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Article

Volatile compounds and palynological analysis from pollen pots of stingless bees from the mid-north region of Brazil

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Samburá is the botanical pollen nectar agglutinated by salivary secretions of bees. Stingless bee pollen samples were collected in three periods of the year in Monsenhor Gil town, PI, Brazil, for extraction of volatile constituents by different techniques, analyzed by gas chromatography–mass spectrometry (GC-MS) and the palynological analysis used to identify the dominant pollen. Among the volatile compounds identified, kaur-16-ene, methyl and ethyl hexadecanoate, methyl linoleate and heneicosane were identified more frequently in the studied parameters: period of sample collection and extraction techniques used. The palynological analysis identified the pollen of *Mimosa caesalpiniifolia* Benth. as the dominant pollen in all samples studied.

Uniterms: Pollen/*Scaptotrigona sp. Mimosa caesalpiniifolia* Benth. Pollen/volatile constituents. Chromatography-mass spectrometry. Palinology.

INTRODUCTION

Pollen of stingless bees (also known as *Samburá*) (Brasil, 2001) consists of botanic pollen combined with nectar and salivary secretions (Dermardersian, Beuther, 2005) and it is collected by worker bees from a variety of plant species. Pollen is the main protein source to bees, for the development of larva (Somerville, 2001), tissues formation (in all bees), to the ovarian development of the queen bee, and the use of which is useful for the increase of the bee community in a beehive (Human *et al*., 2007; Zerbo, Moraes, Brochetto-Braga, 2001).

The chemical composition of pollen is diverse, it is mainly composed by vitamins, minerals, enzymes, free amino acids, crude proteins, carbohydrates, fatty acids and polyphenolic compounds (Dermardersian, Beuther, 2005; Somerville, 2001; Almeida-Muradian *et al*., 2005).

Commercially bee pollen is considered a supplement (Kroyer, Hegedus, 2001) however the scientific literature describes some pharmacological properties and therapeutics: antitumor activity (Yang *et al*., 2007); immunomodulatory action; antioxidant activity (Carpes *et al*., 2008; Leja *et al*., 2007); antianemic action; treatment of respiratory tract infections, endocrine disorders, enteritis, colitis and constipation; poor appetite, decreased blood pressure and prevention of prostate inflammations (Ioirich, 1986).

The aim of this research was to identify, by gas chromatography coupled with mass spectrometry (GC-MS), the volatile constituents of the bee pollen of the genus *Scaptotrigona sp.* (stingless bees) obtained by different extraction techniques and collected at different periods of the year in a microregion of Piauí State, Brazil, and their botanical origin were identified through palynological analysis.

MATERIAL AND METHODS

Pollen samples

The samples of stingless bee pollen were collected manually in pollen pots of hives from Cocal community, in Monsenhor Gil town, Piauí State, Brazil. The collections were made during December 2006 (PAS-1), March (PAS-2) and July (PAS-3), which were located via Global Positioning System (GPS). Coordinates of sampling points: -5° 42'16.64'' S and -42° 38'29.02'' W.

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Extration of volatiles oils

Microhydrodistillation (MD)

About 10 g of bee pollen were subjected by microhydrodistillation during 3 hours. Afterwards, the volatile constituents were extracted from hidrolate (mixture of water and volatile oil) by dichloromethane HPLC grade partition (3x 15 mL). The organic phase was dried with anhydrous Na_2SO_4 P.A, filtered and concentrated (Torres *et al.*, 2008). The product containing the volatile constituents was kept under refrigeration until analysis GC-MS.

Dynamic headspace (HS)

Approximately 10 g of bee pollen was placed in a modified Kitasato flask (with two side outputs). The extraction of volatile constituents from the matrix was done through air suction, which previously passed through a glass tube 10 cm in length and 0.6 mm thick filled with a silica column (6 cm) and activated charcoal (2 cm) and in the outlet a small glass tube (5 cm) containing 150 mg of Porapak-Q® was placed. Both glass tubes contained cotton in its extremities, treated with acetone HPLC grade. Subsequently, the volatile constituents were desorbed with double distilled CH_2Cl_2 P.A (3 mL) and subjected to GC-MS analysis (Andrade *et al*., 2007).

Ultrasound-Assisted extraction (US)

In a 150 mL Erlenmeyer flask 10 g of pollen was placed, then 11 mL of water was added, and also 1.5 g of magnesium sulfate P.A, 15 mL of *n*-pentane /ethyl ether 1:2 (both HPLC grade). Then this mixture was put to be extracted in an ultrasonic bath for 30 minutes. Afterwards, the mixture was transferred to a separation funnel, 10 mL of saturated solution of sodium chloride P.A and 15 mL of solvent (*n*-pentane/ethyl ether) were added. The mixture was centrifuged at 3000 rpm for five minutes and the organic phase was collected and concentrated. The volatile constituents were kept under refrigeration until analysis (Alissandrakis *et al*., 2003).

Solid phase micro extraction (SPME)

The 5 g pollen was conditioned in a proper glass flask closed with rubber septum at room temperature for 1 hour. The SPME fiber (Sulpeco Co. Belfone, PA) was coated with 30 microns of polydimethylsiloxane (PDMS) and it was inserted via the septum in the internal space of the flask becoming exposed the matrix (pollen) for 24 hours. After this time, it was inserted into the injector of the gas chromatograph coupled with the mass spectrometer for 1 minute at 240 °C for the complete desorption of volatiles (Pontes, Marques, Câmara, 2007).

Analysis by Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

After methylation with diazomethane, the identification of volatile constituents was made by GC-MS, on a Shimadzu mass spectrometer model QP5050A equipped with a DB-5 HT column (95% of polymethylsiloxane and 5% of phenyl, 30 m long, 0.25 mm internal diameter, 0.1 microns thick of the stationary phase film). The temperature setting used was the following: injector at 220 °C, detector at 240 °C column 60 °C at 240 °C, with heating rate of 3 °C min^{-1}. The carrier gas selected was helium with a flow rate of 1 mL min^{-1}. The acquisition of mass spectrum was done in the rage 40-650 Daltons by the method of electron impact ionization, with ionization energy of 70 eV and ion source at 200 °C. The mass spectra were compared to the entries from the electronic library Wiley229 to then be identified; Kovats indices were also calculated, it was based on retention times of hydrocarbon standards (C_{10} - C_{25}) counterparts injected under the same conditions of the samples, to compare with literature data (Adams, 2007). All analyses were performed in duplicate.

Palynological analysis

The pollen samples PAS-1, PAS-2 and PAS-3 were identified at the Institute of Botany of São Paulo. For the palynological preparation of microscope slides 2 g of each pollen sample of stingless bees were withdrawn, and then homogenized separately in 10 mL of alcohol at 70%. Basically, the pollen preparation of the samples followed the standard European method (Maurizio, Louveaux, 1965) which consists of washing the material with distilled water and glycerin–water followed by centrifugation. The identification of pollen types was based mainly on the reference collection of microscope slides with pollen from the Palynological Research Center of the Institute of Botany of São Paulo, and also in palynological catalogs. Approximately 500 pollen grains per sample were identified for the class percentage definitions of dominant pollen (D>45%), accessory pollen (A<45 to 15%), isolate pollen (I<15 to 3%) and occasional pollen (O<3%) (Barth, 1989).

Statistical analysis

Statistical analyses were performed using the IBM® SPSS® Statistics software version 21.0. The peak area (percentage) of identified volatile compounds obtained was compared according to the extraction method and period of collection of samples of pollen. Differences between them were established using one-way analysis of

variance (ANOVA) followed by Tukey's test. Differences at the 5% level of significance ($p < .05$) were considered statistically significant.

RESULTS AND DISCUSSION

Along the gathering of pollen samples there was a variation in the amount collected in each period. In December about 180 g of pollen of native bees was obtained, March and July for about 360 g and 1180 g were obtained respectively. Overall this research identified 138 substances (Table I).

The major class of compounds identified were hydrocarbons and esters followed by terpenoids, ketones

TABLE I - Chemical constituents of volatile stingless bee pollen collected in the months of December, March and July and extracted by HS, MD, US and SPME with their relative abundances

COMPOUNDS	Kovats index		PEAK AREA (%)											
			PAS-1 [a]				PAS-2				PAS-3			
	IK_{cal} [b]	IK_{lit} [c]	HS[d]	MD[e]	US[f]	SPME[g]	HS	MD	US	SPME	HS	MD	US	SPME
ALCOHOLS														
Phenylmethanol*	1042	1031	-	3.08	0.32	-	18.16	-	1.00	-	14.4	5.43	-	-
2-phenethyl alcohol*	1111	1107	-	2.28	0.22	-	2.42	0.05	-	-	5.4	2.05	0.28	-
Cyclohexanol	1146	-	-	-	-	-	-	-	-	-	0.43		-	-
Nonan-1-ol	1173	1169	-	0.52	-	-	-	-	-	-	3.4	1.15	-	-
Cinnamyl alcohol	1263	1262	-	-	-	-	-	0.17	-	-	-	-	-	-
4-methoxybenzyl alcohol	1280	1280	-	-	0.13	-	-	0.05	-	-	1.15	-	-	-
Hexadecan-1-ol	1879	1874	0.3	-	-	-	-	-	-	-	-	-	-	-
Octadecan-1-ol	2083	2077	0.66	-	-	-	-	-	-	-	-	-	-	-
Tricosan-1-ol	2271	-	-	-	0.59	-	-	-	-	2.83	-	-	-	-
Icosan-1-ol	2473	-	-	-	2.71	-	-	-	-	3.11	-	-	-	-
KETONES														
Acetophenone	1068	1059	-	-	-	-	-	-	-	-	-	0.45	-	-
Decan-2-one	1193	1192	-	0.38	-	-	0.44	-	-	-	-	-	-	-
Nordavanone	1212	1231	-	0.48	-	-	-	-	-	-	-	-	-	-
Undecan-2-one	1294	1294	-	-	-	-	0.64	-	-	-	0.30	-	-	-
Neryl acetone	1450	1436	-	-	-	-	-	-	-	-	1.21	-	-	-
Geranyl acetone	1450	1455	-	0.36	-	-	-	-	-	-	-	-	-	-
Tridecan-2-one*	1495	1496	0.18	1.48	-	-	2.41	-	-	-	4.20	0.97	-	1.70
Pentadecan-2-one	1748	1760	-	1.25	-	-	-	-	-	-	-	0.14	-	-
2,6-Dibutyl-cyclohex-2,5-dien-1,4-dione	1418	-	-	-	-	-	-	0.70	-	-	-	-	-	-
ALDEHYDES														
5-Methylfurfural	997	964	-	3.07	-	-	-	-	-	-	-	-	-	-
Nonanal	1104	1100	-	-	0.08	-	-	-	-	-	-	-	-	-
Decanal	1204	1201	-	-	-	-	0.46	-	-	-	-	-	-	-
4-Methoxybenzaldehyde	1249	1247	-	0.49	-	-	-	0.06	-	-	-	-	-	-
3,4-Dimetoxybenzaldehyde	1476	1476	0.42	-	-	-	-	-	-	-	-	-	-	-
ESTERS														
Methyl 2-hydroxyhexanoate	1011	-	-	-	0.16	-	-	-	-	-	-	-	-	-
Methyl heptanoate	1035	1025	-	0.33	-	-	-	-	-	-	-	-	-	-
1,2-Diacetoxypropane	1038	-	-	2.65	-	-	12.26	-	-	-	13.48	-	-	-
Furfuryl acetate	1014	990	-	1.12	-	-	-	-	-	-	-	3.57	-	-
1,2-Butanediol acetate	1066	-	-	0.40	-	-	7.43	-	-	-	-	-	-	-
Benzyl formate	1078	1076	-	-	-	-	-	-	-	-	-	0.40	-	-
Methyl benzoate*	1094	1090	-	0.59	1.46	-	4.60	-	14.08	-	0.51	1.08	3.36	-
Methyl octanoate	1124	1127	-	2.55	-	-	-	-	-	-	-	2.51	-	-
Benzyl acetate*	1161	1162	-	0.53	0.04	-	3.98	0.05	-	-	4.5	4.22	-	-
Ethyl benzoate	1167	1173	-	-	-	-	-	-	-	-	0.5	0.90	-	-
Methyl phenylacetate	1175	-	-	-	0.46	-	-	-	-	-	-	-	0.52	-

TABLE I - Chemical constituents of volatile stingless bee pollen collected in the months of December, March and July and extracted by HS, MD, US and SPME with their relative abundances (cont.)

COMPOUNDS	Kovats index		PEAK AREA (%)											
			PAS-1 [a]				PAS-2				PAS-3			
	IK_{cal} [b]	IK_{lit} [c]	HS[d]	MD[e]	US[f]	SPME[g]	HS	MD	US	SPME	HS	MD	US	SPME
Ethyl octanoate	1199	1197	-	-	-	-	-	-	-	-	0.38	0.36	-	-
Methyl nonanoate	1225	1226	-	2.86	-	-	-	-	-	-	0.40	3.30	0.24	-
Ethyl phenylacetate *	1253	1258	-	0.74	-	-	1.16	0.08	-	-	2.88	2.17	-	-
Methyl hydrocinnamate*	1270	-	-	-	0.15	-	1.25	-	7.53	-	0.42	0.24	1.00	-
Ethyl nonanoate	1298	-	-	-	-	-	-	-	-	-	1.85	1.14	-	-
(Z)-Methyl dec-4-enoate	1309	-	-	-	-	-	-	-	-	-	-	0.56	-	-
(E)-Methyl deca-7,9-dienoate	1310	-	-	2.26	-	-	-	-	-	-	-	-	-	-
Nonanyl acetate	1313	1312	-	-	-	-	-	-	-	-	5.2	2.02	-	1.52
Methyl decanoate	1325	1325	-	1.72	-	-	0.31	-	-	-	0.26	0.50	-	-
Ethyl hydrocinnamate	1344	-	-	-	-	-	0.51	0.11	-	-	2.51	2.84	-	-
Methyl 2-hydroxy-3-phenylpropanoate	1361	-	-	-	0.37	-	-	-	-	-	-	-	-	-
Methyl 4-methoxybenzoate	1368	-	-	-	0.34	-	-	-	2.01	-	-	-	0.60	-
(*E*)-Methyl cinnamate*	1376	1378	-	-	1.10	-	0.91	0.05	4.16	-	0.60	0.38	3.11	-
Ethyl decanoate	1397	1395	-	-	-	-	-	-	-	-	0.80	0.22	-	-
4-Methoxybenzyl acetate	1414	1413	-	-	-	-	0.37	-	-	-	0.59	-	-	-
(*E*)-Cinnamyl acetate	1440	1446	-	-	-	-	-	-	-	-	-	0.26	-	-
(*E*)-Ethyl cinnamate	1458	1467	-	-	-	-	-	-	-	-	0.47	0.69	-	-
δ-Decalactone	1488	1494	-	0.56	-	-	-	-	-	-	-	0.53	-	-
Methyl dodecanoate	1525	1525	0.20	2.52	-	-	0.29	-	-	-	-	0.15	-	-
Methyl 3,4-dimethoxybenzoate	1589	-	-	-	0.07	-	-	-	-	-	-	-	-	-
Ethyl dodecanoate	1595	1595	-	-	-	-	-	-	-	-	-	0.13	-	-
Methyl tetradecanoate	1725	1723	2.36	3.19	0.09	-	1.15	-	-	-	0.40	0.31	-	-
Benzyl benzoate	1748	1760	-	-	-	-	-	-	-	-	-	0.14	-	-
Methyl 12-methyl tetradecanoate	1795	-	0.37	-	-	-	-	-	-	-	-	-	-	-
Ethyl tetradecanoate	1796	1796	-	-	-	-	-	-	-	-	0.49	0.46	-	0.65
Methyl pentadecanoate*	1826	-	1.59	2.4	0.28	-	0.57	-	-	-	-	0.29	0.12	-
Methyl hexadec-9-enoate	1900	-	6.56	-	0.25	-	-	-	-	2.52	-	-	-	-
Methyl hexadecanoate*	1927	1921	18.11	28.04	18.35	-	10.4	0.12	33.4	0.64	2.25	3.98	15.10	-
Ethyl hexadecanoate*	1996	1993	0.85	2.43	0.2	6.52	-	-	-	2.88	5.80	22.74	7.15	12.55
Methyl heptadecanoate	2027	-	-	-	0.33	-	-	-	-	-	-	-	-	-
Methyl 14-methyl-hexadecanoate	2027	-	1.12	-	-	-	-	-	-	-	-	-	-	-
Methyl linoleate*	2090	2085	1.37	5.42	25.00	-	0.69	-	9.23	-	-	0.66	9.31	-
Methyl linolenate	2096	-	-	1.67	12.30	-	-	-	9.67	-	-	-	6.90	-
Methyl octadec-9-enoate	2100	-	16.28	-	-	-	2.74	-	-	-	-	-	-	-
Methyl octadecanoate *	2129	2125	7.69	0.73	1.8	-	3.61	-	-	-	1.70	0.19	0.42	-
2-Ethylhexyl 4-methoxycinnamate	2156	-	0.73	-	-	-	-	-	-	-	-	-	-	-
Ethyl linoleate*	2159	-	0.59	2.28	0.34	7.30	-	-	-	1.92	0.60	11.91	6.86	5.9
Ethyl linolenate	2165	-	-	-	-	-	-	-	-	-	-	3.54	4.29	6.13
Ethyl octadec-9-enoate	2166	-	1.31	-	-	-	-	-	-	-	-	-	-	-
Ethyl octadecanoate	2196	2196	0.35	-	0.11	-	-	-	-	-	-	-	0.20	-
Methyl nonadecanoate	2228	-	0.16	-	-	-	-	-	-	-	-	-	-	-
Methyl eicosanoate	2329	-	0.3	-	2.04	-	-	-	-	-	-	-	-	-
Dioctyl hexanedioate	2397	-	0.98	-	-	-	-	-	-	-	-	-	-	-
Methyl tricosanoate	2619	-	0.26	-	0.5	-	-	-	-	-	-	-	-	-
Methyl tetracosanoate	2709	-	0.48	-	2.22	-	-	-	-	-	-	-	0.35	-

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	IK_{cal} [b]	IK_{lit} [c]	HS[d]	MD[e]	US[f]	SPME[g]	HS	MD	US	SPME	HS	MD	US	SPME
HYDROCARBONS														
n-Undecane	1100	1100	-	-	0.14	-	-	-	1.05	-	-	-	0.25	-
n-Dodecane	1200	1200	-	-	0.25	-	-	-	-	-	-	-	0.72	-
n-Tridecane	1300	1300	-	-	-	-	-	-	-	-	-	-	0.66	-
Tetradec-1-ene	1354	1389	-	-	-	-	-	0.15	-	-	-	-	-	-
n-Tetradecane	1401	1400	0.11	-	0.04	-	-	-	-	-	0.8	-	-	-
n-Pentadecane	1500	1500	0.23	-	-	-	0.89	-	-	-	1.05	-	-	-
Hexadec-1-ene	1550	1589	-	-	-	-	-	0.49	-	-	-	-	-	-
n-Hexadecane	1600	1600	0.34	-	-	-	0.72	-	-	-	1.00	-	-	-
Heptadec-8-ene	1675	1675	-	-	-	-	0.42	-	-	-	0.95	-	-	1.87
n-Heptadecane	1700	1700	0.93	-	-	-	0.87	-	-	-	0.6	-	-	-
n-Octadecane	1800	1800	1.08	-	-	-	0.70	-	-	-	0.42	-	-	-
2.6.10.14-Tetramethylhexadecane	1810	-	0.86	-	-	-	-	-	-	-	-	-	-	-
Nonadec-1-ene	1871	-	0.45	0.22	-	-	-	-	-	-	-	-	-	-
Eicos-9-ene	1872	-	-	0.47	-	-	-	-	1.71	-	-	-	-	-
n-Nonadecane	1900	1900	-	1.69	-	-	1.36	-	-	-	-	0.43	-	1.37
n-Eicosane	2000	2000	0.98	-	0.05	-	-	-	-	-	-	-	-	3.45
n-Heneicosane*	2101	2100	-	2.2	-	-	1.12	-	4.00	29.89	0.30	1.14	3.60	2.32
n-Docosane*	2200	2201	1.06	-	0.23	-	-	-	-	1.34	-	-	0.23	3.25
n-Tricosane*	2301	2300	-	1.68	3.15	-	0.46	-	4.52	25.09	-	0.75	6.10	2.30
7-Hexyldocosane	2373	-	-	-	0.18	-	-	-	-	-	-	-	-	-
n-Tetracosane	2400	2400	1.44	-	0.35	-	-	-	-	-	-	-	-	3.23
n-Pentacosane*	2502	2500	1.62	0.49	6.59	-	-	-	5.59	2.88	-	0.29	9.70	2.32
Cyclopentacosane	2658	-	-	-	-	-	-	-	-	-	-	-	8.5	-
11-Decyl-tetracosane	2682	-	-	-	-	-	-	-	-	-	-	-	3.60	-
TERPENOIDS														
(*Z*)-Linalool oxide	1075	1072	-	1.28	-	-	-	-	-	-	-	1.88	-	-
(*E*)- Linalool oxide	1090	1086	-	0.75	-	-	-	-	-	-	-	1.00	-	-
Nerol oxide	1152	1158	-	-	-	-	-	-	-	-	-	0.69	-	-
Pyranoid linalool oxide	1165	1176	-	-	-	-	0.83	-	-	-	-	-	-	-
α-Terpineol	1150	-	-	-	-	-	-	0.07	-	-	-	-	-	-
Terpendiol	1190	-	-	-	0.06	-	1.24	-	-	-	1.6	-	0.88	-
Carvacrol	1302	1299	0.17	-	-	-	-	-	-	-	-	-	-	-
α-Copaene	1368	1376	-	-	-	-	-	-	-	-	0.31	-	-	-
β-Caryophyllene	1408	1419	-	-	-	-	-	-	-	-	0.34	-	-	-
α –Bergamotene	1430	1434	-	-	-	-	-	-	-	-	0.21	-	-	-
α –Farnesene	1506	1505	-	0.47	-	-	-	-	-	-	-	-	-	-
δ- Cadinene	1515	1523	-	-	-	-	-	-	-	-	0.32	-	-	-
(*E*)- Nerolidol	1560	1563	-	0.22	-	-	-	-	-	-	-	-	-	-
Dendrolasin	1574	1571	-	-	-	-	-	-	-	-	-	-	-	3.92
(*Z,Z*)- Farnesol	1675	1698	1.81	-	-	-	-	-	-	-	-	-	-	-
(*E,Z*)-Farnesol	1717	1715	-	1.15	-	-	-	-	-	-	-	-	-	-
Rimuene	1888	1896	0.33	-	-	-	-	-	-	-	0.22	-	-	-
Kaur-15-ene	1961	1997	-	-	-	-	-	-	-	-	-	0.26	-	-
Kaur-16-ene*	2005	2043	1.50	4.12	0.45	13.86	4.67	-	2.05	22.14	5.9	4.60	2.6	22.1
Squalene	2785	-	20.21	-	1.20	-	-	-	-	-	-	-	1.09	22.45
OTHERS														
Phenol	1007	-	-	-	-	-	3.86	-	-	-	-	-	-	-

TABLE I - Chemical constituents of volatile stingless bee pollen collected in the months of December, March and July and extracted by HS, MD, US and SPME with their relative abundances (cont.)

COMPOUNDS	Kovats index		PEAK AREA (%)											
			PAS-1 [a]				PAS-2				PAS-3			
	IK_{cal} [b]	IK_{lit} [c]	HS[d]	MD[e]	US[f]	SPME[g]	HS	MD	US	SPME	HS	MD	US	SPME
p-Cresol	1079	1076	-	1.04	0.05	-	-	-	-	-	-	-	-	-
2-Methoxyphenol	1089	-	-	-	-	-	1.21	-	-	-	-	-	-	-
Hotrienol	1104	-	-	4.25	-	-	-	-	-	-	8.9	5.34	-	-
Glycerol	1117	950	-	-	-	-	-	-	-	-	-	-	2.28	-
Benzenecetonitrile	1134	1138	-	0.31	-	-	-	-	-	-	-	-	-	-
Triethyleneglycol	1168	-	-	-	-	-	4.89	-	-	-	-	-	-	-
Benzoic acid	1161		-	-	-	-	-	0.19	-	-	-	-	-	-
Cyclopentadiene-N-methylamine	1222	-	-	-	-	-	-	0.38	-	-	-	-	-	-
Butylated hydroxytoluene	1475	1514	-	-	-	-	-	96.69	-	-	-	-	-	-
Hexadecanoic acid	1961	1959	-	-	-	4.46	-	-	-	4.76	-	-	-	1.81
UNIDENTIFIED COMPOUNDS (SUM)														
UC[h]	-	-	3.66	1.28	15.25	67.86	-	0.59	-	-	-	-	-	-

[a]PAS - volatile oil in stingless bee pollen; [b]IK cal - calculated Kovats index of substances detected; [c]IK lit - index of Kovats found in the literature of the substances detected; [d]HS – extraction by headspace; [e]MD - extraction by mycrohydrodistillation; [f]US - extraction assisted by ultrasound; [g]SPME - extraction by solid phase microextraction; [h]UC - unidentified compounds; (*) - Nineteen substances most frequently among all the substances identified.

and alcohols. Through statistical tests (ANOVA and Independent T-test), using the null hypothesis less than 0.05 ($p<0.05$), there was a significant difference between the type of extraction technique (HS and SPME) in the obtainment of the volatile compounds, but this didn't happen between collection seasons (PAS-1, 2 and 3). Analyzing the number and relative abundance of compounds identified, the HS technique had more compounds than SPME. It happened because SPME extracts the volatile compounds of samples by adsorption, a passive process, while other extraction processes (HS, MD and US) use assets mechanisms.

The literature of the volatile compounds of stingless bees pollen is scarce, therefore, in order to evidence the source of compounds identified they were compared with compounds indentified in the stingless bees. These bees prepare bee pollen using their bodies and mandibles, mixing secretions (cuticular, cephalic and glandulars) with botanic pollen and the compounds present in secretions and its bodies are purchased for others nestmates as chemical signals, essential for the hive. Age, castes selection, recruitment for several jobs, invaders, predators and nestmates are determined by chemical communication and feed as the bee pollen is a way to transmit the chemical signals. (Pianaro *et al*., 2009). In several studies there was the predominance of hydrocarbons and esters in stingless bee secretions as in our study (Francke *et al*., 2000; Gracioli-Vitti *et al*., 2004; Engels *et al*., 1987; Engels *et al*., 1993; Cruz-Lopes, Patrício, Morgan, 2001; Abdalla *et al*., 2004). The comparison of the volatile compounds identified from the pollen samples with the literature, showed that forty-one volatile compounds were identified from the cephalic part and from mandibular and accessory glands from stingless bees. Nineteen compounds most common in the pollen of stingless bees studied, five are common to the literature (methyl hexadecanoate, heneicosane, ethyl linoleate, tricosane and methyl otcadecanoate) and more related to stingless bees.

Bees of *Melipona* genus use cuticular hydrocarbons to recognize nestmates and invaders (Pianaro *et al*., 2007). *Nanotrigona* and *Plebleia* genus use abdominais secretions for differentiate its species and castes. Workes and males of *Plebeia droryana* excret in abdominal extracts tetradecanal and many quantiftit of fatty acids, like linolenic and linoleic acids, respectvely (Pianaro *et al*., 2009). But another study indicated that hidrocarbons and estheres are the majoritary in cephalic glands of *Scaptotrigona postica* and perfom the comunication in hive (Engels *et al*., 1993).

Seven compounds (methyl linoleate, methyl cinnamate, benzyl acetate, methyl benzoate, methyl hidrocinnamate, ethyl phenylacetate and kaur-16-ene) are not common to the studies of cephalic parts or glandular secretion of stingless bees. These seven compounds are more related to the flora of the region, because shikimate and essential fatty acids derivatives are biosynthesized in plants not in animals (Dewick, 2009). Other studies about

TABLE II - Main pollen types identified in three samples of stingless bee pollen of the genus *Scaptotrigona sp.* from Monsenhor Gil, Piaui, Brazil, collected at December 2006 (PAS-1), March 2006 (PAS-2) and July 2006 (PAS-3)

Percentage class of the pollen type	Frequencies (%) of the pollen types		
	PAS-1	PAS-2	PAS-3
Dominant pollen			
Mimosa caesalpiniifolia Benth.	77.85	53.3	77.0
Accessory pollen			
Piptadenia sp.	-	25.3	-
Isolate pollen			
Piptadenia sp.	8.7	-	10.4
Myrcia sp.	7.5	-	-
Acacia sp.	-	5.3	-
Copaifera sp.	-	-	4

attracting foraging bees demonstrated that the presence of fatty acids odor was an important chemical signal for bees choose a botanic pollen as nutritional supply (Pernal, Currie, 2002; Starratt, Boch, 1971).

According to the palynological analysis the *Mimosa caesalpiniifolia* Benth. was the dominant pollen, frequently found in Monsenhor Gil (Santos *et al*., 2008) and presented frequency of 77.8% 53% and 77% respectively for the samples PAS-1, PAS-2 and PAS-3. This species is a native tree of the Brazilian cerrado and also known as *unha-de-gato* or *sabiá* (Pio Corrêa, 1984). Other significant pollen types were *Piptadenia* sp., *Copaifera* sp., *Acacia* sp. and *Myrcia* sp., which the *Piptadenia* sp. pollen was considered an accessory pollen at the percentage classes whereas the others were considered as isolate pollen (Figure 1 and Table II).

The *Mimosa caesalpiniifolia* Benth. was a plant frequently visited by these bees. Melissopalynological studies observe the *Mimosa caesalpiniifolia* Benth. as an important trophic resource for bees to collect pollen, due to high nutritional value of this pollen type and as it is a typical plant of the mid-north region of Brazil (Barth, Dutra, Justo, 1999; Sodre *et al*., 2008; Luz, Thomé, Barth, 2007; Melo *et al*., 2009). However, in the collection site, this plant and others weren't preserved, due to the presence of many agricultural areas and were constantly burned, common practice in Brazilian cerrado (Ikeda *et al*., 2008) directly affecting the survival of stingless bees.

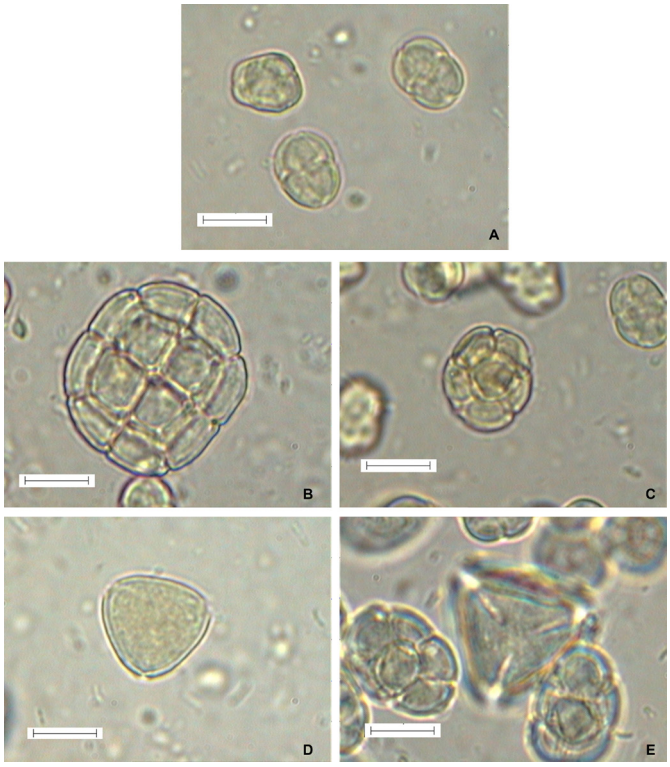

FIGURE 1 - Photographs of pollen grains obtained from optical microscope of the main pollen types identified in samples of stingless bee pollen of Monsenhor Gil-PI, BRA. A – *Mimosa caesalpiniifolia Benth; B – Acacia* sp.*; C – Piptadenia* sp.*; D – Myrcia* sp.*; E - Copaifera* sp. surrounded by *Piptadenia* sp.

CONCLUSION

The palynological analysis demonstrated that the dominant pollen type was from the native tree *Mimosa caesalpiniifolia* Benth. The bee pollen of the genus *Scaptotrigona sp.* from Monsenhor Gil-PI demonstrated a diversified volatile chemical composition.

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