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Repellence of essential oils from tropical and Mediterranean Lamiaceae against *Sitophilus zeamais*

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Abstract

The insect repellent activity of the essential oils of two tropical Lamiaceae, *Hyptis spicigera* Lamarck and *Hyptis suaveolens* (L.) Poitier, and of a Mediterranean one, *Lavandula angustifolia* (Miller), has been measured against adults of the granary weevil, *Sitophilus zeamais* Motschulsky (Coleoptera Dryophthoridae). The chemical composition of the three essential oils was also examinated. Results showed that the three essential oils had repellent activity on *S. zeamais* adults. At the lowest dose (0.001%) *H. suaveolens* essential oil exhibited a significantly higher repellent effect compared with *H. spicigera* and *L. angustifolia*, while no significant differences were observed for the repellent effect of the three essential oils at the highest dose (0.1%). *L. angustifolia* essential oils evidenced a significantly lower repellent activity at the intermediate dose (0.01%) after 1, 3 and 5 h of exposure. Chemical analyses showed that in the essential oil of *H. suaveolens* monoterpene hydrocarbons were the most represented class of volatiles (64.1%), followed by sesquiterpene hydrocarbons (24.0%), oxygenated monoterpenes (8.1%) and oxygenated sesquiterpenes (2.4%). In the essential oil of *H. spicigera* monoterpene hydrocarbons were the most represented class of volatiles (70.4%), followed by sesquiterpene hydrocarbons (22.6%).

Key words: Botanical insecticides, bioassays, stored-food insects, GC/MS analysis, integrated pest management.

Introduction

The most important post harvest activity is the storage of cereals. During storage most losses occurred, due to insect attacks (Ngamo *et al*., 2007b). Insect pests of stored cereals are usually controlled by traditional synthetic insecticides which are currently used to protect stored products and to prevent post-harvest losses. However, many problems are associated with these chemicals, such as toxic residues in the food, worker's safety, insect resistance and the cost of treatments (Sighamony *et al*., 1986).

In the past, leaves or oils extracted from aromatic plants, such as Lamiaceae, have been extensively used in tropical countries to protect stored grain and legumes (Dabiré, 1993; Ladang, 2004; Ngamo *et al*., 2007b). The use as stored grain protection agents of locally available plants and oils obtained from these species is a traditional practice in many developing countries (Pereira, 1983). These substances have proved promising to traditional pesticides in stored products protection (Shaaya *et al*., 1993, 1994, 1997; Liu and Ho, 1999; Ndungu *et al*., 1999; Taponddjou *et al*., 2005; Rajendran and Srirenjini, 2008; Nerio *et al*., 2009, 2010).

Lamiaceae are aromatic herbs with a great socio-economic value, used in flavouring, cosmetics, perfumery and medical preparations (Magness *et al*., 1971). Their essential oils are a complex mixture of aromatic compounds and their composition and bio-activity are a function of species, chemotype, climate, soil conditions and geographical location (Onayade *et al*., 1990; Kini *et al*., 1993; Belanger *et al*., 1994; Jirovetz *et al*., 2000; Sidibe *et al*., 2001; Tchoumbougang *et al*., 2005; Shaaya and Kostyukovysky, 2006; Noudjou *et al*., 2007). Many of them are found in tropical regions such as those of *Hyptis* genus, which included more than 400 species. Also the Mediterranean flora is rich in Lamiaceae and, for example, only in Italy 36 genera and 190 species are present (Penzig, 1974).

Since essential oils could present different chemical composition and, consequently, diverse bio-activity as a function of the habitat were the plants are grown, the aim of the present study was to test the insect repellent activity of the essential oils of two tropical Lamiaceae, *Hyptis spicigera* Lamarck and *H. suaveolens* (L.) Poitier, both cultivated in an experimental field of Pisa University (Tuscany, Italy). The repellent activity of their essential oils and that of a well-known Mediterranean species, *Lavandula angustifolia* (Miller), has been measured against the granary weevil *Sitophilus zeamais* Motschulsky (Coleoptera Dryophthoridae), one of the world's most serious stored maize pest, that also attacks all other cereal grains and cereals products (Tipping *et al*., 1987). Data about the main chemical constituents of the three essential oils were also reported.

Materials and methods

Insect culture and rearing conditions

The strain of *S. zeamais* derived from a laboratory stock culture (25 ± 1 °C, 60-70% R.H.) kept at the Section of Agricultural Entomology of Pisa University since 2000. Insects were reared in plastic boxes (20 x 25 x 15 cm) containing uninfested maize and covered by tops with holes and a thin net for air passage. Adults were removed and transferred each day: in this way, the newly emerged insects were homogeneous in the box (± 3 days). These adults were used in bioassays.

Repellence tests

Essential oils of *H. spicigera*, *H. suaveolens* and *L. angustifolia* were tested for repellence adapting the method suggested by Tapondjou *et al*. (2005), Wang *et al*.

(2006) and Cosimi *et al*. (2009). Half filter paper disks (8 cm diameter) were treated with 0.5 ml of each essential oil solution [0.1, 0.01 and 0.001 % (v:v), corresponding to $2x10^{-2}$, $2x10^{-3}$, $2x10^{-4}$ µl oil/cm^2] in hexane and dried under a fan. Half the bottom of a Petri dish was covered with treated filter paper, while the other half was covered with a half filter paper disk treated with hexane only. Twenty mixed-sex adults were released at the centre of each Petri dish, and the lid was sealed with Parafilm. The test was carried out at 25 ± 1 °C, 70% R.H., natural photoperiod. Four replicates were run for each tested concentration, so that 80 adults/concentration were assayed. Observations were taken after 1, 3, 5 and 24 hrs from the beginning of the test: in each of them, the number of insects on the two half paper disks was recorded. A chi-square test (with Yates correction) was performed to compare the number of adults on each half of filter paper (Sokal and Rohlf, 1981).

Essential oils extraction and analysis

The essential oils of *H. suaveolens* and *H. spicigera* were extracted from fresh leaves, partially dehydrated for 5 d at room temperature. After this period, the leaves were ground and weighed, placed in a round bottom flask and 1000 ml of distilled water were added and hydrodistilled for 2 h in a modified Clevenger apparatus.

Gas chromatography (GC) analyses were accomplished with an HP-5890 Series II instruments equipped with DB-WAX and DB-5 capillary columns (30 m x 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250 °C; the carrier gas was helium (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 µl. The identification of the components was carried out, for both the columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention index (LRI) relative to the series of *n*-hydrocarbons.

Gas chromatography/electron impact mass spectroscopy (GC/EIMS) analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 ml/min; injection of 0.2 µl (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their LRIs with the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Stenhagen *et al*., 1974; Massada, 1976; Jennings and Shibamoto, 1980; Swigar and Silverstein, 1981; Davies, 1990; Adams, 1995). Moreover, the molecular weights of all the identified substances were confirmed by gas chromatography/chemical ionization mass spectrometry (GC/CIMS), using methanol as CI ionizing gas.

Results

Repellence tests

The three essential oils showed repellent activity on *S. zeamais* adults (table 1). It was evident that, at the lowest dose (0.001%), *H. suaveolens* essential oil exhibited a significantly higher repellent effect in comparison to *H. spicigera* and *L. angustifolia*, while no significant differences were observed for the repellent effect of the three essential oils at the highest dose (0.1%). *L. angustifolia* essential oils manifested a lower repellent activity at the intermediate dose (0.01%) after 1, 3 and 5 h from the exposure.

Essential oils: GC/MS analyses

In the essential oil of *H. suaveolens* monoterpene hydrocarbons were the most represented class of volatiles (64.1%), followed by sesquiterpene hydrocarbons (24.0%), oxygenated monoterpenes (8.1%) and oxygenated sesquiterpenes (2.4%) (table 2). Diterpenes and non-terpene derivatives were scarcely represented in this oil (0.3% and 0.2%, respectively). Globally, terpene hydrocarbons constituted 88.1% of the whole oil. The main constituents were sabinene (27.0%), β-caryophyllene (17.1%), terpinolene (11.9%), β-pinene (9.4%), and limonene (6.0%) (table 3).

Also the essential oil of *H. spicigera* was dominated by monoterpene hydrocarbons (70.4%), followed by sesquiterpene hydrocarbons (22.6%) (table 2). Oxygenated monoterpenes and oxygenated sesquiterpenes were detected in comparable amounts (1.3% and 1.4%, respectively). Non-terpene derivatives reached 1.0%, while diterpenes accounted for 0.4% of the whole essential oil. Globally, terpene hydrocarbons reached 93.0% of the whole oil. The main components resulted α-pinene (21.7%), β-caryophyllene (18.4%), sabinene (17.4%), β-pinene (13.8%) and limonene (5.2%) (table 3).

The composition of the essential oil of *L. angustifolia* was previously investigated (Gozzini, 2008) and its main constituents were fenchone (33.9%), camphor (13.8%), camphene (13.7%), α-pinene (6.8%), limonene (4.4%) and 1,8-cineole (2.4%) (table 3).

Discussion and conclusions

From the repellence tests, it became apparent that all the selected essential oils were endowed with repellent activity on *S. zeamais* adults. At the intermediate (0.01%) and highest doses (0.1%), no significant differences on the repellent efficacy of the oils were observed, while it was evident that, at the lowest dose (0.001%), *H. suaveolens* essential oil exhibited a significantly higher repellence in comparison to both *H. spicigera* and *L. angustifolia*, so demonstrating a relatively stronger efficacy.

Many bibliographical data are available on the insecticidal effect of *L. angustifolia* essential oil on different insect species; on the contrary, the data of its repellence properties against *S. zeamais* are not clear. It has been reported that the powdered aerial parts of *L. angustifolia* were the most repellent to *S. granarius* in wheat grain (Ignatowicz, 1997), while when extracts from leaves

Table 1. *S. zeamais* - Repellent effect of three different concentrations of *H. suaveolens, H. spicigera* and *L. angustifolia* essential oils to adults after different exposure times in the filter paper test. Data tested by applying the χ^2-test (with Yates correction); total number of insects for each concentration was 80. Tr = treated half; Un = untreated half; χ^2 r = overall χ^2; * = significantly different at P < 0.05; ** = significantly different at P < 0.01; n.s. = not significant.

Compound	Dose (%)	Trial	Number of beetles on each half after each exposure															
			1 h				3 h				5 h				24 h			
			Tr	Un	χ^2	χ^2 r	Tr	Un	χ^2	χ^2 r	Tr	Un	χ^2	χ^2 r	Tr	Un	χ^2	χ^2 r
Hyptis suaveolens	0.001	1	8	12	0,85		6	14	3,25		6	14	3,25		8	12	0,85	
		2	4	16	7,25	8,45	7	13	1,85	11,26	7	13	1,85	20	5	15	5,05	26,5
		3	6	14	3,25	**	5	15	5,05	**	1	19	16,25	**	1	19	16,25	**
		4	9	11	0,25		7	13	1,85		6	14	3,25		3	17	9,85	
	0.01	1	3	17	9,85		2	18	12,85		2	18	12,85		4	16	7,25	
		2	3	17	9,85	33,8	2	18	12,85	33,8	2	18	12,85	36,5	6	14	3,25	33,8
		3	5	15	5,05	**	4	16	7,25	**	0	20	20,05	**	0	20	20,05	**
		4	3	17	9,85		6	14	3,25		9	11	0,25		4	16	7,25	
	0.1	1	5	15	5,05		1	19	16,25		3	17	9,85		3	17	9,85	
		2	0	20	20,05	39,2	4	16	7,25	51,2	3	17	9,85	42,06	3	17	9,85	36,5
		3	5	15	5,05	**	2	18	12,85	**	1	19	16,25	**	0	20	20,05	**
		4	2	18	12,85		1	19	16,25		4	16	7,25		7	13	1,85	
Hyptis spicigera	0.001	1	10	10	0,05		11	9	0,25		10	10	0,05		7	13	1,85	
		2	9	11	0,25	0,21	9	11	0,25	1,82	10	10	0,05	0,82	5	15	5,05	16,2
		3	8	12	0,85	n.s.	8	12	0,85	n.s.	7	13	1,85	n.s.	9	11	0,25	**
		4	7	13	1,85		6	14	3,25		9	11	0,25		1	19	16,25	
	0.01	1	9	11	0,25		3	17	9,85		5	15	5,05		9	11	0,25	
		2	3	17	9,85	20	1	19	16,25	18,06	3	17	9,85	18,1	0	20	20,05	22,1
		3	5	15	5,05	**	8	12	0,85	**	7	13	1,85	**	5	15	5,05	**
		4	3	17	9,85		5	15	5,05		6	14	3,25		5	15	5,05	
	0.1	1	1	19	16,25		1	19	16,25		1	19	16,25		1	19	16,25	
		2	3	17	9,85	48,01	6	14	3,25	48,01	5	15	5,05	39,2	3	17	9,85	61,3
		3	5	15	5,05	**	1	19	16,25	**	3	17	9,85	**	1	19	16,25	**
		4	0	20	20,05		1	19	16,25		3	17	9,85		0	20	20,05	
Lavandula angustifolia	0.001	1	7	13	1,85		10	10	0,05		9	11	0,25		11	9	0,25	
		2	6	14	3,25	7,21	7	13	1,85	1,82	7	13	1,85	0,82	11	9	0,25	0,06
		3	8	12	0,85	**	9	11	0,25	n.s.	10	10	0,05	n.s.	8	12	0,85	n.s.
		4	7	13	1,85		8	12	0,85		10	10	0,05		9	11	0,25	
	0.01	1	9	11	0,25		7	13	1,85		7	13	1,85		8	12	0,85	
		2	10	10	0,05	1,81	6	14	3,25	5,01	6	14	3,25	7,21	5	15	5,05	11,3
		3	8	12	0,85	n.s.	8	12	0,85	*	8	12	0,85	**	7	13	1,85	**
		4	9	11	0,25		9	11	0,25		7	13	1,85		5	15	5,05	
	0.1	1	6	14	3,25		4	16	7,25		5	15	5,05		5	15	5,05	
		2	7	13	1,85	11,26	5	15	5,05	24,21	4	16	7,25	20	3	17	9,85	18,1
		3	5	15	5,05	**	6	14	3,25	**	7	13	1,85	**	7	13	1,85	**
		4	7	13	1,85		3	17	9,85		4	16	7,25		6	14	3,25	

Table 2. Mean percentages of main chemical classes of the *H. suaveolens* and *H. spicigera* essential oil volatiles.

Main chemical classes	*H. suaveolens* (%)	*H. spicigera* (%)
Monoterpene hydrocarbons	64.1	70.4
Oxygenated monoterpenes	8.1	1.3
Sesquiterpene hydrocarbons	24.0	22.6
Oxygenated sesquiterpenes	2.4	1.4
Non-terpene derivatives	0.2	1.0
Diterpenes	0.3	0.4

Table 3. Main constituents of the three essential oils used in bioassays (data for *L. angustifolia* essential oil from Gozzini, 2008).

	Main constituents					
H. suaveolens	sabinene 27.0%	β-caryophyllene 17.1%	terpinolene 11.9%	β-pinene 9.4%	limonene 6%	4-terpineol 5.4%
H. spicigera	α-pinene 21.7%	β-caryophyllene 18.4%	sabinene: 17.4%	β-pinene 13.8%	terpinolene 7.3%	limonene 5.2%
L. angustifolia	fenchone 33.9%	camphor 13.8%	camphene 13.7%	α-pinene 6.8%	limonene 4.4%	1,8-cineole 2.4%

were tested for their repellent activity against the red flour beetle *Tribolium castaneum* (Herbst) only a small repellent effect was observed (Moharramipour and Rafih, 2008). Furthermore, Lamiri *et al*. (2001) showed ovicidal properties against *Mayeticola destructor* (Say) and identified as main components of the oil 1,8-cineole (48.1%), β-pinene (15.4%), linalool (8.3%) and α-pinene (5.5%), so evidencing substantial differences with the chemical composition of the lavender essential oil used in the present study. Rozman *et al*. (2007) considered 1,8-cineole as the most effective compound of *L. angustifolia* in fumigation tests against different stored-food coleopteran species, followed by camphor (detected in valuable amounts also in the essential oils of our study) and linalool.

Regarding the essential oils extracted from the two tropical Lamiaceae, the present paper improves the knowledge about the repellent activity of *H. spicigera* and *H. suaveolens* against *S. zeamais*. To date, only few published data are available. According to our results, Othira *et al*. (2009) reported that both the whole *H. spicigera* plant and the steam-distilled essential oil exhibited strong insect repellent activity at low doses against *S. zeamais* and *T. castaneum* adults. The repellent and lethal effect of powder and essential oil of *H. spicigera* on adults of *Callosobruchus maculatus* (F.) was reported by Sanon *et al*. (2006). Essential oil extracted from *H. suaveolens* was also assayed by Kéïta *et al*. (2000) on the latter coleopteran species, using kaolin powder as carrier to test the ovicidal activity, obtaining 100% of egg mortality. Iloba and Ekrakene (2006) performed a comparative study on the insecticidal effect of different plant species, and reported that *H. spicigera* powder was the best one in preventing the emergence of both *S. zeamais* and *C. maculatus* adults, having both larvicidal and ovicidal effect. In a work aimed to analyse the chronic toxicity of low doses of the essential oil of *H. spicigera* against *S. zeamais,* it was showed that this approach progressively reduced the survival potential of this insect pest (Ngamo *et al*., 2007a). Moreover, when the crude essential oil of *H. spicigera* was tested against four major stored product insect pests (including *S. zeamais*), it was evidenced a high contact toxicity, even if this essential oil had a very short-lasting toxicity (Ngamo *et al*., 2007c). The analysis of the chemical composition of this essential oil performed by the same authors showed the high content of geranial (>30%) and linalool (>15%) and these compounds, together with α-phellandrene, terpinolene and limonene, were related to the high toxicity towards the stored-food coleopteran species (Ngamo *et al*., 2007c). In a further work, Ngamo *et al*. (2007b) reported that the essential oil obtained from the flowers of *H. spicigera* expressed insecticidal activity against *S. oryzae* and the toxicity was attributed to 1,8-cineole, carvacrol, α-pinene and β-pinene. Our preliminary analyses on both essential oils do not revealed geranial and linalool, while terpinolene and limonene were well represented, partially confirming the indications by Ngamo *et al*. (2007c). Although many of the above mentioned studies suggested that 1,8-cineole, at appropriate doses, was intrinsically toxic to post-harvest pests (cfr. also Obeng-Ofori *et al*., 1997; Aggarwal *et al*., 2001; Rozman *et al*., 2007; Asawalam *et al*., 2008), in our opinion the very low content of this compound in both the assayed *H. spicigera* and *H. suaveolens* essential oils (0.3 % and 0.5%, respectively) suggests that other constituents may be responsible for the bio-activity.

Overall, even if it is not easy to determine which are the compounds responsible for the biological activities of the essential oils used in bioassays, it can be noted that the main differences between the two tropical Lamiaceae oils were that in *H. suaveolens* pinenes summed 9.4%, while in *H. spicigera* they reached 35.5%; in the case of sabinene the percentages were 27.0% and 17.4%, respectively. Furthermore, *H. suaveolens* was richer in oxygenated monoterpenes than *H. spicigera* (8.1% *vs* 1.3%). With the only exception of α-pinene and limonene, the qualitative composition of *L. angustifolia* essential oil was quite different from the two tropical Lamiaceae. Therefore, the abovementioned differences may be responsible of the different repellent efficacy found during our tests. However, the whole composition, comprising also the minor constituents, may play an important role in the biological activities of all the three essential oils (see Nerio *et al*., 2010 and references included). Their repellent effect could be used to prevent insect infestations of cereal products by incorporating an appropiated amount into packaging materials (Cagri *et al*., 2004) and by increasing the repellence activity in appropiate formulations (Nerio *et al*., 2010). In conclusion, even if large-scale trials are necessary to determine an application method for the repellence of these oils against stored product insect pests, in an integrated approach they could represent a possible alternative to chemical insecticides against stored product pests.

References

ADAMS R. P., 1995.- *Identification of essential oil components by gas chromatography-mass spectroscopy*.- Allured Publishing Corporation, Carol Stream, Illinois, USA.

AGGARWAL K. K., TRIPATHI K. A., PRAJAPATI V., SUSHIL K., 2001.- Toxicity of 1,8 cineole towards three species of stored products coleopteran.- *Insect Science and its Application*, 21: 155-160.

ASAWALAM E. F., EMOSAIRUE S. O., HASSANAI A., 2008.- Essential oil of *Ocimum grattissimum* (Labiatae) as *Sitophilus zeamais* (Coleoptera: Curculionidae) protectant.- *African Journal of Biotechnology*, 7 (20): 3771-3776.

BELANGER A., DEXTRAZE L., NACRO M., SAMATÉ A. D., COLLIN G., GARNEAEU E. X., GAGNON H., 1994.- Compositions chimique d'huiles essentielles de plantes aromatiques du Burkina Faso, pp. 300-305. In: *Acte des 13èmes journées internationales huiles essentieles*, Digne-Les-Bains, France.

CAGRI A., USTUNOL Z., RYSER E. T., 2004.- Antimicrobial edible films and coatings.- *Journal of Food Protection*, 67: 833-848.

COSIMI S., ROSSI E., CIONI P. L., CANALE A., 2009.- Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.).- *Journal of Stored Products Research*, 45: 125-132.

DABIRE C., 1993.- Méthode traditionelle de conservation du niébé (*Vigna unguiculata*) au Burkina Faso, pp. 45-55. In: *Protection naturelle des végétaux* (THIAM A., DUCOMMUN G., Eds).- Editions Enda, Dakar, Senegal.

DAVIES N.W., 1990.- Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases.- *Journal of Chromatography*, 503: 1-24.

GOZZINI F., 2008- Attività insetticida di vari oli essenziali nei confronti di larve di *Aedes albopictus* (Diptera Culicidae). 77 pp., *Degree thesis*, University of Pisa, Faculty of Agriculture, Italy.

IGNATOWICZ S., 1997.- Powdered herbs of the mint family (Lamiaceae) as insect repellents for protection of stored wheat grain.- *Polskie Pismo Entomologiczne*, 66 (1/2): 135-149.

ILOBA B. N., EKRAKENE T., 2006.- Comparative assessment on insecticidal effect of *Azadirachta indica*, *Hyptis suaveolens* and *Ocimum gratissimum* on *Sitophilus zeamais* and *Callosobruchus maculatus*.- *Journal of Biological Sciences*, 6 (3): 626-630.

JENNINGS W., SHIBAMOTO T., 1980.- *Qualitative analysis of flavor and fragrance volatiles by glass capillary chromatography*.- Academic Press, New York, USA.

JIROVETZ L., BUCHBAUER G., PUSCHMANN C., NGASSOUM M. B., 2000.- Investigations of aromatic plants from Cameroon: analysis of the essential oils of flowers of *Hyptis spicigera* (L.) by GC, GC/MS and olfactometry.- *Journal of Essential Oil Bearing Plants*, 3: 71-77.

KÉÏTA S. M., VINCENT C., DELANGER A., SCHMIT J. P., 2000.- Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae).- *Journal of Stored Products Research*, 36: 355-364.

KINI F., KAM B., AYCARD J. P., GAYDOU E. M., FAURE R., 1993.- Chemical composition of oils of *Hyptis spicigera* Lam. From Burkina Faso.- *Journal of Essential Oil Research*, 5: 219-221.

LADANG Y. D., 2004.- Contribution à la réussite de stockage de *Penissitum glaucum* et *Sorghum bicolor* par l'analyse phénologique des inféstations et l'utilisation des huiles essentielles de plantes aromatiques, 80 pp., *Mémoire de Maîtrise en Biologie et Physiologie animale*, Faculté des Sciences, Département des Sciences Biologiques, Université de Ngaoundéré, Cameroon.

LAMIRI A., LHALOUI S., BENJILALI B., BERRADA M., 2001.- Insecticidal effects of essential oils against Hessian fly, *Mayeticola destructor* (Say).- *Field Crop Research*, 71: 9-15.

LIU Z., HO S., 1999.- Bioactivities of essential oil extracted from *Evodia rutaecarpa* Hook F. et Thomas against the grain storage insects *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst.).- *Journal of Stored Products Research*, 35: 317-328.

MAGNESS J. R., MARKLE G. M., COMPTON C. C., 1971.- *Food and feed crops of the United States*.- Interregional Research Project IR-4. IR Bull. 1, Bulletin 828 of the New Jersey Agricultural Experimental Station. Revised Edition. Botanical American Council, New Brunswick, USA.

MASSADA Y., 1976.- *Analysis of essential oils by gas chromatography and mass spectrometry*.- J. Wiley & Sons, New York, USA.

MOHARRAMIPOUR S., RAFIH J. N., 2008.- Repellency of *Nerium oleander* L., *Lavandula officinalis* L. and *Ferula assafoetida* L. extracts on *Tribolium castaneum* (Herbst.).- *Iranian Journal of Medicinal and Aromatic Plants*, 23 (4): 443-452.

NDUNGU M., LAWNDALE W., HASSANALI A., MOREKA L., CABRA C. S., 1999.- *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil *Sitophilus zeamais* repellents.- *Entomologia Experimentalis et Applicata*, 76: 217-222.

NERIO L. S., OLIVERO-VERBEL J., STASHENKO E., 2009.- Repellent activity of essential oils from seven aromatics plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera).- *Journal of Stored Products Research*, 45: 212-214.

NERIO L. S., OLIVERO-VERBEL J., STASHENKO E., 2010.- Repellent activity of essential oils: a review.- *Bioresource and Technology*, 101: 372-378.

NGAMO T. S. L., GOUDOUM A., NGASSOUM M. B., MAPONGMESTSEM P. M., LOGNAY G., MALAISSE F., HANCE T., 2007a.- Chronic toxicity of essential oil of 3 local aromatic plants towards *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae).- *African Journal of Agricultural Research*, 2 (4): 164-167.

NGAMO T. S. L., NGATANKO I., NGASSOUM M. B., MAPONGMESTSEM P. M., HANCE T., 2007b.- Insecticidal efficiency of essential oils of 5 aromatic plants tested both alone and in combination towards *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae).- *Research Journal of Biological Sciences*, 2 (1): 75-80.

NGAMO T. S. L., NGATANKO I., NGASSOUM M. B., MAPONGMESTSEM P. M., HANCE T., 2007c.- Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests.- *African Journal of Agricultural Research*, 2 (4): 173-177.

NOUDJOU F., KOUNINKI H., NGAMO L. S. T., MAPONMESTSEM P. M., NGASSOUM M., HANCE T., HAUBRUGE E., MALAISSE F., MARLIER M., LOGNAY G. C., 2007.- Effect of site location and collecting period on the chemical composition of *Hyptis spicigera* Lam. an insecticidal essential oil from North-Cameroon.- *Journal of Essential Oil Research*, 19: 597-601.

OBENG-OFORI D., REICHMUT C. H., BEKELE J., HASSANALI A., 1997.- Biological activity of 1,8-cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles.- *Journal of Applied Entomology*, 121: 237-243.

ONAYADE O. A., LOOMAN A., SCHEFFER J. J. C., BAERHEIM A., 1990.- Composition of the herb essential oil *Hyptis spicigera* Lam..- *Flavour and Fragrance Journal*, 5: 101-105.

OTHIRA J. O., ONEK L. A., DENG L. A., OMOLO E. O., 2009.- Insecticidal potency of *H. spicigera* preparations against *Sitophilus zeamais* (L.) and *Tribolium castaneum* (Herbst) on stored maize grains.- *African Journal of Agricultural Research*, 4 (3): 187-192.

PENZIG O., 1974.- *Flora popolare italiana*.- Edagricole, Bologna, Italy.

PEREIRA J., 1983.- The effectiveness of six vegetables oils as protectants of cowpeas and bambara groundnuts against infestations by *Callosobruchus maculatus* (F.) (Coleoptera Bruchidae).- *Journal of Stored Products Research*, 19 (2): 57-62.

RAJENDRAN S., SRIRANJINI V., 2008.- Plant products as fumigants for stored-product insect control.- *Journal of Stored Products Research*, 44: 126-135.

ROZMAN V., KALINOVIC I., KORUNIC Z., 2007.- Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-products insects.- *Journal of Stored Products Research*, 43: 349-355.

SANON A., ILBOUDO Z., DABIRE C., NEBIE R., DICKO I., MONGE J. P., 2006.- Effects of *Hyptis spicigera* Lam. (Labiatae) on the behaviour and development of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), a pest of stored cowpeas.- *International Journal of Pest Management*, 52 (2): 117-123.

SHAAYA E., KOSTYUKOVYSKY M., 2006.- Essential oils: potency against stored product insects and mode of action.- *Stewart Post Harvest Review*, 4: 5.

SHAAYA E., RAVID U., KOSTYUKOVYSKY M., MENASHEROV M., PLOTKIN S., 1993.- Essential oils and their components as active fumigants against several species of stored-product insects and fungi.- *Acta Horticulturae*, 344: 131-137.

SHAAYA E., KOSTYUKOVYSKY M., RAVID U., 1994.- Essential oils and their constituents as effective fumigants against stored-product pests.- *Israel Agroresearch*, 7: 133-139.

SHAAYA E., KOSTJUKOWSKI M., EILBERG J., SUKPRAKARN C., 1997.- Plant oils as fumigants and contact insecticides for the control of stored-product insects.- *Journal of Stored Products Research*, 33 (1): 7-15.

SIDIBE L., CHALCHAT J. C., GARRY R. P., 2001.- Aromatic plants from Mali (III): chemical composition of essential oils from two *Hyptis* species: *H. suaveolens* (L.) and *H. spicigera* Lam..- *Journal of Essential Oil Research*, 13: 55-57.

SIGHAMONY S., ANEES I., CHANDRAKALA T., OSMANI Z., 1986.- Efficacy of certain indigenous plant products as grain protectans against *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.).- *Journal of Stored Products Resesarch*, 22 (1): 21-23.

SOKAL R. R., ROHLF F. J., 1981.- *Biometry*.- Freeman and Company, New York, USA.

STENHAGEN E, ABRAHAMSSON S., MCLAFFERTY F. W., 1974.- *Registry of mass spectral data*.- J. Wiley & Sons, New York, USA.

SWIGAR A. A., SILVERSTEIN R. M., 1981.- *Monoterpenes*.- Aldrich Chemical Company, Milwaukee, USA.

TAPONDJOU A., ADLER C., FONTEM D., BOUDA H., REICHMUTH C., 2005.- Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val.- *Journal of Stored Products Research*, 41: 91-102.

TCHOUMBOUGANG F., AMVAM ZOLLO P. H., FECAM BOYOM F., NYEGUE M. A., BESSIÈRE J. M., 2005.- Aromatic plants of Tropical Central Africa. XLVIII. Comparative study of the essential oils of four *Hyptis* species from Cameroon: *H. lanceolata* Poit., *H. pectinata* (L.) Poit., *H. spicigera* Lam. and *H. suaveolens* Poit..- *Flavor and Flagrance Journal*, 20: 340-343.

TIPPING P. W., MIKOLAJCZAK K. L., RODRIGUEZ J. G., PONELEIT C. G., LEGG D. E., 1987.- Effects of whole corn kernels and extracts on the behaviour of maize weevil (Coleoptera Curculionidae).- *Journal of Economic Entomology*, 80 (5): 1010-1013.

WANG J., ZHU F., ZHOU X. M., NIU C. Y., LEI C. L., 2006.- Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).- *Journal of Stored Products Research*, 42: 339-347.

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