EFFECT OF SITE LOCATION AND COLLECTING PERIOD ON THE CHEMICAL COMPOSITION OF *HYPTIS SPICIGERA* Lam. AN INSECTICIDAL ESSENTIAL OIL FROM NORTH-CAMEROON

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ABSTRACT: *Hyptis spicigera* essential oil from seven localities in the North-Cameroon (Ngaoundere, Guirvidig, Kodeck, Lara, Toloum, Kaele, Tchecal-baila) was investigated by GC and GC/MS. Results showed differences within harvesting sites and between the different sites of collection but did not revealed clear tendencies in the evolution of essential oil composition as regard the sampling period. The main group of compounds in all the analyzed samples were: α-pinene (11.9%-42.1%), β-pinene + sabinene (6.0%-39.8%) and β-phellandrene + 1,8-cineole (8.8%-27.4%) except in one oil where β-caryophyllene (23.4%) was the principal component. The insecticidal activity of *H. spicigera* and its principal terpenic components was evaluated against the cowpea weevil *Callosobruchus maculatus* F., the major cause of damages of cowpea (*Vigna unguiculata* (L.) Walp) in North Cameroon..

Key words: *Hyptis spicigera*, Lamiaceae, Essential oil composition, α-pinene, α-phellandrene, β-caryophyllene, Insecticidal activity.

INTRODUCTION

Hyptis spicigera Lam. (Lamiaceae) is a strong aromatic, herbaceous annual plant of 0.5 to 1m high with vertical ramifications. Leaves are simple, opposite with 7 to 10cm long and 1 to 3 cm wide. Inflorescences are very small and are assembled in terminal dense ears of 2 to 10cm long (1). Fruits contain each one seed. Although endemic to Brazil, it is widespread in tropical Africa and in Asia where it grows naturally on road sides and on cultivated lands.

Hyptis spicigera is traditionally used as a drug, insecticide and even as a foodstuff (2). Infusions prepared with leaves are used against cough, bronchitis and headaches (11). Soaps, lotions, and perfumes made from the flowers, by people of northern Nigeria, are part of baths

or decoctions for the treatment of various skin diseases (2). In Central and eastern Africa, and also in Guinea, *H. spicigera* is cultivated and the oleaginous seeds are eaten like sesame. It is also recognized as a valuable bio-pesticide, because when mixed with grains (3g of dried leaves powder for 1kg of grains), *H. spicigera* exhibits strong insecticidal and repellent activities against insects devastating stored grains (3). Within a large ethnobotanical survey carried out in northern Cameroon, Ngamo and Mapongmetsem (personal communication, 2004) highlighted that *H. spicigera* is traditionally used by some ethnic groups (Guiziga, Massa, Mofu, Musgum, Mundang…) to protect cowpeas and sorghum against insects infestation. The insecticidal potentiality of *H. spicigera* has been emphasized by some authors (3, 4). Lambert et *al*. (3) managed to control the oviposition and the hatching of weevils by treatment of peas with alcoholic extracts of *H. spicigera*. Niber (4) showed the efficiency of slurries, made with leaves of *H. spicigera* and distilled water, against two major pests: *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and *Sitophilus oryzea* L (Coleoptera: Curculionidae).

There are not many data on the phytochemical characterization of *H. spicigera*. However, seven new labdanes were isolated and structurally characterized (5). One of these natural compounds (15,19-diacetoxy-2α,7α-dihydroxylabda-8(17),13(Z)-diene) significantly inhibited the larval growth of the european corn borer (*Ostrinia nubilalis* Hübner Lepidoptera: Pyralidae) (5). A spicigera δ-lactone (5,6-diacetoxy-1,2-epoxy-1,5'(2'-pentane-5'-olide)-3E-heptene) was isolated from the inflorescences of *H. spicigera* (6).

The chemical composition of *H. spicigera* essential oil published (7-12) suggests the existence of a β-Caryophyllene chemotype. It's found as major compound in oils from Burkina Faso (7) (57.3-65.7%), Nigeria (8) (67.6%) and Mali (9) in lower content (23.5-27.2%). Other compositions have also been reported (10) with α-pinene (43%) and β-pinene (15%) as main compounds. In Cameroon, Jirovetz *et al.* (11) found terpinolene (15.01-27.47%), sabinene (19.69-20.27%) and α-thujene (11.51-12.49%) as main compounds,

whereas Tchoumbougnang *et al.* (12) reported α-pinene 27.3%, β-caryophyllene 20.1%, limonene 13.4% and β-pinene 10.3%.

These papers dealing with the chemical composition of *H. spicigera* essential oil have been published on the basis of the analyses of selected samples from restricted harvesting sites. Therefore, it is noteworthy that no data are available on the effect of geographical location and sampling period on the essential oil composition. Information of this kind is very important when a standardized application of *H. spicigera* as bio-pesticide is of practical concern. Therefore the main target of the present work was to evaluate the variability of essential oil compositions in function of their origin and collecting period. The second part of the work has been designed to better understand the bio-pesticide properties of *H. spicigera* volatile compounds. The activities of the whole essential oil as well as its pure (commercially available) components alone or blended in proportions reflecting those reported for the natural extract has been measured against the cowpea weevil *Callosobruchus maculatus* F., the major cause of damages of cowpea (*Vigna unguiculata* (L.) Walp) in North Cameroon. The present study takes part of an ongoing research focusing on the use of local natural insecticides to limit damages to granaries stored products.

EXPERIMENTAL

Plant material: Dried flowers of *H. spicigera* were collected in seven loacilities of Northern Cameroon: Ngaoundere (H0), Guirvidig (H1), Kodeck (H2), Lara (H3), Toloum (H4), Kaele (H5) and Tchecal-baila (H6) (Figure1). Plant collection took place in May (at the beginning of the rainy season) and in January (during the dry season), except for H0 which was collected only in May. Voucher specimens were deposited at the National Herbarium of Yaounde (Voucher No. 70754/HNC).

Pure α-pinene, β-pinene, α-phellandrene and 1,8-cineole (GC purity ≥ 98%) were purchased from Sigma-Aldrich (Belgium)

Isolation of essential oil: Plant samples (50g) were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus to produce pale yellow oils which were dried with anhydrous sodium sulphate and stored in sealed vials at 4°C before use. To undertake repeated bio-tests directed towards measuring the toxicity of *H. spicigera* oil, larger quantities of plants (H0) were collected during the dry season at Ngaoundere.

Chromatographic analysis: The analysis were performed using an Agilent (HP-6890) gas chromatograph fitted with a fused silica capillary column (30x 0.25; 0.25µm film thickness, coated with a 5% phenyl 95% dimethylpolysiloxane stationary phase HP-5 from Agilent) and a split/splitless injector (splitless mode) at 250°C. The oven temperature was programmed from 40-210°C at 5°C/min, and from 210-280°C at 30°C/min, with a final hold of 5 min at 280°C. Helium was the carrier gas at 1ml/min, and the FID detector was maintained at 280°C. The relative amount of individual components was calculated based upon gas chromatographic peak areas with a common correction factor of one. All the samples were analyzed by GC/MS (Agilent 6890 GC coupled to an Agilent 5973 mass spectrometer) using the same analytical conditions. The mass spectra were recorder in the EI mode at 70 eV, scan mass range from 35 to 350 amu, source temperature: 230°C.

Identification of the oil components was undertaken by comparing fragmentation patterns and retention indices with those of the WILEY 275.L , Adams (13) and Joulain and König (14) databases .

Insecticidal activity: Cowpea weevils (*Callosobruchus. Maculatus* F.) from Cameroon were reared in cowpea seed at 28°C, R.H = 65%. Newly emerged adults of two days old were used to assess the test. The activity of the oil and the four major compounds against *C. maculatus* was measured in closed glass dessicators of 800 mL. To avoid contact between insects and the tested products, twenty-five *C. maculatus* were transferred to perforated petri dishes and 200

µL of essential oil of *H. spicigera* (which represents 215 µg oil/ litre of air); or its equivalent concentrations of α-pinene, β-pinene, α-phellandrene and 1,8-cineole (alone or in combination), were deposited in glass petri dishes at the bottom of the dessicator. The control consisted of a similar set up but without any added product. Each experiment was replicated five times with 25 insects and the observed mortality (fumigant activity) was measured after 24hours. The recorded values were analysed after transformation with arcsine square root by one way ANOVA. Treatment means of untransformed data were compared and separated by Duncan's test at $P<0.05$.

RESULTS AND DISCUSSION

Essential oil yields varied from 0.1 to 0.4%, and regardless of the origin they were higher during the dry season (January) than at the beginning of the rainy season (May). These results are in line with those of the literature (8,9). Differences were also noticed in oil composition between the sample origin and within samples from the same site (Table I). A total of 44 components have been identified. Excepted in H1 (Guirvidig sample collected in May), monoterpenes hydrocarbons represent the principal class of molecules with five main components: α-pinene (11.9%-42.1%), β-pinene + sabinene (6.0%-39.8%) and β-phellandrene + 1,8-cineole (8.8%-27.4%). In the aforementioned analytical conditions these two pairs of molecules co-eluted, the later being not separated from limonene. Although this product has already been reported in oil sample from Mali (9) (percentages ranging from 1.7% to 3.0%) and in a former study (11) of *H. spicigera* dried flowers from Cameroon (4.8-7.6%), careful examination of the reconstructed ion chromatograms (RIC on both Molecular ion and m/z = 68 characteristic of limonene fragmentation) of each compound with a retention index (RI) ca. 1028 revealed only traces of this molecule excepted in H6 collected in the dry season. Using similar analytical conditions, Tchoumbougang et al. (12) reported a limonene percentage of 13.4 % in a batch of *H. spicigera* harvested in Garoua. It seems unlikely to

discriminate between the three co-eluted molecules (RI ca. 1028) therefore, limonene, β-phellandrene and 1,8-cineole were not integrated as separate components. In H1 sample (collected in May), β-Caryophyllene (23.4%) predominated and it was found as the main sesquiterpene hydrocarbon in all the examined samples. Similar proportions have been previously reported (12). β-Caryophyllene has also been mentioned as the major compound in *H. spicigera* oil from Burkina Faso (7) , Nigeria (8) and Mali (9) where it attained much higher levels (23.5%-27.2% , 68% and 62.7%, respectively). From the present study and the literature survey, it should be assumed that chemotypes exist. Nevertheless only systematic and repeated investigations could support this assumption.

From Table I, it did not appear a clear general tendency in the evolution of essential oil composition as regard the sampling period but for some components large differences have been detected: particular increases of α-terpinolene in H2 (0.4 to 15.2) and H6 (2.2% to 17.7%); α-phellandrene in H1 (4.0% to 22.5%), α-pinene in H3 (19.8% to 42.1%) have been observed but still remain un-interpretable. Geographic variability (most likely linked to climatic factors) has been revealed between the different harvesting sites. In all the analyzed samples, 1,8-cineole was the major oxygenated monoterpenes and caryophyllene oxide was the main oxygenated sesquiterpenes specially in H1 (from May) were it represented 10.9%. All other oxygenated compounds have been detected in proportions less than 1%. Most of the non identified molecules were detected in low percentages (< 0.1%) and hardly discernible on the basis of the recorded total ion current. The recording of interpretable mass spectra was impossible for most of them.

Insecticidal trials were performed with the H0 sample of *H. spicigera* from Ngaoundéré. For that purpose repeated hydrodistillations have been performed in order to obtain the required quantities of oil. The main compounds of the pooled oils represented 90.3% of the total oil compounds and were identified as α-pinene (39.0%), 1,8- cineole + β-phellandrene (23.5%), β-pinene + sabinene (14.6%) and α-phellandrene (13.2%), respectively.

The fumigant activities of *H. spicigera* and its principal terpenic components (only α-pinene, 1,8- cineole, β-pinene and α-phellandrene were considered because they were available in pure form) are summarized in Table II. When compared to *H. spicigera* oil (mortalities of 100% and 99.2%, respectively for the two series), the corresponding concentrations of pure chemicals induced variable effects which indicated a structure-concentration - activity relationship. Indeed α-pinene was found to be the more active molecule whereas 1,8- cineole, β-pinene and α-phellandrene led to limited mortalities ranging from 48.8% to 24.8%, respectively. Nevertheless synergistic effects were systematically observed when *C. maculatus* was exposed to terpenes blends made on the basis of oil composition of H0. All the combinations used led to a similar "knock down" effect with very high mortality levels (> 90%) except the 1,8- cineole + α-phellandrene mixture which was significantly different. At this step of the work, the enantiomeric excess of the different chiral molecules has not bee determined. However, it would be valuable in further studies, to perform chiral chromatography in order to establish whether some particular isomers are driving the activity.

CONCLUSION

The present observations confirm the strong bio-pesticide properties of *H. spicigera* oil and evaluate the effect of some its major monoterpenes. To the authors knowledge there are no previous reports on the determination of the chemical compounds responsible of the fumigant activity of *H. spicigera* oil. Although the selected compounds have a significant biocide activity, it is noteworthy that the other components could also contribute to the properties of the oil.

Moreover, taking into account both the composition of the sample H0 oil and the biocidal properties of the tested pure molecules, more extensive studies need to be performed, in particular for samples H3 and H5. Such studies are in progress as well as the evaluation of the phenologic stage of the plant on essential oil composition and properties. This work

constitutes the background of an integrated pest management approach by using simple formulations of *H. spicigera* oils. It also shows that the variability in oil composition is large and has to be considered in practical future developments.

Acknowledgments

The authors gratefully acknowledge the Belgian University Cooperation to Development organisation (CUD: Commission Universitaire pour le Developpement) for financial support through “STOREPROTECT” project.

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Table I. Essential oil composition (%) of *Hyptis spicigera* from North-Cameroon

	KI	H0	H1		H2		H3		H4		H5		H6	
		Jan*	May	Jan*	May	Jan*	May	Jan*	May	Jan*	May	Jan*	May	Jan*
yield (%)		0.4	0.1	0.3	0.2	0.3	0.1	0.2	0.2	0.3	0.2	0.3	0.2	0.3
α-thujene	923	0.2	-	0.7	0.4	0.4	1.1	2.2	1.4	0.4	3.1	0.5	0.1	10.7
α-pinene	930	39.0	14.0	27.1	36.3	39.6	19.8	42.1	31.7	23.8	20.6	34.8	27.8	11.9
camphene	943	-	-	0.1	-	0.2	0.4	0.2	0.7	0.1	0.5	0.2	0.6	0.1
verbenene	949	-	-	-	-	-	0.3	-	0.3	-	0.3	-	0.3	-
sabinene+β-pinene	973	14.6	6.0	9.4	14.1	15.1	13.1	22.7	16.1	10.7	17.6	17.0	10.9	39.8
myrcene	987	-				0.6	0.2	0.7	0.2	0.1	0.2	0.3	0.2	1.1
α-phellandrene	1004	13.2	4.0	22.5	7.8	1.9	0.3	4.4	6.7	14.2	3.0	0.2	1.3	0.3
α-terpinene	1014	0.4	0.2	0.1	0.4		0.4	0.0	0.4	0.1	0.8		0.1	-
p-cymene	1023	1.3	2.6	0.1	3.0	0.1	1.2	0.1	3.1	0.1	3.0	0.1	2.0	0.5
β-phellandrene+ 1,8-cineole	1028	23.5	14.8	20.9	23.2	14.7	27.4	13.7	23.7	20.5	23.5	19.4	21.3	8.8
γ-terpinene	1056	0.3		0.2	0.4	0.3	1.2	0.6	0.8	0.2	1.7	0.3	0.1	1.2
terpinolene	1086	0.3	0.3	0.9	0.4	15.2	1.9	4.5	1.1	3.2	2.3	2.1	2.2	17.7
linalool	1098	-	-	-	-	0.2	-	-	0.7	0.6	0.2	0.6	0.3	-
isoamyl isovalerate	1102	-	2.0	1.2	0.3	0.6	0.9	0.6	0.5	0.8	1.1	0.8	1.1	0.8
β-thujone	1120	-	-	-	-	-	-	-	0.1	-	0.3	-	-	-
α-campholenal	1124	0.1	0.2	0.1	-	0.2	0.7	0.1	0.2	0.2	0.5	0.4	0.4	0.1
nopinone	1134	-	0.1	-	-	-	0.2	-	0.1	-	0.2	-	0.1	-
cis-sabinol	1137	0.3	1.3	0.1	0.7	0.5	1.9	0.1	0.7	0.8	1.3	1.0	1.7	0.7
α-phellandren-8-ol	1160	0.2	0.5	0.1	0.3	0.1	0.7	0.1	0.3	0.1	0.3	0.5	0.6	-
terpinen-4-ol	1175	0.3	0.4	-	2.2	0.6	2.9	0.1	1.6	0.6	3.6	-	0.5	0.3
p-cymen-8-ol	1182	0.1	0.6	-	0.8	0.4	0.8	-	1.6	0.2	3.7	0.2	1.2	-
α-terpineol	1188	0.1	0.3	-	0.3	0.4	0.6	-	0.2	0.5	0.3	0.5	0.3	-
myrtenal+myrtenol	1190	0.2	0.7	0.1	0.6	0.11	1.3	0.1	0.5	0.15	1.0	0.3	0.9	-
verbenone	1200	0.1	0.1	-	-	-	0.1	-	0.1	-	0.1	-	0.1	-

cuminaldehyde	1234	-	0.4	-	-	-	0.2	-	0.1	-	0.3	-	0.2	-
phellandral	1274	-	0.3	-	-	-	0.2	-	0.1	-	0.2	-	0.1	-
thymol	1291	-	0.1	-	0.1	-	-	-	0.1	-	0.1	-	0.4	-
carvacrol	1300	-	0.4	-	-	-	0.1	-		-	0.1	-	0.2	-
α-ylangene	1372	0.2	0.3	0.1	-	0.1	0.2	0.1	-	0.2	0.1	0.2	0.2	-
α-copaene	1377	-	0.1	-	0.2	0.0	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1
β-bourbonene	1385	-	0.1	-	-	-	0.2	-	0.1	-	0.1	-	0.1	-
β-cubebene	1392	0.1	0.2	0.1	-	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
iso-caryophyllene	1407	2.2	0.2	-	-	-	0.1	-		0.1		0.1	0.1	-
β-caryophyllene	1424	-	23.4	10.8	3.6	6.1	9.1	4.9	2.8	13.7	3.2	11.7	12.3	3.3
γ-elemene	1434	-	0.2	0.1	-	-	-	-	-	0.1	-	0.1	0.1	-
guaia-6,9-diene	1444	-	0.2	0.1	-	0.1	0.1	0.1	-	0.1	-	0.1	0.1	-
α-humulene	1456	-	1.3	0.6	0.3	0.3	0.6	0.3	0.3	0.7	0.2	0.6	0.7	0.2
germacrene D	1483	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.1
bicyclosesquiphellandrene	1490	-	0.1	-	0.1	-	-	-	-	-	-	-	-	-
γ-cadinene	1516	0.3	2.2	0.1	0.6		0.8			0.1		0.1	0.8	-
δ-cadinene	1525	0.1	0.6	0.1	0.2	0.1	0.2	-	0.1	0.1	0.1	0.2	0.3	-
α-cadinene	1539	-	0.2	-	-	-	0.1	-	-	-	-	-	-	-
α-calacorene	1551	-	0.5	-	-	-	-	-	-	-	1.2	-	0.2	-
caryophyllene oxide	1587	-	10.9	2.0	1.8	0.5	5.1	0.7	0.8	2.3	-	2.9	4.8	0.8
1-epi-cubenol	1629	-	0.3	0.1	-	-	0.2	-	-	0.1	-	0.1	0.1	-
NI compounds		2.1	9.1	2.3	1.9	1.6	5.2	1.4	2.5	4.7	5.0	4.4	4.7	1.2

H0: Ngaoundere ; H1: Guirvidig ; H2: Kodeck ; H3: Lara ; H4: Touloum ; H5: Kaele ; H6: Tchecal-baïla

May : beginning of the rainy season

*Jan : january, dry season

NI: Non identified