Sublethal Effects of Essential Plant Oils on Biochemical and Ecological Parameters of the Predatory Mite *Amblyseius Swirskii*

Somayyeh Ghasemzadeh (✉ s.gasemzadeh@yahoo.com)

Institute of Plant Protection <https://orcid.org/0000-0002-8112-8377>

Gerben J. Messelink

Business unite greenhouse horticulture

Gonzalo A. Avila

The new Zealand institute for plant and food research limited

Yongjun Zhang

Institute of Plant Protection

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Abstract

The predatory mite *Amblyseius swirskii* is widely used natural enemy of phytophagous pests. Due to negative effects of conventional pesticides on non-target organism, the development of selective natural and eco-friendly pesticides such as essential plant oils are a useful pest control tool to use in synergy with biological control agents. Essential oils of *Nepeta crispa*, *Satureja hortensis* and *Anethum graveolens* showed promising results to control *Tetranychus urticae*. Hence an experiment was carried out to evaluate the effects of these essential oils on biochemical and demographic parameters of *A. swirskii*. Significant reduction of carbohydrate, lipid and protein contents of oil-treated predatory mites was observed. However oils of *S. hortensis* and *A. graveolens* had no negative impact on lipid reserve. Glutathione-S-transferase activity of *A. swirskii* was influenced by *A. graveolens*. In addition, the enzyme activity of the α -esterases was elevated by all treatments. The essential oils showed no effect on β -esterases activity compared to the control treatment. None of the concentrations of the different tested oils affected the population growth parameters of *A. swirskii*. However, a significant reduction was observed in oviposition time and total fecundity of predatory mites. Our observations show that the tested essential plant oils need some caution when applied as alternatives to synthetic pesticides in combination with *A. swirskii* and may have critical implications for Integrated Pest Management (IPM) programs. However, semi-field and field studies are required to evaluate the effects of these products on *T. urticae* and *A. swirskii* for developing IPM strategies.

Introduction

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a major pest of ornamental plants, vegetable crops and fruit trees grown in greenhouses and open fields across the world (Van de Vrie et al. 1972; Jeppson et al. 1975). Although biological control of spider mites is very successful in most cases (Opit et al. 2004; Gigon et al. 2016), application of synthetic pesticides is still the most common form to control arthropod pests in some agricultural production areas (Monteiro et al. 2015). These pesticides have potential to induce pest resistance, increase production cost and could negatively impact the environment, natural enemies, ecological services and human health (Geiger et al. 2010; Attia et al. 2013; Yorulmaz Salman and Ay 2013). Sublethal insecticide exposure can lead to physiological and behavioral changes in the target organism (Hyne and Maher 2003). Population parameters of the target and non-target species (either harmful or beneficial) may be influenced by the lethal or sublethal concentrations of pesticides (Roush 1989; Desneux et al. 2007; Nadimi et al. 2011; Alinejad et al. 2014; Sarbaz et al. 2017; Ghasemzadeh and Qureshi 2018). Due to negative effects of the pesticides on other beneficial mites and insects (Fernandes et al. 2010; Lira et al. 2015; Shahbaz et al. 2019), over the past few years researches number of studies have focused on essential oil's potential for developing natural pesticides which have well shown biocide effects on a variety of arthropods (Birkett et al. 2010; Ali et al. 2016; Camilo et al. 2017; Fatemikia et al. 2017; Mar et al. 2018).

The use of biological control agents in combination with selective natural insecticides, such as essential plant oils that are environmentally sustainable, can be applied as alternatives to conventional insecticides to control pests. These can not only decrease the side effects of synthetic pesticides but also improve the efficiency of natural enemies in prey searching as shown by low toxicity, non-repellence and in particular cases attractiveness to oils (Oliveira et al. 2017; Freitas et al. 2018; Saraiva et al. 2020). However, caution is needed, because models predict pest resurgence when effective natural enemies are present, even when they are less

sensitive to pesticides than the pest (Janssen and van Rijn 2021). Essential oils of some species of *Nepeta*, *Satureja* and *Anethum* have been described to possess insecticidal and acaricidal properties (Çalmaşur et al. 2006; Amizadeh et al. 2013; Ebadollahi et al. 2015; Salman and Bayram 2017).

Insects and mites protect themselves against pesticides through detoxifying enzymes which are probably important in resistance development (Yorulmaz Salman and Ay 2014). General esterases and Glutathione-S-transferases (GSTs) are important detoxifying enzymes in the metabolism of synthetic and non-synthetic insecticides (Motoyama 1980; Mouches et al. 1986; Lamoureux and Rusness 1987; Pizzorno 2014). Activities of GST, carboxylesterase, and other metabolic enzymes can be affected by plant protection products (Ross et al. 2010; Mathieu et al. 2015). Although botanical pesticides show potential to be used in conjunction with biological control agents, supplementary studies are required to evaluate the potential direct and indirect effects of the pesticides on natural enemies before it can be recommended for IPM programs (Tedeschi et al. 2001; Momen and Amer 2003; Tavares et al. 2010; Zancunio et al. 2016; Poderoso et al. 2016; De Araújo et al. 2020). The main goal of this study was to assess whether there are any lethal or sublethal effects of non-commercial essential plant oils of *Nepeta crispa* Willd (Lamiales: Lamiaceae), *Satureja hortensis* L. (Lamiales: Lamiaceae) and *Anethum graveolens* L. (Apiales: Apiaceae) on the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). This predatory mite is a widely used natural enemy for biological control of small soft-bodied pest species including mites, thrips and whiteflies (Messelink et al. 2008; Messelink et al. 2010; Doğramaci et al. 2013; Calvo et al. 2015; Ghasemzadeh et al. 2017). The effects of plant oils were evaluated by assessing the effects of application on the energy contents, the responses of two detoxifying enzymes and the life table parameters of *A. swirskii*.

Material And Methods

Mite colonies

The colony of *T. urticae* started with individuals collected from apple orchards in Urmia (West Azerbaijan province, Iran). Mites were then maintained on potted bean plants (*Phaseolus vulgaris* L. var. Talash) (Fabales: Fabaceae) under conditions of $27\pm 2^{\circ}\text{C}$, $60\pm 5\%$ relative humidity, and 16 h light: 8 h dark photoperiod. *Amblyseius swirskii* was obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) and was reared on *T. urticae* on leaf discs of the bean plants. The leaves were placed upside down on a wet sponge with a layer of cotton on the top in plastic trays with water (23×13 cm) and held in an environmental chamber at $25\pm 1^{\circ}\text{C}$, $70\pm 5\%$ relative humidity, and a photoperiod of 16 h light: 8 h dark. All experiments were conducted under these laboratory conditions.

Plant oils and chemical analysis

Aerial parts of *Nepeta crispa* Willd, *Satureja hortensis* L. (Lamiales: Lamiaceae) and *Anethum graveolens* L. (Apiales: Apiaceae) in flowering stages were harvested from the mountain areas of West-Azerbaijan province (Northwestern Iran) in the middle of August and used for oil extraction.

For a chemical analysis of the plants, aerial parts of plant were dried at room temperature and chopped into small pieces. The samples (100 g) were hydro-distilled using a Clevenger-type apparatus for 4 h. The obtained essential oils were stored in sealed vials at -20°C for subsequent experiments.

For GC/MS analysis an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer (SpectraLab Scientific Inc., Canada) using a HP-5 MS capillary column (5% Phenyl Methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25 μ m film thickness) was used. The oven temperature was programmed as follows: 3 min at 80 °C, subsequently 8°C min⁻¹ to 180 °C, held for 10 min at 180°C. Helium was used as carrier gas at a flow rate of 1 mL min⁻¹ and Electronimpact (EI) was 70 eV. The injector was set in a split mode (split ratio of 1:500) and mass range acquisition was from 40 to 500 m/z. Essential oil constituents were identified by using the calculated linear retention indices (Wiley 2007; NIST 2005) and mass spectra with those reported in the NIST 05 and Wiley 07.

Oil effects on spider mites

To assess contact toxicity of the essential oils on *T. urticae* adults, a leaf disc painting method was used in accordance with the procedure described by Miresmailli et al. (2006) with slight modifications. The obtained essential oils of *N. crisper*, *S. hortensis* and *A. graveolens* were applied at concentration ranges of 19-171 μ l litre⁻¹, 33-297 μ l litre⁻¹ and 23-185 μ l litre⁻¹ of essential oils in carrier solvent (70% methanol + 30% water), respectively. A 20 μ l aliquot of each concentration was painted on the underside of the bean leaf discs ($\varnothing$ 2.5 cm) with a micropipette. After drying under laboratory condition for 10 min, each disc was placed in the bottom of a petri dish on top of a 10 cm diameter disc of filter paper moistened with distilled water. Untreated mites were held on leaf discs painted with the carrier solvent alone. A total of 20 adults of *T. urticae* were added per bean leaf disc. Each concentration assessed was replicated five times. Mortality was recorded after 24 h of exposure and the LC₅₀ value of pesticides and essential oils were estimated. Mites were considered dead if they did not move when prodded with a soft paint brush. The highest concentrations of chemicals calculated for *T. urticae* mortality were applied for further experiments with *A. swirskii*.

Oil side-effects on the biochemical composition of *A. swirskii*

To test the side-effects of the collected oils on the biochemical composition of *A. swirskii*, we exposed twenty-four h old non gravid females of *A. swirskii* to the highest concentrations of *N. crisper*, *S. hortensis* and *A. graveolens*. The bean leaf discs were painted with each essential oil and carrier solvent for control and allowed to dry for 10 min. The surviving predatory mites were collected after 24 h exposure and subsequently used for energy content and enzymes activity determination.

To determine the amount of total carbohydrates, lipid and protein, standard biochemical techniques were used. Protein was measured with the Bradford reagent, lipids with vanillin in phosphoric acid, and carbohydrates with the anthrone reagent.

Total carbohydrates and lipid contents were calculated using the method of Yuval et al. (1998) with slight modifications. Sixty treated adult female individuals were homogenized using a plastic pestle in 62.5 μ l of 2 % sodium sulphate (Na₂SO₄). Thereafter, 469 μ l of chloroform: ethanol (1:2) was added to the homogenate and samples were centrifuged for 10 min at 8000 $\times$ g at 4°C.

To determine carbohydrates content, 150 μ l of the supernatant were mixed with 100 μ l distilled water and then dissolved in 500 μ l of anthrone reagent (500 mg anthrone dissolved in 500 ml concentrated sulphuric acid (H₂SO₄)) for 10 min at 90°C. Samples of 200 μ l were put into wells on ELISA plates (Awareness Technology

INC, USA) and the rate of absorbance was read at 630 nm. Total carbohydrate value was calculated by standard curve using maltose (Sigma) as standard. This experiment was repeated 3 times for each treatment.

To measure lipids content, 125 µl of the supernatant was injected in a micro tube and dried at 40°C. Then, 125 µl of H₂SO₄ (98% Merck) were added to the sample and placed in a hot bath for 10 min at 90°C. Samples of 30 µl were mixed with 270 µl of vanillin solution (600 mg of vanillin in 100 ml distilled water and 400 ml 85% H₃PO₄) and put into wells on ELISA plates (Awareness Technology INC, USA). The plate was shaken for 30 min in shaking incubator at room temperature and subsequently, the absorbance rate was recorded at 545 nm. The amount of total lipid was calculated using cholesterol as standard. This experiment was repeated 3 times for each treatment.

Total protein content was specified based on the Bradford method (1976) using bovine serum albumin as standard. Thirty treated adult female individuals were homogenized with 100 µl of phosphate buffer (pH 7.0) and centrifuged for 10 m at 10000 × *g* at 4°C. The supernatant of 10 µl was mixed in 500 µl Bradford's reagent and the absorbance rate was read at 630 nm in an ELISA reader (Awareness Technology INC, USA). This experiment was repeated 3 times for each treatment.

The activity of general esterase and GST were determined using the methods of Van Asperen (1962) and Habig et al. (1974), respectively. To assay general esterase, 60 adult treated *A. swirskii* females were homogenized with a plastic pestle in 80 µl of phosphate buffer (0.2 M, pH 7.0) prepared with 0.2% Triton X-100 (Sigma). α -naphthyl acetate (α -NA) and β -naphthyl acetate (β -NA) (Fluka) were used as substrates. The homogenized solution was centrifuged at 12,000 × *g* for 10 minutes at 4°C. The supernatant of 13 µl and 112 µl of phosphate buffer was added to a 96-well microplate. The reaction was initiated by addition of 50 µl of substrate solution (0.65 mM in buffer) per well. After 15 min incubation at room temperature, 50 µl fast blue RR salt (Fluka) was added and the microplate left in dark conditions for 30 min. The esterase enzyme activity was calculated at 450 nm and 540 nm for α -NA and β -NA, respectively, with 16 intervals of 30 seconds using microplate reader (Awareness Stat Fax_ 3200).

To determine GST activity, 1-chloro-2,4-dinitrobenzene (CDNB, Merck, Germany) and reduced GSH were used as the substrates. Sixty treated adult females of *A. swirskii* were homogenized in 80 µl of phosphate buffer (0.2 M, pH 7.0) in Eppendorf tubes using a plastic pestle and followed by centrifugation at 12,000 × *g* for 10 min at 4°C. The supernatant of 15 µl was mixed with 110 µl 0.2 M (pH 7.0) of phosphate buffer, 80 µl of CDNB and 100 µl of GSH in buffer. The absorbance rate was continuously measured at 340 nm with 16 intervals of 30 seconds.

Side-effects of oils on the biological parameters of *A. swirskii*

All experiments were conducted on fresh-excised bean leaf disks (2.5×2.5 cm) that were placed upside down in 30-ml transparent plastic cups containing water agar mixture (10%) under conditions of 25±1°C, 70±5% relative humidity, and a photoperiod of 16 h light: 8 h dark. Lids were provided with a vent covered with insect gauze. To study the effect of essential oils of *N. crispera*, *S. hortensis* and *A. graveolens* on *A. swirskii*, bean leaf discs were painted with each essential oil and carrier solvent, or with the carrier solvent alone as a control treatment, and allowed to dry for 10 min (Miresmailli et al. 2006). A cohort of seventy five 24-h old females of *A. swirskii* from untreated bean plants were placed individually on leaf discs of each treatment and control.

After 24 h, forty surviving females from each treatment and control were moved separately to untreated bean leaf discs and individually placed on discs. Following 24 h, the eggs laid by individual females in each experimental arena were stored as per above conditions at one egg per disc per female. The cohort of 0-24 h old eggs from each female was kept through eclosion of nymphs and followed through development to adult. During their development, five adults of spider mites *T. urticae* were provided daily as prey. Experimental arenas were checked daily to record survival and developmental time of the different life stages. The leaves were replaced every three days (in case of necessary). Each newly emerged female from four treatments were coupled with an untreated male for mating as per above conditions. Survival and fecundity was recorded until the death of the last individual.

Statistical analysis

Mortality curves of spider mites were estimated by probit analysis (SPSS 2012). A generalized linear model (GLM) with binomial distribution with logit function was used to investigate differences in mortality rates of *T. urticae* between different oil concentrations for each of the three plant species tested. Results from trials conducted with *A. swirskii* to assess the side effects of the different oils on its biochemical composition were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's test to compare differences among various treatments.

Raw data on the survivorship, longevity, and daily fecundity of individual females were analyzed using the age-stage specific (Chi and Liu 1985; Chi 1988) and TWOSEX life table using both genders in computer program MSChart (Chi 2017). Means and standard errors of the population parameters were estimated by the Bootstrapping procedure with 100,000 replications (Efron and Tibshirani 1993; Reddy and Chi 2015). Bootstrapping uses random resampling, otherwise a small number of replications will generate variable means and large standard errors. Differences between means were compared using the paired bootstrap test at the 5% level of significance (Reddy and Chi 2015).

Following Chi (Chi and Liu 1985; Chi 1988; Chi and Su 2006; Chi 2017), the age-stage specific survival rate (s_{xj}), where x is age and j is the stage; age-stage life expectancy (e_{xj}); the age-specific survival rate (l_x); the age-specific fecundity (m_x); the net reproductive rate (R_0); the intrinsic rate of increase (r); the finite rate of increase (λ); the mean generation time (T) and the doubling time (DT) were calculated:

$$l_x = \sum_{j=1}^{\beta} s_{xj} \quad m_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}}$$

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad \sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad e_{xj} = \sum_{i=x}^{\alpha} \sum_{y=j}^m s'_{iy}$$

Where α is the number of age groups and β is the number of stages.

$$\lambda = e^r$$

$$T = \frac{\ln R_0}{r}$$

$$DT = \frac{\ln 2}{r}$$

Results

Chemical composition of the oils

Chemical components analysis of the essential oils revealed that the predominant chemical compound found on *N. crispa* oil was 1,8-Cineole (57.68%). Predominant essential oil compounds found on *S. hortensis* were carvacrol (34.75%), gamma-terpinene (34.28%) and para-cymene (16.96%). L-phellandrene (34.18%), Carvone (23.68%) and limonene (21.46%) were the main compounds identified in *A. graveolens* oil (Table 1).

Table 1 Chemical composition of the essential oils of *N. crispa*, *S. hortensis* and *A. graveolens*

Plant	Component	^a RRI	Composition %	Plant	Component	^a RRI	Composition %
<i>N. crispa</i>					Para-cymene	1028	16.98
	Alpha-pinene	939	2.50		Limonene	1034	0.74
	Sabinene	974	2.08		1,8-cineole	1038	0.28
	Beta-pinene	982	6.54		Gamma-terpinene	1062	34.28
	Beta-myrcene	993	0.42		Menthol	1176	0.50
	Para-cymene	1028	1.43		Thymol	1298	0.27
	Limonene	1034	1.37		Carvacrol	1308	34.75
	1,8-cineole	1038	57.68		Trans-caryophyllene	1427	0.29
	Gamma-terpinene	1062	1.19		Caryophyllene oxide	1592	0.23
	Linalool	1098	1.26	<i>A. graveolens</i>			
	Terpinene-4-ol	1183	1.74		Beta-myrcene	993	0.74
	Alpha-terpineol	1195	4.45		L-phellandrene	1006	34.18
<i>S. hortensis</i>					Para-cymene	1028	5.53
	Alpha-pinene	939	1.96		Limonene	1034	21.46
	Beta-pinene	982	0.87		Gamma-terpinene	1062	0.77
	Beta-myrcene	993	1.88		Alpha-terpineol	1190	5.57
	Alpha-phellandrene	1007	0.33		Carvone	1251	23.68
	Alpha-terpinene	1019	2.77		Carvacrol	1308	0.79

^a RRI: Relative retention index

Oil effects on *T. urticae*

Mortality rates of *T. urticae* adults were significantly different among oil concentrations of *N. crispa* (Wald $\chi^2 = 115.9$; $df = 4$; $P < 0.001$), *S. hortensis* (Wald $\chi^2 = 102.5$; $df = 4$; $P < 0.001$) and *A. graveolens* (Wald $\chi^2 = 105.2$; $df = 4$; $P < 0.001$). The highest concentrations resulted in an average mortality of about 90% after 24 h exposure (Fig. 1). No mortality was recorded in control group.

Probit analysis data of acute toxicity of *T. urticae* adults in response to the three oils revealed the median lethal concentration (LC₅₀) values, which were the highest for *S. hortensis* and the lowest for *N. crispa* (Table 2).

Table 2 Median lethal concentration (LC₅₀) estimated using probit analysis for adult female *T. urticae* exposed to *N. crispa*, *S. hortensis* and *A. graveolens* for 24 h

Pesticide	LC (50) ($\mu\text{l liter}^{-1}$)	95% Confidence limits	Slope $\pm$ SE	χ^2	df
<i>N. crispa</i>	32.04	29.01–35.47	2.86 $\pm$ 0.24	0.93	3
<i>S. hortensis</i>	136.00	126.07–146.24	3.85 $\pm$ 0.35	1.24	3
<i>A. graveolens</i>	73.07	64.66–82.48	2.32 $\pm$ 0.20	3.05	3

Side-effects on biochemical parameters of *A. swirskii*

Calculation of total available energy as the sum of the energy contents, shows that treatments with the oils of *N. crispa*, *S. hortensis* and *A. graveolens* significantly reduced the total carbohydrates content ($F_{3,8} = 6.16$; $P < 0.018$), lipid content ($F_{3,8} = 6.30$; $P < 0.017$) and protein content ($F_{3,8} = 9.75$; $P < 0.005$) in the females of *A. swirskii* compared to the control treatment. However treatments of *S. hortensis* and *A. graveolens* did not reduce lipid content in comparison to the control treatment (Fig. 2).

Exposure of *A. swirskii* adults to the three essential oils had a significant effect on the detoxification enzyme activity of GST enzyme ($F_{3,8} = 5.15$; $P < 0.028$) and the α -esterase enzyme ($F_{3,8} = 47.56$; $P < 0.0001$), but not on the β -esterase enzyme ($F_{3,8} = 1.16$; $P < 0.38$) (Fig. 3). All oil treatments resulted in a higher α -esterase enzyme activity compared to the control, with the highest levels for *S. hortensia*, whereas the GST enzyme activity was only higher for the *A. graveolis* oil treatment compared to the control treatment (Fig. 3).

Side-effects on demographic parameters of *A. swirskii*

The application of *N. crispa*, *S. hortensis* and *A. graveolens* oils considerably affected developmental time, longevity and total life span of the progeny of *A. swirskii* females (Table 3). Duration of egg and larva stages of female and male from females exposed to the three treatments were not significantly different from the untreated control, but with an exception of *N. crispa* effects on the larva stage of females. There was no significant difference between treatments and control for protonymph and deutonymph duration of female and male (Table 3). However, developmental time of females was significantly prolonged in all treatments compared to the control treatment, but this was not the case for males. Female longevity compared to control was most significantly reduced by oil application of *N. crispa* and *A. graveolens* followed by *S. hortensis*. A similar effect was observed for males. Each of three tested treatments significantly reduced the total life span

of both genders compared to the control treatment, with most impact from *N. crisper* and *A. graveolens* (Table 3).

Table 3 Mean ($\pm$ SE) developmental time, longevity and total life span (days) of offspring from females of *A. swirskii* from control or treatments of *N. crisper*, *S. hortensis* and *A. graveolens*

Treatments					
Sex/stage	<i>N. crisper</i>	<i>S. hortensis</i>	<i>A. graveolens</i>	Control	F value*
Female					
Egg duration	1.76 $\pm$ 0.12a	1.73 $\pm$ 0.10a	1.70 $\pm$ 0.11a	1.48 $\pm$ 0.11a	33.36
Larva duration	1.33 $\pm$ 0.11a	1.27 $\pm$ 0.10ab	1.25 $\pm$ 0.10ab	1.09 $\pm$ 0.06b	30.54
Protonymph	1.81 $\pm$ 0.11a	1.82 $\pm$ 0.13a	1.71 $\pm$ 0.13a	1.74 $\pm$ 0.16a	3.99
Deutonymph	2.00 $\pm$ 0.17a	1.91 $\pm$ 0.17a	1.85 $\pm$ 0.17a	1.87 $\pm$ 0.17a	3.32
Developmental time	6.90 $\pm$ 0.23a	6.73 $\pm$ 0.22b	6.51 $\pm$ 0.179a	6.17 $\pm$ 0.29c	3122.13
Longevity	18.10 $\pm$ 0.22c	19.32 $\pm$ 0.19b	18.20 $\pm$ 0.17c	23.09 $\pm$ 0.22a	3122.13
Total life span	25.00 $\pm$ 0.35c	26.05 $\pm$ 0.28b	24.71 $\pm$ 0.26c	29.26 $\pm$ 0.32a	1026.86
Male					
Egg duration	1.82 $\pm$ 0.15a	1.67 $\pm$ 0.14a	1.61 $\pm$ 0.14a	1.53 $\pm$ 0.12a	13.55
Larva duration	1.41 $\pm$ 0.16a	1.39 $\pm$ 0.14a	1.28 $\pm$ 0.11a	1.24 $\pm$ 0.11a	7.82
Protonymph	1.88 $\pm$ 0.15a	1.83 $\pm$ 0.12a	1.72 $\pm$ 0.14a	1.76 $\pm$ 0.11a	5.53
Deutonymph	1.65 $\pm$ 0.12a	1.61 $\pm$ 0.12a	1.56 $\pm$ 0.12a	1.53 $\pm$ 0.15a	3.04
Developmental time	6.76 $\pm$ 0.34a	6.50 $\pm$ 0.34a	6.17 $\pm$ 0.34a	6.06 $\pm$ 0.20a	19.21
Longevity	17.04 $\pm$ 0.23c	18.33 $\pm$ 0.24b	17.50 $\pm$ 0.32c	22.35 $\pm$ 0.44a	1048.89
Total life span	23.71 $\pm$ 0.43c	24.83 $\pm$ 0.26b	23.67 $\pm$ 0.33c	28.41 $\pm$ 0.49a	598.96

Means followed by different letters in the same row are significantly different by using paired bootstrap test based on CI of difference ($P < 0.05$)

*F(df,n): female (3,82), male (3,66)

Reproduction and population growth parameters of *A. swirskii*

N. crisper and *A. graveolens* treatments resulted in a significantly prolonged pre-oviposition time compared to the control treatment (Table 4). Oviposition time was significantly reduced compared to the control in treatments with an exception of *S. hortensis*. Total fecundity of *A. swirskii* was negatively affected by all oil treatments. A similar effect was observed for the post-oviposition time (Table 4).

Table 4 Mean ($\pm$ SE) reproductive period and total fecundity of offspring from females of *A. swirskii* from control or treatments of *N. crisper*, *S. hortensis* and *A. graveolens*

Treatments					
	<i>N. crisper</i>	<i>S. hortensis</i>	<i>A. graveolens</i>	Control	F value*
Pre-oviposition (day)	3.48 $\pm$ 0.11ab	3.27 $\pm$ 0.13bc	3.65 $\pm$ 0.11a	3.17 $\pm$ 0.08c	80.87
Oviposition (day)	11.38 $\pm$ 0.26b	12.41 $\pm$ 0.21a	10.90 $\pm$ 0.26b	12.91 $\pm$ 0.18a	357.17
Post-oviposition (day)	4.92 $\pm$ 0.10b	5.15 $\pm$ 0.13b	5.35 $\pm$ 0.13b	6.20 $\pm$ 0.11a	278.00
Total fecundity (offspring)	11.57 $\pm$ 0.24c	12.59 $\pm$ 0.22b	11.40 $\pm$ 0.27c	13.57 $\pm$ 0.26a	359.66

Means followed by different letters in the same row are significantly different by using paired bootstrap test based on CI of difference ($P < 0.05$)

*F(df,n): (3,82)

Age-specific survival rate (l_x) and age-specific fecundity of the total population (m_x) exposed to essential oils were compared with populations in the control treatment (Fig. 4). Regardless the developmental stage, l_x represents the probability that an egg will survive to age x , and the curve of the age-specific survival rate is a simplified form of the curves of age-stage survival rate. Total life span averaged 29.26 days for the untreated females and 25.00 days, 26.05 days and 24.71 days for the females treated with highest concentrations of *N. crisper*, *S. hortensis* and *A. graveolens*, respectively. Comparison of the survival (l_x) of untreated mites and those treated with essential oils of *N. crisper* and *A. graveolens* revealed 4.9 and 5.30 % mortality in the immature stages, with 95.1 and 94.7 % chance of reaching adulthood, respectively. However the mites treated with *S. hortensis* showed no mortality in immature stages, with 100 % chances of reaching adulthood.

Maximum m_x of 0.78 eggs/female/day was observed on day 11 for untreated mites. For mites treated with *N. crisper*, *S. hortensis* and *A. graveolens*, m_x was approximately 0.61, 0.60 and 0.63 eggs/female/day, respectively, which occurred on days 15, 13 and 16 of life span, respectively. Based on the age-stage specific survival rate of both untreated and treated individuals of *A. swirskii*, the probability that an egg will survive to age x and develop to stage j was illustrated. Compared to the control, *N. crisper* and *A. graveolens* increased the pre-oviposition period. Male adults emerged simultaneously with females.

The age-stage survival rate (s_{xj}) represents the probability that an egg of *A. swirskii* will survive to age x and stage j . There is an overlap in the curves at different developmental periods among the individuals in all treatments. The highest female survival rate was observed in control compared with other treatments and 57.50% of eggs normally developed to the adult stage (Fig. 5).

There was no significant effect of the highest concentration of essential oils on the population parameters including intrinsic rate of increase (r), the finite rate of increase (λ), the net reproductive rate (R_0), the gross reproductive rates (GRR) and the mean generation time (T) (Table 5).

Table 5 Mean ($\pm$ SE) population parameters of the females of *Amblyseius swirskii* from control or treatments of *N. crisper*, *S. hortensis* and *A. graveolens*

	Treatments				F value*
	<i>N. crisper</i>	<i>S. hortensis</i>	<i>A. graveolens</i>	Control	
Intrinsic rate of increase, r (day^{-1})	0.11 $\pm$ 0.01a	0.12 $\pm$ 0.01a	0.11 $\pm$ 0.01a	0.13 $\pm$ 0.01a	42.11
Finite rate of increase, λ (day^{-1})	1.12 $\pm$ 0.01a	1.13 $\pm$ 0.01a	1.12 $\pm$ 0.01a	1.14 $\pm$ 0.01a	42.56
Net reproductive rate, R_0 (offspring)	6.07 $\pm$ 0.92a	6.92 $\pm$ 0.99a	5.70 $\pm$ 0.90a	7.80 $\pm$ 1.09a	36.55
Gross reproductive rate, GRR (offspring)	6.47 $\pm$ 0.94a	6.95 $\pm$ 0.99a	6.04 $\pm$ 0.93a	7.80 $\pm$ 0.09a	23.10
Mean generation time, T (day)	15.93 $\pm$ 0.23a	15.85 $\pm$ 0.23a	15.58 $\pm$ 0.25a	15.23 $\pm$ 0.29a	60.70

Means followed by different letters in the same row are significantly different by using paired bootstrap test based on CI of difference ($P < 0.05$)

*F(df,n): (3,156)

The age-stage-specific life expectancy (e_{xj} ; the period that an individual of age x and stage j is expected to survive) of *A. swirskii* individuals was affected by treatments. According to the e_{xj} curve of newborns (e_{01}), predatory mite was expected to live 20.00, 21.04, 19.66 and 25.26 days in *N. crisper*, *S. hortensis* and *A. graveolens* and control, respectively (Fig. 6).

Discussion

Over past decade, there has been an increased attention to use extracted oils from plants as alternatives for the conventional synthetic pesticides to control pests and prevent negative effects on natural enemies (Momen et al. 2001; Regnault-Roger et al. 2012; Isman and Grieneisen 2014; Pavela 2015; Nicolle et al. 2016; Atanasova and Leather 2018). The essential oils of *Nepeta sp.*, *Satureja sp.* and *Anethum* proved highly toxic to *T. urticae* (Aslan et al. 2004; Çalmaşur et al. 2006; Miresmailli et al. 2006), as also confirmed in this study. Investigation of the compatibility of plant-based acaricides and biocontrol agents is crucial for execution of IPM strategies (De Araújo et al. 2020). Therefore studies are needed to assess side-effects of biopesticides on beneficial organisms.

The present study evaluated the effects of the highest concentration rates of *N. crisper*, *S. hortensis* and *A. graveolens* on biochemical and demographic parameters of *A. swirskii*. The results demonstrated significant reduction in carbohydrate, lipid and protein contents of *A. swirskii* after exposure to the essential oils, except that lipid content in *S. hortensis* and *A. graveolens* treatments was not significantly different from the control treatment. It is likely that the reduction of carbohydrate content may be due to the antifeedant effect and an

increase in metabolism under toxicant stress (Remia et al. 2008). Lipids are an important source of energy and are reserved in body fat. The reserve of lipids during the feeding period increases but is reduced in non-feeding stage (Chapman 1998). The amount of total lipid levels of oil-treated *A. swirskii* females significantly reduced when compared to the control treatment. This reduction in lipid content of treated insects might have been caused by the effect of insecticide on the adipokinetic hormone that modulates the lipid metabolism (Sak et al. 2006). Proteins play a fundamental role in biochemical reactions and hormonal regulation in all known species (Sugumaran 2010). Decrease in protein content was observed in treated *A. swirskii* female compared to the control treatment. Reduction in protein content is a common phenomenon in insects after a treatment with toxic compounds (Nathan et al. 2008). It is likely that the insect degrades proteins to resultant amino acids in order to let them enter the TCA cycle (tricarboxylic acid cycle) as a keto acid for compensation of lower energy caused by stress (Bizhannia et al. 2005). Protein level reduction might be caused by the destructive effect of IGRs on central nervous system (Baker et al. 2009). Similar biochemical responses were found for insects treated with pesticides. Bashari et al. (2014) showed that hexaflumuron significantly decreased total carbohydrate, lipid and protein content of third instar larvae of *Xanthogaleruca luteola* Müll (Coleoptera: Chrysomelidae). Also clear decreases of total protein levels were observed in fifth instar larvae of *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae) after treatments with pyriproxyfen at 10, 75, 150 and 500 ppm concentrations (Etebari et al. 2007). Sak et al. (2006) also verified the striking decrease in glycogen, lipid and protein reserve of *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) exposed to cypermethrin.

An alternative way to analyze biochemical responses of organisms to pesticides is measuring the detoxification enzyme activity. The enzyme GST plays an important role in biotransformation and degradation of various pesticides (Yang 1976; Motoyama 1980). The activities of GST increased in treated predatory mite with *A. graveolens* as compared to control but not in *N. crisper* and *S. hortensis*. α -Esterase activity substantially increased in treated *A. swirskii* female compared to control, but levels of the enzyme β -esterase were not changed in response to oil treatments. Our results agree with Farahani et al. (2020) who found that the activity of GST and esterase (α -Na) significantly increased in *T. urticae* treated with some essential oils of Lamiaceae family. By contrast, GST, α - and β -esterase activities were reduced by exposure to high concentrations of *Cymbopogon flexuosus* essential oil in *Aedes aegypti* L. (Diptera: Culicidae) (Carreño et al. 2018). Similarly, Liao et al. (2017) and Gao et al. (2019) reported inhibitory effects of essential oils on insect detoxifying enzymes. This suppression of GST activity after exposure to pesticides in target and non-target species, have been extensively studied (Usui et al. 1977; Hayaoka and Dauterman 1982; Hodge et al. 2000; Gholamzadeh-Chitgar et al. 2015). GSTs are involved in detoxification of xenobiotics and protection of organisms from oxidative damage and play an essential role in detoxification of insecticides and thus rendering them less or non-toxic (Hayes and 1995; Rufingier et al. 1999). Esterases are classified as hydrolases, a large and diverse group of enzymes participating in xenobiotic detoxification by surrounding toxic compounds and consequently preventing them from gaining access to the target site (Dauterman 1985; Hayes and 1995). Differences in populations, the mode of action of essential oils, concentrations and time of exposure could explain variability in the results reported in different studies (Liao et al. 2017; Shojaei et al. 2017).

The highest concentrations of *N. crisper*, *S. hortensis* and *A. graveolens* prolonged developmental time of *A. swirskii* female. These effects may eventually result in a reduced population growth of this predator. Similar effects were observed on developmental time duration of *T. urticae* when exposed to sublethal concentrations

of *Cinnamomum zeylanicum* Blume (Laurales: Lauraceae) (Rezaei et al. 2014). Also the longevity and total life span of *A. swirskii* were in all oil treatments reduced. Overall, *S. hortensis* had the strongest negative effect on *A. swirskii* followed by *N. crispa* and *A. graveolens*. Similar effects of aromatic plant oils on female longevity have been reported when *T. urticae* was exposed to *C. zeylanicum* (Rezaei et al. 2014), *Artemisia annua* L. and *Rosmarinus officinalis* L. essential oils (Esmaily et al. 2017). Similar observations have been reported in other studies, where plant extracts also negatively affected the life cycle of insects (Gaspari et al. 2007; Poderoso et al. 2016).

Reproductive variables of *A. swirskii* after exposure to oils from *N. crispa*, *S. hortensis* and *A. graveolens* were reduced compared to untreated mites. Also here similar effects were found for other insects and mites. Fecundity reduced when *Brevicoryne brassicae* (Hemiptera: Aphididae) was treated with sublethal concentration of *Thymus daenensis* pure essential oil and its nanocapsule (Heidary et al. 2020). Fecundity of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) also decreased substantially when exposed to sublethal concentrations of diflovidazin (Havasi et al. 2019). Although a commercial formulation of azadirachtin significantly reduced fecundity of *Neoseiulus baraki* Athias-Henriot (Acari: Phytoseiidae) (Lima et al. 2015), garlic extract compounds did not have a significant effect on fecundity of *P. maculiventris* (Mamduh et al. 2017).

The full rate treatments of tested essential oils significantly reduced the age-specific survival and fecundity of *A. swirskii*, however, *S. hortensis* had negligible impact on the mite survival. Exposure of predatory bug *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) to formulations of citrus essential oils produced similar impact on survival of the predator (Campolo et al. 2020). The essential oil of *Ferula asafoetida* L. (Umbelliferae) led to significant reduction in fecundity and survival rate of parasitoids *Trichogramma embryophagum* (Hartig) and *T. evanescens* Westwood (Hymenoptera: Trichogrammatidae) (Poorjavad et al. 2014). Sublethal concentrations of a natural based acaricide with commercial name of Biomite® caused significant reduction in age-specific fecundity and survival curves of *N. californicus* compared with control treatment (Havasi et al. 2020). These discrepancies could be due to the susceptibility of different species, experiment methods, different chemical constituents, formulations and the concentrations (Obeng-Ofori et al. 1997; Zekri et al. 2013).

The assessments on population growth and reproductive rates of treated and untreated females of *A. swirskii* showed that the examined essential oils did not negatively affect r_m , λ , R_0 and GRR of the predatory mite at the highest concentrations. In accordance to our results, diflovidazin (Havasi et al. 2019) and Biomite® (Havasi et al. 2020) didn't have significant effect on the r_m and λ of *N. californicus*. In contrast to our result, plant essential oils significantly reduced the values of r_m and λ of other arthropods (Esmaily et al. 2017; Mamduh et al. 2017; Saraiva et al. 2020). Differences in species, populations, experimental method, formulations and concentrations could be responsible for different results between studies. Our findings regarding to R_0 and GRR of *A. swirskii* in treatments of essential oils were in partial agreement with other reports indicating that the parameters were not significantly different when arthropods are exposed to biopesticides (Mamduh et al. 2017; Havasi et al. 2020). However, in a similar study looking at the sublethal effects of *Artemisia annua* L. and *Rosmarinus officinalis* L. essential oils on *T. urticae*, authors report that the values of R_0 and GRR demonstrated a declining trend after exposures to sublethal concentrations of essential oils of tested plants (Esmaily et al. 2017).

The age-specific survival rate (l_x), age specific fecundity (m_x), age-stage specific survival rate (s_{xj}) and age-stage life expectancy (e_{xj}) curves were significantly reduced in *A. swirskii* treated with highest concentration of *N. crisper* and *A. graveolens* compared to untreated mites, whereas *S. hortensis* was negligible influential. Similar results were reported on *Podisus nigrispinus*, where survival rates of this predatory stink bug declined at different concentrations of neem oil (Zanuncio et al. 2016). Reduction in *T. urticae* life expectancy (e_{xj}) is an effect of essential oils treatment (Rezaei et al. 2014; Esmaeily et al. 2017). Garlic extract reduced age-stage-specific survival rate (s_{xj}) of *P. maculiventris* (Mamduh et al. 2017). Sublethal concentrations of Biomite® caused significant reduction in the age-specific fecundity (m_x) and survival (l_x) curves of *N. californicus* (Havasi et al. 2020).

Overall, exposure of *A. swirskii* to the highest residual concentrations of *N. crisper*, *S. hortensis* and *A. graveolens* had disruptive effects on the energy reserves, survival, longevity and fecundity of the predatory mite. Nevertheless, the essential oils showed no substantial change in population parameters (r_m , λ and R_0). The intrinsic rate of increase (r) is considered as the best measure for evaluating the total effects of a pesticide (Moscardini et al. 2013) and the use of r has been highlighted as an ecologically bioassay parameter for toxicology studies (Allan and Daniels 1982). According to our observations, the examined essential oils need to be cautiously applied as alternatives to conventional insecticides and may be used in combination with biological control agents within an IPM program. We suggest that shelf life of essential oils and side-effects on natural enemies should be further evaluated under semi-field and field conditions before they can be included as part of holistic approach in pest-control programs.

Declarations

Conflicts of Interest

The authors declare that they have no competing interests. The authors did not receive funds for the submitted work.

Author contribution

SG and YZ conceived and designed the experimental plan. SG and YZ are considered joint corresponding authors. SG performed the experiments and wrote first draft of manuscript. GM, GA assisted with data analysis. All authors commented on manuscript. All authors approved the final manuscript.

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Ethical approval

This study does not contain any experiments using any animal species that require ethical approval.

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Figures

Figure 1

Estimated mean mortality of *T. urticae* adults by residual concentrations of a) *N. crisper*, b) *S. hortensis*, and c) *A. graveolens*. The circles are the back-transformed predicted means by the GLM model and the vertical lines show back-transformed 95% confidence intervals. Non-overlapping confidence intervals within each oil concentration suggest evidence of statistically significant difference

Figure 2

Total energy content (mean $\pm$ SE) of the carbohydrate, lipid and protein reserves of *A. swirskii* females exposed to *N. crisper*, *S. hortensis* and *A. graveolens*. Different letters indicate significant differences based on Tukey's test (P = 0.05)

Figure 3

The enzymes activity (mean±SE) of *A. swirskii* ($\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) females exposed to *N. crispa*, *S. hortensis* and *A. graveolens*. Different letters indicate significant differences based on Tukey's test ($P = 0.05$)

Figure 4

Age-specific survival (l_x) and age-specific fecundity (m_x) of the population of *A. swirskii* females from control or treatments of *N. crista*, *S. hortensis* and *A. graveolens*

Figure 5

Age-stage specific survival rate (sxj) of the population from *A. swirskii* females from control or treatments of *N. crisa*, *S. hortensis* and *A. graveolens*

Figure 6

Age-stage life expectancy (ex_j) of *A. swirskii* females from control or treatments of *N. crista*, *S. hortensis* and *A. graveolens*