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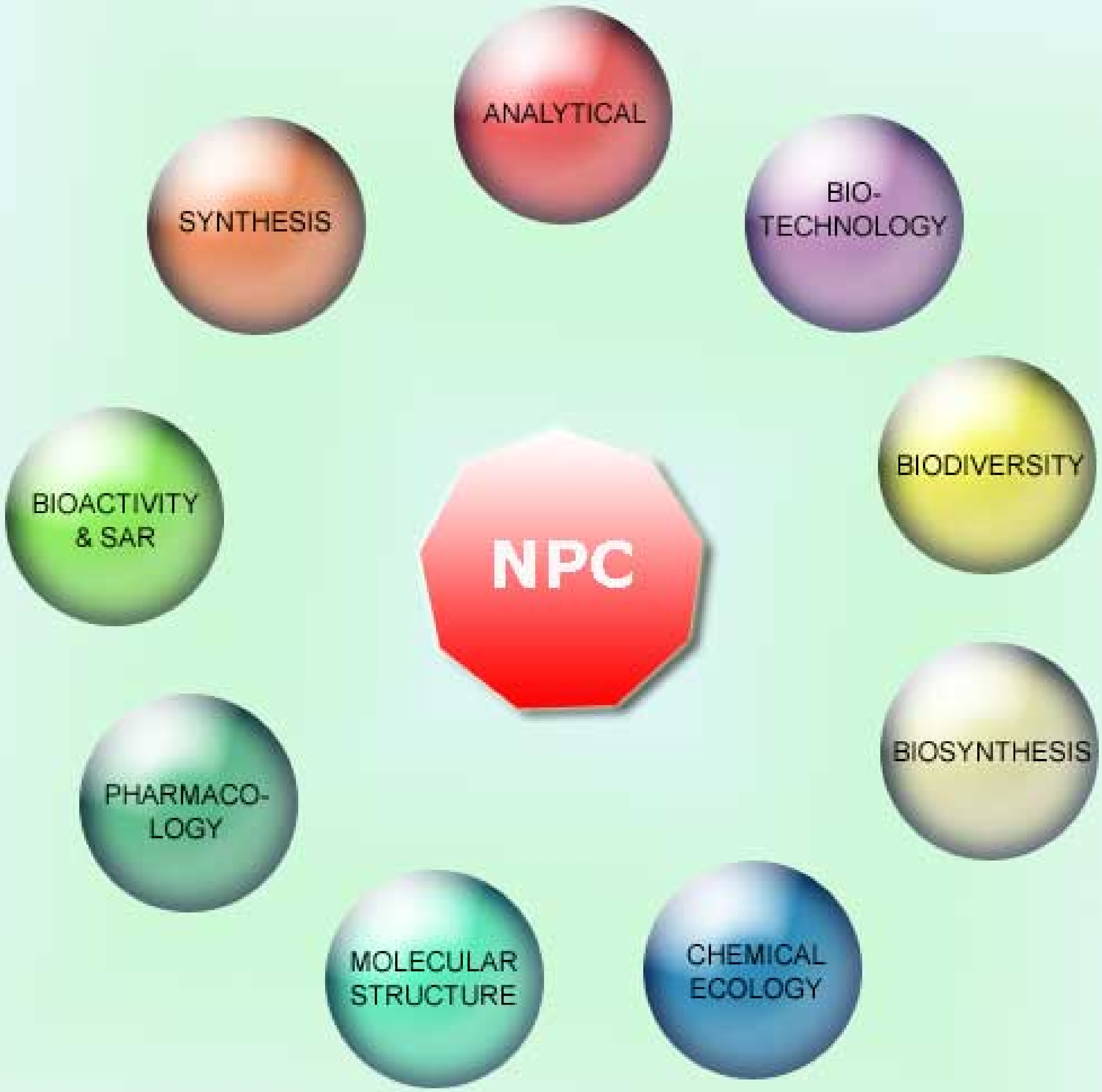

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Chemical Composition, Insecticidal Effect and Repellent Activity of Essential Oils of Three Aromatic Plants, Alone and in Combination, towards *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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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 LD_{50} 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 castaneum* (Coleoptera: Tenebrionidae) [1]. 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

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

Aromatic plant	Part collected	Yield (%)
Vepris heterophylla	leaves	5.8 ± 1.2a
Ocimum canum	Leaves and flowers	3.3 ± 0.9a
Hyptis spicigera	flowers	1.7 ± 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.

Compounds	*Vepris heteropylla*	*Ocimum canum*	*Hyptis 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. camum* 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% 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.

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

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 LD_{50} 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 LD_{50} 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 (McDonald 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 Clevenger-type apparatus. The essential oils 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 LD_{50} 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 µL/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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