Efficacy of two entomopathogenic nematode species as potential biocontrol agents against the rosaceae longhorned beetle, *Osphranteria coerulescens*, under laboratory conditions

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Summary – Rosaceae longhorned beetle, *Osphranteria coerulescens*, is an important pest of fruit trees that attacks all fruit trees belonging to the family Rosaceae in cold regions of Iran. The potential efficacy of two species of entomopathogenic nematodes (EPN), *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, against last instar larvae of this pest was tested under laboratory conditions. In plate assays, the larvae were susceptible to both EPN species but were more susceptible to *S. carpocapsae* (65.0-97.5% mortality) than *H. bacteriophora* (42.5-87.8%). Both nematode species were able to penetrate and reproduce within *O. coerulescens* larvae, but reproduction rate for *H. bacteriophora* was higher than those of *S. carpocapsae*; however, the penetration rate for *S. carpocapsae* was greater than *H. bacteriophora*. In a migration test on agar plate, *S. carpocapsae* showed negligible attraction to the pest or to *Galleria mellonella* cues. However, *H. bacteriophora* was strongly attracted to the sector of Petri dishes containing larvae. In a test using apricot tree branches, both species of EPN passed from mass frass of *O. coerulescens* larvae in the tip of the branches, penetrated into the larval galleries, and located and killed the larvae of *O. coerulescens* in their natural habitat deep inside the branches. Our findings provide the first insight into the biocontrol efficacy of EPN against *O. coerulescens* larvae, and highlight their potential for the control of this pest. Field experiments are needed to evaluate their potential under the environmental conditions in which rosaceae longhorned beetle larvae are found.

Keywords – attraction, behaviour, *Heterorhabditis bacteriophora*, insect pathology, reproduction, *Steinernema carpocapsae*, virulence.

Rosaceae longhorned beetle, *Osphranteria coerulescens* Redtenbacher (Coleoptera: Cerambycidae), is an economically important pest of fruit trees in cold regions of Iran, Turkey and Syria. This beetle attacks all stone and pome fruit trees belonging to the family Rosaceae, as well as Japanese quince, oleaster, willow and elm (Rajabi, 1967). Adult beetles are active from late May to mid-June. Female insects prefer spring twigs for egg laying. Adult beetles lay a single egg into the upper part of twigs. Each insect lay 30-75 eggs and the average number is 52 (Sharifi *et al.*, 1970). When larvae emerge, they bore through the cambium and hardwood (Behdad, 1974) penetrating into the centre of branches where they feed for a year (Rajabi, 1967). In thick branches and trunks, movement of *O. coerulescens* larvae and their feeding cause the branch to become fragile, and easily broken by wind (Esmaeili, 1983). The obvious sign of pest infestation is readily apparent when the tips of infested twigs break off and then larger branches break off as larval boring proceeds. The larva requires 11 months to develop while the pupa completes development during 22-24 days. This pest causes extensive damage to living rosaceous trees in Iran (Sharifi *et al.*, 1970; Esmaeili, 1983).

Due to the cryptic behaviour of the larvae, control methods using chemical pesticides are problematic. Currently the key management tool for *O. coerulescens* consists of cutting the infested branches and destroying them by burning. Heavy infestations and associated economic losses warrant the development of sustainable and environmentally safe strategies, such as the use of biocontrol agents like entomopathogenic nematodes (EPN) against this cerambycid pest, especially in the late season.

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The EPN species of *Steinernema* Travassos and *Heterorhabditis* Poinar are obligate pathogens of insects (Poinar, 1979). These groups of insect pathogens have been studied extensively in different biocontrol programmes (Ehlers, 2001). The free-living infective juveniles (IJ) have to locate a potential host, penetrate through its cuticle or natural openings and establish in the host's body cavity (Koppenhöfer *et al.*, 2007). In the insect haemocoel, the nematode releases its symbiotic bacteria from its intestine. The bacteria proliferate, producing toxins and metabolites that kill the insect host and prevent invasion by secondary organisms. The nematodes feed on the lysed tissues of the host and bacterial colonies (Griffin *et al.*, 2005).

Several species of EPN have the ability to locate and kill insects in cryptic environments (Begley, 1990), and wood-boring larvae of insect pests like cerambycids could be suitable hosts. There are some studies on the biocontrol potential of EPN against wood borer pests. The pathogenicity of EPN has been assayed against larvae of Asian longhorned beetle, *Anoplophora glabripennis* (Liu *et al.*, 1998; Solter *et al.*, 2001; Fallon *et al.*, 2004). Liu *et al.* (1998) stated that application of the Beijing strain of *Steinernema carpocapsae via* existing emergence holes of *A. glabripennis* caused 66.7% decrease in new emergence holes. Solter *et al.* (2001) showed the ability of *Heterorhabditis marelatus* to locate and kill larvae of Asian longhorned beetle within 30 cm larval galleries. Harvey *et al.* (2012) reported that two nematode species, *S. carpocapsae* and *H. downesi*, were able to infect larvae of the two-banded longhorn beetle, *Rhagium bifasciatum*, more than 4 cm deep within the wood.

The limited movement of *O. coerulescens* larvae and their protected environmental niche with high moisture within their galleries indicates that the use of EPN may be feasible, especially for cruiser species of EPN which forage actively for their hosts. There is an absence of data about the infectivity of EPN against *O. coerulescens*, so the main aims of the current project were to assay the EPN pathogenicity, reproduction and foraging behaviour, and to examine the ability of EPN to penetrate, locate and kill pest larvae within their galleries under laboratory conditions.

Materials and methods

Nematodes and insects

Two commercially available EPN products, Larvanem® (*H. bacteriophora*) and Capsanem® (*S. carpocapsae*), were obtained from Koppert (Berkel en Rodenrijs, The Netherlands). The late instar larvae of greater wax moth, *Galleria mellonella*, were reared and selected on the basis of mean weight and head capsule width. The nematodes were reared in the laboratory on *G. mellonella* larvae at $25 \pm 2°C$, using the method described by Woodring & Kaya (1988). The IJ that emerged from *G. mellonella* cadavers were collected using White (1927) traps and stored at 8°C in sterile distilled water in darkness (Kaya & Stock, 1997). Before being used in the experiment, the nematodes were allowed to acclimatise at room conditions for 1 h and their viability was checked under a stereomicroscope; IJ that were 1-2 weeks old were used in the assays. The nematodes were from separate batches to reduce differences in storage period.

The last instar larvae of *O. coerulescens* were collected from Astan Qods orchards on their susceptible hosts, apricot trees, in Mashhad, Razavi Khorasan province, North Eastern Iran (36°4′N, 59°7′E). The larvae were transferred to the laboratory and further checked on the basis of larval morphology and mean weight.

Plate assay

To assess the virulence of *S. carpocapsae* and *H. bacteriophora* against the larvae of *O. coerulescens*, suspensions of IJ of each species were applied in 150 μl of sterile distilled water into a 2.6 cm diam. plastic container lined with two layers of filter papers. An individual last instar larva of *O. coerulescens* was added to each container. Each nematode species was tested at rates of 5, 10, 25 and 50 IJ larva^{-1}. The containers were sealed with parafilm to avoid dehydration and kept in an incubator at $25 \pm 2°C$ and 16 h light/8 h dark photoperiod during the entire experiment. Control treatments received only distilled water. Larval mortality was checked at 24 h intervals up to 72 h and dead larvae were removed, rinsed in distilled water and placed individually in Petri dishes containing moist filter paper. After 3 days each cadaver was dissected to confirm the presence of nematodes. Each treatment was replicated four times with five dishes for each replicate (=20 dishes for each treatment) and the whole experiment was repeated twice.

Penetration and reproduction of EPN on *O. coerulescens*

Forty last instar larvae of *O. coerulescens* were individually placed in separate 2.6 cm diam. plastic Petri dishes padded with a double layer of filter papers. Each larva was inoculated with each nematode isolate at concentra-

tions of 5 or 25 IJ in 150 μl of sterile distilled water. The Petri dishes were kept as described above. After 3 days, the cadavers were picked up from the Petri dishes and ten cadavers that had been killed by nematodes were selected at random. Five cadavers were rinsed with distilled water to remove any nematodes from their surface, dissected in sterile distilled water under a stereomicroscope and the number of nematodes inside each cadaver was determined. Both EPN species complete a generation in a time not less than 4 days, so the numbers of nematodes within a host body were counted after 3 days (Susurluk, 2008; Susurluk *et al.*, 2009), which gave the number of nematodes that had penetrated into the insect host.

The other five dead larvae were used to evaluate nematode reproduction. The larvae were rinsed in sterile distilled water to remove nematodes from their surface body then separately placed on White trap and incubated. The IJ exiting from the cadavers were collected over 20 days. The whole experiment was repeated twice.

MIGRATION BEHAVIOUR OF EPN

To investigate the foraging behaviour of *H. bacteriophora* and *S. carpocapsae*, larvae of *O. coerulescens* and *G. mellonella* were used as hosts. The experimental unit was a 7 cm diam. glass Petri dish filled with a layer of approximately 0.5 cm of 2.5% agar. Each Petri dish was divided into four equal parts and a 1 cm diam. centred circle was drawn on the back of the dishes. In order to provide a chemical gradient, a single larva of *O. coerulescens* or *G. mellonella* was placed on one side of a quadrant of each Petri dish. The Petri dishes were covered with a lid and incubated. After 24 h the larvae were removed and 30 IJ of each EPN species in 10 μl sterile distilled water was added to the centre of the marked circle of each Petri dish. The plates were covered with a glass lid and kept for 3 h in the dark at 23 $\pm$ 2°C to allow the nematodes to migrate. Then the number of IJ in each quadrant and chemical gradient formation point were counted under a stereomicroscope. For each host (*O. coerulescens* and *G. mellonella*) and EPN species, there were ten replicates and the experiment was repeated twice.

BRANCH TEST

The branch test was carried out to assess the ability of *S. carpocapsae* and *H. bacteriophora* to penetrate into the infested branches of apricot trees and infect the larvae of *O. coerulescens* in their larval galleries (their natural habitat). For this purpose, infested branches *ca*

15-17 cm in length were collected from the orchards and transferred to the laboratory. The tips of the branches, where the larvae of *O. coerulescens* deposit their frass, were placed into 45 cm^3 sponge pieces and a 1 cm diam. hole was made inside them. The EPN suspension at a concentration of 10 000 IJ ml^{-1} was inoculated into each sponge piece. Then the branches were incubated horizontally in a controlled growth chamber at 25 $\pm$ 2°C and 16 h light/8 h dark photoperiod. Control treatments received only distilled water. After 5 days, the sponge pieces were removed and mortality of the larvae inside the branches was recorded. In order to confirm the presence of nematodes, cadavers were rinsed with distilled water and dissected. Each treatment had seven replicates with four branches for each replicate (=28 branches for each treatment) and the whole experiment was repeated twice.

STATISTICAL ANALYSIS

Data were corrected for control mortality (Abbott, 1925) and square-root transformed when necessary to meet assumptions of normality and homogeneity of variances. A two-way full factorial ANOVA (EPN species × concentrations, with time as blocking) was used to determine the differences of larva mortality in the plate assay, penetration and reproduction tests. When ANOVA showed a significant effect (P < 0.05), Fisher's Protected LSD test was used to determine significance between mean values. In addition, in the plate assay, the LC$_{50}$ values were determined using the Probit procedure for each EPN species. Parallelism test was applied to regression lines, and LC$_{50}$ differences between nematode species were considered significant when 95% fiducial limits did not overlap.

In the branch test, larval mortality was analysed using the non-parametric Mann-Whitney *U*-test. In the migration test, data comparison was carried out based on the proportion of recovered IJ in the cue areas compared with all recovered IJ by the two-way full factorial ANOVA (EPN species × host species, with time as block). If significant differences were detected, the LSD test was used.

All statistical analyses were carried out using SAS software, version 9.1 (SAS Institute, 2002-2003).

Results

PLATE ASSAY

The pathogenicity of two EPN species against the pest larvae were assessed in plate assays. The results indicated that the larvae of *O. coerulescens* were susceptible to both

nematode species when EPN were applied at a concentration of 5 IJ larva^{-1}, which caused 42.5-65.0% larval mortality after 72 h (Fig. 1C). *Steinernema carpocapsae* had greater virulence to the larvae of *O. coerulescens* and caused larval mortality of between 65.0 and 97.5% after 72 h (Fig. 1C). The maximum mortality of the larvae was caused by *S. carpocapsae* at a concentration of 50 IJ larva^{-1} (97.5%), while the minimum mortality rate was observed in the larvae treated with *H. bacteriophora* at 5 IJ larva^{-1} (42.5%) after 72 h. Both EPN species caused larval mortality 24 h after treatment at all concentrations used, except for the treatment of *H. bacteriophora* in concentration of 5 IJ larva^{-1} (Fig. 1A). The overall mortality of the larvae of *O. coerulescens* 48 h after treatment with EPN ranged between 27.5% and 95.0% (Fig. 1B); 72 h after treatment, the mortality rate was 42.5-97.5% (Fig. 1C),

which demonstrated an increase in larval mortality with increase in exposure time. The differences of larval mortality between the EPN species ($F = 32.85$; df = 1, 48; $P < 0.0001$) and IJ concentrations ($F = 28.36$; df = 3, 48; $P < 0.0001$) were statistically significant. Also, significant differences between mortality were recorded between nematode species and concentrations ($F = 7.83$; df = 3, 48; $P < 0.0002$). The calculated LC$_{50}$ for *S. carpocapsae* and *H. bacteriophora* on the pest larvae after 72 h were 2.7 and 9.0 IJ larva^{-1}, respectively (Table 1). The LC$_{50}$ values implied that *S. carpocapsae* was more virulent against the last instar larvae of *O. coerulescens*. The results of regression analysis showed that the regression lines were parallel ($x^2 = 0.588$, df = 1, $P < 0.44$), which indicates a similar effect of EPN species on *O. coerulescens* larvae.
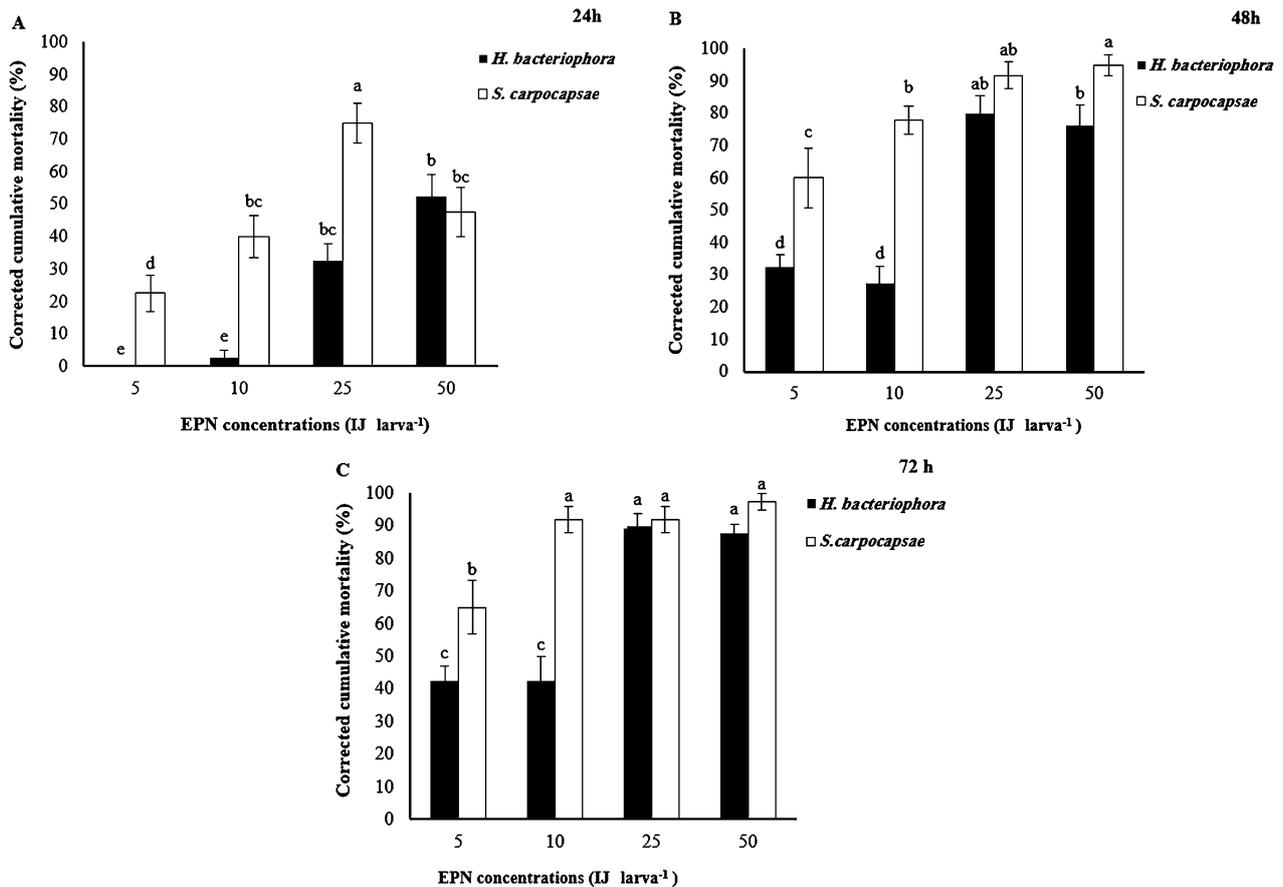
Fig. 1. Mortality of *Ospheranteria coerulescens* larvae after treatment with *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* at concentrations of 5, 10, 25 and 50 IJ larva^{-1}. A, B and C show the results at 24, 48 and 72 h after inoculation, respectively.

Table 1. Calculated LC_{50} values (IJ larva^{-1}) for *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on last instar larvae of *Osphranteria coerulescens*.

EPN species	Time after treatment (h)	LC_{50} (95% fiducial limit)	Intercept $\pm$ SE	Slope $\pm$ SE	x^2 (df = 2)	P
H. bacteriophora	24	43.1 (34.4-60.6)	-4.54 ± 0.75	2.77 ± 0.50	1.75	0.41
	48	15.7 (8.1-28.5)	-2.23 ± 0.54	1.86 ± 0.38	7.55	0.02
	72	9.0 (3.3-16.3)	-1.85 ± 0.45	1.91 ± 0.35	5.21	0.073
S. carpocapsae	24	19.0 (7.1-64.0)	-1.14 ± 0.34	0.87 ± 0.26	13.00	0.0015
	48	3.1 (0.9-5.2)	-0.9 ± 0.43	1.55 ± 0.37	0.59	0.74
	72	2.7 (0.4-6.0)	-0.51 ± 0.46	1.48 ± 0.42	4.25	0.12

Table 2. The number of infective juveniles of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* that penetrated into the larva of *Osphranteria coerulescens* and subsequently reproduced.

Application rate (IJ larva^{-1})	EPN species	Nematode invasion (IJ larva^{-1} $\pm$ SE)	Nematode reproduction (IJ larva^{-1} $\pm$ SE)	Reproduction rate (per invaded IJ)
5	*H. bacteriophora*	1.5 $\pm$ 0.16 c	333 329 $\pm$ 34 431 a	222 219
	S. carpocapsae	2 $\pm$ 0.21 c	45 155 $\pm$ 11 196 a	22 577
25	*H. bacteriophora*	5.8 $\pm$ 0.61 b	273 468 $\pm$ 31 803 b	47 149
	S. carpocapsae	18.3 $\pm$ 0.71 a	71 408 $\pm$ 7145 b	3902

Different letters indicate significant differences at $P < 0.05$.

Host penetration and reproduction

The results of the penetration assay showed that both EPN species were able to penetrate and reproduce within the haemocoel of *O. coerulescens* larvae. The penetration of IJ was significantly affected by the nematode species ($F = 160.19$; df = 1, 32; $P < 0.0001$) and concentration of IJ ($F = 402.24$; df = 1, 32; $P < 0.0001$). Also the interaction between the nematode species and IJ concentration was significant ($F = 136.49$; df = 1, 32; $P < 0.0001$). There was a positive correlation between the applied concentration of EPN and the number of IJ that invaded the pest larvae. By increasing the applied rate of IJ from 5 to 25 IJ larva^{-1}, the number of IJ which penetrated into the larvae was increased. The highest penetration rate of IJ was recorded for *S. carpocapsae* at the concentration of 25 IJ larva^{-1}, while the lowest invasion was observed with *H. bacteriophora* when applied at the concentration of 5 IJ larva^{-1}.

The results of the reproduction test revealed significant differences among the reproduction potential of two nematode species ($F = 97.92$; df = 1, 32; $P < 0.0001$) (Table 1). However, no statistically difference was detected between the IJ concentrations used ($F = 0.46$; df = 1, 32; $P < 0.5024$). Moreover, the interaction between nematode species and IJ concentration was not significant ($F = 3.02$; df = 1, 32; $P < 0.0918$). The number of IJ produced per invaded IJ declined with increasing concentration of IJ from 5 to 25 IJ larva^{-1} for *H. bacteriophora*, while this value increased for *S. carpocapsae*. The highest reproduction rate was recorded for *H. bacteriophora* at the concentration of 5 IJ larva^{-1}, in contrast to the lowest amount for *S. carpocapsae* at the same concentration (Table 2).

Migration test

The results of the migration test showed that there was a significant difference between two EPN species in the proportion of IJ that migrated towards host cues ($F = 455.33$; df = 1, 73; $P < 0.05$). The greatest proportion of migrated IJ was observed for *H. bacteriophora* toward *O. coerulescens* (0.55 ± 0.028), while the lowest proportion of migrated IJ was observed for *S. carpocapsae* toward *O. coerulescens* (0.013 ± 0.0044). In addition, there was a significant difference between the proportion of IJ that migrated towards the two host species ($F = 4.45$; df = 1, 73; $P = 0.038$). Also, significant differences were recorded between the proportion of migrated IJ of the two EPN species and the two host species ($F = 6.37$; df = 1, 73; $P = 0.013$).

The results of this test indicated that both *S. carpocapsae* and *H. bacteriophora* passed from mass frass of *O. coerulescens* larvae in the tip of the branches, penetrated into the larval galleries, located and killed the larvae of *O. coerulescens* in their natural habitat deep inside the branches. IJ of *H. bacteriophora* caused greater mortality (58.9%) than *S. carpocapsae* (42.8%), but there were no statistically significant differences in larval mortality between the two nematode species (Kruskal-Wallis: $x^2 = 2.867$; df $= 1, 55$; $P > 0.05$).

Discussion

Our data provide the first insight into pathogenicity of two species of EPN against larvae of *O. coerulescens*. The laboratory experiments showed the efficacy of *H. bacteriophora* and *S. carpocapsae* to locate, penetrate and kill the larvae of *O. coerulescens* inside the galleries of apricot tree branches. Last instar larvae of *O. coerulescens* were highly susceptible to both species of EPN, causing mortalities of 42.5-97.5% at concentrations of 5, 10, 25 and 50 IJ larva^{-1}. The calculated LC$_{50}$ for *H. bacteriophora* (9.0 IJ larva^{-1}) was greater than these for *S. carpocapsae* (2.7 IJ larva^{-1}) in agar plate assays, indicating a greater virulence of *S. carpocapsae* against *O. coerulescens* larvae. This finding supports the statement of Grewal *et al.* (1993a, b) that *S. carpocapsae* could effectively find pest larvae by cruising horizontally; *H. bacteriophora* is more effective to migrate and infect hosts moving vertically.

Previous work showed differences between virulence of EPN against cerambycid larvae. Fallon *et al.* (2004) examined pathogenicity of *S. feltiae*, *H. marelatus*, *S. glaseri*, *S. riobrave* and *S. carpocapsae* in a filter paper assay against Asian longhorned beetle, *A. glabripennis*. Their results revealed a marked susceptibility of the host larvae to nematode infection; *S. feltiae* and *S. carpocapsae* were the most effective causing 71 and 100% mortality, respectively. In a similar test, Fallon *et al.* (2006) investigated the efficacy of *S. feltiae*, *S. carpocapsae*, *S. glaseri*, *S. riobrave*, *H. indica* and *H. marelatus* on cottonwood borer, *Plectrodera scalator*, at concentrations of 10, 50 and 100 IJ larva^{-1}. They found similar results and stated that *S. carpocapsae* and *S. feltiae* were more virulent, causing 50 and 58% mortality of the larvae, respectively, while the mortality caused by *H. marelatus* was 12.9%. Also Susurluk *et al.* (2009) indicated greater virulence of *S. feltiae* than

H. bacteriopghora against a cerambycid pest, *Dorcadion pseudopreissi*, a result that is in agreement with our data.

The pathogenicity of EPN is related to several biotic and abiotic factors, such as host invasion, penetration and reproduction (Kaya & Gaugler, 1993). In the plate assays of the current work, both EPN and insect host were close to each other and the EPN did not have to move over a great distance. Differences in infectivity potentials of two EPN species might be explained by differences in their ability to penetrate the larvae. Greater virulence of *S. carpocapsae* could be related to the host finding behaviour of this species. However, in the case of *H. bacteriophora*, we could not attribute the difference among the mortalities with high concentrations to its host finding ability. Based on the early theory of host-finding behaviour by EPN (Campbell & Gaugler, 1997), this is a cruiser species that moves and searches for its host (Gaugler & Bilgrami, 2004). Therefore, in our assays at a given concentration the EPN used and searched all available space and a higher IJ concentration did not increase the mortality rates of insect pest larvae.

To be successful as an insect pathogen, the EPN species have to penetrate their host and reproduce. Based on Caroli *et al.* (1996), penetration rates are different between EPN species and are influenced by different hosts and substrates. In our study, *S. carpocapsae* showed greater penetration rates into the host larvae than *H. bacteriophora*. Our results agree with those of Susurluk *et al.* (2009), who showed that the greatest penetration of EPN species into *D. pseudopreissi* larvae was observed for *S. carpocapsae* and the lowest for *H. bacteriophora*. Also, the data indicated that the number of invaded IJ was linked to host mortality. In the present study, both EPN species penetrated and reproduced in the last instar larvae of the host and their IJ emerged from the host cadavers. However, reproduction of *H. bacteriophora* was significantly greater than that of *S. carpocapsae*. The larvae of *O. coerulescens* are large, a condition that provided a suitable host for reproduction of EPN. The potential of pest larvae to support rearing of EPN offspring is an important criterion when considering the possible role of EPN in biocontrol. However, our results of reproductive ability do not agree with the data of Susurluk *et al.* (2009) who reported the highest reproduction potential for *S. carpocapsae* and the lowest for *H. bacteriophora* in *D. pseudopreissi* larvae. Harvey *et al.* (2012) stated that two species of EPN, *S. carpocapsae* and *H. downesi*, reproduced in *R. bifasciatum* larvae successfully and the reproduction rate of *H. bacteriophora* was half that of *S. carpocapsae*.

One possible reason for the difference in progeny results of EPN in larvae of *O. coerulescens* may be related to the type of reproduction of Heterorhabditids. *Heterorhabditis bacteriophora* is a hermaphrodite that produces more offspring than Steinernematids, which are amphimictic (Poinar, 1990; Mannion & Jansson, 1992). Another reason for higher reproduction rate of *H. bacteriophora* in the host larvae might be due to size and behaviour of EPN species. Loya & Hower (2003) also suggested that size and behaviour of nematode species may be a reason for variations in reproduction of *H. bacteriophora* in different life stages of the clover root curculio, *Sitona hispidulus*.

Because of the natural habitat of *O. coerulescens* larvae inside tree branches, investigation into the foraging behaviour of EPN is an important aspect for effective control of this pest. In addition, EPN have to migrate quickly to avoid sunlight and desiccation (Gaugler, 1988). EPN are likely to move in response to host cues like CO_2 (Gaugler *et al.*, 1980; Lewis *et al.*, 1993) or gut fluids (Grewal *et al.*, 1993a, b). They have different host-finding behaviour (Campbell & Gaugler, 1997). Of the two species used in the current study, *H. bacteriophora* was clearly attracted to cues from *O. coerulescens* and *G. mellonella*. However, *S. carpocapsae* showed almost no response to both host cues. Similarly, Fallon *et al.* (2004) indicated that *S. carpocapsae* and *S. riobrave* do not show attraction to frass volatiles of *A. glabripennis*. Grewal *et al.* (1993a) stated that IJ of *H. bacteriophora* move directionally to host cues, so there is no relation between substrate and dispersal patterns of the nematode. By contrast, *S. carpocapsae* did not show a directional response to volatiles and, therefore, their movement pattern is related to the substrate in such way that decreases dispersal on a rough substrate (Grewal *et al.*, 1994). The poor response to chemical cues is characteristic of *S. carpocapsae*. Lewis *et al.* (1992, 1993) showed that *S. carpocapsae* did not respond to *Spodoptera exigua* and *Popillia japonica* frass and host cuticles; nor did it respond to volatiles from *G. mellonella* larvae. Our results support this lack of response by *S. carpocapsae*. In general, higher numbers of *H. bacteriophora* IJ migrated toward *O. coerulescens* larvae than *G. mellonella* larvae. Many studies have showed differences in CO_2 production by different insects (*e.g.*, Gaugler *et al.*, 1991; Ramos-Rodríguez *et al.*, 2007).

In the apricot tree branch test, there was no significant difference between virulence of both EPN species. The late instar larvae of *O. coerulescens* were in a gallery at a depth of approximately 5 cm from the tip of the branch and IJ have to move this distance to reach the larvae. The aggregation of the pest frass at the tip of the branch is a cue for directional movement of the IJ. Wilson *et al.* (2012) re-examined the supposed characteristic foraging behaviour of *S. carpocapsae*. The IJ of this species disperse over short distances, aggregate near the soil surface and react poorly to chemical cues, so occurrence of foraging behaviour may be low. In many papers it was reported that *S. carpocapsae* provided suitable control of wood-boring pest beetles in both laboratory and field experiments (Fallon *et al.*, 2004; Dillon *et al.*, 2006; Harvey *et al.*, 2012). One possible reason for variation between results of attraction and branch tests is the difference between substrate textures. IJ dispersal has been reported to be markedly affected by substrate texture (Georgis & Poinar, 1983; Molyneux & Bedding, 1984; Portillo-Aguilar *et al.*, 1999; Jabbour & Barbercheck, 2008). Many studies were carried out to test mobility of *S. carpocapsae* in sandy soil (*e.g.*, Moyle & Kaya, 1981; Schroeder & Beavers, 1987; Grewal *et al.*, 1994, 1999) or agar (*e.g.*, Lewis *et al.*, 1992; Campbell & Gaugler, 1993; Grewal *et al.*, 1994, 1999; Lewis & Gaugler, 1994). Wilson *et al.* (2012) stated that one reason for the supposed relative immobile behaviour of *S. carpocapsae* was inappropriate test substrates used in those tests. The tree branches used in our work provide more appropriate substrates for movement tests.

One other possible reason for this difference in our data and the previously reported behaviour of *S. carpocapsae* is the existence of the larvae of *O. coerulescens* inside the branch where its feeding causes vibration; this may provide a cue for the nematode. Such a cue would be absent in agar plate assays. Torr *et al.* (2004) showed that *S. carpocapsae* had strong response to a wide range of vibrational cues. Recently Kruitbos *et al.* (2010) stated that *S. carpocapsae* is adapted to being active in habitats other than mineral soils (*e.g.*, peat, leaf litter, bark or wood). Our results support this finding and also fit with the habitat preferences of this species; *S. carpocapsae* prefer woodlands where their soil habitats have much higher organic matter contents (Hominick, 2002).

Overall, EPN have potential to be used against *O. coerulescens* larvae as a management tool in combination with other control methods. One possible method is to apply EPN after cutting the infested branches. While the larvae of *O. coerulescens* showed highly susceptibility to both EPN in the laboratory condition, the feeding niches of this pest occur in twigs or, later, in tree trunks. This could be a challenge due to the high sensitivity

of EPN to UV light and desiccating conditions. Our results show that EPN can enter into insect galleries and kill insect larvae inside but records of successful field control are scarce and limited to those cases of pests that produce galleries in roots. For above ground cases, EPN have to find entry to the galleries before they are killed by adverse environmental conditions. Therefore, implementing EPN as part of control approach for *O. coerulescens* necessitates resolving these questions. What is the best application method of EPN? Which methods will provide suitable condition to protect EPN from extreme conditions? The last method might include some critical issues like application time and using a cover after treatment.

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References

Akhurst, R.J. & Boemaere, N.E. (1990). Biology and taxonomy of *Xenorhabdus*. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press, pp. 75-87.

Begley, J.W. (1990). Efficacy against insects in habitats other than the soil. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press, pp. 215-231.

Behdad, E. (1984). *Pests of fruit trees in Iran*. Tehran, Iran, Plant Pests and Diseases Research Institute.

Burman, M. (1982). *Neoaplectana carpocapsae*: toxin production by axenic insect parasitic nematodes. *Nematologica* 28, 62-70.

Campbell, J.F. & Gaugler, R. (1993). Nictation behaviour and its ecological implication in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* 126, 155-169.

Campbell, J.F. & Gaugler, R.R. (1997). Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? *Fundamental and Applied Nematology* 20, 393-398.

Caroli, L., Glazer, I. & Gaugler, R. (1996). Entomopathogenic nematode infectivity assay: multi variable comparison of pen-

etration into different hosts. *Biocontrol Science and Technology* 6, 227-233.

Dillon, A.B., Ward, D., Downes, M.J. & Griffin, C.T. (2006). Suppression of the large pine weevil *Hylobius abietis* (Coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes with different foraging strategies. *Biological Control* 38, 217-227.

Ehlers, R.-U. (2001). Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology and Biotechnology* 56, 623-633.

Esmaeili, M. (1983). *Important pests of fruit trees*. Tehran, Iran, Sepehr Publishing Center.

Fallon, D.J., Solter, L.F., Keena, M., McManus, M., Cate, J.R. & Hanks, L.M. (2004). Susceptibility of Asian longhorned beetle, *Anoplophora glabripennis* (Motchulsky) (Coleoptera: Cerambycidae) to entomopathogenic nematodes. *Biological Control* 30, 430-438.

Fallon, D.J., Solter, L.F., Bauer, L.S., Miller, D.L., Cate, J.R. & McManus, M.L. (2006). Effect of entomopathogenic nematodes on *Plectrodera scalator* (Fabricius) (Coleoptera: Cerambycidae). *Journal of Invertebrate Pathology* 92, 55-57.

Gaugler, R. (1988). Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. *Agriculture Ecosystems & Environment* 24, 351-360.

Gaugler, R., Lebeck, L., Nakagaki, B. & Boush, G.M. (1980). Orientation of the entomogenous nematode *Neoaplectana carpocapsae* to carbon dioxide. *Environmental Entomology* 9, 649-652.

Gaugler, R., Campbell, J.F. & Gupta, P. (1991). Characterization and basis of enhanced host finding in a genetically improved strain of *Steinernema carpocapsae*. *Journal of Invertebrate Pathology* 57, 234-241.

Grewal, P.S., Gaugler, R. & Lewis, E.E. (1993a). Host recognition by entomopathogenic nematodes during contact with insect gut contents. *Journal of Parasitology* 79, 495-503.

Grewal, P.S., Gaugler, R. & Selvan, S. (1993b). Host recognition by entomopathogenic nematodes: behavioral response to contact with host faeces. *Journal of Chemical Ecology* 119, 1219-1231.

Grewal, P.S., Lewis, E.E., Gaugler, R. & Campbell, J.F. (1994). Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* 108, 207-215.

Grewal, P.S., Converse, V. & Georgis, R. (1999). Influence of production and bioassay methodes on infectivity of two ambush foragers (Nematoda: Steinernematidae). *Journal of Invertebrate Pathology* 73, 40-44.

Griffin, C.T., Boemare, N.E. & Lewis, E.E. (2005). Biology and behaviour. In: Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D. (Eds). *Nematodes as biocontrol agents*. Wallingford, UK, CABI Publishing, pp. 47-64.

Harvey, C.D., Alameen, K.M. & Griffin, C.T. (2012). The impact of entomopathogenic nematodes on a non-target, service-providing longhorn beetle is limited by targeted application

when controlling forestry pest *Hylobius abietis*. *Biological Control* 62, 173-182.

Hominick, W.M. (2002). Biogeography. In: Gaugler, R. (Ed.). *Entomopathogenic nematology*. Wallingford, UK, CABI Publishing, pp. 115-144.

Kaya, H.K. (1985). Susceptibility of early larval stage of *Pseudaletia unipuncta* and *Spodoptera exigua* (Lepidoptera: Noctuidae) to the entomopathogenic nematodes *Steinernema feltiae* (Rhabditida: Steinernematidae). *Journal of Invertebrate Pathology* 46, 58-62.

Kaya, H.K. & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Eentomology* 38, 181-206.

Kaya, H.K. & Stock, S.P. (1997). *Techniques in insect nematology. Manual of techniques in insect pathology*. New York, NY, USA, Academic Press, pp. 281-324.

Koppenhöfer, A.M., Grewal, P.S. & Fuzy, E.M. (2007). Differences in penetration routes and establishment rates of four entomopathogenic nematode species into four white grub species. *Journal of Invertebrate Pathology* 94, 184-195.

Kruitbos, L.M., Heritage, S.E., Hapca, S. & Wilson, M.J. (2010). The influence of habitat quality on the foraging strategies of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis megidis*. *Parasitology* 137, 303-309.

Lewis, E.E. & Gaugler, R. (1994). Entomopathogenic nematode (Rhabdita: Steinernematidae) sex ratio relates to foraging strategy. *Journal of Invertebrate Pathology* 64, 238-242.

Lewis, E.E., Gaugler, R. & Harrison, R. (1992). Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology* 105, 103-107.

Lewis, E.E., Gaugler, R. & Harrison, R. (1993). Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. *Canadian Journal of Zoology* 71, 765-769.

Liu, H.X., Chang, Q., Ma, F. & Liu, H.X. (1998). Field experiment of *Anoplophora glabripennis* and *Melanophila decastigma* control with pathogenic nematode *Steinernema carpocapsae*. *Ningxia Journal of Agricultural and Forestry Science and Technology* 5, 15-17.

Loya, L.J. & Hower, J.A.A. (2003). Infectivity and reproduction potential of the Oswego strain of *Heterorhabditis bacteriophora* associated with life stages of the clover root curculio, *Sitona hispidulus*. *Journal of Invertebrate Pathology* 72, 63-72.

Mannion, C.M. & Jansson, R.K. (1992). Comparison of ten entomopathogenic nematodes for control of sweet potato weevil (Coleoptera: Apionidae). *Journal of Economic Entomology* 85, 1642-1650.

Moyle, P.L. & Kaya, H.K. (1981). Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida: Steinernematidae) in sand. *Journal of Nematology* 13, 295-300.

Poinar Jr., G.O. (1979). *Nematodes for biological control of insects*. Boca Raton, FL, USA, CRC Press.

Poinar Jr., G.O. (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press, pp. 23-61.

Rajabi, G. (1987). *Destructive insect pests of fruit trees in cold regions of Iran*. Tehran, Iran, Research Institute of Plant Pests and Diseases Publication.

Ramos-Rodríguez, O., Campbell, J.F., Lewis, E.E., Shapiro-Ilan, D.I. & Ramaswami, S.B. (2007). Dynamics of carbon dioxide release from insects infected with entomopathogenic nematodes. *Journal of Invertebrate Pathology* 94, 64-69.

Schroeder, W.J. & Beavers, J.B. (1987). Movement of the entomogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. *Journal of Nematology* 19, 257-259.

Sharifi, S., Javadi, I. & Chemsak, J.A. (1970). Biology of the Rosaceae branch borer, *Osphranteria coerulescens* (Coleoptera: Cerambycidae). *Annals of the Entomological Society of America* 63, 1515-1520.

Solter, L.F., Keena, M., Cate, J.R., McManus, M.L. & Hanks, L.M. (2001). Infectivity of four species of nematodes (Rhabditoidea: Steinernematidae, Heterorhabditidae) to the Asian longhorn beetle, *Anoplophora glabripennis* (Motchulsky) (Coleoptera: Cerambycidae). *Biocontrol Science and Technology* 11, 547-552.

Susurluk, A. (2008). Potential of the entomopathogenic nematodes, *Steinernema feltiae*, *S. weiseri* and *Heterorhabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris* (Coleoptera: Curculionidae). *Journal of Pest Science* 81, 221-225.

Susurluk, I.A., Kumral, N.A., Peters, A., Bilgili, U. & Aclkgoz, E. (2009). Pathogenicity, reproduction and foraging behaviours of some entomopathogenic nematodes on a new turf pest, *Dorcadion pseudopreissi* (Coleoptera; Cerambycidae). *Biocontrol Science and Technology* 19, 585-594.

Torr, P., Heritage, S. & Wilson, M.J. (2004). Vibrations as a novel signal for host location by parasitic nematodes. *International Journal for Parasitology* 34, 997-999.

White, G.F. (1927). A method for obtaining infective nematode larvae from cultures. *Science* 66, 302-303.

Wilson, M.J., Ehlers, R.-U. & Glazer, I. (2012). Entomopathogenic nematode foraging strategies – is *Steinernema carpocapsae* really an ambush forager? *Nematology* 14, 389-394.

Woodring, J.L. & Kaya, H.K. (1988). *Steinernematid and Heterorhabditid nematodes: a hand book of techniques*. Fayetteville, AR, USA, Arkansas Agricultural Experiment Station, Southern Cooperative Bulletin.