Fumigant toxicity of *Petroselinum crispum* L. (Apiaceae) essential oil on *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) adults under greenhouse conditions

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Abstract: *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is one of the most harmful, world-wide known pests of greenhouse crops and ornamental plants. This insect feeds on plant sap, produces honeydew, and transmits plant viruses, while causing quantitative and qualitative damage to plants. For controlling this pest in greenhouses, plant essential oils are used as an alternative to chemical insecticidal. So in this study, fumigant toxicity of *Petroselinum crispum* L. (Apiaceae) plant oil on the above-mentioned adult pest was investigated. Dry seeds were ground and subjected to hydrodistillation using a modified Clevenger-type apparatus and the resulting oil contained myristicin (42.65%), *β*-phellandrene (21.83%), *p*-1,3,8-menthatriene (9.97%), and *β*-myrcene (4.25%). All bioassay tests were conducted at 27±2°C, 65±5% relative humidity (RH) and at a photoperiod of 16 : 8 h (light : dark). This research was performed in a completely randomised design with six treatments (five different concentrations of essential oils plus the control). Each concentration included three replicates and each replicate consisted of 20 adult pests. The results showed that the aforementioned essential oil showed significant mortality of adults 24 h after exposure. The value LC_{50} of the mentioned plant oil on *T. vaporariorum* was 2.41 μl/l air. And mortality percentage showed higher sensitivity of *T. vaporariorum* against the application of the essential oil. The value LT_{50} estimated for *T. vaporariorum* in a concentration of 2.41 μl/l air was 8.17 h. The fumigant toxicity of this essential oil had an ordered relationship with the concentration and time exposure. The results of this research showed that the mentioned plant oil had appropriate insecticidal effects on these greenhouse pests. The findings showed that *P. crispum* oil had a high impact on the above-mentioned pest, and its use is suggested because of its high potential fumigant toxicity. The oil of *P. crispum* may be used in integrated pest management programmes in greenhouses.

Key words: bioassay, chemical compounds, essential oils, fumigant toxicity, *Petroselinum crispum*, *Trialeurodes vaporariorum*

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is an important pest that causes damage to greenhouse vegetable crops (Malais and Ravensberg 2004). This pest has been an economically important insect pest for many years. In temperate countries, the most severely affected crops are cucumbers, beans, sweet peppers, tomato plants, and a large number of ornamentals including chrysanthemum, poinsettia, primula, and species of Fuchsia Gerbera, Pelargonium, and Solanum (Capinera 2008). Whiteflies are cosmopolitan phloem-feeding pests that cause serious damage to many crops worldwide due to direct feeding and vectoring of many plant viruses. They also excrete sticky honeydew which may spoil some commodities and they may cause leaf yellowing or death of the host (Skaljac *et al*. 2010). Management of heavy whitefly infestations is very difficult. Crop damage results from phloem feeding and honeydew secretion. Phloem feeding by large populations of *T. vaporariorum* retards plant vigour. The production of copious amounts of honeydew on leaves and fruits stimulates the growth of sooty mould fungi. Large amounts of fungal growth inhibit the photosynthetic processes of leaves and leads to a lowered fruit value (Skaljac *et al*. 2010).

Cucurbits, such as cucumbers, tomatoes, and beans plants are important in the basket of family food harvested fresh daily. The overuse of insecticides can be detrimental to the health of consumers. In any case, the conventional method for controlling whiteflies in many countries, especially Iran, still includes the use of pesticides (Palumbo *et al*. 2001).

To reduce the damage caused by insects, synthetic insecticides are used routinely. These synthetic compounds cause undesirable effects, such as damage to the ozone layer, pollution of the environment, toxicity of non-target organisms, increased resistance in pests, and a misplaced chemical effect (Ogendo *et al*. 2003).

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Such hazards and problems related to the use of chemical pesticides are the cause for increased environmental limitations (Pavela *et al*. 2010). Thus, we need alternative control methods which are environmentally friendly (Tapondjou *et al*. 2002). As a result, natural products have attracted the attention of researchers worldwide (Kebede *et al*. 2010).

At present, approximately 3,000 essential oils are known; 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries (Bakkali *et al*. 2008). Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry, without showing the same secondary effects (Sosa and Tonn 2008). Essential oils and some of their compounds are toxic against a variety of organisms including bacteria, viruses, fungi, protozoan parasites, mites, snails, and insects (Lahlou and Berrada 2001; Papachristos and Stamopoulos 2002; Duschatzky *et al*. 2005; Basile *et al*. 2006; Cavaleiro *et al*. 2006; Liu *et al*. 2006; Moon *et al*. 2006; Priestley *et al*. 2006; Rim and Jee 2006; Schelz *et al*. 2006; Soylu *et al*. 2006; Yazdani *et al*. 2014). Pesticides remain in the environment without degrading. For these reasons, it is better to use botanical pesticides as they do not have secondary effects.

The parsley plant (*Petroselinum crispum* L.) belongs to the Apiaceae (=Umbellifera) family. It is a native of Europe and Western Asia (Jaswir *et al*. 2000). The fresh as well as the dried leaves are used for flavoring food. Essential oil is obtained from the leaves and seeds (Petropoulos *et al*. 2004). Parsley is known as an aromatic, spice as well as herb used for flavoring food (Pandey *et al*. 2009).

Many studies have been done on the insecticidal properties of botanical products, some of which we will point out. Knio *et al*. (2008) studied the fumigant toxicity of essential oil on *Ochlerotatus caspius* (Pallas) (Diptera: Culicidae) larvae. They found out which mentioned oil provided good, effective control of this insect.

Research into the insecticidal effect of different essential oils on whitefly has also been made. For example, the insecticidal effects of *Thymus vulgaris* L. and *Mentha piperita* L. on *T. vaporariorum* have been reported (Arouiee *et al*. 2005). The researchers found that *T. vaporariorum* is sensitive against oil application.

The insecticidal properties of essential oil from lemon peel *Citrus aurantifolia* Hook on *T. vaporariorum* has also been studied (Delkhoon *et al*. 2013). The researchers demonstrated the sensitivity of the mentioned pest when essential oil was applied.

The present study was conducted to determine the efficiency of essential oil from *P. crispum* as a fumigant in the management of *T. vaporariorum*. No previous study has been reported concerning the activity of these compounds as fumigants against this pest. In this study, we wanted to eliminate the negative environmental effects of pesticides and to promote the advantages of using compounds of natural origin for controlling plant pests. For the first time, ajowan *P. crispum* was used on the major greenhouse pest. Fumigation control was done in sealed containers using the method of Kéita (Kéita *et al*. 2001).

Materials and Methods

Collection and drying of the plant sample

Seeds of parsley were collected from Urmia in Western Azerbaijan province. The seeds were taken to the lab and in dark conditions with suitable ventilation provided, were dried. A temperature of –24°C was maintained.

Extraction of essential oil

Parsley seeds were crushed with the help of a compressor apparatus. Each time, 600 ml of distilled water and a Clevenger apparatus was used on 100 g of powdered seed to extract the essential oil. The time of the operation was 120 min. Sodium sulfate was used to bring out water from the extracted oil. The oil was kept in 5 ml glass containers with aluminum covers, in the refrigerator at 4°C and the oil in the containers was kept away from light. The compounds of the essential oil were determined using the method of gas chromatography, at the biotechnology institute of Urmia University.

Rearing of insects

Greenhouse whitefly was grown on tomato plants, at a temperature of 27±2°C, 65±5% relative humidity (RH) and at a photoperiod of 16 : 8 h (light : dark), in a greenhouse.

Making sure that *T. vaporariorum* insects were of the same biological age

In each experiment, the test results needed to be accurate and reliable so that the insects would be of the same biological age. So, according to the method of Muñiz and Nombela (2001), a small cage was used for the bioassay study of *T. vaporiorum*; slight changes were made. Disposable glass was used. To cover the glass doors, a Petri dish with a diameter of 8 cm was used. In the center of the Petri dish, a hole was made and a 50 grid, double mesh was put on to avoid ventilation problems. When the plant reached the four-leaf stage, 30 adult insects that were collected by aspirator from the rearing place, were transferred to cages, until the insects spawned.

After 48 h, whitefly adults were collected from the leaves of the plant by aspirator without damaging the petioles and eggs.

Determining the 50% (LC_{50}) lethal concentration

For the 50% lethal concentration, the mentioned doses of essential oil were put through three replications of filter paper, and into glass dishes which were 305 ml. The dished contained 20 adult insects, along with a nutritional substance (tomato leaf). The glass was sealed with parafilm to prevent any loss of essential oil. Mortality was recorded after 24 h had passed from the time of the exposure to the different concentrations. The insects incapable of moving after a slight touch with a fine brush were considered to be dead (Choi *et al*. 2003).

Determining the 50% (LT_{50}) lethal time

The 305 ml glass contained 10 adults (1–2 days old) along with a nutritional substance (tomato leaf) (Fig. 1). The correct doses of essential oil were put through filter paper and used as the plug for the glass. Parafilm was used to seal the glass. Insect mortality was checked in successive times. The experiment was done in three replicates.

Fig. 1. Glass dishes with nutrition substance to determine LC_{50} and LT_{50}

Data analysis

The mortality was surveyed after 24 h of treatment. The number of dead insects in the treatment and in the control was counted. The mortality percentage was calculated according to Abbott's formula (Abbott 1925). In this experiment, the insects that were incapable of moving after slightly touching their legs and antennae with a hot needle, were considered dead. This research was done at a temperature of 27±2°C and at a relative humidity of 65±5%. The data archived with the SPSS (V. 20) program were analysed (SPSS 2011). After 24 h, the LC_{50} value was calculated for the oil. For the mean comparison of the effect values of different essential oil concentrations, Tukey's test at 1% with a statistical confidence level of 99% was used.

Results

The results of the experiments showed that bioassays determined that the fumigant toxicity of parsley plant essential oil created a significant effect on the greenhouse whitefly. An estimation of the LC_{50} value was done 24 h after exposure of the greenhouse whitefly to 2.41 µl/l air (Table 1).

These values depended on the oil concentration and exposure time. Given the results of research from data of the dose, one-way analysis of variance (ANOVA) obtained F value with four degrees of freedom as 34.230 that showed a significant difference between treatments at a 1% probability level (Table 2).

The mean effect value of *P. crispum* essential oil on *T. vaporariorum* adults is shown on table 3.

The results of bioassay tests showed that with LC_{50} of oil (2.41 µl/l air), the value of LT_{50} for the greenhouse whitefly was reported as 8.17 (Table 4).

Given the results of research from the time data, one-way ANOVA, the obtained F value with four degrees of freedom was 48.737 and showed a significant difference between treatments at a 1% probability level (Table 5).

Table 1. LC_{50} value of *P. crispum* essential oil on *T. vaporariorum* adults during a 24 h time period

Insect species	Insect number	χ^2(df)	Intercept(a)+5	Slope ±SE	LC_{50} [µl/l air]*
T. vaporariorum	300	1.79(3)	3.13	4.91±0.54	2.41 (2.22–2.59)

*lower and upper 95% fiducially limits are shown in parenthesis

Table 2. ANOVA analysis of the effect of *P. crispum* essential oils against *T. vaporariorum* adults in various concentrations

Source	df	Sum of square	Mean of square	F
Concentration	5	10,505.180	2,101.036	34.230**
Error	12	736.560	61.380	–
Total	17	11,241.740	–	–

**statistic level of 1%

Table 3. Comparing the means of the effect value *P. crispum* essential oil on *T. vaporariorum* adults

Concentrations [µl/l air]	Loss of concentration [%]	Classification to groups*
1.31	15.19	C
1.97	36.85	BC
2.62	45.00	B
3.28	62.29	AB
3.93	68.66	A

*using Tukey's test at a statistic level of 1%

Table 4. LT_{50} value of *P. crispum* essential oil on *T. vaporariorum* adults

Insect species	Insect number	χ^2(df)	Intercept(a)+5	Slope ±SE	LT_{50} [h]
T. vaporariorum	300	10.20(3)	3.03	2.16±0.24	8.17 (6.77–9.68)

Table 5. ANOVA analysis of the effect of *P. crispum* essential oils against *T. vaporariorum* adults in different time periods

Source	df	Sum of squared	Mean square	F
Concentration	5	7,871.849	1,574.370	48.737**
Error	12	387.642	32.303	–
Total	17	8,259.491	–	–

**statistic level of 1%

Table 6. Comparing the mean of effect value exposure time of *P. crispum* essential oil on *T. vaporariorum* adults

Time [min]	Loss of concentration [%]	Classification to groups*
120	21.28	C
420	34.21	BC
720	43.56	B
1,080	54.43	AB
1,440	64.46	A

*using Tukey's test at a statistic level of 1%

A comparison of the mean effect value of the exposure time of *P. crispum* essential oil on *T. vaporariorum* adults is shown on table 6. During the first exposure time of 120 min, there was a 21% lethal mortality. When the time was changed, the mortality increased.

According to a gas chromatographic analysis of essential oils (GC/MS), it was determined that the *P. crispum* oil consists of 33 chemical compounds. The most important compounds that have caused fumigation are: myristicin (42.65%), *β*-phellandrene (21.83%), *p*-1,3,8-menthatriene (9.97%), and *β*-myrcene (4.25%). The analysis of substances that exist in *P. crispum* oil is given on table 7. The analysis of the results of this research was conducted on plant parsley by Vokk *et al.* (2011) and is fully consistent with substances that exist in the plant. There is evidence supporting the octopaminergic system as a target for some monoterpenoids (Enan 2001). Several reports suggested that insect mortality was caused by the inhibitory effects of monoterpene on the enzyme acetylcholinesterase (Houghton *et al*. 2006).

Discussion

Experimental results indicate that essential oils have a strong mortality effect on the greenhouse whitefly. Toxicological properties, of the compounds in the essential oils used on different pests, have been reported in scientific resources. The toxicological properties of the compounds can have various effects on the respiratory and gastroenterology systems of the insects. Fahim *et al*. (2012) evaluated the susceptibility of egg, nymph, and adult of *T. vaporariorum* to two botanical oils of peppermint (*Mentha spicata* L.) and cumin (*Cuminum cyminum* L.) *in vitro*. The results showed a mortality value for *M. spicata* oil calculated against egg, larvae, and adult in 0.012 μl/ml, to be 72, 86, and 83%, respectively, and 0.016 μl/ml for *C. cyminum* showed mortality to be 65, 81, and 78%, respectively. The results of the study showed that peppermint had a more insecticidal effect than cumin. First instar nymphs are more sensitive than the eggs and adults to both of the essential oils. This research is in agreement with the results of previous research concerning the sensitivity of *T. vaporariorum* to the application of essential oil. Arouiee *et al*. (2009) investigated the insecticidal activity of the essential oils of three different medicinal plants (caraway, fennel) for the control of *T. vaporariorum*. The results indicated that the most effective essential oils came from caraway (*Carum carvi* L.) and fennel (*Foeniculum vulgare* Mill.). These oils were active at concentrations of 7.5 and 5 ppm, respectively. This study is in agreement with the results of the previous research concerning the sensitivity of *T. vaporariorum* to the application of oils. Also, Delkhoon *et al*. (2013) determined the effect of essential oil derived from lemon peel, *C. aurantifolia* (Hook), on the mortality of egg, first instar nymph, and adult oviposition of *T. vaporariorum* under laboratory conditions. They found that *C. aurantifolia* oil could be used as an effective and environmentally sustainable bioinsecticide for the control of *T. vaporariorum.* This study is in agreement with the results of studies on the sensitivity of *T. vaporariorum* to essential oil application. In another experiment, Soliman (2006) studied the insecticidal effect of *Artemisia herba-alba* (Asso) and *A. monosperma* (Delile) oils on three species of sucking pests: cotton aphid (*Aphis gossypii* Glover; Hemiptera: Aphididae), whitefly (*Bemisia tabaci* Gennadius; Homoptera: Aleyrodidae), and onion thrips (*Thrips tabaci* Lindeman; Thysanoptera: Thripidae) in greenhouse conditions. The results of these tests showed that essential oils have a more lethal effect on *T. tabaci* and *A. gossypii* than *B. tabaci*. This study also shows a lethal effect on

Table 7. Chemical composition of parsley essential oil identified by Gas Chromatography-Mass Spectroscopy

No.	Component	Percentage
1	Sabinene	0.14
2	α-Copaene	0.21
3	2,5-Dimethyl-*p*-cymene	0.66
4	α-Pinene	1.49
5	2,5-Dimethoxy-*p*-cymene	0.90
6	β-Pinene	0.90
7	(E)-β-Caryophyllene	0.31
8	β-Ionone	0.07
9	β-Myrcene	4.25
10	α-Phellandrene	1.22
11	Germacrene D	0.12
12	Limonene	1.97
13	β-Phellandrene	21.83
14	*p*-Cymene	0.35
15	(E)-β-Ocimene	0.27
16	γ-Terpinene	0.21
17	*p*-Cymenene	2.68
18	Terpinolene	0.80
19	*p*-1,3,8-Menthatriene	9.97
20	α-Bergaptene	0.29
21	Myristicin	42.65
22	α-Cadinol	0.06
23	α-Muurolene	0.65
24	Elemicin	0.15
25	Linalool	0.04
26	Germacrene D-4-ol	0.24
27	Aipha-terpineol	0.54
28	δ-Cadinol	0.06
29	Estragol	0.26
30	Bornyl acetate	0.25
31	Apiole	0.11
32	(E,E)-Decadienal	2.65
33	Phthalide isomer	< 0.01

B. tabaci, though this pest belongs to the Aleyrodidae family. There is a similar sensitivity between members of a family. In this respect, our study is similar to this study. The research of Aslan *et al*. (2004) showed the effects of the essential oils of three species of plant: *Satureja hortensis* L., *Ocimum basilicum* L., and *T. vulgaris* on adults and nymphs of *B. tabaci*. While the insecticidal activity of the three plant oils was demonstrated, *S. hortensis* oil showed the most controlling effect in comparison with the other two species. The sensitivity between the members of the family is similar. Thus, the sensitivity of *B. tabaci* is equal to *T. vaporariorum*. Our study agrees with this research. Knio *et al.* (2008) studied the fumigant toxicity of essential oil on *Ochlerotatus caspius* larvae. They studied which of the above-mentioned oils had a good control effect on on *Ochlerotatus caspius* larvae. Our research conforms with the results of these researchers concerning the sensitivity of *T. vaporariorum* to the application of oils.

In our research, for the first time, fumigant toxicity of *P. crispum* on *T. vaporariorum* was studied. The results of this experiment show that this essential oil has a good control effect on the *T. vaporariorum* adult and this natural material is safe for the environment and other mammals. The environment is affected less when using essential oils than when using pesticides and their toxins. The application of the essential oils is easy. The oils are suitable replacements for chemical toxins in the control of pests in small greenhouses.

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