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Effect of fennel oil on *Tetranychus urticae* (Acari: Tetranychidae) and two predatory mites

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Abstract
The acaricidal and repellent effects of fennel oil against *Tetranychus urticae* Koch, were investigated in this study. In addition, the lethal and side effects of the LC_{50}, and LC_{90} (estimated for *T. urticae* females) of fennel oil on *Amblyseius swirskii* (Athias-Henriot) and *Cydnoseius negevi* (Swirski and Amitai) were assessed. The results showed that fennel oil showed acaricidal activity against different stages of *T. urticae*. Repellent and oviposition deterrence effects of fennel oil against *T. urticae* were also demonstrated in this study. However, this oil was found to be less toxic to *A. swirskii* and *C. negevi* than to *T. urticae*. Presently, the oviposition of *A. swirskii* and *C. negevi* were not significantly affected after their females came into contact with LC_{50} (estimated for *T. urticae*) of fennel oil compared to the control. Moreover, this concentration of fennel oil appeared to be harmless for *A. swirskii* and *C. negevi*. On the other hand, the higher tested concentration of this oil was slightly harmful for the two tested predatory mites. Therefore, by combining the lethal and repellent effects of fennel oil against *T. urticae* as well as its slight side effects on the tested predators, the present study suggested that fennel oil may be a promising tool in the management programs of *T. urticae*.

Keywords: acaricidal activity, *amblyseius swirskii*, *cydnoseius negevi*, fennel oil, *tetranychus urticae*, side effects

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a widespread polyphagous mite. It is destructive pest that attacks a wide range of plants (Azadi-Qoort *et al*. 2019) [1]. In different regions around the world, this mite pest can causes severe plant damage and considerable crop losses (James and Price 2002) [2]. The control of *T. urticae* is largely based on using chemical acaricides. Nevertheless, the long term use of chemical acaricides induced resistance in many mite pests, adverse impacts on human health, non-target organisms (e.g., pest's natural enemies), and environment (Naqqash *et al.* 2016) [3]. Moreover, sometimes the chemical acaricides can't keep spider mite populations under economic injury level (Tirello *et al.* 2012) [4]. On the above, it is essential to find safer alternatives in order to minimize the undesirable effects of chemical pesticides. In this regard, plant oils have gained great interest in the field of agriculture pest control. Currently, botanical pesticides (e.g., plant oils) are increasingly recommended as control agents for mite and insect pests. This may be attributed to the multiple mode of action of the plant oils including, contact and/or fumigant toxicity, fecundity reduction, repellent, and neurotoxic effects (Momen *et al.* 2014; Abdel Kader *et al*. 2015; Jankowska *et al.* 2018; Momen *et al.* 2018) [5, 6, 7, 8]. Since plant oils are produced from plants used in food or pharmaceutical industries, they usually show minimal risks to humans and the environment (Pavela 2017) [9]. In addition, botanical oils degraded easily in the environment due to their natural origin (Ebadollahi *et al*. 2020) [10].
Fennel, *Foeniculum vulgare* Mill, is an aromatic plant spread in Mediterranean and Central Europe regions (Aprotosoaie *et al.* 2010) [11]. Fennel oil is used in food flavoring and for pharmaceutical and cosmetic purposes (Bilia *et al.* 2000) [12]. Moreover, this oil found to be exhibited acaricidal and insecticidal activities against mite and insect pests (Kutukoglu *et al.* 2012; Seada *et al.* 2016) [13, 14]. However, the principal components of fennel oil were identified as trans-anethole, estragole, and fenchone (Zhao *et al.* 2012) [15]. This three compounds found to be exhibited acaricidal activity (Lee 2004) [16].
Phytoseiids are the most important predatory mites of mite pests (McMurtry *et al*. 2013) [17]. The phytoseiid mites, *Amblyseius swirskii* (Athias-Henriot) and *Cydnoseius negevi* (Swirski and Amitai) are generalist predators (McMurtry *et al*. 2013) [17] which can feed on different prey types (Abou-Awad *et al.* 2010; Hussein *et al*. 2016) [18, 19]. In the greenhouses, *A. swirskii* is widely utilized for the management of whitefly and thrips (e.g. Calvo *et al.* 2011) [20]. Both *C. negevi* and *A. swirskii* were found to prey, reproduce, and develop well on *T. urticae* (Abdel-Khalek *et al.* 2019; Fahim and El-Saiedy 2021) [21, 22]. Actually, the effective management of *T. urticae* is difficult to achieve by one control technique (Rhodes and Liburd 2006) [23]. Indeed, the integrated pest management (IPM) is based on using different methods of pest control (such as chemical and biological methods) to suppress pest population (Park and Tak 2016) [24]. In this regard, pesticides must be selected from

products with minimal negative effects on the natural enemies (Kaplan *et al.* 2012) [25]. So that, it is necessary not only to investigate the efficacy of pesticides on the target pest, but also to assess their selectivity to the natural enemies. Although several studies were investigated the acaricidal effects of different plant oils (e.g., El-Zemity *et al.* 2006; Kutukoglu *et al.* 2012; Aboelhadid *et al.* 2021) [26, 13, 27], fewer researches were studied the effect of plant oils on predatory mites (e.g., Abdel Kader *et al*. 2015; Oliveira *et al.* 2017) [6, 28]. Therefore, this study aimed to investigate the acaricidal activity of fennel oil against *T. urticae* and to evaluate the lethal and side effects of this oil on *A. swirskii* and *C. negevi*.

Materials and Methods

Mite colonies

Tetranychus urticae

The colony of *T. urticae* was reared on leaves of *Phaseolus vulgaris* L. The leaves were placed on wet cotton layers in Petri-dishes (rearing units). Water was added when required to keep the cotton layers saturated. The rearing units were kept in an incubator at 26 ± 1 ºC, 70-75 % RH and 16 L: 8 D photoperiod.

Predatory mites

Colonies of the tested predatory mites (*A. swirskii* and *C. negevi*) were maintained separately on *P. vulgaris* leaves placed on wet cotton layers in Petri-dishes (rearing units), and fed on *T. urticae*. Wet cotton strips were placed around the leaves margins for preventing mite escaping. Water was added when required to keep the cotton layers saturated. The rearing units were kept in an incubator at 26 ± 1 ºC, 70-75 % RH and 16 L: 8 D photoperiod.

Tested oil

A commercial fennel oil was used in this study. This oil was purchased from El Captain Company for extracting natural oils, plants and cosmetics. The tested concentrations of fennel oil were prepared by using distilled water. Triton X-100 was used as an emulsifier.

Lethal effect of fennel oil against *T. urticae* females

The direct contact toxicity of fennel oil against *T. urticae* females was tested by the direct spray method. The tested concentrations of fennel oil were selected based on preliminary experiments. Females of *T. urticae* were transferred to leaf discs of *P. vulgaris* and sprayed with different tested concentrations of fennel oil using glass atomizer. The leaf discs were placed on wet cotton pads in Petri-dishes (experimental units). However, untreated females were sprayed with distilled water to serve as a control. All the experimental units were kept in an incubator at 26 ± 1 ºC, 70-75 % RH and 16 L: 8 D photoperiod. Mortality of females was recorded at 48 h after treatment. Mites that unable to move after a slight touch with a fine paintbrush were considered as dead. Five concentrations of fennel oil were tested; each tested concentration and control had six replicates (20 females /replicate). The experiment was repeated twice.

Lethal effect of fennel oil against eggs and nymphs of *T. urticae*

In this assay, the toxicity of fennel oil against eggs and nymphs of *T. urticae* were tested using two concentrations of this oil (LC_{50} and LC_{90}, estimated for *T. urticae* females). Ten females of *T. urticae* were transferred per leaf disc and allowed to oviposit for 24 h, then all females were removed. The leaf discs containing newly deposited eggs (0-24 h) of *T. urticae* were used in the experiment.

Leaf discs containing eggs or nymphs of *T. urticae* were sprayed with different concentrations of fennel oil (LC_{50} and LC_{90}, estimated for *T. urticae* females) using glass atomizer. The leaf discs were placed on wet cotton pads in Petri-dishes (experimental units). However, untreated eggs or nymphs were sprayed with distilled water to serve as control. All experimental units were kept in an incubator at 26 ± 1 ºC, 70-75 % 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. Mixed nymphal stages (protonymphs and dutonymphs) were used in this experiment. Mortality of nymphs were recorded at 48 h after treatment. Each tested concentration and control had six replicates (20 eggs or nymphs /replicate). The percentages of mortality were corrected according to Abbott's formula (Abbott 1925) [29] for eggs and nymphs of *T. urticae*. The experiments were repeated twice.

Repellency assay

Repellence effect of fennel oil against *T. urticae* females was evaluated at three evaluation periods (2, 8 and 24 h). Leaf discs of *P. vulgaris* were placed on cotton layers in Petri-dishes. The leaf discs were lined with moist cotton strips to prevent mite escaping. One half of each disc was painted separately with LC_{50} or LC_{90} (estimated for *T. urticae* females) of fennel oil, while the other half was painted with distilled water and served as a control.

After treatment, ten adult females of *T. urticae* were introduced into the middle (leaf midrib) of each leaf disc. Each tested concentration had ten replicates, where each leaf disc represented one replicate. The Petri-dishes were kept in an incubator at 26 ± 1 ºC, 70-75 % RH and 16 L: 8 D photoperiod.

The numbers of mites on the treated and control halves of the leaf discs were counted at 2, 8 and 24 h after treatment. At the last evaluation period (24 h), the numbers of eggs deposited on the treated and control halves of leaf discs were counted.

- Repellency (R) (%) was calculated according to Sundaram and Sloane (1995) [30] as follows:

$$\frac{C-T}{C} x\, 100$$

Where C is the number of mites on the control halves, and T is the number of mites on the treated halves of the Leaves, evaluated at 24 h post-treatment.
- Oviposition deterrence index (ODI) (%) was calculated according to Dimetry *et al*. (1993) [31]; Sundaram and Sloane (1995) [30] as follows:

$$\frac{C-T}{C+T} x\, 100$$

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

Contact effect of fennel oil on the predatory mites

In this assay, the contact effect of fennel oil on eggs and females of *A. swirskii* and *C. negevi* were tested. For each predatory mite, females were transferred to leaf units and allowed to lay eggs for 24 h; then all females were removed. The newly deposited eggs (0-24 h) were used in the experiment.
Leaf discs of *P. vulgaris* were sprayed separately with two concentrations of fennel oil (LC_{50} or LC_{90}, estimated for *T. urticae* females), while distilled water was used to spray the control leaf discs using glass atomizer. Each leaf disc was placed on wet cotton pad in a Petri-dish (experimental unit). Wet cotton strips were surrounded the leaf discs margins to prevent mite escaping. Both treated and control discs were left to dry; then predatory females or eggs (for each predator) were transferred to each treated or control discs. In this experiment, *T. urticae* was add to the treated and control leaf discs to serve as food source for the predatory females. The experimental units were kept in an incubator at 26 ± 1 ºC, 70-75 % RH and 16 L: 8 D photoperiod. For each tested concentration and the control, mortality of predatory eggs (where eggs were assumed dead if not hatched) were recorded. The mortality of predatory females was recorded at 48 h after treatment. Each tested concentration and control had six replicates (15 eggs or females/replicate). The percentages of mortality were corrected according to Abbott's formula (Abbott 1925) [29] for eggs and females of tested predatory mites. The experiments were repeated twice.

Side effects of fennel oil on the predatory mites

In this experiment, the side effects of two concentrations of fennel oil (LC_{50} and $LC_{90,}$ estimated for *T. urticae* females) on reproduction, and sex ratio of progeny of *A. swirskii* and *C. negevi* were investigated. Experimental unites with treated and control leaf discs were prepared as described previously in the section of "Contact effect of fennel oil on the predatory mites". Both treated and control discs were left to dry; then females of each tested predator were transferred individually to the treated or control discs and provided with *T. urticae* as food source. This experiment was continued for one week. The experimental units were kept in an incubator at 26 ± 1 ºC, 70-75 % RH and 16 L: 8 D photoperiod. For each predator, mortality of predatory females and daily number of eggs deposited by each tested female were recorded. Also, the sex ratio of the progeny for each predator were determined. For each predator, 20 replicates were used per tested concentration or control. The effect (*E*) of fennel oil on the tested predatory mites was calculated according to Hassan (1985) [32].

Statistical analysis

The concentrations-mortality response curve was drawn using the Ldp-line computer program to determine the lethal concentrations (LC values) of fennel oil against *T. urticae* females.
The data of repellency assay were subjected to t-test (using SPSS). Also, t-test was used to compare between the lethal effect of each tested concentration of fennel oil on females of *A. swirskii* and *C. negevi*. For each tested predator, the effect of the two tested concentrations of fennel oil on reproduction and sex ratio of predators' progeny 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

Lethal effect of fennel oil against *T. urticae*

In the present study, fennel oil showed acaricidal activity against *T. urticae* females (LC_{50}= 0.727 and LC_{90} = 1.956 %) (Table 1). Using different bioassay techniques, previous studies also reported that fennel oil exhibited acaricidal activity against *T. urticae* (El-Zemity *et al*. 2009; Ebadollahi *et al.* 2014) [33, 34]. The acaricidal activity of fennel oil has also been proven against other mite species (El-Zemity *et al.* 2006; Kutukoglu *et al.* 2012) [26, 13] and ticks (Aboelhadid *et al.* 2021) [27]. Moreover, the insecticidal activity of fennel oil has been demonstrated by previous studies (Zhao *et al.* 2012; Seada *et al.* 2016) [15, 14].

Table 1: Lethal concentrations (LC) (%) of fennel oil for *Tetranychus urticae* females.

LC (%)		Lower – upper limits	Slope ± SE
LC_{30}	0.485	0.421 – 0.546	2.982±0.227
LC_{50}	0.727	0.654 – 0.804	
LC_{90}	1.956	1.692 – 2.353	

Figure (1) showed that fennel oil exhibited ovicidal and nymphicidal activity against *T. urticae*. Presently, *T. urticae* nymphs were more sensitive to fennel oil at its tested concentrations (LC_{50} and LC_{90}, estimated for *T. urticae* females) than eggs. Similarly, previous studies have shown that some plant oils may have ovicidal and adulticidal activity in addition to their toxic effect on immature stages of mite pests (George *et al.* 2010; Momen *et al.* 2014) [35, 5]. The ability of fennel oil to kill the different stages of *T. urticae* could be an important characteristic of an efficacious acaricide based on this oil, as the residual toxicity of the plant oils may be low. In this regard, it can be expected that if plant oil is not able to kill the different stages of mite pest, it will not remain toxic long enough to affect this mite pest once it has reached adulthood (George *et al.* 2010) [35].

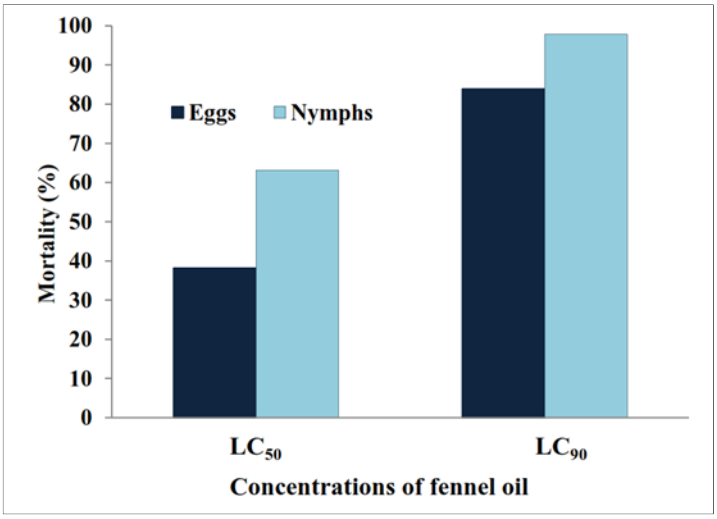

Fig 1: Acaricidal activity of tested concentrations of fennel oil (LC_{50} and LC_{90}, estimated for *T. urticae* females) against eggs and nymphal stages of *Tetranychus urticae*.

Repellency effect of fennel oil against *T. urticae*

For all tested concentrations of fennel oil (LC_{30}, LC_{50}, and LC_{90}, estimated for *T. urticae*) and at all evaluation periods (2, 8, and 24 h post-treatment), the results of repellency assay showed a trend of lower percentages of *T. urticae* on the leaf halves treated with fennel oil compared to the control halves (all *P*'s = 0.000) (Fig. 2). In all evaluation periods, the lowest numbers of *T. urticae* were present on the leaf halves treated with LC_{90} of fennel oil compared to LC_{30} and LC_{50} (Fig. 2). Similarly, it was found that fennel oil showed a repellent effect against ticks (Aboelhadid *et al.* 2021) [27]. At 24 h after treatment, significantly higher numbers of *T. urticae* eggs were deposited on the control halves compared to the leaf halves treated with LC_{30} ($P = 0.000$), LC_{50} ($P = 0.000$), and LC_{90} ($P = 0.000$) of fennel oil (Fig. 3)

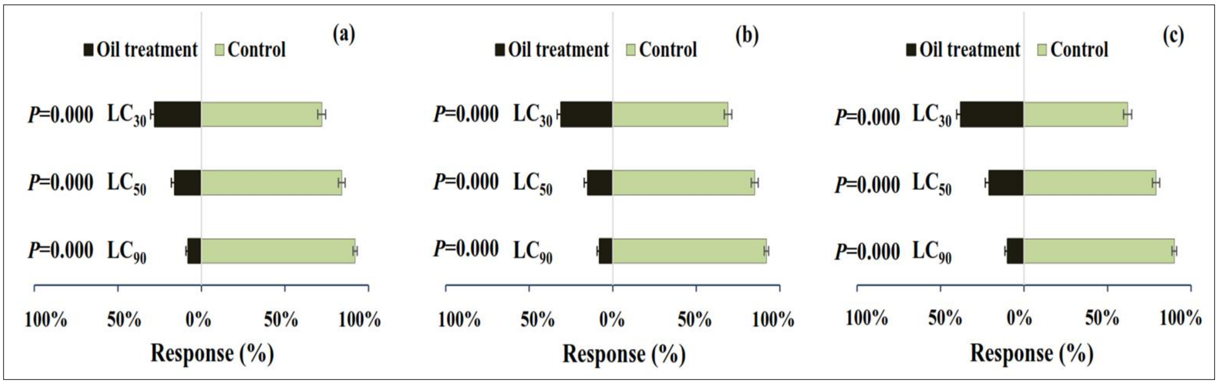

Fig 2: Percentage of *Tetranychus urticae* females choosing between leaf halves that were treated with LC_{30}, LC_{50}, LC_{90} of fennel oil or distilled water (control). The response (%) of mites to the treated or control leaf halves was evaluated at (a) 2, (b) 8, and (c) 24 h after treatment. All the means were displayed with ±SE. For each tested LC, the difference between treatment and control was analyzed by t-test; *P* value was given.

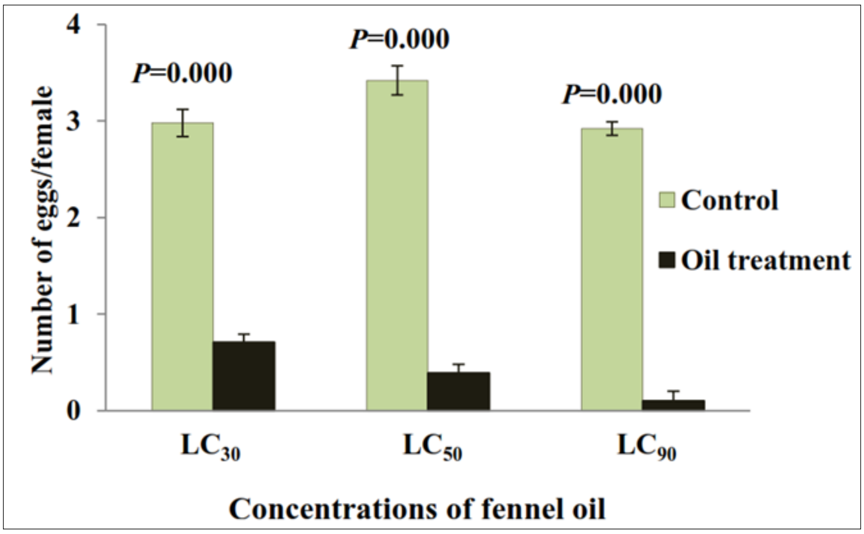

Fig 3: Number of eggs/female of *Tetranychus urticae* on the leaf halves treated with LC_{30}, LC_{50}, or LC_{90} of fennel oil and on the control halves (at 24h after treatment). All the means were displayed with ±SE. For each tested LC, the difference between treatment and control was analyzed by t-test; *P* value was given.

At 24h after treatment, the repellency (R) (%) and oviposition deterrence index (ODI) (%) were calculated for the tested concentrations (LC_{30}, LC_{50}, and LC_{90}) of fennel oil against *T. urticae* and presented in Figure (4). The highest R and ODI values were recorded for LC_{90} (R=88.89% and ODI=93.38%), followed by LC_{50} (R=73.42% and ODI=79.49%), and LC_{30} (R=38.71% and ODI=61.62%) (Fig. 4). Arthropods are capable of detecting the toxic materials and get away from the treated area (Cordeiro *et al.* 2010) [36]. In this context, previous studies have proven the repellency effect of the plant oils against *T. urticae* (Momen *et al*. 2014; 2018) [5, 8].

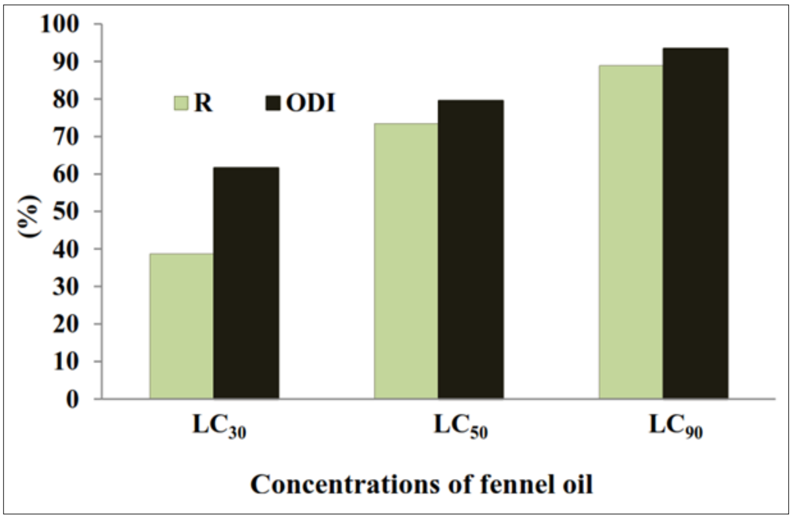

Fig 4: Repellency (R) (%) and oviposition deterrence index (ODI) (%) of LC_{30}, LC_{50}, and LC_{90} of fennel oil against *Tetranychus urticae* (at 24h after treatment).

Contact effect of fennel oil on the predatory mites

The effect of two tested concentrations of fennel oil (LC_{50} and LC_{90}, estimated for *T. urticae*) on eggs and females of *A. swirskii* and *C. negevi* was showed in Figure (5). Herein, although fennel oil showed an ovicidal activity against *T. urticae*, it did not harm predatory eggs. For *A. swirskii* and *C. negevi*, the presence of their eggs on leaf surface treated with LC_{50} or LC_{90}, estimated for *T. urticae*, did not cause mortality in eggs (Figure 5-a). Similarly, Acaridoil 13SL (a plant oil-based pesticide) had no ovicidal activity against *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) eggs (Tsolakis and Ragusa 2008) [37]. Also, *Melissa officinalis* L. oil has been reported to be relatively nontoxic to eggs of the phytoseiid mites, *A. swirskii* (LC_{50}=35.12 %), *Neoseiulus barkeri* (Hughes) (LC_{50}= 40.11 %) (Abdel Kader *et al*. 2015) [6], and *Neoseiulus californicus* McGregor (LC_{50}= 44.48 %) compared to its ovicidal activity against *T. urticae* eggs (LC_{50}=0.38 %) (Momen *et al.* 2014) [5]. Likewise, a high ovicidal effect of Suffoil (a biopesticide based on plant oils) was demonstrated against *T. urticae* eggs, while almost no ovicidal activity was detected against *N. californicus* eggs (Takeda *et al.* 2020) [38]. These variations in the observed ovicidal activity of plant oils/biopestisides against *T. urticae* and predatory mites could be explained by variations in the amount of plant oil/biopestiside sticking to the eggs, where the oily coverage area was greater for *T. urticae* eggs than for predatory eggs (Takeda *et al.* 2020) [38].

Furthermore, Takeda *et al.* (2020) [38] revealed that this oily coverage impedes the hatching of *T. urticae* egg. In the present study, *T. urticae* eggs were sprayed with fennel oil directly, while eggs of predators were exposed to fresh residue of this oil, so this difference in the method of exposure to the oil may be an additional explination for this difference in the ovicidal activity of oil between mite species.

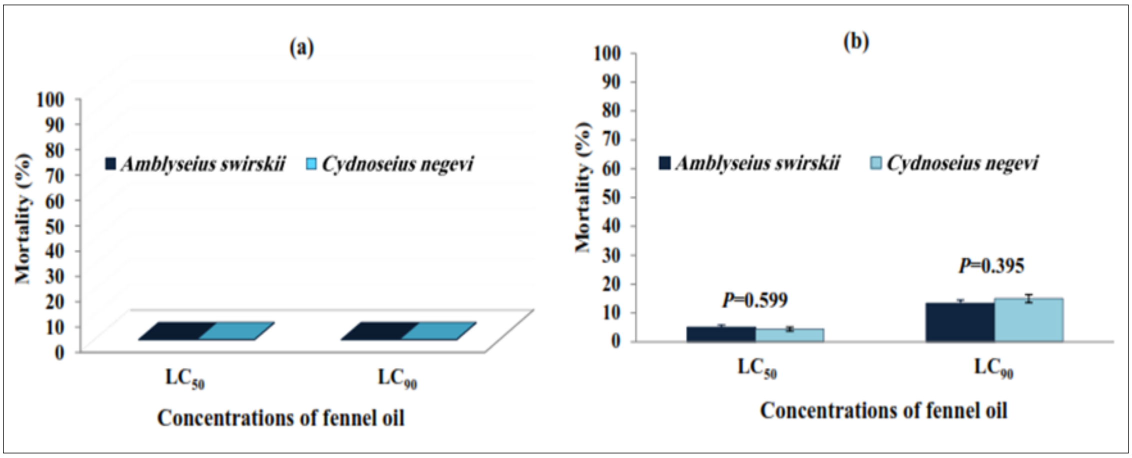

Fig 5: Effect of two concentrations of fennel oil (LC_{50} and LC_{90}, estimated for *T. urticae*) on (a) eggs and (b) females of *Amblyseius swirskii* and *Cydnoseius negevi*. All the means were displayed with ±SE. For each tested concentration, the difference between mortality of females of *Amblyseius swirskii* and *Cydnoseius negevi* was analyzed by t-test; *P* value was given.

Presently, females of *A. swirskii* and *C. negevi* were less sensitive to fennel oil than *T. urticae* females. Figure (5-b) showed that there was no significant difference in the effect of fennel oil on the females of two predatory mites (for LC_{50}: *P*=0.599; for LC_{90}: *P*=0.395). When predatory females came into contact with the LC_{50} and LC_{90} (estimated for *T. urticae*) of fennel oil, the highest mortality (%) was recorded for *C. negevi* females (15.00 % for LC_{90}) (Figure 5-b). In this context, *A. swirskii* showed low susceptibility to sweet basil oil (Momen and Amer 2003) [39]. Also, Mohamed *et al.* (2017) [40] concluded that *C. negevi* could be compatible with two plant oil-formulations in the control strategies of *T. urticae*. Oliveira *et al.* (2017) [28] indicated that some plant oils were less toxic to predatory mites despite their high acaricidal activity against mite pest; which is in line with the present study. The lower sensitivity of predators to different plant oils compared to pests can be attributable to the differences in the morphological and physiological features, metabolism, and foraging behavior (Cloyd *et al*. 2006; Lima *et al*. 2012; Momen *et al.* 2014) [41, 42,5].

Side effects of fennel oil on the predatory mites

During one week, the total and daily oviposition of *A. swirskii* and *C. negevi* were not significantly affected after their females came into contact with LC_{50} (estimated for *T. urticae*) of fennel oil compared to the control (Table 2). On the other hand, the higher tested concentration caused significant reduction in the fecundity of *A. swirskii* and *C. negevi* (Table 2). Momen and Amer (1994) [43] revealed that the fecundity of *N. barkeri* females feeding on prey previously reared on canna extract-treated leaves was approximately close to that of the control females. Also, no significant decrease in the fecundity of the phytoseiid mite, *Typhlodromus athiasae* Porath and Swirski was observed after being treated with sweet marjoram and rosemary oils (Momen and Amer 1999) [44]. As well, there was no significant effect on the oviposition rate of *P. persimilis* females treated with Acaridoil 13SL (Tsolakis and Ragusa 2008) [37]. Moreover, Abdel Kader *et al*. (2015) [6] showed that the oviposition rate of *A. swirskii* did not differ significantly between control females and those treated with LC_{25} (estimated for *A. swirskii*) of Melissacide (plant oil formulation). In contrast, the fecundity of the phytoseiid mites, *Amblyseius zaheri* Yousef and El-Borolossy and *N. barkeri* females fed on prey previously reared on mineral oils-treated leaves was lower than that of the control females (Momen *et al.* 2003) [45]. Besides, Teodoro *et al.* (2020) [46] found that the fecundity of *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) decreased after exposure to degummed soybean oil. The differences in plant oils, tested concentrations, predator species, and experimental conditions may explains these different results.

The LC_{90} (estimated for *T. urticae*) of fennel oil caused 20% mortality of *A. swirskii* and *C. negevi*, while lower mortality was observed for the other tested concentration in one week after treatment (Table 2). The LC_{50} (estimated for *T. urticae*) of fennel oil appeared to be harmless for *A. swirskii* (*E*=21.79 %) and *C. negevi* (*E*=17.43 %) (Table 2). However, the higher tested concentration of this oil was slightly harmful for *A. swirskii* (*E*=35.80 %) and *C. negevi* (*E*=38.50 %) (Table 2). In the same way, Momen and Amer (1999) [44] indicated that sweet marjoram and rosemary oils were harmless to *T. athiasae*, while they were slightly harmful to *N. barkeri* and *A. zaheri*. It was also found that sweet basil oil was harmless to *A. zaheri*, while it was slightly harmful to *T. athiasae* (Momen and Amer 2003) [39]. In all cases, sex ratio of predators' progeny was in favor of females for *A. swirskii* and *C. negevi* (Table 2).

Table 2: Effect of fennel oil on reproduction, female mortality, and sex-ratio of the progeny of *Amblyseius swirskii* and *Cydnoseius negevi*.

Predatory mites	Treatments	[a] Number of eggs/♀	Number of eggs/♀/day	[b] Mortality (%)	Sex ratio (♀ %)	[c] *E*
Amblyseius swirskii	LC_{90}	9.75±0.38b	1.39±0.05b	20	61.12±1.96a	35.80
	LC_{50}	11.18±0.35a	1.60±0.05a	15	63.55±1.51a	21.79
	Control	12.15±0.39a	1.74±0.06a	0	65.60±1.77a	
	P	0.000	0.000		0.223	
Cydnoseius negevi	LC_{90}	8.38±0.38b	1.20±0.05b	20	53.32±0.94b	38.50
	LC_{50}	10.00±0.41a	1.43±0.06a	10	57.83±1.32a	17.43
	Control	10.90±0.32a	1.56±0.04a	0	60.29±1.35a	
	P	0.000	0.000		0.003	

[a] Total number of eggs / female during 7 days after treatment (Mean±SE).
[b] Female mortality (%) during 7 days after treatment.
[c] *E* (effect of fennel oil on *A. swirskii* and *C. negevi*); Based on the value of *E,* Hassan (1985) [32] classified the effect (*E*) of oil on the predator into four categories (harmless, slightly harmful, moderately harmful, and harmful).
Means within a column for each predator followed by different letters are significantly different (Tukey's test; $P < 0.05$).

In pest control programs, acaricides are applied to decrease pest density prior to the release of bio-control agents (Malezieux *et al.* 1992) [47]. Therefore, direct contact toxicity is not an accurate method for measuring toxicity on natural enemies (Park *et al.* 2011) [48]. Accordingly, in the present study the lethal and side effects of fennel oil on tested predators were determined by residual contact bioassay, rather than by direct contact bioassay (direct spraying). In the residual contact bioassay, predators were exposed to fresh residues of fennel oil. Moreover, the prey (*T. urticae*) was placed on the same oil-treated leaves with the predator to be a food source for the predators. Thus, for the predators, ingestion of prey that is present (or that is present and fed) on the same oil-treated leaves might render this prey a potential additional pathway of oil exposure in the current study. However, botanical oils are known to degrade easily in the environment due to their natural origin (Ebadollahi *et al*. 2020) [10]. Thus, their short-term persistence on treated plants could improve their compatibility with predatory mites.

Conclusion

The present results showed that fennel oil had acaricidal and repellent activities against *T. urticae*. In addition, this oil generally had harmless or slightly harmful effects on *A. swirskii* and *C. negevi*. Therefore, by combining the lethal and repellent effects of fennel oil against *T. urticae* as well as its slight side effects on the tested predators, the present results suggested that fennel oil may be a promising tool in IPM programs of *T. urticae*.

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