Entomopathogenic nematodes reduce hibernation success of bumblebee queens, *Bombus terrestris*

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Introduction

Sustainable agriculture increasingly relies on biological control agents as alternatives to synthetic pesticides (Bale et al. 2008). Among these, entomopathogenic nematodes (EPNs) are widely used to control soil-dwelling insect pests. However, many bee species nest and develop in soil, raising concerns about unintended exposure to these biological control agents. EPNs are typically applied from spring to autumn, overlapping with periods when soil-dwelling insects are either nesting or overwintering. Despite their widespread use, the potential risks posed by EPNs—particularly species belonging to the genera of *Steinernema* and *Heterorhabditis*—to non-target organisms remain poorly studied (Cappa et al. 2022; Dutka et al. 2015). Here, we test the effects of two commercially available EPN species on the hibernation success of future bumble bee queens (i.e., gynes).

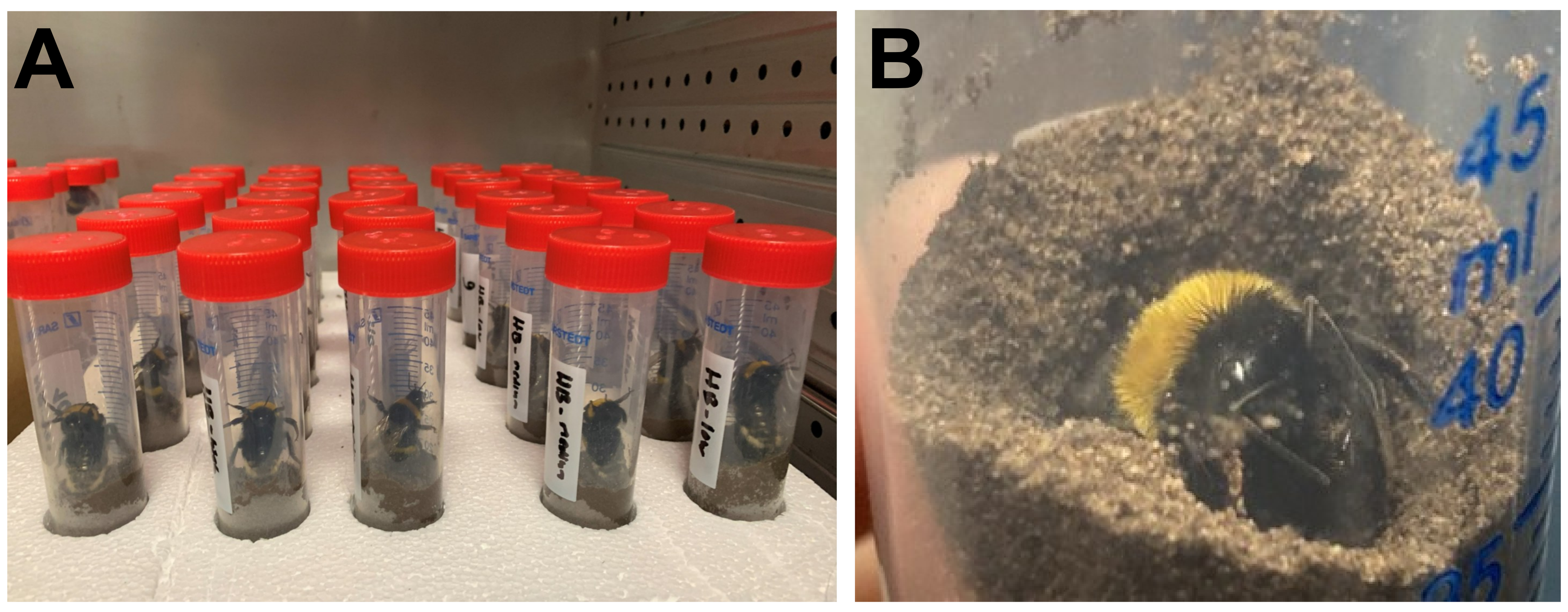

Fig. 1: Hibernation set-up with *Bombus terrestris* gynes inside soil-filled Falcon tubes **(A)**. Partially buried gyne in soil within a Falcon tube, enduring the simulated overwintering period at 14°C **(B)**.

Results

Survival in the control group remained high, with 95% of gynes surviving the 28-day hibernation period. For *Steinernema carpocapsae*, survival differed significantly among treatments (log-rank test: $\chi^2 = 13.0$, df = 3, $P = 0.005$). All exposure levels significantly reduced gyne survival compared to the control (Benjamini–Hochberg–corrected pairwise comparisons, $P < 0.05$), while no significant differences were detected among the exposure levels ($P > 0.05$; **Fig. 4A**). For *Heterorhabditis bacteriophora*, survival also differed significantly among treatments (log-rank test: $\chi^2 = 9.9$, df = 3, $P = 0.02$). Medium and high exposure levels resulted in significantly lower survival compared to the control (Benjamini–Hochberg–corrected pairwise tests, $P < 0.05$), whereas no significant differences were detected among the exposure levels ($P > 0.05$; **Fig. 4B**). Both nematode species successfully reproduced in bee cadavers, with a median emergence of 22,350 infective juveniles (IJ) per host.

Methods

Freshly mated *Bombus terrestris* gynes (age ≈14 days) were exposed to three field-realistic concentrations of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*: 0.25 (low), 0.5 (medium), and 1 million infective juveniles (IJ) m^{-2} (high). Exposure took place in simulated soil hibernation chambers constructed from 50 mL Falcon tubes ($N = 20$ per treatment group; **Fig. 1A,B and 2A**). Gynes were maintained at 14°C for 28 days to mimic initial hibernation conditions. Mortality was recorded daily. Nematode replication was assessed using White traps (White 1927; **Fig. 2B**) for both deceased and surviving individuals (Fig. 3). Survival analyses were conducted in R (v4.2.2) using the survival and survminer packages. Survival curves were estimated with the *survfit()* function, and differences among treatment groups were tested using the log-rank test implemented in *survdiff()* and *pairwise_survdiff()*.

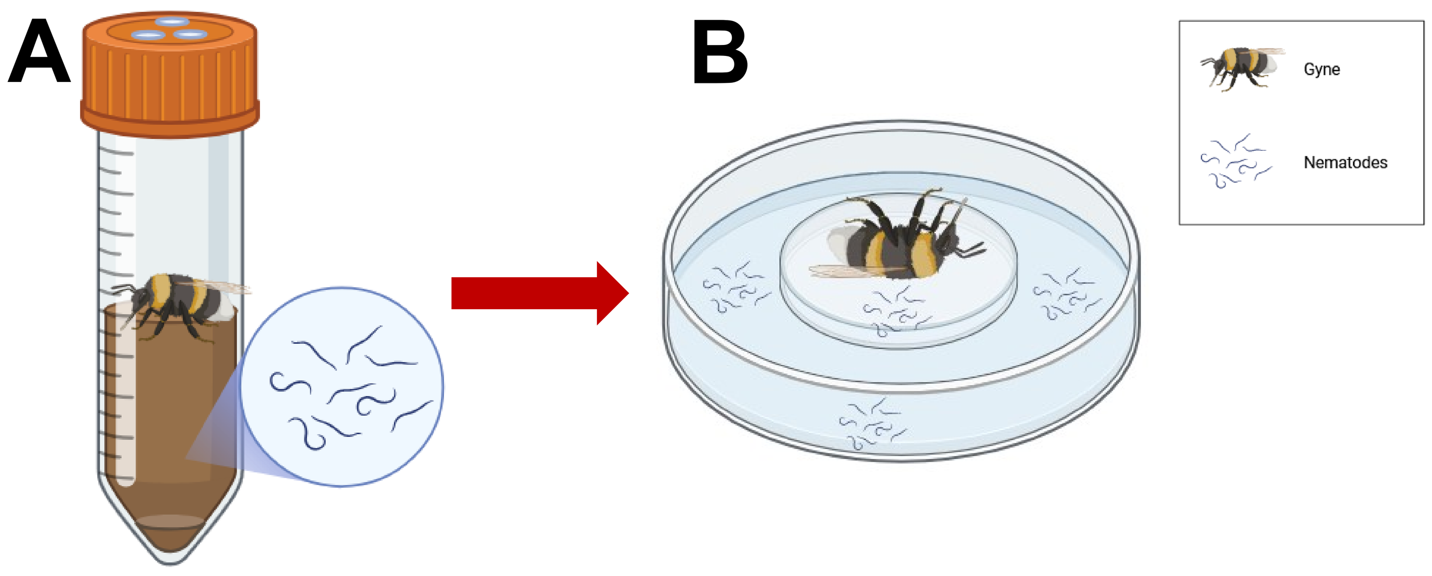

Fig. 2: Schematic representation of the hibernation set-up. Hibernation simulation at 14°C under controlled soil moisture conditions **(A)**. Dead bee on white trap for nematode collection **(B)**.

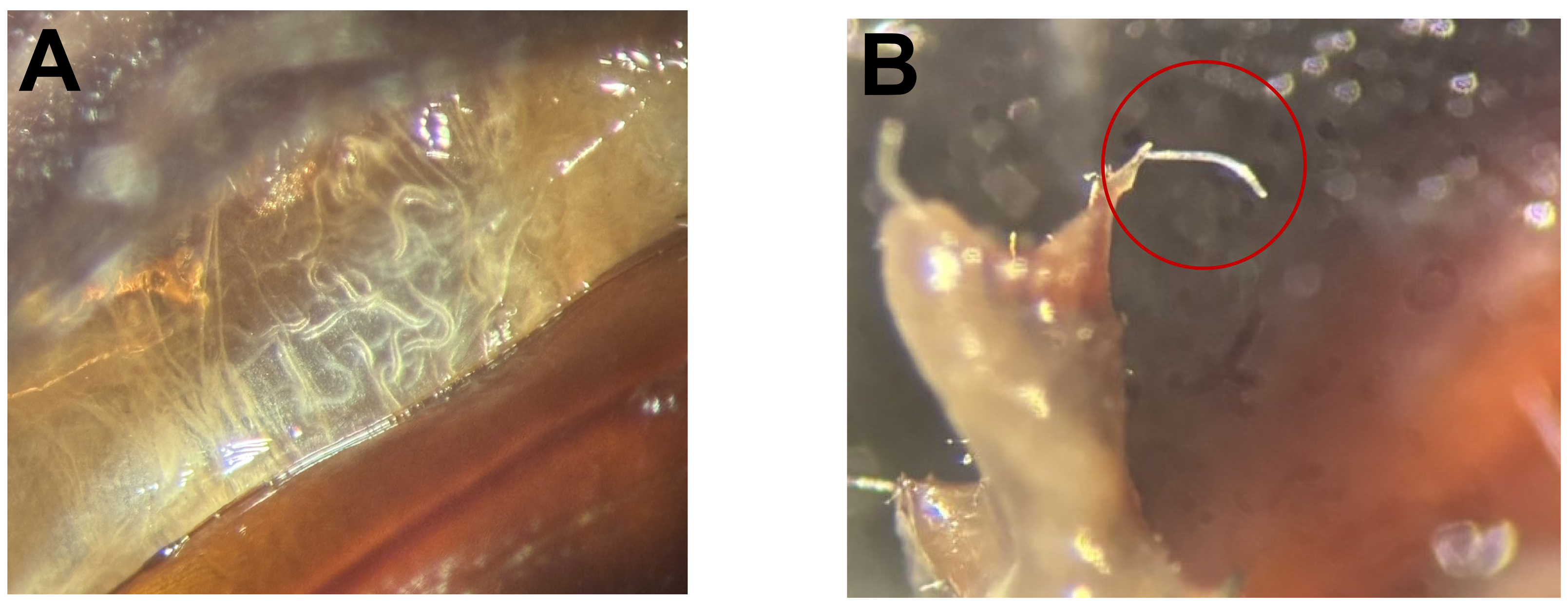

Fig. 3: (A) Entomopathogenic nematodes inside the abdomen of a *Bombus terrestris* cadaver. **(B)** Infective juvenile nematode attached to the leg of a *B. terrestris* queen after emerging from the insect cadaver, searching for a new host.

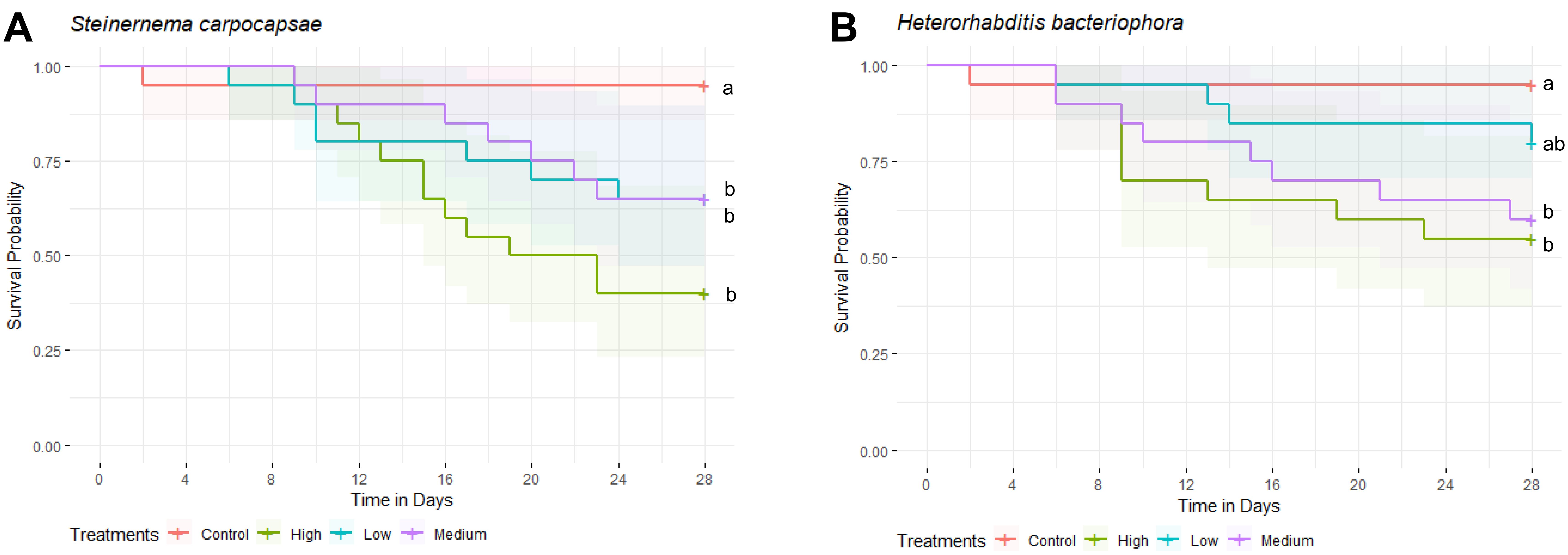

Fig. 4 Kaplan–Meier survival curves of bees: Survival probability of *Bombus terrestris* gynes following exposure to soil treated with **(A)** *Steinernema carpocapsae* and **(B)** *Heterorhabditis bacteriophora*. Pairwise comparisons of survival curves with log-rank tests show significant differences (*P < 0.05*) in survival of treatment groups and are indicated by lower case letters (a and b).

Discussion and conclusion

Our results demonstrate that hibernating bumble bees gynes are vulnerable to EPN exposure, as both *S. carpocapsae* and *H. bacteriophora* successfully infected and reproduced in *B. terrestris* gynes. Given that these EPNs are commonly applied in spring and autumn, exposure during queen hibernation phases represents a realistic yet largely overlooked risk. These findings highlight the need for systematic evaluation of the non-target effects of EPNs on pollinators and other soil-dwelling insects. Our experimental approach provides a novel practical framework that would enable data collection to support future regulatory risk assessments. As many bumble bee populations are declining worldwide, nematode-based biocontrol—if applied without adequate consideration—may represent an additional, unintended stressor. Safeguarding bees will therefore require the careful integration of pest management strategies with biodiversity conservation.

References:

- Bale, J. S., Van Lenteren, J. C., & Bigler, F. (2008). Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1492), 761-776.
- Cappa, F., Baracchi, D., & Cervo, R. (2022). Biopesticides and insect pollinators: Detrimental effects, outdated guidelines, and future directions. *Science of the Total Environment*, 837, 155714.
- Dutka, A., McNulty, A., & Williamson, S. M. (2015). A new threat to bees? Entomopathogenic nematodes used in biological pest control cause rapid mortality in *Bombus terrestris*. *PeerJ*, 3, e1413.
- Hazir, C., Giblin-Davis, R., Keskin, N., Ye, W., Hazir, S., Scheuhl, E., & Thomas, W. K. (2010). Diversity and distribution of nematodes associated with wild bees in Turkey. *Nematology*, 12(1), 65-80.
- White, G. F. (1927). A method for obtaining infective nematode larvae from cultures. *Science*, 66(1709), 302-303.

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