Journal of Mosquito Research, 2013, Vol.3, No.5, 33−44

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Research Report **Open Access**

Efficacy of the Steinernematid and Heterorhabditid Nematodes for Controlling the Mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Journal of Mosquito Research, 2013, Vol.3, No.5 doi: 10.5376/jmr.2013.03.0005

Received: 12 Jan., 2013

Accepted: 23 Jan., 2013

Published: 28 Feb., 2013

Preferred citation for this article:

Zohdy et al., 2013, Efficacy of the Steinernematid and Heterorhabditid Nematodes for Controlling the Mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), Vol.3, No.5 33-44 (doi: 10.5376/jmr.2013.03.0005)

Abstract Entomopathogenic nematodes can be considered effective biocontrol agents of pest insects in aquatic habitat. Larvae of *Culex quinquefsciatus* Say were exposed to infective juveniles of *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, and *S. feltiae* under laboratory conditions. The bioassay studies revealed the suppressive role of *H. bacteriophora* and *H. indica* nematode in controlling the mosquito, *C. quinquefasciatus*. They successfully established themselves in the host cadaver and produced infective juveniles. On the other hand, both *S. carpocapsae* and *S. feltiae* failed to establish in the host larvae or attain significant host mortality values. This is the first report of parasitism of entomopathogenic nematodes isolates from Egypt against larvae of *C. quinquefasciatus*, with promising results. Therefore, further studies must be carried out to determine if these nematodes would be effective as autochthonous agents for the control of *Culex* sp. and other mosquitoes of sanitary interest.

Keywords *Culex quinquefsciatus*; *Heterorhabditis bacteriophora*; *H. indica*; *Steinernema carpocapsae*; *S. feltiae*; bioassay

1 Introduction

Blood-feeding insects, including mosquitoes, are of medical importance because they vector many parasitic and viral diseases of humans and animals. The mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) is the vector of the filarial parasite *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae), which causes human bancroftian filariasis and also an avian malaria parasite.

Numerous studies have been undertaken for unconventional control agents owing to the hazards of conventional pesticides. Several assays were carried out using, for instance, mermithid nematodes against larvae of *Culex* sp. and other genera (Santamarina, 2000; Achinelly et al., 2004 and Perez-Pacheco et al., 2004). Entomopathogenic nematodes (EPN) belonging to the families, Heterorhabditidae and Steinernematidae have been used against insects and arthropods of medical and veterinary significance, including Cat flea (Silverman et al., 1982), spiders (Poinar, 1989), flies, mosquito larvae and black flies (Begley, 1990), body louse (Weiss et al., 1993), ticks (Zhioua et al., 1995), and head louse (De Doucet et al., 1998). The advantage of EPN over the mermithid nematodes is that the former can be grown in vivo in alternate hosts or in vitro on artificial media such as dog food agar (Friedman, 1990). A second advantage is the longevity of the infective stages (3~6 months in water at room temperature) and it is also characterized by short life cycle. They were made exempt from registration and regulation requirement by the US Environmental Protection Agency (EPA) (Grewal et al., 2005).

The infective juveniles (ijs) of those nematodes belonging to the families Heterorhabditidae and Steinernematidae carry cells of the symbiotic bacteria in their intestine belonging to the genera, *Photorhabdus* and *Xenorhabdus*, respectively (Grewal & Georgis, 1998). Upon encountering a susceptible host, the infective stages of both genera enter the host's body via the natural body openings (mouth, anus, spiracles), and then penetrate into the body cavity where they initiate development. The bacteria are released from the infective juveniles soon after the nematode initiated development in the host where they multiply and the

host insect dies rapidly within 2~3 days (Poinar, 1990; Wang and Bedding, 1996). Upon death of the insect, the bacteria digest the host tissues, providing nourishment needed for the nematode to successfully reproduce and provide conditions for the nematode multiplication (Burnell and Stock, 2000; Park et al., 2004). In addition, the bacterial cells produce antibiotics that protect the host cadaver from saprophytes and scavengers (Boemare, 2002; Hazir, 2004). The infective juveniles of the next generations leave the cadaver and enter the environment seeking for infecting new host (Kaya and Gaugler, 1993).

Studies on the potential of the entomopathogenic nematodes for the control of mosquitoes are rare. Welch & Bronskill (1962), Dadd (1971) and Poinar & Kaul (1982) were the first to study the potentiality of the rhabditoid nematode and its associative bacterium against mosquitoes. Recently, a laboratory colony of the mosquito, *C. apicinus* was parasitized by the nematode *S. rarum* (Cagnolo & Walter, 2010).

The fitness of a parasite can be adversely affected by increasing population density within the host. This has been noted in vertebrate intestinal helminthes, and mermithid nematodes in mosquito larvae. Density dependent effects on parasite include reduced fecunddity, increased mortality, change in sex ratio, reduction in adult size, increased generation time and reduced oviposition (Selvan & Muthukrishnan, 1988). Although all entomopathogenic nematodes have the same general life histories, species difference in host utilization (Selvan & Blackshaw, 1990) and reproductive strategies (Poinar, 1990) may influence the nematode response to increasing density.

The present investigation aimed to evaluate and compare virulence of four nematode species belonging to the genera, *Heterorhabditis* and *Steinernema* against *Culex quinquefasciatus* larvae and to estimate the different factors that can improve the beneficial traits of the infective juveniles.

2 Materials & Methods

2.1 Insects

The mosquitoes, *Culex quinquefasciatus* were kindly supplied by Prof. Dr. Fatma Kamel Adham, Faculty of Science, Cairo University, Giza, Egypt.

2.2 Nematode isolates

The nematode isolates representing four species, *Heterorhabditis bacteriophora* (HB), *Heterorhabditis indica* (HI), *Steinernema carpocapsae* (SC), *Steinernema feltiae* (SF) were supplied by Prof. Dr. Muhammed Mostafa Shamseldean, Zoology and Nematology Department, Applied Center of Entomonematode, (ACE), Faculty of Agriculture, Cairo University, Giza, Egypt. They were mass reared according to Bedding & Akhurst (1975) using last instar larvae of the greater wax moth, *Galleria mellonella*.

2.3 Susceptibility to EPN

The one-on-one assay according to Miller (1989) was used with some modifications related to the water habitat of the mosquito larvae. *C. quinquefasciatus* 4th instar larvae were placed individually in a 1.5 cm diameter plates (24-well plates). Each well contained only one infective juvenile of the four tested nematode species in distilled water. Each treatment consisted of 20 replicates and repeated 3 times (n=60). The plates were incubated at 25℃ in the darkness. Mortality records were taken after 72 h and corrected according to Abbot's formula (Abbott, 1925) versus a control treatment contained only *Culex* larvae in distilled water.

2.4 Infective juvenile virulence

The virulence of the infective juveniles of *H. bacteriophora* and *H. indica* was estimated in relation to:
a- The exposure period. b- Concentration of the infective juveniles (Dose res- ponse).

2.5 Exposure period assay

In this assay, both of nematode invasion ability and host larval mortality were evaluated in response to varying exposure periods. Fifty 4th instar mosquito larvae were exposed to infective juveniles of the different nematode species in 9 cm diameter Petri-dish half filled with distilled water containing the desired infective juvenile concentration. There were three replicates per treatment. Different batches of nematodes and insects were used for each replicate. Controls contained only mosquito larvae in distilled water. 1500 infective juveniles of *H. bacteriophora* and *H. indica* nematode were incubated at 25℃ in the dark with host larvae for 6,9,12,24 and 48 hr. After each exposure time, the insect larvae were washed from nematode and

transferred to new Petri dishes and were incubated for further 72 hr. The Percentage of larval mortalities was determined and the number of nematodes present in each cadaver was determined by dissection after 3,6,9 and 12 hr according to Mauleon et al.(1993).

2.6 Dose response assay

In the present assay, the effect of different concentrations of infective juvenile nematodes of *H. bacteriophora* and *H. indica* on the mortality of 4th instar mosquito larvae was monitored and LC_{50} values were calculated for both nematode species. The nematode concentrations used include 0 (control),100,150,200, 250 and 300 ij/larva. The insect mortality was recorded 48 hr post-inoculation.

To study the effect of host larval instar on infection by nematode and hence the resulted host mortality, the experiment was repeated using younger host instar larvae (2nd, 3rd) using the infective juveniles of *H. bacteriophora*.

2.7 Nematode fecundity

Infective juvenile production was used as an indirect measure to first-generation nematode fecundity. The used nematode concentrations were (50,100,150,200, 250 and 300 ij/larva). Host cadavers (three replicates) were transferred to individual White dishes (Woodring & Kaya, 1988). All infective juveniles that emerged from a single host at 48, 72 and 96 hr post exposure were collected and counted.

The first generation of *H. bacteriophora* and *H. indica* within the host is represented by hermaphrodites. Because body length is correlated with fecundity (Selvan et al., 1993), the total length of 12 hermaphrodites at variable infective juvenile concentrations (50, 100,150,200,250 and 300 ij/larva) was measured at 48, 72 and 96 hr post exposure. The control value (0 nematode in host) is based on hermaphrodite length that emerged from the rearing host, *Galleria mellonella* larvae. Since both species showed similar recovery pattern, only *H. bacteriophora* was selected in this assay. Infective juvenile length was also measured for each concentration. Prior to measurements, nematodes were heat killed on a glass slide.

2.8 Statistical analyses

In bioassay studies, host larval mortalities were calculated according to Abbott (1925). Values of LC_{50} and LT 50 were calculated according to Finney (1971) using Probit analysis (SPSS, v11). Hermaphrodite length and female fecundity were analyzed by general linear models procedures. There were five replicates per treatment.

All comparisons were made at the 0.05 level of significance. Data were presented as means ± standard error. Comparisons by means were done by student t-test and one-way analysis of variance.

3 Results and Analysis

3.1 Susceptibility to EPN

A significant increase in host larval mortality was achieved by using both *Heterorhabditis* species, where *H. bacteriophora* was significantly more virulent than *H. indica* ($P \leqslant 0.05$) (Figure 1). Both *Heterorhabditis* species raised the host mortality levels above the normal control values by a magnitude of 2~4 times. In contrast, the mortality levels did not exceed 7% in case of using both *Steinernema* strains, with no significant differentce in host mortality between both strains ($P \geqslant 0.05$). The host mortality levels achieved by applying both *Steinernema sp*. were nearly doubled when *H. indica* was used. In the same time, *H. bacteriophora* was proved to be the most virulent species, where the recorded host mortality levels were 2 times the mortality values ach-

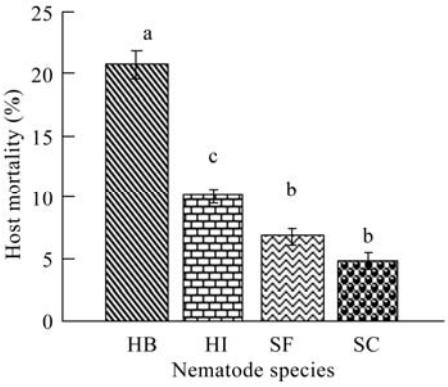

Figure 1 The percentage mortality of *C. quinquefasciatus* larvae following exposure for 72 hr to individual ijs of *H. bacteriophora* (=HB), *H. indica* (=HI), *S. carpocapsae* (=SC) and *S. feltiae* (=SF) in the one-on-one assay. Means with the same letter are not significantly different ($P \geqslant 0.05$)

ieved by *H. indica* and 3~4 times the mortality values achieved by *S. feltiae* and *S. carpocapsae*, respectively.

When using *Steinernema* species, nematode individuals were melanized and were obviously seen through the host cuticle (Figure 2). In contrast, *Heterorhabditis* species successfully completed their life cycle within the host larvae till adult stage (Figure 3) and infective juvenile emergence. For this reason, only *Heterorhabditis* species were used to estimate their role in controlling the mosquito larvae.

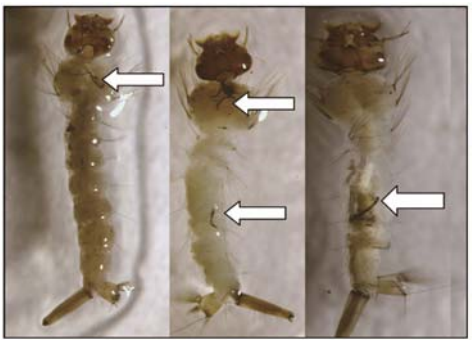

Figure 2 *Culex quinquefasciatus* larvae showing the melanized *Steinernema carpocapsae* nematode in thorax and abdomen

Figure 3 *Culex quinquefasciatus* larvae showing the adult stage of *Heterorhabditis bacteriophora* in head and thorax (showed by an arrow)

3.2 Exposure period assay

Increasing the exposure period from 3~12 hr resulted in a significant increase in nematode entry to the host in both *H. bacteriophora* and *H. indica* (r=0.97, P≤ 0.01), (Figure 4), but the difference between numbers of *H. bacteriophora* juveniles and those of *H. indica* was not quite significant during the first 3 hr of incubation (P≥0.05). The difference between them becomes more pronounced as the duration of exposure increased from 6~12 hr (P≤0.01) ; where the number of the infective juveniles that entered the hosts was doubled.

Considering host mortalities, it was found that, increasing the exposure period resulted in a significant increase in host mortality (r=0.9, P≤0.01). Also, it is worthy to mention that, at all of the tested exposure periods, *H. bacteriophora* caused significantly higher host mortalities than those obtained by using *H. indica* (Figure 5). After 6 hr, the recorded host mortality values due to *H. bacteriophora* infection were nearly double the mortality levels recorded due to *H. indica* infection. By increasing the exposure time from 6 to 24 hr, the increase in host mortality became gradual in both nematode species. Maximum mortality (96.0%, 80.0%) was achieved when insects were exposed to nematodes from the species *H. bacteriophora* and *H. indica* for 48 hr, respectively (Figure 5).

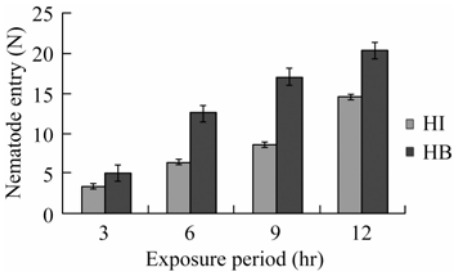

Figure 4 The effect of the exposure of *C. quinquefasciatus* larvae to 300 ijs of *H. bacteriophora* (=HB) and *H. indica* (=HI) per larva for different time periods on the average number of nematodes found in the insect cadaver (=N)

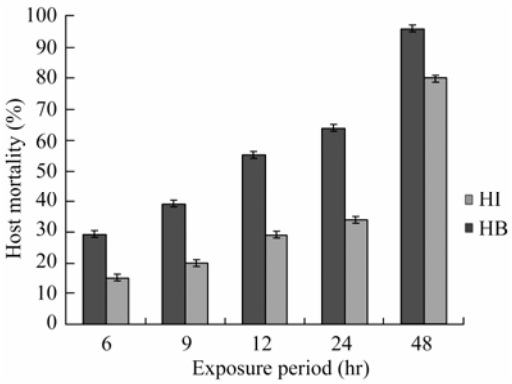

Figure 5 The effect of the exposure of *C. quinquefasciatus* larvae to 300 ijs of *H. bacteriophora* (=HB) and *H. indica* (=HI) per larva for different time periods on percentage host larval mortality in the exposure period assay

Considering lethal time, it was found that *H. bacteriophora* was proved to be a faster killer than *H. indica*, where the Probit analysis showed a deduced LT_{90}

values for *H. bacteriophora* of 43.12 hr in comparison with 82.64 hr for *H. indica*. It is worthy to mention that 12 hr of exposing the host larvae to infective juveniles of *H. bacteriophora* was sufficient time to kill 50% of the host population. Meanwhile, *H. indica* needed not less than a day to achieve the same control levels.

3.3 Dose response assay

In the present experiment, increasing the infective juvenile nematode concentration of both *H. bacteriophora* and *H. indica* from 100 to 300 infective juvenile/ 4th instar host larva resulted in a significant increase in host mortality (r=0.9, P≤0.01, Figure 6). The increase in host mortality was gradual up to 200 ij/host. Further increase in juvenile concentration (250, 300 ij/host) resulted in a sharp increase in host mortality. The increase in host mortality was significant only when 300 infective juvenile of both species were used per host larva, where the maximum host mortality levels were 96.0%, 80.0%, respectively. The LC_{50} value was 121.5 ij/host for *H. bacteriophora* as compared with 141.7 ij/host for *H. indica*.

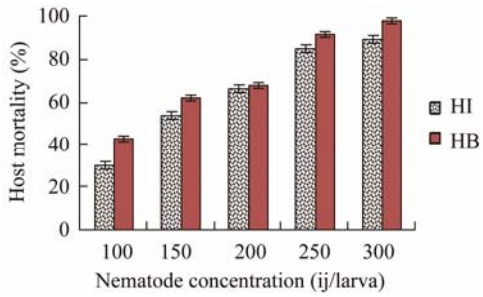

Figure 6 The percentage mortality of 4th insatr *C. quinquefasciatus* larvae following exposure to different concentrations of ijs of *H. bacteriophora* (=HB) and *H. indica* (=HI)

The experiment was repeated using *H. bacteriophora* only and 2nd and 3rd instar larvae as host to detect the effect of host instar on the control process (Figure 7).

Increasing nematode concentration from 100 to 300 ij/ larva resulted in significantly increasing the levels of mortality in 3rd and 4th larval instars (r=0.9, P≤0.05). By using 2nd instar mosquito larvae as hosts; no significant change in larval mortality was recorded by increasing the infective juvenile concentration from 100 to 300 (P≥0.05).

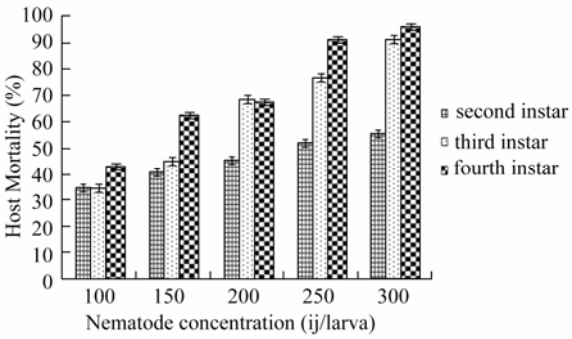

Figure 7 Percentage mortality of second, third, and fourth-instar larvae of *C. quinquefasciatus* at different ij concentrateions of *H. bacteriophora*. Mortality was recorded after 48 hr of incubation

The effect of increasing host instar from 2nd to 3rd instar was delayed up to 200 ij/larva, where there was no significant difference between 2nd and 3rd instar larval mortalities at lower nematode concentrations (P≥0.05). The 3rd and 4th instar larvae were more susceptible to infection than 2nd instar and the recorded host mortalities were 90.9%, 96.0% and 55%, respectively.

Considering LC_{50}, it was found that 121.5 ij/larva and 143.5 ij/larva were sufficient to kill 50% of 4th and 3rd instar larval populations, respectively. Meanwhile, to achieve 50% decreases in population of 2nd instar larvae, 231.8 ij/larva were needed.

Since both of the tested *Heterorhabditis* species completed their life cycle within the mosquito larvae, the nematode recovery was measured for both species as an indication of the ability of the nematode species to persist and multiply in this host. The infective juvenile production (recovery) for both species increased with increasing initial infective juvenile concentration up to approximately 200 infective juveniles/ host (r=0.9, P≤0.01), where a total average of 68.3 and 97.1 infective juveniles of *H. bacteriophora* and *H. indica*, respectively, were produced (Figure 8). The maximum number of infective juveniles produced, was 136.6 ij/host for *H. bacteriophora* (df=14, F=37.77, P≤0.01) and 134.3 ij/host for *H. indica* (df=14, F= 42.25, P≤0.01). Increasing nematode concentration to more than 200 ij resulted in a decline in nematode recovery (r=−0.9, P≤0.01, Figure 8). However, the number of infective juveniles produced per host dropped to levels comparable with low initial densities when nematode initial densities were raised to 300.

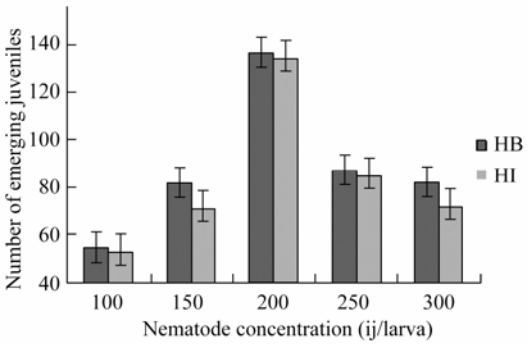

Figure 8 Mean number of individual infective juveniles of *H. bacteriophora* (=HB) and *H. indica* (=HI) emerged from a *C. quinquefasciatus* cadaver as a function of changing initial nematode density

Generally, both species were similar in their recovery from cadavers as they were both interacting with the same manner to increasing nematode concentration/ host with no significant difference between numbers of their produced juveniles ($P \geqslant 0.05$).

The effect of increasing the initial nematode concentration of *H. bacteriophora* on the length of the resulted hermaphrodites (produced at the first generation within the host) was determined. Since both species showed similar recovery pattern, only *H. bacteriophora* was selected in this assay. Changing concentration of nematodes/host resulted in a significant change in hermaphrodite length after 48, 72 and 96 hr of exposure. Adult length increased with increasing initial nematode concentration up to 150 infective juvenile/host then declined with further increase in nematode concentration, $P \leqslant 0.05$ (Figure 9).

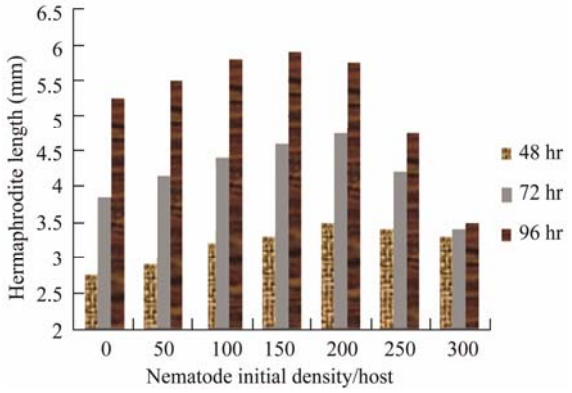

Figure 9 Mean adult hermaphrodite length of *H. bacteriophora* at 48, 72 and 96 hr post exposure

Initially, after 48 hr of exposure, only hermaphrodites at low densities (below 100) were small. The effect of high density on hermaphrodite length was delayed till 72 and 96 hr. The growth rate was nearly constant during the 1st 2 days and did not change by changing initial nematode concentration, i.e. it is not affected by crowding yet. Further increase in concentration did not negatively affect the adult length after 2 days; i.e. the nematodes can tolerate this level of crowding. After 48 hr, the adult length (3.3 mm) was still greater than those of control (2.76 mm) even at maximum nematode density of 300. The maximum adult length was 3.4 mm at nematode concentration of 200.

The effect of crowding was obvious after 3 to 4 days post infection, where the adult length changed signifycantly upon increasing nematode concentration from 0 to 250 and decreased to a size smaller than that of control at 300 nematode/host. The maximum adult length was 4.7 mm and 5.9 mm when using 200 and 150 ij/host after 72 and 96 hr, respectively.

After 3 days of infection, adults were significantly shorter when concentration of nematodes infective juveniles was raised to 250 and 300. Raising the concentration from 50 up to 200 resulted in a gradual increase in adult length reaching maximum length at 150~200 nematodes/host, after which adult length decreased sharply.

After 72 and 96 hr, further increase in initial nematode density resulted in a significant decrease in hermaphrodite length comparable to control levels after 72 hr (3.7 and 3.4, respectively) and less than control after 96 hr (3.4 and 5.3, respectively) ($r=-0.9$, $P \leqslant 0.01$).

Increasing initial nematode concentration up to 300 ij/host resulted in a decrease in the length of the emerging juveniles of the 1st generation that were migrating out of the cadaver. Maximum infective juvenile length (651.6 μm) for *H. bacteriophora* occurred at low densities (50 nematode/host), not at the densities producing the maximum number of infective juveniles (Figure 10). Infective juvenile length declined sharply upon treatment until initial densities of 200 for, approximately 546.6 μm ($r=-0.97$, $P \leqslant 0.01$). The decline in length was more gradual at higher initial densities ranging from 250 to 300 nematode/host ($P \geqslant 0.05$).

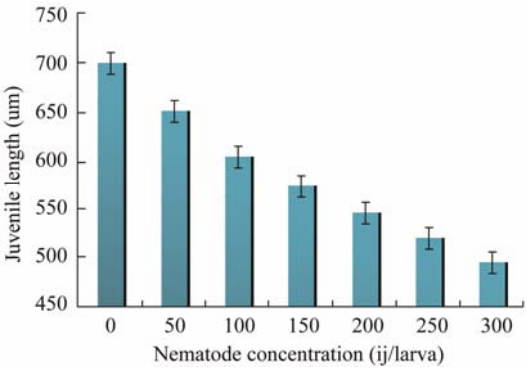

Figure 10 Mean length of individual infective juveniles of *H. bacteriophora* emerged from a *C. quinquefasciatus* cadaver as a function of changing initial nematode density

4 Discussion

Mosquito insecticide-resistance at larval stages is extremely high compared to adult stage. For this reason, another biological, safe, and effective control method is favored over the chemical one. In the present study, the nematode impact in biological control of mosqueitoes was ranked according to various factors affecting the infection process. The exposure period assay indicated, indirectly, how quickly insects were infected by the nematodes. The one-on-one and dose response assays measured the overall infection process in regards to nematode species and doses which affects the control levels as well as the nematode characteristics as fecundity.

In the present study, one-on-one assay indicated that *H. bacteriophora* is more virulent to mosquito larvae than *H. indica* and the recorded mortality values give a promising spot light on the possibility of using both species to control mosquito larvae. Our results were in agreement with the early work of Poinar & Kaul (1982), where they investigated the parasitism of *C. pipiens* by the nematode, *H. bacteriophora* and they recorded high levels of host mortality.

On the other hand, both tested *Steinernema* species failed to establish in the *C. quinquefasciatus* mosquito larvae and were melanized. Bronskill (1962) recorded melanization of Neoaplictanid rhabditoid nematode (DD136) by *A. aegypti* larvae.

In the exposure period assay, both *Heterorhabditis* species entered the host with nearly the same rate during the first 3 hr. Increasing exposure time separated the efficiencies of both species as fast invader (*H. bacteriophora*) and slower invader (*H. indica*). By the end of 12 hr exposure period, *H. bacteriophora* was proved to be still more active than those of *H. indica* and this might be the reason for the higher *H. bacteriophora* numbers that entered the larvae (20 ij) in comparison with (14 ij) in case of *H. indica*. These two parameters (invasion rate and nematode numbers) are closely related, and are considered characteristic for specific nematode species. On the other hand, Abd El Rahman & Hussein (2007) recorded that *H. indica* invaded *G. mellonella* larvae in higher numbers than *H. bacteriophora* only at lower initial nematode concentration while *H. bacteriophora* entered in higher numbers than *H. indica* at high nematode concentrations.

Concerning the effect of exposure period on host mortality, *H. bacteriophora* was found to be a faster killer for *C. quinquefasciatus* (with high invasion rate) than *H. indica*. This was clear from the LT90 values. In this context, *H. bacteriophora* needed 43.12 hr to kill 90% of the host population which is nearly half of the time needed by *H. indica* to attain the same host mortality values. These results support the previous ones concerning nematode entrance as a measure of invasion rate. However, low penetration level and slow invasion rate may not necessarily mean that a nematode has lower efficacy. In the present work, *H. indica* raised the host mortality percentages very efficiently reaching 80%, which is a good indication of virulence towards this host species. This contrast may be attributed to variation in infection strategies. Since the infective juveniles of heterorhabditid nematodes develop into hermaphrodites, a single invader can potentially reproduce. For this reason, low number of nematodes entering to their hosts will be sufficient to establish the next generation. In contrast, steinernematids are amphimictic and mating is necessary to reproduce, thus an invasion of high numbers of individuals increases the probability of mating and further reproduction (Koltai et. al., 1995).

The data obtained in the exposure period assay suggest that this assay may be used to compare different species or production batches of nematodes with different penetration ability. However, the biological impact of

this assay and its relationship to nematode activity in the field is yet to be determined.

The dose response assay could be another way to determine nematode virulence. The increase in nematode infective juvenile numbers/ host larva resulted in an increase in mortality of 3rd and 4th instars of mosquito larvae after using both *Heterorhabditis* nematode species. These results were in agreement with the effect of host size recorded by (Poinar & Kaul, 1982). From the factors that governed the degree of infection is the stage of the host. Parasitism in general was highest in fourth instar larvae. This is due to the fact that larger hosts could more readily ingest nematodes without damaging them. In contrast, second-instar larvae rarely ingested whole nematodes, more often crushing them with their mandibular teeth because of their smaller oral aperture. Once the nematode cuticle was broken, the parasite perished.

Our results and early work of Dadd (1971) and Poinar & Kaul (1982) were following the same pattern of response to changing nematode concentration, but their work showed the inability of the rhabditoid nematode-bacteria system to complete their life cycle in *C. pipiens* mosquitoes. However, the mortality records were in agreement with our records concerning the host instar effect. They observed that although 4th instar larvae may ingest hundreds of nematodes within few hours, very few individual successfully established themselves in the hemocoel and that those worms remaining within the peritrophic membrane commence to disintergrate within a few hours, often leaving no recognizable remains after a day. But the benefit of our nematode strains is that they were able to complete their life cycle in their host larvae till infective juvenile emergence. The same pattern of dose response was found in their experiment.

These results were in agreement with those of Capinera et al., (1988) who found a positive correlation between numbers of infective juvenile used and the number of nematodes which successfully invaded the host. Meanwhile, Abd El Rahman & Hussein (2007) did not found a significant effect of increasing nematode concentration from 250 to 500 ij/host neither on mortality of *L. decemlineata* larvae nor on the nematode invasion rate. They also did not find a significant difference in 3rd and 4th instar larval mortality upon exposure to *H. bacteriophora* infective juveniles for 24 to 72 hr.

Considering nematode fecundity after changing initial nematode concentration per host, the results showed that *H. bacteriophora* was able to tolerate high densities within the host than *H. indica* but both species responded with the same pattern to increasing nematode concentration. Metabolic rate, processing of host tissues by symbiotic bacteria, and the physiological parameters required for growth differ between nematode species. Differences between both heterorhabditid species may result from the differences in host utilization. Also, the reason for the differences in the effect of viable bacterial cells of *P. luminiscens* (*H. bacteriophora* symbionts) may be that these bacteria could survive more the host immune response and present toxic components that killed the host while the bacterial symbionts of *H. indica* were susceptible to host immune system (Selvan & Blackshaw, 1990).

The effect of initial infection density was noticed clearly in the quality of the produced nematode adults as well as the produced juveniles which in turns had an important influence on the population dynamics of parasites. Hermaphrodite length is often used as an indirect measure of nematode fecundity (Selvan et al., 1993). The decline in length which is an indication of reduced fecundity at low density may be probably due to either the decreased level of bacterial inoculums causing slower break down of host tissues, or conversely, insufficient bacterial culling by the nematode resulting in unrestrained bacterial growth. In contrast, at high densities, host utilization by nematode and bacteria is more rapid and may result in inadequate nutrition resulting from competition for limited nutrients within the host. Because *H. bacteriophora* was able to survive at high densities, this could explain how it successfully reproduced at all of the studied densities.

The effect of the initial infection density on juvenile length was clearly obvious, where the longest infective juveniles were produced at lowest densities, not at the densities producing the largest number of infective juveniles. Abd El Rahman & Hussein (2007) recorded a negative correlation between infective juvenile

length of both *H. bacteriophora* and *H. indica* with increasing infection density. In this respect, the larger host supports more the development of higher numbers of juveniles without competitions and constrains that were found in small hosts, where crowding effect appears as a great factor affecting juvenile growth and hence juvenile length.

The tradeoff between quantity and quality of emerging infective juveniles (in respect to length) has been reported early for other parasites (Kino, 1984). Because taller infective juveniles harbor more nutrients, they can be expected to survive for a longer period than shorter nematodes. These juveniles were expected to be more active and hence have higher searching capacity than shorter, less active ones. In contrast, producing large numbers of small, short-lived infective juveniles may decrease the probability of rapidly locating a new host. The explanation of these results may rely on the lipid content of the nematode juveniles. Lewis et al. (1995) studied the relationship between the metabolic rate, energy reserves, and foraging behavior in three species of entomopathogenic nematodes; *S. carpocapsae*, *S. glaseri*, and *H. bacteriophora*, each species is characterized by differing in juvenile length. Their studies showed that lipids, the major components of nematode energy reserves, were stored in larger quantities in longer juveniles than in shorter ones. These lipids were declined at species-specific rates.

The density-dependent factors may play an important role in entomopathogenic nematode fecundity. The density dependent effect may be important in regulating nematode populations either by acting directly through affecting the numbers of infective juveniles produced from each cadaver, or indirectly, by changing the infective juvenile longevity. In laboratory culturing and biological control applications where recycling and persistence is advantageous, the impact of infection density may be of critical importance in maximizing nematode efficacy.

The dose response bioassays has been used many times previously and probit analysis has been used to analyze the data to calculate LC_{50} values. However, when a parasite is highly virulent, the applicability of probit analysis is questionable, since a single steinernematid or heterorhabditid nematode is often capable of killing an insect (Ricci et al., 1996).

Although similar ranking was observed in the present bioassays, the ability to separate the species statistically varied among assays. One-on-one assay effectively separated *H. bacteriophora* and *H. Indica* from each other and from *S. carpocapsae* and *S. feltiae*. The later two species could not be separated from each other by this assay. This assay was conducted in multi-well plates, so, nematodes and insects were kept in close contact and the influence of foraging strategies was limited. Differences in nematode ability to penetrate into the insect and complete its life cycle served as the main factor distinguishing between species. Also, the dose response assay could not separate both heterorhabditid species from each other except in the low and high doses of 100 and 200 ij/insect after 48 hr. These treatments were the best for separating both species. The LC_{50} or LC_{90} values also separated both species.

The present work dealt with demonstrating the variations of entomopathogenic nematode species performance in different bioassays. The differences in the activity of nematodes in the exposure period assay made a spot light on the potential of measuring some behavioral responses as specific criteria for nematode virulence. The presented data support the fact that, since nematodes vary in their behavior, one bioassay cannot be used as a unique measure of virulence for all species (Caroli et al., 1996).

In general, particular bioassays may be used for other purposes: for the selection of a specific population for use against an insect, a variable assay measures which are more laborious but simulate natural environmental conditions or invasion by nematode (e.g. nematode entrance) should be considered. In cases where production batches of the same nematode strain are compared, a simple rapid assay is needed (e.g. One-on-one or exposure period assay). The obtained results may add much to our information concerning the use of nematode-bacteria system to control *C. quinquefasciatus* larvae. Its significance is that it is the first attempt in Egypt to get benefits of augmenting host-specific, lethal bacteria within the nematode to the aquatic larvae to reduce the mosquito population before adult

emergence as well as producing nematode progeny that can re-infect another bottom-feeding host nearby in the system.

The use of laboratory pathogenecity bioassays in these experiments has been relevant in showing consistently the pathogenic capability of the heterorhabditid nematodes over steinernematid one and hence their symbiotic bacteria, *P. luminiscens* over that of *X. nematophilus* to *C. quinquefasciatus* larvae, although, these laboratory bioassays do not provide an assurance of field efficacy, so, field application studies should be done. However, they guaranteed the promising value of using entomopathogenic nematodes of the genus *Heterorhabditis* in controlling the aquatic larvae. These aquatic systems do not introduce the detrimental effect of sun light and ultraviolet radiation as well as desiccation on these types of nematodes as it usually happens in agricultural systems which are considered the most important factors that lower the efficacy of applying these entomopathogenic nematodes in agricultural systems. This was because the bacteria are susceptible to sunlight and U.V. radiation, also, desiccation is a major concern for the nematode itself, where all nematodes need at least a water film to move through it and infect a host. In this system of aquatic habitat, many of the mosquito larvae are bottom feeders, and hence they were protected from sunlight and U.V. radiation.

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