J. BIOL. ENVIRON. SCI.,
2007, 1(2), 67-71

# A Review on Entomopathogenic Nematodes in Turkey

**İ. Alper Susurluk***
Uludag University, Agriculture Faculty, Plant Protection Department, 16059 Görükle-Bursa, Turkey.

## ABSTRACT

**Entomopathogenic nematodes (EPNs) are considered as potential biological control agents against soil-borne insect pests in the world. There are also some studies on EPNs in Turkey. The purpose of this review is to clarify the current situation of EPNs in Turkey. Initial study on EPNs has started in 1995 in Turkey and several survey studies have been performed primarily on the extraction and identification of EPNs. Five steinernematid and two heterorhabditid species have been identified to date from Turkey. There have been also studies examining the activity of EPNs against several insect. Furthermore, ecological studies including host finding behaviour and reproductive potential of EPNs are also performed. In conclusion, the potential of EPNs in biological control should be evaluated, and policies of government on EPNs should be also regulated in agricultural strategies.**

**Key Words: Entomopathogenic nematodes, general review, Turkey**

## INTRODUCTION

Biological control had its scientific beginning in 1889 with the introduction of the vedalia beetle, *Rodolia cardinalis*, and a parasitic fly, *Cryptochaetum iceryae*, from Australia to control the cottony-cushion scale, *Icerya purchasi*, in California citrus groves (Ehler 1990; DeBach 1964). Since that time, arthropod predators and parasites, the primary natural enemies have been used against insect pests. In addition to microbial control agents (i.e. viruses, bacteria, fungi and protozoa), beneficial nematodes are also important in biological control of pests (Tanada and Kaya 1993).

The entomogenous nematodes belonging to the families, *Steinernematidae* and *Heterorhabditidae* are intensively infective for a wide range of insect hosts. The infective juveniles (IJs) of these families are successfully used for the control of soil-borne insect pests (Ehlers 1996). IJs enter the host larva, release their symbiont bacteria, *Xenorhabdus* or *Photorhabdus*, and kill the insect larvae within 36-48 hours (Poinar 1979; Griffin et al 1991; Brown and Gaugler 1997; Adams and Nguyen 2002). Following the two or three generations in the host haemocoel, they emerge from the cadaver and search for new hosts in the soil.

Entomopathogenic nematodes (EPNs) possess also many desirable characteristics of, such as searching ability of hosts, safety to non-target organisms and the potential to survive in the environment. EPNs show considerable promise especially in the soil environment (Akhurst and Bedding 1986). Additionally, these agents are produced commercially and used against a broad range of soil insect pests and insects in cryptic habitats worldwide. Glaser and Fox (1930) discovered a nematode parasitizing the Japanese beetle (*Popillia japonica*) in 1929. This is the first field trial and culturing of EPNs worldwide. From 1930s to date, lots of research was conducted, new species and strains were isolated. Today, many biological control studies by EPNs have been planned in a lot of country (Mracek 1980; Blackshaw 1988; Hominick and Briscoe 1990; Nguyen and Smart 1990; Ozer et al 1995; Miduturi et al 1996; Elawad et al 1999; Susurluk et al 2001; Hazir et al 2003b; Mracek et al 2003).

Turkey has very suitable climatic conditions for cultivation, resulting in a broad range of insect pests on plants. Farmers have difficulties with the control of these pests and need intensive control methods. In addition to chemical control strategies, biological control has also an important role in IPM (Integrated Pest Management). However, the roles and effects of microbial agents except *Bacillus thuringiensis* are not well-known in such control strategies. On the other hand, several institutes and universities just started several studies on this subject. However, these are in a start point and needs to be improved. Researches with the Turkish EPNs have only recently been initiated. In this review, a general overview of the studies and the current situation of EPNs research in Turkey are discussed.

***Nematode sampling and identifications***

Samples were collected from cultivation fields and several natural habitats in different regions of Turkey. Positive soil samples varied from 1 to 11.5% of the collected samples (Susurluk et al 2001; Hazir et al 2003b). These results indicated that Turkish fields were very suitable for establishment of EPNs. In all studies the nematodes were extracted according to the greater wax moths, *Galleria mellonella* L. (Lepidoptera: Galleridae), bait technique. Extracted EPNs were then stored at low temperatures (3-4ºC).

* Corresponding author: susurluk@uludag.edu.tr

Most of the isolated nematodes in Turkey were identified by molecular and cross-breeding methods using the described species at University of Arizona-USA and University of Kiel-Germany.

***Described Turkish nematodes***

In Turkey, 5 surveys have been conducted to date. Özer et al (1995) initially stated that they had recovered *Steinermema feltiae* from the coast of the Black Sea.

Susurluk et al (2001) found two *Heterorhabditis bacteriophora* isolates and one *S. feltiae* from natural areas in Ankara.

Kepenekci (2002) extracted and identified *S. carpocapsae* in a natural habitat of southern Turkey. This species was described by the using of morphological characters.

The most detailed survey was performed by Hazir et al (2003b). Hazir and co-workers collected 1080 soil samples, resulted in 22 positive sites. The isolated nematodes were identified as *H. bacteriophora, S. feltiae, S. affine* and a new *Steinernema* species. The new species was extracted from grassland in Kars (East Anatolia) and described as *Steinernema anatoliense* (Hazir et al 2003a). The most commonly found species was reported as *S. feltiae*, isolated from ten sites in six regions. On the other hand, *H. bacteriophora* was isolated from seven sites in five regions, while *S. affine* was isolated from four sites in two regions.

Susurluk and Toprak (2006) detected two steinernematids and one heterorhabditid species from 130 soil samples collected in Ankara according to the PCR-RFLP technique. While the *Steinernema* species were identified as *S. feltiae* and *S. carpocapsae,* the *Heterorhabditis* species was identified as *H. bacteriophora*.

Unlu et al (2007) isolated and identified *S. weiseri* by the using of cross-breeding and molecular method-PCR-RFLP in Ankara.

Distribution of the nematodes was shown in Figure 1 and the identified Turkish nematodes were summarized in Table 1.

***Ecological studies***

Susurluk et al (2001) performed an ecological characterization of 3 Turkish EPNs (2 heterorhabditis and 1 steinernematid isolates) isolated from the Experimental Field of Ankara University Agricultural Faculty Campus. Heat tolerance and the infectivity at different soil moistures of these isolates (TUR-H1 and TUR-H2 of *H. bacteriophora* and *S. feltiae* TUR-S3) were compared. Infection rate was found significantly higher for either isolate at 10% water-soil content. Moreover, *S. feltiae* invaded the host insects higher than that of two *Heterorhabditis* strains at 7% upwards water content. Additionally, the isolates were exposed to 28, 32 and 36 ºC in water suspension, in order to detect their heat tolerance. *S. feltiae* was reported as the most tolerant nematode at 32 ºC. However, no nematodes were able to survive at 36 ºC 4-5 hour post exposure.

Hazir et al (2003b) compared the development of 5 geographic isolates of *S. feltiae* at different temperatures. While one of the species was from Sinop (North Turkey), other species were from SN-France, Monterey-California, Rafaela-Argentina and MG-14-Hawaii. Isolates were exposed to 5, 8, 10, 15, 20, 25 and 28 ºC in greater wax moth, *G. mellonella* larvae. Mortality and progeny of the isolates were examined. All isolates caused 100% mortality of wax moth larvae and produced a progeny between 8-25 ºC. Mortality was found 100% at 28 ºC. However, no progeny was detected. The highest infective juvenile production was found at 15 ºC for all isolates. Emergence of infective juveniles from the host cadaver varied in different times. The Turkish Sinop isolate emerged earlier than the other species.

Oguzoglu and Ozer (2003) compared the emergence time of *H. bacteriophora* TUR-H2 (isolated in Ankara) and a *S. feltiae* (isolated in Rize) from *G. mellonella* cadavers. The results of the experiment revealed that the isolates emerged from the hosts in 6-9 days at 25 ºC. In opposite, Poinar (1979) and Woust (1984) reported that steinernematids emerge earlier than heterorhabditids at the same temperatures. On the other hand, reproductive potentials of the *H. bacteriophora* and *S. feltiae* were also found approximately 141,600 and 13,800 IJS/cadaver, respectively. The reproductive potential is based on exposing *G. mellonella* larvae that weighing 200 mg to 100 infective juveniles. In competition studies between the two species, their results indicated that the first species inoculated was responsible for the highest mortality rates.

Susurluk et al (2003) examined the host finding behaviours of two different *H. bacteriophora* isolates (TUR-H1 and TUR-H2) isolated in Ankara. Both species revealed a significant movement towards *G. mellonella* larvae. 0.26, 3.20, 52.38, 12.52, 8.20 and 3.73%, of the IJs of TUR-H2 moved towards the *G. mellonella* larvae at 24, 48, 72, 96, 118 and 148 h at 25 ºC, respectively. However, 0.52, 3.28, 28.16, 4.34, 3.90 and 1.82% of the IJs of TUR-H1 moved towards the *G. mellonella* larvae at the same time intervals, respectively. An interesting result of this study was that although these nematodes belong to the same species, they appeared to have different foraging strategies on the same host.

**Table 1**. EPN species described in Turkey.

| Nematodes | Isolation regions | Authors |
|---|---|---|
| *Steinernema feltiae* | Ankara, Kirsehir, Eskisehir (Central Anatolia), Rize and Sinop (North of Turkey), Burdur (South of Turkey), Van (East of Turkey), Canakkale (West of Turkey). | Özer et al (1995); Susurluk et al (2001); Hazir et al (2003b) |
| *S. anatoliense* | Kars (East Anatolia) | Hazir et al (2003a) |
| *S. carpocapsae* | Antalya and Icel (South of Turkey) | Kepenekci (2002) |
| *S. affine* | Icel, Adana (South of Turkey), Mardin (Southeast Anatolia), Tokat (North of Turkey), Tekirdag, Kirklareli (Northwestern Turkey) | Hazir et al (2003b) |
| *S. weiseri* | Ankara (Central Anatolia) | Unlu et al (2007) |
| *Heterorhabditis bacteriophora* | Ankara, Aksaray (Central Anatolia), Istanbul, Kirklareli, Tekirdag (Northwestern Turkey), Kayseri (Central Anatolia) | Susurluk et al (2001); Hazir et al (2003b) |
| *H. megidis* | Eastern Black Sea Region | Yılmaz et al (2007) |


**Figure 1.** Distribution of Entomopathogenic Nematodes in Turkey.

Aydin and Susurluk (2005) examined the competitive abilities of *S. feltiae* and *H. bacteriophora* in meal worm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) at different temperatures. Mortality rates of host larvae due to EPNs infections were reported at separate or mixed inoculations. Experiments were conducted in sterile-sand at 12, 18 and 25 ºC for 5 days. According to the results, efficiency of the *H. bacteriophora* was found 52.5, 93.5 and 97.5% at 12, 18 and 25 ºC, respectively. While, efficiency of *S. feltiae* was found 85, 95.6 and 97.5% at the same temperatures, respectively. In mixed infections, these rates were 3 and 72% at 12 ºC; 8 and 85% at 18 ºC and 12.5 and 80% at 25 ºC for *H. bacteriophora* and *S. feltiae*, respectively.

Susurluk (2008) studied that the vertical movement of Turkish isolates of *Steinernema feltiae* (TUR-S3) and *Heterorhabditis bacteriophora* (TUR-H2) was compared at different temperatures in the presence and absence of larvae of the host insect, *G. mellonella*. The nematodes that had migrated different distances were compared for their infectivity to *G. mellonella* and the positive correlations between distance travelled, and infectivity indicate that there is a link between host searching behaviour and infection behaviour in *S. feltiae* and, to a lesser extent, also in *H. bacteriophora*.

***Efficiency studies***

The current information on the effectiveness of EPNs is limited studies in our country. The effects of two Turkish *S. feltiae* strains (isolates from Rize and Ankara) were examined on mealy plum aphid, *Hyalopterus pruni*, in the first one, and on Cherry Fruit Fly, *Rhagoletis cerasi* and Mediterranean Fruit Fly, *Ceratitis capitata*, in the second study. Both experiments were conducted at 10, 15 and 25 ºC and the following doses were used: 25, 50 and 100 IJs/larva. The highest mortalities of *H. pruni* at 25ºC were 83.3 and 74.9% for Ankara and Rize isolates, respectively. Mortalities varied from 38.8 to 83.3%. *S. feltiae* from Ankara was found more effective than the *S. feltiae* from Rize (Kepenekci and Susurluk 2006).

In the other study, *R. cerasi* and *C. capitata* pupae were used. Mortality rates were very lower than that reported in the first study mentioned above. As a result of this, pup stage could not be useful for EPN penetration. Mortalities varied from 0 to 40% at these experiments. *S. feltiae* from Ankara was found more active than the one from Rize also in this study. Both efficiency studies indicated that ecologic variability could play an important role in the infectivity abilities of EPNs (Kepenekci and Susurluk 2006).

Susurluk (2006) examined that reproduction capacities and effectiveness of *S. weiseri* and *S. feltiae* isolated from Turkey were compared in variable conditions. The reproduction capacities were examined at the following temperatures; 10, 15 and 20 ºC and the doses of 10, 50 and 100 IJs in the last instar larvae of greater wax moth, *G. mellonella*. The results showed that *S. weiseri* was more productive than *S. feltiae* in all experimental conditions. Effectiveness of the both species on the last instar *G. mellonella* larvae as was calculated 48 h after penetration. According to the results, *S. weiseri* was more productive than *S. feltiae* in all experimental conditions, and *S. weiseri* was more effective ($LD_{50}$= 25.68 IJs) than *S. feltiae* ($LD_{50}$= 80.70 IJs).

Susurluk (2006) investigated that the efficiency of *S. feltiae* TUR-S3 and *H. bacteriophora* TUR-H2, against larvae of *T. molitor* L. was studied in different soil type and temperature conditions. Sterilized and non-sterilized silver sand, clay-loam soil, and compost soil were tested, each at 12, 18, and 24 °C. Temperature had the greatest effect on the mortality of *T. molitor* larvae caused by both nematode species. The efficiency of the 2 nematodes was greater in sterile than in nonsterile conditions, and was greater in sandy soils than in clay soils. The results showed that *S. feltiae* was more efficient than *H. bacteriophora* at all temperatures tested, especially at 12 °C.

Unlu et al (2007) studied that the efficacy of *S. weiseri* (BEY) against last instars of the black cutworm *Agrotis segetum* Denis & Schiffermüller (Lepidoptera: Noctuidae) was compared with *S. feltiae* (TUR-S3) and *S. carpocapsae* isolated in Turkey (Kepenekci 2002). Each nematode species was applied at 10, 25, 50 and 100 IJS per larvae of the pest in 10 ml water. The results showed that *S. weiseri* (BEY) was more effective than *S. feltiae* (TUR-S3) at doses of 50 and 100 IJS per larva but was less effective than *S. carpocapsae.*

## CONCLUSIONS

Researches on EPNs in Turkey have been just initiated. Species found in Turkey could provide an important potential for biological control strategies. Therefore, on their ecology, pathogenicity and efficiency studies should be described in Turkey. Especially, field-application of these isolates should be tried in selected pilot regions. Growers should be informed about the EPNs, their application, advantages and disadvantages. In addition to scientific studies, regulatory policies of the government should also aim at supporting the further introduction of EPNs based products into biological control practices.

The interplay between environment and economic forces in agriculture is creating a tremendous demand for products that are effective and economical, while at the same time safe and compatible with the environment. EPNs are ideally suited to fit this need. After the required coordination in government, EPNs having a great potential for insect biological control should be improved and adapted with IPM strategies in Turkey.

## REFERENCES

Adams B.J., and Nguyen K. B. (2002). Taxonomy and systemtics. In: Entomopathogenic Nematology, (Eds.: R. Gaugler). CABI Publishing, Oxon, UK. Pp.1–33.
Akhurst R.J., and Bedding R.A. (1986). Natural occurance of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. J Austral Entomologi Soci 25: 241–244.
Aydin H., and Susurluk A. (2005). Competitive Abilities of Entomopathogenic Nematodes, Steinernema feltiae and Heterorhabditis bacteriophora in the Same Host at Different Temperatures. Turk J Biol 29: 35-39.
Blackshaw R.P. (1988). A survey of insect parasitic nematodes in Northern Ireland. Ann Appl Biol 113: 561-565.
Brown I., and Gaugler R. (1997). Temperature and humidity influence emergence and survival of entomopathogenic nematodes. Nematologi 43: 363–375.
DeBach P. (1964). Biological Control of Insect Pests and Weeds. Rheinhold Publishing Corp. New York.
Ehler L.E. (1990). Some contemporary issues in biological control of insects and their relevance to the use of entomopathogenic nematodes. In: Entomopathogenic Nematodes in Biological Control. (Eds.: Gaugler, R. and Kaya, H. K.). CRC Boca Raton, FL; Pp: 1-19.
Ehlers R.U. (1996). Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontr Sci and Technol 6: 303-316.
Elawad S.A., Gowen S.R., and Hague N.G.M. (1999). The life cycle of Steinernema abbasi and S. riobrave in Galleria mellonella. Nematol 1: 762- 764.
Glaser R.W., and Fox. H. (1930). A nematode parasitic of the Japanese beetle (Popillia japonica Newman). Sci 71:16-17.
Griffin C.T., Moore J.F., and Downes M.J. (1991). Occurence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in The Republic of Ireland Nematologi 37: 92-100.
Hazir S., Keskin N., Stock S.P., and Kaya H. (2003a). A new entomopathogenic nematode, Steinernema anatoliense n. sp. (Rhabditida: Steinernematidae), from Turkey. System Parasitol 55: 211–220.
Hazır S., Stock S.P., and Keskin N. (2003b). Diversity and distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey. Biodiver. Conserv 12: 375–386.
Hominick W.M., and Briscoe B.R. (1990). Survey of 15 sites over 28 months for entomopathogenic nematodes (Rhabditida: Steinernematidae). Parasitol 100: 289–294.
Kepenekci I. (2002). Entomopathogenic nematodes (Rhabditida) in the Mediterranean Region of Turkey. Nematologia Mediter 30: 13–16.
Kepenekci I., and Susurluk A. (2006). Infectivity of Two Turkish Isolates of Steinernema feltiae (Rhabditida: Steinernematidae) Against Rhagoletis cerasi and Ceratitis capitata. Nematologia Mediter 34: 95-97.
Kepenekci I., and Susurluk A. (2006). Susceptibility of The Mealy Plum Aphid, Hyalopterus pruni (Geoffroy) (Homoptera: Aphididae) of Two Isolates of Steinernema feltiae (Rhabditida: Steinernematidae) in Laboratory Studies. Pak J Nematol 24: 49–55.
Miduturi J.S. Moens M., Hominick W.M., Briscow B.R., and Reid A.P. (1996). Naturally occuring entomopathogenic nematodes in the province of West-Flanders, Belgium. J Helminthol 70: 319-327.
Mracek Z. (1980). The use of Galleria traps for obtaining nematode parasites of insects in Czechoslovakia (Lepidoptera: Nematoda, Steinernematidae). Acta entomologica bohemoslovaka 77: 378- 382.
Mracek Z. Sturhan D., and Reid A. (2003). Steinernema weiseri n. sp. (Rhabditida, Steinernematidae), a new entomopathogenic nematode from Europe. Syst. Parasitol 56: 37- 47.
Nguyen K.B., and Smart G.C. (1990). Steinernema scapterisci n. sp. (Rhabditida: Steinernematidae). J Nematol 22: 187- 199.
Oguzoglu Unlu I., and Ozer N. (2003). Evaluation of the reproductive potential and competition between two entomopathogenic nematodes, Steinernema feltiae Filipjev, 1934 (Rhabditida: Steinernematidae) and Heterorhabditis bacteriophora, Poinar 1976 (Rhabditida: Heterorhabditidae). Turk J Biol 27: 149-155.
Ozer N. Keskin N., and Kirbas Z. (1995). Occurrence of entomopathogenic nematodes (Steinernematidae: Heterorhabditidae) in Turkey. Nematologi 41: 639–640.
Poinar G.O. Jr. (1979). Nematodes for Biological Control of Insects. CRC Press. Boca Raton, FL.
Susurluk I. A. (2008). Influence of temperature on the vertical movement of the entomopathogenic nematodes, Steinernema feltiae (TUR-S3) and Heterorhabditis bacteriophora (TUR-H2) and infectivity of the moving nematodes. Nematology, in press.
Susurluk I. A. (2006). Comparison of Some Biological Characterizations of the Entomopathogenic Nematodes, Steinernema weiseri and S. feltiae (Rhabditida: Steinernematidae), Isolated in Turkey. Turk J of Agricultural Science 12: (4), 340-344.
Susurluk A. (2006). Effectiveness of the Entomopathogenic Nematodes, Heterorhabditis bacteriophora and Steinernema feltiae against Tenebrio molitor (Yellow Mealworm) Larvae at Different Temperature and Soil Types. Turk J of Biol 30 (4): 199-205.
Susurluk A.. Dix I., Stackebrandt E., Strauch O., Wyss U., and Ehlers R.U. (2001). Identification and ecological characterization of three entomopathogenic nematode-bacterium complexes from Turkey. Nematol 3: 833–841.
Susurluk, I. A., and Toprak U. (2006). Molecular Identification of Three Entomopathogenic Nematodes from Turkey by PCR-RFLP of the ITS Regions. Phytoparasit 34 (1): 17-20
Susurluk I.A., Ünlü I., and Kepenekci I. (2003). Host finding behaviour of two different Turkish isolates of entomopathogenic nematode species, Heterorhabditis bacteriophora. Poinar 1976 (Rhabditida: Heterorhabditidae). Turk. J. Biol. 27, 203–207.
Tanada Y., and Kaya H.K. (1993). Insect Pathology. Academic Press. San Diego CA.
Unlu I., Ehlers R.-U., and Susurluk A. (2007). Additional data and first record of the entomopathogenic nematode Steinernema weiseri from Turkey. Nematol 9 (5): 739-741.
Woust W.M. (1984). Nematode parasites of lepidopterans. In: Plant and Insects Nematodes, (Eds.: R. W. Nickle), Marcel Deckler, Inc. New York; Pp: 655-696.
Yılmaz H., Waeyenberge L., Demirbağ Z., and Moens M. (2007). First record of Heterorhabditis megidis (Rhabditida: Heterorhabditidae) from Turkey. 59. International Crop Protection Symposium, May 22, 2007, Gent, Belgium.