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Investigations on Both Chemical Composition and Insecticidal Activities of Essential Oils of *Vepris heterophylla* (Rutaceae) from Two Localities of Northern Cameroon Towards *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract: Leaves of *Vepris heterophylla* (Rutaceae) are used traditionally as medicine and in crop protection for the reduction of post harvest losses due to insect pests. The present research aims to investigate the chemical composition, the toxicity and the repellent activities of the essential oil leaves of *V. heterophylla* collected in 2 localities (Mokolo and Meri). The research pointed out that, the 2 essential oils differ in their chemical composition. Sabinene and E-β-ocimene are the most common important compounds. Sabinene is particular to the essential oil from Meri (19.1%). E-caryophyllene, ą-humulene, elemol and germacrene, are the comon lowest compounds. E-carryophyllene (3.1%) and safrole (3%) are active compounds more present in the essential oil from Mokolo. Evaluation of the insecticidal properties of these 2 oils pointed out that the volume of essential oil killing half of the insect population tested (VL50) is not the same for the 2 oils. That from Mokolo is 49.44 μL from Meri is the highest, 61.2 μL. This useful plant is also cited as endangered, it is therefore an emergency to search strategy of exploitation that preserves this important component the local biodiversity.

Key words: *Vepris heterophylla, tribolium castaneum*, essential oil, Cameroon, toxicity, repellence

INTRODUCTION

In tropical countries, plants have been used and are still used to prevent post harvest losses. Most of these botanicals are used as insecticides or repellent agent (Boeke, 2002). However, the liberalisation of the insecticide market increases the availability of synthetic products for untrained farmers. One of the most important consequences of this is the loss of traditional knowledge on the use of botanicals in crop protection (Szafranski, 1991).

Important damages occur in untreated crops and the farmers are obliged to sell them at low price if possible. Stored products are treated many times during the conservation till the consumption or the selling. Many pests of stored products are Coleopterans and the most damaging species are from *Sitophilus* and *Tribolium* genus (Dal Bello *et al.*, 2001) for cereals. In developing countries as in Cameroon, many synthetic insecticides are used to prevent post harvest losses (Ngamo, 2004). In most of the researches, they seem not to be used properly, consumers are therefore exposed to intoxication by their residues.

Moreover, synthetic insecticides are subject to dynamic trends. Target insects are able to develop resistance against single insect pesticide (Schmutterer and Ascher, 1986; Boeke, 2002). This can also enhance the development of non target pests, allowing a secondary pest in the past to be an important one with high tolerance towards popular pesticides. *T. castaneum* occurs in all infested grain during storage and is one of the most resistant pest to treatment.

The use of botanicals in crop protection may be a solution to prevent the environment and the consumer from the effects of the synthetic pesticides (Tapondjou *et al.*, 2002; Regault-Roger and Hamraoui, 1995). One of the most used plants to protect grains in northern Cameroon is *Vepris heterophylla* (Engl.) Letouzey. This plant is not regularly distributed on the area, it occurs in patches from place to place. Its habitat

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is at the sides of mountains, where it is not as present in sub Saharan Africa as observed in the past time (Geerling, 1982), this plant is signaled today in the UICN redlist as endangered plant with the reference EN A1c, B1+ 2c.

Botanicals produce secondary compounds which are concentrated in their essential oils. Their composition depends on many factors like the phenology of the plant and the ecological conditions. These factors influence the quantity and the quality of the essential oil and could explain the difference observed in activities of essential oils from different localities.

The present research combines investigations on the chemical composition of essential oils from 2 localities in northern Cameroon against *T castaneum*, in order to come to a user friendly method of protection of crops from the attacks of resistant pest.

MATERIALS AND METHODS

Production of essential olis

Sampling sites: Meri and Mokolo are located in the dry savana, where only 900 to 400 mm of rain are observed during the year. The dry season can be in seven months period. The mean temperature for the year is 28±7°C. In each of the towns Meri, Mokolo, a patch of *V. heterophylla* was located and the leaves cut. Meri and Mokolo are in the Far North province, in the dry savana. The dry season is observed only during five months. In the Far North, peasant are mostly farmers cultivating sorghum, maize cowpea and others staple crops. *V. heterophylla* is known as a very important post harvest botanical insecticide.

Extraction of essential oils: The leaves of the plants of patch were collected, taken to the laboratory and dried without sun light at ambient temperature during 4 days. The essential oils were extracted by hydro distillation during 4 h using a Clevenger type apparatus. The oil obtained were collected and kept in brown bottles at 4 °C until use for bio assays or for analysis.

Chemical analysis of the essential oils GC/MS and GC/FID: The GC/MS was done with HP-5MS column (5% phenyl methyl siloxane) with 30m length and 250 μm in diameter. The carrier gas was helium, the temperature program applied was from 40 to 230°C with a speed of 5°C min^{-1} with a stay at 280°C during 5 min. The pressure of the carrier gas was 49.9 KPa and the flux at 74.1 mL min^{-1}. The ion-source-eating at 230°C, the ion scan range was 50-350 amu. Mass spectra correlations for the identification of the compounds were done using Wiley 275 L, (Joulain and König, 1998; Adams Robert, 2001). After this qualitative analysis, the quantitative analysis was made using the GC/FID procedure in similar conditions.

Bio assays: A strain of *T. castaneum* associated with infected grains collected from farmer's granaries and reared in an incubator at 30 °C since 1999 was used for the tests. For the assays, only one-month aged adults were used.

Toxicity: Insecticidal activity of any oil was accessed through a contact and inhalation test. For each essential oil, precise volumes ranking from 0.5 to 3.2 mL were tested. The oil was diluted in 10 mL of acetone. For each trial, 0.5 mL of the preparation was deposited on a 9 cm diameter filter disk (Whatman n°1) put in a petri disk of 80 mL, 5 min after application, evaporation of the acetone was completed. Once acetone was evaporated, 20 *T. castaneum* were introduced in the petri disk, each one covered and sealed with parafilm. For each preparation, 5 replications were made. The control was the same volume of acetone without addition of essential oil. The mortality of insects was recorded 24 h after the treatment. Dose-response curves were analysed by the log-probit method. The mortalities observed were compared to that of control with an ANOVA test.

Repellence: The repellent activity of the essential oils was tested by using a linear olfactometer made of 30 cm glass tube having 2 cm diameter with a hole at its middle. At each end a small container was place with 2 g of flour of *Sorghum bicolour*. One contained a piece of filter paper with essential oil dilute in acetone and the control in the other hand had only acetone.

The hole in the middle was used to introduce insects there after it was covered with gauze. The choice of insects was observed for a period of one hour. Only the insect within the flour in either end were considered to have made a choice. Insect used was one month old. A group of 10 insects was used for each trial five replications were made.

The attractiveness via the Percentage of Repellence (PR) was evaluated according to a formula from Talukder and Howse (1993, 1995); Liu and Ho (1999).

PR = 2 x (C-50) (where C = Percentage of insects choosing the untreated end or the control). If PR>0 the oil is repellent and if PR<0 the oil is attractive.

RESULTS AND DISCUSSION

Yield and chemical composition of the essential oils: The yield of essential oil obtained by hydro distillation was not the same for all the dried leaves of *V. heterophylla* from the 2 regions sampled. The production of essential oil is important with *V. heterophylla* leaves from Mokolo (1.12%) (Table 1) Those of Meri produced 0.65% of essential oil.

Table 1: Production of essential oil in relationship with the origin of the plant material

Sampling site	Amount of samples	Yield	SD
Meri	4	0.65 ^{b}	0.19
Mokolo	3	1.12 ^{a}	0.61

In the same column, the values followed by the same letter do not differed significantly (p = 0.001)

Table 2: Chemical composition of essential oils of *Vepris heterophylla* from northern Cameroon

		% in essential oil of each locality	
Compounds	IK	Mokolo	Meri
A-thujene	930	0.4	0.4
A-pinene	938	0.1	0.1
Sabinene	977	14.0	19.1
β-pinene	982	0.3	0.3
Myrcene	992	4.2	2.8
A-phellandrene	1009	0.1	tr
A-terpinene	1021	0.8	0.5
Cymene (p/o)	1028	0.5	0.2
Limonene	1033	4.3	4.1
Z-β-ocimene	1039	0.7	0.5
E-β-ocimene	1049	14.0	9.2
γ-terpinene	1063	1.3	0.9
Terpinolene	1093	1.6	1.3
Allo-ocimene	1131	0.3	0.3
Pregeijerene	1288	0.2	0.2
Hydrocarbonated monoterpenes		42.8	40.1
Cis-sabinene hydrate	1072	0.11	0.3
Linalool	1100	0.9	0.6
Cis-p-menth-2-en-1-ol	1127	0.1	0.1
Trans-p-menth-2-en-1-ol	1145	tr	tr
Terpinen-4-ol	1184	3.3	2.1
A-terpineol	1196	1.7	1.2
Methyl salicylate	1201	0.2	
Safrole	1296	3.0	1.7
Neryl acetate	1364	tr	tr
Geranyl acetate	1395	tr	
Methyl eugenol	1406	0.3	
Methyl-n-methyl anthranilate		1407	tr
Oxygenated monoterpenes		9.7	6.2
Monoterpenes NI		tr	0.5
Bicycloelemene	1348	tr	tr
A-cubebene	1360	tr	tr
A-ylangene	1383	0.4	0.1
A-copaene	1389	0.3	0.1
β-bourbonene	1400	tr	tr
β-elemene	1402	0.8	0.6
E-caryophyllene	1438	3.1	1.7
β-copalene	1445	0.3	0.3
Alloaromadendrene	1451		0.1
γ-elemene	1457	tr	
A-humulene	1473	1.9	1.2
Acora-3(10).14-diene	1479	0.1	tr
γ-muurolene	1490	0.2	tr
Germacrene D	1499	3.4	2.3
Ledene	1504	0.1	tr
γ-amorphene	1512	0.4	0.4
Δ-amorphene	1517	0.1	tr
γ-cadinene	1521	0.1	tr
Δ-cadinene	1536	0.6	0.5
β-cadinene	1540	0.2	0.4
Germacrene D-4-ol	1593		tr
Hydrocarbonated sesquiterpenes		12.3	8.1
Cubebol	1531	0.16	0.25
Elemol	1562	14.37	23.99
Caryophillene oxide	1604	0.05	0.12
Guaiol	1613	12.85	13.47
Humulene epoxide II	1630	0.11	0.21
10-epi-γ-eudesmol	1641	0.11	0.07
γ-eudesmol	1650	1.26	0.61
a-muurolol	1658	0.08	0.08
β-eudesmol	1671	0.73	0.7
A-eudesmol + valerianol	1673	1.78	1.63
Bulnesol	1684	1.7	1.59
Oxygenated sesquiterpenes	33.20	42.72	
Sesquiterpenes NI		1.95	2.06

KI = Kovalt index; NI = non identified

These values indicate the low content of the 3 strains of *V. heterophylla* in essential oils. Even with this low yields observed, the range of the production of the essential oil is not the same for all these plants.

According to Fields *et al.* (2001), ecological factors like: water content in the soil, the quality and quantity of nutriments available, the luminosity, the temperature and relative humidity can influence the metabolism of the synthesis of essential oils. For Fields *et al.* (2001), Geographical factors is a significant parameter with respect to the essential production of oil and its composition (Azevedo *et al.*, 2002).

The samples studied contain merely equal composition in monoterpenes and sesquiterpenes. That from Mokolo contains 53.42% monoterpenes, the most important compounds being elemol (14.41%) and E-ßocimene (13.92%). That of Meri contains 52.90% of sesquiterpenes. Elemol (23.99%) and sabinene (19.11%) being most important compounds.

The essential oil of *V. heterophylla* from northern Cameroon contains mainly: Sabinene, E-ß ocimene, E-caryophyllene, α-humulene, germacrene and E-nerolidol (Table 2). The proportion of these compounds varies from a sample to another. Several chemotytes of *Hyptis suaveolens* and showed that differences among sesquiterpenes (Azevedo *et al.*, 2002) this come from the fact that the plants were from different latitude and altitude. Other differences were explained by the growing conditions of the plants as light quality and intensity, availability in water and nutrients (Schoonhoven *et al.*, 1998). These conditions may act on the accumulation of the secondary metabolites as it may be the case between plants from Meri and Mokolo. They seem to form 2 ecotypes of the plant in the soudano sahelian area of the Far North Cameroon.

Toxicity of the essential oils: The mortality of adult *T. castaneum* due to exposition to essential oil of *V. heterophylla* from 2 localities of northern Cameroon is shown the Table 3. The relationship between the volume of each essential oil and the insect mortality associated showed that, the volume of oil killing half of the insect population tested (VL50) for essential oil from Mokolo is 49.44 µL and that from Meri is the highest 61.2 µL. The required volume of essential oil to kill all *T. castaneum* also changed in relationship with the origin of plants. A

Table 3: Insecticidal effect of the essential oils of *Vepris heterophylla* from 2 localities of the northern Cameroon on *Tribolium castaneum*

Volume of the essential oil (µL)	Mortality (%) of adult *T.castaneum* Mokolo	 Meri
24	1±0	3.8±0.4
32	7.6±1.7	3±0
40	22.4±6.11	8.6±2.5
48	49±6.96	7.8±3.2
56	54.6±4.04	14.8±5.81
64	52.4±8.11	30.8±9.47
72	86±6.4	41.21±5.7
80	93±3.2	42.8±13.2
88	100±0	50.6±17.04
96	100±0104	67±13
112	100±0	80±8.54
120	100±0	82.6±9.6
VL50	49.44 µL^b	61.21 µL^b

In the same line the values followed by the same letter does not differed significantly ($p = 0.001$)

Table 4: Repellence of essential oil in relationship with the origin of the plant material toward *Tribolium castaneum*

Origin of the oil	PR (%)	Observation
Meri	- 32	Attractive
Mokolo	- 32	Attractive

maximum of 88 µL could kill 100% of the insect tested with oil from leaves collected at Mokolo. But with that of Meri, to kill all these insects, more than 120 µL of essential oil is required.

In general, results obtained by contact and inhalation test confirm the toxicity of *V. heterophylla* towards *T. castaneum.* The 2 oils tested contain toxic compound like limolene which is highly toxic to *T. castaneum* (Prates *et al.,* 1998). A variation of efficiency between chemotypes of *V. heterophylla* would be due to difference in the amount of compound present such as sabinene which is most present amongst the plants collected at Meri (19.1%) than in that collected at Mokolo (14.0%). Sabinene is toxic to insects (Park *et al*., 2003). Other differences are observed with compounds such as α-pinene, myrcene, α-terpinene toxic to insects especially *Callosobruchus maculatus* (Park *et al*., 2003) occur most in the oil from Mokolo which is the most toxic to the pest.

Repellent properties of the essential oils: The essential oil of *V. heterophylla* expressed attractiveness or repellence depending on the origin of the leaves used for the extraction. Those from Meri and Mokolo are repellent to *T. castaneum*. The percentage of the attractive is 32% (Table 4). The essential oil from Ngaoundéré is repellent to *T. castaneum* at 20%.

The essential oils are both attractive and toxic to *T. castaneum.* This observation is the same as that made with *Hyptis suaveolens* which is very attractive to *Callosobruchus maculatus* (Boeke, 2002).

Leaves of *V. heterophylla* are used in traditional medicine against rheumatism, malaria and also as anthelminthic, parasiticide or purgative. They are promising for their use in protection of grains during storage from the attack of post harvest insect pests. This useful plant is also endangered, it is therefore an emergency to search strategy of exploitation that preserve this important component the local biodiversity.

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