1. Introduction

Certain kinds of beetles (Buchidae and Cucurbitidae) attack grains. They are a major threat to a wide range of seeds [1-4]. Without specific protection, stored cereals are destroyed by pests. Furthermore, feeding on seeds, insect pests can cause quantitative and qualitative losses leading to a low market value. Chemical control using conventional insecticides with serious drawbacks are considered as unfriendly to the environment. These problems lead to increasingly stringent environmental regulation of pesticides [5,6]. The essential oils are being assessed as potential candidates for pests and disease management. Their toxicities as well as arresting and repellent effects on pests were of special interest during the last decade. All these gentle properties of essential oils allow their use even in responsive areas. Peasant farmers traditionally use spices and other aromatic plant material against insect pests of stored products. *Artemisia herba-alba* (A. herba-alba) is one of the aromatic herbs widely distributed in Tunisia in old days. It was used as a folk medicine to delight diseases such as cough, stomach and intestinal disturbances, common cold, measles, diabetes, yellowed skin (jaundice), anxiety, irregular heartbeat and muscle weakness. It is also used for parasitic infections as an antihelminthic agent.

The target of this study was to investigate the composition of *A. herba-alba* essential oil and to evaluate their toxic, antifeedant activities and their insecticidal activity to control stored product insects *Tribolium castaneum* (*T. castaneum*) and *Trogoderma granarium* (*T. granarium*). The *Artemisia* essential oil offers an interesting potential insecticide that could be studied more deeply to isolate and identify the active substances, to study their physiological impact on other insects.
of Chatt Meriem during the period from December 2011 to April 2012. They were cultured in a nutrient medium consisting of wheat semolina and corn meal. The rearing conditions were 26 °C, a photoperiod of 15:9 h (L:D) and 60% relative humidity.

2.2. Plant material

A. herba-alba leaves were collected in February 2012 in the region of Boughrara Medenine, Southeast Tunisia, 33°32’16” N, 10°40’34” E. The harvested material was air-dried (20–25 °C) and stored at 4 °C.

2.3. Chemical characterization of the essential oil

A total of 100 g of dry matters were subjected to hydrodistillation using a modified Clevenger-type apparatus to remove remaining water. Na₂SO₄ was added after extraction. The distilled oil was then stored at 4 °C.

The essential oil was analyzed by gas chromatography-mass spectrometer with electron impact ionization (70 eV) coupled with an HP-5890 series II gas chromatograph using a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with a flame ionization detector and an electronic pressure control injector. A polar HP Innowax column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The oven temperature was programmed to rise from 50 to 240 °C at a rate of 5 °C/min. Analyses were performed using the following temperature program. Injector and detector temperatures were held at 250 and 300 °C, respectively.

Essential oil volatile compounds based on the comparison of mass with other published mass spectra (Adams, 2001).

2.4. Fumigant toxicity

To test the toxicity of essential oils on insects, 10 specimens (of one or other of the insect) were put into a 44 mL plexiglas bottle. Doses of the essential oils were applied on a filter-paper attached to the screw cap. Each concentration and control was replicated four times. The mortality was recorded when no insect movements were observed and was calculated using Abbott’s formula correction.[7]

The second experiment was designed to assess 50% and 90% lethal doses. A series of dilutions was prepared to evaluate the mortality of insects after an initial dose-setting experiment. T. castaneum and T. granarium were exposed to the essential oil vapors 1, 2, 4 and 8 μL for 24 h. The lethal concentration of the essential oil needed to kill 50% or 90% of the insects was determined. The mortality data were corrected for control mortality by using Abbott formula[7] and probit analysis[8] was used to estimate the LC₅₀ and LC₉₀ values.

2.5. Repellent activity

This activity has been tested only on T. castaneum. The repellent effect of the essential oil against adults of T. castaneum was evaluated using the method of the preferred area on filter papers as described by McDonald et al.[9]. The filter paper discs of 9 cm in diameter used for this purpose have been cut into two equal parts. Four doses were prepared (1, 2, 4 and 8 μL/mL) and diluted with acetone. Then, 0.5 mL of each solution thus prepared was spread evenly over one half of the disc. After 15 min, the time required for completing evaporation of the solvent dilution, the two halves of the discs were glued together using adhesive tape. The filter paper disc was restored and placed in a box and kneaded a batch of 20 adult insects was placed in the center of each disk. Three repetitions were performed for each dose. After 2 h, the number of insects on the part of filter paper treated with essential oil (Nt) and the number of those present on the treated only with acetone (Nc) part were identified. The percentage of repulsion (RP) was calculated using the following formula:

\[
PR = \frac{Nc - Nt}{Nc + Nt} \times 100
\]

The average percentage of essential oil repellency was calculated and assigned as ranked by McDonald et al.[9] by a repulsive different classes varying from 0 to V [Class 0 (RP < 0.1%), class I (RP = 0.1%–20.0%), class II (RP = 20.1%–40.0%), class III (RP = 40.1%–60.0%), class IV (RP = 60.1%–80.0%) and class V (RP = 80.1%–100.0%)].

2.6. Statistical analysis

Analysis of variance was performed by ANOVA. The comparison of means was based on Duncan’s test using SPSS 11 software for Windows. The LC₁₀ values were calculated using the method of probit analysis program by Finey.

3. Results

3.1. The major components of the essential oil

Gas chromatography-mass spectrometer analysis of A. herba-alba essential oil led to the identification of 58 compounds (Table 1). The oil profile is characterized by β-thujone (12.50%), α-thujone (8.78%), sabiinyl acetate (8.56%), terpinen-4-ol (8.51%), 1,8-cineole (5.45%), γ-terpinene (4.82%), camphor (4.52%), dimethyl-ethylbenzene (3.93%) and α-terpinene (3.35%).

3.2. Fumigant toxicity

The essential oil was more toxic to T. granarium than to T. castaneum. No mortality was observed in the control. The lowest oil concentration (2 μL) achieved 5.0% of T. castaneum and 77.5% mortality of T. granarium after 24 h of exposure (Figures 1 and 2). Furthermore, at the concentration of 8 μL, 70.0% mortality was recorded for T. castaneum, by cons 75.0% mortality was recorded at 4 μL after 24 h of exposure for T. granarium adult and only 35% for T. granarium larvae (Figure 3).

T. granarium was more susceptible to A. herba-alba essential oil than T. castaneum. The corresponding LC₁₀ and LC₉₀ were 2.09 and
4.12 mg/mL for *T. granarium*, respectively, and 6.39 and 10.10 mg/mL for *T. castaneum*, respectively. The corresponding LC50 and LC90 were 10.14 and 19.50 mg/mL for *T. granarium* larvae, respectively.

Table 1

| Number | Retention time | Peak area (%) | Compounds                        |
|--------|----------------|---------------|----------------------------------|
| 1      | 4.33           | 0.08          | 1,3-Cyclopentadiene              |
| 2      | 4.72           | 0.17          | 7-Methyl-1-octene-3-heptene      |
| 3      | 5.79           | 0.09          | Terpinolene                      |
| 4      | 6.11           | 0.11          | Tricyclene                       |
| 5      | 6.22           | 0.23          | Alpha-thujene                    |
| 6      | 6.37           | 0.64          | Alpha-pinene                     |
| 7      | 6.71           | 1.79          | Camphene                         |
| 8      | 7.25           | 1.03          | Sabinene                         |
| 9      | 7.32           | 0.22          | 1-Beta-pinene                    |
| 10     | 7.63           | 0.46          | Myrcene                          |
| 11     | 7.94           | 0.19          | Alpha-phellandrene               |
| 12     | 8.23           | 3.35          | Alpha-terpinene                  |
| 13     | 8.43           | 3.80          | O-cymene                         |
| 14     | 8.58           | 5.45          | 1,8-Cineole                      |
| 15     | 9.19           | 4.82          | Gamma-terpinene                  |
| 16     | 9.40           | 0.41          | Trans-sabinene hydrate           |
| 17     | 9.82           | 1.43          | Terpinolene                      |
| 18     | 10.32          | 12.50         | Beta-thujone                     |
| 19     | 10.36          | 0.19          | 1,3,8-Purashmentatriene          |
| 20     | 10.56          | 8.78          | Alpha-thujone                    |
| 21     | 10.64          | 1.49          | cis-p-2-menthen-1-ol             |
| 22     | 10.70          | 2.68          | Chrysanthenone                   |
| 23     | 10.90          | 0.23          | Camphene                         |
| 24     | 11.03          | 2.00          | P-menthe-2-en-1-ol               |
| 25     | 11.14          | 4.52          | Camphor                          |
| 26     | 11.36          | 0.19          | Trans-chrysantheal               |
| 27     | 11.48          | 0.62          | 3-Nopinenone                     |
| 28     | 11.60          | 1.89          | Borneol                          |
| 29     | 11.90          | 8.51          | Terpinene-4-ol                   |
| 30     | 12.09          | 0.47          | L-alpha-terpinole                |
| 31     | 12.30          | 1.11          | Piperitol                        |
| 32     | 13.07          | 0.55          | Alpha-terinene                   |
| 33     | 13.50          | 3.93          | Dimethylethylbezene              |
| 34     | 13.98          | 0.78          | L-bornylacetate                  |
| 35     | 14.15          | 8.56          | Sabinylacetate                   |
| 36     | 14.27          | 0.67          | Gamma-terpinene                  |
| 37     | 14.32          | 0.05          | Chrysanthenone                   |
| 38     | 14.37          | 0.06          | Phenol,2-ethyl-4,5-dimethyl      |
| 39     | 15.20          | 0.30          | Camphene                         |
| 40     | 15.72          | 0.52          | Ethylcinnamate                   |
| 41     | 16.16          | 0.37          | Cis-jasnone                      |
| 42     | 16.59          | 0.48          | Beta-caryophyllene               |
| 43     | 17.37          | 0.94          | Ethylcinnamate                   |
| 44     | 17.72          | 3.47          | Germacrene-d                     |
| 45     | 18.19          | 2.09          | Davanaether                      |
| 46     | 17.98          | 2.10          | Bicyclogermacrene                |
| 47     | 18.40          | 0.25          | Delta-cadinene                   |
| 48     | 19.03          | 0.28          | Farnesol                         |
| 49     | 19.45          | 2.12          | Cis-davanone                     |
| 50     | 19.50          | 0.52          | (-)-Caryophylleneoxide           |
| 51     | 19.66          | 0.17          | Virdiflor                        |
| 52     | 20.14          | 0.28          | Beta-myrcene                     |
| 53     | 20.38          | 0.29          | Isopulethanol                    |
| 54     | 20.65          | 0.40          | T-muurolol                       |
| 55     | 20.88          | 0.42          | Ethanone                         |
| 56     | 21.17          | 0.36          | Gamma-trans-2-aequi-cyclocitrall |
| 57     | 25.09          | 0.34          | Palmiticacid                     |
| 58     | 27.10          | 0.26          | Phytol                           |

Figure 1. *T. castaneum* adult mortality.

Figure 2. *T. granarium* adult mortality.

Figure 3. *T. granarium* larvae mortality.

3.3. Repellent activity

The repellent activity is a physiological phenomenon that occurs in insects as a defense mechanism against toxins secreted by plants. Studying this phenomenon allows us to identify potential repellents in *A. herba-alba* essential oil. These repellents can be used to fight against this insect-grains damage. In this study, this test was applied only on *T. castaneum* adult. The maximum repellency rate is 73.33% with a dose of 4 μL (Figure 4). According to McDonald et al.[9], this plant belongs to the repulsive class IV.
Several studies confirm our results showing that the selected aromatic plant essential oils have a repellent effect on the stored grain insects including T. castaneum[10-13].

The repellent activity of the essential oil of the genus Artemisia could be attributed to its major constituents. For example, camphor (24.81%) for A. absinthium has been reported as a toxic repellent against some stored product beetles[14]. Many authors have reported that the Artemisia genus could have insecticidal properties and/or repellent against many insects in stored cereals[15,16].

Thujone (β and α) is the major component of absinthe essential oil causing "central nervous system cholinergic receptor binding activity" in the brain, which improves the brain’s cognitive functions claimed by scientists. They have been reported to be toxic to several insect species. The sabinyl acetate used in veterinary medicine, also has been used to induce abortion in humans. Furthermore, this terpene is the main component of Juniperus sabina essential oil and it has an implantation inhibiting effect. The terpinen-4-ol representing the major component of A. herba-alba essential oil was reported to be toxic to several insect species. Cavalcanti et al.[20] mentioned that monoterpenoids eugenol and 1,8-cineole from Ocimum gratissimum showed a good larvicidal activity against Aedes aegypti. Prates also reported that the monoterpenic element demonstrated insecticidal activity by penetrating the insect cuticle (contact effect), by respiration (fumigant effect) and through the digestive system (digestion effect)[21]. The camphor is a terpenic substance found in a number of essential oils extracted from aromatic plants (such as Eucalyptus sp., Cinnamomum camphora (L.) Sieb., Rosmarinus officinalis L., Chrysanthemum coronarium L., Artemisia sp., Carum carvi L., Thymus sp., etc.). It is one of the highly biologically active substances that possess insect, fungi and bacteria control management[22-25]. Essential oils are known to possess both antifeedant and larval growth inhibiting potential[26]. All of these data can explain the effectiveness of A. herba-alba oil on T. granarium and T. castaneum development.

Furthermore, results from this study demonstrated that the selected aromatic plant essential oil has excellent larvicidal activity. However, there is little information about the mechanism of essential oils action. One of the hypotheses is that the components of the essential oils act on other vulnerable sites, such as nervous system. However, it would be difficult to link the insecticide and repellent activities of this oil only to some of their major constituents; it could be due to the synergistic effect of several elements of the oil. Thus, the use of natural products may be considered as an important alternative insecticide for the control of stored-product pests.

This study has highlighted a bioinsecticide activity of A. herba alba on two insect pests of stored foodstuffs (T. castaneum and T. granarium). This bioinsecticide action is manifested by a repulsive toxicity of the selected plant essential oil activity. In this study, adults of T. granarium seems to be more sensitive to essential oil than those of T. castaneum. The T. granarium larvae are much less affected by the essential oil than their adults. This result is confirmed by mortality rate and lethal doses tests.

These results show that the Artemisia genus offers an interesting potential insecticide that could be studied more deeply to isolate and identify the active substances to study their physiological impact on other insects.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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