RESEARCH/INVESTIGACIÓN

SUSCEPTIBILITY OF DIFFERENT LARVAL STAGES OF THE TOMATO LEAF MINER, *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) TO SELECTED ENTOMOPATHOGENIC NEMATODE SPECIES

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ABSTRACT

Saleh, A. 2023. Susceptibility of different larval stages of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: gelechiidae) to selected entomopathogenic nematode species. Nematropica 53:58-66.

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is a key threat to tomato production worldwide. Current management methods remain insufficient to ensure effective control of this insect pest. Hence, more scientific research is needed towards finding more effective and sustainable solutions. In the present study, the efficacy of the entomopathogenic nematode (EPN) species *Steinernema feltiae* UTP-5, *S. carpocapsae* E-76, and *Heterorhabditis bacteriophora* AVB-15 was tested against the 1st/2nd and 3rd/4th larval stages of *T. absoluta* at different concentrations (5 infective juveniles (IJs) cm^{-2}, 10 IJs cm^{-2}, 20 IJs cm^{-2}) under laboratory conditions (25 ± 1°C, RH 60%).

Key words: Biological control, *Heterorhabditis* sp., *Steinernema* sp., tomato leaf miner

RESUMEN

Saleh, A. 2023. Susceptibilidad de diferentes estadios larvales del minador de la hoja del tomate, *Tuta absoluta* (Meyrick) (Lepidoptera: gelechiidae) a especies seleccionadas de nematodos entomopatógenos. Nematropica 53:58-66.

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) es una amenaza clave para la producción de tomate en todo el mundo. Los métodos de manejo actuales siguen siendo insuficientes para garantizar un control eficaz de esta plaga de insecto. Por lo tanto, se necesita más investigación científica para encontrar soluciones más efectivas y sostenibles. En el presente estudio, la eficacia de diferentes especies de nematodos entomopatógenos (EPN); como, *Steinernema feltiae* UTP-5, *S. carpocapsae* E-76, y *Heterorhabditis bacteriophora* AVB-15 se probaron contra los estadios larvales 1°/2° y 3°/4° de *T. absoluta* a diferentes concentraciones (5 JsI cm^{-2}, 10 JsI cm^{-2}, 20 JsI cm^{-2}) en condiciones de laboratorio (25 ± 1°C, HR 60%).

Palabras clave: Minador de la hoja de tomate, control biológico, *Steinernema* sp., *Heterorhabditis* sp.

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest that affects tomato (*Solanum lycopersicum* L.) grown in the greenhouse and field (Desneux *et al*., 2011). Although the pest is considered to originate from South America, its

present wide geographical distribution has made it the most devastating tomato pest across other continents (Tropea Garzia *et al*., 2012). Over the past decade, *T. absoluta* has become a serious threat to the world's leading tomato-producing countries (Biondi *et al*., 2018; Mansour *et al*., 2018; Zhang *et al*., 2020; EPPO, 2021). The larval stages of *T. absoluta* can feed on all the aboveground parts of the tomato plant including the fruits, leaves, and stems (Tropea Garzia *et al*., 2012). The larvae start feeding by mining into the leaves, stems, and immature fruits. Then, the larvae tend to stay inside the galleries they made during feeding until pupation (Urbaneja *et al*., 2013). At high population densities, the larvae can cause severe yield losses, ranging between 50 and 100% (EPPO, 2021).

The control of *T. absoluta* is quite challenging due to the internal feeding habits of larvae, high reproductive capacity, and acquired resistance against various insecticides (Garzia *et al*., 2012; Inak *et al*., 2021). Chemical insecticides used against this pest generally fail to satisfy growers' expectations. Moreover, the increasing use of insecticides leads to various environmental and marketing problems (Arnó and Gabarra, 2011; Shalaby *et al*., 2012). Therefore, many researchers are in search of sustainable control methods against *T. absoluta* (Batalla-Carrera *et al*., 2010; Yüksel *et al*., 2017; Ndereyimana *et al*., 2019). In this context, the entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are obligate parasites of insects, which have been successful in controlling a great variety of insects (above and underground) of agricultural importance (Shapiro-Ilan *et al*., 2017; Yuksel and Canhilal, 2018; Öğretmen *et al*., 2020; Özdemir *et al*., 2021). Infective juveniles (IJs) can search for a suitable host in the application environment (Lewis *et al*., 2006). Under favourable conditions, they are capable of killing the target host within a short period with the help of the symbiotic bacteria (*Xenorhabdus* sp. and *Photorhabdus* sp.) that they carry in their intestine (Boemare, 2002; Lortkipanidze *et al*., 2016; Akhurst and Boemare, 2018). Foliar application of EPNs was studied against many insect pests and some of these studies showed that EPNs can be used effectively if applied under optimal conditions (Batalla-Carrera *et al*., 2010; Beck *et al*., 2013).

However, earlier studies showed that the virulence of EPNs varies remarkably among EPN species and their isolates (Yüksel *et al*., 2018; Husin and Port, 2021). Therefore, it is crucial to evaluate the geographical species and isolates of EPN on target pests in the laboratory before conducting field studies to achieve successful control. This study was conducted with the most virulent EPN isolates that had demonstrated effectiveness against various agricultural pests in earlier studies. The susceptibility of different larval stages of *T. absoluta* was evaluated to native EPN species and the capability of infective juveniles (Ijs) to find the larvae inside leaf galleries of tomato.

MATERIALS AND METHODS

Entomopathogenic nematodes

The three EPN species (*Steinernema feltiae* UTP-5, *S. carpocapsae* E-76, and *Heterorhabditis bacteriophora* AVB-15) obtained from the Cappodoccia region, Turkey were evaluated in the pathogenicity bioassays (Canhilal *et al*., 2017; Yuksel and Canhilal, 2019). The infective juveniles (IJs) of the EPN species were propagated using the last larval instar of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) under laboratory conditions (25 ± 1°C and 60% RH) (Kaya and Stock, 1997). The population of *G. mellonella* was provided by the Entomology Department of Erciyes University. The larvae of *G. mellonella* were cultured in glass jars using the artificial diet described by Metwally *et al*. (2012) and kept at 30 ± 1°C and 60% RH. The last larval instars of *G. mellonella* were handpicked and 10 larvae were added to each Petri dish containing 2 filter papers. The IJs of each EPN species in 1-ml aqueous solution were inoculated to Petri dishes at the concentrations of 200 IJs/dish and kept at 25 ± 1°C and 60% RH for three days. After three days of exposure to the IJs, the dead larvae were placed in modified White Traps and observed for 10 days for the emergence of IJs. The IJs that emerged from the cadavers of *G. mellonella* were collected in beakers and rinsed several times. After rinsing, the IJs were stored horizontally in cell culture flasks (250 ml) containing distilled water (50 ml) at 9°C (Kaya and Stock, 1997; Öğretmen *et al*., 2020). Only two-week-old IJs were used in these experiments. The identification of EPN to species level was conducted under the Zeiss Axio A1 light

microscope (Carl Zeiss AG, Oberkochen, Germany). Photographs and measurements from the specimens were taken using ZEN light microscopy software.

The culture of Tuta absoluta

The population of *T. absoluta* larva was collected from infested tomato fields from the provinces of Mersin and Antalya in the Mediterranean region of Turkey and transported to the Laboratory of the Erciyes University in plastic containers (45 x 30 x 25 cm). To attain a sufficient number of larvae for pathogenicity bioassays, the larvae were reared on potted tomato plants under controlled conditions (25 ± 1°C, 60% RH, and 16:8 h L: D photoperiod). To increase the reproduction of adult *T. absoluta*, several pieces of cotton wool submerged in a 10% honey solution were placed in a cage (60 x 60 x 60 cm^3) as a source of food. The larval instars of *T. absoluta* were differentiated by measuring the head capsules of the larvae (Gullan and Cranston, 2005).

Pathogenicity bioassays

Petri dish bioassay. The contact efficacy of three native EPN species against the $1^{st}/2^{nd}$ (L1 and L2) and $3^{rd}/4^{th}$ (L3 and L4) larval instar of *T. absoluta* was evaluated in Petri dishes with 25 g of sterilized air-dried sandy soil with distilled water (10%, w/w) (surface area 8.8 cm^2). Different concentrations of the IJs of each EPN species (0, 5, 10, 20 IJs cm^{-2}) in 1 ml tap water were applied onto the soil surface using a micropipette and one healthy larva (L1/L2 or L3/L4) was added to each Petri dish. Petri dishes were maintained at 25 ± 1°C and 60% RH for three days following the application of IJs, and mortality rates were recorded on a daily basis. The dead larvae were dissected and examined under a stereo microscope to confirm the infection of EPN IJs. Each EPN concentration was tested against 10 larvae and replicated four times. Only 1 ml of tap water was applied to the control treatments.

Leaf bioassay. In the leaf bioassay, the IJs of each EPN were tested for their ability to infect the larvae inside the leaf galleries. One-month-old tomato seedlings (SC-2121) infected with larvae of *T. absoluta* were collected from different greenhouses and brought to the laboratory in pots. The leaves were observed for two days and sorted based on the presence of the pest at different larval stages. To confirm the larval instars, 10 leaves were chosen randomly and removed using a fine needle and brush. Subsequently, the head capsules of the larvae were measured. The IJs of each EPN species were applied to the surface of a leaf containing only one larval stage of *T. absoluta* at the concentrations of 0, 5, 10, and 20 IJs cm^{-2}, and the treated leaves were put into Petri dishes containing two filter papers. To keep the leaves fresh, the petioles were covered with a paper towel dampened with tap water. The Petri dishes were sealed with parafilm and kept at 25 ± 1°C, 16:8 L: D photoperiod and 60% RH for three days. Each concentration was evaluated using 10 larvae, applying only tap water to the leaves as a control. The mortality of larvae was monitored daily by observing and gently nudging the larvae with a fine needle. Nematode infection was confirmed following the same aforementioned protocol. Each treatment was replicated 4 times, and there were 10 larvae for each replication. Only 1 ml of tap water was applied to the control treatments.

Statistical analysis

The experiments were laid out in a completely randomized design (RCD) with four replicates. Each bioassay was repeated twice on different dates and pooled for analysis. Data were analyzed using SPSS (Version 18.0) statistical software package following normality tests. The repeated measures analysis of variance (RM ANOVA) was performed after arcsine transformation (Rangaswamy, 2013). No corrections were made to the mortality rates before analysis since there was no mortality in the control treatments. Tukey's multiple range tests ($P \leq 0.05$) were performed to separate the mean of mortality rates.

RESULTS

Petri dish bioassay. The EPN treatments and their associated interactions significantly ($P < 0.05$) affected the mortality rates of *T. absoluta* in the Petri dish bioassays except for the interactions between nematode concentration and larval stage of *T. absoluta* (C*L) ($P = 0.112$). The mortality rates of *T. absoluta* increased as the exposure time and the concentration of IJs increased. The maximum mortality (100%) for L1/L2 was obtained by only one EPN (*S. carpocapsae* E-76)

at the highest application concentration. In general, *S. carpocapsae* E-76 caused the highest mortality against L1/L2 and L3/L4 of *T. absoluta* at all application concentrations and exposure times except for 72 hr of exposure at the 10 EPN IJs cm^{-2} concentration. All EPN species caused mortalities over 90% in both bioassays against L1/L2 and L3/L4 of *T. absoluta* after 72 hr of exposure. *S. carpocapsae* E-76 induced remarkable mortalities at 20 IJs cm^2 concentration against L1/L2 and L3/L4 of *T. absoluta* 48 hr after treatment. (Table 1).

Leaf bioassay. In the leaf bioassay, the differences in mortality rates of *T. absoluta* larvae were significantly affected by all main treatments [EPN species (N), IJs concentrations (C), and exposure times (T)]. In the leaf bioassay, mortality rates were lower than in the Petri dish bioassay. However, increasing the concentrations of and exposure time to EPN IJs had a positive effect on mortality rates. The highest mortality rates against larval stages of *T. absoluta* did not exceed 62.5% after 72 hr of exposure. The highest mortalities (52.5%) against the L1/L2 of *T. absoluta* were obtained from two EPN species (*S. carpocapsae* E-76 and *H. bacteriophora* AVB-15). *Steinernema carpocapsae* was the most virulent isolate against L3/L4 of *T. absoluta* at the highest concentration of nematodes and caused 62.5% mortality after 72 hr of exposure (Table 2). Although, in general, there were no significant differences in mortalities among the EPN species tested, notable differences occurred in the mortality rates depending on the exposure time. *S. carpocapsae* E-76 caused 5% mortality against L1/L2 at the lowest concentration after 24 hr of exposure. However, the mortality rates in L1/L2 reached 62.5% after 72 hr of exposure for the same application concentration.

DISCUSSION

In the current study, the pathogenicity of

Table 1. Effects of the applications of entomopathogenic nematodes (EPN) infective larvae (IJ) on mortality rates (mean ± SE) of $1^{st}/2^{nd}$ and $3^{rd}/4^{th}$ larval instars of *Tuta absoluta* after one, two and three days after treatment in Petri dish bioassays under laboratory conditions (25 ± 1°C, R.H. 60%).

			EPN[x]		
Larval Stages	Dose	Time (hr)	UTP-5	E-76	AVB-15
$1^{st}/2^{nd}$	5 IJs cm^2	24	10.0 ± 8.1 x[y] a[z]	17.5 ± 5.0 x a	5.0 ± 5.7 x a
		48	42.5 ± 5.0 y a	58.3 ± 14.6 y a	37.5 ± 5.0 y a
		72	60.4 ± 17.7 z a	80.0 ± 11.2 z b	62.5 ± 5.0 z a
	10 IJs cm^2	24	15.0 ± 5.7 x a	42.5 ± 5.0 x b	22.5 ± 5.0 x a
		48	57.5 ± 5.0 y a	62.5 ± 5.0 y a	57.5 ± 5.0 y a
		72	82.5 ± 5.0 z a	85.0 ± 5.7 z a	82.5 ± 5.0 z a
	20 IJs cm^2	24	20.0 ± 0.0 x a	57.5 ± 5.0 x b	37.5 ± 5.0 x a
		48	75.0 ± 5.7 y a	92.5 ± 5.0 y b	67.5 ± 5.0 y a
		72	90.0 ± 8.1 z a	100.0 ± 0.0 y a	97.5 ± 5.0 z a
$3^{rd}/4^{th}$	5 IJs cm^2	24	15.0 ± 5.7 x a	22.5 ± 5.0 x a	15.0 ± 5.7 x a
		48	50.0 ± 0.0 y a	60.0 ± 8.1 y a	54.1 ± 10.8 y a
		72	82.5 ± 5.0 z a	82.5 ± 5.0 z a	76.6 ± 8.8 z a
	10 IJs cm^2	24	20.0 ± 0.0 x a	47.5 ± 5.0 x b	27.5 ± 5.0 x a
		48	62.5 ± 5.0 y a	80.0 ± 8.1 y b	65.0 ± 5.7 y a
		72	95.0 ± 5.7 z a	87.5 ± 5.0 y a	82.5 ± 5.0 z a
	20 IJs cm^2	24	27.5 ± 5.0 x a	60.0 ± 5.7 x b	37.5 ± 5.0 x a
		48	92.5 ± 5.0 y b	97.5 ± 5.0 y b	80.0 ± 0.0 y a
		72	95.0 ± 5.7 y a	100.0 ± 0.0 y a	100.0 ± 0.0 z a

[x]UTP-5: *Steinernema feltiae*; E-76: *S. carpocapsae*; AVB-15: *Heterorhabditis bacteriophora*. DAT: Days after treatment.

[y]Different xyz letters show statistically significant differences among infective juvenile doses for each exposure time level ($P < 0.05$, Tukey test).

[z]Different abc letters show statistically significant differences among entomopathogenic nematode species for the same exposure time levels ($P < 0.05$, Tukey test).

Table 2. Effects of the applications of entomopathogenic nematodes (EPN) infective juveniles (IJ) on mortality rates (mean ± SE) of 1st/2nd and 3rd/4th larval instars of *Tuta absoluta* at one, two and three days after treatment in leaf bioassays under laboratory conditions (25 ± 1°C, R.H. 60%).

			EPN[x]		
Larval stages	Dose	Time (hr)	UTP-5	E-76	AVB-15
1st/2nd		24	0.0 ± 0.0 x[y] a[z]	5.0 ± 5.7 x a	0.0 ± 0.0 x a
	5 IJs cm^2	48	22.5 ± 5.0 y a	20.0 ± 0.0 y a	17.5 ± 5.0 y a
		72	42.5 ± 5.0 z a	62.5 ± 5.0 z b	32.5 ± 5.0 z a
		24	2.5 ± 5.0 x a	7.5 ± 5.0 x a	2.5 ± 5.0 x a
	10 IJs cm^2	48	32.5 ± 5.0 y a	35.0 ± 5.7 y a	32.5 ± 5.0 y a
		72	52.5 ± 5.0 z a	52.5 ± 5.0 z a	42.5 ± 5.0 z a
		24	2.5 ± 5.0 x a	10.0 ± 8.1 x a	5.0 ± 5.7 x a
	20 IJs cm^2	48	35.0 ± 5.7 y a	45.0 ± 5.7 y a	42.5 ± 5.0 y a
		72	52.5 ± 5.0 z a	57.5 ± 5.0 y a	57.5 ± 5.0 y a
3rd/4th		24	2.5 ± 5.0 x a	0.0 ± 0.0 x a	0.0 ± 0.0 x a
	5 IJs cm^2	48	17.5 ± 5.0 x a	25.0 ± 5.7 y a	22.5 ± 5.0 y a
		72	37.5 ± 5.0 y a	37.5 ± 5.0 y a	35.0 ± 5.7 y a
		24	5.0 ± 5.7 x a	2.5 ± 5.0 x a	2.5 ± 5.0 x a
	10 IJs cm^2	48	22.5 ± 5.0 y a	35.0 ± 5.7 y a	32.5 ± 5.0 y a
		72	47.5 ± 5.0 z a	50.0 ± 0.0 y a	42.5 ± 5.0 y a
		24	10 ± 0.0 x a	2.5 ± 5.0 x a	7.5 ± 5.0 x a
	20 IJs cm^2	48	40.0 ± 0.0 y a	50.0 ± 8.1 y a	42.5 ± 5.0 y a
		72	55.0 ± 5.7 z a	62.5 ± 5.0 y a	52.5 ± 5.0 y a

[x]UTP-5: *Steinernema feltiae*; E-76: *S. carpocapsae*; AVB-15: *Heterorhabditis bacteriophora*. DAT: Days after treatment.

[y]Different xyz letters show statistically significant differences among exposure time levels for the same doses (P <0.05, Tukey test).

[z]Different abc letters show statistically significant differences among entomopathogenic nematode species for the same exposure time levels (P <0.05, Tukey test).

different EPN species was evaluated against the L1/L2 and L3/L4 of *T. absoluta* at varying EPN IJs concentrations under laboratory conditions. The results showed that all tested EPN species were able to infect and kill the different larval instars of *T. absoluta*. The virulence of EPNs was significantly correlated with EPN species, IJs concentrations, and exposure time in Petri dish and leaf bioassays. In both bioassays, mortality rates were relatively higher for the 3rd /4th larval instars than the 1st /2nd larval instars. These findings echo the results of earlier studies (Gözel and Kasap, 2015; Ndereyimana *et al*., 2019; Husin and Port, 2021). In a laboratory study conducted by Van Damme *et al*. (2016), the last instar larvae were reported to be more susceptible to the tested EPNs, reaching the maximum mortality rate of 97% for the last instar larvae, compared to the maximum of 60% mortality for the first instar larvae. In the same study, *S. feltiae* and *S. carpocapsae* performed better than *H. bacteriophora*. In a similar study, Gözel *et al*. (2015), reported higher pathogenicity of *S. feltiae* (75%) and *H. bacteriophora* (70%) against the last larval instars of *T. absoluta* at 30 IJs/larva.

Other studies have shown that EPNs can locate and kill the larvae inside the leaf galleries (Batalla-Carrera *et al*., 2010; Van Damme *et al*., 2016; Dlamini *et al*., 2020; El Aimani *et al*., 2021). In these studies, the mortality rates were positively affected by exposure time and EPN species, which is in line with our findings. Similar to the current study, El Aimani *et al*. (2021) tested the pathogenicity of different *H. bacteriophora* and *S. feltiae* isolates against the L4 of *T. absoluta* in 24-well plates and leaf bioassays. In this study, the mortality rate varied between 40 and 60% for *H. bacteriophora* isolates, and higher mortality rates were recorded for *S. feltiae* isolates, ranging between 60 and 80%. Differences in larval mortality among these studies may be a result of differences in the pathogenicity of EPN species, IJs concentrations, and application environment along with other environmental factors. Previous studies

have shown that there is great variation in the pathogenicity of EPN species and their isolates (Yüksel and Canhilal, 2020; Özdemir *et al*., 2021). To a certain extent, mortality rates tend to increase with increasing concentrations of EPN IJs as higher concentrations increase the likelihood of the host getting infected and exposed to higher concentrations of symbiotic bacteria. Host size plays an important role in the pathogenicity of EPNs since this affects the penetration and host location capability of EPNs. Also, it has been reported that young instars of the potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) were able to hide from EPNs better compared to older instars (Lewis *et al*., 1993; Malan and Manrakhan, 2009; Hassani-Kakhki *et al*., 2013; Bastidas *et al*., 2014). Relative humidity (RH) is a significant factor influencing both the survival and penetration capability of IJs. Earlier studies reported that the penetration rate of IJs into the galleries formed by the larvae of *T. absoluta* varies greatly by RH levels. *S. feltiae* and *S. carpcapsae* IJs were able to enter the leaves between 6 and 12 hr after application at 75% RH (Husin and Port, 2021). In the current study, EPNs were applied in an aqueous suspension in leaf bioassay and kept at 60% RH during the experiment and the highest mortality did not exceed 63%. Low RH in the current study might have led to a decrease in both the survival and mobility of EPN IJs, thus reducing mortality rates.

In the current study, although *S. carpocapsae* have a 'sit and wait' foraging strategy, *S. carpocapsae* E-76 generally induced higher mortality compared to *S. feltiae* (intermediate-foraging) and *H. bacteriophora* (active-foraging cruiser). The reason behind this might be the differences in the pathogenicity and desiccation tolerance of EPN species. *S. carpocapsae* IJs were reported to have a higher desiccation tolerance compared to S. *felitae* and *H. bacteriophora* (Kagimu *et al*., 2017; Husin and Port, 2021). Moreover, studies have shown that *S. carpocapsae* might adopt a cruiser strategy depending on the circumstances (Wilson *et al*., 2012). This hypothesis is in line with our study. *S. carpocapsae* IJs were able to locate the larvae inside galleries more effectively and caused relatively higher mortality rates. Different application environments and host volatile chemical cues may have led *S. carpocapsae* E-76 to shift its foraging strategy from a 'sit and wait' foraging to a cruiser strategy (Wilson *et al*., 2012).

The tested EPNs were remarkably effective in Petri dish bioassay and achieved mortality rates over 60% at the lowest concentration (5 IJs cm^{-2}) 72 hr after application. However, in the leaf bioassay, the mortality rate did not exceed 63%. *S. carpocapsae* generally performed better than *S. feltiae* and *H. bacteriophora* in both bioassays. The results showed that tested EPN species have great potential in the control of *T. absoluta*. Nevertheless, additional trials under field conditions are still required to confirm the effectiveness of the tested EPNs.

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