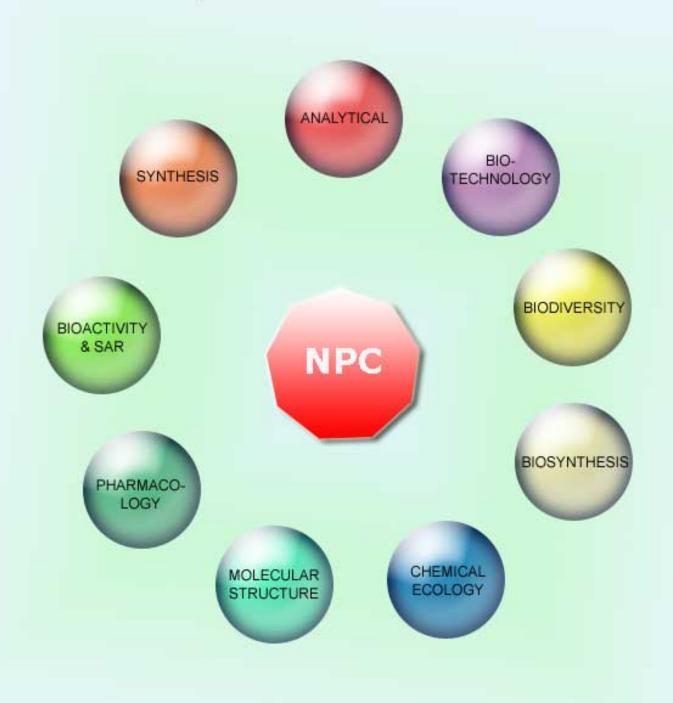
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Chemical Composition, Insecticidal Effect and Repellent Activity of Essential Oils of Three Aromatic Plants, Alone and in Combination, towards *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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Essential oils of aromatic plants with insecticidal properties are nowadays considered as alternative insecticides to protect stored products from attack by insect pests. A combination of some of these plants in the granaries is a current practice in certain localities of northern Cameroon. The aim of the present work was to analyze the impact of the combinations of the essential oils of *Vepris heterophylla* (Rutaceae), *Ocimum canum*, and *Hyptis spicigera* (both Lamiaceae), the three most used local aromatic plants because of their insecticidal activity and their repellent effect on *Sitophilus oryzae*. The present work revealed that these plants are rich in monoterpenoids. The GC/MS analyses have shown that monoterpenoids represented 65.5% for *H. spicigera*, 92.1% for *O. canum* and 47.0% for *V. heterophylla*. The crude essential oil of *O. canum* was the most insecticidal with a LD₅₀ of 42.9 ppm. The most repellent effect was obtained by a combination of the essential oils of *H. spicigera* and *O. canum*, with a repellent percentage at 77.5%. These results suggest a suitable strategy for pest management of stored products.

Key words: Aromatic plants, combination, essential oils, repellent effect, stored products.

In northern Cameroon, the most important insect grain pests are Sitophilus zeamais and S. oryzae (Coleoptera: Curculionidae), Callosobruchus maculatus (Coleoptera: Bruchidae) and Tribolium (Coleoptera: Tenebrionidae) castaneum Smallholders lose up to 80% of their stock each year because of insects [2]. To prevent the losses, producers usually rely on a relish of chemical insecticides. These tools, used frequently and abusively, consequently result in pollution of the environment and intoxication of consumers. There is, therefore, an urgent need to develop user-friendly storage methods with minimal adverse effects on the environment and on consumers. Essential oils of aromatic plant that have insecticidal properties could be considered as alternative insecticides [3,4]. These oils are volatile with high insecticidal efficiency and very low persistence. Most of the active compounds of the essential oil are specific to particular insect groups and not to mammals [5], and, therefore, should be considered in pest management strategies. One of the most important qualities of aromatic plants is their odors, which confer them their repellent effects. To maximize the effects of these

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Table 1: Yields of essential oil from 3 aromatic plants of Northern Cameroon.

Aromatic plant	Part collected	Yield (%)
Vepris heterophylla	leaves	$5.8 \pm 1.2a$
Ocimum canum	Leaves and flowers	$3.3 \pm 0.9a$
Hyptis spicigera	flowers	$1.7 \pm 0.2a$
Chi square		2.4 (df=2)

The yields followed by the same letter do not differ significantly (p<0.01)

Table 2: Major components of the three essential oils.

	Vepris	Ocimum	Hyptis
Compounds	heteropylla	canum	spicigera
α-Thujene		0.2	0.5
α-Pinene	0.2	2.1	9.1
β-Pinene		8.8	5.7
Sabinene	17.3		
Myrcene	1.9	1.6	
Cymene (p/o)	0.2		
Limonene	4.0	49.2	
(E)-β-ocimene	10.2		
γ-Terpinene	0.7		
1,8-Cineol			24.5
Terpinolene	1.4		
Linalool	0.9		8.4
Sabinol			1.1
Terpinen-4-ol	1.5		4.7
α-Terpineol	1.2		8.3
Safrole	3.0		
(E)-Caryophyllene	2.3	8.6	22.2
Carvacrol			1.9
Germacrene D	1.6		
γ-Amorphene	0.4		
δ-Cadinene	3.2		
Elemene		3.2	1.2
Elemol	19.4		
Guaiol	15.2		
Humulene epoxide II	1.6		
α-Eudesmol + Valerianol	1.1		

plants, farmers in the past utilized many of them in the same granary. This present work investigates the insecticidal and repellent efficiency of three local aromatic plants, *Vepris heterophylla* (Engl.) Letouzey (Rutaceae), *Ocimum canum* Sims (Lamiaceae), and *Hyptis spicigera* Lam. (Lamiaceae), frequently used alone and in combination.

The essential oil yields obtained ranged from 1.7 to 5.8% (Table 1). Flowers of *H. spicigera* produced less essential oil than the leaves of *V. heterophylla* and *O. canum*.

The GC/MS analyses of each of the three essential oils showed that they contain abundant monoterpenes (Table 2): 65.5% for *H. spicigera*; 92.1% for *O. canum* and 47.0% for *V. heterophylla*. The amount of sesquiterpenes observed was also different between the essential oils. That of *V. heterophylla* had the highest percentage, 51%, and that of *O. canum* the lowest, 7%. The most abundant active compounds in these essential oils differed from one oil to another. Thus, 49.2% of *O. canum* was composed of limonene, 8.8% of α -pinene and 3.2%

Table 3: Chemical composition of combinations of the essential oils.

Compounds	Vh + Oc	Oc + Hs	Vh + Hs
α-Pinene		4.3	4.5
β-Pinene		6.1	2.6
Sabinene	8.6		
Myrcene	2.1		
Limonene	27.6	26.2	
(E)-β-Ocimene	5.2		5.7
1,8-Cineol		14.4	11.8
Linalool		54.1	3.0
Terpinen-4-ol		2.9	2.1
α-Terpineol		4.1	
Safrole	1.6		
(E)-Caryophyllene	5.4	17.8	
Elemene		1.3	
Elemol	9.5		10.2
Guaiol	7.8		9.8

of elemene. The essential oil of H. spicigera had two main components, 1,8-cineol (24.0%) and (E)-caryophyllene (22.2%). Other active compounds found in this essential oil were α -pinene (9.1%), β -pinene (5.7%), α -terpineol (8.3%) and linalool (8.4%). The essential oil of V. heterophylla contained elemol (19.4%), sabinene (17.3%), (E)- β -ocimene (10.6%), guiaol (15.3%), limonene (4.0%), (E)-caryophyllene (2.3%) and additional compounds such as myrcene and terpinolene.

The chemical composition of combinations of essential oils (Table 3), as expected, represent averages of the percentages of each of the components in the individual oils. The LD_{50} values obtained for each of the essential oils, as well as their combinations, are presented in Table 4. The most active essential oil, with the lowest LD_{50} value, was that of *O. canum* oil.

The insecticidal activity of an essential oil depends on its chemical composition and the sensitivity of the target pest to the active compounds [6]. The essential oil of *O. canum*, which is the most toxic, contains 49% limonene, according to the GC/MS analysis. It has been shown that limonene is highly toxic to Coleopterans [7]. All the essential oils tested showed remarkable insecticidal activity, the least active of which was *Vepris heterophylla* with an LD₅₀ of 349.8 ppm. *H. spicigera* oil showed a high concentration of 1,8 cineol (24.5%) and (*E*)-caryophyllene (22.2%).

These compounds, along with α -phellandrene, terpinolene, and (+)-limonene have shown high toxicity towards S. oryzae [8]. The insecticidal efficiency observed is due to both major and minor components of each active oil [4,7-9]. These synergistic effects could explain the differences between observed LD₅₀ values and what would be expected based on average activities of the individual

Table 4: Insecticidal activity (LD_{50}) of the three essential oils and their combinations towards *Sitophilus oryzae*.

	LD ₅₀ ((ppm)	
Plant species	Observed	Expected	CHI ²
Hyptis spicigera	112.0		
Ocimum canum	42.9		
Vepris heterophylla	349.8		
Hyptis + Ocimum	75.8	77.5	0.017 ns
Hyptis + Vepris	182.1	230.9	5.76*
Ocimum + Vepris	103.8	196.0	28.3***

Table 5: Duration of insecticidal potency of the essential oils tested alone and in combination towards *Sitophilus oryzae*.

	LD_{50}			
Plant species	Observed	Expected	CHI ²	
Hyptis spicigera	6h 2 min			
Ocimum canum	5h 4 min			
Vepris heterophylla	14h 5 min			
Hyptis +Ocimum	4h 2 min	5h 5 min	16.8***	
Hyptis + Vepris	13h 4 min	10h 5 min	18.7***	
Ocimum + Vepris	7h 4 min	10h 2 min	23.5***	

Table 6: Insect repellent activity of the essential oils tested alone and in combination towards *Sitophilus oryzae*.

	Repellent rate (M	cDonald class)	
Plant species	Observed	Expected	CHI^2
Hyptis spicigera	62.5 (IV)		
Ocimum canum	33.7 (II)		
Vepris heterophylla	42.5 (III)		
Hyptis +Ocimum	77.5 (IV)	48.1 (III)	6.9***
Hyptis + Vepris	41.2 (III)	52.5 (III)	1.3 ns
Ocimum + Vepris	62.5 (IV)	38.1 (II)	9.5***

essential oils (Table 4). This synergistic effect has already been demonstrated between essential oils of five aromatic plants used in north Cameroon [10].

The activity of the essential oils decreased with time due to their high volatility, although the decrease was not the same for the three oils tested (Table 5). Those oils with a high proportion of hydrocarbon components lost their activity more rapidly than those composed mainly of oxygenated compounds [4,11].

The essential oil that exhibited the most repellent activity was *H. spicigera*, with a repellent percentage (RP) of 62.5% (Table 6). The least repellent oil, however, was O. canum, which had an RP of 33.7%. For the essential oil combinations, Hyptis + Ocimum was the most repellent (RP >77%), whereas the combination was expected to have an RP of 48%. The synergy between O. canum and H. spicigera has increased their repellent effects. Comparable results were observed for O. canum + V. heterophylla. The repellent effect of V. hetrophylla has previously been shown on S. oryzae. [8]. Leaves of V. heterophylla, H. spicigera and O. canum are used in traditional medicine against diseases and as purgatives. Their use in combinations in granaries could prove to be beneficial to prevent attack of post harvest insect pests.

Experimental

Plant collection: Leaves of V. heterophylla and flowers of O. canum were collected at Maroua, far north of Cameroon (10° 39.214' N, 14° 24.145' E, 375 m elevation). Flowers of H. spicigera were collected near the campus of the University of Ngaoundéré (7° 25.609' N, 13° 33.549' E, 1100 m elevation). These data were recorded with a GPS Garmin Geko 301. The collection of all plant materials was made in December 2005. After collection, the plant material was dried in the shade under laboratory conditions for 24 h, cut in pieces, weighed, and hydrodistilled for 4 h using a The essential oils Clevenger-type apparatus. obtained were stored at 4°C until their use for the bioassays.

GC/MS chemical analysis: GC/MS analysis utilized an HP-5MS column (5% phenyl methyl siloxane), 30 m long and 250 μm in diameter. The carrier gas was helium; the temperature program applied was from 40°C to 230°C at a rate of 5°C/min and then maintained at 230°C for 5 min. The pressure of the carrier gas was 49.9 KPa with a flux of 74.1mL/min. The ion-source temperature was 230°C and the ion scan range was 50-350 amu. The mass spectrum of each compound was compared with those of the Wiley 275 L library [12,13].

Insects: Insects used for the test were reared in the *in vivo* collection at the Storeprotect laboratory at the University of Ngaoundéré in Cameroon. They were derived from a strain collected in November 2003 from a granary in Beka hosséré (Ngaoundéré, Cameroon).

Insecticidal activity: In preliminary tests, several doses were chosen between those having no killing effect on the experimental population to the minimal one killing 100% of this population, in order to establish the LD₅₀ of each essential oil. With a micropipette (Rainin Magnetic-assist), the precise volume of essential oil was added to acetone and diluted to 5 mL. From this, 0.5 mL of solution was uniformly applied to a 9 cm disk of filter paper (Whatman N°1) and placed in a Petri dish. Twenty adult insects, less than one month old, were introduced into the dish 5 min later and the dish was covered. A control with acetone alone, was made. For each preparation, 5 replications were made. The number of dead insects was determined 24 h after the application.

Insect repellent activity: Repellent effects of essential oils and their combinations were evaluated at doses of 0.031, 0.062, 0.125, and 0.251 μ L/cm². The test was conducted in a 9-cm diameter Petri dish in which two half circles of filter paper were introduced. One half was treated with either essential oil or a combination of essential oils, while the second half was treated with acetone. Twenty insects were placed in the middle of the Petri dish and, after two h, the distribution of insects on each part of the

paper was noted. The repellent percentage of the different oils, their combinations and the class were calculated according to the McDonald formula [14,15].

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Volatile Constituents of <i>Calamintha origanifolia</i> Boiss. Growing Wild in Lebanon Carmen Formisano, Daniela Rigano, Francesco Napolitano, Felice Senatore, Nelly Apostolides Arnold, Franco Piozzi and Sergio Rosselli	1253
Essential Oil from <i>Chenopodium ambrosioides</i> as a Promising Antileishmanial Agent Lianet Monzote Fidalgo	1257
Selective Cytotoxic Activities of Leaf Essential Oils from Monteverde, Costa Rica Debra M. Moriarity, Anita Bansal, Ramona A. Cole, Sayaka Takaku, William A. Haber and William N. Setzer	1263
Chemical Composition of Leaf Essential Oil of <i>Hedyosmum arborescens</i> and Evaluation of Its Anticancer Activity Muriel Sylvestre, André Pichette, Angélique Longtin, Marie-Anna Couppé De Ker Martin, Sylvie Rodin Bercion and Jean Legault	1269
Volatile Leaf Constituents and Anticancer Activity of <i>Bursera simaruba</i> (L.) Sarg. Essential Oil Muriel Sylvestre, André Pichette, Angélique Longtin and Jean Legault	1273
Antibacterial and Cytotoxic Activity of Nepeta cataria L., N. cataria var. citriodora (Beck.) Balb. and Melissa officinalis L. Essential Oils Ulrike Suschke, Frank Sporer, Jürgen Schneele, Heinrich Konrad Geiss and Jürgen Reichling	1277
Chemical Composition, Antiradical and Antifungal Activities of Essential Oil of the Leaves of <i>Cinnamomum zeylanicum</i> Blume from Cameroon Pierre M. Jazet Dongmo, Léopold N. Tatsadjieu, François Tchoumbougnang, Modeste L. Sameza, Bernadin Ndongson Dongmo, Paul H. Amvam Zollo and Chantal Menut	1287
Antifungal and Anti-insect Activities of Three Essential Oils on Aspergillus flavus Link and Sitophilus zeamais Motsch Leopold N. Tatsadjieu, Martin B. Ngassoum, Elias N. Nukenine, Augustin Mbawala and Aoudou Yaouba	1291
Review /Account	
Biological Activities of Selected Essential Oils Lawrence. A. D. Williams, Roy B. Porter and Grace O. Junor	1295
Antifungal Activity of the Volatile Phase of Essential Oils: A Brief Review Heather M. A. Cavanagh	1297
The Medicinal Use of Essential Oils and Their Components for Treating Lice and Mite Infestations	1202
Elizabeth M. Williamson A Review of Aromatic Herbal Plants of MedicinalImportance from Nigeria Isiaka A. Ogunwande, Tameka M. Walker and William N. Setzer	1303 1311
The Biology of Essential Oils in the Pollination of Flowers Leland J. Cseke, Peter B. Kaufman and Ara Kirakosyan	1317

Natural Product Communications 2007

Volume 2, Number 12

Contents

Original paper	<u>Page</u>
Composition and Antinociceptive Activity of the Essential Oil from <i>Protium heptaphyllum</i> Resin Vietla S. Rao, Juliana L. Maia, Francisco A. Oliveira, Thelma L.G. Lemos, Mariana H. Chaves and Flavia A. Santos	1199
Cruzain Inhibitory Activity of Leaf Essential Oils of Neotropical Lauraceae and Essential Oil Components William N. Setzer, Sean L. Stokes, Ashley F. Penton, Sayaka Takaku, William A. Haber, Elizabeth Hansell, Conor R. Caffrey and James H. McKerrow	1203
Cruzain Inhibitory Activity of the Leaf Essential Oil from an Undescribed Species of <i>Eugenia</i> from Monteverde, Costa Rica Sean L. Stokes, Ramona A. Cole, Mariana P. Rangelova, William A. Haber and William N. Setzer	1211
Biological Activities of Essential Oils from Monteverde, Costa Rica Jennifer Schmidt Werka, Amelia K. Boehme and William N. Setzer	1215
Composition and Antibacterial Screening of the Essential Oils of Leaves and Roots of Espeletiopsis angustifolia Cuatrec Gina Meccia, Luis B. Rojas, Judith Velasco, Tulia Díaz and Alfredo Usubillaga	TY 1221
GC-MS Analysis of the Leaf Essential Oil of <i>Ipomea pes-caprae</i> , a Traditional Herbal Medicine in Mauritius Daniel E.P. Marie, Brkic Dejan and Joëlle Quetin-Leclercq	1225
Chemical Composition, Insecticidal Effect and Repellent Activity of Essential Oils of Three Aromatic Plants, Alone and in Combination, towards Sitophilus oryzae L. (Coleoptera: Curculionidae) Martin B. Ngassoum, Leonard S. Ngamo Tinkeu, Iliassa Ngatanko, Leon A. Tapondjou, Georges Lognay, François Malaisse and Thierry Hance	SI ₁₂₂₉
Chemical Composition and Larvicidal Activity against Aedes aegypti of Essential Oils from Croton zehntneri Hélcio S. Santos, Gilvandete M. P. Santiago, João P. P. de Oliveira, Angela M. C. Arriaga, Délcio D. Marques and Telma L. G. Lemos	1233
Composition and Larvicidal Activity of Essential Oil from Stemodia maritima L. Angela M. C. Arriaga, Francisco E. A. Rodrigues, Telma L. G. Lemos, Maria da C. F. de Oliveira, Jefferson Q. Lima, Gilvandete M. P. Santiago, Raimundo Braz-Filho and Jair Mafezoli	1237
Cytotoxic Leaf Essential Oils from Neotropical Lauraceae: Synergistic Effects of Essential Oil Components Brenda S. Wright, Anita Bansal, Debra M. Moriarity, Sayaka Takaku and William N. Setzer	1241
Chemical Composition and Antibacterial Activity of the Essential Oil of <i>Baccharis latifolia</i> Pers. and <i>B. prunifolia</i> H. B. &K. (Asteraceae) Janne Rojas, Judith Velasco, Luis B. Rojas, Tulia Díaz, Juan Carmona and Antonio Morales	1245
Biological Activity and Composition of the Essential Oil of <i>Tetrataenium nephrophyllum</i> (Apiaceae) from Iran Ali Sonboli, Mohammad Reza Kanani, Morteza Yousefzadi and Mehran Mojarrad	1249