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## Introduction

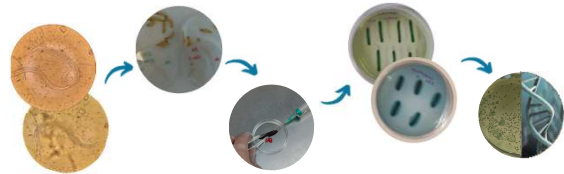
- *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is the most important pest threatening tomato production in Mediterranean countries, causing 80-100% production loss
- Biological control of *T. absoluta* using the predator *Nesidiocoris tenuis* Reuter and entomopathogenic nematodes (EPN) is a sustainable approach
- The use of EPNs bacterial symbionts and their products could be an alternative solution to conventional agrochemicals and the base of forming new biopesticides and other new naturally derived green molecules

The aim of this study is to assess the effectiveness of nematodes, their associated bacteria, and their cell free filtrates against larvae of *T. absoluta* and investigate the potency of these factors on its non-target predator *N. tenuis*

## Materials and methods

1. Experimental organisms:
  - *Steinernema carpocapsae* Weiser and *Heterorhabditis bacteriophora* Poinar
  - *Tuta absoluta*
  - *Nesidiocoris tenuis*

2. Identification of *Xenorhabdus* and *Photorhabdus* bacteria:



3. Preparation of cell-free liquid filtrate and bacterial cell suspension:

4. Pathogenicity bioassays



For *T. absoluta* → 1<sup>st</sup>+2<sup>nd</sup> instar larvae and 3<sup>rd</sup>+4<sup>th</sup> instar larvae



For *N. tenuis* → at the adult stage (2-4 days old)

Nematodes, bacterial cell suspensions and crude cell-free liquid filtrates were sprayed to assess the mortality each of the experimental insects

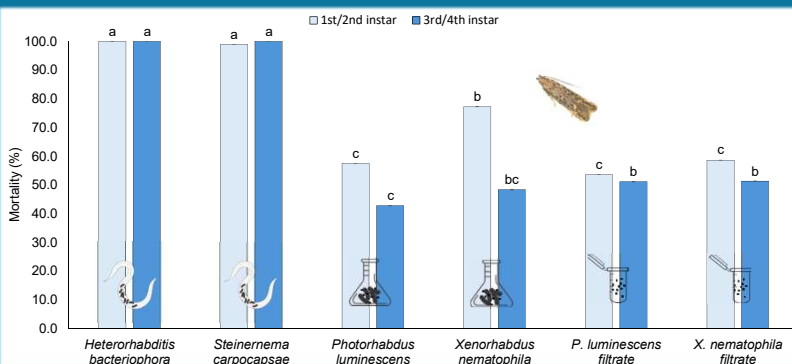
## Results

Molecular identification of *X. nematophila* and *P. luminescens*

Isolate information					Accession numbers (GenBank)	
Bacterial species	Isolate number	Isolation source	Symbiotic host	Date	16S	RecA
<i>Xenorhabdus nematophila</i>	XN1	<i>Galleria mellonella</i> larvae	<i>Steinernema carpocapsae</i>	June 2023	OR782825	OR791744
<i>Photorhabdus luminescens</i>	PL1	<i>Galleria mellonella</i> larvae	<i>Heterorhabditis bacteriophora</i>	June 2023	OR782824	OR791746

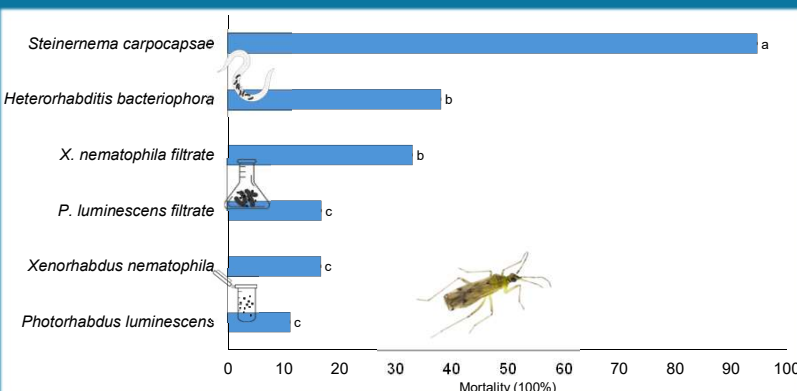
*T. absoluta* bioassays

- The 1<sup>st</sup> and 2<sup>nd</sup> instar were more susceptible than the 3<sup>rd</sup> and 4<sup>th</sup> instars
- Mortality rates increased with the extension of exposure time
- Bacterial cell suspensions of *X. nematophila* demonstrated a significant mortality rate



*N. tenuis* bioassays

- Nematodes *S. carpocapsae* caused high mortality rate
- Bacterial cell suspensions of *X. nematophila* and *P. luminescens* and crude cell-free liquid filtrates of *P. luminescens* caused low mortality



## Conclusions

- The symbiotic bacteria of EPNs and their cell-free filtrates affected *T. absoluta* larvae, surpassing their effect on *N. tenuis* adults
- Bacterial cells of *X. nematophila* were the most effective against young *T. absoluta* larvae, whereas they were marginally harmful to *N. tenuis* adults
- The products of nematodes (bacteria and their secreted metabolites) and *N. tenuis* could be used in IPM programs against *T. absoluta*, as their compatibility is feasible

## Acknowledgements

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