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Research Article

Efficacy of two entomopathogenic nematode species as potential biocontrol agents against the diamondback moth, *Plutella xylostella* (L.)

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ABSTRACT: The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is an important pest of cruciferous crops in Iran. The susceptibility of *P. xylostella* larvae to two species of entomopathogenic nematodes (EPNs) (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) was examined under laboratory conditions. Leaf bioassays were conducted to evaluate the nematode's capability to reach the larvae and kill them. High larval mortality (72.6–96%) was observed in laboratory experiments. The ET_{50} of *H. bacteriophora* was higher than that of *S. carpocapsae*. The ET_{50} of entomopatpgenic nematodes, *H. bacteriophora* and *S. carpocapsae* tested ranged from 21 to 139.7 and 11.3 to 71.4 hours, respectively. The effect of both factors, infective juveniles (IJs) and exposure time of 50% (ET50) on the larval mortality was significant ($df = 6$; $P < 0.001$) and ($df = 2$; $P < 0.001$), respectively. This study revealed that entomopatogenic nematodes have great potential that should be exploited in diamondback moth, *P. xylostella* management.

KEY WORDS: Exposure time fifty (ET50), infective juveniles (IJs), *Heterorhabditis bacteriophora, Plutella xylostella, Steinernema carpocapsae*

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INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is major pest of cruciferous crops. The pest is commonly assumed to be native to the Palaearctic or Mediterranean area, but due to its ability to migrate over distances of more than 3000 km (Talekar and Shelton, 1993), today the moth is distributed worldwide. *Brassica* crops and brassicaeous weeds are common hosts for *P. xylostella*. The overuse of insecticides to control this pest has caused development of resistance (Shelton *et al*., 1993; Tabashnik *et al*., 1990; Tabashnik, 1994). Broad-spectrum insecticides not only lost their efficacy but also lowered the potential of invertebrate antagonists. While the potential of beneficial antagonists decreased, *P. xylostella* developed resistance against every insecticide applied. Decades later the call for sustainable biological control strategies arose due to loss of susceptibility of *P. xylostella* to all kinds of active ingredients in chemical insecticides to *P. xylostella* (Zhao *et al.,* 2002; Ninsin, 2004). Use of entomopathogenic nematodes (EPNs) against crop pests is one such alternative which has shown promise in recent years. Wide spectrum of insecticidal activity, ability to kill most hosts within short periods, and efficient mass culturing techniques are some of the attributes which has stimulated interest in nematodes as biological insecticides (Kaya and Gaugler, 1993). EPNs are important biological control agents for a variety of economically important pests (Grewal *et al*., 2005). These nematodes, belonging to the families Steinernematidae and Heterorhabditidae, are obligate parasites that kill insects with the help of mutualistic bacteria that inhabit the intestine of the infective juveniles (IJs) (Poinar, 1990; Boemare, 2002). They have been used with variable success against insects occupying different habitats. Most success has been achieved against soil dwelling pests or pests in cryptic habitats such as inside galleries in plants where IJs find higher protection from environmental factors (Begley, 1990; Klein, 1990; Williams and Walters, 2000; Tomalak *et al*., 2005). The success against foliar pests, where IJs are exposed to abiotic factors (UV radiation, desiccation and extreme temperatures), has been limited in some cases (Begley, 1990). However, the addition of adjuvant to increase leaf coverage and persistence of the IJs has enhanced the use of EPNs against foliar pests (Head *et al*., 2004; Williams and Walters, 2000; Batalla-Carrera *et al*., 2010).

High susceptibility of *P. xylostella* larvae to *Steinernema* spp. and to a lesser amount to *Heterorhabditis* spp. has been observed (Ratnasinghe and Hague, 1994, Baur *et al*., 1995). Mason and Wright (1997) recorded infectivity

of different nematode species against the *P. xylostella* ranging from 3 to 100%. Cherry *et al*. (2004) summarized the efficacy of entomopathogenic nematodes indicating that *Steinernema carpocapsae* is the most appropriate nematode species to control *P. xylostella.*

The aim of this work was to study the susceptibility of larval stages of *P. xylostella* to EPNs and the capacity of EPNs to infect larvae on the cabbage leaves under controlled conditions.

MATERIALS AND METHODS

Source of nematodes

Two different EPNs species *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* obtained from Koppert B.V (Netherlands, Berkel en Rodenrijs) were tested in the present study. The nematodes were reared at 25 ± 1°C, 65 ± 5 % relative humidity in the dark condition on the late instar larvae of the greater wax moth, *Galleria mellonella* (Lep.: Galleridae), according to the method of Woodring and Kaya (1998). The IJs that emerged from cadavers were recovered using modified white traps (Kaya and Stock 1997). After storage at 7°C for one week, they were allowed to acclimatize at room temperature for 1 h and their viability was checked under a stereomicroscope.

Insect rearing

Plutella xylostella pupae were collected from cabbage fields of Miami road in Mashhad city of Iran in the early summer of 2013. After identifying the samples collected by morphological characteristics, they were transported to the laboratory for rearing and colonization. For rearing of *P. xylostella* wooden cage sizes (50×50×70 cm) were used. In order to make adequate ventilation, side walls of the cages were covered with netting fabric. Cauliflower plants were used in experiments (both for moth rearing and for egg laying) and rearing was done at 25 ± 5°C, 65 ± 5% relative humidity and 16L:8D photoperiod.

Leaf bioassay

Virulence of *S. carpocapsae* and *H. bacteriophora* was determined against third-instar larvae of *P. xylostella* in 10-cm-diameter petri dishes lined with two moistened filter papers (Whatman No. 2). Ten individuals of *P. xylostella* larvae were released in a Petri dish, with 1 ml suspension of 50 dauer juveniles squirted onto the moistened filter paper. Similar treatments were performed with 50, 100, 200, 400, 800, 1600 and 3200 dauer juveniles. Petri dishes containing insect larvae and nematodes were sealed with parafilm, as described above, and kept at 25 ±2˚C and relative humidity of 65 ± 5% for 72 hours. A control was maintained without nematodes. Three replications were maintained. The number of surviving larvae and dead were counted at intervals of 24, 48 and 72 hours after treatment.

Statistical analysis

To evaluate the effectiveness of EPNs against *P. xylostella* larval percentage mortalities were square-root transformed before analysis. General linear model (GLM) was used to test significant differences between treatments. Afterwards a Tukey's multiple range test was performed to separate means. A level of significance of $P < 0.05$ was used for all tests.

RESULTS AND DISCUSSION

The results revealed that the third larval instars of the diamondback moth were highly susceptible to two nematode species tested. When a dose of 50 IJ/ml was applied the mortality of larvae reached 14% with *H. bacteriophora* and 54% with *S. carpocapsae*. At the dose of 1600 IJ/ml mortality reached 84% with *H. bacteriophora* and 100% with *S. carpocapsae* (Fig. 1).

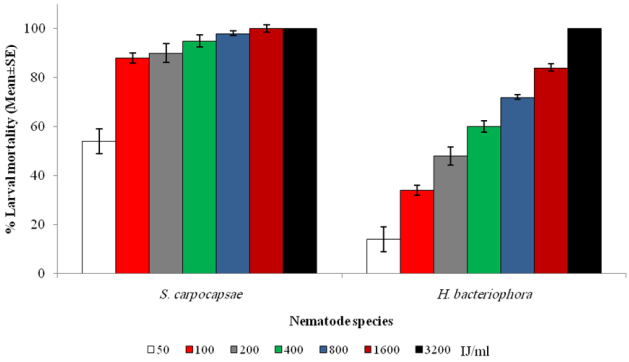

Fig. 1. Mean percentage mortality of third instars of *Plutella xylostella* at different concentrations of entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* at 5 days after treatment (DAT). Different letters on the top of error bars indicate statistically different values for different nematode concentrations at ($P < 0.05$) using Tukey's test. Error bars indicate standard error (n = 10).

The results illustrated a positive relationship between insecticidal activity and nematode concentration, with a cumulative increase in mortality as nematode concentrations increased (Fig. 1). At a concentration of 50 IJ/host, 54% and 14% mortality was obtained for *S. carpocapsae* and *H. bacteriophora*, respectively which was significantly higher than the control ($P < 0.001$). When the concentration of nematodes increased from 50-3200 IJ/host, insect mortality also increased from 14 to 100% for *H. bacteriophora* and 54 to 100% for *S. carpocapsae* (Fig. 1).

The differences between doses and nematode species were significant and *S. carpocapsae* species was more effective than *H. bacteriophora* (GLM: $F = 1.288$; df = 2, 48; $P < 0.05$).

The effect of factors infective juveniles stage and exposure time of 50% (ET_{50}) on the larval mortality for both species was significant (df = 6, $F = 122.65$, $P < 0.001$) and (df = 2, $F = 11.04$, $P < 0.001$), respectively. Both entomopathogenic nematode species caused mortality on *P. xylostella* larvae. Interaction effects between IJs and exposure time of 50% (ET_{50}) on the larval mortality was significant (Table 1).

Table 1. ANOVA of *Plutella xylostella* larval mortality exposed to different concentrations of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* at laboratory conditions

	Source of variation	df	MS	F	P
S. carpocapsae	IJ	6	7183.81	122.65	<0.001
	ET_{50}	2	16940.95	289.24	<0.001
	IJ× ET_{50}	12	574.28	9.80	<0.001
	E	84	58.57		
H. bacteriophora	IJ	6	8777.46	111.04	<0.001
	ET_{50}	2	6046.67	76.49	<0.001
	IJ× ET_{50}	12	148.89	1.88	0.0480
	E	84	6640.00		
	Total	104			
CV= 11%					

A one-way ANOVA for pooled mortality across time intervals caused by each species showed that *S. carpocapsae* caused significantly higher average mortality (54% ± 5.10%) than did *H. bacteriophora* (14% ± 2.44%) ($P < 0.001$). Low levels of mortality (14–54%) and (8-14%) were recorded when *P. xylostella* larvae were exposed to *S. carpocapsae* and *H. bacteriophora* respectively for 24–72 h. The highest mortality levels were observed after 72 h exposure in 3200 IJs and ranged between 100% and 80% for *S. carpocapsae* and *H. bacteriophora* respectively.

The ET_{50} of *H. bacteriophora* was significantly higher than of *S. carpocapsae*. The ET_{50} of *H. bacteriophora* and *S. carpocapsae* tested ranged from 139.7 to 21 hours and 71.4 to 11.3, respectively (Table 2 and Fig. 2).

Both the entomopathogenic nematode species caused mortality of *P. xylostella* larvae. These findings are in agreement with those of early workers who have also reported the efficacy of different *Steinernema* spp. and *Heterorhabditis* spp. against *P. xylostella* (Ratansinghe and Hauge, 1995; Lello *et al*. 1996; Mason and Wright, 1997; Belair *et al*., 2003; Mahar *et al*., 2004; Somvanshi *et al*., 2006). Park *et al*. (2012) also have shown that *P. xylostella* larvae were most susceptible to *Rhabditis blumi* Sudhaus with a mortality rate of 88.0 %.

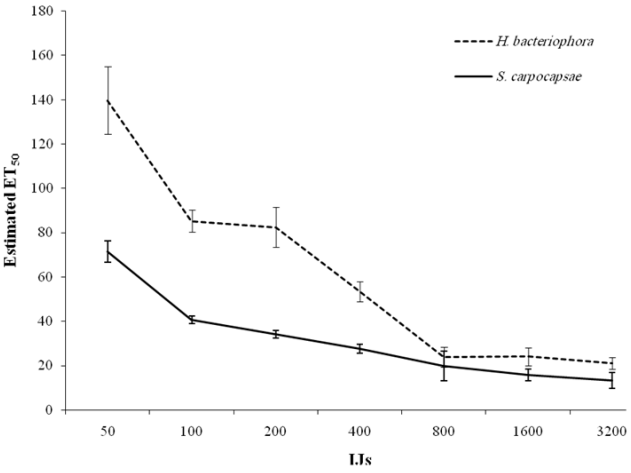

Fig. 2. ET_{50} index for infective juvenile stage both entomopathogenic nematode isolate. Vertical lines are standard error, overlap in the sense that there is no significant difference in the 0.05. ET_{50} Number of hours that the pest larvae exposed to each level of the IJ is to be achieved 50% fatality.

Table 2. Levels of exposure time (ET50) in the exposure time assay with two entomopathogenic nematodes isolates versus larvae of *Plutella xylostella*

Group	IJs	ET_{50}	S.E.	CI	
				Lower 95%	Upper 95%
H. bacteriophora	50	139.7	15.16	105.8	173.6
	100	85.3	4.91	74.3	96.3
	200	82.4	8.98	62.3	105.2
	400	53.5	4.51	43.4	61.6
	800	33.8	4.42	23.9	42.7
	1600	24	3.98	15.1	32.9
	3200	21	2.63	15.1	26.9
S. carpocapsae	50	74.1	4.87	60.5	82.2
	100	40.7	1.74	36.8	44.5
	200	34.1	1.73	30.2	35.6
	400	27.5	2.04	23	29.1
	800	19.9	2.67	13.9	25.8
	1600	15.8	2.52	10.2	21.5
	3200	11.3	3.68	3	19.5

The results of this study clearly illustrate that *P. xylostella* larvae had a nematode- concentration-dependant susceptibility to both *S. carpocapsae* and *H. bacteriophora* The pooled mortality of both species illustrated the high-

est percentage mortality when *P. xylostella* larvae were inoculated with the highest concentration of 3200 IJ/host. The lowest mortality was recorded for both *S. carpocapsae* and *H. bacteriophora* at 24 h of exposure time and highest was at 72 hours. The ET_{50} for *H. bacteriophora* and *S. carpocapsae* ranged from 139.7 to 21 hours and 71.4 to 11.3, respectively. This indicated that infection by *S. carpocapsae* was more rapid than *H. bacteriophora.*

The exposure time has an implication on the efficacy of the entomopathogenic nematode isolate to control diamondback moth in the field as field effectiveness of entomopathogenic nematodes is limited by desiccation, extreme temperatures, UV radiation and relative humidity in the microclimate (Baur *et al*., 1995; Mason and Wright, 1997). Therefore, a nematode species that is able to cause mortality in the *P. xylostella* larvae in the shortest time possible is desired to overcome the above mentioned limitations in the field. Based on these studies, *S. carpocapsae* was selected for use in the field trial.

The period of time that nematodes need to infect the insect is a relevant factor that must be considered to determine the nematodes' efficacy after a foliar application. Schroer and Ehlers (2005) found that nematodes invaded *P. xylostella* within 1 h after foliar application of *S. carpocapsae* and obtained a mean survival of IJ greater than 3 h. Kim *et al.* (2006) also showed a 12 h survival time of IJ of *S. carpocapsae* in foliar application on Chinese cabbage leaves. Consequently, a foliar application of EPNs against larvae of *P. xylostella* would allow the survival of the IJ long enough to find and infect the larvae on the surface of the leaf.

To evaluate the time required (hours) that the diamondback moth larvae in contact with any of the third instar larvae of the nematode are to be achieved 50% fatality index (ET_{50}), different levels of exposure time per each Infective juvenile nematodes were fitted. As the Table 2 and Figure 1 shows that *H. bacteriophora* in the lower infective juveniles (80 IJs), performance is much weaker than the other nematode. Another point that the IJ above 80, ET_{50} Index increases in both nematodes were almost constant trend. Based on these results we can conclude that the IJ_{80}, ET_{50} values in both the nematode would be desirable and necessary to increase the level of Infective juvenile. The study further showed that the effect of *S. carpocapsae* more than *H. bacteriophora* may be due to differences in toxicity and host foraging strategies. It should be noted that for the first time in the early 1990s, the differences in the distribution and foraging behavior of nematodes was detected (Lewis 2002).

This study is a clear demonstration that EPNs have great potential that should be exploited in diamondback moth management. A series of field trials using these entomopathogenic nematodes would be appropriate to verify the laboratory results and to see if the laboratory results can be extrapolated to the field. More work also needs to be done on the economics of using entomopathogenic nematodes in the management of *P. xylostella.*

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