



Acaricidal activity of fenugreek and parsley oils against *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch, is a key pest of numerous plants worldwide. The acaricidal and oviposition deterrence activity of fenugreek and parsley oils against *T. urticae* were evaluated in the present study. In addition, the sublethal effects of these oils on female longevity, fecundity and sex ratio of progeny of *T. urticae* were also assessed. Fenugreek and parsley oils showed acaricidal activity against eggs and females of *T. urticae*. However, *T. urticae* females were more susceptible to fenugreek oil (LC₅₀ = 0.562 and LC₉₀ = 1.659 %) as compared to parsley oil (LC₅₀ = 1.011 and LC₉₀ = 2.739 %). Herein, both tested oils showed oviposition deterrence activity against *T. urticae*. When *T. urticae* females were treated with LC₂₅ of fenugreek and parsley oils, the female laid 0.95 and 1.22 eggs/day, respectively as compared to 4.63 eggs/day for the control female. However, female laid 0.76 and 0.89 eggs/day when treated with the higher tested concentration of the abovementioned oils, respectively. The longevity of *T. urticae* females treated with LC₅₀ of fenugreek (10.60 days) and parsley oils (12.25 days) was shorter than that of control females (16.00 days). The sex ratio (♀ %) of the progeny produced by *T. urticae* females treated with tested oils was lower than that of the control. The present results suggested that fenugreek and parsley oils may be promising tools in the control programs of *T. urticae*.

Keywords: acaricidal activity, fenugreek oil, oviposition deterrence activity, parsley oil, sublethal effects, *Tetranychus urticae*

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a key pest of numerous plants. This mite species infests more than a thousand plant species (van Leeuwen *et al.* 2010; 2015) ^[1, 2] causing yield losses in many, ornamental, horticultural and agricultural crops around the world (Hoy 2011; Vacante 2016) ^[3, 4]. However, heavy mite infestation can lead to the plant death in several cases (van der Geest 1985) ^[5]. In fact, chemical control applications have been predominant in the management of *T. urticae* for many years. On the other hand, *T. urticae* resistance to most chemical acaricides has turned into a global problem (Van Leeuwen *et al.* 2009) ^[6]. This problem leads to the frequent application of the acaricides; that are mostly not economically viable as well as causing negative environmental effects (Navia *et al.* 2013) ^[7] and leading to outbreaks of this pest. In addition, pesticide residues on treated plants, especially the fresh fruits and vegetables, increase the risk of cancer in humans (Fitriasari and Prijono 2009) ^[8]. Consequently, the urgent needs to use new products with minimal environmental and health risks has shifted the focus to botanical pesticides as an alternative to chemicals. Botanical oils usually represent little danger to humans and environment (Pavela 2017) ^[9] in addition to being easily degraded in the environment because of their natural origin (Ebadollahi *et al.* 2020) ^[10]. In agriculture, botanical pesticides are gradually becoming important elements of integrated pest management (IPM) and resistance management techniques (Medeiros *et al.* 2006) ^[11]. In this regard, several studies have showed the acaricidal activity of different plant oils against spider mites (Roh *et al.* 2013; Momen *et al.* 2014; Esmaily *et al.* 2017; Elsadany *et al.* 2020; Aissaoui *et al.* 2021; Hamed, *et al.* 2021) ^[12, 13, 14, 15, 16, 17].

Fenugreek, *Trigonella foenum-graecum* L., and parsley, *Petroselinum crispum* (Mill.), are plant species widely used in food and medicinal purposes (Zandi *et al.* 2015; Agyare *et al.* 2017) ^[18, 19]. It was found that parsley oil and fenugreek extract exhibited acaricidal activity against house dust mites (Song *et al.* 2011; Al-Akhdar *et al.* 2015) ^[20, 21]. The insecticidal activity of fenugreek extract has also been reported (Pemonge *et al.* 1997; Al-Akhdar *et al.* 2015; Ali and Ali 2019) ^[22, 21, 23].

However, the efficient pest management strategy must use pesticides that have lethal and sublethal effects on the target pest (Aissaoui *et al.* 2021) ^[16]. Several botanicals are able to influence the development and reproduction of mite pests (Tsolakis and Ragusa 2008; Silva *et al.* 2013) ^[24, 25] as sublethal effects of these botanicals. The plant oils may display multiple modes of action such as contact and/or fumigant toxic effects, repellent activity, neurotoxic effects and fecundity reduction (Momen *et al.* 2014; Abdel Kader *et al.* 2015; Jankowska *et al.* 2018; Momen *et al.* 2018) ^[13, 26, 27, 28]. Knowledge of the biological activity of any botanical oil/pesticide against target

pest by conducting the laboratory bioassays is essential precondition for its practical use (Musa *et al.* 2017) [29]. Thus, besides assessing the lethal effect of any pesticide, the evaluation of its effects on the important biological traits of pest individuals that survived treatment is also essential to evaluate the overall effect of this pesticide (Musa *et al.* 2017) [29]. Therefore, the present study aimed to obtain basic data about the lethal effects of fenugreek and parsley oils against *T. urticae* and to evaluate their sublethal effects on female longevity, fecundity and sex ratio of progeny of *T. urticae*. In addition, the oviposition deterrence activity of these tested oils against *T. urticae* was also assessed.

Materials and methods

Colony of *T. urticae*

The colony of *T. urticae* was reared on leaves of *Phaseolus vulgaris* L. The leaves were placed on water-saturated cotton layers in Petri-dishes (rearing units). Water was added when necessary to keep the cotton layers wet. The rearing units were kept in an incubator at 27 ± 1 °C, 60–70% RH and 16 L: 8 D photoperiod.

Tested Oils

Commercial fenugreek and parsley oils were used in this study. These oils were purchased from El Hawag Company for Extraction & Packing of Natural Oils.

Lethal Effect of Fenugreek and Parsley Oils Against *T. urticae* females

The lethal effect of fenugreek and parsley oils against *T. urticae* females was assessed by the direct spray application. The tested concentrations of fenugreek and parsley oils were chosen after preliminary experiments. The tested concentrations of tested oils were prepared by using distilled water. Triton X-100 was used as an emulsifier.

Females of *T. urticae* were transferred to leaf discs of *P. vulgaris* and sprayed with different tested concentrations of fenugreek and parsley oils separately using glass atomizer. The leaf discs were placed on wet cotton pads in Petri-dishes (experimental units). Control female individuals of *T. urticae* were sprayed with distilled water. All the experimental units were kept in an incubator at 27 ± 1 °C, 60–70% RH and 16 L: 8 D photoperiod. Mortality of females was recorded at 48 h after treatment. Mite females that unable to move after a slight touch with a fine paintbrush were considered as dead. Five concentrations of each oil were tested; each tested concentration and control had six replicates (20 females /replicate). The experiment was repeated twice.

Ovicidal Activity of Fenugreek and Parsley Oils Against *T. urticae*

In this experiment, the ovicidal effect of fenugreek and parsley oils against *T. urticae* eggs were assessed using two concentrations of each oil (LC50 and LC90, estimated for *T. urticae* females). Ten *T. urticae* females were transferred per leaf disc of *P. vulgaris* and allowed to lay eggs for 24 h, then all females were removed. The leaf discs containing newly laid eggs (0-24 h) of *T. urticae* were used in the experiment. The leaf discs carried *T. urticae* eggs were sprayed with different concentrations of each tested oil (LC50 and LC90, estimated for *T. urticae* females) separately using glass atomizer. The leaf discs were placed on wet cotton pads in Petri-dishes (experimental units). Control eggs were sprayed with distilled water. All experimental units were kept in an incubator at 27 ± 1 °C, 60–70% RH and 16 L: 8 D photoperiod. For each tested concentration and the control, mortality of eggs (where eggs were assumed dead if not hatched) were recorded. For each tested oil, each tested concentration and control had six replicates (20 eggs/replicate). The percentages of mortality of *T. urticae* eggs were corrected according to Abbott's formula (Abbott 1925) [30]. The experiment was repeated twice.

Oviposition Deterrence Activity of Fenugreek and Parsley Oils

The oviposition deterrence activity of fenugreek and parsley oils against *T. urticae* females was assessed at 24 h after treatment. Leaf discs of *P. vulgaris* were placed on cotton layers in Petri-dishes. The leaf discs were surrounded with wet cotton strips to prevent mites from escaping. For each tested oil, one half of each disc was painted separately with LC25, LC50 or LC90 (estimated for *T. urticae* females) of oil, while the other half was painted with distilled water and served as a control.

After oil treatment, ten adult females of *T. urticae* were placed on the middle (leaf midrib) of each leaf disc. For each tested oil, each tested concentration had ten replicates, where each leaf disc represented one replicate. The Petri-dishes were kept in an incubator at 27 ± 1 °C, 60–70% RH and 16 L: 8 D photoperiod.

The numbers of mites and oviposited eggs on the treated and control halves of leaf discs were counted at 24 h after treatment.

- Oviposition deterrence index (ODI) (%) was calculated according to Dimetry *et al.* (1993) [31]; Sundaram and Sloane (1995) [32] as follows:

$$\frac{C-T}{C+T} \times 100$$

Where C is the number of eggs deposited on the control halves, and T is the number of eggs deposited on the treated halves of the leaves, evaluated at 24 h after treatment.

Sublethal Effects of Fenugreek and Parsley Oils on Female Longevity, Fecundity and Sex Ratio of Progeny of *T. urticae*

The sublethal effects of fenugreek and parsley oils were evaluated by comparing some biological parameters of *T. urticae* exposed to LC25, LC50 of each oil separately or distilled water (control). For each tested oil, newly emerged females of *T. urticae* were transferred to leaf discs of *P. vulgaris* and sprayed with distilled water (control), LC25 or LC50 of tested oil. The leaf discs were placed on wet cotton pads in Petri-dishes. All Petri-dishes were kept in an incubator at 27 ± 1 °C, 60–70% RH and 16 L: 8 D photoperiod.

At 48 h after spraying, surviving females from each treatment were transferred individually to new leaf discs placed on wet cotton pads in Petri-dishes (experimental units); where each surviving female on one leaf disc was considered as one replicate. A male was added to each surviving female on each experimental unit. These females were observed daily until their death to record longevity and number of eggs laid per female. In addition, the sex ratio of their progeny were determined. Twenty surviving *T. urticae* females (replicates) were investigated per tested concentration for each tested oil or control.

Statistical Analysis

The concentrations-mortality response curves were drawn using the Ldp-line computer program to determine the lethal concentrations (LC values) of fenugreek and parsley oils for *T. urticae* females.

In the oviposition deterrence activity bioassay, t-test was used to compare the numbers of eggs deposited on treated or control leaf halves. The effect of tested LC of fenugreek and parsley oils on female longevity, daily number of eggs/female and sex ratio (♀ %) of *T. urticae* offspring were analyzed by one-way analysis of variance (ANOVA) using SPSS, where significant differences between means were detected by Tukey's test ($P < 0.05$).

Results and Discussion

Acaricidal activity of fenugreek and parsley oils against *T. urticae*

Presently, fenugreek and parsley oils were firstly studied for their lethal effects against *T. urticae* females when applied by direct spray method. The present results revealed that both tested oils showed acaricidal activity against *T. urticae* (Table 1). Similarly, parsley oil and fenugreek extract exhibited acaricidal activity against house dust mites (Song *et al.* 2011; Al-Akhdar *et al.* 2015) [20, 21]. Also, the acaricidal activity of parsley oil has been proven against cattle tick (Camilotti *et al.* 2015) [33]. In accordance with the current results, Alkot *et al.* (2020) [34] indicated that fenugreek oil had acaricidal activity against the acarid mite, *Tyrophagus putrescentiae* (Schrank). However, *T. urticae* was more susceptible to fenugreek oil (LC50 = 0.562 and LC90 = 1.659 %) as compared to parsley oil (LC50 = 1.011 and LC90 = 2.739 %) (Table 1). Additionally, both tested oils showed ovicidal activity against *T. urticae* eggs; with parsley oil displayed the highest ovicidal effect (Fig. 1). The results obtained by previous studies have revealed that some plant oils may have both adulticidal and ovicidal activity against mite species (George *et al.* 2010; Momen *et al.* 2014) [35, 13].

Table 1: Acaricidal activity of fenugreek and parsley oils against *T. urticae* females.

Tested oils	*LC (%) (Confidence limits)			Slope ± SE
	LC ₂₅	LC ₅₀	LC ₉₀	
Fenugreek	0.318 (0.262-0.370)	0.562 (0.496-0.629)	1.659 (1.426-2.010)	2.727± 0.216
Parsley	0.598 (0.518-0.677)	1.011 (0.908-1.125)	2.739 (2.344-3.329)	2.962±0.213

* LC: lethal concentration.

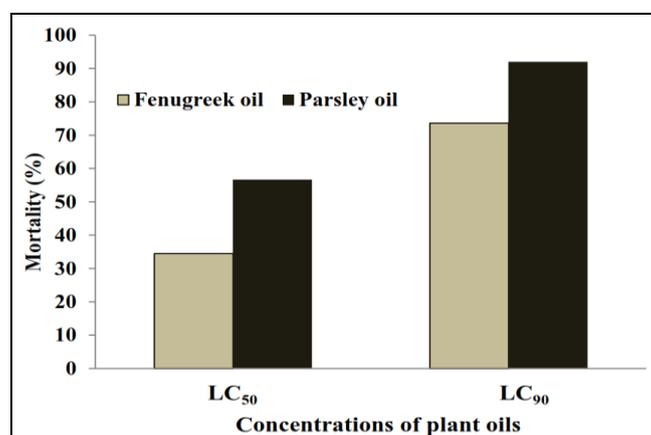


Fig 1: Ovicidal activity of tested concentrations of fenugreek and parsley oils (LC50 and LC90, estimated for *T. urticae* females) against *T. urticae* eggs.

Oviposition Deterrence Activity of Fenugreek and Parsley Oils Against *T. urticae*

Results represented in Figures (2-3) showed that females of *T. urticae* generally preferred to deposit their eggs on the control leaf halves as compared to the treated halves. At 24 h after treatment, *T. urticae* females deposited statistically higher numbers of eggs on the control halves comparing with the halves treated with LC25, LC50, and LC90 of fenugreek (all P 's = 0.000; Fig. 2) and parsley oils (all P 's = 0.000; Fig. 3). At 24h after treatment, the oviposition deterrence index (ODI) (%) were estimated for LC25, LC50, and LC90 of each tested oil against *T. urticae* (Fig. 4). For fenugreek and parsley oils, the highest ODI (%) values were observed for LC90, while the lowest values were recorded for LC25 (Fig. 3). Similarly, an oviposition deterrent activity was reported for *Datura stramonium* L. seed extract (Kumral *et al.* 2010) [36], sweet marjoram and rosemary oils (Amer *et al.* 2001) [37] against *T. urticae*. Also, the current results are compatible with other studies which revealed that some plant oils possess oviposition deterrence activity against *T. urticae* (Momen *et al.* 2014; 2018) [13, 28]. Previously, a study was conducted by El-Yamani *et al.* (2017) [38] to assess the repellent effect of fenugreek oil against females of *T. urticae*. Their results indicated that this oil is able to repel *T. urticae*. The authors, however, did not assess the oviposition deterrence activity of this oil against *T. urticae* females.

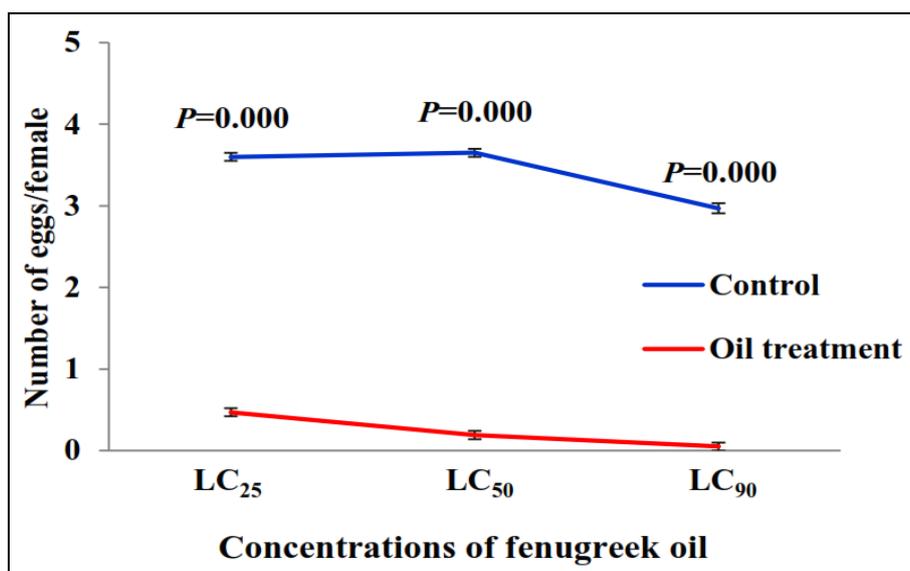


Fig 2: Number of eggs/female of *T. urticae* on the leaf halves treated with LC25, LC50, or LC90 of fenugreek oil and on the control halves (at 24h after treatment). All the means were showed with \pm SE. For each tested LC, the difference between control and treatment was analyzed by t-test; P value was given.

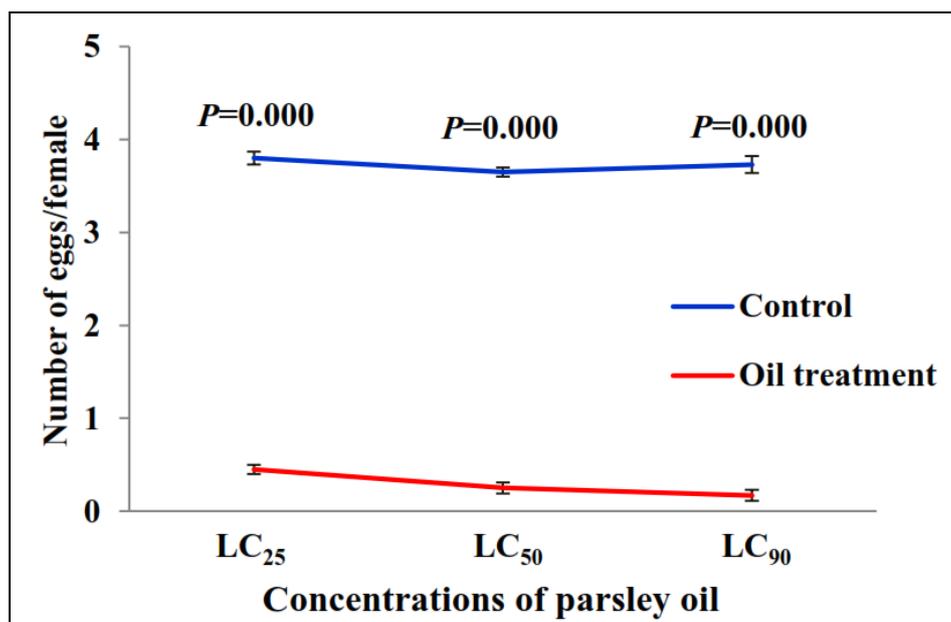


Fig 3: Number of eggs/female of *T. urticae* on the leaf halves treated with LC25, LC50, or LC90 of parsley oil and on the control halves (at 24h after treatment). All the means were showed with \pm SE. For each tested LC, the difference between control and treatment was analyzed by t-test; P value was given.

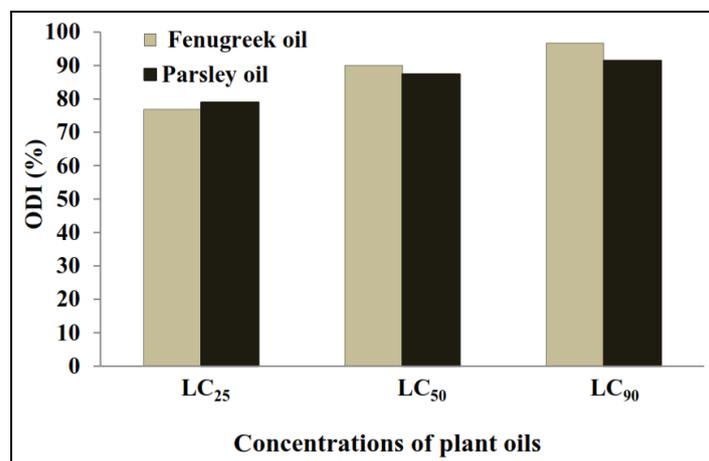


Fig 4: Oviposition deterrence index (ODI) (%) of LC₂₅, LC₅₀, and LC₉₀ of fenugreek and parsley oils against *T. urticae* (at 24h after treatment).

Sublethal Effects of Fenugreek and Parsley Oils on Female Longevity, Fecundity and Sex Ratio of Progeny of *T. urticae*

Two concentrations (LC₂₅ and LC₅₀, estimated for *T. urticae* females) were chosen for each tested oil to study their sublethal effects on female longevity, reproduction and sex ratio of *T. urticae* progeny. Direct spray application with LC₂₅ and LC₅₀ of fenugreek and parsley oils significantly reduced female longevity ($F=174.113$; $P=0.000$) and daily number of eggs/female ($F=17.258$; $P=0.000$) of *T. urticae* as compared to control.

When *T. urticae* females were treated with LC₂₅ of fenugreek and parsley oils, the female laid 0.95 and 1.22 eggs/day, respectively as compared to 4.63 eggs/day for the control female. However, female laid 0.76 and 0.89 eggs/day when treated with the higher concentration (LC₅₀) of the abovementioned oils, respectively. Similarly, *Rosmarinus officinalis* L. oil has been found to reduce the fecundity of *T. urticae* females (Esmaily *et al.* 2017; Aissaoui *et al.* 2021) [14, 16]. Also, an egg reduction was observed after exposing *T. urticae* females to basil (Elsadany *et al.* 2020) [15], neem and eucalyptus oils (Hamed *et al.* 2021) [17].

The longevity of *T. urticae* females treated with LC₅₀ of fenugreek (10.60 days) and parsley oils (12.25 days) was shorter than that of control females (16.00 days); however the difference between two tested oils were insignificant (Table 2). In the same way, LC₅₀ of neem oil reduced fecundity and longevity of *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) as compared to control (Silva *et al.* 2013) [25]. The change in longevity of individuals may influence fecundity and affect arthropod population (Croft 1990) [39]. Mite pests exposed to pesticides/plant oils have been reported to suffer non-lethal effects (Saryazdi *et al.* 2013; Wang *et al.* 2014; Esmaily *et al.* 2017; Elsadany *et al.* 2020; Hamed *et al.* 2021) [40, 41, 14, 15, 17] that cannot be observed in mortality bioassays which usually performed in lethal toxicity studies. Here, both tested oils proved to act as oviposition deterrent to *T. urticae*, which may play a role in reducing fecundity. Roh *et al.* (2013) [12] demonstrated that treatment with *Eucalyptus bicostata* oil cause reduction in fecundity of *T. urticae*, which in agreement with the current findings. However, the lower performance of *T. urticae* in terms of decreased female longevity and fecundity may be due to the antifeeding effect of tested oils. Sabelis (1985) [42] indicated that the increase in the feeding rate of mites leads to a subsequent increase in egg laying. In this context, Sousa *et al.* (2015) [43] indicated that parsley oil displayed feeding and development inhibitory effects against the armyworm moth. Recently, Elmadawy and Omar (2022) [44] revealed that parsley oil can reduce the feeding of adult and larval stages of coleopteran insect pests. The sex ratio (♀ %) of the progeny produced by *T. urticae* females treated with LC₅₀ of tested oils was statistically lower than that of the control ($F=3.411$; $P=0.012$) (Table 2).

Table 2: Sublethal effects of fenugreek and parsley oils on female longevity, fecundity and sex ratio of progeny of *T. urticae*.

Treatments	*LC (%)	Female Longevity	Daily number of eggs/female	Sex ratio (♀ %)
Fenugreek oil	LC ₂₅	14.45±0.55ab	0.95±0.05b	65.87±2.38ab
	LC ₅₀	10.60±0.41d	0.76±0.06b	62.08±3.50b
Parsley oil	LC ₂₅	13.85±0.51bc	1.22±0.07b	68.23±3.13ab
	LC ₅₀	12.25±0.38cd	0.89±0.07b	60.92±3.39b
Control		16.00±0.61a	4.63±0.25a	74.24±1.56a
F		174.113	17.258	3.411
P		0.000	0.000	0.012

* LC: lethal concentration.

Means within a column followed by different letters are significantly different (Tukey's test; $P < 0.05$).

The adaptive strategy of *T. urticae* as a colonizing mite species is depend on the high reproductive potential of adult females (Sabelis 1985) ^[42]. The treatment with LC50 of fenugreek and parsley oils had a negative influence on fecundity and longevity of *T. urticae* females and exhibited a considerable reduction in these parameters. These results revealed the acaricidal potential of tested oils against *T. urticae*. These acaricidal activity of tested oils including the combined results of lethal (direct toxicity of oils against females and eggs of *T. urticae*) and sublethal effects (reduction in longevity and oviposition of females in addition to reducing female proportion in the progeny) could reduce population growth of *T. urticae*. All this suggested the possibility of using fenugreek and parsley oils in the control of *T. urticae* as an alternative technique to chemical acaricides. However, for the practical use of tested oils in the management of *T. urticae*, further research is needed in the future to assess the efficiency of fenugreek and parsley oils under semi-field and field conditions.

Conclusion

The present study revealed that fenugreek and parsley oils exhibited acaricidal activity against *T. urticae* eggs and adult females. Both tested oils also showed sublethal effects on some biological aspects of *T. urticae*. In the light of integrating both lethal and non-lethal effects of the tested oils, the present results suggested that fenugreek and parsley oils may be promising tools in the control programs of *T. urticae*.

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