albiceps*	*Chrysomya marginalis*	
1	Cyclic Alkane	Hydroxymethylcyclododecane	18.78	8.13 ± 0.70	7.12 ± 0.22	—	0.000
2	Alkane	heneicosane	18.87	—	—	1.04 ± 0.18[ab]	0.000
3	Alkane	Dodecane	19.65	2.36 ± 0.32	1.88 ± 0.66	—	0.000
4	Alkane	Triacontane	19.75	—	—	1.85 ± 0.38[ab]	0.000
5	Cyclic Alkane	Tetracosamethyl-cyclododecasiloxane	19.83	—	—	0.83 ± 0.11[ab]	0.000
6	Alkane	Tricosane	20.60	—	—	2.43 ± 0.36[ab]	0.000
7	Ester	Oxalic acid, allyl pentadecyl ester	20.98	—	0.99 ± 0.21[a]	—	0.000
8	Alkane	Tetracosane	21.41	—	—	4.12 ± 0.64[ab]	0.000
9	Alkane	Heptacosane	22.21	—	—	7.60 ± 2.17[ab]	0.000
10	Halogenated Cycloalkane	9-*t*-Butyl-4-iodo-2,2-dimethyladamantane	22.41	—	1.04 ± 0.14[a]	—	0.000
11	Ester	Oxalic acid, allyl octadecyl ester	22.50	2.50 ± 0.93	—	—	0.000
12	Alcohol	2-Ethyl-1-decanol	22.52	3.06 ± 1.10	—	—	0.000
13	Alkane	Pentatriacontane	22.94	—	—	5.94 ± 0.57[ab]	0.000
14	Alcohol	2-Butyl-1-octanol	23.53	4.42 ± 0.17	2.69 ± 0.41	—	0.000
15	Alkane	Octacosane	23.67	—	—	6.75 ± 0.77[ab]	0.000
16	Cycloalkane	1-(2-Octyldecyl)octahydropentalene	25.33	5.68 ± 0.58	0.86 ± 0.04[a]	0.88 ± 0.25[a]	0.000
17	Alkane	Hexatriacontane	24.36	—	—	5.91 ± 0.73[ab]	0.000
18	Alcohol	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (2E,7R,11R)-Phytol	24.53	3.25 ± 0.35	2.35 ± 0.46[a]	—	0.000

Peak No.	Hydrocarbon class	Compound	RT	Percent composition			p-Value
				Lucilia sericata	*Chrysomya albiceps*	*Chrysomya marginalis*	
19	Ketone	N-[4-Bromo-*n*-butyl]-2-piperidinone	24.61	4.07 ± 0.27	3.37 ± 0.25[a]	—	0.000
20	Alkane	Nonacosane	25.03	—	—	5.57 ± 0.32[ab]	0.000
21	Alkane	2-Methyltetracosane	25.05	5.53 ± 0.99	4.92 ± 0.33	—	0.000

According to one-way ANOVA test, $P < 0.000$, represent significant effect of the studied factor. According to post-hoc least significant difference (LSD) test a, b represent significant differences ($P < 0.05$) as compared to *Lucilia sericata* and *Chrysomya albiceps*, respectively.

Table 2
b. Continue

Peak No.	Hydrocarbon class	Compound	RT	Percent composition			p-Value
				Lucilia sericata	*Chrysomya albiceps*	*Chrysomya marginalis*	
22	Halogenated alkane	1-Bromohexadecane	25.18	4.87 ± 0.05	2.89 ± 0.29[a]	—	0.000
23	Alkane	Dotriacontane	25.68	—	—	3.53 ± 0.35[ab]	0.000
24	Ketone	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	25.98	3.85 ± 0.09	2.99 ± 0.24[a]	—	0.000
25	Alkane	2,6,10,14-Tetramethylhexadecane (phytan)	26.31	—	—	2.65 ± 0.18[ab]	0.000
26	Alcohol	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (T-phytol)	26.59	5.46 ± 1.10	—	—	0.000
27	Alkane	4-Methyldocosane	26.91	—	—	2.21 ± 0.17[ab]	0.000
28	Acid	3,5-Di-tert-butyl-4-hydroxy hydrocinnamic acid	27.01	—	—	3.03 ± 0.46[ab]	0.000
29	Acid anhydride	2-Dodecen-1-yl(-)succinic anhydride	27.11	3.84 ± 0.91	1.38 ± 0.13[a]	—	0.003
30	Aldehyde	7,11-Hexadecadienal	27.16	4.00 ± 2.82	—	—	0.000
31	Alkene	Squalene	27.50	—	—	1.57 ± 0.23[ab]	0.000
32	Halogenated alkane	1,2-Dibromododecane	27.66	3.21 ± 0.51	1.74 ± 0.28	—	0.000
33	Alkane	Tritetracontane	28.07	—	—	1.13 ± 0.18[ab]	0.000
34	Alkane	Tetratetracontane	28.63	—	—	0.89 ± 0.08[ab]	0.000
35	Alcohol	12-Methyl-E,E-2,13-octadecadien-1-ol	29.08	1.88 ± 0.27	4.73 ± 0.18[a]	—	0.003
36	Ketone	3-(Dodecenyl)dihydro-2,5-furandione	23.73	1.06 ± 0.21	—	5.73 ± 1.48[ab]	0.000
37	Alcohol	1-Eicosanol	29.88	5.19 ± 1.79	—	—	0.000
38	Ester	Undec-10-ynoic acid, dodecyl ester	30.23	2.77 ± 0.02	1.90 ± 0.44[a]	—	0.000

Peak No.	Hydrocarbon class	Compound	RT	Percent composition			p-Value
				Lucilia sericata	*Chrysomya albiceps*	*Chrysomya marginalis*	
39	Epoxide	1,2–15,16-Diepoxyhexadecane	31.15	2.90 ± 0.30	1.38 ± 0.18[a]	—	0.000
40	Ether	1-(Ethenyloxy)octadecane	33.50	1.57 ± 0.34	3.74 ± 0.02	—	0.001
41	Ether	Oxirane, [(hexadecyloxy)methyl]-	35.03	—	1.58 ± 0.24[a]	—	0.000

According to one-way ANOVA test, $P < 0.000$, represent significant effect of the studied factor. According to post-hoc least significant difference (LSD) test a, b represent significant differences ($P < 0.05$) as compared to *Lucilia sericata* and *Chrysomya albiceps*, respectively.

Table 3
Summary of Canonical Discriminant Functions

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	18355.136[a]	83.8	83.8	1.000
2	3537.382[a]	16.2	100.0	1.000

Table 4
Canonical Discriminant Function Coefficients (unstandardized)

Characteristic peaks	Function	
	1	2
C1	− .867	3.551
C2	17.835	74.332
C3	-37.899	29.267
C4	1.052	4.382
C5	-3.858	-16.078
C6	-10.439	-43.508
C7	112.916	-75.184
C10	135.229	20.004
C11	− .093	1.607
C12	-7.051	-3.120
C14	-12.785	10.309
C18	46.312	-9.549
(Constant)	-74.299	-33.121

Discussion

Cuticular hydrocarbons are proven to be species-specific in many insect taxa including Diptera (Carlson, 1988; Braga et al., 2013; Moore et al., 2022). It is expected to be a promising tool when comes into the field of forensic entomology especially in cases where only empty puparia are available in a scene. Analysis of CHCs provides very helpful information in identifying ambiguous specimens due to physical damage, degradation of the genetic material or even in case of sexually dimorphic or morphologically similar species (Braga et al., 2013; Moore, et al., 2021). Moreover, various studies confirmed the reliability of this technique in assigning individuals to certain geographic population (Charabidze, et al., 2017; Moore et al., 2022) which in turn can reveal the presence of non- native population on a cadaver, hence cadaver movement from original death location. The main aim of this study was to establish if a distinction could be made between the empty puparial cases of the three blow fly species (*Lucilia. sericata*, *Chrysomya albiceps* and *Chrysomya marginalis*) using cuticular hydrocarbon analysis. As far as we know, this is the first study that deals with the cuticular chemical composition of some Egyptian flies of forensic importance. More investigation should be done on the cuticle of necrophagous flies as it could greatly facilitate species identification and accelerate solving forensic cases without the need to rear larvae or pupae to adult stage (Paula et al., 2017)

Morphological differentiation can be noticed among the adults of those flies (Lutz et al., 2018), while identification of larvae is time consuming and challenging specially in early instars (Szpila et al. 2014). When comes into pupae, usual morphological distinction is very difficult or even impossible for scientists other than taxonomists due to deformation or weathering conditions (Ye et al., 2007; Moore et al., 2022). Despite being known in many insect species, the chemical composition of the cuticle of many Egyptian species is still unknown and requires a thorough investigation. Our results showed that the three fly species have a distinct fingerprint profile. Their CHCs are like those of other insects and consisted of alkanes, methylalkanes, halogenated alkanes and cyclic hydrocarbons (Byrne et al., 1995; Ye et al., 2007; Braga et al., 2013; Galhoum, 2018; Moore et al., 2022). We also included all compounds obtained from the chromatogram like alcohols, ketones, aldehydes, esters and acids into our analysis. As detected previously (Frederickx et al., 2012; Kranz et al, 2017); those compounds yielded distinct peaks that can be used to distinguish between the three species. The classes of the chemical compounds obtained from the chromatogram of *L. sericata* and *Ch. albiceps* included hydrocarbons and alcohols, ketones, esters, ethers, acid anhydrides, epoxides and an aldehyde. Similar results were recorded by many authors as (Al-Dawsary, 2014) who found that the most prevalent chemical groups in the cuticle of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) are alcohols then hydrocarbons, carboxylic acid, esters, aldehydes and ketones respectively. While, (Alnajim et al., 2019) found the most abundant classes in the cuticle of *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (Fabricius) are hydrocarbons, fatty acids and a sterole. Same was obtained by (Elshaier, 2021) who found the cuticle of *Anthidium amabile* (Alfken) dominated by fatty acids then hydrocarbons sterols, glycerides, one ketone and one alcohol. Although the number of hydrocarbons in the cuticle of *L. sericata* (6 compounds) and *Ch. albiceps* (7 compounds) recorded in this work was significantly smaller than those obtained from previous studies on the same species (Ye et al., 2007; Braga et al., 2013; Moore et al., 2014; Paula et al., 2017; Moore et al., 2022), some authors like (Elshaier, 2021) recorded only five hydrocarbons from the cuticle of the wool-carder bees *Anthidium amabile* from Egypt. However, (Drijfhout et al., 2009) estimated the total number of hydrocarbons in the cuticle of insects as ranging from five to fifty compounds. Similar to many other dipteran flies, the chemical profile of the cuticle of *Ch. marginalis* composed mainly of *n*-alkanes, with the most abundant compound is heptacosane (C27: H56) (Goodrich, 1970; Ye et al., 2007; Moore et al., 2022; Kula *et al*., 2022). Squalene was found only in the profile of *Ch. marginalis* and most likely was ingested during feeding as insects don't produce this compound (Braga et al., 2013).

Our results showed that, the only shared compound between the three flies is 1-(2-Octyldecyl)octahydropentalene (C26: H50). This compound was encountered in essential oils extracted from medicinal plants for cytotoxic, antimicrobial

and insecticidal activities (Mohamed et al., 2015; Al-Mazroa et al., 2015; Hamada et al., 2018; Sadiq et al., 2018; Mamza et al., 2021; Kewlani et al., 2022). Also, was detected in ground water samples used for drinking and irrigation in Egypt (Abd-Elgawad et al., 2022). So, the presence of this substance may be due to the feeding habits of the three fly species. Kranz et al., (2017), found that diet outmost the impact of any other abiotic factors on the structure of insects cuticle, resulting in significant influence on their profiles. Until now, there is no study reported the presence of such compound in insect cuticle and the exact role of it is still unknown.

Declarations

All authors certify that there is no conflict of interests and no funding was received for conducting this study. This manuscript does not involve human and/or animals research.

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Figures

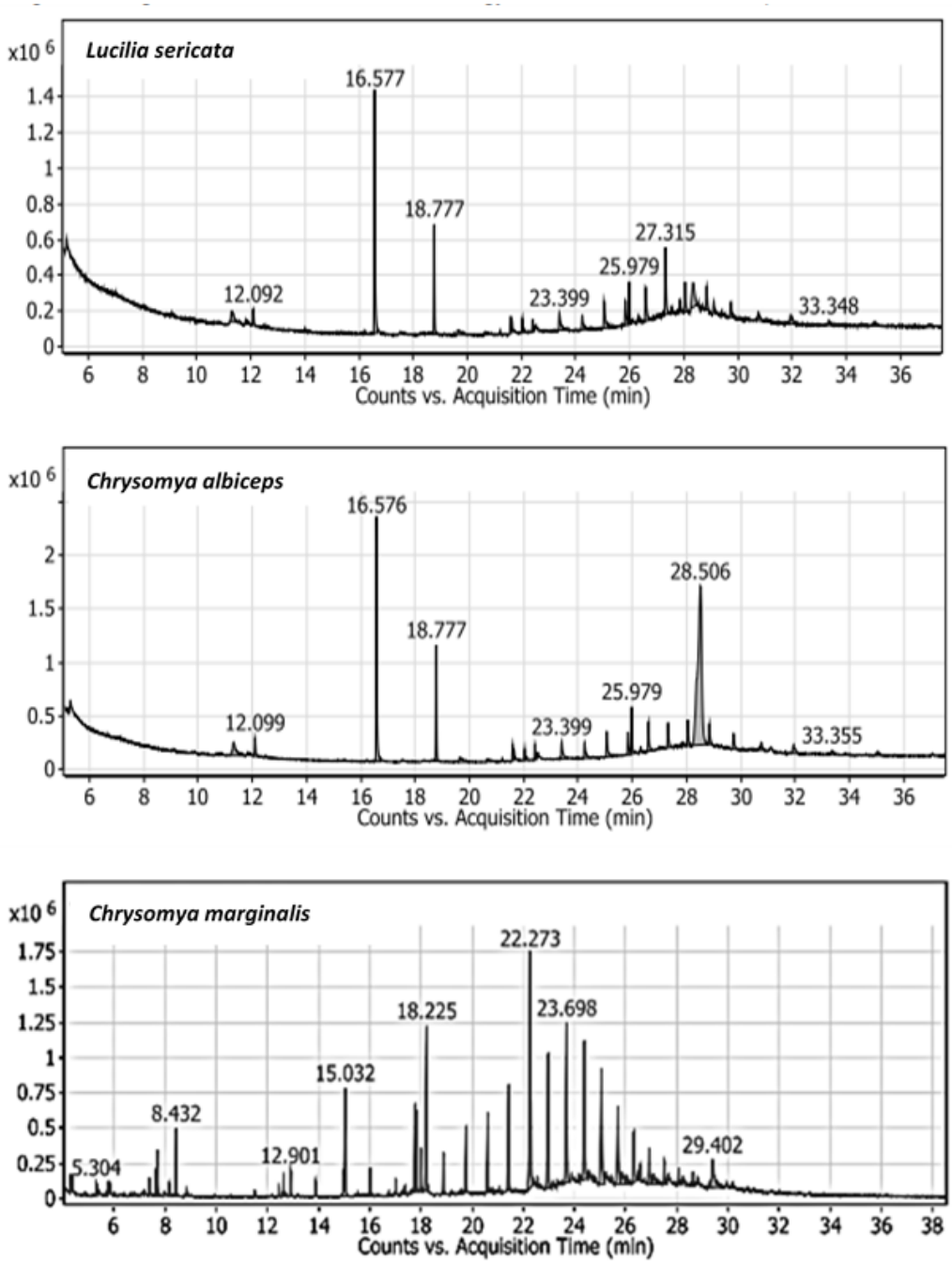

Figure 1

Representative gas chromatographs of cuticular compounds of *Lucilia sericata, Chrysomya albiceps* and *Chrysomya marginalis*

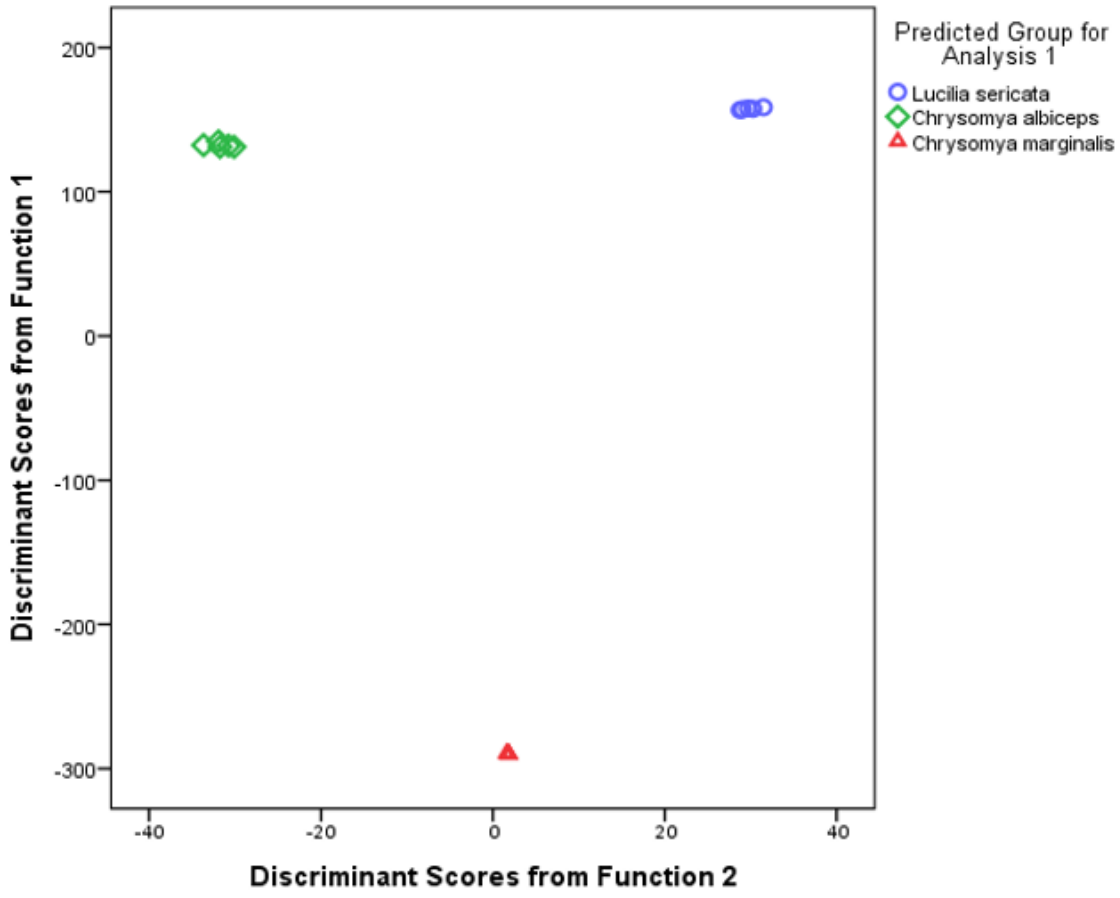

Figure 2

Cuticular hydrocarbon composition of *Lucilia sericata, Chrysomya albiceps* and *Chrysomya marginalis*distributed in the space of discriminant functions 1 and 2