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SHORT COMMUNICATION

Chemical constituents and insecticidal activity of the essential oil from fruits of *Foeniculum vulgare* Miller on larvae of Khapra beetle (*Trogoderma granarium* Everts)

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S u m m a r y

The objective of current study was to determine the chemical constituents and fumigant toxicity of essential oil isolated by hydro-distillation from dry fruit of bitter fennel (*Foeniculum* vulgare Miller). The chemical composition of the essential oil was assessed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Constituents of the oil were determined as α-pinene (1.6%) and limonene (3.3%), fenchone (27.3%), estragol (3.9%), and (*E*)-anethole (61.1%). The fumigant toxicity of the essential oil was tested on larvae of the stored product insect *Trogoderma granarium* Everts. The mortality of larvae was tested at different concentrations ranging from 31.2 to 531.2 μl/l air and at different exposure times (24 and 48 h). Probit analysis showed that LC_{50} and LC_{90} following a 48 h-exposure period for essential oil were 38.4 and 84.6 μl/l, respectively. These results showed that the essential oil from *F. vulgare* may be applicable to the management of populations of stored-product insects.

Key words: *Foeniculum vulgare, Apiaceae, essential oil vapours, Trogoderma granarium, fumigant toxicity, (E)-anethole, fenchone*

INTRODUCTION

Fumigants are considered as one of the most economical and convenient tools for managing stored-grain insect pests, not only because of their ability to kill a broad spectrum of pests, but also because of their easy penetration into the commodity while leaving minimal residues [1]. Therefore, the use of safe, less toxic botanical pesticides is now emerging, as protection of crops, their products and the environment from pesticide pollution is a crucial problem [2]. In general, botanicals should cause less damage to human and environmental health than conventional insecticides.

Among stored-product insect pests, khapra beetle *Trogoderma granarium* Everts is a very destructive insect of stored grains and cereals in hot and dry climates of the world. It has been known as one of the 100 worst invasive species worldwide [3]. Development rates and survival vary considerably depending on temperature, light, moisture, season, and host species [4]. Khapra beetle may have one to nine or more generations per year with high humidity resulting in depressing effect on population buildup [5]. The larval stage may survive between a month to several years under diapause condition [6]. It is present in Syria, and the prevailing climatic conditions in the area are conducive to serious outbreaks [7, 8].

Currently, phosphine and methyl bromide are two common fumigants used for stored product protection [9]. Insect resistance to phosphine is a global issue now, and failure in the control of several insects have been reported in some countries [9-11]. The use of methyl bromide will be prohibited because of its ozone depletion potential and high toxicity [9, 12].

These problems have highlighted the need for the development of selective insect-control alternatives with fumigant action. Many plant extracts and essential oils may be an alternative source of stored-product insect control agents [13-15] because they constitute a rich source of bioactive chemicals. Contemporary research showed that essential oils and their constituents may have potential as compounds alternative to currently used fumigants [15-18].

Major constituents of aromatic plants, mainly monoterpenes, have been of special interest to industrial markets because of their content of potent biologically active constituents in addition to their toxicity to insects. *Foeniculum vulgare* Miller (*Apiaceae*), is a perennial hemicryptophyte, common in the Mediterranean region, which is used in traditional medicine and as a spice. Herbal drug preparations, of numerous wild types, are active against dyspeptics, bloating and flatulence [19]. Diuretic, analgesic and antipyretic activities have also been found in *F. vulgare* fruit [20] as well as antioxidant activity [21]. The leaves and fruits are used mainly to flavour fish and meat, giving them a strong aroma and taste, and as an ingredient of cosmetics. Essential oils are concentrated mainly in mericarps (fruits) and provide a unique aroma and taste [22]. The chemical composition of volatile oil fraction has been well described in the literature [23-25]. Also, some of its compounds are considered to be toxic for rats [26-28]. Earlier investigation of *F. vulgare* fruit led to the isolation of phenolic components with antihypertensive activity [29, 30].

The objective of current work was to carry out a chemical study to identify the major constituents of the essential oil distilled from *F. vulgare* fruits, and to carry out a toxicity study of total essential oil on *T. granarium* larvae.

MATERIALS AND METHODS

Plant material

Fruits of *F. vulgare* were collected in 2009 from the coastal area (Bustan al-Basha – Latakia, Syria). The fruits were dried naturally on laboratory benches at room temperature (23–27°C) and in a shaded place until became crisp, and then hydro-distilled to isolate their essential oil.

Isolation of essential oils

Essential oil was distilled from plant samples according to Tayoub et al. [31]. Plant material was subjected to hydro-distillation using a Clevenger-type apparatus with following conditions of distillation: 100 g of air-dried, ground sample was immersed in distilled water at a ration of 1:10 (plant material/water, w:v). Hydro-distillation was carried out for 3 h. Anhydrous sodium sulphate was used to remove water after distillation. Oil yield (5.44 % v/w) was calculated on a dry weight basis. Distilled oil was stored in a refrigerator at 4°C until tested.

Gas chromatography

Essential oil was analyzed using an Agilent (6890N) GC system. The capillary column used was DB-5 (30m × 0.25mm i.d., 0.25 μm film thickness) with helium as a carrier gas at 1 ml/min. The initial temperature of column was 45°C (held for 2 min) and then heated to 175°C with a 3°C/min rate (held for 5 min) and then heated to 275°C with a 4°C /min rate (held for 10 min).

Injector temperature was 275°C, and flame ionization detection temperature was 300°C.

Gas chromatography – mass spectrometry

Constituents of the essential oils were identified using GC-MS. The GC-MS analysis was carried out using an Agilent GC-MS model GC-6890, with an inert mass selective detector 5973.

The capillary column was DB-35 (30x0.2bmm, film thickness 0.25 μm). The operating conditions were as follows; carrier gas, helium with a flow rate of 1 ml /min; injected volume was 1 μl of the essential oil and ionization mode was electron impact. The GC-MS system was operated under following conditions: injection temperature 250°C, source temperature 250°C, fragment energy of 70 eV mass spectra were acquired using ionization voltage 70 eV. The initial temperature of the column was 50°C (held 2 min), then heated to 170°C at a rate of 2°C/min (held for 7 min), then heated to 250°C at a rate of 4°C/min (held for 10 min). The same conditions of temperature programming were used for oil samples in order to calculate the retention index (RI). Identification of components in the oil was based on RI.

Individual components were identified by comparison of mass spectra and their corresponding GC retention data. Identifications were made by comparison of obtained mass spectra with those in data system libraries installed in the GC-MS system and with those cited in the literature [32]. The quantitative analysis of percentages was performend according to reference materials and standards obtained from Aldrich. Calculations were made with the use of gas chromatography chemstation software.

Biological material

Insects

A culture of *T. granarium* insects was reared in the lab in 3 liter glass jars covered with a piece of muslin and placed in an incubator in continuous darkness at 37°C. Larvae were isolated using a sieve that allowed their separation from wheat grains.

Treatment of larvae with essential oil

For *F. vulgare* Miller treatment, isolated larvae were divided into 11 groups. Each group consisted of 5 replicates, 10 larvae per replicate. One group was used as a control, other 10 groups were used for treatment with different concentrations of *F. vulgare* essential oil. These were 5, 10, 20, 30, 40, 50, 60, 70, 80, 85 μl of essential oil.

Treatments were carried out by placing larvae in small glass Petri dishes (9 cm in diameter) with wheat as a source of food. Each dish contained 10 larvae The small Petri dish with larvae in it was placed in a larger glass Petri dish (11 cm in diameter). The essential oil droplets were deposited on the inner glass surface of the larger Petri dish using a micropipette, the volume of the large Petri dish was

160 cm^3 air. Hence, the essential oil vapour filled this volume and the concentration of essential oil vapour was calculated on the basis of μl essential oil/l air and amounted to 5, 10, 20, 30, 40, 50, 60, 70, 80, 85 μl/160 cm^3 and 31.25, 62.5, 125, 187.5, 250, 312.5, 375, 437.5, 500, 531.25 ul/l air, respectively [35]. The whole system was sealed by parafilm. Controls were treated with distilled water only. Mortality percentage was observed after 24 and 48 h. Mortality was verified by observing any sign of larvae movement in response to poking with a needle [33].

Mortality data were corrected for natural mortality in controls and were subjected to probit analysis to estimate LC_{50}, LC_{90}. Slopes were also generated [38].

RESULTS

Figure 1 shows a typical chromatogram of *F. vulgare* fruit volatiles using a DB-5 capillary column. The substances in the fruit essential oil are shown in table 1.

The GC analysis of the crude oil isolated from *F. vulgare* fruits resulted in the identification of 12 compounds representing 100% of the essential oil. The compounds are listed in the order of their elution from the BD-5 column (fig. 1). The major constituents of the oil were α-pinene (1.6%) and limonene (3.3%), fenchone (27.3%), estragol (3.9%), and (*E*)-anethole (61.1%). The oil contained a relatively high amount of phenylpropanoids (65 %) and monoterpenes (35 %) as presented in table 1.

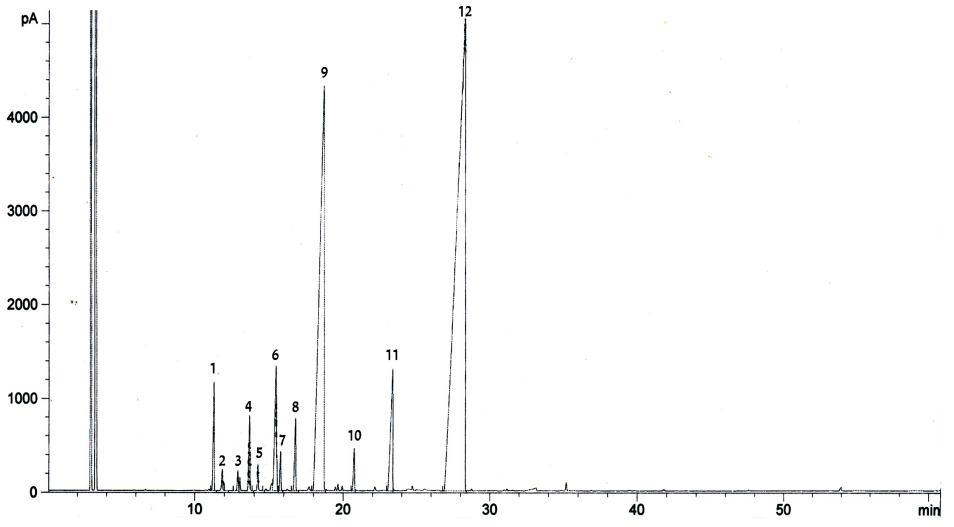

Figure 1.
Chromatogram of *F. vulgare* essential oil

Table 1

Chemical composition of *F. vulgare* fruits oil

No	Constituents	RI	Percentage (%)
1	α-Pinene	943	**1.6**
2	Camphene	961	tr
3	Sabinene	978	0.3
4	α-Phellandrene	1000	tr
5	α-Terpinene	1016	tr
6	Limonene	1034	**3.3**
7	(*Z*)-β-Ocimene	1041	0.6
8	γ-Terpinene	1063	**1.3**
9	Fenchone	1105	**27.3**
10	Camphor	1150	0.7
11	Estragole	1208	**3.9**
12	(E)-Anethole	1316	**61.1**
Total			**100**
Monoterpenes identified			**35**
Phenyl propanoids identified			**65**

tr – traces, <0.1%

Vapours of essential oil of *F. vulgare* showed a variable toxicity to larvae of *T. granarium* (fig. 2). Exposure of larvae to the highest dose of 85 μl/160 cm^3 air (531.25 μl/l air) resulted in 94% and 98 % mortality after 24 h and 48 h-exposure period, respectively. Whereas, the lowest concentration used, i.e. 5 μl/160cm^3 air (31.25μl/l air) had no effect at 24 h-exposure period and resulted in 2% mortality only at 48 h-exposure period A positive dose-effect relationship was evident for the range of applied doses (fig. 2).

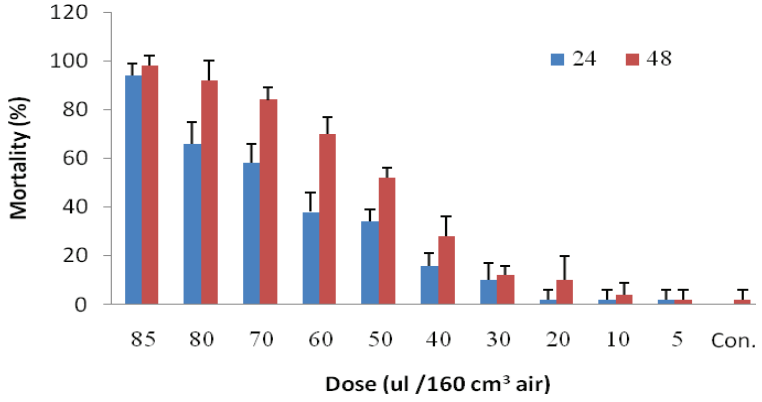

Figure 2.

Percent mortality (mean values ±SD) in larvae of *T. granarium* exposed to *F. vulgare* essential oil at different concentrations and exposure times

Table 2 shows the LC_{50} and LC_{90} values for *F. vulgare* essential oil as calculated from mortality data using probit analysis (34). At 48 h-exposure period, LC_{50} for *F. vulgare* essential oil on larvae was 38.4 μl/l air, whereas LC_{90} was 84.6 μl/l air. However, at 24h LC_{50} was 60.3 μl/l air and LC_{90} was 123.2 μl/l air. (tab. 2).

Table 2.

Fumigant toxicity of essential oil distilled from *F. vulgare* fruit against *Trogoderma granarium* Everts larvae

Exposure time	Exposure time	LC_{90} μL/l air	Slope ±SE	Fcal	F 05	*d.f.*	Chi square χ^2
48 h	48 h	84.6	3.7 ± 1.0	13.7	5.3	8	15.5
24 h	24 h	123.2	4.1± 1.0	16.1	5.3	8	15.5

DISCUSSION

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids [35-37]. Monoterpenoids are volatile and rather lipophilic compounds, making them able to penetrate into insects rapidly and interfere with their physiological functions [38].

Due to their high volatility, essential oils have fumigant and gaseous action and might be important for stored-product insects [37]. The toxic effects of *F. vulgare* essential oil on larvae of *T. granarium* recorded in present study could be attributed to one or more of constituents of monoterpene compounds (representing 35% of constituents), or to phenylpropanoids (representing 65% of constituents, see tab. 1) of the essential oil. For example the monoterpene β-pinene has an insecticidal activity against *S. oryzae* [39]. Limonene showed fumigant toxicity to *T. castaneum* [38]. Also, fumigation of *Tribolium castaneum* (Herbst) adults with camphor at a dose of 120 μl/350 ml resulted in 93.5% mortality [40]. The phenylpropanoid anethole showed fumigant activity against the greenhouse pests, the carmine spider mite *Tetranychus cinnabarinus* (Boisd.) and the cotton aphid *Aphis gossypii* Glov. [41]. The time required for 99% mortality in females of *T. cinnabarinus* was 79 h at 1.7 mg/l air, while in females of *A. gossypii* it was 50 h at 1.7 mg/l air [45]. Therefore, insecticidal activity of *F. vulgare* essential oil may be related to these components. The toxicity exhibited by essential oils and their monoterpene constituents marks them as potential alternative compounds to currently used fumigants [15].

It is interesting to compare toxicity effects of *F. vulgare* essential oil found in the present study to toxicity effects of essential oils extracted from other sources on different stored product insects. Tunç et al. [17] tested the essential oils of anis and cumin, close relatives to *F. vulgare*, on eggs of confused flour beetle *T. confusum* and Mediterranean flour moth *E. kuechniella*. They found that the essential oil of anis was capable of causing 100% mortality to eggs of both insects at a concentration of 196.9 μl/l, whereas, cumin essential oil caused 100% mortality only on eggs

of *E*. ku*echniella*. A recent study on the insecticidal activity of *F. vulgare* oil against wheat weevil, *Sitophilus granarius* and rice weevil *Sitophilus oryzae*, both *Curculionidae*, showed a dose dependent mortality [42]. Fumigation of adult *S. granarius* with the highest tested concentrations of 50 ul/l air for 24 and 48 h resulted in 75% and 69% mortality, respectively. In the present study, the LC_{50} exhibited by *F. vulgare* essential oil (38.4 μl/l air, 48 h-exposure period) was comparable to that of bay laurel *Laurus nobilis* (LC_{50} = 37.9 ul/l air) reported by Tayoub et al. [43]. It was suggested that for fumigants, the active stages (adults and non-diapausing larvae) of insects are more susceptible than the sedentary stages (eggs and pupae) due to difference in their respiratory rate [9]. In present study, only larvae were tested and were found more tolerant to fumigation effect of *F. vulgare* essential oil than adults of wheat weevil *S. granarius* and rice weevil *S. oryzae* [42]. Also, larvae of the khapra beetle *T. granarium* were found to be the most tolerant of all other developmental stages to fumigation effect of the essential oil of *Myrtus communis* L [33]. It was reported that tolerance of certain developmental stages of a pest to fumigation with an essential oil is dependent on the pest species and the type of essential oil or component [9]. A study on the essential oil constituent isolated from aromatic plants showed that two natural terpenes termed ZP-51 and SEM-76 isolated and cultivated from unidentified cultivated aromatic plants belonging to *Labiatae* family have an outstanding fumigant toxicity effect on *T. granarium* larvae, at 1.5 μl/l air they showed 87% and 99% mortality for SEM-76 and ZP-51, respectively [44].

Present study showed that the essential oil of this *F. vulgare* plant may be used as a fumigant to khapra beetle as a representative of stored product insects. *F. vulgare* is used as culinary and medicinal plant, and its essential oil is considered less harmful than conventional insecticides and may pose fewer or lesser risks to human health and the environment. [42] Therefore, the possibility of employing such natural fumigant to control insects in stored products may warrant further investigation.

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ZWIĄZKI CHEMICZNE ORAZ AKTYWNOŚĆ OWADOBÓJCZA OLEJKU ETERYCZNEGO Z OWOCÓW *FOENICULUM VULGARE* MILLER NA LARWY SKÓROJADKI ZBOŻOWEJ (*TROGODERMA GRANARIUM EVERTS*)

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Streszczenie

Celem pracy było określenie składników chemicznych i właściwości owadobójczych olejków eterycznych wyizolowanych za pomocą hydrodestylacji z suchych owoców kopru włoskiego (*Foeniculum vulgare* Miller). Skład chemiczny olejku eterycznego oznaczono za pomocą chromatografii gazowej (GC) i chromatografi gazowej sprzężonej ze spektrometrem gazowym (GC-MS). Oznaczono następujące składniki olejku eterycznego: α-pinen (1,6%) i limonen (3,3%), fenchon (27,3%), estragol (3,9%) i (*E*)-anetol (61,1%).
Toksyczność oparów olejku eterycznego przetestowano na larwach *Trogoderma granarium* Everts, szkodnika produktów magaznowych. Śmiertelność larw sprawdzono przy różnych stężeniach od 31,2 do 531,2 μl/l powietrza i różnych czasach ekspozycji (24 i 48 godzin). Analiza probitowa wykazała, że LC_{50} i LC_{90} po 48-godzinnej ekspozycji na olejek eteryczny wynosiły odpowiednio 38,4 i 84,6 μl/l. Wyniki pokazują, że olejek eteryczny z *F. vulgare* może być stosowany w populacjach szkodników produktów magazynowych.

Słowa kluczowe: *Foeniculum vulgare, Apiaceae, opary olejku eterycznego, Trogoderma granarium, toksyczność oparów, (E)-anetol, fenchon*