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Efficacy of entomopathogenic nematodes present in Việt Nam against brown marmorated stink bug (*Halyomorpha halys*) under laboratory conditions

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Summary

The brown marmorated stink bug (*Halyomorpha halys*), a native of Asia, has become an invasive pest in North America and Europe. Given the severity of *H. halys* and the need for better and environmentally-friendly control methods for this pest, we evaluated the virulence four entomopathogenic nematode species (*Heterorhabditis indica*, *Oscheius myriophilus, Steinernema eapokense* and *Steinernema pakistanense*) that occur naturally in soils in southern Việt Nam and compared them with that of a commercially available strain of *S. carpocapsae*. We report for the first time the pathogenicity of *Oscheius myriophilus* towards BMSB. The commercial strain of *S. carpocapsae* was most virulent against BMSB. However, the strain of *S. pakistanense* isolated from soil in southern Việt Nam has potential for the biological control of this pest. To achieve this potential, further work is needed including evaluation in the field and with the formulation of the nematode inoculum.
Keywords: Biological control; nematode virulence; *Heterorhabditis*; *Oscheius; Steinernema*; entomopathogenic nematodes

Introduction

Entomopathogenic nematodes (EPNs) are obligate parasites of insects and are used for biological control of important insect pests (Shapiro-Ilan *et al*., 2022). The two major genera are *Heterorhabditis* (Rhabditida: Heterorhabditidae) and *Steinernema* (Rhabditida: Steinernemadidae) with 121 species described to date (Bhat *et al*., 2020). The species have symbiotic associations with pathogenic bacteria with species of *Heterorhabditis* associated with species of *Photorhabdus* (Enterobacterales: Morganellaceae) and species of *Steinernema* with species of *Xenorhabdus* (Morganellaceae) (Poinar, 1990). Virulence depends on the species and strain of the nematodes and the symbiotic bacteria they contain (Sharmila *et al*., 2018).

Recently, several EPNs belonging to genus *Oscheius* (Rhabditida: Rhabditidae) have been recorded in China, Iran, Iraq, and Mexico (del Ochio Castro-Ortega *et al*., 2019; Al-Zaidaw *et al*., 2019; Ghavamabad *et al*., 2021). They include *Oscheius myriophilus* (Poinar), which is associated with two symbiotic bacteria, *Serratia nematodiphila* Zhang *et al*. (Zhang *et al*., 2009) and *S. marcescens* Bizio (Enterobacterales: Yerssiniaceae) (Zhang *et al*., 2009; Ghavamabad *et al*., 2021). These nematodes can infect insects belonging to the orders, Lepidoptera, Coleoptera, Orthoptera, Diptera, Thysanoptera and Siphonaptera (Puza & Mracek, 2010).

The brown marmorated stink bug (*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is thought to be native to Asia. Since 2007, it has become a serious invasive pest in Europe where it was first recorded in Switzerland in 2007 (Wermelinge *et al*., 2008; Heckmann, 2012; Callot & Brua, 2013; CABI/EPP, 2013; Haye *et al*., 2014; Vétek *et al*., 2014). It is also found across North America in five states in Canada and 46 in the United States, where it was

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Fig. 1: Vinh Hai village, Ninh Hai district, Ninh Thuan province.

Fig. 2. Hon Ba Nature Reserve, Khanh Son district,Khánh Hòa province.

present in Pennsylvania as early as 1999 (Rice *et al*., 2014). It feeds on more than 100 plant species (Schumm *et al*., 2020) and causes significant economic losses to agriculture and forestry (Rice *et al*., 2014; Valentin *et al*., 2017; Kistner, 2017) as it feeds on fruit, nut, vegetable, ornamental, and field crops (Hodgson & Leskey, 2014). Moreover, the aggregation of *H. halys* in buildings and houses (Acheampong, 2023), a characteristic of *H. halys* in winter, can cause a nuisance in indoor living spaces (Inkley, 2012). Several studies (Guide *et al*., 2019; Burjanadze *et al*., 2020; Nanzer *et al*., 2021; Cecconello *et al*., 2022) have demonstrated the virulence of EPNs against stink bugs. In Việt Nam, most of EPN species have been described from natural forests; 14 EPN species belonging to the genera *Steinernema* and *Heterorhabditis* have been found (Trinh *et al*., 2021). In order to identify native species and their exploitation for insect pest management, a survey EPN were carried out on in coastal region of Central Việt Nam, located in the tropical savanna climate zone in 2022. Given the severity of *H. halys* and the need for better and environmentally-friendly control methods for this insect, we evaluated the virulence four EPN species, including one belonging to the genus *Oscheius*, that isolated naturally from soil in southern Việt Nam and compared them with that of a commercially available strain of *S. carpocapsae* All strainThe study was undertaken under laboratory conditions at the Plant Protection Research Institute (PPRI), Hà Nội.

Material and Methods

<u>*Brown marmorated stink bug sources and rearing techniques*</u>

Halyomorpha halys adults were collected in citrus orchards in Kim Bôi (20.6901°N, 105.5240°E 70 m ASL), in Hòa Bình province,

Table 1. Locations where the EPNs were collected in southern Việt Nam. These nematodes were collected in 2022.

EPN Species	Location	GPS coordinates
Heterorhabditis indica Poinar, Karunakar & David BNN2-02	Vinh Hai village, Ninh Hai district Ninh Thuan province	11.7748°N, 109.2048°E, 9 m ASL
*Oscheius myriophilus** (Poinar) BNN1-07	Vinh Hai village, Ninh Hai district Ninh Thuan province (Fig. 1)	11.7858°N, 109.1899 °E, 9 m ASL
Steinernema eapokense Phan, Spiridonov, Subbotin & Moens HB33	Hòn Ba Nature Reserve Khanh Son district Khanh Hoa province (Fig. 2)	12.2684°N, 109.1406°E, 580 m ASL
*Steinernema pakistanense** Shahina, Anis, Reid, Rowe & Maqbool BNN2-12	Vinh Hai village, Ninh Hai district Ninh Thuan province	11.7748 °N, 109.2048 °E, 9 m ASL

* First recorded in Viet Nam

Table 2. Median lethal concentrations (LC_{50}) of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae*, *S. eapokense* and *S. pakistanense* against third instar BMSB nymphs. df = degrees of freedom.

EPN species	Slope	LC_{50}	Lower 95% limit	Upper 95% limit	χ^2-test	df	Sig.
Heterorhabditis indica	1.015	248	113	380	9.638	16	0.885
Oscheius myriophilus	0.845	247	79	412	4.349	16	0.998
Steinernema carpocapsae	2.062	167	112	217	11.622	16	0.77
Steinernema eapokense	1.012	193	72	303	3.304	16	1
Steinernema pakistanense	1.484	118	53	178	4.878	16	0.996

northern Việt Nam. A colony was maintained in the laboratory at 25°C, 60 % RH, and 16:8 (L:D) photoperiod using French bean (*Phaseolus vulgaris* L.) pods as food.

Nematode strains and production

With the exception of *S. carpocapsae*, nematodes were isolated from soil samples collected at the locations in southern Việt Nam listed in Table 1. The nematodes were baited from soil samples using the methods described in Kaya HK, Stock P (1997). *Steinernema carpocapsae* All strain was sourced from South Korea.

Cultures of EPNs were maintained at the Entomology and Nematology Department at PPRI using late instar larvae of the greater wax moth (*Galleria mellonella* (L.) (Lepidoptera: Pyralidae)) maintained at 25 °C as described in Shapiro-Ilan *et al*. (2016). After being harvested from the moth larvae, the nematodes were stored at 10 – 18 °C depending on the species of EPN. EPNs were used in the bioassays within 2 weeks of being harvested.

Bioassay conditions

The bioassays to determine virulence were conducted in the laboratory at 25°C, 70 % RH for each of the EPN species as listed in Table 1.

Halyomorpha halys stages

Third instar nymphs and adults were used to evaluate the virulence of *H. indica*, *O. myriophila*, *S. carpocapsae*, and *S. pakistanense*. Third instar nymphs only were used to evaluate the virulence of *S. eapokense*.

EPN concentrations

For both the third instar nymphs and adult stages of *H. halys*, six EPN concentrations were used: 1000; 800; 600; 400; 200; and 100 infective juveniles (IJs) /insect.

Bioassay arena

For adults, 90 mm diameter plastic Petri dishes were used. Each

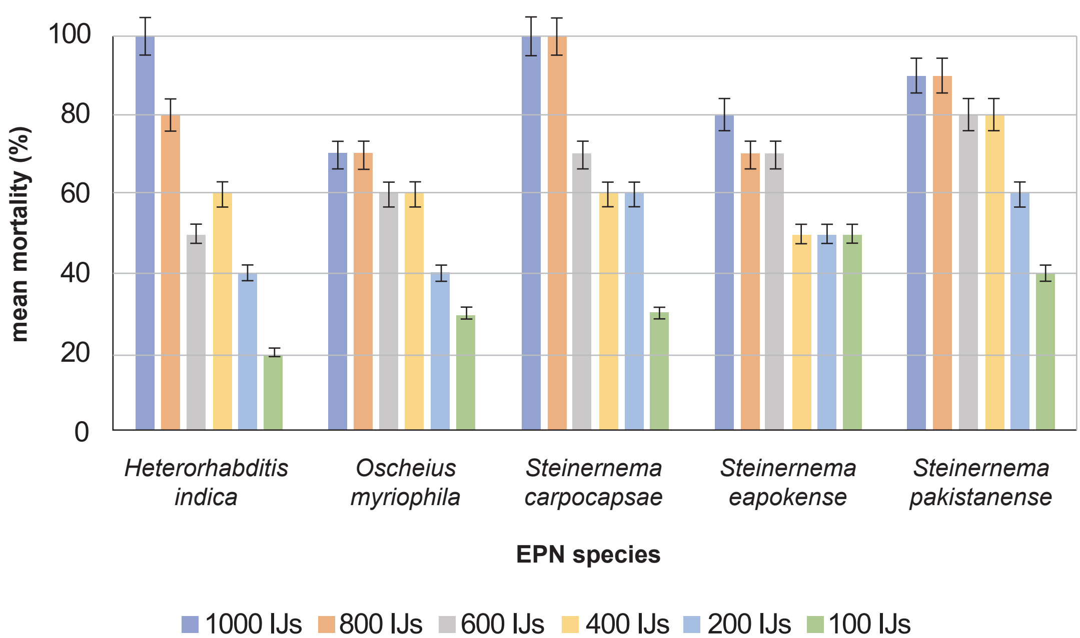

Fig. 3. Virulence of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae*, *S. eapokense*, and *S. pakistanense*, and against third instar *H. halys* nymphs under laboratory conditions. Bars represent the standard errors of the means. IJs = infective juveniles.

Table 3. Median lethal times (LT_{50}) for *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae*, *S. eapokense* and *S. pakistanense* against third instar BMSB nymphs.

EPN	Slope	LT_{50}	Lower 95% limit	Upper 95% limit	χ²-test	df	Sig.
Heterorhabditis indica	1.185	6.195	5.188	8.594	11.379	16	0.785
Oscheius myriophilus	1.367	5.45	4.711	6.727	1.733	16	1
Steinernema carpocapsae	0.62	3.787	2.204	5.38	1.678	16	1
Steinernema eapokense	0.69	6.645	4.966	18.019	1.228	16	1
Steinernema pakistanense	0.554	2.955	0.702	4.136	1.944	16	1

Petri dish was lined with a filter paper and moistened with 1 mL of water containing EPN inoculum. Fine nylon mesh was used to cover 40 mm holes cut in the lids of each dish. A control Petri dish received 1 mL of water only. A 20 mm piece of French bean pod was placed in each Petri dish to provide the food for the insects.

For third instar nymphs, 60 mm diameter plastic Petri dishes were used. Each Petri dish was lined with a filter paper and moistened with a volume of 0.6 mL of water containing EPN inoculum. Fine nylon mesh was used to cover 30 mm holes cut in the lids of each dish. Control Petri dishes received 0.6 mL of water only. A 20 mm piece of French bean pod was placed in each Petri dish to provide the food for the insects. The piece of bean pot was replaced every two days.

Fig. 4. BMSB adult cadaver after infected by EPNs (scale bar= 5mm).

At each concentration of IJs, 10 insects were used. Each insect was placed in a separate Petri dish. The number of alive and dead adult and third instar *H. halys* were recorded 2, 3, 4, 5, 6, 7 days after application. Each cadaver was dissected using forceps and needles in a plastic 90 mm Petri dish. The contents of each dish were then transferred to a Doncaster counting dish (Doncaster, 1962) and counted under a Labomed CZM4 Stereo Binocular Microscope fitted with a light/dark field base. All nematodes from each cadaver were counted.

Statistical analysis

Data were corrected for control mortality (Abbott, 1925) and log(x + 1) transformed when necessary to meet assumptions of normality and homogeneity of variances. A two one-way full factorial analysis of variance (ANOVA), and Duncan multiple comparison method e.g., nematode species × exposure dose, with time intervals (as blocking) was used to determine the differences of nymph mortality in the plate assay and reproduction tests. In addition, in the plate assays, LC_{50} and LT_{50} values were determined using the Probit procedure for each EPN species, and the LC_{50} and LT_{50} differences between nematode species were considered significant when the 95 % fiducial limits did not overlap. Data are presented as means ± standard errors of the mean. All statistical analyses were conducted using SPSS software. (SPSS Statistics, SPSS Inc., Chicago, IL, USA), using $p < 0.05$ to assess the statistical differences.

Ethical Approval and/or Informed Consent

Ethics approval and consent to participate are not applicable. The entomopathogenic nematodes were isolated from soil samples following the methods described in Kaya HK, Stock P (1997).

Results

Heterorhabditis . indica, *O. myriophila*, *S. carpocapsae*, *S. eapokense*, and *S. pakistanense* caused significant mortality of third instar *H. halys* nymphs. A *H. halys* was dead after infected with EPNs and the EPNs found when the cadaver was dissected are shown in Figures 4 & 5. Insect mortality increased as nematode concentrations in treatments increased (Table. 6). Concentrations of 800 and 1000 IJs/nymph of *S. carpocapsae* caused 100 % mor-

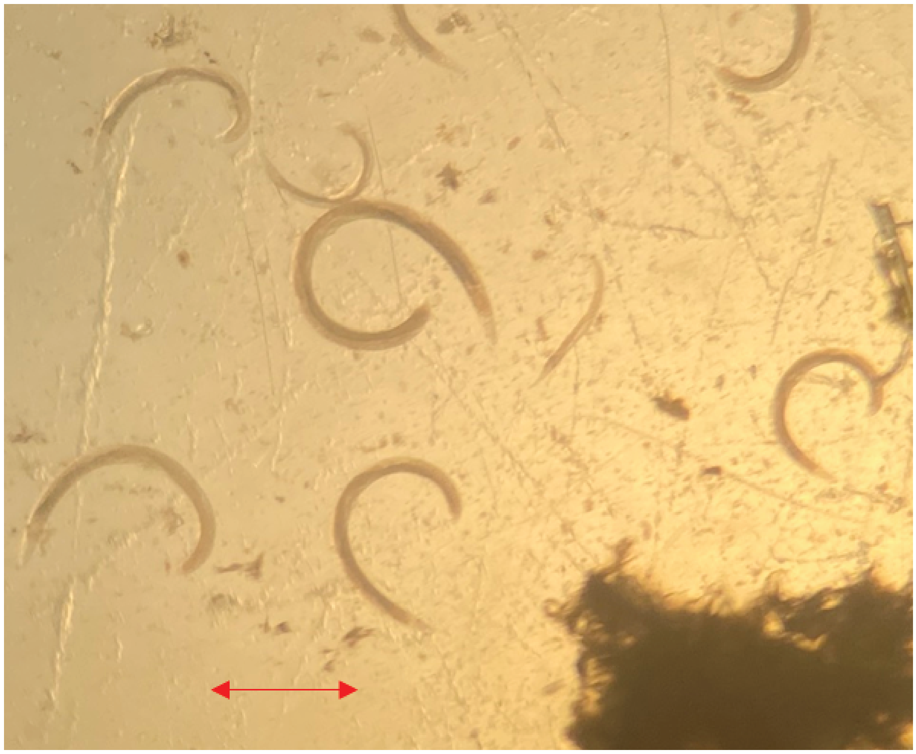

Fig. 5. EPNs dissected from *H. halys* cadaver infected by EPNs (scale bar=1mm).

tality. The virulence of the other nematodes was lower. *H. indica* caused 100 % mortality but only at a dose of 1000 IJs/nymph. At this dose, *S. pakistanense* killed ~90 % of nymphs, *S. eapokense* ~80 % and *O. myriophila*~70 %.

Concentrations of *H. indica*, *O. myriophila*, *S. carpocapsae* and *S. pakistanense* required to cause 50 % mortality of third instar *H. halys* nymphs are presented in Table 2. The LC_{50} was lowest for *S. pakistanense*. The LC_{50} values for *H. indica* and *O. myriophilus* were higher than the values recorded for the two *Steinernema* species. The results indicate that the two *Steinernema* species have higher virulence against third instar *H. halys* nymphs than *H. indica* and *O. myriophilus*.

The lethal times (LT_{50}) for treatment concentrations of 200 IJs to cause 50 % mortality of third instar *H. halys* nymphs was lowest for *S. pakistanense* (2.955 days), followed by *S. carpocapsae* (3.787 days) (Table 3). The LT_{50} value for *H. indica* was highest (6.195 days).

Heterorhabditis indica, *O. myriophila*, *S. carpocapsae* and *S. pakistanense* all caused 100 % mortality of *H. halys* adults at 800 and 1000 IJs/adult (Fig. 3, and 100 % was also recorded for 400 and 600 IJs of *S. carpocapsae*. With the exception of *H. indica*, all nematode concentrations caused ≥ 60 % mortality. The highest mortality was caused by *S. carpocapsae* followed by *S. pakistanense*. *O. myriophila* was less effective than the other species (Fig. 6).

The concentrations of *H. indica*, *O. myriophila*, *S. carpocapsae* and *S. pakistanense* required to cause 50 % mortality of BMBS adults are presented in Table 4. For each of the four species, the LC_{50} values were lower than those for third instar nymphs. The lowest LC_{50} value was recorded for *S. pakistanense*–followed by *S. carpocapsae*. The LC_{50} values recorded for *H. indica* and *O. myriophilus* were higher than for the two *Steinernema* species. For *H. halys* adults, the LT_{50} value for the *O. myriophilus* was longest, followed by *S. pakistanense*. The LT_{50} was lowest for *S. carpocapsae* (1.618 days) (Table 5).

Table 4. Median lethal concentrations (LC_{50}) of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae* and *S. pakistanense* against BMSB adults.

EPN species	Slope	LC_{50}	Lower 95% limit	Upper 95% limit	χ²-test	df	Sig.
Heterorhabditis indica	1.833	46	9	84	12.401	16	0.716
Oscheius myriophilus	1.447	122	53	183	5.865	16	0.989
Steinernema carpocapsae	1.383	31	0	63	1.791	16	1
Steinernema pakistanense	1.656	49	9	91	9.826	16	0.876

Table 5. Median lethal time (LT_{50}) of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae* and *S. pakistanense* against adult BMSB.

EPN species	Slope	LT_{50}	Lower 95% limit	Upper 95% limit	χ^2-test	df	Sig.
Heterorhabditis indica	2.099	2.975	2.577	3.322	16.2	16	0.639
Oscheius myriophilus	0.795	5.606	4.418	9.349	0.904	16	1
Steinernema carpocapsae	2.106	1.618	0.969	1.994	5.73	16	0.991
Steinernema pakistanense	1.252	3.362	2.701	3.93	3.191	16	1

The mean numbers of nematodes in the cadavers are presented in Tables 6 and 7. The higher the concentrations of inoculum the more nematodes were found in the cadavers of both the nymphs and adults. For both stadia, numbers were significantly highest for *S. carpocapsae* and lowest for *H. indica* for all concentrations of inoculum. Numbers for the other two species were intermediate with little difference occurring between the two species.

Discussion

All five nematode species used in this study, including those isolated from soils samples collected in various locations in southern Việt Nam, showed virulence against third instar nymphs and adults of *H. halys* under laboratory conditions. The highest virulence was shown by the commercial strain of *S. carpocapsae*; this species had the lowest LC_{50s}, LT_{50s} and highest numbers of nematodes in cadavers for both nymphs and adults. However, our results suggest that the strain of *S. pakistanense* isolated from Việt Nam has the potential for highly effective biological control of *H. halys*, as the LC_{50s}, LT_{50s} and numbers of nematodes in cadavers were only slightly different from those of *S. carpocapsae*. The variation of the efficacy of the EPN species in this and other studies demonstrates that the selection of appropriate EPN species and strains is important for achieving effective control of *H. halys*.

The potential of entomopathogenic nematodes for the control of *H. halys* was evaluated in experimental trials in Georgia by Burjanadze *et al*. (2020). The study indicated that Italian strains of *Heterorhabditis bacteriophora* Poinar and *S. apuliae* Triggiani, Mrácek & Reid had higher efficacy against *H. halys* than Georgian strains of *H. bacteriophora* and *S. borjomiense* Gorgadze *et al*. (2017), reaching 95.5 % and 60 % mortality at concentration of 1000 IJs/adults. In our bioassays, *H. indica*, *O. myriophilus*, *S. carpocapsae* and *S. pakistanense* caused 100 % mortality against *H. halys* adults at 1000 IJs/adult. In addition, the LC_{50} values of the four species in our study were higher than for the EPNs evaluated by Burjanadze *et al*. (2020). The differences between the current study and the one by Burjanadze *et al*., (2020) are likely due to the species of EPN used and the assay conditions. A second study by Gorgadze *et al*.,(2017) tested five EPNs (three local, Georgian strains of *S. gurgistana* Gorgadze & Lortkipanidze, *S. tbilisiensis* Gorgadze *et al*., and *S. thesami* Gorgadze and two introduced strains of *S. carpocapsae and H. bacteriophora*) against third and fifth instar nymphs of *H. halys* at concentrations of 100 and 200 nematodes per insect. At the higher concentration, the nematodes were applied in combination with the entomopathogenic fungus, *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae). The results of this study indicated that the introduced strains of *S. carpocapsae* and *H. bacteriophora* were more effective against third and fifth instar nymphs than the local Georgian species. The results of the current study are similar in that the local Vietnamese strains were not as virulent as the introduced strain of *S. carpocapsae.* The study by Gorgadze *et al*. (2017) also suggested the combination of EPNs and entomopathogenic fungi gives higher efficacy than when applied separately.

In Slovenia, *O. myriophilus* has been shown to be pathogenic towards the slugs, *Arion vulgaris* Moquin-Tandon and *Deroceras reticulatum* (Müller) and the snail, *Cernuella virgata* (Da Costa) (Mollusca: Gastropoda) (Laznik *et al*., 2023). In Iran, Ghavamabad

Table 6. Mean numbers of nematodes recorded in cadavers of third instar BMSB nymphs following death of nymphs treated with 100 to 1000 infective juveniles (IJs) of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae*, *S. eapokense* and *S. pakistanense*.

EPN species	Nematode concentration (IJs) applied					
	1000	800	600	400	200	100
Heterorhabditis indica	6.27aC	4.07aBC	3.4aBC	2.8aAB	1.73aAB	0.87aA
Oscheius myriophilus	10.47aC	5.4aB	2.33aAB	1.93aA	1.4aA	0.8aA
Steinernema carpocapsae	28.8bE	20.07bDE	12.33bCD	9.2bBC	5.47bB	2.13bA
Steinernema eapokense	13.13aC	8.13aBC	4.8aAB	3.071aAB	3.27abcAB	2.53A
Steinernema pakistanense	38.33bF	31.2bEF	16.53bCD	11.07bBC	6.27bAB	2.47bA

Note: Means followed by lowercase letters within columns and uppercase letters within rows are significantly different.

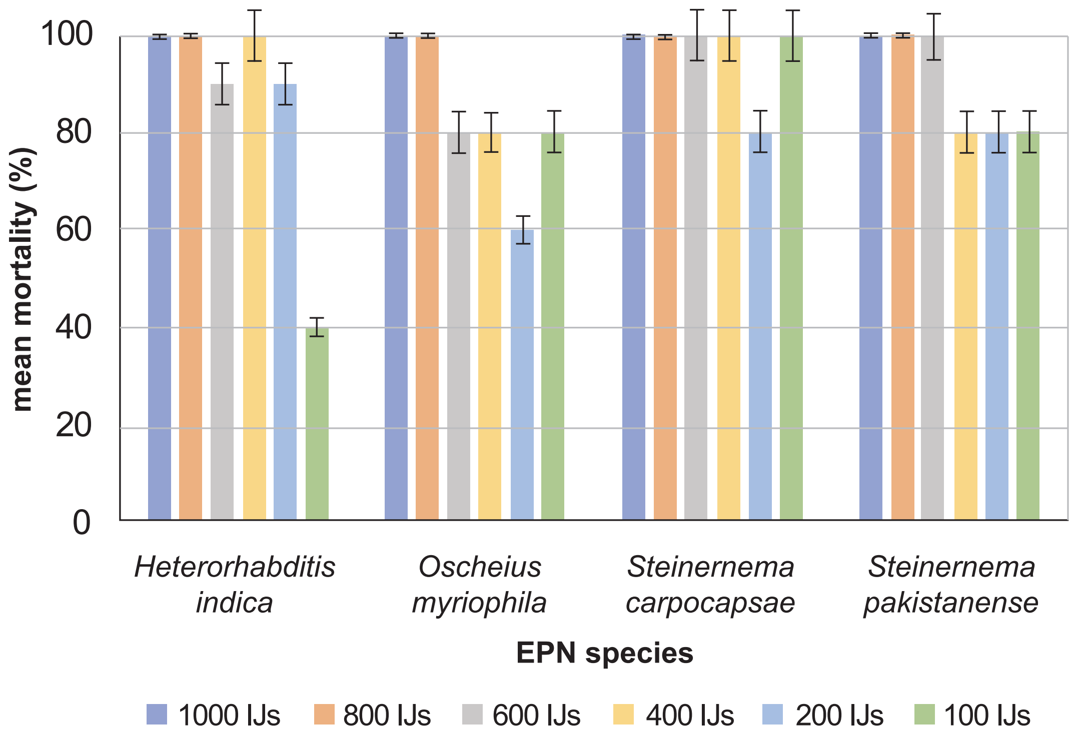

Fig. 6. Virulence of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae*, and *S. pakistanense* against BMSB adults under laboratory conditions. Bars represent the standard errors of the means. df = degrees of freedom.

et al. (2021) have found this nematode affecting the forest tree pests, *Cydalima perspectalis* (Walker) (Lepidoptera: Crambidae) and *Hyphantria cunea* (Drury) (Lepidoptera: Erebidae). Onwong *et al*. (2023) showed that a Thai strain of *O. myriophilus* was pathogenic towards *Galleria mellonella* L. (Lepidoptera: Pyralidae), *Spodoptera litura* F., and *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). However, the strain was not as effective at killing fifth-instar larvae of *G. mellonella* as strains of *S. carpocapsae* and *H. bacteriophora.* We report for the first time the pathogenicity of *O. myriophilus* towards *H. halys*, but like the study by Onwong *et al*. (2023), it was not as effective as *S. carpocapsae* and was the least effective of the four species isolated from soil in southern Việt Nam.

The efficacy of EPNs depends on various abiotic factors such as ultraviolet radiation, adequate soil moisture, relative humidity, and temperatures (Gaugler & Boush, 1978; Shapiro-Ilan *et al*., 2022), and there are ways to optimise the applications conditions and increase the efficacy of EPN for use in the field. Early morning or afternoon application have been recommended to eliminate the impact of the ultraviolet radiation on the virulence of EPNs under field application (Shapiro-Ilan *et al*., 2022). In addition, the successful application of EPNs is more likely to be achieved when applying to the soil or cryptic habitats (Shapiro-Ilan *et al*., 2022). This suggests that the application of EPNs to control overwintering *H. halys* populations could be of high potential and feasible. For field conditions, particularly foliar application, the manner in which the inoculum is formulated has been used improve to enhance the efficacy of EPNs (Schroer & Ehlers., 2005; Fallet *et al*., 2022). Of

Table 7. Mean numbers of nematodes recorded in cadavers of adults following death of adults treated with 100 to 1000 IJs of *Heterorhabditis indica*, *Oscheius myriophila*, *Steinernema carpocapsae* and *S. pakistanense*

EPN species	Nematode concentration (IJs) applied					
	1000	800	600	400	200	100
Heterorhabditis indica	8.8aC	6.2aBC	9.6C	6BC	4.93aB	0.93aA
Oscheius myriophilus	16.27aD	8.07aCD	12.6CD	7.33CD	5.47abB	1.27aA
Steinernema carpocapsae	47.67bC	38.47bC	11.73B	10.33B	8.27bB	3.07bA
Steinernema pakistanense	11.67aD	8.87aCD	14.8CD	7CD	4.6abB	1.67abA

Note: Means followed by lowercase letters within columns and uppercase letters within rows are significantly different.

the nematode species used in this study, the commercial strain of *S. carpocapsae* was most virulent against *H. halys*. However, our results suggested that the strain of *S. pakistanense* isolated from soil in southern Việt Nam has potential for the biological control of *H. halys*. To achieve this potential however, further work is needed, in particular trials in the field and the evaluation different formulations of inoculum.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgements

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