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Comparison of virulence, reproductive potential, and persistence among local *Heterorhabditis indica* populations for the control of *Temnorhynchus baal* (Reiche & Saulcy) (Coleoptera: Scarabaeidae) in Egypt

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Abstract

The scarab beetle, *Temnorhynchus baal* (Reiche & Saulcy) (Coleoptera: Scarabaeidae), becomes a key pest of strawberry, especially after increasing its cultivated area and economic importance in Egypt. Few entomopathogenic nematode (EPN) species/strains were tested against this pest, where only a foreign species had good effect comparable to the native populations tested previously. Thirty-eight indigenous *Heterorhabditis indica* populations were tested against the most damaging, third instar larvae of *H. baal* in two soil types, where strawberry cultivation prevails. The corrected mortality induced by indigenous *H. indica* population in *T. baal* larvae was (99.52 and 98.57%) for 15 populations in the sandy soil and 23 in loamy sand soil, respectively. Overall average of infective juveniles (IJs) per *T. baal* larva was about (41,000). The average of emerged nematode-IJs from the infected grubs for the 15 EPN populations in sandy soil, (46,960 IJs/grub), was significantly ($P < 0.001$) higher than that (36,502 IJs/grub) of 23 EPN populations in loamy sand soil. The insignificant difference was detected in the reproductive capacity among nematode populations in *T. baal* larvae in sandy or loamy sand soil. A highly significant difference was found among total IJ numbers collected 10, 18, 26, and 30 days post-inoculation in sandy or loamy sand soil. The persistence of the *H. indica* populations in the soil varied greatly. Obtained results suggest further use of at least ten populations of such indigenous nematodes under field conditions.

Keywords: Biocontrol, Entomopathogenic nematodes, Beneficial traits, Scarab beetle

Background

Strawberry, *Fragaria ananassa* Duchesne, cultivated area in Egypt has highly expanded in recent years. Early fruiting, long harvest season, good quality, low production costs, and closeness of export markets offer good opportunities (Abd-Elgawad 2019). Egypt occupied the fourth largest producer of strawberries in the world in 2017 (FAO 2019). In Egypt, strawberry pests and diseases that were not of economic importance have spread and are threatening the productivity of the crop. Among those pests, the strawberry white grub or rootgrub (*Temnorhynchus baal* Reiche & Saulcy) (Coleoptera: Scarabaeidae) ranks high. The serious phytophagous immature stages of *T. baal* cause heavy economic losses to strawberry in Egypt (El-Shemy et al. 2013). Like other scarabaeids, *T. baal* larvae undergo subterranean life and therefore their control is difficult. On the other hand, because of the problems associated with environmental and regulatory concerns due to the excessive use of chemical insecticides, research on developing alternative control strategies is desperately needed. In this vein, entomopathogenic nematodes (EPNs) are known to infect the soil-dwelling development stages of insect pests since soil is their basic habitat. So, the grubs are

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excellent candidates for biocontrol by EPNs (e.g., Koppenhöfer and Fuzy 2008; Abdel-Razek and Abd-Elgawad 2013 and Kajuga et al. 2018). They are one of the potential alternatives worldwide and in Egypt (Abd-Elgawad 2017a, 2017b). Nematode genera *Heterorhabditis* and *Steinernema* with their mutualistic bacteria *Photorhabdus* spp. and *Xenorhabdus* spp., respectively, have been commercially used against many economically important insect pests worldwide (MMM et al. 2017). Steinernematids are amphimictic, whereas heterorhabditids are hermaphroditic. Infective juveniles (IJs), the only free-living stage, penetrate into the hemocoel of their hosts, usually through the gut or spiracles. *Heterorhabditids* have a dorsal tooth that might also facilitate penetration in other areas (e.g., intersegmental membranes). Then, mutualistic bacteria from the nematode's digestive system are released within the insect host, and the host dies from septicemia, usually within 24–72 h. The nematodes feed, develop, mate, and reproduce within the host cadaver, and, often after multiple generations, IJs are again produced and leave the cadaver to seek new hosts (Kaya and Stock 1997). Biological control via EPNs has been particularly successful against certain insect species that spend a large portion of their life cycle in the soil, particularly the strawberry white grub, *T. baal* (Shamseldean and Atwa 2004; Atwa 2009 and Atwa and Hassan 2014).

However, research on the potential of EPNs to suppress *T. baal* has been limited. Only few EPN species/strains have been tested for pathogenicity to *T. baal* larvae, which are usually found in the soil and therefore are potential targets. A few indigenous and introduced EPNs have been used for biocontrol of *T. baal* larvae. Foreign EPN strains were more effective (Shamseldean and Atwa 2004; Atwa 2009 and Atwa and Hassan 2014) but indigenous nematodes are more appreciated because they are likely more adaptive without any risk to Egyptian fauna and flora. So, laboratory testing of additional species/isolates may lead to the identification of nematodes with superior beneficial traits for biocontrol of *T. baal*.

The study primary objective was to determine the relative susceptibility of *T. baal* larvae to 38 indigenous isolates of *H. indica* under laboratory conditions. Also, the reproductive potential of the nematodes in *T. baal* as well as IJs persistence in two soil types that are common for growing strawberry in Egypt were investigated.

Materials and methods

Thirty-seven EPN isolates were obtained from soil planted with citrus alone or mixed with mango trees from seven locations in July, 2017, and additional six isolates were extracted from 5 other locations in August, 2017, both surveys were at Giza Governorate, Egypt. So, a total of 43 EPN isolates were identified herein according to the morphological and molecular tests as *H. indica*. Nematode-infective juveniles (IJs) of these 43 EPN populations were reared in vivo, via the greater wax moth (*Galleria mellonella* L.) last instar larvae at 24 ± 2 °C. After 3 cycles in *G. mellonella*, the IJ culturing and harvesting procedures (Kaya and Stock 1997) could only maintain 38 isolates, which were separately kept in 500-ml flasks containing approximately 150 ml distilled water at 12 °C. In order to avoid anoxic conditions for the IJs, their suspensions were aerated almost weekly by an aquarium oxygenator. The IJs were used within a week after harvesting of the last cycle, when the nematode survival was checked first by observing their mobility under stereomicroscope (Majić et al. 2019).

The grub (*T. baal*) larvae were collected from strawberry field at Arab El-Ghadeer, Tukh district, El-Qalioubia Governorate, Egypt, via tracking a plow, driven by the tractor, to gather them at season-end late in March. Active and full grown *T. baal* third instar larvae were identified (Voglar et al. 2019) and selected in the laboratory to test them for the nematode-IJ virulence and reproductive potential. Two types of soil similar to those were selected from which EPNs were isolated, i.e., each isolate was tested in a soil similar to that isolated from it. Four larvae were placed over 250 g soil within each plastic semi-cone cup (12.8 cm diam., 9 cm height with perforated lids), and 4 cups were used as a replicate for each EPN population. A total of 640 *T. baal* larvae were used for 40 treatments; 38 nematode populations and 2 untreated cups as checks (without nematodes) for the 2 soil types in a completely randomized block design. One day was left for the grubs to penetrate the cup soil before adding (2000 IJs in 5 ml) water on the soil surface within a cup at room temperature (27 ± 5 °C and 65% RH). Soon, the cups were delicately shacked handily to distribute the nematodes within the soil. Such a test was carried out in each of two 10% moistened sterilized soil types; sandy (sand 88.8%, silt 4%, clay 7.2%, pH 7.7, CaCO3 1.88%, OM 0.68%) and loamy sand (sand 82.8, silt 6, clay 11.2, pH 7.8, CaCO3 13.8%, OM 8.7%) soil. Both soil types were collected from strawberry field plots; sandy soil from Badr district, El-Beheira Governorate and loamy sand from Tukh district, El-Qalioubia Governorate, Egypt. Five days after the addition of IJs, EPN infectiousness to the grubs was screened where the grubs were transferred to White traps (Kaya and Stock 1997). The infectiousness was defined here as the insect mortality caused by EPNs. Therefore, all data were corrected in order to compensate for natural mortality by Abbott's formula (Abbott 1925). For each nematode population, each infected *T. baal* larva was placed on a White trap, where IJs were collected every other day, starting 10 days after IJs addition, until emergence ceased or was considered negligible (30 days post-inoculation). At

each collection time, the number of IJs produced per insect was determined through dilution counts (Kaya and Stock 1997). Soon after getting the grubs out of the cups, 4 *G. mellonella* last instar larvae were replaced the grubs per cup to test the persistence of the remaining EPNs. There were 4 insects also in each replicate (cup) with 4 cups for each check of the soil types (16 insects/check). About 2–3 days later, *G. mellonella* cadavers exhibiting the characteristic signs and symptoms of EPN infection were transferred to modified White traps (Kaya and Stock 1997). The IJs emerging in the traps were used to reinfect additional *Galleria* to confirm pathogenicity and to fulfill Koch's postulates (Pelczar and Reid 1972). Addition of new *G. mellonella* larvae to replace previous ones in these cups was done for 3 additional times so typically that infected *G. mellonella* cadavers were counted also after 1, 2, and 3 months post-inoculation of IJs.

For EPN identification, nematode-IJs were concentrated in approximately (200 μl) water in Eppendorf Centrifuge tube and DNA was extracted, using the UltraClean™ Soil DNA Extraction Kit (MoBio). All the samples were checked for quality and quantity using 1 μl per duplicate in a Nanodrop ND−1000 v3.3.0 (Thermo Scientific, Wilmington, DE) and adjusted to the appropriate concentration, 0.2 ng μl. Species-specific primers and TaqMan probes were used to conduct real time PCR according to the protocols and conditions described by Atkins et al. (2005) and Campos-Herrera et al. (2011). Optical 96-well reaction plates (USA Scientific, Orlando, FL, USA) were used on an ABI Prism 7000 (Applied Biosystems) for the qPCR identification. The reactions were carried out in duplicate in a final volume of 20 μl, using 1 μl of the corresponding DNA template, 10 μl of Master Mix (Applied Biosystems, manufactured by Roche, Branchburg, NJ, USA), and the appropriate concentration of primers and probe. Sterile de-ionized water as a negative control instead of template DNA and a positive control from pure identified EPN species corresponding to its standard curve as described by Campos-Herrera et al. (2011) was used.

Statistical analysis

An analysis of variance (ANOVA) was used to analyze the data of nematode virulence, reproductive capacity, and persistence among nematode populations. A comparison of means was done using Duncan's new multiple range test (DNMRT). The average of emerging IJ numbers in the 2 soil types were compared by the two-tailed Student's *t* test. Because of using original counts of nematodes with aggregated distribution violates assumptions necessary for parametric statistical analysis, transformed nematode counts, $\log_{10}$ (X + 1), were subjected to the analysis.

Results and discussion

Comparing virulence and reproductive potential among nematodes

Each of the tested EPN populations attained high percentage mortality in the scarab grub (Table 1). Averages (± standard errors) of nematode-induced mortality in *T. baal* larvae were (99.58 ± 0.42 and 98.64 ± 0.55%) for 15 EPN populations in sandy soil and 23 populations in loamy sand soil, respectively. The corresponding corrected mortality was (99.52 and 98.57%), respectively. Insignificant difference in virulence was detected among nematode populations in sandy ($F = 1$; df = 14; $P = 0.47$), or loamy sand ($F = 1$; df = 22; $P = 0.48$) soil. The highest numbers of IJs emerging from the grub cadavers were obtained at 14, 16 and 18 post-inoculation days (Fig. 1). Emerging IJs were counted every other day (Fig. 1) but Table 1 contains combined data at 10, 18, 26, and 30 days. An infected grub cadaver produced a total of about 41,300 to 59,700 and 32,300 to 41,300 IJs per *T. baal* larva at 30 days post-inoculation in sandy and in loamy sand soil, respectively. Overall average of IJs per *T. baal* larva was about (41000). Based on *t* test, the average of such emerged IJs for the 15 EPN populations in sandy soil (46,960 IJs/grub) was significantly ($P < 0.001$) higher than that of 23 EPN populations in loamy sand soil (36,502 IJs/grub). Insignificant difference was detected in reproductive capacity among nematode populations in *T. baal* larvae in sandy ($F = 1.39$; df = 14; $P = 0.20$) or loamy sand ($F = 0.87$; df = 22; $P = 0.64$) soil. Highly significant difference was found among total IJ numbers collected 10, 18, 26, and 30 days post-inoculation in sandy ($F = 664.3$; df = 14; $P = 2.12E-35$; Fig. 2) or loamy sand ($F = 2080.8$; df = 22; $P = 2.9E-65$; Fig. 3) soil.

Persistence in the nematode populations

Since the percentage mortality of grubs was similar in both soil types, the efficacy of remained EPN populations in the cups was pooled together for further calculations of virulence against *G. mellonella* larvae at extended periods as an evidence of nematode persistence in soil. Over a period of 3 months in soil, the number of infected *G. mellonella* larvae varied greatly ($F = 8.64$; df = 37; $P = 3.98E-19$) according to the applied nematode population (Table 2). These infected larvae ranged from 15.6 to 56.3%. In a descending order, the following code numbers of *H. indica* populations caused greater mortality and could persist longer in the soil compared to others: 25, 21, 44, 5, 33, 22, 6/2, 47, 18/2, 20. Nematode persistence, measured by ability to infect *G. mellonella* larvae in soil at extended baiting times, significantly ($P \leq 0.05$) differed among the tested periods (Fig. 4). Only about 3% of *G. mellonella* larvae in soil were infected at 3 months post-inoculation.

Table 1 Mortality of *Temnorhynchus baal* larvae after exposure to different populations of *Heterorhabditis indica* and numbers of emerging nematode-infective juveniles using 2 different types of soil as substrates (mean of four replicates (cups) each has four insects. The control has the grubs without nematodes)

H. indica population code	Soil type	Mortalities of *T. baal* larvae (*5 days post-treatment*)		Number of emerging nematode-infective juveniles/grub larva				
				Days of post-infestation				
		Mean ± SE	Mortality %	10 days (7 April 2018)	18 days (15 April 2018)	26 days (23 April 2018)	30 days (1 May 2018)	Total IJs rate/larva
20	Sandy	4 ± 0	100	324	39,606	4522	4	44,456
16	Loamy sand	3.75 ± 0.43	93.75	294	36,005	1455	0	37,754
46	Loamy sand	3.75 ± 0.43	93.75	199	33,855	1460	0	35,514
15	Loamy sand	3.75 ± 0.43	93.75	124	35,864	1328	0	37,316
17	Loamy sand	3.75 ± 0.43	93.75	151	38,157	1457	0	39,765
32	Loamy sand	3.75 ± 0.43	93.75	102	30,787	1405	0	32,294
19	Loamy sand	4 ± 0	100	114	30,228	2492	0	32,834
18	Loamy sand	4 ± 0	100	127	32,363	1437	0	33,927
35	Loamy sand	4 ± 0	100	131	31,148	1491	2	32,772
33	Sandy	4 ± 0	100	211	37,455	3640	0	41,306
27	Loamy sand	4 ± 0	100	100	30,515	2462	0	33,077
26	Loamy sand	4 ± 0	100	187	31,356	1487	0	33,030
39	Sandy	4 ± 0	100	174	36,718	7669	17	44,578
37/2	Sandy	4 ± 0	100	155	38,987	2528	0	41,670
31	Sandy	4 ± 0	100	389	41,018	12,575	3	53,985
4//2	Sandy	4 ± 0	100	166	42,757	1573	2	44,498
34	Loamy sand	4 ± 0	100	103	34,036	1438	5	35,582
5	Sandy	4 ± 0	100	129	44,308	3588	7	48,032
44	Sandy	4 ± 0	100	209	43,817	2554	9	46,589
48	Loamy sand	4 ± 0	100	151	36,834	1701	2	38,688
11	Loamy sand	4 ± 0	100	101	39,789	1448	0	41,338
18/2	Sandy	4 ± 0	100	156	44,393	4172	0	48,721
47	Sandy	4 ± 0	100	177	43,688	15,812	0	59,677
60	Sandy	4 ± 0	100	188	42,540	2773	0	45,501
40/2	Loamy sand	4 ± 0	100	114	34,334	2295	0	36,743
41	Loamy sand	4 ± 0	100	107	37,599	1826	0	39,532
40	Loamy sand	4 ± 0	100	122	35,288	1368	0	36,778
21	Sandy	4 ± 0	100	184	43,585	3229	0	46,998
6 //2	Sandy	4 ± 0	100	177	42,715	2856	0	45,748
50	Loamy sand	4 ± 0	100	218	38,150	1560	0	39,928
22	Sandy	4 ± 0	100	139	41,894	2778	7	44,818
43	Loamy sand	4 ± 0	100	190	35,828	2816	2	38,836
25	Sandy	3.75 ± 0.43	93.75	248	41,196	9491	8	50,943
58	Loamy sand	4 ± 0	100	112	38,005	805	0	38,922
7	Loamy sand	4 ± 0	100	110	34,113	1632	11	35,866
38	Loamy sand	4 ± 0	100	97	37,989	1470	9	39,565
29	Loamy sand	4 ± 0	100	162	33,661	1534	0	35,357
6	Loamy sand	4 ± 0	100	137	34,702	1519	0	36,358
Control 1	Loamy sand	0.25 ± 0.43	6.25	0	0	0	0	0
Control 2	Sandy	0.5 ± 0.5	12.5	0	0	0	0	0

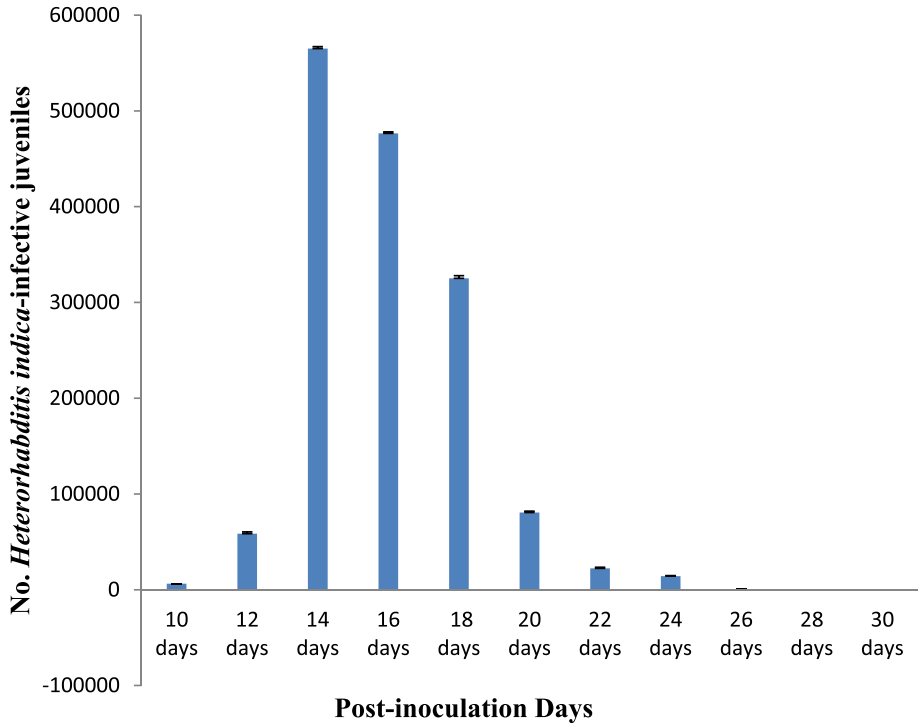

Fig. 1 Total numbers of nematode-infective juveniles for 38 nematode populations emerging from *Temnorhynchus baal* larvae at different days

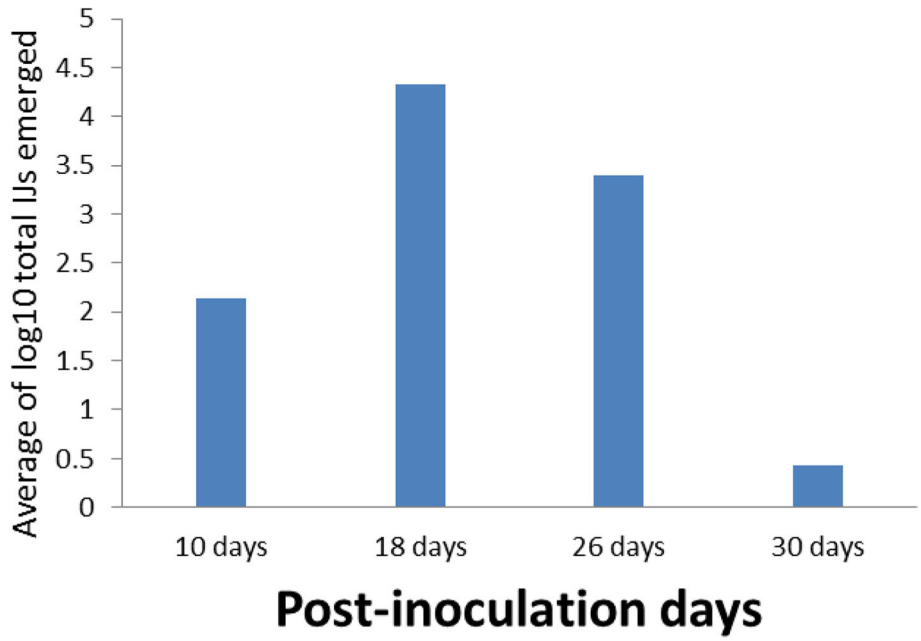

Fig. 2 Average numbers of emerging infective juveniles (IJs)/ *Temnorhynchus baal* larva for 15 *Heterorhabditis indica* populations in sandy soil. Numbers among all post-inoculation days are significantly ($P \leq 0.05$) different according to DNMRT

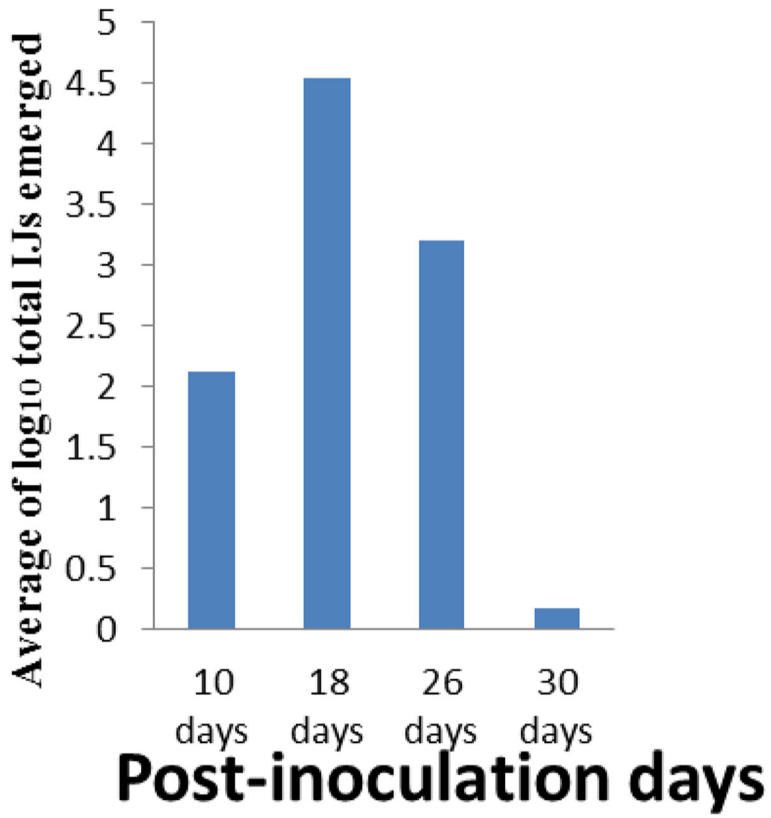

Fig. 3 Average numbers of emerging infective juveniles (IJs)/ *Temnorhynchus baal* larva for 23 *Heterorhabditis indica* populations in loamy sand soil. Numbers among all post-inoculation days are significantly ($P \leq 0.05$) different according to DNMRT

Initially, since a nematode population comprises individuals that inhabit a specific unit of substratum (Caveness 1964), the primary thrust was to examine the novel populations herein to look for any new nematode strain which is a subgroup within a species differing in one or more of such significant traits as virulence and reproductive potential within the third instar larvae of *T. baal*, and/or persistence from the rest of the species. Significant beneficial traits have been recorded to differ among strains within an EPN species, e.g., virulence (Griffin 2015; Baiocchi et al. 2017), reproductive potential (Testa and Shields 2017; Abd-Elgawad 2017a), persistence (Shields 2015), and environmental tolerance (Somasekhar et al. 2002). Therefore, studies to estimate fitness and quality of nematodes reared in *T. baal* such as measuring nematode viability (percentage of emerged living infective juveniles), infectivity (power to invade), reproductive capacity (yield per insect), and virulence (power to kill) (Abd-Elgawad et al. 2012) could upgrade the grub biocontrol.

Also, Somasekhar et al. (2002) examined virulence and reproductive potential among 14 freshly isolated natural populations of *Steinernema carpocapsae* and got significant variations among these traits. However, the present investigation diverged from that of Somasekhar et al. (2002) as the virulence tests targeted a specific pest (*T. baal*) whereas their virulence tests used the factitious host, *G. mellonella*. An economic pest of interest was chosen because superior virulence to *G. mellonella* or any other host would not necessarily indicate superior virulence to the target pest. Shapiro-Ilan et al. (2003) mentioned similar debate on their comparing such beneficial traits among strains of *S. carpocapsae* for control of *Curculio caryae*; another target pest.

Clearly, the 3 beneficial traits investigated herein may expand the potential to control *T. baal* larvae, using indigenous EPNs. Foremost, EPN virulence toward the targeted and most economically important larval stage of *T. baal* on strawberry plants appears to be great (Table 1). Also, reproductive potential, and persistence among local *H. indica* populations were generally valid in the 2 tested soil types, where strawberry cultivation prevails in Egypt. While the foreign EPN *Steinernema glaseri* (NJ strain) proved to have superior efficacy on *T. baal*, such an efficacy had only 50% control or even less when Egyptian isolates were previously used (Shamseldean and Atwa 2004; Atwa 2009 and Atwa and Hassan 2014). In this vein, the native populations tested herein

Table 2 Percentage mortality of *Galleria mellonella* last instar larvae exposed to 38 *Heterorhabditids indica* populations at 4 baiting times post-inoculation

Population no.	1 week	1 month	2 months	3 months	Overall average*
20	93.75	62.5	25	6.25	1.875ghij
16	81.25	25	0	0	1.063abcde
46	87.5	18.75	0	0	1.063abcde
15	68.75	31.25	0	0	1abcd
17	62.5	25	6.25	0	0.938abcd
32	87.5	43.75	12.5	0	1.438efg
19	68.75	37.5	6.25	0	1.125bcde
18	68.75	31.25	6.25	0	1.063abcde
35	37.5	31.25	12.5	0	0.813ab
33	100	68.75	31.25	12.5	2.125ij
27	50	31.25	12.5	0	0.938abcd
26	56.25	18.75	6.25	0	0.813ab
39	87.5	50	31.25	6.25	1.75fghi
37/2	87.5	62.5	25	0	1.75fghi
31	81.25	37.5	18.75	6.25	1.438efg
4//2	81.25	43.75	18.75	6.25	1.5efgh
34	62.5	56.25	12.5	0	1.313cdef
5	93.75	62.5	37.5	18.75	2.125ij
44	100	68.75	31.25	12.5	2.125ij
48	56.25	31.25	12.5	0	1abcd
11	43.75	25	0	0	0.688ab
18/2	81.25	68.75	31.25	6.25	1.875ghij
47	87.5	62.5	31.25	6.25	1.875ghij
60	68.75	43.75	25	0	1.375def
40/2	75	25	6.25	0	1.063abcde
41	50	25	0	0	0.75ab
40	56.25	25	6.25	0	0.875abc
21	100	75	31.25	12.5	2.188ij
6 //2	87.5	62.5	37.5	6.25	1.938hij
50	62.5	25	0	0	0.875abc
22	100	68.75	25	12.5	2.063ij
43	62.5	25	0	0	0.875abc
25	100	75	31.25	18.75	2.25j
58	56.25	25	0	0	0.813ab
7	43.75	18.75	0	0	0.625a
38	56.25	37.5	12.5	0	1.063abcde
29	56.25	31.25	6.25	0	0.938abcd
6	75	50	12.5	0	1.375def

*Average number of infected insects out of four; a cup contained 4 insects with 4 cups (replicates) per a population at each baiting time. Averages followed by same letter(s) are not significantly ($P \leq 0.05$) different according to the Duncan's multiple range test

had good effect comparable to or even better than *S. glaseri* NJ. Successful biocontrol with EPNs should rely on nematode-host matching and economics (e.g., Laznik et al. 2010 and Abd-Elgawad 2017b). Large size of *S. glaseri*, however, reduces its mass-production yield, making this species significantly more expensive to produce than other species. Also, tendency to occasionally "lose" its bacterial symbiont is bothersome. Moreover, the

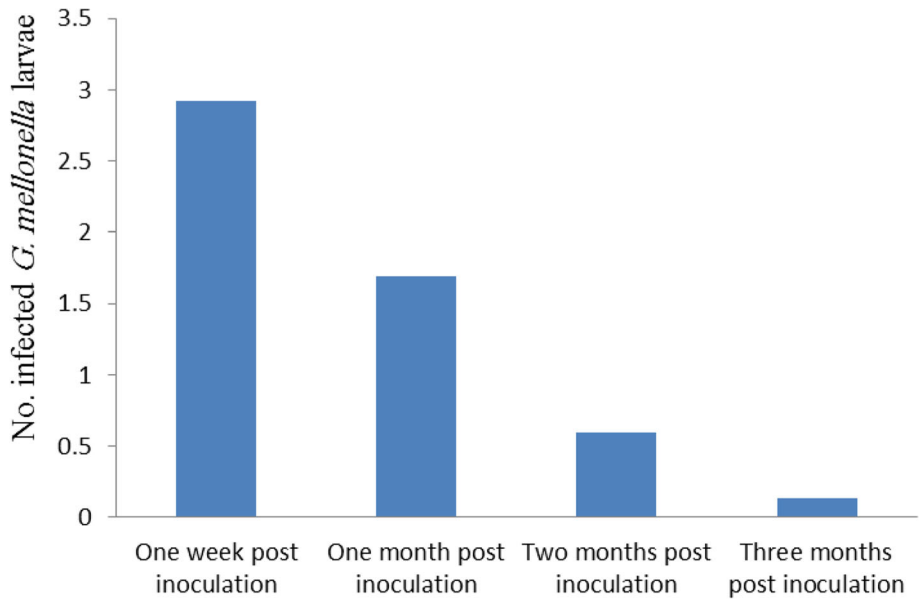

Fig. 4 Average mortality out of four *Galleria mellonella* larvae exposed to 38 *Heterorhabditids indica* populations in soil at increased post-nematode inoculation periods. Numbers among all post-inoculation days are significantly ($P \leq 0.05$) different according to DNMRT

highly active and robust *S. glaseri*-IJs are difficult to contain within formulations that rely on partial nematode dehydration (Shapiro-Ilan and Gaugler 2019). On the other hand, indigenous EPNs should be favored since they are likely more adaptive without any risk to Egyptian fauna and flora. Also, *H. indica* is considered to be a heat tolerant nematode (infecting insects at 30 °C or higher). It has high yields in vivo and in vitro (Shapiro-Ilan and Gaugler 2019). This latter can compensate its short shelf life via local production, which would likely reduce transport, packaging, formulation and storage costs. For *H. indica*, only "fresh" biological agents would be applied, providing improved efficacy.

More research is needed to overcome substantial barriers to nematode control of this scarab pest, especially under field conditions (e.g., Coupland et al. 2017). Also, nematode application methods and costs may be reduced through further improvement of mass production, formulation and delivery technology (MMM et al. 2017).

Conclusions

The beneficial traits, i.e. virulence and reproductive capacity within the third instar larvae of *T. baal*, as well as persistence of *H. indica* populations in soil investigated herein, may expand the potential to control *T. baal,* using indigenous EPNs. Clearly, *H. indica* virulence toward the most economically important larval stage of *T. baal* on strawberry plants appears promising. Reproductive potential and persistence of *H. indica* populations were valid in the 2 tested soil types where strawberry commonly grows. Also, other well-known beneficial traits of *H. indica* would support its local application rather than foreign nematodes. Yet, more research is warranted to overcome barriers to nematode control of this scarab pest under field conditions.

Abbreviations

DNMRT: Duncan's new multiple range test; EPN: Entomopathogenic nematode; IJs: Infective juveniles

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors participated in the development and implementation of the research plan and subsequently written it. All authors read and approved the final manuscript.

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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