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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS OF ORIGANUM MAJORANA L. (LAMIACEAE) AND SALVIA OFFICINALIS (L.) (LAMIACEAE) AGAINST BRUCHUS LENTIS (COLEOPTERA, CHRYSOMELIDAE)

Mostafa El IDRISSI; Ghita HARMOUCH; Ali AMECHROUQ

Laboratory of Molecular Chemistry and Natural Substances, Moulay Ismail University, Faculty of Science, B.P 11201 Zitoune, Meknes (Morocco).

*Corresponding author. E-mail: aamechrouq@yahoo.fr

Abstract- To assess the biological activity of origanum majorana essential oils and salvia officinalis, 6 concentrations were tested as fumigation against Bruchus lentis (Coleoptera, Chrysomelidae) high on lentil seeds. Essential oils from two plants affect biological parameters weevil. The main components are marjoram: Trans Sabinene hydrate (26,64%), Terpinen-4-ol (17,08%) and o-Cymene (6,29%), while those of sage are: α-Thujone (20,52%), Eucalyptol (12,39%) and γ-Gurjunene (11,13%). The survival time of 50% of adults exposed to different concentrations of essential oils ranges from less than 24 hours to about 4 days depending on the concentration, while in the control group, in live adult average of 7 to 11 days. After 48h the letal concentration LC_{50} fumigation marjoram is 10,28µl/ l while that of the sage is 13,42µl/l. The intensity of the impact of the essential oil is directly dependent on their concentrations. The essential oils of officinal sage and marjoram studied can be integrated into management weevils, stored and associated with the pulses thus replace the phosphine and methyl bromide.

Keywords: Origanum majorana; Salvia officinalis; Fumigants; Bruchus lentis; Chemical composition; LT_{50}; LC_{50}

I. INTRODUCTION

Control of stored-product insect populations is primarily dependent upon continued applications of liquid and gaseous insecticides. Although effective, their repeated use for several decades has disrupted biological control system by natural enemies and led to outbreaks of insect pests, widespread development of resistance, undesirable effects on non-target organisms, and environmental and human health concerns [1; 2; 3].

Fumigants such as methyl bromide and phosphine are still the most effective for the protection from insect infestation of stored food, feedstuffs, and other agricultural commodities [4]. These compounds are so effective and lead to very satisfactory results, but they cause the destruction of the environment [5]. Additionally, some stored-product insects are found to have developed resistance to methyl bromide and phosphine [2; 3]. Therefore, essential oils act an important role as potential resources for the natural fumigation because they constitute a rich source of bioactive chemicals. [7]. Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals [8]. Since these are often active against a limited number of species including specific target insects, are often biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management, they could lead to the development of new classes of safer insect-control agents. Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents. Little work has been done to manage stored product insects by using aromatic medicinal plants despite their excellent pharmacological actions [9-11]. In this work, we present the effects of essential oils of marjoram and sage on Bruchus lentis.

II. MATERIALS AND METHODS

2.1 Vegetal material

The plants used in this work are: marjoram (Origanum majorana L.) and sage (Salvia officinalis L.) They come from the region of Meknes, Morocco. Only the aerial parts of the plants were collected, they were then dried in the laboratory and stored protected from light.

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2.2 Insects

Bruchus lentis is a species of insects belonging to the family Chrysomelidae. It has a size of 3 to 3.5 mm, reddish gray hairs on the back, marked with several white spots. Bruchus lentis used for bioassays have emerged from lentil seeds from a warehouse in Azrou, Morocco. Insects are placed in a ventilated laboratory room where the temperature is between 24 ° C and 28 ° C.

2.3 Extraction of essential oils

The extraction of essential oils was performed by steam distillation at atmospheric pressure in a Clevenger-type apparatus. The distillation lasted 3 hours. The essential oil collected was dried over anhydrous sodium sulfate and stored at 4 ° C in the dark.

2.4 Gas chromatography–mass spectrometry

The chromatographic analyzes were performed on gas chromatograph type GC Ultra equipped with a column of type VB-5 (95% methylpolysiloxane 5% phenyl) 25m length, 0.25mm in diameter and 0,25µm of film thickness, a flame ionization detector (FID) is set at 260 ° C and fed with a gas mixture of H2 / air and a split-splitless injector set at 250 ° C. The injection mode is split. The device is controlled by a "HP ChemStation" type computer system managing the operation of the device and to monitor the chromatographic analyzes. The identification of the components was carried out on the gas chromatography coupled to mass spectrometry (MS Polaris Q ion trap). Fragmentation is done by electron impact in a field of 70 eV. The column temperature is programmed from 50 ° C to 350 ° C at a rate of 4 ° C / min. The carrier gas was helium with a flow rate set at 1,4ml / min. the unit is connected to a computer system running a mass spectra library NIST 98. The identification of the compounds was determined by comparing their mass spectra with those of computer databases.

2.5 Bioassays

The concentrations used are: 5, 10, 15, 20, 25 and 30µl / l. Fumigation was carried out in transparent plastic boxes, 1l capacity. Every concentration was made on filter paper and placed in the box containing 5 boxes of kneaded 5.5cm in diameter, containing five adult insects. The closed dishes were kept in the laboratory, an identical treatment without essential oil was used as a control, and each concentration was repeated 5 times. After 24 hours of fumigation, control of mortality in each box was increased every day until the last dead insect.

2.6 Statistical analysis

To compare the effects of essential oils tested on biological parameters measured, an analysis of variance followed by the Scheffe test at 5% was achieved using the Excel version 2007 software. LC 50 and LC 99 were determined by probit according to Finney [12]. Mortalities were corrected by Abbott's formula [13] The lethal time required to kill 50% (LT 50) of adults exposed to different concentrations of the essential oil was estimated using linear regression equations expressing the mortality corrected time.

III.RESULTS

3.1 Chemical composition

The average yield of essential oil of marjoram is 2.19%. Chromatographic analysis has identified 13 constituents representing approximately 77% of the essential oil (Table 1). In fact, the majority compounds are obtained: Trans-Sabinène hydrate (26,64%), Terpinèn-4-ol (17,08%) and o-Cymène (6,29%). The chemical profile of the essential oil of Marjoram is quite different from what has been reported in the literature. Indeed, Vera and Chane-Ming (1999) [14] studied the chemical composition of the essential oil of marjoram and French origin. The majority compound are: Sabinène (4,94%), α-Terpinène (2,75%), p-Cymène (7,01%), Trans-Sabinène hydrate (3,49%), Cis-Sabinène hydrate (14,95%), Terpinèn-4-ol (38,40%) and α-Terpinéol (4,88%). Ben Hamada-Ben Ezzeddine et al., (2001) [15] carried back a key oil Original Tunisia: Sabinène (3,9%), α-Terpinène (5,1%), p-Cymène (3,8%), γ-Terpinène (9,9%), Cis-Sabinène hydrate (8,6%) and Terpinèn-4-ol (32,8%). Yong et al., (2005 et 2009) [16 ; 17] found in Korea mainly Sabinène (3,25%), α-Terpinène (3,49%), 1,8-Cinéol (41,50%), γ-Terpinène (6,48%), Linalool (11,11%) and Terpinèn-4-ol (10,6%). Busatta et al., (2008) [18] analyzed the essential oil of Egyptian origin which proved rich Sabinène (5,11%), α-Terpinène (7,7%), γ-Terpinène (13,94%), Cis-Sabinène hydrate (9,64%), Terpinèn-4-ol (30,41%) and α-Terpinéol (4,47%).

Whereas the essential oil of sage, twelve compounds were identified and represent more than 76% of the total chemical composition of the essential oil (Table 2). The majority compounds are: α-Thujone (20,52%), Eucalyptol (12,39%) and γ-Gurjunène (11,13%). Lima et al., (2004) [19] have studied the chemical composition of sage from Portugal: 1,8-Cinéole (12,7%), cis-Thujone (17,4%), Bornéol (8,3%), α-Humulène (13,3%), E-Caryophyllène (8,5%), Veridiflorol (6,2%). Valeria et al., (2004) [20] found in Romania: 1-Octèn-3-ol (8,5%), α-Thujone (21,85%), Camphor (11,25%), β-Thujone (5,51%), α-Humulène (4,51%), Veridiflorol (11,71%). Vukovic´-Gacic´ et al., (2006) [21] analyzed an essential oil of origin Belgrade: α-Pinène (5%), 1,8-Cinéole (14,42%), α-Thujone (37,51%), Camphor (13,77%), α-Humulène (4,99%). Eugénia et al., (2007) [22] carried back home Portugal essential oil: Camphène (3,8%), 1,8-Cinéole (13%), cis-Thujone (32,9%), Camphor (23,4%), α-Humulène (3,7%).

Table 1: Main components of the essential oil of Origanum majorana.

RT (min)	Constituents	Composition	Mass spectrum	%
7,82	α-Pinène	$C_{10}H_{16}$	136(3), 121(10), 105(15), 91(100), 77(45), 65(7)	0,56
9,17	γ-Terpinène	$C_{10}H_{16}$	136(14), 107(9), 91(100), 77(64), 65(8)	4,89
10,95	**o-Cymène**	$C_{10}H_{14}$	136(30), 119(100), 103(3), 91(41), 77(6)	**6,29**
11,10	Cyclohexane, 1-methyl-4-(1-methylethylidiène)	$C_{10}H_{16}$	136(17), 121(16), 119(48), 107(14), 91(100), 77(52), 65(48)	3,39
13,59	**trans-Sabinène hydrate**	$C_{10}H_{18}O$	154(5), 139(30), 135(24), 121(41), 106(25), 93(100), 91(75), 81(50), 79(82), 77(50), 67(36)	**26,64**
13,73	Santolina triène	$C_{10}H_{16}$	136(13), 121(50), 105(20), 93(100), 91(90), 79(61), 77(48), 67(45), 55(43)	2,12
16,37	**Terpinèn-4-ol**	$C_{10}H_{18}O$	154(17), 136(25), 111(45), 93(100), 91(65), 77(30), 71(45), 67(34)	**17,08**
16,84	p-Menth-1-èn-8-ol	$C_{10}H_{18}O$	154(0), 136(60), 121(80), 107(16), 93(100), 91(64), 81(35), 79(45), 67(35)	3,50
19,18	3-Carène	$C_{10}H_{16}$	136(8), 121(31), 105(20), 93(100), 91(80), 79(46), 67(25)	4,77
24,38	Caryophyllène	$C_{15}H_{24}$	204(2), 189(26),175(10), 161(42), 147(25), 133(75), 119(41), 105(80), 93(45), 91(100), 79(50), 67(21)	3,33
26,75	γ-Gurjunène	$C_{15}H_{24}$	204(23), 189(26), 176(4), 161(75), 147(15), 133(26), 121(100), 105(82), 93(90), 91(92), 79(60), 67(20)	1,40
29,08	(-)-Spathulénol	$C_{15}H_{24}$	220(0), 205(100), 187(55), 159(80), 145(46), 119(57), 105(70), 91(75), 79(38), 67(25)	2,79
32,95	1-Methyl-3-(3,4-dimethoxyphenyl)-6,7-dimethoxyisochromène	$C_{20}H_{22}O$	342(25), 340(48), 327(74), 281(60), 253(31), 207(82), 187(60), 162(87), 147(65), 119(64), 105(75), 91(100), 79(58), 67(41)	0,29
Total				**77,05%**
Bicyclic monoterpenes				**22,02%**
Oxygenated monotepenes				**47,22%**
Bicyclic sesquiterpenes				**4,73%**
Oxygenated sesquiterpenes				**2,79%**
Diterpenes oxygenated				**0,29%**

can clearly observe a toxic effect which depends on their concentrations. Adult survival to decrease as the

Table 2: Main components of the essential oil of Salvia officinalis

RT (min)	Constituants	Composition	Mass spectrum	%
7,82	α-Pinène	$C_{10}H_{16}$	136(5), 121(10), 105(15), 93(62), 92(45), 91(100), 77(45), 65(9)	3,79
8,27	Santolina triène	$C_{10}H_{16}$	136(3), 121(41), 107(17), 93(100), 91(67), 79(45), 77(32), 67(24)	2,56
9,23	3-Carène	$C_{10}H_{16}$	136(9), 121(20), 107(10), 93(85), 91(100), 79(40), 77(55), 67(16)	3,65
11,15	**Eucalyptol**	$C_{10}H_{18}O$	154(22), 139(54), 125(33), 121(25), 111(28), 108(30), 93(100), 81(59), 67(39)	**12,39**
14,2	**α-Thujone**	$C_{10}H_{16}O$	152(1), 135(8), 110(35), 109(30), 95(99), 81(43), 79(40), 67(100)	**20,52**
15,08	(-)-Camphre	$C_{10}H_{16}O$	152(20), 137(7), 108(85), 95(100), 93(42), 81(48), 67(65)	6,62
20,09	Exobornyl acétate	$C_{12}H_{20}O_2$	196(0), 154(10), 136(41), 121(66), 108(24), 95(100), 93(85), 79(35), 67(50)	0,15
24,38	Trans-Caryphyllène	$C_{15}H_{24}$	204(5), 189(23), 175(15), 161(40), 147(25), 133(81), 119(50), 105(82), 91(100), 79(50), 67(26)	6,78
25,43	α-Guaiène	$C_{15}H_{24}$	204(20), 189(21), 175(3), 161(22), 147(60), 121(37), 105(65), 93(100), 91(90), 79(45), 77(41), 67(26)	5,99
29,22	(-)-Caryophyllène oxyde	$C_{15}H_{24}O$	220(0), 187(16), 121(40), 105(60), 93(65), 91(100), 79(75), 67(47)	1,31
29,5	**γ-Gurjunène**	$C_{15}H_{24}$	204(35), 189(64), 175(15), 161(100), 147(35), 133(40), 119(55), 105(71), 93(50), 81(35), 67(47)	**11,13**
39,4	Verticellol	$C_{20}H_{34}O$	290(0), 272(9), 257(100), 229(30), 189(34), 161(40), 133(46), 121(56), 95(100), 81(82), 67(38)	1,16
Total				**75,87%**
Bicyclic monoterpenes				**10%**
Oxygenated monotepenes				**39,68%**
Bicyclic sesquiterpenes				**23,9%**
Oxygenated sesquiterpenes				**1,13%**
Diterpenes oxygenated				**1,16%**

3.2 *Effect of essential oils on adult B. lentis*

Figures 1 and 2 provide an overview of the survival curves Bruchus lentis treated with essential oils of marjoram and sage.Each essential oil of two plants, one

concentration of essential oils increases. The survival time of 50% of adults exposed to different concentrations of essential oils range from 1 to 4 days, whereas in the control group, in live adult average of 7 to 11 days. The

TL50 are negatively correlated with concentrations of essential oils tested (**Table 3**).

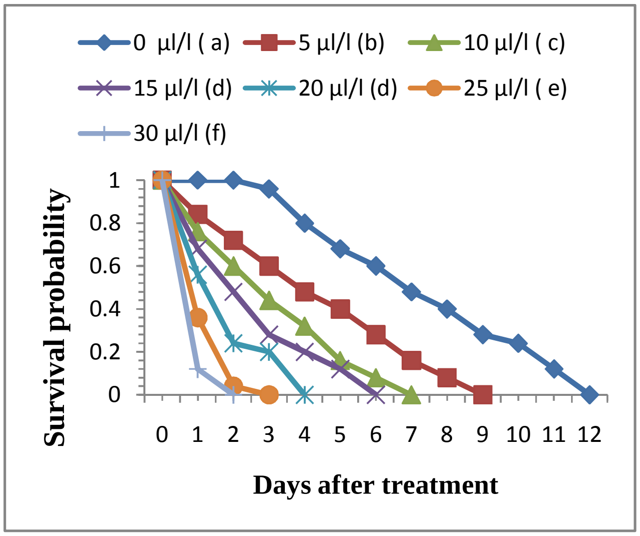

Figure 1: *Survival of adult B. lentis exposed to the essential oil of* O. majorana.

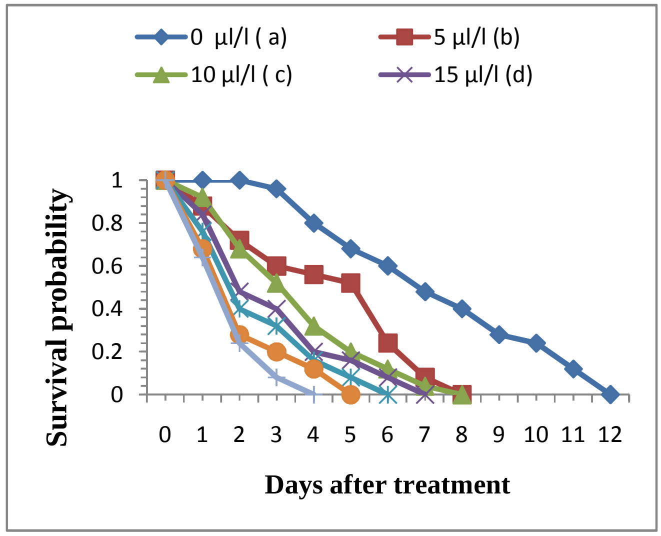

Figure 2: *Survival of adult B. lentis exposed to the essential oil of S. officinalis.*

Table 3: LT_{50} of Bruchus lentis adults fumigated with marjoram and sage essential oils.

Plant	Concentrations (µl/l air)	LT_{50}	$r>r_{(0,05;5)}$	LT_{99}	$r>r_{(0,05;5)}$
Witness	**0**	7,06		11,94	
	5	4,64		8,57	
	10	3,56		6,64	
Marjoram	**15**	2,89	-0,97	5,87	-0,98
	20	1,89		4,18	
	25	-		2,91	
	30	-		-	
	5	4,59		8,02	
	10	3,33		7,27	
Sage	**15**	2,44	-0,93	6,68	-0,94
	20	2,19		5,77	
	25	1,44		5,06	

The toxicity of essential oils of marjoram and sage parameters vis-à-vis adults Bruchus lentis are summarized in Table 4. Indeed, extreme LC50 range from about 17.66 to 5,02µl / l air to marjoram and between 66.96 to 4,69µl / l air to the sage, while ranging CL99 to marjoram about 155.35 16,34µl / l and to 28,23µl 4230.49 / l to sage.

Table 3: LT_{50} of Bruchus lentis adults fumigated with marjoram and sage essential oils.

Plant	Days	Slope [confidence interval]	$\chi^2_{calculé} < \chi^2_{(0,05;5)}$ =9,488	LC_{50} (µl/l air) [confidence interval]	LC_{99} (µl/l air) [confidence interval]
Marjoram	1	2,46 [1,52; 3,39]	7,604	17,66 [14,34; 22,27]	155,35 [81,79; 629,41]
	2	3,04 [0,89; 5,19]	10,36	10,28 [3,77; 15,48]	59,67 [30,21; 2124,56]
	3	2,88 [1,86; 3,89]	6,985	8 [5,47; 10,12]	51,26 [34,49; 111,35]
	4	3,72 [2,16; 5,28]	6,02	7,58 [4,51; 9,97]	31,96 [23,02; 63,80]
	5	4,87 [2,40; 7,34]	1,834	6,12 [3,43; 8,05]	18,37 [13,41; 39,73]
	6	4,53 [1,81; 7,25]	0,741	5,02 [2,14; 6,91]	16,34 [11,31; 52,16]
Sage	1	1,29 [0,30; 2,27]	2,144	66,96 [34,23; 5869,43]	4230,49 [381,76; 21535986,04]
	2	1,78 [0,95; 2,61]	1,619	13,42 [9,48; 17,74]	270,76 [105,73; 3370,90]
	3	1,88 [1,00; 2,76]	3,104	9,56 [5,46; 12,93]	164,18 [73,91; 1389,71]
	4	2,65 [1,43; 3,86]	2,3	8,29 [4,24; 11,49]	62,54 [38,16; 211,46]
	5	3,69 [1,91; 5,46]	2,41	8,06 [4,06; 10,99]	34,44 [24,21; 80,41]
	6	2,98 [1,27; 4,68]	2,234	4,69 [1,30; 7,30]	28,23 [17,81; 110,71]

IV. DISCUSSION AND CONCLUSION

In the literature, the essential oil of Origanum acutidens obtained by steam distillation caused 37% mortality of adult Tribolium confusum after 96h exposure [23]. The Origanum majorana oil and its components showed ovicidal and adulticide against the pyrethroid insecticide [17]. In addition, the insecticidal activity of oregano oil against the female Blattella germanica L. was comparable to that of permethrin and propoxur as shown LC50 values after 24 hours of exposure [16]. Oregano oil also has a strong fumigant toxicity adult Tribolium castaneum by the action of a vapor phase [5].

The essential oils of Salvia triloba and Salvia officinalis are used for commercial production of sage in Turkey [24]. Salvia species, called "adaçayi" in Anatolia, are used as antiseptics, stimulants, diuretics and wound healing in Turkish traditional medicine and herbal teas and food flavorings [24-26]. Oil Salvia hydrangea showed 68.3 to 75.0% mortality against adult Sitophilus granarius and Tribolium confusum [27]. However, there is so far no insecticidal effect of the essential oil of sage.

The mode of action of natural plant fumigants on insects has largely focused on the monoterpenoid [28]. Treated insects several reports indicate that monoterpenoid cause their deaths by inhibiting the enzyme acetylcholinesterase (AChE) [29; 30]. Monoterpenes also inhibit octopamine [31; 32]. In this study, the essential oils of marjoram and sage can be used as biopesticides in integrated management of weevils polyvoltine and pests of agricultural commodities stored. They can therefore replace phosphine, vis-à-vis that most stored product pests have developed resistance [33], and methyl bromide which agricultural use will be removed in

2015 in developing countries. The adoption by farmers essential for the control of pests of stored oils should not a priori be a problem, insofar as people are accustomed to using plant products to protect their crops.

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