Pathogenicity of three entomopathogenic nematodes against the onion thrips, Thrips tabaci Lind. (Thys.; Thripidae)

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Pathogenicity of three entomopathogenic nematodes against the onion thrips, *Thrips tabaci* Lind. (Thys.; Thripidae)

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Pathogenicity of a native isolate of *Steinernema feltiae* (H1) and two exotic strains, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* was assessed under laboratory conditions using different concentrations i.e. 4000, 6000, 8000 and 10,000 infective juveniles/ml against second instar larvae, prepupa and pupa of *Thrips tabaci* Lindeman. The mortality data were recorded 24 and 48 h post-inoculation. The highest mortality rate was recorded for prepupa (62%) than second instar (12.5%) by *H. bacteriophora* and *S. carpocapsae*, respectively, 24 h after treatment. No significant differences were found in mortality between prepupa and pupa with increasing the nematodes concentrations (from 4000 to 10,000 nematode/ml) but increasing nematode concentrations increased the mortality of second instar. At the end of the experiment (48 h), *S. feltiae* H1 caused the highest mortality on second instar larvae (74%), whereas all other species caused 80–83% mortalities on pupa. This study suggests that native isolate of *S. feltiae* (H1) had high potential to infect soil-dwelling stages of *T. tabaci*.

**Keywords:** Entomopathogenic nematodes; *Steinernema feltiae*; *Heterorhabditis bacteriophora*; *S. carpocapsae*; onion thrips; pathogenicity

1. **Introduction**

Onion thrips, *Thrips tabaci* Lind. (Thys.: Thripidae) is an important greenhouse pest worldwide. This polyphagous and cosmopolitan pest attacks a wide range of host plants including onion, garlic, cucurbits, potato, leek, bean, eggplant and ornamentals (Capinera 2001) and has high economic importance especially in Iran. Furthermore, it damages indirectly through transmitting some viruses like sowbane mosaic virus and tomato spotted wilt virus (Hardy & Teakle 1992). The current control method for managing *T. tabaci* on most crop plants is spraying of chemical insecticides but, hidden life habits and short life cycle increase *T. tabaci* population and necessitate frequent applications of chemical treatments unavoidable (Maninia et al. 2003). Therefore, addressing alternative control methods specially the environmental friendly methods like biocontrol agents might be a suitable option (Griffin et al. 2005). Among different natural enemies, entomopathogenic nematodes (EPNs) can successfully attack insect pests in different habitats including soils as well as foliage (Ansari et al. 2006, 2009; McGraw & Koppenhöfer 2008; Tradan et al. 2009) and could be suitable alternatives for management of soil-dwelling as well other pests on canopy.

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There are a few surveys about biocontrol potential of EPNs against thrips species and mostly they are limited to pathogenicity of EPNs against western flower thrips (WFT), Frankliniella occidentalis (Thys.: Thripidae) (Tomalak 1994; Helyer et al. 1995; Chyzik et al. 1996; Ebssa et al. 2001a, 2001b; Premachandra et al. 2003; Buitenhuys & Shipp 2005). In addition, introduction of the EPNs as biological control agents in a particular area requires previous knowledge of their presence in the region and appropriate identification of native species (Emelianoff et al. 2008). Due to insufficient information about pathogenicity of native isolates of EPNs against different stages of the T. tabaci in Iran, we evaluated efficiency of a native EPN and exotic species against different developmental stages of T. tabaci.

2. Materials and methods

2.1. Nematodes

EPNs used in the experiments were Steinernema feltiae (Hamedan 1) isolated from soil in Hamedan, Iran, Steinernema carpocapsae (Capsanem®) and Heterorhabditis bacteriophora (Larvaneem®) supplied by the Koppert B. V. (Berkel en Rodenrijs, The Netherlands) that cultured on Galleria mellonella (Kaya & Stock 1997).

2.2. Onion thrips

The colony of T. tabaci was established on cucumber plants (Cucumis sativa L. var. Negeen) in greenhouse at the Department of Plant Protection, Bu-Ali Sina University, Hamedan. The cucumber leaf discs were prepared according to Brodeur and Cloutier method (1992) with minor modifications. Briefly, the healthy cucumber leaves were cut to round pieces (diameter: 6 cm). Then, leaf disc surfaces were disinfected with sodium hypochlorite 5% solution and sterile distilled water. Finally, the leaf discs were put on the water agar medium (7 g/L). To obtain cohort, T. tabaci females were transferred to new leaf discs every 12 h. Then, all females were removed and sealed containers were kept in the controlled growth chamber (23 ± 1 °C, 55 ± 5% R.H. with 16:8 L:D). Under these conditions, the cohort of second instar larvae, prepupae and pupae appeared after 8, 11 and 12 days, respectively.

2.3. Pathogenicity test

The EPNs isolates with less than two weeks old were used in the test. They were acclimatised for at least 5 h at room temperature before application. Experimental units consisted of sterile plastic containers (6 cm × 5 cm) with their bottom covered by filter papers. The IJs tested concentrations were 4000, 6000, 8000 and 10,000/ml. Control treatment received only distilled water. Twenty individuals of each developmental stage (second instar larvae, prepupae and pupae) were introduced into each container. Each treatment was replicated four times. The container was sealed by parafilm and placed in the controlled growth chamber (23 ± 1 °C, 50 ± 10% R.H. and 16:8 L:D). Thrips mortality was recorded after 24 and 48 h post-inoculation. Thrips were considered dead when there was no response to mechanical stimulus and showed discolouration symptoms of infection. Due to the short growth period of prepupa, mortality of this stage was assessed only after 24 h. Whole experiment was repeated twice in the same condition.
2.4. Reproduction potential of EPNs on thrips cadaver

To evaluate the reproduction potential of different EPN species/stains within thrips haemocoel, the cadavers were rinsed with distilled water and dissected under stereomicroscope. Dissection was conducted 3–4 days after inoculation to check the possibility of EPNs reproduction within the host. For this purpose, the cadavers were sterilised using 70% ethanol. After which they were dissected in a physiological serum prepared by NaCl.

2.5. Statistical analysis

Thrips mortality data were corrected using Schneider-Orelli’s corrected mortality (CM) formula (Püntener 1981). The efficacy of EPNs strains was evaluated using the CM data. The CM data were square root of X + 1, transformed before subjecting to statistical analysis. Normality and homogeneity of variance were sought by Shapiro–Wilcoxon test and Leven’s test (SAS Institute 2004), respectively. The experiment was carried out in a completely randomised factorial design with adequate repetition of experiments. Significant differences of means were evaluated by Duncan’s multiple range test (PROC GLM, SAS Institute 2004).

3. Result
3.1. Pathogenicity of EPNs

Overall, all EPNs species and concentrations caused significantly higher mortality than control treatment in different developmental stages of T. tabaci. Mortality rates in control treatments were low through the whole experiments. Analysis of variance indicated that thrips developmental stage was the most important factor affecting the pathogenicity of nematodes (24 h after treatment). Single and interactive effects between the treatments are shown in Table 1. At the end of the experiment (48 h after treatment), thrips developmental stage, different EPNs species and their interaction were significantly different (Table 1).

Table 1. ANOVA results for corrected mortalities of different developmental stages of onion thrips (second instar larva, prepupa and pupa) caused by EPNs, Heterorhabditis bacteriophora, Steinernema carpocapsae and S. feltiae at concentrations 4000, 6000, 8000 and 10,000 IJs/ml after 24 and 48 h.

<table>
<thead>
<tr>
<th>Time</th>
<th>Dependent variable</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Hour</td>
<td>Stage</td>
<td>2</td>
<td>0.076</td>
<td>0.038</td>
<td>4.21</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>2</td>
<td>0.011</td>
<td>0.005</td>
<td>0.63</td>
<td>0.532</td>
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<tr>
<td></td>
<td>Concentration</td>
<td>3</td>
<td>0.031</td>
<td>0.010</td>
<td>1.14</td>
<td>0.334</td>
</tr>
<tr>
<td></td>
<td>Stage × species</td>
<td>4</td>
<td>0.080</td>
<td>0.020</td>
<td>2.21</td>
<td>0.072*</td>
</tr>
<tr>
<td></td>
<td>Species × concentration</td>
<td>6</td>
<td>0.039</td>
<td>0.006</td>
<td>0.72</td>
<td>0.638</td>
</tr>
<tr>
<td></td>
<td>Stage × concentration</td>
<td>6</td>
<td>0.102</td>
<td>0.017</td>
<td>1.87</td>
<td>0.092*</td>
</tr>
</tbody>
</table>

48 Hour

<table>
<thead>
<tr>
<th>Time</th>
<th>Dependent variable</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage</td>
<td>1</td>
<td>0.304</td>
<td>0.304</td>
<td>26.65</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>2</td>
<td>0.128</td>
<td>0.064</td>
<td>5.41</td>
<td>0.006**</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
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<td>0.064</td>
<td>0.021</td>
<td>1.81</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>Stage × species</td>
<td>2</td>
<td>0.124</td>
<td>0.062</td>
<td>5.20</td>
<td>0.008**</td>
</tr>
<tr>
<td></td>
<td>Species × concentration</td>
<td>6</td>
<td>0.051</td>
<td>0.008</td>
<td>0.72</td>
<td>0.637</td>
</tr>
<tr>
<td></td>
<td>Stage × concentration</td>
<td>3</td>
<td>0.005</td>
<td>0.001</td>
<td>0.14</td>
<td>0.934</td>
</tr>
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</table>

**, * and ′ indicated significant and tends to significant at α = 0.01, 0.05 and 0.1, respectively.
After 24 h, significant differences were observed between different thrips developmental stages in terms of mortality (Anova, $F=4.21$, $df=2$, $p=0.01$). Prepupa was recognised as the most susceptible stage, while the second instar larvae showed the least susceptibility to EPNs. Interaction effect between life stages and EPNs species, besides life stages and different concentrations was nearly significant ($\alpha=0.1$). *H. bacteriophora* caused the highest mortality in prepupae ($54\%\pm6.4$) while its effect was significantly reduced on second instar larvae ($24.7\%\pm7.26$). Similarly, the *S. carpocapsae* was most effective against prepupae and significantly ineffective against second instar larvae. Generally, 24 h after treatment, this species caused $20\pm4.45\%$, $49.7\pm1.56\%$ and $50\pm4.63\%$ mortalities in second instar larvae, prepupae and pupae, respectively. In contrast, *S. feltiae* H1 had same effect on all developmental stages. No significant differences were found between mortalities of different developmental stages (Figure 1). 

No significant difference was observed between various concentrations ($F=1.07$, $df=2$, $p=0.3$), but interaction effect between various concentrations and different developmental stages was significant at $\alpha=0.1$ (Table 1). Irrespective of species/strains, mortality of the second instar larvae significantly increased with increasing concentration from 4000–10,000 IJs/ml, whereas, no clear scenario was found for prepupae and pupae. During 24 h, about half of the prepupa and pupa were killed by 8000 and

Figure 1. Susceptibility of different developmental stage of *T. tabaci* to EPNs after 24 h. Bars (mean ± SE) with different letters indicate statistical significances ($n=4$).
10,000 IJs/ml concentrations, while for second instar larvae, mortality rate was not exceeded from 40% (Figure 2).

Both *S. carpocapsae* and *H. bacteriophora* caused higher mortalities in pupae than second instar larvae after 48 h. While, the efficacy of *S. feltiae* (H1) against both stages was similar, all species of EPNs reduced number of pupae at a similar level (70–80%) (Figure 3).

3.2. Reproduction potential of EPNs in the thrips body

Infected thrips by EPNs showed discoloration symptoms of infection. It proves that onion thrips mortality is caused by both physical injury and virulence of symbiotic bacteria. Dissection of cadavers also confirmed inability of EPNs to reproduce inside immature stages of thrips.

4. Discussion

Control mortality was comparable with other related studies e.g. Madadi et al. (2005) who reported that 84% of *T. tabaci* population survived on cucumber leaf discs until pupal stage. Hence, low mortality in control treatment enabled us to study the efficacy of EPNs against onion thrips developmental stages. The results indicated that all three species had high ability to kill different developmental stages of onion thrips.

![Figure 2. Different concentrations of EPNs against developmental stages of *T. tabaci* after 24 h. Bars (mean ± SE) with different letters indicate statistical significances (*n* = 4).](image-url)
Furthermore, second instar larva was less susceptible to EPNs, while prepupa was known as most susceptible stage and pupal mortalities significantly ranked higher than second instar larvae. Differences in susceptibility of developmental stages have been observed for other pest species. Buitenhuis and Shipp (2005) investigated susceptibility of different developmental stages of *F. occidentalis* (WFT) to various concentrations of *S. feltiae*. They reported prepupa and pupa had the highest susceptibility to the pathogen. Ebssa et al. (2001a) also reported higher pupal mortality than late second instar larvae after infection by *S. feltiae*. In another study, Ebssa et al. (2001b) found that mortality of second instar larvae, prepupa and pupa varied significantly in WFT populations. Conversely, Premachandra et al. (2003) expressed that second larvae were most susceptible to EPNs relative to pupae. These inconsistent results may be explained by differences between nematode isolates or experimental conditions.

Unlike the second instar larvae, prepupae and pupae of *T. tabaci* are relatively quiescent and do not feed (Capinera 2001). In the present study, we observed that second larvae were highly active and roved to different parts of the container e.g. walls and lid. In contrast, prepupae and pupae were immobile and just responded to mechanical stimulus slowly. Similar results have been reported by Buitenhuis and Shipp (2005), which suggest that differences in susceptibility of *T. tabaci* developmental stages to EPNs arise

![Figure 3. Efficacy of *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* against different developmental stages of *T. tabaci* after 48 h. Bars (mean ± SE) with different letters indicate statistical significances (*n* = 4).](#)
from differences in their mobility. North et al. (2006) found T. palmi larvae is more susceptible to S. feltiae than adults. Therefore, it can be argued that EPNs may be more effective against settled stages such as immature stage of Bemisia tabaci (Cuthbertson et al. 2003) and/or leafminer larvae (Williams & Walters 2000).

Moreover, some mechanisms like difference in aggressive defence behaviour (Drees et al. 1992) and physical barriers to penetration (Gaugler 1988) can be considered for difference in variable susceptibility of host developmental stages. Larvae and adult thrips showed escape or defence behaviours against nematodes. During EPNs infection, second instar larvae had jerking behaviour, fast moving its abdomen to remove pathogen from its body surface. This was confirmed through studies of Bakker and Sabelis (1989) who found less attack rate of predators like phytoseiid mites against second larval stages of T. tabaci due to same behaviour. Current research confirmed significant difference in susceptibility of second instar larvae to various EPNs species. The obtained data about susceptibility of pupae were similar to Ebssa et al. (2001b), who found the highest mortality on second instar larvae (69%) caused by S. feltiae OBSIII and the lowest rate (41%) by S. carpocapsae DD136. In spite of us, they showed that S. carpocapsae DD 136 caused less mortality on prepupa rather than H. bacteriophora HK3 and S. feltiae Sylt.

Additionally, various EPNs efficiency against different host developmental stages may have occurred due to different host finding ability or virulence of EPNs species/strain (Kurtz et al. 2009). H. bacteriophora is a cruiser forager and responds to host cues; S. carpocapsae is ambusher, S. feltiae is known to have an intermediate strategy between cruiser and ambusher (Lewis 2002). Our result indicates that S. feltiae use this advantage against soil-dwelling stages of thrips, which move when disturbed. When some IJs disturb prepupa and pupa during searching, other ambusher IJs can encounter with mobile immature stages of thrips. This could be suggested as a reason for why S. feltiae provides better thrips control continuously comparing to other species.

Here, we used the EPNs strains from both native and commercial origins. There are some reports about comparison between above-mentioned isolates. Premachandra et al. (2003) showed that there were no significant differences between commercial isolate (S. feltiae Nemaplus®) and native isolate (H. bacteriophora HD01) efficacies against WFT. Helyer et al. (1995) compared the efficacy of some EPNs strains against F. occidentalis using commercial products and endemic strains, but their results were not consistent with ours. Thus, it seems that there is no clear relation between rearing method of EPNs and their pathogenicity. Moreover, difference in virulence can be attributed to innate factors such as ability of invasion to their hosts, ability to overcome host immune system (e.g. avoidance of encapsulation), difference in proliferation and pathogenicity of symbiotic bacteria associated with different EPNs (Forst et al. 1997).

Most studies generally have been shown that there is no direct relationship between different IJs concentration levels and mortality rate, and IJs concentration is a less important factor in EPNs efficacy e.g. Buitenhuys and Shipp (2005) research on pathogenicity of S. feltiae against different developmental stages of F. occidentalis and Chyzik et al. (1996) study about efficacy of S. riobravis, S. feltiae strains Ger and H. bacteriophora strain HP88 against F. occidentalis. Small body size is a limiting factor for EPNs entrance to host (Peters & Ehlers 1994).

The current work confirmed the results of Tomalak (1994) who showed that there is no possibility for EPNs reproduction within thrips body. Ebssa et al. (2001a) also supposed similar results and stated that due to small size of thrips, there is no possibility
for EPN reproduction within haemoceol. In general, EPNs are unable to proliferate reproduction in small insects (English-Loeb et al. 1999).

The results indicate high levels of control obtained by native *S. feltiae* H1 on all three life stages of onion thrips. Therefore, application of native strain can provide desirable effect on *T. tabaci*. High efficacy of this strain on foliage feeding stage of onion thrips promises a new window towards biological control programmes. This issue implied by other researchers including Bennison et al. (1998) recommending foliar applications of *S. feltiae* on *Verbena* leaves against WFT as well as Wardlow et al. (2001). Moreover, foliar applications of *S. feltiae* every three days reduced the population of *F. occidentalis* (Arthurs and Heinz 2006). While soil applications can be useful in integrated thrips management programmes, but the critical concern is that the target stages contained small portion of thrips population. Alternatively, one strategy could be multiple applications of aerial predators like *Neoseiulus cucumeris* or different species of *Orius* bugs, effective against thrips larvae plus soil dwelling EPNs. Furthermore, because onion thrips are dispersed by adult stage and initial pest damages caused by thrips adults on the plants, infection may spread and cause damage to flowers and foliage before thrips can be controlled. Hence, it seems that foliar applications of EPNs on vegetables with low aesthetic values, against target thrips feeding stage might control infection early, before thrips population build-up. The results reported here indicate substantial potential of pathogenicity of EPNs against *T. tabaci* under laboratory conditions. This study is an initial step of the potential use of EPNs against *T. tabaci* and the efficacy of EPNs against the *T. tabaci* should now be examined in the field.

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**References**


