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Full Length Research Paper

Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon

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This study compared the chemical composition of the essential oils obtained by hydrodistillation of different tissues of *Piper capense*, *Piper guineense*, *Piper nigrum* and *Piper umbellatum* grown in Cameroon. The GC and GC/MS analysis showed qualitative and quantitative differences between these oils. Oils from the fruits were rich in α-pinene (5.6 - 12.3%) and β-pinene (6.7 - 59.3%). The other major constituents were sabinene (14.7%) for *P. capense*, limonene (15.8%) and β-caryophyllene (20.8%) for *P. guineense*. The oil from the fruits of *P. nigrum* contained sabinene (11.2%), δ-3-carene (18.5%), limonene (14.7%) and β-caryophyllene (12.8%) while that of *P. umbellatum* content linalool (14.4%) and (E)-nerolidol (10.0%) as major constituents. The essential oil obtained from the leaves of *P. capense* was largely composed of α-pinene (12.8%), β-pinene (50.1%) and β-caryophyllene (12.4%). The most abundant constituents identified in the oil from the leaves of *P. guineense* were limonene (10.3%) and germacrene B (25.1%) while that from *P. nigrum* was characterized by its high amount of α-selinene (16.5%) and β-selinene (14.6%). β-pinene (10.8%), β-caryophyllene (28.2%) and (E)-nerolidol (16.5%) were the quantitative important constituents of the essential oils from the leaves of *P. umbellatum*. The oils from the lianas of *P. guineense* was rich in (Z, E)-α-farnesene (28.7%), limonene (19.7%) and myristicine (10.9%), while those from *P. nigrum* contained δ-3-carene (14.4%) and β-caryophyllene (36.0%). The oil from the stems of *P. capense* contained mostly α-pinene (14.3%) and β-pinene (61.4%). The distillation of those from *P. umbellatum* did not produce any essential oil. Oils from the three fruits showed variable contact toxicity against *Sitophilus zeamais* with *P. guineense* being more toxic (LD_{50} = 10.0 ± 0.3 µl/g) than *P. capense* (LD_{50} = 16.1 ± 0.6 µl/g) and *P. nigrum* (LD_{50} = 26.4 ± 1.5 µl/g). Poudrox (5%) used as a standard insecticide exhibited 100% mortality.

Key words: *Piper capense* L., *P.guineense* Schum. Et Thonn., *P. nigrum* L., *P. umbellatum* L, essential oils, α-pinene, β-pinene, β-caryophyllene, insecticidal, *Sitophilus zeamais* Motsch.

INTRODUCTION

Piperaceae is one of the most widely distributed families of flowering plants. It consists of over 12 genera comprising about 1400 species distributed throughout tropical and sub-tropical region in both hemispheres (Sengupta and Ray, 1987). They are erect, climbing, lianescent herbs or shrubs or infrequent trees that can be easily recognized by the simple and entire alternate leaves, rarely opposite or verticillate (Letouzey, 1972). This large and heterogeneous family is represented in Cameroon by the genera *Peperomia* and *Piper*. Of the 700 species of

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Table 1. Essential oil yields of four *Piper* species from Cameroon.

Plant tissues	Essential oil yields (%)			
	P. capense	***P. guineense***	***P. nigrum***	***P. umbellatum***
Fruits	1.51	4.71	1.10	0.02
Leaves	0.30	0.47	0.24	0.01
Lianas or stems	0.03	0.10	0.07	-

Piper, only three are recognized to be indigenous to Cameroon (Hutchinson and Dalziel, 1963). The first, *Piper guineense*, commonly known as the African black pepper, is a forest liana with gnarled branchlets spiraling up to shrubs to about 10 m. The leaves, elliptic in shape, have a pleasant aroma when they are crushed. Its small spherical fruits in racemes are yellow becoming orange, red and finally black (Iwu, 1993). The second species, *Piper capense*, is an under storey shrub common in gallery forest, with V-shaped branchless and pointed ovate leaves bearing seven basal nerves. Their fruits occur around January and March (Hutchinson and Dalziel, 1963). The third species is *Piper umbellatum*. It is a large shrub of about 1.5 to 2 m height with broad circular leaves deeply cordate at the base. The last *Piper* species which has been examined is *Piper nigrum*. This plant originates from India and has been introduced to Cameroon where it is now cultivated for the production of spices (Noumi, 1984).

The *Piper* species have high commercial, economical and medicinal value. The aromatic fruits of some *Piper* are used as spices. The ripened fruit of *P. nigrum* is the source of white pepper while the unripe fruit is the source of black pepper. In West Cameroon, the seeds of *P. guineense* (usually dried or sometimes fresh) are used as flavor in “Nah poh” (yellow sauce) widely eaten by the “Bamileke”. In the South, leaves of *P. umbellatum* are eaten as vegetables and sometimes, the “Betis” peoples use them as condiment for cough, the “Ndomba” soup (Noumi, 1984). The genus *Piper* is also reputed for the medicinal proprieties of its plants. The preparations of leaves, roots and seeds of *P. guineense* are used internally as medical agents for the treatment of bronchite, gastrointestinal diseases, veneral diseases and rheumatism. The powder obtained from the ground seeds is used for its stimulating properties (Sofowora, 1982).

In the literature, there are several studies on the essential oils composition of *P. nigrum* and *P. guineense* (Menut et al., 1998; Amvam et al., 1998; Jirovetz et al., 2002; Asawalam et al., 2007), and only one paper on the volatile composition of *P. umbellatum* (Vogler et al., 2006), whereas the oil of *P. capense* seem not to have been reported before.

After our previous work on *P. guineense* from Cameroon and Congo (Menut et al., 1998), it was confirm that the chemical composition of the essential oils from different part of this plant depends on various factors such as geographical and climatic conditions. The purpose of the present study was to compare the chemical composition of different tissues of the four *Piper* species grown in Cameroon in order to provide more data on the oils composition of these spices. Their seeds are transformed in powders and used for the protection of grains by communities of the Western highlands of Cameroon. Since these grains are also powerful source of essential oils, the insecticidal activity of their oils were then evaluated by contact toxicity against *Sitophilus zeamais*, one of the pests of stored grain in Cameroon.

MATERIALS AND METHODS

Collection of plant material

The plant samples were collected in April 2005 (Table 1). Their identification was carried out at the National Herbarium in Yaoundé where voucher specimens are kept under the following numbers: *P. capense* (2461/SRFK), *P. guineense* (3615/SRFK), *P. nigrum* (25818/SFR/Cm) and *P. umbellatum* (2854/SFR/Cm).

Extraction of essential oils

Fractions of 300 g of plant materiel of each *Piper* species were submitted for hydrodistillation in 1.5 L of water using a Clevenger-type apparatus for 5 h. The obtained oil was dried over anhydrous sodium sulfate and, after filtration, stored at 4°C until tested and analyzed one or two months later. The yields were calculated according to the weight of plant material before distillation.

Chemical analysis of essential oils

Gas chromatography: The oils were analyzed on a Varian CP-3380 GC with flame ionization detectors and fitted with a fused silica capillary column (30 m x 0.25 mm coated with DB-1, film thickness (0.25 µm), the oven temperature was programmed from 50 - 200°C at 5°C/min, injector temperature 220°C, detector temperature 250°C, carrier gas N_2 0.8 mL/min. The linear retention indices of the components were determined relative to the retention times of a series of n-alkanes, and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

Gas Chromatography-Mass Spectrometry: GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with an HP-1 fused silica column (30 m x 0.20 mm, film thickness 0.25 µm) and interfaced with a quadrupole detector (Model 5970). The oven temperature was programmed from 70 - 200°C at 10°C/min; injector temperature was 220°C. Helium was used as carrier gas at a flow rate of 0.6 mL/min; the mass spectrometer was operated at 70 eV.

Identification of components: The identification of the constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature (Joulain and Köning, 1998; Adams, 2001).

Insecticidal activity of the oils *against Sitophilus zeamais*

Adult of *S. zeamais* used for the study were obtained from infested maize grains collected in Santchou farmers granaries. These insects were identified by Dr M. Tindo (entomologist) on the basis of the morphological key of Delobel and Tran (1993). From this stock, new generations were reared in the laboratory on dry weevil susceptible white maize at room temperature 30°C. Freshly emerged adults of *S. zeamais* were then used for the experiments. Essential oils from the fruits of *P. capense*, *P. guineense* and *P. nigrum* which were in great amount were tested for contact toxicity on *S. zeamais*. Each oil applied to the grains at different doses (5 – 50 µl/g of grain) was dissolve in 2 ml of acetone and stirred thoroughly with a glass rod for 5 min, to ensure uniform distribution over the grain surface. The treated grains were kept for 60 min to allow the solvent to evaporate completely before bioassays were conducted. 20 insects were introduced into vials of 125 ml containing the different treated and untreated maize grains. The lids of vials were perforated with holes. Muslin cloth held with rubber band was used to secure the mouth of the plastic vials to ensure aeration and avoid entry or exit of insects. Each treatment was replicated three times in a completely randomized design. The mortality was evaluated after 24 h of contact in each trial and compare to that of the control.

Statistical analysis

The results were given as means ± s.d. Percentages of mortality were calculated from the overall number of death insects. Raw data were compared using Fisher's exact-test (StatXact 2.05 software). Appropriate probabilities were adjusted for the number of simultaneous tests, using the sequential Bonferroni procedure (Rice, 1989): at the significance level (α = 0.05), statistical probabilities *P* were determined for *k* total number of pairwise tests and were ranked from smallest (P_1) to largest (P_k). For independent samples (our situation), the test corresponding to *Pi* indicated significance if $Pi \leq (1-[1-\alpha]1/1+k-i)$.

RESULTS AND DISCUSSION

The yields of essential oils varied with plant species and plant tissues (Table 1). Whatever the plant part considered, *P. guineense* appears to be richer in oil (0.10 - 4.71% w/w) than the other plant species, while *P. umbellatum* was poorer (0.01-0.02%).

The results of CG and CG/MS analysis of the essential oils are given in Table 2 where constituents are listed according to their elution order on DB-1 column. Monoterpenes (63.7 - 89.1%) were the predominant constituents in the essential oils obtained from the fruits. Among these, α-pinene (10.5 - 12.3%) and β-pinene (12.1 - 59.3%) were the most abundant compounds in the oils from seeds of *P. capense*, *P. guineense* and *P. umbellatum*. In addition to these constituents, sabinene (14.7%) as well as linalol (14.4%) were noted in the oil from fruits of *P. capense* and *P. umbellatum* respectively. The major monoterpenes from the seed oil of *P. nigrum* were δ-3-carene (18.5%), limonene (14.7%) and sabinene (11.2%). These results are different from the earlier published data on *P. guineense* collected in Cameroon riched in β-caryophyllene (57.59%). They are also different from those reported by Jirovetz et al. (2002) on *P. nigrum* dominated by germacrene D (11.01%) and β-pinene (10.02%). These compounds were present in relatively low concentration in our samples. It is also important to mention the small amount of myristicine (1.5%) in the seed oil of *P. umbellatum*.

The oils from the leaves were in general different from those of the fruits because of the high content of sesquiterpenes (65.2 - 89.5%). The main constituents of this group were germacreme B (25.1%) for *P. guineense*, α-selinene (16.5%) and β-selinene (14.6%) for *P. nigrum*, β-caryophylene (28.2%) and (E)-nerolidol (16.5%) for *P. umbellatum*. Safrole (49.0%) has been found to be the major constituent of the leaf oil of *P. umbellatum* from Cuba, with smaller amount of germacrene D (8.0%) (Pino et al., 2005) while those from Sao Tomé e Príncipe show an abundance of α-and β-pinene (18.0 and 27.0% respectively) (Martins et al., 1998). Our sample of *P. umbellatum* essential oil is different from that from Costa Rica, dominated by β-caryophyllene (28.0%), germacrene D (17.0%), and (E, E)-α-farnesene (15.0%) (Vogler et al., 2006). Interesting to notice is the small amount of myristicine (2.3% and 1.7% respectively) in the oils from the leaves of *P. guineense* and *P. umbellatum*. This component is absent in the oils from *P. capense* and *P. nigrum*. Analysis of the oils from the leaves of *P. capense* from Cameroon however, revealed the most prominent compounds to be α-pinene (12.8%) and β-pinene (50.1%); consequently, this oil is quantitatively rich in monoterpenes (80.8%).

Due to the high proportion of the two main constituents α-pinene (14.3%) and β-pinene (61.4%), the oil obtained from the stem of *P. capense* is monoterpene predominate. The percentages of most of the remaining components are then below 5.0%. The essential oil of *P. guineense* and *P. nigrum* liana exhibits high amounts of sesquiterpenes (57.3 - 61.5%). The dominating constituent was (Z, E)-α-farnesene (28.7%) which was abundantly found only in the volatile oil from *P. guineense* (liana). It is also in lesser amounts in the other parts of the plant (1.9 - 3.9%). Additionally, the presence of myristicine (10.9%) was observed. The dominating constituents in the oils of lianas from *P. nigrum* were β-caryophyllene (36.0%) and δ-3-carene (14.4%).

A comparison of the chemical profile of the oils from each species shows that only *P. capense* is fairly similar to the composition of oil from different tissues compared to those exhibited by others. In this study, β-pinene and β-caryophyllene were found in all species and were also identified as one of the major component in six and five samples respectively. The above results show that *Piper* species growing in Cameroon could serve as good sources of these industrial useful compounds. The diffe-

Table 2. Comparative percentage composition of the essential oils from the fruits, leaves, and lianas or stems of *P. capense (1), P. guineense (2)*, *P. nigrum (3)* and *P. umbellatum* (4) grown in Cameroon.

Components	RI	Percent composition on DB-1 type column										
		Fruits				Leaves				Lianas or stems		
		1	2	3	4	1	2	3	4	1	2	3
MT		89.1	63.7	72.7	64.3	80.8	21.2	9.7	32.8	84.4	30.5	37.8
MTH		87.7	62.6	69.5	43.1	78.9	15.9	7.7	26.6	83.0	29.4	33.5
α-Thujene	924	0.1	1.0	1.8	t	2.1	0.2	-	t	0.7	0.3	0.8
α-Pinene	931	10.5	10.6	5.6	12.3	12.8	-	-	7.4	14.3	2.3	2.1
Camphene	945	0.3	t	0.1	0.7	0.1	-	t	0.3	0.5	0.1	-
Sabinene	965	14.7	5.6	11.2	-	8.8	0.3	3.5	0.7	0.1	0.9	2.8
β-Pinene	970	59.3	12.1	6.7	21.2	50.1	1.1	1.2	10.8	61.4	0.5	1.1
Myrcene	983	1.0	1.8	2.5	1.7	1.3	0.2	-	2.3	3.3	1.1	1.2
α-Phellandrene	1001	-	3.4	4.5	t	0.6	0.6	-	t	t	1.2	2.8
δ-3-Carene	1005	t	4.2	18.5	-	t	1.1	0.3	0.8	t	1.2	14.4
α-Terpinene	1012	0.1	4.9	0.9	-	-	0.7	-	t	0.1	t	0.8
p-Cymene	1016	1.2	0.2	0.7	0.5	0.4	0.1	-	0.8	t	t	0.5
Limonene	1020	-	15.8	14.7	5.5	1.8	10.3	2.3	2.1	2.0	19.7	4.4
β-Phellandrene	1021	-	1.0	-	-	-	0.3	-	-	-	0.4	-
(Z)-β-Ocimene	1025	0.1	0.1	-	0.3	0.4	-	-	0.8	0.2	0.1	-
(E)-β-Ocimene	1045	0.2	0.6	0.1	t	0.5	0.5	0.4	0.3	0.2	-	0.4
γ-Terpinene	1050	0.1	0.4	1.0	0.3	-	0.5	-	0.2	0.1	0.1	1.2
Terpinolene	1082	0.1	0.9	1.2	0.6	-	-	-	0.1	0.1	1.5	1.0
OCM		1.4	1.1	3.2	21.2	1.9	5.3	2.0	6.2	1.4	1.1	4.3
Sabinene hydrate	1060	0.1	0.2	0.3	-	t	-	-	-	0.2	-	-
Linalol	1090	0.1	0.6	0.7	14.4	1.6	5.3	2.0	4.7	-	1.0	1.9
Camphor	1127	0.1	t	-	1.4	-	-	-	-	0.2	t	-
Borneol	1154	t	-	-	1.9	-	-	-	0.2	0.1	-	-
Terpinen-4-ol	1167	0.3	0.3	2.0	1.4	0.1	-	-	0.4	0.1	0.1	2.2
α-Terpineol	1178	0.2	t	0.2	2.1	0.2	t	-	0.5	t	-	0.2
Thymol	1284	0.5	-	-	-	-	-	-	-	0.5	-	-
Bornyl acetate	1301	0.1	-	-	-	-	-	-	0.4	0.3	-	-
ST		9.3	34.9	25.8	32.6	18.2	74.9	89.5	65.2	14.7	57.3	61.5
STH		7.7	33.9	24.7	20.6	17.1	62.4	77.0	43.6	14.0	55.5	57.9
δ-Elemene	1337	-	0.8	1.7	-	-	8.8	6.4	0.4	-	0.7	1.1
α-Cubebene	1352	t	1.0	0.2	0.2	-	3.0	1.4	0.7	0.5	-	0.2
α-Copaene	1378	0.2	-	1.4	-	0.6	0.7	2.5	1.2	0.3	0.1	0.4
β-Cubebene	1387	0.1	1.7	t	1.0	0.5	t	t	-	-	t	t
β-Elemene	1388	0.2	4.3	1.3	-	0.8	0.3	4.6	2.2	0.6	1.3	1.5
α-Gurjunene	1411	-	-	0.2	-	-	0.8	3.0	-	-	-	0.4
β-Caryophyllene	1419	3.4	20.8	12.8	4.2	12.4	4.1	8.9	28.2	4.1	6.4	36.0
β-Gurjunene	1429	-	-	-	t	t	1.4	-	0.4	0.4	t	-
α-Bergamotene	1432	-	t	0.2	-	-	0.1	3.4	-	-	t	1.2
(E)-β-Farnesene	1445	t	0.1	-	-	-	t	-	-	0.2	4.2	-
(Z)-β-Farnesene	1448	-	t	-	0.1	0.1	-	1.2	0.5	-	0.1	-
α-Humulene	1452	0.1	-	1.3	0.9	0.2	3.7	6.2	2.0	1.2	0.1	3.5
Valencene	1458	0.1	-	-	-	t	-	-	0.9	0.5	-	-
Allo-Aromadendrene	1472	-	t	0.2	7.5	-	0.5	2.4	-	-	-	0.7
Germacrene D	1480	2.5	0.5	0.2	-	1.4	2.7	2.4	2.0	1.7	1.0	0.7
α-Selinene	1483	-	0.8	2.2	-	-	0.9	16.5	-	2.9	0.8	6.5
γ-Muurolene	1484	-	0.3	-	t	0.1	-	-	0.4	0.8	t	-

Table 2. Contd.

α-Curcumene	1485	-	1.0	-	0.9	-	1.4	-	-	-	2.5	-
β-Zingiberene	1488	-	-	-	0.8	-	-	-	-	-	1.6	-
Bicyclogermacrene	1490	-	-	-	0.9	-		-	-	-	-	-
α-Guaiene	1491	-	-	-	-	-	-	-	1.0	-	-	-
β-Selinene	1492	-	-	2.2	2.0	-	-	14.6	1.0	-	-	4.6
(Z,E)-α-Farsenene	1493	-	1.9	-	-	-	3.9	-	-	-	28.7	-
β-Bisabolene	1500	0.3	0.6	t	-	0.1	1.0	1.4	-	t	4.2	t
γ-Cadinene	1509	t	-	-	0.3	t	-	-	0.2	-	-	-
Calamenene	1514	-	0.1	0.2	-	-	t	0.9	0.4	-	-	0.7
δ-Cadinene	1516	0.1	-	0.6	0.9	0.5	2.0	1.2	1.0	0.3	0.2	0.4
(E,E)-α-Farsenene	1521	0.6	-	-	0.9	0.4	2.0	-	1.1	0.2	3.5	-
Germacrene B	1554	0.1	-	-	-	-	25.1	-	-	0.3	0.1	-
OCS		1.6	1.0	1.1	12.0	1.1	12.5	12.5	21.6	0.7	1.8	3.6
Elemol	1541	-	-	-	-	-	0.1	-	1.1	-	-	-
(E)-Nerolidol	1550	0.2	1.0	-	10.0	0.7	0.1	2.3	16.5	0.4	0.1	0.4
Spathulenol	1571	t	-	t	-	-	2.0	t	-	0.1	-	-
Caryophyllene oxide	1575	-	-	-	-	-	2.2	1.0	0.9	0.1	0.1	1.0
Humulene oxide	1595	-	-	-	-	-	-	0.8	-	-	-	-
Epi α-bisabolol	1618	1.4	-	-	0.4	-	4.5	-	0.3	0.1	-	-
Torreyol	1621	-		-	0.3	-	0.4	2.6	0.2	-	-	-
γ-Eudesmol	1630	-	-	-	0.4	-	0.5	-	1.8	-	1.5	-
T-Cadinol	1631	-	-	0.8	0.3	0.2	-	1.3	0.3	-	-	0.5
α-Cadinol	1645	t	-	0.1	0.6	0.2	2.7	0.6	0.5	-	0.1	0.8
(E,E)-Farnesol	1720	-	-	0.2	-	-	-	3.9	-	-	-	0.9
AC		-	-	-	1.5	-	2.3	-	1.7	-	10.9	-
Myristicine	1532	-	-	-	1.5	-	2.3	-	1.7	-	10.9	-
% of total identified		98.4	98.6	98.5	98.4	99.0	98.4	99.2	99.7	99.1	98.7	99.3

t (<0.1%) = trace. **RI** = Retention indices on DB-1-type column., **MT**: Monoterpenes, **MTH**: Monoterpene hydrocarbons, **OCM**: Oxygen-containing monoterpenes, **ST**: Sesquiterpenes, **STH**: Sesquiterpenes hydrocarbons, **OST**: Oxygen-containing sesquiterpenes, **AC**: Aromatic compounds.

rences of the essential oils content and composition between the leaves, lianas and fruits of these *Piper* may be attributed to the different plant tissues from which they were isolated.

The results of the bioassays (Table 3) suggest that essential oils from the three *Piper* species analysed exhibited different inhibition levels against *S. zeamais*. The percentages of mortality increased with oil doses. Volatile oil from *P. guineense* exhibited a strong activity (LD_{50} = 10.0 ± 0.3 µl/g) than those of *P. capense* (LD_{50} = 16.1 ± 0.6 µl/g). The pairwise comparisons of different doses of essential oils of *P. guineense* with corrected significance level using the sequential Bonferroni procedure (Table 4) show that they were significantly different ($P < 0.001 < \alpha' = 0.001$) from (A/C to A/K) and from (B/D to B/K). But no significant difference was observed for other comparison. The oil from *P. nigrum* showed a lower activity (LD_{50} = 26.4 ± 1.5 µl/g). Poudrox 5% used as a standard insecticide exhibited 100% mortality (significant differences with all other doses of oils from *P. capense* and *P. nigrum*; Table 4). As secondary metabolites contain in essential oils play an important role in plant resistance to insects (Prates et al., 1998), the results of this study suggest that the activity of these oils can be attributed to the monoterpenes (63.7 - 98.1%) found in the oils which appears to possess similar activities against all of the tested pest organisms. Similarly, essential oils from other Cameroonian plants and extract from *P. guineense* have been shown to possess high levels of insecticidal activity (Tapondjou et al., 2003; Ngamo et al., 2005; Asawalam et al., 2007). Our result lends some support to the empirical use of the grain powder from these plants as grain protectants by small scale farmers in rural area of Cameroon.

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Table 3. Insecticidal activity of the essential oils from the fruits of *P. capense, P. guineense* and *P. nigrum* and positive control.

Test group		Dose (µl/g of grain)	Insecticidal activity		
			P. capense	***P. guineense***	***P. nigrum***
			Percentage of mortality		
Experimental	A	5	10.0±0.5	20.0±1.0	0.0±0.0
	B	10	25.3±0.7	50.0±1.0	6.7±0.5
	C	15	46.9±1.1	76.7±1.5	17.0±0.6
	D	20	63.5±0.8	100.0±0.0	27.0±1.5
	E	25	70.1±0.9	100.0±0.0	40.1±2.0
	F	30	78.3±1.8	100.0±0.0	56.7±3.5
	G	35	87.0±1.1	100.0±0.0	57.0±3.0
	H	40	100.0±0.0	100.0±0.0	70.0±3.0
	I	45	100.0±0.0	100.0±0.0	78.8±1.5
	J	50	100.0±0.0	100.0±0.0	100.0±0.0
Positive control (Poudrox 5%)	K	/	100.0±0.0	100.0±0.0	100.0±0.0
			LD_{50} (µl/g of grain)		
			16.1±0.6	10.0±0.3	26.4±1.5

Table 4. Statistical pairwise comparisons of different group (doses of oils) with corrected significance level using the sequential Bonferroni procedure.

Comparisons	***P. capense***		***P. guineense***		***P. nigrum***	
	α'	**P-values**	**α'**	**P-values**	**α'**	**P-values**
A/B	0.005	0.180 ns	0.002	0.029 ns	0.008	0.491 ns
A/C	0.002	0.003 ns	0.001	<0.001*	0.003	0.052 ns
A/D	0.001	<0.001*	0.001	<0.001*	0.003	0.046 ns
A/E	0.001	<0.001*	0.001	<0.001*	0.002	<0.001 *
A/F	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
A/G	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
A/H	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
A/I	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
A/J	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
A/K	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
B/C	0.004	0.179 ns	0.002	0.059 ns	0.007	0.423 ns
B/D	0.002	0.008 ns	0.001	<0.001*	0.003	0.079 ns
B/E	0.001	0.001*	0.001	<0.001*	0.002	0.005 ns
B/F	0.001	<0.001*	0.001	<0.001*	0.002	<0.001 *
B/G	0.001	<0.001*	0.001	<0.001*	0.002	<0.001 *
B/H	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
B/I	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
B/J	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
B/K	0.001	<0.001*	0.001	<0.001*	0.001	<0.001 *
C/D	0.006	0.299 ns	0.001	0.010 ns	0.010	0.532 ns
C/E	0.004	0.115 ns	0.001	0.010 ns	0.003	0.084 ns
C/F	0.003	0.032 ns	0.001	0.010 ns	0.002	0.003 ns
C/G	0.002	0.002*	0.001	0.010 ns	0.002	0.001*
C/H	0.001	<0.001*	0.001	0.010 ns	0.002	<0.001 *
C/I	0.001	<0.001*	0.002	0.010 ns	0.002	<0.001 *
C/J	0.001	<0.001*	0.002	0.010 ns	0.001	<0.001 *
C/K	0.001	<0.001*	0.001	0.010 ns	0.001	<0.001 *
D/E	0.012	0.784 ns	0.002	0.010 ns	0.006	0.412 ns

Table 4. Contd.

D/F	0.007	0.398 ns	0.002	0.010 ns	0.002	0.035 ns
D/G	0.003	0.071 ns	0.002	0.010 ns	0.002	0.018 ns
D/H	0.001	<0.001*	0.002	0.010 ns	0.002	0.002 *
D/I	0.001	<0.001*	0.003	0.010 ns	0.002	<0.001 *
D/J	0.001	<0.001*	0.003	0.010 ns	0.001	<0.001 *
D/K	0.001	<0.001*	0.003	0.010 ns	0.001	<0.001 *
E/F	0.010	0.771 ns	0.003	0.010 ns	0.005	0.301 ns
E/G	0.005	0.210 ns	0.003	0.010 ns	0.004	0.196 ns
E/H	0.001	0.001*	0.003	0.010 ns	0.003	0.037 ns
E/I	0.002	0.001*	0.004	0.010 ns	0.002	0.008 ns
E/J	0.002	0.001*	0.004	0.010 ns	0.001	<0.001 *
E/K	0.001	0.001*	0.004	0.010 ns	0.001	<0.001 *
F/G	0.008	0.506 ns	0.005	0.010 ns	0.050	1.000 ns
F/H	0.002	0.010 ns	0.005	0.010 ns	0.016	0.614 ns
F/I	0.002	0.010 ns	0.006	0.010 ns	0.004	0.170 ns
F/J	0.002	0.010 ns	0.007	0.010 ns	0.002	<0.001 *
F/K	0.002	0.010 ns	0.007	0.010 ns	0.002	<0.001 *
G/H	0.003	0.112 ns	0.008	0.010 ns	0.012	0.589 ns
G/I	0.003	0.112 ns	0.010	0.010 ns	0.005	0.267 ns
G/J	0.003	0.112 ns	0.012	0.010 ns	0.002	<0.001 *
G/K	0.003	0.112 ns	0.012	0.010 ns	0.002	<0.001 *
H/I	0.015	1.000 ns	0.016	0.010 ns	0.025	0.771 ns
H/J	0.016	1.000 ns	0.025	0.010 ns	0.002	0.002 *
H/K	0.025	1.000 ns	0.025	0.010 ns	0.002	0.002 *
I/J	0.050	1.000 ns	0.050	0.010 ns	0.002	0.010 ns
J/K	0.500	1.000 ns	0.050	0.010 ns	0.002	0.010 ns

* = Significant differences: $P < \alpha'$; ns = not significant.

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