

TOXICITY OF ESSENTIAL OIL FROM *ARTEMISIA*
HERBA-ALBA (ASSO.) AGAINST TWO INSECT PESTS OF
STORED PRODUCTS

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Abstract

This study aimed to examine the insecticide properties of *Artemisia herba-alba* essential oil (EO) against two main insect pests of stored products *Tribolium castaneum* and *Sitophilus zeamais*. The toxicity of EO was tested using contact and fumigant bioassays. Repellent activity was also studied using the preference zone method on filter paper. Using GC/MS analysis, the major compounds were, respectively, camphor (23.61%), α -thujone (13.67%), chrysanthenone (11.21%), 1,8-cineole (10%) and β -thujone (7.65%). In both insects, the toxicity tests applied showed a significant increase in the percentage of mortality according to the doses tested ($p \leq 0.05$). Moreover, the probit analysis exposed lower values of doses and lethal concentrations (LD₅₀, LD₉₀, LC₅₀, and LC₉₀), which justified the toxic effect of EO on the two insects. In addition, EO caused a significant repulsion after 1 h and 2 h of exposure. The results obtained suggest that *A. herba-alba* EO can be an excellent alternative to chemical insecticides in the conservation of stored products.

Key words: insecticidal properties, repellent activity, *Tribolium castaneum*, *Sitophilus zeamais*, *Artemisia herba-alba* essential oil

Introduction. Cereals are considered the main sources of micro and macronutrients, representing between 50 to 60% of the human diet [1]. During the post-harvest and storage stages, these products can be damaged, mainly by insect

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pests, affecting the quality and quantity of grains [2]. In storage industries, controlling insects is managed by chemical insecticides, which affect human health, pollute the environment leaving non-biodegradable toxic residues and enhancing insect resistance [3]. Aromatic plants from the Asteraceae family, especially *Artemisia* species, have been known for their essential oils largely used in medicine, pharmaceuticals, food and cosmetic industries [4]. Among these species, *Artemisia herba-alba* (Asso.), commonly known as the desert wormwood and Sheeh in Arabic [5].

The use of essential oils is one of the most important available and biodegradable methods to control insect pests. The development of new control methods using plant extracts possessing insecticidal characteristics constitutes the objective of this work. This study focuses on Algerian *Artemisia herba-alba* (Asso.) essential oil, its chemical composition, insecticidal properties and its repellent activity against two main insect pests of stored food stuffs, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motschulsky).

Material and methods. Plant material and essential oil extraction. The aerial parts of *Artemisia herba-alba* (Asso) were collected in November 2021 from the M'sila region located in the southeast of Algeria (latitude: 35°42'07" N; longitude: 04°32'49" E). A reference specimen (No. 03/AST/2022) was deposited at the research laboratory of conservation and valorization of plant biodiversity at the University of Sidi Bel Abbes, Algeria. The dried areal parts were subjected to hydrodistillation.

Essential oil analysis. The resulting essential oil with a yield of 1.15% was analyzed by gas chromatography-mass spectrometry (GC-MS) with capillary column HP-5MS (5% Phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID, 0.25 μm film thickness); helium (N 6.0 purity) as a carrier gas (0.5 ml.min⁻¹); oven temperature programme, initially 60 °C rising to 250 °C; injector temperature of 250 °C with split mode of injection. The identification of compounds was based on comparison of their Kovats retention indices (KI) with those of literature data [6] and confirmed by matching their mass spectra of peaks with those stored on MS databases NIST 02 and Wiley 7N libraries.

Insects culture. The insects were collected from infested storage wheat, at the cereals and dry vegetables cooperative of Sidi Bel Abbes (Algeria). The insects were isolated by sieving and then identified by the identification key. The red flour beetle *Tribolium castaneum* (Herbst) was reared on wheat flour mixed with yeast (5%, w/w) previously frozen at -5 °C for 24 h, whereas, the rearing of *Sitophilus zeamais* (Motschulsky) was done in glass jars filled with healthy maize grains not treated with any chemical insecticide. Adults of both insects were reared under constant conditions of temperature (28 ± 2 °C) and relative humidity (65 ± 5%). Only young adults (7–14 days old) were used for the tests. In all toxicity bioassays, insects were considered dead when no leg or antennal movements were observed.

Contact toxicity bioassay. Insecticidal effect of EO was determined by contact toxicity using filter paper discs, method as described by [5]. Oil was dissolved in acetone at concentrations of 2, 4, 8, and 10 $\mu\text{L}/\text{mL}$. One ml of each solution was dispensed on the surface of the filter paper that was then placed in glass petri dishes. Control was treated only with one ml of acetone. After 10 min, once the solvent had evaporated, ten unsexed adults were placed into each dish and stored in darkness at $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. For each dose, the test was repeated three times with control. Mortality was recorded after 24 h.

Fumigant toxicity bioassay. Glass petri dishes of 85 ml volume were used as fumigation rooms. Whatman filter papers No. 1 were cut into circular discs of 3 cm diameter and placed on the underside of the lid of glass petri dish, which contained a group of ten insects. Circular discs attached were impregnated with different doses of EO (2, 4, 8, and 10 μL). The tested doses were converted to the volume of the petri dishes to give the corresponding concentrations 23.5, 47.1, 94.1, and 117.6 $\mu\text{L}/\text{L}$, respectively. The petri dishes were quickly closed and sealed with parafilm [3]. The control groups were placed in the same conditions and did not receive any treatment. The fumigation test was carried out at 26°C , with three replications. Mortality was determined 24 h after the treatment.

Repellency test. To evaluate the repellent effect of EO, the method of preference zone was used as described by [7]. The filter paper discs of 8 cm diameter were cut into two equal parts. Four doses of EO (2, 4, 8, and 10 $\mu\text{L}/\text{mL}$) were tested. Using a micropipette, a volume of 0.5 ml of each solution was applied uniformly to a half filter paper, while the other half was treated by 0.5 ml of acetone (negative control). After evaporation of the solvent, the treated and untreated half discs were attached with an adhesive and placed in the petri dishes. Twenty unsexed adults were placed in the centre of the filter paper discs. The petri dishes were covered and sealed with parafilm. Three replications were performed for each essential oil dose. The number of insects presented on each half-paper disc was counted after 1 h and 2 h of exposure. Percentage repellency (PR) was calculated according to the following formula:

$$\text{PR}\% = [(N_C - N_T)/(N_C + N_T)] * 100,$$

where N_C and N_T are the number of insects in the negative control half-paper disc and in the treated half filter paper disc, respectively.

Data analysis. Mortality percentages were calculated using the Abbott correction formula [8]:

$$\text{MC} = (M_0 - M_t/100 - M_t) * 100.$$

Here (MC) designates the correct mortality, (M_0) is the mortality in the treated population, and (M_t) is the insect mortality in controls. Correct mortalities were subjected to probit analysis [9] to estimate lethal doses (LD_{50} and

LD₉₀) for contact bioassay, lethal concentrations (LC₅₀ and LC₉₀) for fumigant bioassay, and median lethal time LT₅₀. The data were subjected to the analysis of variance (ANOVA) for all treatments, followed by Tukey's multiple comparisons test to assess the comparison between mean values at the significance level 5%. All statistical analyses were made by IBM SPSS Statistics version 21 and Graph Pad Prism 8.

Results. Chemical composition of EO. The chemical composition of EO analyzed by GC-MS is presented in Table 1. *Artemisia herba-alba* EO was dominated by the oxygenated monoterpenes, among them, camphor (23.61%), α -thujone (13.67%), chrysanthenone (11.21%), 1,8-cineole (10%) and β -thujone (7.65%). Monoterpenes hydrocarbons were found as the second main chemical class, with camphene (5.36%) as the principal component of this class.

T a b l e 1
Major chemical compounds of essential oil of *Artemisia herba-alba*

Components*	Kovats retention index (KI)	Percentage (%)
Santolina triene	907	2.90
α -Pinene	930	1.86
Camphene	944	5.36
Sabinene	970	1.99
β -Pinene	972	0.94
β -Myrcene	990	0.99
1,8-Cineole	1030	10
α -Thujone	1107	13.67
β -Thujone	1117	7.65
Chrysanthenone	1127	11.21
Camphor	1146	23.61
Pinocarvone	1160	0.86
Borneol	1165	3.56
Terpinen-4-ol	1176	1.01
(Z)-Jasmone	1397	3.49
Total		89.18

*Compounds listed in order of elution

Insecticidal activity (Contact test). The data revealed that EO showed an interesting insecticidal effect on adults of *T. castaneum* and *S. zeamais*. In fact, the mortality percentages increased significantly with increasing the doses of EO ($p \leq 0.05$), of which mortality rates of 13.33 ± 5.77 , 30 ± 10 , 66.67 ± 15.27 and 86.67 ± 5.77 , and 40 ± 10 , 56.67 ± 11.54 , 80 ± 10 and 93.33 ± 5.77 were recorded for *S. zeamais* and *T. castaneum*, respectively. In this test, *T. castaneum* was more sensitive to the effect of EO with an LD₅₀ value of 2.81 and LD₉₀ value of 9.88 $\mu\text{l/mL}$ compared to *S. zeamais*, with LD₅₀ and LD₉₀ values of 5.13 $\mu\text{l/mL}$ and 13.81 $\mu\text{l/mL}$, respectively.

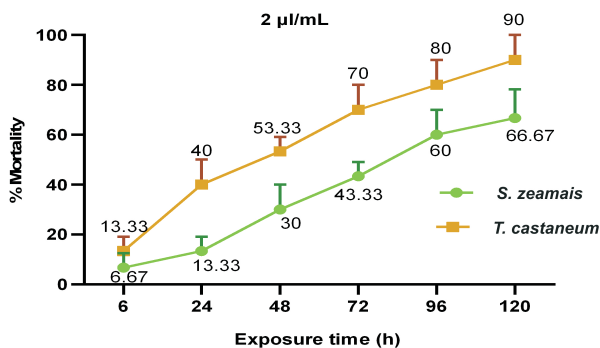


Fig. 1. Percentage of corrected mortality (mean $\pm$ SD) of the adult insects

Results of mortality rate evolution after 6, 24, 48, 72, 96, and 120 h for the lowest dose tested in the contact test (2 μ l/mL) are presented in Fig. 1. Results showed an increase in mortality rates with increasing exposure times in both insects. According to the ANOVA analysis, the exposure time significantly affected mortality rates ($p \leq 0.05$). Mortality rate values ranged from 13.33 to 90% and from 6.67 to 66.67%, for *T. castaneum* and *S. zeamais*, respectively. The time required to kill 50% of the insect population by applying the dose 2 μ l/mL was 80.91 and 31.2 h, respectively, for *S. zeamais* and *T. castaneum*.

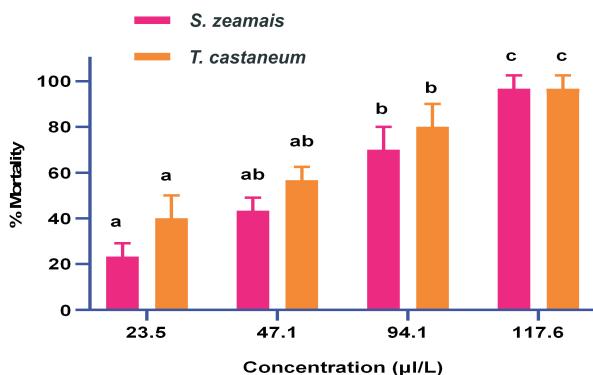


Fig. 2. Percentage of corrected mortality (mean $\pm$ SD) of *T. castaneum* and *S. zeamais* after exposure to various concentrations of *A. herba-alba* EO in fumigant toxicity bioassay (different letters over columns indicate significant differences according to Tukey's test at $\alpha = 0.05$)

Insecticidal activity (Fumigant test). The percentages of mortality (%) of *A. herba-alba* EO against *S. zeamais* and *T. castaneum* using fumigant toxicity bioassay and the lethal concentrations (LC₅₀ and LC₉₀ values) obtained are shown in Fig. 2. The results obtained showed an increase in the percentage of mortality

with an increase in the concentrations applied. A significant difference ($p \leq 0.05$) in mortality rate was observed between concentrations of EO in the two insects. The EO concentrations resulted in mortalities of around 23.33 ± 5.77 , 43.33 ± 5.77 , 70 ± 10 , and 96.67 ± 5.77 for *S. zeamais*, against mortality rates of 40 ± 10 , 56.67 ± 5.77 , 80 ± 10 , and 96.67 ± 5.77 for *T. castaneum*. In this test, EO was more effective against *T. castaneum*. Probit analysis revealed LC₅₀ values of 50.12 and 33.73 $\mu\text{l/L}$, and LC₉₀ of 114.82 and 102.33 $\mu\text{l/L}$, respectively, for *S. zeamais* and *T. castaneum*.

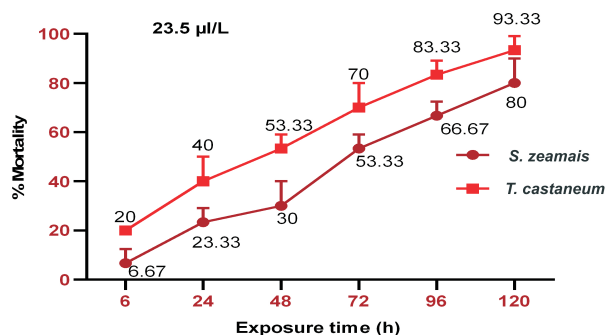


Fig. 3. Percentage of corrected mortality (mean $\pm$ SD) of the adults of *T. castaneum* and *S. zeamais* exposed for various durations to the lowest concentration tested (23.5 $\mu\text{l/L}$) of EO

Results of mortality rate evolution after 6, 24, 48, 72, 96, and 120 h for the lowest concentration tested in the fumigant test (23.5 $\mu\text{l/L}$) are presented in Fig. 3. The lowest concentration tested of EO (23.5 $\mu\text{l/L}$) showed high mortality rates (%) depending on the exposure time (h). According to the ANOVA analysis, mortality rates increased significantly depending on the increase in the exposure time ($p \leq .05$). For the exposure times monitored, the percentage mortality of *T. castaneum* adults ranged from 20 to 93.33%, and from 6.67 to 80% for adults of *S. zeamais*. According to the probit analysis, after 55.6 h, half (50%) of the adults of *S. zeamais* died exposed to the lowest concentration (23.5 $\mu\text{l/L}$), while a duration of 26.55 h was sufficient to kill 50% of the adult population of *T. castaneum* exposed to the same concentration.

Repellent activity of essential oil. It was found that the EO exhibited obvious repellent activity towards *T. castaneum* and *S. zeamais*. There were significant differences in repellency between the tested doses of EO for each insect after 1 h and 2 h of exposure ($p \leq 0.05$). For each dose, the percentages of repellency increased with prolonged exposure time, except at the doses of 2 $\mu\text{l/mL}$ and 8 $\mu\text{l/mL}$ percentages were constant in *T. castaneum* and *S. zeamais*, respectively. The highest repellent activity against both insects was given at 10 $\mu\text{l/mL}$. This last provided 70 and 76.67% of repellency after 1 h against *S. zeamais* and

T. castaneum, respectively. At this dose, the percentages of repulsion increased up to 73.33 and 93.33%, respectively, in *S. zeamais* and *T. castaneum* after 2 h. Based on the mean repellency rate, *A. herba-alba* EO was moderately repellent (class III) for *S. zeamais*, and repellent (class IV) for *T. castaneum*.

Discussion. Our findings are in agreement with [10], who reported the dominance of oxygenated monoterpenes mostly represented by camphor and α -thujone in Algerian *A. herba-alba* EOs. In Tunisia, the main chemical class were oxygenated monoterpenes and the most abundant constituents were β and α -thujone [11]. Moreover, in Morocco, [12] reported the major compound to be cis chrysanthenyl acetate (26.7–30%). Nevertheless, [13] who conducted their study in Egypt, revealed an exceptional composition with a high percentage of piperitone (26.5%) and ethyl cinnamate (9.5%). The diversity in the chemical composition of *A. herba-alba* EOs was attributed to geographical location, plant genotype, seasonality, and physiological harvesting age [14]. The toxicity EO against the two important stored products pest insects, was evaluated using contact and fumigation bioassays. The results obtained showed a significant insecticidal effect of the essential oil on the two insect species, of which the adults of *T. castaneum* were more sensitive to this effect compared to the adults of *S. zeamais*. The results found in this study were in good agreement with previous studies that confirmed the insecticidal potential of *A. herba-alba* essential oil against several pests of stored products [15]. In our study, based on LD₅₀ and LC₅₀ values obtained after 24 h of exposure, the EO was less effective than those of [16] (LC₅₀; 27.76 μ l/L) using fumigant bioassay. Moreover, our EO was found to be very toxic toward *T. castaneum* compared with the study of [5] (LC₅₀; 758.2 μ l/L, fumigant bioassay), and [17] (LC₅₀; 66.17 μ l/L). However, to our knowledge, there are few studies that have evaluated the insecticidal effect of *A. herba-alba* EO against the insect pest *S. zeamais*, of which, [18] reported super toxic effect of *A. herba-alba* EO against the adults of *S. zeamais* after seven days of exposure with LC₅₀ value of 15.41 mg/mL.

Several studies confirm our results showing that the selected aromatic plant essential oil has a repellent effect on the stored product insects [3]. The toxicity of our EO is likely associated with the high percentage of camphor, thujone, chrysanthenone and 1,8-cineole, known for their insecticidal potential [19].

Conclusions. Our results indicated that *A. herba-alba* EO possessed strong contact and fumigant toxicity against adults of the two stored product insects. Indeed, adults of *T. castaneum* seem to be more sensitive to EO than those of *S. zeamais*. In addition, our EO showed a significant repellent activity confirmed by the mean repellent rate. The strongest effects seem to be attributed to its richness in compounds with insecticidal effects and repellent activity (camphor, α/β thujone, chrysanthenone and 1,8-cineole). Results of the current study suggest that the *A. herba-alba* EO is an excellent bioinsecticide for reducing insect contamination of stored products while avoiding the use of chemical insecticides.

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