Studies on the Chemical Profile of Some Melliferous Plant from Cluj Area, Romania, Using Different Extraction Techniques

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In this paper chemical compounds collected from melliferous plants (Cluj, Transylvania-area flora) using two methods and identified by GC-MS were presented. The melliferous plant studied : acacia, rape, linden and sun flower, represent a rich source of nectar and pollen used in the honey production. The methods were SPME (solid phase microextraction) coupled with GC-MS (gas chromatography and mass spectrometry) and alcoholic extraction of flowers followed by GC-MS. It was pursued in special the identifying of components with semiochimical role. They were identified a total of 158 compounds, from which some semiochemical compounds were already known, but also new compounds were discovered.

Kewords: SPME, rape, acacia, sun flower, linden flowers, semiochemicals

The melliferous plants are of great interest for honey production by bees.The composition of honey is tightly associated to its botanical source and also to the geographical area from where it originated, because soil and weather determines melliferous flora development [1]. In this paper are presented the melliferous potential of the Forest Tree Species: *Robinia pseudoacacia* and *Tilia cordate* and Agricultural Crops: *Brassica napus* L ssp. Oleifera D.C. and *Helianthus annus.*

The volatile compounds found in these plants give taste and flavor to the honey. The purpose of this work is to identify specific volatile compounds from each analyzed melliferous plant and this result can be used in the future to monitor changes in the composition of honey volatiles. Besides the volatile compounds, alcoholic extracts of these flowers are analyzed and the results was compared. Some of the floral compounds have a semiochemical role, they are involved in interspecific communication from the hive. Most plants in their natural habitat can attract or repel certain insect species, by releasing chemical signals. This is a very complex process if we consider the multitude of chemicals involved and the very varied species receivers.

The conventional analytical techniques are limited in identifying volatile compounds and their correlation with honey flavors. SPME is a simple, sensitive, time-efficient, cost-effective, reliable, easy-to-automate, portable sample-preparation and solventless extraction technique [2]. SPME is an alternative method to conventional analytical technique. SPME sampling can be performed in three basic modes: direct extraction (the analytes were transported directly from matrix to the extracting phase), headspace extraction (the analytes are extracted from the gas phase equilibrated with the sample) and extraction with membrane protection (the fibre is separated from the sample with a selective membrane). The selection of the sampling mode is dependent on the nature of the compounds to be analysed and the sample type. In our study, the headspace extraction mode was the most appropriate.

Experimental part

Material and methods

a.The volatiles from melliferous plants sampled by SPME technique and GC-MS analyzed

Plant material**:** The four samples melliferous flower were obtained from a rape culture, sunflower culture, acacia flower and linden flower parkland located in Cluj county, Romania. The flowers were collected during their full flourishing, when they are most visited by bees.

SPME adsorbtion: In each box was placed (50 gr) flower for 30 min. Then the fiber attachment needle was inserted and exposed SPME fiber in the headspace for 30 min during collection/adsorption. At the end of time, the fiber is retracted, holder with the saturated fiber was analyzed by injection in GC injection port. The fiber used was Carboxen / PDMS 75μm (Supelco USA). Before used, the fiber was preconditioned at 300°C during 60 min in the GC injector port.

GC-MS Analysis**:** The GC-MS analysis of the volatile was done using an instrument Model Agilent 7890 & 5975 Series MSD, equipped with a HP-5MS column (30 m, 0.25 mm, 0.25 μM). The carrier gas was helium with a flow rate of 1mL/min. Oven temperature was programmed as 60°C for 5 min and an increase by 6°C /min to 200°C. From 200°C to 250°C, increase with 15°C /min. It is maintained at 250 ° C for 5 min. The injector temperature was 250°C, splitless mode. The detector temperature: 230°C. The software adopted to handle mass spectra and chromatograph was a Chemstation and mass spectrum Library: NIST L14. In addition, a C_8-C_{20} standards alkans (Alkane Standard Solution C_8-C_{20} Sigma Aldrich) was used for calculation of the linear retention index (RI), comparing obtained values with those reported in the literature, in the same condition.

b.The compounds from melliferous plants sampled by alcoholic extraction technique and GC-MS analyzed

Plant material**:** The four samples melliferous flower were obtained from a rape culture, sunflower culture, acacia flower and linden flower parkland located in Cluj county, Romania. The flowers were collected during their full flourishing, when they are most visited by bees.

The extraction technique in alcohol: In each vial (20 mL) was placed 5 gr flower in 10 mL ethanol. Extraction time was 20 h followed by filtration and concentration of the solvent in a current of nitrogen.

GC-MS Analysis**:** The GC-MS analysis of the alcoholic extracts was done using an instrument Model Agilent 7890 & 5975 Series MSD, equipped with a HP-5MS column (30 m, 0.25 mm, 0.25 μM). The carrier gas was helium with a flow rate of 1mL/min. Oven temperature was programmed as 50°C for 5 min and an increase by 8°C /min to 220°C. From 220°C to 280°C, increase with 20°C /min. It is

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maintained at 280 ° C for 5 min. Injector temperature was 260ÚC, detector: 230ºC, splitless. Software adopted to handle mass spectra and chromatograph was a Chemstation and mass spectrum Library: NIST L14. In addition, a C_8-C_{20} standards alkans (Alkane Standard Solution C_8-$C_{20,}$ Sigma Aldrich) was used for calculation of the linear retention index (RI), comparing obtained values with those reported in the literature, in the same condition.

Results and discussions

The volatiles composition sampled by SPME from melliferous flower and their GC-MS analysis is summarized in table 1.

In total **72** compounnds were found in the emissions of the four types of flowers, but there is no common compound. There are no common compounds to all types of flowers. Floral markers were identified and their semiochemical role established.

Table 1

THE SAMPLE AND ANALYSIS BY SPME –GC-MS

No.	Compounds	RT	RI	Rape flower[%]	Acacia flower [%]	Linden flower [%]	Sun flower [%]
1	1,3-pentadiene,2-methyl	1.655	627	-	18.67	-	-
2	Butanoic acid ,2-methyl, methyl ester	2.717	780		0.56		
3	3-Hexen-1-ol	4.477	851	-	-	0.04	
4	α pinene	6.883	940	-	1.1	0.58	72.65
5	Bicyclo[3.1.0]hexane,4-methylene-1-(methylethyl)	7.932	973	-	-	0.05	-
6	Sabinene, (β-Thujene)	8.019	975	-	-	-	2.76
7	Lavandulyl acetate	8.101	978	-	-	0.16	-
8	β -Pinene	8.140	979	-	0.13	-	-
9	β-Myrcene	8.557	992	-	0.35	1.27	-
10	(Z)-3-Hexen-1-ol acetate	9.034	1008	0.06	-	-	-
11	α- Phellandrene	9.064	1009	-	1.65	1.09	0.69
12	α- Terpinene	9.354	1018	-	-	0.64	-
13	4-Carene	9.437	1021	-	-	-	0.57
14	*D-limonene*	9.788	1033	-	0.73	18.79	7.21
15	β- ocimene (E)	10.009	1040	-	1.75	-	-
16	Benzenacetaldehyde	10.178	1046	1.96	-	-	-
17	β- ocimene (Z)	10.369	1052	-	9.76	16.60	-
18	γ - terpinene	10.677	1062	-	-	2.72	0.43
19	α-terpynolene	11.547	1091	-	0.62	2.89	0.15
20	Benzoic acid, methyl ester	11.678	1094	-	2.39	-	-
21	Undecane	11.825	1100	0.03	-	-	-
22	Linalool	11.843	1101	-	18.68	-	-
23	Nonanal	11.938	1105	0.11	-	-	-
24	Phenyl ethyl alcohol	12.237	1116	1.92	-	-	-
25	2,6-dimethyl-1,3,5,7-octatetraene, E,E	12.441	1123	-	0.32	0.08	-
26	Verbenol	13.087	1147	-	-	-	0.06
27	4,8-dimethylnona-1,3,7-triene	12.315	1119	-	-	-	0.27
28	(E,Z)-Allo ocimene	12.684	1132	-	3.19	4.8	-
29	Pinocarveol	12.940	1142				0.06
30	(E,E) -Allo ocimene	13.026	1145	-	4.16	6.98	-
31	Pinocarvone	13.577	1165	-	-	-	0.03
32	Endo-borneol	13.668	1169	-	-	-	0.03
33	Benzoic acid ethyl ester	13.802	1174	0.94	0.04	0.05	-
34	1,3,8-p-Menthatriene	13.967	1177	-	-	0.07	-
35	Terpinen-4-ol	13.967	1180	-	-	0.1	-
36	Myrtenol	14.479	1199	-	-	-	0.05
37	Dodecane	14.509	1200	0.04	-	-	-
38	Decanal	14.678	1207	003	-	-	-
39	cis-Verbenone	14.817	1212	-	-	0.07	-
40	Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl	14.821	1213	-	-	-	0.11
41	para-Methylpyridine	14.960	1218	-	1.2	-	-
42	2,6,6-Trimethyl-cyclohexene-1-carboxaldehyde	15.094	1224	-	-	0.09	-
43	Anisole	15.671	1247	-	-	0.1	-
44	Benzene acetic acid,ethyl ester	15.701	1248	0.10	-	-	-
45	(E)-Cinnamalaldehyde	16.330	1274	0.19	-	-	-
46	Bornyl acetate	16.711	1290	-	-	-	0.42
47	Tridecane	16.976	1300	0.01	-	-	-
48	Trans-Pinocarvyl acetate	17.041	1303	-	-	-	0.04
49	3-Phenyl-2-propen-1-ol	17.158	1308	0.07	-	-	-
50	Methyl anthranilate	17.986	1345	-	0.06	-	-
51	Benzenepropanoic acid, ethyl ester	18.155	1353	0.07	0.06	0.08	-
52	α-Cubebene	18.211	1355	-	-	-	0.01

53	(E)-Longipinene	18.255	1357	0.07	-	-	0.03
54	α-Copaene	18.823	1382	-	-	-	0.02
55	Z- β-Elemene	19.165	1397	-	-	-	0.06
56	Caryophyllene	19.798	1427	-	-	-	0.13
57	β- Copaene	19.993	1436	-	-	-	0.03
58	β- Calarene	20.088	1441				0.99
59	Trans-Muurola-3,5-diene	20.353	1453				0.02
60	Humulene	20.531	1462	-	-	-	0.03
61	2,6-Di-tert-butylbenzoquinone	20.756	1472	0.01	-	-	-
62	gamma Muurolene	20.986	1483	-	-	-	0.02
63	Germacrene D	21.099	1489	-	-	-	0.02
64	α -Murolene	21.463	1506	-	-	-	0.02
65	Cis-Calamenene	21.944	1530	-	-	-	0.03
66	2,2,4-Trimethyl-1,3-pentanedioldiisobutyrate	23.362	1602	0.06	-	-	-
67	Tetradecanal	23.596	1615	0.01	-	-	-
68	Hexadecanal	27.298	1819	0.01	-	-	-
69	Dimethyl palmitamine	28.759	1907	0.09	-	0.02	-
70	Ethyl Palmitate	29.930	1996	0.03	-	-	-
71	Ethyl Oleate	31.625	2174	0.01	-	-	-
72	Cyclohexanone,2,6-bis(phenylmethylene)	33.498	2406	0.2	-	-	-

The most common floral compounds are used in chemical communication of species. The pollinating insects are attracted by visual and olfactory cues. There are a large number of compounds which are produced by flowers and blended into complex mixtures to guide and stimulate pollinating insects [3]. Referring to the behavior modifying action on bees, suggest that the volatile floral compounds acts as attractants or repellents**.**

In the volatiles collected from ***Acacia flower*** there are eight specific compounds**. Linalool** - floral compound and major constituent of many scents is known to cause behavioral changes to the bee [4]. It is an attractant for the Queen honeybee. **β- ocimene E** is a constituent of the pheromones of many pollinators (e.g.*Liostenogaster veckti)*.

In the volatiles collected from ***Sun flower*** there are twenty-four specific compounds. The major component belonging to the *sun flower* volatiles is **α--pinene.** It is emitted by many species of plants including those belonging to the Order Asterales and Family *Asteraceae*. Many species of the pollinating insect utilize α-pinene in its chemical communication system. They belong to the orders: *Hymenoptera* (bees, wasps, and ants), *Lepidoptera* (butterflies and moths), *Diptera* (flies) and *Coleoptera* (beetle). The last identified compounds from sun flower composition, **markers**, belong to the Sesquiterpenes class. They are found naturally in plants and insects, as semiochemicals, e.g. defensive agents or pheromones.

In the volatiles collected from ***Linden flower*** there are eleven specific compounds**. D-limonene**, is the major component from *Linden flower* volatiles. It is a floral compound emitted by many species of plants including *Malvales –Malvaceae:* genus*: Tilia cordata***.**

β-- ocimene Z (Z)-3,7-Dimethyl-1,3,6-octatriene)- floral compound emitted by some of the flowers from the Malvales, Malvaceae species. It is an attractant and constituent of the pheromones of many pollinators (e.g.*Macropis fulvipes*, *Liostenogaster veckti).*

For ***Rape flowers, t***here are eighteen specific compounds. The floral markers are: benzeneacetaldehyde and phenyl ethanol. It has a pleasant floral odor and many species utilize 2-Phenylethanol in its chemical communication system. It is a part of the worker bee alarm pheromone. Further evidence for their role are identified in interspecific and intraspecific defence and aggression pheromone and against their role in food source signalling [5].

The chemicals composition for alcoholic extracts from melliferous flower and their GC- MS analysis is summarized in table 2.

Table 2

THE MELIFEROUS PLANTS IN ALCOHOLIC EXTRACTS AND ANALYZED BY GC-MS

No	Compounds	RT	RI	Rape flower [%]	Acacia flower [%]	Linden flower [%]	Sun flower [%]
1	1-Butanol-3-methyl	2.772	730	2.56	-	1.43	-
2	Butanoic acid -3-methyl	3.123	864	-	-	2.90	0.02
3	2-Furanmethanol	4.025	875	-	-	0.55	0.03
4	Cyclopent-4-ene-1,3-dione	4.402	901	-	0.69	-	
5	Oxime, methoxy phenyl	4.584	907	-	-	-	0.13
6	1,3-Dihydroxyacetone	4.814	926	1.76	0.31	0.69	0.13
7	Alpha – pinene (isomers)	5.057	941	-	-	-	22.77
8	2-Cyclopenten-1-one, 2-hydroxy	5.126	945	0.62	2.81	1.43	-
9	Sabinene	5.646	577	-	-	-	1.68
10	Heptane-2,2,4,6,6-pentamethyl	5.841	989	0.29	0.22	-	0.21
11	Phenol	5.950	995	-	-	0.20	-
12	4-Carene	6.379	1020	-	-	-	0.03
13	2-Hydroxy-gamma-butyrolactone	6.539	1029	-	3.01	-	0.14
14	D-Limonene	6.552	1030	-	-	-	0.22
15	3-Methylcyclopentane-1,2-dione	6.804	1044	-	0.38	0.21	-
16	Benzenemethanamine,N,N-dimethyl	6.899	1049	-	-	-	0.19
17	Gamma-terpinene	7.124	1062	-	-	-	0.04

18	Furaneol	7.289	1071	0.31	-	1.20	-
19	2,5-dimethylfuran-3,4-(2H,5H) dione	7.476	1081	-	1,13	-	-
20	Phenylethyl alcohol	8.217	1123	0.27	-	-	-
21	Maltol (3-Hydroxy-2-methyl-4H-pyran-4-one)	8.273	1126	-	0.41	0.06	-
22	Alpha-campholenal	8.408	1134	-	-	-	0.02
23	Trans-Verbenol	8.755	1149				0.07
24	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.811	1156	1.48	3.23	1.61	0.05
25	Pinocarvone	9.088	1172	-	-	-	0.01
26	Benzoic acid	9.171	1176	0.51	1.11	0.38	0.05
27	Cyclobutane-1,1-dicarboxamide,N,N'-di-benzoyloxy	9.383	1188	-	0.57	-	-
28	(-)-Myrtenol	9.678	1205	-	-	-	0.03
29	Catechol (1,2-Benzenediol)	9.717	1207	-	-	1.60	
30	Benzofuran-2,3-dihydro	10.020	1224	-	0.23	0.21	
31	5-hydroxymethylfurfural	10.194	1234	0.39	3.51	0.80	0.04
32	1,2-Benzenediol, 3-methyl	10.831	1271	-	0.31	-	
33	Isobornyl acetate	11.213	1293	-	-	-	0.04
34	(E)-2-Propen-1-ol, 3-phenyl	11.538	1313	0.24	-	-	
35	Alpha-Cubenene	12.296	1358	-	-	-	0.02
36	Alpha-Copaene	12.752	1386				0.05
37	Tetradecane	12.986	1400	-	-	0.07	-
38	Beta-Ylangene	13.476	1432	-	-	-	0.13
39	Germacrene D	13.640	1442				0.11
40	Beta-Calarene	13.705	1446	-	-	-	0.51
41	Humulene	14.031	1467	-	-	-	0.11
42	Undecanal-2-methyl	14.091	1471	0.07	-	-	-
43	Bicyclosesquiphellandrene	14.169	1476	-	-	-	0.10
44	Gamma-Muurolene	14.356	1488	-	-	-	0.05
45	Benzeneacetonitrile-4-hydroxy	14.408	1491	3.32	-	-	-
46	Benzenepropanol-4-methoxy	14.425	1492	-	-	0.05	-
47	Apocynin (Ethanone,1-(4-hydroxy-3 methoxyphenyl)	14.473	1495	0.11	-	-	-
48	Pentadecan	14.555	1500	0.15	-	-	-
49	Tridecanal	14.755	1514	0.17	0.24	-	-
50	Methyl vanillate (Benzoic acid,4-hydroxy-3-methoxy-methyl ester)	14.941	1526	-	0.10	-	-
51	Trans-Calamenene	15.075	1535	-	-	-	0.09
52	Sulphoraphane nitrile	15.140	1540	0.60	-	-	
53	E-5-Hexadecene	15.938	1593	0.25	-	-	
54	Hexadecane	16.038	1600	0.08	-	-	0.02
55	Tridecanoic acid	16.918	1662	0.14	-	-	0.19
56	Pentadecanal	17.685	1718	0.12	1.01	-	-
57	Tetradecanoic acid	18.323	1765	0.28	-	-	-
58	Benzoic acid,4-hydroxy-3,5-dimethoxy,hydrazide (syringic acid hydrazide)	18.583	1785	1.83	-	-	-
59	1-Octadecene	18.704	1793	-	-	-	0.05
60	E-15-Hexadecenal	18.713	1794	0.30	-	-	-
61	Ethyl tetradecanoate	18.734	1796	-	-	0.06	-
62	Octadecane	18.791	1800	-	0.10	-	-
63	Isopropyl miristate	19.146	1828	0.11	-	-	0.01
64	2-Pentadecanone-6,10,14-trimethyl	19.415	1849	-	0.14	-	
65	Caffeine	19.563	1860	-	-	0.21	
66	n-Hexadecanoic acid (Palmitic acid)	20.911	1969	1.44	0.73	0.32	0.15
67	Hexadecanoic acid, ethyl ester	21.249	1997	1.14	0.91	1.14	0.07
68	Kaur-15-ene	21.513	1998				0.16
69	Heneicosane	22.315	2100	-	-	0.44	-
70	Phytol (2-Hexadecen-1-ol, 3,7,11,15-tetramethyl)	22.463	2111	0.53	0.44	0.22	-
71	9,12-Octadecadienoic acid (Z,Z) Linoleic acid	22.654	2129	0.34	0.19	0.13	-
72	9,12,15-Octadecatrienoic acid (Z,Z,Z) (Linolenic acid)	22.723	2135	1.68	0.18	0.37	-
73	9,12-Octadecadienoic acid, ethyl ester	22.862	2148	0.74	0.57	0.96	0.05
74	9,12,15-Octadecatrienoic acid (Z,Z,Z) ethyl ester	22.922	2154	1.73	1.17	2.04	0.06
75	Octadecanoic acid, ethyl ester	23.078	2168	0.25	0.19	0.15	0.04
76	Docosane	23.785	2200	-	-	1.76	-

77	Nonadecanoic acid, ethyl ester	24.336	2287	0.08	-	-	-
78	Tricosane	24.362	2300	-	-	0.16	-
79	Docosyl methyl ether	24.904	2340	0.40		0.17	-
80	2-Momopalmitin (Hexadecanoic acid,2-hydroxy-1-hydroxymethyl)ethyl ester	25.047	2345	-	0.91	0.84	-
81	Eicosanoic acid, ethyl ester	25.541	2405	-	-	0.09	-
82	Beta-Monolinolein 9,12-Octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl)ethyl ester	26.282	2503	0.57	0.99	0.91	-
83	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	26.369	2478	1.24	-	0.62	-
84	13-Docosenamide	26.998	2537	0.15	-		-
85	1-Hexacosene	28.073	2643	-	-	0.25	-

In total **85** compounds were found in the alcoholic extracts of the four types of flowers.

9 common compounds were identified to the four types of flowers, namely: *esters of fatty acids*, *fatty acid*, *5-hydroxymethylfurfural, benzoic acid*, *4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl*. The smallest percentage is always found in the sunflower.

Esters of fatty acids: *ethyl linolenat, ethyl linoleat, ethyl stearate, ethyl palmitate*- identified in all 4 types of flowers have semiochemicals role. There are pheromones for some of the species belonging to the Hymenoptera order, all pollinating insects [6, 7].

Fatty acids: *C16(Palmitic) and C18(stearic, oleic, linoleic, and linolenic*) The most studied acids are those of *Apis* wich play an important roles in stabilizing swarm clusters, production of queen, and a variety of other behavioral roles.

5-Hydroxymethylfurfural: present as minor constituents in the secretion of the sternal glands of *P. sericea* (Olivier) (*Hymenoptera: Vespidae*).

2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one- the major constituent in the secretion of the sternal glands of *P. sericea* (Olivier) (*Hymenoptera: Vespidae*). The behaviour of those polistine wasps which found their nests by swarming, suggests that these species use trail pheromones for leading the swarm to the location chosen for the new nest [8].

*Dyhydroxyaceton*e- monosaccharide of trioze category, may be obtained from plant sources. Recently, exceptionally high levels of the antimicrobial compound methylglyoxal (MGO) have been found in manuka honey. In general, MGO is formed from sugars during heat treatment or prolonged storage of carbohydrate-containing foods and beverages. However, the high levels of MGO in manuka honey are formed by conversion of dihydroxy-acetone (DHA) present at exceptionally high concentrations in the nectar of L. scoparium flowers [9].

For rape flowers 13 specific compound were identified and the most abundant is Benzeneacetonitrile-4-hydroxy followed by Benzoic acid, 4-hydroxy-3,5-dimethoxy,-hydrazide and ethyl linolenate. Among the species that utilizes ethyl linolenat in its chemical communication system is foundFrieseomelitta trichocerata-pollinating insect as well as Apis mellifera belong to the same class, family and subfamily. Volatile compound in cephalic secretions of workers of Brazilian stingless bees (Frieseomelitta trichocerata) acts as pheromone [7]. Benzeneacetonitrile-4-hydroxy has biological role: plant metabolite - Any eukaryotic metabolite produced during a metabolic reaction in plants, including flowering plants, conifers and other gymnosperms.

For Linden Flower, 7 specific compounds were identified and the most abundant are*: 9, 12, 15-Octadecatrienoic acid (Z,Z,Z) ethyl ester* - Linolenic acid ethylester- the largest amount isolated of the four types of melliferous flowers.

Docosane- used in chemical communication system of Apis mellifera - Pheromone component, cuticular hydrocarbons, for Worker and queen [10].

1-Hexacosene - found in green vegetables and used in chemical communication of *Bombus cryptarum* (*Hymenoptere, Apidae, Apinae*) [11].

3-Methylbutanoic acid- the most abundant compound is floral compound, used by some species of insects in their chemical communication system.e.g: allomone-defensive substances, for *Polistes dominulus* (*Hymenoptera, Vespidae, Polistinae, Polistini*) [12].

For Acacia flowers, 7 specific compounds were identified.

Compounds found in higher percentages, over 3%, 5 or 6-membered heterocyclic rings are respectively *4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* and hydroxy-methyl furfural derived from dehydration of sugars. *3,4-Dihydro-8-hydroxy-3-methylisocoumarin* (mellein) and ***2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one*** are among the volatile constituents identified from the hindgut of the formicine ant *Lasius* (*Dendrolasius*) *fuliginosus (Hymenoptera, Formicidae)*. Mellein induces **trail-following behavior** in worker ants of this species and evokes electrophysiological responses from their antennae [13].

The principal constituents of hydrocarbons with functional groups in the headspace of honeybee workers are **aldehydes** from chain length C9 to C17 (***pentadecanal***). Due to their high volatility these aldehydes might also play a role in olfactory kin or nestmate discrimination in honeybees [14].

For Sun flowers, 22 specific compounds were identified.

The major component of sunflower extracts is *alpha-pinene*, as in the case of volatiles collected by SPME from this flower. Plant terpenes, as ***alpha-pinene*** and *beta-myrcene*, are the scents that bees look for to locate food sources. Sabinene- floral compound, monoterpene, used in chemical communication system for many insect [15]. Most are flower compounds, belonging to the sesquiterpenes class and have a semiochemical role for many polinating insects [16].

Conclusions

This study is the first part of an investigation searching in honey sorts previously identified compounds in flowers. Interesting observation are that majority of these compounds are found in volatiles emitted by the bee brood too [unpublished experiments yet].Volatile compounds identified in melliferous plants by SPME specific to these plants are likely responsible for bees attraction to nectar and pollen source. The pollen has a specific aroma, taste and shape for each plant.

Organic compounds identified in alcoholic extracts of melliferous plants are generally common, less specific and these compounds give the base note of the fragrance for each flower. They are large and heavy molecules that evaporate slowly.

Demonstrating the semiochimic role of *newly* identified components require biological experiments using electrophysiological methods, by testing the response of an organism to some volatiles stimuls.

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