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# The Tunisian *Artemisia* Essential Oil for Reducing Contamination of Stored Cereals by *Tribolium castaneum*

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## SUMMARY

Essential oils of three species of *Artemisia* genus (*A. absinthium* L., *A. campestris* L. and *A. herba-alba* (Asso)) were analyzed by gas chromatography–mass spectrometry (GC-MS) and their potential insecticidal and repellent activities against the stored grain insect *Tribolium castaneum* (Herbst) was investigated. Fumigant and repellent activity bioassays were investigated *in vitro*. Chemical characterisation of essential oils showed that the bicyclic monoterpenes were predominant in all *Artemisia* essential oils, *A. absinthium* essential oil having the highest content of bicyclic monoterpenes, bicycloheptanes, naphthalenes and cycloalkenes. *A. campestris* had the highest content of sesquiterpenoids and acyclic monoterpenoids. *A. herba-alba* was characterised by the highest amounts of menthane monoterpenoids, oxanes, cumenes, oxolanes, ketones, benzenoids and monocyclic monoterpenes. Fumigant bioassay demonstrated that the three types of oil applied separately caused significant insect mortality. The lowest median lethal dose, $LC_{50}$=142.8 µL/L, was observed with *A. herba-alba*. In repellency test, essential oil of *A. absinthium* was more potent with more rapid action than all other species. The mixture of *Artemisia* sp. essential oils showed an antagonistic effect in all the tested combinations. This study highlighted an important potential of *Artemisia* sp. especially *A. herba-alba* and *A. absinthium* in the control of the pests of stored products.

**Key words:** *Artemisia* sp., essential oil, chemical composition, insecticidal activity, repellency

## INTRODUCTION

Insects are considered as the major source of damage in stored grains. They often cause significant economic damage of 5 to 10 % in the temperate zone and 20 to 30 % in the tropical one (*1*). In modern storage technologies, controlling insects is managed by chemical insecticides, including both fumigants and contact insecticides, which present serious threat to human health and environment, leave residues and enhance insect resistance. Besides, the high cost of the treatment requires new alternatives for insect control (*2*). Fumigation is still among the most effective and widespread techniques for the control of stored product. Methyl bromide and phosphine are the two most common and widely used fumigants (*3,4*). In addition, carbon dioxide and sulfuryl fluoride are also used for fumigation of stored grain as alternatives to phosphine (*5*). However, the current fumigants are a cause for some concerns since methyl bromide has been phased out in many countries including Tunisia because it has been found to cause stratospheric ozone layer depletion (*4*).

Contrary to chemical pesticides, natural aromatic products are less harmful to humans and the environment. Essential oils are recognized as alternatives to chemical fumigants. They are isolated from non-woody plant material by steam or hydrodistillation. They are composed essentially of terpenoids, represented by monoterpenes ($C_{10}$) and sesquiterpenes ($C_{15}$) and a minority of aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones that can attribute to the aromatic profile of the plant. The chemicals in essential oil play a crucial function in plant defense against fungal and insecticidal attacks (*6*). Besides their use

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in food products as preservatives (*7*), antioxidants (*8*) and antimicrobials (*9*), essential oil can also be applied as repellent or insecticide for treatment of stored products (*10,11*). These constitute effective ecofriendly alternatives to synthetic pesticides (*10*). They have been considered as pesticides and they have been used since 1947. At least 24 essential oil-based pesticides are registered in the United States (*12*). Botanicals are considered safe to humans due to their relatively high median lethal dose ($LD_{50}$) values to mammals so they have an important role in natural control strategies (*13*). The *Artemisia* genus is one of the most diversified among the Asteraceae family, which contains more than 500 species including a wide number of aromatic species (*14*) among which several species have an economic impact by their use in fragrance industry, medicine, food, forage, ornamentals or soil stabilizers (*15*). Our study focused on *Artemisia* species growing in Tunisia, especially on three species. The first is *Artemisia herba-alba,* which is the most commercially viable crop for industrial purposes in Tunisia. It constitutes 3 % of the main essential oils destined for exportation (*16*). Moreover, it showed important antimicrobial and insecticidal effects (*17,18*). The second species is *Artemisia absinthium,* which has been revealed to have several biological activities such as antimicrobial (*19*), acaricidal (*20*), insecticidal (*18*), anthelmintic, antiseptic, antispasmodic (*21*) and antioxidant (*19*). The third one is *Artemisia campestris,* showing several pharmacological activities such as antioxidant, antimicrobial and insecticidal (*22*).

In various studies essential oils have been extracted to screen their insecticidal activity without studying the relationship between the chemical composition and the relative activity. Moreover, few studies focused on the interactive effect of essential oils on their insecticidal activity.

In the present study, the insecticidal and repellent activities of *A. absinthium*, *A. campestris* and *A. herba-alba* essential oils are investigated against *Tribolium castaneum* in relation to chemical composition. Furthermore, the assessment of binary combinations of the essential oils was performed to detect their interactive effects against the tested insect.

## MATERIALS AND METHODS

### *Plant material and essential oil extraction*

In this study the upper part of *Artemisia absinthium*, *A. campestris* and *A. herba-alba* was used. These plants were collected from the region of Boughrara, Medenine, Tunisia (33°32´16˝N and 10°40´34˝E) during February 2012. Fresh shoots (200 g) were subjected to steam distillation by means of Clevenger apparatus (flask capacity 1000 mL, model TF-1000ml; TEFIC BIOTECH CO., Xi'an, PR China) with 400 mL of distilled water and boiled for 4 h at 100 °C. The extracted oil was weighed and stored at 4 °C until used.

### *GC-MS analysis of the essential oils*

The essential oils were analysed with HP 6890N gas chromatograph (GC; Agilent Technologies, Palo Alto, CA, USA) coupled with HP 5975B mass spectrometrer (MS; Agilent Technologies), equipped with a flame ionization detector and capillary column with HP-5 MS 5 % phenylmethyl siloxane (30 m×0.25 mm, film thickness 0.25 µm) under the following conditions: temperature program: 50 °C for 2 min and raised at 7 °C/min to 250 °C, then held for 2 min, injector temperature was 240 °C, the carrier gas was helium, with a flow rate of 1.2 mL/min, injected volume was 1 µL, with split mode at ratio of 1:50, transfer line temperature was 150 °C, and ion source temperature was 230 °C. Identification of the individual oil components was performed by comparison of retention times and mass spectral data with those of literature data (*23,24*) and the Wiley 275.L library (*25,26*).

### *Insect rearing*

*T. castaneum* adults were cultured on food medium composed of maize and wheat flour. The colony was reared in plastic jars at 26 °C and 60 % humidity in the dark. All experiments were carried out in a climate chamber under the same laboratory conditions.

### *Bioassay of fumigant activity*

The fumigant effect of the three essential oils of *Artemisia* species and their combinations was evaluated against adults of *T. castaneum*. Whatman filter paper no. 1 circular discs (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK) were cut to 3 cm and placed in the underside of the lid of a 40-mL glass vial, which contained a group of 10 insects. Paper discs attached to the inside top of the container lid were impregnated with different doses of essential oil (25, 50, 100 and 200 µL/L) and vials were quickly closed. The fumigation test was carried out at 26 °C, with five replications. Percentage of mortality was determined 24 h after the treatment. The fumigation test was divided into two bioassays: the first one was performed at various volume fractions of the oils tested individually and the second one was used to evaluate the synergism/antagonism among the three species of *Artemisia*. For this, different mixtures of essential oils were prepared in the same proportion, named AB, AC, BC and ABC, where A is the essential oil of *A. absinthium*, B is the essential oil of *A. campestris* and C the essential oil of *A. herba-alba*. The joint action of essential oil mixture was determined on the basis of probit analysis (*27*). The toxicity indices of different essential oils were lethal volume fractions causing 50 and 95 % mortality of exposed insects ($LC_{50}$ and $LC_{95}$). For the toxicity determination of essential oil mixtures, we used the synergistic ratio (SR) model (*28*):

$$SR=LC_{50}(\text{essential oil alone})/LC_{50}(\text{mixture}) \qquad /1/$$

where SR is 1 for additive effect, SR<1 for antagonistic effect and SR>1 for synergistic effect.

### *Bioassay for repellency activity*

Repellency degrees of the phytochemicals against *T. castaneum* were evaluated using some modifications of the area preference method (*29*). Essential oils in different doses were applied on 9-cm Whatman filter paper no. 1 circular discs cut in half. Tested solutions were adjusted by a dilution of 1, 2, 4 and 8 μL of each essential oil in 1 mL of acetone providing corresponding concentrations of 0.03, 0.06, 0.12 and 0.25 μL/cm$^2$. A volume of 0.5 mL of each essential oil solution was applied uniformly to a half filter paper and the second half was soaked with 0.5 mL of acetone using a micropipette (single-channel mechanical micropipette (1000 μL, model DG1120; Labomoderne, Paris, France). All filter papers and control were dried for 15 min. Twenty unsexed adults aged seven days were placed at the centre of the filter paper disc and the number of insects on each half paper was counted after 2 h of exposure. Three replicates were set for each treatment. Percentage repellency (PR) after 2 h of exposure was calculated according to the formula:

$$PR=[(N_c-N_t)/(N_c+N_t)]\cdot 100 \qquad /2/$$

where $N_c$ and $N_t$ were the number of insects in the negative control half and in the treated half, respectively.

### *Statistical analysis*

Statistical analyses were conducted using SPSS software, v. 20.0 (*30*). Duncan's multirange test was used to assess the comparison between the mean values at $p<0.05$. The correction using Abbott's formula (*31*) was applied to correct mortality data for control response. Data from all replicates were used in probit analysis (*27*) to calculate $LC_{50}$ and $LC_{95}$. Principal component analysis (PCA) was applied to analyze the interdependence between *Artemisia* species and their chemical constituents.

## RESULTS AND DISCUSSION

Our data indicate that essential oil yields varied within *Artemisia* species. The highest yield (in %) was recorded in leaves of *A. campestris* (0.31 %) compared to that of *A. herba-alba* (0.27 %) and *A. absinthium* (0.16 %); data not shown. In total, 48, 72 and 51 components were identified representing 100, 99.91 and 95.54 % *A. absinthium*, *A. campestris* and *A. herba-alba* oil, respectively (**Table 1**). Common major compounds in all oils (in %) were: camphor 31.3, β-pinene 14.9, γ-terpinene 14.06 and germacrene 12.15, whereas the major components of *A. absinthium* essential oil (in %) were: camphor 24.81, chamazulene 13.71, bornyl acetate 5.89 and myrcene 5.83; however, *A. campestris* essential oil was characterized by (in %): β-pinene 14.49, germacrene 7.15 and *trans*-β-ocimene 6.78 as major components. The dominant components detected in *A. herba-alba* essential oil were (in %): β-thujone 12.5, α-thujone 8.78, sabinyl acetate 8.56 and terpinene-4-ol 8.51. The type and proportion of various monoterpenoids in the oil are characteristic of the genus and species. The composition of essential oils obtained from the tested *Artemisia* species in the current investigation showed a significant similarity to previous reports (*32-37*): the major component of the Tunisian *A. absinthium* was chamazulene, thujones are similar components from the essential oil of *A. herba-alba* and β-pinene was found in *A. campestris* collected from different geographic localities of Tunisia (*33-35*). Moreover, our results were in accordance with those of Riahi *et al.* (*38*) who found that the main components of *A. absinthium* in the semi-arid areas of Tunisia were camphor, chamazulene and bor-

**Table 1.** Identified volatile constituents of the essential oils of areal parts of *Artemisia absinthium*, *A. campestris* and *A. herba-alba* from Tunisia

| Compound | RI | *w*(compound)/% | | | |
|---|---|---|---|---|---|
| | | *A. absinthium* (47) | *A. campestris* (67) | *A. herba-alba* (50) | Total |
| Compounds whose total abundance is greater than 1 | | | | | |
| Acyclic monoterpenoids | | | | | |
| Myrcene | 7.63 | 5.83 | 2.84 | 0.46 | 9.13 |
| *trans*-β-Ocimene | 8.81 | - | 6.78 | - | 6.78 |
| Linalool | 10.07 | 3.53 | - | - | 3.53 |
| Bicyclic monoterpenoids | | | | | |
| α-Pinene | 6.37 | 3.02 | 3.94 | 0.64 | 7.6 |
| Sabinene | 7.24 | 0.27 | - | 1.03 | 1.3 |
| *trans*-Sabinene hydrate | 9.37 | 1.52 | - | 0.41 | 1.93 |
| Trimethylnaphtalene | 20.03 | 3.23 | - | - | 3.23 |
| β-Pinene | 7.32 | 0.19 | 14.49 | 0.22 | 14.9 |
| β-Thujone | 10.26 | - | 5.73 | 12.5 | 18.23 |
| α-Thujone | 10.49 | - | 4.15 | 8.78 | 12.93 |
| Camphor | 11.13 | 24.81 | 1.77 | 4.52 | 31.3 |
| Borneol | 11.55 | 0.26 | 0.68 | 1.89 | 2.83 |
| Bornyl acetate | 13.97 | 5.89 | 0.67 | 0.78 | 7.34 |
| Bicycloheptanes | | | | | |
| Camphene | 6.69 | 3.74 | 0.81 | 1.79 | 6.34 |
| Cumenes | | | | | |
| *o*-Cymene | 8.39 | 0.57 | 3.09 | 3.8 | 7.46 |
| Cycloalkenes | | | | | |
| Trimethyl-dihydrocyclopropainden-6(6a)-one | 20.64 | 3.12 | - | - | 3.12 |
| Ketones | | | | | |
| Chrysanthenone | 10.64 | - | 0.62 | 2.68 | 3.3 |

**Table 1.** – continued

| Compound | RI | *w*(compound)/% | | | |
|---|---|---|---|---|---|
| | | *A. absinthium* (47) | *A. campestris* (67) | *A. herba-alba* (50) | Total |
| 2-Undecanone | 14.06 | 1.27 | - | - | 1.27 |
| Menthane monoterpenoids | | | | | |
| α-Terpinene | 8.2 | 2.09 | 0.89 | 3.35 | 6.33 |
| Limonene | 8.48 | 1.16 | 5.56 | - | 6.72 |
| γ-Terpinene | 9.15 | 3.59 | 5.65 | 4.82 | 14.06 |
| α-Terpinolene | 9.81 | 0.67 | 0.72 | 1.43 | 2.82 |
| *p*-Menth-2-en-1-ol | 10.58 | 0.27 | 0.31 | 1.49 | 2.07 |
| 1-4-Terpineol | 11.78 | 4.97 | - | - | 4.97 |
| Terpinene-4-ol | 11.90 | - | - | 8.51 | 8.51 |
| α-Terpineol | 12.06 | 0.33 | 1.46 | 0.74 | 2.53 |
| Sabinyl acetate | 14.15 | - | 2.33 | 8.56 | 10.89 |
| Benzenoids | | | | | |
| Dimethyl ethyl benzene | 13.5 | - | - | 3.93 | 3.93 |
| Monocyclic monoterpenes | | | | | |
| Piperitol | 12.30 | - | - | 1.11 | 1.11 |
| Naphthalenes | | | | | |
| Trimethyldihydronaphthalene | 18.25 | 1.03 | - | - | 1.03 |
| Trimethylnaphtalene | 20.45 | 5.09 | - | - | 5.09 |
| Oxanes | | | | | |
| 1,8-Cineole | 8.55 | 0.31 | 2.24 | 5.45 | 8 |
| Oxolanes | | | | | |
| Davana ether | 17.79 | - | 0.45 | 2.09 | 2.54 |
| *cis*-Davanone | 19.45 | - | - | 2.12 | 2.12 |
| Sesquiterpenoids | | | | | |
| Germacrene | 17.73 | 1.53 | 7.15 | 3.47 | 12.15 |
| Bicyclogermacrene | 17.98 | - | 2.58 | 2.1 | 4.68 |
| Spathulenol | 19.4 | - | 2.33 | - | 2.33 |
| Δ-Cadinene | 20.43 | - | 1.07 | - | 1.07 |
| β-Eudesmol | 20.62 | - | 3.42 | - | 3.42 |
| Chamazulene | 21.47 | 13.71 | - | - | 13.71 |
| Compounds whose total abundance is less than 1 | | | | | |
| Terpinolene | 5.79 | - | 0.16 | 0.09 | 0.25 |
| Tricyclene | 6.11 | 0.12 | 0.25 | 0.11 | 0.48 |
| α-Thujene | 6.21 | 0.43 | 0.15 | 0.23 | 0.81 |
| 6-Methyl-5-hepten-2-one | 7.55 | 0.23 | - | - | 0.23 |
| α-Phellandrene | 7.93 | 0.45 | 0.09 | 0.19 | 0.73 |
| 2-Nonanone | 9.87 | 0.53 | - | - | 0.53 |
| Butanoic acid | 10.12 | 0.56 | - | - | 0.56 |
| Filifolone | 10.16 | - | 0.33 | - | 0.33 |
| 1,3,8-*para*-Menthatriene | 10.36 | - | - | 0.19 | 0.19 |
| (e)-4,8-Dimethyl-1,3,7-nonatriene | 10.42 | - | 0.27 | - | 0.27 |
| Cycloheptane,1,3,6-trimethylene | 10.86 | - | 0.1 | - | 0.1 |
| α-Phellandrene epoxide | 10.98 | - | 0.61 | - | 0.61 |
| γ-Terpinene | 10.98 | 0.18 | - | - | 0.18 |
| Menthone | 11.26 | - | 0.08 | - | 0.08 |
| *trans*-Chrysanthemal | 11.36 | - | - | 0.19 | 0.19 |
| Pinocarvone | 11.46 | - | 0.21 | - | 0.21 |
| 3-Nopinenone | 11.48 | - | - | 0.62 | 0.62 |
| Myrcenol | 11.93 | 0.53 | - | - | 0.53 |
| Myrtenal | 12.17 | - | 0.18 | - | 0.18 |
| Verbenone | 12.46 | - | 0.13 | - | 0.13 |
| Citronellol | 12.78 | - | 0.17 | - | 0.17 |
| 2-Methylheptyl acetate | 12.9 | 0.8 | - | - | 0.8 |
| Cuminaldehyde | 13.06 | - | 0.17 | - | 0.17 |
| Citronellyl formate | 13.1 | - | 0.25 | - | 0.25 |

**Table 1.** – continued

| Compound | RI | *w*(compound)/% | | | |
|---|---|---|---|---|---|
| | | *A. absinthium* (47) | *A. campestris* (67) | *A. herba-alba* (50) | Total |
| Chrystanthenyl acetate | 13.46 | - | 0.6 | - | 0.6 |
| Perilla aldehyde | 13.77 | 0.17 | - | - | 0.17 |
| Phenol,2-ethyl-4,5-dimethyl | 14.37 | - | - | 0.06 | 0.06 |
| α-Coapene | 15.74 | - | 0.3 | - | 0.3 |
| Nerylacetate | 15.78 | - | 0.22 | | 0.22 |
| *cis*-Jasmone | 16.16 | - | - | 0.37 | 0.37 |
| Methyl eugenol | 16.21 | 0.16 | - | - | 0.16 |
| β-Caryophyllene | 16.58 | 0.71 | 0.74 | 0.48 | 1.93 |
| α-Dodecylene | 16.65 | 0.2 | - | - | 0.2 |
| *trans*-β-Fanesene | 17.14 | - | 0.17 | - | 0.17 |
| α-Humulene | 17.2 | - | 0.18 | - | 0.18 |
| Ethyl cinnamate | 17.37 | - | 0.26 | 0.52 | 0.78 |
| α-Ylangene | 17.61 | - | 0.51 | - | 0.51 |
| (E,E)-α-Farnesene | 18.05 | - | 0.66 | - | 0.66 |
| α-Amorphene | 18.26 | - | 0.34 | - | 0.34 |
| Methylpatchenol | 18.95 | - | 0.18 | - | 0.18 |
| Nerolidol | 19.03 | - | 0.51 | | 0.51 |
| Farnesol | 19.03 | - | - | 0.28 | 0.28 |
| Citronnellylpropanoate | 19.18 | - | 0.31 | - | 0.31 |
| Caryophyllene oxide | 19.49 | 0.16 | - | - | 0.16 |
| Alloaromadendrene | 19.5 | - | 0.92 | - | 0.92 |
| (-)-Caryophullene oxide | 19.5 | - | - | 0.52 | 0.52 |
| γ-Gurjunene | 19.66 | - | 0.41 | - | 0.41 |
| Viridiflorol | 19.66 | - | - | 0.17 | 0.17 |
| Geranyl isovalerate | 19.73 | 0.13 | 0.77 | - | 0.9 |
| Bicyclo (5,5,2) nonane-4,8,8-trimethyl-2-methylene | 19.95 | - | 0.5 | - | 0.5 |
| β-Maaliene | 20.1 | - | 0.68 | - | 0.68 |
| β-Myrcene | 20.14 | - | | 0.28 | 0.28 |
| Diethyl-dimethyl-tricyclo-hexane | 20.19 | 0.27 | - | - | 0.27 |
| β-Cadinene | 20.27 | - | 0.43 | - | 0.43 |
| Isospathulenol | 20.38 | - | | 0.29 | 0.29 |
| (2S,5E)-Caryophyll-5-en-12-al | 20.45 | 0.6 | - | - | 0.6 |
| α-Amorphene | 20.5 | - | 0.39 | - | 0.39 |
| 1,2-Dimethyl-4-methylene-3-phenyl-cyclopentene | 20.58 | 0.25 | - | - | 0.25 |
| T-Muurolol | 20.65 | - | - | 0.4 | 0.4 |
| Ethanone | 20.88 | - | - | 0.42 | 0.42 |
| Calamenene | 21.17 | - | 0.67 | - | 0.67 |
| γ-*trans*-aeaqui-Cyclocitral | 21.17 | - | - | 0.36 | 0.36 |
| (8)Paracyclophane-2,4-diene | 21.18 | 0.51 | - | - | 0.51 |
| Geranyl butyrate | 21.23 | - | 0.28 | - | 0.28 |
| Mintsulfide | 22 | - | 0.17 | - | 0.17 |
| N-Methylsuccinimide | 23.43 | - | 0.22 | - | 0.22 |
| Palmiticacid | 25.09 | - | - | 0.34 | 0.34 |
| 2,4-Dimethylfuran | 25.48 | 0.22 | - | - | 0.22 |
| Bromoacetonitrile | 25.79 | 0.99 | - | - | 0.99 |
| Phytol | 27.1 | - | 0.25 | 0.26 | 0.51 |
| 2-Acetyl-4-(2,5-dichlorophenyl) furan | 30.13 | - | 0.17 | - | 0.17 |

Number in the brackets next to the plant name indicates the total number of identified compounds in its oil, RI=retention index

nyl acetate. To evaluate the chemotaxonomic significance of the essential oils of the three *Artemisia* species, a total number of 40 components whose total abundance is more than 1 % were classified in 13 chemical families (**Table 1**), which were processed with PCA.

Among the 13 chemical families investigated from the three *Artemisia* species, the most abundant were bicyclic monoterpenes, followed by menthane monoterpenoids, sesquiterpenoids, acyclic monoterpenoids and the least abundant chemicals were monocyclic monoterpenoids (**Fig. 1**).

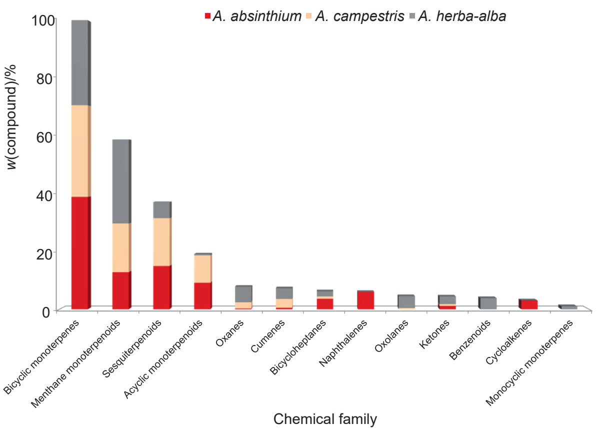

**Fig. 1.** Chemical families and relative contents of essential oils from *Artemisia absinthium, A. campestris* and *A. herba-alba*

It is important to note the absence of oxolanes, benzenoids and monocyclic monoterpenes from *A. absinthium*. Naphthalenes, benzenoids, cycloalkenes and monocyclic monoterpenes were not identified in *A. campestris* while *A. herba-alba* did not contain naphthalenes and cycloalkenes.

The results of PCA for *Artemisia* species are shown in **Fig. 2**. Based on this analysis, a higher variability within the essential oils of *Artemisia* species is observed. First PCA axis explained 75.8 % of the total variance whereas the second axis revealed 24.1 % of the total variance (**Fig. 2**). The first principal component separates bicyclic monoterpenes, sesquiterpenoids, acyclic monoterpenoids, naphthalenes, bicycloheptanes and cycloalkenes from menthane monoterpenoids, oxanes, oxolanes, cumenes, benzenoids, ketones and monocyclic monoterpenes. The second principal component distinguishes sesquiterpenoids, acyclic monoterpenoids, oxanes and cumenes from the other compounds.

Menthane monoterpenoids, oxanes, oxolanes, cumenes, benzenoids, ketones and monocyclic monoterpenes distinctly overlap in a separate group in the PCA, represented by *A. herba-alba*. The other groups overlap and are divided into two subgroups; the first one is represented by sesquiterpenoids and acyclic monoterpenoids, and is related to *A. campestris*. The second one is made of sesquiterpenoids, acyclic monoterpenoids, bicyclic monoterpenes, naphthalenes, bicycloheptanes and cycloalkenes, and is related to *A. absinthium*. These findings are in agreement with those cited by Dib *et al.* (*39*) indicating that *A. campestris* collected from four different localities in Southern Tunisia contains a high level of sesquiterpenes.

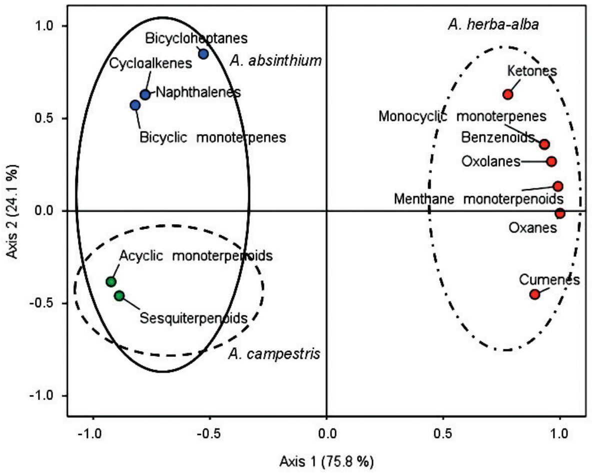

**Fig. 2.** Principal component analysis of 13 major chemical families in essential oils of three Artemisia species

The results of fumigation bioassay are shown in **Fig. 3**. At the two lowest volume fractions, the three *Artemisia* sp. did not show statistical differences. At 100 µL/L, essential oil from *A. herba-alba* was more toxic than the other two *Artemisia* sp. after 24 h of exposure. At the highest volume fraction (200 µL/L), all three *Artemisia* sp. reached the highest toxic effect. The fumigant toxicity test showed that *A. herba-alba* was more toxic than the other two *Artemisia* species. These results were confirmed by $LC_{50}$ values shown in **Table 2.** In a fumigant test, a dosage of 142.8 µL/L of *A. herba-alba* was sufficient to kill 50 % of insects after 24 h of treatment, followed by *A. absinthium* and *A. campestris* with $LC_{50}$ of 147.6 and 151.3 µL/L respectively. These results show clearly the effectiveness of *A. herba-alba* in comparison with the other two *Artemisia* species. In a previous study of Titouhi *et al.* (*35*) *A. herba-alba* exhibited the best insecticidal effect against the two stored grain insects, *Callosobruchus maculatus* and *Bruchus rufimanus,* with $LC_{50}$ of 7.7 and 8.3 µL/L, respectively.

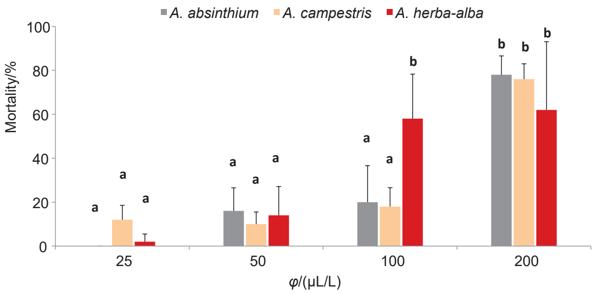

**Fig. 3.** Percentage of mortality of *Tribolium castaneum* after 24 h of exposure to various volume fractions of *Artemisia absinthium*, *A. campestris* and *A. herba-alba* essential oils

**Table 2.** Median lethal dose ($LC_{50}$) and 95 % mortality ($LC_{95}$) values of fumigant bioassay with *Artemisia absinthium*, *A. campestris* and *A. herba-alba* essential oils after 24 h

| *Artemisia* oil | *N* | Slope±SE | $LC_{50}$ µL/L | $LC_{95}$ µL/L | $\chi^2$ | df |
|---|---|---|---|---|---|---|
| *A. absinthium* | 200 | (0.16±0.01) | 147.6 | 262.7 | 13.67 | 2 |
| *A. campestris* | 200 | (0.14±0.01) | 151.3 | 289.7 | 9.81 | 2 |
| *A. herba-alba* | 200 | (0.13±0.01) | 142.8 | 304.6 | 42.39 | 2 |

*N*=number of tested insects, SE=standard error of mean, $\chi^2$=Pearson chi-square value, df=degrees of freedom

Aromatic plants contain essential oils that are a complex mixture of acyclic and/or cyclic monoterpenoids used in perfume, cosmetic and pharmaceutical industries. Application of essential oils to manage insects and diseases in agriculture is the recently emerging trend (*40*). Monoterpenoids have a promising role in pest control due to their acute toxicity to insects and their repellent (*41*) and antifeedant potency (*42*). Moreover, among monoterpenes, ketones have higher insecticidal effect than alcohols or hydrocarbons (*43-46*) and even among ketones, toxicity may be of varying degrees (*47,48*). The main cause of this variation may be due to either geographical or physicochemical characteristics (*49*). Numerous studies have shown the high toxicity of ketones against some stored pests like *Sitophilus* in fumigant and contact assays (*44,47,50*). These results show that among the three *Artemisia* oils, the one from *A. herba-alba* has the best effect ($LC_{50}$=142.8 μL/L). Such finding suggests that the presence of ketone groups increases toxicity since *A. herba-alba* contains the highest mass fraction (58.64 %) of ketones (chrysanthenone and 2-undecanone). Other studies revealed insecticidal and repellent effect of terpenes on several stored grain pests, with a much more pronounced effect of ketone (*48,51*). Moreover, *A. herba-alba* contains more menthane monoterpenoids, among them terpinen-4-ol, which was present only in this species. According to Chu *et al.* (*52*) terpinen-4-ol has insecticidal activity against *Sitophilus zeamais* (Motschulsky). Other researchers reported the effective contact toxicity of *A. herba-alba* against *Tribolium castaneum* (*18*).

In the interest of the improvement of the effectiveness of essential oil as pest management method, combined activities were examined to analyse interactions among the three essential oils. The joint effects of the three oils were assessed by mixtures adjusted at a ratio of 1:1 for binary mixtures and 1:1:1 for tertiary mixtures against *Tribolium castaneum* adults. In the present study, combinations of *Artemisia* essential oils exhibited lower insecticidal activity than single oils. The activity did not exceed 60 % of mortality at the highest volume fraction (200 μL/L). **Fig. 4** shows that there are no statistical differences among the four combinations tested at four volume fractions, although the toxic effect of all the tested combinations at the highest volume fraction was relatively higher.

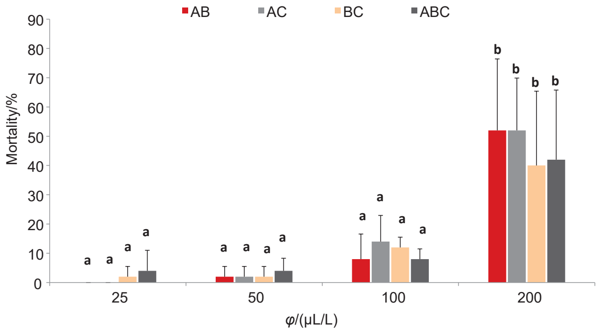

**Fig. 4.** Percentage of mortality of *Tribolium castaneum* exposed to various volume fractions of combinations of *Artemisia absinthium* (A), *A. campestris* (B) and *A. herba-alba* (C) essential oils after 24 h of exposure

The synergistic ratio (SR) shows antagonistic effects of all mixtures (**Table 3**), which indicates that oils tested alone have the most toxic effect. It has been known that mixtures of compounds increase the insecticidal effect, because insect sensitivity differs from one compound to another (*53*). In our study, the combination of the three essential oils had antagonist effect, showing that the combined application led to the decrease in insecticidal activity. In agreement with our results, Benelli *et al.* (*54*) showed antagonistic insecticidal activity of binary mixtures of *Satureja montana* and *Pinus nigra* essential oils against *Culex quinquefasciatus.* These findings show the importance of testing combined effects of essential oils used as control tools against pests, because positive or negative interactions between major components of the essential oil (alcoholic, phenolic, terpenic or ketonic compounds), minor components and biological activities can occur.

**Table 3.** Synergistic ratio (SR) of three combined *Artemisia* sp. essential oils against *Tribolium castaneum* adults after 24 h of exposure

| *Artemisia* oil | Combined oils | Combined $LC_{50}$/(μL/L) | Synergistic ratio (SR) | Effect |
|---|---|---|---|---|
| *A. absinthium* (A) | A | 147.6 | | |
| | A+B | 195.8 | 0.75 | Antagonism |
| | A+C | 192.4 | 0.76 | Antagonism |
| | A+B+C | 221.3 | 0.66 | Antagonism |
| *A. campestris* (B) | B | 151.3 | | |
| | B+A | 195.8 | 0.77 | Antagonism |
| | B+C | 221.8 | 0.68 | Antagonism |
| | B+A+C | 221.3 | 0.68 | Antagonism |
| *A. herba-alba* (C) | C | 142.8 | | |
| | C+A | 192.4 | 0.74 | Antagonism |
| | C+B | 221.8 | 0.64 | Antagonism |
| | C+A+B | 221.3 | 0.64 | Antagonism |

$LC_{50}$=median lethal dose

The results of the repellency test of the essential oils from three *Artemisia* species against *Tribolium castaneum* are shown in **Table 4**. It was found that the three samples exhibited obvious repellent activity towards *T. castaneum*. There were significant differences in repellency among the tested oils ($p<0.05$). Both *A. absinthium* and *A. herba-alba* oils provided ≥80 % protection for 1 h against *T. castaneum* at 0.08 $μL/cm^2$. After 2 h of exposure, *A. absinthium* and *A. campestris* oils showed the highest repellent activity against *T. castaneum* adults at the same dose. Among the three essential oils, *A. absinthium* repelled rapidly and strongly at all tested exposure times, while *A. herba-alba* showed highest repellency by the first hour and its effect declined with prolonged exposure time. Contrary, *A. campestris* exerted its highest effect by the second hour of exposure. *A. absinthium* also showed quite promising insecticidal activity with $LC_{95}$ of 262.7 μL/L. In terms of repellency, it exhibited the highest effect at 0.08 $μL/cm^2$ after 1 and 2 h of exposure. The chemical analysis shows that this species contains more bicyclic monoterpenes, bicycloheptanes, cycloalkenes and naphthalenes than the two other *Artemisia* sp. The above data show that fumigant and repellent activities of *A. campestris* are the weakest. The results of the repellency bioassay are in agreement with those reported by Jemâa (*55*) in that a higher repellency was recorded with *A. absinthium* essential oil than with *A. herba-alba* against both stored insects, *T. castaneum* and *Oryzaephilus surinamensis*. Moreover, previous study has demonstrated the repellent activity of *A. absinthium* oil against *Phthorimaea operculella* (*56*). Lower or higher amounts of bioactive ingredients in essential

**Table 4.** Repellent activity of *Artemisia absinthium*, *A. campestris* and *A. herba-alba* essential oils on *Tribolium castaneum* adults after different exposure times using the filter paper test

| *t*(exposure)/h | Dose/(μL/cm$^2$) | Repellent activity/% | | |
|---|---|---|---|---|
| | | *A. absinthium* | *A. campestris* | *A. herba-alba* |
| 1 | 0.01 | (36.6±3.3)$^{abc}$ | (56.6±8.8)$^{abc}$ | (20.0±15.2)$^{ab}$ |
| | 0.02 | (63.3±6.6)$^{bc}$ | (43.3±27.2)$^{abc}$ | (43.3±8.8)$^{abc}$ |
| | 0.04 | (83.3±6.6)$^{c}$ | (76.3±14.4)$^{bc}$ | (70.0±11.5)$^{bc}$ |
| | 0.08 | (90.0±5.7)$^{c}$ | (70.0±5.7)$^{bc}$ | (80.0±5.7)$^{c}$ |
| 2 | 0.01 | (40.0±5.7)$^{abc}$ | (76.6±3.3)$^{bc}$ | (6.66±18.5)$^{a}$ |
| | 0.02 | (56.6±12)$^{abc}$ | (60.0±11.5)$^{bc}$ | (40.0±5.7)$^{abc}$ |
| | 0.04 | (60.0±5.7)$^{bc}$ | (73.3±14.5)$^{bc}$ | (73.3±6.6)$^{bc}$ |
| | 0.08 | (90.0±5.7)$^{c}$ | (83.3±8.8)$^{c}$ | (63.3±12.0)$^{bc}$ |

Data are presented as mean value±standard deviation, mean values with different letters in superscript are statistically different ($p<0.05$)

oil may be responsible for reducing the insect repellency and toxicity. In this context, our study shows that *A. campestris* essential oil is richer in sesquiterpenoids than the other two *Artemisia* species.

## CONCLUSION

*Artemisia* essential oils showed promising effect in protecting the stored grains from *Tribolium castaneum* attacks. However, the effect varied significantly depending on species and chemical composition of each oil. In general, the strong insecticidal activity of *A. herba-alba* was associated with a high content of menthane monoterpenoids, whereas *A. absinthium* exhibited the highest repellent activity, based on its richness of bicyclic monoterpenes. Moreover, we observed that the combination of essential oils did not improve their insecticidal effect. Consequently, chemical composition affects the effectiveness of the desired essential oil or their mixtures. The above findings suggest that *A. absinthium* and *A. herba-alba* oils have a potential to be used separately as alternatives to chemical fumigants in the protection of stored cereals. *A. herba-alba* causes mortality of more than 60 % of insect population at volume fraction of 200 μL/L within 24 h and *A. absinthium* repel 90 % of insects at the dose of 0.08 μL/cm$^2$ after 2 h of exposure. The oils from these plant species may have an interesting potential as natural repellents and insecticides considering their noticeable effects at low applied volume fractions and short times of exposure. However, the evaluation of these activities under industrial conditions is mandatory to prove their practicable application.

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