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Sublethal effects of plant essential oils toward the zoophytophagous mirid *Nesidiocoris tenuis*

Luis C. Passos[1,2] · Michele Ricupero[1] · Antonio Gugliuzzo[1] · Marianne A. Soares[2] · Nicolas Desneux[3] · Orlando Campolo[4] · Geraldo A. Carvalho[2] · Antonio Biondi[1] · Lucia Zappalá[1]

Abstract

Zoophytophagous hemipteran predators provide relevant biological control services and their applications are consolidated in greenhouse pest management. The use of plant essential oils (EOs) for sustainable crop protection is being currently promoted. However, further knowledge of the potential side effects of EOs on predatory mirids (Hemiptera: Miridae) is required. Here, we evaluated the non-target impact of four EOs (anise, fennel, garlic and lavender) on the generalist predator *Nesidiocoris tenuis* (Reuter) in the laboratory. The baseline toxicity of EOs was firstly assessed on *N. tenuis* adults following topical contact exposure. Then, the predator reproduction and orientation behavior was tested following the exposure to three estimated EO lethal concentrations (LC_1, LC_{10} and LC_{30}). Garlic EO had the lowest estimated LCs (e.g., LC_{30} 1.34 mg mL^{-1}), being thus the most toxic compound among the tested EOs. The estimated LC_{30}s for lavender, anise and fennel EOs were 2.75, 4.55 and 5.17 mg mL^{-1}, respectively. The fertility and the orientation behavior of *N. tenuis* females was negatively affected by all the EOs at the highest tested concentration. Nevertheless, anise EO at LC_1 and LC_{10} caused no sublethal effects on *N. tenuis*. Our findings suggest that careful attention should be given when EOs are used in combination with *N. tenuis* in pest management programs.

Keywords Biocontrol · Botanicals · Ecotoxicology · Integrated pest management · Non-target effects · Predator

✉ Antonio Biondi
antonio.biondi@unict.it

1 Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, 95123 Catania, Italy

2 Laboratório de Ecotoxicologia e MIP, Departamento de Entomologia, Universidade Federal de Lavras, Lavras, MG CEP 37200-900, Brazil

3 Université Côte d'Azur, INRAE, CNRS, UMR ISA, 06000 Nice, France

4 Department of Agriculture, University of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria, Italy

Introduction

The use of zoophytophagous hemipteran predators is a consolidated practice for the biological control of agricultural pests (Thomine et al. 2020; Pérez-Hedo et al. 2021a; van Lenteren et al. 2021). Also their role as plant defense elicitors has been recently exploited in several horticultural systems (Pérez-Hedo et al. 2021b). Among predatory mirid bugs (Hemiptera: Miridae), *Nesidiocoris tenuis* (Reuter) is used for suppressing arthropod pest populations in Mediterranean protected crops because it can spontaneously inhabit various crops or easily purchased in bio-factories for augmentative releases (Castañé et al. 2011; Calvo et al. 2012; Zappalà et al. 2013; Naselli et al. 2017). The mirid predator has the capacity to be highly predaceous and the ability to survive on plants when prey is scarce, although plant damage can occur due to its herbivory occur at high population levels and increasing temperatures (Madbouni et al. 2017; Siscaro et al. 2019; Ingegno et al. 2021).

Albeit this controversial aspect, *N. tenuis* is routinely used in tomato crops as a biocontrol agent against leafminers,

whiteflies and spider mites (Biondi et al. 2016). In fact, the introduction of the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in the Mediterranean became one of the main purposes of *N. tenuis* releases in tomato crops, as this invasive pest can cause serious yield losses in tomato (Biondi et al. 2018; Desneux et al. 2021; Mansour and Biondi 2021).

Conventional management of arthropod pests often relies on repeated applications of synthetic pesticides which frequently have adverse effects on non-target organisms, such as physiological and behavioral (Desneux et al. 2007). As eco-friendly alternative, botanicals have been recognized as efficient pest control tools, with plant essential oils (EOs) being their most emphasized category (Regnault-Roger et al. 2012; Rathore 2017). EOs are secondary plant metabolites constituted by volatile and semi-volatile compounds involved in defense mechanisms against biotic and abiotic factors (Walling 2000; Hare 2011; Miresmailli and Isman 2014). With a low toxicity toward mammalians and negligible persistence in the environment, EOs have been increasingly suggested for application in organic and IPM programs (Campolo et al. 2017; Giunti et al. 2019; Pavela et al. 2020).

Despite their potential benefits, EOs have constitutive drawbacks linked to their properties, such as phytotoxicity, low solubility in water, high volatility and fast degradation (Moretti et al. 2002; Regnault-Roger et al. 2012; Krzyżowski et al. 2020). Nevertheless, recent advances in nanotechnology can remedy these inconveniences through a constant release of the active ingredients, a reduction in degradation, and by improving their solubility and stability patterns (Kah et al. 2013; de Oliveira et al. 2014; Campolo et al. 2017, 2020a; Maroofpour et al. 2021; Cherif et al. 2022).

The integration between EOs and the predator *N. tenuis* can be an interesting strategy to improve the effective management of arthropod greenhouse pests, such as *T. absoluta*. However, the insecticidal activity of EOs might be also harmful toward *N. tenuis* by causing lethal and sublethal effects, such as the reduction in population growth parameters and biological control capacity (Soares et al. 2019). In fact, EOs have often broad-spectrum activity, resulting in direct insect mortality or side effects, such as antifeedant and/or repellent behavior, oviposition deterrence and growth regulation, both to pests and beneficial organisms, such as pollinators and biological control agents (Isman and Tak 2017; Rathore 2017; Kim et al. 2021).

Although numerous studies have focused on the EOs bioactivity toward greenhouse insect pests (Campolo et al. 2017; Dunan et al. 2021; Sciortino et al. 2021; Tortorici et al. 2022), limited knowledge on EOs non-target effect toward beneficial arthropods has been produced. Earlier laboratory studies assessed the non-target impact of *Citrus* spp., *Mentha pulegium* L. and garlic (*Allium sativum* L.) EOs on *N. tenuis* in terms of acute toxicity and sublethal

effects (Papadimitriou et al. 2019; Soares et al. 2019; Campolo et al 2020b; Ricupero et al. 2022). Most of these studies evaluated EO side effect by testing the pest recommended label rate or the lethal concentrations (LCs) estimated on the target pest. However, there is a lack of data concerning the dose–response relationship of EOs on *N. tenuis* and their effects at low concentrations (i.e. that cause low mortality) on the fertility and orientation capacity of this predator.

The aim of this study was to assess in the laboratory the baseline toxicity caused by different concentrations of four commercial EOs on the predator *N. tenuis*. We also investigated the behavioral and physiological response of the predator under the effect of these compounds in low concentrations.

Materials and methods

Biological materials

The *N. tenuis* individuals used in the bioassays were obtained in the laboratory rearing according to the methodology described by Passos et al. (2022). Adults of *N. tenuis* (~150 individuals) were kept in entomological cages (32×40×70 cm) covered by fine net mesh. Potted seedlings (~30 cm high) of *Sesamum indicum* L. (variety T-85 Humera) were added to the cages as water and oviposition sources, and the commercial mixture of the alternative prey *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and *Artemia* spp. cysts (i.e., Entofood®, Koppert, the Netherlands) was offered *ad libitum* to the predators (spread over the leaves) as an additional food source (Biondi et al. 2016). Individuals were maintained in entomological cages for three days for mating and oviposition, and subsequently they were collected with a mechanical aspirator and transferred into new cages as previously described. The sesame seedlings containing *N. tenuis* eggs were kept in the cages for egg hatching and the development of newly hatched nymphs until adulthood. Insect rearing and experiments were carried out under laboratory controlled conditions (25 ± 1 °C, 55 ± 5% RH, and 14L:10D photoperiod) at the Department of Agriculture, Food and Environment of the University of Catania (Italy).

EO nanoemulsions preparation and characterization

Anise (*Pimpinella anisum* L.-Apiaceae), fennel (*Foeniculum vulgare* Mill.-Apiaceae), lavender (*Lavandula angustifolia* Miler.-Lamiaceae) and garlic (*A. sativum*) commercial food grade EOs (Esperis s.p.a. Milano, Italy) were used to develop insecticide nanoemulsions. For complete analytical procedures and chemical characterization (GC/FID and GC/

MS analyses) of anise, fennel, lavender EOs see Campolo et al. (2020a), and for garlic EO see Ricupero et al. (2022).

All the developed EO-based nanoemulsions were obtained following the procedures proposed by Giunti et al. (2019). In details, 15% of each EO and 5% Tween 80 (w/w) were mixed for 30 min (8000 RPM) using a magnetic stirrer; then distilled water (80%) was dropwise (1 mL×min^{-1}) added and then stirred for 60 min. To reduce the particle size and improve the stability, the resulted raw emulsions were then sonicated for 5 min using an UP200ST ultrasonic immersion homogenizer (Hielsher$^{©}$, Teltow, Germany) at 100 W power (frequency: 26 kHz) (Campolo et al. 2020a). The physical characterization of the developed formulations was carried out by a dynamic light scattering (DLS) apparatus (Zetasizer Nano, Malvern). The quality and the stability patterns over time of the developed EO-based nanoformulations were evaluated through the measurement of the following parameters: droplet dimension, expressed in terms of Z-average size (nm), polydispersity index (PDI) and zeta (ζ) potential (droplet surface charge expressed as mV).

Baseline toxicity of EOs toward *Nesidiocoris tenuis* females

The concentration-mortality bioassay aimed at assessing the topical contact toxicity of anise, fennel, lavender and garlic EOs on *N. tenuis* adult females. *Nesidiocoris tenuis* females were topically exposed from five to six serial dilutions of each EO completely dispersed in a solution of distilled water. The EO dilutions were chosen according to preliminary observations aimed at identifying the minimum concentration necessary to cause 100% mortality of *N. tenuis* females and the maximum concentration that cause no mortality similarly to the untreated control. Additionally, a stock solution containing 15% of EO (i.e., concentrated nanoemulsion) was used as the highest concentration, and an untreated control with only distilled water was included for all the treatments as the "zero concentration".

For the bioassay, *N. tenuis* females (2-day old) from the laboratory rearing were isolated in groups of five individuals in conical ventilated plastic tubes (Falcon®—50 mL) and maintained at low temperature (~8 °C) inside a thermic box containing ice packs for 3 h to reduce their mobility. Thereafter, each group of *N. tenuis* females was placed in a plastic cup (100 mL) and immediately sprayed with 2 mL of each concentration of EO solutions through a hand-sprayer (50 mL). The inner part of plastic cups was covered in absorbent paper to avoid the formation of solution droplets and the potential drowning of sprayed insects. The absorbent paper was replaced among replicates for each EO-concentration. After spraying, each group of *N. tenuis* females was transferred in an acrylic ventilated arena (5.5 cm diameter, 3 cm ht) containing Entofood® (scattered on the arena) and a zucchini leaf disc (3 cm in diameter) as food and water sources. The number of dead females was recorded after 48 h. Each arena containing five *N. tenuis* females was considered a replicate and the experiment was replicated eight times per each EO tested concentration and the untreated control.

Effects of EOs on *Nesidiocoris tenuis* fertility

This bioassay aimed at evaluating whether previously determined decreasing EO concentrations can affect the progeny production of *N. tenuis* females. Predators were exposed to LC$_1$, LC$_{10}$ and LC$_{30}$ of anise, fennel, lavender and garlic EOs in order to emulate a potential field scenario in which different insecticide concentration may occur in field after environmental degradation, including a lethal range from negligible mortality (LC$_1$) to moderate mortality (LC$_{30}$).

Nesidiocoris tenuis adults (2 days old) were sprayed with LC$_1$, LC$_{10}$ and LC$_{30}$ of anise, fennel, lavender and garlic EOs in the same process previously described in "Baseline toxicity of EOs toward *Nesidiocoris tenuis* females". Thereafter, each *N. tenuis* male and female was placed into a ventilated arena (400 mL) containing 1 g of *E. kuehniella* eggs as food supply and a green bean pod (*Phaseolus vulgaris* L., cv. "Garrafal enana") as oviposition substrate (Passos et al. 2022). *Nesidiocoris tenuis* couples were kept into experimental arenas to mate successfully and allow females to oviposit into green bean pods. After 3 days, *N. tenuis* adults were removed from the arenas. Green bean pods bearing *N. tenuis* eggs were thus maintained at the aforementioned described controlled laboratory conditions. Approximately ten days after, newly emerged *N. tenuis* nymphs were daily counted (=fertility) and removed with a soft paintbrush under the stereomicroscope. The evaluation was conducted for 20 days until no nymph emerged. For each EO-concentration combination and the control, the fertility of 25 *N. tenuis* females (each female being a replicate) was evaluated.

Effects of EOs on *Nesidiocoris tenuis* orientation

The aim of this bioassay was to assess the potential effects of EOs at low-lethal concentrations on the orientation capacity of *N. tenuis* females. For this, adult females (~2 days old) were topically exposed to LC$_1$, LC$_{10}$ and LC$_{30}$ of anise, fennel, lavender and garlic EOs following the same methodology described in "Baseline toxicity of EOs toward *N tenuis* females". Briefly, *N. tenuis* females collected from the rearing were starved for 24 h in transparent vials with a moistened cotton wad as water source. Thus, after being sprayed topically, each *N. tenuis* female was individually transferred into a two-way olfactometer (main arm and lateral arms 15 cm long and 4 cm internal diameter) and observed. The odor sources were clean air and a sesame plant (~20 cm

height), since prior studies reported high attractiveness of this plant species to *N. tenuis* (Biondi et al. 2016). Sesame plant was placed inside one of the cylindrical glass jars (5 L) connected to the lateral arms of the olfactometer. An air pump (Airfizz®, Ferplast, Italy) produced a unidirectional flow (150 mL×min^{-1}) that passed through a water filter before entering the olfactometer system, conducting the air through the olfactometer lateral arms and reaching the main arm. The olfactometer was placed vertically on the bench surface and illuminated by 22 W cool-white fluorescent lamps positioned 80 cm above. The observations were conducted between 9:00 a.m. and 6:00 p.m. in a dark room at the aforementioned laboratory conditions. The olfactometer was inverted for eliminating environmental interference to insect response every two replicates. Both the olfactometer and glass jars were cleaned with pure grade acetone between the tested EOs and their different concentrations.

Each *N. tenuis* female was observed for 5 min and the time spent by the predator for choosing between the odor sources was recorded. The choice was considered valid when each *N. tenuis* female crossed 2/3 of the lateral arm. Per each EO-concentration combination 30 valid replicates, i.e., females that made a choice within the 5 min of observations, were considered.

Statistical analyses

The dependent variables (i.e., mortality, fertility and olfactory response data) were checked for normality of variance and homoscedasticity through Shapiro's test and Wilk's test, respectively, and dataset was log-transformed whenever needed. The baseline toxicity of anise, fennel, lavender and garlic EOs on *N. tenuis* by topical contact exposure was determined by a log-probit regression model. The concentration-mortality relationships were considered valid when

no significant deviation occurred between the observed and the expected values at $p < 0.05$ level. The preference of *N. tenuis* females that made a choice between sesame plant and clean air was analyzed through the chi-squared goodness-of-fit to determine whether the orientation behavior toward each odor source diverged from an equal (50:50) distribution. The effect of the factors "*EO*", "*Concentration*" and their interaction "*EO*"×"*Concentration*" on mortality, fertility and time spent data was analyzed through a Generalized Linear Model (GLM). Means were separated by a post-hoc Tukey HSD test. Probit analyses were carried out in IBM® SPSS® Statistics for Macintosh, Version 23.0.0.0 (IBM Corp. Released 2015. Armonk, NY: IBM CorpSPSS v. 21.0). Statistical analyses for fertility and olfactory response data were performed in "R" 3.6.0 (R Core Team 2019), using the packages "car" and "MASS" for model fitting and the package "multcomp" to separate means.

Results

Baseline toxicity of EOs to *Nesidiocoris tenuis* females

Probit models fit the observed data for all treatments ($p > 0.05$), validating the concentration–response curve and therefore estimated low-lethal concentrations for all EOs (Table 1). During the evaluation period, no death was recorded in insects treated only with distilled water ("zero concentration"). The lowest low-lethal concentrations were estimated for garlic EO which hence presented the highest toxicity toward *N. tenuis* females. The highest LC_1 and LC_{10} were estimated for anise EO, while the highest LC_{30} was recorded for fennel EO.

Table 1 Baseline toxicity of four nanoemulsioned essential oils toward *Nesidiocoris tenuis* females 48 h after topical contact exposure

Essential oil	Slope ± SE	χ^2 (df)	P	Lethal concentration (mg×mL^{-1})	95% Confidence limits (mg×mL^{-1})
Anise	2.058 ± 0.288	57.649 (46)	0.116	$LC_1 = 0.606$	0.212–1.065
				$LC_{10} = 1.949$	1.127–2.693
				$LC_{30} = 4.547$	3.450–5.772
Fennel	1.000 ± 0.220	61.942 (47)	0.071	$LC_1 = 0.081$	0.001–0.354
				$LC_{10} = 0.903$	0.124–1.862
				$LC_{30} = 5.166$	2.931–8.673
Garlic	1.368 ± 0.194	62.340 (47)	0.066	$LC_1 = 0.065$	0.011–0.159
				$LC_{10} = 0.375$	0.149–0.628
				$LC_{30} = 1.340$	0.856–1.899
Lavender	2.271 ± 0.282	48.430 (38)	0.120	$LC_1 = 0.443$	0.176–0.755
				$LC_{10} = 1.277$	0.747–1.785
				$LC_{30} = 2.751$	2.013–3.516

Effects of EOs on *Nesidiocoris tenuis* fertility

There was a significative interaction between "*EO*" × "*Concentration*" ($\chi^2 = 22.25$, df = 8, $p < 0.001$), indicating a higher reduction in *N. tenuis* fertility when EO concentration increased. For all EOs, the reduction in fertility was higher at LC_{30} (progeny/female of 1.33, 1.25, 1.20 and 1.36 for anise, garlic, fennel and lavender EOs, respectively; while the progeny for untreated control was 7.89) in comparison with LC_1 and LC_{10} (progeny/female of 3.42, 2.33, 2.25 and 2.78 at LC_1 and 3.00, 3.73, 3.06 and 2.93 at LC_{10} for anise, garlic, fennel and lavender EOs, respectively). Within the three concentrations, treatments with EOs significantly reduced the fertility of *N. tenuis* females in comparison with the untreated control (progeny/female of 7.35, 8.17 and 7.89 for the tests within LC_1, LC_{10} and LC_{30}) (Fig. 1).

Effects of EOs on *Nesidiocoris tenuis* orientation

In the untreated control *N. tenuis* females showed significant attraction toward sesame plants rather than clean air. Conversely, all three low-lethal estimated concentrations of lavender, fennel and garlic EOs affected the attraction of *N. tenuis* females toward sesame plants, resulting in no difference in the choices between sesame and clean air. The orientation capacity of *N. tenuis* females sprayed with anise EO was affected only at its LC_{30} (Fig. 2).

The time spent by *N. tenuis* females for choosing between the odor sources was also significantly affected by the EOs
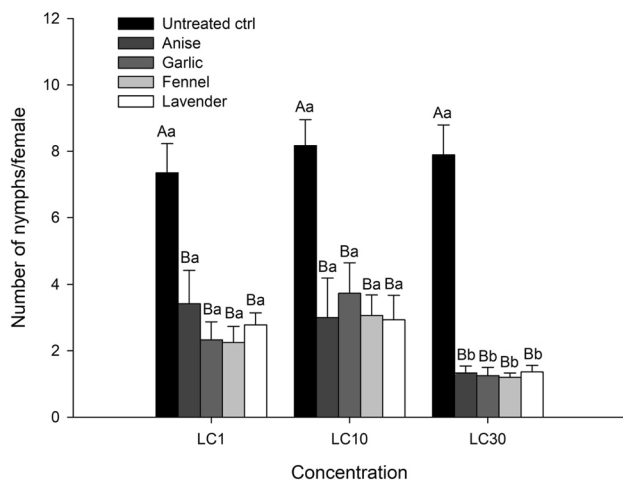

Fig. 1 Mean number (± SE) of nymphs generated by *Nesidiocoris tenuis* females during three days after being topically exposed to anise, fennel, garlic and lavender essential oils at three low-lethal concentrations (LC_1, LC_{10} and LC_{30}). Different capital letters indicate significant differences among treatments in a concentration, while different lower-case letters indicate significant differences in the concentrations for a treatment (GLM—Poisson distribution, Tukey HSD test, $p \leq 0.05$)

in LC_1 ($\chi^2 = 16.29$, df = 4, $p < 0.001$) and LC_{10} ($\chi^2 = 12.23$, df = 4, $p = 0.002$). *Nesidiocoris tenuis* females treated with garlic EO significantly spent more time for choosing in comparison with untreated insects. In LC_{30}, all EOs increased the time spent by insects to make a choice in comparison with untreated insects, except the anise EO ($\chi^2 = 28.92$, df = 4, $p < 0.001$). Regarding each EO, time spent by *N. tenuis* females to make a choice was not affected by the concentration for anise ($\chi^2 = 2.25$, df = 2, $p = 0.320$), garlic ($\chi^2 = 1.24$, df = 2, $p = 0.540$) and fennel ($\chi^2 = 4.64$, df = 2, $p = 0.100$); however, for lavender an increase was observed at LC_{30} in comparison with LC_1 ($\chi^2 = 6.04$, df = 2, $p = 0.040$) (Fig. 3). Nevertheless, no interaction among EOs and their concentrations was highlighted in *N. tenuis* choice time between the two odor sources ($\chi^2 = 9.39$, df = 8, $p = 0.310$).

Discussion

The interest of EOs as pest control tools has been increased in the past decades. These compounds are considered eco-friendly, with low impact on the environment and low toxicity to mammalians. EOs derived from plants of different species are have been studied in combination with different formulation technologies to obtain more stable and efficient plant protection products, in order to be incorporated in pest management programs (Campolo et al. 2017; 2020a). In our experiment, we found various toxicity levels caused by EOs toward *N. tenuis* behavior (orientation) and physiology (reproduction).

Among the tested EOs, garlic EO was the most toxic compound with the lowest lethal concentrations estimated. It also negatively affected the progeny produced by *N. tenuis* females especially that the concentration of LC_{30}. Also, the three tested LCs of garlic EO impaired the orientation capacity of *N. tenuis* since the predator spent significantly more time and it was not able to distinguish between attractive and non-attractive odor source. Although, the insecticidal properties of garlic EO have been widely recognized for numerous insect pests (Regnault-Roger 1997; Ricupero et al. 2022) and several commercial formulations are currently marketed in different world countries (Anwar et al. 2014), to the best of our knowledge, we reported for the very first the side effect of garlic EO on the behavior of a zoophytophagous mirid predator.

The high toxicity recorded for garlic EO can be attributed to its composition consisting in more than 90% of organosulfides. Several studies report the acute toxicity and sublethal effects of garlic EO and its components on different development stages of arthropod pests (Ho et al. 1996; Meriga et al. 2012; Yang et al. 2012; Plata-Rueda et al. 2017). The same nanoemulsioned garlic EO of the current study at the concentrations of 0.12 and 3% was

Fig. 2 Olfactory response of *Nesidiocoris tenuis* females topically exposed to anise, fennel, garlic and lavender essential oils at three low-lethal concentrations, (LC$_1$, LC$_{10}$ and LC$_{30}$ toward *Sesamum indicum* emitted volatiles and clean air. "n.c." = no choice, indicate the number of non-responder females. "n" = *N. tenuis* females making a choice within the 5 min of observations. Asterisks indicate significant differences in the attraction toward *S. indicum* plant and clean air (likelihood chi-squared test $p \leq 0.05$)
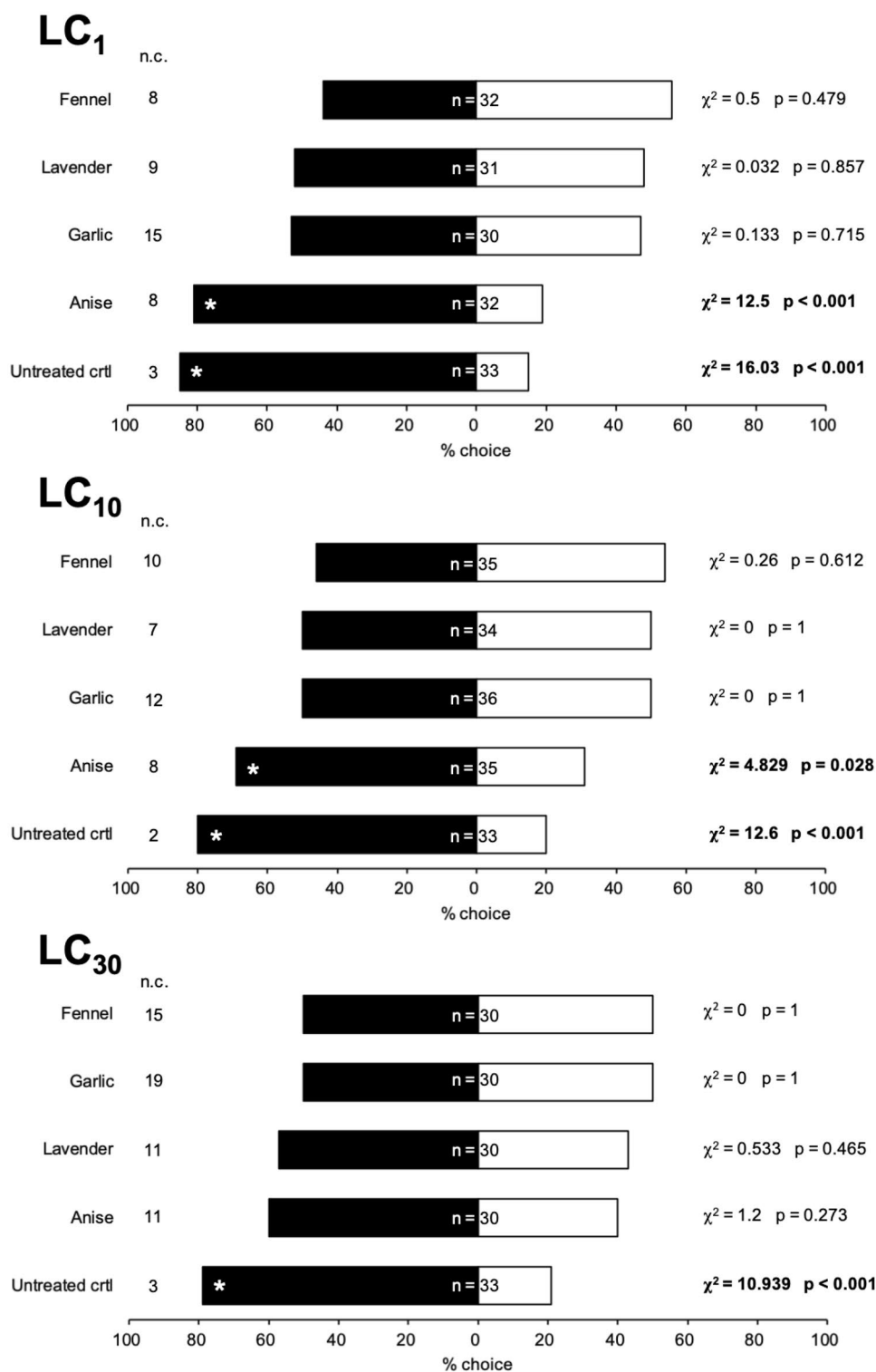
effective in controlling *T. absoluta* but reduced the fertility in *N. tenuis*, although low mortality of the predator occurred (Ricupero et al. 2022). The difference in the EO toxicity might be related to different exposure route, i.e., topical contact in the present study, while previously dry residue was the exposure route. Conversely, although topical application of sublethal doses of garlic extract disrupted some reproductive life table parameters of the predatory bug *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), this compound was considered a safe botanical when the predator plays a key role in pest control (Mamduh et al. 2017). In this case, different concentrations and/
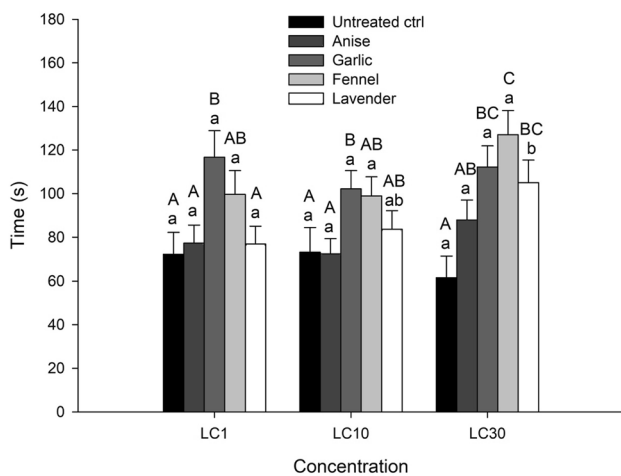
Fig. 3 Mean (± SE) time spent (*s*) by *Nesidiocoris tenuis* females for choosing between volatiles emitted by a *Sesamum indicum* plant or clean air after topical contact exposure to anise, fennel, garlic and lavender essential oils at three low-lethal concentrations (LC_1, LC_{10} and LC_{30}). Different capital letters indicate differences among treatments in a concentration, while different lower-case letters indicate differences in the concentrations for a treatment (GLM—Negative Binomial distribution, Tukey HSD test, $p \leq 0.05$)

or diverse compounds present in different types of garlic extracts might be related to the changing toxicity.

Anice EO showed the highest LCs (lower toxicity) among the tested EOs but decreased the fertility and the orientation capacity of *N. tenuis*, with the exception of LC_{10} and LC_1 for the olfactory response of the predator. Acute toxicity by anise EO on *N. tenuis* might be due to the high proportion of phenylpropanoids with anethole and estragole constituting approximately the 80% in oil composition. Toxic effects of anice EO in the literature include increase in mortality of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), reduction in progeny at increasing concentrations, irritations and damages to different body parts, including cells of the midgut, which was due probably to its lipophilicity (Hashem et al. 2018). Similar acute toxicity and repellency against *Tribolium confusum* were recorded for nanoemulsioned anice EO delivered by cold aerosol and gel formulation in laboratory trials (Palermo et al. 2021). Conversely, testing the anise EO in the laboratory, Benelli et al. (2018) detected scarce and no toxicity on larvae and adults of the aphid predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), respectively.

Among the EOs evaluated in this experiment, topical application of lavender EO showed an intermediate toxicity toward *N. tenuis,* resulting in progeny reduction and adverse effect on its orientation at the olfactory bioassay. The toxicity of lavender EO has been demonstrated in the laboratory by topical application on several agricultural and veterinary pests including Hemiptera (Faraone et al. 2015; Grul'ová

et al. 2017) and Diptera (Shalaby et al. 2016). Amer et al. (2002) observed that French lavender EO decreased the oviposition rate and food consumption of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), albeit the EO was considered less toxic toward the predacious mite. The toxicity of lavender EO has been attributed to linalool (Erland et al. 2015), its major component with recognized acetylcholinesterase inhibition activity; linalool constituted over the 40% of the tested lavender EO.

Lethal toxicity on *N. tenuis* caused by fennel EO in its lower LCs was slightly lower in comparison with garlic EO but higher if compared to anise and lavender EOs. In our laboratory trials, fennel EO also affected *N. tenuis* progeny production as also recorded for the other differently concentrated EOs we tested. Besides, *N. tenuis* females were not able to discriminate between the attractive and nonattractive odor sources when treated topically with different LCs of fennel EO and they spent more time for choosing under the influence of LC_{30}. The insecticidal properties of fennel EO have recognized for its insecticide effects against many insects, such as *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae), adults of *Musca domestica* Linnaeus (Diptera: Muscidae) (Pavela et al. 2016), including *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Digilio et al. 2008). Pavela (2018) found that fennel EO showed toxicity on *M. persicae* in the range of 0.3–3.7 mL × L^{-1} but, these concentrations had no mortality toward the predator *H. axyridis*. The most present compound in fennel EO is cis-anethole, a phenylpropanoid that can cause insecticidal effect. Phenylpropanoids can neutralize insect defense mechanisms, i.e., P450, glutathione-S-transferases and esterases (Jankowska et al. 2018). Fennel EO also has a considerable proportion of monoterpenes (approximately 40%), mainly limonene and fenchone. Therefore, we have reasons to believe that the high proportion of phenylpropanoids and monoterpenes was associated with the deleterious effects observed to *N. tenuis* in the present experiment.

Most EOs can potentially cause behavioral and physiological effects on insects at a given dose, however, behavioral effects are more likely to be observed at concentrations below those necessary to produce acute toxicity and secondary effects on physiology (Isman and Tak 2017). In the present bioassays, the EOs probably penetrated *N. tenuis* tegument due to topical treatment, causing physiological and behavioral effects at low concentrations on fertility and orientation.

Healthy insects can detect fragrant and chemosensoryactive compounds such as plant volatile compounds through odorant binding proteins and chemosensory proteins, located on the periphery of sensory receptors, with the function to capture and transport molecular stimuli (Picimbon 2005).

In our experiment, we were able to observe untreated *N. tenuis* females highly attracted to sesame plants, as previously reported (Biondi et al. 2016; Passos et al. 2022). After treatment with the EOs, even at very low concentrations, the orientation of *N. tenuis* toward a host plant was compromised. The neurotoxic effects of EOs might have affected the capacity of the predator to guide themselves toward the stimuli. Moreover, the direct treatment with the oils might have caused confusion to the insect, because many compounds can interfere with proteins responsible for perception.

The persistence of EOs (i.e., the time that the product remains biologically active against pests after application) is often considered low, although field trials showed that one single spray can provide up to three weeks of protection, presumably for the repellent effect of residual concentrations (Isman et al. 2011). Low persistence of EOs in the environment is due to their high volatility (Hu and Coats 2008; Rathore 2017). Nanotechnology can improve their stability patterns, susceptibility to oxidation and prevent the phytotoxicity (Rathore 2017; Isman 2020). By contrast, for zoophytopaghous mirids which may also survive on host plants such as *N. tenuis*, the increased persistence provided by the nanoformulation can be harmful, since the deleterious effects of EOs might be prolonged. The contact with lower concentrations is likely to happen in field conditions even when higher doses of insecticides are used, due to natural degradation of the compounds (Eijaza et al. 2015). This can negatively affect their survival, development and their efficiency as biological control agents (Desneux et al. 2007; Soares et al. 2019). As a consequence, careful evaluation should be taken before using nanoemulsioned EOs when the predation exerted by *N. tenuis* is desirable. However, in situation when *N. tenuis* populations need to be reduced, namely when prey is lacking and injury on tomato plants may occur (Castañé et al. 2011), the EOs can be used as a management strategy to mitigate plant damage by *N. tenuis*.

In conclusion, we observed that *N. tenuis* was susceptible to anise, fennel, garlic and lavender EOs even in low concentrations since these compounds negatively affected its fertility and orientation behavior, with garlic and lavender EOs being the most harmful compounds. Therefore, the use of these EOs should be carefully evaluated when the presence of *N. tenuis* is suitable because they might impair the predator's capacity to efficiently exert biological control activity.

Future studies on the insect olfactory perception under the influence of EOs through electroantennography or transcriptome analyses are warranted to unveil unknown mechanisms that cause detrimental effects on insect nervous system (Campolo et al. 2020b). The assessment of reproductive parameters of the mirid predator under the influence of EOs in combination with environmental stressors is another point that should be considered (Ricupero et al. 2020). Finally, spraying of these EOs under field conditions should be carried in order to assess how the biological control provided by *N. tenuis* is affected by these compounds. Overall, EOs are likely to be increasingly used in the future, accelerating changes in the way pest control is provided. For this reason, researchers and policy makers have thus an exciting and challenging opportunity to use them to improve the IPM systems worldwide.

Author contributions

LCP: contributed to conceptualization, methodology, execution of experiments, data collect, statistical analysis, investigation, writing; MR: contributed to conceptualization, methodology, execution of experiments, data collect, statistical analysis, investigation, writing; AG: contributed to conceptualization, methodology, execution of experiments, data collect, statistical analysis, investigation, writing; MAS: contributed to statistical analysis, writing—review and editing; ND: contributed to conceptualization, writing—review and editing; OC: contributed to conceptualization, writing—review and editing; GAC: contributed to conceptualization, writing—review and editing; AB: contributed to conceptualization, methodology, supervision, writing—review and editing; LZ: contributed to conceptualization, writing—review and editing, project administration.

Data availability Not applicable.

Declarations

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