

Orijinal araştırma (Original article)

Effects of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser), in biological control of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae)

Agrotis segetum (Denis & Schiffermüller) (Lepidoptera: Noctuidae)' un biyolojik mücadelesinde entomopatojen nematodlar, *Heterorhabditis bacteriophora* (Poinar) ve *Steinernema carpocapsae* (Weiser)' in etkinlikleri

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Summary

Agrotis segetum (Denis & Schiffermüller) (Lepidoptera: Noctuidae) is one of the most serious pests in Iran that attacks nearly all vegetables. Using synthetic insecticides is the main controlling method of this pest. Human and environmental health hazards on insecticides usage encourage scientists to search for alternative safer methods. This research is devised to evaluate the potential of two indigenous entomopathogenic nematodes against this pest. Ability of different concentrations of infective juveniles to infect penultimate and last instars larvae, pre-pupa and pupa was assessed under laboratory condition after 12, 24 and 48 h. Susceptibility of larval stages, pre-pupa and pupa to different concentrations of both entomopathogenic nematodes was evaluated in a pot experiment. Final instar larvae was the most susceptible stage in both laboratory and greenhouse condition. Pre-pupa was more vulnerable to entomopathogenic nematodes as compared with pupa. The mortality increased with increasing in the time of exposure. After 12 hours, the LD₅₀ of *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser) on final instar larvae were 34 and 56 infective juveniles per 10 cm Petri dish respectively. About 98 and 90% of final instar larvae were parasitized five days after exposing to *H. bacteriophora* and *S. carpocapsae* in the greenhouse. According to the results, these two indigenous entomopathogenic nematodes have good potentials in managing *A. segetum*.

Keywords: Biocontrol, common cutworm, lethal dose, pest management, turnip moth

Özet

Agrotis segetum (Denis & Schiffermüller) (Lepidoptera: Noctuidae), İran'da hemen hemen tüm sebze bitkilerine saldıran en önemli zararlılardan birisidir. Zararlıının mücadelesinde sentetik kimyasallar kullanılmaktadır. İnsektisit kullanımının çevreye insan hayatına vermiş olduğu olumsuz etkileri ortadan kaldırmak için bilim insanları zararlılar ile mücadele için alternatif ve daha güvenli metotlar araştırmaktadırlar. Bu çalışma bu zararlıya karşı iki entomopatojen nematod türünün potansiyelini belirlemek için planlanmıştır. Farklı konsantrasyondaki nematod juvenillerinin zararlıının son bir önceki, son, pre-pupa ve pupa dönemleri üzerindeki etkisi laboratuvar koşullarında uygulamadan 12, 24 ve 48 saat sonra değerlendirilmiştir. Larva dönemlerinin, pre-pupa ve pupa dönemlerinin her iki nematodun farklı konsantrasyonlarına uygunluğu saksı denemeleri ile belirlenmiştir. Zararlıının son larva dönemi hem laboratuvar hem de sera koşullarında nematodlar için en uygun dönem olarak belirlenmiştir. Pupa dönemi ile karşılaştırıldığında pre-pupa dönemi nematodlara karşı daha hassas bulunmuştur. Ölüm oranı zararlıının nematodla muamele süresi arttıkça artmıştır. Uygulamadan 12 saat sonra *Heterorhabditis bacteriophora* (Poinar) ve *Steinernema carpocapsae* (Weiser)' nin son larva dönemine karşı 10 cm çapındaki Petri kabında LD50 değeri sırasıyla 34 ve 56 juvenil bulunmuştur. Serada *H. bacteriophora* ve *S. carpocapsae* uygulamalarından beş gün sonra son larva dönemlerinin yaklaşık % 98 ve 90'nın nematodlar tarafından parazitlendiği tespit edilmiştir. Araştırma sonuçlarına göre her iki entomopatojen nematod türünün *A. segetum* mücadelesinde önemli bir potansiyele sahip olduğu belirlenmiştir.

Anahtar sözcükler: Biyolojik mücadele, bozkurt, letal doz, zararlılarla mücadele, şalgam güvesi

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Introduction

Larvae of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) have a widespread host range and can attack the roots and lower stems of their host plants. Their presence is often revealed only when the plants are already damaged severely (Bourner et al., 1992; Bowden et al., 1983). This moth is considered as one of the most serious pests on vegetables and cereals throughout Asia, Europe and parts of Africa that could impose a considerable economic loss (Jakubowska et al., 2005). Management of this pest is difficult due to their soil-dwelling habit. The current control method is mainly based on different kinds of insecticides that have met varying success (Sevim et al., 2010). Hazards on environment and human health have encouraged researchers to find some alternative safe measures for chemicals (Moosavi & Zare, 2012).

Biological control by entomopathogenic nematodes (EPNs) has attracted much attention in the last few decades (Hunt, 2007). Among all EPNs, members of the families Steinernematidae and Heterorhabditidae are considered effective (Hominick, 2002; Adams et al., 2006) with a great potential for biological control, especially against soil-inhabiting insects (Ehler, 1990; Koppenhöfer, 2000; Sharma et al., 2011). Rapid death of the host insects, searching ability of hosts, easy to use, long-term effect, surviving ability in environment, being safer to non-target organisms and compatibility with many chemical insecticides are some of EPNs' favorable features that make them suitable as biocontrol agents (Koppenhöfer & Kaya, 2002; Vashisth et al., 2013). There is significant variation among different species/isolates of EPNs in their host range, environmental requirement for survival and pathogenicity (Bedding, 1990; Lacey & Georgis, 2012). Indigenous isolates of EPNs may have greater potential in biocontrol as a result of their compatibility to native habitats (Griffin et al., 2005); therefore, it is rational to evaluate the ability of locally adapted species or isolates in controlling significant pests of that region (Moosavi & Zare, 2015).

Lepidopterans are considered as a susceptible host for Steinernematids and Heterorhabditids (Vashisth et al., 2013). Additionally, existence of the common cutworm's larval stages below ground, make them an appropriate target for biocontrol by EPNs (Georgis et al., 2006). Thus the present study was designed to evaluate the effect of two Iranian species of EPNs (Damani Zamani et al., 2015) on biological control of the common cutworm, *Agrotis segetum*, in laboratory and greenhouse.

Materials and Methods

Preparation of entomopathogenic nematodes inoculum

Two indigenous nematode species maintained alive in the Marvdasht branch, Islamic Azad University EPN Collection were used in this research. Enough population of nematodes were reared on *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. The greater wax moth larvae was collected from infected hives and reared on an artificial medium (Metwally et al., 2012).

Petri dishes (100 × 15 mm) were lined with two pieces of filter papers and were moistened with 2 or 3 ml of water containing 200-300 infective juveniles (IJs) of *Steinernema carpocapsae* (Sc) or *Heterorhabditis bacteriophora* (Hb). Ten late instar larvae of the greater wax moth were exposed to IJs of nematodes by putting on filter papers (Nguyen, 2007). The IJs migrated away from the host cadaver upon emergence and were harvested on the White trap (White, 1927; Ehlers & Shapiro-Ilan, 2005). IJs were stored at 12°C (Stock & Goodrich-Blair, 2012) for one week and were permitted to acclimatize at room temperature for 1 h before using as inoculum. The viability of IJs in nematode suspension was assessed by observation of movement under a stereomicroscope.

Collecting and rearing of *Agrotis segetum*

The common cutworm larvae were collected from different tomato fields near Marvdasht, south west of Iran. In order to prepare enough larvae for the experiment, sufficient population of *Agrotis segetum* was established in growth chamber at a temperature of 23°C, relative humidity 60% and a photoperiod of 17:7 (L:D) (Rosen, 2002). Different healthy larval stages and pupae were selected to be used in pathogenicity test.

Pathogenicity test

Laboratory experiment

The virulence of two indigenous nematode species against pre-pupa, pupa and two last larval stages (4th and 5th instar larvae) of common cutworm was evaluated in a laboratory experiment. Ten larvae in fourth or fifth instar stage were put on two moist filter papers in a 100 × 15 mm Petri dish inoculated with 1 ml of water containing different concentrations of IJs. The experiment was established in a completely randomized design with a factorial treatment arrangement consisting of two nematode species (Hb and Sc) and 4 application rates (25, 50, 75, and 100 IJs/dish). The number of dead larvae was recorded at 3 different exposure times (24, 48 and 72 hour). Control plates were treated with distilled water only. Five replicates were considered for each treatment and Petri dishes were kept at 27 ± 1 °C. Dead larvae were recognized according to change in their body color. Cadavers were transferred to White trap to confirm nematode infection.

The susceptibility of pre-pupal and pupal stages of the common cutworm was also assessed in soil. Ten insect's pre-pupa or pupa was placed at the bottom of a dish with 3 cm depth and the dish was filled with 23 g of moistened sterile sandy loam soil (sand 67.3%, clay 12.1%, silt 20.6%, organic matter 3.5% and pH 7.5). The experiment was conducted at 27 ± 1 °C in a factorial arrangement consisting of two nematode species (Hb and Sc) and 3 application rates (50, 100 and 200 IJs/ cm² of soil) with five replicates. IJs of two nematode species were applied on the soil surface. The amount of water in nematode suspension was adjusted that the final soil moisture level reached to 10% (w/w). Control plates were treated with distilled water only. Mortality of the pre-pupae and pupae was recorded 48 and 72 h after inoculation by transferring on individual White trap to verify the mortality was due to nematode infection.

Greenhouse experiment

500 g plastic pots (15 cm diameter, 15 cm depth) were filled with sterile sandy loam soil (sand 67.3%, clay 12.1%, silt 20.6%, organic matter 3.5% with pH 7.5) and two disinfected tomato seeds (*cv.* Early Urbana) were sown in each pot. After two weeks, one seedling was selected and the other was eliminated. Each nematode species was evenly applied on to the soil surface of each pot at the rate of 8, 10 and 20 IJs/g soil (respectively equal to 25.5, 28.3 and 56.6 IJs cm⁻² soil). After 48 h, the pots were separately inoculated with ten larvae (2nd to 5th larval stages), pre-pupae and 3-days-old pupae of the common cutworm. Different developmental stages of insect were put at depth of 2 cm and were covered with soil. After five days, the number of dead insects was counted. Cadavers were transferred to White trap to confirm nematode infection. The experiment was carried out in a completely randomized design with five replicates.

Statistical analysis

The mortality percentage of each treatment was corrected according to the control treatment values by Abbott's formula (Abbott, 1925). Statistical analyses were carried out by SAS software (version 9.1.3; SAS Institute, Cary, NC) (1990). Mean values were separated using Duncan's Multiple Range Test ($P < 0.05$). Polynomial regression was performed on larval mortality data in the laboratory experiment to determine the lethal dosage which kills 50% (LD₅₀) and 90% (LD₉₀) of insect's population for each nematode species (Sigmaplot 11, Systat Software Inc., San Jose, CA). The effect of same concentrations of *H. bacteriophora* and *S. carpocapsae* on mortality of each developmental stage of *A. segetum* in greenhouse was compared by the Independent-Samples T Test.

Results

Laboratory experiment

The mortality of insect's larvae was significantly influenced by nematode species (N), nematode concentration (C), and their interaction (N × C) (Table 1). At all exposure times, *H. bacteriophora* caused a significantly greater mortality in 4th and 5th larval stages of *A. segetum* than *S. carpocapsae* did. Similarly, the highest larval mortality was achieved when EPNs were applied at a dose of 100 IJs / dish.

Table 1. Analysis of variance for mortality of 4th and 5th instar larvae of the common cutworm (*Agrotis segetum*) on filter paper when they exposed to four different concentrations (25, 50, 100 and 200 IJs / Petri dish) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in a factorial design

Source ^a	Mean squares					
	L4 mortality percent ^b			L5 mortality percent ^b		
	12 h	24 h	48 h	12 h	24 h	48 h
N	390.6**	1322.5**	1232.5**	1050.6**	1500.6**	1322.5**
C	3377.3**	3407.5**	4026.7**	3029**	3914**	4689.2**
N × C	167.3**	174.2**	142.5**	139**	174**	149.2**
Error	31.87	24.37	22.2	35.3	20.6	20
C.V. (%)	18.3	32.7	24.5	15.2	27.4	29.2

** : significant at 1% probability levels

^a N = nematode species, C = nematode concentration

^b The corrected mortality percents were considered for analysis.

The mortality percent of 4th and 5th instar larvae was increased with increase in exposure time. When L₄ and L₅ were exposed to EPNs for similar time span, L₅ was more vulnerable than L₄ was. After 48 h, *H. bacteriophora* and *S. carpocapsae* caused 98% and 87% mortality respectively in last instar larvae when they were applied at a dose of 100 IJs / dish (Table 2).

Table 2. The effect of different concentrations of *Heterorhabditis bacteriophora* (Hb) and *Steinernema carpocapsae* (Sc) on mortality of 4th and 5th instar larvae of the common cutworm (*Agrotis segetum*) on filter paper

EPN species	No. of IJs ^a	% dead L4 (mean ± SE) ^b			% dead L5 (mean ± SE) ^b		
		12 h	24 h	48 h	12 h	24 h	48 h
Hb	25	33 ± 2.5 e E	39 ± 1.8 d CD	41 ± 1.9 d BC	39 ± 1.9 c CD	46 ± 1.8 e AB	49 ± 1.9 e A
	50	55 ± 3.5 c C	70 ± 2.2 b AB	75 ± 2.3 b A	65 ± 4.5 b B	73 ± 2.5 bc AB	79 ± 1.9 c A
	100	72 ± 2.5 a D	83 ± 1.2 a BC	89 ± 2.9 a B	77 ± 2.5 a CD	88 ± 1.2 a B	98 ± 1.2 a A
	200	61 ± 1.9 bc D	70 ± 2.2 b BC	76 ± 1.7 b AB	67 ± 2.5 b C	73 ± 1.2 bc BC	80 ± 2.2 c A
Sc	25	23 ± 2.5 f B	29 ± 1.9 e AB	32 ± 2.6 e A	27 ± 2.5 d AB	28 ± 2.5 f AB	34 ± 1.9 f A
	50	41 ± 1.8 d D	49 ± 1.8 c BC	54 ± 1.9 c AB	46 ± 1.9 c CD	53 ± 2.5 d B	60 ± 2.2 d A
	100	66 ± 2.9 ab D	69 ± 2.7 b CD	76 ± 1.8 b BC	68 ± 2.5 b D	79 ± 1.9 b B	87 ± 2.5 b A
	200	66 ± 1.7 ab C	69 ± 2.9 b BC	73 ± 1.2 b B	66 ± 1.8 b C	71 ± 1.9 c BC	79 ± 1.9 c A

^a the number of infective juveniles in each 100 × 15 mm Petri dish.

^b Mean values followed by different lowercase letters in the same column, or followed by different uppercase letters on the same row are significantly different according to Duncan's test (P < 0.05). Each treatment had five replications.

After 48 h, the mortality of pre-pupa and pupa of *A. segetum* significantly differed according to nematode species (N), nematode concentration (C), and their interaction (N × C) (Table 3). However, no significant effect of nematode species and nematode concentration was observed after 72 h of exposure (Table 3). After 48 or 72 h, *H. bacteriophora* caused greater mortality to pre-pupa and pupa than did *S. carpocapsae*. At the similar time of exposure, the highest level of parasitizing occurred at the highest dose of EPNs (200 IJs cm⁻² soil).

Pre-pupae were more vulnerable to both EPN species than pupae. Mortality percent increased as the time of exposure increased (Table 4). When IJs of *H. bacteriophora* were applied at a dose of 200 IJs cm⁻² of soil, 80 and 66 % of pre-pupa and pupa were respectively killed after 72 h. Infection rate of pre-pupa and pupa by *S. carpocapsae* was respectively 76 and 50 percent 72 h after inoculation with a 200 IJs cm⁻² soil (Table 4).

Table 3. Analysis of variance for mortality of pre-pupae and pupae of *Agrotis segetum* in soil when they exposed to three different concentrations (50, 100 and 200 IJs/cm² soil) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in a factorial design

Source ^a	Mean squares			
	Pre-pupae mortality percent ^b		pupae mortality percent ^b	
	48 h	72 h	48 h	72 h
N	907.5**	163.3*	1333.3**	213.3*
C	6070**	5923.3**	6107.5**	2822.5**
N × C	270**	3.3 ^{ns}	425.8**	55.8 ^{ns}
Error	20	29.2	22.9	27.1
C.V. (%)	35.6	19.1	21.8	33.4

^{ns}, *, **: Non significant, significant at 5% and 1% probability levels, respectively.

^a N = nematode species, C = nematode concentration

^b The corrected mortality percents were considered for analysis.

Table 4. The effect of different concentrations of *Heterorhabditis bacteriophora* (Hb) and *Steinernema carpocapsae* (Sc) on mortality of pre-pupa and pupa of the common cutworm (*Agrotis segetum*) in soil in laboratory experiment

EPN species	No. of IJs ^a	% dead pre-pupa (mean ± SE) ^b		% dead pupa (mean ± SE) ^b	
		48 h	72 h	48 h	72 h
Hb	50	15 ± 1.6 d B	32 ± 2.5 c A	5 ± 1.6 e C	20 ± 3.2 d B
	100	35 ± 2.2 c B	50 ± 2.2 b A	24 ± 2.9 c C	40 ± 1.6 b B
	200	73 ± 2.5 a AB	80 ± 2.2 a A	50 ± 1.6 a C	66 ± 2.9 a B
Sc	50	10 ± 1.6 d B	28 ± 2.5 c A	2 ± 1.2 e C	13 ± 1.2 e B
	100	30 ± 2.2 c B	44 ± 1.9 b A	15 ± 2.3 d C	31 ± 1.9 c B
	200	50 ± 1.6 b B	76 ± 2.9 a A	38 ± 1.2 b C	50 ± 3.5 a B

^a the number of infective juveniles per cm² of soil. IJs were applied onto the soil surface in Petri dish.

^b Mean values followed by different uppercase letters on the same row, or followed by different lowercase letters in the same column are significantly different according to Duncan's test (P < 0.05). Each treatment had five replications.

The estimated LD₅₀ of each nematode species on two developmental stages (L₄ and L₅) of the common cutworm at certain time after inoculation is presented in table 5. The LD₉₀ was calculated when it was applicable. The required number of both EPNs for killing 50% of each developmental stage of the pest decreased with increase in exposure time.

Table 5. The calculated LD₅₀ or LD₉₀ of *H. bacteriophora* and *S. carpocapsae* on the penultimate and last instar larvae of the common cutworm at different exposure times

Growth stage	Time (h)	<i>Heterorhabditis bacteriophora</i>			<i>Steinernema carpocapsae</i>		
		LD ₅₀	LD ₉₀	R ^{2a}	LD ₅₀	LD ₉₀	R ^{2a}
L ₄	12	43	–	0.87	65	–	0.94
	24	32	–	0.95	51	–	0.92
	48	30	–	0.94	45	–	0.95
L ₅	12	34	–	0.84	56	–	0.93
	24	28	–	0.95	47	–	0.95
	48	25	65	0.96	39	114	0.96

^aR² shows the coefficient of determination for fitting data in each regression model.

Greenhouse experiment

The virulence of the both EPNs against the common cutworm was similar in greenhouse. No significant difference was found between *H. bacteriophora* and *S. carpocapsae* when the same concentration of them was applied on the same developmental stage of *A. segetum*.

The last instar larvae of *A. segetum* were more vulnerable to both nematode species than other developmental stages were (Figures 1 and 2). The least susceptible developmental stages to *H. bacteriophora* were second and third instar larvae while the least susceptible developmental stage to *S. carpocapsae* was pupa. The best nematode dose for control of nearly all developmental stages of the common cutworm was 10 IJs per g of soil (equal to 28.3 IJs cm² soil). Higher or lesser concentrations of EPNs resulted in lesser efficacy against *A. segetum*; however, 8 IJs g⁻¹ soil (= 25.5 IJs cm² soil) was more effective compared with 20 IJs g⁻¹ soil (= 56.6 IJs cm² soil).

Application of *H. bacteriophora* on soil surface of tomato pots resulted in a maximum 98% kill of fifth instar larvae five days after inoculation with a dose of 10 IJs g⁻¹ soil. Only 54% of 2nd instar larvae were infected by *H. bacteriophora* when it was applied at a dose of 20 IJs g⁻¹ soil (Figure 1).

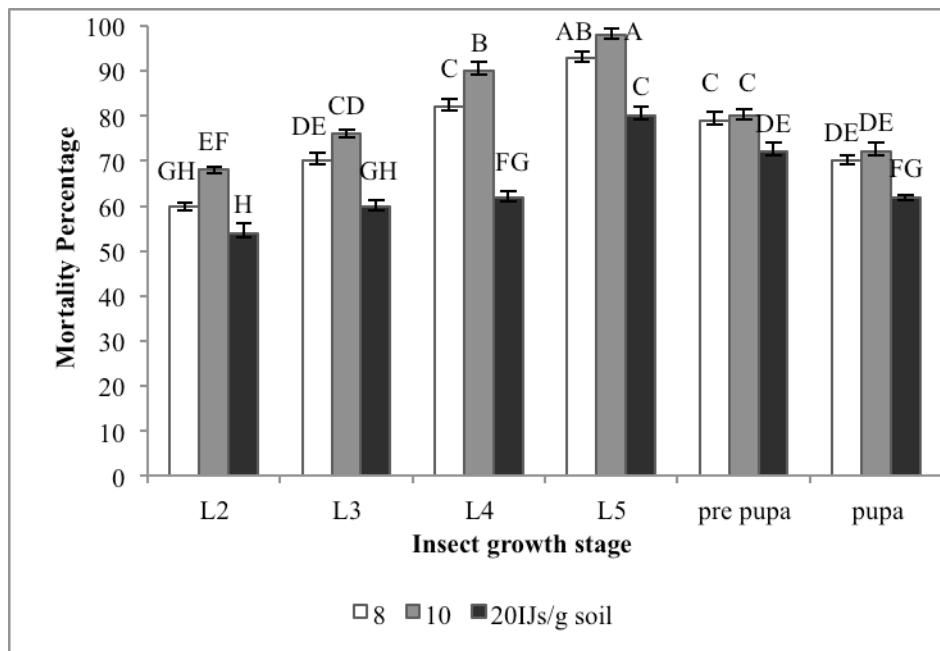


Figure 1. Corrected mortality percent of different developmental stages of the common cutworm five days after inoculation with three concentrations of *Heterorhabditis bacteriophora* in tomato pots. Bars on the columns correspond to standard error. Each treatment had five replications. Columns with the similar letter(s) are not significantly different ($P < 0.05$).

The *S. carpocapsae* respectively caused 90% and 85% mortality in 5th and 4th instar larvae when it was applied at a dose of 10 IJs g⁻¹ soil. Eight IJs g⁻¹ soil was more virulent to all insect's developmental stages than 20 IJs g⁻¹ soil was, except for pupal stage. Contrary to other treatments, pupal's infection by *S. carpocapsae* increased when the application dose of IJs per gram of soil was increased (Figure 2).

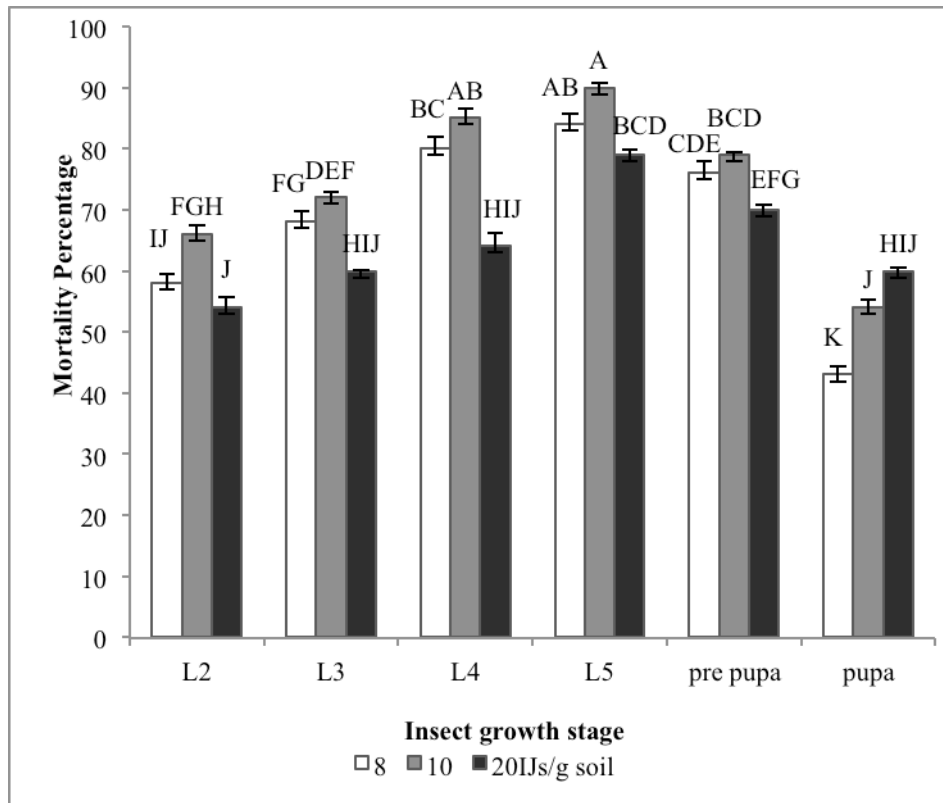


Figure 2. Corrected mortality percent of different developmental stages of the common cutworm five days after inoculation with three concentrations of *Steinerinema carpocapsae* in tomato pots. Bars on the columns correspond to standard error. Each treatment had five replications. Columns with the similar letter(s) are not significantly different ($P < 0.05$).

Discussion

EPNs have been successfully employed as efficient biocontrol agents against the larvae of some noctuids, including *Agrotis ipsilon* (Hufnagel) and *A. segetum* in some Asian or European countries (Georgis et al., 2006; Kaya et al., 2006; Lacey & Georgis, 2012). Both *Heterorhabditis* spp. and *Steinerinema* spp. are considered as promising biological control agents of important insect pests due to their ability to seek target hosts, kill hosts rapidly, being safe to non-target organisms and having no threat to the environment-as compared to chemical insecticides (Vashisth et al., 2013).

On filter paper, *H. bacteriophora* provided a more efficient control of the common cutworm's larvae. Increasing in time of exposure caused more mortality in 4th and 5th instar larvae for both nematode species (Table 2). Similar results have been repeatedly reported in which increasing exposure time raised the mortality rate of most developmental stages (Capinera et al., 1988; Jackson & Brooks, 1995; Ansari et al., 2006; Ebssa & Koppenhöfer, 2012; Gökçe et al., 2013; Kamali et al., 2013) due to increasing the possibility of encountering with and penetrating into the insect by IJs.

Insect's larval (L₄ and L₅) mortality was IJs-does-dependant. It is suggested that exposing insect hosts to higher dose of EPNs may obfuscate host recognition and decrease host mortality (Lewis, 2002). Though the filter paper technique cannot simulate field conditions accurately, it is usually used as a quick method of efficacy assessment (Capinera et al., 1988).

The common cutworm pre-pupa was more susceptible to both EPNs rather than pupa. *H. bacteriophora* appeared to act better since it inflicted higher mortality in pre-pupal and pupal stages of insect than *S. carpocapsae* did. The soil technique surely is more similar to natural conditions (Stock & Goodrich-Blair, 2012). Pre-pupa further susceptibility may be due to the fact that the natural opening of this developmental stage is not completely sealed yet and its cuticle is thinner.

The LD₅₀ of *H. bacteriophora* on fourth and fifth larval stages of *A. segetum* was lower than those of *S. carpocapsae* (at similar time span). Because of decline in larval mortality at doses more than 100 IJs, LD₉₀ was only calculable after 48 h and on L₅ where the mortality percent surpassed 90%. The pathogenicity of EPNs may greatly differ according to different species / isolates (Bedding, 1990; Lacey & Georgis, 2012). The LD₅₀ of *Steinernema kraussei* (Steiner) against *A. segetum* third instar larvae was 99 IJs g⁻¹ dry sand seven days after treatment (Gökçe et al., 2013). Application of a Japanese isolate of *S. carpocapsae* against *A. segetum* larvae at a rate of 5×10^5 and 10^6 IJs/m² soil respectively caused 67% and 80% mortality (Yokomizo & Kashio, 1996). Among three Turkish *Steinernema* species, *S. feltiae* (Filipjev), *S. weiseri* (Mráček, Sturhan & Reid) and *S. carpocapsae*, the latter was more effective when applied at 10, 25, 50 and 100 IJs per larva (Unlu et al., 2007). The LD₅₀ of two isolates of *S. carpocapsae* (in the original article the species of the nematode was identified as *S. feltiae* according to confusion on the nomenclature at that time) against *A. ipsilon* was determined as 16 and 486 IJs per 100 g soil (Capinera et al., 1988). Significant variation was also reported for different strains of *S. carpocapsae* against *Curculio caryae* (Horn) (Shapiro-Ilan et al., 2003).

Mortality of successive larval stages of common cutworm progressively increased in pot experiment by both EPN's species with the maximum value seen in 5th instar larvae. Thereafter the susceptibility reduced for pre-pupae and pupae. This pattern of susceptibility is consistent with earlier studies (Shannag et al., 1994; Jackson & Brooks, 1995; Kim et al., 2004; Ebssa & Koppenhöfer, 2012). Variation in vulnerability of different developmental stages of insect hosts has been chiefly ascribed to differences in the size or accessibility of body natural openings (mouth, spiracles and anus) which serve as a gate for entrance of nematode IJs (Dowds & Peters, 2002). On the other hand, older larvae with larger body size may attract more IJs (Smits et al., 1994). For noctuids, stage susceptibility may also be affected by variation in feeding activity. Susceptibility of unfed insect larvae was more than fed ones at lower nematode inoculum doses. Emergence of IJs from unfed insect larval cadaver was also faster and more frequent than from cadavers of fed ones (Kondo, 1987).

Pre-pupa and pupa were less susceptible to tested EPNs than most larval stages were. Less available natural opening for entering IJs to pupae can reduce pupal vulnerability. Only spiracles remain open at pupal stage and the mouth and anus are sealed. The cuticle of pupae is also thicker than in the larval stages and resists more against IJs penetration (Dowds & Peters, 2002). Pupae are considered as a relative inactive stage with minimum metabolism. At this stage, volatile cues emission (especially CO₂) by pupae is significantly decreased than by larval stages making pupae less attractive to IJs (Lewis et al., 1993, 1995).

There was no significant difference between virulence of *H. bacteriophora* and *S. carpocapsae* against the common cutworm in pot experiment. Search tactics and dispersal pattern of IJs in soil govern infectivity level (Griffin et al., 2005). Foraging strategy of *S. carpocapsae* IJs is ambushing (Campbell & Gaugler, 1993) while *H. bacteriophora* IJs are cruiser (Lewis, 2002). *S. carpocapsae* IJs usually inhabit the upper 1-2 cm of the soil while *H. bacteriophora* IJs are distributed evenly in the top 8 cm of soil (Ferguson et al., 1995; Campbell et al., 1996). As well, IJs of *S. carpocapsae* tend to move toward the surface of the soil (Georgis & Poinar, 1983) but IJs of *H. bacteriophora* move both upwards and throughout the soil column (Schroeder & Beavers, 1987). Though *S. carpocapsae* is a sit-and-wait strategist (ambusher), it can efficiently parasitize mobile host species (like cutworms) near the soil surface (Hazir et al., 2003). *H. bacteriophora* is highly mobile and orients to volatile host cues (Hazir et al., 2003). In this study the different developmental stages of the pest were put at the depth of 2 cm. Movement of the common cutworm's larval stages in the soil enhances the chance of confronting with the infective juveniles of the both species of EPNs. These factors along with dispersal pattern of both EPNs may be the reason of similar virulence against *A. segetum*.

The efficacy of *S. carpocapsae* and *H. bacteriophora* against cutworms were inconsistent in previous studies. *S. carpocapsae* was highly virulent (95%) to *A. ipsilon*, while *H. bacteriophora* did not provided sufficient control (62%) (Georgis & Poinar, 1994). When four commercial products containing *H. bacteriophora*, *S. carpocapsae*, *S. feltiae* and *S. riobrave* (Cabanillas, Poinar & Raulston) were applied against the fourth-instar of *A. ipsilon* in turfgrass, *S. carpocapsae* was the best performing species. Though the efficacy of *H. bacteriophora* and *S. feltiae* was often like to *S. carpocapsae*, their overall consistency were lesser (Ebssa & Koppenhöfer, 2011). Six isolates of EPNs (*S. carpocapsae* All, *S. carpocapsae* Mex, *S. feltiae* T319, *S. longicaudum* X-7 (Shen & Wang), *H. bacteriophora* H06 and *H. indica* LN2 (Poinar, Karunakar & David)) were tested against *A. ipsilon* in a laboratory and field experiments in china. *S. carpocapsae* Mex and *Heterorhabditis indica* LN2 were the most effective species and could respectively kill 80 and 83.3 % of 3rd instar larvae after 72 h (Yan et al., 2014). The pathogenicity of ten Canadian (from three species: *S. carpocapsae*, *S. feltiae*, and *S. kraussei* ((Steiner) Travassos)) and two commercial (from two species: *S. carpocapsae* and *S. feltiae*) isolates of EPNs were tested against *A. ipsilon* in a laboratory experiment. Both commercial (98% at the 1000 IJs concentration after 72 h) and a Canadian (94% at 250 IJs / larva / Petri dish after 72 h) isolates of *S. carpocapsae* could effectively control the pest (Bélair et al., 2013). When *S. carpocapsae* and *H. bacteriophora* were sprayed on above ground of corn at a rate of 5×10^5 IJs / 150 plants, they could respectively kill 96.7 and 68.7% of *A. ipsilon* larvae (Saleh et al., 2015). *Steinernema websteri* was recently isolated from *A. segetum* in Turkey and could efficiently control this pest. The nematode killed 100% of third instar larvae in 5 days when it was applied with the concentration of 500 IJs / g dry sand (Gökçe et al., 2015).

The results of current research suggest that both *H. bacteriophora* and *S. carpocapsae* can be used to control the common cutworm. Future work in an open field situation should be done to emphasize the potential of these two candidates in biological control of *A. segetum*.

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