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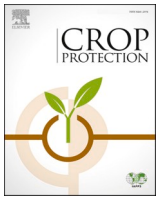

Host-foraging strategies of five local entomopathogenic nematode species in South Africa

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ABSTRACT

Entomopathogenic nematodes (EPNs) are obligate parasites of soil-dwelling insects and are used as biological control agents for many insect pests. These nematodes have a free-living third growth stage called infective juveniles (IJs), which are responsible for foraging and infecting suitable insect hosts. Infective juveniles exhibit three host-foraging strategies: cruising, ambushing, and intermediate foraging strategies. The foraging strategy of EPNs is important for successful infection but is poorly understood. The current study investigated the host-foraging strategies of five local South African EPN species including *Heterorhabditis noenieputensis*, *H. safricana*, *Steinernema fabii*, *S. jeffreyense,* and *S. yirgalemense* by assessing their dispersal behavior. Of the five EPN species, *H. noenieputensis, H. safricana, S. jeffreyense*, and *S. yirgalemense* showed a positive response to the presence of the wax moth larvae, whereas *S. fabii* showed a negative response. The four EPN species that showed a positive response to the presence of the host also caused 100% mortality of wax moth larvae that were buried in sand at a depth of 10 cm, whereas *S. fabii* caused the lowest mortality of 34%. The average distance traveled by all five EPN species decreased on rough textured substrate compared with smooth textured substrate. The observed behavioral patterns suggested that *H. noenieputensis, H. safricana, S. jeffreyense*, and *S. yirgalemense* use a cruiser foraging strategy whereas *S. fabii* uses an ambusher foraging strategy.

1. Introduction

Entomopathogenic nematodes (EPNs) are obligate parasites of many soil-dwelling insects (Jansson et al., 1990; Belien, 2018). These nematodes belong to the families Steinernematidae and Heterorhabditidae, which have a symbiotic relationship with bacteria from the genus *Xenorhabdus* and *Photorhabdus,* respectively (Poinar, 1990). The third growth stage of EPNs is called the infective juveniles (IJs) and is the only free-living stage of the nematodes. It is responsible for surviving the harsh environmental conditions in the soil (Smart, 1995; Glazer, 2002). These include various abiotic and biotic factors that have a negative impact on the efficacy of EPNs (Stuart et al., 2015). The main abiotic factors include ultraviolet radiation, temperature, soil moisture, and soil texture, whereas the biotic factors include an array of antagonist organisms, the interaction of EPNs with insect hosts, and the behavior and ecology of EPNs (Kaya, 2002; Stuart et al., 2015; Skowronek et al., 2020).

The IJs play an important role in searching for, locating and infecting suitable insect pests (Gaugler et al., 1997). Infective juveniles exhibit variation in behavior when searching for insect hosts (Campbell et al., 2003). This difference in host-searching behavior is categorized into two major host-foraging strategies: cruising and ambushing (Campbell et al., 2003; Lewis et al., 2006; Laznik and Trdan, 2016). EPNs that use the cruising foraging strategy actively seek out the insect host by following the change in carbon dioxide gradient, volatiles emitted by the host and volatiles from plant roots induced by insect damage (Choo et al., 1989; Lewis et al., 1993; Adams and Nguyen, 2002). EPNs that use an ambushing foraging strategy tend to use a sit and wait mechanism whereby they stand with their tails and wave part of their body to attack insect hosts passing within their striking range (Lewis et al., 2006). Unlike the cruising EPNs, the ambushing EPNs seldom use volatiles and do not disperse to a greater area (Grewal et al., 1993; Laznik and Trdan, 2016). The third foraging strategy is the intermediate foraging strategy which includes EPNs that display characteristics of both cruiser and ambusher nematodes (Grewal et al., 1994).

A poor understanding of the EPN's behavioral ecology can result in inadequate pest control (Hominick and Reid, 1990; Gaugler et al., 1997). Each category of the host-foraging strategy influences the type of

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insect hosts that the EPNs infect successfully (Campbell et al., 2003). Matching the host-foraging strategy of EPNs with the target insect pest increases the efficacy of EPNs (Lewis et al., 1992). For instance, EPNs that use the cruising foraging strategy are effective against cryptic and sedentary insect hosts within the soil, while EPNs that use the ambushing foraging strategy are effective against mobile insect hosts found on or near the soil surface (Campbell et al., 2003).

The host-foraging strategies of EPN species are often inferred based on related species where the strategy has been studied (Lewis, 2002). However, this is not an accurate approach and it is important to assess the host-foraging strategies of newly isolated EPN species to inform their use in pest management strategies and to broaden the existing knowledge of EPN host-foraging strategies. The aim of the current study was to investigate the host-foraging strategies of five EPN species that were isolated from South Africa (Malan et al., 2008, 2011, 2014, 2016; Abate et al., 2016). A few selected behavioral patterns that are linked to the host-foraging strategies were assessed. These were the responsiveness of the IJs of EPNs to an insect host (wax moth larvae), the ability of EPNs to detect and infect the insect host buried in sand at a depth of 10 cm and the effect of different substrates (smooth versus rough) on the dispersal of the EPNs. The predictions were that cruiser foraging EPNs would be attracted to the host volatiles and thus show greater movement towards the insect host; they would result in high larval mortality in sand bioassays, as they are able to move in the sand to locate the insect host; and that the difference in their movement on smooth and rough substrates will not be significantly different, because of they rarely nictate (standing with the tails and body wave). On the contrary, it was predicted that ambushing foraging EPNs will not be attracted to host volatiles and thus will show less movement toward the host insects; they will cause low larval mortality in sand bioassays; and the difference in their movement on smooth and rough substrates will be significant.

2. Materials and methods

2.1. Source of EPNs

The EPN species used in this study were sourced from the EPN collection of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria. These included *Heterorhabditis noenieputensis* Malan, Knoetze, and Tiedt; *Heterorhabditis safricana* Malan, Nguyen, De Waal, and Tiedt; *Steinernema fabii* Abate, Malan, Tiedt, Wingfield, Slippers, and Hurley; *Steinernema jeffreyense* Malan, Knoetze, and Tiedt; and *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler, and Adams (Table 1). Standard procedures for the rearing and storage of EPNs were followed. In brief, each of the five EPN species was separately inoculated in a 9 cm diameter Petri dish lined with filter paper at a concentration of 1000 IJs in 800 µl (equivalent to 200 IJs/larva), followed by the introduction of 10 wax moth larvae, *Galleria mellonella* Linnaeus, (Kaya and Stock, 1997). After 24 h, the dead wax moth larvae were removed from the Petri dish, rinsed with water, and transferred into a new Petri dish lined with filter paper that was incubated for 48 h. The dead larvae were then transferred into the White trap and the new batch of nematodes that emerged and migrated into the water were harvested and stored in the culture flasks at 12 °C (White, 1927). A new batch of nematodes was used for the different repeats of experiments within three weeks after harvesting.

Table 1
The local EPN species used in the study, their associated bacteria, place of origin, and GenBank accession number.

EPN species	Associated bacteria	Origin (province/ town)	GenBank accession no.	Reference
Heterorhabditis noenieputensis	*Photorhabdus luminescens* subsp. *Noenieputensis*	Northern Cape	JN620538	Malan et al. (2014)
H. safricana	*P. luminescens* subsp. *Laumondii*	Western Cape	EF488006	Malan et al. (2008)
Steinernema fabii	*X. khoisanae*	Mpumalanga	KR527216	Abate et al. (2016)
S. jeffreyense	*X. khoisanae*	Jeffreys Bay, Eastern Cape	KC897093	Malan et al. (2016)
S. yirgalemense	*Xenorhabdus indica*	Nelspruit, Mpumalanga	EU625295	Malan et al. (2011)

2.2. Source of insects

The wax moth larvae used in the study were sourced from the biocontrol and insect rearing facility at FABI, University of Pretoria. Wax moth larvae were reared following the adjusted method described by Birah et al. (2008). In brief, the newly hatched larvae were fed with a defined diet consisting of the following ingredients: powdered milk (200 g), wheat bran (400 g), oat bran (100 g), nutty wheat bran (200 g), yeast (100 g), honey (300 ml), and glycerol (400 ml). The larvae were reared on this diet in jars kept in an incubator (Memmert IPS 750) until they reached the fourth instar. The fourth instar larvae were then collected for the experiments. The incubator was set at a temperature of 27 ± 1 °C, relative humidity of 65 ± 5%, and 16:8 day: night photoperiod.

2.3. Responsiveness of EPNs to the presence of a host

The responsiveness of IJs to the presence of wax moth larvae was assessed using 9 cm diameter Petri dishes (Fig. 1). The methodology used by Grewal et al. (1994), Glazer and Lewis (2000) and Noosidum et al. (2010) was adjusted for this experiment. The lids of the Petri dishes were marked with two perpendicular lines to make four equal quadrants, followed by four concentric circles of 1, 2, 3 and 4 cm in diameter from the center (Grewal et al., 1994). A 2% agar was prepared and 60 ml

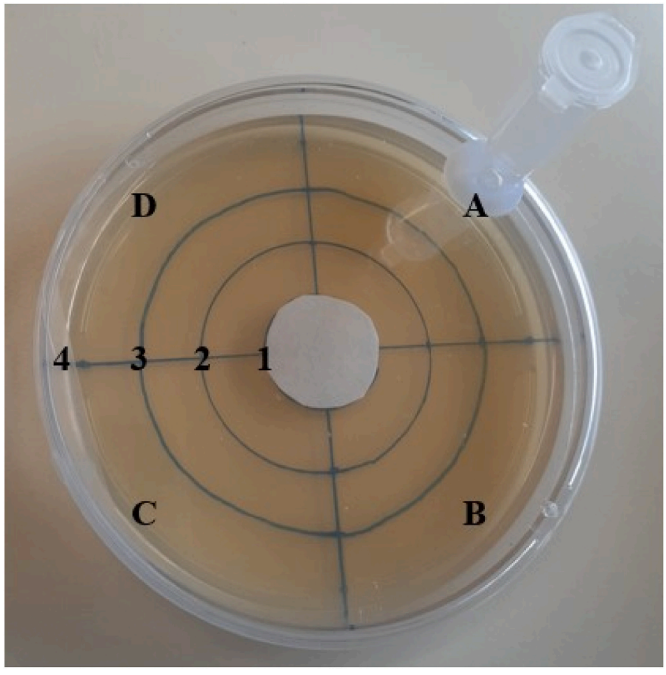

Fig. 1. Petri dish marked with four quadrants and four concentric circles of 1, 2, 3 and 4 diameters in centimeters from the center. A 1.5 ml tube with one wax moth larvae is placed at the edge of quadrant A. Infective juveniles are transferred to the center on top of the filter paper.

of agar was poured into each Petri dish and allowed to cool for 1 h (Glazer and Lewis, 2000). Two holes of 3 mm diameter were made on each lid, one hole at the edge of quadrant A and the other hole at the center (Fig. 1). The hole at the edge was used to accommodate a 1.5 ml Eppendorf tube. Four holes were made at the bottom of the Eppendorf tube using a hot needle. The Eppendorf tube was fixed on the lid with a glue gun such that the bottom of the Eppendorf tube was suspended 3 mm above the agar when the prepared lids were used to close the Petri dishes. The hole at the center of the lid of each Petri dish served as an entry port for IJs. A small filter paper disc of 15 mm diameter was placed at the center of each Petri dish on top of the agar to absorb water from the suspension of IJs. The Petri dishes were closed with the prepared lids and sealed with parafilm.

The Eppendorf tube on each lid received one wax moth larva and this setup was kept at room temperature for 1 h to allow larval volatiles to form. During this time the hole at the center was closed with transparent tape to avoid evaporation and drying out of agar. After 1 h, 100 IJs were pipetted at the center, on top of the filter paper disc, in 50 μl of water. IJs located in each section of quadrants A and C were counted every 10 min for a duration of 30 min after inoculation (Fig. 1). This was done with the aid of a stereomicroscope. The mean distance traveled by IJs toward the larva was calculated by the following formula (Noosidum et al., 2010):

$$\frac{\{(2*A)+(3*B)+(4*C)\}-\{(2*D)+(3*E)+(4*F)\}}{100}$$

The letters A, B, and C represent the number of IJs in the second, third, and fourth arc of quadrant A, respectively (Fig. 1). The letters D, E, and F represent the number of IJs in the second, third, and fourth arc of quadrant C, respectively (Fig. 1). The numbers in the equation, 2, 3 and 4 are distances in centimeters from the center (Glazer and Lewis, 2000). One Petri dish was treated as a replicate and thus replicated five times for each EPN species. The control had a similar setup but without wax moth larva. The experiment was repeated twice on a different date.

2.4. Movement behavior of IJs on a substrate with a smooth and rough texture

A similar setup as explained above was used here, except that the movement behavior of IJs was investigated on a smooth and rough textured substrate in the absence of the host following the methodology by Noosidum et al. (2010). A cooled 2% agar contained in the Petri dish was regarded as a smooth substrate, whereas the rough textured substrate was prepared by evenly sprinkling 0.5 g of sifted river sand on top of the cooled agar (Grewal et al., 1994). A small filter paper disc of 15 mm diameter was placed (before sprinkling the sand particles for the rough substrate) at the center of each Petri dish on top of the agar to absorb water from the suspension of IJs. The lids were secured on the Petri dish with parafilm. One hundred IJs in 50 μl of distilled sterile water were pipetted on the filter paper in each Petri dish through the hole on the lid. The hole was then closed with transparent tape. The number of IJs in each section of all four quadrants was counted every 10 min for a duration of 30 min with the aid of a stereomicroscope. The distance traveled by nematodes in any direction was calculated by the following formula (Noosidum et al., 2010):

$$\frac{\{(2*A)+(3*B)+(4*C)\}}{100}$$

The letters A, B, and C represent the number of IJs in the Petri dish's second, third, and fourth circles, respectively. The numbers in the equation 2, 3 and 4 are distances in centimeters from the center. Each EPN species/substrate combination was replicated five times and the experiment was repeated on a different date using a different batch of IJs.

2.5. Sand columns bioassay

Ten plastic test tubes (2 cm diameter × 15 cm height) were used as an arena. One wax moth larva was placed inside each test tube at the bottom and buried with sifted river sand at a depth of 10 cm (Grewal et al., 1994). Stamping of the sand was minimized to keep compaction low, which allowed airflow and partial movement of the larva. A nematode suspension of 100 IJs in 0.6 ml of distilled sterile water was pipetted on top of the sand and the test tubes were closed with the lid and kept at 25 °C. The number of dead larvae was recorded after 48 h. The dead larvae were washed with a spray bottle to remove surface nematodes and incubated at 25 °C for 24 h in a 9 cm diameter Petri dish lined with moist filter paper. The mortality by EPNs was confirmed by dissecting the dead larvae and checking for the presence of the EPNs with the aid of the stereomicroscope. The ten test tubes were treated as a replicate and this was repeated five times per EPN species. The control setup received water without nematodes. The experiment was repeated on a different date.

2.6. Data analysis

The effect of two test dates on the movement of the nematodes was first checked with a *t*-test in R-studio (RStudio Team, 2020). In the absence of a significant effect of the test dates on the movement of nematodes, data were pooled and analyzed in one-way ANOVA to determine the difference in the responsiveness of EPN species to the presence of the host. Tukey post hoc test was used to separate the mean distance traveled by nematodes toward the host post-detecting the difference in the responsiveness to the presence of the host. The student t-test was used to determine the difference in mean distance traveled by EPNs between the smooth textured and rough textured substrate. One-way ANOVA was used to determine the difference between the mean larval mortality caused by EPN species in the sand column bioassays.

3. Results

3.1. Responsiveness of EPNs to the presence of a host

There was a significant difference in the responsiveness of IJs of EPN species to the presence of wax moth larvae for all three-time intervals, namely 10 min ($F_{4,45} = 27.42$, $p < 0.001$), 20 min ($F_{4,45} = 24.75$, $p < 0.001$) and 30 min ($F_{4,45} = 24.23$, $p < 0.001$). Four EPN species (*H. noenieputensis, H. safricana, S. jeffreyense,* and *S. yirgalemense*) had a positive net average distance (cm) per IJ towards the wax moth larvae at all three-time intervals, whereas *S. fabii* had a negative average distance away from the wax moth larvae (Table 2). *Steinernema jeffreyense* (1.21 ± 0.15 cm, 1.15 ± 0.10 cm, 1.13 ± 0.09 cm) provided the highest average distance at 10 min, 20 min, and 30 min respectively, followed by *H. noenieputensis* (0.56 ± 0.11 cm) at 10 min, and *S. yirgalemense* (0.67 ± 0.16 cm and 0.73 ± 0.16 cm) at 20 and 30 min, respectively. There was no significant difference in the average distance traveled by

Table 2
Net average movement ($\bar{x}$ ± SE) per IJ of five EPN species towards/away from wax moth larvae over time. The different superscript letters indicate the significant difference between results within a column.

EPN species	Net average distance (cm/IJ) traveled towards (+) or away (−) from the host		
	10 min	20 min	30 min
Heterorhabditis noenieputensis	0.56 ± 0.11^{B}	0.55 ± 0.10^{B}	0.63 ± 0.11^{B}
H. safricana	0.04 ± 0.06^{CD}	0.09 ± 0.05^{C}	0.14 ± 0.04^{C}
Steinernema fabii	-0.38 ± 0.10^{D}	-0.25 ± 0.11^{C}	-0.31 ± 0.12^{D}
S. jeffreyense	1.21 ± 0.15^{A}	1.15 ± 0.10^{A}	1.13 ± 0.09^{A}
S. yirgalemense	0.48 ± 0.13^{BC}	0.67 ± 0.16^{B}	0.73 ± 0.16^{AB}

IJs of each EPN species (*S. jeffreyense*: $F_{2,27} = 0.12$, $p = 0.89$; *S. yirgalemense*: $F_{2,27} = 0.72$, $p = 0.50$; *H. noenieputensis*: $F_{2,27} = 0.18$, $p = 0.84$; *H. safricana*: $F_{2,27} = 1.02$, $p = 0.37$; and *S. fabii*: $F_{2,27} = 0.32$, $p = 0.73$) towards or away from the wax moth larvae between the time intervals.

3.2. Movement behavior of nematodes on a substrate with a smooth and rough texture

There was a difference in the net average distance traveled by IJs of each EPN species between a smooth and rough textured substrate. *Steinernema yirgalemense* showed a significant decrease in the net average distance traveled on the rough substrate (0.49 ± 0.04 cm, 0.54 ± 0.05 cm, 0.54 ± 0.06 cm) in comparison to the distance traveled on the smooth substrate (0.93 ± 0.16 cm, 1.12 ± 0.20 cm, 1.23 ± 0.22 cm) at 10 min ($t = -2.55$, $df = 10.32$, $p = 0.03$), 20 min ($t = -2.76$, $df = 10.13$, $p = 0.02$) and 30 min ($t = -3.01$, $df = 10.31$, $p = 0.01$), respectively. A similar trend was observed with *H. safricana* at 10 min ($t = -5.13$, $df = 11.14$, $p < 0.001$), 20 min ($t = -6.19$, $df = 9.59$, $p < 0.001$) and 30 min ($t = -5.51$, $df = 9.59$, $p < 0.001$). *Steinernema jeffreyense*, *S. fabii*, and *H. noenieputensis* also showed a similar trend, but in at least two time intervals (Table 3).

3.3. Sand columns bioassay

There was a significant difference in the mortality of wax moth larvae caused by the five EPN species 48 h post-inoculation (pi) ($F_{4,45} = 182.7$, $p < 0.001$). Four of these EPN species (*H. noenieputensis, H. safricana, S. jeffreyense*, and *S. yirgalemense*) caused the highest mean (±SE) larval mortality and there was no significant difference between the EPN species (Tukey multiple comparisons of means 95% family-wise confidence level, Fig. 2). *Steinernema fabii* caused the lowest mean larval mortality. There was no larval mortality in the control.

4. Discussion

This was the first study in South Africa to report on host-foraging strategies of local EPN species. The results suggested that four of the EPN species studied, namely *H. noenieputensis, H. safricana, S. jeffreyense* and *S. yirgalemense,* use a cruiser foraging strategy, whereas *S. fabii* possibly uses an ambusher foraging strategy. *Heterorhabditis noenieputensis, H. safricana, S. jeffreyense* and *S. yirgalemense* showed a positive average distance per nematode movement towards the wax moth larvae, in comparison to *S. fabii* which showed a negative average distance. The same EPN species that showed a positive average distance were also able to locate and infect wax moth larvae buried at a depth of 10 cm and induce mortality of 100%. IJs of all five EPN species included in the study showed a decreased average distance traveled on the rough textured substrate compared to the average distance traveled on the smooth textured substrate.

A positive average distance towards the insect host by the four EPN species used in the study suggests that IJs of EPNs are attracted to the wax moth larvae. During the experiments, IJs of these EPNs were observed gathering underneath the Eppendorf tubes carrying the wax moth larvae, suggesting an intentional movement toward the host. This is similar to the results reported by Lewis et al. (1993) who demonstrated that *Steinernema glaseri* Steiner, a species that uses cruiser foraging strategy, locates its host by tracking associated cues. Grewal et al. (1994) also reported similar results on a few other EPN species, including *Heterorhabditis bacteriophora* Poinar*; Heterorhabditis megidis* Poinar, Jackson, and Klein; and *Steinernema anomaly* Kozodoi. *Steinernema carpocapsae* Weiser and *S. scapterisci* Nguyen and Smart, EPNs that use an ambusher foraging strategy, seldom respond to the volatiles of an insect host and they travel less distance on a rough textured substrate (Grewal et al., 1994, 1997). Similar results were observed in the current study where *S. fabii* rarely responded to the presence of wax moth larvae and traveled less distance on a rough textured substrate compared to the smooth textured substrate.

The ability of *H. noenieputensis, H. safricana, S. jeffreyense,* and *S. yirgalemense* to cause 100% mortality of wax moth larvae buried at a depth of 10 cm suggested that these EPN species actively seek out their host. This was expected as the four EPN species showed directional responsiveness to the presence of wax moth larvae. Grewal et al. (1994) reported the same behavioral pattern exhibited by *H. bacteriophora, H. megidis*, and *S. anomali*, which located and infected wax moth larvae buried at a depth of 10 cm. Similarly, an undescribed *Steinernema* spp. (isolate K8) showed positive responsiveness to the presence of the wax moth larvae and infected larvae buried at a depth of 9 cm (Noosidum et al., 2010). Additionally, *H. safricana* was often observed burrowing in the agar and reaching the bottom of the Petri dish, which may indicate that the nematode is adapted to deep soil. *Steinernema yirgalemense* was able to infect pupae of *Gonipterus* sp. n. 2 buried at 5 cm depth (Rakubu, 2023). This further supports the cruising foraging behavior of this nematode observed in the current study. *Steinernema fabii* did not show directional movement towards the host and it also caused the lowest mortality (34%) of wax moth larvae in the sand column experiment, which are characteristics associated with an ambusher foraging nematodes (Grewal et al., 1994). Grewal et al. (1994) reported similar results wherein *S. carpocapsae* and *S. scapterisci* showed lack in directional response to the *G. mellonella* and poor establishment rate in hosts in sand column experiments.

IJs of all five EPN species used in the study showed a decreased net average distance traveled on the rough textured substrate compared to the average distance traveled on the smooth textured substrate. This indicates an ambusher foraging strategy, as the rough textured substrate allows the nematodes to nictate (body wave), a behavior associated with ambusher nematodes. Thus, ambusher nematodes are expected to reduce the distance traveled on a rough substrate, due to time spent nictating, whereas cruiser nematodes are expected to continue moving (Gaugler and Campbell, 1993). However, these results are contrary to those from the other behaviors assessed in the study which suggested that the four EPN species (*H. noenieputensis, H. safricana, S. jeffreyense,* and *S. yirgalemense)* use cruiser foraging strategy. It is possible for EPN species to exhibit traits that are intermediary between the cruiser and ambusher foraging strategies but are categorized into a particular foraging strategy depending on the importance of the observed traits. For example, *Steinernema ceratophorum* Jian, Reid, and Hunt showed a high jumping rate and short duration standing bouts, suggesting that it is an ambusher forager, but was categorized as a cruiser forager as it was effective against sedentary insect hosts (Campbell and Kaya, 2002). The reduced average distance traveled on the rough textured substrate by *S. fabii*, the negative response to the presence of the hosts and the low larval morality in sand bioassays suggest that this nematode uses an

Table 3
The net average distance ($\bar{x}$ ± SE) per IJ traveled by EPN species on smooth and rough substrates at 10 min, 20 min, and 30 min * indicates the significant difference between the average distance on smooth substrate and rough substrate per EPN species at each time interval. Ns = non-significant.

EPN species	Average distance (cm) traveled by IJs between a smooth and rough substrate		
	10 min	20 min	30 min
Heterorhabditis. Noenieputensis	0.65 ± 0.10/ 0.36 ± 0.05*	0.88 ± 0.15/ 0.52 ± 0.07*	1.08 ± 0.22/ 0.60 ± 0.07ns
H. safricana	0.68 ± 0.08/ 0.22 ± 0.03*	0.78 ± 0.09/ 0.23 ± 0.02*	0.81 ± 0.10/ 0.25 ± 0.02*
Steinernema fabii	0.73 ± 0.06/ 0.49 ± 0.07*	0.68 ± 0.09/ 0.55 ± 0.08ns	0.80 ± 0.06/ 0.58 ± 0.05*
S. jeffereyense	0.42 ± 0.06/ 0.36 ± 0.03ns	0.60 ± 0.07/ 0.40 ± 0.05*	0.76 ± 0.10/ 0.41 ± 0.05*
S. yirgalemense	0.93 ± 0.16/ 0.49 ± 0.04*	1.12 ± 0.20/ 0.54 ± 0.05*	1.23 ± 0.22/ 0.54 ± 0.06*

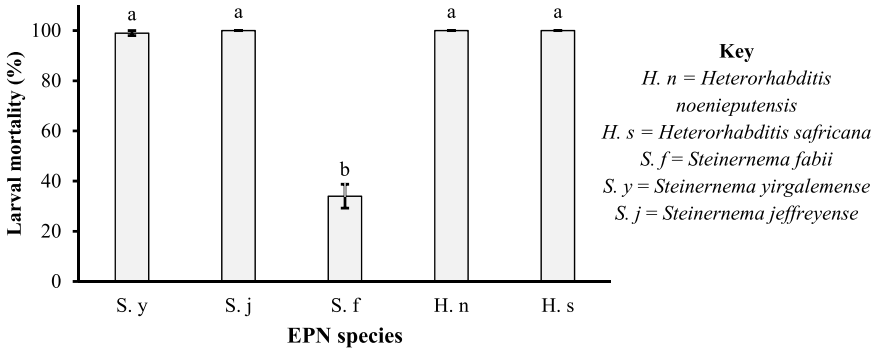

Fig. 2. Larval mortality ($\bar{x} \pm$ SE) of wax moth larvae buried at a depth of 10 cm by five EPN species 48 h pi. Different letters above the bars indicate the significant larval mortality.

ambusher foraging strategy.

Steinernema glaseri (NC strain), which belongs to the *Steinernema* spp. Group with IJs of average length of ≥1000 μm (Nguyen et al., 2007; Poinar 1990), is characterized as a cruiser-foraging nematode, whereas *Steinernema carpocapsae* (All strain), which belongs to the *Steinernema* spp. Group with IJs of average length of ≤600 μm (Nguyen et al., 2007; Poinar 1990), is characterized as an ambusher-foraging nematode. In addition, Campbell and Kaya (2002) noted that cruiser-foraging nematodes tend to be larger than ambusher-foraging nematodes. In contrast, *Steinernema carpocapsae* (IJ average length: 558 μm (Poinar 1990) is slightly longer than *H. bacteriophora* (IJ average length: 527 μm (Bhat et al., 2019)), but the former is characterized as an ambusher foraging nematode, whereas the latter is characterized as a cruiser foraging nematode (Grewal et al., 1994; Lewis et al., 1995). We observed a similar trend wherein *S. fabii* (IJ average length: 641 μm (Abate et al., 2016)), which is characterized as an ambusher-foraging nematode, is longer than *H. noenieputensis* (IJ average length: 536 μm (Malan et al., 2014)), *H. safricana* (IJ average length: 600 μm (Malan et al., 2008)), and *S. yirgalemense* (IJ average length: 635 μm (Nguyen et al., 2004)), which were characterized as cruiser-foraging nematodes. Thus, it becomes apparent that the size of IJs may not be a reliable criterion to infer a host foraging strategy of EPNs.

Increasing our knowledge about the biology of EPNs, particularly the foraging behaviors of their IJs, leads to an improved understanding of the potential of EPNs in pest management (Lewis et al., 2006). The EPNs that showed cruiser foraging behavior in this study, namely *H. noenieputensis, H. safricana, S. jeffreyense,* and *S. yirgalemense*, are likely to be more effective against sedentary insect hosts in the soil depths. This would include the pupal stage for forest insect pests that pupate in the soil, such as the Eucalypt snout beetle, *Gonipterus* sp. n. 2, a major pest of *Eucalyptus* spp. (Rakubu et al., 2023). *Steinernema fabii*, which showed ambush foraging behavior, will be less effective against sedentary insect hosts in the soil depths but will likely be more effective against mobile insect hosts found on/near the soil surface. This could include larvae of several insect pests that crawl on the soil surface before pupating.

Declaration of competing interest

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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