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Original Article

Fumigant toxicity and sublethal effects of *Teucrium polium* essential oil on *Aphis fabae* scopoli A

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ABSTRACT

Objective: Black bean aphid (*Aphis fabae*) is one of the most important greenhouse and crop pests with a wide range of hosts, which causes damages through feeding on vegetable sap and transmitting viral diseases. Currently, chemical methods are mainly used to control this pest. Considering the adverse effects of pesticides, it is essential to apply less chemical pesticide in pest control programs. The lethal and sublethal effects of the essential oil (EO) of *Teucrium polium* leaves on one-day-old adults of black bean aphid were investigated under laboratory conditions.
Method: The bioassay was evaluated at the concentrations of 0.88−12 μL/L air, for 24 h after treatment. Reproductive life table parameters of new emerged aphid surveyed at sub-lethal concentrations (LC_{20} and LC_{40}) of EO and the biological reproductive table was calculated by Jackknife method.
Results: The mortality rate increased significantly with the increasing of EO concentration. The estimated LC_{50} value was 4.5 μL/L air. Laboratory exposure to sublethal concentrations of EO caused significant decrease in adult female longevity and fertility of surviving aphids and as a result caused significant reduction in the intrinsic rate of natural increase (r_m value).
Conclusion: The results of this study revealed that EO of *T. polium* could be used as a potential control agent for the aphid.

1. Introduction

The common method of aphid management in cucumber greenhouses is the use of chemical pesticides. Of course, their excessive use can lead to high costs and result in health hazards of producers and consumers, and cause serious problems, such as direct toxicity for nontarget organisms, including parasitoid and predator insects, pollinators and human. It can also increase the pests' resistance to pesticides, and remain in food products, and leave severe environmental impacts and re-emerge the pests (Isman, 2006; Libs & Salim, 2017). Moreover, because of repeated use of pesticides, many species of the aphids have become resistant to a great number of such chemical compounds, so alternative methods are needed to control these aphids (Pavela, 2007).

Most plants are rich in bioactive compounds which can be used to develop environmentally compatible factors. Herbal essences act as complex mixtures of natural materials made by the plant. These compounds include secondary metabolites, such as alkaloids, terpenoids, flavonoids, polyacetylenes, steroids, amino acids, and sugars which increase resistance against vegetarian animals (Wink, 2018). Of course, most of these compounds have evolved over the course of plants' evolution to repel plant pests and pathogens, so that the secondary metabolites do not play a significant role in the biochemical processes of the plant, but it affects the plant ecological relationships, especially plant and insect interactions, and even results in inducing plant resistance against insect. An important part of these compounds is terpenoid which is contained in the essential oil (EO) of the plant, so it seems to be a good alternative to chemical pesticides in pest control (Mischko et al., 2018). Some essences exhibit a wide range of activities used to act against pest infestations, reversible inhibitors, anti-nutrients, growth regulators and antiviral activity, and can also affect the physiological processes of insects (Cox, 2004).

Teucrium polium L. (locally called kalpooreh in Iran) is a perennial, pubescent, aromatic, and medicinal plant of Lamiaceae family that grows naturally in most parts of Iran. The flowering branches and leaves of the plant contain essential oil (Mahmoudi & Nosratpour, 2013). Various biological activities have been reported for *T. polium*, such as fumigant toxicity (Khani & Heydarian, 2014) and repellent properties (Khani & Heydarian, 2014; Nabavi, Talebi-Jahromi, & Goldansaz, 2010) on insect.

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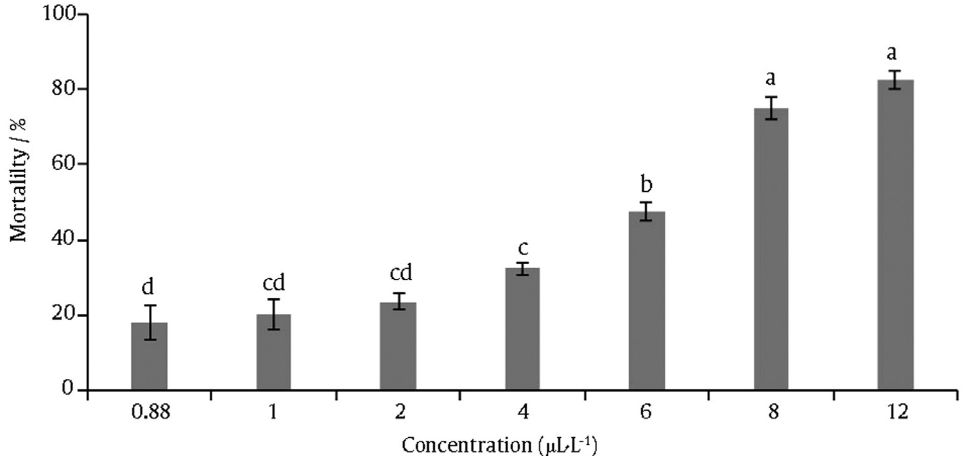

Fig. 1. Mortality percentage of *A. fabae* exposed to different concentrations of *T. polium* essential oil for 24 h (mean ± SE). Means above columns followed by different letters were significantly different ($P < 0.05$; Tukey post-hoc test after analysis of variance).

Phytochemical investigations have shown that *Teucrium* genus contains various compounds such as terpenoids, flavonoids, and iridoids (Mahmoudi & Nosratpour, 2013; Piozzi, Bruno, Rosselli, & Maggio, 2005). The major constituents of *T. polium* EO were α-pinene (12.52%), linalool (10.63%), and caryophyllene oxide (9.69%) (Moghtader, 2009). The results of Khani and Heydarian (2014) showed that the major compounds in *T. polium* EO were the sesquiterpene hydrocarbons α-cadinol (46.2%) and caryophyllene oxide (25.9%).

In this research, the lethal and sublethal effects of *T. polium* (Lamiaceae) on *A. fabae* Scopoli (Hemiptera: Aphididae) were investigated.

2. Materials and methods

2.1. Preparation of EO

T. polium (Lamiaceae) were collected from medicinal plants fields located in Baghyat-o-laah-e-azam (Chah-nime) organization, Zabol, Iran and transferred to the laboratory under appropriate ventilation and dried in shade conditions at room temperature (Mahmoudi & Nosratpour, 2013). The flowering branches (flowers and buds) of plant were powdered by an electric mill. 50 g of the powdered parts with 500 mL of distilled water were extracted using a clevenger apparatus at 100 °C each time. The extraction time was 120 min. The EO collected from plant was dewatered using sodium sulfate and stored in a capped microtube container (2 mL) with an aluminium foil in a refrigerator at 4 °C. In each set of extracting EO, 1 mL essence was taken from 50 g of *T. polium*, and EO extraction yield was 2% volume/weight.

2.2. Rearing of black bean aphid

In order to create the initial population of the black bean aphid, the infected leaves were collected from a greenhouse unit in the city of Zabol and transmitted on the cucumber plants. The aphids were kept on pots containing cucumber plant in a growth chamber with the temperature of (25 ± 1) °C and the relative humidity (RH) of 70% and the light period of 16 h light: 8 h darkness. Biological experiments were performed on non-winged one-day-old adult aphids.

2.3. Fumigant toxicity of herbal EO

According to the method used in Khani and Heydarian (2014), this experiment was carried out in a 100 mL cylindrical container whose cap was covered with a paper lid. A certain volume of different concentrations of EO in acetone was poured on the filter paper, and for uniform distribution of EO in the canister space, a filter paper was placed inside the cap of the plastic container. For control treatment, a filter paper impregnated with acetone without EO was used. The lid was opened for 5 min to evaporate the solvent completely. A total of 10 black bean adult aphids (*Aphis fabae*) of the same age (one-day-old) were placed in the container and the lid was closed, and the area around the opening was tightly sealed with cellophane so that EO could not penetrate the outside. After 24 h, the number of dead insects was counted. In this experiment, insects unable to move their legs and tentacles were considered dead.

This experiment was conducted in a completely randomized design with four replicates with control at (25 ± 1) °C and (70 ± 5)% RH and darkness. The mortality rate of the insects was assessed to calculate the LC_{50} and obtain optimal concentrations, and the LC_{50} was first determined by performing several initial tests. After carrying out the necessary tests, the amount of LC_{50} was determined. Concentrations of 0.88, 1, 2, 4, 6, 8, and 12 μL/L air were tested in 100 mL containers on one-day-old adult insects.

2.4. Estimating parameters of reproductive life table

To estimate the effects of sub-lethal concentrations (LC_{20} and LC_{40}) of EO on the biological parameters of fertility, the black bean aphids of the same age (one-day-old) were treated with LC_{20} and LC_{40} concentrations, and after 24 h, the survived insects (minimum 30 individuals) were selected as a single-replicated insect, from each of EO and of control treatments. Then, these insects were placed on the cucumber leaves (8 cm in diameter) in special containers (8 cm diameter petri dishes) containing 1% agar gel. The agar culture was also used to maintain the medium moist and keep the leaves of the host plant for a longer period.

For ventilation of the petri dishes, a hole was designed on top of each petri bin and then it was covered with a lace. The petri dishes were kept in an incubator at (25 ± 1) °C, (70 ± 5)% RH and light period of 16 h light: 8 h darkness. The number of the appeared nymphs and the probable mortality rate of the adult insects were calculated, and the born nymphs were removed from the petri dishes each day. Moreover, the cucumber leaves on the Petri's floor were replaced every day. It continued until the death of aphids. The parameters of the biological reproductive table were calculated by Jackknife method (Maia, De, Luiz, & Campanhola, 2000).

Table 1
Fumigant toxicity of *T. polium* essential oil on *A. fabae* after 24 h (Number of insects tested = 232).

LC_{50}	95% CL	Probability	Slope ± SE	χ^2(df)
4.5	(2.86–8.23)	0.07	1.56 ± 0.21	10.1 (5)

Note: Ten individuals per replicate, four replicates per concentration, six concentrations per assay; LC: lethal concentration μL/L air; CL: confidence limits; df: degrees of freedom.

2.5. Statistical analysis

Normality of data was tested using Kolmogorov–Smirnov test, a non-parametric test. Statistical analysis of the mortality data was performed by Univariate analysis of variance and one-way analysis of variance (ANOVA) with a post-hoc Tukey test after the assessment homogeneity of variance by Levene's test using IBM SPSS Statistics 24 software. The results were expressed as mean ± SE and considered significantly different at $P < 0.05$.

3. Results

3.1. Fumigant toxicity of T. pilosum *EO*

The EO of *T. polium* was toxic for *Aphis fabae* adults and the mortality increased with rising concentration ($F_{6,21} = 78.7$, $P < 0.001$, Fig. 1).

EO of *T. polium* at lowest concentration (0.88 μL/L air) caused 18% mortality after 24 h compared to 82.5% mortality at the highest concentration (12 μL/L air) (Fig. 1). The LC_{50} of *T. polium* EO on the adult black bean aphid 24 h after applying the extract was obtained 4.5 μL/L air (Table 1).

3.2. Sublethal effect of T. pilosum EO on reproductive parameters

Laboratory exposure of new emerged females to LC_{20} and LC_{40} of *T. polium* EO caused significant decreases in longevity and fertility of surviving aphids (Table 2). Adult longevity in control was 11.3 d. However, the adult longevity in LC_{20} and LC_{40} of *T. polium* EO was 8 and 7.6 d, respectively ($F_{2,61} = 8.52$, $P < 0.001$). Population increase parameters were also adversely affected by EO application. Life table assays revealed that treatment of aphids with LC_{20} or LC_{40} of *T. polium* EO caused significant reduction in the intrinsic rate of natural increase (r_m value) compared to control ($F_{2,61} = 9.59$, $P < 0.001$).

Based on the results, the net reproductive rates (R_0) in control was 49.64 nymphs/female. While the net reproductive rates in LC_{20} and LC_{40} of *T. polium* EO was 30.15 and 29.41 nymphs/female, respectively. Also, the intrinsic rate of population was increased (r_m) in control, which was 0.328 nymphs/female/d. While r_m in LC_{20} and LC_{40} of *T. polium* EO were 0.289 and 0.286 nymphs/female/d, respectively. The mean generation time (T) was estimated as 11.91, 11.81, and 11.85 d in control and LC_{20} and LC_{40} treatment group, respectively (Table 2).

The course of the age-specific survival rate (l_x) and the age-specific number of progeny per day (m_x) of *Aphis fabae* for treated and untreated aphids were presented in Fig. 2. The developmental success of *Aphis fabae* from birth to adult was 100%. The mean longevity of untreated aphids was 20 d and did not reach their peak of reproduction (m_x = 6 nymphs/female/d) until the adults were 9 days old, at the same time the probability of reaching this age was 60%. The mean longevity of treated females with LC_{20} and LC_{40} concentrations of EO reduced to 16 and 14 d, respectively and both groups reached their peak of reproduction (m_x = 5 nymphs/female/d), 9 and 6 d after reaching adulthood, respectively.

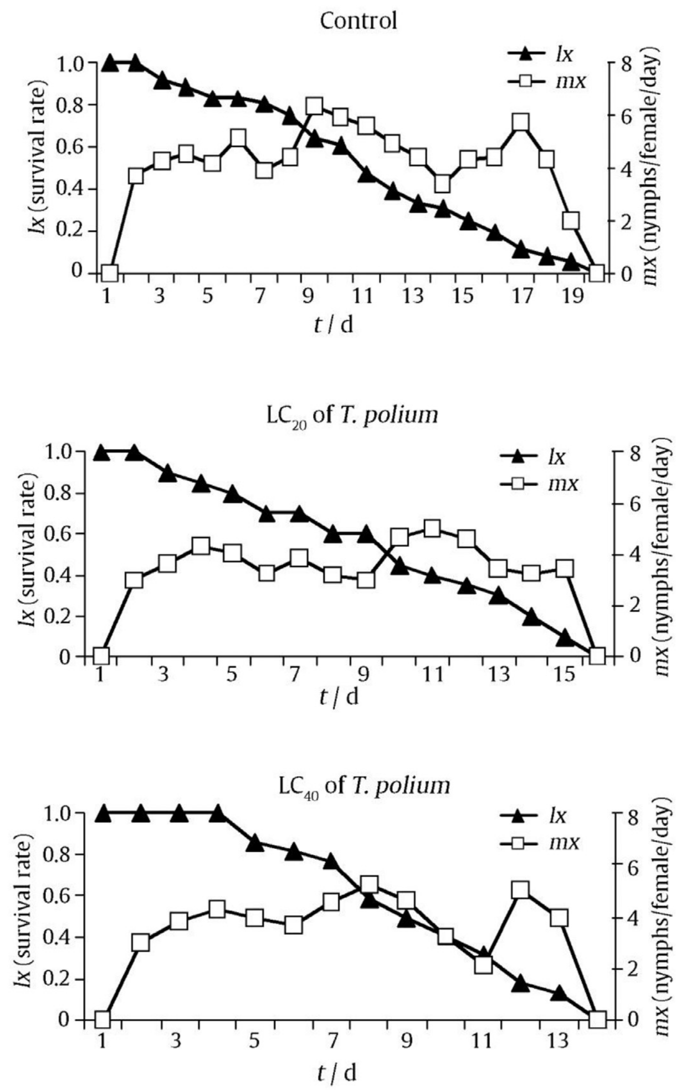

Fig. 2. Effect of sublethal concentration of *T. polium* essential oil on survivorship and age-specific fecundity curves of *A. fabae* (l_x: fraction of females surviving to age x; m_x: age-specific fecundity at age x).

4. Discussion

The insecticide properties of herbal essences on various pests have been proven by many researchers. The use of fumigant toxicity of EOs is one of the most important methods for using these compounds (Isman, 2006). The results indicated that mortality of aphid was related to the concentration of plant EO. By increasing the concentration, the mortality rate of the black bean aphid rose. The result was also in consistent with other researchers' reports (Khani & Heydarian, 2014).

So far, no reports have been made to show the lethal effects of *T. polium* EO on aphids, but some studies have been done for the insecticidal activity of this plant on other insects. The results of Samareh Fekri, Samih, Shahouzahi, Imani, and Zarabi (2014) showed that, the LC_{50} of the *T. polium* extract for *Bemisia Tabaci* (Gennadius) adults reared on susceptible and resistant cultivars, were 93.88 and 68.36 (g/lit), respectively and sublethal effect of the extract was significant on esterase enzyme activity compared with control group. Khani and Heydarian (2014) found that the EO of *T. polium* was toxic against two stored product pests, *Callosobruchus maculatus* (Fabricius) (LC_{50} = 148.9 μL/L air) and *Tribolium castaneum* (Herbst) (LC_{50} = 360.2 μL/L air).

Table 2
Parameters of black bean aphid's fertility life table under influence of sublethal concentrations of *T. polium* (mean ± SE).

Parameters	Groups			F[1]
	Control	LC_{20}	LC_{40}	
R_0 (nymphs/female)	49.64±6.45 a	30.15±4.45 b	29.41±3.23 b	5.52**
r_m (nymphs/female/d)	0.328±0.0083 a	0.289±0.0086 b	0.286±0.0062 b	9.59**
T / d	11.91±0.27	11.81± 0.38	11.85±0.22	0.97
Adult longevity / d	11.31±0.71 a	8.00±0.83 b	7.59±0.63 b	8.52**

Means in a row followed by different letters were significantly different by Tukey test at $P < 0.05$.
[1] At all cases df = 2, 61.
** $P < 0.01$.

To evaluate the effects of pesticides on their physiology and behavior, their sub-lethal concentrations are used. These concentrations influence insect populations differently and change the length of different growth stages, reduce weight and fertility rate and result in behavioral changes. The results of this research indicated that *T. polium* not only caused reduced longevity of mature stages but also caused decreased female density (R_0 and r_m). Few studies have been carried out on the reproductive effect of *T. polium* on insects. In agreement with our results, Jafarbeigi, Samih, Zarabi, and Esmaeily (2012) showed that *T. polium* extract caused increased egg duration, decreased nymphal and pupa longevity and developmental time of the cotton whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae).

The results of our previous study showed that α-cadinol and caryophyllene oxide were the main volatile compounds found in EO extracted from aerial parts of *T. polium* collected from Kashmar located in Khorasan Razavi Province, Iran (Khani & Heydarian, 2014). The main chemical constituents of *T. polium* EO were E-piperitenone oxide, α-pinene, and carvone and showed contact toxicity with LC_{50} values of 1263.09 and 1469.72 μL/m^2 on adult males and females of *C. maculatus*, respectively (Hydarzade & Moravvej, 2012). Studies showed that monoterpenoid compounds had insecticidal properties, fumigant toxicity, and anti-nutritional effects on insects (Mischko et al., 2018). Probably, the sublethal effects of the EO may be related to the effect on enzymitc systems of insects, particularly digestive enzymes. For example, an LC_{50} of 80 μL/L was estimated with the larvae of *Musca domestica* in bioassays when the EO of *T. polium* was admixed with artificial diet. Furthermore, a reduction of carbohydrase activity of α-amylase, α-glucosidase and β-glucosidase were detected in larval midgut extract (Bigham, Hosseininaveh, Nabavi, Kh., & Esmaeilzadeh, 2010). It also was shown that *T. polium* decreased α-amylase activity and had antifeedant activity against *M. domestica* (Bigham et al., 2010) and *Ephestia kuehniella* (Lepidoptera: Pyralidae) (Shahriari, Sahebzadeh, & Zibaee, 2017). Also, methanolic extracts of *T. polium* significantly decreased relative growth rate (RGR), relative consumption rate (RCR), efficiency of digested food (ECD), and efficiency of conversion of ingested food (ECI) of third instar larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Nakhaie Bahrami, Mikani, & Moharramipour, 2018).

Although plant EOs have long been recognized to possess insecticidal actions, physical properties of EOs such as high boiling point, high molecular weight, and low vapour pressure are limitations for application in large scale fumigation (Hu & Coats, 2008). Thus, the formulation of EOs pesticides such as new formulations with nanotechnology "Nanoformulation" seems to improve its permanence and to obtain better activity (Libs & Salim, 2017; Negahban, Moharramipour, Zand, & Hashemi, 2014). In addition, phytotoxicity of EOs requires serious attention when formulating products for agricultural and landscape use (Miresmailli & Isman, 2006).

5. Conclusion

Considering the good effect of plant EO tested in this study, which can lead to a reduction in the population growth rate of the aphid, after further studies and determination of appropriate concentrations and evaluating it in greenhouse conditions (Miresmailli & Isman, 2006), as well as determination its effect on useful insects, it can be considered as an appropriate plant in controlling the aphids.

Conflict of interest

We declare that we have no conflict of interest.

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