Entomologia Generalis, Vol. 40 (2020), Issue 4, 421–435
Published online 12 November 2020

Article

Phenolic monoterpene-rich essential oils from Apiaceae and Lamiaceae species: insecticidal activity and safety evaluation on non-target earthworms

Roman Pavela[1,2], Mohammad Reza Morshedloo[3], Hasan Mumivand[4], Ghazaleh Jafari Khorsand[3], Akbar Karami[5], Filippo Maggi[6,*], Nicolas Desneux[7], and Giovanni Benelli[8]

[1] Crop Research Institute, Drnovska 507, 161 06 Prague 6, Ruzyne, Czech Republic
[2] Department of Plant Protection, Czech University of Life Sciences Prague, Kamycka 129, 165 00 Praha 6, Suchdol, Czech Republic
[3] Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, 55136-553, Maragheh, Iran
[4] Department of Horticultural Sciences, Faculty of Agriculture, Lorestan University, PO Box 465, Khorramabad, Iran
[5] Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran
[6] School of Pharmacy, University of Camerino, via Sant'Agostino 1, 62032 Camerino, Italy
[7] Université Côte d'Azur, INRAE, CNRS, UMR ISA, 06000 Nice, France
[8] Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy
* Corresponding author: filippo.maggi@unicam.it

With 1 figure and 6 tables

Abstract: The genera *Oliveria* (Apiaceae), *Thymus* and *Satureja* (Lamiaceae) encompass important medicinal and aromatic plants of Iran, which contains noteworthy amounts of essential oils (EOs) that are worthy of exploitation on an industrial level, including pesticide formulation development. In this study, the efficacy of five EOs obtained from *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri*, was evaluated against three insects of economic relevance, the mosquito *Culex quiquefasciatus*, the housefly *Musca domestica*, and the moth *Spodoptera littoralis*. Potential non-target effects of these EOs were assessed on earthworms, *Eisenia fetida*. The chemical composition of the five EOs was determined by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). EOs from *O. decumbens, T. daenensis, S. khuzistanica* and *S. rechingeri* were characterized by oxygenated monoterpenes (70.3, 91.6, 89.7 and 89.4%, respectively), whereas the EO from *S. sahendica* was dominated by monoterpene hydrocarbons (63.3%) followed by oxygenated monoterpenes (35.3%). In all cases, the biogenetically related thymol (0.3–36.7%), carvacrol (1.3–89.6%), *p*-cymene (2.1–13.7%) and γ-terpinene (1.5–41.3%) were found as the characteristic volatile compounds of the five EOs, with high quantitative variation according to the species considered. The five EOs exhibited high toxicity on the three target insects, with LC_{50}/LD_{50} values in the range of 16.2–29.3 μg mL^{-1}, 18.1–48.1 μg $adult^{-1}$ and 7.4–23.1 μg $larva^{-1}$ on *C. quinquefasciatus, M. domestica* and *S. littoralis*, respectively. Toxicity of the five EOs on non-target soil invertebrates was minimal, leading to no or very low (5.0–7.5%) mortality on *E. fetida* when tested at the concentration of 200 mg kg^{-1}, at variance with the positive control α-cypermetrin, which caused 100% mortality at 0.1 mg kg^{-1} of soil. Overall, our study discloses new opportunities to farmers growing these Iranian endemic plants, shedding light on the potential of these EOs to formulate effective and eco-friendly insecticides.

Keywords: *Culex quinquefasciatus, Musca domestica, Spodoptera littoralis,* essential oils, toxicity, eco-friendly insecticides

1 Introduction

A major challenge in entomological research is the effective management of pest and vector populations within the Integrated Pest/Vector Management (IPM/IVM) criteria (Cook et al. 2007; Mouden et al. 2017; Wilke et al. 2020). Indeed, the frequent and indiscriminate employ of chemical pesticides led to quick resistance development (Naqqash et al. 2016; Aponte et al. 2019; Senthil-Nathan, 2019; Yavaşoglu et al. 2019; Guz et al. 2020), as well as to

www.schweizerbart.de
DOI: 10.1127/entomologia/2020/1131 0171-8177/2020/1131 $ 6.75

detrimental consequences for human health and the environment, including severe damages to insect pollinators and other non-target species (Wood & Goulson 2017; Varikou et al. 2019), even though at sub-lethal effects (Desneux et al. 2007; Gul et al. 2019; Ullah et al. 2019a,b,c). Therefore, developing new and eco-friendly insecticides is a key goal for pest/vector management science (Palacios et al. 2009a,b; Chaieb et al. 2018; Petrovic et al. 2019; Isman 2020).

Iran is one of the largest countries, characterized by 11 out of the 13 climates which have been identified so far in the world. This huge surface encompasses different ecosystems giving birth to about 8000 plant species belonging to 156 botanical families, among which 1725 species are classified as endemic (Jalili & Jamzad, 1999; Mozafarian, 2012). Notably, more than one thousand species growing in Iran have been classified as aromatic, being valuable sources of essential oils (EOs), i.e. liquid mixtures of hydrophobic, volatile compounds secreted into specific tissues of epidermal or parenchymatous origin. Two of the most important EO-bearing families growing in Iran are Apiaceae and Lamiaceae (Vitali et al., 2016; Esmaeili et al., 2018; Pavela et al., 2019a, 2020a; Bistgani et al., 2018; Morshedloo et al., 2017, 2018; Machiani et al., 2018). These two families are worthy of industrial utilization for the manufacture of valuable insecticides against mosquitoes and other arthropods of medical or agricultural importance (Evergetis et al., 2013; Benelli et al., 2018, 2019a; Pavela et al., 2019a,b, Rizzo et al., 2020). Within these families, the genera *Oliveria* Vent., *Thymus* L. and *Satureja* L. encompass important medicinal and aromatic plants in Iran (Fig. 1) whose potential on an industrial level is yet to explore (Pirbalouti et al., 2013; Nazari et al., 2013).

Oliveria decumbens Vent. (Apiaceae) is an annual plant commonly growing in the mountain areas of south of Iran as well as south-western of Syria and Iraq (Motamedi et al., 2010). The characteristic of this aromatic plant is the showy umbrellas, which are made up of purple flowers (Fig. 1). The EO from its aerial parts is reported to be rich in phenolic monoterpenes such as thymol and carvacrol (Karami et al., 2019). Recently, the antibacterial, anti-*Helicobacter pylori* and insecticidal properties of this EO have been documented (Eftekhari et al., 2019).

Thymus daenensis Celak (Lamiaceae) is an endemic perennial herb, 6–30 cm tall, with narrow, triangular-shaped leaves, multi-stems and small purple-white flowers (Fig. 1) (Ghahreman, 2001). The plant is used in the traditional medicine as well as in foodstuffs as a spice, to make an herbal tea and as an additive for livestock and poultry feed (Nickavar et al., 2005; Heydari et al., 2019). Its EO, which is rich of thymol, has been reported for its notable antimicrobial, antioxidant, hypolipidemic and anti-inflammatory properties (Emami Bistgani et al., 2019; Amirghofran et al., 2012; Nazari et al., 2013; Moazeni et al., 2014; Moghimi et al., 2016).

The genus *Satureja* (Lamiaceae) encompasses 14 species naturally growing in Iran, among which 9 are considered endemic. Among them, *S. sahendica* Bornm., *S. khuzistanica* Jamzad and *S. rechingeri* Jamzad are perennial and bushy aromatic herbs growing in the mountain regions, mostly on rocky walls (Fig. 1) (Mozafarian, 2012; Jamzad, 2009). They are used as a spice for flavoring foods and beverages and to make herbal teas (Nooshkam et al., 2017). As a traditional remedy, they are employed for their stimulant, carminative, stomachic and expectorant properties and for treatment of diarrhea and infectious diseases (Sefidkon et al., 2004; Mozafarian, 2012; Saharkhiz et al., 2016). These species are rich in EOs containing phenolic monoterpenes and have shown relevant potential as antimicrobial and insecticidal agents (Momtaz & Abdollahi, 2008; Hadian et al., 2011; Yousefzadi et al., 2012; Taban et al., 2017). Notably, *S. khuzistanica* and *S. rechingeri* are currently used by local pharmaceutical companies to make antidiabetic herbal teas, dental anesthetic solutions and toothpastes (Nooshkam et al., 2017).

In the framework of novel and eco-friendly pesticide development, plants represent a major source of effective compounds, which can be used to manage arthropod populations, formulating novel insecticides, acaricides (Govindarajan et al. 2016a,b; Tabari et al. 2017; Benelli & Pavela 2018a; Isman 2017; Pavela et al. 2019c) and repellents (Pavela et al., 2016; Adenubi et al. 2018a,b; Benelli & Pavela 2018b; Benelli et al., 2019b). In particular, the use of botanical-based insecticides strongly reduces the chance of resistance development in targeted pests and vectors due to their multiple modes of action, including – but not limited to – inhibition of acetylcholinesterase, GABA and octopamine receptors (Pavela & Benelli, 2016; Jankowska et al., 2017, 2019).

Therefore, in the continuation of our research studies on medicinal and aromatic plants from Iran, with the aim of finding new applications for their EOs on an industrial level (Pavela et al., 2018a, 2020), in this study we focus on the chemical composition and insecticidal potential of five Iranian endemic species, namely *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri*. For the purpose, the chemical composition of their EOs, obtained from the aerial parts by hydro-distillation, was analyzed by GC-FID and GC-MS. The insecticidal activity of the five EOs was evaluated on three insects of economic relevance, the mosquito *Culex quiquefasciatus* Say (Diptera: Culicidae), a competent vector of lymphatic filariasis and West Nile virus, among others, the housefly, *Musca domestica* L. (Diptera: Muscidae) and the African cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Besides, the non-target effects of the five EOs were evaluated on the earthworm *Eisenia fetida* (Savigny), to assess their impact on beneficial soil invertebrates.

Fig. 1. The five Iranian species investigated for the chemical composition and insecticidal activity of their essential oils: **(a)** *Satureja khuzistanica*, **(b)** *Satureja rechingeri*, **(c)** *Satureja sahendica*, **(d)** *Oliveria decumbens* and **(e)** *Thymus daenensis.*

2 Materials and methods

2.1 Plant material

The aerial parts of *T. daenensis* and *O. decumbens* were collected at full flowering stage from their natural habitat in Sepidan city, Fars province (E 51°52'42.1", N 30°30115.4", 2376 m a.s.l.) and Nourabad city, Fars province (E 51°33'16.3", N 30°8'21.6", 1268 m a.s.l.), Iran, in June–July 2017, respectively. The voucher specimens (No 14508 and 55079, respectively) were identified and deposited at the Herbarium of Fars Research Center for Agriculture and Herbarium of Shiraz University, Shiraz, Iran.

The flowering aerial parts of *S. sahendica* were collected from Maragheh, East Azerbaijan, Iran in September 2018 (N 37°17', E 46°18', 1624 m a.s.l.). A voucher specimen (no 4001) was deposited at the herbarium of Department of Horticultural science, University of Maragheh, Maragheh, Iran.

The aerial parts of *S. khuzistanica* and *S. rechingeri* were collected in September 2018 from a 3-years-old crop at the Garden of Khorraman CO, Khorramabad, Iran (N 33°44', E 48°26' E and 1170 m a.s.l). Both species were grown in the open field from the elite cuttings; the average density was 4 plants per m^2; plants were irrigated at 15-day intervals; no pesticide or fertilizer was used during the plant growth; weeds were controlled manually. The two species (*S. rechingeri* and *S. khuzestanica*) were identified at the Department of Botany of the Research Institute of Forests

and Rangelands (TARI), Tehran, Iran, and archived with the voucher numbers no 75587 (*S. rechingeri*) and no. 58416 (*S. khuzestanica*).

After collection, the plant aerial parts were dried in the shade at ambient temperature and stored in paper bags until distillation.

2.2 Distillation of EOs

After drying at room temperature, the plant material was roughly powdered (30 g) and extracted using a Clevenger device for 3 h following the British Pharmacopoeia (1993). The extracted EOs were dried over anhydrous Na_2SO_4 and stored in dark vials at 4°C. The EO yield was expressed as mean percentage [volume (mL)/amount (g) of dry material] of 3 independent extractions.

2.3 GC-FID analysis

The analytical method was consistent with that earlier detailed by Maggi et al. (2010). A gas chromatograph Agilent 4890D equipped with FID (flame ionization detector) was used. The stationary phase was a HP-5 (25 m × 0.32 mm, 0.17 μm; 5% phenylmethylpolysiloxane) purchased from Agilent. The oven was programmed at 60°C isotherm for 5 min, then ramp of 4°C/min up to 220°C, and ramp of 11°C/min up to 280°C. The injection (1:100 in *n*-hexane, 1 μL) was in split mode (1:34). The injector and detector temperature were 280°C. A C_8-C_{30} alkane mixture from Supelco (Bellefonte, CA) was analyzed under the same conditions to determine the temperature-programmed RI (retention index) (Van den Dool & Kratz, 1963). The relative peak area percentages were obtained by FID peak area normalization and represent mean of 3 replications.

2.4 GC-MS analysis

Qualitative data of the *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* EOs were obtained on an Agilent 6890N gas chromatograph coupled with a 5973N mass spectrometer (MS). The column used as stationary phase was a HP-5MS (5% phenylmethylpolysiloxane, 30 m × 0.25 mm, 0.1 μm) from Agilent. The temperature program was the same used for GC-FID analysis, as well as the injection conditions except for the split ratio (1:50) and the carrier gas (He, 99.99%). Injector and transfer line temperatures were 280 and 250°C, respectively. MS were acquired in the 29–400 *m/z* interval. The identification of the main components relied on the RT, RI and MS overlapping with respect to those of available commercial standards (Sigma-Aldrich, Milan, Italy). For the other peaks, we compared the MS and RI with those recorded in ADAMS (Adams, 2007), NIST 17 (NIST 17, 2017), FFNSC3 (FFNSC3, 2015) and WILEY 275 libraries.

2.5 Insect and earthworm rearing

The mass-rearing of *C. quinquefasciatus* larvae, *M. domestica* adults and *S. littoralis* larvae was carried out following Benelli et al. (2019c), maintaining them at 25±1°C, 70±3% R.H. and 16:8 h (L:D). The same laboratory conditions were applied also in the experiments described below.

Eisenia fetida earthworm adults with well-developed clitella were also reared in laboratory (> 20 generations; out-crossed once) in artificial soil following OECD (1984). Maximum water-holding soil capacity (35%) was monitored weekly; the room temperature was 20±1°C.

2.6 Insecticidal activity on *Culex quinquefasciatus*

The EOs of *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* were diluted in dimethyl sulfoxide (DMSO) and tested against *C. quinquefasciatus* 3rd instar larvae following WHO (1996) slightly modified by Ammar et al. (2020). The larvae were obtained from an established laboratory colony. The EOs were diluted in DMSO to prepare a serial dilution of test concentrations (10, 15, 20, 25, 30, 35 and 40 μg mL^{-1}). For the experimental treatment, 1 mL of serial dilutions was added to 224 mL of distilled water in a 500-mL glass bowl and shaken lightly to ensure a homogenous test solution.

The selected mosquito larvae were transferred in distilled water into a bowl of prepared test solution with final surface area of 125 cm^{-2} (25 larvae/beaker). Each concentration was replicated 4 times in groups of 25 larvae each. Distilled water with the same amount of DMSO used for dissolving the EO was the negative control. *α*-Cypermethrin (Vaztak®) was the positive control (tested concentrations: 0.008, 0.01, 0.02, 0.03 and 0.05 μg mL^{-1}). Larval mortality was recorded after 24 h, during which time no food was offered to the larvae. The boxes were placed for 24 h in a growth chamber (16:8 (L:D), 24±1°C). All larvae that did not respond to mechanical stimuli were considered dead.

2.7 Insecticidal activity on *Musca domestica*

Acute topical toxicity was examined with *M. domestica* adults, obtained from an established laboratory colony. The EOs of *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* EOs were tested on adult females and males (3–6 day-old) through topical application, as detailed by Benelli et al. (2019c); briefly, 1 μL of acetone (Sigma-Aldrich, Germany) plus the EO at 10, 20, 30, 50, 70, 80 and 100 μg $adult^{-1}$ (each concentration was tested on 4 groups of 20 males or females each), was applied using a microelectric applicator to the pronotum of *M. domestica* individuals anesthetized with CO_2. Acetone was the negative control. α-Cypermethrin (Vaztak®) at 0.05, 0.1, 0.2, 0.3, 0.5, 0.7 and 0.9 μg $adult^{-1}$ was the positive control. Then, *M. domestica* flies were moved to a recovery box (10 × 10 × 12 cm) for 24 h. Mortality was recorded in males and females. The boxes were placed for 24 h in a growth chamber (16:8 (L:D), 24±1°C). Flies that did not respond were considered dead.

2.8 Insecticidal activity on *Spodoptera littoralis*

The acute toxicity, measured as mortality after 24 h of exposure, was determined by topical application to *S. littoralis* early 3rd instar larvae (by weight 20–25 mg/larva), obtained from an established laboratory colony. EOs of *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* diluted in acetone were topically applied on larvae of *S. littoralis* (Pavela et al. 2017). Each larva was treated on the dorsum with 1 µL of acetone containing 5, 10, 15, 20, 30, 40, 50 or 60 µg larva^{-1} of the *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* EOs. 4 R (n=20 larvae per replicate) for each concentration were done. Acetone was the negative control and α-cypermethrin (Vaztak®) at 0.005, 0.01, 0.02, 0.03 and 0.04 µg larva^{-1} was the positive control. All treated larvae from each replicate were transferred to the relevant diet in plastic boxes (10 × 10 × 7 cm), which were closed using perforated caps to make sure that the experiment was not affected by the fumigation effect of the acetone, EO or α-cypermethrin. The boxes were placed for 24 h in a growth chamber (16:8 (L:D), 24±1°C). Death was recorded when the larvae did not respond to prodding with forceps (Ammar et al. 2020).

2.9 Toxicity on non-target earthworms, *Eisenia fetida*

The EOs of *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* EOs were tested on *E. fetida* adults (weight 350–500 mg) following OECD (1984). Soil composition and pH was the identical to the one of *E. fetida* rearing. The artificial soil was comprised of 70% quartz sand, 20% kaolin clay, 10% sphagnum peat, and $CaCO_3$ to regulate the pH to 6.0, water-holding soil capacity 35%. The five EOs were tested at 200 mg kg^{-1} of soil, while distilled water plus the same amount of Tween 80 (Sigma Aldrich, Czech Republic) used to solve the EO was the negative control. The positive control was α-cypermethrin (Vaztak®, Czech Republic) tested at 0.1 mg kg^{-1} of soil.

An aqueous formulation containing the selected EO (200 mg kg^{-1} of soil emulsified using 200 mg of Tween 80) or water (as a negative control, only Tween 80 in dose 200 mg kg^{-1} of soil) or α-cypermethrin (positive control) at dose of 0.1 mg kg^{-1} of soil was mixed into the soil (650 g) and 10 *E. fetida* adults were added; 4 replicates per tested concentration were done. Treated and control soils were stored in glass pots (1 L) covered with gauze to ensure aeration. Earthworm mortality was checked after 7 and 14 days of exposure. Death was recorded when the *E. fetida* earthworms did not respond to prodding with forceps. All trials were carried out in an air-conditioned room at 20 ± 1°C, 50 ± 5% R.H. and 16:8 h (L:D).

2.10 Statistical analysis

If control mortality < 20%, the observed insect mortality was corrected with the Abbott's formula (Abbott, 1925). Therefore, $LD_{50(90)}$ and $LC_{50(90)}$ values and their 95% confidence limits (CI_{95}) were estimated through probit analysis (Finney 1971). *E. fetida* mortality data were transformed by arcsine√ and analysed using one-way ANOVA with EO as a factor followed by Tukey's HSD test (p<0.05).

3 Results and discussion

3.1 EOs composition

The hydro-distillation of the flowering aerial parts of *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* yielded 3.3, 1.2, 2.4, 2.2. and 3.7% of yellowish EOs, respectively. These values, which are consistent with those previously reported in literature (Sefidkon et al., 2004, 2009; Taban et al., 2017; Bistgani et al., 2018; Esmaeili et al., 2018; Eftekhari et al., 2019; Karami et al., 2020), make these Iranian species worthy of industrial exploitation, for instance as a source of botanical insecticide ingredients.

A total of 48 volatile components were identified in the EOs from the five Iranian MAPs, accounting to 99.7–99.9% of the total compositions (Table 1). The ones owning the highest number of identified components were those from *S. khuzistanica* and *S. rechingeri* (44 and 37 compounds, respectively), followed by *T. daenensis* (26), *S. sahendica* (20) and *O. decumbens* (12).

From a phytochemical perspective, the EOs of *O. decumbens, T. daenensis, S. khuzistanica* and *S. rechingeri* were characterized by the class of oxygenated monoterpenes, accounting for 70.3, 91.6, 89.7 and 89.4%, respectively, with the monoterpene hydrocarbons giving a lower contribution (25.9, 7.3, 8.4 and 9.2%, respectively). On the other hand, the EO of *S. sahendica* displayed a different profile, with the group of monoterpene hydrocarbons (63.3%) being more abundant than that of oxygenated monoterpenes (35.3%) (Table 1).

In the EO from *O. decumbens* the major constituents were thymol (36.7%) and carvacrol (33.6%), followed by γ-terpinene (13.6%), *p*-cymene (9.4%) and myristicin (3.4%). The oils from *T. daenensis, S. khuzistanica* and *S. rechingeri* were quali-quantitatively similar, being dominated by carvacrol (89.6, 86.6 and 86.5%, respectively), with little amounts of *p*-cymene (3.0, 2.1 and 2.2%, respectively) and γ-terpinene (1.5, 2.9 and 3.0%, respectively). In the EO of *S. sahendica* the most abundant components were γ-terpinene (41.3%), followed by thymol (33.8%), *p*-cymene (13.7%) and α-terpinene (4.1%).

The chemical profiles detected in the EOs of *O. decumbens* and the three *Satureja* species were consistent with those reported in previous studies (Sefidkon et al., 2009; Taban et al., 2017; Esmaeili et al., 2018; Eftekhari et al., 2019; Karami et al., 2020). On the other hand, the composition of *T. daenensis,* as determined in our study, showed some differences with the ones previously described, for instance by Nickavar et al. (2005), Pirbalouti et al. (2013) and Alizadeh et al. (2013). These authors found a different

Table 1. Chemical composition of the essential oils from *Oliveria decumbens, Thymus daenensis, Satureja sahendica, S. khuzistanica* and *S. rechingeri*.

No	Component a	RI b	RI Lit. c	*O. decumbens* (%)	*T. daenensis* (%) d	*S. sahendica* (%)	*S. khuzistanica* (%)	*S. rechingeri* (%)	ID e
1	α-thujene	921	924	0.1±0.0	0.6±0.2	1.2±0.3	0.6±0.2	0.8±0.2	RI,MS
2	α-pinene	926	932	0.1±0.0	0.3±0.0	0.5±0.1	0.3±0.0	0.4±0.1	Std
3	camphene	940	946		Tr[f]	Tr	0.1±0.0	0.1±0.0	Std
4	β-pinene	969	974	1.1±0.2	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	Std
5	1-octen-3-ol	976	974				Tr		Std
6	myrcene	989	988	0.1±0.0	1.0±0.2	1.5±0.2	1.0±0.2	1.1±0.2	Std
7	α-phellandrene	1003	1002		0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.0	Std
8	δ-3-carene	1008	1008		Tr	Tr	0.1±0.0	0.1±0.0	Std
9	α-terpinene	1014	1015	0.1±0.0	0.3±0.0	4.1±0.6	0.7±0.1	0.8±0.1	Std
10	p-cymene	1022	1020	9.4±0.8	3.0±0.5	13.7±1.0	2.1±0.4	2.2±0.4	Std
11+12	β-phellandrene + limonene	1025	1024,1025	1.4±0.3	0.2±0.0	0.4±0.1	0.2±0.0	0.3±0.0	Std
13	1,8-cineole	1028	1026				Tr	Tr	Std
14	(*Z*)-β-ocimene	1037	1032			0.1±0.0			Std
15	(*E*)-β-ocimene	1047	1044			0.1±0.0	0.1±0.0	0.1±0.0	Std
16	γ-terpinene	1055	1054	13.6±1.8	1.5±0.3	41.3±3.2	2.9±0.6	3.0±0.5	Std
17	*cis*-sabinene hydrate	1064	1065		0.3±0.1	Tr	0.3±0.1	0.4±0.1	RI,MS
18	terpinolene	1085	1086		Tr		0.1±0.0	0.1±0.0	Std
19	*trans*-sabinene hydrate	1096	1098		Tr		0.1±0.0	0.1±0.0	RI,MS
20	linalool	1100	1095		0.6±0.2		0.5±0.1	0.5±0.1	Std
21	*cis-p*-menth-2-en-1-ol	1118	1118				0.1±0.0	0.1±0.0	RI,MS
22	*trans-p*-menth-2-en-1-ol	1137	1136				Tr	Tr	RI,MS
23	borneol	1161	1165		Tr		0.1±0.0	0.1±0.0	Std
24	terpinen-4-ol	1173	1174		0.3±0.1	0.1±0.0	0.4±0.1	0.5±0.1	Std
25	α-terpineol	1188	1186		0.3±0.0		0.4±0.0	0.1±0.0	Std
26	*cis*-dihydro carvone	1194	1191				0.1±0.0	0.1±0.0	RI,MS
27	*trans*-dihydro carvone	1202	1200				Tr	Tr	RI,MS
28	*trans*-piperitol	1207	1207				Tr	Tr	RI,MS
29	carvacrol, methyl ether	1243	1241		0.2±0.0		0.1±0.0	0.2±0.0	RI,MS
30	carvone	1244	1239				0.1±0.0		Std
31	geranial	1275	1264				Tr		Std
32	(*E*)-anethole	1282	1282		0.3±0.1				Std
33	thymol	1295	1289	36.7±2.9	0.2±0.0	33.8±2.2	0.3±0.1	0.3±0.0	Std
34	carvacrol	1302	1298	33.6±2.1	89.6±1.4	1.3±0.3	86.6±2.0	86.5±1.9	Std
35	α-terpinyl acetate	1346	1346				Tr		RI,MS
36	thymol acetate	1353	1349			0.1±0.0			RI,MS
37	eugenol	1355	1356				Tr	Tr	RI,MS

Table 1. continued.

No	Component a	RI b	RI Lit. c	*O. decumbens* (%)	*T. daenensis* (%) d	*S. sahendica* (%)	*S. khuzistanica* (%)	*S. rechingeri* (%)	ID e
38	carvacrol acetate	1371	1370		0.1±0.0		0.4±0.1	0.4±0.1	RI,MS
39	(*E*)-caryophyllene	1407	1417		0.1±0.0	1.3±0.3	0.2±0.0	0.4±0.0	Std
40	α-*trans*-bergamotene	1427	1432				Tr		RI,MS
41	α-humulene	1439	1452				Tr		Std
42	α-zingiberene	1489	1493				Tr		RI,MS
43	β-bisabolene	1498	1505		0.6±0.2		0.6±0.2	0.5±0.1	RI,MS
44	(*E*,*E*)-alpha-farnesene	1501	1505				0.4±0.1	Tr	Std
45	myristicin	1511	1517	3.4±0.4					Std
46	(*E*)-α-bisabolene	1534	1532				0.1±0.0	0.1±0.0	RI,MS
47	caryophyllene oxide	1575	1583				0.1±0.0	0.1±0.0	Std
48	*epi*-α-bisabolol	1673	1683				Tr	Tr	Std
	Total identified (%)			99.7±0.2	99.9±0.0	99.9±0.0	99.7±0.1	99.8±0.0	
	Oil yield (%, w/w)			3.3	1.2	2.4	2.2	3.7	
	Grouped compounds (%)								
	Monoterpene hydrocarbons			25.9±1.5	7.3±0.9	63.3±2.0	8.4±0.7	9.2	
	Oxygenated monoterpenes			70.3±2.5	91.6±1.8	35.3±1.7	89.7±1.4	89.4±1.1	
	Sesquiterpene hydrocarbons				0.7±0.1	1.3±0.4	1.4±0.3	1.0±0.1	
	Oxygenated sesquiterpenes						0.1±0.0	0.1±0.0	
	Others			3.4±0.9	0.3±0.0		0.1±0.0	Tr	

[a] The order of elution is according a HP-5MS capillary column (30 m × 0.25 mm, 0.1 mm). [b] Linear retention indices calculated according the Van den Dool & Kratz (1963) formula using a mixture of C_8-C_{30} alkanes. [c] Retention index value taken from Adams (2007). [d] Relative peak area percentage (mean of three determinations) ± standard deviation. [e] Identification method used: RI, retention index value overlapping with those of Adams, FFNSC3 and NIST 17; MS, mass spectrum fragmentation similarity compared with WILEY 275, ADAMS, NIST 17 and FFNSC3 libraries; Std, co-injection with analytical standards purchased from Sigma-Aldrich (Milan, Italy).

chemical profile with thymol being the predominant compound over carvacrol. Rowshan and colleagues (2013) analyzed the EO of *T. daenensis* as processed in different storage conditions finding comparable percentages of thymol and carvacrol. Instead, Bahreininejad et al. (2010) analysed 11 populations of *T. daenensis* subsp. *daenensis* finding significant chemical variability at variance with geographic origin and altitude. They found out that one out of eleven EOs showed carvacrol as the predominant volatile component (80.1%), being this profile overlapping with the one detected by us. Thus, we hypothesize that genetic variability (e.g. different subspecies, inter-population variability etc.) along with changes in the pedoclimate and altitude may affect significantly the EO composition of *T. daenensis* giving different chemotypes.

Overall, the volatile fraction of the five Iranian species was characterized by the phenolic monoterpenes thymol and carvacrol together with their precursors γ-terpinene and *p*-cymene. Indeed, the synthesis of the two phenolics starts from γ-terpinene that undergoes aromatization to give *p*-cymene; the latter is subjected to hydroxylation to give thymol and/or carvacrol (Alizadeh et al., 2013).

3.2 Insecticidal activity and effects on non-target *Eisenia fetida*

Earlier reports on the insecticidal potential of the five EOs extracted from the Iranian plants studied in this research are extremely limited. Taban et al. (2017) showed that *S. khuzestanica* and *S. rechingeri* growing wild in Iran can be sources of EOs toxic to the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). The authors reported that *S. khuzestanica* EO was the most efficient both in contact and fumigation assays (LD_{50}=20.11 µg adult^{-1}; LD_{50}=2.51 mg L^{-1} of air, respectively). The same EO at 1% (v/v) also had high repellent activity (98% to 100%) after 4 h of exposure (Taban et al. 2017). Eftekhari et al. (2019) assayed the EO of *O. decumbens* against larvae of the cabbage looper *Trichoplusia ni* (Hübner) by topical application finding a LD_{50} of 52 µg larva^{-1}, and showing myristicin as the most active constituent.

In the present study, the larvicidal activity of *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* EOs on 3rd instar larvae of *C. quinquefasciatus* is provided in Table 2. The highest toxicity was achieved by *S. sahendica* EO, with a LC_{50} of 16.2 µg mL^{-1}; however, the 95% confidence intervals for this LC_{50} value overlapped with those of *T. daenensis* and *S. rechingeri* EOs, therefore the mosquitocidal activity of these three EOs was comparable. The lowest mosquito larvicidal toxicity among the tested EOs was observed for the *S. khuzistanica* EO, which achieved a LC_{50} of 29.3 µg mL^{-1} (Table 2). Of note, all the tested EOs showed LC_{50} values lower than 50 µg mL^{-1}, which has been earlier proposed by Pavela (2015a) as a threshold to identify EOs with promising mosquito larvicidal potential. Notably, the LC_{50} values of the Iranian *Satureja* EOs tested here are comparable with the results achieved testing *S. montana* L. from Italy against *C. quinquefasciatus* larvae (LC_{50}=25.6 µg mL^{-1}) (Benelli et al., 2017b). This very good efficacy was probably achieved thanks to a high ratio of phenols – thymol and carvacrol – in the EOs. Given that these substances were present in all tested EOs, the efficacy levels were similar. The higher efficacy determined for the EO from *S. sahendica* could probably be attributed to a higher content of *p*-cymene and γ-terpinene, since both compounds have been reported earlier as major constituents of EOs highly toxic to mosquito larvae (Pavela, 2015b). For instance, they contribute to the high toxicity observed for ajwain (*Trachyspermum ammi* (L.) Sprague) EO on *C. quinquefasciatus* larvae (Benelli et al., 2017), and γ-terpinene is also toxic to *Culex pipiens* L. larvae (Evergetis et al., 2009), with LC_{50} values always lower than 30 mg L^{-1}. Further research assessing the potential synergistic effects of γ-terpinene and *p*-cymene binary combinations on insect pests and vectors considered in the present study is still needed.

The insecticidal efficacy of the EOs extracted from five Iranian plants on *M. domestica* adult females and males is summarized in Table 3. LD_{50} values ranged from 18.1 µg adult^{-1} (*T. daenensis*) to 35.8 µg adult^{-1} (*S. khuzistanica*) for housefly males and from 30.5 µg adult^{-1} (*S. khuzistanica*) to 48.1 µg adult^{-1} (*T. daenensis*) for housefly females. LD_{50} values tended to be lower on males over females. However, within each sex, 95% confidence intervals mostly overlapped, therefore no major significant differences can be drawn (Table 3). Houseflies males are known to be more susceptible to EOs if compared with females (Pavela et al., 2020b, Pavela, 2011). The lethal doses determined by us (LD_{90}) correspond approximately to 5–10% application concentration, which is acceptable also in terms of occupational safety where EO formulations up to 10% can be considered as relatively safe (Tisserand & Young, 2014). However, even sub-lethal EO concentrations are known to be able to significantly reduce insect longevity and fertility, which applies also to all the three tested target species (Hummelbrunner & Isman, 2001; Desneux et al., 2007; Pavela, 2007; Pavela et al., 2020b). Thus, even much lower concentrations or doses can result in a significant reduction of the insect populations and their damages to public health and agriculture.

Table 4 shows the insecticidal activity of the *O. decumbens, T. daenensis, S. sahendica, S. khuzistanica* and *S. rechingeri* EOs on 3rd instar larvae of *S. littoralis*. The EOs from *S. khuzistanica, S. rechingeri*, and *T. daenensis* were the most toxic (LD_{50}=8.9, 9.4 and 9.6 µg larva^{-1}, respectively), with overlapping 95% confidence intervals. The least toxic EO was that obtained from *S. sahendica* (LD_{50}=23.1 µg larva^{-1}) (Table 4); this EO was the only one to contain significantly

Table 2. Insecticidal activity of the essential oils extracted from the five Iranian species against 3rd instar larvae of *Culex quinquefasciatus*.

Essential oil	LC_{50} (µg mL^{-1})	CI_{95}	LC_{90} (µg mL^{-1})	CI_{95}	χ^2(*df*)	*p*-value
O. decumbens	26.8±0.8	25.3–28.6	43.6±3.1	39.1–51.3	4.049 (4)	0.395 ns
T. daenensis	23.1±1.1	20.7–24.9	36.1±1.9	33.1–40.1	0.758 (3)	0.684 ns
S. sahendica	16.2±1.7	12.8–21.6	35.5±2.8	33.8–42.7	3.523 (3)	0.152 ns
S. khuzistanica	29.3±0.7	27.8–30.6	39.3±1.2	37.2–42.2	0.037 (3)	0.998 ns
S. rechingeri	22.6±0.5	21.5–23.7	29.4±1.4	27.4–32.1	0.642 (3)	0.725 ns
α-cypermethrin	0.02±0.00	0.01–0.03	0.05±0.01	0.04–0.06	1.231 (3)	0.578 ns

ns=not significant (p>0.05)

Table 3. Insecticidal activity of the essential oils from the five Iranian species against adults of *Musca domestica*.

Essential oil	*Musca domestica* females						*Musca domestica* males					
	LD_{50} (µg adult^{-1})	CI_{95}	LD_{90} (µg adult^{-1})	CI_{95}	χ^2	*p*-value	LD_{50} (µg adult^{-1})	CI_{95}	LD_{90} (µg adult^{-1})	CI_{95}	χ^2 *(df)*	*p*-value
O. decumbens	37.3±0.5	29.3–42.8	107.4±5.9	103.2–126.7	6.818 (4)	0.146 ns	27.8±2.4	22.9–35.8	62.5±7.2	55.3–78.1	6.489 (3)	0.392 ns
T. daenensis	48.1±3.3	32.9–56.8	95.4±2.1	89.2–102.7	2.602 (3)	0.271 ns	18.1±2.8	12.3–29.8	61.6±5.2	58.3–72.9	2.582 (3)	0.235 ns
S. sahendica	36.5±0.9	29.8–42.1	84.1±5.2	78.9–98.2	3.529 (3)	0.293 ns	33.5±2.6	28.5–38.1	75.7±5.6	63.9–86.5	0.921 (3)	0.631 ns
S. khuzistanica	30.5±2.3	26.2–32.1	73.3±1.3	65.9–77.8	3.609 (3)	0.306 ns	35.8±1.5	32.1–39.5	67.5±5.5	59.9–78.3	2.639 (3)	0.451 ns
S. rechingeri	35.3±2.1	30.7–42.8	60.8±5.5	55.9–82.3	1.535 (3)	0.465 ns	32.7±1.2	29.8–35.3	54.9±0.8	49.2–62.1	0.146 (3)	0.985 ns
α-cypermethrin	0.18±0.2	0.17–0.21	0.91±0.1	0.82–1.15	1.538 (3)	0.597 ns	0.10±0.1	0.06–0.12	0.61±0.2	0.51–0.75	1.923	0.843 ns

ns=not significant ($p>0.05$)

Table 4. Insecticidal activity of the essential oils from the five Iranian species against *Spodoptera littoralis* larvae (3rd instar).

Essential oil	LD_{50} (µg larva^{-1})	CI_{95}	LD_{90} (µg larva^{-1})	CI_{95}	χ^2 *(df)*	*p*-value
O. decumbens	7.4±1.1	6.1–9.3	21.8±1.5	18.7–29.6	0.468 (3)	0.925 ns
T. daenensis	9.6±1.2	7.6–12.8	28.9±7.4	20.1–38.6	0.222 (3)	0.894 ns
S. sahendica	23.1±2.5	18.1–28.3	60.1±3.9	48.6–91.5	1.446 (3)	0.485 ns
S. khuzistanica	8.9±1.1	7.1–11.2	33.93±3.2	30.4–47.1	2.569 (4)	0.632 ns
S. rechingeri	9.4±1.2	6.5–12.3	31.1±3.6	22.2–42.8	0.709 (3)	0.701 ns
α-cypermethrin	0.01±0.005	0.01–0.02	0.05±0.01	0.03–0.08	1.562 (3)	0.628 ns

ns=not significant ($p>0.05$)

lower amounts of phenols (thymol and carvacrol) (35.1%) if compared with the other EOs, where these phenols were contained in percentages ranging from 70.3 to 89.8%. Our results indicate that the efficacy against *S. littoralis* larvae correlates with thymol and carvacrol contents in the EOs, as stressed in our previous research (Pavela et al., 2019a).

Thymol and carvacrol are bioactive components occurring mainly in aromatic plants belonging to the Lamiaceae and, to a minor extent, Apiaceae families (Benelli et al., 2017a, b; Caprioli et al., 2019; Karami et al., 2019; Rizzo et al., 2020). They were proven as effective insecticidal, antileishmanial, anthelmintic, acaricidal and antimicrobial agents (Tabari et al., 2017; Benelli et al., 2019d; López et al., 2019; Youssefi et al., 2019; Gagliano Candela et al., 2019; Merino et al., 2019). In particular, they exhibited a notable toxicity against several mosquito species (Pavela, 2015; Maggi & Benelli, 2018), with LC_{50} values in the range of 15.1–48.9 µg mL^{-1} (Table 5).

This activity can be modulated by other components such as *p*-cymene and γ-terpinene, for instance in the EOs from *O. decumbens* and *S. sahendica*, and by myristicin in the one from *O. decumbens* (Table 5). Thymol and carvacrol can inhibit the cholinergic system as well as interacting with the octopamine and GABA receptors leading to insecticidal efficacy (Enan, 2001; Jankowska et al., 2017; López et al., 2018). From an ecotoxicological perspective, thymol and carvacrol are considered safe for beneficial organisms such as honeybees (Giacomelli et al., 2016) as well as for human consumption being listed among the US GRAS substances (Varel, 2002).

When tested on *E. fetida* earthworms, four out of five tested EOs showed no toxicity at 200 mg kg^{-1} of soil, while mortality achieved testing the fifth EO, i.e. *T. daenensis* (200 mg kg^{-1} of soil), was limited, i.e. < 8% after 14 days of exposure (Table 6). On the other hand, the positive control α-cypermethrin tested on *E. fetida* tested at a concentration 2000-folds lower (0.1 mg kg^{-1} of soil) led to 85% earthworm mortality after 7 days, and 100% mortality after 14 days of exposure (Table 6).

As reported earlier, EOs can be very friendly to some non-target organisms including aquatic crustaceans or aphid biocontrol agents (Benelli et al., 2018, 2019a, c). This makes them suitable candidates for the development of novel, environmentally safe botanical insecticides. Accordingly, the tested EOs not only showed very good insecticidal activity, but they were also friendly to representatives of non-target soil organisms, such as earthworms. Nevertheless, we are aware that further experiments will be needed to confirm environmental safety of the tested EOs. Additionally, the relatively high yields of the EOs (around 3%), which can be probably increased even further using appropriate elicitation products (Pavela et al., 2018b), indicate good prospects for using the tested aromatic plant species as a biomass suitable for obtaining active substances for botanical insecticides.

Table 5. Insecticidal activity, expressed as LC_{50}/LD_{50} values, of the main volatile components of the five Iranian species on insect vectors and pests.

Compound	Target species	Stage	LC_{50}/LD_{50}	References
Thymol	*Anopheles stephensi*	Larva	48.9 ppm	Pandey et al., 2009
	Aedes aegypti	Larva	17.5 ppm	Tabanca et al., 2013
	Culex pipiens biotype *molestus*	Larva	36.0 ppm	Traboulsi et al., 2002
	Culex quinquefasciatus	Larva	15.1 ppm	Pavela et al., 2018
	Musca domestica[a]	Adult	30.0 ppm	Pavela, 2011
	Spodoptera littoralis[a]	Larva	42.1 µg larva^{-1}	Pavela, 2010
	Spodoptera litura[a]	Larva	25.4 µg larva^{-1}	Hummelbrunner & Isman, 2001
	Trichoplusia ni[a]	Larva	50.1 µg larva^{-1}	Eftekhari et al., 2019
Carvacrol	*Culex pipiens* biotype *molestus*	Larva	37.6 ppm	Traboulsi et al., 2002
	Anopheles stephensi	Larva	21.2 ppm	Govindarajan et al., 2016
	Anopheles subpictus	Larva	24.1 ppm	
	Culex quinquefasciatus	Larva	26.1 ppm	
	Culex tritaeniorhynchus	Larva	28.0 ppm	
	Musca domestica[a]	Adult	36.0 ppm	Pavela, 2011
	Spodoptera littoralis[a]	Larva	38.3 µg larva^{-1}	Benelli et al., 2019b
	Spodoptera litura[a]	Larva	42.7 µg larva^{-1}	Hummelbrunner & Isman, 2001
	Trichoplusia ni[a]	Larva	68.8 µg larva^{-1}	Eftekhari et al., 2019
p-Cymene	*Aedes aegypti*	Larva	19.2 ppm	Cheng et al., 2009
	Aedes albopictus	Larva	46.7 ppm	
	Culex quinquefasciatus	Larva	20.6 ppm	Pavela et al., 2017
	Musca domestica[a]	Adult	282.1 µg larva^{-1}	Pavela, 2008
	Spodoptera littoralis[a]	Larva	108.8 µg larva^{-1}	Pavela, 2010
	Trichoplusia ni[a]	Larva	202.8 µg larva^{-1}	Eftekhari et al., 2019
γ-Terpinene	*Aedes aegypti*	Larva	30.7 ppm	Cheng et al., 2009
	Aedes albopictus	Larva	29.8 ppm	
	Culex quinquefasciatus	Larva	16.7 ppm	Pavela et al., 2017
	Musca domestica[a]	Adult	248.3 µg larva^{-1}	Pavela, 2008
Myristicin	*Culex quinquefasciatus*	Larva	16.3 ppm	Pavela et al., 2017
	Trichoplusia ni[a]	Larva	61.7 µg larva^{-1}	Afshar et al., 2017

[a] Data obtained by topical application

Moreover, given the changing climate, farmers welcome any new crops not demanding in terms of water supply and growing conditions. All these facts indicate highly promising prospects of using the tested plants as new crops cultivable even in arid regions or in regions where the climate has deteriorated due to global warming.

Lastly, estimating a realistic dose of EOs and their main constituents (especially thymol and carvacrol) that may be used in an insecticide formulation for crop protection or vector control programs is a crucial challenge for future research (Pavela & Benelli, 2016; Isman, 2020). However, a realistic dose to be used in the field can be estimated only after proper formulation enhancing activity and stability (e.g. micro- and nanoemulsions, see Pavela et al. 2019c,d; Pavoni et al. 2019); a dedicated study on this issue is still needed.

Conflict of Interest: The Authors declare no competing interests.

Table 6. Lack of toxicity of the essential oils from the five selected Iranian plants on non-target earthworms, *Eisenia fetida*.

Essential oil (200 mg kg^{-1})	Mortality (%)* ± SE	
	7th day	14th day
Thymus daenensis	5.0±5.0^{a}	7.5±4.3^{b}
Satureja sahendica	0.0±0.0^{a}	0.0±0.0^{a}
Satureja khuzestanica	0.0±0.0^{a}	0.0±0.0^{a}
Satureja rechingeri	0.0±0.0^{a}	0.0±0.0^{a}
Oliveria decumbens	0.0±0.0^{a}	0.0±0.0^{a}
Negative control**	0.0±0.0^{a}	0.0±0.0^{a}
Positive control (0.1 mg kg^{-1}) α-cypermethrin	85.0±5.0^{b}	100.0±0.0^{c}
ANOVA $F_{6,21}$, p-value	426.5, <0.001	1 569.0, <0.001

* Average mortality of *E. fetida* (±SE) achieved on the 7th and 14th day after application of EOs; means±SD followed by the same letter do not differ significantly (Tukey's HSD test).
% = arcsine square root transformed data.
** Negative control = distilled water + Tween 80 (200 mg kg^{-1}).

Acknowledgements: R. Pavela would like to thank the Ministry of Agriculture of the Czech Republic for its financial support concerning botanical pesticide and basic substances research (Project QK1910103).

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*(2), 265–267. https://doi.org/10.1093/jee/18.2.265a

Adams, R. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry* (4th ed.). Carol Stream, IL, USA: Allured Publishing Corp.

Adenubi, O. T., Ahmed, A. S., Fasina, F. O., McGaw, L. J., Eloff, J. N., & Naidoo, V. (2018a). Pesticidal plants as a possible alternative to synthetic acaricides in tick control: A systematic review and meta-analysis. *Industrial Crops and Products, 123*, 779–806. https://doi.org/10.1016/j.indcrop.2018.06.075

Adenubi, O. T., McGaw, L. J., Eloff, J. N., & Naidoo, V. (2018b). In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties: A critical review. *Veterinary Parasitology, 254*, 160–171. https://doi.org/10.1016/j.vetpar.2018.03.008

Afshar, F. H., Maggi, F., Iannarelli, R., Cianfaglione, K., & Isman, M. B. (2017). Comparative toxicity of *Helosciadium nodiflorum* essential oils and combinations of their main constituents against the cabbage looper, *Trichoplusia ni* (Lepidoptera). *Industrial Crops and Products, 98*, 46–52. https://doi.org/10.1016/j.indcrop.2017.01.004

Alizadeh, A., Alizadeh, O., Amari, G., & Zare, M. (2013). Essential oil composition, total phenolic content, antioxidant activity and antifungal properties of Iranian *Thymus daenensis* subsp. *daenensis* Celak. as in influenced by ontogenetical variation. *Journal of Essential Oil-Bearing Plants, 16*(1), 59–70. https://doi.org/10.1080/0972060X.2013.764190

Amirghofran, Z., Ahmadi, H., & Karimi, M. H. (2012). Immunomodulatory activity of the water extracts of *Thymus vulgaris, Thymus daenensis*, and *Zataria multiflora* on dendritic cells and T cells responses. *Journal of Immunoassay & Immunochemistry, 33*(4), 388–402. https://doi.org/10.1080/15321819.2012.655822

Ammar, S., Noui, H., Djamel, S., Madani, S., Maggi, F., Bruno, M., … Benelli, G. (2020). Essential oils from three Algerian medicinal plants (*Artemisia campestris, Pulicaria arabica* and *Saccocalyx satureioides*) as new botanical insecticides? *Environmental Science and Pollution Research International, 27*(21), 26594–26604. https://doi.org/10.1007/s11356-020-09064-w

Aponte, A., Penilla, R. P., Rodríguez, A. D., & Ocampo, C. B. (2019). Mechanisms of pyrethroid resistance in *Aedes (Stegomyia) aegypti* from Colombia. *Acta Tropica, 191*, 146–154. https://doi.org/10.1016/j.actatropica.2018.12.021

Bahreininejad, B., Mirza, M., & Arzani, A. (2010). Essential oil variation in *Thymus daenensis* subsp. *daenensis* Cleak populations. *The Journal of Essential Oil Research, 22*(1), 48–51. https://doi.org/10.1080/10412905.2010.9700263

Benelli, G., & Pavela, R. (2018a). Repellence of essential oils and selected compounds against ticks – A systematic review. *Acta Tropica, 179*, 47–54. https://doi.org/10.1016/j.actatropica.2017.12.025

Benelli, G., & Pavela, R. (2018b). Beyond mosquitoes – Essential oil toxicity and repellency against bloodsucking insects. *Industrial Crops and Products, 117*, 382–392. https://doi.org/10.1016/j.indcrop.2018.02.072

Benelli, G., Pavela, R., Iannarelli, R., Petrelli, R., Cappellacci, L., Cianfaglione, K., … Maggi, F. (2017a). Synergized mixtures of Apiaceae essential oils and related plant-borne compounds: Larvicidal effectiveness on the filariasis vector *Culex quinquefasciatus* Say. *Industrial Crops and Products, 96*, 186–195. https://doi.org/10.1016/j.indcrop.2016.11.059

Benelli, G., Pavela, R., Canale, A., Cianfaglione, K., Ciaschetti, G., Conti, F., … Maggi, F. (2017b). Acute larvicidal toxicity of five essential oils (*Pinus nigra, Hyssopus officinalis, Satureja montana, Aloysia citrodora* and *Pelargonium graveolens*) against the filariasis vector *Culex quinquefasciatus*: Synergistic and

antagonistic effects. *Parasitology International, 66*(2), 166–171. https://doi.org/10.1016/j.parint.2017.01.012

Benelli, G., Pavela, R., Petrelli, R., Cappellacci, L., Canale, A., Senthil-Nathan, S., & Maggi, F. (2018). Not just popular spices! Essential oils from *Cuminum cyminum* and *Pimpinella anisum* are toxic to insect pests and vectors without affecting non-target invertebrates. *Industrial Crops and Products, 124*, 236–243. https://doi.org/10.1016/j.indcrop.2018.07.048

Benelli, G., Pavela, R., Maggi, F., Wandjou, J. G. N., Koné-Bamba, D., Sagratini, G., … Caprioli, G. (2019a). Insecticidal activity of the essential oil and polar extracts from *Ocimum gratissimum* grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. *Industrial Crops and Products, 132*, 377–385. https://doi.org/10.1016/j.indcrop.2019.02.047

Benelli, G., Maggi, F., Canale, A., & Mehlhorn, H. (2019b). Lyme disease is on the rise – How about tick repellents? A global view. *Entomologia Generalis, 39*(2), 61–72. https://doi.org/10.1127/entomologia/2019/0787

Benelli, G., Pavela, R., Zorzetto, C., Sánchez-Mateo, C. C., Santini, G., Canale, A., & Maggi, F. (2019c). Insecticidal activity of the essential oil from *Schizogyne sericea* (Asteraceae) on four insect pests and two non-target species. *Entomologia Generalis, 39*(1), 9–18. https://doi.org/10.1127/entomologia/2019/0662

Benelli, G., Pavela, R., Petrelli, R., Cappellacci, L., Bartolucci, F., Canale, A., & Maggi, F. (2019d). *Origanum syriacum* subsp. *syriacum*: From an ingredient of Lebanese 'manoushe' to a source of effective and eco-friendly botanical insecticides. *Industrial Crops and Products, 134*, 26–32. https://doi.org/10.1016/j.indcrop.2019.03.055

Bistgani, Z. E., Siadat, S. A., Bakhshandeh, A., Pirbalouti, A. G., Hashemi, M., Maggi, F., & Morshedloo, M. R. (2018). Application of combined fertilizers improves biomass, essential oil yield, aroma profile, and antioxidant properties of *Thymus daenensis* Celak. *Industrial Crops and Products, 121*, 434–440. https://doi.org/10.1016/j.indcrop.2018.05.048

British Pharmacopoeia Commission. (1998). *British Pharmacopoeia* (Vol. 4, pp. 137–138). London: HMSO.

Caprioli, G., Lupidi, G., & Maggi, F. (2019). Comparison of chemical composition and antioxidant activities of two Winter savory subspecies (*Satureja montana* subsp. *variegata* and *Satureja montana* subsp. *montana*) cultivated in Northern Italy. *Natural Product Research, 33*(21), 3143–3147. https://doi.org/10.1080/14786419.2018.1516661

Chaieb, I., Zarrad, K., Sellam, R., Tayeb, W., Hammouda, A. B., Laarif, A., & Bouhachem, S. (2018). Chemical composition and aphicidal potential of *Citrus aurantium* peel essential oils. *Entomologia Generalis, 37*(1), 63–75. https://doi.org/10.1127/entomologia/2017/0317

Cheng, S. S., Huang, C. G., Chen, Y. J., Yu, J. J., Chen, W. J., & Chang, S. T. (2009). Chemical compositions and larvicidal activities of leaf essential oils from two *Eucalyptus* species. *Bioresource Technology, 100*(1), 452–456. https://doi.org/10.1016/j.biortech.2008.02.038

Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. *Annual Review of Entomology, 52*(1), 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*(1), 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Eftekhari, M., Ardekani, M. R. S., Amin, M., Attar, F., Akbarzadeh, T., Safavi, M., … Khanavi, M. (2019). *Oliveria decumbens*, a bioactive essential oil: Chemical composition and biological activities. *Iranian Journal of Pharmaceutical Research, 18*, 412.

Emami Bistgani, Z. E., Hashemi, M., DaCosta, M., Craker, L., Maggi, F., & Morshedloo, M. R. (2019). Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis* Celak. *Industrial Crops and Products, 135*, 311–320. https://doi.org/10.1016/j.indcrop.2019.04.055

Enan, E. (2001). Insecticidal activity of essential oils: Octopaminergic sites of action. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP, 130*(3), 325–337. https://doi.org/10.1016/S1532-0456(01)00255-1

Esmaeili, H., Karami, A., & Maggi, F. (2018). Essential oil composition, total phenolic and flavonoids contents, and antioxidant activity of *Oliveria decumbens* Vent. (Apiaceae) at different phenological stages. *Journal of Cleaner Production, 198*, 91–95. https://doi.org/10.1016/j.jclepro.2018.07.029

Evergetis, E., Michaelakis, A., Kioulos, E., Koliopoulos, G., & Haroutounian, S. A. (2009). Chemical composition and larvicidal activity of essential oils from six Apiaceae family taxa against the West Nile virus vector *Culex pipiens*. *Parasitology Research, 105*(1), 117–124. https://doi.org/10.1007/s00436-009-1370-8

FFNSC 3(2015). Mass Spectra of Flavors and Fragrances of Natural and Synthetic Compounds, 3rd Edition. John Wiley & Sons, Inc., Hoboken, NJ.

Finney, D. J. (1971). *Probit Analysis* (pp. 68–78). London: Cambridge University.

Gagliano Candela, R., Maggi, F., Lazzara, G., Rosselli, S., & Bruno, M. (2019). The Essential Oil of *Thymbra capitata* and its Application as A Biocide on Stone and Derived Surfaces. *Plants, 8*(9), 300. https://doi.org/10.3390/plants8090300

Ghahreman, A. (2001). *Flor of Iran* (Vol. 22). Tehran: Research Institute of Forest and Rangeland.

Giacomelli, A., Pietropaoli, M., Carvelli, A., Iacoponi, F., & Formato, G. (2016). Combination of thymol treatment (Apiguard®) and caging the queen technique to fight *Varroa destructor*. *Apidologie, 47*(4), 606–616. https://doi.org/10.1007/s13592-015-0408-4

Govindarajan, M., Rajeswary, M., Hoti, S. L., & Benelli, G. (2016a). Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi, Anopheles subpictus, Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). *Research in Veterinary Science, 104*, 77–82. https://doi.org/10.1016/j.rvsc.2015.11.011

Govindarajan, M., Rajeswary, M., Hoti, S. L., Bhattacharyya, A., & Benelli, G. (2016b). Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. *Parasitology Research, 115*(2), 807–815. https://doi.org/10.1007/s00436-015-4809-0

Gul, H., Ullah, F., Biondi, A., Desneux, N., Qian, D., Gao, X., & Song, D. (2019). Resistance against clothianidin and associated fitness costs in the chive maggot, *Bradysia odoriphaga*. *Entomologia Generalis, 39*(2), 81–92. https://doi.org/10.1127/entomologia/2019/0861

Guz, N., Cagatay, N. S., Fotakis, E. A., Durmusoglu, E., & Vontas, J. (2020). Detection of diflubenzuron and pyrethroid resistance mutations in *Culex pipiens* from Muğla, Turkey. *Acta Tropica, 203*, 105294. https://doi.org/10.1016/j.actatropica.2019.105294

Hadian, J., Akramian, M., Heydari, H., Mumivand, H., & Asghari, B. (2012). Composition and *in vitro* antibacterial activity of essential oils from four *Satureja* species growing in Iran. *Natural Product Research, 26*(2), 98–108. https://doi.org/10.1080/14786419.2010.534734

Heydari, A., Hadian, J., Esmaeili, H., Kanani, M. R., Mirjalili, M. H., & Sarkhosh, A. (2019). Introduction of *Thymus daenensis* into cultivation: Analysis of agro-morphological, phytochemical and genetic diversity of cultivated clones. *Industrial Crops and Products, 131*, 14–24. https://doi.org/10.1016/j.indcrop.2019.01.033

Hummelbrunner, L. A., & Isman, M. B. (2001). Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). *Journal of Agricultural and Food Chemistry, 49*(2), 715–720. https://doi.org/10.1021/jf000749t

Isman, M. B. (2017). Bridging the gap: Moving botanical insecticides from the laboratory to the farm. *Industrial Crops and Products, 110*, 10–14. https://doi.org/10.1016/j.indcrop.2017.07.012

Isman, M. B. (2020). Botanical Insecticides in the Twenty-First Century – Fulfilling Their Promise? *Annual Review of Entomology, 65*(1), 233–249. https://doi.org/10.1146/annurev-ento-011019-025010

Jalili, A., & Jamzad, Z. (1999). *Red Data Book of Iran*. Tehran: RIFR.

Jamzad, Z. (2009). *Thymus and Satureja species of Iran* (pp. 1–76). Teheran, Iran: Research Institute of Forests and Rangelands.

Jankowska, M., Rogalska, J., Wyszkowska, J., & Stankiewicz, M. (2017). Molecular targets for components of essential oils in the insect nervous system – A review. *Molecules (Basel, Switzerland), 23*(1), 34. https://doi.org/10.3390/molecules23010034

Jankowska, M., Lapied, B., Jankowski, W., & Stankiewicz, M. (2019). The unusual action of essential oil component, menthol, in potentiating the effect of the carbamate insecticide, bendiocarb. *Pesticide Biochemistry and Physiology, 158*, 101–111. https://doi.org/10.1016/j.pestbp.2019.04.013

Karami, A., Kavoosi, G., & Maggi, F. (2019). The emulsion made with essential oil and aromatic water from *Oliveria decumbens* protects murine macrophages from LPS-induced oxidation and exerts relevant radical scavenging activities. *Biocatalysis and Agricultural Biotechnology, 17*, 538–544. https://doi.org/10.1016/j.bcab.2019.01.015

Karami, A., Khoshbakht, T., Esmaeili, H., & Maggi, F. (2020). Essential Oil Chemical Variability in *Oliveria decumbens* (Apiaceae) from Different Regions of Iran and Its Relationship with Environmental Factors. *Plants, 9*(6), 680. https://doi.org/10.3390/plants9060680

López, V., Cascella, M., Benelli, G., Maggi, F., & Gómez-Rincón, C. (2018). Green drugs in the fight against *Anisakis simplex* – Larvicidal activity and acetylcholinesterase inhibition of *Origanum compactum* essential oil. *Parasitology Research, 117*(3), 861–867. https://doi.org/10.1007/s00436-018-5764-3

López, V., Pavela, R., Gómez-Rincón, C., Les, F., Bartolucci, F., Galiffa, V., … Benelli, G. (2019). Efficacy of *Origanum syriacum* Essential Oil against the Mosquito Vector *Culex quinquefasciatus* and the Gastrointestinal Parasite *Anisakis simplex*, with Insights on Acetylcholinesterase Inhibition. *Molecules (Basel, Switzerland), 24*(14), 2563. https://doi.org/10.3390/molecules24142563

Machiani, M. A., Javanmard, A., Morshedloo, M. R., & Maggi, F. (2018). Evaluation of yield, essential oil content and compositions of peppermint (*Mentha piperita* L.) intercropped with faba bean (*Vicia faba* L.). *Journal of Cleaner Production, 171*, 529–537. https://doi.org/10.1016/j.jclepro.2017.10.062

Maggi, F., & Benelli, G. (2018). Essential Oils from Aromatic and Medicinal Plants as Effective Weapons Against Mosquito Vectors of Public Health Importance. In G. Benelli & H. Mehlhorn (Eds.), *Mosquito-borne Diseases. Parasitology Research Monographs* (Vol. 10). Cham: Springer; https://doi.org/10.1007/978-3-319-94075-5_6

Maggi, F., Cecchini, C., Cresci, A., Coman, M. M., Tirillini, B., Sagratini, G., … Vittori, S. (2010). Chemical composition and antimicrobial activity of the essential oils from several *Hypericum* taxa (Guttiferae) growing in central Italy (Appennino Umbro-Marchigiano). *Chemistry & Biodiversity, 7*(2), 447–466. https://doi.org/10.1002/cbdv.200900091

Merino, N., Berdejo, D., Bento, R., Salman, H., Lanz, M., Maggi, F., … Pagán, R. (2019). Antimicrobial efficacy of *Thymbra capitata* (L.) Cav. essential oil loaded in self-assembled zein nanoparticles in combination with heat. *Industrial Crops and Products, 133*, 98–104. https://doi.org/10.1016/j.indcrop.2019.03.003

Moazeni, N., Khajeali, J., Izadi, H., & Mahdian, K. (2014). Chemical composition and bioactivity of *Thymus daenensis* Celak (Lamiaceae) essential oil against two lepidopteran stored-product insects. *The Journal of Essential Oil Research, 26*(2), 118–124. https://doi.org/10.1080/10412905.2013.860412

Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & McClements, D. J. (2016). Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food Chemistry, 194*, 410–415. https://doi.org/10.1016/j.foodchem.2015.07.139

Momtaz, S., & Abdollahi, M. (2008). A systematic review of the biological activities of *Satureja* L. species. *Pharmacologyonline, 2*, 34–54.

Morshedloo, M. R., Craker, L. E., Salami, A., Nazeri, V., Sang, H., & Maggi, F. (2017). Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono- and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.) subspecies. *Plant Physiology and Biochemistry, 111*, 119–128. https://doi.org/10.1016/j.plaphy.2016.11.023

Morshedloo, M. R., Salami, S. A., Nazeri, V., Maggi, F., & Craker, L. (2018). Essential oil profile of oregano (*Origanum vulgare* L.) populations grown under similar soil and climate conditions. *Industrial Crops and Products, 119*, 183–190. https://doi.org/10.1016/j.indcrop.2018.03.049

Motamedi, H., Darabpour, E., Gholipour, M., & Nejad, S. M. (2010). Antibacterial effect of ethanolic and methanolic extracts of *Plantago ovata* and *Oliveria decumbens* endemic in Iran against some pathogenic bacteria. *International Journal of Pharmacology, 6*(2), 117–122. https://doi.org/10.3923/ijp.2010.117.122

Mouden, S., Sarmiento, K. F., Klinkhamer, P. G., & Leiss, K. A. (2017). Integrated pest management in western flower thrips: Past, present and future. *Pest Management Science, 73*(5), 813–822. https://doi.org/10.1002/ps.4531

Mozaffarian, V. (2012). *Identification of Medicinal and Aromatic Plants of Iran*. Tehran, Iran: Farhang Moaser Press.

Naqqash, M. N., Gökçe, A., Bakhsh, A., & Salim, M. (2016). Insecticide resistance and its molecular basis in urban insect pests. *Parasitology Research, 115*(4), 1363–1373. https://doi.org/10.1007/s00436-015-4898-9

Nazari, M., Monajemi, R., Ghasemi Pirbalouti, A., Jafarian Dehkordi, M., & Dehkordi, M. (2013). Effects of essential oils of *Thymus deanensis* and *Satureja bachtiarica* on plasma lipoproteins in rats feeding with a fatty diet. *Journal of Herbal Drugs, 3*, 243–248.

Nickavar, B., Mojab, F., & Dolat-Abadi, R. (2005). Analysis of the essential oils of two *Thymus* species from Iran. *Food Chemistry, 90*(4), 609–611. https://doi.org/10.1016/j.foodchem.2004.04.020

NIST 17(2017). Mass Spectral Library (NIST/EPA/NIH). National Institute of Standards and Technology, Gaithersburg, USA.

Nooshkam, A., Mumivand, H., Hadian, J., Alemardan, A., & Morshedloo, M. R. (2017). Drug yield and essential oil and carvacrol contents of two species of *Satureja* (*S. khuzistanica* Jamzad and *S. rechingeri* Jamzad) cultivated in two different locations. *Journal of Applied Research on Medicinal and Aromatic Plants, 6*, 126–130. https://doi.org/10.1016/j.jarmap.2017.04.002

OECD. (1984). Guideline for testing of chemicals no. 207. Earthworm, acute toxicity tests. OECD-guideline for testing chemicals, Paris, France.

Palacios, S. M., Bertoni, A., Rossi, Y., Santander, R., & Urzua, A. (2009a). Efficacy of essential oils from edible plants as insecticides against the house fly, *Musca domestica* L. *Molecules (Basel, Switzerland), 14*(5), 1938–1947. https://doi.org/10.3390/molecules14051938

Palacios, S. M., Bertoni, A., Rossi, Y., Santander, R., & Urzua, A. (2009b). Insecticidal activity of essential oils from native medicinal plants of central Argentina against the house fly, *Musca domestica* (L.). *Parasitology Research, 106*(1), 207–212. https://doi.org/10.1007/s00436-009-1651-2

Pavela, R. (2007). Lethal and Sublethal Effects of Thyme Oil (*Thymus vulgaris* L.) on the House Fly (*Musca domestica* Lin.). *Journal of Essential Oil-Bearing Plants, 10*(5), 346–356. https://doi.org/10.1080/0972060X.2007.10643566

Pavela, R. (2010). Acute and synergistic effects of monoterpenoid essential oil compounds on the larvae of *Spodoptera littoralis. Journal of Biopesticides, 3*, 573–578.

Pavela, R. (2011). Insecticidal properties of phenols on *Culex quinquefasciatus* Say and *Musca domestica* L. *Parasitology Research, 109*(6), 1547–1553. https://doi.org/10.1007/s00436-011-2395-3

Pavela, R. (2015a). Essential oils for the development of eco-friendly mosquito larvicides: A review. *Industrial Crops and Products, 76*, 174–187. https://doi.org/10.1016/j.indcrop.2015.06.050

Pavela, R. (2015b). Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. *Parasitology Research, 114*(10), 3835–3853. https://doi.org/10.1007/s00436-015-4614-9

Pavela, R., & Benelli, G. (2016). Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends in Plant Science, 21*(12), 1000–1007. https://doi.org/10.1016/j.tplants.2016.10.005

Pavela, R., Canale, A., Mehlhorn, H., & Benelli, G. (2016). Application of ethnobotanical repellents and acaricides in prevention, control and management of livestock ticks: A review. *Research in Veterinary Science, 109*, 1–9. https://doi.org/10.1016/j.rvsc.2016.09.001

Pavela, R., Maggi, F., Lupidi, G., Cianfaglione, K., Dauvergne, X., Bruno, M., & Benelli, G. (2017). Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.). *Industrial Crops and Products, 109*, 603–610. https://doi.org/10.1016/j.indcrop.2017.09.013

Pavela, R., Maggi, F., Cianfaglione, K., Bruno, M., & Benelli, G. (2018a). Larvicidal activity of essential oils of five Apiaceae taxa and some of their main constituents against *Culex quinquefasciatus. Chemistry & Biodiversity, 15*(1), e1700382. https://doi.org/10.1002/cbdv.201700382

Pavela, R., Žabka, M., Vrchotová, N., & Tříska, J. (2018b). Effect of foliar nutrition on the essential oil yield of Thyme (*Thymus vulgaris* L.). *Industrial Crops and Products, 112*, 762–765. https://doi.org/10.1016/j.indcrop.2018.01.012

Pavela, R., Bartolucci, F., Desneux, N., Lavoir, A. V., Canale, A., Maggi, F., & Benelli, G. (2019a). Chemical profiles and insecticidal efficacy of the essential oils from four *Thymus* taxa growing in central-southern Italy. *Industrial Crops and Products, 138*, 111460. https://doi.org/10.1016/j.indcrop.2019.06.023

Pavela, R., Maggi, F., Iannarelli, R., & Benelli, G. (2019b). Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. *Acta Tropica, 193*, 236–271. https://doi.org/10.1016/j.actatropica.2019.01.019

Pavela, R., Benelli, G., Pavoni, L., Bonacucina, G., Cespi, M., Cianfaglione, K., … Maggi, F. (2019c). Microemulsions for delivery of Apiaceae essential oils – Towards highly effective and eco-friendly mosquito larvicides? *Industrial Crops and Products, 129*, 631–640. https://doi.org/10.1016/j.indcrop.2018.11.073

Pavela, R., Pavoni, L., Bonacucina, G., Cespi, M., Kavallieratos, N. G., Cappellacci, L., … Benelli, G. (2019d). Rationale for developing novel mosquito larvicides based on isofuranodiene microemulsions. *Journal of Pest Science, 92*(2), 909–921. https://doi.org/10.1007/s10340-018-01076-3

Pavela, R., Morshedloo, M. R., Lupidi, G., Carolla, G., Barboni, L., Quassinti, L., … Benelli, G. (2020a). The volatile oils from the oleo-gum-resins of *Ferula assa-foetida* and *Ferula gummosa*: A comprehensive investigation of their insecticidal activity and eco-toxicological effects. *Food and Chemical Toxicology, 140*, 111312. https://doi.org/10.1016/j.fct.2020.111312

Pavela, R., Maggi, F., Petrelli, R., Cappellacci, L., Buccioni, M., Palmieri, A., … Benelli, G. (2020b). Outstanding insecticidal activity and sublethal effects of *Carlina acaulis* root essential oil on the housefly, *Musca domestica*, with insights on its toxicity on human cells. *Food and Chemical Toxicology, 136*, 111037. https://doi.org/10.1016/j.fct.2019.111037

Pavoni, L., Pavela, R., Cespi, M., Bonacucina, G., Maggi, F., Zeni, V., … Benelli, G. (2019). Green micro-and nanoemulsions for managing parasites, vectors and pests. *Nanomaterials (Basel, Switzerland), 9*(9), 1285. https://doi.org/10.3390/nano9091285

Petrović, M., Popović, A., Kojić, D., Šućur, J., Bursić, V., Aćimović, M., … Vuković, G. (2019). Assessment of toxicity and biochemical response of *Tenebrio molitor* and *Tribolium confusum* exposed to *Carum carvi* essential oil. *Entomologia Generalis, 38*(4), 333–348. https://doi.org/10.1127/entomologia/2019/0697

Pirbalouti, A. G., Hashemi, M., & Ghahfarokhi, F. T. (2013). Essential oil and chemical compositions of wild and cultivated

Thymus daenensis Celak and *Thymus vulgaris* L. *Industrial Crops and Products, 48*, 43–48. https://doi.org/10.1016/j.indcrop.2013.04.004

Rizzo, R., Verde, G. L., Sinacori, M., Maggi, F., Cappellacci, L., Petrelli, R., … Benelli, G. (2020). Developing green insecticides to manage olive fruit flies? Ingestion toxicity of four essential oils in protein baits on *Bactrocera oleae. Industrial Crops and Products, 143*, 111884.

Rowshan, V., Bahmanzadegan, A., & Saharkhiz, M. J. (2013). Influence of storage conditions on the essential oil composition of *Thymus daenensis* Celak. *Industrial Crops and Products, 49*, 97–101.

Saharkhiz, M. J., Zomorodian, K., Taban, A., Pakshir, K., Heshmati, K., & Rahimi, M. J. (2016). Chemical Composition and Antimicrobial Activities of Three *Satureja* Species Against Food-borne Pathogens. *Journal of Essential Oil-Bearing Plants, 19*, 1984–1992.

Sefidkon, F., Jamzad, Z., & Mirza, M. (2004). Chemical variation in the essential oil of *Satureja sahendica* from Iran. *Food Chemistry, 88*, 325–328.

Sefidkon, F., & Akbari-nia, A. (2009). Essential oil content and composition of *Satureja sahendica* Bornm. at different stages of plant growth. *The Journal of Essential Oil Research, 21*, 112–114.

Senthil-Nathan, S. (2019). A Review of Resistance Mechanisms of Synthetic Insecticides and Botanicals, Phytochemicals, and Essential Oils as Alternative Larvicidal Agents Against Mosquitoes. *Frontiers in Physiology, 10*, 1591. https://doi.org/10.3389/fphys.2019.01591

Taban, A., Saharkhiz, M. J., & Hooshmandi, M. (2017). Insecticidal and repellent activity of three *Satureja* species against adult red flour beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Acta Ecologica Sinica, 37*, 201–206.

Tabanca, N., Avonto, C., Wang, M., Parcher, J. F., Ali, A., Demirci, B., … Khan, I. A. (2013). Comparative Investigation of *Umbellularia californica* and *Laurus nobilis* Leaf Essential Oils and Identification of Constituents Active against *Aedes aegypti*. *Journal of Agricultural and Food Chemistry, 61*, 12283–12291.

Tabari, M. A., Youssefi, M. R., Maggi, F., & Benelli, G. (2017). Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). *Veterinary Parasitology, 245*, 86–91.

Tisserand, R., & Young, R. (2014). *Essential Oil Safety. A Guide for Health Care Professionals* (2nd ed., p. 784). Elsevier Ltd.; https://doi.org/10.1016/C2009-0-52351-3

Traboulsi, A. F., Taoubi, K., El-Haj, S., Bessiere, J. M., & Rammal, S. (2002). Insecticidal properties of essential plant oils against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). *Pest Management Science, 58*, 491–495.

Ullah, F., Gul, H., Desneux, N., Gao, X., & Song, D. (2019a). Imidacloprid-induced hormetic effects on demographic traits of the melon aphid, *Aphis gossypii. Entomologia Generalis, 39*, 325–337.

Ullah, F., Gul, H., Desneux, N., Tariq, K., Ali, A., Gao, X., & Song, D. (2019b). Clothianidin-induced sublethal effects and expression changes of vitellogenin and ecdysone receptors genes in the melon aphid, *Aphis gossypii. Entomologia Generalis, 39*, 137–149.

Ullah, F., Gul, H., Desneux, N., Qu, Y., Xiao, X., Khattak, A. M., … Song, D. (2019c). Acetamiprid-induced hormetic effects and vitellogenin gene (Vg) expression in the melon aphid, *Aphis gossypii. Entomologia Generalis, 39*, 259–270.

Van den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas – Liquid partition chromatography. *Journal of Chromatography. A, 11*, 463–471.

Varel, V. H. (2002). Carvacrol and thymol reduce swine waste odor and pathogens: Stability of oils. *Current Microbiology, 44*(1), 38–43. https://doi.org/10.1007/s00284-001-0071-z

Varikou, K., Garantonakis, N., & Birouraki, A. (2019). Exposure of *Bombus terrestris* L. to three different active ingredients and two application methods for olive pest control. *Entomologia Generalis, 39*(1), 53–60. https://doi.org/10.1127/entomologia/2019/0706

Vitali, L. A., Beghelli, D., Biapa Nya, P. C., Bistoni, O., Cappellacci, L., Damiano, S., … Bramucci, M. (2016). Diverse biological effects of the essential oil from Iranian *Trachyspermum ammi*. *Arabian Journal of Chemistry, 9*(6), 775–786. https://doi.org/10.1016/j.arabjc.2015.06.002

WHO (1996). Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES /IC /96.1. p. 69

Wilke, A. B. B., Beier, J. C., & Benelli, G. (2020). Filariasis vector control down-played due to the belief the drugs will be enough – not true! *Entomologia Generalis, 40*(1), 15–24. https://doi.org/10.1127/entomologia/2019/0776

Yavaşoglu, S. İ., Yaylagül, E. Ö., Akıner, M. M., Ülger, C., Çağlar, S. S., & Şimşek, F. M. (2019). Current insecticide resistance status in *Anopheles sacharovi* and *Anopheles superpictus* populations in former malaria endemic areas of Turkey. *Acta Tropica, 193*, 148–157. https://doi.org/10.1016/j.actatropica.2019.02.003

Yousefzadi, M., Riahi-Madvar, A., Hadian, J., Rezaee, F., & Rafiee, R. (2012). *In vitro* cytotoxic and antimicrobial activity of essential oil from *Satureja sahendica. Toxicological and Environmental Chemistry, 94*(9), 1735–1745. https://doi.org/10.1080/02772248.2012.728606

Youssefi, M. R., Moghaddas, E., Tabari, M. A., Moghadamnia, A. A., Hosseini, S. M., Farash, B. R. H., … Sut, S. (2019). *In vitro* and *in vivo* effectiveness of carvacrol, thymol and linalool against *Leishmania infantum. Molecules (Basel, Switzerland), 24*(11), 2072. https://doi.org/10.3390/molecules24112072

Manuscript received: 3 July 2020
Revisions requested: 14 September 2020
Modified version received: 16 September 2020
Accepted: 22 September 2020