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Traits of the entomopathogenic nematode, *Heterorhabditis bacteriophora* (Hb-EG strain), for potential biocontrol in strawberry fields

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Abstract

Entomopathogenic nematodes (EPNs) can provide a substantial control, especially of the root feeding larval pests. The objective of the present study was to examine beneficial traits of an indigenous entomopathogenic nematode species, *Heterorhabditis bacteriophora* Poinar (Hb-EG strain), and to identify superior biocontrol candidates for suppression of the scarab beetle *Temnorhynchus baal* (Reiche & Saulcy) (Coleoptera: Scarabaeidae: Dynastinae) larvae as serious pests of strawberry in Egypt. The nematodes were applied to infect the greater wax moth, *Galleria mellonella* L. cadavers, to represent natural emergence from the host in laboratory and under field conditions. Its average yield was 49604 infective juveniles (IJs) per *G. mellonella* larva. Overall averages of nematode-induced mortality in *G. mellonella* larvae were (4.63, 3.12, and 1.92) at 4, 8, and 12 weeks, respectively, after continuous weekly baiting with 5 cadavers. Nematode capability for infection and reproduction ranged from 72 to 26% one to five months, respectively, after field inoculation of the infected larvae into the strawberry rhizosphere. The number of IJs, moved to the North of the rhizosphere, was significantly ($P \leq 0.05$) less than that moved to the South or the East as reflected by the numbers of infected insects. The factors that may have a marked influence on EPN foraging behavior, persistence, and movement direction with implications for harnessing them as biological pest control agents were discussed.

Keywords: Biological control, Entomopathogenic nematodes, Indigenous traits, Strawberry, Egypt

Background

White grubs (larvae of scarab beetles) are serious pests of strawberry (Shehata et al. 2019). Control recommendations for the white grubs currently consist solely of applications of chemical insecticides (El-Shemy et al., 2013). Due to the health hazards and environmental pollution problems associated with such chemicals, research on developing alternative and safe control strategies is desperately needed (Trdan et al. 2019). Entomopathogenic nematodes (EPNs), in the two families Steinernematidae and Heterorhabditidae, are potential environmentally friendly biological alternatives.

They possess virtually many attributes of ideal biological control agents. Dozens of important insect pests are susceptible yet non-target populations are mostly undisturbed (Abd-Elgawad et al. 2017). Despite their high pathogenicity to insects, their lack of threat to the environment facilitated their commercialization without registration requirements in many countries.

Therefore, a number of studies have examined the persistence of these biological control agents (Khan and Javed 2018 and Shehata et al. 2019). Interestingly, to survive in the native soil of the tested strain, EPNs possess a motile infective stage (IJs), which occupies soil habitats until they infect a host. Thus, critical points in the EPNs' life history are their reproductive capacity within the host, persistence, dispersal, and movement direction.

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Such beneficial traits have been reported to differ among strains/species (Bal et al., 2017; Hussaini 2017 and Ruan et al. 2018). Their dispersal has been studied in terms of biotic and abiotic factors that affect nematode travel within the soil profile, but a dearth of information related to foraging patterns and movement direction, particularly in the absence of their hosts' remains.

The objective of the present study was to examine beneficial traits of an indigenous strain of *Heterorhabditis bacteriophora* Poinar that has a high potential for biocontrol of white grub in the strawberry fields.

Materials and methods

Insect culture and nematode strain

Last instar larvae of the greater wax moth, *Galleria mellonella* (L.), reared on artificial diet according to Abd-Elgawad (2001), and were obtained from the Nematology Laboratory (National Research Centre, Egypt). *Heterorhabditis bacteriophora* (Hb-EG strain) was obtained from USA-Egyptian Mutual Research Project. The nematode strain was reared at ≈ 25 °C in the *G. mellonella* larvae according to procedures described in Woodring and Kaya (1988).

Laboratory tests

Heterorhabditis bacteriophora-infected *G. mellonella* larvae were also used for soil inoculation under laboratory and field conditions. To prepare cadavers, 400 healthy *G. mellonella* last instar larvae were equally released into 20 12-cm petri dishes (sterilized with 70% alcohol and lined with filter paper): 20 larvae per dish. One milliliter of nematode suspension of the Egyptian strain was sprayed directly into the dish, using a plastic syringe; the nematodes were counted so that about 200 infective juveniles (IJs) were used per each insect larva. All sprayed dishes were incubated at 25 ± 1 °C for 3 days. Then, 200 infected larvae, which appeared to have active infection, immobile with reddish-brown color, were selected. These selected larvae were divided into 3 groups, i.e., 20, 30, and 150 infected hosts (*G. mellonella*). The first 20 cadavers were placed singly on 20 White traps (White, 1929) at the rate of one cadaver per 5-cm petri dish to measure the reproductive capacity (nematode yield per insect) of Hb-EG strain (Woodring and Kaya 1988). The mean number of IJs emerged from *H. bacteriophora*-infected *G. mellonella* larvae was compared with IJs for each of 2 groups, comprising 23 and 15 *H. indica* populations emerging from the scarab grub, *Temnorhynchus baal* (Reiche & Saulcy) (Coleoptera: Scarabaeidae: Dynastinae) in a previous study (Shehata et al. 2019), using the two-tailed Student's *t* test. Transformed nematode counts, $\log_{10}$ (X), were subjected to the analysis

because using original counts of IJs with aggregated distribution violates assumptions necessary for parametric statistical analysis (Ferris, 1974).

Other 30 cadavers were used to measure nematode persistence in a laboratory test. The soil was transferred to the laboratory from a strawberry field at El-Dare Village, Tokh Centre, El-Qalioubia governorate, Egypt, using a hand shovel up to 10 cm of soil depth. The soil samples were sterilized in the oven at 320 °C for 1 h to kill all live organisms; thereafter, all samples were moistened by addition of 200 ml of distilled water for each kg of the soil. Experimental units consisted of plastic semicone cups (12.8 cm diam., 9 cm deep) filled to a depth of ≈ 8 cm with the loamy sand (sand 82.8, silt 6, clay 11.2, pH 7.8, CaCO3 13.8%, OM 8.7%) soil (about 1 kg/cup). Two of the infected larvae were placed randomly within each of 15 cups at 5 cm away of the soil surface and kept at 25 ± 2 °C and 70% RH during the extraction cycles. Ten days after adding the infected insects, 5 live last instar *G. mellonella* larvae per cup were similarly added and examined after 1 week in order to (i) isolate any of the 5 added insects, which showed symptoms of EPN infection. These cadavers were transferred to White traps to fulfill Koch's postulates (Pelczar and Reid, 1972) and/ or (ii) discard the other dead insects. A new *Galleria* baiting cycle began with replacing the infected cadavers/ dead insects by new living *G. mellonella* larvae. Examining larval mortality to be replaced by living ones occurred weekly for 12 weeks. Suspect cadavers that failed to produce infectives were considered negative. An analysis of variance (ANOVA), followed by Duncan's new multiple range test (DNMRT), was used to compare the weekly data of nematode persistence among the 12 weeks. Also, data of the 4 consecutive weeks were pooled together so that mean numbers of infected larvae after 4, 8, and 12 weeks (almost 1, 2, and 3 months) of exposure in the soil were also compared.

Field experiments

Likewise, nematode persistence was determined by measuring mortality of *G. mellonella* larvae over time in the above-mentioned strawberry "cv. Festival" field (longitude of 31.21° North latitude, 30.18° East longitude, and 180 feet elevation). The strawberry was transplanted on beds on 20 September 2018. Each bed (25 m in length, 95 cm in width, 25 cm height) had 2 longitudinal tubes to irrigate 4 strawberry rows. The field was not previously treated with any fumigants or insecticides. It had previously negative samples for EPNs. Plants in a row were spaced 20 cm apart. All agricultural practices were carried out as recommended (El-Shemy et al. 2013). On 7 November 2018, 30 consecutive strawberry-planted beds were selected. Ten infected larvae of the remaining 150 ones were equally placed inside every

other selected bed, i.e., 15 beds (2 larvae per a root system of every other plant in a randomly selected row of a bed). Six days post EPN infection, the infected larvae were inoculated at 10 cm deep in the rhizosphere soil where each 2 larvae were placed in the 2 opposite directions around a root system. So, inoculated root systems were spaced 40 cm apart (10 larvae/1.6 m long row in each bed). Five soil samples were taken from each bed (a sample per an inoculated root system) at 1, 2, 4, and 5 months after inoculating the cadavers into the field beds. Each 0.5 kg sample was put in cups of half the size of the above-mentioned ones, baited with 5 live *G. mellonella* larvae per cup and subjected to the similar above-mentioned laboratory conditions and procedures. The larvae, which had EPN infection after a week of baiting, were recorded and transferred to White traps to confirm infection.

Five of the inoculated beds that had the highest insect mortality rates, due to EPN infection after one month of inoculation, were selected for exploring the direction of EPN movement in the soil after insect inoculation. Additional 4 samples were taken per an inoculated plant from the 4 directions (South, North, West, and East), each about 5–15 cm away from the center of the root system at 5–15 cm depth. Three inoculated plants were randomly selected for sampling per bed by hand shovel at about 5, 8, and 16 weeks post insect inoculation. At each time, 0.5 kg sample was put in a cup (12.8 cm diam., 4.5 cm deep) with 5 live *G. mellonella* larvae and transferred to the laboratory as mentioned above. So, 12 cups (samples) per bed (3 plants × 4 directions per each plant) were taken. The larvae which had EPN infection after a week of baiting were similarly transferred to White traps. Means of the infected larvae for each of the

above-mentioned samples were calculated and used as an indicator for persistence and direction in movement of Hb-EG strain in the soil under strawberry field conditions. Similar statistical comparison among the means was also done.

Results and Discussion

The average (± standard deviation) yield of IJs per *G. mellonella* larva was 49604 ± 20257 herein. The corresponding average of 15 populations (Shehata et al., 2019) related to Egyptian *H. indica* IJs emerged from the infected scarab grub, *T. baal* (Coleoptera: Scarabaeidae), in sandy soil, 46,960 IJs/grub, was not significantly ($P \leq 0.05$) different from that of Hb-EG strain reported herein ($t = 0.99$; $df = 33$). Yet, the average (36,502 IJs/grub) of other 23 Egyptian *H. indica* populations (Shehata et al. 2019) in loamy sand soil was significantly different ($t = 2.05$; $df = 41$) from that of Hb-EG strain reported herein. However, when these 2 groups of populations were combined together (38 populations), the average (41,000 IJs/grub) of their released IJs was lower ($P \leq 0.05$) than that of Hb-EG strain researched herein ($t = 4.05$; $df = 56$). This difference is possibly due to the used insect host, EPN species/strain, reproductive capacity, and/or genetic constitution, given the other aspects of the general identical procedure applied in the laboratory for IJs extraction and counting.

Insect mortality caused by Hb-EG strain was the greatest after the first baiting, but began to decrease over time (Fig. 1). Interestingly, some successive weeks had significant differences among numbers of the infected host such as weeks 5, 6, and 7, contrary to weeks 8, 9, 10, and 11. This might be due to the relatively low numbers of IJs over time. Overall averages (± standard
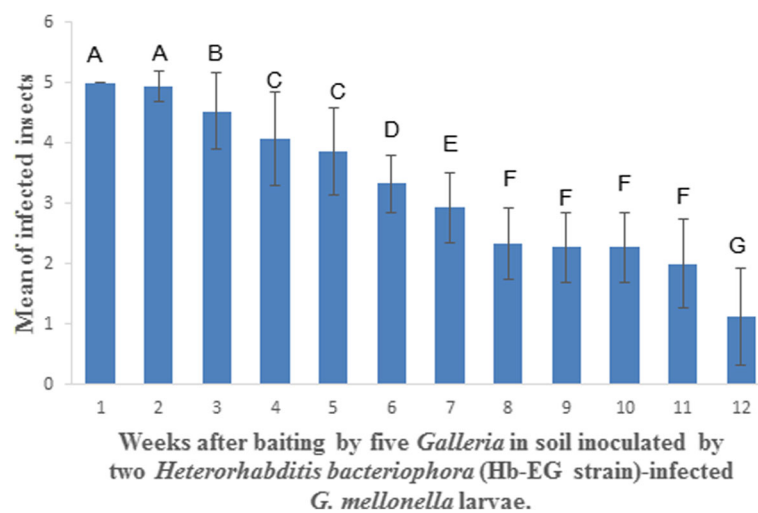

Fig. 1 *Heterorhabditis bacteriophora* (Hb-EG strain) persistence measured by ability to infect *G. mellonella* larvae in soil at extended baiting times. Means (± SD) of five insects per cup for 15 replicates (cups). Different letters above bars indicate statistical significance ($P \leq 0.05$) using DNMRT

deviations) of nematode-induced mortality in *G. mellonella* larvae were 4.63 ± 0.36 (for combined 1–4 weeks), 3.12 ± 0.47 for 5–8 weeks, and 1.92 ± 0.62 for 9–12 weeks after the start of baiting, respectively. Their corresponding percentages were 92.67, 62.33, and 38.33%, respectively. Nematode persistence, measured by the ability to infect *G. mellonella* larvae in soil over these baiting times, differed significantly ($P \leq 0.0001$) among the 3 periods. Shehata et al. (2019) also found that the persistence of *H. indica* populations in the soil varied greatly concerning their total IJ numbers collected 10, 18, 26, and 30 days post-inoculation in sandy or loamy sand soil.

In contrast, under field condition, nematode capability for infection and reproduction ranged from 72 to 26%, 1–5 months post its inoculation via the infected wax moth larvae in the strawberry rhizosphere, respectively (Fig. 2). Obtained results showed that 92.7 and 62.3% of the exposed larvae were infected under laboratory conditions compared to only 72 and 50.4% in the field one and 2 months after inoculations, respectively. Generally, such an EPN efficacy gap is usually apparent between controlled and actual conditions due to harsh field events such as changes in soil temperature. Although EPN species/strains differ in their persistence in soil (e.g., Abd-Elgawad 2017a, b; Khan and Javed 2018 and Labaude and Griffin 2018), it is assumed that many biotic and physical factors can affect their persistence too. Biotic factors as availability of host insects (Hussaini, 2017), predation by other organisms such as mites and collembola (Wilson and Gaugler, 2004), and competition with other pathogens including even other EPNs

Fig. 2 *Heterorhabditis bacteriophora* (Hb-EG strain) persistence over 5 months. Means (± SD) of 5 insects/cup (375 insects per 75 root systems; 5 cups per a bed) in 15 strawberry beds. Different letters above bars indicate statistical significance ($P \leq 0.05$) using DNMRT

(Labaude and Griffin, 2018) can play a significant role. Physical factors such as soil mulching (Leite et al. 2015), soil type and moisture (Hussaini, 2017), application pattern of the introduced EPNs (Wilson et al., 2003), ultraviolet radiation, and desiccation and temperature extremes (Baiocchi et al. 2017) can also affect the EPN persistence. So, the significant ($P \leq 0.05$) differences found between beds ($F = 4.41$; $df = 14$) in the infected numbers of insects are likely attributed to one or more of these factors and should be considered for more effective biocontrol potential (Abd-Elgawad, 2019).

Non-significant ($P \leq 0.05$) differences were found among the 5 beds in the number of infected insects at each of the tested time, i.e., 5 ($F = 0.28$, $P = 0.89$), 8 ($F = 0.19$, $P = 0.94$), and 16 ($F = 1.38$, $P = 0.26$) weeks post-inoculation ($df = 4$). When the data of the 5 beds were combined together for statistical analyses, non-significant ($P \leq 0.05$) difference in the mean numbers of infected insects was found between 5th and 8th weeks ($\bar{x} = 0.65$ vs 0.9, $n = 75$) post-inoculation. Yet, each of these numbers differed ($P \leq 0.05$) from the mean number ($\bar{x} = 0.05$, $n = 75$) of the infected insects, 16 weeks post-inoculation. Significant ($P \leq 0.05$) differences were found among the cardinal directions of EPN movement (Fig. 3). The nematodes moved to the North had the least numbers. Their mean number over the 3 tested times was significantly ($P \leq 0.05$) less than the corresponding number moved to the South or the East. Many factors may have a marked influence on EPN foraging behavior and movement direction. Kapranas et al. (2017) found that 2 levels of soil compaction and media with varying ratio of peat: sand could influence dispersal success of 3 EPN species, with different foraging strategies. Increasing peat content and compaction generally decreased IJ dispersal for *Steinernema carpocapsae* (ambusher), *Heterorhabditis downesi* (cruiser), and *S. feltiae* (intermediate). Of the 3 species, the least affected by peat content was *H. downesi*, whereas *S. carpocapsae* was the most adversely influenced by compaction. They found that dispersal of male and female IJs is also differentially affected by soil parameters and that this differentiation is species-specific. Other studies revealed that the EPNs respond directionally to insect host exudates (Dillman et al., 2012), plant volatiles (Jagodič et al., 2017), carbon dioxide (Ramos-Rodríguez et al. 2007), vibration (Torr et al. 2004), and magnetic and electric fields (Ilan et al. 2013). Likewise, various biotic and abiotic factors can affect movement behavior (Perony and Townsend, 2013) such as group size (Bonnell et al. 2013), environmental spatial structure and resource availability (Reeve and Cronin 2010), avoidance of predators (Beauchamp, 2017) and genetic diversity (Johnson et al. 2016). Bal and Grewal (2015) found that the cruise foraging *H. bacteriophora* differ in its dispersal and foraging behavior from
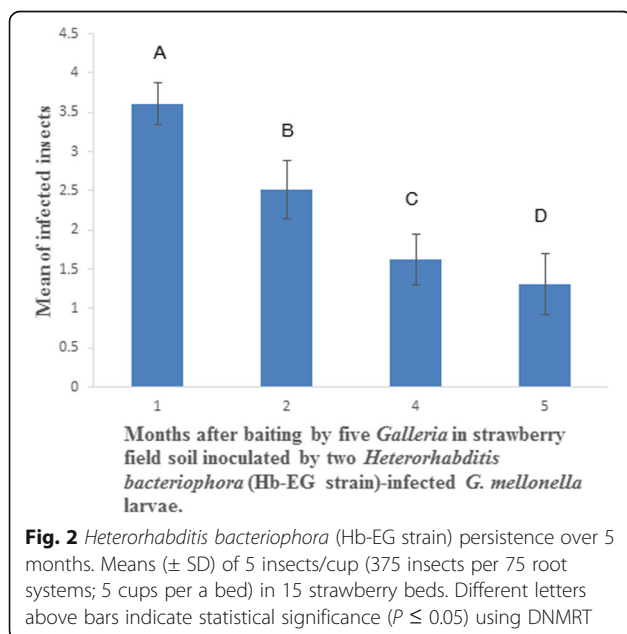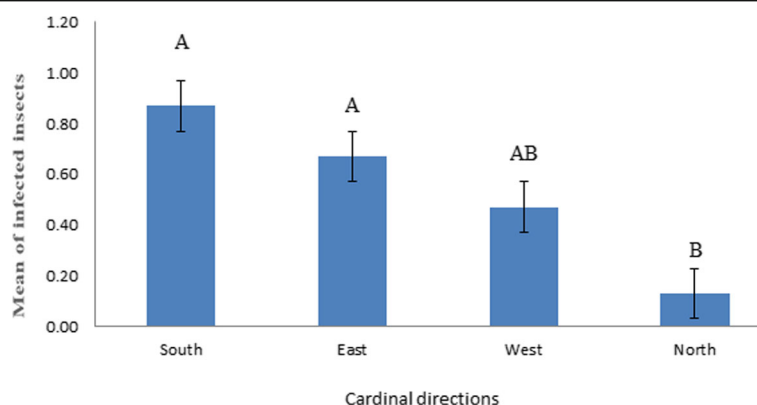
Fig. 3 Mean mortality of *Galleria mellonella* larvae (75 insects per 15 root systems in 5 strawberry beds for each direction in each of 3 times, a total of 900 baiting insects). Different letters above bars indicate statistical significance ($P \leq 0.05$) using DNMRT

ambush foraging *S. carpocapsae* at the population level, and this behavior is affected by both the presence and absence of hosts and by their mobility. Ruan et al. (2018) reported that EPN dispersal continuously exhibited an aggregative pattern (independent movement was not observed). Given the narrow experimental range examined herein in addition to many unmeasured, hidden, physical, and biotic variables affecting EPN numbers, viability, and movement/dispersal, further studies on the effect of various edaphic and biotic factors on EPN efficacy and application for biological control are warranted with implications of using them for harnessing EPNs as biological pest control agents.

Conclusions

A beneficial trait of an indigenous nematode, *Heterorhabditis bacteriophora* Poinar (Hb-EG strain), was examined in various scenarios and compared with other heterorhabditis nematodes for the suppression of the scarab beetle larvae attacking strawberry plant roots in Egypt. The persistence of Hb-EG strain reported herein was significantly better than that of 23 Egyptian *H. indica* populations but did not differ from other 15 Egyptian *H. indica* populations. The infected insects at the North of the rhizosphere were significantly ($P \leq 0.05$) less than that moved to the other directions. Further research is required to determine the cause of such trends as the variability in nematode persistence and cardinal directions as well as the applicability of these findings under different field conditions.

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

Author's contributions
Dr. Fahiem E. El-Borai helped Dr. Larry Duncan in the parts of work done at University of Florida.

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

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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