Virulence of entomopathogenic nematodes isolated from Australian soils

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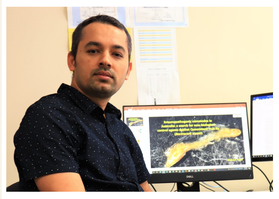

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BACKGROUND INFORMATION

Queensland fruit fly (*Bactrocera tryoni*) is Australia's most significant horticultural pest. Eggs are laid in fruit of plants from more than 40 families including many important crop species, where the larvae develop causing substantial damage. Eventually, the larvae leave the fruit to pupate in the soil, a key stage for control using biological control agents. Several chemical insecticides for fruit fly control have recently been abolished, and there are few alternative control strategies. Entomopathogenic nematodes (EPNs) with their bacterial symbionts (*Photorhabdus* and *Xenorhabdus* in *Heterorhabdus* and *Steinernema*, respectively) are lethal pathogens of important insect pests and are promising biocontrol agents of *B. tryoni* (Langford et al., 2014; Aryal et al., 2022a; Aryal et al., 2022b).

SCOPE AND OBJECTIVES

Australia is a continent with diverse climates and soils which could host yet unknown EPNs and parasitic nematodes which might be more virulent and effective against *B. tryoni* than commercially available EPNs due to their better adaptation to local climate and population regulators. Akhurst & Bedding (1986) have characterised some of the EPN diversity in Australia; however very little research has been undertaken on Australian EPNs and their genetic and symbiont diversity since.

Thus, this study aims to:

- characterize the natural diversity of EPNs and their symbiotic bacteria that attack different developmental stages of fruit fly in the soil across Australia.
- investigate the virulence of isolated EPN strains against larvae and pupae of *B. tryoni*.

MATERIALS AND METHODS

- A total of 198 soil samples were collected from citrus orchards, grasslands and forests across temperate, subtropical and tropical climates and baited with *Tenebrio molitor*, *Galleria mellonella* and *B. tryoni*.
- DNA sequence analysis was carried out to identify the EPN species.
- The virulence of the 32 newly isolated and 4 commercial EPN strains was assessed on larval and pupal stages of *B. tryoni* using sand plate assay.

Fig.1: Life stages of *B. tryoni* used in this study. a) Late instar larvae and early-stage pupae and b) Pupae (> 3 days).

RESULTS

- EPNs were found in 48% of total samples.
- *Heterorhabditis indica* (14 isolates) was the most frequently encountered species followed by *H. zealandica* (8 isolates), *S. feltiae* (8 isolates), *H. marelatus* (6 isolates) and *H. bacteriophora* (2 isolates).
- The mean LD50 value of *B. tryoni* ranged from 25.32 ± 4.22 to 113.88 ± 16.79 for larvae, and 114.24 ± 43.46 to 360.22 ± 104.36 for pupae.
- The lowest and highest LD50 were found for Hz.NAR1 and Hz.NAR4 for *B. tryoni* larvae, and for Sf.Y13 and Sf.YNG for *B. tryoni* pupae.

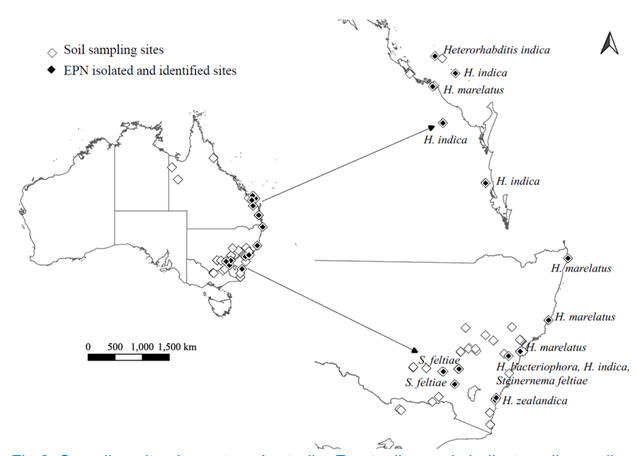

Fig.2. Sampling sites in eastern Australia. Empty diamonds indicate soil sampling sites and filled diamonds sites from where EPNs were successfully extracted and identified (Aryal et al., 2022a).

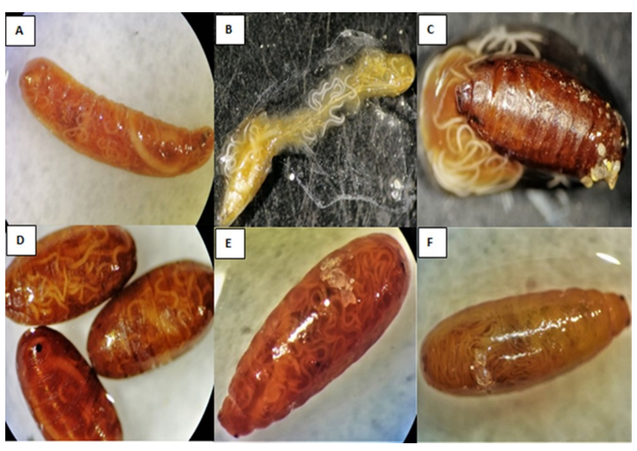

Fig.3. *Bactrocera tryoni* packed with EPNs: A) larva infected with Hi.HRN ; B) larva infected by Sf.ECCS; C) pupae infected with Hi.HRN; D) pupae infected with Hb.HIE; E-F): larvae that pupated after infection with Hz.NAR1 and Sf.Y13, respectively (Aryal et al., 2022b).

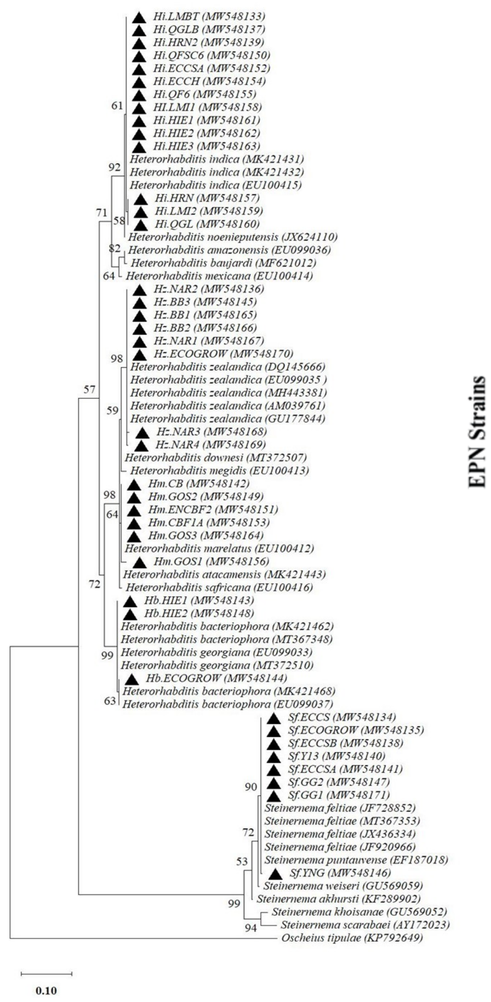

Fig.4. Phylogenetic tree (Maximum Likelihood) of EPN isolates from eastern Australia and other known *Heterorhabditis* spp. and *Steinernema* spp. based on the analysis of the D2-D3 segment of the 28S rRNA gene (512bp – 3,493 bp) (Aryal et al., 2022a).

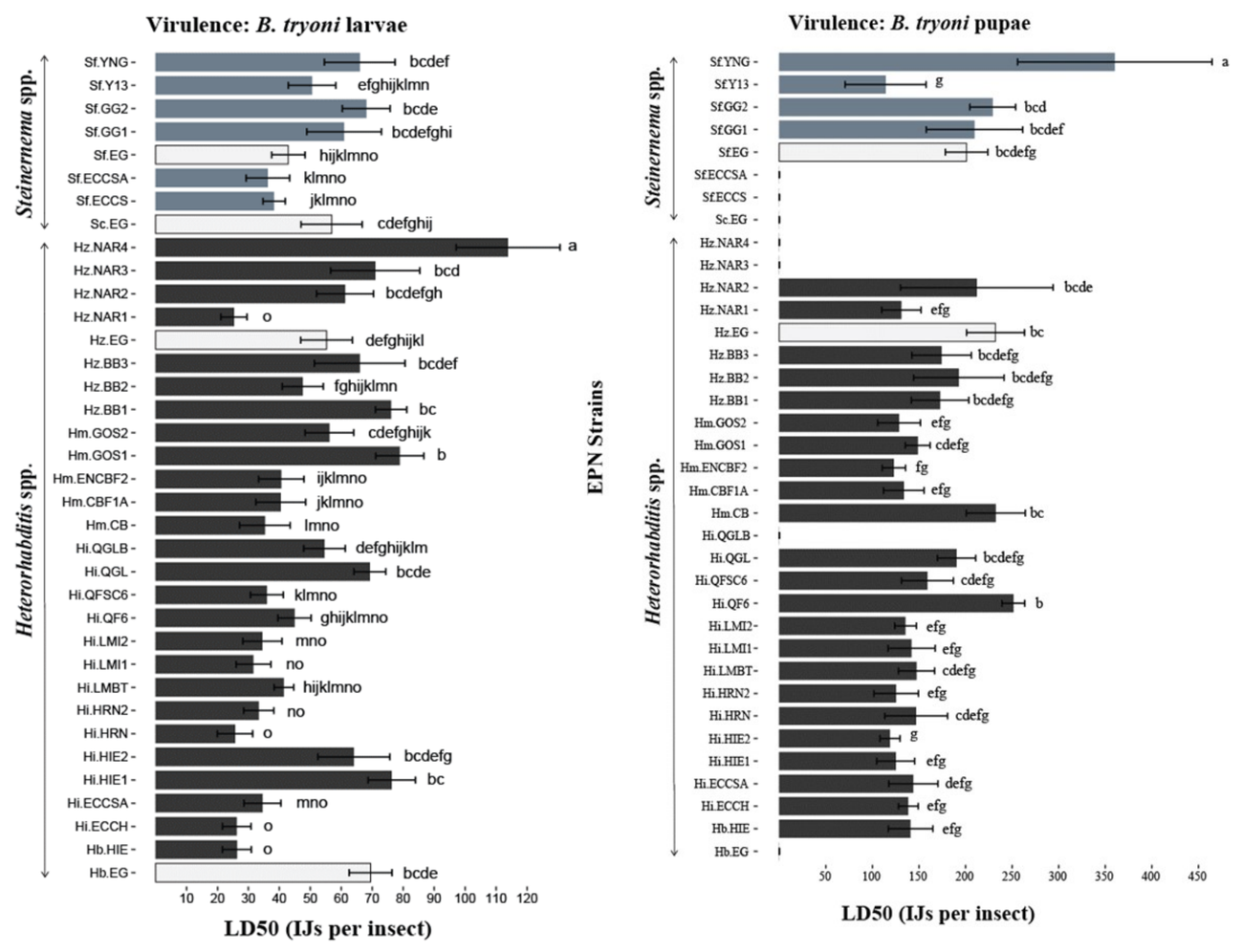

Fig.5-6. LD_{50} of EPN isolates tested against *Bactrocera tryoni* larvae and pupae after one week of exposure to IJs at 25 °C. Error bars indicate SD across five replicates. Lower values resemble higher virulence .The commercial strains are represented by white bars, the newly isolated *Steinernema* strains by grey bars and the newly isolated *Heterorhabditis* strains by black bars (Aryal et al., 2022b).

CONCLUSIONS

- We obtained 36 isolates which, according to DNA sequence analyses, represented five species, *Heterorhabditis bacteriophora*, *Heterorhabditis indica*, *Heterorhabditis marelatus*, *Heterorhabditis zealandica* and *Steinernema feltiae*.
- This study provides the first record of *H. marelatus* from Australia, and *H. indica* and *H. zealandica* from New South Wales.
- All 32 EPN strains were virulent against larvae while 29 strains remarkably caused pupal mortality.
- *Heterorhabditis indica* (Hi.HIE2, Hi.ECCH, Hi.HRN), *H. bacteriophora* (Hb.HIE), *H. marelatus* (Hm. ENCBF2), *H. zealandica* (Hz.NAR1) and *S. feltiae* (Sf.Y13) will be further tested in *B. tryoni* under semi-field and field conditions

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