Heterorhabditis brevicaudis: A New Nematode Species Isolated from El-Dakhla Oasis in Egypt and its efficiency against some lepidopteran pests

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Abstract

Background: Entomopathogenic nematodes have been used as biopesticides for controlling insect pests in niche markets. Many new isolates have been recovered from soil of natural habitats in many parts of the world. Native EPNs may be more suitable for controlling local pests. Therefore, isolation and identification of native nematode-bacterial associations are necessary for the successful control of endemic pests. ***Methods:*** The occurrence of an EPN, *Heterorhabditis brevicaudis* and its symbiotic bacteria was newly isolated from Egypt and recorded for the first time in this report. Diagnosis was based on morphometric characteristics and cross-hyperdization. Biochemical characterization and antibiotic activity of its bacteria were investigated. The new species activity was studied against four lepidopteran pests under laboratory conditions. ***Results:*** The isolate was proved to be a new species and was congruently belongs to *H. brevicaudis*. The bacteria produced very strong bioluminescence and its antimicrobial activity is of medium level. *H. brevicaudis* was highly pathogenic to *G. mellonella*, *T. absoluta* and *A. ipsilon* larvae when mature larvae were treated with doses of 150, 100, and 50 IJs/ml, respectively. ***Conclusion:*** This is the first report of *H. brevicaudis* from Egypt. Data showed that the isolate is a new heterorhabtid species. Its symbiotic bacteria belong to ideal criteria and parameters that support nematode growth and pathogenicity. The antibiotics production is an advantage for the successful culturing of EPNs. This new EPN had a good impact against the four tested economic insect pests and could be positively enrolled in an integrated pest management program.

Keywords: Entomopathogenic nematode, *Heterorhabditis brevicaudis*, *Photorhabdus luminescens*, *Tuta absoluta*, *Spodoptera littoralis*, *Agrotis ipsilon*, Egypt.

Abbreviations: EPNs, Entomopathogenic nematodes; *H. brevicaudis, Heterorhabditis brevicaudis; G. mellonella, Galleria mellonella; T. absoluta, Tuta absoluta; S. littoralis, Spodoptera littoralis; A. ipsilon, Agrotis ipsilon*; IJs, Infective juveniles; TAF, Triethanolamine–formalin; *P. luminescens*, *Photorhabdus luminescens*; BTB, Bromthymol blue; NBTA, triphenyltetrazolium chloride and bromothymol blue; LB, Luria–Bertani; *B. subtilis*, *Bacillus subtilis*; Fl, First generation; F2, Second generation.

Introduction

Entomopathogenic nematodes (EPNs) of the families *Steinernematidae* and *Heterorhabditidae* have been used as biopesticides for controlling insect pests in niche markets (Bedding, 1998). With the increasing commercial use of these nematodes as biological control agents, many new isolates have been recovered from soil of natural habitats in many parts of the world, including Europe (Burman et al., 1986; Deseö et al., 1988; Downes et al., 1991; Hernandez & Mracek, 1984; Hominick & Briscoe, 1990; Vänninen et al., 1989); North America (Akhurst & Brooks, 1984; Hara et al., 1991),

Significance | Entomopathogenic nematodes in eco-friendly pest management

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Edited by Dr. Ashfaqul Muid Khandaker, University of Dhaka, Bangladesh, and accepted by the Editorial Board December 25, 2022 (Received for review December 9, 2022)

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Please cite this article:
Hussein MA (2022). *Heterorhabditis brevicaudis*: A New Nematode Species Isolated from El-Dakhla Oasis in Egypt and its efficiency against some lepidopteran pests. *Microbial Bioactives, 5*(2), 211-218.

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Australia (Akhurst & Bedding, 1986), South America (Stock et al., 1996), Asia (Hsieh et al., 2009), Palestine (Iraki et al., 2000) and Egypt in Africa (Hussein, 2004; Hussein & El-Souud, 2006). However, most of these isolates remain unidentified at the species level. The need for more effective EPNs for controlling insect pests have stimulated efforts on EPNs surveys worldwide (Hussein et al., 2018; Webster et al., 2002). This has resulted in the recovery of many new species of EPNs. Native EPNs may be more suitable for inundative release against local insect pests because of adaptation to local climate and other population regulators (Bedding, 1998). In addition, many countries are concerned about the introduction of exotic EPNs because they may have negative impact on non-target organisms (Bathon, 1996).

A survey for EPNs was carried out recently in Egypt, which has resulted in the recovery of more than 21 isolates of insect parasitic nematodes. At the beginning, three isolates were identified (Hussein & El-Souud, 2006) based on the morphological and cross-breeding studies. The isolate coded MH1 identified as *Heterorhabditis* sp. and herein the morphmetric characterization of this isolate is described. Moreover, the efficiency of *H. brevicaudis* (MH1 strain) against some common insect pests was tested.

Methodology

Origin of the nematode used in this study

The isolate designated MH1was recovered from soil samples collected from El- Dakhla Oasis, El-Wadi El-Gadid Governorate, in the western area of Egypt using *G. mellonella* larvae as bait as described by Woodring and Kaya (Woodring & Kaya, 1988). This nematode was isolated from sandy loamy soils.

Identification of strain

For identification of the nematodes, the infective juveniles (IJs) were examined for the presence of a tooth on the head and the structure of the cuticle. In a next step the total length of 20 IJs was measured. For morphological studies, nematodes were examined live or heat-killed in 60°C Ringer's solution. The heat-killed nematodes were placed in Triethanolamine–formalin (TAF) fixative (7% ml Formalin 40%, 2 % ml Triethanol-amine, 91% ml Distilled water) (Kaya & Patricia Stock, 1997) and processed to anhydrous glycerine for mounting (Seinhorst, 1959). Observations were made from live and mounted specimens using an Olympus BX81 (Olympus Trade, Japan) microscope equipped with differential interference contrast optics. For morphological characterization of the isolate, 20 first-generation males and 20 IJs were randomly selected from different *G. mellonella* cadavers. According to their morphological characteristics, all isolates were placed into similar species-groups using taxonomic criteria suggested (Kaya & Patricia Stock, 1997; Stock, 1997; Stock et al., 1997; Uribe-Lorío et al., 2005).

Cross-Hybridization

Although this technique is time consuming and too laborious; it is important to insure the identification of the species. The Egyptian isolate, *Heterorhabditis* sp. (MH1) was cross-bred with the following species of described EPNs: *H. bacteriophora* HB1-3; *H. indica* SAA2; *H. marilatus* HM and *H. zealandica* HZ. Amphimictic adults for cross-breeding were obtained as described by Iraki (Iraki et al., 2000). *G. mellonella* larvae were put into Petri dishes filled with moist sand (10% water w/w) and infested with 100 IJs per insect. Dead *G. mellonella* larvae were dissected 6 days after infestation and thoroughly washed in sterile Ringer's solution. To separate the different adult and juvenile stages, gradient centrifugation was used. The gradient consisted of 30, 20, 15 and 10% (w/w) Ficoll 70000 (Sigma, St. Louis, MO, USA) in distilled water. All nematodes together with the *G. mellonella* haemolymph were put on top of the Ficoll gradient and the suspension was centrifuged at 0.1 g for 2 mm. After centrifugation, debris and hermaphrodites were at the top of the 20% Ficoll gradient. The next layer contained mainly fertilized and unfertilized female phenotypes of the filial generation, and on top of the lower layer (10%), males and juvenile stages were found. Each of these layers was transferred to sterile Ringer's solution with 0.1% streptomycin sulphate. This technique provided almost clean nematode suspensions from which the unfertilized females and males were removed and for crossbreeding experiments unfertilized females were used. For crossbreeding, amphimictic females from one strain and males from the other strain were combined in in vitro cultures of the symbiotic bacterium *P. luminescens* in cell wells which were lined with an agar medium containing the following components in g/l: 12g agar, 10 g trypticase soy, 5 g yeast, 5 g nutrient broth, 1g NaCl, 0.5g $MgSO_4.6H_2O$, 0.2 g $CaCl_2$ and 0.5% sunflower oil adjusted to pH 7 with NaOH. Each well was inoculated with 10 µl of a 24 h *P. luminescens* culture in YS broth (Iraki et al., 2000). The symbionts always originated from the female nematode strain. The space between the wells was filled with sterile water and the wells were incubated at 25 °C. From each strain 5 to 10 female individuals were combined in one well with approximately the same number of males. Controls contain males and females from the same strain and additional control cultures contained females of one strain only without any male nematodes. The production of offspring was recorded 4 days later.

Bacteria isolation and characterization

Isolation of the endosymbiotic bacteria followed procedures described by Akhurst with a small modification in the sterilization process (Akhurst, 1980). Approximately, 100 IJs were immersed in 85% sodium hypochlorite solution for 8min until larvae sheath was completely digested. The unsheathed IJs were rinse twice in sterile Ringer solution and crushed in fresh sterile Ringer solution and then transferred to sterile nutrient broth. Single colonies were successively extracted and streaked on a new NBTA plate until no contamination

was identified. Bacterial colonies maintained on these NBTA plates were incubated at 28 °C for 48 h to be used for further study. Cellular morphology was examined under a light microscope, and motility on 0.25% (w/v) Luria–Bertani (LB) agar was monitored after 16 h. LB cultures were spotted on plates as described by Vivas and Goodrich-Blair (Vivas & Goodrich-Blair, 2001). Dye adsorption of bromothymol blue was assessed on NBTA agar (Akhurst, 1980). Pigmentation was test as described by Boemare (N. Boemare et al., 1997). Bioluminescence was also tested as described by Peel and the colleagues (Peel et al., 1999).

Conventional phenotypic criteria were used for verifying generic identity of the bacterial isolate (N. E. Boemare & Akhurst, 1988). Probes of hemolymph were grown on Standard I agar (peptone-15.0g, yeast agar-3.0g, NaCl-6.0,g, D(+)-glucose-1.0g, agaragar- 12.0g, demi water-1 liter, pH=7.5; Merck) for 24 hours at 25-27ºC. Each colony was transferred in an agar plate as a single strain.

Cellular morphology was assessed by macro morphological tests of 24-h-old nutrient broth cultures to check the possibility of the strains to grow on different media, Standard I agar and Mac Conkey were used. The cultivation conditions were 27 ºC and pH=7.0. Based on these tests, size, consistence, color, form, margin of the colony and pigmentation were described. Dye adsorption of bromothymol blue was tested on nutrient agar supplemented with 0.004% triphenyltetrazolium chloride and 0.0025 bromothymol blue (NBTA medium). Dye adsorption of neutral red was tested on MacConkey agar which is specific to *Photorhabdus* isolates (Kaya & Patricia Stock, 1997). Micro morphological tests 24-hours old cultures were used to describe shape of the cells and motility and to differentiate between both primary and secondary phase on solid and liquid culture media (LCM) (Hussein, 2004).

Antibiotics production

The production of antimicrobial substances by *P. luminescens* strain MH1 was determined as described by Akhurst (Akhurst, 1982). The test organism was *B. subtilis*. A 5 µl of an overnight culture of *Photorhabdus* MH1 was pipetted into the center of an LB agar plate. After a 2-5 days incubation at 30 °C, the plate were overlaid with 5ml soft agar (0.6 g agar/100 ml LB solution) included 1 ml suspension of *B. subtilis* spores (containing about 10^8 spores). The plates were incubated overnight at 37 °C. The diameter of the clear (inactivation) zone, as well as that of the bacterium colony were determined.

Virulence assays

In laboratory bioassay, The IJs of the indigenous EPN, *H. brevicaudis* MH1was tested to check their pathogenicity against four lepidopteran insect pests, namely greater wax moth, *Galleria mellonella,* tomato leafminer, *Tuta absoluta,* cotton leafworm, *Spodoptera littoralis,* and black cutworm, *Agrotis ipsilon*. The final larval instars of tested insects were exposed to 1 ml nematode suspension at 50, 100 and 150 IJs separately onto a moist Whatman's No.1 filter paper lined in a Petri plate (9 cm dia.) and allowed them to infect for 48 h. Ten larvae per each replicates and three replicates were maintained for each treatment of all the three insects with maintaining appropriate untreated control treatments separately. The entire experiment was repeated twice to confirm the results. After 2 days of exposure, the treated larva was washed twice in distilled water and transferred onto White trap (White, 1927) in a fresh Petri plate containing a moist filter paper. Observations were made on larval mortality caused by *H. brevicaudis* MH1 at 48 h post-treatment.

Results

Description

Morphometrics of the holotype (infective juvenile), and all stages of the new nematode are presented in Tables 1–3. *H. brevicaudis* MH1 sp. n. was separated from other recognized *Heterorhabditis* species by morphological characters. Light microscopy revealed that the excretory pore of nematode is located posterior to the nerve ring. Morphologically the average length of *H. brevicaudis* MH1 sp. n. infective juveniles (582 µm) is different (Table **1**) from those of *H. megedis* (768 µm), *H. zealandica* (685 µm), *H. argentinensis* (657 µm), *H. indicus* (528 µm) and *H. bacteriophora* (588 µm). However, the length range of *H. brevicaudis* sp. n. infective juveniles (540-600 µm) somewhat overlaps with those of *H. bacteriophora* (588 µm) and *H. zealandica* (570-740 µm) and *H. indicus* (479-573 µm); but no overlapping occurs with those.

Infective third-stage juveniles

In this report, the IJ morphometrics and features of the isolate were consistent with those original species descriptions for *H. brevicaudis* (Table 1) (Adams et al., 2006; Hsieh et al., 2009; Nguyen & Smart Jr, 1996; Poinar Jr, 1990). Total length of the IJs was: 582± 21.6, Greatest width: 25.4 ± 1.6, Distance from anterior end to excretory pore: 94.16 ± 5.5, Distance from anterior end to nerve ring: 75.8 ± 9.2, Distance from anterior end to oesophegus base: 121.4± 13, Tail length: 71.5±3.4, Ratio A: 23±0.7, Ratio B: 4.9± 0.74, Ratio C: 8.15 ± 0.41, Ratio 4: 0.79± 0.1, Ratio 5: 1.3±0.07 and Ratio 6: 0.35± 0.15.

Males

As recorded in Table (2) the total length of *H. brevicaudis* MH1 males were 900 ± 109, Greatest width: 47.5 ± 5.5; Distance from anterior end to excretory pore: 87.7 ± 9.5; Distance from anterior end to nerve ring: 74 ± 3.6; Distance from anterior end to oesophegus base: 103.9±6.2, Tail length: 53.3±3.6; Spicule length: 41.9±5.2; Spicule width: 6.23±1.5; Gubernaculum length: 22.1±2.08; Gubernaculum width: 1.04±0.13; Width at anus: 28.6±2.4; Guber. length/ Spic. length: 0.56 ± 0.06.

Females

The measurments (in micrometers) of both Hermaphroditic and Amphimictic females are recordes in Table (3). Hermaphroditic females (n=20): L=4300 (3550-5040), W= 25 (200-312); stoma length

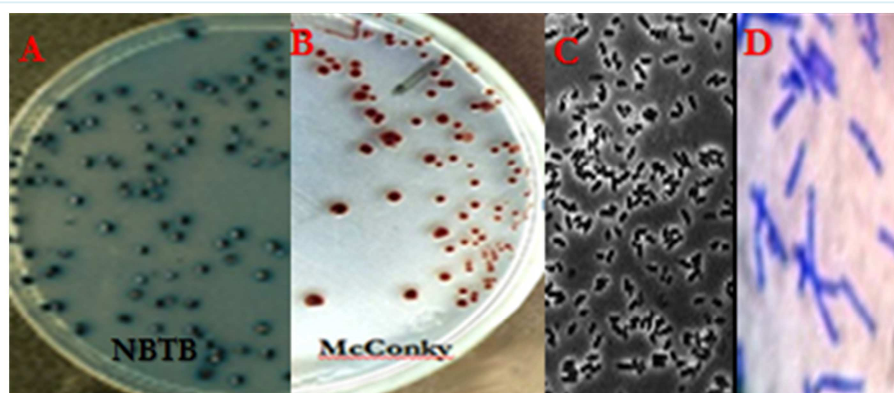

Figure 1| **Colonies and bacterial cells of *Photorhabdus luminescens* associated with the entomopathogenic nematodes, *Heterorhabditis brevicaudis* MH1 on different media, A: on NBTB plate; B: on McConky; C: PhaseI and D: PhaseII.**

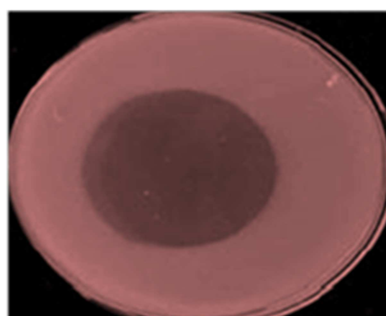

Figure 2| **Antibiotics activities of the new *Photorhabdus* isolates against *B. subtilis.***

Table 1| **Comparative measurements of infective juveniles of the Egyptian isolate of *Heterorhabditis brevicadus* and that of the original description.**

Character	Measurement in μm[a,b]	
	Egyptian *Heterorhabditis brevicadus* (MH1)	Original *Heterorhabditis brevicadus* (Hsieh et al., 2009)[e]
L[b]	582± 21.6[d]	572 (528–632)
W	25.4 ± 1.6	22 (20–24)
EP	75.8 ± 9.2	111 (104–116)
NR	94.16 ±5.5	101 (96–104)
ES	121.4 ± 13	124 (120–136)
T	71.5 ± 3.4	76 (68–80)
Ratio A	23 ± 0.7	26
Ratio B	4.9 ± 0.74	4.6
Ratio C	8.15 ±0.41	7.6 (6.6–8.6)
D%	79 ± 0.1	90
E%	130 ± 0.07	147

Measurement ranges are given in parentheses following the mean (N = 20); L[b], Body length, W, Max.body diameter EP, distance from anterior end to excretory pore; NR, distance from anterior end to nerve ring; ES, distance from anterior end to the base of esophagus.T, Tail length A=Body length÷maximum body diameter; B=Body length÷oesophageal length; C=Body length÷tail length; D%=Distance from anterior end to excretory pore÷oesophageal length X100; E%=Distance from anterior end to excretory pore÷tail length X100.[e]Morphometric characters (range) of *H. brevicaudis* described in Liu (1994); [d]Means±SD and range in μm.

= 10.4 (8.8-12) ; stoma width = 18.2 (16.20); head-excretory pore = 190 (160-200) ; head-nerve ring = 164.4 (144-176) ; esophagus length= 218 (192-240); tail length= 86 (72-128); ABW= 70.4 (56-88); V%= 43.2 (37-50). Amphimictic females (n=20): L=2350 (2100-2500); W = 149 (128-168); stoma length = 6 (6-6); stoma width = 10 (10-10); head excretory pore = 135 (124-160); head-nerve ring = 103 (100-108); esophagus length = 154 (144-160); tail length = 86 (76-92); ABW = 39 (36-48); V%=48.4 (45-53).

Cross- Hybridization

The isolate of EPN (MH1) did not cross or produce progeny in the F1 generation with all different species tested (Table 4). In the controls containing only females, no progeny was observed. Control crosses using individuals of the same isolate were always successful. The Fl offspring resulting from crosses of the Egyptian isolate MH1 produced F2 offspring. Both the morphological and cross-breeding data presented in this paper showed congruently that the new Egyptian nematode isolate belong to *H. brevicadus,* which is characterized by the presence of 9 bursal papillae (Hsieh et al., 2009).

Bacteria isolation and characterization

One bacterial strain, designated *Photorhabdous* sp. MH1, was isolated from IJs, the first to be isolated from this species. The bacterial culture produced bioluminescence which was very strong and detected by the naked eye after adaptation to the dark for approximately 5 min. The colonies absorbed dye from both McConkey agar plates and NBTA plates (Fig. 1).

Unlike other *Photorhabdus* sp., the bacterial symbionts of this isolate was able to divide and multiply on NBTB plates over period of 5 days as primary phase (1°). The colony morphology was as regular *Photorhabdus*sp., granulated, convex, opaque and circular with irregular margin. The colonies were very sticky and gives dark orange colonies when cultured in liquid culture media.

The cell morphology of the phase I was also distinct with small to middle sized cells, majority with ovoid and /or rhombic or rectangular inclusion bodies (Fig. 1C). The cells absorbed BTB and were olive and green colonies, with clear zone around colony absorb and were able to absorb Neutral red from McConky plates. However, the cell morpholgy of phase II was different from that of phase I. the cells were flat, translucent with regular margin and usually have a greater diameter (Fig.1D). The cells were long and thei nclusion bodies rarely found. The cells also were not able to absorb BTB from NBTB plate and the same as well for Neutral red from McConky agar plates.

Antibiotics production

The antimicrobial activity of the Egyptian strain MH1is of medium level (Fig.2). It was found that the antibiotics production of the new species (*H. brevicaudis*) is reproducibly much higher than those of the others. The antibiotics production is an advantage for a successful liquid cultures of EPNs.

Virulence assays

In the laboratory, *H. brevicaudis* was highly pathogenic to *Galleria* larvae, with all *Galleria* larvae killed within 48 h when mature larvae were exposed to 150 IJs in a 9 Cm Petri dish and 73.3% and 50% mortality recorded when treated with 100 and 50 IJs. The mortality % of the tomato leaf miner, *Tuta absoluta* reached 30, 43.3 and 50% when treated with doses of 50, 100 and 150 IJs/ml. For the cotton leaf worm, *S. littoralis* treated with the same concentrations, the mortality % recorded 26.7, 36.7 and 50 % within 48 hours post treatment with *H. brevicaudis.* The cut worms, *Agrotis ipsilon* were moderately affected with the new species within 48 hours after treatment (Table 5). The mortality recorded 56.7%, 33.3 % and 13.3% when treated with 150, 100 and 50 IJs, respectively.

Discussion

The genus *Heterorhabditis* has a widespread distribution in tropical and temperate regions of the world. Because of the increasing importance of EPNs in biological control, a number of surveys have been undertaken in different parts of the world in attempts to isolate promising new isolates of *Heterorhabditis* and *Steinernema* (Akhurst & Bedding, 1986; Akhurst & Brooks, 1984; Blackshaw, 1988; Deseö et al., 1988; Downes et al., 1991; Downes & Griffin, 1991; Hara et al., 1991; Hernandez & Mracek, 1984; Hominick & Briscoe, 1990; Hussein, 2021; Hussein & El-Souud, 2006).

This is the first report on the isolation of *H. brevicaudis* from Egypt; Africa and the Middle East. The original type specimen of *H. brevicaudis* was described initially in Fujian Province of China in 1994 by Liu (Hsieh et al., 2009). In this study, the identification of *H. brevicaudis* was mainly based on morphometrics by comparing with the data from original descriptions (Hsieh et al., 2009) and on Cross-Breeding data as well. Our results indicated that nematodes of the genus *Heterorhabditis* are present in El- Dakhla Oasis, El-Wadi El-Gadid Governorate. As it has been stated by several authors (Damgaard et al., 1998; Dix et al., 1992; Griffin et al., 1994), morphological characterization of *Heterorhabditis* species provides useful tools for diagnostic studies at the population and species level (Damgaard et al., 1998; Stock et al., 1996). At the same time, the identification of presumed biological species in the genus *Helerorhabditis* by cross-breeding of second-generation amphimictic females has confirmed that *H. brevicaudis* (MH1 strain) is a distinct biological species, unique and does not fit the description of currently recognized species of the genus *Heterorhabditis,* therefore it is considered a new species. The measurements of IJs of the isolate shown in Table 1, prove obviously that the tail length is less than 80 µm (Table 1), shorter than that of other species (Adams et al., 2006). Light microscopy revealed that the bursa peloderan of male *H. brevicaudis* MH1 has nine pairs of genital papillae (data not shown), which is a distinct character of the species (Hsieh et al., 2009). Unlike other pathogenic bacteria, the symbiotic bacteria *Photorhabdus* sp. of

Table 2| **Comparative measurements of adult males of the Egyptian isolates and the original population of *Heterorhabditis brevicadus.***

Character	*Heterorhabditis brevicadus*	
	Egyptian	Original
L	[d]900 ± 109	900 (840-950)
W	47.5 ± 5.5	42.6 (40-48)
Stoma length	1.7±0.4	1.9 (1.2-2)
Stoma width	4.1±0.7	3.98(3.2-4.8)
EP[a]	74 ± 3.6	95 (92-100)
NR[b]	87.7 ± 9.5	82.4 (80-88)
ES[c]	103.9 ± 6.2	108 (104-112)
T	53.3 ±3.6	32 (28-36)
SL	41.9 ± 5.2	46.8 (44.48)
SW	6.23 ± 1.5	6.6 (6-8)
GL	22.1 ± 2.08	22.2 (20-24)
GW	1.04 ± 0.13	3.8 (3.2-4)

Table 3| **Measurements of adult hermaphroditic and amphimictic females of the Egyptian isolates of *Heterorhabditis brevicadus.***

Character	Measurements (in micrometers)	
	Hermaphroditic females	*Amphimictic females*
L*	4300 (3550-5040)	2350 (2100-2500)
W	25(200-312)	149 (128-168)
Stoma length	10.4 (8.8-12)	6 (6-6)
Stoma width	18.2 (16.20)	10(10-10)
EP	190 (160-200)	135 (124-160)
NR	164.4 (144-176)	103 (100-108)
ES	218 (192-240)	154(144-160)
T	86(72-128)	86(76-92)
ABW*	70.4 (56-88)	39(36-48)
V%	43.2(37-50)	48.4(45-53)

Table 4| **Results of crossBreeding experiments between different *Heterorhbditis* species and the Egyptian isolate of *Heterorhabditis brevicadus* MH1 on lipid agar plates.**

Male Female	MH1	HB1-3	SAA2	HM	HZ.
MH1*	+**	-	-	-	-
HB1-3	-	+	-	-	-
SAA2	-	-	+	-	-
HM	-	-	-	+	-
HZ	-	-	-	-	+

*MH1: *Heterorhabditis brevicadus;* HB1-3: *H. bacteriophora*; SAA2: *H. indica*; HM: *H. marilatus,* and HZ: *H. zealandica*
** + Fertile infective juveniles -No infective juveniles

Table 5| **Mortality of 4 different species of lepidopteran larvae to different concentrations of infective juveniles of *Heterorhabditis brevicaudis* MH1.**

Test insects	Mortality (%)*			
	IJs**X 50	IJs X 100	IJs X 150	Control***
Galleria mellonella	50	73.3	100	0
Tuta absoluta	30	43.3	50	0
Spodoptera littoralis	26.7	36.7	50	0
Agrotis ipsilon	13.3	33.3	56.7	0

*Ten larvae/replicate, 3 replicates/ trial. **IJs, infective juveniles. ***Distilled water

the genus *H. brevicaudis* were unique in their characters. The primary form was stable for five days and their pigments were very remarkable.

Parasitic nematodes were used widely to control different insect pests (Abd El Azim, 2022; Grewal et al., 2005; Sobhy et al., 2020; Thakur et al., 2022). Steinernematidae and Heterorhabditidae nematodes have been used against several coleopteran pests however, less information is available on their use against lepidopterans pests (Grewal et al., 2005; Sun et al., 2021). The cotton leaf worm, *Spodoptera littoralis* (Boisd.), and the black cutworm, *Agrotis ipsilon* (Hufnagel), and the tomato leafminer, *Tuta absoluta* (Meyrick) consider the most important pests of many crops in different areas all around the world (Biondi et al., 2018; Labaude & Griffin, 2018; Paniagua Voirol et al., 2018; Thakur et al., 2022). In Egypt, these pests cause economic losses not only to cotton, corn, and tomatoes but also to about over 90 different economic crops (Ibrahim et al., 2017). In this study, the larval mortality induced by *H. brevicaudis* MH1 against the four tested larvae was concentration-dependent; that is, the mortality in larvae increased as the EPNs concentration increased. Data proved that *H. brevicidus* is a new promising species that could be an excellent bio-control agent against the cotton leaf worm, the black cutworm, the tomato leaf miner, and the great wax moth.

Author Contribution

Conceptualization, experimentation, analysis and interpretation of data, drafting and critical revision of the manuscript were done by MAH.

Acknowledgment

This study was funded in part by a CSSP grant from Bibliotheca Alexandrina and supported by grant No. 12050137 from the National Research Centre. Deep appreciation to Prof. Suey-Sheng Kao for providing the original key of the Chinese species.

Competing financial interests

The authors declare that they have no potential conflict of interest in publishing this research output.

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