Composition and insecticide potential against *Tribolium castaneum* of the fractionated essential oil from the flowers of the Tunisian endemic plant *Ferula tunetana* Pomel ex Batt.

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ABSTRACT

For several years, plant derived substances, in particular essential oils, have been the subject of increasing attention in their safe and ecofriendly application to crops, as a powerful alternative to chemical insecticides. For this reason, the essential oil isolated from flowers of *Ferula tunetana*, a Tunisian endemic plant, was investigated for the first time for its chemical profile, and its toxicity and repellency effects against *Tribolium castaneum* adults. The analysis by GC/MS led to determine 92.8-99.1% of the total oil (FEO) and its fractions (F_1-F_{12}) obtained every 15 minutes during the hydrodistillation process. The chemical analysis

allowed to identify 77 compounds. α-Pinene (14.3%), a monoterpene hydrocarbon, was the major compound of the raw essential oil. Relatively high amounts of oxygenated sesquiterpenes (44.9-76.8%) were detected, consisting mainly of *epi*-α-muurolol (3.6-9.5%), himachalol (6.8%) and β-chenopodiol (5.1-7.1%). Regarding the repellency assay, results demonstrated that flowers essential oil of *F. tunetana* and its fractions displayed interesting repellent property (93%). The LD_{50} of the topical application of the oil was 10.44%. Fumigation with the raw essential oil gave a LD_{50} of 161.89 µL/L air. The overall data suggest that the *F. tunetana* essential oil might be used to protect stored products from pest attacks, but further studies are needed in order to better understand the synergistic relationship between the phytochemicals contained in the essential oil.

Keywords

Ferula tunetana, endemic plant, essential oil, chemical composition, *Tribolium castaneum*, insecticidal activity

1. Introduction

A major issue in production and storage of cereal products and grains is insect infestation, principally with moths and beetles. They affect the grains quantity and quality. Post-harvest damages caused by stored grains pests have been estimated at 10-40% worldwide (Matthews and Hislop, 1993). It is evident that *Tribolium castaneum* (Coleoptera: Tenebrionidae), the red flour beetle, is a cosmopolitan and polyphagous pest, which nourishes on a wide range of stocked products comprising grains, pulses, cacao and spices (Mahroof and Hagstrum, 2012), and is, thus, listed as a severe pests of stored grains and many other products throughout the world. To date, chemical insecticides are employed for controlling *T. castaneum*. However, because of their constant use, resistance has been established in the pest population (Hu et al., 2018). Furthermore, synthetic pesticides led to serious menace to human health and environment by perturbing the ecosystem. Besides, the elevated cost of treatments

necessitates novel alternatives to control insects. Different researches recommended essential oils as effective substitutes to synthetic insecticides for controlling a huge range of insects. In this context, *Ferula* is the 3rd biggest genus of the Apiaceae family, which includes about 180 species (Yaqoob and Nawchoo, 2016), mainly spread from central-west Asia to North Africa. In Tunisia, the genus *Ferula* is represented by only four taxa: *F. communis* L., *F. lutea* (Poir.) Maire, *F. tingitana* L., and *F. tunetana* Pomel ex Batt, which is endemic. The importance of the *Ferula* genus is related to its usage in traditional and modern medicine. In classical Persian medicine, people ratify that *F. assafoetida* L. was effective for the treatment of a wide range of disorders and diseases, which is why it was named "food of God" (Mohammadhosseini et al., 2019). *F. gummosa* Boiss has been used to treat constipation, diarrhea and stomachache (Miyazawa et al., 2009). *F. communis* L. has been utilized to treat rheumatism, skin diseases, foot cracks and dysentery (Nguir et al., 2016). Several new medicinal and biological activities have been reported for different species of this genus: these comprise antioxidant (Zhang et al., 2015), anticancer (Perveen et al., 2017), antifungal (Bashir et al., 2014), antitumor (Bagheri et al., 2017), antispasmodic (Upadhyay et al., 2017), antidiabetic (Yarizade et al., 2017), anti-inflammatory (Moosavi et al., 2015) and antiviral (Ghannadi et al., 2014) properties.

Only one previous investigation on *F. tunetana* described the detailed composition of seeds essential oil (Znati et al., 2017). This latter was dominated by monoterpene hydrocarbons (77.3%) and α-pinene was the major compound (39.8%) followed by β-pinene (11.5%) and *(Z)*-β-ocimene (7.5%). To the best of our knowledge, this is the first investigation on the flowers essential oil composition of *F. tunetana*. In the present study, the chemical profiles of the essential oil isolated from flowers of *F. tunetana* and of its fractions have been characterized for the first time, to get herwith their repellency against the adults of *T. castaneum*. Moreover, the insecticidal activity by fumigant and toxic contact tests was

assessed. Only the raw essential oil was tested for these last bioassays due to the available volume of the fractions.

2. Material and methods

2.1. Plant material

The flowers of *F. tunetana* were gathered from the region of Kroussia (Sousse, Tunisia) in March 2018. The botanical identification was accomplished by Pr. *F. Harzallah-skhiri* (Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia). A voucher specimen (FT-18) was deposited at the Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity (LR11ES39), Faculty of Sciences of Monastir, Tunisia for further reference.

2.2. Extraction and fractionation of the essential oil

The essential oil was obtained by the hydrodistillation of 2.8 kg of fresh flowers of *F. tunetana* using a Clevenger-type apparatus for 180 min. Moreover, twelve samples (F_1-F_{12}) were collected, at intervals of 15 min between each fraction, during the hydrodistillation process (3 h). Anhydrous sodium sulfate (Na_2SO_4) was employed to dry the raw oil and its fractions that were maintained in the darkness into a freezer until used.

2.3. Chromatographic Analysis

The compositions of the essential oil and its twelve fractions were determined by GC/MS using a Varian CP-3800 gas chromatograph fitted with an HP-5 capillary column (30 m, 0.25 mm, 0.25 μm film thickness), coupled with a Varian Saturn 2000 ion-trap mass detector. Operating conditions were as follows: injector temp. = 220°C, transfer line temp. = 240°C, oven temp.= 60 to 240°C (3°C/min), carrier gas: He at 1 mL/min , injection of 0.2 μL of 10% HPLC hexane solution, split ratio 1:30. The comparison of retention times with those of authentic references permitted the identification of constituents, this implied paralleling their LRIs with reference to a homologous series of *n*-hydrocarbons (C_8 to C_{30}) and employing

computer matching against commercial (ADAMS and NIST 14) and home-made MS libraries obtained from pure substances and components of known EOs and MS literature values (Stenhagen et al., 1974; Massada 1976; Jennings and Shibamoto 1980; Swigar and Silverstein 1981; Davies 1990; Adams 1995; Benelli et al., 2013).

2.4. Insect rearing

T. *castaneum* adults were grown on alimentation medium consisted of wheat flour and maize. In plastic pots, the colony was reared at 25°C and 60% humidity in darkness. All tests were performed under the climate laboratory condition.

2.5. Repellent activity bioassay

Repellency potential of the phytochemicals against *T*. *castaneum* adults were assessed using the area preference method with some modifications (McDonald et al., 1970). *F*. *tunetana* EO and its fractions (F_1-F_{12}) were put on a 9 cm Whatman filter paper no. 1 circular disks sheared into semicircles. Tested samples were adjusted by a dilution of 4 µL in 1 mL of acetone providing correspondent concentration of 0.12 µL/cm^2. A measure of 0.5 mL of each solution was evenly distributed on the first half filter paper, while the other half was steeped with 0.5 mL of acetone as a control using a 1000 µL micropipette (single-channel mechanical micropipette; DG1120 model; Labo moderne, France). The filter papers were dried for 10 min. 20 unsexed bugs seven days-old were deposited in the middle of the filter paper disc and the number of insects on each half was recorded after 15 min, 30 min, 60 min and 120 min of exposure. Five replicates were performed for each treatment. Percentage repellency (PR) after 120 min of exposure was calculated as follows:

$$PR(\%) = [(Nc-Nt)/(Nc+Nt)]\times 100$$

Where Nc was the number of adults in the control half and Nt was their number in the treated one. The mean values are then ranged to various classes (0-V), based on the scale suggested

by Mc DONALD (1970): Class 0, PR < 0.1%; Class I, PR (%) = 0.1-20%; Class II, PR = 20-40%; Class III, PR = 40-60%; Class IV, PR = 60-80%; Class V, PR = 80-100%.

2.6. Fumigant activity bioassay

To evaluate the fumigant toxicity of *F. tunetana* essential oil against the adults of *T. castaneum*, 3 cm circular disks of Whatman filter paper No. 1 (GE Health care Life Sciences, Little Chalfont, UK) were prepared and tightly laid in the lower part of the lid of a 40 mL glass vial containing a group of 10 insects. These paper disks were soaked with different doses of EO (25, 50, 100 and 200 µL/L air) and the vials quickly closed. The fumigation test was performed in triplicate for each dose at 26 ± 5°C. The percentage of mortality was evaluated after 24 hours of exposure: when no antennal or leg response was observed upon soft pressing with a paintbrush, insects were designed dead (Zarrad et al., 2015).

2.7. Contact toxicity bioassay

Contact toxicity assays were conducted testing *F. tunetana* flowers essential oil on *T. castaneum* adult. Aliquots of 1 µL of essential oil at different concentrations (1, 5 and 10% of FEO diluted with acetone) were topically applied onto the pronotum of newly hatched *T. castaneum* adults by means of a Hamilton micro-syringe (ten insects per replicate, three replicates per dose). The solvents were left to evaporate, and the insects were transferred to 9 cm diameter Petri dishes. Insects treated with acetone alone were used as negative controls. Mortality of insect was noted 24 hours after treatment. Adults are considered dead in absence of any movement of legs and antenna (Janaki et al., 2018).

2.8. Statistical analysis

Statistical analyses were performed employing SPSS software, v. 20 (Ho, 2014). Duncan's multirange test was applied to estimate the difference between the means at $p < 0.05$. The correction employing Abbott's formula (Abbott, 1925) was used to correct mortality data for

control response. Results from all replicates were employed in probit analyses (Finney, 1971) to calculate LD_{50}.

3. Results and discussion

3.1. Chemical composition of the flowers essential oil (FEO) and its fractions (F_1-F_{12})

The fresh flowers of *F. tunetana* obtained by hydrodistillation gave a pale yellow colored EO in a 0.05% (w/w) yield. Applying a fractional hydrodistillation method, the raw essential oil was separated into 12 fractions (F_1-F_{12}), based on the difference in boiling points of their main components, with yields of 0.009%, 0.008%, 0.005%, 0.005%, 0.005%, 0.003%, 0.004%, 0.002%, 0.003%, 0.001%, 0.002%, 0.003% (w/w) respectively for: F_1 [0-15 min], F_2 [15-30 min], F_3 [30-45 min], F_4 [45-60 min], F_5 [60-75 min], F_6 [75-90 min], F_7 [90-105 min], F_8 [105-120 min], F_9 [120-135 min], F_{10} [135-150 min], F_{11} [150-165 min], F_{12} [165-180 min]. The composition of the essential oil and its volatile fractions (F_1-F_{12}) are listed in Table 1. As shown, a total of seventy-seven compounds were identified, accounting for 92.8-99.1% of FEO and F_1-F_{12}, from them, 57 compounds were identified as sesquiterpenes, whereas only 17 were monoterpenes. We noted that the major chemical class of all samples was that of oxygenated sesquiterpenes (44.9-76.8%): they were mainly illustrated by guaiol (**46**) (0.8, 11.9%), α-cadinol (**57**) (3.6, 10.7%), bulnesol (**59**) (4.1, 10.0%), *epi*-α-bisabolol (**64**) (5.7, 16.9%), β-chenopodiol (**72**) (5.1, 7.1%).

Fractional hydrodistillation of the essential oil was accomplished with two purposes: amelioration of the identification of the constituents and evaluation of the fractions and/or the components responsible for the insecticidal activity. In this study, the fractionation during the extraction process failed to isolate a pure compound, but it allowed to concentrate some constituents in certain fractions. This aided the identification of some undetected components on the chromatogram of the raw EO due to the complexity of its chemical composition, in particular bicyclogermacrene (**30**), germacrene B (**39**), α-acorenol (**52**), *α*-cadinol (**57**) and

176 *epi*-α-bisabolol (**64**). α-Pinene (**1**) (14.3%) was identified as the major compound of the
177 essential oil of *F*. *tunetana* flowers.
178 To the best of our knowledge, this is the first report on the itemized chemical characterization
179 of *F*. *tunetana* flowers EO. In spite of the many investigations about the chemical
180 composition of EOs from other species of *Ferula* genus, the *F*. *tunetana* flowers EO presented
181 a total distinct chemical profile. *F*. *szovitsiana* (Dehghan et al., 2007), *F*. *persica* (Javidnia et
182 al., 2005) and *F*. *cupularis* (Alipour et al., 2015) EOs contain neryl acetate (33.0%), dillapiole
183 (57.3%) and limonene (25.0%) at elevated concentrations, respectively. On the contrary, *F*.
184 *tingitana* (Elghwaji et al., 2017) EO was characterized by high percentages of α-thujene
185 (13.5%). A very slight similarity can be noted between the present EO and the ones extracted
186 from *F*. *vesceritensis* (Labed-Zouad et al., 2015) and *F*. *communis* (Maggi et al., 2016), which
187 contained α-pinene as a major compound. It is apparent that oxygenated sesquiterpenes
188 constitute the main chemical class of compounds of *F*. *tunetana* flowers EO, while in the
189 essential oil obtained from the seeds of the same species, monoterpene hydrocarbons (77.3%)
190 dominated. Results from this investigation also differ from those of the literature. In effect,
191 other species from the same genus were characterized by the abundance of monoterpene
192 hydrocarbons, remarkably *F*. *lutea* (Ben Salem et al., 2016) and *F*. *gummosa* (Fatemikia et al.,
193 2017), whereas *F*. *glauca* (Maggi et al., 2009) and *F*. *communis* (Rahali et al., 2016) were
194 found to contain sesquiterpene hydrocarbons at high concentrations.

195 ***3.2. Repellency activity of the essential oil and its fractions***

196 The essential oil from *F*. *tunetana* flowers was very effective as a repellent against *T*.
197 *castaneum* adults, after 120 min exposure, obtaining Class V evaluation for F_2, F_3, F_4, F_5, F_6,
198 F_9 and F_{12}. With the exception of F_8, the repellent effect was fundamentally steady (Class IV).
199 The results proved that the repellent activity was related to the exposure time. The average
200 percentage of repellency of the studied EO and its fractions are shown in Table II.

Usually, a mixture of sesquiterpenes and monoterpenes present in various essential oils cannot be disregarded as repellent agents (Tavares et al., 2018). More precisely, many pure compounds were investigated for their repellent efficiency, for example among monoterpenes, α-pinene, the major compound of *F. tunetana* flowers EO, is considered to be responsible for a repellent effect (Kim et al., 2010).

Limonene and linalool, contained in the present EO, were also reported as repellent monoterpenes (Müller et al., 2009; Tavares et al., 2018). Sesquiterpenes, such as elemol, 10-*epi*-γ-eudesmol,and especially *β*-caryophyllene and caryophyllene oxide, which were present in the EO and almost in all its fractions at significant levels, are endowed with a good repellent activity (Paluch et al., 2009a; Paluch et al., 2009b; Ashitani et al., 2015; Cao et al., 2018). The above-mentioned constituents may be the responsible for the observed powerful insecticidal activity, but the synergistic action of other compounds cannot be underrated. Their presence in different proportions can explain the slight dissimilarity between the tested samples. In addition, the presence of oxygenated sesquiterpenes in highest amount in fraction **F_2** (76.8%) could be at the origin of its important repulsive effect (93% from the first hour of exposure) compared to all the other tested samples (Ngassoum et al., 2007; Adjalian et al., 2015; Kouninki et al., 2017). On the other hand, the lowest activity of eighth fraction (37% after 2 hours of exposure) could probably be due to the presence of some minor compounds causing a feasible phenomenon of antagonism.

The results of repellent activity test are greatly encouraging, the synergistic effect of the monoterpenes and sesquiterpenes may provide important products. Further studies are required to develop a suitable formulation of this oil: as a consequence its efficiency could be extremely enhanced.

3.3. Fumigant toxicity of the raw essential oil

The essential oil of *F*. *tunetana* was toxic for *T*. *castaneum* adults and the mortality increased with increasing of concentration. At the lowest concentration (25 µL/L air), the EO caused 6.6% mortality after 24 h compared to 73.3% mortality at the highest concentration (200 µL/L air) (Figure 1). The LD_{50} of flowers EO of *F*. *tunetana* on the adult of red flour beetle 24 hours after applying the sample was 161.89 µL/L air.

To this day, no reports have been performed to prove the lethal effects of *F*. *tunetana* EO on *T*. *castaneum*. Generally, fumigant toxicity of the genus *Ferula* essential oils against insect has been poorly investigated up to here. However, the fumigant results of the Iranian *Ferula gummosa* oil gathered from hydrodistillation indicated a LD_{50} = 76.44 µL/L air against the Mediterranean flour moth (Ghasemi et al., 2014). EO of *F*. *gumosa* having its major components similar to those of *F*. *tunetana* in the present study, such as *α*-pinene (14.9%), showed high toxicity on *Tetranychus urticae* eggs and adults, with a LD_{50} = 6.98 and 6.52 µL/L, respectively (Fatemikia et al., 2017).

Terpenes have been extensively reported as active insecticides, particularly as fumigants, against stored-product pests. For this reason, the insecticidal activity of the essential oil observed in the current study may be due to its high terpenoids content. There are some other reports on the toxicity of the essential oils from various plants species having major components similar to *F*. *tunetana*, such as *α*-pinene (**1**), *β*-pinene (**3**), limonene (**7**), and elemol (**38**) which are ranged as active toxic constituents. These compounds might play a crucial role in the bioactivity of the FEO (García et al., 2005; Paluch et al., 2009b; Kim et al., 2010).

Furthermore, insecticidal activity of some of the major constitutes of the tested oil were previously reported. *α*-Pinene (**1**) killed the same studied insects after 24 h with a LD_{50} = 0.114 mg/cm^3. *β*-Pinene (**3**) possessed a strong toxic effect, with LD_{50} = 0.23 mg/cm^2 on *T*. *castaneum* after 72 h exposure. In addition, caryophyllene oxide (**42**) showed interesting

insecticidal activity (LD_{50} = 0.00018 mg/cm^3) towards *T. castaneum* (Kim et al., 2010). Many studies report insecticidal activity or fumigant toxicity of this latter compound. Its elevated toxicity could be the result from the affection of respiration rate of *T. castaneum* by the modification in the concentration of oxygen or CO_2 and consequently the inhibition of the mitochondrial electron transport system, thus provoking fumigant toxicity actions (Emekci et al., 2002). The high volatility of the previous mentioned compounds and many others, probably handed over fumigant toxicity by vapor action through the respiratory system, although further studies are required to establish their exact mechanism of action.

3.4. Contact toxicity of the essential oil

The *F. tunetana* EO was toxic to *T. castaneum* adults. In the contact toxicity bioassay, the LD_{50} was 10.44% after one day of exposure. As shown in Figure 2, the higher the concentration, the more evident the effect. For the highest concentration (10%) the attained mortality was 44%. The noticeable effects displayed by the essential oil of *F. tunetana* can be related with its main terpenes components, especially monoterpene hydrocarbons and oxygenated sesquiterpenes. The efficiency of the EO was comparable to other EOs known as very promising botanical pesticides. The essential oil from *Eucalyptus grandis* can be mentioned as an example. This essential oil, containing *α*-pinene (**1**) as major compound (52.71%), showed LD_{50} of 32.4 ppm against *Aedes aegypti*. Used as a pure compound, *α*-pinene showed LD_{50} values of 15.4 ppm on larvae of *A. aegypti* (Lucia et al., 2007). Interestingly, its larvicidal effects may be antagonized by other compounds present in the mixture. Guaiol (**46**), as a main component from *Ferula ferulaeoides*, was identified to be toxic to larvae of *Mythimna separate* and larvae of *Plutella xylostella*, showing a LD_{50} of 0.07 and 8.9 mg/larvae (Liu et al., 2013). Limonene (**7**) and *β*-pinene (**3**), the main components of *Citrus* essential oil, had toxic effects on *Aedes albopictus* (34% and 42% respectively) (Giatropoulos et al., 2012). These substances may be important for the level of insecticidal

potency of FEO against *T*. *castaneum* adults, even though they are less effective than others. Based on the above, the mechanism of death in adults of *T*. *castaneum* treated with FEO may be due to the synergistic effect between different phytochemical groups of the tested complex mixture of compounds, which can react with various molecular targets. Moreover, the possible effect of other compounds, although in small concentrations, should not be neglected. Indeed, they could synergize the effect of terpenoids. Regarding the mechanism of action, essential oils has been found to exhibit neurotoxicity on insects by attacking GABA, cholinergic and octopamine receptors (Pavela and Benelli, 2016).

4. Conclusion

This work aims to contribute to the valorization of *F*. *tunetana* as an endemic species, which can represent an interesting source of effective biological compounds. The essential oil of *F*. *tunetana* flowers (FEO) collected in Tunisia was reported for its chemical composition for the first time. The fractionation during the hydrodistillation process gave twelve fractions (F_1-F_{12}) that helped to identify more compounds in the complex chemical composition of the studied essential oil. A total of 77 compounds have been identified. The oil and its fractions were found to be rich in oxygenated sesquiterpenes (44.9-76.8%), followed by monoterpene hydrocarbons. Notably, the current work represents the first study of the insecticidal efficacy of *F*. *tunetana* essential oil. In fact, the samples were investigated for their repellent, fumigant and contact toxicity against *T*. *castaneum* adults. Despite the small applied volume and short times of exposure, the results demonstrate that they have promising effect in defending the stored grains from the red flour beetle attacks. According to these results, the essential oil of *F*. *tunetana* can be employed as an eco-friendly natural alternative for chemical insecticides to protect the stored products against insects. However, further research is needed to characterize the bioactive compounds contained in the essential oil for a better practical use.

Conflict of interest

None declared**.**

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461 properties of the seed oil from the Tunisian endemic *Ferula tunetana* Pomel ex Batt. Chem.
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Table

Table I. Chemical composition of the flowers essential oil (FEO) of *Ferula tunetana* and its fractions (F_1-F_{12}) collected during the extraction process.

No	Compounds	R.I[a]	Composition (%)													
			FEO	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	Identification
1	*α*-Pinene	941	**14.3**	**12.1**	4.2	1.7	3.6	2.2	3.9	1.1	2.1	3.6	1.6	1.4	0.4	GC–MS, RI
2	Sabinene	976	1.0	2.0	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
3	*β*-Pinene	982	3.9	**5.7**	1.8	0.7	1.3	0.8	1.0	-	0.5	0.8	0.4	-	-	GC–MS, RI
4	Myrcene	993	1.4	1.3	0.7	-	-	-	0.5	-	-	0.6	-	-	-	GC–MS, RI
5	*δ*-3-Carene	1011	0.8	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
6	*p*-Cymene	1027	0.5	1.0	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
7	Limonene	1032	1.4	2.4	1.2	0.5	0.5	0.4	0.5	-	-	-	-	-	-	GC–MS, RI
8	1-Octanol	1071	-	0.9	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
9	Fenchone	1087	-	0.8	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
10	Linalool	1101	0.6	1.0	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
11	Isopentyl isovalerate	1105	-	2.1	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
12	*trans*-Pinocarveol	1139	-	0.8	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
13	*trans*-Verbenol	1144	0.7	1.1	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
14	4-Terpineol	1178	0.8	1.6	0.7	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
15	*p*-Cymen-8-ol	1183	-	1.1	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
16	Myrtenal	1194	-	1.4	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
17	Verbenone	1205	0.5	0.8	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
18	*endo*-Fenchol acetate	1223	3.9	**11.0**	3.4	1.1	0.8	0.5	-	-	-	-	-	-	-	GC–MS, RI
19	1-Decanol	1272	-	2.0	0.8	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
20	Isobornyl acetate	1285	-	1.9	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
21	*α*-Copaene	1376	0.7	0.9	-	-	-	0.5	0.5	0.7	0.6	0.8	0.8	0.6	0.7	GC–MS, RI
22	*β*-Bourbonene	1384	0.5	0.9	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
23	*β*-Cubebene	1390	0.5	0.8	-	-	-	0.5	-	-	-	0.5	-	-	-	GC–MS, RI
24	*β*-Caryophyllene	1420	1.1	-	1.5	1.9	2.2	2.6	2.2	2.8	2.5	3.1	3.2	2.6	3.1	GC–MS, RI
25	Aromadendrene	1441	-	-	-	-	-	0.5	0.5	0.6	0.5	0.7	0.7	0.5	0.7	GC–MS, RI
26	*α*-Himachalene	1448	-	-	-	-	-	0.6	0.6	0.8	0.7	0.9	1.0	0.8	1.0	GC–MS, RI
27	*α*-Humulene	1456	-	-	-	0.6	0.6	0.7	0.6	0.8	0.6	0.7	0.8	0.6	0.7	GC–MS, RI
28	*γ*-Himachalene	1475	-	-	-	-	-	0.5	0.5	0.8	0.6	0.8	0.9	0.8	1.1	GC–MS, RI
29	Germacrene D	1478	0.5	-	3.3	**7.9**	**8.7**	**8.8**	**9.0**	**11.0**	**9.2**	**10.2**	**10.6**	**9.4**	**10.3**	GC–MS, RI
30	Bicyclogermacrene	1495	-	-	1.0	2.5	2.8	2.7	2.6	3.4	2.7	3.0	3.0	2.5	2.8	GC–MS, RI

31	*α*-Muurolene	1498	-	-	-	1.0	1.1	1.2	1.2	1.8	1.5	1.8	-	-	-	GC–MS, RI
32	*β*-Himachalene	1501	-	-	-	-	-	-	-	-	-	-	1.9	1.8	2.1	GC–MS, RI
33	*δ*-Amorphene	1505	-	-	-	-	0.6	-	0.7	1.1	0.9	1.0	1.3	1.1	1.3	GC–MS, RI
34	*trans*-*γ*-Cadinene	1513	-	-	-	0.5	0.6	0.5	-	1.1	0.7	0.8	1.1	0.9	1.1	GC–MS, RI
35	Cubebol	1516	-	1.0	0.8	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
36	Isobornyl isovalerate	1523	-	-	0.9	1.2	1.0	0.9	0.7	0.7	0.7	0.6	0.5	-	-	GC–MS, RI
37	*δ*-Cadinene	1524	0.6	-	2.6	**5.0**	**5.4**	**5.6**	**5.4**	**7.2**	**6.3**	**6.7**	**7.1**	**6.4**	**7.1**	GC–MS, RI
38	Elemol	1549	3.4	2.2	4.0	3.9	3.6	3.7	3.4	3.4	3.4	2.9	2.8	2.3	2.4	GC–MS, RI
39	Germacrene B	1554	-	-	1.2	1.5	1.5	1.6	1.4	2.1	1.7	1.9	1.9	1.3	1.8	GC–MS, RI
40	Germacrene D-4-ol	1575	-	-	**5.0**	4.4	4.1	3.7	3.6	3.0	2.5	2.3	2.1	1.6	1.6	GC–MS, RI
41	Spathulenol	1576	1.8	3.0	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
42	Caryophyllene oxide	1581	2.6	**5.9**	1.2	0.5	-	0.4	-	-	-	-	-	-	-	GC–MS, RI
43	Globulol	1583	-	-	-	-	-	0.4	-	-	-	-	-	-	-	GC–MS, RI
45	Viridiflorol	1590	-	-	-	1.1	-	0.5	-	-	-	-	-	-	-	GC–MS, RI
46	Guaiol	1595	2.7	0.8	**10.7**	**11.9**	**10.7**	**9.4**	**9.4**	**8.0**	**8.8**	**7.3**	**6.5**	**6.1**	**5.3**	GC–MS, RI
47	Humulene epoxide II	1607	0.8	2.2	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
48	*β*-Himachalene oxide	1612	0.5	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
49	10-*epi*-γ-Eudesmol	1623	0.9	-	2.5	2.9	2.4	0.6	1.0	0.6	-	-	0.6	-	-	GC–MS, RI
50	1-*epi*-Cubenol	1628	4.0	1.4	1.5	-	-	1.4	-	0.8	0.4	-	-	-	-	GC–MS, RI
51	*γ*-Eudesmol	1630	1.1	-	-	0.8	0.8	0.9	0.9	1.1	1.2	1.2	1.2	1.2	1.2	GC–MS, RI
52	*α*-Acorenol	1631	-	-	-	1.9	2.0	2.2	2.1	2.5	2.7	2.6	2.9	2.8	2.8	GC–MS, RI
53	Himachalol	1638	**6.8**	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
54	*epi*-*α*-Cadinol	1640	4.1	2.2	3.1	3.9	4.1	4.4	4.7	**5.0**	**5.4**	**5.0**	**5.4**	**5.1**	**5.1**	GC–MS, RI
55	*epi*-*α*-Muurolol	1642	**9.5**	-	-	3.6	4.0	4.6	4.9	**5.4**	**6.1**	**5.9**	**6.3**	-	-	GC–MS, RI
56	*α*-Muurolol	1645	1.7	1.8	3.3	-	-	-	-	-	-	-	-	7.0	6.5	GC–MS, RI
57	*α*-Cadinol	1654	-	3.6	**6.0**	**5.9**	**7.2**	**7.4**	**8.5**	**8.8**	**10.4**	**9.8**	**10.1**	**10.7**	**10.0**	GC–MS, RI
58	*cis*-Calamenen-10-ol	1661	-	1.2	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
59	Bulnesol	1666	4.1	-	**10.0**	**9.9**	**9.0**	**8.7**	**8.6**	**7.9**	**8.5**	**7.5**	**7.1**	**7.6**	**6.9**	GC–MS, RI
60	Intermedeol	1667	-	-	-	-	-	0.8	-	-	-	-	-	-	-	GC–MS, RI
61	Aromadendrene epoxide I	1673	0.6	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
62	Guaia-3,10(14)-dien-11-ol	1679	-	1.4	-	-	-	-	-	-	-	-	-	-	0	GC–MS, RI
63	*α*-Bisabolol	1683	2.8	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
64	*epi*-*α*-Bisabolol	1686	-	-	**16.1**	**16.9**	**15.5**	**13.5**	**12.4**	**10.8**	**10.5**	**8.8**	**7.7**	**6.9**	**5.7**	GC–MS, RI
65	Oplopanone	1720	2.2	4.2	1.6	-	-	-	-	-	-	-	-	-	-	GC–MS, RI

66	14-Hydroxy-α-muurolene	1760	-	-	-	-	1.0	1.2	1.0	-	-	-	-	-	-	GC–MS, RI
67	*(Z)*-Lanceol	1765	**5.6**	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
68	*(Z)*-*α*-Santalol acetate	1788	-	**5.0**	3.6	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
69	Drimenone	1793	-	-	1.3	1.1	-	-	-	1.3	1.5	1.5	1.8	1.5	1.4	GC–MS, RI
70	Isovalencenyl formate	1799	0.6	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
71	8-*α*-Acetoxyelemol	1805	0.7	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
72	*β*-Chenopodiol	1810	**6.0**	**7.1**	**5.1**	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
73	Khusinol acetate	1828	-	-	-	-	-	-	-	-	-	-	-	**6.0**	**6.3**	GC–MS, RI
74	Platambin	1867	0.5	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
75	Ledene oxide I	1890	1.5	-	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
76	Acetoxyeudesman-4-*α*-ol	1940	-	1.8	-	-	-	-	-	-	-	-	-	-	-	GC–MS, RI
77	*α*-Chenopodiol-6-acetate	1960	-	-	-	3.7	3.1	3.2	2.9	3.5	3.9	3.7	4.2	4.0	3.6	GC–MS, RI
Monoterpene hydrocarbons			23.2	24.6	7.9	2.8	5.4	3.4	5.9	1.1	2.6	4.9	2.0	1.4	0.4	
Oxygenated monoterpenes			6.6	21.5	4.1	1.1	0.8	0.5	-	-	-	-	-	-	-	
Sesquiterpene hydrocarbons			4.0	2.6	9.5	20.9	23.5	26.1	24.9	34.1	28.3	32.8	34.2	29.3	33.8	
Oxygenated sesquiterpenes			64.5	44.9	76.8	73.2	68.6	67.9	64.2	62.7	65.9	58.9	59.2	62.9	58.7	
Other non-terpene derivatives			-	4.9	0.8	-	-	-	-	-	-	-	-	-	-	
Total identified (%)			98.3	98.5	99.1	98.1	98.4	97.8	94.9	97.9	96.8	96.6	95.4	93.5	92.8	

Table

Table I. Repellent activity of *Ferula tunetana* essential oil on *Tribolium castaneum* adults after different exposure times using the filter paper test

Test sample	T (exposure)/min	Repellent activity/%	class
FEO	15	(26±0.29)[a]	II
	30	(40±0.35)[ab]	III
	60	(40±0.28)[ab]	III
	120	(71±0.21)[b]	**IV**
F1	15	(57±0.12)[b]	III
	30	(63±0.06)[b]	IV
	60	(67±0.15)[c]	IV
	120	(73±0.09)[b]	**IV**
F2	15	(83±0.17)[b]	V
	30	(87±0.12)[b]	V
	60	(93±0.12)[c]	V
	120	(93±0.05)[b]	**V**
F3	15	(47±0.00)[b]	III
	30	(60±0.10)[b]	IV
	60	(70±0.10)[c]	IV
	120	(80±0.05)[b]	**V**
F4	15	(77±0.00)[b]	IV
	30	(80±0.10)[b]	V
	60	(80±0.12)[c]	V
	120	(87±0.12)[b]	**V**
F5	15	(67±0.10)[b]	IV
	30	(73±0.29)[b]	IV
	60	(80±0.21)[c]	V
	120	(90±0.08)[b]	**V**
F6	15	(73±0.21)[b]	IV
	30	(67±0.15)[b]	IV
	60	(80±0.10)[c]	V
	120	(80±0.16)[b]	**V**
F7	15	(40±0.20)[a]	III
	30	(53±0.15)[a]	III
	60	(60±0.10)[b]	IV
	120	(67±0.06)[b]	**IV**
F8	15	(27±0.15)[a]	II
	30	(30±0.17)[a]	II
	60	(33±0.21)[a]	II
	120	(37±0.25)[a]	**II**
F9	15	(63±0.35)[b]	IV
	30	(73±0.21)[b]	IV
	60	(77±0.23)[c]	IV
	120	(80±0.10)[b]	**V**
F10	15	(40±0.10)[a]	III
	30	(60±0.17)[b]	IV
	60	(60±0.26)[b]	IV
	120	(60±0.20)[a]	**IV**
F11	15	(57±0.35)[b]	III
	30	(57±0.31)[b]	III
	60	(70±0.00)[c]	IV
	120	(70±0.00)[b]	**IV**
F12	15	(77±0.10)[b]	IV
	30	(80±0.23)[b]	V

	60	$(80\pm0.17)^{c}$	V
	120	$(80\pm0.17)^{b}$	**V**

Figure

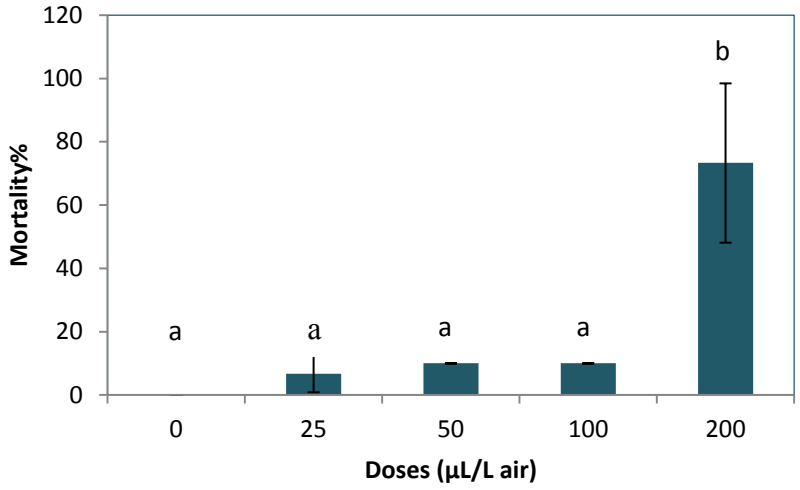

Fig. 1. Percentage of mortality of *Tribolium castaneum* after 24 h of exposure to various volume fractions of *Ferula tunetana* essential oil using the fumigation bioassay

Figure

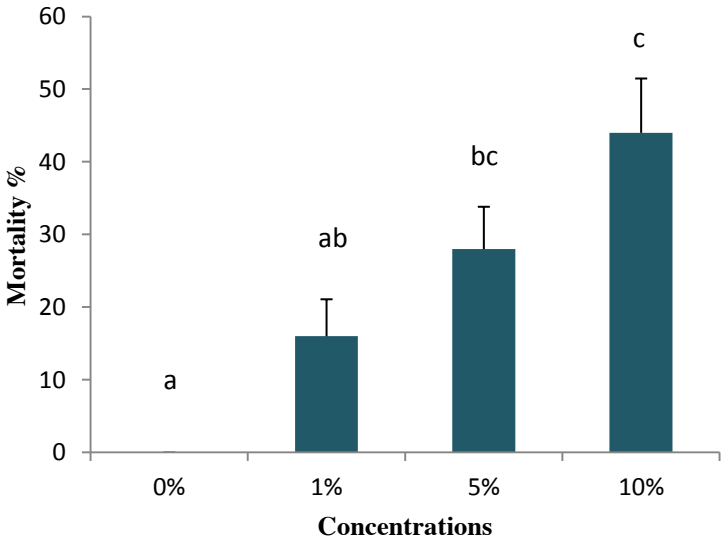

Fig. 2. Toxicity of *Tribolium castaneum* exposed to *Ferula tunetana* flowers essential oil using the topical application bioassay