



Tarım Bilimleri Dergisi
Tar. Bil. Der.

Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences

Journal homepage:
www.agri.ankara.edu.tr/journal

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

Çiğdem GÖZEL^a, İsmail KASAP^a, Uğur GÖZEL^a

^aÇanakkale Onsekiz Mart University Faculty of Agriculture Department of Plant Protection

ARTICLE INFO

Research Article

Corresponding Author: Çiğdem GÖZEL, E-mail: cigdemgunes@comu.edu.tr, Tel: +90 (286) 218 00 18/1385

Received: 30 January 2019, Received in Revised Form: 27 March 2019, Accepted: 07 April 2019

AUTHORS ORCID ID

(Çiğdem GÖZEL: 0000-0002-0742-7205), (İsmail KASAP: 0000-0002-0015-4558), (Uğur GÖZEL: 0000-0003-1363-1189)

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 (IJs) were inoculated to a single *T. absoluta*

larva for each nematode species. After nematode inoculation, larvae were checked on the 3rd, 5th, 7th days and mortalities were recorded. All nematode species used in the study showed the lowest efficacy on the 3rd control day at 10 °C and the highest efficacy on the 7th 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

© Ankara Üniversitesi Ziraat Fakültesi

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

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 *Photorhabdus* (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 nontarget 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 (%) \pm SE

Temperature (°C)	3 rd day	5 th day	7 th day	F value
10	8.33 \pm 4.81 Aa	12.50 \pm 4.17 Aab	25.00 \pm 4.81 Ab	F _(3,15) = 3.55*
15	20.83 \pm 4.17 ABa	29.16 \pm 4.17 Bab	41.67 \pm 4.81 Bb	F _(3,15) = 5.70
20	37.50 \pm 4.17 Ba	45.83 \pm 4.17 Ca	66.66 \pm 0.00 Cb	F _(3,15) = 19.49
25	70.83 \pm 4.17 Ca	75.00 \pm 4.81 Dab	83.33 \pm 0.00 Db	F _(3,15) = 3.00
	F _(3,15) = 39**	F _(3,15) = 37.77	F _(3,15) = 58.00	

*, 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 (%) \pm SE

Temperature (°C)	3 rd day	5 th day	7 th day	F value
10	12.50 \pm 7.98 Aa	12.50 \pm 4.17 Aa	25.00 \pm 4.81 Aa	F _(3,15) = 1.50*
15	20.83 \pm 4.17 ABa	29.16 \pm 4.17 Bab	41.67 \pm 4.81 Bb	F _(3,15) = 5.70
20	41.67 \pm 4.81 Ba	54.17 \pm 4.17 Cb	66.66 \pm 0.00 Cc	F _(3,15) = 11.57
25	62.50 \pm 4.17 Ca	75.00 \pm 4.81 Dab	87.50 \pm 4.17 Db	F _(3,15) = 8.10
	F _(3,15) = 16.53**	F _(3,15) = 40.24	F _(3,15) = 47.53	

*, 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 (%) \pm SE

Temperature (°C)	3 rd day	5 th day	7 th day	F value
10	8.33 \pm 4.81 Aa	16.66 \pm 6.80 Aa	16.66 \pm 0.00 Aa	F _(3,15) = 1.0*
15	20.83 \pm 4.17 Ba	29.16 \pm 4.17 ABab	33.33 \pm 0.00 Bb	F _(3,15) = 3.5
20	50.00 \pm 0.00 Ca	54.17 \pm 4.17 Bab	70.83 \pm 4.17 Cb	F _(3,15) = 10.5
25	75.00 \pm 4.81 Da	83.33 \pm 0.00 Cab	91.67 \pm 4.81 Db	F _(3,15) = 4.5
	F _(3,15) = 56.28**	F _(3,15) = 42.87	F _(3,15) = 54.06	

*, 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).

Table 4- Mortality of *Tuta absoluta* larvae caused by *Heterorhabditis bacteriophora* 1144 at four different temperatures and three control days Mean (%) \pm SE

Temperature (°C)	3 rd day	5 th day	7 th day	F value
10	0.00 \pm 0.00 Aa	8.33 \pm 4.81 Aa	8.33 \pm 8.33 Aa	F _(3,15) = 0.75*
15	8.33 \pm 4.81 ABa	16.66 \pm 6.80 Aa	25.00 \pm 4.81 Ba	F _(3,15) = 2.25
20	33.33 \pm 0.00 Ba	41.67 \pm 4.81 Bab	50.00 \pm 0.00 Cb	F _(3,15) = 9.00
25	70.83 \pm 4.17 Ca	75.00 \pm 4.81 Cab	87.50 \pm 4.17 Db	F _(3,15) = 3.90
	F _(3,15) = 99.87**	F _(3,15) = 31.00	F _(3,15) = 43.10	

*, 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 & Kaşkavalcı 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 SilwetL-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.

References

- Batalla-Carrera L, Morton A & Garcia-del-pino F (2010). Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *Biocontrol* 55: 523-530
- Bedding R A & Akhurst R J (1975). A simple technique for the detection of insect parasitic Rhabditid nematodes in soil. *Nematologica* 21: 109-110
- Ben Husin T O A (2017). Biological control of tomato leaf miner *Tuta absoluta* using entomopathogenic nematodes. PhD Thesis, Faculty of Science, Agriculture and Engineering, Newcastle University, United Kingdom, pp. 196
- Boemare N (2002). Biology, taxonomy and systematic of *Photorhabdus* and *Xenorhabdus*. In: R. Gaugler (Ed.), *Entomopathogenic Nematology*. Wallingford, UK: CAB International, pp. 35-56
- Boemare N E, Akhurst R J & Mourant R G (1993). DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. *International Journal of Systematic Bacteriology* 43(2): 249-255
- Cáceres S (1992). La polilla del tomate en Corrientes. *Biología y control*, Estación Experimental Agropecuaria Bella Vista INTA, pp. 19
- Desneux N, Wajnberg E, Wyckhuys K A G, Burgio G, Arpaia S, Narvaez-Vasquez C A, Gonzalez-Cabrera J, Ruescas D A, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T & Urbaneja A (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geo-graphic expansion and prospects for biological control. *Journal of Pest Science* 83: 197-215
- Ehlers R U (1996). Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. *Biocontrol Science Technology* 6: 303-316
- Estay P (2000). Polilla del tomate *Tuta absoluta* (Meyrick), URL <http://alerce.inia.cl/docs/Informativos/Informativo09.pdf> (Date accessed: September 2014)
- Garcia-del-Pino F, Alabern X & Morton A (2013). Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect. *BioControl* 58(6): 723-731
- Gözel Ç (2016). Domates güvesi *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'nin mücadelesinde entomopatogen nematodların kullanım olanakları. Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü Doktora Tezi, 83 s
- Gözel Ç & Kasap İ (2015). Efficacy of entomopathogenic nematodes against the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field. *Turkish Journal of Entomology* 39(3): 229-237
- Gözel U & Güneş Ç (2013). Effect of entomopathogenic nematode species on the corn stalk borer (*Sesamia cretica* Led. Lepidoptera: Noctuidae) at different temperatures. *Turkish Journal of Entomology* 37(1): 65-72
- Kamali S, Karimi J & Koppenhöfer A M (2018). New Insight into the Management of the Tomato Leaf Miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with Entomopathogenic Nematodes. *Journal of Economic Entomology* 111(1): 112-119
- Kasap İ, Gözel U & Özpınar A (2011). "Yeni bir zararlı; Domates güvesi *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), 284". *Çanakkale Tarımı Sempozyumu (Dünü, Bugünü, Geleceği) Bildirileri*, 10-11 Ocak Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi, Çanakkale pp: 717
- Kaya H K & Gaugler R (1993). Entomopathogenic nematodes. *Annual Review of Entomology* 38: 181-206
- Kaya H K & Stock S P (1997). "Techniques in Insect Nematology, 281-324" In: L A Lacey (Ed.), *Manual of Techniques in Insect Pathology Biological Techniques Series*, Academic Press, San Diego, California pp: 409
- Kılıç T (2010). First record of *Tuta absoluta* in Turkey. *Phytoparasitica* 38: 243-244
- Lacey L A & Unruh T R (1998). Entomopathogenic nematodes for control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): Effect of nematode species, concentration, temperature, and humidity. *Biological Control* 13: 190-197

- Olthof ThHA & Broadbent A B (1990). "Control of a chrysanthemum leafminer, *Liriomyza trifolii* with the entomophilic nematode, *Heterorhabditis heliothidis*, 379". Abstracts of Papers, Posters and Films Presented at the Second International Nematology Congress, 11-17 August, Veldhoven the Netherlands, *Nematologica* 36(1990): 327-403
- Roditakis E, Papachristos D & Roditakis N E (2010). Current status of the tomato leafminer *Tuta absoluta* in Greece. *EPPO Bulletin* 40: 163-166
- Shamseldean M, Abd-Elbary N A, Shalaby H & Ibraheem H I H (2014). Entomopathogenic nematodes as biocontrol agents of the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato plants. *Egyptian Society for Biological Control of Pests* 24: 503-513
- Siqueira H A A, Guedes R N C & Picanço M C (2000). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology* 2: 147-153
- Susurluk A & Ehlers R U (2008). Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. *BioControl* 53: 627-641
- Susurluk A, Dix I, Stackebrandt E, Strauch O, Wyss U & Ehlers R U (2001). Identification and ecological characterisation of three entomopathogenic nematode-bacterium complex from Turkey, *Nematology* 3(8): 833-841
- Türköz S & Kaşkavalcı G (2016). Determination of the efficacy of some entomopathogenic nematodes against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions. *Turkish Journal of Entomology* 40(2): 175-183
- Urbaneja A, Vercher R, Navarro V, Porcuna J L & Maria F G (2007). La polilla del tomate, *Tuta absoluta*. *Phytoma España* 194: 16-23
- Van Damme V M, Beck B K, Berckmoes E, Moerkens R, Wittemans L, Vis R D, Nuyttens D, Casteels H F, Maes M, Tirry L & Clercq P D (2016). Efficacy of entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory. *Pest Management Science* 72: 1702-1709
- Vargas H C (1970). Observaciones sobre la biología y enemigos naturales de la polilla del tomate, *Gnorimoschema absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Idesia* 1: 75-110
- White G F (1927). A method for obtaining infective nematode larvae from cultures. *Science* 66: 302-303