

LARVICIDAL EFFECTS OF ESSENTIAL OILS FROM *Artemisia herba-alba*, *Juniperus oxycedrus*, AND *Pelargonium graveolens* AGAINST *Tribolium castaneum* LARVAE

Mounir KHERROUBI*, Izzeddine Zakarya ZERROUK**, Ahmed Diya Elhak GUETTOUCHE*, Mounir SAIFI***

*Laboratory of Ecobiologie animals, Higher Normal School of Kouba, vieux Kouba, Algeria

**Laboratory of Biotechnology and Conservation of the Biodiversity and Didactic of the Sciences, Training School of Teachers Assia Djebbar, Constantine, Algeria

***Department of biology, University of Djilali Bounaama, khemis miliana, Algeria

Corresponding author: Mounir Kherroubi, address: Laboratory of Ecobiologie animals, Higher Normal School of Kouba, 16006 vieux Kouba, Algiers, Algeria, phone number: 00213661266146, e-mail: kherroubi.m@hotmail.fr

Abstract. The chemical composition of the essential oils from *Artemisia herba-alba*, *Juniperus oxycedrus*, and *Pelargonium graveolens* (Geranium) and their insecticidal activity against *Tribolium castaneum* (Herbst) larvae were investigated. The essential oils from the aerial parts of these three plants were obtained by hydro-distillation and analyzed by gas chromatography–mass spectrometry (GC–MS). These plant species were selected because they are widely distributed in Algeria and have been traditionally used for their aromatic and medicinal properties. Previous studies have reported that their essential oils contain high levels of bioactive monoterpenoids, such as camphor, α -pinene, citronellol, and geraniol, which are known to possess insecticidal and repellent activities. The main compounds in *Artemisia herba-alba* EO's were chrysanthenone (31.40%), camphor (15.97%), and alpha -thujone (14.90%), in *Juniperus oxycedrus* were alpha-pinene (64.80%), myrcene (5.03%), and delta- cadinene (2.62%), and the major compounds in *Pelargonium graveolens* EO's were citronellol (36%), geraniol (16.63%), and isomenthone (8.82%). The insecticidal effect of EO's extracts were evaluated by means of contact toxicity. After 24 h exposure time, it was found that the *Artemisia herba-alba*, *Juniperus oxycedrus* and *Pelargonium graveolens* EO's exhibited contact activity against *T. castaneum* larvae (LD50 = 0.003%, LD50 = 0.095%, LD50 = 0.006%, respectively). The results showed that *Artemisia herba-alba* and *Pelargonium graveolens* oils more toxic against *T. castaneum* larvae and may have potential as a control agent.

Keywords: essential oil; insecticid; larvae; *Tribolium castaneum*.

INTRODUCTION

Food grain losses caused by storage pests represent a serious global problem. High levels of insect contamination make grains unfit for human consumption and lead to both qualitative and quantitative losses of food commodities. Insect infestations in storage environments also create favorable conditions for the growth of storage fungi, resulting in additional damage. It is estimated that more than 20,000 species of field and storage pests destroy about one-third of the world's food production each year, with losses valued at over 100 billion USD, of which the highest proportion (43%) occurs in developing countries [26, 27]. The red flour beetle, *Tribolium castaneum* (Herbst), is one of the most common and destructive pests of stored grains and grain-based products in tropical regions [24]. Although chemical control remains the most widely used and effective method of pest management, increasing evidence in recent years has revealed serious limitations of this approach. Repeated use of chemical insecticides often leads to the artificial selection of resistant individuals within pest populations. The offspring of these resistant mutants form new pest strains that are more difficult to control than the original population [29]. Growing concerns about environmental pollution and human health have stimulated the search for biodegradable and environmentally safe insecticides [25, 26]. Plants provide an alternative source of pest control agents because they produce a wide range of bioactive compounds that are selective and usually have minimal or no harmful effects on non-target organisms and the environment [17]. Among these, essential oils secondary metabolites of plants have shown promising

insecticidal properties. The toxicity of many essential oils and their components has been evaluated against numerous stored-product insect species [1, 24]. These oils contain active compounds that exhibit significant insecticidal effects and have the advantage of being cost effective and naturally derived [3, 16, 27]. The main objective of the present study is to determine the chemical composition and to evaluate, under laboratory conditions, the insecticidal effects of essential oils extracted from *Artemisia herba alba*, *Juniperus oxycedrus*, and *Pelargonium graveolens* (Geranium) against the major stored grain pest *Tribolium castaneum*.

MATERIALS AND METHODS

The red flour beetle, *Tribolium castaneum* is a species of beetle in the family Tenebrionidae on which our tests were carried. We used larvae, from a mass breeding carried out in an oven (35°C) at the laboratory of animals eco biology. The test larvae pests of *Tribolium castaneum* were obtained from the infected grain (durum wheat grains) at the granary, these pests (First instar) were reared without exposure to any insecticide at the laboratory. *Tribolium castaneum* were reared in glass test tubes (covered by a cotton to ensure better ventilation) containing wheat flour. The operation was maintained in the dark, in a growth chamber set at 27±1 °C and 65±5 relative humidity. All experiments were carried out under the same environmental conditions. The tubes are checked daily and noted over time.

The aerial parts (stems and leaves) of *Artemisia herba-alba*, *Juniperus oxycedrus*, and *Pelargonium graveolens* were collected from different regions in central Algeria. Plant samples were dried naturally on

laboratory benches at room temperature (24-25 °C) for two months and kept in paper bags. The dried plant materials were ground to a homogenous powder using a RECOCHET blender (Réf.CSL04).

Essential oils were obtained by aqueous distillation in a Clevenger device. The applied method consists of adding 100 g of plant material to 800 mL of distilled water in a 2-liter flask. The set is placed in a balloon heater installed on the refrigerator to ensure that the essential oils condense for 3 hours. At the end of the distillation process, two stages are observed, aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The essential oil was collected, dried under anhydrous sodium sulfate, and stored in sealed vials in the dark, at 4 °C, until use [9].

The insecticidal effect of essential oils extracts against the first instar larvae of *Tribolium castaneum* was evaluated by means of contact toxicity. For this purpose, we used an impregnated paper assay, following the method described by Stefanazzi *et al.* [32]. A range of volumic doses: 10% (1 mL), 20% (0.1 mL), 30% (0.01 mL) and 40% (0.001 mL) were formulated. Each filter paper was then placed inside a glass Petri dish with 20 larvae of *Tribolium castaneum* at optimal laboratory condition.

One of the ways to estimate the effectiveness of a product is to calculate the LD50 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 according to the method of Finney [10].

We performed the statistical analyses using SAS software (version 9). We used analysis of variance (ANOVA) to detect differences between treatments, and considered P-values < 0.05 as statistically significant. We compared the mean values of the different plant extracts using Tukey's least significant difference (LSD) test for pairwise comparisons.

RESULTS

The results of the gas chromatography-mass spectrometry (GC-MS) analysis of *Artemisia herba alba*, *Juniperus oxycedrus*, and *Pelargonium graveolens* are presented in Table 1. The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids. Because of their high volatility, these compounds exhibit fumigant activity, which can be important for the control of stored-product insects [6, 12]. The toxic effects of *A. herba alba* may be attributed to its major constituents, including chrysanthenone (31.40%), camphor (15.97%), and α -thujone (14.90%). The monoterpene chrysanthenone is known to have broad insecticidal activity against stored-product insects [7, 20]. Chromatographic analysis of *J. oxycedrus* essential oil (GC-MS) showed that the main compound was α -pinene, which accounted for 64.80% of the total composition. Similarly, analysis of *P. graveolens*

essential oil revealed that it mainly contained citronellol (36%), geraniol (16.63%), and isomenthone (8.82%). The monoterpenoid compounds citronellol and geraniol were identified as the predominant and most significant components in *P. graveolens* oil.

After 24 hours of exposure, the effects of *Artemisia herba-alba*, *Juniperus oxycedrus*, and *Pelargonium graveolens* essential oils at different concentrations on *Tribolium castaneum* larvae are summarized in Table 2. The contact bioassays revealed that the extracts of *A. herba-alba* and *P. graveolens* were highly toxic to the larvae, indicating strong insecticidal potential. These two extracts caused mean mortality rates of 100% and 90%, respectively, at dose 1 (10%, 1 mL); 85% and 75% at dose 2 (20%, 0.1 mL); 60% and 50% at dose 3 (30%, 0.01 mL); and 40% and 35% at dose 4 (40%, 0.001 mL). In contrast, the extract of *J. oxycedrus* exhibited lower toxicity against *T. castaneum* larvae. Mortality rates recorded were 60% at dose 1 (10%, 1 mL), 55% at dose 2 (20%, 0.1 mL), 40% at dose 3 (30%, 0.01 mL), and 20% at dose 4 (40%, 0.001 mL). These results indicate that *J. oxycedrus* oil possesses moderate insecticidal activity compared to the other two plant extracts. Overall, the first concentration (dose 1, 10%) showed the highest insecticidal efficiency after 24 hours, with mortality averages of 100%, 90%, and 60% for *A. herba-alba*, *P. graveolens*, and *J. oxycedrus* extracts, respectively. The strong activity observed for *A. herba-alba* and *P. graveolens* may be associated with their high contents of monoterpenoid compounds such as chrysanthenone, camphor, citronellol, and geraniol, which are known for their fumigant and contact toxicity. These findings suggest that the essential oils tested, particularly those from *A. herba-alba* and *P. graveolens*, could be promising candidates for the development of natural insecticidal formulations to control stored-product pests like *T. castaneum*.

Table 3 summarizes the ANOVA test of *Tribolium castaneum* larvae mortality after 24 h of exposure on different concentrations of the plant extracts from *A. herba-alba*, *J. oxycedrus* and *P. graveolens*. The ANOVA analyses by GLM proceeding shows a highly significant difference between the variables, extracts (DF = 2, F = 10.31, P < 0.0001), hours (DF = 11, F = 62.64, P < 0.0001), dose (DF = 3, F = 91.90, P < 0.0001), that are in accordance with the mortality rate.

The results of the multiple comparisons (two by two) of the averages and the toxicity grouping are presented respectively in Table 4. The multiple comparisons (two by two) results (Table 4) 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 the extracts: *Artemisia* and *Geranium*, *Artemisia* and *Juniperus*, *Geranium* and *Juniperus* with differences between means respectively of 0.4479, 0.8125, 0.3646, and a confidence limits of (0.0950 - 0.8009), (0.4595 - 1.1655), (0.0116 - 0.7175) respectively.

Table 1. Chemical composition of *A. herba-alba*, *J. oxycedrus* and *P. graveolens* essential oils

Components	Compounds rate (%)		
	<i>A. herba-alba</i>	<i>J. oxycedrus</i>	<i>P. graveolens</i>
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
guaiaiene-6.9			0.50 ± 0.06

Table 2. Descriptive analysis of the mortality averages (24 h).

Mortality averages (%) after 24 h			
Dose	<i>A. herba-alba</i>	<i>J. oxycedrus</i>	<i>P. graveolens</i>
1 mL (10%)	100%	60%	90%
0.1 mL (20%)	85%	55%	75%
0.01 mL (30%)	60%	40%	50%
0.001 mL (40%)	40%	20%	35%

Table 3. Variance analyses of *Tribolium castaneum* larvae mortality.

Source	DF	Type III SS	Mean	F	P > F
Extract	2	31.798611	15.899306	10.31	< 0.0001
Hour	11	1062.986111	96.635101	62.64	< 0.0001
Dose	3	425.291667	141.763889	91.90	< 0.0001

Table 4. The multiple comparisons (two by two) of the averages.

Extract Comparison	Differences between means	Simultaneous 95% confidence limits
<i>Artemisia</i> - <i>Geranium</i>	0.4479	0.0950 - 0.8009
<i>Artemisia</i> - <i>Juniperus</i>	0.8125	0.4595 - 1.1655
<i>Geranium</i> - <i>Juniperus</i>	0.3646	0.0116 - 0.7175

Table 5. LD50 values of extracts of *A. herba-alba*, *J. oxycedrus* and *P. graveolens* after 24 h.

Treatment	LD 50%	95% Fiducial limits
E1 (<i>Artemisia</i>)	0.00307	0.0001 - 0.0125
E3 (<i>Geranium</i>)	0.00651	0.0000 - 0.0397
E2 (<i>Juniperus</i>)	0.09523	0.0952 - 0.0993

Results of the LD50 of the insecticide activity of three plants, *A. herba-alba*, *J. oxycedrus* and *P. graveolens* against *Tribolium castaneum* larvae after 24 h are shown in Table 5. The LD50 results for the *Tribolium castaneum* larvae showed that two of the plant extracts tested, *Artemisia* and *Geranium* extract were interesting in terms of toxicity. They presented the weakest value of LD50 at 0.00307% and 0.00651% respectively. On the other hand, the extract of *Juniperus* was less toxic, with a value of 0.09523%.

DISCUSSION

Based on the chemical composition analysis, *Artemisia herba-alba* essential oil was found to contain chrysanthenone (31.40%), camphor (15.97%), and α -thujone (14.90%) as its major constituents. These compounds are well-documented for their neurotoxic effects on insects, particularly through the inhibition of acetylcholinesterase (AChE), which leads to disrupted neural transmission and ultimately insect mortality. The insecticidal potential of *A. herba-alba* essential oil has been confirmed by several studies. For example, Amara *et al.* [2] reported significant toxicity of *A. herba-alba* oil against *Tribolium castaneum* and *Sitophilus zeamais*, identifying camphor (23.61%), α -thujone (13.67%), and chrysanthenone (11.21%) as key active compounds. In another investigation, the essential oil caused over 60% mortality in *T. castaneum* at a concentration of 200 μ L/L within 24 hours. Similarly, Kherroubi *et al.* [18] highlighted the significant insecticidal activity of essential oils from *A. herba-alba*, *Juniperus oxycedrus*, and *Pelargonium graveolens* against *Callosobruchus maculatus*.

Geographic variation also appears to influence the chemical profile and bioactivity of *Artemisia* species [4]. For instance, the composition of *A. sieberi* essential oil varies depending on the region, which may impact its effectiveness against insect pests. Dunkel and Sears [13] reported potent toxic effects of camphor derived from *Artemisia tridentata* Nutt. against *T. castaneum*. Likewise, Obeng-Ofori *et al.* [22] demonstrated that 1,8-cineole exhibits strong repellency and toxicity against stored-product beetles.

Collectively, these findings reinforce the potential of essential oils as viable alternatives to synthetic insecticides for the management of stored-product pests. Our results confirm the strong insecticidal activity of *A. herba-alba* EO against *T. castaneum* larvae. Previous reports by Hu *et al.* [13] and Chaubey [5] also document the acute toxicity, developmental disruption, repellency, and antifeedant properties of essential oils from various *Artemisia* species against *T. castaneum*. Additional studies, including those by Safi and Sidi [30], El Bakkali and Afifi [8], and Bachrouh *et al.* [3], have further validated the contact toxicity of *A. herba-alba* against *T. castaneum*.

In support of our findings, Guesmi *et al.* [11] reported the repellency and lethal effects of *Anethum graveolens* essential oil on *Tribolium confusum*. They observed LD50 values of 1.23 μ L/cm² for larvae and 2.14 μ L/cm² for adults, with significantly higher susceptibility in larvae ($P < 0.05$). These results suggest a broad-spectrum efficacy of essential oils, including *A. herba-alba* and *A. graveolens*, as promising agents for integrated pest management.

The GC/MS analysis revealed α -pinene (64.80%) as the predominant compound in *J. oxycedrus* essential

oil. α -pinene is known for its fumigant properties, which interfere with the respiratory system of insects. While specific studies on the insecticidal activity of *J. oxycedrus* essential oil against *T. castaneum* are limited, research on related species provides insight Semerdjieva *et al.* [31]. Jbilou *et al.* [15] found that *Peganum harmala* extract caused 58% death of *Tribolium castaneum* larvae within 10 days after treatment. At the same time, mortality rates for extracts from *Aristolochia baetica*, *Ajuga iva* and *Raphanus raphanistrum* were 34%, 31% and 26% of mortality. Liu & Ho [19] studied the toxic activity of the essential oil extracted from *Evodia rutaecarpa* against *Sitophilus zeamais* adults and *Tribolium castaneum* larvae and adults. Also, the analysis identified citronellol (36%), geraniol (16.63%), and isomenthone (8.82%) as the main components of *P. graveolens* essential oil. Both citronellol and geraniol have been extensively studied for their potential insecticidal and repellent activities [31].

The contact toxicity of essential oils evaluated by topical application revealed a clear difference in susceptibility among insect species. Notably, *Sitophilus zeamais* adults were significantly more susceptible to the essential oil of *Evodia rutaecarpa*, with an LD50 of 0.043 μ g/mg body weight, compared to *Tribolium castaneum* adults and larvae, which had LD50 values of 0.118 μ g/mg and 0.093 - 0.126 μ g/mg body weight, respectively [19]. This finding suggests species-specific sensitivity and highlights the potent bioactivity of certain plant-derived compounds.

Pandey and Singh [23] investigated the chemical composition and biological activities of *Artemisia* species essential oils. Their results showed that the LD50 values for contact toxicity against *T. castaneum* were 0.209 μ L/L air for *Artemisia absinthium* and 7.432 μ L/L air for *A. herba-alba*, further confirming the insecticidal potential of this genus. These findings corroborate our results, where *A. herba-alba* demonstrated significant toxic effects, albeit less potent than some other *Artemisia* species.

In contrast, the essential oil of *Juniperus oxycedrus* showed minimal insecticidal activity against *T. castaneum* larvae. Although it is rich in α -pinene a compound known for its repellent and antimicrobial properties its contact toxicity appears to be relatively weak when compared to other essential oils [14, 21].

On the other hand, *Pelargonium graveolens* (*Geranium*) essential oil displayed remarkable insecticidal efficacy. Our findings are consistent with those of Kabera *et al.* [16], who reported that *P. graveolens* oil exhibited a 90% mortality rate against *S. zeamais* at a 1/5 dilution after just 24 hours of exposure. The strong bioactivity of this oil is attributed to the high concentration of monoterpenoids such as citronellol and geraniol, which are known to disrupt insect nervous systems [28].

Overall, the essential oils examined in this study particularly those of *A. herba-alba* and *P. graveolens* exhibit promising insecticidal properties and could

serve as effective botanical alternatives to synthetic insecticides. However, further investigation is essential to assess their safety and stability under practical storage conditions. Additionally, it is important to study their effects not only on larvae but also on adult stages of *T. castaneum*, as developmental stages may respond differently to treatments. Future research should also include large-scale toxicological assessments and pharmacological evaluations to enable the safe formulation of natural insecticides based on these plant extracts.

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