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Chemical composition, toxicity and acetylcholinesterase inhibitory activity of *Salvia officinalis* essential oils against *Tribolium confusum*

Khemais Abdellaoui, Meriem Miladi, Iteb Boughattas, Fatma Acheuk, Nizar Chaira and Monia Ben Halima-Kamel

Abstract
The present study was aimed to evaluate the contact toxicity of *Salvia officinalis* essential oils against *Tribolium confusum*. Essential oils were obtained by Clevenger-type water distillation and their contact toxicities were tested at concentrations of 0.06, 0.12, 0.25 and 0.5 µL/cm^2. β-Thujone (20.1%), 1,8-Cineole (15.91%), Camphor (14.79%) and Viridiflorol (9.06%) were determined to be the major constituents of the oil. Biotests showed that essential oils exhibited contact toxicity against *T. confusum* larvae and adults with median lethal concentrations values of 0.13 and 0.16 µL/cm^2, respectively after 7 days of treatment. Toxicity was also demonstrated by a significant repellent activity in binary choice bioassays. Results showed that essential oils caused significant inhibition in the acetylcholinesterase activity with a dose-response relationship. The highest concentration used (0.5 µL/cm^2) was the most effective inhibitor of *T. confusum* AChE activity (59.88 ± 4.71%) after 72 h of exposure.

Keywords: Botanical insecticide, *Tribolium confusum*, toxicity, enzymatic activity

1. Introduction
The global post-harvest grain losses caused by insect damage and other bio-agents range from 10 to 40% [1]. Insect pests are the major problem in storage products throughout the world because they reduce the quantity and quality of stored products [2]. Insects belonging to the family Tenebrionidae and especially to the genus *Tribolium* are important pests of stored substances such as flour, cereals, meal, beans, spices and even dried museum specimens [3]. The confused flour beetle, *Tribolium confusum* (Jacquelin Du Val) (Coleoptera: Tenebrionidae) is a rather significant pests in stored grain and related products [4]. It is a polyphagous species that easily penetrates products, has a relatively short development cycle, decreases the quality of colonized products and causes enormous economic losses each year. Furthermore, this species is resistant to several traditional insecticides, which are commonly used as grain protectants [5]. In Tunisia and North Africa, Jarraya [6] reported that this insect is amid the most important and destructive pests in the mills. Besides contamination with the bodies and frass of *T. confusum*, the food substrate will be exposed to quinones that are released from the thoracic and abdominal defence glands [7]. The measures used worldwide to control stored product insect infestations rely mainly on the use of fumigants such as methyl bromide and phosphine. These insecticides bring about such serious problems as contamination of the environment, lethal effects in non-targeted organisms which can lead to a failure of biological control programs, and insect resistance. Toxic residues on stored grain for human consumption are other problems related to chemical pesticides [8]. Therefore, efforts are being made worldwide to find a series of alternative methods to control pests, such as the employment of naturally derived chemical compounds (biopesticides), which are less toxic to the environment [9, 10]. Among current alternative strategies aiming at decreasing the use of classical insecticides, economical control based on plant-insect relationships is one of the most promising methods [11]. Many scientists believe that plant extracts can be one of the most efficient alternatives to pest control. Indeed, plants may provide a potential option to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Nicotine (from *Nicotiana tabacum*), piretrins (from *Tanacetum cinerariifolium*) and rotenone (from *Derris* and *Lonchocarpus*) being good examples of natural compounds used long ago to control agricultural pests [12].

Recently, plant essential oils have been receiving global attention and considered as potential alternatives to conventional insecticides. Plant essential oils, abundant in aromatic plants families such as Lamiaceae and Apiaceae, are easily obtained by steam-distillation and contain many bioactive compounds having insecticidal, nematicidal, or antifungal properties [13]. Many researchers highlighted their action against insects as: larvicidal, antifeedant, growth inhibitory, adulticidal, fertility reducer, oviposition deterrent and repellent [14, 15, 16]. Furthermore, they are characterized by a low toxicity to human and animals, high volatility, and toxicity to stored grain insect pests [17]. The aim of this research was to evaluate the insecticidal activity and repellency of *Salvia officinalis* (Lamiaceae), medicinal plant which occurs in the Tunisia flora, essential oils in the control of the confused flour beetle *T. confusum*.

2. Materials and Methods

2.1 Insect culture

Insects used for testing came from the culture maintained in the entomological laboratory at the Higher Institute of Agronomy of Chott Mariem, Sousse University. *T. confusum* was reared in 2-L plastic containers containing wheat flour mixed with yeast (10:1 w/w). The cultures were maintained in darkness in a growth chamber set at 30±1 °C and 60-70% r.h.

2.2 Plant materials

Leaves of *S. officinalis* were collected on April 2014 from the experimental farm of the Higher Agronomic Institute of Chott-Mariem (N: 36.81°, E: 10.18°) in the Sahel of Tunisia. The samples were dried naturally in the shade at room temperature (23-27 °C) for two weeks. The dried materials were ground to a fine powder used for the extraction of essential oils.

2.3 Extraction and analysis of essential oils

The essential oils were extracted from 100 g dried-leaves of *S. officinalis* by hydrodistillation for 4 hours, using a Clevenger-type apparatus. Anhydrous sodium sulphate was utilized to remove water after extraction. Essential oils samples were than stored in sterile tubes at 4 °C until analyses.

Chemical compounds of the essential oils were determined using GC-MS (GCMS-QP 2010 Plus Shimadzu, Japan) equipped with a RTX-5 ms capillary column (30m x 0.25mm x 0.25µm film thickness). The column temperature was initially programmed at 50 °C for 2 min, then gradually increased at 7 °C/min until the final temperature of 250 °C was reached, where it was held for 5 min. The injector and detector temperatures were 250 and 280 °C respectively, using helium as the carrier gas, at a flow rate of 1.2 mL/min. The injection volume was 1 µL with a split ratio of 1:50. The identification of the components separated by GC-MS was made by comparing the obtained mass spectra for each component with the values stored in NIST Mass Spectral Library (NIST 08). The percentage composition of the oils was calculated in peak areas using the normalization method.

2.4 Contact toxicity bioassays

The contact toxicity of *S. officinalis* essential oils against *T. confusum* was evaluated on filter paper discs (Whatman No. 1, cut into 5 cm diameter pieces) which were treated with the substances diluted in acetone. 500 µL of each solution was applied to the surface of the filter papers and homogeneously distributed, giving a range of concentration of 0.06, 0.12, 0.25 and 0.5 µL/cm^2. After drying under a room temperature for 10 min, each filter paper disc was placed in the bottom of a Petri dish, and then 20 *T. confusum* adults (1-7 days old) or larvae (1.88 ± 0.15 mg) were introduced. Untreated controls used acetone alone (with the same 10 min-delay before deposition of the insects on the filter paper disc). In each experimental box, 0.5 g of food (artificial diet of wheat flour mixed with beer yeast: 10/1 w/w) was added. Control and treated groups were kept under the same conditions described above for mass rearing. Each treatment was replicated four times. The mortality was assessed daily via direct observation for a period of 7 days. Insects were considered dead when no leg or antenna movements were observed after prodding with a fine brush. The mortality was calculated using the Abbott correction formula for natural mortality in untreated controls [18]. Probit analysis [19] was conducted to estimate the LC_{50} and LC_{95} values with their 95% fiducial limits (FL). Time-mortality data for each experiment were analyzed via the method developed by Finney [19], with time as the explanatory variable to derive the estimated time for 50% mortality (LT_{50}).

2.5 Repellency bioassay

Repellency assays of *S. officinalis* essential oils were carried out using the area preference method [20] at 27 ± 1 °C and 60-70% r.h. Experiments were conducted in glass Petri dishes (diameter 8.5 cm and height 1.2 cm) using concentrations of 0.06, 0.12, 0.25 and 0.5 µL/cm^2 prepared in acetone. The Whatmann filter paper was cut into two equal halves and each test solution (500 µL) was applied to filter paper half as uniform as possible using micropipette. The other half of filter paper was treated with acetone alone as a control. The treated and control half discs were air-dried to evaporate solvent completely. Both treated and untreated halves were then attached using adhesive tape and placed in Petri dishes. Twenty adults of *T. confusum* (7-14 days-old) were released at the centre of each filter paper disc and then Petri dishes were covered and sealed with parafilm. Five replicates were used for each tested concentration. Observations on the number of insects present on both the treated and untreated halves were recorded after 2, 4 and 8 h of exposure. Percentage repellency (PR) values were computed as follows: PR = [(Nc - Nt)/(Nc + Nt)] x 100, where Nc was the number of insects on the untreated area and Nt was the number of insects on the treated area. Results were presented as the mean of percentage repellency ± the standard deviation. To categorize the repellent effect of the essential oils, the method of Tapondjou *et al.* [21] was used. Five groups were formed based on the mean of percent repellency (PR): Class 0: PR= 0-0.1%; Class I: PR = 0.1-20%; Class II: PR = 20.1-40%; Class III: PR = 40.1-60%; Class IV: PR = 60.1-80%, and Class V: PR = 80.1-100%.

2.6 Acetylcholinesterase activity

Larvae were homogenized in 1 mL of 0.1 M phosphate buffer (pH 7.4) using a Teflon glass tissue homogenizer. Homogenates were centrifuged (15,000 g for 20 min at 4 °C) and supernatants were used for enzyme assays. The acetylcholinesterase activity (AChE) was determined according to the method of Ellman *et al.* [22] using the Jenway 6105 spectrophotometer. The reaction medium included sodium phosphate buffer (0.1 M, pH 7.2), DTNB (1.6 mM), AcSChI (156 mM) and sample (S9). Kinetics was recorded at 412 nm and the assay was carried out at 25 °C. The enzymatic activity was expressed as nM of acetylthiocholine hydrolyzed per min per mg of proteins. The AChE inhibition assay was

replicated 6 times. The percentage inhibition rate of each treatment was calculated using the following formula [23]: %Inhibition rate = 100- (Enzyme activity of treatment/Enzyme activity of control*100). The IC_{50} was estimated by probit analysis.

2.7 Data analysis

Statistical analysis was performed using Probit analysis [19] to determine LC_{50} and LC_{95} (Lethal Concentration for 50% or 95% mortality) values with their 95% confidence limits. Lethal concentration values were considered significantly different when their respective 95% fiducial limits did not overlap [24]. Data from repellency and enzyme activities were expressed as means ± standard deviation (SD) and subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS: version 18.0). The significance between control and treated series was made by the Student-Newman-Keuls (*SNK*) test at the 5% level.

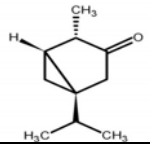
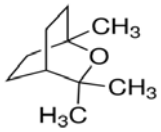
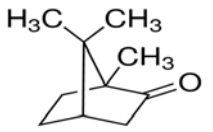
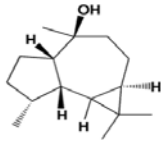
3. Results

3.1 Chemical composition of the essential oils

The yield of the essential oils extracted from the leaves of *S. officinalis* growing in Tunisia and obtained by hydrodistillation was 1.03 ± 0.2%. Data of the chemical composition analysis (Table 1) revealed that the essential oil of *S. officinalis* contains, mainly β-Thujone (20.1%), 1,8-Cineole (15.91%), Camphor (14.79%) and Viridiflorol (9.06%). A total of 53 compounds amounting to 97.66 were identified by GC-MS.

Table 1: Chemical constituents of the essential oils from *S. officinalis* leaves collected from Chott Meriam, Tunisia.

Peak No.	Compounds	RT	Peak area (%)
1	α-thujene	7. 990	0.23
2	α-pinene	8.164	0.03
3	Camphene	8.585	0.18
4	Sabinene	9.354	0.44
5	β-pinene	9.414	2.28
6	1-Octen-3-ol	9.708	0.07
7	β-Myrcene	9.919	1.35
8	1-Phellandrene	10.273	0.06
9	Cymol	10.906	0.13
10	1,8-Cineol	11.095	15.91
11	Cis-Ocimene	11.336	0.22
12	Trans- β- Ocimene	11.645	0.05
13	Ɣ-Terpinene	11.931	0.56
14	Trans-Sabinene hydrate	12.240	0.27
15	α-Terpinolene	12.83	0.34
16	Cis-Sabinene-hydrate	13.205	0.22
17	Linalool L	13.265	0.19
19	β-Thujone	13.393	20.1
20	α-thujone	13.687	6.23
21	Isothujol	14.327	0.2
22	Camphor	14.501	14.79
23	Piperitenone	14.802	0.13
24	Pinocamphone	14.938	0.26
25	Borneol L	15.201	3.22
26	4-Terpineol	15.480	0.74
27	α- Terpineol	15.895	0.53
28	2-Pinen-10-ol	16.121	0.06
29	1-Bornyl acetate	18.479	0.88
30	(-)-Myrtenyl acetate	19.557	0.03
31	Copaene	20.190	0.04
32	Ylangene	20.891	0.03
33	β-Bourbonene	21.132	0.12
34	Ɣ-Muurolen	21.260	0.02
35	Caryophyllene	22.028	3.5
36	β-Cubebene	22.262	0.04
37	α-Humulene	22.887	1.99
38	(+-)-isoseychellene	23.053	0.07
39	α-Amorphene	23.445	0.09
40	Germacrene D	23.566	0.13
41	Ledene	23.912	0.08
42	Junipene	24.440	0.03
43	δ-Cadinene	24.583	0.13
44	Dodecanoic acid	25.766	0.11
45	Caryophyllene oxide	26.022	0.5
46	Viridiflorol	26.278	9.06
47	Ɣ-Gurjunene	26.504	0.08
48	Tetradecanoic acid	30.084	0.61
49	Palmitic acid	34.077	1.72
50	13-Epitorulosol	35.803	4.57
51	o-Xylene-d10	39.932	1.15

52	Bicyclo[10.1.0] tridec-1-ene	42.751	1.96
53	Olealdehyde	42.826	1.93
EO yields			1.03 ± 0.2
Total (%)			97.66
Major components structure			
			
β-Thujone	1,8-Cineole	Camphor	Viridiflorol

3.2 Contact Toxicity

This experiment was conducted in order to determine the insecticidal activity of *S. officinalis* essential oils on *T. confusum*. Results showed that these oils exhibited contact toxicity against *T. confusum* larvae and adults with median lethal concentrations values of 0.13 and 0.16 µL/cm^2, respectively after 7 days of treatment. The Probit analysis also demonstrated that both larvae and adults manifested similar toxicity to *S. officinalis* essential oils. Indeed, the comparison of the LC_{50} and LC_{90} values and their upper and lower 95% confidence limits using the Preisler method, showed no significant differences between the two biological stages (Table 2).

Table 2: Toxicity of *T. confusum* larvae and adults treated with *S. officinalis* essential oils in contact toxicity bioassay.

Insects	LC_{50} [a, b]	LC_{95} [a, b]	Chi square (χ2)	df	Comparison using the Preisler method
Larvae	0.13 (0.1- 0.17)	0.28 (0.23 - 0.42)	110.78	10	A*
Adults	0.16 (0.11 - 0.22)	0.3 (0.23 - 0.52)	204.73	10	A

[a] Units LC_{50} and LC_{95}=µL/cm^2.
[b] 95% lower and upper fiducial limits are shown in parenthesis. *Based on the Preisler method and comparing the upper and lower confidence limits, these values overlap, and no significant difference is observed and same letter (A) is used.

Experiment was also designed to assess median effective time to cause mortality of 50% of treated insects (LT_{50}). LT_{50} values for concentrations of 0.12, 0.25 and 0.5 µL/cm^2 were regrouped in table 3. Probit analysis showed that the LT_{50} for *T. confusum* larvae ranged from 5.09 days (95% FL = 4. 85 to 6.99 days) for the lowest dose (0.12 µL/cm^2) to 0.91 day (95% FL = 0. 36 to 1.28 days) for the highest dose (0.5 µL/cm^2). The LT_{50} values for *T. confusum* adults ranged from 6.59 days (95% FL = 5.78 to 7.97 days) to 2.22 days (95% FL = 0.93 to 2.99 days) for the lowest and highest doses, respectively (Table 3). Generally, the lethal time values decreased when the essential oils concentrations increased. In all cases, increased susceptibility of both stages was directly associated with oil concentration.

Table 3: LT_{50} values of *S. officinalis* essential oils against larvae and adults of *T. confusum.*

Insects	Concentration (µL/cm^2)	LT_{50} (days)[a]	df	Chi square (χ2)
Larvae	0.12	5.09 (4. 85-6.99)	5	4.61
	0.25	1.09 (0.51-1.5)	5	3.06
	0.5	0.91 (0. 36-1.28)	5	4.26
Adults	0.12	6.59 (5.78-7.97)	5	5.35
	0.25	4.07 (3.37-4.76)	5	18.12
	0.5	2.22 (0.93-2.99)	5	23.21

[a] 95% lower and upper fiducial limits are shown in parenthesis.

3.3 Repellency bioassay

The results of repellency effects are presented in Table 4. Results showed that *S. officinalis* essential oils were found to be repellent against *T. confusum* adults in binary choice bioassays. Oils were repellent even at low concentrations. Indeed, the lowest concentration (0.06 µL/cm^2) led to percentage repellency of 46.6 ± 11.5% after 2h of exposure, but it increased to 63.3 ± 5.7% after 8h. The repellent activity becomes more evident by increasing the concentration of the oil and the maximum activity (100% repellency) was observed at the highest concentrations (0.12 and 0.5 µL/cm^2) after 8 h of exposure (Table 4). The analysis of variance, with the oil concentrations as classification criteria, showed a significant difference among treatments for all exposure time tested (F = 6.33, df = 3, P = 0.016; F = 5.12, df = 3, P = 0.028; F = 28.25, df = 3, P<0.001), respectively for 2, 4 and 8 hours of exposure and the *SNK*-test gives heterogeneous groups represented by different letters in table 4.

Table 4: The results of the repellency effect (mean percentage repellency ± SD) of *S. officinalis* essential oils against *T. confusum* adults.

Concentrations (µL/cm^2)	2h	4h	8h
0.06	46.6 ± 11.5^a	53.3 ± 15.2^a	63.3± 5.7^a IV
0.12	60± 10^{ab}	66.6 ± 11.5^{ab}	80 ± 10^b IV
0.25	80 ± 17.3^b	86.6 ± 15.2^b	100 ± 0^c V
0.50	83 ± 5.7^b	90 ± 10^b	100 ± 0^c V
F-value	6.33	5.12	28.25
P-value	0.016	0.028	<0.001

The mean of percent repellency (PR): Class 0: PR = 0-0.1%, Class I: PR = 0.1-20%, Class II: PR = 20.1-40%, Class III: PR = 40.1-60%, Class IV: PR = 60.1-80%, and Class V: PR = 80.1-100%. The mean with the same letter for each column are not significant (SNK test at p<0.05).

3.4 AChE inhibition assay and IC_{50} estimation

Data from enzyme activitiy were subjected to two-way ANOVA followed by SNK test at $P = 0.05$. Results of the AchE determination are shown in Figure 1. In control, the mean AChE activity remained constant during the two first days of treatment and increased significantly ($F = 4.66$, df = 2, $P = 0.02$) at day 3. AChE values of the control group ranged from 1.46 ± 0.12 to 1.66 ± 0.06 nM/min/mg proteins after 24 and 72 h, respectively (Figure 1). Treatment with the essential oils affected the AChE activity. ANOVA revealed a significant effect of the essential oils on the AChE activity which varied as a function of the concentration and the duration of treatment. As compared to controls, the mean values of AChE activity recorded during the experimental period decreased significantly with the all tested concentrations mostly after 72 h of exposure ($F = 190.7$, df = 3, $P<0.0001$). Data also indicated that the two highest concentrations (0.25 and 0.5 µL/cm^2) had the greatest inhibitory activity at all the time periods evaluated and there was no significant difference between them. Indeed, the AChE activity recorded in the treated group at these concentrations is 0.80 ± 0.11 and 0.66 ± 0.07 nM/min/mg proteins after 72 h of exposure, respectively (Figure 1).

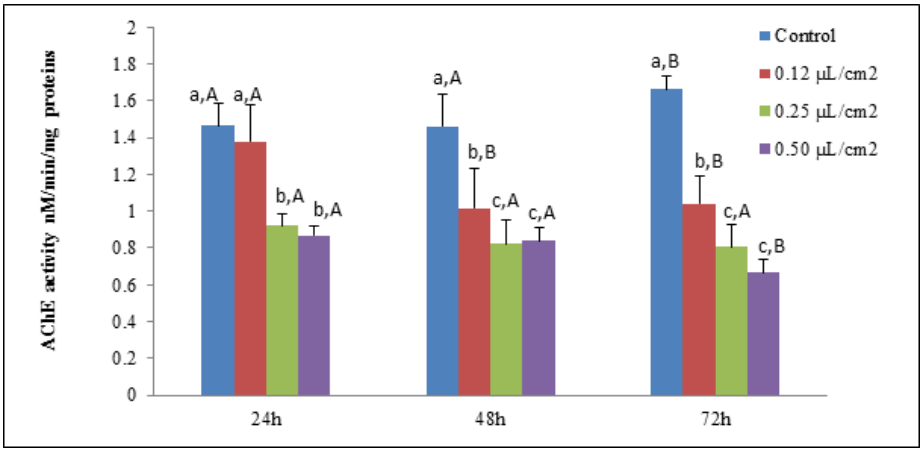


Fig 1: Effect of *S. officinalis* essential oils on AChE activity of *T. confusum* larvae. Data are means ± SD of sex replicates. Different letters are significantly differen at 0.05 level. Comparison was made between concentrations (letter in lowercase) and exposure times (letter in uppercase).

As summarized in table 5, we determined the AChE inhibition rates and the IC_{50} of the *S. officinalis* essential oils against *T. confusum* larvae after 72 h of exposure. Essential oils caused significant inhibition in the AChE activity with a dose-response relationship. The highest concentration used (0.5 µL/cm^2) was the most effective inhibitor of *T. confusum* AChE activity (59.88 ± 4.71%). The analysis of variance with the oil concentrations as classification criteria shows a significant difference ($F = 12.68$, df = 16, $P = 0.001$) among treatments and the SNK-test gives heterogeneous groups represented by different letters in table 5. Probit analysis showed that the concentration needed for the essential oils to cause IC_{50} for *T. confusum* larvae was 0.29 µL/cm^2 (95% FL = 0.21 - 0.38 µL/cm^2) (Table 5).

Table 5: AChE inhibition and IC_{50} values of *S. officinalis* essential oils against *T. confusum* larvae.

Concentrations (µL/cm^2)	Inhibition rate ± SD (%)	IC_{50} (µL/cm^2)	95% FL[a]	df	$\chi 2$
0.12	37.15 ± 10.85[a]	0.29	0.21 - 0.38	16	45.53
0.25	51.47 ± 6.87[b]				
0.50	59.88 ± 4.71[b]				

[a]Fiducial limits. Mean inhibition rate values corresponding to each treatment with different letters are significantly different ($F_{2,15} = 12.68$, $P = 0.001$, SNK test).

4. Discussion

Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment [10]. In this context, essential oils have received much attention of the scientific communities in a pest management programme as potentially useful bioactive compounds [25, 26]. The present study demonstrated that essential oils of *S. officinalis* (Lamiaceae) possess contact toxicity as well as repellency effects against the confused flour beetle, *T. confusum*. It has been shown that different concentrations of the essential oils from *S. officinalis* exhibited toxicity against *T. confusum* larvae and adults with an LC_{50} value of 0.13 and 0.16 µL/cm^2, respectively. In previous studies, the insecticidal activity of many essential oils from Lamiaceae has been evaluated against a number of stored product insects. In fact, *Ocimum basilicum* essential oils showed insecticidal activity against *Sitophilus oryzae*, *Stegobium paniceum*, *T. castaneum* and *Bruchus chinensis* [11]. Oils from *Thymus vulgaris* were toxic to *Rhizopertha dominica* [27]. Plant essential oils of Lamiaceae have also been documented to show insecticidal and repellent activity against several insect pests. Petrakis *et al.* [28] reported the effect of three Lamiaceae family members: *Origanum majorana*, *Mentha pulegium*, and *Melissa offcinalis*, on longevity and fecundity of the aphid *Myzus persicae*. Ogendo *et al.* [29] noted the fumigant and repellent effects of *Ocimum gratissimum* essential oils and its constituents, β-(Z)-ocimene and eugenol, on adults of *S. oryzae*, *T. castaneum*, *Oryzaephilus surinamensis*, *R. dominica*, and *Callosobruchus chinensis*. Essential oils extracted from some Lamiaceae aromatic plants, especially *Satureja*, *Origanum* and *Mentha*, prevented egg hatching and provoked prohibition or malformation of the puparium of the flies *Drosophila auraria* [30]. Aslan *et al.* [31] showed insecticidal activities of essential oils from the plant species *Micromeria fruticosa*, *Nepata racemosa* and

Origanum vulgare (Lamiaceae) against the adults of *S. granarius* and larvae (third instar) of *Ephestia kuehniella*. In the same context, Kim *et al.* [32] have recently evaluated the fumigant toxicity of eight Lamiaceae essential oils and their constituents against the adult rice weevil *S. oryzae*. Of the eight species tested, *Hyssopus offcinalis*, *O. majorana*, and *Thymus zygis* essential oils showed strong fumigant toxicity against *S. oryzae* adults at 25 mg/L air concentration. Among the test compounds, pinocamphone and isopinocamphone showed the strongest fumigant toxicity against *S. oryzae*. Sabinene hydrate, linalool, α-terpineol, and terpinen-4-ol exhibited 100% fumigant toxicity against *S. oryzae* at 3.9 mg/L air concentration. Another essential oil from *Hyptis spicigera* (Lamiaceae) were tested on *C. maculates*. Essential oil had a dose-dependent insecticidal effect while sub-lethal doses were only repellent to adults, also reduced oviposition eggs viability with increasing doses [33].

Salvia officinalis is one of the most important species of the genus *Salvia* which comprises nearly 1000 species, and represents one of the largest genera in the Lamiaceae family. *S. officinalis* essential oils have been the subject of many studies and for many of them certain biological activities are proved. Souguir *et al.* [34] reported interesting insecticidal properties against *Spodoptera littoralis* third instar larvae with an LC_{50} = 23.05 µl/L air. The insecticidal properties displayed by *S. officinalis* against *T. confusum* seems to be related to the major constituents detected in plant essential oils principally monoterpenes such as 1,8-Cineole and Camphor. Several previous studies reported that the insecticidal activities of plant oils were attributed to some of their major chemical constituents [35]. There are numerous reports regarding the insecticidal activity of 1,8-Cineole and Camphor against stored-product insects [36]. In a detailed study, Dunkel and Sears [37] demonstrated potent toxic effects of Camphor from *Artemisia tridentate* against *T. castaneum.* Also, Obeng-Ofori *et al.* [38] found that 1,8-Cineole to be highly repellent and toxic against some stored product beetles. In the same context, Negahban *et al.* [39] attributed the higher toxicity of the *A. sieberi* oils to higher concentrations of the Champhor. Rozman *et al.* [40] reported that 1,8-Cineole and Camphor produced 92.5% and 77.5% mortality, respectively against *T. castaneum* after 7 days exposure. Similarly, Suthisut *et al.* [41] also showed that 1,8-Cineole and Camphor from *Curcuma zedoaria* rhizome essential oils exhibited fumigant toxicity against *S. zeamais* and *T. castaneum*.

In a second series of experiments, we noted that the contact toxicity of *S. officinalis* essential oils was also demonstrated by reducing significantly the activity of AChE. This enzyme has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides. Our results agree with the reports of others regarding the inhibitory activity of essential oils and monoterpenes on AChE activity. Bessette *et al.* [42] noted that in direct contact, essential oils may penetrate via the insects cuticle and contact the nerve endings in the invertebrate pest's trachea, and cause neurotoxic activity and more rapid death. Several reports indicated that monoterpenoids was lethal to the insects through inhibition of the activity of the AChE enzyme [43]. Picollo *et al.* [44] noted that among four monomterpenes (1,8-Cineole, (−)-carvone, (−)-limonene, and (−)-linalool), the 1,8-Cineole was the most inhibitory effect on AChE activity. However, it was also observed that the AChE inhibition was not necessarily related to insect mortality levels. Lee *et al.* [35] did not find a direct correlation between insect toxicity and AChE inhibition. Menthone from *M. arvensis* was highly toxic (LC_{95} = 25 ml/l) to *S. oryzae* but it had a relatively small inhibitory effect on AChE activity (*ki* = 0.39 mM) whilst less toxic *β*-pinene (LC_{95} = 107 ml/l) showed high-level inhibition (*ki* = 0.0028 mM). These findings suggest that AChE may be a target for monterpenes but does not rule out that there may be other targets like cytochrome P450-dependent monooxygenases [8].

5. Conclusion

The observed contact activity demonstrates that essential oils are a source of biologically active compounds which may potentially prove to be efficient insecticides. Furthermore, the essential oils may have the advantage over conventional insecticides in terms of low toxicity to human and the environment, rapid degradation and local availability. Therefore, the possibility of employing these botanical insecticides to control insects in stored products seems to be practical. Indeed, Essential oil-based aerosol and dust formulations for household insect pest control are already marketed in the USA.

6. References

1. Papachristos DP, Stamopoulos DC. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* Say Coleoptera: Bruchidae. Journal of Stored Products Research. 2002; 38:117-128.
2. Madrid FJ, White NDG, Loschiavo SR. Insects in stored cereals, and their association with farming practices in southern Manitoba. Canadian Entomologist. 1990; 122:515-523.
3. Jovanović Z, Kostić M, Popović Z. Grain-protective properties of herbal extracts against the bean weevil *Acanthoscelides obtectus* Say. Industrial Crop and Products. 2007; 26:100-104.
4. Mediouni Ben Jemaa J, Tersim N, Toudert KT, Khouja ML. Insecticidal activities of essential oils from leaves of *Laurus nobilis* L. from Tunisia, Algeria and Morocco, and comparative chemical composition. Journal of Stored Products Research. 2012; 48:97-104.
5. Arthur FH. Grain protectants: current status and prospects for the future. Journal of Stored Product Research. 1996; 32:293-302.
6. Jarraya A. Principaux nuisibles des plantes cultivées et des denrées stockées en Afrique du Nord: leur biologie, leurs ennemis naturels, leurs dégâts et leur contrôle. Edn1, Climat Pub, Tunisia, 2003, 415.
7. Hodges RJ, Robinson R, Hall DR. Quinone contamination of dehusked rice by *Tribolium castaneum* Herbst Coleoptera: Bostrichidae. Journal of Stored Products Research. 1996; 32:31-37.
8. Rajendran S, Sriranjini V. Plant products as fumigants for stored-product insect control. Journal of Stored Products Research. 2008; 44:126-135.
9. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: low-risk products in a high-stakes world. Annual Review of Entomology. 2012; 57:405-424.
10. Ebadollahi A. Essential oils isolated from Myrtaceae family as natural insecticides - a review. Annual Review and Research Biology. 2013; 3(3):148-175.
11. Regnault-Roger C. The potential of botanical essential oils for insect pest control. Integrated Pest Management. 1997; 2:25-34.
12. Abdellaoui K, Ben Halima-Kamel M, Acheuk F, Soltani

N, Aribi N, Ben Hamouda MH. Biochemical and histological effects of gibberellic acid on *Locusta migratoria migratoria* fifth instar larvae. Pesticide Biochemistry and Physiology. 2013; 107:32-37.
13. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils. A review. Food and Chemical Toxicology. 2008; 46:446-475.
14. Werdin-González JO, Gutiérrez MM, Murray AP, Ferrero AA. Composition and biological activity of essential oils from Labiatae against *Nezara viridula* Hemiptera: Pentatomidae soybean pest. Pest Management Science. 2011; 67:948-955.
15. Cardiet G, Fuzeau B, Barreau C, Fleurat-Lessard F. Contact and fumigant toxicity of some essential oil constituents against a grain insect pest *Sitophilus oryzae* and two fungi, *Aspergillus westerdijkiae* and *Fusarium graminearum*. Journal of Pest Science. 2012; 85:351-358.
16. Licciardello F, Muratore G, Suma P, Russo A, Nerín C. Effectiveness of a novel insect-repellent food packaging incorporating essential oils against the red flour beetle *Tribolium castaneum*. Innovative Food Science and Emerging Technologies. 2013; 19:173-180.
17. Batish DR, Singh HP, Kohli RK, Kaur S. *Eucalyptus* essential as natural pesticide. Forest Ecology and Management. 2008; 256:2166-2174.
18. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of economic entomology. 1925; 18:265-267.
19. Finney DJ. Probit analysis, Edn3. Cambridge University, London, 1971, 333.
20. Mc Donald LL, Guy RH, Speirs RD. Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. Marketing Res. Report No. 882. Agric. Res. Serv., US. Dept. Agric. Washington DC. 1970, 8.
21. Tapondjoua AL, Adlerb C, Fontem DA, Boudaa H, Reichmuthb C. 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. 2005; 41:91-102.
22. Ellman GL, Courtney KD, Andres JV, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 1961; 7:88-95.
23. Yeom HJ, Jaesoon J, Kim SW, Park IK. Fumigant and contact toxicity of Myrtaceae plant essential oils and blends of their constituents against adults of German cockroach *Blattella germanica* and their acetylcholinesterase inhibitory activity. Pesticide Biochemistry and Physiology. 2013; 107:200-206.
24. Robertson J, Russell R, Preisler H, Savin N. Bioassay with Arthropods. Edn 2, CRC Press Taylor and Francis, New York, 2007, 224.
25. Mahdi KRA, Behnam AB. Insecticidal potential of essential oil from the leaves of *Citrus aurantium* L. against *Oryzaephilus surinamensis* F. *Lasioderma serricorne* L. and *Sitophilus oryzae* L. Journal of Entomology and Zoology Studies. 2016; 4(5):865-869.
26. Idoko JE. Toxicity and Sub-lethal effect of essential oils of *Eugenia aromatica* and *Piper guineense* on cowpea seed beetle *Callosobruchus maculatus* Coleoptera: Chrysomelidae. Journal of Entomology and Zoology Studies. 2017; 5(2):895-899.
27. Kurowska A, Kalemba D, Gora J, Majda T. Analysis of essential oils: influence on insects. Part IV. Essential oil or garden thyme *Thymus vulgaris* L. Pestycydy. 1991; 2:25-9.
28. Petrakis EA, Kimbaris AC, Perdikis DC, Lykouressis DP, Tarantilis PA, Polissiou MG. Response of *Myzus persicae* Sulzer to three Lamiaceae essential oils obtained by microwave-assisted and conventional hydrodistillation. Industrial Crop and Products. 2014; 62:272-279.
29. Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, *et al.* Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. Journal of Stored Products Research. 2008; 44:328-334.
30. Konstantopoulou I, Vassilopoulou L, Mauragani-Tsipidov P, Scouras ZG. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. Experientia. 1992; 48:616-19.
31. Aslan I, Çalmaşur O, Şahin F, Çağlar O. Insecticidal effects of essential plant oils against *Ephestia kuehniella* Zell. *Lasioderma serricorne* F. and *Sitophilus granarius* L. Journal of Plant Diseases and Protection. 2005; 12:257-267.
32. Kim SW, Lee HR, Jang MJ, Jung CS, Park IK. Fumigant toxicity of Lamiaceae plant essential oils and blends of their constituents against adult rice weevil *Sitophilus oryzae*. Molecules. 2016; 21, 361. doi:10.3390/molecules21030361.
33. Sanon A, Iboudo Z, Dabire CLB, Nebie RCH, Dicko IO, Monge JP. 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. 2006; 52:117-123.
34. Souguir S, Chaieb I, Ben Cheikh Z, Laarif A. Insecticidal activities of essential oils from some cultivated aromatic plants against *Spodoptera littoralis* Boisd. Journal of Plant Protection Research. 2013; 53:388-391.
35. Lee SE, Lee BH, Choi WS, Park BS, Kim JG, Campbell BC. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* L. Pest Management Science. 2001; 57:548-553.
36. Lee BH, Annis PC, Tumaalii F, Lee SE. Fumigant toxicity of *Eucalyptus blakelyi* and *Melaleuca fulgens* essential oils and 1,8-cineole against different development stages of the rice weevil *Sitophilus oryzae*. Phytoparasitica. 2004; 32:498-506.
37. Dunkel FV, Sears LJ. Fumigant properties of physical preparations from mountain big sagebrush, *Artemisia tridentata* Nutt. ssp. *vaseyana* Rydb. beetle for stored grain insects. Journal of Stored Products Research. 1998; 34:307-321.
38. Obeng-Ofori D, Reichmuth CH, Bekele J, Hassanali A. Biological activity of 1,8-cineol, a major component of essential oil of *Ocimum kenyense* Ayobangira against stored product beetles. Journal of Applied Entomology. 1997; 121:237-243.
39. Negahban M, Moharramipour S, Sefidkon F. Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored product insects. Journal of Stored Products Research. 2006; 43:123-128.
40. Rozman V, Kalinovic I, Korunic Z. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to

three stored-product insects. Journal of Stored Products Research. 2007; 43:349-355.
41. Suthisut D, Fields PG, Chandrapatya A. Fumigant toxicity of essential oils from three Thai plants Zingiberaceae and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. Journal of Stored Products Research. 2011; 47:222-230.
42. Bessette S, Lindsay A, Enan E. Pesticidal compositions containing rosemary oil and wintergreen oil, US. Patent US 20130142893; 2013.
43. Houghton PJ, Ren Y, Howes MJ. Acetylcholinesterase inhibitors from plants and fungi. Natural Products Reports. 2006; 23:181-199.
44. Picollo MI, Toloza AC, Mougabure Cueto G, Zygadlo J, Zerba E. Anticholinesterase and pediculicidal activities of monoterpenoids. Fitoterapia. 2008; 79:271-278.