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Orijinal araştırma (Original article)

Efficacy of entomopathogenic nematodes against the Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field[1]

Entomopatojen nematodların Domates güvesi *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'ya karşı domates tarlasındaki etkinliği

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Summary

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a very challenging pest that causes economical losses in tomato production. This devastating pest originated from South America was the first time detected in İzmir province of Turkey in August 2009. The efficacy of the infective juveniles (IJs) of four native entomopathogenic nematode (EPN) species, *Steinernema affine* (Bovien) (isolate 46), *S. carpocapsae* (Weiser) (isolate 1133), *S. feltiae* (Filipjev) (isolate 879) and *Heterorhabditis bacteriophora* (Poinar) (isolate 1144) was investigated against the larvae of *T. absoluta* in the field during the tomato production seasons of 2012-2013 in Çanakkale. Individuals of *T. absoluta* were collected from infested tomato fields in Çanakkale and mass produced on tomato plants in a climate controlled room. EPNs were isolated from different parts of Turkey and mass produced by using *Galleria mellonella* larvae in the laboratory. The tomato leaf miners were exposed to each nematode species at the rate of 50 IJs/cm^2 on tomato plants in cages. *T. absoluta* were susceptible to all EPNs tested but the degree of susceptibility of the larvae to EPN infection varied according to the species. The most effective nematode species on *T. absoluta* larvae was *S. feltiae* (isolate 879) with 90.7% and 94.3% mortality in 2012 and 2013, respectively, whereas the least effective species was *S. affine* (isolate 46) with 39.3% and 43.7% mortality in 2012 and 2013, respectively. EPNs can be potential canditates to control tomato leafminer, so the integration possibility of these biological agents into the *T. absoluta* management programme is discussed.

Keywords: Biological control, entomopathogenic nematodes, *Heterorhabditis, Steinernema, Tuta absoluta*

Özet

Domates güvesi, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) domates üretiminde ekonomik kayıplara neden olan, mücadelesi zor bir zararlıdır. Tahrip gücü yüksek bu zararlı Güney Amerika orijinli olup, ülkemizde ilk olarak 2009 Ağustosu'nda İzmir'de tespit edilmiştir. Dört yerel entomopatojen nematod türü; *Steinernema affine* (Bovien) (izolat 46), *S. carpocapsae* (Weiser) (izolat 1133), *S. feltiae* (Filipjev) (izolat 879) ve *Heterorhabditis bacteriophora* (Poinar) (izolat 1144)'nın *T. absoluta*'ya karşı etkinliği tarlada 2012-2013 Çanakkale domates üretim sezonu süresince araştırılmıştır. *T. absoluta* bireyleri Çanakkale'deki bulaşık domates tarlalarından toplanmış ve iklim odasında domates bitkileri üzerinde kitle üretimi yapılmıştır. EPN'ler ise ülkemizin farklı bölgelerinden elde edilmiş ve laboratuarda *Galleria mellonella* larvalarında kitle üretimi yapılmıştır. Her bir nematod türü domates güvesine kafeslerdeki domates bitkileri üzerinde 50 IJs/cm^2 olacak şekilde uygulanmıştır. *T. absoluta*'nın, denemede kullanılan tüm EPN'lere karşı duyarlı olduğu tespit edilmiş, ancak larvaların infeksiyona karşı gösterdiği duyarlılık nematod türüne bağlı olarak değişiklik göstermiştir. *S. feltiae* (izolat 879) 2012 ve 2013 yıllarında sırası ile meydana getirdiği %90.7 ve %94.3 ölüm oranları ile en etkili tür olarak tespit edilmişken, *S. affine* (izolat 46) 2012 ve 2013 yıllarında sırası ile meydana getirdiği %39.3 ve %43.7 ölüm oranları ile en az etkili tür olarak tespit edilmiştir. EPN'ler domates güvesini kontrol etmek için potansiyel adaylar olabilir, bu nedenle bu biyolojik ajanların *T. absoluta*'nın mücadele programına dahil edilme olasılığı üzerinde durulmalıdır.

Anahtar sözcükler: Biyolojik mücadele, entomopatojen nematodlar, *Heterorhabditis, Steinernema, Tuta absoluta*

[1] This study is a part of PhD thesis of the first author.

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Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a Neotropical, oligophagous pest of solanaceous crops that originates from South America (Lietti et al., 2005; Urbaneja et al., 2007). This devastating pest has spread throughout the Mediterranean Basin, dispersing to other European and Middle East Asian countries, and within a 15-year period, it is expected to reach the Pacific Asian Coast (Potting, 2009; Desneux et al., 2011; Germain et al., 2009). It has been listed with the code GNORAB in the A1 quarantine list of the European and Mediterranean Plant Protection Organization (EPPO, 2009). In Turkey it was first recorded in 2009 in the Urla District of Izmir Province (Kılıç, 2010) and has been a serious problem to tomato production in Çanakkale since the first detection in Turkey (Kasap et al., 2011).

Tuta absoluta is a holometabolous insect with a high rate of reproductive capacity. It can overwinter in the egg, pupal, or adult stage, is multivoltine and can complete 12 generations per year depending on environmental conditions. Adults are silvery gray with black spots on the forewings and a wingspan reaching 10 mm. Their activity is concentrated in the early morning and dusk; during the rest of the day, they remain hidden among the leaves. Adult lifespan ranges between 10 and 15 days for females and 6-7 days for males. The number of eggs per female is usually between 40 and 50 and may reach 260. Eggs are small, 0.35 mm long, cylindrical and creamy white to yellow. Egg hatching takes 4-6 days. Larval development goes through four stages and pupation may take place in the soil, on the leaves and even within the galleries or other parts of the plant. The pupa is cylindrical and greenish when recently formed, later turning brown. It may be protected by a silky white cocoon (Anonymous, 2010).

Females generally prefer to lay eggs on leaves (73%), leaf veins and stem margins (21%), sepals (5%) or green fruits (1%) (Estay, 2000). After hatching, larvae enter the plant tissue and begin feeding. These feeding mines affect the photosynthetic capacity of the plant and enable attacks by plant pathogens. The galleries produced by young larvae may be confused with those produced by leafminers (*Liriomyza* spp.), but the gallery produced by *T. absoluta* subsequently widens and the damaged tissue dries. During development the larvae may change gallery several times. Young larvae can mine leaves, stems, shoots, flowers, and developing fruit; later instars can attack mature tomato fruit and infested fruit usually falls to the ground (Vargas, 1970). This devastating pest can attack all parts and stages of the tomato plant, overwinter in the egg, pupal, or adult stage and can cause up to 100% losses in tomato crops (EPPO, 2005).

Tomato leaf miner primarily attacks cultivated and non-cultivated tomato plants and other members of the family Solanaceae but it can also feed, develop and reproduce on other naturally available host-plants such as *Datura ferox* L., *D. stramonium* L. and *Nicotiana glauca* Graham (Garcia & Espul, 1982; Larrain, 1986). Different plant species have been reported as alternative hosts of this insect as Cape gooseberry (*Physalis peruviana* L.), bean (*Phaseolus vulgaris* L.), *Lycium* sp. L. and *Malva* sp. L. (Caponero, 2009; EPPO, 2009; Tropea Garzia, 2009). This range indicates that *T. absoluta* shows a high propensity to use various plants as secondary hosts.

Since its dispersal in the 1970s, chemical control has been the main method of controlling this pest. Farmers have tried to reduce its damages by applying insecticides two times a week during a single cultivation period, sometimes every 4-5 days/season with minimum and maximum numbers of 8 to 25 sprays (Temerak, 2011). Even with the numerous applications of chemicals, effective control is difficult to achieve due to the mine-feeding behaviour of the larvae. Furthermore, the use of pesticides in crop production has many disadvantages such as pesticide residues on human health and on the environment. Thus, biological control can be considered as an alternative method to chemical control. In this respect, entomopathogenic nematodes (EPNs), which have great potential as biological control agent of insects, can be an alternative to chemicals.

EPNs are a group of soil-dwelling organisms that attack soilborne insect pests that live in, on, or near the soil surface and can be used effectively to control important pests. EPNs of the families Steinernematidae and Heterorhabditidae are symbiotically associated with bacteria in the genera *Xenorhabdus* (Thomas and Poinar) and *Photorhabdus* (Boemare, Akhurst and Mourant), respectively (Boemare et al., 1997; Burnell & Stock, 2000). The bacteria kill the host by producing toxins, provide nematodes with nutrition, and prevent secondary invaders from contaminating the host cadaver (Forst & Clarke, 2002). Infective juveniles (IJs) enter the host body mainly through natural openings such as the mouth, spiracles, anus or thin parts of the host cuticle and release their bacteria inside the hemocoel. Most biological agents require days or weeks to kill the host, yet nematodes can kill insects usually in 24-48 hours.

EPNs have many advantages; they are easy and relatively inexpensive to culture, live from several weeks up to months in the infective stage, are capable of infecting a broad range of insect species, occur in soil and have been isolated from most regions of the world except Antarctica (Griffin et al., 1990; Kaya & Gaugler, 1993). Foliar applications of nematodes have been successfully used to control the quarantine leaf eating caterpillars on various crops and have the potential for controlling various other insect pests. Application of EPNs does not require masks or other safety equipment as chemicals. EPNs and their associated bacteria have no detrimental effect to mammals or plants (Poinar et al., 1982; Boemare et al., 1996; Akhurst & Smith, 2002).

Discovery and development of new nematode species and strains and further improvement in formulation to enhance the biological control potential of entomopathogenic will further expand the options for implementation of nematodes against a wider range of targeted pests and also improvements in production technology, distribution, and application will be key to increasing nematode use.

The aims of the work were to determine the efficacy of EPNs against *T. absoluta* and to reduce the use of pesticides. This paper covers the efficacy of native EPNs against *T. absoluta* larvae in a tomato field in Çanakkale.

Materials and Methods

Entomopathogenic nematodes culture

Four native species of nematodes; *Steinernema affine* (Bovien) (isolate 46) *S. carpocapsae* (Weiser) (isolate 1133), *S. feltiae* (Filipjev) (isolate 879) and *Heterorhabditis bacteriophora* (Poinar) (isolate 1144) were evaluated against the tomato leaf miner larvae. Each isolates was reared in the last instar of wax moth larvae *Galleria mellonella* L., which is the most commonly used insect host for in vivo production of EPNs (Bedding & Akhurst, 1975; Lindegren et al., 1993; Kaya & Stock, 1997). *G. mellonella* was preferred because of its high susceptibility to the most nematodes, wide availability, ease in rearing, and high yields (Shapiro-Ilan & Gaugler, 2002; Woodring & Kaya, 1988).

Nematode-killed *G. mellonella* larvae were placed on White traps (White, 1927) at 25 ^{o}C and IJs that emerged from cadavers were harvested. These IJs were rinsed in distilled water and used within a week for the experiments. Before using the nematodes, their viability was checked under the stereomicroscope.

Tuta absoluta culture

Larvae, pupae and adults of *T. absoluta* used in the trials were obtained from infested tomato fields in Çanakkale. They reared in wooden rearing cages (50x50x50 cm) covered with organza on tomato plants at 25±1 ^{o}C, 65±5% RH, with a 16:8 L:D photoperiod in climate room. Male and female adults of *T. absoluta* were used to establish larval infestation on the tomato plants for the trials. A continuous mass-

rearing of all development stages of *T. absoluta* was maintained on tomato plants in a climate room in cages.

Field trials

Field trials were carried out in the training and research area of Agriculture Faculty in Dardanos Campus in Çanakkale in 2012-2013. In both seasons, approximately 1000 m^2 area was cultivated with tomato. The tomato cultivar, Troy F1, was used in the trials because it is the most suitable for the Çanakkale climate. Seedlings were watered and fertilized periodically and closed by a cage when they reached 20 cm height. Each tomato plant was grown in a single cage (50x50x50 cm) covered with organza to prevent the entry of natural enemies and other unwanted organisms. An iron frame structure was used for the cages to prevent them from falling over. When the plants were 30 days old, 2 males and 2 females were released into each cage.

EPNs were applied at dusk to utilise the higher air humidity for the nematodes using a conventional airblast-sprayer at a rate of 50 IJs/cm^2. This application rate was calculated based on the ground surface area of the cage and recommended dose of a commercial company called e-nema. No adjuvants were added while spraying and no IJs were sprayed on control plants, only water was sprayed with the same volume as in nematode suspension on the tomato plants. Tomato plants remained wet in cages after application for a couple hours and that provides EPNs enough time with perfect condition to find and infect the target pest.

A zip was sewn up on the organza to control the leaves, fruits inside of the cages easily. Periodic observation of the cages allowed to control the damages of *T. absoluta* on tomato plants. The experiment was carried out with 2 replicates per nematode species and exposure day and repeated twice. Three plants were cut to determine the leaf miner mortality on each control days.

After releasing the adults of *T. absoluta*, EPNs were sprayed on tomato plants at the 7^{th}, 14^{th}, and 21^{st} days. Tomato plants were cut from the soil line at the 3^{rd}, 5^{th} 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} days after EPN applications and analysed to determine the mortality of *T. absoluta.* Dead *T. absoluta* larvae were immediately dissected and checked for nematode infection.

Statistical analysis

To evaluate the efficacy of EPNs against *T. absoluta,* larval percentage mortalities were Arcsine transformed before analysis (Anscombe transformation) (Zar, 1999). Factorial Design ANOVA was used to test significant differences among treatments. Afterwards a Tukey's multiple range test was performed to separate means. A level of significance of $P < 0.05$ was used. All statistical analyses were performed using Minitab 16 software version (Minitab Inc., State College, PA, USA).

Results

Efficacy of entomopathogenic nematodes in the first year

The efficacy of EPNs in field in 2012 varied between 0 and 90.7±1.5%. The least efficient day post treatment was found on the 3^{rd} and the most efficient day was found on the 15^{th}. After the emergence of *T. absoluta* adults, the lowest mortality occurred on the 7^{th} day and the highest mortality was observed on the 21^{st} day. The least efficient species was *S. affine* (isolate 46) and the most efficient species was *S. feltiae* (isolate 879) with the mortality of 39.3±1.5% and 90.7±1.5%, respectively (Table 1). The temperature and humidity was recorded from June to October and found between 21 and 26 ^{o}C and 57 and 65%, respectively in 2012.

Steinernema affine caused 0-39.3±1.5% mortality and found as the least efficient species. *S. carpocapsae* caused 0-43.7±1.5% mortality while *S. feltiae* caused 0-90.7±1.5% mortality. Among the

Steinernema species, *S. feltiae* was found to be the most efficient species. *H. bacteriophora* caused 0-81±3.5% mortality and was the second efficient species after *S. feltiae* against *T. absoluta* in tomato field in 2012. The differences between control days and EPNs application days were significant (*F*= 28.40; *df*= 12; *P*<0.000). The differences between control days and EPN isolates (*F*= 11.88; *df*= 18; *P*<0.000), EPN application days and EPN isolates (*F*= 63.65; *df*= 6; *P*<0.000), control days, EPN application days and EPN isolates were also found significant (*F*= 2.50; *df*= 36; *P*<0.000).

Table 1. Mortality of *Tuta absoluta* larvae caused by EPNs in field in 2012[a, b, c] Mean (%)±SE

Day	*Heterorhabditis bacteriophora* (isolate 1144)*			*Steinernema affine* (isolate 46)			*Steinernema carpocapsae* (isolate 1133)			*Steinernema feltiae* (isolate 879)		
	7**	14	21	7	14	21	7	14	21	7	14	21
3***	0±0 C f I	15.7±1.5 B e II	33.0±1.7 A d II	0±0 B c I	9.7 ±0.9 A c II	14.7±1.8 A c III	0±0 B e I	16.0±1.2 A d II	17.3±2.3 A d III	0±0 C e I	42.0±1.7 B e I	58.3±2.3 A e I
5	23.3±1.2 B e I	21.7±2.6 B e II	38.3±1.5 A d II	10.3±1.5 B b II	11.0±1.7 AB c III	17.0±2.3 A c III	9.3±0.9 B d II	19.7±2.3 A cd II	21.3±2.0 A cd III	14.3±2.0 C d II	54.3±2.3 B d I	65.7±1.8 A de I
7	28.7±2.3 B de I	33.7±2.6 B d II	44.7±2.3 A cd II	13.3±1.5 B ab II-III	20.7±1.8 A b III	19.7±1.2 AB bc III	11.0±1.7 B d III	21.3±1.8 A cd III	22.3±1.5 A cd III	20.3±2.0 B d II-III	66.7±2.3 A c I	70.3±3.5 A cd I
9	35.0±2.7 B cd I	41.0±2.3 B cd II	52.3±2.6 A c II	14.3±2.3 B ab II	23.0±2.3 A ab III	21.3±2.3 AB bc III	16.3±2.3 B cd II	29.3±1.8 A bc III	29.7±2.3 A bc III	36.3±2.6 B c I	77.7±2.0 A b I	77.3±3.5 A bc I
11	44.3±2.6 B bc I	48.3±2.6 B bc II	64.3±2.6 A b II	17.3±2.6 B ab II	24.0±2.3 AB ab III	28.3±2.6 A ab III	20.7±2.3 B bc II	32.0±1.7 A ab III	36.3±3.2 A ab III	49.7±2.6 B b I	81.3±4.1 A ab I	81.0±2.9 A b I
13	51.0±3.5 B b I	53.7±3.5 B b II	71.3±3.2 A ab II	20.0±2.1 B a II	31.7±3.2 A a III	34.3±2.3 A a III	28.0±2.7 B ab II	39.0±2.1 A ab III	41.0±2.1 A ab III	59.7±2.6 B b I	84.7±3.8 A ab I	85.7±3.2 A ab I
15	64.0±2.3 B a I	73.7±2.6 A a II	81.0±3.5 A a II	22.0±1.7 B a III	32.7±2.6 A a III	39.3±1.5 A a III	37.7±2.6 A a II	41.3±2.0 A a III	43.7±1.5 A a III	72.3±2.6 B a I	86.3±5.4 A a I	90.7±1.5 A a I

[a] The EPN isolate (*) means within column followed by the same capital letter for the control day are not statistically different by Tukey's multiple range test $P < 0.05$

[b] The EPNs application day (**) means within column followed by the same small letter for each EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$

[c] The control day (***) means in a row followed by the same roman numeral for the EPN application day and EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$

Efficacy of entomopathogenic nematodes in the second year

The efficacy of entomopathogenic nematodes in field in 2013 varied between 0-94.3±2.0%. Similar to the results obtained in 2012, the least efficient day was found as the 3rd and the most efficient day was found as the 15th. After the emergence of *T. absoluta* adults, the lowest mortality occurred on the 7th day and the highest mortality occurred on the 21st day. The least efficient species was *S. affine* (isolate 46) and the most efficient species was *S. feltiae* (isolate 879) with the mortality of 43.7±2.3% and 94.3±2.0%, respectively (Table 2). The temperature and humidity was recorded from June to October and found between 19.9-25.5 °C and 50.4-60.3%, respectively in 2013.

Steinernema affine caused from 0 to 43.7±2.3% mortality and was the least efficient species. *S. carpocapsae* caused from 0 to 49.3±2.4% mortality, whereas *S. feltiae* caused from 0 to 94.3±2.0% mortality. Among the *Steinernema* species, *S. feltiae* was the most efficient species. *H. bacteriophora* caused from 0 to 83.0±2.1% mortality and was the second efficient species after *S. feltiae* against *T. absoluta* in field in 2013. The differences between control days and EPNs application days were significant (*F*= 37.79; *df*= 12; *P*<0.000). The differences between control days and EPN isolates (*F*= 15.47; *df*= 18; *P*<0.000), EPN application days and EPN isolates (*F*= 78.35; *df*= 6; *P*<0.000), control days, EPN application days and EPN isolates were also found significant (*F*= 2.94; *df*= 36; *P*<0.000).

Table 2. Mortality of *Tuta absoluta* larvae caused by EPNs in field in 2013 [a, b, c] Mean (%)±SE

Day	*Heterorhabditis bacteriophora* (isolate 1144)*			*Steinernema affine* (isolate 46)			*Steinernema carpocapsae* (isolate 1133)			*Steinernema feltiae* (isolate 879)		
	7**	14	21	7	14	21	7	14	21	7	14	21
3***	0±0 C f I	17.3±1.5 B f II	34.3±2.3 A e II	0±0 B d I	12.3±0.9 A d II	16.3±1.5 A d III	0±0 B f I	18.0±1.7 A d II	20.3±2.0 A b III	0±0 C f I	48.0±4.0 B d I	61.0±2.3 A e I
5	21.0±2.3 B e I	25.3±2.0 B ef II	39.7±2.0 A de II	11.3±1.5 B c II	13.7±0.9 AB cd III	18.0±1.7 A d III	10.3±0.9 B e II	21.3±1.8 A d II	22.0±1.7 A b III	14.3±2.0 B e I-II	58.3±3.2 A d I	66.7±2.0 A de I
7	31.0±2.1 B d I	34.7±2.0 B de II	46.0±2.1 A cd II	15.3±2.0 A bc II-III	20.7±1.9 A bc III	21.7±1.5 A cd III	12.3±1.5 B de III	23.7±0.9 A cd III	23.3±2.0 A b III	21.7±1.5 B e II	70.3±3.8 A c I	74.3±3.2 A cd I
9	36.7±2.0 B cd I	43.7±2.0 B cd II	53.7±2.0 A c II	15.7±2.0 B bc II	23.0±1.2 A b III	24.7±2.0 A cd III	18.3±2.0 B cd II	31.3±2.0 A bc III	29.3±2.3 A b III	38.7±2.0 B d I	80.0±2.7 A b I	79.7±3.2 A bc I
11	46.0±2.3 B bc I	51.0±2.7 B bc II	65.7±2.6 A b II	19.0±2.1 B abc II	25.3±2.0 AB ab IV	30.3±1.5 A bc IV	22.0±1.2 B bc II	35.3±2.0 A ab III	41.3±2.0 A a III	51.7±2.6 B c I	84.3±2.6 A b I	82.7±3.5 A bc I
13	53.7±2.3 B b II	57.7±3.5 B b II	72.7±4.1 A b II	20.7±1.8 B ab IV	29.7±1.5 A ab IV	36.7±2.6 A ab III	31.7±2.0 B ab III	40.3±1.5 A ab III	44.7±2.3 A a III	63.3±1.5 B b I	86.0±3.8 A ab I	85.7±2.6 A b I
15	67.7±2.6 B a I	78.0±3.5 A a II	83.0±2.1 A a II	24.7±2.0 C a III	34.0±1.7 B a III	43.7±2.3 A a III	41.3±2.0 A a II	42.3±2.0 A a III	49.3±2.4 A a III	75.0±2.3 B a I	92.0±2.3 A a I	94.3±2.0 A a I

[a] The EPN isolate (*) means within column followed by the same capital letter for the control day are not statistically different by Tukey's multiple range test $P < 0.05$

[b] The EPNs application day (**) means within column followed by the same small letter for each EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$

[c] The control day (***) means in a row followed by the same roman numeral for the EPN application day and EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$

Discussion

Tuta absoluta is considered as one of the most important lepidopterous pests associated with tomato crops and because of its biology and behavior, it is a very challenging pest to control. At high densities and without adequate controls, infestations of *T. absoluta* can result in 90 to 100% loss of field-produced tomatoes by losing their commercial value (Estay, 2000; Vargas, 1970). Effective chemical control is difficult because *T. absoluta* feeds internally within the plant tissues. Also resistance to insecticides is another significant problem in chemical control of *T. absoluta* because of its high reproduction capacity, short generation cycle and intensive use of insecticides (Salazar & Araya, 1997, 2001; Siqueira et al., 2000, 2001). Additionally, the widespread use of pesticides disturbs populations of natural enemies and consequently reduces natural control of this pest. Due to these negative aspects of chemical insecticides other approaches need to be found for this pest.

Some insects may be controlled by a combination of practices that are not fully effective when used alone. *T. absoluta* is one of these insects that require more than one practice to be controlled successfully. Therefore, integrated pest management (IPM) programs are being developed in several countries to manage infestations of *T. absoluta*. EPN species belonging to the families Steinernematidae and Heterorhabditidae have been considered as potential control agents for leafminers in recent years (Olthof & Broadbent, 1990). EPNs can be applied, in combination with other biological and chemical pesticides, fertilizers and soil amendments and in the form of adjuvants or antidesiccants (Glazer & Navon, 1990; Baur et al., 1997). Progress in nematode commercialization during the 1990s was substantial. Development of large-scale production technology and easy-to-use formulations led to the expanded use of nematodes. These developments led to the use of nematodes against various insect species (Georgis et al., 2006).

Many studies on EPNs have been conducted throughout the world, but little research has been conducted on the efficacy of EPNs against tomato leaf miner. This is the first study conducted both in Çanakkale and in Turkey that focused on the efficacy of native EPNs against *T. absoluta* in a tomato field. EPNs most likely entered feeding canals in the leaves of tomatoes. Many larvae of *T. absoluta* died inside these galleries, which indicate that IJs were able to find and infect them.

In a similar study by Batalla-Carrera et al. (2010), the efficacy of the three nematode species after foliar application to potted tomato plants was evaluated under greenhouse conditions. They reported high larval mortality (78.6-100%) and low pupal mortality (<10%) in laboratory experiments. In the leaf bioassay a high level of larval parasitisation (77.1-91.7%) was recorded. In the pot experiments, they determined that nematode treatment reduced insect infestation of tomato plants by 87-95%. Their findings demonstrate the suitability of EPNs for controlling *T. absoluta*.

In another study, the efficacy of soil treatments of three native EPNs (*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*) against *T. absoluta* larvae, pupae and adults was determined under laboratory conditions (Garcia-del Pino et al., 2013). They also evaluated the effect of three insecticides commonly used against *T. absoluta*, in the survival, infectivity and reproduction of these nematode species. When the larvae dropped into the soil to pupate, soil application of nematodes resulted in a high mortality of larvae: 100%, 52.3% and 96.7% efficacy for *S. carpocapsae*, *S. feltiae* and *H. bacteriophora,* respectively. No mortality of pupae was observed and mortality of adults emerging from soil was 79.1% for *S. carpocapsae* and 0.5% for *S. feltiae*. They reported that the insecticides tested, had a negligible effect on nematode survival, infectivity and reproduction. They didn't observe any sublethal effects. Their results suggest that larvae of *T. absoluta*, falling from leaves following insecticide application, could be suitable hosts for nematodes, thereby increasing their concentration and persistence in the soil.

Kaya & Gaugler (1993) emphasized that there is a need for more in-depth basic information on EPNs biology, including ecology, behavior, and genetics, to help understand the underlying reasons for their successes and failures as biological control agents. Selecting the most appropriate nematode species and/or strain is important for efficacy and abiotic factors such as soil type, soil temperature and moisture. Proper match of the nematode to the host entails virulence, host finding, and ecological factors are essential before application to the field. There is little hope of success if a nematode does not possess a high level of virulence toward the target pest. In rare cases, persistence may compensate for moderate virulence (Shields et al., 1999). Matching the appropriate nematode host-seeking strategy with the pest is also essential. Poor host suitability has been the most common cause of failure in EPN applications (Gaugler, 1999). Furthermore, high virulence under laboratory conditions has often been inappropriately extrapolated to field efficacy (Georgis & Gaugler, 1991). Application strategies, including field dosage, volume, irrigation and appropriate application methods, are very important. Besides, crop morphology and phenology must be considered in predicting whether nematodes are viable control candidates (Georgis et al., 2006).

Our results clearly demonstrate that larvae of *T. absoluta* were highly susceptible to the EPNs tested and these EPNs can be used as efficient biological control agents against *T. absoluta*. All four EPNs tested showed efficacy at different rates against *T. absoluta*. EPNs were able to find and infect *T. absoluta* larvae both inside and outside of the tomato leaf. In conclusion, it could be suggested that EPNs have a great potential to use as biocontrol agents for the management of *T. absoluta*. Typical feeding galleries made by *T. absoluta* larvae provide EPNs an excellent environment to penetrate the pest easily and also avoid negative factors (desiccation, ultraviolet light, etc.). However, to control *T. absoluta* effectively, it is critical to combine all available control measures including cultural methods, other biological control agents, and the proper and judicious use of registered pesticides.

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