Research Article

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Does the dose make the poison? Neurotoxic insecticides impair predator orientation and reproduction even at low concentrations

Luis C Passos,[a,b] Michele Ricupero,[b] Antonio Gugliuzzo,[b] Marianne A Soares,[a] Nicolas Desneux,[c] Geraldo A Carvalho,[a] Lucia Zappalà[b] and Antonio Biondi[b*]

Abstract

BACKGROUND: Pesticides can be noxious to non-target beneficial arthropods and their negative effects have been recently recognized even at low doses. The predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) plays an important role in controlling insect pests in solanaceous crops, but its concurrent herbivory often poses relevant concerns for tomato production. Although insecticide side effects on *N. tenuis* have been previously studied, little is known on the potential implications of neurotoxic chemicals at low concentrations. We assessed the baseline toxicity of three neurotoxic insecticides (lambda-cyhalothrin, spinosad and chlorpyrifos) on *N. tenuis* by topical contact exposure. The behavioral and reproduction capacity of the predator was then investigated upon exposure to three estimated low-lethal concentrations (LC_1, LC_{10} and LC_{30}).

RESULTS: Predator survival varied among insecticides and concentrations, with LC_{30}/label rate ratios ranging from 8.45% to 65.40% for spinosad and lambda-cyhalothrin, respectively. All insecticides reduced the fertility of *N. tenuis* females at all estimated low-lethal concentrations. Chlorpyrifos seriously compromised predator orientation towards a host plant even at LC_1, while the same effect was observed for lambda-cyhalothrin and spinosad solely at LC_{30}. Lambda-cyhalothrin (at all concentrations) and chlorpyrifos (at LC_{10} and LC_{30}) also affected the time taken by *N. tenuis* females to make a choice.

CONCLUSION: The results indicate that all three insecticides can be detrimental to *N. tenuis* and should be avoided when presence of the predator is desirable.

Keywords: ecotoxicology; sublethal effects; predatory mirid; pesticides; integrated pest management

1 INTRODUCTION

Pesticides have been incriminated for their negative consequences on biodiversity and its functioning, although their relevance in controlling plant pests effectively remains undeniable.[1,2] Pesticides can potentially affect non-target organisms present in the agroecosystem, leading to disruption of the ecological services they provide, such as pollination, nutrient cycling and biological control.[3,4] For this reason, studies on the side effects of pesticides are encouraged to provide new insights to mitigate their negative impacts on non-target beneficial arthropods.[5–9] This is especially relevant in integrated pest management (IPM) programs in which natural enemies are often deliberately released and/or conserved to reduce pest populations.[10–14]

Ecotoxicological screenings are usually based on guidelines developed by non-governmental institutions, and in the European Union (EU) the ecotoxicological risk assessment of pesticides towards non-target arthropods was developed in the Guidance Document on Terrestrial Ecotoxicology,[15] following the recommendation of the European standard characteristics of beneficials regulatory testing (ESCORT) of the Society of environmental toxicology and chemistry (SETAC) for non-bee arthropods.[16,17] Most ecotoxicology studies consist of laboratory trials aimed at testing the highest pesticide dose recommended by manufacturers. However, pesticides are naturally degraded by biotic and abiotic factors,[18,19] and their drift may also occur in the field resulting in lower doses compared with their initial application.[20,21] Therefore, non-target organisms present in the agroecosystem can be exposed to chemical residues at low

[*] *Correspondence to: Antonio Biondi, Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: antonio.biondi@unict.it*

[a] *Laboratório de Ecotoxicologia e MIP, Departamento de Entomologia, Universidade Federal de Lavras, Lavras, Brazil*

[b] *Department of Agriculture, Food and Environment, University of Catania, Catania, Italy*

[c] *Université Côte d'Azur, INRAE, CNRS, UMR ISA, Nice, 06000, France*

concentrations and, surviving individuals may experience related sublethal effects.[19,22–24] These effects may include lower fertility and a reduction in predation/parasitism ability, which can negatively affect the establishment of natural enemies in the field and bias their efficiency in reducing pest populations.[3,25,26]

Hemipteran predators are of paramount importance for the biological control of insect pests in greenhouse crops because they are able to control populations of several arthropod pests.[26–31-31]Among mirid predators (Hemiptera: Miridae), the zoophytophagous *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is one of the most used species for biological control in the Palaearctic. *Nesidiocoris tenuis* has a multifaceted role for greenhouse pest control due to its high efficacy against a number of pests including aphids, whiteflies and lepidopterans, such as the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).[32] Moreover, the use of *N. tenuis* has been fostered because of its ability in priming induced plant defense mechanisms.[33–38] However, owing to its plant-feeding activity when prey is scarce, *N. tenuis* can become a pest because this predator can cause plant damage at high population levels.[39–41] Despite this drawback, IPM programs still rely on the biological control provided by *N. tenuis*. Therefore, the predator can be often exposed to organic and/or synthetic insecticides routinely adopted in these programs.[42–45]

Earlier studies investigated the impact of insecticides on *N. tenuis* in terms of lethal and sublethal effects, by exposing *N. tenuis* adults to synthetic and organic neurotoxic compounds via different exposure routes (i.e., contaminated prey, direct spray and residual contact).[27,28,46,47] Nevertheless, most studies investigated only the maximum label rate of these compounds. To the best of our knowledge, there is no information regarding the effects of low insecticide concentrations on *N. tenuis* orientation capacity, which may ultimately affect the success of this predator as a biological control agent.

In this study, we hypothesized that low concentrations of neurotoxic insecticides might have detrimental effects on the physiology and behavior of *N. tenuis*. We tested this hypothesis through laboratory trials aiming to assess the fertility and olfactory response of *N. tenuis* adults topically exposed to three low-lethal concentrations (LC_{30}, LC_{10}, LC_1) of insecticides, previously estimated for this mirid predator. Our findings may help in understanding the convolutions of pesticide side effects at low concentrations on natural enemies and provide new useful insights into the association between the predator *N. tenuis* and chemical insecticides in pest control.

2 MATERIALS AND METHODS

2.1 Biological materials

Nesidiocoris tenuis for laboratory rearing were obtained from periodic collections in organic open tomato greenhouses located in Fiumefreddo (Catania, Italy). Collected specimens were morphologically identified and reared in the laboratory as follows. Briefly, adults of *N. tenuis* (~150 individuals) were kept in entomological cages (32 × 40 × 70 cm) covered by fine net mesh and containing pesticide-free sesame (*Sesamum indicum* L., variety T-85 Humera) potted seedlings (~30 cm in height), as water and oviposition sources, according to the methodology described by Biondi *et al.*[48] The commercial mixture of the alternative prey *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and *Artemia* spp. cysts (i.e., Entofood® Koppert) was offered *ad libitum* to the predators as an additional food source. *Nesidiocoris tenuis* adults were kept on sesame plants for 3 days to allow mating and oviposition events; subsequently, *N. tenuis* adults were collected with a mechanical aspirator and transferred to new cages as described above. Sesame plants bearing *N. tenuis* eggs were isolated inside the cages for egg hatching and the development of newly hatched nymphs to adulthood. Half of the newly molted *N. tenuis* adults were collected with a mechanical aspirator and used for the bioassays, whereas the remainder were added to the rearing. New sesame plants and Entofood® were added to each cage twice a week. The rearing was maintained under laboratory conditions (25 ± 1°C, 55% ± 5% relative humidity, and a 14:10 h light/dark photoperiod) at the Department of Agriculture, Food and Environment of the University of Catania (Italy).

2.2 Insecticides

To assess the potential physiological and behavioral effects on *N. tenuis*, three neurotoxic insecticides were evaluated in this study. The insecticides, followed by their tradename, manufacturer, chemical group and mode of action, were: lambda-cyhalothrin (Karate Zeon®, Syngenta Italia S.p.a.), a pyrethroid, Na^+ channel modulator; spinosad (Laser®, Dow AgroSciences S.r.l.), a spinosyn, nicotinic acetylcholine receptor allosteric modulator; and chlorpyrifos (Dursban®, Dow AgroSciences S.r.l.), an organophosphate, acetylcholinesterase (AChE) inhibitor. Lambda-cyhalothrin and chlorpyrifos are both synthetic insecticides used in conventional tomato crops in many countries, whereas spinosad is a naturally derived insecticide, therefore its use is allowed in both conventional and organic crops. These insecticides were selected due to their potential use in tomato crops to control hemipteran and lepidopteran pests (such as aphids, whiteflies and *T. absoluta*), which are also *N. tenuis* prey.

2.3 Insecticides baseline toxicity toward *Nesidiocoris tenuis*

In this bioassay, we assessed the concentration–mortality response relationship of *N. tenuis* adult stage to lambda-cyhalothrin, spinosad and chlorpyrifos by topical contact exposure. Newly emerged females (~2 days old) were exposed by topical spray to different concentrations of the insecticides. For each insecticide, six or seven concentrations, including of the highest label rate, were tested (see Table 1). Stock solutions were prepared with dilution of insecticidal formulations in distilled water, according to the manufacturer's recommendations. In addition, an untreated control with distilled water was included for all the insecticides (referred to as "zero concentration"). The insecticide dilutions were based on preliminary observations aimed at identifying the minimum dose needed to cause 100% mortality of *N. tenuis* females and the maximum dose that does not significantly affect the mortality of the treated insects in comparison with the untreated control.

An adapted methodology for insecticide topical contact application on *N. tenuis* adult stage was developed. Briefly, five *N. tenuis* females were isolated together in conical ventilated plastic tubes (Falcon®, 50 ml) and maintained at low temperature inside an insulated thermic box with ice packs for 3 h to reduce insect mobility. Thereafter, each group of *N. tenuis* females was placed in a plastic cup (100 ml) and topically sprayed with insecticide solutions using a hand-sprayer (50 ml). The inside of the plastic cups was covered by absorbent paper to prevent the formation of insecticide droplets in the arena, preventing insect mortality via drowning. Clean and new absorbent paper was changed in each replicate for every insecticide–concentration combination. After spraying, each group

TABLE 1. Baseline toxicity of three insecticides toward *Nesidiocoris tenuis* females 48 h after topical contact exposure by spraying

Insecticide	Tradename	% a.i.	Label rate (ppm)	Slope ± SE	χ^2 (*df*)	*p*-value	Lethal concentration (ppm)	95% Confidence limits (ppm)	% LC/LR[a]
Spinosad	Laser®	44.20	0.3315	1.974 ± 0.260	33.355 (31)	0.353	$LC_1 = 3.37 \times 10^{-3}$	1.35×10^{-3} to 5.88×10^{-3}	1.08
							$LC_{10} = 1.14 \times 10^{-2}$	6.71×10^{-3} to 1.62×10^{-2}	3.32
							$LC_{30} = 2.75 \times 10^{-2}$	2.00×10^{-2} to 3.60×10^{-2}	8.44
Lambda-cyhalothrin	Karate Zeon®	9.48	0.0236	1.301 ± 0.201	42.901 (36)	0.201	$LC_1 = 6.39 \times 10^{-4}$	1.10×10^{-4} to 1.68×10^{-3}	2.70
							$LC_{10} = 4.06 \times 10^{-3}$	1.49×10^{-3} to 7.33×10^{-3}	17.13
							$LC_{30} = 1.55 \times 10^{-2}$	9.24×10^{-3} to $2.22 \cdot 10^{-2}$	65.40
Chlorpyrifos	Dursban®	44.53	0.3340	0.948 ± 0.202	35.563 (33)	0.349	$LC_1 = 8.87 \times 10^{-4}$	2.30×10^{-4} to 4.09×10^{-4}	0.27
							$LC_{10} = 1.12 \times 10^{-2}$	1.72×10^{-3} to 2.58×10^{-2}	3.35
							$LC_{30} = 7.05 \times 10^{-2}$	3.34×10^{-2} to 1.14×10^{-1}	21.11

[a] % LC/LR is percentage of the estimated low-lethal concentration in comparison with the highest label rate recommended in tomato crop.

of five *N. tenuis* females was transferred to an acrylic ventilated pot (5.5 cm in diameter × 3 cm height), along with a zucchini (*Cucurbita pepo* L.) leaf disc and Entofood®. Each pot containing five females was considered one replicate. Mortality caused by the insecticides on *N. tenuis* females was evaluated after 48 h. Eight replicates were performed for each insecticide–concentration combination.

2.4 Sublethal effects of insecticides on *Nesidiocoris tenuis* fertility

The aim of this bioassay was to evaluate whether low concentrations of lambda-cyhalothrin, spinosad and chlorpyrifos could affect the fertility of the predator *N. tenuis*. Based on the results of the previous bioassay, newly molted *N. tenuis* males and females (2 days old) from the rearing were exposed to three low-lethal concentrations (LC_1, LC_{10} and LC_{30}) of the aforementioned insecticides. These concentrations were chosen to expose the predators to low concentrations that can occur under field conditions after environmental degradation of a full label spray, including a lethal range from very low mortality (LC_1) to moderate mortality (LC_{30}).

Adult females (2 days old) were sprayed with the low-lethal concentrations mentioned above and distilled water, as described in Section 2.3. Sprayed couples were kept in a ventilated plastic cup (400 ml) containing a green bean pod (*Phaseolus vulgaris* L., cv. 'Garrafal enana') as a water source and oviposition substrate,[28,49] and *E. kuehniella* eggs (1 g) as food supply in the arena. Each *N. tenuis* couple was kept in the aforementioned arena for 3 days to increase mating success and let the females oviposit into the bean. The experimental arenas containing green bean pods with *N. tenuis* eggs were maintained under laboratory conditions as described above, and the number of newly emerged nymphs was recorded daily under a stereomicroscope and removed with a soft paintbrush. The evaluation was conducted for 20 days until no new nymph emerged. For each pesticide–concentration combination and the control, the fertility of 25 *N. tenuis* couples (i.e., 25 replicates) was evaluated.

2.5 Sublethal effects of insecticides on *Nesidiocoris tenuis* orientation

The aim of this bioassay was to evaluate whether the orientation of the predator *N. tenuis* could be affected by the three low-lethal concentrations (LC_1, LC_{10} and LC_{30}) of lambda-cyhalothrin, spinosad and chlorpyrifos. Adult females (2 days old) were sprayed with the low-lethal concentrations mentioned above and distilled water, as described in Section 2.3. After being sprayed topically, *N. tenuis* females were starved for 24 h in transparent vials (1.5 cm in diameter × 6 cm height) with a wet cotton wad as the only water source. Thereafter, each *N. tenuis* female was transferred in a two-way olfactometer (main arm and lateral arms 15 cm long and 4 cm internal diameter). The odor sources used were clean air and a sesame plant (~20 cm height). Sesame plant was chosen because previous studies demonstrated that this plant is highly attractive to *N. tenuis*.[48,50] A sesame plant was placed inside one of the cylindrical glass jars (5 L volume) connected to the lateral arms of the olfactometer. An air pump (Airfizz®, Ferplast) 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 thus the main arm. The olfactometer was placed vertically on the bench surface and *N. tenuis* females were placed individually on the central arm. The bioassays were performed in a dark room, with controlled environmental conditions (25 ± 1°C, 60 ± 10% R. H.) and were conducted between 9:00 a.m.

and 6:00 p.m. The olfactometer was illuminated by 22 W cool-white fluorescent lamps, positioned 80 cm above the olfactometer arms, according to Naselli *et al.*[50]

The choice of each female was considered when it crossed half of the lateral arm. Each predator was observed for 5 min and, if no choice was made after that time, non-responder *N. tenuis* females were discarded from the data set. After every two tested females, the olfactometer was inverted to reduce environmental interference in the insect response. For each insecticide–concentration combination, at least 30 replicates, each composed of an insect that have did a choice, were carried out. The time taken for *N. tenuis* females to make a choice (for insects that made a choice) was also recorded.

2.6 Statistical analyses

The baseline toxicity of lambda-cyhalothrin, spinosad and chlorpyrifos on *N. tenuis* by topical contact exposure was carried out through a log-probit regression model.[51] The preference data of *N. tenuis* towards sesame plants were analyzed using a chi-squared goodness-of-fit to determine whether the female attraction to sesame plants was different from a 50:50 distribution.

Data regarding time taken by *N. tenuis* females to make a choice and fertility were tested for normality and homoscedasticity;[52,53] however, these assumptions were not met. Therefore, these data were fitted to generalized linear models (GLMs),[54] and potential interaction between factors (four treatments × three concentrations) was tested. The models were fitted using the Poisson family for fertility and negative binomial family for time taken by *N. tenuis* females to make a choice (Poisson and quasi-Poisson families were first tested, but the negative binomial model presented a better fit). Means were separated by a post-hoc Tukey's HSD test ($p < 0.05$). Probit analyses were performed in the statistical program SPSS v. 21.0 (IBM Corp.), whereas the analyses for the fertility and olfactory response bioassays were performed in R v. 3.6.0 (R Foundation for Statistical Computing), using the packages *car* and *MASS* for model fitting and the package *multcomp* to separate means.[55–57]

3 RESULTS

3.1 Insecticides baseline toxicity toward *Nesidiocoris tenuis*

The probit models were fitted to observed data for all the treatments (i.e., there were no significant differences between the observed and the expected data), validating the low-lethal concentrations for all the tested neurotoxic insecticides (Table 1). All insects treated with distilled water only ("zero concentration") survived throughout the evaluation period. Lambda-cyhalothrin was the insecticide with the lowest values of LC_1, LC_{10} and LC_{30}, being the most lethal active ingredient for *N. tenuis* females. Spinosad and chlorpyrifos also presented high toxicity to the predator as highlighted by the low LC_{30} values estimated for these compounds. Nevertheless, despite lambda-cyhalothrin being the most toxic active ingredient, it was observed that the proportion values between the estimated LC_{10} and LC_{30} and the maximum label rate were higher for this insecticide (17.13% and 65.40%) in comparison with those observed for spinosad (3.32% and 8.45%) and chlorpyrifos (3.35% and 21.11%) (Table 1).

3.2 Sublethal effects of insecticides on *Nesidiocoris tenuis* fertility

Although the GLM revealed no significant insecticide × concentration interaction ($\chi^2 = 12.023$, $df = 6$, $p = 0.061$), all the tested insecticides significantly reduced the fertility of *N. tenuis* females at all the evaluated concentrations (LC_1: $\chi^2 = 64.642$, $df = 3$, $p < 0.001$; LC_{10}: $\chi^2 = 73.707$, $df = 3$, $p < 0.001$; LC_{30}: $\chi^2 = 118.560$, $df = 3$, $p < 0.001$). The reduction in fertility was higher for chlorpyrifos at LC_{30} ($\chi^2 = 9.939$, $df = 2$, $p = 0.007$), whereas no differences were observed among the concentrations for lambda-cyhalothrin ($\chi^2 = 2.659$, $df = 2$, $p = 0.265$), spinosad ($\chi^2 = 1.008$, $df = 2$, $p = 0.604$) and the control ($\chi^2 = 0.427$, $df = 2$, $p = 0.808$) (Figure 1).

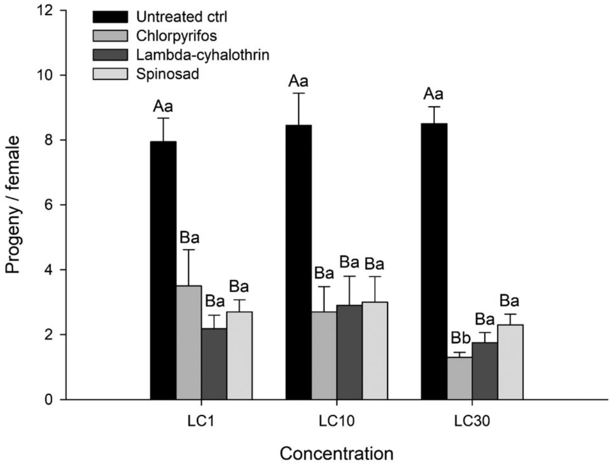

FIGURE 1. Mean (±) fertility values for *Nesidiocoris tenuis* females after topical contact exposure to three insecticides at three low-lethal concentrations and distilled water (untreated control). Different upper case letters indicate significant differences among treatments in a concentration, whereas different lower case letters indicate significant differences in the concentrations for a treatment (GLM – Poisson distribution, Tukey's HSD test, $p < 0.05$).

3.3 Sublethal effects of insecticides on *Nesidiocoris tenuis* orientation

A significant attraction towards sesame plants was expected for insects that did not experience any insecticide exposure,[50] and it was confirmed for all control treatments. Therefore, this was taken as a reference for the percentage of insects orienting toward sesame compared with clean air for the treatments with insecticides. The preference of *N. tenuis* females for sesame plants instead of clean air was not affected by lambda-cyhalothrin or spinosad at LC_1 and LC_{10}. However, the choices of insects treated with chlorpyrifos did not differ between sesame and air for these two low-lethal concentrations. At LC_{30}, all insecticides affected *N. tenuis* orientation, resulting in no difference between the proportion of choices for sesame and clean air (Figure 2).

Differences in the time taken by *N. tenuis* females to make a choice were observed in all low-lethal concentrations (LC_1: $\chi^2 = 9.358$, $df = 3$, $p = 0.024$; LC_{10}: $\chi^2 = 22.566$, $df = 3$, $p < 0.001$; LC_{30}: $\chi^2 = 33.291$, $df = 3$, $p < 0.001$) (Figure 3). Insects treated with all the tested concentrations of lambda-cyhalothrin took longer to make a choice in comparison with the control treatment. The same was observed for insects treated with chlorpyrifos at LC_{10} and LC_{30}. For females treated with all concentrations of spinosad time taken to make a choice was not affected in comparison with the control treatment. No differences were observed among concentrations for any of the treatments (control: $\chi^2 = 0.508$, $df = 2$, $p = 0.777$; lambda-cyhalothrin: $\chi^2 = 0.634$, $df = 2$, $p = 0.729$; chlorpyrifos: $\chi^2 = 4.981$, $df = 2$, $p = 0.083$; spinosad: $\chi^2 = 3.589$, $p = 0.166$) (Figure 3). There was no interaction between

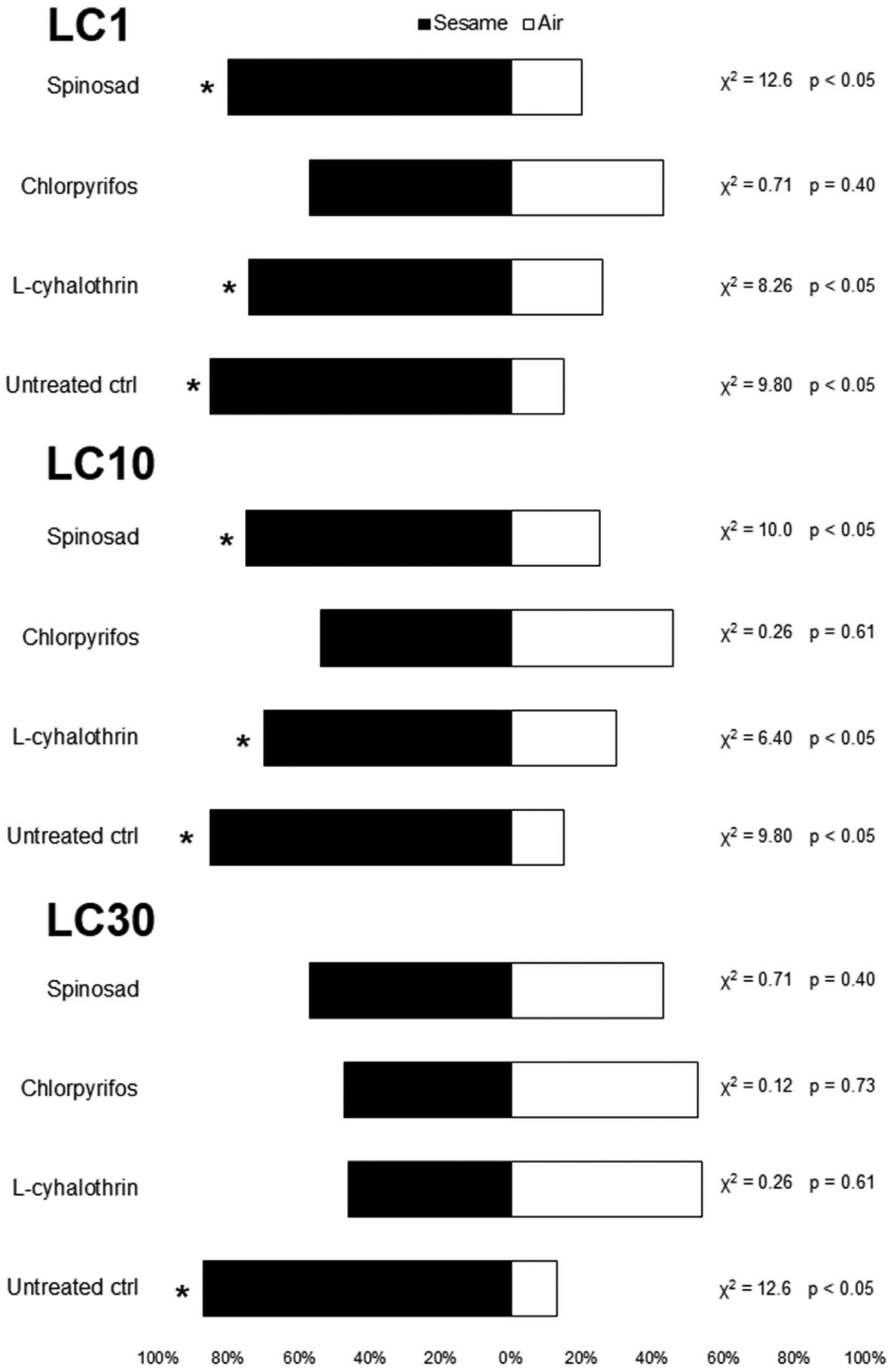

FIGURE 2. Response of *Nesidiocoris tenuis* females topically exposed to three insecticides at three low-lethal concentrations (LC_1, LC_{10} and LC_{30}) and distilled water (untreated control) towards the volatiles produced by a *Sesamum indicum* plant. The percentages indicate the proportion of choices for sesame and clean air. Asterisks indicate differences in the attraction to *S. indicum* and clean air according to the likelihood chi-squared ($p < 0.05$).

treatments and concentrations for the time taken by *N. tenuis* females to make a choice ($\chi^2 = 9.066$, $df = 6$, $p = 0.170$), therefore the data were evaluated separately.

4 DISCUSSION

In many systems, broad-spectrum insecticides, such as neurotoxic insecticides, are the most used compounds for pest control because of their effectiveness in controlling pests. However, a vast literature has documented concerning detrimental effects on beneficial organisms caused by these effective tools.[3,58,59] To preserve the ecological functions of beneficial organisms in the agroecosystem (including biological control) selective insecticides should be preferred in pest management.[5,22,26,60–62] Additionally, insecticides can cause sublethal effects that can bias the biological control provided by predators and parasitoids.[3,24] These alterations can be observed in individuals that survived both full label rates of selective compounds and lower

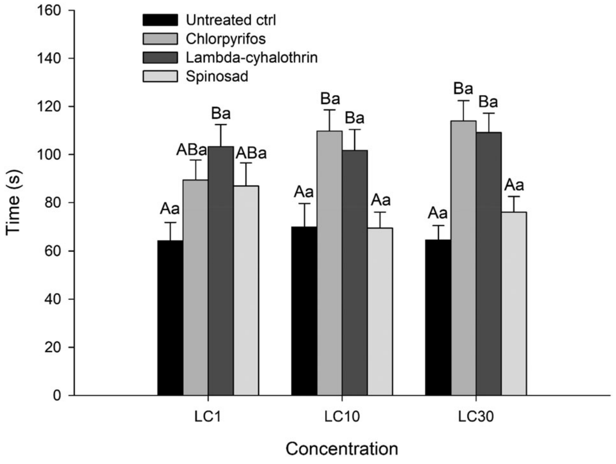

FIGURE 3. Mean (±SE) time taken (s) by *Nesidiocoris tenuis* females to make a choice between the volatiles emitted by a *Sesamum indicum* plant or clean air after topical contact exposure to three insecticides at three low-lethal concentrations and distilled water (untreated control). Different upper case letters indicate differences among treatments in a concentration, whereas different lower case letters indicate differences in the concentrations for a treatment (GLM – Negative Binomial distribution, Tukey's HSD test, $p < 0.05$).

concentrations of broad-spectrum insecticides, which can occur after natural degradation under field conditions.[22,63–65]

Probit models are often used to estimate concentration–mortality of pesticides to pests and natural enemies, in order to select efficient and safe compounds, respectively.[49,65–68] In our observations, lambda-cyhalothrin was the most toxic compound for the predator, because lower values were estimated for all lethal concentrations. Spinosad was the least toxic insecticide at LC_1 towards *N. tenuis*; however, at LC_{30} this insecticide was more toxic than chlorpyrifos. The highest slope estimated for spinosad treatment indicates that a slight increase in insecticide concentration may lead to high predator mortality.[69] Moreover, due to the lower active ingredient concentration in the lambda-cyhalothrin based insecticide, the difference between the maximum label rate and the estimated LC_{10} and LC_{30} are lower for lambda-cyhalothrin than for the spinosad and chlorpyrifos commercial products. Therefore, despite the higher LC_{10} and LC_{30} values observed for spinosad and chlorpyrifos, these insecticides might be even more toxic than lambda-cyhalothrin under field conditions.

Besides mortality, all insecticides at the three evaluated concentrations reduced the fertility of the predator *N. tenuis*. Reproductive parameters are among the most sensitive biological characteristics to insecticides and the most important in terms of population dynamics.[70] Similar to our results, a reduction in *N. tenuis* progeny was also observed for the pyrethroids cypermethrin and deltamethrin.[28,46] Additionally, pyrethroids can be used at sublethal concentrations to contaminate insect-proof nets, and Biondi *et al*. found that the continuous exposure of *T. absoluta* adults to such nets can cause a variety of chronic sublethal effects rather than acute toxicity.[71]

Lower concentrations of the organophosphate chlorpyrifos were also frequently reported as causing negative effects on the reproduction of natural enemies. At LC_{30} several sublethal effects were observed on the hemipteran *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae), such as reduction in fertility and enzyme activity, and alterations in life table parameters.[72] Fernandes *et al*. observed negative effects on reproduction after chlorpyrifos exposition at LC_{20} for the predator *Orius insidiosus* (Say) (Hemiptera: Anthocoridae).[67] Moreover, spinosad reduced the offspring of the predatory bugs, such as *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae),[22] *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae)[27,73] and *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae).[74]

Negative effects were also observed in the behavioral traits of *N. tenuis*. In the insecticide treatments, spinosad and lambda-cyhalothrin at LC_{30} and chlorpyrifos at all concentrations affected the orientation ability of the predator. Moreover, the two synthetic insecticides also increased the time taken by *N. tenuis* females to make a choice. Because of their neurotoxic action, all three insecticides can affect the capacity of the nervous system to react to external stimuli.[59,63,72,75]

The behavioral results in this study are consistent with neurotoxicity associated with lambda-cyhalothrin. The deleterious effects caused by pyrethroids result from a blockage in electrical stimulus conduction as a consequence of the permanent opening of sodium channels while the insecticide is acting, leading to behavioral and physiological impacts.[59,76] Desneux *et al.* also observed that sublethal doses of lambda-cyhalothrin ($LD_{0.1}$) affected the orientation behavior of the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae).[63] The parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) exhibited a reduction in parasitism and longevity after treatment with sublethal concentrations of lambda-cyhalothrin.[77] Soares *et al.* also observed alterations in *N. tenuis* behavior caused by lambda-cyhalothrin.[26]

Similarly, a lack of coordination is associated with chlorpyrifos intoxication.[72] The biased orientation of *N. tenuis* females treated with chlorpyrifos at LC_1, LC_{10} and LC_{30} indicates that this insecticide can affect the predator behavior even at very low concentrations. The time taken by *N. tenuis* females to make a choice also increased after treatment with LC_{10} and LC_{30}. Fernandes *et al.* observed negative effects on predation rate after chlorpyrifos exposure at LC_{20} for the predator *O. insidiosus*.[67] The predator *M. pygmaeus* also showed behavioral alterations (reduced attack rate and increased handling time) after treatment with chlorpyrifos at LC_{30}.[78]

By contrast, studies regarding the side effects of spinosad on the behavior of beneficial insects are scarce. For example, Barbosa *et al.* observed alterations in walking activity in the stingless bee *Melpona quadrifasciata* Depeleiter (Hymenoptera: Apidae) after exposure to spinosad.[79] Nevertheless, owing to the toxic effect on the nervous system, insects intoxicated by spinosad may present symptoms like a lack of coordination, trembling of appendages and a compromised perception of external stimuli, which can ultimately result in reduced predatory capacity for a natural enemy.[75,80–82]

Plants from different botanical families, such as Asteraceae, Solanaceae and Pedaliaceae, are suitable for *N. tenuis* biological development. These plants could serve as water and oviposition sources, and are also plants where *N. tenuis* prey can be found in the field.[39,48] For this reason, disrupting the predators' capacity to locate host plants directly influences their survival and success as biological control agents. The misorientation caused by lower concentrations of insecticides could also compromise *N. tenuis* capacity to locate plants infested with herbivorous prey, as observed for the predator *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) after exposure to the pyrethroid deltamethrin.[83] Further study is needed on this point of the system.

In summary, we observed that spinosad, chlorpyrifos and lambda-cyhalothrin can be toxic to the predator *N. tenuis,* even at low concentrations, with effects on fertility and orientation. In addition to the laboratory results, field trials should be performed to confirm the toxicity of the compounds exploring different exposure routes (i.e., residual contact and ingestion of contaminated prey) and/or by testing the potential side effects toward insect parasitoids exploited in tomato crops.[13,84,85]

5 CONCLUSIONS

The baseline toxicity showed that the insecticides were toxic to *N. tenuis* females. The sublethal effects caused by the tested concentrations of the three insecticides were also relevant. Even at LC_1 and LC_{10} the fertility of *N. tenuis* females was compromised by all the insecticides. In addition, sublethal effects on predator orientation were observed. We concluded that the three insecticides were noxious to *N. tenuis* and should be avoided when the presence of the predator is desirable. Nevertheless, field trials must be carried out to confirm their sublethal toxicity and overall risk (interaction of exposure, hazard and other factors).

Additionally, the negative effects on *N. tenuis* orientation observed in the current study provide a basis for further research aiming to elucidate how neurotoxic insecticides impair *N. tenuis* capacity to locate host plants or herbivorous prey, by investigating alterations in the gene expression of odorant-binding and chemosensory proteins that might be involved in plant volatile reception by employing electro-antennography and quantitative real-time polymerase chain reaction bioassays.[86–88] Moreover, the results highlight the importance of investigating other insecticides that might have a narrow spectrum and that would be more compatible with sustainable IPM.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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