EVALUATION OF SUSCEPTIBILITY OF THE BROWN TAIL MOTH, *EUPROCTIS CHRYSORRHOEA* (L.), TO ENTOMOPATHOGENIC NEMATODES UNDER LABORATORY CONDITIONS

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Summary. The brown tail moth, *Euproctis chrysorrhoea*, is an important foliar pest of forest trees in most areas of Europe and Asia. Therefore, the potential of the entomopathogenic nematodes *Heterorhabditis bacteriophora* isolate IRAZ5 and *Steinernema carpocapsae* isolate IRAZ9 was assessed for biological control of third, fourth and fifth instar larvae of *E. chrysorrhoea* under laboratory conditions. The two entomopathogenic nematodes were tested at the rates of 0, 500, 1000, 1500, 3000, and 5000 IJs per ml of distilled water using 150 ml per well plate containing three larvae of third or fourth instar or two fifth instar larvae of the pest. Both nematode species were highly effective on last instar larvae of the pest. The greatest mortality of *E. chrysorrhoea*, by both nematodes, was achieved with last instar larvae at the rate of 5,000 IJs of the nematodes/ml suspension. Mean mortalities for *H. bacteriophora* and *S. carpocapsae* on the third, fourth and fifth larval stages of the pest at over all rates were 44.7, 44.0 and 51.1 and 42.2, 57.5 and 67.8, respectively. *Steinernema carpocapsae* caused significantly greater mortality of fourth and fifth instars of the insect compared to *H. bacteriophora*. LC_{50}s were estimated at 2236, 2138 and 1592 IJs/ml for *H. bacteriophora* and 4472, 652 and 423 IJs/ml for *S. carpocapsae* on third, fourth and fifth instars, respectively.

Keywords: Bioassay, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae.*

The brown tail moth (hereafter referred as to BTM), *Euproctis chrysorrhoea* (L.), is a univoltine pest that causes severe damage to forest trees, especially oaks, in central and southern Europe, north Africa, central Asia, north America and in some areas of England (Alford, 1995). The pest has also been reported from northern provinces and north-west areas of Iran (Abaei, 2000). In Arasbaran forests, north-west Iran, the moth is the most important defoliator of oak trees (Nikdel *et al*., 2002). The pest overwinters as larvae in colonies that are enclosed within webbed nests of white silk tightly woven around a leaf in trees or shrubs. The nests are spun in the early autumn, contain 250 to 400 larvae, and remain firmly attached to twigs or small branches through the winter and early spring. The webs are often confused with silken structures formed by other less serious species of moths (Grill, 1986; Bertucci, 1994). The larval stage of this insect feeds on the foliage of hardwood trees and shrubs including oak, shadbush, apple, cherry, beech, plum, and dogrose. Larval feeding causes reduction of growth and occasional mortality of valued trees and shrubs.

Entomopathogenic nematodes are attractive for use in biological control programmes of insects because numerous species are commercially available and have been used successfully for the control of a variety of insect pests (Georgis *et al*., 2006). Due to their sensitivity to UV light and desiccation, nematodes are most effective against pests in soil or other protected environments (Kaya and Gaugler, 1993). Based on available literature, there are no data on the infectivity of Steinernematidae and Heterorhabditidae nematodes against the BTM, but a few records show the infection of the pest with mermithids (Demirbag and Yaman**,** 1999; Nikdel *et al*., 2002).

Despite the occurrence of many insect pests in environmentally sensitive forest areas, the use of EPNs in forest pest management has not been widely established (Sanders and Webster, 2000). The infectivity of EPN is influenced by temperature, moisture and host finding by the nematode (Lewis *et al*., 2006). Therefore, EPNs are ideally suited to attack overwintering larvae in a cryptic habitat and nematode infectivity can be optimized by applying the IJs to the most vulnerable insect stage, which usually is the larval stage that occurs in soil or in a similar moist and cryptic habitat. Such environmental conditions during the life cycle of BTM are found when the third instar larvae remain for about 8 months of the year (from late August through May) aggregated within the overwintering silky nests. At other times, appropriate formulations of the nematodes can be used against individual larvae (fourth and fifth instars) of the pest that are not in the nests.

In this study, we evaluated the susceptibility of different larval instars of BTM collected from Arasbaran forests against two Iranian isolates of EPN under laboratory conditions.

MATERIALS AND METHODS

Nematodes. Two Iranian species of EPNs, *Heterorhabditis bacteriophora* Steiner, isolate IRAZ5, and

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Steinernema carpocapsae Weiser, isolate IRAZ9, recovered from soil samples collected from Arasbaran forests in the north-west of Iran, were cultured in *Galleria mellonella* L. last instar larvae at 24 °C. Infective juveniles (IJ) were extracted in White traps, according to the procedure described by Woodring and Kaya (1988), and stored at 7 °C. A maximum concentration of 5,000 IJs per ml of distilled water was prepared four to five days before use. To adjust nematode concentrations to the tested rates, serial dilutions were made (Glazer and Lewis, 2000). At the time of application, nematode viability was at least 95% in all experiments.

Insects. Overwintering third instar larvae, enclosed within the webbed nests, were collected on the dominant species of oak trees, *Quercus petraea* (Mattuschka) Liebl. from Arasbaran forests in early spring. The nests were placed in polystyrene boxes (20 cm × 10 cm × 5 cm) with new leaves of the host tree at 22-23 °C and 65% RH. After two days of feeding on the leaves, the required number of third instar larvae were taken from the boxes and the remainder were reared for longer in order to obtain fourth and fifth instar larvae.

Pathogenicity of EPNs. *Heterorhabditis bacteriophora* and *S. carpocapsae* were tested against third, fourth and fifth instar larvae of *E. chrysorrhoea*. Wells of 24-well plates lined with filter paper were used for all treatments in the evaluation of EPN pathogenicity. Six rates of the EPN (0; 500; 1,000; 1,500; 3,000 and 5,000 IJs/ml) as aqueous suspension (150 µl) were deposited on the filter paper, using an Eppendorf micropipette. Control wells of each treatment received only 150 µl of distilled water. The browntail moth larvae were transfered to the wells of the plates according to each treatment and were covered with caps (three larvae of third or fourth instar and two larvae of fifth instar larvae were added to the wells, separately). Data on insect mortality were recorded after 24, 48, 60 and 72 hours. Such that dead insects were dissected to be sure that they had been killed following nematode infection.

Each experiment was replicated three times. In each replicate, 216 third and fourth instars and 144 fifth instar larvae were used for each nematode species and rate combinations.

Statistical analysis. Mortality data were normalized by square root transformation. The quantity (concentration) of IJ per ml of distilled water was transformed logarithmically. The significance of the effects of the factors species, larval instar and concentration were analyzed by analysis of variance (ANOVA) using the SAS programme. The level at which all analyses were considered significant was $P<0.05$. Probit analysis of percent mortalities was also performed to estimate LC_{50}s.

RESULTS AND DISCUSSION

The results showed that, on the fourth and fifth larval instars of *E. chrysorrhoea*, all concentrations of *S. carpocapsae* and, on the third larval instar, only its 500 IJs/ml concentration were more virulent than treatments with *H. bacteriophora* (Fig. 1). Greatest mortality by both EPNs occurred with fifth instar larvae with 5,000 IJs/ml (Fig. 1C). Analysis of variance revealed that the factors larval instar ($F = 202$, $df = 2$, $P<.0001$),

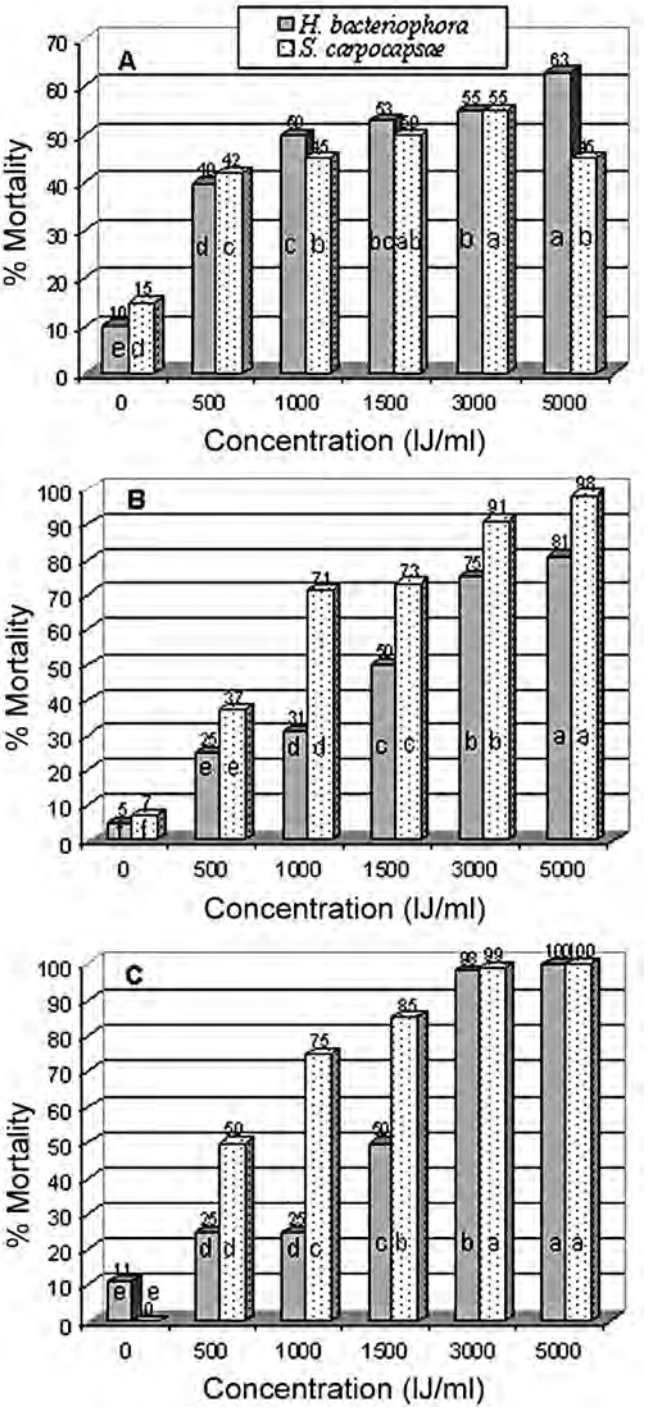

Fig. 1. Nematode induced mortality of A) third instar, B) fourth instar and C) fifth instar larvae of the brown tail moth, *Euproctis chrysorrhoea* (L.). Within each trial, bars (mean) of each nematode species followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range Test for mortality.

Table I. LC_{50} of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* applied against three larval instars of *Euproctis chrysorrhoea* at different concentrations of nematodes carried out in the 24-well plates.

Nematode	Larval instar	LC_{50}	R^2	Mean mortality (%)
H. bacteriophora	third	2236	0.17	51.62
	fourth	2138	0.87	52.06
	fifth	1592	0.82	59.23
S. carpocapsae	third	4472	0.03	47.71
	fourth	652	0.22	67.49
	fifth	423	0.79	81.31

nematode species (F = 457, df = 1, P<.0001), and concentration (F = 4514, df = 5, P<.0001), had significant effects on larval mortality. With increasing concentrations of the nematodes larval mortality increased accordingly. From probit analysis, LC_{50}s of *H. bacteriophora* and *S. carpocapsae* for third, fourth and fifth instar larvae of *E. chrysorrhoea* were obtained. Regression analysis of the dose × mortality response showed significant relationships for both nematodes (Table I). Total mortality percentages of third, fourth and fifth instar larvae were 49.7, 59.8 and 70.3, respectively. The overall mean larval mortality caused by *S. carpocapsae* was 65.5% compared to 54.3% for *H. bacteriophora*.

Cumulative mortality percentage of *E. chrysorrhoea* larvae 60 h after treatment was significantly larger with *S. carpocapsae* than with *H. bacteriophora*. No mortality by any rate of *H. bacteriophora* was recorded until 24 h, whereas 97% of total mortality occurred between 24 and 48 h, while *S. carpocapsae* caused 75% of total mortality between 48 and 72 h, thus indicating that mortality of the pest larvae occurred faster with *H. bacteriophora*.

A mean of 5.5 *S. carpocapsae* adults (female) and four adults (female) of *H. bacteriophora* were recovered from each dissected BTM larva cadaver after 72 h (Fig. 2). Furthermore, the monitoring of the emergence of EPN IJs from some of the dead BTM larvae indicated that *S. carpocapsae* and *H. bacteriophora* were able to complete their life cycles in the BTM host. However, the total developmental time from infection to emergence of IJs was different for the two nematode species: 5 and 7 days after the death of the BTM larvae by *S. carpocapsae* and *H. bacteriophora,* respectively.

All the above demonstrates that *S. carpocapsae* is more virulent to *E. chrysorrhoea* larvae than *H. bacteriophora*, especially to fourth and fifth instar larvae (F = 457.17, P<0.0001). However, in addition to nematode species, symbiotic bacteria, insect age and other factors are involved in the virulence of EPNs. It is likely that the different host-finding strategies exhibited by the ambusher *H. bacteriophora* and the cruiser *S. carpocapsae* is one of the most important reasons for the observed differences. *Steinernema carpocapsae*, having cruiser behaviour, has more contact with the host as compared to *H. bacteriophora*. On the other hand, the mean mortality (51.6%) in third instar larvae caused by *H. bacteriophora* was greater than that caused by *S. carpocapsae* (47.7%) in the same instar.

Steinernema carpocapsae and *H. bacteriophora* have been evaluated against and are infective toward a wide range of insect pests (Kaya and Gaugler, 1993). However, there are no documented reports on the infectivity of EPNs upon BTM larvae with which to compare our results. EPNs have been found highly infective against a number of other Lepidoptera (Williams *et al.*, 2002; Cottrell and Shapiro-Ilan, 2006; McKern *et al.*, 2007). Shapiro-Ilan and Cottrell (2006) found that steinernematids were more virulent toward the lesser peach tree borer, *Synanthedon pictipes* Grote *et* Robinson (Lepidoptera: Sesiidae), than the heterorhabditids under laboratory conditions, and field applications of *S. carpocapsae* and *H. bacteriophora* reduced the numbers of raspberry crown borer, *Pennisetia marginata* Harris (Lepidoptera: Sesiidae).

The results of several studies such as those of Belair *et al.* (1999) and Bruck *et al.* (2008), who assayed the activity of EPNs against *Choristoneura rosaceana* (Harris) (Tortricidae) and *Synanthedon bibionipennis* (Bioduval) (Sesiidae), respectively, are similar to ours. These authors found that, for most of the lepidopteran larvae, insect

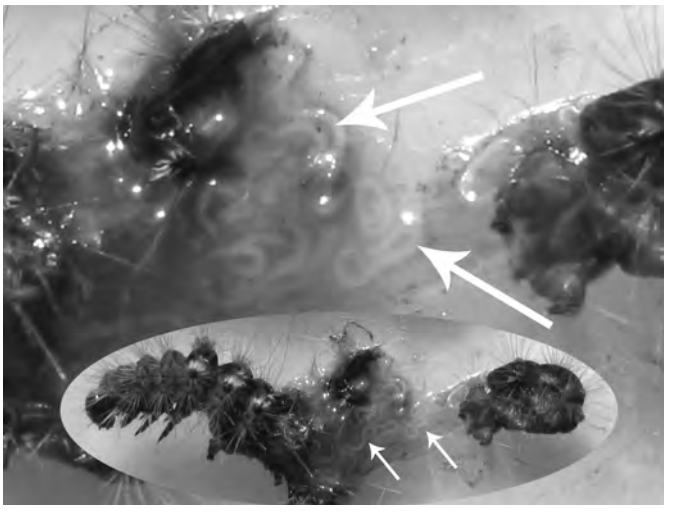

Fig. 2. Dissected dead fifth instar larva of *E. chrysorrhoea* exposed to *H. bacteriophora* that shows many of adult (female) nematodes 72 hours after death.

death rates also increased with increasing larval instar.

Based on the results of this study, both nematode species should be effective at controlling BTM larvae. However, while *S. carpocapsae* was highly effective against fourth and fifth instar larvae, *H. bacteriophora* was highly effective on third instar larvae. Since the pest larvae of the fourth and fifth instars are active and found individually on the host trees, their control using the cruiser *S. carpocapsae* would probably be better. On the other hand, third instar larvae overwinter in non-motile colonies that are enclosed within webbed nests of white silk tightly woven around a leaf in trees or shrubs (each nest containing 250 to 400 larvae), from early autumn to early the following spring. Therefore, using *H. bacteriophora* with ambusher behaviour should be better against third instar larvae.

ACKNOWLEDGMENTS

We are thankful to Dr. S.A. Mohammadi from Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tabriz, IRAN and Dr. A. Razban from Research Center of Agriculture and Natural Resources of East Azarbaijan, Tabriz, IRAN for assistance with the analysis of the data.

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Accepted for publication on 4 December 2009.