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Efficacy of entomopathogenic nematode, *Steinernema carpocapsae* against the diamondback moth, *Plutella xylostella* (L.) in laboratory condition

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In vitro studies were carried out on the diamondback moth, *Plutella xylostella* larvae using an insect entomopathogenic nematode isolate, *Steinernema carpocapsae* obtained from the Koppert company, the Netherlands. Larvae of *P. xylostella* were collected from cabbage farms around Mashhad city of Iran. During the study, the responses of larvae at 25 °C for three periods of 24, 48 and 72 h with different concentrations of 0, 5, 10, 20, 40, 80, 160 and 320 third instar larvae of nematode (infective stage = IJs) per insect into 10 cm Petri dishes containing filter paper soaked with 1 ml of nematodes suspension were compared. Maximum mortality caused by *S. carpocapsae* nematode was 88% at 24 h, and it was 100% at 48 and 72 h. With increasing nematode population level and exposure time (ET in hour), mortality of *P. xylostella* larvae was increased. Based on probit analysis, LC_{50} values of *S. carpocapsae* nematode in three test periods were 45.61, 12.02 and 40.80 IJs per insect, respectively. Initial ANOVA was performed for *S. carpocapsae* nematode. The effect of both nematode population levels (IJ) and ET on third instar larvae of the diamondback moth, *P. xylostella* and interaction between IJ and ET were significant. In general, it is recommended to apply this nematode in suitable condition for controlling diamondback moth.

Keywords: *Plutella xylostella*; nematode; *Steinernema carpocapsae*; LC_{50}; laboratory condition

Introduction

Among the important species of the *Plutella* genus, *Plutella xylostella* (Linnaeus, 1758) is the only species which is widespread throughout world (Kfir 1998). In recent years, diamondback moth, *P. xylostella* is the most destructive pest of plants under the family Brassicaceae in the world and its management costs are estimated about one million dollars (Talekar 1992; Talekar & Shelton 1993; Verkerk & Wright 1996) and a major threat to crucifer crops in many parts of the world and sometimes more than 90% of the product is reduced (Iqbal et al. 1996). Pest damage by larvae usually occurs when feeding on different host plants, such as cabbage, cauliflower, kohlrabi, radishes and turnips. Larvae feed on about 62–78% of the lower leaves and leaf surface-irrelevant paths are created. Larvae feed on the upper leaf epidermis making holes on the leaves and the final instar larvae cause greater damage than the initial larval stages. This pest is due to

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a strong preference for *Brassicae oleracea* (L.) plant among other cruciferous and also due to direct larvae feed and injury to the leaves, which are marketable, caused the most severe damage (Harcourt 1957; Mitchell et al. 1997). It is believed that the lack of natural enemies, especially parasitoids, is the main factor pest being this insect in most parts of the world (Lim 1986). This is perhaps due to the widespread power of the diamondback moth (Mohan & Gujar 2003) that has better establishment in compare to parasitoids in newly planted cruciferous plants areas. Another reason for the lack of effective biological control of this pest may be due to disruption of natural enemy activity by using broad-spectrum insecticides. In the late 1940s, before the use of synthetic insecticides , there were no reports on the pest moth being a major pest of cruciferous plants and with increasing use of synthetic insecticides in the mid-1950s, important natural enemies were excluded, which in turn led to greater use of synthetic insecticides and subsequently led to resistance of the pest to insecticides and the failure of the control programmes (Talekar & Shelton 1993). There are reports of insect resistance to 36 chemical insecticides of 14 countries (Georghious 1981). Considering restrictions on the use of chemical pesticides in the fields of natural resources, it is necessary to find and use the biological pest control elements, especially factors such as entomopathogenic nematodes (EPNs). Use of EPNs to control insect pests for many years been common in most European and American countries and has had great success in controlling many insects (Ellers-Kirk et al. 2000; Mráček & Sturhan 2000; Schroer & Ehlers 2005; Shapiro-Ilan & Cottrell 2005). Several formulations of the nematode are produced in terms of pests living place, application location or product type produced (Smith 2007). Also, natural and applied control of some Diptera, Coleoptera and Lepidoptera pests by *Heterorhabditis* and *Steinernema* species in different countries such as China, America, Denmark, Australia and the UK is the use of EPNs. In America, *Steinernema feltiae* and *Heterorhabditis bacteriophora* nematodes caused 67 and 83% mortality in larvae of *Lycoriella auripila* (Winn.) (Dip.: Sciaridae) (Nickle & Cantelo 1991). In Iran, studies on insect parasitic nematodes and their pathogenicity started in the last few years, but so far practical use of these nematodes is not found. But there are numerous cases of EPNs that have been reported across the country from soil of different regions (Nikdel et al. 2002; Parvizi 2003; Tanha Maafi et al. 2006). Considering the mentioned issues, there is possibility to use EPNs to control insect pests in Iran. Therefore, in this study, the effectiveness of a species of nematode, *Steinernema carpocapsae* against third instar larvae of diamondback moth, *P. xylostella* was investigated in laboratory condition.

Materials and methods

Insect rearing

The diamondback moth, *P. xylostella* pupae were collected from cabbage fields of Miami road in Mashhad city of Iran in the early summer of 2013. After identifying and ensuring the samples collected by morphological characteristics, they were transported to the laboratory for rearing and colonisation. For colony forming of diamondback moth, wooden cage sizes ($50 \times 50 \times 70$ and $40 \times 40 \times 50$ cm) were used. In order to make adequate ventilation, side walls of the cages were covered with netting fabric. In this research, cauliflower plant was used in experiments (both for moth rearing and for egg laying). All stages of the diamondback moth rearing was conducted at 25 ± 5 °C, 65 ± 5% relative humidity and 16L:8D photoperiod.

Bioassay of pathogenic nematode on third instar larvae of P. xylostella

In this study, the commercial strain of EPN, *S. carpocapsae* was used. Commercial isolate from Koppert BV (Netherlands, Berkel en Rodenrijs) was prepared. To perform the pathogenicity test of nematode on *P. xylostella* larvae, Petri dishes, bottom-covered with 10 cm diameter filter paper were used in all treatments. For each concentration of 0, 5, 10, 20, 40, 80, 160 and 320 third instar larvae of nematode (infective stage = IJs) per insect, five Petri dishes were selected. Ten third instar larvae of *P. xylostella* were placed in each Petri dish. Control Petri dishes were prepared with 1 ml of distilled water. Prepared Petri dishes were maintained for three days in germinator with 25 ± 5 °C and a relative humidity of 65 ± 5%; mortality of treated, dead and survived individuals larvae were counted in the interval of 24, 48 and 72 h.

Statistical analysis

In this study, the percentage of mortality per unit of test and control treatments was calculated based on the Henderson–Tilton formula (Henderson & Tilton 1955) and was calculated using SAS software (SAS Institute 1997). Given the significance of the interactions between two factors, ANOVA analysis was performed (Soltani 1979) in

Table 1. ANOVA of *P. xylostella* larval mortality exposed to different concentrations of *S. carpocapsae* at 25 °C.

Source of variation	DF	MS	*F*	*P*
IJ[a]	6	7183.809	122.65**	<0.001
ET[a]	2	16940.9523	289.24**	<0.001
IJ × ET	12	574.2857	9.80**	<0.001
Error	84			
		CV = 11%		

**Significant difference at 1% level.
[a]IJ: infective juveniles, ET: exposure time.

Table 2. Analysis variance of *P. xylostella* larval mortality exposed to different concentrations of *S. carpocapsae* at 25 °C.

IJ	DF	MS	*F*	*P*
Effect sliced IJ × ET				
5	2	2060.00	35.17**	<0.001
10	2	5460.00	93.22**	<0.001
20	2	5806.66	99.14**	<0.001
40	2	3926.66	67.04**	<0.001
80	2	2006.66	34.26**	<0.001
160	2	886.66	15.14**	<0.001
320	2	240.00	4.10*	0.0200
Effect sliced ET × IJ				
24	6	3769.5238	64.36**	<0.001
48	6	3080.00	52.59**	<0.001
72	6	1482.857	25.32**	<0.001

*Significant difference at 5% level.
**Significant difference at 1% level.

order to calculate interaction effects slicing using SAS. LC_{50} (third instar larvae of nematode required to obtain 50% mortality) and ET_{50} (the time required to obtain 50% mortality) was calculated using probit test by SPSS software (SPSS 2006). Graphs were drawn in the 2010 version of Excel software.

Results

The bioassay results showed that with increasing levels of nematode (IJ) and exposure time (ET), mortality rate was increased. The overall effect of both the level of third instar larvae of the nematode (IJ) and ET and also IJ and ET interaction were significant (Table 1).

Results of ANOVA analysis about interaction effects of slicing are given in Table 2. As it is shown in Table 2, the effect of ET levels in all levels of IJ of *S. carpocapsae* (except 320 IJ level) was significant at 1% level. Interaction effect of ET in 320 IJ was also significant at 5% level. The results showed that the effect of infective juvenile stage

Table 3. Probit analysis results of *P. xylostella* larval mortality by *S. carpocapsae* nematode.

		LC_{50}*			LC_{90}		
Nematode	Time (h)	IJ	SE**	CI[a]	IJ	SE**	CI[a]
S. carpocapsae	24	45.61	3.58	37.60–53.62	513.58	71.889	352.13–674.33
	48	12.02	1.16	9.42–14.62	110.93	11.82	84.50–137.36
	72	4.80	0.35	4.01–5.59	10.02	0.55	8.78–11.26

*LC index, number of third instar larvae of nematode (IJ) required for 50 and 90% mortality of *P. xylostella*.
**Standard error.
[a]Upper and lower limits of 95% confidence level.

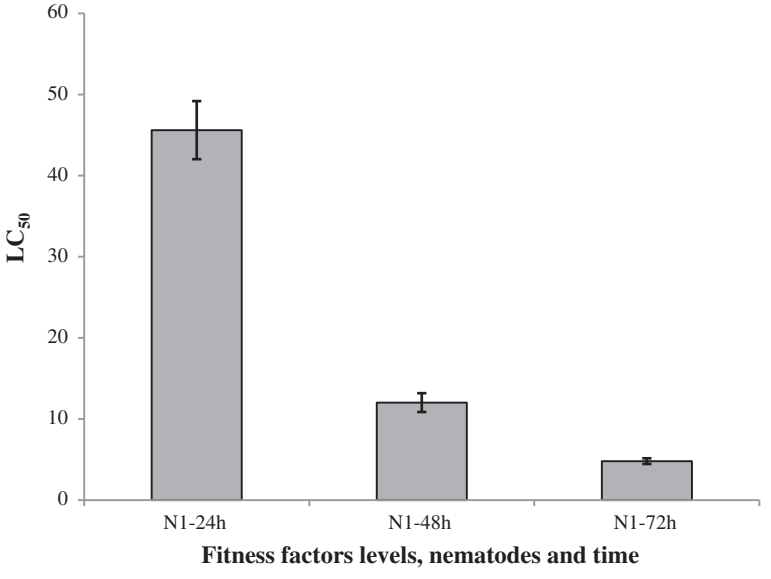

Figure 1. Number of third instar larvae of nematodes (IJs) are required to obtain 50% mortality (LC_{50}) at three levels of nematode (N1), *S. carpocapsae* nematode and h, are ET levels.

of *S. carpocapsae* on mortality depends on ET that pest larvae exposed to the nematode larvae and the converse is true. The effect of duration of ET of pest larvae to the nematode in compare to control was significantly related to the number of nematode larvae. LC index of number of third instar larvae of *S. carpocapsae* nematode, required to obtain 50 and 90% mortality of the diamondback moth larvae as compared to control, is shown in Table 3. Probit analysis of the data and the calculated LC_{50} of *S. carpocapsae* nematode at three general timeframes showed that over time, the number of nematode larvae required to obtain 50 and 90% mortality decreased. Table 3 also shows LC_{50} trend of change in the *S. carpocapsae* nematode. As can be seen, this trend is declining over time. Confidence interval, 95% in different times indicating that the difference between the LC_{50} levels was statistically significant (Table 3 and Figure 1).

Discussion

Test results of *in vitro* bioassays showed that *S. carpocapsae* nematode species have a suitable pathogenic potential on the diamondback moth, *P. xylostella* larvae. Conclusions of this research data showed that the diamondback moth larvae susceptibility to the EPN larvae depends on the infective juvenile stage and ET. Considering that EPN are moving at the level of filter paper in the Petri dishes (one dimension) and third instar larvae of the diamondback moth can bind to different parts of Petri dishes (move in multiple dimensions), as a result of which Larvae out of reach and mortality are recorded in random order. In this research, the proper effect of nematode species of *S. carpocapsae* could be due to differences in toxicity and host-foraging strategies in this species compared to other species of pathogenic nematodes. It should be noted that for the first time in the early 1990s, difference in the distribution and foraging behaviour of nematodes were detected (Lewis 2002). Although EPNs are usually preferred for increasing the contact with and identifying the insect host, a range of searching behaviours are used that can affect nematode pathogenicity; therefore, foraging behaviour could be nematode selection criteria to be used in biological control programmes (Lezama-Gutiérrez et al. 2006). In terms of foraging behaviour, the *Steinernema* genus was placed in the ambushed group (Campbell et al. 1996). Ambush nematode species usually are in monitored practice (Nictation) and in this way they are separated from the substrate and are attached to the passing insect. Nictation behaviour is usually in three forms: stand, without stirring, jump (up slightly from the bed) and a swing forward and back (Lewis 2002). The ambush species such as *S. carpocapsae* and *S. feltiae* are more effective on the active hosts (Lewis 2002). These findings are in agreement with Saurav Gupta et al. (2008) who have also reported the lethal dose of *S. carpocapsae* nematode on third instar larvae of the diamondback moth, 6.72 pathogen larvae per insect that determines close to the LC_{50} obtained in the present study. In another similar study, lethal dose of *S. carpocapsae* nematode was reported as 24.5 pathogen larvae per insect (Be′lair et al. 2003). The value of LC_{50} of *S. carpocapsae* nematode in this study was a paradox, because this contradiction is probably due to the difference in temperature (20 ± 1) that has decreased the nematode efficacy. Salem et al. (2007) suggested a greater effect of *S. carpocapsae* nematode on the diamondback moth larvae.

It may be too early to conclude that *S. carpocapsae* nematode can be used as a powerful biocontrol agent. However, its remarkable pathogenicity and low LC_{50} and ET values make the nematode a potential biological control agent against insect pests. Further studies are required on the ability of *S. carpocapsae* nematode to sustain and survive under environmental conditions, also to study its persistence and shelf life.

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