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EFFECTS OF ACETAMIPRID AND FLUPYRADIFURONE-BASED INSECTICIDES ON SOIL FAUNA: RESEARCH ON NEMATODES AND EARTHWORMS

Short communication

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ABSTRACT

The extensive use of neonicotinoid and butenolide insecticides in horticultural systems has raised concerns about their potential effects on nontarget soil organisms, including plant-parasitic nematodes and earthworms. This study evaluates the effects of two commonly used insecticides, a neonicotinoid (acetamiprid) and butenolide (flupyradifurone), on the survival of plant pathogenic nematodes *Hirschmanniella baltica*, *Ditylenchus laurae*, and the earthworm *Eisenia fetida* in horticultural soils. A four-parameter Weibull model was used to analyze the survival dynamics following exposure of nematodes to one concentration of acetamiprid and *Eisenia fetida* to two concentrations. Both insecticides exhibited similar toxicity to earthworms, emphasizing the need for careful consideration of their use in sustainable pest management practices. These findings highlight the importance of evaluating the ecological risks of insecticides in horticulture, particularly regarding their unintended consequences on soil fauna. Further research is recommended to optimize the use of these insecticides while minimizing potential harm to beneficial soil organisms.

Key words: *Ditylenchus laurae, Eisenia fetida, Hirschmanniella baltica,* Sivanto Prime, Mospilan, pest management

INTRODUCTION

Neonicotinoids, a common class of insecticides, adversely affect nontarget species such as earthworms, affecting their survival, growth, and reproduction (Miglani et al. 2020; Barathi et al. 2024). The use of neonicotinoid insecticides has raised concerns about their adverse effects on pollinators. These insecticides act on the nervous system of insects, but their persistence in soil and water raises concerns about wider ecological impacts (El-Naggar & Zidan 2013). Studies indicate that neonicotinoid seed treatments affect soil nematode communities, affecting their abundance and diversity (Douglas & Tooker 2015; Parizadeh et al. 2023; Cao et al. 2024). Mospilan (acetamiprid) is widely used to control insect pests in horticultural crops such as fruits, vegetables, and ornamentals. Although effective against aphids, whiteflies, and thrips, its persistence in soil and water and its potential effects on beneficial soil organisms have become a key area of interest in environmental toxicology and sustainable agriculture research (Martin et al. 2024). Impacts on beneficial soil organisms like earthworms and nematodes, essential for soil fertility and productivity, require careful consideration (Jouquet et al. 2010; Griffiths et al. 2021).

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Sivanto Prime (flupyradifurone), a novel butenolide insecticide developed by Bayer Crop Science, was introduced as an alternative to neonicotinoids, offering rapid control of sucking pests in a variety of horticultural crops (Nauen et al. 2015; Hesselbach & Scheiner 2018). At recommended rates, it is promoted for its safety to a range of nontarget organisms in soil, water, and air (Nauen et al. 2015). However, recent studies have raised concerns about its potential effects on nontarget soil organisms (Scheibli et al. 2023, 2024) and pollinators, including effects on cognition and taste perception in honeybees (Hesselbach & Scheiner 2018). Notably, research on the effects of Sivanto Prime on earthworms remain scarce, creating a knowledge gap in assessing its ecological safety. Because it shares a mode of action with neonicotinoids, concerns have been raised about its potential effects on soil invertebrates, including earthworms (El-Naggar & Zidan 2013). Flupyradifurone disrupts neural signaling in insects via nicotinic acetylcholine receptors (nAChRs), similar to neonicotinoids, raising the question of whether it could have a similar effect on nontarget species such as earthworms (Nauen et al. 2015).

Earthworms (Opisthopora) are vital to soil ecosystems, constituting 60–80% of soil biomass (Jouquet et al. 2010). Their feeding and burrowing activity facilitates the decomposition of organic matter, enhances soil aeration, and improves soil structure and nutrient cycling (Griffiths et al. 2021). Their role in soil fertility and health underscores the need to assess the impact of insecticides on their populations. The introduction of new insecticides, such as Sivanto Prime, for pest control poses potential ecological threats to soil-dwelling organisms essential for soil health. Given the environmental significance of soil organisms such as nematodes and earthworms in sustaining soil fertility and productivity, it is imperative to assess the impact of insecticides on these nontarget species. In this study, the effects of two insecticides from different chemical groups, acetamiprid and flupyradifurone (neonicotinoid and butenolide, respectively), on the plant-parasitic nematodes *H. baltica* and *D. laurae* and the earthworm *E. fetida* was evaluated. By comparing the survival rates of these organisms after exposure to selected insecticides, we aim to contribute to the overall understanding of the ecological risks associated with their use.

MATERIALS AND METHODS

Study location

The experiment was conducted in two research facilities: the Nematology Laboratory of the National Institute of Horticultural Research in Skierniewice, Poland, and the Laboratory of Pesticide Monitoring of the National University of Life and Environmental Sciences in Ukraine.

Insecticides

Acetamiprid (Mospilan, Sumi Agro) and flupyradifurone (Sivanto Prime, Bayer) were used at the concentrations recommended by the manufacturers. Sivanto Prime was also evaluated at a concentration 50% higher than the standard dose for *Eisenia fetida* (flupyradifurone II) to see whether this wider exposure range would reveal more apparent dose-dependent effects that might be missed at a single, lower concentration.

Aqueous working solutions were prepared from commercial formulations according to the label concentrations for all bioassays, but are reported here solely for their active ingredients. Mospilan (20% w/w soluble powder) was dissolved at 0.25 g formulation per L, obtaining 0.05 g a.i.$\cdot$L^{-1}. Sivanto Prime (200 g a.i.$\cdot$L^{-1} suspension concentrate) was used in two concentrations: a standard dose of 0.375 mL$\cdot$L^{-1} (0.075 g a.i.$\cdot$L^{-1}) and a higher dose of 0.5625 mL$\cdot$L^{-1} (0.1125 g a.i.$\cdot$L^{-1}) to extend the exposure range for dose–response analysis.

Experimental design

Nematodes

H. baltica and *D. laurae* specimens were isolated from *Potamogeton perfoliatus* rhizome spacers collected from the coast of the Bay of Puck [54°63′25″N; 18°49′82″E]. Rhizomes were cut into 1-cm sections and immersed in water on a 150-μm sieve for 24 hours. Nematodes were placed in 5-cm Petri dishes containing 30 ml of distilled water. Groups of 20 *H. baltica* and 10 *D. laurae* were exposed to 10 ml of Mospilan or Sivanto Prime at the doses recommended by the manufacturers. Control groups received 10 ml of distilled water. Mortality was assessed at 1, 7, 14, and 21 days after application. Each treatment was replicated three times. Although peak activity of acetamiprid (Mospilan) is reported within 10 days, we extended the observation period to 21 days to detect any delayed or sublethal effects that are ecologically relevant and may occur outside the immediate exposure window. This duration also allowed consistency among test organisms.

Earthworms

Adult *E. fetida* specimens were obtained from a compost system enriched with peat, apple, kitchen waste, and granulated cattle manure. Groups of 10 earthworms were placed in plastic cups containing 150 ccm of ecological substrate (ProLine Potgrond, Klasmann) and treated with one of the agents at the recommended doses. Control groups received distilled water. Survival was monitored after 1, 7, 14, and 21 days. The temperature was maintained at 21 °C by adding 20 ml of distilled water daily.

Statistical analysis

To model the survival dynamics of nematodes and earthworms under insecticide exposure conditions, a nonsymmetric Weibull type 1 model was used:

$$Y = c + \frac{d - c}{1 + exp\,\{b[log(x) - log(ET_{50})]\}};$$

where Y is the survival rate (response variable); x – insecticide dose; c – lower limit (when dose x→∞); d – upper limit (when dose x→0); b – slope around the inflection point, which is ET_{50}; ET_{50} – inflection points of the dose–response curve, analogously to ED_{50}, but here x is the exposure time and not the dose concentration. ET_{50} values (adequate exposure time at which 50% mortality occurs) were derived using the ED function from the “drc” package in R (Ritz et al. 2009), after fitting a Weibull dose–response model with the “drm” function. Mortality data were modeled as a function of exposure time, and ET_{50} values were computed with their 95% confidence intervals using the delta method.

Data exploration

Normality and homogeneity of variance were assessed using Shapiro–Wilk and Levene’s tests (package car). The effects of treatment, exposure time, and interactions were analyzed using two-way ANOVA followed by Tukey’s post hoc test for multiple comparisons (α = 0.05) implemented using the agricolae package. R version 4.2.2 software was used (R Core Team 2022).

RESULTS

Weibull type 1 models effectively described the survival dynamics of nematodes and earthworms exposed to insecticides (Fig. 1). Data presented in Table 1 reveal distinct differences in the sensitivity of *E. fetida* to the tested insecticides. The highest sensitivity of *E. fetida*, as indicated by the lower limit parameter of the Weibull model, demonstrated a greater adverse effect compared to nematodes. Both insecticides significantly reduced ED_{50} values, confirming their toxic effects. However, increasing the dose of flupyradifurone did not cause further significant toxicity (P = 0.37), suggesting a plateau in its effect. Additionally, no significant differences were observed between the effects of acetamiprid and flupyradifurone (P = 0.42), indicating comparable levels of toxicity. Among the nematodes tested, *D. laurae* showed higher tolerance than *H. baltica*, with no statistically significant changes in ET_{50} values after exposure to insecticides (P = 0.96 for acetamiprid, P = 0.68 for flupyradifurone). In contrast, pesticides significantly reduced ET_{50} values for *H. baltica* (P = 0.001 for acetamiprid, P = 0.002 for flupyradifurone). These findings underscore the potential ecological risks of both insecticides – Mospilan and Sivanto Prime, in particular their impact on earthworm populations, which could have broader implications for sustainable pest management strategies.

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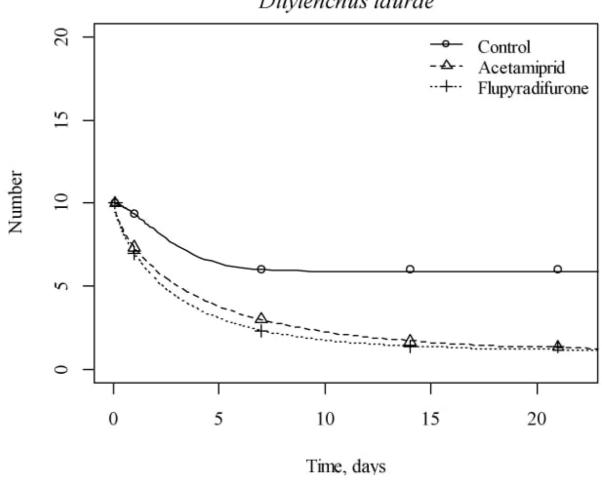

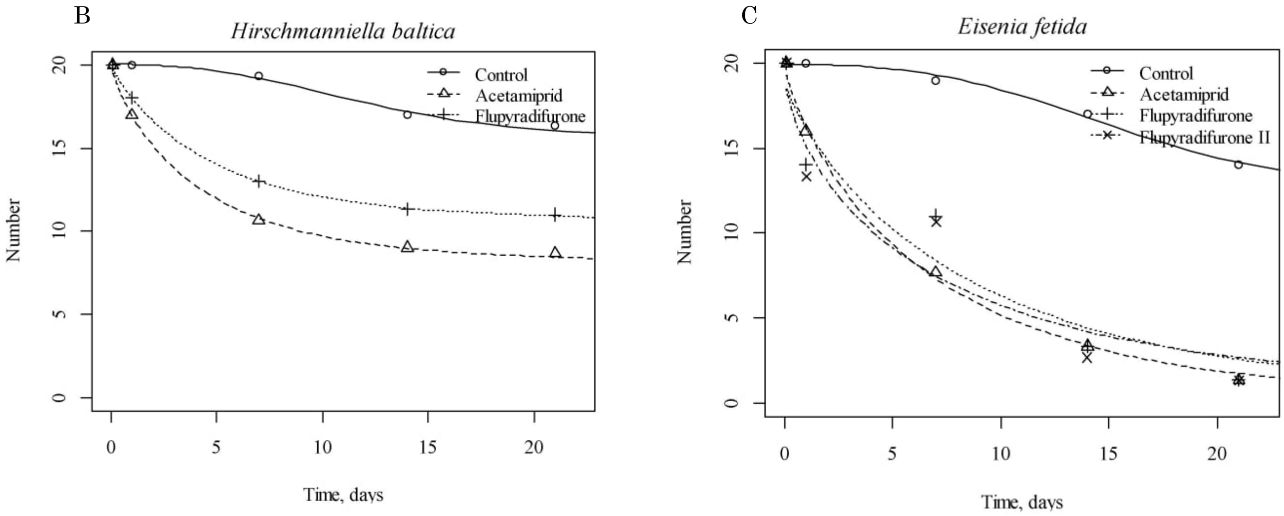

Figure 1. Survival dynamics fitted using a four-parameter Weibull model: *Ditylenchus laurae* (A) lack of fit test F = 0.12, P = 0.99, *Hirschmanniella baltica* (B) lack of fit test F = 0.21, P = 0.97, *Eisenia fetida* (C) lack of fit test F = 17.9, P < 0.001 50% higher concentration of flupyradifurone tested only for *Eisenia fetida* and not applied to other test species

Table 1. Weibull model parameters of nematode and earthworm survival dynamics over time depending on the insecticide used

Species	Treatment	Steepness (b)	Lower limit (c)	Upper limit (d)	Inflection (e)	ET_{50}
Ditylenchus laurae	control	1.55±0.67	5.89±0.22	10.00±0.37	3.06±1.42	2.41±1.05
	acetamiprid	0.75±0.11	1.01±0.33	10.00±0.37	4.03±0.74	2.47±0.55
	flupyradifurone	0.80±0.13	1.1±0.29	10.00±0.37	3.01±0.56	1.91±0.36
Hirschmanniella baltica	control	2.32±1.13	15.82±0.49	20.04±0.3	13.30±2.41	11.36±1.74
	acetamiprid	0.83±0.11	8.17±0.37	20.02±0.43	4.25±0.64	2.73±0.43
	flupyradifurone	0.90±0.15	10.70±0.36	20.00±0.43	4.81±0.84	3.20±0.61
Eisenia fetida	control	2.68±1.46	12.94±0.89	19.91±0.64	16.99±2.52	14.83±2.41
	acetamiprid	0.83±0.14	0.13±0.85	19.86±0.91	6.89±1.19	4.44±0.88
	flupyradifurone	0.86±0.30	0.55±0.82	18.88±1.32	8.41±1.85	5.51±1.87
	flupyradifurone II	0.70±0.15	0.49±0.84	19.30±1.06	7.08±1.53	4.22±1.21

flupyradifurone II – concentration 50% higher than the standard dose

DISCUSSION

This study shows that Sivanto Prime (flupyradifurone), especially at higher doses, has a strong biocidal effect on plant-parasitic nematodes. However Mospilan (acetamiprid) was slightly more effective against *H. baltica*, while *D. laurae* responded better to flupyradifurone. These findings confirm that insecticide efficacy varies between species, emphasizing the importance of species-specific pest control strategies.

A key finding of this study is the first documented evidence of nematicidal activity of Sivanto Prime (flupyradifurone). Given its mode of action through nAChRs, which are not considered primary targets in nematodes (Schleker et al. 2022), potential off-target effects warrants careful consideration. In particular, although nAChRs are not directly involved in plant physiology, secondary mechanisms such as modulation of plant defense responses, stress signaling pathways, or alterations in root exudate composition may indirectly affect nematode behavior or survival. Furthermore, indirect effects resulting from shifts in the composition and function of the soil microbiota should not be overlooked (Hawk & Faske 2020).

The insecticides tested were particularly harmful to *E. fetida*, aligning with previous studies showing oxidative stress. Both acetamiprid and flupyradifurone significantly reduced earthworm populations, indicating potential adverse effects on nontarget soil organisms (Hesselbach & Scheiner 2018). DNA damage and disruption of antioxidant enzyme activity in earthworms exposed to neonicotinoid insecticides (Li et al. 2018; Gill et al. 2021). These effects may impair essential soil functions such as nutrient cycling and organic matter decomposition, posing risks to soil health and ecosystem stability (Qiao et al. 2022).

The results highlight the need for long-term studies to assess the persistence of these insecticides in soil and their broader ecological consequences. Future research should investigate sublethal effects on earthworm reproduction and behavior (Siregar et al. 2024), potential development of resistance in nematodes, and effects on soil microbial communities and fertility to ensure sustainable agricultural pest management practices.

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