Efficacy of Native Entomopathogenic Nematodes on the Larvae of Tomato Leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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**ABSTRACT**

This study was conducted to determine the efficacy of native entomopathogenic nematodes (EPNs); *Steinernema affine* 46 (Bovien, 1937), *S. feltiae* 879 (Filipjev, 1934), *S. carpocapsae* 1133 (Weiser, 1955) and *Heterorhabditis bacteriophora* 1144 (Poinar, 1976) on the larvae of tomato leafminer *Tuta absoluta* (Meyrick). Bioassays were conducted in the laboratory at four different temperatures (10, 15, 20 and 25±1 °C) in the plates and 30 infective juveniles (IJ$s$) were inoculated to a single *T. absoluta* larva for each nematode species. After nematode inoculation, larvae were checked on the 3\textsuperscript{rd}, 5\textsuperscript{th}, 7\textsuperscript{th} days and mortalities were recorded. All nematode species used in the study showed the lowest efficacy on the 3\textsuperscript{rd} control day at 10 °C and the highest efficacy on the 7\textsuperscript{th} day at 25 °C. *S. feltiae* 879 was found as the most efficient species with the highest mortality (91.67%) among EPNs used in the study. The results proved that *T. absoluta* larvae are highly susceptible to EPNs and the control of the pest by EPNs on this stage is successful.

Keywords: Tomato; *Tuta absoluta*; Larva; Biological control; Entomopathogenic nematodes

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**1. Introduction**

Tomato (*Solanum lycopersicum*) is the most popular home garden and one of the most widely cultivated and important vegetables grown around the world both outdoors and in greenhouses for fresh consumption and processing due to its taste, color, flavor, and nutrient contents, but requires protection from a variety of pests.

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is highly damaging to tomato plants causes nearly 100% yield losses in absence of control methods applied. *T. absoluta*, originated in South America, entered Europe (Spain) at the end of 2006. Spread rapidly in many other countries, was included in the EPPO A2 list due to its current distribution in the region (Roditakis et al 2010). In Turkey, *T. absoluta* was first recorded in 2009 in Urla (İzmir) and July-August of the same year it was also reported in Çanakkale. It caused significant crop losses in tomato production (Kılıç 2010; Kasap et al 2011).

*Tuta absoluta* has four main growth stages as egg, larvae, pupa and adult. The life cycle of the pest is completed 29-38 days with 10-12 generations per year based on environmental conditions (Vargas 1970; Urbaneja et al 2007). Females generally deposit their eggs on leaves, leaf veins; stem margins, sepals and green fruits. Its fecundity ranges from 60-120 eggs (Estay 2000). It has four larval instars and last instar larvae generally drop on the ground and pupate in soil on the leaves or other parts of the plants. Pupae complete their development in soil and adults

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emerge from soil after few days (Urbaneja et al 2007). Young larvae of *T. absoluta* start feeding on the plant by penetrating the tissues. They produce several mines in leaves, burrowing into stalks, apical buds, green and ripening fruits. These mines reduce the rate of photosynthesis and enable attacks by pathogens (Cáceres 1992).

Chemical control has been the first preferred method against *T. absoluta* since the pest was reported. However, successful chemical control of tomato leafminer is complicated due to its feeding behaviour because *T. absoluta* feeds internally within host mesophyll tissues. Chemicals may increase the cost of production but decrease the number of natural enemies of the pest (Desneux et al 2010). Also resistance to various insecticides has been occurred in different countries, adding further difficulties in the pest management (Siqueira et al 2000).

Alternatively, biological control may be considered using entomopathogenic nematodes (EPNs), due to their potential as biological control agents. EPNs are parasites of soil-borne organisms that infect pests occurring in, close or on top of the soil surface. In recent years, they have been used effectively to control many severe pests such as leafminers (Olthof & Broadbent 1990).

EPNs belong to the Steinernematidae and Heterorhabditidae families are identified by carrying symbiotic bacteria of the genus *Xenorhabdus* (Thomas & Poinar 1979) and *Photobacteroides* (Boemare Akhurst & Mourant 1993) in their intestine, respectively (Ehlers 1996; Boemare 2002). These specific bacteria have an important role in the pathogenicity; they multiply and kill the host insect by an induced septicemia. EPNs have many advantages such as their high reproductive potential, the ability to kill hosts quickly, high virulence, broad host range, easy mass rearing, and safety to plants, vertebrates, and other non-target organisms (Kaya & Gaugler 1993). Due to these advantages of EPNs, many researchers from different countries conducted important studies on *T. absoluta*, which is a serious pest in tomato production, both under laboratory and field conditions to evaluate the potential of EPNs against this pest (Garcia-del-Pino et al 2013; Shamseldean et al 2014; Van Damme et al 2016; Ben Husin 2017; Kamali et al 2018).

We aimed at determining the efficacy of native EPNs on the larvae of *T. absoluta* in the laboratory. Unlike other studies, the efficacy of non-commercial native isolates on *T. absoluta*, obtained from different provinces of Turkey; *S. affine* 46, *S. feltiae* 879, *S. carpocapsae* 1133 and *H. bacteriophora* 1144 were determined at different temperatures in this study.

2. Material and Methods

2.1. Source and rearing of entomopathogenic nematodes

*Steinernema affine* 46, *S. feltiae* 879, *S. carpocapsae* 1133 and *H. bacteriophora* 1144, isolated from different provinces of Turkey in a previous project were reared at 25±1 °C, 65±5% RH in the dark condition on the last instar larvae of greater wax moth *Galleria mellonella* L., (Lepidoptera: Pyralidae) (Bedding & Akhurst 1975; Kaya & Stock 1997). Nematode-infected *G. mellonella* larvae were put to White traps (White 1927). Freshly emerged IJs were harvested, rinsed in distilled water and stored at 8-10 °C in tissue culture flasks within a week. Before being used for bioassays, the viability of EPNs was checked by a stereomicroscope.

2.2. Source and rearing of *Tuta absoluta*

Larvae, pupae and adults of *T. absoluta* were collected from different tomato production areas in Çanakkale and maintained in rearing cages (50x50x50 cm), covered with netting fabric for ventilation on tomato plants in a climate room at 25±1 °C and 65±5% RH with a 16:8 h L:D photoperiod. Healthy larvae were collected for laboratory bioassays.

2.3. Laboratory bioassays

The efficacy of native nematode species against larvae of the tomato leafminer was evaluated in the laboratory. In each well of the 12 well plates one last instar larva of *T. absoluta* was placed and filled with moistened sterile sandy soil. The bioassays were conducted at 10, 15, 20 and 25±1 °C with 30 IJs per each larva with two replicates. EPNs were applied on the soil surface, with only distilled water added to the control plates.
The larvae were checked on the 3rd, 5th and 7th days, counting the number of alive and dead larvae. Mortalities were recorded and infected larvae were transferred to White traps. Data were analyzed by one-way ANOVA followed by Duncan’s multiple range test (P<0.05).

3. Results and Discussion

The findings of the study showed that the larvae of *T. absoluta* were susceptible to four EPN isolates used in this study. Different mortalities were obtained on the larvae based on the EPN species, temperatures and control days (Table 1-4). Among the species used in the study the most efficient species was found as *S. feltiae* 879 with 91.67% and the least efficient species was found as *S. affine* 46 with 83.33% (Table 1-3).

### Table 1- Mortality of *Tuta absoluta* larvae caused by *Steinernema affine* 46 at four different temperatures and three control days Mean (%) ± SE

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>3rd day</th>
<th>5th day</th>
<th>7th day</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8.3±4.81 Aa</td>
<td>12.5±4.17 Aab</td>
<td>25.0±4.81 Ab</td>
<td>F1,15=3.55*</td>
</tr>
<tr>
<td>15</td>
<td>20.8±4.17 Aa</td>
<td>29.1±4.17 Bab</td>
<td>41.6±4.81 Bb</td>
<td>F1,15=5.70</td>
</tr>
<tr>
<td>20</td>
<td>37.5±4.17 Ba</td>
<td>45.8±4.17 Ca</td>
<td>66.6±0.00 Cb</td>
<td>F1,15=19.49</td>
</tr>
<tr>
<td>25</td>
<td>70.8±4.17 Ca</td>
<td>75.0±4.81 Dab</td>
<td>83.3±0.00 Db</td>
<td>F1,15=3.00</td>
</tr>
</tbody>
</table>

* means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); ** means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

### Table 2- Mortality of *Tuta absoluta* larvae caused by *Steinernema carpocapsae* 1133 at four different temperatures and three control days Mean (%) ± SE

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>3rd day</th>
<th>5th day</th>
<th>7th day</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>12.5±7.98 Aa</td>
<td>12.5±4.17 Aa</td>
<td>25.0±4.81 Aa</td>
<td>F1,15=1.50*</td>
</tr>
<tr>
<td>15</td>
<td>20.8±4.17 Aa</td>
<td>29.1±4.17 Bab</td>
<td>41.6±4.81 Bb</td>
<td>F1,15=5.70</td>
</tr>
<tr>
<td>20</td>
<td>41.6±4.81 Ba</td>
<td>54.1±4.17 Cb</td>
<td>66.6±0.00 Cc</td>
<td>F1,15=11.57</td>
</tr>
<tr>
<td>25</td>
<td>62.5±4.17 Ca</td>
<td>75.0±4.81 Dab</td>
<td>87.5±4.17 Db</td>
<td>F1,15=8.10</td>
</tr>
</tbody>
</table>

* means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); ** means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

### Table 3- Mortality of *Tuta absoluta* larvae caused by *Steinernema feltiae* 879 at four different temperatures and three control days Mean (%) ± SE

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>3rd day</th>
<th>5th day</th>
<th>7th day</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8.3±4.81 Aa</td>
<td>16.6±6.80 Aa</td>
<td>16.6±0.00 Aa</td>
<td>F1,15=1.0*</td>
</tr>
<tr>
<td>15</td>
<td>20.8±4.17 Ba</td>
<td>29.1±4.17 ABab</td>
<td>33.3±0.00 Bb</td>
<td>F1,15=3.5</td>
</tr>
<tr>
<td>20</td>
<td>50.0±0.00 Ca</td>
<td>54.1±4.17 Bab</td>
<td>70.8±4.17 Cb</td>
<td>F1,15=10.5</td>
</tr>
<tr>
<td>25</td>
<td>75.0±4.81 Da</td>
<td>83.3±0.00 Cab</td>
<td>91.6±4.81 Db</td>
<td>F1,15=4.5</td>
</tr>
</tbody>
</table>

* means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); ** means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

In the current study high mortalities occurred on the larvae by high temperatures. The lowest efficacy was observed on the 3rd control day at 10 °C and the highest efficacy was observed on the 7th control day at 25 °C for all nematode species used in the study. *H. bacteriophora* 1144 caused no mortality on the larvae on the 3rd control day at 10 °C and the efficacy started on the 5th control day at the same temperature. This is an expected situation as *H. bacteriophora* requires high temperatures for successful development and to infect and kill the host insect (Susurluk et al 2001; Susurluk & Ehlers 2008).
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Table 4- Mortality of *Tuta absoluta* larvae caused by *Heterorhabditis bacteriophora* 1144 at four different temperatures and three control days Mean (%) ± SE

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>3rd day</th>
<th>5th day</th>
<th>7th day</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.00±0.00 Aa</td>
<td>8.33±4.81 Aa</td>
<td>8.33±8.33 Aa</td>
<td>F(3,15)= 0.75*</td>
</tr>
<tr>
<td>15</td>
<td>8.33±4.81 ABA</td>
<td>16.66±6.80 Aa</td>
<td>25.00±4.81 Ba</td>
<td>F(3,15)= 2.25</td>
</tr>
<tr>
<td>20</td>
<td>33.33±0.00 Ba</td>
<td>41.67±4.81 Bab</td>
<td>50.00±0.00 Cb</td>
<td>F(3,15)= 9.00</td>
</tr>
<tr>
<td>25</td>
<td>70.83±4.17 Ca</td>
<td>75.00±4.81 Cab</td>
<td>87.50±4.17 Db</td>
<td>F(3,15)= 3.90</td>
</tr>
</tbody>
</table>

..., means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); **, means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

Similarly to our result, Van Damme et al (2016) reported that while *S. carpocapsae* and *H. bacteriophora* showed higher efficacy at 25 °C (55.3 and 97.4% mortality) than at 18 °C (12.5 and 34.2% mortality), *S. feltiae* caused 100% mortality at both temperatures. In the laboratory, *S. feltiae* and *S. carpocapsae* have high potential on tomato leaf miner larvae inside the mines of tomato leaves.

Among Steinernema species *S. affine* 46 showed the lowest efficacy on the larvae with 83.33 and followed by *S. carpocapsae* 1133 with 87.5 and *S. feltiae* 879 with 91.67%. *S. feltiae* and *S. carpocapsae* were successful species against various pests in many studies based on the EPNs efficacy (Lacey & Unruh 1998; Gözel & Güneş 2013; Gözel 2016).

In similar studies; (78.6-100%) (Batalla-Carrera et al 2010), 100, 52.3 and 96.7% (Garcia-del-Pino et al 2013), low pupal mortality (7%), caused by *S. feltiae* were reported (Türköz & Kaskaivalç 2016). Also Shamseldean et al (2014) reported that *H. bacteriophora* caused higher mortality than *S. monticolum* both in the laboratory and field; 80-100%, 60-80%; 80-89%, 58-67% respectively against to *T. absoluta* larval stage using two different EPN applying dosages. Our results are consistent with the results of these studies.

In a study that was carried out in a tomato field, high efficacy was occurred with the same isolates on the larvae of *T. absoluta* by *S. feltiae* 879 90.7, 94.3%; *S. affine* 46 39.3, 43.7%; *S. carpocapsae* 1133 43.7, 49.3% and *H. bacteriophora* 1144 81, 83% in 2012 and 2013, respectively (Gözel & Kasap 2015).

4. Conclusions

This study focused on the potential of four native EPNs on the larvae of *T. absoluta* under laboratory conditions. *Tuta absoluta* is a devastating pest, difficult and expensive to control solely by chemicals; so IPM should be considered. EPNs are gaining attention in biocontrol researches day by day because they are highly pathogenic organisms and cause high mortality in a broad range of insects, so it is important to use these beneficial organisms in managing pests. In conclusion based on the literature knowledge and the results we obtained from this study EPNs are excellent candidates for biological control and have potential to control *T. absoluta*. All isolates tested in current study performed high efficacy on the larvae of *T. absoluta* particularly at high temperatures. Studies should be carried out with these species showing high efficacy in the laboratory against *T. absoluta* in IPM program as Kamali et al 2018 reported in their study.

It is necessary to carry out the conformity tests with EPNs and pesticides particularly the ones intensively used in tomato production to minimize the effects of these chemicals. Also, studies should be done using adjuvants compatible with EPNs by the most effective application method on the most effective application time against *T. absoluta* in the field, as EPNs encounter many different factors that affect their efficacy and survival adversely in foliar application. Furthermore, it could be useful to carry out studies in tomato fields by using the combinations of different adjuvants as Silwet-L-77, used in Van Damme et al (2016), and Barricade® II, used in Ben Husin (2017), both in terms of less EPNs per area and high efficacy. Previous studies indicated that addition of adjuvants to nematode suspension increases EPNs efficacy by providing perfect conditions for nematode host finding and invasion, also increases nematode survival and reduces the time required by nematodes to enter a leaf, tissue, etc.
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