EVALUATION OF THE POTENTIAL INSECTICIDE ACTIVITY OF THREE PLANTS ESSENTIAL OIL AGAINST THE CHICKPEA SEEDS BEETLES, *Callosobruchus maculatus*

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Abstract. Loses caused by *Callosobruchus maculatus* in stored legumes are significant. In this study we were carried out in order to determine the insecticidal potential of *Artemisia herba-alba*, *Juniperus oxycedrus* L. and *Pelargonium graveolens* essential oils. Environmental and health concerns of synthetic insecticides highlight the need for new strategies to protect stored grains insect infestation. The contact effect of the three essential oils against adults of *Callosobruchus maculatus* (Coleoptera: Bruchidae) was investigated. While the oils of *P. graveolens* and *J.* oxycedrus *essential* oils showed the same strong insecticidal activity against *C. maculatus* (LD50 = 2.270% and 8.017%, respectively), the oil of *A. herba-alba* revealed poor activity against the insect (LD50 = 11.670%). The components of the essential oils were obtained and identified. Chromatographic analysis (GC-MS) of *A. herba-alba* essential oil demonstrated that chrysanthenone (31.40%) is the main constituent. And as for the *J. oxycedrus* L. the highest components were Alpha-pinene (64.80%). And the main components of the *P. graveolens* essential oil were determined as citronellol (36.00%).

Key words: *Callosobruchus maculatus*; biocontrol; essential oil.

INTRODUCTION

Legumes are an immense family of plants, and are among the most widely cultivated food crops in human life. Many species are cultivated for their seeds, which are rich in starch [12]. Chickpea is an important food legume for millions of people [27]. Stored food can be attacked by insects, fungi and rodents. The insect damage is the most significant [16]; the *Callosobruchus maculatus* F (Coleoptera: Chrysomelidae: Bruchidae) is the major and the most serious pest of stored chickpea, cowpea, lentil and soybean seeds. Insect infests chickpea in the field and the subsequent population built up in storage can cause complete weight quantitative losses and qualitative losses of stored chickpeas within six months if no prophylactic measures are put in place [5]. Infestation of grain by various storage-product pests may occur at various stages from the time of harvest to consumption by consumers. Thereover, the management of insect pests in many storage systems relies principally on applying synthetic insecticides. Use of synthetic insecticides is currently the most effective way to prevent the infestation of stored product pests. However, continuous and heavy use of these chemicals has caused adverse effects on non-target organisms, and the development of pesticide resistance in some stored product pests. To solve this problem, many researchers have discovered alternative pest management products derived from plants. Several plant products have been investigated for insecticidal activities, including *Artemisia herba-alba*, *Juniperus oxycedrus* L. and *Pelargonium graveolens* [28]. Fumigation is one of the main chemical methods used in the world to control insect infestations from stored products. Only volatiles of the applied constituents has been studied. Fumigation of some essential oils has inhibited larval development in pupae and pupae in adults, which has also led to malformations at different stages of insect development [7]. Synthetic organic chemicals have been used as an effective means of stored-product pest control for many years. Many compounds have been and will be phased out because of their toxicity to humans, resistance problems in insects and environmental concerns. Fumigation has played a significant role in controlling stored-product insect pests [11]. In the present investigation, three essential oils of *A. herba-alba*, *J. oxycedrus* and *P. graveolens* was tested as a contact insecticide to protect stored chickpea from insect pest, *Callosobruchus maculatus* F. (Bruchidae) in laboratory conditions.

MATERIALS AND METHODS

Callosobruchus maculatus F. (Bruchidae) is the insect on which were carried out our study because of the important harm caused against the stored commodities. We used adults of *C. maculatus* from a mass breeding carried out in an oven (35ºC) at the laboratory of animals eco-biology. The mass breeding of *C. maculatus* F. was achieved in a glasses container on chickpea grains. The glasses were maintained in the dark, in a laboratory oven set at 27 ± 1 °C and 65 ± 5% of relative humidity. All experiments were carried out under the same environmental conditions. The glasses are checked daily and noted over time.

We have selected three plants from Algeria steppe area for this work: *A. herba-alba* (Asteraceae), *J.*

oxycedrus (Cupressaceae) and *P. graveolens* (Geraniaceae). The aerial parts "stem and leaves" of plants were collected during the year from a different region of Algeria. Plant samples were dried naturally on laboratory benches at room temperature (24-25ºC) for two months and kept in paper bags. The dried plant materials were ground to a homogenous powder using a RECOCHET blender (Réf. CSL 04).

In this work, the extraction was carried out by hydrodistillation.250 g of the plant were hydrodistilled for 3 hours in an all-glass Clevenger apparatus in accordance with the description of the phytopharmacology laboratory protocol (ENSA). Heat was supplied to the heating mantle (50ºC) and the essential oil was extracted with 4 L of water for 3 hours (until no more essential oil was recovered). The essential oil was collected and analysed immediately [29].

The insecticidal effect of essential oils extracts against of adults *C. maculatus* F. was evaluated by means of contact toxicity. For this purpose, we used an impregnated paper assay, following the method described by Stefanazzi *et al.* [31]. Based on preliminary results, a range of volumic doses: 40% (1 mL), 30% (0.1 mL), 20% (0.01 mL) and 10% (0.001 mL) were formulated using acetone solvent and applied to Whatman filter paper (no. 1) (9 cm diameter). The extracted solvent was air-dried for 10 minutes. Each filter paper was then placed inside a glass Petri dish with five adults of *C. maculatus* F.(The insects emerged after four weeks were used in entire investigation). The dishes were kept in optimal laboratory condition (27 ± 2ºC, 70 ± 5% relative humidity and in darkness). Following exposure, the mortality adults were determined over time. Petri dishes with sterile water (T0) were used as controls. Three replicates were used for each dose and exposure time combination.

One of the ways to estimate the effectiveness of a product is to calculate the LD50 by SAS software 9, which corresponds to the quantity of toxic substance causing the death of 50% of individuals in the same batch. It is deduced by plotting the mortality / dose regression line. Therefore, the corrected percentages of mortality are transformed into probit.

The constituents of *A. herba-alba*, *J. oxycedrus* L. and *P. graveolens* essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS) using a GC system 7890A with a HP5MS capillary column (HP 5. L 50 m. d 0.20 mm x 0.50 µm). The carrier gas was hydrogen and the oven temperature was held at 50ºC to 250ºC (5ºC / min), split mode was 1:1500. The injector temperature was 250ºC and the compounds were identified by comparison to NF ISO 4731 standard [4].

Statistical analysis was conducted using SAS software 9. The differences between treatments were detected by analysis of variance (ANOVA). P–values < 0-05 were regarded as significant. The multiple comparisons (two–two) of the averages between the different plant extracts were performed using Tukey's test of least significant differences (LSD).

RESULTS

The toxicity results of the extracts *A. herba-alba*, *J. oxycedrus* and *P. graveolens* at different concentrations against adults *C. maculatus* F. are presented in the table 1. Important efficiency after 48 h in accordance with dose 1 (1 mL) (40%) and dose 2 (0.1 mL) (30%) with a mortality average of 100% and 100%, respectively for the *P. graveolens* and *A. herba-alba* extract. The dose dose 3 (0.01 mL) (20%) caused 100% of mortality for the extract *P. graveolens*, 86.6% for the *J. oxycedrus* extract and 80% for *A. herba-alba* extract. *J. oxycedrus* extract reached 100% and 93.3% respectively for dose 1 and dose 2. Dose 4 (0.001 mL) (10%) caused 93% of mortality for *P. graveolens* extract, 73.3% for the *J. oxycedrus* extract and 46.67% for the *A. herba-alba* extract. We found that the extracts of *J. oxycedrus* and *P. graveolens* were highly toxic compared with *A. herba-alba* extract, which shows less toxicity against adult's *C. maculatus* F.

Table 2 summarizes the ANOVA test of *C. maculatus* F. mortality after 48 h of exposure on different concentrations of the plant extracts from *A. herba-alba*, *J. oxycedrus* and *P. graveolens*. The ANOVA shows a highly significant difference between the variables, extracts (DF = 2, F = 40.93, P < 0.0001), hours (DF =23, F = 25.13, P < 0.0001), dose (DF = 3, F = 411.34, P < 0.0001). That are in accordance with the mortality.

Table 1. Mortality average of *C. maculatus* adu1lts after 48 h

	Mortality averages (%) after 48 h		
Dose	***A. herba-alba***	***J. oxycedrus***	***P. graveolens***
1 mL (10%)	100	100	100
0.1 mL (20%)	100	93.3	100
0.01 mL (30%)	80	86.6	100
0.001 mL (40%)	46.67	73	93

Table 2. Variance analyses of *C. maculatus* F. mortality

Source	DF	Anova SS	Mean	F	P > F
Extract	2	100.673611	50.336806	40.93	<0.0001
Hour	23	710.847222	30.906401	25.13	<0.0001
Dose	3	1517.634259	505.878086	411.34	<0.0001

Note: The F–value is the ratio produced by dividing the Mean Square for the Model by the Mean. The Anova SS is the sum of squares for a balanced test of each effect adjusted for all other effects. (DF is the degrees of freedom).

The results of the multiple comparisons (two–two) (***: p = 0.05) of the averages and the toxicity grouping presented respectively in the table 3. The multiple comparisons (two–two) of the averages between the different plant oils using the test of least significant differences (LSD) at p = 0.05 show a significant difference between extracts: *P. graveolens* and *J. oxycedrus*, *A. herba-alba* and *J. oxycedrus*, respectively with a differences between means of 0.79514 and 0.62153. In contrast, there is no significant difference between the extracts of *A. herba-alba* and *P. graveolens*.

Table 4 summarize the lethal amounts with 50% (LD50) of the extracts of *A. herba-alba*, *J. oxycedrus* and *P. graveolens*. LD50 results for Adult *C. maculatus* F. showed that two of the extracts plant tested were interesting in terms of toxicity: the *P. graveolens* extract and the *J. oxycedrus* extract. They presented the weakest concentration of LD50 at 2.270% and 8.017% respectively. On the other hand, *A. herba-alba* extract was less toxic, with value of 11.670%. This result confirmed the grouping test results of toxicity and show that the extracts of *P. graveolens* and *J. oxycedrus* have an important insecticidal activity overlooked *C. maculatus* then *A. herba-alba* extract.

The constituents of essential oils for each test plant were analysed by gas chromatography-mass spectrometry (GC/MS) and presented in the table 5. The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. The toxic effects of *A. herba-alba* could be attributed to major constituents such as chrysanthenone (31.40%), camphor (15.97%) and alpha-Thuyone (14.90%). The monoterpene chrysanthenone might have broad insecticidal activity against stored-product insects [18]. The chromatographic analysis of the essential oil *J. oxycedrus* by CG/SM allowed to record that, the majority compound of this oil is alpha-pinene with a rate of (64.80%). Chromatographic analysis conducted on *P. graveolens* essential oil, consists mainly of a high rate of citronellol (36%), geraniol (16.63%) and isomenthone (8.82%). The monoterpenoid compounds, citronellol and geraniol were the main components in the *P. graveolens* oil and the most important components.

DISCUSSION

The use of natural products can be considered as an important alternative control of stored product pests. The results from this study indicated that the essential oil of *A. herba-alba*, *J. oxycedrus* and *P. graveolens* exhibited effective toxicity against *C. maculatus* F. Moreover, essential oils can affect insects in several ways also had a good fumigation effect on adult insects [1]. Similar observations about other plant extracts have also been made. Wang *et al.* [32] showed that the Artemisia specie, strongly repelled *Tribolium castaneum* and *Callosobruchus maculatus* has also been demonstrated to be repellent to three stored product insects.

Kim *et al.* [15] have shown that the essential oils of *Brasica juncea* and *Cinnamomum cassica*, have a mortality percentages of 84.2% and 98%, respectively. Similarly, Nondenot *et al.* [19] showed that the essential oils of *Ageratum conyzoides*, *Citrus aurantifolia* and *Melaleuca quenrvia* have insecticidal activity against *Callosobruchus maculatus* F. They stipulate that the highest dose of 33.3 µL/mL cause 100% mortality. In addition, Kellouche & Soltani [14] have shown that clove essential oil greatly reduces the longevity of *Callosobruchus maculatus* F. and that a dose of 4 to 5 µL/g is sufficient to achieve total mortality within three days of exposure. The leaves and seeds of *Azadirachta indica* [24] as well as the leaves and fruits of *Boscia senegalensis* (Capparaceae) [25] caused 80-100% mortality of *C. maculatus* adults at doses ranging from 2-4%. The essential oils tested on grain insects *Citrus limon* was the most effective for *C. maculatus* since the dose of 7 mL/kg caused 100% mortality after 1 hour of treatment [22].

In another study, three essential oils were tested for their fumigant toxicity and repellency against adults of *C. maculatus*. The insecticidal activity varied with plant-derived material and concentration. The chemical constituents of many plant essential oils are mainly composed of monoterpenoids [8]. Monoterpenoid compounds have been considered as potential pest control agents because they are acutely toxic to insects and possess repellent and antifeedant properties [33], we can say that the several studies have shown that essential oils have a broad spectrum of action on insects in stored foods [13]. The study conducted by Shaaya *et al.* [26] on fumigation toxicity of 26 essential

Table 3. The multiple comparisons (two–two) of the averages

Extract comparison	Differences between means	95% Confidence limits
P. graveolens – A. herba-alba	0.17361	0.00011 - 0.39059
P. graveolens – J. oxycedrus	0.79514	0.57816 - 1.01212 ***
A. herba-alba – J. oxycedrus	0.62153	0.40455 - 0.83851 ***

Table 4. LD50 values of extracts of *A. herba-alba*, *J. oxycedrus* and *P. graveolens* against *C. maculatus* F. adults after 48h

Treatement	DL50	R^2
A. herba-alba	11.670	0,8438
J. oxycedrus	8.017	0,7565
P. graveolens	2.270	0,7768

Table 5. Chemical composition of *A. herba-alba*, *J. oxycedrus* and *P. graveolens* essential oils

Components	**Compounds rate (%)**		
	A. herba-alba	***J. oxycedrus***	***P. graveolens***
Ethyl isobutyrate	0.02 ± 0.01		
1-octene	0.04 ± 0.01		
Ethyl isovalerate	0.05 ± 0.01		
1-Nonene	0.03 ± 0.02		
Artemisia-Triene	0.20 ± 0.01		
Tricyclene	0.28 ± 0.01		
Alpha-Thuyene	0.07 ± 0.01		
Alpha-Pinene	1.35 ± 0.02	64.80 ± 0.01	
Camphene	3.95 ± 0.01		
Verbenene	0.11 ± 0.01		
Sabinene	1.52 ± 0.02	0.22 ± 0.01	
Beta-pinene	0.21 ± 0.01	1.80 ± 0.01	
Beta-Mycene	0.21 ± 0.01		
1.2.4-trimethyl-Benzene	0.23 ± 0.06		
Alpha-Terpinene	0.14 ± 0.02		
Para-cymene	0.51 ± 0.01	0.48 ± 0.01	
Lemonene	0.14 ± 0.01	1.27 ± 0.01	
1.8-cineole	4.57 ± 0.02		
Gamma-Terpinene	0.26 ± 0.01	1.38 ± 0.01	
Trans-4-Thuyanol	0.19 ± 0.01		
Alcohol Artemisia	0.03 ± 0.01		
Alpha-Terpinolene	0.08 ± 0.02		
CIS-4-thuyanol	0.15 ± 0.01		
Filifolone	0.69 ± 0.01		
Alpha-Thuyone	14.90 ± 0.01		
Beta-Thuyone	7.17 ± 0.01		
Chrysanthenone	31.40 ± 0.03		
Trans-pinocarcinol	0.94 ± 0.01		
Camphor	15.97 ± 0.04		
Pinocarvone	0.95 ± 0.01		
Borneol	1.05 ± 0.01		
1-Terpinene-4-ol	0.43 ± 0.01		
Alpha-Terpineol	0.16 ± 0.05		
Estragole	0.30 ± 0.01		
Trans-Piperitol	0.17 ± 0.04		
Verbenone	0.15 ± 0.02		
Carvone	0.11 ± 0.01		
Piperitone	0.24 ± 0.04		
Cis-Chrysanthenyl acetate	0.45 ± 0.03		
Bornyl acetate	0.19 ± 0.01		
Trans-4-Thuyanyl acetate	0.17 ± 0.03		
Alpha-Copaene	0.15 ± 0.01		
Jasmone	0.20 ± 0.05		
Germmermaid D	1.31 ± 0.01		
Bicyclogermacrene	0.48 ± 0.03		
Delta-cadinene	0.07 ± 0.01	2.62 ± 0.02	
Caryophyllene oxide	0.05 ± 0.02		
Viridiflorol	0.10 ± 0.01		
Myrcene		5.03 ± 0.01	
Cis-beta-ocia		0.55 ± 0.01	
Beta-Caryophyllene		1.61 ± 0.02	
Cis-beta-ocia		0.90 ± 0.04	
Caryophyllene oxide		1.01 ± 0.01	
Linalool			4.37 ± 0.07
Rose cis-Oxide			1.83 ± 0.04
Trans-Oxide			0.73 ± 0.01
Menthone			0.42 ± 0.01
Isomenthone			8.82 ± 0.10
Alpha-Terpineol			0.36 ± 0.01
Citronellol			36.00 ± 0.90
Geraniol			16.63 ± 0.12
Citronellyl formate			7.97 ± 0.02
Geranyl formate			2.58 ± 0.01
Geranyl butyrate			0.79 ± 0.01
Geranyl tiglate			1.12 ± 0.01
Phenylethyl tiglate			0.71 ± 0.09
10-epi-gamma-eudesmol			5.57 ± 0.01
Guaiadiene-6.9			0.50 ± 0.06

oils showed that only laurel sage, and lavender caused 100% mortality on *T. castaneum* and *C. maculatus* F. Also in another study [30], it was observed that *camphor, eucalyptus*, *rosemary* and *wintergreen* essential oils showed 100% egg mortality of *C. maculatus* for 40 µL/mL after 48 h. Džamić *et al.* [10] demonstrated that the essential oils obtained via steam distillation extraction using solvent and hydrodistillation of the geranium plant are often used as fragrances in the perfume industry and more recently for aromatherapy and as herbal medicines. Earlier attempts to explore the toxicity of geranium essential oil against the cowpea beetle. *Callosobruchus maculatus* has been made and proved that essential oils affect insects by repellent, oviposition inhibitory, fumigant [2]. The fumigant effect of essential oils has been researched, bringing new perspectives in the control of *C. maculatus*. Mahmoudvand *et al.* [17] observed that the essential oils of *Lippia citrodora* Kunth, *Rosmarinus officinalis* L., *M. piperita* L. and *Juniperus* caused mortality to *C. maculatus*. Aboua *et al.* [3] testing the essential oils of *Melaleuca quinquenervia* L.*, Citrus aurantifolia* (Christm.) and *Ageratum conyzoides* L. against *C. maculatus* F. Also, they are obtained fumigation toxicity effects for this pest. The oils of *Carum copticum* C.B. Clarke and *Vitex pseudo-negundo* Hand were efficient in different stages of *C. maculatus* F. development [23].

The findings of this study indicate that essential oils can be a promising tool in insect pest management. However, before its application, it must be kept in mind that essential oil should be toxic to target insects but not toxic to non-target organisms such as other beneficial insects and other animals such as fish, birds, and humans. Several other factors must be considered during the evaluation of insecticides like those that the risk associated with users, mode of exposure, degradation in the environment and chronic toxicity to be used effectively for control of stored-product insect populations [6].

The essential oil of *Artemisia sieberi* demonstrated fumigant toxicity against *C. maculatus* and *T. castaneum*. The insecticidal activity varied with insect species, concentrations of the oil and exposure time. The results showed high mortality rates in *C. maculatus compared* to *T. castaneum*. In addition, Papachristos and Stamopoulos [21] have reported the higher susceptibility of the *C. maculatus* than *T. castaneum*.

The monoterpene Camphor might be a broad insecticidal activity against stored insects, since in more detailed study. Dunkel and Sears [9] demonstrated potent toxic effects of Camphor from *Artemisia tridentata* Nutt against *T castaneum*. Also, Obeng-Ofori *et al.* [20] found that 1.8-Cineol to be highly repellent and toxic against some stored product beetles. Therefore, higher toxicity of the Qum oil as part, could be attributed to higher concentrations of the Camphor. These results showed that the chemical composition of the essential oil from other Artemisia specie (sieberi) could be changed according with geographical distribution and might be an effective factor on its insecticidal activity.

This investigation affirms that the three essential oils studied can ensure the protection of chickpea grains. Consequently, they can be exploited as biocidal agents. This study, therefore, opens up a wide range of possibilities for assessing the insecticidal and toxic effects of the essential oils studied on adults of *Callosobruchus maculatus*. Additional experiments are needed to clarify the nature of the compound responsible for this activity to optimize effective doses. At the end of this work, we offer some reflections and recommendations under the form of prospects for good exploitation of the by-product of this forest species so preserved. Also, establish the chemical composition of these studied EO's.

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