Origanum syriacum subsp. *syriacum*: from an ingredient of Lebanese 'manoushe' to a source of effective and eco-friendly botanical insecticides

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Abstract

Origanum syriacum subsp. *syriacum*, also known as 'Za'tar' is an aromatic shrub native to Lebanon and cultivated in other Middle East countries. The plant leaves enjoy a high reputation as a traditional remedy against cardiovascular, respiratory and infectious diseases. In addition, they are a famous component of the Lebanese pizza ("manoushe"). Starting from its safety for humans, here *O. syriacum* subsp. *syriacum* was selected to assess the insecticidal efficacy of its leaf essential oil (EO) and its major constituent carvacrol against two deleterious agricultural pests, namely the noctuid *Spodoptera littoralis* and the aphid *Myzus persicae*, as well as on the fly pest *Musca domestica*. Furthermore, Za'tar EO impact on beneficial organisms such as the aphid predator *Harmonia axyridis* and the earthworm *Eisenia fetida,* which is used in the vermicomposting process, was assessed as well. GC-MS analysis highlighted the phenolic monoterpene carvacrol as the predominant component (83%) of Za'tar EO. Toxicity of *O. syriacum* subsp. *syriacum* EO was noteworthy, showing LC_{50}/LD_{50} of 103.3 µg $larva^{-1}$, 2.1 mg L^{-1} and 58.7 µg $adult^{-1}$ on *S. littoralis*, *M. persicae* and *M. domestica*, respectively, which were partly consisted with those of its major component carvacrol (38.3 µg $larva^{-1}$, 1.6 mL L^{-1} and 59.3 µg $adult^{-1}$, respectively). When tested up to 3.8 mL L^{-1} and 200 mg kg^{-1} on *H. axyridis* and *E. fetida,* this EO was not toxic, at variance to α-cypermethrin, which caused 100% mortality at 1 ml L^{-1} and 25 mg kg^{-1}, respectively. Taken together, these results promote carvacrol-rich Za'tar EO as a promising reservoir of green insecticides to be used for managing insect pests and vectors of economic relevance.

Keywords: agricultural pest; essential oil toxicity; *Musca domestica*; *Myzus persicae*; *Spodoptera littoralis*; non-target species

1. Introduction

Synthetic pesticides used until the second half of 20th century produced deleterious effects on the environment and human health pushing agrochemical companies to reduce or delete the use of harmful substances from their arsenal (Koul and Dhaliwal, 2003; Isman, 2006; Benelli, 2015). Among them, DDT and methyl parathion, previously used against malaria vectors and as potent pesticides in crop protection, respectively, were banned from the market in the 1970s after the discovering of their dangerous effects (Morgan, 2004). To counterbalance this trend, research and the public opinion in developed countries watched natural alternatives from plant sources with rising interest (Koul et al., 2008; Benelli et al., 2015; Pavela et al., 2019). Among the latter, plant-borne essential oils (EOs) represent new effective and eco-friendly tools to be used in integrated pest management (IPM) strategies (Isman and Machial, 2006; Benelli and Pavela, 2018a,b).

EOs are liquid mixtures obtained by steam distillation or hydrodistillation from several medicinal and aromatic plants (MAPs). They are made up of volatile and lipophilic compounds, mostly belonging to monoterpenoids, sesquiterpenoids and phenylpropanoids. In the Mediterranean area, the main EO sources belong to the families of Apiaceae, Asteraceae, Lamiaceae, Lauraceae and Myrtaceae (Lubbe and Verpoorte, 2011). EOs have been consumed for a long time as flavorings and fragrances, spices and drugs. Among other uses, EOs and extracts allow protection from various deleterious insects and preservation of stored foodstuffs have been documented (Giatropoulos et al., 2013; Duarte etal., 2015; Pavela et al., 2017, 2019; Hashem et al., 2018; Benelli et al., 2018a,b,c; Ribeiro et al., 2018).

An important ecological role played by EOs is defending plants from several enemies such as phytophagous insects (Paré and Tumlinson, 1999). In this respect, scientific evidence has documented their efficacy against larvae and adults of pests of medical and agricultural importance (Isman, 2000; Koul et al., 2008; del Carmen Romero et al., 2012; Isman, 2017; Benelli et al., 2018d, 2019). EOs are multiple-component mixtures displaying several modes of action and wide spectrum of efficacy, thus having the unlikely capacity to induce episodes of resistance in insects (Pavela and Benelli, 2016). Besides, their low toxicity on non-target organisms and environment makes them ideal candidate ingredients in green formulations to be used in organic agriculture and IPM as well (Rattan, 2010; Stevenson et al., 2017). Among the various bioactive components characterizing EOs, the phenolic monoterpene carvacrol is definitely one of the most studied. This volatile compound is very common in several Lamiaceae EOs and proved to be effective against a wide spectrum of harmful insects and pests (Koc et al., 2013; Tong et al., 2013; Park et al., 2017; Campos et al., 2018).

In the present study, we paid attention to an EO extracted from a species widely cultivated for food and medical purposes in Lebanon, but still unexplored for insecticidal activity, namely that from *Origanum syriacum* L. subsp. *syriacum*. This species is a shrub up to 130 cm tall with leaves and flowers emitting a pleasant aroma (Arnold et al., 2000). *Origanum syriacum* subsp. *syriacum* grows on dry rocky soils and is native to Lebanon although is found in other Middle East countries such as Syria, Jordan, Israel, Egypt and Turkey (Greuter et al., 1986). In addition, due to its several applications as a food and medicine, it is also intensively cultivated in the above countries (Zein et al., 2011; Khoury et al., 2016).

The plant is known in Lebanon as ‘Za’tar’ and enjoys a good reputation as a traditional remedy against metabolic, neurodegenerative, respiratory, cardiovascular, gastrointestinal and infectious diseases (Yaniv et al., 1987; Alkofahi and Atta, 1999; Aburjai et al., 2001; Abu-Irmaileh and Afifi, 2003; Hamdan and Afifi, 2004; Salah and Jager, 2005; El Beyrouthy et al., 2008; Hudaib et al., 2008; Darwish and Aburjai, 2010; Khoury et al., 2016). Noteworthy, in Lebanon, leaves of *O. syriacum* are mixed with cheese and consumed to combat parasitic diseases (Khoury et al., 2016). Besides, *O. syriacum* is a famous component of the ‘manoushe’ recipe, a sort of Lebanese pizza (Khoury et al., 2016).

Several studies addressed the important antimicrobial, antioxidant and anticancer activity of *O. syriacum* subsp. *syriacum* EO (Loizzo et al., 2009; Viuda-Martos et al., 2010; El Gendy et al., 2015). However, despite the safety of this species on humans, its insecticidal capacity has been poorly investigated. In this work, we evaluated its efficacy as insecticide against two important agricultural pests, namely *Spodoptera littoralis* (Boisduval) and the aphid *Myzus persicae* (Sulzer), and the housefly *Musca domestica* L. Furthermore, the toxicity to non-target organisms, such as the ladybeetle *Harmonia axyridis* (Pallas) and the earthworm *Eisenia fetida* (Savigny), has been assessed to demonstrate its eco-friendliness.

Spodoptera littoralis (Boisd.) is a noctuid caterpillar feeding on more than 90 species of economic importance such as horticultural crops and ornamental plants (Sut et al., 2017). The European and Mediterranean Plant Protection Organization (EPPO) defined this moth as a A2 quarantine pest since it is capable of spreading to the temperate zone due to the transport of ornamental plants and vegetables (OEPP/EPPO, 2015). Recently, this pest acquired resistance against some synthetic insecticides that are overwhelming its natural predators causing damages to the environment and human health (Abo Elghar et al., 2005). *Myzus persicae* Sulzer is an important agricultural pest. It also vectors plant viruses and crop diseases in temperate regions (Blackman and Eastop, 2000). Because of a long history of use of insecticides, this pest developed high resistance to various classes of pesticides such as pyrethroids, carbamates, organophosphorous and neonicotinoids (Devonshire et al., 1998). *Musca domestica* L., also known as housefly, is a vector of more than one hundred pathogen diseases (Benelli et al., 2018c). Since most of insecticides present on the market have a unique mode of action on houseflies, frequent episodes of resistance due to genetic changes have been observed (Walsh et al., 2001; Naqqash et al., 2016).

On the above, a different approach in the fight of agricultural pests and insect vectors relying on multitasking substances endowed with low impacts on the environment and human health is urgently needed. EOs seem to satisfy the above criteria, thus they are expected to be used as effective ingredients for replacing or reducing the use of conventional insecticides in the years to come (Isman, 2000; Pavela and Benelli, 2016; Stevenson et al., 2017).

2. Material and methods

2.1. Plant material

Leaves of *O. syriacum* subsp. *syriacum* were manually collected in a site around Tayibe (33°16′35″N; 35°31′14″E, 800 m a.s.l.), South Lebanon, in May 2017. The botanical identification was performed by one of us (F. Bartolucci) using literature available (Ietswaart 1980, 1982, 1985). A voucher specimen was stored in the herbarium of the Floristic Research Centre of the Apennines under the voucher codex APP No 59012.

2.2. Distillation of Origanum syriacum subsp. syriacum essential oil

Air-dried leaves (420 g) of *O. syriacum* subsp. *syriacum* were crushed and inserted in a 10 L flask filled with 6 L of distilled water, then subjected to hydrodistillation for 3 h a Clevenger-type apparatus. Once decanted for 30 min, EO, of orange colour, was separated from the water layer and collected in a 10 mL vial sealed with a PTFE-silicon cap and stored at +4°C until further analyses. The oil yield (4.3%, w/w) was determined on a dry weight matter as the average of two independent distillations.

2.3. GC-MS analysis

The *O. syriacum* subsp. *syriacum* EO chemical analysis was performed by an Agilent 6890N gas chromatograph equipped with a 5973N mass spectrometer and an auto-sampler 7863 (Agilent, Wilmingotn, DE). The column was a HP-5MS (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness) which was purchased from Agilent (Folsom, CA, USA). Helium was used as the mobile phase with a flow of 1 mL/min. The temperature of injector and detector was 280°C.

The EO samples, diluted in hexane (1:100) were injected (2 μL) in split mode with a 1:50 ratio. The single quadrupole detector operated in electron impact (EI) full scan mode with acquisition in the mass range of 29–400 *m/z*. The temperature of the oven was programmed as follows: 60°C held for 5 min, then rise to 220°C at 4°C/min, rise to 280°C at 11°C/min. The quali-quantitative analysis was performed according to our previously published procedure (Benelli et al., 2017, 2018c, 2018d, 2018e).

2.4. Insect rearing

Myzus persicae and *M. domestica* adults, *S. littoralis* 3rd instar larvae, as well as *H. axyridis* and *E. fetida* were reared as recently reported by Benelli et al. (2018e). All the tested species were maintained and subsequently tested at 25±1 °C, 70±3% R.H. and 16:8 h (L:D).

2.5. Insecticidal activity on Myzus persicae

The insecticidal efficacy of *O. syriacum* subsp. *syriacum* EO and its main constituent carvacrol was tested on adult aphids feeding on cabbage, following the method by Pavela (2018). *Origanum syriacum* subsp. *syriacum* EO or carvacrol was mixed with Tween 80 (1:1, v:v). We tested the mixture at 15.0; 10.0; 8.0; 5.0; 3.0; 2.0 and 1.0 mL L^{-1}, i.e., 7.5; 5.0; 4.0; 2.5, 1.5; 1.0 and 0.5 mL L^{-1} of the EO or carvacrol.

The product was applied on cabbage at 50 mL m^{-2} (about 500 L ha^{-1}). Water + Tween 80 at 7.5 mL L^{-1} was the negative control (50 mL.m^{-2}). Positive control was α-cypermethrin (Vaztak®) at 0.02, 0.015, 0.01, 0.007, 0.004 and 0.002 mL L^{-1}. For each concentration, 4 groups, each composed by 50 aphid adults, were tested. *Myzus persicae* mortality was noted after 48 h.

2.6. Insecticidal activity on Spodoptera littoralis

The insecticidal efficacy of the *O. syriacum* subsp. *syriacum* EO and its main constituent carvacrol on *S. littoralis* larvae was studied via topical assays. Following Sut et al. (2017), the dorsum of each larva was treated with 1 µL of acetone + *O. syriacum* subsp. *syriacum* EO or carvacrol at 20, 40, 50, 70, 90, 120, 150, 180 and 200 µg larva^{-1}, (4 groups, each composed by 20 larvae, were tested per each dose). Acetone without *O. syriacum* subsp. *syriacum* EO was the negative control. Positive control was α-cypermethrin (Vaztak®) at 0.01, 0.008, 0.006, 0.004, 0.002 and 0.001 µg larva^{-1}. *Spodoptera littoralis* larvae were stored as reported by Sut et al. (2017) and mortality was noted after 24 h.

2.7. Insecticidal activity on Musca domestica

Topical application tests were done with *O. syriacum* subsp. *syriacum* EO and its main constituent carvacrol on *M. domestica* females (3–6 days old). In agreement with the method recently detailed by Benelli et al. (2019), 1 µL of acetone + *O. syriacum* subsp. *syriacum* EO or carvacrol at 10, 20, 40, 60, 80 and 100 µg adult^{-1} (4 groups of 20 flies each were tested for each dose), was applied on the pronotum of CO_2-anesthetized flies. Acetone without *O. syriacum* subsp. *syriacum* EO was the negative control. Positive control was α-cypermethrin (Vaztak®) at 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg adult^{-1}. Flies were moved to a recovery box (10×10×12 cm) for 24 h. Therefore, mortality was noted.

2.8. Toxicity on the non-target ladybug Harmonia axyridis

Following Pavela (2018), 3rd instar larvae and adults (3-7 days old) of *H. axyridis* were tested to evaluate the non-target impact of *O. syriacum* subsp. *syriacum* EO. The EO was tested at 3.8, 2.0 and 1.0 mL L^{-1} following the testing procedure showed in 'Insecticidal activity on *Myzus persicae*'.

Only a difference needs to be highlighted. The *O. syriacum* subsp. *syriacum* EO was applied on ladybug larvae and adults in open Petri dishes (diameter 9 cm, 10 ladybugs per replicate; n=4 per tested concentration). Vaztak® was applied as recommended on aphids: 1.0 mL L^{-1}, i.e., 0.05 g L^{-1} of α-cypermethrin. 20 mL m^{-2} of liquid were applied (200 L ha^{-1}). Negative control was water + 7.5 mL L^{-1} of Tween 80; 50 mL were applied per m^2 (500 L ha^{-1}). Post-treatment, ladybugs were moved to clean Petri dishes, fed with *M. persicae*, thus mortality was checked 48 h post-treatment.

2.9. Toxicity on non-target Eisenia fetida earthworms

The protocol by OECD (1984) was used to assess the impact of the *O. syriacum* subsp. *syriacum* EO on *E. fetida* adults. The artificial soil with same composition and pH used for *E. fetida* rearing was employed. *Origanum syriacum* subsp. *syriacum* EO at 400.0; 200.0 and 100.0 mg kg^{-1} + Tween 80 (ratio 1:1 v:v) was added to the soil, (=200.0; 100.0 and 50.0 mg of *O. syriacum* subsp. *syriacum* EO a.i. per kg of dry soil). Positive control: *α*-cypermethrin at 50.0; 25.0 and 12.5 mg kg^{-1} of dry soil [i.e., Vaztak® at 1,000.0; 500,0 and 250.0 µL kg^{-1} (v/v)]; negative control: distilled water. The mixture containing *O. syriacum* subsp. *syriacum* EO, water or *α*-cypermethrin was mixed in the soil described above (650 g), thus 10 *E. fetida* adults were added. Each experiment was repeated four times. All soil samples were moved to glass pots (1 L) covered with gauze. Mortality of non-target earthworms was monitored till 14 days of exposure.

2.10. Statistical analysis

When mortality in the control ranged from 1 to 20%, we corrected experimental mortality with Abbott's formula (Abbott, 1925); if control mortality was >20%, experiments were repeated. Therefore, lethal doses, LD_{50} and LD_{90}, as well as lethal concentrations, LC_{50} and LC_{90}, were estimated by probit analysis (Finney, 1971). In non-target assays, mortality rates (%) transformed by arcsine√ were analyzed by ANOVA and Tukey's HSD test ($P \leq 0.05$).

3. Results and Discussion

3.1. *Origanum syriacum* subsp. *syriacum* essential oil composition

Hydrodistillation of Lebanese Za'tar gave 4.3% of leaf EO. This value was similar to that obtained from an Egyptian accession of *O. syriacum* (4.6%) (Gendy et al., 2015) and slightly lower than that determined in a Lebanese population (5.9%) (Arnold et al., 2000). On the other hand, lower yield values (0.5-0.6%) were obtained for other accessions from Lebanon and Egypt (Loizzo et al., 2009; Viuda-Martos et al., 2010). These differences may depend on the different collection times and plant part processed, as well as environmental factors and genetics.

The EO chemical profile of Za'tar is depicted in Fig. 1, while the chemical constituents identified (30) are listed in Table 1. As it can be observed in Fig. 1, the EO composition was dominated by the phenolic monoterpene carvacrol (82.6%). The remaining fraction was made up of γ-terpinene (5.7%), *p*-cymene (3.7%), thymol (2.4%), α-terpinene (1.3%) and myrcene (1.0%) and other 24 components occurring in percentages below 1%.

On the above, this Lebanese accession of *O. syriacum* subsp. *syriacum* belonged to the carvacrol chemotype. Previously, Zein et al. (2011) reported that this chemotype is more abundant in cultivated accessions whereas the thymol chemotype is more spread in spontaneous populations. Thus, our results seem to contradict their proposal. On the other hand, intermediate forms with similar levels of thymol and carvacrol have been also reported in the literature (Baser et al., 2003; Lukas et al., 2009; Zein et al., 2011; El Gendy et al., 2015; Al Hafi et al., 2016).

3.2. Insecticidal activity and lack of toxicity on non-target species

In our experiments, the insecticidal activity of *O. syriacum* subsp. *syriacum* EO was compared with that of the positive control, *α*-cypermethrin, a pyrethroid widely used to control agricultural pests as well as insect vectors. Results pointed out that the tested EO achieved a relevant toxicity towards three insects of economic importance selected as representative study species (Table 2). Concerning *M. persicae* adult aphids, the LC_{50} and LC_{90} values were 2.1 and 3.4 mL L^{-1}, while on *M. domestica* adults we obtained LD_{50} and LD_{90} of 58.7 and 98.3 µg adult^{-1}, respectively. Concerning the moth pest *S. littoralis*, LD_{50} and LD_{90} estimated on 3rd instar larvae were 103.3 and 173.7 µg adult^{-1} (Table 2). As expected, LC/LD_{50} and LC/LD_{90} values obtained testing the positive control *α*-cypermethrin on the three insect species were lower if compared with those calculated for the *O. syriacum* subsp. *syriacum* EO, being 0.005 and 0.012 mL L^{-1}, 0.18 and 0.73 µg adult^{-1}, 0.003 and 0.009 µg larva^{-1}, for *M. persicae, M. domestica* and *S. littoralis*, respectively (Table 2). The LC/LD_{50} and LC/LD_{90} values displayed by *O. syriacum* subsp. *syriacum* EO were consistent with those of its major component carvacrol on the first two target insects, i.e., 1.6 and 2.7 mL L^{-1} and 59.3 and 102.3 µg adult^{-1}, respectively. On the other hand, the EO toxicity to *S. littoralis* larvae was lower than that of carvacrol, showing LD_{50} and LD_{90} of 38.3 and 98.7 µg larva^{-1}. The presence of other minor components with possible antagonistic effects (Pavela, 2015b) may be the cause of the weaker activity of the EO on *S. littoralis* larvae compared with its major component carvacrol.

Although for *O. syriacum* subsp. *syriacum* EO, the efficacy did not reach a level close to the positive control, it was comparable with other EOs such as those obtained from *Rosmarinus officinalis* L., *Foeniculum vulgare* L. and *Thymus vulgaris* L. (Faraone et al., 2015; Pavela, 2018; Pavela and Sedlák, 2018; Murcia-Meseguer et al., 2018), which are currently used to produce commercial botanical insecticides (Pavela, 2016). Our results thus indicate high prospects of using the EO from *O. syriacum* subsp. *syriacum* as an active substance in botanical insecticides. Moreover, these prospects are enhanced by the fact that *O. syriacum* subsp. *syriacum* is currently grown as a commercial crop, and provided that a suitable growing technology is used, more than

4,500 kg of dry mass can be obtained from one hectare, yielding about 180 kg of EO (Jaafar et al., 2015). Thus, this crop may provide a well available and relatively inexpensive source of active substances for potential botanical insecticides, in agreement with the ideal criteria that should characterize the plant sources of biopesticides, as outlined by Pavela and Benelli (2016).

Actually, the noteworthy toxicity of *O. syriacum* subsp. *syriacum* EO on the two agricultural pests and one insect vector assayed can be assigned to its main component, the phenolic monoterpene carvacrol, although a little contribution by *p*-cymene and γ-terpinene cannot be disregarded. These molecules were previously found to be toxic to several insect pests and vectors, including *Anopheles*, *Aedes* and *Culex* mosquitoes, as well as houseflies and moth pests (Table 3). Notably, *p*-cymene toxicity towards larval instars of several mosquito vectors was comparable to that of carvacrol (Table 3). Earlier, it has been also outlined that carvacrol showed detrimental effects on longevity and fecundity of the green peach aphid *M. persicae* when tested at 500 μl L^{-1} (Petrakis et al., 2014). In our previous experiments, carvacrol showed to be highly toxic to larvae of *S. littoralis*, showing an LD_{50} of 15 μg $larva^{-1}$ (Pavela, 2014). Noteworthy, its effect was found to be synergized by other components occurring in the Za'tar EO like *p*-cymene (Pavela, 2010). Similar results were obtained studying the effects of mixtures of carvacrol and *p*-cymene on *M. domestica* (Pavela, 2008). However, the low amount of *p*-cymene in *O. syriacum* subsp. *syriacum* EO (3.7%) may be unable to boost the insecticidal effects of the major compound carvacrol (Table 2).

Concerning the possible mechanism of action of *O. syriacum* subsp. *syriacum* EO on insects, the main component carvacrol has been reported to inhibit the acetylcholinesterase (AChE) enzyme, affecting the synaptic transmission in insects (Lopez et al., 2018). At CNS level, carvacrol is also capable of interacting with the GABA and octopamine receptors leading to toxic effects on parasites and pests (Tong and Coats, 2010; Enan, 2001; Jankowska et al., 2017).

Besides, in the present work, non-target tests pointed out the lack of toxicity of the *O. syriacum* subsp. *syriacum* EO on terrestrial invertebrates, such as *H. axyridis* ladybugs (Table 4) and *E. fetida* earthworms (Table 5). We did not observe suffering of *H. axyridis* larvae and adults exposed to the Za'tar EO at concentrations ranging from 1.0 to 3.8 mL L^{-1}, whereas ladybeetles exposed to the positive control, α-cypermethrin [0.015 mL L^{-1} (w/v)], showed 100% mortality (Table 4).

In EO-contaminated soil experiments, *E. fetida* adult earthworms exposed to concentrations of *O. syriacum* subsp. *syriacum* EO ranging from 50.0 to 200.0 mg kg^{-1} failed to show relevant mortality rates (maximum mortality 5.0% after 14^{th} days of exposure to the EO at 50 mg kg^{-1}), while earthworms exposed to α-cypermethrin (12.5-50.0 mg kg^{-1}) showed mortality rates ranging from 89.5 to 100% and from 95.5% to 100%, after 7 and 14 days of exposure, respectively (Table 5). Insecticides – pyrethroids, neonicotinoids and organophosphates – are known to be highly toxic to earthworms even at very low doses (Yuguda et al., 2015). On the contrary, earthworms and other non-target organisms are tolerant to EOs as previously demonstrated (Pavela and Govindarajan, 2017; Pavela, 2018). This is another significant benefit arising from the use of EOs, which we believe to be more important than the lower insecticidal efficacy achieved compared with the positive control.

Besides, from a safety perspective, the Za'tar EO seems to not pose risk for environment and human health. Indeed, carvacrol is a food additive that is classified as a Generally Recognized as Safe (GRAS) compound by the US Food and Drug Administration (FDA) (Tabari et al., 2017; Pavela and Benelli, 2016). Its toxicity (LD_{50}) in rats after gavage administration has been estimated as 810 mg kg^{-1} (Lee et al., 2003). Therefore, a possible formulation containing the Za'tar EO at low percentages is expected to have a LD_{50} above 5 g kg^{-1}, which is the threshold to comply with the requirements of regulatory agencies (e.g., EPA) (Isman and Machial, 2006). In addition, carvacrol did not produce toxicity on non-target invertebrates such as mealworm beetles, honeybees, shellfish and the mosquito fish *Gambusia affinis* Baird & Girard (Mattila et al., 2000; George et al., 2009; Lahlou, 2002). Overall, our results for the carvacrol-rich *O. syriacum* subsp. *syriacum* EO confirmed its eco-friendliness, outlining a perspective of use in IPM and organic agriculture.

4. Conclusions

In summary, this work showed for the first time that the EO from Za'tar, a Lebanese plant used traditionally as herbal remedy, as well as a food, may be a candidate ingredient for effective, safe and eco-friendly botanical insecticides to be employed in IPM and organic agriculture. Its effectiveness was mostly due to the major component carvacrol. From an industrial standpoint, the production of green insecticides based on Za'tar EO can be considered scalable, since the raw material may be afforded by both wild and cultivated accessions of *O. syriacum* that are diffused in several Middle East countries. Further field studies on the development of effective and safe formulations based on this EO for real-world applications in IPM programs are urgently needed.

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Conflict of interest

Authors declare they have no conflict of interest.

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Table

Table 1. Chemical composition of the *Origanum syriacum* subsp. *syriacum* essential oil.

No	Component[a]	RI[b]	RI lit.[c]	%[d]	ID[d]
1	α-Thujene	920	924	0.4±0.1	RI,MS
2	α-Pinene	926	932	0.4±0.1	RI,MS,Std
3	Camphene	939	946	tr[e]	RI,MS,Std
4	β-Pinene	968	974	tr	RI,MS,Std
5	3-Octanone	975	979	0.1±0.0	RI,MS
6	Myrcene	988	988	1.0±0.2	RI,MS,Std
7	3-Octanol	996	988	0.2±0.0	RI,MS
8	α-Phellandrene	1002	1002	0.2±0.0	RI,MS,Std
9	δ-3-Carene	1007	1007	tr	RI,MS,Std
10	α-Terpinene	1013	1014	1.3±0.3	RI,MS,Std
11	*p*-Cymene	1021	1020	3.7±0.6	RI,MS,Std
12	Limonene	1024	1024	tr	RI,MS,Std
13	β-Phellandrene	1024	1025	0.2±0.0	RI,MS
14	(*E*)-β-Ocimene	1046	1044	tr	RI,MS,Std
15	γ-Terpinene	1055	1054	5.7±0.9	RI,MS,Std
16	*cis*-Sabinene hydrate	1063	1065	0.1±0.0	RI,MS
17	Terpinolene	1084	1086	0.1±0.0	RI,MS,Std
18	Linalool	1096	1095	tr	RI,MS,Std
19	*trans*-Sabinene hydrate	1101	1098	tr	RI,MS
20	Borneol	1160	1165	tr	RI,MS,Std
21	Terpinen-4-ol	1172	1174	0.4±0.1	RI,MS,Std
22	α-Terpineol	1189	1186	tr	RI,MS,Std
23	Cumin aldehyde	1236	1238	tr	RI,MS
24	Carvacrol methyl ether	1242	1241	tr	RI,MS
25	Thymol	1294	1289	2.4±0.4	RI,MS,Std
26	Carvacrol	1308	1298	82.6±2.9	RI,MS,Std
27	Carvacrol acetate	1372	1370	0.1±0.0	RI,MS

28	(*E*)-Caryophyllene	1408	1417	0.9±0.2	RI,MS,Std
29	α-Himachalene	1443	1449	tr	RI,MS
30	γ-Eudesmol	1632	1630	tr	RI,MS
	Total identified (%)			99.9	
	Grouped compounds (%)				
	Monoterpene hydrocarbons			13.1	
	Oxygenated monoterpenes			85.8	
	Sesquiterpene hydrocarbons			0.9	
	Oxygenated sesquiterpenes			tr	
	Others			0.2	

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[a] Order of compounds is according to elution from a HP-5MS column. [b] Van den Doll and Kratz (1963) linear retention index. [c] Retention index taken from ADAMS (2007) or NIST 17 (2017) libraries. [d] Relative percentages values are mean of two replicates ± standard deviation. [f] Method of identification: RI, coherence of the RI values with those of ADAMS, NIST 17 and FFNSC2 (2012) libraries; MS, matching with the ADAMS, NIST 17, FFNSC 2, and MAGGI libraries; Std, comparison with available analytical standard. [g] tr, % < 0.1.

Table

Table 2. Insecticidal efficacy of the *Origanum syriacum* subsp. *syriacum* essential oil and its main constituent carvacrol on selected insect pests.

Target insect	**Unit**	**LC_{50}/LD_{50}**	**CI95**	**LC_{90}/LD_{90}**	**CI95**	χ^2
O. syriacum subsp. *syriacum* essential oil						
Myzus persicae adults	mL L^{-1}	2.1	1.8-2.2	3.4	3.1-3.8	5.538 *ns*
Musca domestica adults	µg $adult^{-1}$	58.7	52.7-65.5	98.3	88.3-99.7	4.544 *ns*
Spodoptera littoralis 3rd instar larvae	µg $larva^{-1}$	103.3	87.6-122.1	173.7	143.3-197.8	1.261 *ns*
Positive control, α-cypermethrin						
Myzus persicae adults	mL L^{-1}	0.005	0.004-0.009	0.012	0.011-0.015	3.235 *ns*
Musca domestica adult females	µg $adult^{-1}$	0.18	0.15-0.21	0.73	0.68-0.91	2.524 *ns*
Spodoptera littoralis 3rd instar larvae	µg $larva^{-1}$	0.003	0.002-0.006	0.009	0.008-0.012	3.524 *ns*
Carvacrol						
Myzus persicae adults	mL L^{-1}	1.6	1.3-1.9	2.7	2.4-2.9	2.258 *ns*
Musca domestica adult females	µg $adult^{-1}$	59.3	51.7-62.5	102.3	95.7-110.1	3.251 *ns*
Spodoptera littoralis 3rd instar larvae	µg $larva^{-1}$	38.3	32.1-48.6	98.7	85.2-112.5	3.582 *ns*

ns = not significant (P>0.05)

Table

Table 3. Current knowledge on the insecticidal activity of the three major constituents of *Origanum syriacum* subsp. *syriacum* essential oil: carvacrol, *p*-cymene and γ-terpinene.

Compound	**Insect species**	**LC$_{50}$ (ppm)**	**References**
Carvacrol	*Anopheles stephensi*	21.2	Traboulsi et al., 2002 Pavela, 2008 Pavela, 2014 Govindarajan et al., 2016
	Anopheles subpictus	24.1	
	Culex quinquefasciatus	26.1	
	Culex tritaeniorhynchus	28.0	
	Culex pipiens molestus	$^{-1}$37.6	
	Musca domestica	78.3 μg adult^{-1}	
	Spodoptera littoralis	15 μg larva^{-1}	
p-cymene	*Aedes aegypti*	19.2	Pavela, 2008 Cheng et al., 2009 Pavela et al., 2017
	Aedes albopictus	46.7	
	Culex quinquefasciatus	20.6	
	Musca domestica	282.1 μg adult^{-1}	
γ-terpinene	*Aedes aegypti*	30.7	Pavela, 2008 Cheng et al., 2009 Pavela et al., 2017
	Aedes albopictus	29.8	
	Culex quinquefasciatus	16.7	
	Musca domestica	248.3 μg adult^{-1}	

Table

Table 4. Lack of toxicity of the essential oil from *Origanum syriacum* subsp. *syriacum* on non-target third instar larvae and adults of *Harmonia axyridis*.

Concentration of *O. syriacum* subsp. *syriacum* essential oil (mL.L^{-1})	Larval mortality (%±SD)	Adult mortality (%±SD)
3.8	0.0±0.0^{a}	0.0±0.0^{a}
2.0	0.0±0.0^{a}	0.0±0.0^{a}
1.0	0.0±0.0^{a}	0.0±0.0^{a}
α-cypermethrin (positive control)	100.0±0.0^{b}	100.0±0.0^{b}
Water (negative control)	0.0±0.0^{a}	0.0±0.0^{a}
ANOVA	$F_{4,15}$=1228; P<0.0001	$F_{4,15}$=1218; P<0.0001

* Means±SD within a column followed by the same letter do not differ significantly (Tukey's HSD test, P<0.05)
% = arcsine square root transformed data
Positive control = 1 mL.L^{-1} Vaztak® (0.05 mL.L^{-1} (w/v)) of *α*-cypermethrin. $F_{d.f.}$ = F-value and *d.f.*=numerator and denominator degrees of freedom.

Table

Table 5. Lack of toxicity of the essential oil from *Origanum syriacum* subsp. *syriacum* on non-target *Eisenia fetida* earthworms.

Tested substance and concentration ($mg.kg^{-1}$)	7th day* (mortality % ± SD)	14th day* (mortality % ± SD)
O. syriacum subsp. *syriacum* essential oil 200.0	$0.0±0.0^a$	$0.0±0.0^b$
O. syriacum subsp. *syriacum* essential oil100.0	$0.0±0.0^a$	$0.0±0.0^b$
O. syriacum subsp. *syriacum* essential oil 50.0	$5.0±2.5^a$	$5.0±2.5^b$
α-cypermethrin 50.0	$100.0±0.0^a$	$100.0±0.0^b$
α-cypermethrin 25.0	$100.0±0.0^b$	$100.0±0.0^b$
α-cypermethrin 12.5	$89.5±2.5^b$	$95.5±2.5^b$
Control	$0.0±0.0^a$	$5.0±2.5^a$
ANOVA	$F_{6,21}$=398.36; *P*<0.0001	$F_{6,21}$=542,18; *P*<0.0001

*Average mortality of *E. fetida* (±SD) achieved on the 7th and 14th day after application of essential oil from *O. syriacum* subsp. *syriacum* and *α*-cypermethrin (positive control).

Means±SD within a column followed by the same letter do not differ significantly (Tukey's HSD test, *P*<0.05).

% = arcsine square root transformed data.

Negative control = water.

Figure

Fig. 1. GC-MS chromatogram of *Origanum syriacum* subsp. *syriacum* leaf essential oil.