# Underestimated adverse effects of entomopathogenic nematodes to honey bees

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## Introduction

There is a great interest in finding sustainable plant protection products to safeguard biodiversity and our ecosystem. Entomopathogenic nematodes (EPNs) have received much attention as alternative biological-control agents to conventional synthetic agrochemicals (Erler et al., 2022) . EPNs live parasitically and are mainly applied as soil treatments or foliar sprays where they infect various insect pests (Labaude & Griffin, 2018). However, as nematodes are considered natural enemies, authorities often approve commercial products based on limited or no data (EU Commission, 2001). Here, we assess whether foliar application of a commerical EPN can pose a risk to honey bees, *Apis mellifera*.

## Methods

Newly emerged worker honey bees and larvae of the greater wax moth (*Galleria mellonella*) were exposed to dry and wet spray residues on foliage at a low (0.25 Mio/m$^2$) and high (0.5 Mio/m$^2$) field-realistic concentration of *Steinernema carpocapsae*. Four treatment groups (**a**: direct over spray wax moth larvae (wet), **b**: dried residue wax moth larvae (dry), **c**: direct over spray honey bees (wet), **d**: dried residue honey bees (dry)) per Nematode concentration (low & high), control and three replicates were prepared. Mortality was assessed over 96 h and nematode reproduction (i.e., total number offspring) was evaluated in all dead individuals (**Fig. 1**). Generalized linear regression models (GLMs) were applied to analyse survival and nematode reproduction using STATA 17 statistical software.

## Results

EPN exposure resulted in an 80% increase in mortality rate for wax moth larvae ($p$<0.001), while honey bee survival was also significantly reduced ($p$<0.001). The effect was dose-dependent, with the high concentration leading to a significantly higher mortality rate (55%) than the lower dose (43%) compared to the control in honey bees (**Fig. 2 A&C**). Nematode reproduction was significantly higher in wax moths than in honey bees ($p$<0.001). Irrespective of the treatment group, mean nematode reproduction per individual wax moth larvae and honey bee was 1,127 and 41, respectively; representing a 27-fold increase in wax moths. (**Fig. 2 B&D**). Wet treatments at a high concentration lead to a significant increase in nematode reproduction compared to the remaining honey bee treatments ($p$<0.01; **Fig. 2 B&D**). Here we show for the first time that foliar exposure to a commercial EPN product can significantly reduce honey bee survival and that the nematodes can successfully replicate within the carcasses of adult bees.

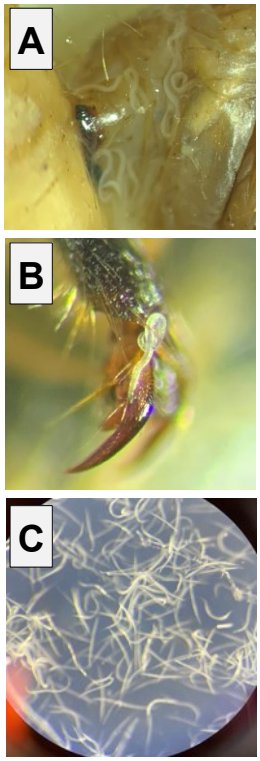


**Fig. 1** Detected nematodes 15 days post infection. Nematodes were found, in wax moth larvae (**A**), on honey bee tarsus-claw (**B**) and in White traps (**C**).

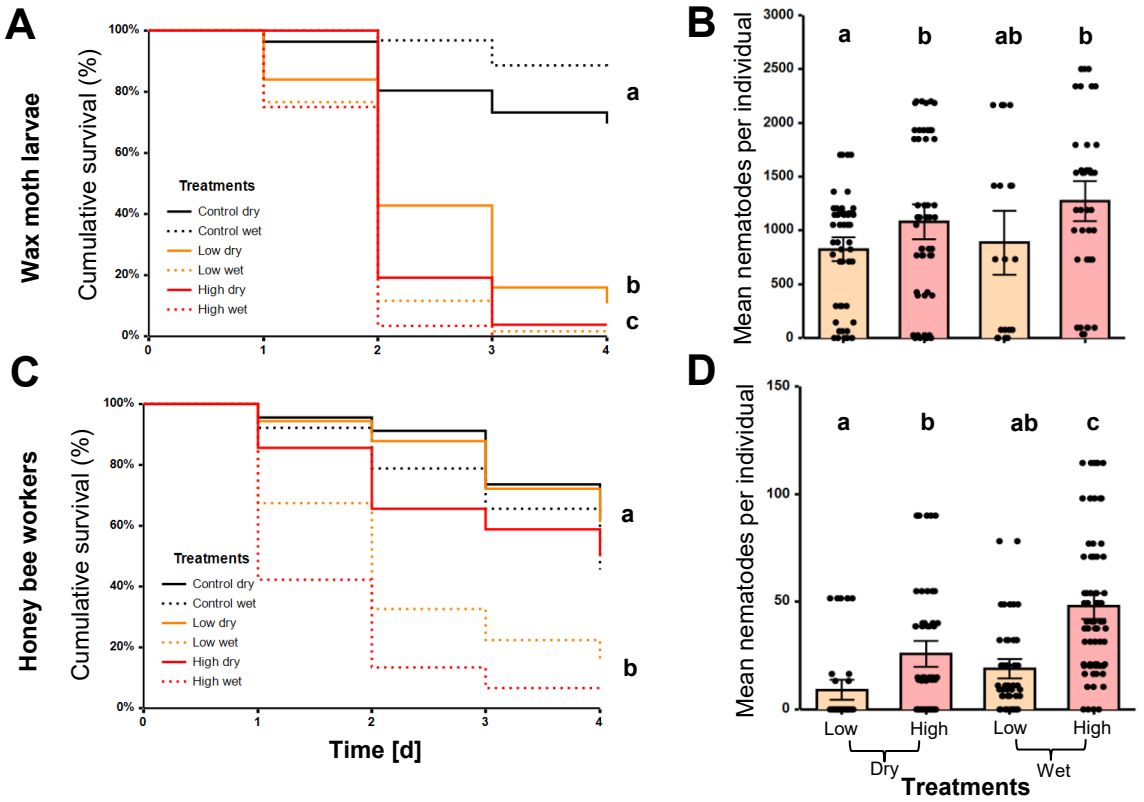


**Fig. 2 Kaplan-Meier survival and *Steinernema carpocapsae* reproduction analyses.** Survival was recorded over 96 h post *S. carpocapsae* exposure where as nematode reproduction was assessed in dead individuals 15 days post exposure. Survival and mean nematode reproduction post exposure per individual wax moth larvae (**A&B**) and honey bee worker (**C&D**).

## Discussion and conclusion

The data provide clear evidence that exposure to *S. carpocapsae* can cause lethal effects and proliferation of nematode is possible in honey bees. Given the lack of data on potential adverse effects of EPNs on non-target pollinating insects, our results highlight the urgent need to be cautious when applying foliar application of EPNs to crops. As dry residues of our EPN treatments imposed lower lethality to honey bees and decreased nematode proliferation when compared to direct (wet) exposure, foliar treatments with EPNs should ideally be applied when bees are not foraging (i.e., early evenings) to reduce the likelihood of exposure. Additional research is urgently required to adequately investigate the potential risk of EPNs to ground-nesting bees and other non-target insect species during foliar and soil application.

**References:**
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