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RESEARCH REPORT

Virulence of four entomopathogenic nematode species for plum sawfly, *Hoplocampa flava* L. (Hymenoptera: Tenthredinidae)

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Abstract

The yellow sawfly *Hoplocampa flava* is an important pest of plum all around the world. Larvae feed on the seed with damaged fruit falling prematurely. There are many studies on the control of other pests with entomopathogenic nematode (EPN), but few on the control of plum sawfly. The present study was conducted to determine virulence of four EPN species: *Heterorhabditis bacteriophora*, *H. marelatus*, *Steinernema carpocapsae* Tur-S4 and *S. feltiae* Tur-S3 against plum sawfly *Hoplocampa flava* L. (Hymenoptera: Tenthredinidae) under laboratory conditions. For each nematode species, six different doses (3, 5, 7, 10, 15, 20 Infective Juveniles (IJs) /larva) were applied against last instar larvae of *H. flava*. Assays were done in 24 well tissue culture plates filled with 10 % (w/v) moist silver sand. The most virulent species was *H. bacteriophora* which had LD_{50} and LD_{90} values of 6.51 and 15.46 IJs, respectively. The least virulent was *S. carpocapsae* Tur-S4 with LD_{50} and LD_{90} values of 16.617 and 33.779 IJs, respectively.

Key Words: Entomopathogenic nematode; *Hoplocampa* spp.; sawfly; virulence

Introduction

The yellow plum sawfly *Hoplocampa flava* is a member of the Hymenopteran suborder Symphyta. The name of the group is coming from saw-like shape of ovipositors of the female. Generally, adults are 4 - 7 mm length with reddish-brown in color and black legs. Eggs are white, slim-long-elliptic, 0.4 - 0.5 mm length and larvae is cream, caterpillar-like, when the larvae mature length is 10 - 15 mm. The species has one generation per year in Turkey, and the wasps overwinter within cocoon as larvae stage, in soil of a few centimeters deep. In the spring, matures emerge from cocoon while plum trees about to bloom and emerging continues during blossoming period. *H. flava* is considered to a major pest of plum fruits and heavy infestations can cause considerable economic losses. This monovoltine species spends most of its life-cycle as diapausing larvae in the soil and adult emergent flight usually coincides with the start of flowering of early plum varieties. The females lay eggs on the calyx and larvae emerge when the fruit begin to develop. Larva can destroy up to several fruitlets during the course of feeding and damaged fruitlets fall off.

Sawflies are one of the important pest groups on pear, apple and plum. Larvae of the sawflies have direct damage and fruits and apple sawfly can cause up to 100 % product loss. Although economic importance of the pest, there are very few permitted agricultural chemicals for the control of sawflies. In addition, pollinator bees were exposed to these chemicals due to the spraying period, which is done while blossoming. Moreover, zero tolerance policies and strict rules of importer countries are big obstacles for chemical control. Herewith, biological control becomes an alternative method and because of sawflies overwinters in soil, EPNs can be considered as a suitable biocontrol agent.

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae have been using to control especially soil-dwelling insect pests (Ehlers, 1996; Lacey and Georgis, 2012). EPNs have several beneficial features, which make them an impotant bio-control agents. EPNs can inhabit almost all around the world with different geographical and climatic conditions (Griffin *et al.*, 1990; Poinar, 1990; Hominick *et al.*, 1996; Hominick, 2002), they are effective on more than 250 insects pests (Peters, 1996) and safe for non-target organisms and environment (Boemare *et al.*, 1996; Ehlers 2003), they can seek for their hosts (Lewis *et al.*, 1992;

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Grewal *et al.*, 1994) up to 50 cm soil depth (Susurluk, 2008), and they can be mass produced in liquid culture (Lunau *et al.*, 1993; Strauch and Ehlers, 1998; Ehlers, 2001) for widespread outdoor applications. Several commercial formulations of EPNs have being used on agricultural pests such as Western Flower Thrips, Sciarid flies, Vine Weevils, Codling Moths, Cut Worms etc. Common characteristic of these pests is that they spend at least one biological stage within soil.

Since sawflies are overwintering within cocoon as larvae in the soil, controlling sawflies with EPNs are highly possible. EPNs provide long time effectiveness on the pest in applied areas, because of their long term persistence (Susurluk and Ehlers, 2008). Therefore, an alternative control method for sawflies with EPNs is expected to be successful especially within integrated pest management.

The aim of this study was to identify virulent strain of EPN for the control of sawfly larvae and to determine virulence of four EPNs and to calculate their lethal doses as LD_{50} and LD_{90} on plum sawfly larvae in order to detect possibility of using these EPNs in plum orchards in Turkey.

Materials and Methods

Nematode species

Four different entomopathogenic nematode species, *Heterorhabditis bacteriophora*, *H. marelatus*, *Steinernema carpocapsae* and *S. feltiae* were used in the present study. Species were isolated from different regions of Turkey (Bursa, Ankara, Bursa and Izmir, respectively) and cultured *in vivo* on the last instar of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae as described by Kaya and Stock (1997). The species were identified by molecular methods (PCR-RFLP) (Unpublished data). All cultures were stored at +4 °C and they were reproduced *in vivo* on *G. mellonella* larvae before inoculations. Two weeks old EPN populations were used for the experiments.

Hoplocampa flava *larvae*

Over 2000 last instar sawfly larvae were collected from decayed plum fruits brought from Aydin, Turkey.

Virulence experiments

In order to determine virulence and calculate lethal doses of EPN species on sawfly larva, six doses 3, 5, 7, 10, 15, 20 IJs/larva were applied. All trials were conducted in 24-well tissue culture plates and replicated three times with 20 larvae for each replicate. Distilled water was applied on 10 larvae with three replicates for control. For each well of the plate, a larva was put within 10 % humid sterile silver sand (particle size 300 - 400 µm). All doses were applied on sand, plates were sealed with parafilm and incubated at 24 °C for 4 days. After incubation, the cadavers inside the sand were washed with distilled water. Each cadaver was dissected under a stereomicroscope to ensure nematode presence. After dissection, dead and alive larvae were counted and mortality ratios calculated. For determining lethal doses more precisely, higher doses were applied on sawfly larva to reach 100 % mortality.

Statistical analysis

Data of virulence experiments were analyzed by analysis of variance (ANOVA) and followed by a Least Significant Difference (LSD) test as post-hoc comparisons with $p < 0.05$ significance level using JMP® 7.0 software. Probit analysis performed for lethal dose calculations by XLSTAT® software.

Results and Discussion

Results showed that sawfly larvae were susceptible to EPN, however, there were no significant differences for virulence at the low doses (3 and 5 IJs). At 7 IJs and above, *H. bacteriophora* had significantly higher lethal effect than both *Steinernema* species. Mostly, *S. carpocapsae* had the lowest effect among the other species (Fig. 1).

As it can be estimated from the virulence experiments, *H. bacteriophora* had the lowest LD_{50} (6.514) and LD_{90} (15.455) among the other species while. *S. carpocapsae* had the highest LD_{50} (16.617) and LD_{90} (33.779). *Heterorhabditis bacteriophora* were more effective than *Steinernema* species on *H. flava* larvae at higher doses (>7 IJs/larva) (Table 1).

Besides effectiveness of EPNs, their safety also supports their usings in plant protection or IPM systems. This aspect is endorsed by the European and Mediterranean Plant Protection Organisation (EPPO), which has produced the document PM 6/3 (2), containing a positive list of invertebrate biocontrol agents. The list contains five EPNs used in biological control (EPPO, 2002). Therefore, they are encourged to farmers who especially do organic farming in many countries. Consequently, EPNs as successful biological control agents have potential for use in biological control of many economically important insect pests in glasshouses, lawns, turfgrasses, pasture, nurseries, trees, mushrooms, orchards, soft fruits and vegetables. In these crops,

Table 1 LD_{50} and LD_{90} values of the species on sawfly *H. flava* larvae

	LD_{50}	Confidence Interval (LD_{50})	LD_{90}	Confidence Interval (LD_{90})
H. bacteriophora	6.514	3.991-8.361	15.455	13.07518.933
H. marelatus	8.617	6.941-10.008	18.946	16.835-21.931
S. feltiae	12.958	10.626-14.685	25.120	22.521-29.485
S. carpocapsae	16.617	13.410-18.956	33.779	30.102-40.250

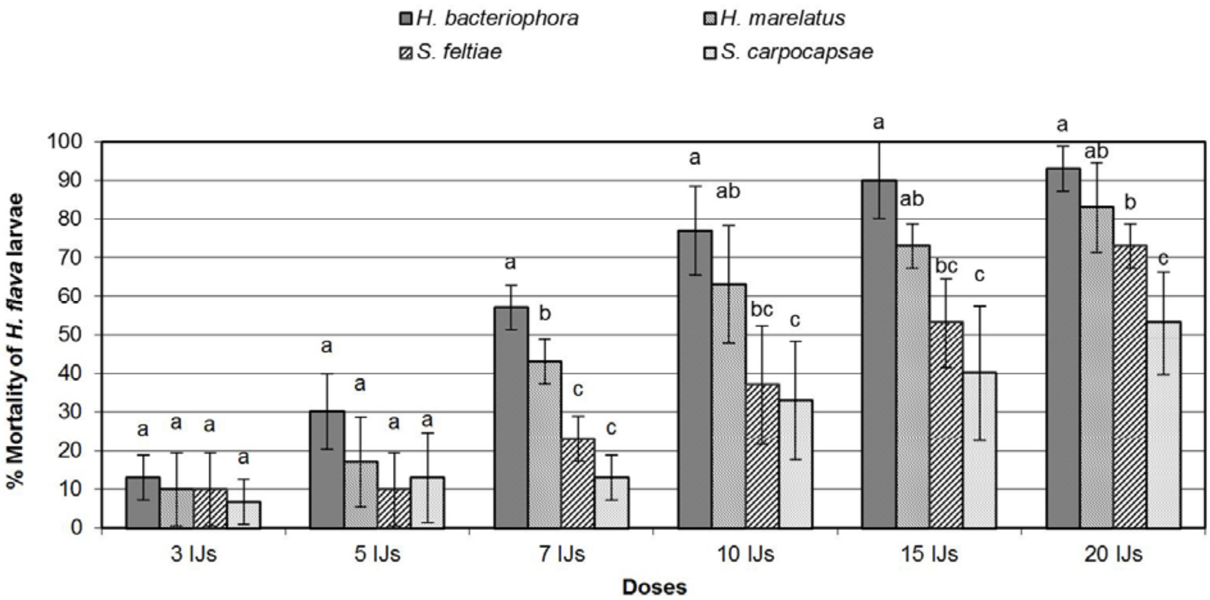

Fig. 1 Virulence of four different entomopathogenic species with six different doses on *H. flava* larvae. For each dose, letters not connected by same letter are significantly different (F = 21.757; df = 23, 48; $p < 0.0001$)

EPNs can commercially used against many insect pests such as; *Otiorhynchus sulcatus*, *Frankliniella occidentalis*, *Agrotis* spp., *Agriotes* spp., *Dorcadion pesudopreissi*, Sciarids, *Cydia pomonella*, *Tuta absoluta*, *Bothynoderes punctiventris*, *Diabrotica virgifera virgifera* and *Cassida vittata* (Lemma *et al.*, 2004; Susurluk, 2008; Susurluk and Ehlers, 2008b; Saleh *et al.*, 2009; Toepfer *et al.*, 2010; Carrera *et al.*, 2010; Susurluk *et al.*, 2011).

A study was conducted by Bélair *et al.* (1998) on foliar application of *S. carpocapsae* against early season apple pests. In 1992 and 1993, damage caused by apple sawyfly *H. testudinea* larva was reduced by 98 % and 100 %, respectively. Oppositely, treatments in 1994 were not effective as first two years. In addition, Curto *et al.* (2006) performed a study to determine virulence of *S. feltiae* on pear sawfly *H. brevis.* Results of the two years study showed that, in the first year, application of *S. feltiae* significantly reduced the adult population of *H. brevis* in the next spring. In the second year, EPNs managed to reproduce and control sawfly larvae, but they could not reduce the fruit damage. Both studies showed that *S. carpocapsae* and *S. feltiae* can have high virulence of sawfly larvae, which is inconsistent with the present study.

On the other hand, there are some studies that have similar result with the present study. Tomalak (2006) studied on potential of 2 EPN species (*H. megidis* and *S. feltiae*) for control of 13 different orchard and urban tree sawfly species including *H. flava*. Semi-field trials of the study revealed that, for all sawfly species (excluding *Arge berberidis*), *H. megidis* was found more effective than *S. feltiae*. Additionaly, Nježić and Ehlers (2014) performed a new study to control two plum sawfly species (*H. minuta* and *H. flava*) with *S. feltiae*, *S. carpocapsae* and *H. bacteriophora*. The study had both laboratory and field trials, and methodology for laboratory trials were similar with the present study. However, doses were higher (50, 100 and 200 IJs/larva) and different ages of the larva (1, 10, 20, 40 days) were considered. Results of the laboratory trials showed that mortality was 92 - 100 % for 1 day old larvae. whereas no mortality was observed for older larvae. Moreover, field trials showed that *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* reduced adult population by 62 %, 47 % and 85 %, respectively. The data obtained from the both studies were consistent with the present study.

As it can be estimated from the studies, different results obtained from same EPN species under similar conditions, except target host. *S. carpocapsae* and *S. feltiae* were found effective against *H. testudinea* and *H. brevis*, while same EPN species were found less effective against *H. flava*. It is thought that the main reason for the differences between effectiveness of the species was the target host. Generally, *Heterorhabditis* species tend to be more effective then *Steinernema*, which can be described by cruising behaviour of *Heterorhabditis*.

The present study was one of the first applications of EPNs on sawfly. Besides having significant virulence, most effective species was found as *H. bacteriophora*, which is the most common EPN species in Turkey (Susurluk *et al.*, 2001). Even though further virulence studies and field trials are necessary, the result of the study showed hopeful results for controlling sawflies with EPNs. There is very limited scientific data about EPNs and the pest relationships, yet. Thus, the study can provide additional information on the pest controlling by EPNs for the future.

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