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Ecological aspects of three strains of entomopathogenic nematodes from the department of Lambayeque-Peru

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Abstract

Background Entomopathogenic nematodes (EPN) are used as a biological control agent for different insect pests in agriculture. The genera *Heterorhabditis* and *Steinernema* are the most used commercially. For an EPN species to be used as a biological controller, it is necessary to know its ecological aspects, including reproductive potential, movement capacity, and mean lethal concentration (LC_{50}). These aspects were evaluated in three EPN strains isolated in *Galleria mellonella* larvae collected in the Lambayeque-Peru region, to determine if they are promising as biological controllers. The strains of EPN studied are *Heterorhabditis* sp. (PC9 strain), *H. bacteriophora* Poinar (PM10 strain), and *Steinernema diaprepesi* Nguyen y Duncan (SV19 strain).

Results *Heterorhabditis* sp. (PC9 strain) and *H. bacteriophora* (PM10 strain) had high production of infective juveniles (IJs): 217.750 and 186.800, respectively, while *S. diaprepesi* (SV19 strain) only reached 84.150 IJs. The movement capacity of *Heterorhabditis* sp. (PC9 strain) and *H. bacteriophora* (PM10 strain) reached a depth of 15 cm to parasitize *G. mellonella* larvae, while *S. diaprepesi* (SV19 strain) only reached 10 cm. In decreasing order, the LC_{50} value of *S. diaprepesi* (SV19 strain), *Heterorhabditis* sp. (PC9 strain) and *H. bacteriophora* (PM10 strain) were: 24.03, 13.74, and 8.19 IJs/ml, respectively.

Conclusions *Heterorhabditis* sp. PC9 and *H. bacteriophora* PM10 are promising a biological control agent because they present great production of IJs, great displacement capacity, and high pathogenicity against *G. mellonella*. Additionally, both strains present a mixed search strategy or seeker-hunter (seeker-browser).

Keywords Entomopathogenic nematodes, Ecological aspects, Reproductive potential, Displacement capacity, Lc_{50} value

Background

Entomopathogenic nematodes (EPN) are among the most numerous organisms on Earth and can be found in different types of soils, areas, and environments (Guide et al. 2022). They are important biological control agent for various soil pests, especially lepidopteran larvae, and pupae (Nouh 2021). The EPN of the families Steinernematidae and Heterorhabditidae present a symbiotic association with enteric bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively. These bacteria provide virulence to EPN against insects (Campos-Herrera et al.

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2005). These bacteria are specifically associated with the third juvenile stage of EPN, characterized by being infective and the only one found in the soil (Varela et al. 2021). The persistence of EPN in soil is determined by several factors such as texture, humidity, soil temperature and pH, ultraviolet radiation, and target host (Ratnakala et al. 2023). The parasitic mode of action of EPN is well-known and has been confirmed in previous studies (Sayed et al. 2022). When they locate a host, they penetrate it through the mouth, anus, or spiracles, and once inside they release the bacteria into the host's hemocoel. These bacteria multiply and generate several toxins with high insecticidal potential, which can kill the insect in less than two days (Tomar et al. 2022). Toxins degrade tissues and produce immunosuppressive factors in the insect host, such as antimicrobial compounds, hydrolytic enzymes, toxin complexes, and hemolysins (Mohamed and Shairra 2023).

Knowledge of the biology and ecology of EPN can improve their effectiveness and usefulness as a biological control agent (Flores et al. 2021). However, numerous EPN species have been described without providing the necessary ecological information to continue with other studies (Del Valle et al. 2014). Ecology is the study of the interaction between organisms with each other and the environment around them. The ecological information allows us to expand the study of the characteristics of these microorganisms, among them the capacity of movement, reproductive potential, survival, and host range stand out (Argotti et al. 2010a).

The displacement capacity of EPN is related to the fact that infective juveniles (IJs) search for a suitable host in the soil. This leads to the conceptual model distinguishing seekers, navigators (hunters), and seeker-navigator EPN species. Seekers remain on the soil surface and raise their bodies to jump and attack passing insects. Navigators actively move on the soil to seek their host. Seeker-navigator presents characteristics of both distinctions (Labaude and Griffin 2018).

Reproductive potential, survival, and virulence are determined by the characteristics of the species, including the population, and are influenced by the environment (Campos-Herrera et al. 2005). Furthermore, the reproductive potential of EPN may depend on the strain and the quality of the host used, in such a way that in the same species, there may be differences due to the behavior and physiology of the individuals (Jiménez and Del Pozo 2010). This value is high in EPN, which allows them to be raised "in vivo" and "in vitro" (Cedano 2019). Additionally, the mean lethal concentration (LC_{50}) is a measure that allows measuring the capacity of EPN to kill 50% of insects (Kour et al. 2021). The CL_{50} is also part of the ecological information.

This study aimed to determine the ecological aspects of three EPN strains native to certain soils in the department of Lambayeque-Peru.

Methods

Entomopathogenic nematodes

Three EPN strains were used (Table 1). The strains were located and isolated in soils from the Reque-Lambayeque district, Peru, and reproduced in the last instar larvae of the greatest wax moth (*Galleria mellonella* L., Lepidoptera: Pyralidae) in the Biological Control laboratory of the Vista Florida Agrarian Experimental Station, belonging to the National Institute of Agrarian Innovation of Peru (INIA).

EPN juvenile infectives emerged from *G. mellonella* cadavers and were collected in distilled water using a White trap modified by Kaya and Stock (1997). The IJs were stored at 20 °C for 20 h before performing the three assays described below. Before the experiment, the viability of the IJs of each strain was checked, and only IJs populations with survival rates greater than 95% were used (Laznik et al. 2012).

Reproduction potential of the three EPN strains in *G. mellonella*

The procedure of Argotti et al. (2010a) was followed. For this purpose, 250 ± 20 mg of *G. mellonella* larvae were placed in 0.5 L plastic containers and parasitized with 100 IJs of each strain under study. Each treatment was repeated 30 times. After five days, each parasitized larvae were removed and placed in a humid chamber to allow the IJs to fall. Once the IJs began to fall, three inter-daily counts were carried out under the stereoscope. The test was repeated thrice with a temperature range of 25–29 °C and a relative humidity of 60–70%.

Table 1 Three strains of the entomopathogenic nematode used in the experiment

N°	EPN	Strain	Crops from which EPN was isolated
1	*Heterorhabditis* sp.	PC9	*Coriandrum sativum* (coriander)
2	*Heterorhabditis bacteriophora* Poinar	PM10	*Pouteria sapota* (mamey), adjacent to a *Beta vulgaris* crop (beet)
3	*Steinernema diaprepesi* Nguyen y Duncan	SV19	*Medicago sativa* (alfalfa)

Displacement capacity of the three EPN strains

In this experiment, the procedure of Argotti et al. (2010b) was followed. Polyvinyl chloride (PVC) tubes measuring (15 cm long × 5 cm diameter) were used. The tubes were filled with soil with a sandy loam texture (63:20:17 sand, silt, and clay, respectively). The soil was sterilized with 5% formaldehyde. *G. mellonella* larvae were placed at 5, 10, and 15 cm depth. Then 100 IJ of each strain was placed on the top of each tube (Table 2). Each treatment was repeated three times. After a week, the tubes were disassembled and the *G. mellonella* larvae were collected to verify the mortality caused by EPNs. The *G. mellonella* cadavers were placed in a humid chamber to allow the IJs to fall. The test was repeated thrice with a temperature range of 25–29 °C and relative humidity of 60–70%.

Table 2 Displacement capacity (cm) of the three entomopathogenic nematode strains

Treatment numbers	EPN	Strain	Depth (cm)	Treatments
1	*Heterorhabditis* sp.	PC9	15	PC9:15
2			10	PC9:10
3			5	PC9:5
4	*Heterorhabditis bacteriophora*	PM10	15	PM10:15
5			10	PM10:10
6			5	PM10:5
7	*Steinernema diaprepesi*	SV19	15	SV19:15
8			10	SV19:10
9			5	SV19:5

Mean lethal concentration (LC_{50} value) of the three EPN strains in *G. mellonella*

The Sánchez (2002) procedure was used. Five concentrations (C) of 1, 5, 10, 15, and 20 IJs/ml were applied to plastic containers containing 4.0 oz of soil and the last instar larvae of *G. mellonella* (Table 3). Each treatment was repeated 30 times. After 48 h, the LC_{50} capacity of each strain was verified. The test was carried out three times over time, at a temperature of 25–29 °C and a relative humidity of 60–70%.

Statistical analysis

A complete randomized design (CRD) was implemented in all experiments. Data were analyzed using Infostat software version 2020e SPSS 26. Excel 2019 was used to prepare the figures. The data from the reproductive potential assay of the three EPN strains in *G. mellonella* did not present a normal distribution ($P < 0.05$), so the Kruskal–Wallis test was performed to calculate IJs production. Spearman correlation analysis was carried out between the weight (mg) of *G. mellonella* and IJs production.

In the displacement capacity test of the three strains, a factorial arrangement (A × B) was used, where: A is the strain type and B is the depth (cm) at which the *G. mellonella* larvae were placed. The data did not present a normal distribution ($P < 0.05$), so the Kruskal–Wallis test was performed to calculate the number of dead *G. mellonella* larvae and IJ production.

In the LC_{50} assay of the three strains in *G. mellonella*, a factorial arrangement (A × B) was used, where: A is the type of strain and B is the concentration or number of IJs/

Table 3 Treatments of the experiment to determine the mean lethal concentration (LC_{50} value) of three entomopathogenic nematode strains in *Galleria mellonella*

Treatment numbers	EPNs	Strains	IJs/ml concentrations	Treatments
1	*Heterorhabditis* sp.	PC9	1	PC9:1
2			5	PC9:5
3			10	PC9:10
4			15	PC9:15
5			20	PC9:20
6	*Heterorhabditis bacteriophora*	PM10	1	PM10:1
7			5	PM10:5
8			10	PM10:10
9			15	PM10:15
10			20	PM10:20
11	*Steinernema diaprepesi*	SV19	1	SV19:1
12			5	SV19:5
13			10	SV19:10
14			15	SV19:15
15			20	SV19:20

ml. The average number of dead *G. mellonella* larvae was calculated, as well as the LC_{50} value. The average number of dead *G. mellonella* larvae presented a normal distribution ($P > 0.05$), so an analysis of variance was performed and a probit analysis was performed for the LC_{50}.

Results

Reproduction potential of the three EPN strains in *G. mellonella*

The Kruskal–Wallis test allowed us to identify significant differences in the IJ production of the three strains under study (H (2) = 62.157, $P \leq 0.001$). Dunn's post-hoc multiple comparisons analysis showed that strains PC9 and PM10 had high IJs production (Table 4). Furthermore, the Spearman correlation analysis obtained an R-value of 0.05 and a *P*-value of 0.39 (data not shown).

Displacement capacity of the three EPN strains

The Kruskal–Wallis test allowed us to identify significant differences among treatments for IJs production per larva (H (8) = 19.49; $P = 0.01$) and for the number of dead *G. mellonella* larvae (H (8) = 17.39; $P = 0.02$). Dunn's post-hoc analysis of multiple comparisons showed that treatments PC9:10, PC9:15, PM10:15, PC9:5, PM10:5, and PM10:10 presented the highest production of IJs and the highest number of dead larvae, without being significantly different from each other using Dunn's multiple comparisons test (Table 5).

Mean lethal concentration (LC_{50}) of the three EPN strains in *G. mellonella*

Regardless of the concentration of IJs/ml, the mortality of *G. mellonella* caused by the three strains was statistically different (F = 10.67; df = 2, 30; $P = 0.0001$). *H. bacteriophora* (PM10 strain) caused great mortality, without significant difference with the *Heterorhabditis* sp. (PC9 strain) (Fig. 1a). Likewise, for the concentration (C) of IJs/ml (Fig. 1b), there are also significant differences (F = 33.64; df = 4, 30; $P = 0.0001$). The C20 surpassed all other concentrations. No differences were found between C10 and C15, and C5 was superior to C1 (Fig. 1b). When analyzing the interaction between strain and IJs concentration, a non-significant difference was found (F = 1.08; df = 8, 30; $P = 0.4055$), therefore, the 15 treatments in (Table 3) are similar.

The LC_{50} value of S. *diaprepesi* (SV19 strain) (48 h; $LC_{50} = 24.03$ IJs/ml; $P = 0.028$) was higher than the LC_{50} of *Heterorhabditis* sp. (PC9 strain) (48 h; $LC_{50} = 13.74$

Table 4 Median and interquartile range (IQR) of infective juveniles production of three entomopathogenic nematode strains per last instar larva of *Galleria mellonella*

EPNs	Strains	IJs production by *G. mellonella* larva				
		Median	IQR		H	*p*
Heterorhabditis sp.	PC9	217,750	172.14	a	62.157	< 0.001
Heterorhabditis bacteriophora	PM10	186,800	150.33	a		
Steinernema diaprepesi	SV19	84,150	84.03	b		

Different letters represent significant differences using Dunn's multiple comparisons test

Table 5 Median and interquartile range (IQR) of the infective juveniles production of three entomopathogenic nematode strains and the number of dead *Galleria mellonella* larvae, under three displacement depths

Treatments	IJs Production per larva *G. mellonella*								Number of dead larvae *G. mellonella*						
	Median	IQR					H	*p*	Median	IQR				H	*p*
PC9:10	303,500	21.67	a				19.49	0.01	2	18.33	a	b		17.39	0.02
PC9:15	304,000	21.33	a						2	18.33	a	b			
PM10:15	259,000	18.00	a	b					2	18.33	a	b			
PC9:5	247,000	17.33	a	b					3	24.00	a				
PM10:5	244,500	16.67	a	b	c				2	15.67	a	b			
PM10:10	189,500	16.00	a	b	c				2	12.83	a	b	c		
SV19:5	35,000	8.00		b	c	d			1	7.50		b	c		
SV19:10	10,500	4.50			c	d			1	8.50		b	c		
SV19:15	0	2.50				d			0	2.50			c		

Different letters in the vertical represent significant differences using Dunn's multiple comparisons test

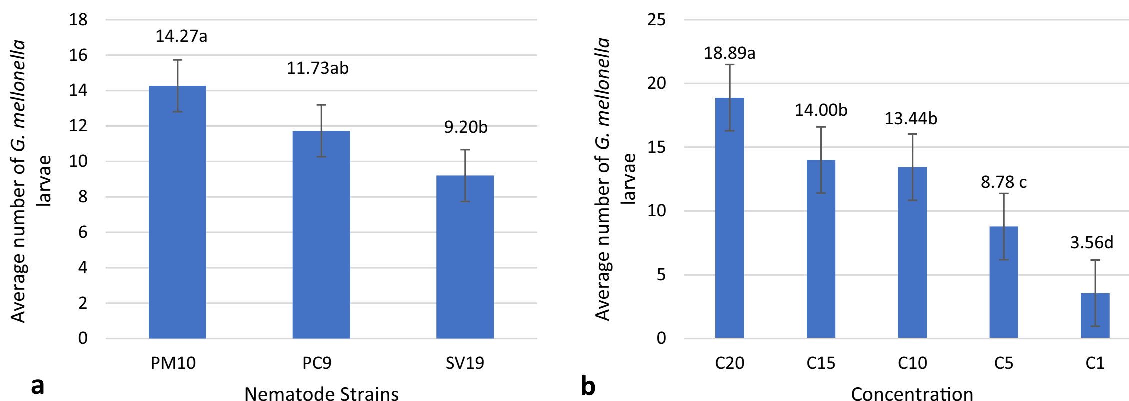

Fig. 1 Average number of dead *Galleria mellonella* larvae per strain (**a**) and per IJs/ml concentration (**b**)

Table 6 Mortality caused by three entomopathogenic nematode strains against the last stage of *Galleria mellonella*, at different concentrations, after 48 h of exposure

EPN Strains	Trust limits	Regression equation	X^2	CL_{50}	R^2	Correlation	*p*
SV19	14.91 – 68.37	y = − 1.449 + 1.05x	24.23	24.03	0.76	0.86	0.028
PC9	8.75 – 29.30	y = − 1.234 + 1.075x	31.56	13.74	0.59	0.78	0.003
PM10	6.47 – 10.43	y = − 1.111 + 1.22x	3.04	8.19	0.87	0.98	0.998

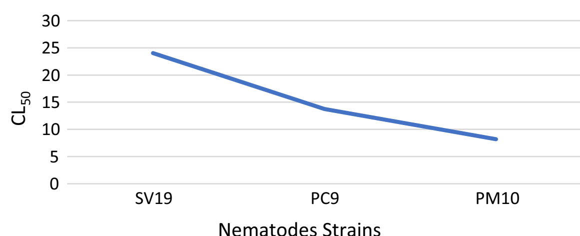

Fig. 2 Mean lethal concentration (LC_{50} value) of three entomopathogenic nematode strains in *Galleria mellonella*

IJs/ml; $P = 0.003$) and *H. bacteriophora* (PM10 strain) (48 h; $LC_{50} = 8.19$ IJs/ml; $P = 0.998$) (Table 6 and Fig. 2). Furthermore, the concentration of nematodes showed a positive correlation concerning the mortality of the last instar of *G. mellonella* (Table 6).

Discussion

The reproduction potential, the displacement capacity, and the LC_{50} value are parts of the ecological aspects of the *Steinernema diaprepesi* (SV19 strain), *Heterorhabditis bacteriophora* (PM10 strain), and *Heterorhabditis* sp. (PC9 strain). These complement the aspects of time, storage temperature, humidity range, and optimal density per application surface. These factors allowed to establish specific attributes of these strains, which translates into making better decisions for their use in agricultural pest control.

It is necessary to indicate that to determine the reproduction potential, IJs production per insect was considered, and to determine the LC_{50} value, infectivity, and virulence were considered. These parameters along with viability estimate the quality of the nematodes as pointed out by Shehata et al. (2019). The study of the three described ecological characteristics of EPNs would increase the possibility of commercial success and avoid economic losses (Del Valle et al. 2014).

Reproduction potential of the three EPN strains in *G. mellonella*

The IJs production of *Heterorhabditis* sp. (PC9 strain) and *H. bacteriophora* (PM10 strain) was more than double that of *S. diaprepesi* (SV19 strain). Therefore, this great yield potential makes them easier to produce commercially. Oúuzoúlu and Ozer (2003) obtained 13,829 IJs of *Steinernema feltiae,* and 141,562 IJs of *H. bacteriophora*, in 200 mg of *G. mellonella* with an inoculum of 100 IJs. In the present study, 249.62 mg of *G. mellonella* larvae were used, parasitized with 100 IJs, obtaining 84,150 IJs for *S. diaprepesi* (SV19 strain), 186,800 IJs for *H. bacteriophora* (PM10 strain) and 217,750 IJs for *Heterorhabditis* sp. (PC9 strain). That is, using the genus *Steinernema*, with a similar inoculum concentration, obtained result was high. However, since it is not the same species

since Oúuzoúlu and Ozer (2003) used *S. feltiae*, it can be attributed that the production of IJ is due to the species and the average weight of *G. mellonella*, coinciding with Argotti et al. (2010a).

The Spearman correlation coefficient (r) between the weight (mg) of *G. mellonella* and IJs production was 0.05 and a P value of 0.39 ($P > 0.05$) (data not shown), indicated that there was no correlation between these two variables, making it impossible to establish that the greatest the weight of *G. mellonella* there will be the greatest the production of IJs. This statement contrasts with the result of Cedano (2019) who found $r = 0.30$ and $P = 0.004$ ($P < 0.05$), being able to affirm that the greatest the weight of *G. mellonella* there was great the production of IJs. In the present study, the number of IJs that penetrated the host was lower than those recorded by Cedano (2019) and, as indicated by Argotti et al. (2010a), the reproductive potential depends on the type of species and EPN strain to be used.

Displacement capacity of the three EPN strains

In general, the number of dead *G. mellonella* larvae was minimal (1.7), which translates into low IJs production. At 15 cm depth, the IJ production of the *S. diaprepesi* (SV19 strain) was null, without producing mortality of *G. mellonella* larvae. This result contrasts with Cagnolo and González (2017), who found *G. mellonella* larvae infected with *Steinernema rarum* (OLI) infective juveniles at 14 cm depth. They point out that if an EPN species goes deeper, it had a great displacement to find its host, and therefore, its search strategy is that of a hunter. In the present study, the SV19 strain produced mortality only up to 10 cm, therefore, it can be assumed that it is an ambush EPN. On the other hand, *Heterorhabditis* sp. (PC9 strain) and *H. bacteriophora* (PM10 strain) produced more IJ per larva and parasitized at 5, 10, and 15 cm, therefore, it can be deduced that their search strategy was mixed or ambusher-hunter. Furthermore, the results achieved with the *S. diaprepesi* (SV19 strain) agree with the results of Del Valle et al. (2014), who studied vertical columns with *S. diaprepesi* up to 10 cm, without finding significant differences with heights of 2 and 5 cm.

Koppenhöfer and Kaya (1999) experimented up to 10 cm with the Sargento Cabral strain of *S. rarum* and point out that the strain had an intermediate strategy, between seeker and hunter. They add that it didn't disperse as quickly as a typical hunter like *S. glaseri,* and that the mortality generated was lower than the latter. The results obtained in the present study agree with those of Argotti et al. (2010b) and Labaude and Griffin (2018), who pointed out that *Heterorhabditids* are hunters and can search for the host up to 90 cm deep in sandy soils, that is, they are active. On the other hand, most species of the genus *Steinernema* conserved their energy and wait for their host, although some species of *Steinernema* can be hunters, such as those reported by Desch and Pablo (2016): CAT3 and Flor strains, which generated 100% mortality of G. *mellonella* at 20 cm depth in sandy soil. In the present study, sandy loam soil was used and it is known that in this type of soil *H. bacteriophora* moves more than in salty clayey sandy loam soils (Campos-Herrera et al. 2005) and, as indicated by Del Valle et al. (2014), *S. diaprepesi* had a great survival in sandy loam soils. The results achieved in this study agree with the analyses of these investigations and leave only the movement capacity and the type of EPN species as influencing variables.

Mean lethal concentration (LC_{50} value) of the three EPN strains in *G. mellonella*

The mean lethal concentration (LC_{50}) of the three strains studied fluctuated between 8 and 25 IJs/ml This indicates that all three had a good pathogenicity and were selectable for studies with other pest hosts. Argotti et al (2010a) mentioned that a laboratory test with 100 IJs/ml was sufficient to select EPN strains as a biological control agent. In the present study, the 1:1 test was included (an IJ of EPN and a larva of the host), which ensures the nematodes quality (Sánchez 2002). As reported, the three strains under study managed to kill *G. mellonella* larvae with a single IJ.

Several studies have shown that LC_{50} varies depending on the host type and EPN species (Ghoneim and Hassan 2024). This is possible due to the reactions of insects to nematode infection as an immune response mechanism (Askary 2022). Therefore, the LC_{50} of the strains in this study would vary depending on the host. Additionally, the LC_{50} depends on the virulence of the EPN, which in turn is affected by environmental parameters (abiotic and biotic) and application techniques. The abiotic parameters are temperature, soil humidity, UV radiation, and osmotic pressure, among the most important. The biotic parameters are the EPN species, the age of the target insect, among others (Ghoneim and Hassan 2024).

On the other hand, the three strains presented significant differences on the average number of dead *G. mellonella* larvae and also for the IJ/mL concentrations. This means that the difference that causes a great or less mortality in *G. mellonella* larvae was due to the concentration of IJs and the type of EPN strain. It was also highlighted that there was a high positive correlation between the concentration and the mortality of *G. mellonella* larvae. This result agrees with what Ghoneim and Hassan (2024) points out that insect mortality depends on the EPN concentration. Since the highest the concentration of EPNs there will be more symbiotic bacteria that

multiply rapidly producing a large amount of toxins that kill the host quickly.

In the linear regression analysis, where the slope of the strains studied was less than 2, *H. bacteriophora* (PM10 strain) had a correlation coefficient close to 1 and a determination coefficient of 0.87. This result differs from that obtained by Khashaba et al. (2020) for *H. indica* obtained slopes of 2.2 and 2.3 for 48 and 72 h, respectively, after exposure of the IJs to *G. mellonella* larvae. These values explain why, apart from the fact that the species used are different, Khashaba et al. (2020) used concentrations of 10, 25, and 50 IJs/larva, while in the present investigation, 1, 5, 10, 15, and 20 IJs/larva were used. Furthermore Khashaba et al. (2020) used two exposure times (24 and 48 h) while in the present study only 48 h according to Ghoneim and Hassan (2024) who recorded a high mortality at great increase in the time of EPN exposure due to the highest penetration of the infective juveniles.

Conclusions

Native strains of the entomopathogenic nematode, *Heterorhabditis* sp. (PC9 strain) and *H. bacteriophora* (PM10 strain) are promising biological control agent because they present a great production of IJs, great displacement capacity, and high pathogenicity against *G. mellonella*. Due to their displacement capacity, the native strains of the EPN, *H. bacteriophora* (strain PM10) and *Heterorhabditis* sp. (strain PC9) presented a mixed search strategy, as they are of the searcher-hunter type. Instead, the strategy of *S. diaprepesi* (strain SV19) was of the search type.

Abbreviations

INIA	National Institute of Agrarian Innovation of Peru
IQR	Interquartile range
IJs/mL	Infective juveniles per milliliter
IJ	Infective juveniles
EPN	Entomopathogenic nematodes
CRD	Completely randomized design
CL_{50}	Median lethal concentration
C	Concentration

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Author contributions
Edgar Darwin Pérez Tesén conceived the research, designed the experiments, and performed the statistical analysis. Jennifher Elizabeth Rodas Adrianzén ran the experiment, recorded the data, and contributed to the writing. Raúl Samuel Cueva Dávila contributed to the execution of the experiments and data recording. Carmen Patricia Calderón Arias contributed to the supervision, visualization, and administration project. Alexander Chávez Cabrera contributed to the manuscript's original writing, revision, and final editing. The final manuscript was read and accepted by all authors.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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