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Characterization of volatile compounds from three *Cymbopogon* species and *Eucalyptus citriodora* from Benin and their insecticidal activities against *Tribolium castaneum*

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ABSTRACT

The chemical volatile profiles of four plant species from Benin were established via SDE/GC–MS and SPME/GC–MS, and were compared to chemical profiles obtained by hydrodistillation/GC–MS. Essential oils and selected identified compounds were used to perform fumigant tests and contact "no choice" tests against adults of the red flour beetle *Tribolium castaneum*, an important pest insect in stored products worldwide. The chemical profiles and the concentrations of the main compounds varied with the method used. Geranial (hydrodistillation, SPME) and geraniol (SDE) for *Cymbopogon citratus*, piperitone for *Cymbopogon schoenanthus* (hydrodistillation, SPME, SDE), *E*-*p*-mentha-1(7), 8-dien-2-ol for *Cymbopogon giganteus* (hydrodistillation, SPME, SDE), neo-isopulegol (SDE) and citronellal (hydrodistillation and SPME) for *Eucalyptus citriodora,* were identified as main volatile compounds. By fumigation, the LC_{50} values obtained after 24 h for essential oils of *C. citratus*, *C. giganteus*, *C. schoenanthus*, *E. citriodora,* and for piperitone and citronellal were 4.2 mL/L air, 2.3 mL/L air, 2.1 mL/L air, 2.0 mL/L air, 0.5 mL/L air and 1.2 mL/L air, respectively. Furthermore, mortalities of 100%, 82%, 75%, 72%, 68% and 42%, respectively, were found for piperitone at 2.4 mL/L air, citronellal at 2.1 mL/L air, and essential oil extracted from *E. citriodora, C. schoenanthus, C. giganteus* and *C. citratus*, at 4 mL/L air. By contact "no choice" test, the LC_{50} values for the crude oils after 72 h were: 15% (w/v), 6% (w/v), 6% (w/v) and 5% (w/v) for *C. citratus, C. giganteus,* C. *schoenanthus* and *E. citriodora*, respectively. After 72hours, mortalities of 18%, 67%, 73%, 82%, 87% and 40% for essentials oils (8% w/v) of *C. citratus, C. giganteus, C. schoenanthus, E. citriodora*, piperitone at 4.7% w/v, and citronellal at 4.2% w/v, respectively, were obtained. The essential oil from *C. schoenanthus* and *E. citriodora* as well as piperitone and citronellal represent potential valuable alternative pesticides to conquer the resistance of *T. castaneum* to synthetic insecticides.

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1. Introduction

The objective of the present study was to evaluate the efficacy of some essential oils against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), the red flour beetle. The choice for *T. castaneum* was based on its economic importance as an insect pest expressed by its worldwide distribution and its preference for several stored products and grocery stores (Entomology and nematology/FDACS/EDIS, 2015). For a very long time, the control of *T. castaneum* has required the use of chemicals and has promoted the appearance and development of high levels of insecticide resistance in the genus *Tribolium* (Keys and factsheet, 2015) as well

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Table 1
Locations, soils and organs extracted from selected plant species.

Plant species	*Cymbopogon citratus*	*Cymbopogon giganteus*	*Cymbopogon schoenanthus*	*Eucalyptus citriodora*
Locations	Cotonou	Koudo	Nalohou 2	Abomey Calavi
Latitude/longitude	N06°21.424′E02°23.520′	N06°37.599′E01°43.000′	N09°45.626′ E01°36.153′	N06°24.946′E02°20.564′
Soils	Sandy	Ferralitic	Gravelly ferruginous	Ferralitic
Organs	Leaves	Leafy stems	Leafy stems	Leaves
Voucher numbers	AA6463/HNB	AA6464/HNB	AA6465/HNB	AA6468/HNB

as adverse effects in animals and humans (Okonkwo and Okoye, 1996). Botanical extracts and essential oils have been successfully evaluated against this pest as natural repellent or natural insecticide (Caballero-Gallardo et al., 2011; Goudoum, 2013; Khani and Asghari, 2012; Liu et al., 2011; Mishra et al., 2012).

In order to find alternative bioactive compounds to control *T. castaneum*, which demonstrates insecticide resistance, the current work has screened the efficacy of essential oils of *C. citratus*, *C. giganteus*, *C. schoenanthus* and *E. citriodora* from Benin, as well as piperitone and citronellal as potential natural pesticides against this pest.

2. Material and methods

2.1. Plant material

Four plant species, namely *Cymbopogon citratus* (DC.) Stapf (Poaceae), *Cymbopogon schoenanthus* (L.) Spreng. (Poaceae), *Cymbopogon giganteus* Chiov. (Poaceae), and *Eucalyptus citriodora* Hook. (Myrtaceae) were collected in various areas of Benin and a specimen of each plant species was subjected to classification (Akoègninou et al., 2006) and was deposited at the National Herbarium of Benin. Collected plant material was dried, free from light, at room temperature. A sample of each dried organ was transformed into powder using a traditional mortar. Locations of collection, organs extracted, nature of soil and voucher numbers are summarized in Table 1.

2.2. Essential oils extracted by simultaneous distillation extraction (SDE)

Essential oils were extracted by simultaneous distillation extraction (SDE) using a Likens–Nickerson-type apparatus. Practically, one-hundred grams of each dried plant material was added to one liter of distilled water into a round-bottom flask, whereas 10 mL of diethyl ether was added to a conical flask. Both flasks were connected to the extremities of the apparatus, then water and diethyl ether were added to the central arm of the Likens–Nickerson apparatus. The round-bottom flask was heated using an oil bath at 120 °C, whereas the conical flask was heated with a water bath at 35 °C. Simultaneous distillation was carried out for one hour after reaching the boiling point. Mixtures obtained (essential oil and diethyl ether) were dried over anhydrous magnesium sulfate, concentrated using a rotavapor and the extracted oils obtained were stored at 4 °C, away from light, until the analysis. For this purpose, essential oils were diluted at 10% with diethyl ether and one milliliter of each diluted solution was charged into a sampler flask for GC–MS analysis.

2.3. Essential oils extracted by hydrodistillation

Essential oils from all the plant species were extracted by hydrodistillation using a Clevenger-type apparatus for two to four hours. The extracted oils were dried over anhydrous magnesium sulfate and stored at 4 °C, away from light, before use. The chemical profiles obtained by hydrodistillation were published elsewhere (Bossou et al., 2013). The essential oils obtained were used to perform insecticidal tests on *T. castaneum*.

2.4. Procurement of identified compounds

To perform the tests with the identified compounds, the isolation of piperitone has been achieved by purification of the essential oil of *C. schoenanthus* via flash chromatography on SiO_2 with hexane:diethyl ether 4:1. 7.26 g of the crude oil was used during the process of purification at the end of which four fractions were obtained. Each fraction was analyzed by GC–MS following the conditions described below. Fraction 1 (0.89 g), contained δ-2-carene (72.8%) and limonene (13.8%). Fraction 2 (3.21 g) contained only pure piperitone (99.9%), while Fraction 3 (1.37 g) contained mainly piperitone (93.7%). Fraction 4 (0.68 g) contained elemol (50.8%) and α-eudesmol (23.6%), *E*-*p*-menth-2-en-1-ol (5.8%), α-terpineol (10.5%), γ-eudesmol (0.4%) and eremoligenol (6.26%). The combined yield of piperitone obtained was 63%. The NMR data of the extracted piperitone were in correspondence with literature data. The R_f obtained of this isolated pure piperitone was 0.25 (Hex:Et_2O 4:1) and its optical rotation was $[\alpha]^{24}_D$ +21.4 (*c* 0.427 $CHCl_3$). The optical rotation values of piperitone obtained from literature were *R*-piperitone −52.4 (neat), *S*-piperitone +57.0 (neat) (Klein and Ohloff, 1963) and *R*-piperitone -18.8 (*c* 0.313 $CHCl_3$, nature-derived sample), *R*-piperitone −14.6 (*c* 0.504 $CHCl_3$, Tokyo Chemical Industry) (Yaguchi et al., 2009). It follows that the piperitone extracted from *C. schoenanthus* was identified as *S*-piperitone. The fraction 2 was used to perform the tests on *T. castaneum* because of its purity in piperitone (99.9%).

Concerning (±)−citronellal 80–90%, citronellol 97% and citronellyl acetate 97%, they were purchased from Fluka Chemie AG CH-9471 Buchs, Aldrich Europe-Belgium and ACROS Organics-Belgium, respectively.

2.5. Gas Chromatography coupled with mass spectrometry

GC–MS analysis of the essential oils was performed on an Agilent 6890 GC Plus automatic sampler system, coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium) and equipped with a capillary HP-5MS column fused with silica (length: 30 m; diameter: 0.25 mm, film thickness: 0.25 μ) in split mode 1:100. The oven temperature was programmed at 60 °C for 3 min and to 350 °C at a rate of 5 °C/min. The injector was kept at 250 °C programmed with a rate of 10 °C/s. Helium was used as carrier gas at a flow rate of 1 mL/min. All analyses were performed at constant flow. The mass detector conditions were: transfer line temperature 260 °C; ionization mode electron impact: 70eV. The identification of sample compounds was carried out in single runs. The Kovats retention indices were calculated for all volatile constituents using a series of *n*-alkanes C_7–C_{17} (Adams, 2012). Quantification of each compound was performed using percentage peak area calculations. The identification of volatile compounds of the oil was done by comparing their retention indices with those of reference compounds in the literature and confirmed by GC–MS by comparison of their mass spectra with those of reference compounds (Adams, 2012; Flavornet and human,

2015; The Pherobase-Database, 2015; Chemistry WebBook, 2015). The relative concentration of each compound in the essential oil was quantified according to the peak area integrated by the analysis program (Chemstation data analysis).

2.6. Headspace solid-phase microextraction (HS-SPME/GC–MS)

SPME extraction and desorption were performed automatically by means of an MPS-2 autosampler (Gerstel). One gram of powder of each sample was incubated at 30 °C for 2 min with an agitation speed of 250 rpm. Extraction of volatiles by solid-phase microextraction were carried out using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco, Bornem, Belgium) appropriate for the analysis of mixtures with polar and non-polar components (Balasubramanian and Panigrahi, 2010), and flavor compounds: volatiles and semi-volatiles (Selection Guide, 2015). The determination of each component was done by exposure of the fiber to the headspace for 60 min at 35 °C, followed by desorption for 120 s at 250 °C. The GC–MS analyses of the SPME extracts were performed with an Agilent 6890 GC Plus, coupled to a mass selective-quadrupole spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium), equipped with a Varian CP-PoraBOND Q capillary column (25 m length; 0.32 mm id; coating 5 μm film thickness) and a CIS-4 Programmed Temperature Vaporization (PTV) injector. Working conditions were as follows: transfer line to MSD 250 °C, injector 250 °C, carrier gas (He) 1.2 mL/min, SPME desorption in a CIS-4 PTV injector (Gerstel) in split mode; ionization: EI 70eV. The oven temperature was programmed from 60 to 350 °C and analyses were performed in SCAN mode. Each analysis was done with three repetitions.

2.7. Stock of T. castaneum

The rearing of *T. castaneum* was done in the Laboratory of Agrozoology at Ghent University, Belgium. Beetles were reared on whole-wheat nutritionally mixed with 5% (w/w) brewer's yeast and maintained under standard conditions (Yu et al., 2013).

2.8. Contact "no choice" test

Essential oils were diluted in acetone at 0.5% (w/v), 1% (w/v), 2% (w/v), 4% (w/v) and 8% (w/v) whereas piperitone, citronellal, citronellol and citronellyl acetate were tested at the concentrations of 4.7%, 4.2%, 1.6% and 0.7%, respectively. Contact tests were performed as described by Zapata and Smagghe (2010). Aliquots of 0.7 mL of various doses of solutions were used for the impregnation of filter paper Whatman # 1, 8.5 cm diameter. After drying during 5 min, filter papers were inserted into the petri dishes, ten unsexed adult beetles were added and six replicates were tested. Acetone and permethrin 0.75% (i.e., the discriminating concentration for adult anopheline mosquitoes (Bossou et al., 2013)) were used as negative control and positive control, respectively. Permethrin is an insecticide from the class of pyrethroids recommended by WHO in agriculture and public health applications because of its efficiency at lower dose, its safety for humans, its relative longevity and its low cost (WHO, 2011).

Permethrin impregnated papers were obtained from the WHO reference center of "the Vector Control Research Unit of The University Sains Malaysia". Acetone was purchased from Sigma–Aldrich (Bornem, Belgium). Mortality rates were recorded at 24 h, 48 h, and 72 h of exposure. Lethal concentrations (LC_{50}), at which 50% of beetles died, were calculated as described (Zapata and Smagghe, 2010). The beetle was considered dead when no antenna or leg movement was detected when gently touched with a brush.

2.9. Fumigant test

Filter paper (Whatman # 1) discs of 2 cm in diameter were impregnated with 30 μL, 60 μL or 120 μL of the crude essential oil or the pure natural compound without any solvent, and attached to the undersurface of the screw cap of a 30 mL glass vial, as described by Zapata and Smagghe (2010). The cap was screwed on the vial to generate concentrations of 1 mL/L air (C_1), 2 mL/L air (C_2), and 4 mL/L air (C_3), respectively. Concerning identified compounds, piperitone was tested at 0.60 mL/L air (C_1), 1.2 mL/L air (C_2) and 2.4 mL/L air (C_3), citronellal was tested at 1.1 mL/L air (C_2) and 2.1 mL/L air (C_3), citronellol was tested at 0.4 mL/L air (C_2) and 0.8 mL/L air (C_3) whereas the concentrations for citronellyl acetate were 0.2 mL/L air (C_2) and 0.4 mL/L air (C_3). Negative and positive controls were acetone and permethrin 0.75%, respectively. Six replicates of batches of ten adult beetles were prepared and incubated as described during the rearing, i.e., petri dishes were maintained in total darkness, at 30 °C, with 60% relative humidity. Mortality was recorded after 24 h of exposure and LC_{50} values were calculated as described (Zapata and Smagghe, 2010).

2.10. Data analysis

LC_{50} values were calculated by means of the log probit regression using SPSS statistics 20.0 software for Windows and expressed with 95% confidence limits as described (Zapata and Smagghe, 2010). Differences among lethal concentrations (LC) were considered significant when the 95% CL of the LC_{50} values failed to overlap.

3. Results and discussion

3.1. Chemical composition of extracts

The chemical profiles of the selected plant species were investigated using essential oils extracted by simultaneous distillation extraction (SDE) as well as by solid-phase microextraction (SPME). These chemical profiles were compared to those obtained for essential oils extracted by hydrodistillation, by Bossou et al. (2013) and published elsewhere. The chemical profiles obtained, and essential oil yields expressed as oil wt./wt. of dried organ extracted, are presented in Tables 2–5 . The structures of the major compounds are indicated in Fig. 1.

3.1.1 C. citratus

In total 56 volatile compounds were identified in *C. citratus* by SPME with a total of 92.5% of total peak areas, while 37 compounds were identified for the sample obtained through SDE covering 92.5% of total peak areas (Table 2). Twenty five compounds were detected in both the SPME and SDE sample. The main compounds (that have a relative peak area higher than 5% for at least one method) were neral (19.7%, 23.6%), geraniol (2.0%, 41.2%), geranial (23.9%, 0%) and selina-6-en-4-ol (0%, 7.2%) covering 45.6% and 72.0%, respectively (Fig. 1). The presence of several minor compounds (1-5%) can be noticed as well. The GC–MS data of the main compounds of *C. citratus* were obtained as follows:

Myrcene: (RT 6.949) EI-MS *m/z*(rel. int.): 136 [M] (5), 121 (6), 108 (5), 93 (100), 91 (24), 79 (16), 69 (65), 53 (11), 41 (55).

Neral: (RT 16.504) EI-MS *m/z*(rel. int.): 152 [M] (2), 137 (10), 123 (6), 119 (17), 109 (38), 95 (28), 94 (38), 91 (15), 84 (32), 83 (23), 82 (21), 81 (24), 79 (13), 69 (100), 68 (26), 59 (15), 41 (81).

Geranial: (RT 17.744) EI-MS *m/z*(rel. int.): 152 [M] (7), 137 (14), 123 (11), 109 (14), 94 (19), 84 (30), 69 (100), 53 (11), 41 (61).

Geraniol: (RT 17.081) EI-MS *m/z*(rel. int.): 154 [M] (1), 139 (4), 123 (14), 111 (8), 93 (23), 69 (100), 55 (9), 53 (10), 41 (52).

E-Caryophyllene: (RT 23.726) EI-MS *m/z*(rel. int.): 204 [M] (22), 189 (38), 175 (12), 161 (65), 148 (32), 147 (45), 134 (25), 133 (100),

Table 2
Chemical composition and essential oil yields of *Cymbopogon citratus*.

KI_{exp}[a]	KI_{lit}[b]	ID[c]	Compounds[d]	Hydro.	SDE	SPME
				%	%	%
978	988	MS, RI	6-Methyl-5-hepten-2-one	0.5	0.4	1.6
987	988	MS, RI	**Myrcene**	**12.4**	1.2	1.2
999	1002	MS, RI	δ-2-Carene			1.2
1013	1014	MS, RI	α-Terpinene			1.6
1021	1020	MS, RI	*p*-Cymene			2.1
1024	1024	MS, RI	Limonene			0.8
1028	1026	MS, RI	Eucalyptol			0.1
1033	1032	MS, RI	*Z*-β-Ocimene	0.3		0.1
1039	1044	MS, RI	*E*-β-Ocimene	0.2		
1050	1051	MS, RI	2,6-Dimethyl-5-heptenal			0.1
1054	1054	MS, RI	γ-Terpinene			0.1
1069	1067	MS, RI	*Z*-linalool oxide			0.1
1084	1084	MS, RI	*E*-linalool oxide			0.1
1085	1090	MS, RI	6,7-Epoxymyrcene	0.2		0.3
1093	1095	MS, RI	Linalool	1.1	1.1	3.0
1141	1144	MS, RI	Neo-Isopulegol		0.4	0.4
1149	1148	MS, RI	Citronellal	0.1	0.7	3.8
1150	1155	MS, RI	iso-Isopulegol		0.1	0.6
1171	1173	MS, RI	Rose furan epoxide	0.2	0.3	2.0
1175	1177	MS, RI	*E*-Isocitral		0.6	
1188	1190	MS, RI	Methyl salicylate		0.9	1.7
1216	1224	MS, RI	2,3-Epoxyneral	0.1		0.3
1222	1223	MS, RI	Citronellol		1.1	0.6
1234	1234	MS, RI	Ascaridole			0.2
1237	1235	MS, RI	**Neral**	**33.1**	**23.6**	**19.7**
1250	1249	MS, RI	Piperitone			1.6
1252	1249	MS, RI	**Geraniol**	1.0	**41.2**	2.0
1268	1264	MS, RI	**Geranial**	**44.3**		**23.9**
1289	1293	MS, RI	2-Undecanone	0.1	0.2	0.6
1298	1298	MS, RI	Geranyl formate		0.2	0.2
1301	1299	MS, RI	Isoascaridole			2.0
1351	1359	MS, RI	Geranic acid	0.1		
1364	1369	MS, RI	Cyclosativene			0.2
1368	1374	MS, RI	α-Copaene			0.1
1376	1379	MS, RI	Geranyl acetate	0.8	0.5	
1384	1389	MS, RI	β-Elemene		0.2	0.9
1411	1417	MS, RI	***E*-Caryophyllene**	0.1	1.0	**5.0**
1419	1424	MS, RI	2,5-dimethoxy-p-Cymene			0.1
1428	1432	MS, RI	α-*E*-Bergamotene		0.9	2.3
1431	1437	MS, RI	α-Guaiene			0.6
1433	1439	MS, RI	Aromadendrene		0.4	
1436	1438	MS, RI	Iso-caryophyllene			0.6
1445	1452	MS, RI	α-Humulene			0.7
1448	1451	MS, RI	*E*-Muurola-3,5-diene			0.4
1450	1454	MS, RI	*E*-β-Farnesene		0.2	0.6
1455	1451	MS, RI	*E*-Muurola-3,5-diene			0.1
1466	1461	MS, RI	*Z*-Cadina-1(6), 4-diene		0.2	
1466	1465	MS, RI	*Z*-Muurola-4(14), 5-diene			0.1
1469	1471	MS, RI	Dauca-5,8-diene			0.3
1473	1478	MS, RI	γ-Muurolene		0.5	1.4
1476	1477	MS, RI	γ-Selinene			1.4
1484	1492	MS, RI	δ-Selinene			0.9
1487	1492	MS, RI	*Z*-β-Guaiene			0.2
1489	1495	MS, RI	2-Tridecanone	0.1	0.6	0.3
1493	1500	MS, RI	α-Muurolene		0.8	0.6
1498	1496	MS, RI	Viridiflorene			0.5
1502	1505	MS, RI	β-Bisabolene		0.2	0.3
1506	1513	MS, RI	γ-Cadinene		1.4	1.2
1516	1522	MS, RI	δ-Cadinene		1.3	1.1
1525	1527	MS, RI	Cadina-1,4-diene			0.2
1527	1537	MS, RI	α-Cadinene		0.2	
1576	1582	MS, RI	Caryophyllene oxide	0.1	1.4	0.3
1581		MS, RI	3-Tetradecanone		0.3	
1594	1607	MS, RI	5Epi, 7Epi-α-Eudesmol		0.3	
1609	1615	MS, RI	**Selina-6-en-4-ol**	0.4	**7.2**	
1637	1638	MS, RI	epi-α-Cadinol		1.2	0.1
1639	1644	MS, RI	α-Muurolol		0.1	
1648	1652	MS, RI	α-Cadinol		1.6	
			Yield	1.7	1.2	
			Total identified	95.2	92.5	92.5

Extraction methods: Hydro. = hydrodistillation; SDE = simultaneous distillation extraction; SPME = solid phase microextraction.

[a] KI_{exp} = retention indices are determined using *n*-alkanes (C_7–C_{17}).

[b] KI_{lit} = retention indices of reference compounds from literature.

[c] ID = identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2012); RI = comparison of calculated RI with those reported in the literature.

[d] Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.

Table 3
Chemical composition and essential oil yields of *Cymbopogon schoenanthus*.

KI_{exp}[a]	KI_{lit}[b]	ID[c]	Compounds[d]	Hydro.	SDE	SPME
				%	%	%
984	988	MS, RI	2,3-Dehydro-1,8-cineole	0.1	0.1	
987	988	MS, RI	Myrcene			0.1
999	1001	MS, RI	**δ-2-Carene**	**15.5**	4.4	1.3
1003	1002	MS, RI	α-Phellandrene	0.2		0.1
1013	1014	MS, RI	α-Terpinene	0.2		0.4
1021	1020	MS, RI	*p*-Cymene	0.1		0.4
1024	1024	MS, RI	Limonene	3.6	0.8	0.5
1033	1032	MS, RI	*Z*-β-Ocimene	0.1		
1039	1044	MS, RI	*E*-β-Ocimene	0.1		
1081	1083	MS, RI	L-Fenchone	0.2		0.1
1118	1118	MS, RI	*Z*-*p*-Menth-2-en-1-ol	1.4	1.1	1.6
1136	1136	MS, RI	*E*-*p*-Menth-2-en-1-ol	0.7	0.7	0.6
1141	1144	MS, RI	Neo-Isopulegol		0.6	
1148	1148	MS, RI	Citronellal		1.6	1.0
1152	1155	MS, RI	iso-Isopulegol		0.4	0.1
1160	1166	MS, RI	α-Phellandren-8-ol	0.1		
1173	1169	MS, RI	Lavandulol			0.1
1179	1178	MS, RI	Naphthalene			0.1
1180	1179	MS, RI	*p*-Cymen-8-ol	0.1		
1182	1184	MS, RI	Dill ether			0.1
1183	1186	MS, RI	α-Terpineol	1.5	1.4	1.5
1188	1195	MS, RI	*Z*-Piperitol	0.3	0.6	
1192	1190	MS, RI	Methyl salicylate			2.8
1205	1207	MS, RI	*E*-Piperitol	0.2		0.1
1253	1249	MS, RI	**Piperitone**	**58.9**	**53.1**	**56.6**
1268	1264	MS, RI	*E*-Citral (Geranial)			0.1
1302	1299	MS, RI	Isoascaridole			1.0
1343	1345	MS, RI	α-Cubebene			0.1
1368	1374	MS, RI	α-Copaene			0.1
1378	1378	MS, RI	α-Bourbonene			0.6
1382	1389	MS, RI	**β-Elemene**	0.4	1.3	**5.1**
1400	1407	MS, RI	α-Barbatene			0.1
1408	1417	MS, RI	***E*-Caryophyllene**	1.1	2.3	**6.5**
1419	1424	MS, RI	2,5-Dimethoxy-*p*-cymene			0.1
1424	1431	MS, RI	β-Gurjunene	0.1	0.3	0.9
1428	1436	MS, RI	Isobazzanene			0.1
1433	1440	MS, RI	β-Barbatene			0.1
1443	1452	MS, RI	α-Humulene	0.1	0.2	0.6
1455	1451	MS, RI	*E*-Muurola-3,5-diene			0.1
1460	1458	MS, RI	allo-Aromadendrene			0.1
1469	1474	MS, RI	*Z*-Prenyl limonene		0.3	0.6
1470	1471	MS, RI	Dauca-5,8-diene		0.9	
1475	1475	MS, RI	γ-Gurjunene		0.3	
1484	1489	MS, RI	β-Selinene			3.0
1487	1498	MS, RI	α-Selinene			0.7
1490	1500	MS, RI	α-Muurolene	0.1	0.5	0.6
1494	1496	MS, RI	Valencene		1.5	
1497	1504	MS, RI	Cuparene			1.0
1507	1513	MS, RI	γ-Cadinene	0.1	0.8	1.1
1513	1519	MS, RI	β-Bazzanene			0.1
1517	1522	MS, RI	δ-Cadinene	0.4	2.0	1.2
1525	1529	MS, RI	*E*-γ-Bisabolene		0.3	0.7
1530	1537	MS, RI	α-Cadinene			0.2
1545	1546	MS, RI	**Elemol**	**5.3**	**9.3**	2.2
1576	1582	MS, RI	Caryophyllene oxide			0.4
1600	1607	MS, RI	5-Epi-7-epi-α-eudesmol	0.3	0.6	0.1
1614	1622	MS, RI	10-Epi-γ-eudesmol	0.2	0.3	0.1
1623	1630	MS, RI	γ-Eudesmol	1.1	1.9	0.1
1624	1630	MS, RI	β-Muurola-4,10(14)-dien-1-ol			0.1
1626	1629	MS, RI	Eremoligenol	1.9	0.3	
1634	1640	MS, RI	Hinesol	0.7	2.7	0.2
1643	1649	MS, RI	β-Eudesmol	1.2	2.6	0.5
1646	1652	MS, RI	α-Eudesmol	2.1	3.5	0.5
			Yield	2.6	1.4	
			Total identified	98.4	96.7	96.5

Extraction methods: Hydro. = hydrodistillation; SDE = simultaneous distillation extraction; SPME = solid phase microextraction.

[a] KI_{exp} = retention indices are determined using *n*-alkanes (C_7–C_{17}).

[b] KI_{lit} = retention indices of reference compounds from literature.

[c] ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2012); RI = comparison of calculated RI with those reported in the literature.

[d] Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.

Table 4
Chemical composition and essential oil yields of *Cymbopogon giganteus*.

KI_{exp}[a]	KI_{lit}[b]	ID[c]	Compounds[d]	Hydro.	SDE	SPME
				%	%	%
800	801	MS, RI	Hexanal			0.2
984	988	MS, RI	Dehydro-1,8-cineole	0.1		
999	1001	MS, RI	δ-2-Carene	0.1		
1021	1020	MS, RI	*p*-Cymene	0.5	1.2	2.2
1024	1024	MS, RI	**Limonene**	**9.6**	3.7	0.2
1085	1089	MS, RI	*p*-Cymenene	0.2	0.3	1.7
1104	1108	MS, RI	1,3,8-*p*-Menthatriene		0.3	
1119	1119	MS, RI	***E*-*p*-Mentha-2,8-dienol**	**19.3**	**15.3**	**17.7**
1131	1133	MS, RI	***Z*-*p*-Mentha-2,8-dienol**	**10.2**	**10.4**	**6.8**
1133	1137	MS, RI	*E*-Limonene Oxide			0.2
1138	1144	MS, RI	Neo-Isopulegol	0.1		
1145	1148	MS, RI	Citronellal	0.7		
1148		MS	4-Isopropenylcyclohexanone	0.4	0.4	
1161		MS	6-Methyl-Bicyclo[3.3.0]oct-2-en-7-one		0.5	
1178	1179	MS, RI	*p*-Methyl-acetophenone		0.3	
1186	1187	MS, RI	***E*-*p*-Mentha-1(7),8-dien-2-ol**	**19.6**	**28.3**	**22.8**
1193	1200	MS, RI	*E*-Dihydrocarvone			0.2
1194		MS	*p*-Menth-6-en-2,3-diol	3.2		
1195	1196	MS, RI	***E*-4-Caranone**		**6.9**	
1214	1215	MS, RI	***E*-Carveol**	**6.0**	**8.4**	**6.0**
1224	1226	MS, RI	***Z*-Carveol**	**17.0**		0.5
1226	1227	MS, RI	***Z*-*p*-Mentha-1(7), 8-dien-2-ol**	2.1	**17.3**	**15.9**
1239	1239	MS, RI	Carvone	3.2	3.2	3.9
1247	1249	MS, RI	Piperitone	0.1		
1269	1269	MS	Perilla aldehyde	0.8	0.5	0.6
1273	1273	MS, RI	*E*-Carvone oxide			0.2
1284		MS	2,6-Dimethyl-2,4-heptadiene			0.3
1297	1299	MS, RI	**Isoascaridole**		0.5	**6.8**
1571	1582	MS, RI	Caryophyllene oxide	0.1		
1636	1640	MS, RI	2-Phenylethyl hexanoate	0.1		
			Yield	1.4	0.3	
			Total identified	93.4	97.5	86.2

Extraction methods: Hydro. = hydrodistillation; SDE = simultaneous distillation extraction; SPME = solid phase microextraction.

[a] KI_{exp} = retention indices are determined using *n*-alkanes (C_7–C_{17}).

[b] KI_{lit} = retention indices of reference compounds from literature.

[c] ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2012); RI = comparison of calculated RI with those reported in the literature.

[d] Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.

121 (30), 120 (55), 119 (49), 107 (46), 105 (75), 93 (86), 91 (93), 81 (34), 79 (65), 77 (41), 69 (58), 67 (33), 55 (26), 41 (56).

Selina-6-en-4-ol: (RT 31.482) EI-MS *m/z*(rel. int.): 222 [M] (6), 204 (100), 189 (63), 179 (13), 161 (96), 147 (23), 137 (38), 135 (43), 121 (51), 109 (31), 105 (51), 95 (32), 93 (46), 91 (36), 81 (80), 79 (25), 71 (21), 69 (19), 55 (18), 43 (50), 41 (24).

Both SPME and SDE have permitted the identification of the same main volatile components in *C. citratus* from Benin, which are neral and geranial but with significant differences in their relative amounts. To these main compounds *E*-caryophyllene (5.0%) can be added based on analysis of the SPME sample, whereas geraniol (41.2%) and selina-6-en-4-ol (7.2%) were identified in the SDE sample. By hydrodistillation, the essential oil from *C. citratus* was characterized by myrcene (12.4%), neral (33.1%) and geranial (44.3%) (Bossou et al., 2013) These results corroborate the analyses of essential oils extracted by hydrodistillation from Brazil, Portugal, Colombia, Burkina Faso, Ivory Coast, Peruvian Amazon and Togo, in which myrcene, geraniol, neral and geranial have been also detected as main compounds (Andrade et al., 2009; Bassole et al., 2011; Kanko et al., 2004; Koba et al., 2004; Leclercq et al., 2000; Machado et al., 2012; Menut et al., 2000; Oliveira et al., 2009; Olivero-Verbel et al., 2010 Olivero-Verbel et al., 2010).

3.1.2 C. schoenanthus

52 and 31 compounds have been detected, respectively, via SPME and SDE for a total of 96.5% and 96.7% (Table 3). 23 components were detected via both methods and the main compounds detected were piperitone (56.6%, 53.1%), β-elemene (5.1%, 1.3%), *E*-caryophyllene (6.5%, 2.3%) and elemol (2.2%, 9.3%) for a total of 70.4% and 66%, respectively, via SPME and SDE (Fig. 1). In addition, δ-2-carene (1.3%, 4.4%), *Z*-*p*-menth-2-en-1-ol (1.6%, 1.1%), citronellal (1.0%, 1.6%), α-terpineol (1.5%, 1.4%), γ-cadinene (1.1%, 0.8%) and hinesol (0.2%, 2.7%) are significantly present in both profiles of *C. schoenanthus*. Methyl salicylate (2.8%), isoascaridole (1.0%), cuparene (1.0%) and β-selinene (3.0%) have been detected only via SPME, whereas valencene (1.5%) was identified only via SDE. The essential oil extracted by hydrodistillation from this plant species exhibited the presence of two major constituents namely δ-2-carene (15.5%) and piperitone (58.9%) (Bossou et al., 2013).

The detailed GC–MS data of the main compounds of *C. schoenanthus* were obtained as follows:

δ−2-Carene: (RT 7.277) EI-MS *m/z*(rel. int.): 136 [M] (58), 121 (100), 108 (13), 105 (22), 93 (94), 91 (52), 79 (36), 77 (36), 67 (6), 65 (7), 53 (7), 41 (12).

Piperitone: (RT 17.165) EI-MS *m/z*(rel. int.): 152 [M] (24), 137 (32), 124 (7), 110 (88), 109 (27), 95 (37), 91 (4), 82 (100), 77 (6), 67 (7), 54 (12), 41 (18).

β−Elemene: (RT 22.608) EI-MS m/z (rel. int.): 204 [M] (3), 189 (42), 175 (10), 161 (43), 147 (61), 135 (22), 133 (40), 121 (50), 119 (38), 108 (37), 107 (71), 105 (44), 95 (29), 94 (30), 93 (100), 91 (43), 81 (90), 79 (56), 77 (28), 68 (59), 67 (62), 55 (29), 53 (31), 41 (36).

Elemol: (RT 28.916) EI-MS *m/z*(rel. int.): 204 (12), 189 (41), 175 (7), 161 (94), 149 (21), 147 (21), 135 (39), 133 (23), 121 (53), 119 (33), 109 (24), 108 (24), 107 (65), 105 (34), 95 (35), 94 (26), 93 (100), 91 (31), 81 (50), 79 (42), 71 (17), 69 (25), 68 (24), 67 (41), 59 (91), 55 (24), 53 (18), 43 (31), 41 (32).

Table 5
Chemical composition and essential oil yields of *Eucalyptus citriodora*.

KI_{exp}[a]	KI_{lit}[b]	ID[c]	Compounds[d]	Hydro.	SDE	SPME
				%	%	%
832		MS	3-Methylcyclopentanol		0.5	
911	908	MS, RI	Isobutyl isobutyrate			0.1
930	932	MS, RI	α-Pinene	0.1		0.2
969	969	MS, RI	Sabinene			0.1
973	974	MS, RI	β-Pinene	0.2	0.1	0.7
982	788	MS, RI	Myrcene	0.1		0.3
999	1001	MS, RI	δ-2-Carene			0.6
1009	1007	MS, RI	Isoamyl isobutyrate			0.1
1013	1014	MS, RI	α-Terpinene	0.1		0.2
1017	1020	MS, RI	*p*-Cymene	0.1		0.4
1020	1024	MS, RI	Limonene	0.2		0.5
1027	1026	MS, RI	1,8-Cineole	1.5	0.8	0.9
1033	1032	MS, RI	*Z*-β-Ocimene			0.1
1049	1051	MS, RI	2,6-dimethyl-5-Heptenal	0.2	0.2	0.3
1050	1054	MS, RI	γ-Terpinene	0.1		0.1
1085	1086	MS, RI	Terpinolene			0.1
1093	1095	MS, RI	Linalool	0.4	0.6	0.7
1102	1103	MS, RI	Isoamyl isovalerate			0.1
1104	1106	MS, RI	*Z*-Rose oxide	0.1	1.0	0.1
1120	1122	MS, RI	*E*-Rose oxide		0.4	
1143	1144	MS, RI	**Neo-Isopulegol**	**7.8**	**31.5**	**15.2**
1150	1148	MS, RI	**Citronellal**	**52.8**	**28.4**	**48.8**
1152	1155	MS, RI	**iso- Isopulegol**	3.0	**15.7**	3.9
1188	1186	MS, RI	α-Terpineol	0.3	0.1	0.1
1192	1190	MS, RI	Methyl salicylate			0.5
1224	1223	MS, RI	**Citronellol**	**20.0**	**11.8**	4.5
1246	1249	MS, RI	Piperitone		0.6	
1256	1257	MS, RI	Methyl citronellate			0.2
1268	1274	MS, RI	Neo-Isopulegyl acetate	0.3	0.2	0.3
1301	1299	MS, RI	Isoascaridole			0.1
1330	1335	MS, RI	δ-Elemene			0.1
1349	1350	MS, RI	**Citronellyl acetate**	**9.0**	3.5	**5.8**
1354	1356	MS, RI	Eugenol			0.6
1384	1389	MS, RI	β-Elemene			0.4
1390	1393	MS, RI	2-Phenylethyl isobutanoate			0.1
1391	1392	MS, RI	*Z*-Jasmone	0.2		0.1
1412	1417	MS, RI	***E*-Caryophyllene**	0.4	1.6	**9.2**
1435	1442	MS, RI	6,9-Guaiadiene			0.1
1445	1452	MS, RI	α-Humulene			0.5
1452	1458	MS, RI	allo-Aromandendrene			0.1
1478	1482	MS, RI	Citronellol isobutanoate			0.1
1485	1500	MS, RI	Bicyclogermacrene	0.1		
1568	1577	MS, RI	Spathulenol	0.1		
1576	1582	MS, RI	Caryophyllene oxide			0.2
			Yield	4.6	1.5	
			Total identified	97.1	97.0	96.5

Extraction methods: Hydro. = hydrodistillation; SDE = simultaneous distillation extraction; SPME = solid phase microextraction.

[a] KI_{exp} = retention indices are determined using *n*-alkanes (C_7–C_{17}).

[b] KI_{lit} = retention indices of reference compounds from literature.

[c] ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2012); RI = comparison of calculated RI with those reported in the literature.

[d] Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.

In samples from Togo and Burkina Faso, piperitone has also been detected as major compound in the essential oils extracted by hydrodistillation (Koba et al., 2004; Ketoh et al., 2005; Ketoh et al., 2006; Onadja et al., 2007), in addition to the presence of the isomers α-, β- and γ-eudesmol, which were identified in the current work by all the tree methods.

3.1.3 C. giganteus

The characterization of volatiles of *C. giganteus* has resulted in the identification of 17 and 16 compounds in SPME and SDE samples, respectively, for a total of 86.2% and 97.5% of the total peak areas (Table 4). Eleven compounds were identified via both methods and the main volatiles were *E*-*p*-mentha-2,8-dienol (17.7%, 15.3%), *Z*-*p*-mentha-2,8-dienol (6.8%, 10.4%), *E*-*p*-mentha-1(7), 8-dien-2-ol (22.8%, 28.3%), *Z*-*p*-mentha-1(7), 8-dien-2-ol (15.9%, 17.3%), *E*-carveol (6.0%, 8.4%), and isoascaridole (6.8%, 0.5%) (Fig. 1), followed by three minor compounds, namely carvone (3.9%, 3.2%), *p*-cymene (2.2%, 1.2%) and *p*-cymenene (1.7%, 0.3%). In addition, *E*-4-caranone (6.9%) has been identified only via SDE. By hydrodistillation this sample revealed the presence of limonene (9.6%) and the set of monoterpene alcohols: E-p-mentha-1(7), 8-dien-2-ol (19.6%), E-p-mentha-2,8-dienol (19.3%), Z-p-mentha-2,8-dienol (10.2%), Z-p-mentha-1(7), 8-dien-2-ol (2.1%), Z-carveol (17.0%) and E-carveol (6.0%) together with p-menth-6-en-2,3-diol (3.2%) and carvone (3.2%) (Bossou et al., 2013).

The GC–MS data of the main compounds of *Cymbopogon giganteus* were obtained as follows:

Limonene: (RT 8.111) EI-MS *m/z*(rel. int.): 136 [M] (34), 121 (40), 107 (35), 94 (41), 93 (100), 92 (34), 91 (38), 79 (53), 77 (31), 68 (97), 67 (84), 53 (26), 41 (22).

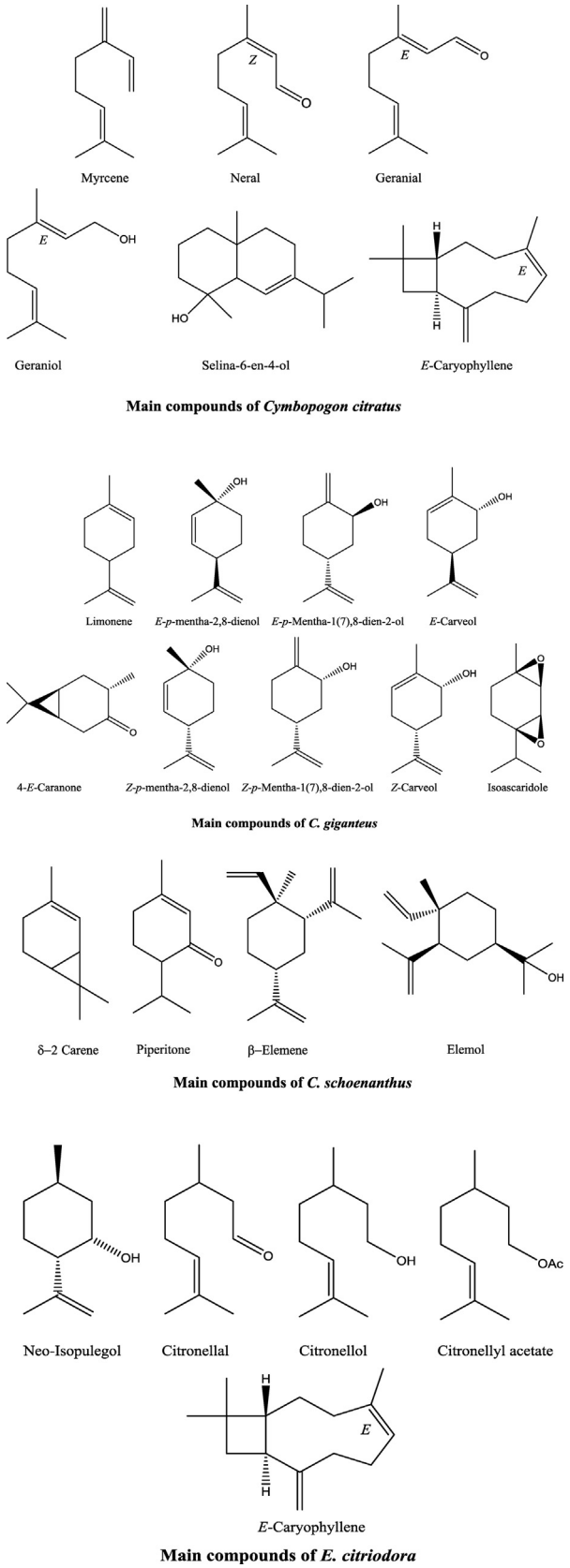

Fig. 1. Main volatile compounds of studied plant species.(Adams, 2012; Cavalli et al., 2004; Delort et al., 2015; Satyala et al., 2012).

E-*p*-mentha-2,8-dienol: (RT 11.549) EI-MS *m*/*z*(rel. int.): 152 [M] (6), 137 (56), 134 (19), 124 (20), 121 (50), 119 (31), 109 (100), 107 (12), 105 (15), 95 (37), 94 (73), 91 (44), 84 (12), 79 (73), 77 (31), 69 (18), 67 (26), 55 (14), 53 (13), 43 (43), 41 (22).

Z-*p*-mentha-2,8-dienol: (RT 12.060) EI-MS *m*/*z*(rel. int.): 152 [M] (4), 137 (60), 134 (98), 123 (21), 119 (41), 109 (100), 107 (10), 105 (15), 95 (31), 93 (24), 91 (41), 84 (10), 81 (28), 79 (56), 77 (28), 69 (15), 67 (23), 55 (13), 53 (13), 43 (37), 41 (20).

E-*p*-mentha-1(7), 8-dien-2-ol: (RT 14.283) EI-MS *m*/*z*(rel. int.): 152 [M] (3), 134 (78), 123 (11), 119 (73), 109 (100), 106 (21), 105 (24), 95 (28), 93 (32), 92 (27), 91 (70), 83 (17), 81 (27), 79 (39), 77 (26), 69 (35), 67 (39), 55 (38), 53 (21), 43 (17), 41 (43).

E-4-caranone: (RT 14.776) EI-MS *m*/*z*(rel. int.): 152 [M] (50), 134 (29), 124 (15), 121 (77), 119 (82), 109 (95), 105 (29), 95 (42), 93 (50), 91 (69), 84 (74), 83 (58), 81 (100), 79 (75), 77 (40), 69 (29), 67 (94), 55 (41), 53 (35), 41 (60).

E-carveol: (RT 15.540) EI-MS *m*/*z*(rel. int.): 152 [M] (12), 137 (15), 123 (10), 119 (19), 109 (100), 105 (9), 95 (18), 93 (17), 91 (30), 84 (78), 81 (15), 79 (19), 77 (19), 69 (20), 67 (17), 65 (9), 56 (18), 55 (23), 53 (13), 43 (11), 41 (24).

Z-*p*-Menta-1(7), 8-dien-2-ol: (RT 16.004) EI-MS *m*/*z*(rel. int.): 152 [M] (5), 137 (16), 134 (28), 123 (20), 119 (50), 109 (100), 106 (24), 105 (26), 96 (21), 95 (42), 93 (46), 91 (59), 84 (15), 83 (23), 81 (35), 79 (46), 77 (27), 69 (43), 67 (52), 65 (14), 55 (52), 53 (25), 43 (23), 41 (51).

Z-carveol: (RT 15.979) EI-MS *m*/*z*(rel. int.): 152 [M] (5), 137 (16), 134 (28), 123 (20), 119 (50), 109 (100), 106 (24), 105 (26), 95 (42), 93 (45), 91 (57), 84 (15), 83 (25), 81 (33), 79 (46), 77 (26), 69 (41), 67 (51), 65 (14), 55 (50), 53 (25), 43 (21), 41 (51).

Isoascaridole: (RT 19.112) EI-MS *m*/*z*(rel. int.): 168 [M] (2), 150 (2), 140 (8), 139 (8), 135 (8), 125 (25), 119 (11), 110 (12), 107 (23), 97 (96), 95 (32), 91 (15), 85 (25), 82 (28), 81 (18), 79 (30), 71 (34), 69 (39), 60 (16), 55 (38), 43 (100), 41 (56).

Except for isoascaridole, the same main compounds have been detected in essential oils obtained by hydrodistillation from previous research in Benin and other West and Central African countries such as Togo, Ivory Coast, Mali, Burkina Faso, Togo and Cameroon (Kanko et al., 2004; Menut et al., 2000; Boti et al., 2006; Jirovetz et al., 2007; Noudogbessi, 2009; Nyamador et al., 2010; Sidibé et al., 2001).

3.1.4 E. citriodora

Sixteen and 39 compounds, for a total of 97.0% and 96.5% of the total peak areas, were detected via SDE and SPME, respectively (Table 5). Six main compounds, namely neo-isopulegol (31.5%, 15.2%), citronellal (28.4%, 48.8%), iso-isopulegol (15.7%, 3.9%), citronellol (11.8%, 4.5%), citronellyl acetate (3.5%, 5.8%) and *E*-caryophyllene (1.6%, 9.2%), were detected via both methods (Fig. 1).

The detailed GC–MS data of the main compounds of *Eucalyptus citriodora* were obtained as follows:

Neo-isopulegol: (RT 12.522) EI-MS *m*/*z*(rel. int.): 154 [M] (18), 139 (34), 136 (48), 121 (100), 112 (23), 111 (52), 110 (33), 109 (38), 108 (33), 98 (23), 97 (38), 95 (74), 93 (71), 84 (60), 83 (42), 81 (88), 79 (43), 77 (18), 71 (69), 69 (76), 68 (66), 67 (85), 56 (38), 55 (60), 53 (33), 43 (33), 41 (71).

Citronellal: (RT 12.815) EI-MS *m*/*z*(rel. int.): 154 [M] (13), 139 (14), 136 (13), 121 (43), 111 (29), 110 (21), 109 (21), 95 (77), 84 (20), 82 (17), 69 (100), 56 (25), 55 (45), 41 (79).

Citronellol: (RT 15.888) EI-MS *m*/*z*(rel. int.): 156 [M] (8), 138 (14), 123 (25), 109 (21), 95 (53), 82 (55), 81 (58), 71 (27), 69 (100), 67 (58), 56 (24), 55 (48), 41 (72).

Citronellyl acetate: (RT 21.062) EI-MS *m*/*z*(rel. int.): 138 (50), 123 (79), 109 (37), 95 (97), 82 (70), 81 (100), 69 (88), 68 (39), 67 (65), 55 (44), 43 (71), 41 (60).

E-Caryophyllene: (RT 23.726) EI-MS *m*/*z*(rel. int.): 204 [M] (22), 189 (38), 175 (12), 161 (65), 148 (32), 147 (45), 134 (25), 133 (100),

121 (30), 120 (55), 119 (49), 107 (46), 105 (75), 93 (86), 91 (93), 81 (34), 79 (65), 77 (41), 69 (58), 67 (33), 55 (26), 41 (56).

In the essential oil extracted via hydrodistillation from *E. citriodora*, the main compounds detected by GC–MS were citronellal (52.8%), citronellol (20.0%), citronellyl acetate (9.0%) and neo-isopulegol (7.8%) (Bossou et al., 2013). Citronellal, citronellol and isopulegol were also detected in *E. citriodora* essential oil analyzed in Colombia (Olivero-Verbel et al., 2010; Maciel et al., 2010), the Democratic Republic of Congo (Cimanga et al., 2002) and Ethiopia (Dagne et al., 2000). On the contrary, the volatile profiles obtained in Brazil and India were quite different since their main compounds were (−)- isopulegol (7.3%), β-citronellal (71.7%) (Maciel et al., 2010), and α-thujene (11.9%), α-pinene (18.3%), sabinene (19.6%), β-pinene (25.7%) (Mohammed and Abhilasha, 2011), respectively.

3.2. Insecticide activity by contact against Tribolium beetles

The lethal concentrations (LC_{50}) obtained by contact "no choice" test are summarized in Table 6. Lethal concentrations varied depending on the times of exposure. Indeed the LC_{50} values after 24 h, 48 h and 72 h were 22%, 20%, 15% for *C. citratus*, 10%, 9%, 6% for *C. giganteus*, 8%, 6%, 6% for *C. schoenanthus*, and 7%, 5%, 5% for *E. citriodora*. It follows that the most efficient crude oil against *T. castaneum* was *E. citriodora* followed by *C. schoenanthus*. The LC_{50} values obtained after 24 h, 48 h, and 72 h of exposure for *E. citriodora* essential oil and *C. schoenanthus* essential oil were not significantly different based on the non-overlapping 95% CL for doses.

Regarding the mortalities (Table 7), the same tendencies were observed since after 72 h, the most efficient essential oil was the one extracted from *E. citriodora* (82% mortality at 8%), followed by *C. schoenanthus* essential oil (73% at 8%), *C. giganteus* essential oil (67% at 8%) and *C. citratus* essential oil (18% at 8%).

Piperitone, the major compound isolated from *C. schoenanthus* essential oil, was more efficient than the crude essential oil, since the mortality values obtained after 24 h, 48 h and 72 h were 63%, 77% and 87%, respectively, for piperitone at a concentration of 4.7% versus 50%, 65% and 73%, respectively, for the crude oil (8% concentration) extracted from *C. schoenanthus* (Table 7). The results obtained with piperitone were better than those of permethrin 0.75% as positive control (60%, 75% and 83%). From these results, it can be confirmed that the major compound of *C. schoenanthus* (piperitone) is responsible for the insecticidal properties against *T. castaneum*.

The tested compounds of *E. citriodora* were not as effective as the crude oil since no or mortality less than 4% was obtained for citronellyl acetate (at 0.7%) and citronellol (at 1.6%), whereas for citronellal at 4.2% a mortality of 40% could be obtained after 72 h (Table 7). Since the isolated compounds are not as efficient as the crude oil, the insecticidal properties of this essential oil could be rationalized by the synergistic action of its major compounds (citronellal and citronellol) and some other minor compounds such as 1,8-cineole, α-pinene and *p*-cymene, whose insecticidal properties have been already reported. Indeed, the insecticidal properties of 1,8-cineole, α-pinene and *p*-cymene have been demonstrated against *Aedes aegypti* and correlated to the amount of 1,8-cineole in the extract (Lucia et al., 2009; Lucia et al., 2008). The pronounced contact toxicity of limonene, an important compound in the essential oil of *C. giganteus* and *C. schoenanthus*, with LD_{50} value of 14.97 μg/adult for *T. castaneum*, better than 1,8-cineole (LD_{50} = 18.83 μg/adult) was recently shown (Wang et al., 2014). The insecticidal property of citronellol has not been demonstrated according to our knowledge, but the repellent properties of (+)-citronellol against *T. castaneum* has been already reported (Zhang et al., 2011). The nature of the biological properties of this compound as a repellent might explain its inefficiency in the contact "no choice" test.

Biological properties of essential oils extracted from plant species are related to their chemical profiles which depend on the extracted organ, its age, the geographical area of collection, the method of extraction (Olivero-Verbel et al., 2010; Negahban et al., 2006) and the method used to test the extract. The essential oil extracted from the whole plant of *C. citratus*, collected in Colombia, was rich in neral (28.4%), geraniol (11.5%) and geranial (34.4%). This composition is a bit different from the one obtained in the current study in view of the high amount of geraniol (11.5% in Colombia versus 1% in Benin) and the absence of myrcene whose relative peak area was 12.4% in the current work (Olivero-Verbel et al., 2010). The mortality obtained at 40 mL/L against *T. castaneum* by Olivero-Verbel et al. (2010) with the essential oil, originating from Colombia (65% after 24 h, 70% after 48 h and 75% after 72 h of exposure) might be explained by the different chemical profiles of this plant originating from different areas, combined with the different method of dilution used, i.e., w/v in the current work and v/v by Olivero-Verbel et al. (2010).

The chemical profile obtained from the extract of the leaves of *E. citriodora* in Colombia revealed as main compounds citronellal (40%), isopulegol (14.6%), and citronellol (13.0%). Its chemical profile revealed the presence of two minor compounds, namely 1,8-cineole (3.4%) and citriodiol [2-(2-hydroxy-2-propyl)-5-methylcyclohexanol] (4.7%). In adults population of *T. castaneum*, this crude oil has induced mortalities of 55%, 57% and 60%, at 40 mL/L (4%) after 24 h, 48 h and 72 h, respectively (Olivero-Verbel et al., 2010).

The insecticidal property of 1,8-cineole against stored grain insects (Maciel et al., 2010; Lucia et al., 2009; Palacios et al., 2009), fumigant property against *Lutzomyia longipalpis* (Lee et al., 2004), acaricidal and repellent properties against *Tetranychus urticae* and *Culex pipiens* (Roh et al., 2013; Traboulsi et al., 2005) have been already reported. Citriodiol has also demonstrated repellent properties against *Ixodes ricinus* tick (Dautel et al., 2013) and land leeches of the genus *Haemadipsa* (Kirton, 2005). The presence of citriodiol and 1,8-cineole in the extract from Colombia might explain the differences in response of *T. castaneum* to these essential oils extracted from *E. citriodora*.

To our knowledge, no research on the insecticidal activity of *C. schoenanth*us and *C. giganteus* against *T. castaneum* by contact "no choice" test has been already published.

3.3. Fumigant activity against Tribolium beetles

The screening against *T. castaneum* has demonstrated the susceptibility of this beetle to the essential oil tested by fumigation, especially to essential oil from *C. schoenanthus* and *E. citriodora* with LC_{50} values ranged respectively as followed: 4.2 mL/L air for *C. citratus*, 2.3 mL/L air for *C. giganteus*, 2.1 mL/L air for *C. schoenanthus* and 2.0 mL/L air for *E. citriodora* (Table 6). The results obtained for *C. giganteus*, *C. schoenanthus* and *E. citriodora* are not significantly different according to the non-overlapping 95% CL for doses.

Concerning the isolated compounds, the LC_{50} values were 0.5 mL/L air for piperitone, and 1.2 mL/L air for citronellal (Table 6).

Piperitone, the major compound of *C. schoenanthus* was more than three times more efficient than the crude oil, whereas citronellal was about two times more efficient than the crude essential oil extracted from *E. citriodora*.

Regarding the mortalities, the more efficient essential oils were the same as for the contact assays. Indeed at the highest concentration (Table 8), the mortalities obtained were 42% for *C. citratus*, 68% for *C. giganteus*, 72% for *C. schoenanthus*, 75% for *E. citriodora,* 82% for citronellal and 100% for piperitone. Citronellol has no insecticidal effect by fumigation (0% mortality) whereas citronellyl acetate (2%) and permethrin (8%) have demonstrated very weak mortalities (Table 8).

Table 6
LC_{50} values of essential oils and identified compounds against *Tribolium castaneum*, by contact and fumigation.

Exposure	Essential oil/product	LC_{50} (% for contact; ml/l air for fumigant)(95% CL)	Slope ± SE	Chi-square	df	Sig.
Contact, after 24 h	*C. citratus*	21.8* (15.1 – 45.0)[a]	0.10 ± 0.03	47.60	40	0.191
	C. giganteus	10.3* (9.1 – 12.0)[b]	0.25 ± 0.03	30.76	34	0.627
	C. schoenanthus	7.5 (6.7 – 8.7)[c]	0.26 ± 0.02	54.49	34	0.014
	E. citriodora	6.9 (6.1 – 7.9)[c]	0.29 ± 0.03	38.03	34	0.291
Contact, after 48 h	*C. citratus*	20.0* (13.49 – 42.91)[a]	0.10 ± 0.03	54.68	40	0.061
	C. giganteus	8.5* (7.46 – 10.03)[b]	0.23 ± 0.02	47.16	34	0.066
	C. schoenanthus	6.4 (5.75 – 7.14)[c]	0.32 ± 0.02	57.80	34	0.007
	E. citriodora	5.1 (4.29 – 6.07)[c]	0.29 ± 0.03	50.00	34	0.038
Contact, after 72 h	*C. citratus*	15.0* (11.0 – 22.0) [a]	0.12 ± 0.02	37.30	40	0.594
	C. giganteus	6.1 (5.4 – 7.0) [b]	0.26 ± 0.02	56.70	34	0.009
	C. schoenanthus	5.6 (5.1 – 6.2) [b]	0.35 ± 0.02	48.04	34	0.056
	E. citriodora	4.5 (3.8 – 5.5) [b]	0.29 ± 0.03	53.50	34	0.018
Fumigant, after 24 h	*C. citratus*	4.2* (3.5 – 5.4)[a]	0.37 ± 007	28.2	22	0.168
	C. giganteus	2.3 (1.7 – 2.9)[b]	0.46 ± 0.06	40.06	22	0.011
	C. schoenanthus	2.1 (1.5 – 2.8)[b]	0.49 ± 0.06	55.43	22	0.000
	E. citriodora	2.0 (1.6 – 2.5)[b]	0.49 ± 0.07	32.14	22	0.075
	piperitone	0.5 (0.34 – 0.74)[c]	2.94 ± 0.34	85.87	22	0.000
	citronellal	1.2 (0.9 – 1.4)[d]	1.29 ± 0.16	25.02	16	0.069

LC_{50} = concentration of essential oil required to kill 50% of *T. castaneum*. LC_{50} values within an exposure condition followed by the same letter are not significantly different, based on non-overlapping 95% CL for the LC_{50}

* calculated LC_{50} is obtained by extrapolation because the% mortality at the highest concentration tested was <50%.

Table 7
Mortalities obtained by contact "no choice" test against *Tribolium castaneum.*

Exposure	Plant EO/identified compounds	Mortality[a] at 0.5% EO	Mortality[a] at 1% EO	Mortality[a] at 2% EO	Mortality[a] at 4% EO	Mortality[a] at 8% EO
24 h	*C. citratus*	1.5% ± 0.9	1.6% ± 1.0	1.7% ± 1.0	4.9% ± 2.8	6.7% ± 2.8
	C. giganteus	0.8% ± 0.7	0.8% ± 0.7	0.8% ± 0.7	10.0% ± 1.7	26.7% ± 5.6
	C. schoenanthus	1.6% ± 1.5	3.1% ± 0.9	9.9% ± 2.8	24.6% ± 4.6	50.4% ± 7.0
	E. citriodora	1.7% ± 1.5	3.3% ± 1.9	13.3% ± 5.1	26.7% ± 7.7	58.3% ± 2.8
	Piperitone 4.7%	ND	ND	ND	ND	63.3% ± 8.4
	Citronellal 4.2%	ND	ND	ND	ND	40.0% ± 3.3
	Citronellol 1.6%	ND	ND	ND	ND	0.0% ± 0.0
	Citronellyl acetate 0.7%	ND	ND	ND	ND	0.0% ± 0.0
	Permethrin 0.75%	60.0% ± 7.8				
	Acetone	0% ± 0				
48 h	*C. citratus*	2.3% ± 0.9	2.5% ± 1.6	5.8% ± 2.2	6.6% ± 3.2	10.0% ± 3.9
	C. giganteus	2.5% ± 1.0	4.2% ± 1.8	9.2% ± 1.8	20.8% ± 1.4	41.7% ± 9.5
	C. schoenanthus	1.6% ± 1.5	3.1% ± 1.4	10.7% ± 4.3	30.6% ± 5.1	65.1% ± 4.1
	E. citriodora	11.7% ± 3.7	13.3% ± 3.8	25.0% ± 6.1	41.7% ± 7.2	76.7% ± 8.4
	Piperitone 4.7%	ND	ND	ND	ND	76.7% ± 6.5
	Citronellal 4.2%	ND	ND	ND	ND	40.0% ± 3.3
	Citronellol 1.6%	ND	ND	ND	ND	1.7% ± 1.5
	Citronellyl acetate 0.7%	ND	ND	ND	ND	0.0% ± 0.0
	Permethrin 0.75%	75.0% ± 7.0				
	Acetone	0% ± 0				
72 h	*C. citratus*	3.8% ± 1.2	8.9% ± 1.9	10.8% ± 2.2	11.5% ± 2.2	18.3% ± 3.0
	C. giganteus	4.9% ± 1.6	12.5% ± 4.7	20.8% ± 2.2	30.8% ± 4.9	66.7% ± 5.2
	C. schoenanthus	2.4% ± 1.5	3.9% ± 1.3	9.2% ± 1.6	44.0% ± 5.0	72.9% ± 3.1
	E. citriodora	15.0% ± 3.9	16.7% ± 3.8	30.0% ± 8.2	45.0% ± 8.4	81.7% ± 6.4
	Piperitone 4.7%	ND	ND	ND	ND	86.7% ± 4.5
	Citronellal 4.2%	ND	ND	ND	ND	40.0% ± 3.3
	Citronellol 1.6%	ND	ND	ND	ND	3.3% ± 1.9
	Citronellyl acetate 0.7%	ND	ND	ND	ND	0.00% ± 0.0
	Permethrin 0.75%	83.3% ± 7.3				
	Acetone	0% ± 0				

[a] Mortality values = mean ± SE of the six replicates (SE = standard error); EO = essential oil.

Table 8
Mortalities obtained by fumigation against *Tribolium castaneum*.

Plant essential oil/identified compounds	Mortality [a] at C_1	Mortality [a] at C_2	Mortality [a] at C_3
C. citratus	15.0% ± 5.7	28.3% ±5.5	41.7% ±4.4
C. giganteus	38.3% ± 5.5	63.3% ± 6.5	68.3% ± 3.7
C. schoenanthus	40.0% ± 7.8	66.7% ± 3.8	71.7% ± 8.0
E. citriodora	48.3% ± 4.4	60.0% ± 2.4	75.0% ± 3.1
Piperitone	78.3% ± 6.8	91.7% ± 6.0	100% ± 0.0
Citronellal	–	60.0% ± 2.4	81.7% ± 5.5
Citronellol	–	0.0% ± 0.0	0.0% ± 0.0
Citronellyl acetate	–	0.0% ± 0.0	1.7% ± 1.5
Permethrin 0.75%	8.3% ± 3.7		
Acetone	0% ± 0		

[a] C_1, C_2 and C_3 values for crude oils and identified compounds are indicated in the protocol of each test.

The strong fumigant toxicity of 1,8-cineole was demonstrated. Indeed against *T. castaneum*, LC_{50} values obtained were 5.5 mg/L air for 1,8-cineole by fumigation versus 6.2 mg/L air for limonene (Lucia et al., 2008). In an earlier study, limonene was more effective against *T. castaneum* than 1,8-cineole (Prates et al., 1998).

To our knowledge, except for the essential oil of *C. citratus* for which no fumigant toxicity on larvae and adults of *T. castaneum* was found (Stefanazzi et al., 2011), no result on the fumigant properties of essential oils from the investigated plant species tested against *T. castaneum* has been already reported.

Nevertheless, the good insecticidal properties of piperitone have been already reported. Indeed, piperitone, isolated from the essential oil of *Artemisia judaica,* has demonstrated a complete antifeedant activity at a concentration of 1000 μg/mL against the third instar larvae of *Spodoptera littoralis* using non-choice leaf disc assay (Abdelgaleil et al., 2008). Furthermore when tested against *Callosobruchus maculatus*, piperitone, isolated from *C. schoenanthus,* was more toxic by fumigation to adults with a LC_{50} value of 1.6 μL/L vs. 2.7 μL/L obtained with the crude extract (Ketoh et al., 2006). The crude oil of *E. citriodora,* rich in beta-citronellal (71.8%) has demonstrated good insecticidal activity against the adult of *Lutzomya longipalpis* by fumigation. Indeed, at a concentration of 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL and 10 mg/mL, the following mortalities have been respectively recorded after 24 h: 11%, 31%, 48%, 68%, and 88% (Maciel et al., 2010).

Despite the observed potential of some of the essential oils or identified compounds as alternative pesticides against *T. castaneum*, complementary research is needed to evaluate the possible toxic effects of these extracts and compounds. Some of the studied extracts, i.e., the essential oil of *C. citratus*, were reported to demonstrate cytotoxic effects on mammalian cells (Koba et al., 2009; Kpoviessi et al., 2014; Sanchez-Suarez et al., 2013).

4. Conclusion

The chemical volatile profiles of four plant species from Benin have been established by SDE/GC–MS and SPME/GC–MS, and compared to the profiles obtained by hydrodistillation/GC–MS. Profiles obtained via SPME/GC–MS were the most complete, which indicates that this method is the best choice for the chemical profiling of volatiles extracted from plant species. The insecticidal tests against *T. castaneum* revealed the essential oils of *C. schoenanthus* and *E. citriodora*, as well as their main identified compounds piperitone and citronellal, respectively, as promising bio-insecticides against this pest.

Competing interests

The authors declare that they have no competing interests.

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