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RESEARCH ARTICLE

Efficacy of entomopathogenic nematodes against potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) under laboratory conditions

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The susceptibility of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) to native and commercial strains of entomopathogenic nematodes (EPNs) was studied under laboratory conditions. Native strains of EPNs were collected from northeastern Iran and characterised as *Steinernema feltiae* and *Heterorhabditis bacteriophora* (FUM 7) using classic methods as well as analysis of internal transcribed spacer (ITS) and D2/D3 sequences of 28S genes. Plate assays were performed to evaluate the efficiency of five EPN strains belonging to four species including *Steinernema carpocapsae* (commercial strain), *S. feltiae*, *Steinernem glaseri* and *H. bacteriophora* (FUM 7 and commercial strains). This initial assessment with 0, 75, 150, 250, 375 and 500 IJs/ml concentrations showed that *S. carpocapsae* and *H. bacteriophora* caused the highest mortality in both larval and prepupal stages of *P. operculella*, PTM. Thereafter, these three strains (i.e. *S. carpocapsae*, *H. bacteriophora* FUM 7 and the commercial strains) were selected for complementary assays to determine the effects of soil type (loamy, loamy–sandy and sandy) on the virulence of EPNs against the second (L2) and fourth instar (L4) larvae as well as prepupa. A soil column assay was conducted using 500 and 2000 IJs in 2-ml distilled water. Mortality in the L2 larvae was not affected by the EPN strain or soil type, while there was a significant interactive effect of nematode strains and soil type on larval mortality. The results also showed that EPN strains have higher efficiency in lighter soils and caused higher mortality on early larvae than that in loamy soil. In L4 larvae, mortality of PTM was significantly influenced by nematode strain and applied concentrations of infective juveniles. The larval mortality induced by *S. carpocapsae* was higher than those caused either by a commercial or the FUM 7 strain of *H. bacteriophora*. Prepupa were the most susceptible stage.

Keywords: potato tuber moth; *Steinernema*; *Heterorhabditis*; insect pathology; soil type

1. Introduction

Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera, Gelechiidae) is a primary pest of Solanaceae crops which contributes to potato loss in field and storage (Larraín, Guillon, Kalazich, Graña, and Vásquez 2007; Sporleder, Zegarra, Rodriguez Cauti, and Kroschel 2008). PTM larvae attack leaves, petioles and stems and infest tubers during plant senescence (Rondon et al. 2007). Severe damage (up to

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100% in some cases) can occur in storage (Sileshi and Teriessa 2001; Sporleder et al. 2008).

Among diverse groups of biocontrol agents, entomopathogenic nematodes (EPNs) are important in suppressing populations of several economically important insect pests (Lacey, Knight, and Huber 2000; Grewal, Ehlers, and Shapiro-Ilan 2005) and have been used with variable success against insects occupying different habitats (Batalla-Carrera, Morton, and García-del-Pino 2010). However, most success has been achieved in insect pests that spend some stages in the soil or those in cryptic habitats such as galleries in plants where infective juveniles (IJs) are protected from environmental extremes (Shapiro-Ilan, Mizell, and Campbell 2002; Almeida, Batista Filho, Oliveira, and Raga 2007; Batalla-Carrera et al. 2010).

EPNs can effectively control several lepidopteran species (Kaya 1985; Siegel, Lacey, Fritts, Higbee, and Noble 2004; Batalla-Carrera et al. 2010) but matching the most suitable nematode with the target host is a critical component for success in any biocontrol programme (Shapiro-Ilan and Cottrell 2006). The primary objective of our current work was to evaluate the susceptibility of PTM immature stages [(i.e. second instar (L2), fourth instar larvae (L4) and prepupa] to five strains of EPNs species (native and commercial strains) in Petri dishes. The second objective was to determine the efficiency of the most virulent strains in the soil column assays to evaluate which soil type provides the best condition for EPN pathogenicity.

2. Materials and methods

2.1. Insect

Adult and larval stages of PTM were obtained from the laboratory colony maintained at the entomology division, University of Mohaghegh Ardabili in Ardabil, Iran. The larvae were reared on potato tubers in rearing room at $28 \pm 2^{\circ}$C, 14:10 L:D, following the procedures described by Arthurs, Lacey, Pruneda, and Rondon (2008) with minor modification. Briefly, infested tubers were placed in plastic storage containers ($25 \times 12 \times 10$ cm) with a thin layer of the sterile sand as a pupation substrate. The pupae were separated daily from the sand using a sieve. Pupae were placed in mating cups (750 ml) covered with gauze. A filter paper (Whatman No. 1) on the gauze provided an oviposition site for PTM adults who were fed with a 20% sugar solution. A potato slice was placed on filter paper to stimulate oviposition. For the larval experiments, filter papers containing eggs were changed daily and stored at 10°C before use. For the prepupal experiments, the sand was sieved daily to collect cocoons containing prepupa, followed by storage at $28 \pm 2^{\circ}$C for 1 day before use.

2.2. EPNs

Between July and October 2010, a survey for native EPNs was conducted in Masshad, Razavi Khorasan province, northeastern Iran (36°4′N, 59°7′E). Two hundred soil samples were collected from two peach orchards. Subsamples from each sample were baited with seven *Galleria mellonella* (L) (Lepidoptera: Pyralidae) larvae in 200-ml plastic containers (Bedding and Akhurst 1975). After one week, the

infected cadavers were collected, and individually placed on White traps to collect emerging nematodes (White 1927). Harvested nematodes were then stored in distilled water at 8°C for further study.

Nematodes were characterised on the basis of morphologic and morphometric traits (Nguyen and Smart 1996). For molecular identification, sequence analysis of internal transcribed spacer (ITS) rDNA and D2/D3 expansions of 28S genes were used. After DNA extraction, both genes were amplified and sequenced with the BigDye Terminator Kit (Macrogen Inc., Korea). Both DNA chains of each strain were sequenced separately with the corresponding primers. The primers set were Vrain, Wakarchuk, Levesque, and Hamilton (1992) (for ITS) and Stock, Campbell, and Nadler (2001) (for 28S). Each PCR mixture was carried out in a reaction volume of 25 µl, containing 2.5 µl of 10 × PCR buffer, 15.7 µl of H_2O, 1 µl of $MgCl_2$ (25 mM), 0.5 µl of dNTPs (10 mM), 0.3 µl *Taq* polymerase (5 units/µl), 1 µl of forward primer (10 pmol/µl), 1 µl of reverse primer (10 pmol/µl) and 3 µl of DNA. All PCRs were conducted in a T-Personal thermocycler (Biometra). For phylogenetic analysis on the basis of ITS and D2/D3 regions, valid and verified sequences were retrieved from GenBank. For phylogenetic analyses, two methods were used: Maximum Parsimony (MP) using PAUP*4.0b (Swofford 2002) and neighbour-joining (NJ) method by MEGA 5 software (Tamura, Dudley, Nei, and Kumar 2007).

A laboratory culture of *Steinernema glaseri* IRAZ19 (Nikdel, Niknam, Griffin, and Eivazian Kary 2010) was kindly provided by Dr. Gh. Niknam (University of Tabriz, Iran). Moreover, two commercially available products of EPNs, including Larvanem® (*Heterorhabditis bacteriophora*) and Capsanem® (*Steinernema carpocapsae*) were provided by Koppert B. V. (Berkel en Rodenrijs, The Netherlands). Native and commercial EPN strains were cultured in parallel on last instar wax moth, *G. mellonella*. The emerging IJs were harvested from White traps and stored in tap water at 12°C (Kaya and Stock 1997) for less than 4 weeks.

2.3. Pathogenicity assays

Assays were conducted to determine the susceptibility of immature stages of PTM, i.e., L2 larvae (5 days old), L4 larvae (12 days old) and prepupa (1 day old) to *H. bacteriophora* (FUM 7 and commercial strains), *S. carpocapsae* commercial strain, *S. feltiae* FUM 2 strain and *S. glaseri* IRAZ 19 strain. Preliminary tests were conducted to determine appropriate doses for the assays using 0, 10, 25, 50, 100, 250 and 500 IJs/ml of distilled water (data not shown). Subsequently, the pathogenicity assays were carried out in 60-mm-diameter Petri dishes lined with a filter paper (Whatman No. 1). The nematode IJs concentrations tested were 0, 75, 150, 250, 375 and 500 IJs in 1-ml distilled water uniformly distributed on the surface of each Petri dish. After inoculation of nematodes, 10 PTM larvae or prepupae (with cocoon) were introduced to each dish along with potato slices as food. Due to low number of *S. glaseri* IJs, assay on prepupa stage was not performed. The plates were covered, sealed with parafilm and incubated at controlled growth chamber, 26 ± 1°C and 65 ± 10% relative humidity (RH). Insect infectivity and mortality was checked after 48 h. Dead larvae and prepupae were dissected under the stereomicroscope to confirm that the mortality resulted from EPNs. The cadavers were also kept on White traps to observe nematode emergence. Each treatment combination was replicated four times and the experiment was conducted two times.

2.4. Soil experiments

Soil column assays were conducted to evaluate the effect of three soil types (loamy, sandy–loamy and sandy soil), which are dominant soil textures in potato fields, on the virulence of effective EPN species. At first, a loamy soil sample (clay 13%, silt 40% and sand 42%) was obtained from a potato field. Soil had been autoclaved and sieved through a 1-mm-pore sieve. River sand was used for preparing sandy–loamy soil (clay 6.6%, silt 19.42% and sand 74.1%) and sandy soil. Three EPN strains, including *S. carpocapsae*, *H. bacteriophora* FUM 7 and *H. bacteriophora* (commercial strain), with the highest pathogenicity in the first experiment were selected for the determination of the effects on the same developmental stages of PTM as described in the first experiment. The experiments were carried out in 80-ml plastic, 4-cm in diameter cups.

Cohorts of larvae or prepupae were started from eggs deposited at the same time. For larval experiments, individual tubers were placed in each cup, and then filter papers containing 20 eggs were added. Cups were incubated at $28 \pm 1^{\circ}C$ and $65 \pm 10\%$ RH. In the case of prepupa, ten 12-h-old pupae were placed into each cup. The cups were filled with 52 g of sterilised soil which received equal moisture (10%). Each cup was inoculated with 2 ml of distilled water containing 500 IJs (low concentration) and 2000 IJs (high concentration) of the EPN species/strains. Control treatments received 2-ml distilled water only. The cup was closed with a plastic cover perforated with a 9-mm hole covered with a fine sieve and incubated at $26 \pm 1^{\circ}C$ and $65 \pm 10\%$ RH in a growth chamber until the emergence of PTM adult. Prepupal assays were similar to the larval assays except that the assay was initiated after 12 h of adding soil. Each treatment combination was replicated four times and the experiment was conducted two times.

2.5. Statistics

Data were corrected for control mortality (Abbott 1925) and square-root transformed when necessary to meet assumptions of normality and homogeneity of variances. In the plate assays, the influence of nematode strain and concentration on the mortality of PTM in each stage were analysed by two-way full factorial ANOVA (nematode strain $\times$ nematode concentration). When ANOVA indicated a significant effect ($P < 0.05$), Fisher's protected Least Significant Difference (LSD) test was used to determine the significance between mean values. The LC_{50} values were also determined using the Probit procedure for each nematode strain in early and late larva stages of PTM. Parallelism test was applied to regression lines, and LC_{50} differences between nematode strains were considered significant when 95% fiducial limits did not overlap.

In the soil experiments, three-way full factorial ANOVA were performed by nematode strain, nematode concentration and soil type, and were followed by Fisher's protected LSD test to compare PTM mortality in each stage caused by interactive effects of nematode strain, nematode concentration and soil type. SAS software, version 9.1 was used for all statistical analyses (SAS Institute 2003).

3. Results

3.1. EPNs

Three out of two hundred soil samples were positive for EPNs, *Steinernema* or *Heterorhabditis*. EPNs strains were identified as *S. feltiae* (FUM 1 and FUM 2) and

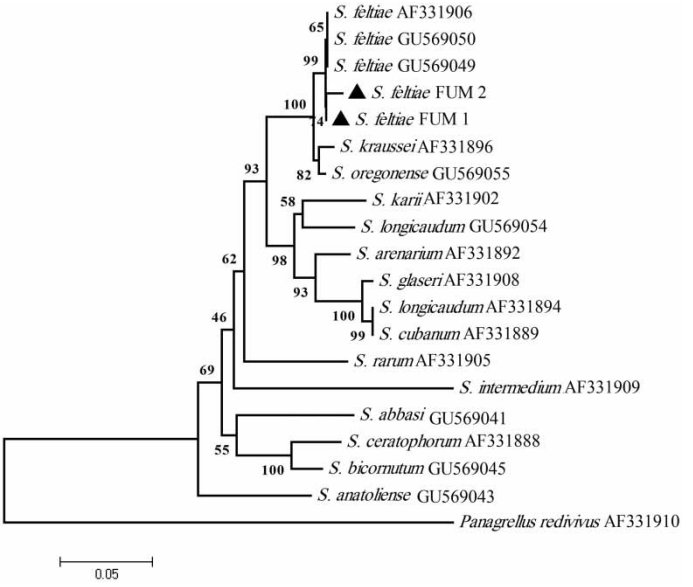

Figure 1. Phylogenetic relationships among *Steinernema* spp. based on D2/D3 expansion segments of the 28S rRNA gene sequences inferred by neighbour-joining analysis, using the Kimura two-parameter. *Panagrellus redivivus* was used as an outgroup. Numbers represent bootstrap frequencies (10,000 replicates).

H. bacteriophora FUM 7. GenBank accession numbers for ITS sequences of *S. feltiae* FUM1, *S. feltiae* FUM2 and *H. bacteriophora* FUM7 are JF920964, JN039363 and JX164230, respectively. Sequences of the 28S gene of *S. feltiae* FUM1, *S. feltiae* FUM2 and *H. bacteriophora* FUM7 were deposited in Genbank under accession numbers JF920966, JX436334 and JX183539, respectively. Phylogenetic position of Iranian strains of *S. feltiae* among their relatives in NJ tree based on ITS and D2/D3 sequences are represented in Figures 1 and 2. The topology of NJ and MP trees resulted from two methods were similar (the MP tree is not shown).

3.2. Pathogenicity assays

The susceptibility of the prepupal stage of PTM was significantly greater than that of larvae to *H. bacteriophora* FUM 7 ($F=22.9$; df $=2$, 114; $P<0.01$), *H. bacteriophora* ($F=16.76$; df $=2$, 117; $P<0.01$), *S. carpocapsae* ($F=3.34$; df $=2$, 117; $P<0.05$) and *S. feltiae* FUM 2 ($F=128.14$; df $=2$, 114; $P<0.01$) (Figure 3).

There were significant differences among nematode strains ($F=119.5$; df $=4$, 168; $P<0.01$) and concentrations ($F=181.75$; df $=4$, 168; $P<0.01$) on mortality of the L2 larvae. However, the interactive effect of nematode strains and concentrations on the mortality of early larvae was not significant ($F=1.42$; df $=16$, 168; $P=0.11$). *S. carpocapsae* and *H. bacteriophora* (commercial strain) were the most effective species causing higher mortality. Among the EPN strains, *S. glaseri* had the lowest infectivity (Figure 3). Commercial strains of *H. bacteriophora* and *S. carpocapsae* produced the lowest LC_{50} values with 81 and 84 IJs per early PTM larvae with a

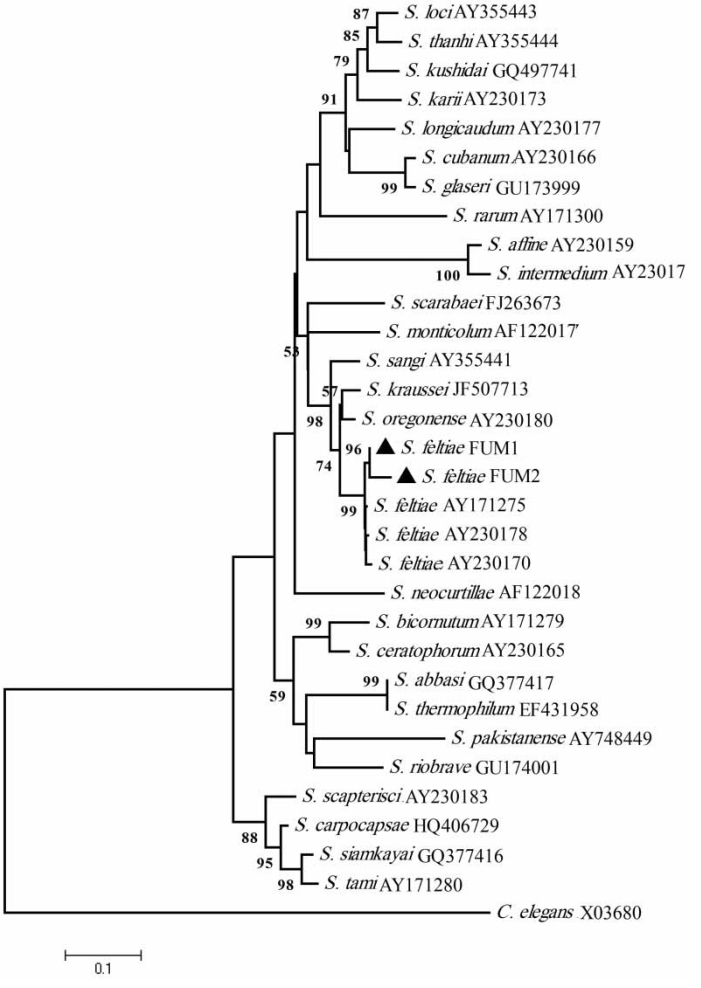

Figure 2. Unrooted neighbour-joining tree (with 10,000 replicate) constructed with the Kimura two-parameter distance calculation based on ITS sequence data of *Steinernema* spp. *Caenorhabditis elegans* used as an outgroup. Bootstrap support $\geq 50\%$ is indicated at branches.

confidence intervals ($\alpha = 0.05$) of 61–99 and 71–95, respectively (Table 2). LC_{50} for FUM 7, *S. feltiae* and *S. glaseri* were 323 (267–409), 392 (313–550) and 427 (364–531) IJs per early larvae, respectively (Table 1). Analysis of $\chi 2$ proved that the regression lines were not considered parallel ($\chi^2 = 51.8$, df $= 4$; $P < 0.05$), which indicates the qualitative and quantitative differential effect of nematode strains on the L2 larvae mortality.

In L4 larval assays, significant differences were observed among nematode strains ($F = 68.39$; df $= 4$, 175; $P < 0.01$) and concentrations ($F = 145.06$; df $= 4$, 175; $P < 0.01$) on larval mortality. However, the interaction between nematode strains and concentrations was not significantly different ($F = 1.32$; df $= 16$, 175; $P = 0.18$). *S. carpocapsae* and *H. bacteriophora* (commercial and FUM 7 strains) were significantly more virulent on late larvae, whereas *S. feltiae* and *S. glaseri* were less

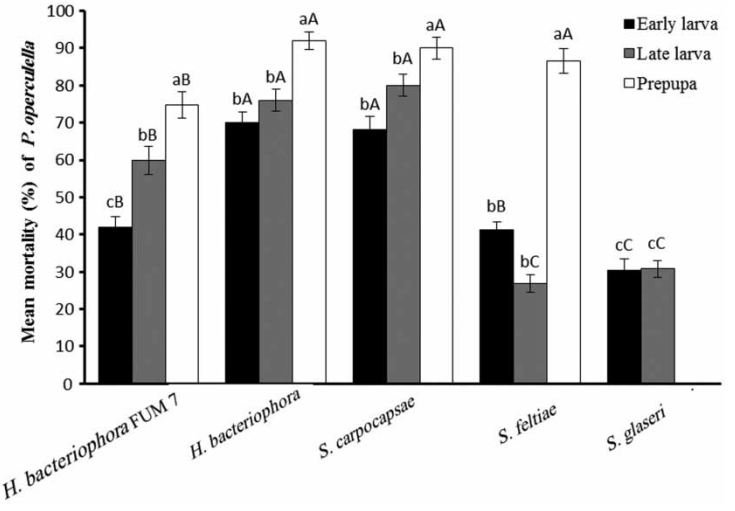

Figure 3. Mean mortality percentage (±SE) of *P. operculella* in different stages treated with five strains of EPN, applied at five dose concentrations in Petri dish assays. Bars topped by the different lower- and uppercase letters represent means that are statistically different among *P. operculella* stages and EPN strains, respectively (one-way ANOVA, followed by Fisher's protected LSD: $P < 0.05$) ($n = 8$).

virulent (Figure 3). *S. carpocapsae* had the lowest LC_{50} value with 64 IJs per late larva of PTM with a confidence interval ($\alpha = 0.05$) of 48–78 (Table 1). LC_{50} for *H. bacteriophora*, FUM 7, *S. glaseri* and *S. feltiae* were 101 (82–118), 153 (121–183),

Table 1. LC_{50} values for five EPNs species against the second (upper part) and fourth larval stages of PTM (*Phthorimaea operculella*) ($n = 8$).

Nematode species	LC_{50} (95% fiducial limits)	Intercept ± SE[a]	Slope ± SE	χ^{2b} (df = 3)	*P*-value[c]
Second larval stage					
S. feltiae	392 (313–550)	−2.61 ± 0.39	1.01 ± 0.16	2.35	0.51
S. glaseri	427 (364–531)	−4.22 ± 0.44	1.61 ± 0.18	1.94	0.58
H. bacteriophora	81 (61–99)	−3.01 ± 0.39	1.57 ± 0.17	5.34	0.15
H. bacteriophora FUM7	323 (267–409)	−2.95 ± 0.38	1.18 ± 0.16	5.67	0.13
S. carpocapsae	84 (71–95)	5.03 ± 0.46	2.61 ± 0.21	0.59	0.89
Fourth larval stage					
S. feltiae	642 (521–929)	−5.54 ± 0.81	1.97 ± 0.32	1.82	0.61
S. glaseri	530 (412–803)	−3.25 ± 0.46	1.19 ± 0.19	5.23	0.15
H. bacteriophora	101 (82–118)	−3.78 ± 0.42	1.69 ± 0.37	0.31	0.95
H. bacteriophora FUM7	153 (121–183)	−2.84 ± 0.40	1.30 ± 0.17	4.12	0.24
S. carpocapsae	64 (48–78)	−3.44 ± 0.43	1.91 ± 0.19	5.96	0.11

[a]SE, standard error.
[b]Pearson χ^2 of the slope.
[c]*P*-values represent the probability of the slope.

Table 2. Mortality (mean ± SE) of the second (L2) and fourth (L4) larval stages of *P. operculella* in three soil types including loamy, sandy–loamy and sandy after exposure to *H. bacteriophora* FUM 7, *H. bacteriophora* (commercial strain) and *S. carpocapsae* ($n = 8$).

			S. carpocapsae		*H. bacteriophora* **concentration (IJs)		*H. bacteriophora* FUM 7	
Stage	*n**	Soil type	500	2000	500	2000	500	2000
L2	80	Loamy	63 ± 8d***	96 ± 2ab	62 ± 1.8d	92 ± 2.2ab	59 ± 1.8d	92 ± 2.1ab
	80	Sandy	84 ± 3.8b	100 ± 0a	82 ± 4.3b	98 ± 2a	82 ± 6.5b	98 ± 3.5a
	80	Sandy–Loamy	71 ± 7.2c	100 ± 0a	70 ± 6.7c	96 ± 5.4ab	71 ± 2c	94 ± 2.8ab
L4	80	Loamy	82 ± 4b	96 ± 3.9a	52 ± 5.8e	92 ± 3.4ab	62 ± 6.4d	91 ± 1.8ab
	80	Sandy	80 ± 2.4b	96 ± 3a	68 ± 5.1c	89 ± 4.3ab	68 ± 5.6c	94 ± 3.7ab
	80	Sandy–Loamy	78 ± 5.6b	98 ± 1.8a	76 ± 3.4b	90 ± 3.6ab	74 ± 6.8 b	87 ± 5.1ab

*Number of individuals for each tested concentration.
**Commercial strain.
***Different letters for percent mortality show significant differences after Factorial ANOVA and Fisher's protected LSD test ($P < 0.05$).

530 (412–803) and 642 (521–929) IJs per late larva, respectively (Table 2). There was no evidence of any parallelism between the regression lines of nematode strains in L4 larvae stage ($\chi^2 = 21.68$, df = 4; $P < 0.05$).

The mortality of prepupa was significantly influenced by nematode strains ($F = 5.58$; df = 3, 137; $P < 0.01$). However, neither nematode concentration ($F = 1.08$; df = 4, 137; $P = 0.37$) nor the interactive effect of nematode strains and concentrations ($F = 1.37$; df = 12, 137; $P = 0.19$) on the mortality of prepupae was significant (Figure 3).

3.3. Soil experiments

S. carpocapsae, *H. bacteriophora* and FUM 7 strains which were the most efficient strains in the plate assays were used in soil experiments. The mortality of young PTM larvae was not significantly influenced by the effect of nematode strains ($F = 1.23$; df = 4, 126; $P = 0.31$) or by soil type ($F = 0.57$; df = 2, 126; $P = 0.52$). However, there was a significant interactive effect of nematode strain and soil type on the mortality of young larvae ($F = 3.98$; df = 4, 126; $P < 0.01$) and in general, nematode strains induced lower level of larval mortality in loamy soil, whereas sandy soil type was better for EPN pathogenicity (Table 2). The effect of applied concentration of IJs on larval mortality was significant ($F = 68.9$; df = 1, 126; $P < 0.01$), and larval mortality (96 ± 5.1) with 2000 IJs (160 IJs/cm^2) was higher than that with 500 IJs (40 IJs/cm^2; 72 ± 8.5%). There were no interactive effects for nematode strain × concentration ($F = 1.46$; df = 2, 126; $P = 0.22$), concentration × soil type ($F = 1.69$; df = 2, 126; $P = 0.19$), as well as concentration × nematode strain × soil type ($F = 2.12$; df = 4, 126; $P = 0.12$).

In older larvae, mortality was significantly influenced either by nematode strain ($F = 6.89$; df = 2, 122; $P < 0.01$) or IJ concentration ($F = 64.2$; df = 1, 122; $P < 0.01$). The larval mortality induced by *S. carpocapsae* (88 ± 3.4) was higher than those caused either by the commercial strain of *H. bacteriophora* (79 ± 4.1) or by

H. bacteriophora FUM 7 (78 ± 5.2). In addition, larval mortality (92 ± 9.6) with 2000 IJs (160 IJs/cm^2) was greater than that with 500 IJs (40 IJs/cm^2; 71 ± 6.8). The effect of soil types on larval mortality was not significant ($F = 1.81$; df = 2, 122; $P = 0.17$). However, there was a significant interactive effect of soil type and applied concentration of IJs on the mortality of older larvae ($F = 5.17$; df = 2, 122; $P < 0.01$) (Table 2). While the mortality caused by higher IJs concentration (2000 IJs) was not significantly different among soil type, the mortality of older larvae induced by lower IJs concentration (500 IJs) was significantly lower in loamy soil than that in other soil types (Table 2).

The mortality of PTM pupae was not significantly affected by nematode strain ($F = 0.89$; df = 2, 126; $P = 0.42$), soil type ($F = 2.71$; df = 2, 126; $P = 0.09$) or nematode concentration ($F = 2.23$; df = 2, 126; $P = 0.12$). Also, there was no interactive effects for nematode strain × concentration × soil type on the mortality of prepupae ($F = 1.61$; df = 4, 126; $P = 0.19$).

4. Discussion

This study presents the first data on the susceptibility of PTM larva and prepupa to native and non-native strains of EPNs in three different soil profiles. In general, *S. carpocapsae* and *H. bacteriophora* had high efficacy among all species/strains of studied EPNs. Ivanova, Borovaya, and Danilov (1994) also showed that *S. carpocapsae*, *H. bacteriophora* and *S. bibionis* caused significant mortality in larval stages of PTM. In agreement with Lacey and Kroschel (2009), who reported that EPNs have good potential in controlling stages of PTM that enter or emerge from the soil, the results of our study also indicate that EPNs are able to cause high mortality in soil dwelling stages of PTM. As shown in this study, high effectiveness of *S. carpocapsae* and *H. bacteriophora* on most lepidopteran pests than other Steinernematid and Heterorhabditid nematodes has been previously demonstrated (Siegel et al. 2004; Barbosa-Negrisoli, Negrisoli, Dolinski, and Bernardi 2010).

The susceptibility of PTM to EPN infection depended on different factors such as the developmental stage of insect, the age of the host insect within a given stage, soil type, EPN species/strain and IJ concentration as well as foraging behaviour strategy of the EPN. Thus, responsible elements that cause differences among insect stages and nematode species/strains effectiveness need to be addressed.

The stage of insect development has a significant effect on vulnerability to EPNs (Kaya and Hara 1980). Prepupa was the most susceptible stage, showing the highest mortality across all EPNs concentrations. Similar results were obtained by Kaya and Hara (1980) during their work on several species of lepidopterans, on *Spodoptera exigua* (Hubner) (Kaya and Grieve 1982) and on *Mocis latipes* (Guenée) (González-Ramírez et al. 2000). It seems that developmental events during the pupal stage might influence infective juvenile penetration rates (Dolinski, Del Valle, and Stuart 2006).

In relation to susceptibility among larval stages, the plate assays showed that young and older larvae have different mortality rates. This could be related to experimental conditions in the Petri dish assay. Because of the smaller size of young larvae, they could burrow into potato pieces but there was no such shelter for older larvae. The age-related susceptibility of host insects to EPNs is variable as some studies showed enhancing infectivity with increasing larval instars, while others

indicate an opposite trend (Kaya 1985; Glazer and Navon 1990; Journey and Ostlie 2000).

In the first experiment, both genera of EPNs from different species/strains with three types of foraging strategy were used. Virulence of EPNs towards the PTM larvae differed among species. In general, pathogenicity of *S. carpocapsae* and *H. bacteriophora* (commercial strain) was significantly higher in all stages while efficacy of *S. feltiae* FUM3, *H. bacteriophora* FUM7 and *S. glaseri* IRAZ19 had poor to moderate virulence to PTM larvae. One probable reason for higher mortality with *S. carpocapsae* and *H. bacteriophora* was the temperature that the experiment was carried out. *S. carpocapsae* and *H. bacteriophora* have optimum infection at 25°C, whereas other species are capable of infecting insects at different temperatures, *S. glaseri* at higher temperatures and *S. feltiae* at lower temperatures (Lacey, Neven, Headrick, and Fritts 2005). Of note are that differences observed between EPNs species from separate sources, native and commercial, which are evaluated under laboratory conditions may or may not be attributed directly to virulence (Lacey et al. 2005).

The second part of our experiment was designed to determine the effects of soil type on the pathogenicity of EPNs against PTM in tubers. A positive relationship was observed between decreased moth emergence and increase in nematode concentration. *S. carpocapsae* and both strains of *H. bacteriophora* caused high mortality on larval and prepupal stages in all tested soil types. The reason for the high efficacy of nematodes in all soils might be the small size of test containers, and consequently the shorter distance which nematodes must move to reach the host insect.

The interaction between species/strain and soil type on young larvae was significant. Possible explanation for this interactive effect might be the ecological niche of early larvae that remain in the tuber, while older larvae emerge from tubers and move through the soil profile, therefore, the soil type would not have as an important role on effectiveness of the EPN. The results showed that *S. carpocapsae* and *H. bacteriophora* strains in sandy and sandy–loamy soils caused higher mortality than loamy soil. This observation is consistent with early reports that showed infectivity of *S. carpocapsae* and *H. bacteriophora* were higher in lighter soils (Kung, Gaugler, and Kaya 1990; Choo and Kaya 1991) and it supposed that mobility of EPNs reduces as soil particle size decreases (Shapiro, McCoy, Fares, Obreza, and Dou 2000). In addition, it was noted that sandy soils are appropriate for nematode survival, host finding behaviour and infectivity (Kung et al. 1990). However, some strains/populations might be adapted to heavier soils. Georgis and Gaugler (1991), Shapiro et al. (2000), Lezama-Gutiérrez et al. (2006) and Toepfer, Kurtz, and Kuhlmann (2010) stated that infectivity of EPNs was higher in heavier soils than that in sandy ones. Differences between observations could be attributed to other characters of the soils tested, e.g., pH, nutrient content, insect species and experimental methods (Shapiro et al. 2000).

Foraging strategy of EPNs can affect their efficacy in controlling pests (Lezama-Gutiérrez et al. 2006). Some species such as *S. carpocapsae* display a nictation or ambusher foraging strategy which tends to remain near the soil surface waiting for its host, whereas a number of species like *H. bacteriophora* display a cruiser foraging strategy, which seek their host actively and are more effective against less mobile insects in soils (Lewis 2002). In this study, we expected that *H. bacteriophora*, because

of its foraging strategy, would be more virulent than *S. carpocapsae* but both species caused similar mortality. In spite of ambusher behaviour of *S. carpocapsae*, there are several reasons for high pathogenicity of *S. carpocapsae* in our experiments. The nictation strategy of *S. carpocapsae* allows it to be more effective longer in soil (Yee and Lacey 2003). Reduced movement of *S. carpocapsae* may be the reason for higher energy reserves in this species. This energy is adequate to provide sufficient chance for *S. carpocapsae* to penetrate and kill the PTM larvae. A similar explanation was proposed by Yee and Lacey (2003).

To promote the use of EPNs as biological control agents, locally adapted species need to be identified and their unique characteristics, such as pathogenicity and survival under extreme conditions, documented (Stock, Pryor, and Kaya 1999). In this study, we used two native strains of EPNs, however, a significant difference in efficacy between native and commercial strains of *H. bacteriophora* was not observed. EPNs must usually be applied to soil at rates of 25 IJs/cm^2 or higher (Shapiro-Ilan, Gouge, Piggott, and Fife 2006). In this study, however, the rates of 40 and 160 IJs/cm^2 appear to be a requirement to reduce larval numbers by 70% and 90% or greater, respectively. This variation between recommended application rate of IJs in soil and applied concentration in our study may be due to cryptic habitat of PTM larvae which inhabit galleries in potato tubers in soil.

The effectiveness of *S. carpocapsae* and *H. bacteriophora* against PTM larvae and prepupae in loamy and sandy–loamy soils is important because potatoes grow well in these soil types. On the other hand, EPNs can proliferate within the host cadavers, an advantage over insecticides, and thus can respond to high pest densities (Gaugler 2002). In this respect, the overlap between generations of PTM result in high populations (Yathom 1986) providing suitable conditions for use of EPNs against larval and prepupal stages of PTM during the growing season. Further studies on biocontrol potential of EPNs against *P. operculella* in field studies are warranted.

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