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Research Article

Chemical Composition and Acaricidal Effects of Essential Oils of *Foeniculum vulgare* Mill. (Apiales: Apiaceae) and *Lavandula angustifolia* Miller (Lamiales: Lamiaceae) against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Utilization of synthetic acaricides causes negative side-effects on nontarget organisms and environment and most of the mite species such as two spotted spider mite, *Tetranychus urticae* Koch, are becoming resistant to these chemicals. In the present study, essential oils of fennel, *Foeniculum vulgare* Mill., and lavender, *Lavandula angustifolia* Miller, were hydrodistilled using Clevenger apparatus and chemical composition of these oils was analyzed by GC-MS. Anethole (46.73%), limonene (13.65%), and α-fenchone (8.27%) in the fennel essential oil and linalool (28.63%), 1,8-cineole (18.65%), and 1-borneol (15.94%) in the lavender essential oil were found as main components. Contact and fumigant toxicity of essential oils was assessed against adult females of *T. urticae* after 24 h exposure time. The essential oils revealed strong toxicity in both contact and fumigant bioassays and the activity dependeds on essential oil concentrations. Lethal concentration 50% for the population of mite (LC_{50}) was found as 0.557% (0.445–0.716) and 0.792% (0.598–1.091) in the contact toxicity and 1.876 μL/L air (1.786–1.982) and 1.971 μL/L air (1.628–2.478) in the fumigant toxicity for fennel and lavender oils, respectively. Results indicated that *F. vulgare* and *L. angustifolia* essential oils might be useful for managing of two spotted spider mite, *T. urticae*.

1. Introduction

Spider mites belong to the family Tetranychidae and are named because many members of this family produce silk webbing on the host plants. Some 1,200 species of spider mites belonging to over 70 genera are known in the world especially in the Southern Hemisphere [1]. Two-spotted spider mite, *Tetranychus urticae* Koch, is widely distributed globally and a common pest of many plant species in greenhouses, orchards and field crops. To date, 3877 host species have been reported around the world in both outdoor crops and greenhouses [2]. *T. urticae* feeding causes graying or yellowing of the leaves and necrotic spots occur in advanced stages of leaf damage. Mite damage to the open flower causes a browning and withering of the petals that resembles spray burn. In addition, small chlorate spots can be formed at feeding sites as the mesophyll tissue collapses due to the destruction of 18–22 cells per minute [2]. The importance of this mite pest is not only due to direct damage to plants but also due to indirect damage to plants which decreases photosynthesis and transpiration [3]. Because of their high reproductive rates, its management can be difficult. When mites begin to feed on a plant, they produce webbing that can protect both motile and egg stages from the acaricide [3].

Synthetic acaricides have been used as the main strategy for *Tetranychus* species resulting in an increased cost for production and environmental impacts as well as resistance development to even the newly synthesized molecules such abamectin [2, 4]. In addition, control methods based on the use of synthetic acaricides sometimes fail to keep the number

of spider mites below economic threshold levels [5]. It is therefore necessary to find alternatives that can minimize negative effects of synthetic acaricides.

Essential oils obtained by hydrodistillation, steam distillation, dry distillation, or mechanical cold pressing of aromatic plants [6] have long been used as fragrances and flavorings in the perfume and food industries, respectively, and recently for aromatherapy and medicines [2]. The essential oils can play major roles in pollination by attracting insects and in water loss's prevention due to excessive evaporation. Repellence is another property of essential oils, as some contain numerous secondary metabolites that can deter attacks from pests [7]. Most essential oil constituents degrade quickly in the environment or are rapidly lost from plant foliage through volatilization, which minimizes residual contact. They have short residual activities due to temperature and UV light degradation and, with a few exceptions, their mammalian toxicity is low [8, 9]. Therefore, essential oils can be applied to both field and greenhouse crops in the same manner as current synthetic acaricides [2, 9].

The fennel, *Foeniculum vulgare* Mill. [Apiales: Apiaceae (Umbelliferae)], is indigenous to the Mediterranean and is largely used to impart flavor to a number of foods, such as soups, sauces, pickles, breads, and cakes. It is an annual, biennial, or perennial herbaceous plant, depending on the variety, which grows in good soils from sunny mild climatic regions and is a well-known aromatic plant species. Traditionally in Europe and Mediterranean areas, fennel is used as antispasmodic, diuretic, anti-inflammatory, analgesic, secretolytic, galactagogue, eye lotion, and antioxidant remedy [10, 11]. The lavender, *Lavandula angustifolia* Miller [Lamiales: Lamiaceae (Labiatae)], is an evergreen bushy shrub with straight, woody branches; the lower parts are leafless, putting out numerous herbaceous stems to a height of about 1 m [12]. It is native to southern Europe and the Mediterranean area and is commercially cultivated in France, Spain, Portugal, Hungary, UK, Bulgaria, Australia, China, and USA [13].

This paper describes a laboratory study examining the contact and fumigant toxicity of essential oils of *F. vulgare* and *L. angustifolia* grown in Iran against of *T. urticae* followed by evaluation of their chemical constituents by Gas chromatography-Mass spectrometry (GC-MS).

2. Materials and Methods

2.1. Rearing of Two-Spotted Spider Mite. The two-spotted spider mites were collected from infested leaves of some wildly grown weeds in the yard of University of Mohaghegh Ardabili which did not have any exposure to acaricides. The *Tetranychus urticae* Koch species after separation, and slide preparation was identified according to introduced keys by Zhang [1]. Spider mites were reared on navy bean (*Vigna unguiculata* Walp. [Fabales: Fabaceae]) plants for one year. The infested plants were held in cages (120 × 300 × 100 cm) covered with mesh cloth. To synchronize the adult stage of *T. urticae* for adulticidal bioassays, 50 adult female mites were transferred to the leaves of trifoliate bean plants (held individually in cylindrical glass containers with appropriate aeration) with a hair brush and allowed to lay eggs for 24 h, after which the adults were removed. The infested leaves were held at the above-mentioned conditions to allow the eggs to hatch and the larvae to develop into synchronized adults. All experiments were carried out at 25 ± 2°C, 60 ± 5% relative humidity (RH) and a photoperiod of 16 : 8 (light : dark) in a growth chamber.

2.2. Plant Materials and Essential Oil Extraction. Aerial parts 5 cm from the top of *L. angustifolia* at flowering stage and seeds of *F. vulgare* were collected from Ardabil, Ardabil province, Iran from June to August 2013. The specimens were air dried in the shade at room temperature and chopped into small pieces with electric grinder. The essential oils were extracted using a Clevenger-type water steam distillation apparatus within 3 h. To carry out the extraction, 100 g of powdered plant material was used along with 1200 mL distillated water. Anhydrous sodium sulphate was used to remove excess water after extraction. The essential oils were transferred to dark brown glass vials covered with aluminum foil and stored in refrigerator at 4°C until used in the experiments.

2.3. Analysis of Essential Oil. One μL of prepared essential oil was injected to GC-MS (HP Agilent 6800N/(61530N) with CPSil5CB column (Chrompack, 100% dimethyl polysiloxane 60 m, 0.25 mm (ID) film thickness 0.25 l). The analysis was performed under temperature programming from 100°C (3 min) to 250°C (5 min) with the rate of 3°C/min. Injector temperature was 230°C. Identification of spectra was carried out by study of their fragmentation and also by comparison with standard spectra present in the library of the instrument. Area normalization was used for determination of composition percentage.

2.4. Contact Toxicity. Contact toxicity was conducted in Petri dishes (6 cm diameter). Concentrations ranged from 0.12% to 2.8% for *L. angustifolia* and 0.11% to 1.7% for *F. vulgare*, using a spreader sticker adjuvant (20 μL Tween, 0.02%) diluted in distilled water. Leaf discs (3 cm diameter) were cut from leaves of greenhouse-grown *V. unguiculata* and immersed in solutions of the each essential oil for 20 seconds. After drying at room temperature for 45 min, each disc was individually placed at the bottom of a Petri dish atop a 6 cm diameter disc of filter paper wetted with distilled water. The wet cotton pads were then placed on excised leaves and ten adult females were transferred thereafter. The lids of Petri dishes were pierced (1 cm in diameter) and their openings were covered with mesh cloth in order to evade of fumigant toxicity. Control mites were held on leaf discs immersed in dilutions without essential oils. Mortality was noted after 24 h and there were tree replicates for each treatment. Mites were considered dead if appendages did not move when prodded with a fine paintbrush.

2.5. Fumigant Toxicity. For evaluation of fumigant toxicity, 750 mL plastic containers with tight lids were used as the test chambers. Each treatment consisted of five concentrations of the essential oil and a control. Based on preliminary experiments, ranges of concentrations tested against the adult

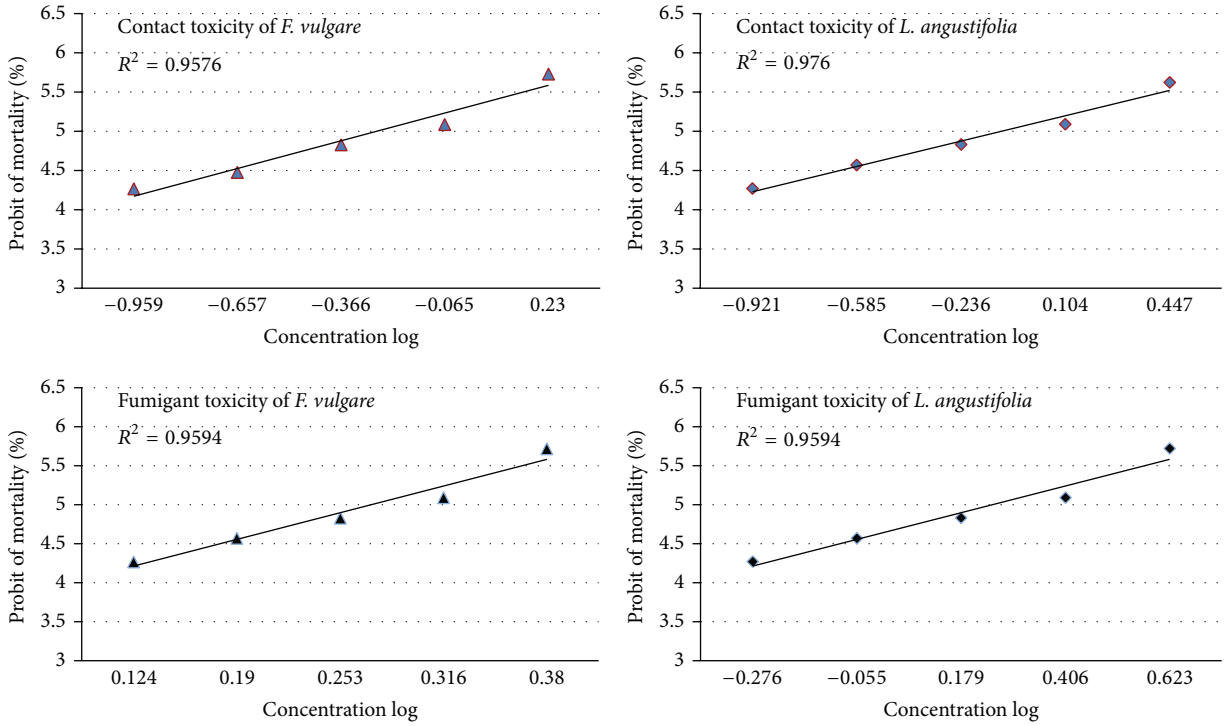

Figure 1: Concentration-mortality response lines for adult females of *Tetranychus urticae* exposed to different concentrations of *Foeniculum vulgare* and *Lavandula angustifolia* essential oils.

females were 0.53 to 4.2 and 1.33 to 2.4 μL/L air for *L. angustifolia* and *F. vulgare*, respectively. For each concentration, three replicates were used. Each replicate consisted of sixty 24 h old adult females on each leaf plant disc. The discs (3 cm in diameter) punched from leaves of bean plants were placed inside a 6 cm diameter plastic Petri dishes without lids lined with water-soaked cotton. The Petri dishes were then put into plastic containers used as fumigation chambers. To achieve the desired concentration of the oil in the fumigation chambers, using a micropipette, the appropriate volume of the oil was applied on a 2 × 2 cm strip of Whatman no. 1 filter paper adhered to the inner surface of the fumigation chamber. The exposure period for assessing the adulticidal effect of the essential oils was 24 h. To determine mortality, the mites were touched with the tip of a fine hair brush. If the mite did not move, it was considered dead. The controls consisted of the same number of mites as the treatments; and were kept under the same conditions on leaf disks left untreated.

2.6. Analysis of Data. Experiments were arranged in a completely randomized design and data were analyzed by ANOVA. The mortality data were subjected to probit analysis using SPSS software to estimate LC_{50} values of the essential oils against *T. urticae*.

3. Results

Using hydrodistillation process, fennel seed yielded 2.15% essential oil while lavender leaf yielded 2.01%. Results of analysis of the essential oils are presented in Table 1. Twenty six compounds were identified in the essential oil of fennel, representing 99.94% of the total essential oil sample while twenty five compounds were found in the lavender essential oil, representing 99.97% of the total essential oil sample. The major components were found to be anethole (46.73%), limonene (13.65%), α-fenchone (8.27%), carvone (6.12%), and estragole (5.26%) for *F. vulgare* essential oil and Linalool (28.63%), 1,8-Cineole (18.65%), 1-Borneol (15.94%), Camphor (8.20%), and Terpineol-4 (4.27%) for *L. angustifolia* essential oil. Amounts of monoterpenes hydrocarbon in the essential oils of *F. vulgare* and *L. angustifolia* were 20.43% and 8.55%, respectively. Monoterpenoids content of *F. vulgare* and *L. angustifolia* essential oils was 20.27% and 83.36% and the sesquiterpenes were 0.44% and 2.38%, respectively.

F. vulgare and *L. angustifolia* essential oils revealed strong significant toxicity on the adult females of *T. urticae* in both contact and fumigant assays. The activity depended on essential oil concentrations in both contact and fumigant bioassays and increased susceptibility of mite was directly associated with oil concentration (Table 2 and Figure 1).

In the contact toxicity, lethal concentration 50% mite mortality (LC_{50}) was 0.557% and 0.792% with *F. vulgare* and *L. angustifolia*, respectively, with *F. vulgare* essential oil being the most toxic the adult females of *T. urticae* (Table 2). On the other hand, fumigant toxicity was 1.876 and 1.971 μL/L air for *F. vulgare* and *L. angustifolia* essential oils, respectively (Table 2).

4. Discussion

In the present study, anethole, limonene, α-fenchone, and carvone were the major compounds of essential oil of

Table 1: Chemical analysis of essential oils of *Foeniculum vulgare* and *Lavandula angustifolia* grown in Iran by GC-MS.

Compound	*F. vulgare*		*L. angustifolia*		Formula	Molecular weight (g/mol)	Classification
	RT	Percentage	RT	Percentage			
α-Pinene	3.93	3.53	4.80	1.46	$C_{10}H_{16}$	136.23	Monoterpene
Camphene	4.17	0.32	5.08	0.68	$C_{10}H_{16}$	136.23	Monoterpene
Sabinene	4.51	0.67	5.54	0.96	$C_{10}H_{16}$	136.23	Monoterpene
β-Pinene	4.61	0.20	5.64	2.16	$C_{10}H_{16}$	136.23	Monoterpene
Myrcene	4.74	1.09	5.84	1.39	$C_{10}H_{16}$	136.23	Monoterpene
α-Phellandrene	5.05	0.54	—	—	$C_{10}H_{16}$	136.23	Monoterpene
Limonene	5.61	13.65	—	—	$C_{10}H_{16}$	136.23	Monoterpene
1-Octen-3-ol	—	—	5.73	0.46	$C_8H_{16}O$	128.21	Alcohol
γ-Terpinene	6.06	0.43	—	—	$C_{10}H_{16}$	136.23	Monoterpene
δ-3-Carene	—	—	6.27	0.72	$C_{10}H_{16}$	136.23	Monoterpene
α-Fenchone	6.83	8.27	—	—	$C_{10}H_{16}O$	152.23	Monoterpenoid
1,8-Cineole	—	—	6.87	18.65	$C_{10}H_{18}O$	154.25	Monoterpenoid
trans-β-Ocimene	—	—	7.10	0.98	$C_{10}H_{16}$	136.23	Monoterpene
Terpineol-4	—	—	7.58	4.27	$C_{10}H_{18}O$	154.25	Monoterpenoid
Terpinolene	—	—	7.99	0.50	$C_{10}H_{16}$	136.23	Monoterpene
Linalool	—	—	8.64	28.63	$C_{10}H_{18}O$	154.25	Monoterpenoid
cis-Sabinenehydrate	—	—	8.99	0.51	$C_{10}H_{18}O$	154.25	Monoterpenoid
Camphor	7.92	0.28	9.58	8.20	$C_{10}H_{16}O$	152.23	Monoterpenoid
Estragole	9.12	5.26	—	—	$C_{10}H_{12}O$	148.20	Aromatic hydrocarbon
trans-Dihydrocarvone	9.24	0.47	—	—	$C_{10}H_{16}O$	152.23	Monoterpenoid
D-Fenchyl alcohol	9.49	0.18	—	—	$C_{10}H_{18}O$	154.25	Monoterpenoid
Fenchol	9.83	1.78	—	—	$C_{10}H_{18}O$	154.25	Monoterpenoid
1-Borneol	—	—	10.17	15.94	$C_{10}H_{18}O$	154.25	Monoterpenoid
Carvone	10.18	6.12	—	—	$C_{10}H_{14}O$	150.22	Monoterpenoid
l-Carvone	10.26	3.17	—	—	$C_{10}H_{14}O$	150.22	Monoterpenoid
Hexyl butyrate	—	—	10.43	1.56	$C_{10}H_{20}O_2$	172.26	Fatty ester
Cryptone	—	—	10.51	0.58	$C_9H_{14}O$	138.21	Ketonic chelate
α-Terpineol	—	—	10.61	3.25	$C_{10}H_{18}O$	154.25	Monoterpenoid
Anethole	11.23	46.73	—	—	$C_{10}H_{12}O$	148.21	Aromatic hydrocarbon
Bornyl formate	—	—	11.29	0.61	$C_{11}H_{18}O_2$	182.26	Ethyl ester
Linalyl acetate	—	—	11.83	2.18	$C_{12}H_{20}O_2$	196.29	Monoterpenoid
Geraniol acetate	—	—	12.55	1.05	$C_{12}H_{20}O_2$	196.29	Monoterpenoid
Eugenol	12.72	3.75	—	—	$C_{10}H_{12}O_2$	164.20	Carboxylic Acid
Thymol	—	—	12.80	0.68	$C_{10}H_{14}O$	150.28	Monoterpenoid
cis-Jasmone	13.09	0.69	—	—	$C_{11}H_{16}O$	164.24	Fatty Acid
Anisyl acetone	13.17	0.36	—	—	$C_{13}H_{16}O_2$	204.27	Ketonic ether
β-Caryophyllene	13.90	0.22	—	—	$C_{15}H_{24}$	204.35	Sesquiterpene
Germacrene d	15.11	0.22	—	—	$C_{15}H_{24}$	204.35	Sesquiterpene
Eugenyl acetate	15.96	0.91	—	—	$C_{12}H_{14}O_3$	206.24	Carboxylic Acid
β-Farnesene	—	—	16.05	0.81	$C_{15}H_{24}$	204.35	Sesquiterpene
Benzeneacetic acid	17.07	0.17	—	—	$C_8H_8O_2$	136.15	Carboxylic Acid
cis-isoapiole	17.85	0.28	—	—	$C_{12}H_{14}O_4$	222.24	Phenylpropanoids
α-Bisabolol	—	—	20.56	1.57	$C_{15}H_{26}O$	222.37	Sesquiterpenoid
α-ethyl-4,4-dimethoxy-Stilbene	30.91	0.64	—	—	$C_{16}H_{16}O_2$	240.30	Ketonic chelate
1,2-Benzenedicarboxylic acid	—	—	33.67	2.17	$C_8H_6O_4$	166.13	Carboxylic Acid

Table 1: Continued.

Compound	*F. vulgare*		*L. angustifolia*		Formula	Molecular weight (g/mol)	Classification
	RT	Percentage	RT	Percentage			
Monoterpene hydrocarbons		20.43		8.85			
Oxygenated monoterpenes		20.27		83.36			
Sesquiterpene hydrocarbons		0.44		0.81			
Oxygenated sesquiterpenes		0		1.57			
Others		58.8		5.38			
Total		99.94		99.97			
Yield		2.15		2.01			

RT: retention time (min).

Table 2: Contact and fumigant toxicity of the essential oils isolated from *Foeniculum vulgare* and *Lavandula angustifolia* against the adult females of *Tetranychus urticae*.

Bioassay	Essential oil	Results of ANOVA		Results of probit analysis				Toxicity index
		F (df = 4, 10)[a]	P value	24-h LC_{50} with 95% confidence limits[b]	Slope ± SE	χ^2 (df = 3)	Sig.[c]	
Contact toxicity	*F. vulgare*	49.75	1.4446	0.557 (0.445–0.716)	1.181 ± 0.144	3.327	0.351	100.00
	L. angustifolia	33.30	9.3842	0.792 (0.598–1.091)	0.936 ± 0.124	1.620	0.655	0.703
Fumigant toxicity	*F. vulgare*	37.70	0.000005	1.876 (1.786–1.982)	5.335 ± 0.670	3.086	0.379	100.00
	L. angustifolia	18.167	0.0001	1.971 (1.628–2.478)	1.377 ± 0.187	3.975	0.264	0.951

[a]Calculated values are greater than values in F table ($\alpha = 0.05$, F credit = 3.4780). Therefore, they are significant.
[b]% v/v and μL/L air for contact and fumigant toxicity, respectively.
[c]Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits.

F. vulgare while linalool, 1,8-cineole, 1-borneol and camphor were the main compounds of essential oil of *L. angustifolia*. In the study of Chowdhury et al. [14], anethole (58.5% in seed oil and 51.1% in leaf oil) and limonene (22.9% in leaf oil and 19.6% in seed oil) determined as main components of the seeds and leaves of *F. vulgare*. In the other study, borneol, α-terpinene, linolool, and geranyl proprionate were found as major constituents in the *L. angustifolia* essential oil. The variations could be due to differences in location, elevation, and genetic makeup of the plant or due to an adaptive process to particular ecological conditions [15].

Acaricidal activity of *F. vulgare* and *L. angustifolia* essential oils reported in the present study has been reported earlier by other authors. For example, the essential oils from *F. vulgare* and *L. angustifolia* were toxic against *Varroa destructor* Anderson and Trueman (a major pest of honey bees, *Apis mellifera* L.) [16]. The fumigant toxicity of essential oil extracted from seeds of *F. vulgare* was tested against adult females of *T. urticae* by Amizadeh et al. [17].

Essential oils are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. The components include two groups of distinct biosynthetical origin. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight [6]. They are made from combinations of several 5-carbon-base (C_5) units called isoprene. The monoterpenes are formed from the coupling of two isoprene units (C_{10}). They are the most representative molecules of the essential oils and allow a great variety of structures. A terpene containing oxygen is called a terpenoid. Hence, monoterpenes are found as two forms; monoterpenes hydrocarbon and oxygenated monoterpenes or monoterpenoids. The sesquiterpenes are formed from the assembly of three isoprene units (C_{15}) [6]. Monoterpenes hydrocarbon, monoterpenoids, and sesquiterpenes are present in our tested study too. Regarding their biological properties, essential oils are complex mixtures of numerous molecules, and their biological effects are the result of a synergism of all components or reflect only those of the main components present at the highest levels according to gas chromatographical analysis [6, 18]. It is suggested that the variability of biological activities of essential oils extracted from different plant species against *T. urticae* could be due to chemical components, differences in their chemical composition, and even in synergic and antagonistic interactions between these components [2, 19, 20].

One of the most attractive features of essential oils is that they are low-risk products and they are relatively well-studied experimentally and clinically because of their use as medicinal products [11]. In terms of ecotoxicology, essential oils are safe to use but not without potential problems. For example, constituents of essential oils are biodegradable, with short half-lives ranging from 30 to 40 h for α-terpineol [21] and in contrast to some synthetic insecticides; no biomagnification has been reported to date [11]. Their short residual half-lives on plants also enhance their compatibility with biological control agents and indigenous natural enemies of pests and reduce risks to honeybees and other foraging pollinators [2].

As cost effective commercial problems, large quantities of plant material must be processed to obtain sufficient quantities of essential oils for commercial-scale tests, situation which also requires breeding these plants in great quantities.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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