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UNDERSTANDING THE IMPACT OF ACETAMIPRID-BASED INSECTICIDES ON THE BIOLOGICAL FITNESS OF ENTOMOPATHOGENIC NEMATODES: IMPLICATIONS FOR BIOLOGICAL CONTROL

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ABSTRACT

The impact of acetamiprid-based insecticides on the survival and activity of entomopathogenic nematodes (EPNs) was evaluated in laboratory, focusing on two species, *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Despite variations in sensitivity, with S. feltiae showing greater susceptibility, both species maintained their ability to infect *Galleria mellonella* larvae after exposure. Exposure to Mospilan 20 SP® significantly decreased the reproductive capacity of S. feltiae ($F = 443.215$, $p < 0.001$), while H. bacteriophora showed greater resilience, especially when exposed to and Kobe 20 SP®. The ED50 values for *H. bacteriophora* increased over time with Kobe 20 SP® (0.46 ±0.04 at 24 h to 0.60 ±0.01 at 96 h), while Mospilan 20 SP® decreased the ED50 for *S. feltiae* (0.55 ±0.02 at 24 h to 0.64 ±0.03 at 96 h). The study highlights that the effects of systemic insecticides extend beyond immediate mortality, influencing reproductive potential and long-term viability, particularly for more sensitive species like *S. feltiae*. These findings raise important considerations for integrating EPNs into pest management strategies, especially in systems reliant on chemical pesticides. Further research is recommended to explore the broader ecological impacts of neonicotinoids on beneficial nematodes and their potential interactions with other biocontrol agents, aiming to enhance the sustainability of integrated pest management systems.s.

Keywords: biocontrol agents, *Heterorhabditis bacteriophora*, pest management, reproductive capacity, *Steinernema feltiae*

INTRODUCTION

Neonicotinoids are active ingredients widely used in plant protection products for pest control [Kundoo et al. 2018]. These systemic pesticides are absorbed by plants and distributed throughout their tissues, targeting the central nervous system of insects and causing paralysis and eventual death [Simon-Delso et al. 2015, Casida 2010]. Neonicotinoids are classified into two main groups: N-cyanoamidines and N-nitroguanidines [Jeschke and Nauen 2008, Ligtelijn et al. 2024]. Acetamiprid,

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a member of the N-cyanoamidines group, is comparatively less studied for its ecotoxicity [Morrissey et al. 2015]. It is primarily used as a foliar insecticide spray, leading to direct exposure of various soil organisms, including entomopathogenic nematodes (EPNs) [El-Ashry et al. 2020, Özdemir et al. 2021]. Although EPNs reside in the soil, foliar insecticide sprays can indirectly impact them. The chemicals from the spray can run off into the soil, affecting the habitat and overall environment of the nematodes. This indirect exposure can influence their survival, behavior, and ability to control pest populations effectively. In integrated pest management (IPM), neonicotinoids, particularly acetamiprid, are commonly used and often combined with biological control agents like EPNs to enhance pest suppression [Özdemir et al. 2020].

EPNs are essential biological control agents for managing soil-dwelling pests and belong to the families *Steinernematidae* and *Heterorhabditidae*. These nematodes infect and kill insect pests during their larval stages through a symbiotic relationship with specific bacteria (*Xenorhabdus* spp. in *Steinernema* and *Photorhabdus* spp. in *Heterorhabditis*). Once the nematodes enter the insect host, they release bacteria that multiply rapidly within the host. The bacteria produce toxins that break down tissues and suppress the insect's immune system, ultimately leading to the host's death within 24–48 hours. Once the insect host dies, the nematodes complete their lifecycle by reproducing inside the cadaver, producing new infective juveniles that are released into the environment to seek out and infect new hosts [Stefanovska et al. 2023]. EPNs are critical components of soil ecosystems, contributing to pest population regulation and maintaining ecological balance.

Given the environmental risks associated with acetamiprid and its extensive application, as well as the role of EPNs as biocontrol agents, it is imperative to evaluate the interactions between EPNs and neonicotinoids. This understanding is crucial to assessing the impact of these pesticides on beneficial soil organisms. Most research to date has focused on imidacloprid and thiamethoxam, members of the N-nitroguanidine class [Polavarapu et al. 2007, Miranda et al. 2016, Koppenhöfer et al. 2020, Koppenhöfer and Foye 2024]. However, comprehensive data on the effects of acetamiprid on EPNs remains scarce.

This study aims to bridge this gap by evaluating the effects of two acetamiprid-based formulations (Mospilan 20 SP® and Kobe 20 SP®) on two EPN species (*Steinernema feltiae* and *Heterorhabditis bacteriophora*). The potential risks of disrupting biological control agents when combining insecticides with IPM strategies are assessed through the following objectives:

1. Evaluate the survival of infective juveniles (IJs) of *S. feltiae* and *H. bacteriophora* after direct exposure to two neonicotinoid insecticides at three concentrations over 24, 48, 72, and 96 hours.
2. Assess the virulence of *S. feltiae* and *H. bacteriophora* IJs against *Galleria mellonella* larvae after exposure to two neonicotinoids at varying concentrations.
3. Investigate the reproductive potential of *S. feltiae* and *H. bacteriophora* on *G. mellonella* following exposure to insecticides.

MATERIAL AND METHODS

This study used two acetamiprid-based neonicotinoids, Mospilan 20 SP® and Kobe 20 SP®, to assess their impact on the survival and performance of *S. feltiae* and *H. bacteriophora*. The insecticides were tested in a controlled laboratory setting using three concentrations – 0.5%, 1%, and 1.5% – based on the manufacturers' recommended doses for pest control. These concentrations were based on the average recommended field doses for neonicotinoids: Mospilan 20 SP at 275 g/ha and Kobe 20 SP at 100 g/ha, which were then converted into the appropriate laboratory concentrations. The application volume was standardized to match typical field conditions, ensuring that the experimental setup reflected the pesticide's use under real conditions. The control treatment (0% insecticide concentration) consisted of distilled water.

Commercial strains of EPNs in the infective juvenile (IJ) stage were employed: *H. bacteriophora* (*B-Green*, Biobest Group NV, Belgium) and *S. feltiae* (*Steinernema-System*, Biobest Group NV, Belgium). These biopreparations were sourced simultaneously, ensuring high quality. The greater wax moth, *G. mellonella*, served as the host insect. The laboratory culture of *G. mellonella* was maintained at 25°C with 60–70% relative humidity under a 16:8 h light/dark

cycle. Larvae were reared according to the protocol described by Stefanovska et al. [2024], ensuring proper development. Healthy fifth instar larvae were selected for inoculation with nematodes. The study consisted of three bioassays, as described in Kaya and Stock [1997].

Study design

Nematode viability. The survival of IJs of *S. feltiae* and *H. bacteriophora* exposed to various concentrations of Mospilan 20 SP ® and Kobe 20 SP ® was evaluated. For each treatment, 1000 IJs were placed in 50 mm Petri dishes containing 5 cm³ of insecticide solution. The dishes were kept at 20°C, and nematode mortality was assessed every 24 hours over four days using a stereomicroscope. Distilled water was used as the control.

Nematode virulence. IJs previously exposed to insecticides (subsection Nematode viability) were rinsed three times with deionized water and transferred to 100 mm Petri dishes containing filter paper. Ten *G. mellonella* larvae were placed in each dish (30 insects per treatment variant, with three replicates per concentration). The ability of the nematodes to infect hosts after exposure to insecticides was recorded. Control samples consisted of IJs exposed to distilled water.

Reproductive potential. To assess reproductive capacity, *G. mellonella* cadavers were placed in dish traps as described by White [1927]. The experiment was conducted at 24°C, observing the migration of infective larvae from newly emerged IJ generations into the aquatic environment. Migration occurred after 10 days for *S. feltiae* and 14 days for *H. bacteriophora*. Experimental conditions were consistent across all treatments and nematode species.

Data analysis

The symmetric log-logistic model was employed to evaluate the response of nematode survival over time under the influence of various doses of insecticides [Vanegas and Paula 2016]. This model is well-suited for analyzing dose-response relationships, as it provides estimates for key parameters such as the lower asymptote (c), the upper asymptote (d), the slope (b), and the median effective dose (ED50). The mathematical representation of the model is as follows:

$$Y = \mathrm{c} + \frac{\mathrm{d} - \mathrm{c}}{1 + exp(\mathrm{b}(\log(\mathrm{x}) - log(ED50)))},$$

where Y is the response, c denotes the lower limit of the response when the dose x approaches infinity, d is the upper limit when the dose x approaches 0, b denotes the slope around the point of inflection, which is the ED50, i.e. the dose required to reduce the response half-way between the upper and lower limit. Nematode virulence (time to infect) was analysed using beta regression (β), while reproductive potential (log-transformed IJ counts) was assessed with a general linear model (GLM) including insecticide, concentration, and species as factors. Calculations were performed using the drc package [Ritz et al. 2015]. Descriptive statistics and GLM parameters were calculated using the Statistica software [StatSoft Inc. 2014].

RESULTS

Impact of insecticides on EPNs viability

The data obtained indicate that the effect of insecticides on nematode mortality depends on both the insecticide brand and the duration of exposure (Fig. 1). Symmetric log-logistic models effectively described the relationship between insecticide concentration and nematode mortality, which is confirmed by the high correspondence of the obtained curves to the experimental data (Table 1). With an increase in the duration of exposure, a decrease in the lower limit of the mortality curves was noted, indicating a decrease in the baseline level of nematode mortality. At the same time, there was a decrease in the slope of the curves, which indicates a decrease in the rate of change in mortality with increasing insecticide concentration. The smallest decrease in the lower limit of mortality was recorded for *H. bacteriophora* after treatment with Kobe 20 SP®, which may indicate increased resistance of this species to this insecticide.

Analysis of ED50 values revealed significant differences between insecticides. In the case of Kobe 20 SP®, an increase in ED50 was observed over time, indicating a decrease in the insecticide effectiveness, probably due to the adaptation of nematodes to the toxic effect. On the other hand, the use of Mospilan 20 SP® led to a decrease in ED50, indicating an increase

Table 1. Parameters of the symmetric log-logistic models of nematode survival response to insecticide exposure depending on exposure times

Parameter	Time (h)	Species					
		Steinernema feltiae			*Heterorhabditis bacteriophora*		
		b ±SE	*t*-value	*P*-level	*b* ±SE	*t*-value	*P*-level
		Kobe 20 SP®					
Slope	24	3.94 ±0.63	6.3	<0.001	5.51 ±6.72	0.8	0.418
	48	3.38 ±0.43	7.9	<0.001	3.07 ±0.74	4.2	<0.001
	72	2.45 ±0.30	8.2	<0.001	5.01 ±0.86	5.8	<0.001
	96	2.69 ±0.25	10.8	<0.001	3.06 ±0.20	15.2	<0.001
Lower limit	24	0.67 ±0.01	72.1	<0.001	0.92 ±0.00	257.2	<0.001
	48	0.62 ±0.01	54.6	<0.001	0.86 ±0.01	138.9	<0.001
	72	0.53 ±0.02	26.9	<0.001	0.77 ±0.00	204.9	<0.001
	96	0.47 ±0.02	26.6	<0.001	0.64 ±0.01	95.2	<0.001
Upper limit	24	1.00 ±0.01	172.3	<0.001	1.00 ±0.00	347.4	<0.001
	48	0.99 ±0.01	170.7	<0.001	0.99 ±0.00	345.7	<0.001
	72	0.98 ±0.01	170.1	<0.001	0.98 ±0.00	340.5	<0.001
	96	0.95 ±0.01	164.6	<0.001	0.96 ±0.00	336.0	<0.001
ED50	24	0.55 ±0.02	31.8	<0.001	0.46 ±0.04	10.3	<0.001
	48	0.57 ±0.02	29.3	<0.001	0.50 ±0.02	22.7	<0.001
	72	0.57 ±0.03	21.2	<0.001	0.53 ±0.01	51.0	<0.001
	96	0.64 ±0.03	24.0	<0.001	0.60 ±0.01	43.5	<0.001

Mospilan 20 SP®							
Slope	24	3.60 ±1.44	2.5	0.018	3.94 ±0.63	0.81 ±0.82	1.0
	48	2.32 ±0.55	4.2	<0.001	3.38 ±0.43	0.59 ±0.50	1.2
	72	1.34 ±0.43	3.1	0.004	2.84 ±0.30	0.45 ±0.27	1.6
	96	2.25 ±0.58	3.9	0.001	2.69 ±0.25	0.51 ±0.30	1.7
Lower limit	24	0.76 ±0.01	67.4	<0.001	0.67 ±0.01	0.46 ±2.53	0.2
	48	0.60 ±0.02	27.8	<0.001	0.62 ±0.01	0.45 ±1.42	0.3
	72	0.46 ±0.07	6.8	<0.001	0.53 ±0.02	-0.11 ±1.64	–0.1
	96	0.46 ±0.02	21.5	<0.001	0.49 ±0.02	-0.31 ±2.03	–0.2
Upper limit	24	1.00 ±0.01	152.6	<0.001	1.00 ±0.01	1.00 ±0.02	41.7
	48	0.99 ±0.01	152.1	<0.001	0.99 ±0.01	0.99 ±0.02	41.6
	72	0.99 ±0.01	151.9	<0.001	0.98 ±0.01	0.98 ±0.02	41.3
	96	0.99 ±0.01	151.6	<0.001	0.95 ±0.01	0.98 ±0.02	41.2
ED50	24	0.47 ±0.02	25.1	<0.001	0.55 ±0.02	7.40 ±63.63	0.1
	48	0.46 ±0.02	21.7	<0.001	0.56 ±0.02	7.46 ±52.73	0.1
	72	0.43 ±0.07	6.1	<0.001	0.57 ±0.03	7.57 ±44.25	0.2
	96	0.37 ±0.02	20.8	<0.001	0.64 ±0.03	6.42 ±34.67	0.2

b – the slope coefficient of the model, indicating the steepness of the dose-response curve; SE – standard error

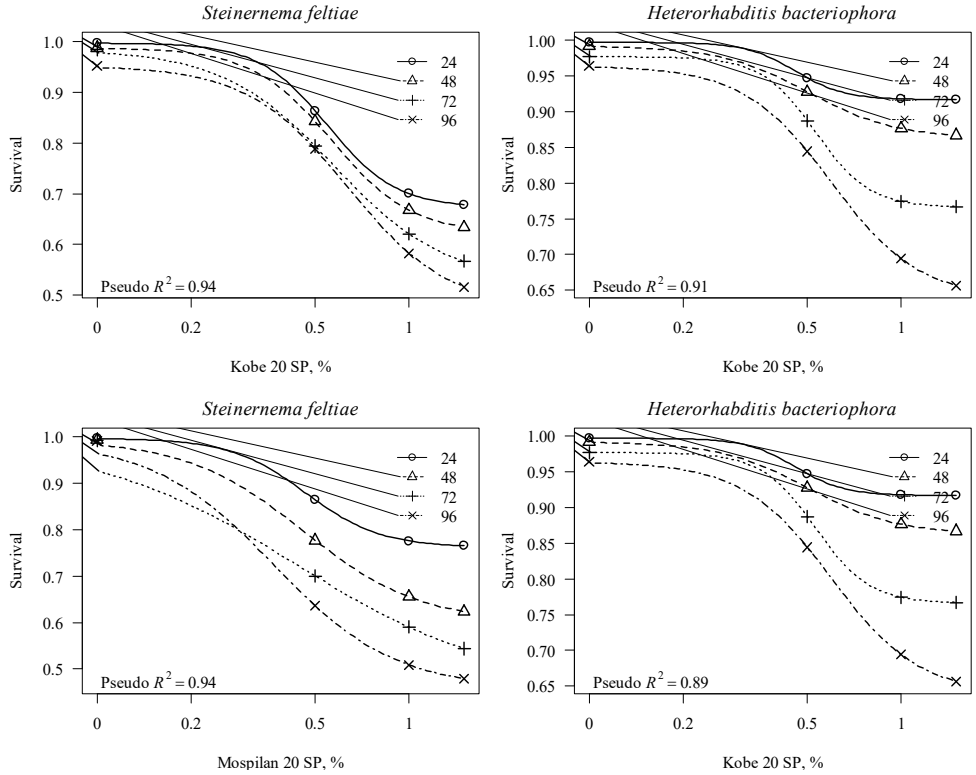

Fig. 1. The symmetric log-logistic models of the response of nematode (IJs) survival to insecticide exposure depending on exposure time (24, 48, 72, and 96 hours). The ordinate axis is the concentration of insecticides, the abscissa is the proportion of surviving IJs

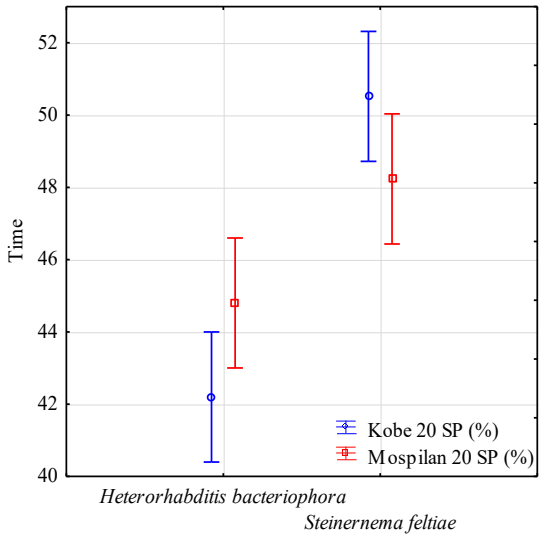

Fig. 2. Variation in the time of infection of G. mellonella by entomopathogenic nematodes previously stored in solutions of the tested insecticides (Mospilan 20 SP® and Kobe 20 SP®) at concentrations of 0.5%, 1%, and 1.5%. The dose of pesticides was used as a covariate in the analysis

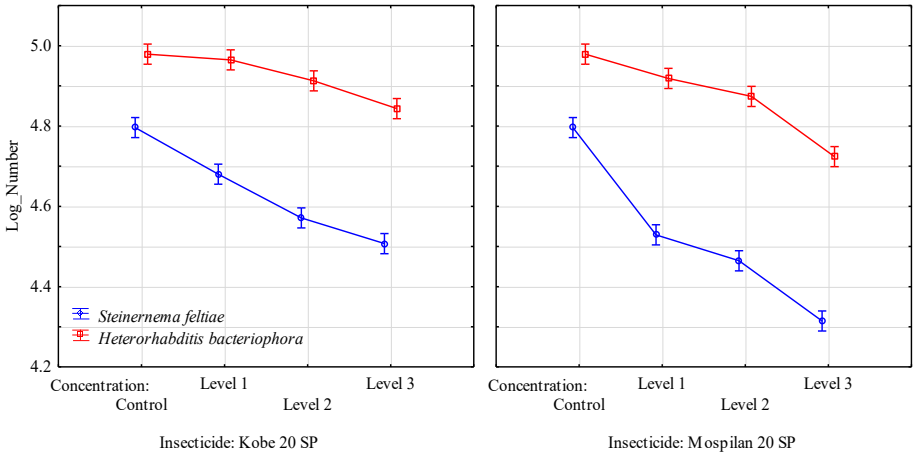

Fig. 3. Reproductive capacity of EPNs depending on the applied insecticide. The x-axis represents the applied pesticide: Mospilan 20 SP® or Kobe 20 SP®, and the species of nematodes: *H. bacteriophora* or *S. feltiae*. Level 0 corresponds to the control (0% insecticide), while Levels 1, 2, and 3 correspond to concentrations of 0.5%, 1.0%, and 1.5%, respectively. The y-axis represents the log-transformed number of infective juveniles (IJs)

in insecticide efficacy and raising nematode mortality even at lower doses.

Effect of insecticides on *Galleria mellonella* virulence

Entomopathogenic nematodes (EPNs) demonstrated the ability to significantly delay the infection process in the greater wax moth larvae (Fig. 2). In the control group, infection of *G. mellonella* occurred within 35.2 ±0.3 hours. The inhibitory effects of pesticides on nematode virulence were species-specific and dose-dependent ($R_{adj}^2 = 0.89$, $F = 82.4$, $p < 0.001$). However, within the tested dose ranges, the insecticides did not differ significantly in their overall impact on infection rates by nematodes ($F = 0.04$, $p = 0.84$).

Application of Mospilan 20 SP® led to stronger inhibition of virulence in *H. bacteriophora* compared to *S. feltiae* (Planned comparison $F = 4.2$, $p = 0.04$). In contrast, Kobe 20 SP ® exhibited no significant species-specific inhibitory effect on infection rates (Planned comparison $F = 3.3$, $p = 0.08$). Increasing pesticide doses significantly delayed the infection of *G. mellonella* by nematodes ($\beta = 0.88 \pm 0.05$, $t = 18.7$, $p < 0.001$).

The sensitivity of nematodes to pesticide doses varied between species. Delays in infection caused by *H. bacteriophora* at increasing pesticide doses were less pronounced compared to those caused by *S. feltiae* (β for species * dose interaction = –0.28 ±0.08, t = –3.5, $p = 0.001$). At the highest tested pesticide concentration, *H. bacteriophora* infected *G. mellonella* within 51.2 ±1.2 hours, whereas *S. feltiae* required 61.9 ±1.0 hours.

EPN reproductive capacity

The reproductive potential of entomopathogenic nematodes, measured as the total number of infective juveniles (IJs) emerging from *G. mellonella* cadavers, varied significantly based on experimental factors (Fig. 3). Nematode species, insecticide type, and concentration collectively accounted for 86% of the variation in reproductive capacity ($R_{adj}^2 = 0.86$, $F = 611.6$, $P < 0.001$) (Table 2).

A significant reduction in the reproductive capacity of *S. feltiae* was observed, particularly following exposure to Mospilan 20 SP® (Planned comparison $F = 443.215$, $p < 0.001$). Increasing concentrations of both tested insecticides led to a substantial reduction in the reproductive capacity of nematodes (β for concentration = –0.46 ±0.02, t = –30.9, $p < 0.001$).

The effects of increasing pesticide concentrations were less pronounced for *S. feltiae* compared to *H. bacteriophora* (β for concentration * species interaction = 0.07 ±0.01, t = 4.5, $p < 0.001$). For *H. bacteriophora*, the number of larvae (IJs) emerging

Table 2. General linear model (GLM) of the effect of insecticides, insecticide concentration, and species on the EPNs reproductive capacity

Source of variation	Sum of squares	df (degrees of freedom)	Mean sum of squares	*F*-ratio	*p*-value
Intercept	4085.2	1	4085.2	663757.1	<0.001
Insecticide	0.8	1	0.8	130.7	<0.001
Species	2.2	1	2.2	355.1	<0.001
Insecticide × species	0.1	1	0.1	18.4	0.002
Concentration (continuous)	5.3	1	5.3	859.2	<0.001
Species × concentration	0.6	1	0.6	90.1	<0.001
Error	2.9	474	0.0	–	–

from insect cadavers remained similar to control levels, particularly following exposure to Kobe 20 SP®. This suggests that *H. bacteriophora* exhibited greater resilience to pesticide exposure compared to *S. feltiae*.

DISCUSSION

Soil fauna encompasses a rich diversity of taxonomic groups, including nematodes [Ramirez et al. 2015]. Intensive agricultural and horticultural practices often rely on chemical pesticides to protect crops; however, these pesticides can adversely affect beneficial organisms such as EPNs. Entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae play a crucial role in regulating pest populations and are widely used as biocontrol agents. However, pesticide contamination in soils can compromise EPN survival, reproduction, and efficacy in pest control, with responses varying depending on pesticide type, concentration, and nematode species [Ulu 2023].

In this study, the effects of two acetamiprid-based neonicotinoids (Mospilan 20 SP® and Kobe 20 SP®) on *S. feltiae* and *H. bacteriophora* were evaluated. Our findings revealed varying sensitivity among the two EPN species, with *S. feltiae* exhibiting greater sensitivity to acetamiprid exposure compared to *H. bacteriophora*. Significant reductions in fecundity and reproductive potential were observed for both species, although the ability to infect the insect host *G. mellonella* remained largely unaffected.

These results align with previous studies, which reported species-specific tolerances to pesticides among EPNs [Ulu et al. 2016, Kruk and Dzięgielewska 2020]. While acetamiprid exposure reduced fecundity, the virulence of *S. feltiae* and *H. bacteriophora* remained unchanged. This suggests that acetamiprid primarily affects reproduction rather than the infection capability of EPNs.

Interestingly, studies on imidacloprid, another neonicotinoid, have shown more complex interactions with EPNs. For example, imidacloprid has been reported to enhance the efficacy of *Steinernema* species in some cases by promoting host attraction or infection rates [Atwa et al. 2013]. Synergistic effects have also been observed between imidacloprid and *Steinernema* spp., wherein the pesticide increases nematode efficiency in pest control. However, our study revealed that acetamiprid causes higher nematode mortality than imidacloprid, a finding consistent with previous research on EPN compatibility with these pesticides [Koppenhöfer and Grewal 2005]. Greenhouse and field studies on scarab larvae demonstrated that imidacloprid enhanced EPN efficacy, whereas acetamiprid and thiamethoxam exhibited weaker interactions.

Koppenhöfer et al. [2002]. reported that imidacloprid has stronger adverse effects on entomopathogenic nematodes than acetamiprid, but our results suggest that acetamiprid has more persistent negative effects

on *S. feltiae* reproductive capacity. This limitation could reduce the long-term biocontrol potential of *S. feltiae*. The higher toxicity of acetamiprid, compared to imidacloprid, may result from differences in their chemical structures, modes of action, or toxicity profiles. Acetamiprid appears to have a more potent effect on nematode fecundity by regulating immune pathways, as previously suggested in related studies.

The effects of pesticides on EPNs are also influenced by exposure duration and nematode species. For instance, Laznik and Trdan [2014] demonstrated that prolonged exposure to chemical solutions exacerbates nematode sensitivity. Our findings support these observations, as exposure duration significantly influenced EPNs survival and reproduction.

Species-specific sensitivity was also evident in this study. *S. feltiae* exhibited lower resistance to acetamiprid compared to *H. bacteriophora*. This suggests that *S. feltiae* may be more vulnerable to pesticide contamination in agricultural soils where acetamiprid is commonly applied. Conversely, the higher resistance of *H. bacteriophora* indicates its potential as a more robust biocontrol agent in environments with acetamiprid exposure.

CONCLUSIONS

The results showed that *S. feltiae* was more vulnerable to acetamiprid than *H. bacteriophora*, with significantly higher mortality rates observed in *S. feltiae* after four days of exposure. However, pesticide exposure did not significantly affect the nematodes' ability to infest *G. mellonella*, suggesting their parasitizing ability remained intact.

Steinernema feltiae also exhibited reduced fecundity and reproductive capacity after exposure, indicating lower resistance compared to *H. bacteriophora*. This raises concerns about the long-term viability of *S. feltiae* in areas with frequent pesticide use. The study highlighted that EPN species can show different sensitivities to the same insecticide, complicating their use in integrated pest management (IPM) systems involving chemical pesticides.

Further research is needed to assess the long-term impacts of acetamiprid and other neonicotinoids on EPN reproductive capacity and population dynamics, particularly *S. feltiae* and *H. bacteriophora*. Such studies are essential for evaluating their sustainability as biocontrol agents in pest management. Additionally, exploring potential synergistic interactions between neonicotinoids and other biocontrol agents, such as fungi or bacteria, could improve pest control while minimizing pesticide impact on EPN populations. This research will be crucial for developing sustainable pest management strategies that protect both crops and beneficial soil organisms.

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