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Phytochemical and Insecticidal Study of the Avishan-e-denaii (*Thymus daenensis* Celak.) Essential Oil against the Melon Aphid (*Aphis gossypii* Glover)

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Abstract: *Aphis gossypii* Glover (Hemiptera: Aphididae) is one of the most important pests of agriculture worldwide. To control the pest population, research on the use of the environmental and plant-based compounds has increased in recent decades. Essential oils due to volatility and very short-term persistence in the environment, as biocompatible pesticides can be considered as one of the best alternatives to chemical pesticides in aphid's control. In the current study, phytochemical and insecticidal toxicity of the essential oil from Avishan-e-denaii, *Thymus daenensis* Celak. was studied against the melon aphid, *Aphis gossypii* Glover. in the laboratory conditions under 25±2°C, 60±5 % RH and 16L:8D photoperiods. The essential oil was obtained by hydrodistillation method, using Clevenger-type apparatus. Mortality was evaluated at different concentrations that ranging from 0.1 to 0.9 µL/L air, and with three replications at the interim of 24 hours. Also nymph production deterrent effect of the oil at sublethal concentration was studied against parthenogenesis form of aphid. Results indicated that essential oil of *T. daenensis* is toxic to the *A. gossypii*. The major components in the oil were 3-Methyl-4-isopropylphenol (26.94 %), thymol (22.40 %), p-cymene (13.81 %), γ-terpinene (6.74 %), borneol (5.24 %) and linalool (4.31 %). Probit analysis showed that the LC_{50} values for nymphs and adults of *A. gossypii* were 0.0087 and 0.0016 µl./L^{-1} air, respectively. Also, degree of nymph production deterrent effect was calculated for essential oil of *T. daenensis* as 46.49 ± 4.71 %. The overall results showed that the Avishan-e-denaii essential oil has high potential in controlling the melon aphid especially in protected cultivation.

Key words: Chemical composition, *Thymus daenensis,* fumigant toxicity, nymph production deterrent, *Aphis gossypii.*

Introduction

The melon aphid, *Aphis gossypii* Glover is one of the major pests of greenhouse cucumber production in Iran. According to Hasanshahi *et al*.[1] about 2.5 million tons of the different pesticides are used for pest control on crops each year and damage caused by pesticides reaches $100 billion annually in the worldwide [2]. The frequent use of chemical insecticides results in negative consequences. The botanical pesticides could be consider as alternative for control of pests in modern ecological technologies. They do not carry the threat for the environment and human health. The spectrum of these products continuously expands that requires recognition of the mechanism of their action [3, 4]. The plant extracts contain alkaloids, esters, glycosides etc. and they have phytopesticide properties [5]. Some of plant substances are used as antifeedants and repellents in pest control management [4].

Herbal essential oils have a wide range of ecologically safe ingredients because their active ingredients are rapidly degraded and can be used as an alternative to traditional chemical pesticides [1,6]. Also, Blair *et al*.[7] found that, the smallest pests, for instance, immature, soft-bodied or delicate

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arthropods, can be killed or weakened once the lipophilic plant oils penetrate their waxy cuticle and the aliphatic and aromatic components of the oils begin to block specific neurotransmitters, growth hormones or digestive enzymes. In previous studies, extensive researches has been done on the biological activity of essential oils, and the results indicate that these compounds have insecticidal, fungal, antibacterial and antimicrobial properties [6, 8, 9, 10].

Many plants essential oils show a broad spectrum of activity against pest insects ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and anti-vector activities. Recent investigations indicate that some chemical constituents of these oils interfere with the octopaminergic nervous system in insects. They cover the criteria for "reduced risk" pesticides. These plant oils are well accepted in the agricultural practice as "green pesticides" that could be effective enough particularly for biological foods production. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential-oil-based pesticides owing to the complex mixtures of constituents that characterize many of these oils [11].

The plant products possess a range of priorities that make them preferable in modern biological agriculture. It is studied the effect of different essential oils and water extracts towards *A. gossypii* [12, 13]. Gorski and Tomczak [14] has examined the efficacy of natural essential oils, such as basil, citronella, eucalyptus, juniper, and patchouli in the control of foxglove aphid, *Aulacorthum solani* Kalt. Insecticidal effects of 23 essential oils against adults of turnip aphid, *Lipaphis pseudobrassicae* Davis. have been studied by Sampson *et al*.[15]. Hori and Komatsu [16] studied repellency of rosemary oil against onion aphid, *Neotoxoptera formosana* Takahashi.

In spite of considerable research efforts in many laboratories around the world and the increase of scientific articles regarding the pesticides properties of herbal essential oils and their compounds, unfortunately, a few pests of agricultural products are controlled on the basis of essential oils. Since application of plant essential oils in greenhouses and fields is not possible in pure form, therefore, it is necessary to prepare commercial formulations of these compounds [17]. As part of future strategies for aphid control, essential oils with deterrence and/or insecticidal properties should be studied. Therefore, the aim of the present work was to study the fumigant toxicity of the Avishan-e-denaii, *Thymus daenensis* Celak. essential oils against nymphs so adults of *A. gossypii*.

Materials and methods

Rearing of aphid

Cucumber plant (*Cucumis sativus*) for rearing aphids and preparing leaf discs for testing was planted in pots containing soil, peat moss and perlite (two thirds of soil and one third of peat moss and perlite) in a research greenhouse of the Khorramabad Technical and Professional Training Center, Iran was planted in pots containing soil, petite mas and perlite (two thirds of soil and one third of pit moss and perlite). The pots were then transported under a Grid Scaffolding to prevent the transmission of any infestation. The pots were kept in greenhouse conditions at 25±5°C and relative humidity of 50±20 %. Irrigation was carried out every day, one month later, the plants were used for the rearing of aphids and from leaves for bioassay experiments.

To create an initial population of aphid, infested cucumber leaves with the melon aphid were collected from cucumber fields. The aphids were transferred to the cucumber pots. The pots were placed in a cage with net cover and kept in a greenhouse at 25±5°C, 50± 5 % relative humidity, and a light period of 16 L: 8D hours of photoperiods until the colony reached an acceptable level. In order to maintain the population of aphids and having a high population, three healthy plants entered the colony of the aphid in the cage every three days. This method was continued until 6 to 7 generations of aphid.

Preparation of essential oil

Tested plant, the Avishan-e-denaii, *Thymus daenensis* Celak. (Lamiaceae) was collected from Shahrekord habitat, Chaharmahal and Bakhtiari Province of Iran with Latitude N 32°21'06.1"

during summer 2016. The collected plant material was authenticated by a plant taxonomist (Dr. A. Rezaei Nejad) of the Department of Horticultural Sciences, Lorestan University of Iran. Plant materials were dried in ambient temperature (23±3°C) in laboratory for one week.

Extraction of essential oils was carried out by hydro-distillation using Clevenger apparatus [9]. For extraction of essential oils, 100 g of ground leaves with one liter of water was poured in the Clevenger and extraction of essential oils was done at three hours. Oil extraction was repeated ten times for one week. Extracted oils were dried over anhydrous sodium sulphate and in microtubes of 2 ml which were covered with aluminum coating were kept in a conventional refrigerator in 4°C and away from light until used in experiments.

Gas chromatography-Mass spectrometry analysis

The oil was analyzed utilizing a GC model: 7890B- MS model: 5977A Agilent HP-5973 chromatograph (Agilent Technologies, www.agilent.com). Gas chromatographic (GC) analysis was carried out using a Shimadzu (www.shimadzu.com) GC-9A with helium as a carrier gas, with a linear velocity of 1.1 ml/s on HP-5 Agilent Column (30 m × 250 nm i.d, 0.25 µm film thickness). Injection mode was split (100:1). The oven was programmed to rise at 60°C (10 min) isotherm, and then to 250°C at a rate of 5°C/min. Injector and detector temperatures were 250 and 280°C, respectively.

The GC mass analysis was performed on a Varian 3400 equipped with an HP-5 column with similar characteristics as the one employed in GC. The transfer line temperature was 260°C. The ionization energy was 70 eV with a scan time of 1 s and mass range of 50-500 amu.

Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra. Quantitative analysis is performed by normalizing the level of the spectrum (the concentration of each component is equal to the peak level associated with that composition divided by the sum of the levels corresponding to other compounds).

Fumigant toxicity bioassay

In the preliminary test, two or three primary concentrations were selected initially, these concentrations were selected as upper and lower concentrations, resulting in mortality between 5 and 95 %. Then, the main concentrations of essential oil were tested with logarithmic intervals between minimum and maximum concentrations. Finally, the essential oil of the Avishan-e-denaii was five concentrations including 0.1, 0.3, 0.5, 0.7 and 0.9 µL/L air and each concentration in three replicates and mortality was evaluated at the interim of 24 hours. In each container using 15 cm diameter (Whatman No. 1 or equivalent) filter paper, 10 adult and nymph aphids were released on the cucumber leaf with dimensions of 10 × 10 cm^2. The desired concentration of essential oil was released on the filter paper in the inner surface of containers. After that, the treated insects were placed in a growth chamber at 25±5°C, 50±5 % relative humidity, and a light period of 16 L: 8D hours of photoperiods. Insects those were not able to move the legs and antenna were considered dead. Control container had no essential oil. To prevent the release of volatiles, around the cap was covered with parafilm [1].

Nymph production deterrent

Nymph production deterrent effect of essential oil of the Avishan-e-denaii, *T. daenensis* was calculated according to sublethal LC_{50} concentration calculated in previous experiments. For this experiment, one 12 h old fundatrix adult aphid was placed on cucumber leaf. The desired essential oil was released on the filter paper in the inner surface of containers. The number of nymphs produced were recorded daily until 72 h. After each daily counts, the nymphs were removed from the test containers. The nymph production deterrent effect was calculated using the following formula [18]:

$$\text{Nymph production deterrent} = (1-NN_t/NN_c) \times 100$$

NN_t= Number of nymph on the treatment
NN_c= Number of nymph on the control

Statistical analysis

Polo-Pc software was used to calculate LC_{50}

and LC_{90} concentration values. Statistical analysis was performed in a completely randomized design with SPSS 19 software [19]. The experiments were arranged in a completely randomized design and the data from nymph production deterrent were subjected to (PROC GLM) ANOVA ($p < 0.05$) after checking for normality. The means were separated using the Duncan Multiple Range test at the 5 % level [1].

Results

Chemical compositions of essential oil

Chemical constituents of the Avishan-e-denaii, *T. daenensis* from the results of the component analyzes by gas chromatography-mass spectrometry (GC-MS) are summarized in Table 1. In total, 48 compounds were identified by GC-MS, representing 100 % of essential oil and 26 of them which were important and representing 90.54 % of composed ingredients are presented in Table 1. The major components include 3-methyl-4-isopropylphenol (26.94 %), thymol (22.40 %), p-cymene (13.81 %), γ-terpinene (6.74 %), borneol (5.24 %) and linalool (4.31 %).

Fumigant toxicity assay

Fumigant toxicity of *T. daenensis* essential oil noted on the nymphs and adults of *A. gossypii* after 24 hours is shown in Table 2. LC_{50} and LC_{90} values of the essential oil against nymphs and adults were 0.0087, 0.555 and 0.0016, 0.304 µl/l, respectively. The results showed that by increasing dose and time, the mortality rate of nymphs ($F= 8.16$, $df= 4$, $P<0.01$) and adults ($F= 4.11$, df=

Table 1. Volatile compounds in steam-distilled oil of the leaf from the Avishan-e-denaii, *Thymus daenensis* identified by gas chromatography–mass spectrometry

No.	Compound	RT	Start time (min)	End time (min)	% Area
1	α-Thujene bicyclo[3.1.0	4.627	4.588	4.690	0.23
2	α-Pinene	4.776	4.690	4.854	0.83
3	d-Camphene	5.072	5.010	5.127	0.86
4	β-Pinene	5.626	5.579	5.689	0.16
5	β-Myrcene	5.852	5.798	5.899	0.81
6	α-Terpinene 1,3-cyclohe	6.469	6.391	6.555	0.92
7	p-Cymene	6.750	6.555	6.773	13.81
8	Limonene	6.789	6.773	6.874	0.34
9	γ-Terpinene	7.514	7.350	7.600	6.74
10	*cis*-Sabinenene hydrate	7.655	7.600	7.740	0.34
11	α-Terpinolene cyclohexe	8.177	8.037	8.287	0.19
12	Linalool	8.489	8.357	8.583	4.31
13	Borneol	10.253	10.104	10.417	5.24
14	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	10.495	10.424	10.627	0.68
15	2,6-Octadienal, 3,7-dimethyl-, (Z)-	12.531	12.414	12.601	0.11
16	Phenol 5-methyl-2-(1-methylethyl)-	13.288	13.194	13.358	0.40
17	Thymol	13.662	13.358	13.686	22.40
18	Phenol, 2-methyl-5-(1-methylethyl)-	13.709	13.686	13.725	0.30
19	3-Methyl-4-isopropylphenol	14.037	13.725	14.107	26.94
20	2-isopropyl-5-methyl phenyl acetate	15.113	15.043	15.168	0.63
21	Carvacryl acetate	15.581	15.496	15.628	0.67
22	Caryophyllene	16.845	16.744	16.908	1.74
23	Cyclohexene, 1-methyl-4-(1-methylethyl)	18.921	18.843	18.983	0.15
24	1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl	20.629	20.505	20.668	0.20
25	Caryophyllene oxide	20.778	20.668	20.902	1.54

4, P<0.01) was significantly increased (Fig. 1). The mortality rate of the nymphs and adults of *A. gossypii* at the highest concentration (0.9 µl/l) of the Avishan-e-denaii was 96.66 %. At the lowest concentration (0.1 µl/l) of oil, the mortality of *A. gossypii* was recorded as 80 % for nymphs and 86 % for adults, respectively.

Effect of essential oils on nymph production deterrent

The effect of the Avishan-e-denaii essential oil on nymph production and nymph production deterrent of the melon aphid is shown in Table 3. The results of the analysis of variance showed that there is a significant difference between nymph production in 24, 48 and 72 hours (F= 13.61, df= 2, P<0.01). Also, with increasing time after treatment, the number of produced nymphs was decreased. Mean comparison of produced nymphs of aphid (Table 3) compared to the control showed a significant difference.

Based on the analysis of variance (Table 3), there was a significant difference between treatments for the percentage of nymph production deterrent, at 24, 48 and 72 hours, respectively (F= 14.36, df= 2, P<0.01). Also, with increasing time

Table 2. LC_{50} and LC_{90} values of essential oil of the Avishan-e-denaii, *Thymus daenensis* against the adults and nymphs of the melon aphid, *A. gossypii*.

Growth stage	LC_{50} (µl/l)	LC_{90} (µl/l)	Slope ± SE	Intercept ± SE	χ^2 (df)	Sig.
Nymph	0.0087	0.555	0.71±0.38	1.46±0.22	0.70	1.41 (3)
Adult	0.0016	0.304	0.57±0.41	1.57±0.24	0.79	1.04 (3)

PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10 logarithm).

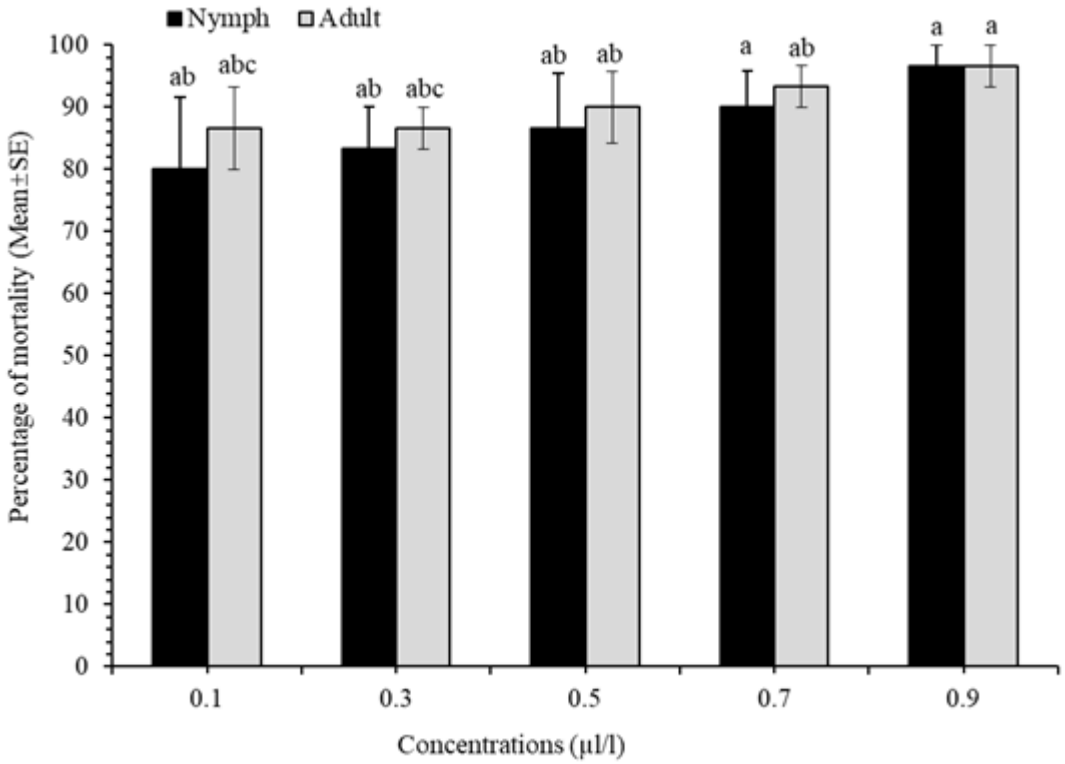

Fig. 1. Percentage of mortality (±SE) of nymphal and adult stages of the melon aphid, *Aphis gossypii* in different essential oil concentrations of the Avishan-e-denaii, *Thymus daenensis*. The similar letters indicate no significant difference

Table 3. Mean (±SE) of nymph production and nymph production deterrent of the melon aphid, *Aphis gossypii* caused by essential oil of the Avishan-e-denaii, *Thymus daenensis*

	Essential oil		
Time (h)	Control	Nymph production	Nymph production deterrent
24	5.0±0.0 [a]	3.1±0.72 [a]	48.0±9.98 [a]
48	7.0±0.0 [b]	3.8±0.42 [b]	45.7±5.95 [b]
72	3.0±0.0 [c]	2.1±0.48 [c]	46.67±8.89 [b]
Total	5.0±0.32	3.0±0.34	46.79±4.71

* Comparison was performed at each column, the means with the same letters based on Duncan's test did not have a significant difference at 5 % level

after treatment, the percentage of deterrence was also increased. Mean comparison of the nymph production deterrent of the aphid (Table 3) compared to the control showed a significant difference. The degree of nymph production deterrent effect was calculated for the Avishan-e-denaii, *T. daenensis* as 46.49 ± 4.71.

Discussion

According to GC/MS analysis, it was found that most of the detected volatile compounds in plant essential oil are monotropine which have insecticidal, fumigant toxicity, repellency, and anti-feeding properties for insects. In the research of Akbarinia and Mirza [20], twenty four components were identified. The main constituents of the essential oil were thymol (74.61 %), p-cymene (4.6 %), γ-terpinene (4.48 %) carvacrol methyl ether (4.27 %), 1,8 cineol (1.64 %), borneol (1.61 %), and carvacrol (1.40 %). In our findings, while confirming this research, more compounds were identified in *T. daenensis* essential oil. In the studies of Moazeni *et al*. [21], the main components of the essential oil were carvacrol (37.0 %), thymol (12.8 %), β-caryophyllene (7.6 %), and germanium (5.74 %). Also, the fumigant toxicity of *T. daenensis* essential oil on the flour moth, *Ephestia kuehniella* and the Indian meal moth, *Plodia interpunctella* was confirmed and LC_{50} for these two pests was calculated as 0.191 and 0.27 µl/l, respectively.

In this study, the fumigant toxicity effect of essential oils of the Avishan-e-denaii on the melon aphid was different in relation to the life stage of the insect, and with increasing concentrations, mortality also increased. Previous experiments conducted on nymph and adult stages of aphids showed that adults are the most tolerant stage against plant essential oils. Due to the application of very low concentrations of the essential oil of the above plant, the mortality rate was found to be comparable to the concentrations used for the adult stage. Many studies have been carried out on the toxicity of essential oils of various plants on aphids. In the study of Riazi *et al*. [22], the concentration of 50 % lethal concentration (LC_{50}) of the peppermint, *Mentha spicata* L. essential oil on the 1st, 3rd nymphs and adult stages of the melon aphid were 2.70, 3.41 and 5.24 µl/1 of air, respectively. However, in the present study, the essential oil of *T. daenensis* in much lower values and with the lethal concentration (LC_{50}) on the nymph and adult stages of the aphid were 0.0087 and 0.0016 µl/l, respectively. In a study by Mahmoudi [23] on the melon aphid, *A. gossypii*, the LC_{50} values of essential oil of parsley (*Petroselinum crispum* L.) and ajowan (*Carum copticum* L.) (Apiaceae) on the adult insects of aphid was calculated as 2.71 and 21.2 µl/l, respectively.

In the study of Mousavi [24], the fumigant toxicity of tarragon (*Artemisia dracunculus* L.) and dill (*Anethum graveolens* L.) plants on the adult insects of the melon aphid was investigated. Based on the results, the calculated LC_{50} values after 24 hours of essential oil exposure was 18.63 and 28.84 µl/l, respectively. Razmjou *et al*. [25] studies on the insecticidal activity of essential oil of two species of eucalyptus (*Eucalyptus microtheca* Muell.) and (*E. spathulata* Hook) on the cotton aphid, *A.*

gossypii showed that the aphid had a high susceptibility to these essential oils, and the LC_{50} values were 366.12 and 15.952 µl/l, respectively. The results related to the LC_{50} value of studied essential oil showed that oil toxicity effect of this essential oil on *A. gossypii* in low quantities. Jahan *et al*. [26] were studied insecticidal effects of *Artemisia dracunculus* L. and *Satureja isophylla* Rech against the cabbage aphid, *Brevicoryne brassicae* were concluded that *A. dracunculus* (LC_{50}=6.25 µL/L air) possesses the highest lethal activity whereas *S. isophylla* the lowest (LC_{50}= 45.60 µL/L air). Insecticidal effect of essential oils of *Thymus carmanicus* Jalas and *Elettaria cardamomum* L. were tested against the 3rd nymphal instars of *B. brassicae* by Jahan *et al*. [27] and it was found that the highest toxic effect was recorded for *E. cardamomum.*

Also, fumigant toxicity of five essential oils on *B. brassicae* was studied by Hasanshahi *et al*. [1, 28] and they found that the highest toxicity was observed in *Artemisia dracunculus* L. essential oil. In addition, LC_{50} value for *A. dracunculus* oil was calculated equal to 6.25 µL/L air in the laboratory conditions. Toxic activities of 10 essential oils against *Myzus persicae* Sulzer was studied by Hori [29] and it was found that spearmint, thyme, pennyrol, mint and peppermint oils have high activity. In other study, effect of essential oils of *Thymus vulgaris*, *Veronica officinalis* L. and *Agrimonia eupatoria* L. on the cabbage aphid, *B. brassicae* showed that essential oil of *T. vulgaris* caused about 85 % mortality in aphid population [30]. Jaastad [31] showed that rapeseed oil significantly reduced damage by the black cherry aphid, *Myzus cerasi* (Fabricius). Furthermore, essential oils of *Eucalyptus globules* against *Aphis gossypii* had insecticidal property and its LC_{50} value was obtained equal to 2000 PPM [32]. Digilio *et al*. [33] also showed that application dose of various essential oils resulted in significant differences in mortality rate for *M. persicae* and *Acyrthosiphon pisum* Harris. Based on the results obtained from the current research, it can be concluded that studied essential oil had the good effect on the melon aphid, *A. gossypii*. The Avishan-e-denaii is the most promising and could be considered for practical applications for aphid control. But before use, it is necessary to study their toxicity in humans and their persistence in the environment.

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