Assessing the Impact of Entomopathogenic Nematodes on Bees: Risks and Recommendations

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Introduction

There is growing interest in sustainable plant protection products to safeguard biodiversity. Entomopathogenic nematodes (EPNs) are promising biological control agents, that target mainly soil-dwelling insect pests and are therefore applied on soil. Recently, EPNs have also been applied as foliar treatments (Erler et al., 2022; Labaude & Griffin, 2018) to control pests that attack above-ground plant parts. However, limited ecotoxicological data is available for regulatory approval (EU Commission, 2001). This study investigates the effects of *Steinernema carpocapsae* on adult honey bees (*Apis mellifera*) and Mason bees (*Osmia bicornis*) and presents novel foliar and soil exposure methods for testing the risks of EPN products to bees in the laboratory.

Methods

Newly emerged honey bees (Fig. 1A) were exposed to field realistic dry (4 hours of drying before exposing to bees) or wet foliar residues of *S. carpocapsae* at low (0.25 million/m²) and high (0.5 million/m²) doses. Mason bees were exposed to soil treated with low (0.5 million) and high (1 million) nematode doses (Fig. 1B). Mortality was recorded over 48 hours, and nematode reproduction in deceased bees was measured using white traps and dissection of cadavers (Fig. 1C-E). The tests were conducted in an incubator at 36 °C.

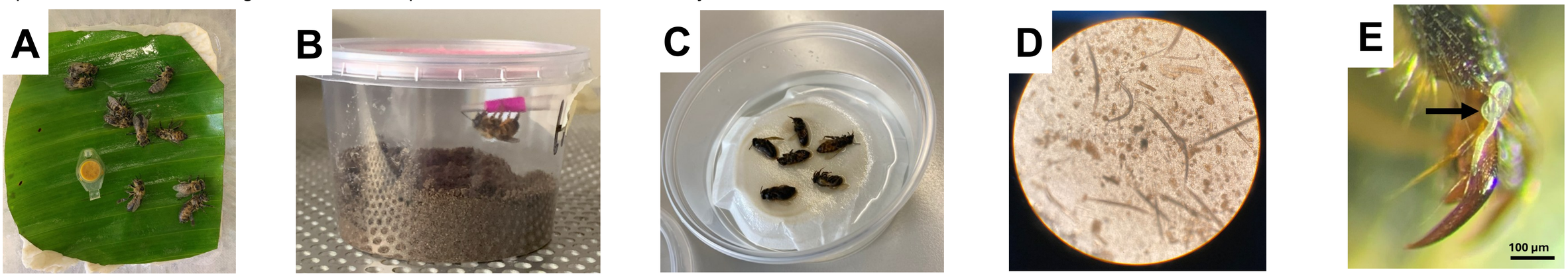

Fig. 1: Honey bee wet and dry exposure to *Steinernema carpocapsae* residues on leaves **(A)**. Mason bee EPN soil exposure **(B).** Bees on white trap. Nematode infection and replication in dead test bees was assessed 15 days post exposure **(C)**. Nematodes were counted in water of white traps **(D).** Infective juveniles of *S. carpocapsae* were found on cadavers (e.g. tarsus-claw) **(E)**.

Results

Foliar spray exposure of honey bees: Controls treated only with water (dry and wet, *N=91* and *N=90*) maintained high survival (91% & 79%). Under dry conditions, EPN exposure reduced survival only moderately (low dose 88%, *N=90* & high dose 66%, *N=90*). In contrast, the survival of exposed honey bees to wet EPN residues dropped to only 33% (*N=89*) with low dose and to 13% with high dose treatment (*N=90*; Fig. 2A). Nematode reproduction in honey bee carcasses was highest in the high wet treatment (Median ~45 nematodes per individual), (Fig. 2C).

Soil exposure of mason bees: The control group maintained a survival rate of 53% (*N=34*), while EPN exposure caused sharp declines after 24h and death of almost all test bees after 48h. At low EPN concentration, survival dropped to 0 % (*N=28*), whereas high concentration resulted in a survival of only 9% (*N=32*) by 48h (Fig. 2B). No significant difference in nematode reproduction was observed between the EPN low and high concentration treatments (Medians ~2200 and ~ 2700 nematodes per individual, respectively), however groups showing high variability in nematode counts as reflected by large error bars (Fig. 2D).

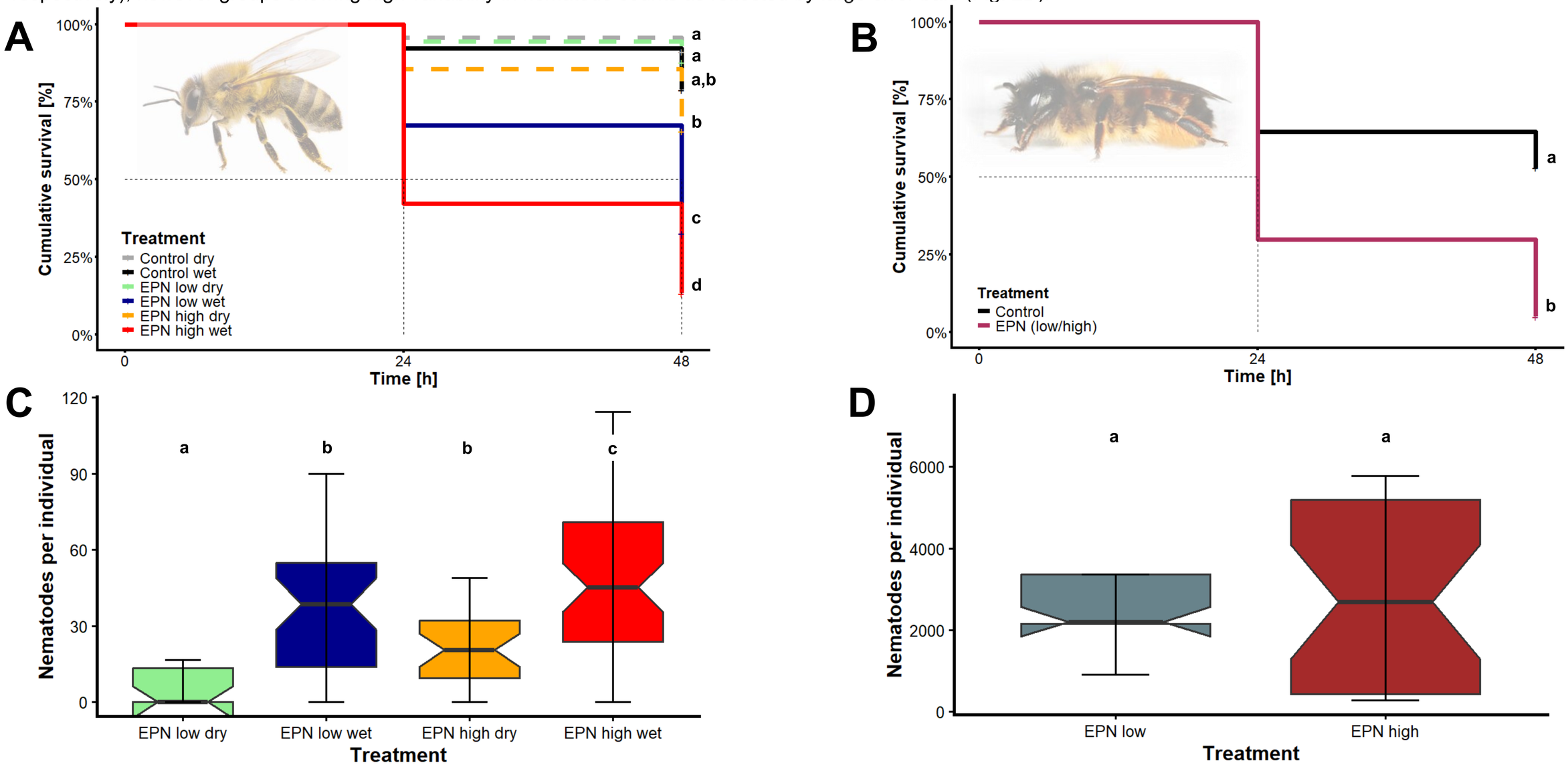

Fig. 2 Kaplan–Meier survival curves of bees: Survival of adult *A. mellifera* following exposure to dried and wet spray residues on leaves **(A)**, and survival of adult *O. bicornis* following exposure to soil treated with the EPN *S. carpocapsae*. Multiple pairwise comparisons of survival curves with log-rank tests show significant differences ($P<0.05$, post-hoc Bonferroni corrected) in survival of treatment groups. For visualization, low and high EPN treatment groups were pooled (EPN low/high) because binomial GLM at 48-h found no difference in survival between doses. Lower case letters indicate significant differences between survival of bees treated only with water (Controls) and bees treated with EPNs **(B)**. **Number of nematodes counted in dead bees:** after exposure to wet and dry EPN residues after foliar application **(C)**, and after exposure to treated soil **(D)**. Differences in EPN replication were tested by non-parametric test on medians. Significant differences (*P<0.05*) are indicated by lowercase letter. No EPNs were detected any cadaver of control bees treated only with water.

Discussion and conclusion

Our results provide clear evidence that both foliar and soil exposure to *Steinernema carpocapsae* used in plant protection can cause lethal effects to adult honey and mason bees, and nematodes are able to successfully replicate within their carcasses. Due to the limited data on EPN impacts on non-target organisms, caution is advised when applying EPN plant protection products, particularly if applied as foliar treatments. Since dry residues caused lower lethality compared to exposure to wet residues on leaves, foliar applications should ideally occur when bees are inactive (e.g., early evenings after sunset) to minimize exposure. However, as the majority of wild bee species is ground-dwelling, exposure to EPNs when applied as soil treatment cannot be fully prevented. Hence further research is urgently needed to evaluate the effects of EPNs to these important non-target organisms and to ensure the safe use of EPN plant protection products. This study represent an important step toward developing reliable bioassays for assessing the risks of EPNs to bees, which is critical and should be included as a standard requirement for regulatory approval of EPN-products for plant protection.

References:

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