TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

Occurrence and Distribution of Entomopathogenic Nematodes (*Steinernematidae* and *Heterorhabditidae*) in Ordu Province, Turkey

Ömer ERTÜRK[a]* Fikret USTAOĞLU[b] Faruk AKYAZI[c]
[a]Department of Biology, Faculty of Scienses, University of Ordu, Turkey
[b]Department of Biology, Faculty of Scienses, University of Giresun, Turkey
[c]Department of Plant Protection, Faculty of Agriculture, University of Ordu, Turkey

*Corresponding author: oseerturk@hotmail.com

Received: 07.07.2014 Received in Revised Form: 28.08.2014 Accepted: 30.08.2014

Abstract

During 2009-2010, a survey was conducted to investigate the presence of entomopathogenic nematodes (EPNs) and to characterize the species in Ordu province, Turkey. In total, 156 soil samples were collected randomly from different cultivated and non-cultivated areas. Soil samples were tested for the presence of Steinernematid and Heterorhabditid nematodes by baiting with *Galleria mellonella* larvae. From the total of 156 soil samples, 8 (5.1%) samples tested were found positive for the presence of EPNs, containing the genera *Steinernema* and *Heterorhabditis*. Morphological characterization and sequence analysis of the ITS regions of ribosomal DNA allowed the identification of EPN isolates. Four *Steinernema* spp. were recovered including *S. feltiae*, *S. carpocapsae*, *S. kraussei* and one new undescribed species. Among the Steinernematids, *S. feltiae* was found the most common species. *Heterorhabditis bacteriophora* was the only Heterorhabditid recovered during this survey. *H. bacteriophora* was only found in a corn field while *Steinernema* species were present in hazelnut orchard, forest, potato field, rangeland and wheat field.

Keywords: *Heterorhabditis bacteriophora*, ITS, *Steinernema feltiae*, *Steinernema carpocapsae, Steinernema kraussei*

Ordu ilinde Entomopatojenik Nematodlar (*Steinernematidae* and *Heterorhabditidae*)'ın Varlığı ve Dağılımları

Özet

Ordu ilinde entomopatojenik nematodların (EPNs) varlığını ve bulunan türlerin özelliklerini ortaya çıkarmak için 2009-2010 yıllarında bir sürvey yapılmıştır. Farklı ürünlerin yetiştirildiği alanlardan ve kültür yapılmayan alanlardan rastgele toplam 156 toprak örneği toplanmıştır. Toprak örneklerindeki Steinernematid ve Heterorhabditid nematodların varlığı *Galleria mellonella* larvalarının kullanıldığı tuzak böcek yöntemi ile tespit edilmiştir. Toplam incelenen 156 örnekten 8 (%5.1) tanesi *Steinernema* ve *Heterorhabditis* cinslerine ait entomopatojen nematodlar yönünden pozitif olarak bulunmuştur. EPN izolatlarının teşhisi morfolojik karekterler ve ribozomal DNA'nın ITS bölgesinin sekans analizi yapılarak sağlanmıştır. *Steinernema feltiae, Steinernema carpocapsae, Steinernema kraussei* ve bir tane teşhis edilemeyen 4 tür bulunmuştur. Steinernematid nematodlar arasında en yaygın tür *Steinernema feltiae* bulunmuştur. Yapılan surveyde Heterorhabditid tür olarak sadece *Heterorhabditis bacteriophora* tespit edilmiştir. *Steinernema* türleri fındık bahçelerinde, ormanlarda, patates alanlarında, meralarda ve buğday alanlarında bulunabilirken *Heterorhabditis bacteriophora* yalnızca mısır yetiştirilen alanlarda bulunmuştur.

Anahtar kelimeler: *Heterorhabditis bacteriophora, ITS, Steinernema feltiae, Steinernema carpocapsae, Steinernema kraussei*

Introduction

Entomopathogenic nematodes (EPNs) (genera *Heterorhabditis* and *Steinernema*) are excellent biocontrol agents for a wide range of insect pests (Grewal *et al*., 2005; Georgis *et al*., 2006; Ansari *et al*., 2009). They offer a benign alternative to chemical insecticides, mainly because of their ability to locate insects in cryptic habitats, their high reproductive ability, the simplicity of mass producing them, and their safety to humans and other vertebrates (Gaugler, 2007).

Currently, over 90 species of EPN have been described worldwide belonging to *Steinernema* and *Heterorhabditis* genera. Recently, these nematodes were successfully used as biological control agents of noxious insect species in agriculture, forestry etc. At least one dozen of the EPN species have been commercialized for use as biological control agents (Shapiro-Ilan *et al*., 2014). Therefore, there is a great scientific interest in understanding the nematode natural incidence. Several studies have been published concerning their distribution in America (Mracek and Webster, 1993), Australia (Akhurts and Bedding, 1986) and Europe. Europen entomopathogenic nematode mapping projects have provided a lot of data on nematode distribution mainly in western and northern Europe (Ehlers *et al*., 1991, Hominick and Briscoe, 1990; Steiner, 1996; Vanninen *et al*., 1989; Haukeland, 1993). Recently, these nematodes also have been found in Turkey. Seven species of *Steinernema* including *Steinernema affine*, *S. carpocapsae*, *S. feltiae*, *S. weiseri*, *S. anatoliense*, *S. websteri, S. krausse* and three *Heterorhabditis* species including *Heterorhabditis bacteriophora*, *H. marelata* and *H. megidis* (Özer *et al*., 1995; Kepenekçi *et al*., 1999; Kepenekçi and Susurluk, 2000, 2003; Susurluk *et al*., 2001, 2003; Kepenekçi, 2002; Hazır *et al*., 2003a, b; Ünlü *et al*., 2007, Yılmaz *et al*., 2009; Gökce *et al*., 2013; Erbaş *et al*., 2014) were identified in Turkey. A few EPN surveys reported the presence of these nematodes, but until now, not much has been known about the distribution of Heterorhabditids and Steinernematids in Ordu.

The objective of this study was to survey entomopathogenic nematodes in Ordu Provinces of Turkey, to identify the species present and to distinguish their ecosystem, habitat and soil type preferences.

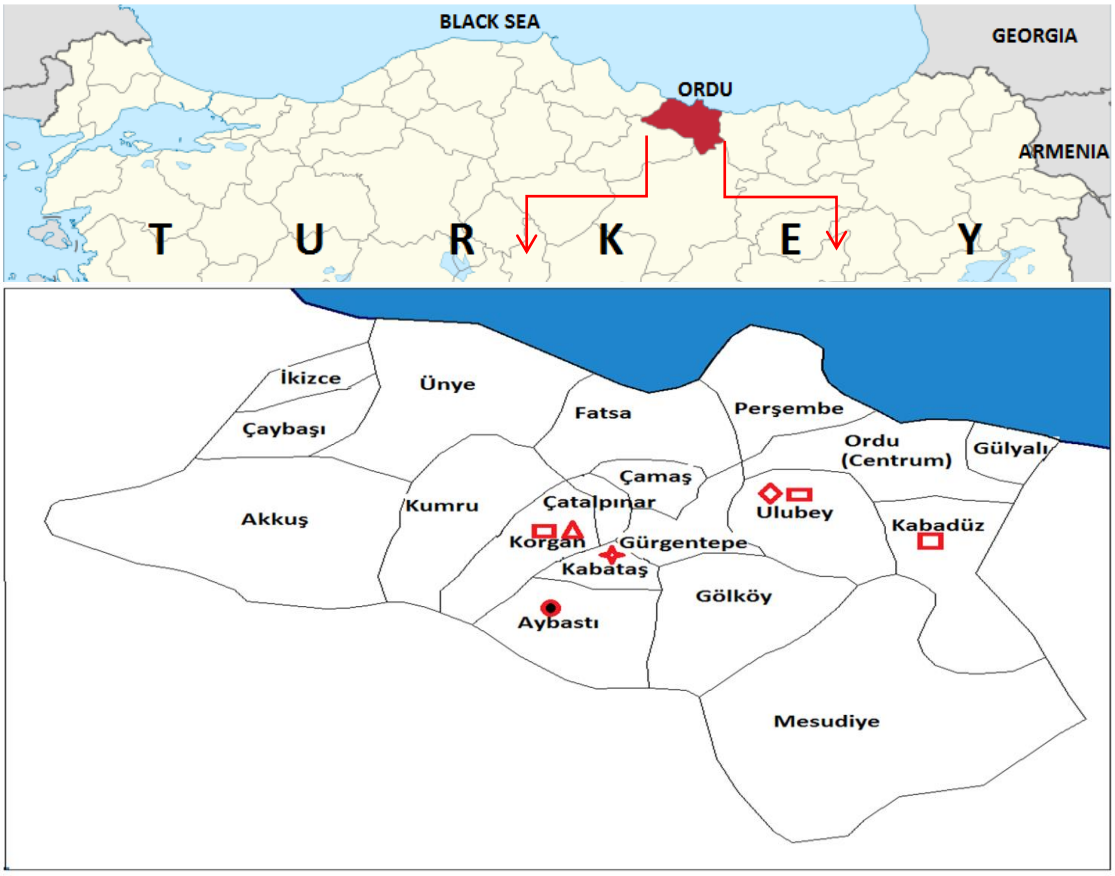

Figure 1. Occurance and distribution of entomopathogenic nematodes in Ordu province. Δ *Steinernema carpocapsae*, □ *Steinernemafeltiae,* ⊙ *Steinernema kraussei,* ◊ *Steinernema* sp., ⋆ *Heterorhabditis bacteriophora.*

Table 1. Environmental and soil characteristics of positive samples for EPN species

Isolates	Species	Locations	Vegetation	Soil temp.	pH	Elevation (m)	Sampling date
52-25	*S. feltiae*	Ulubey	Hazelnut	19	6,58	850	09.06.2009
52-26	*Steinernema sp.*	Ulubey	Forest	18	6,35	1080	09.06.2009
52-100	*S. feltiae*	Korgan	Wheat field	16	7,03	1265	07.08.2009
52-150	*S. feltiae*	Korgan	Rangeland	12	6,46	1300	10.09.2009
52-153	*S. feltiae*	Kabadüz	Rangeland	10	6,50	1737	18.04.2010
52-118	*S. carpocapsae*	Korgan	Potato field	15	6,96	1228	07.08.2009
52-126	*S. kraussei*	Aybastı	Rangeland	13	6,35	1530	10.09.2009
52-60	*H. bacteriophora*	Kabataş	Cornfield	21	7,61	479	02.07.2009

Table 2. Sequence similarity of 8 isolates identified as known species with previously published sequences of other populations of the same species

Isolates		GenBank Code	Overlapping base number	Similarity Rate(%)
52-25	*S. feltiae* (SCM isolate)	JF728857.1	685/693	(99)
	S. feltiae (SNC isolate)	JF728856.1	685/693	(99)
	S. feltiae (B30 Slovenia isolate)	EU914855.1	685/693	(99)
52-26	*S. feltiae* (Z8 isolate)	JN886631.1	693/732	(95)
	S. feltiae (32A isolate)	JN886598.1	679/718	(95)
	S. feltiae (93E isolate)	JN886609.1	673/712	(95)
52-100	*S. feltiae* (SCM isolate)	JF728857.1	798/803	(99)
	S. feltiae (SNC isolate)	JF728856.1	798/803	(99)
	S. feltiae (SSp60 isolate)	JF728859.1	798/802	(99)
52-118	*S. carpocapsae* (Az20 isolate)	GQ421607.1	696/696	(100)
	S. carpocapsae (Az143isolate)	GQ421608.1	696/696	(100)
	S. carpocapsae (IRA18 isolate)	EU598239.1	696/696	(100)
52-126	*S. kraussei* (Westfalia isolate)	AY230175.1	687/690	(99)
	S. kraussei (C46 isolate)	EU914856.1	686/690	(99)
	S. kraussei (Russian isolate)	AY171264.1	686/690	(99)
52-150	*S. feltiae* (SCM isolate)	JF728857.1	682/699	(98)
	S. feltiae (SNC isolate)	JF728856.1	682/699	(98)
	S. feltiae (B30 isolate)	UE914855.1	679/696	(98)
52-153	*S. feltiae* (SSp60 isolate)	JF728859.1	673/677	(99)
	S. feltiae (IRAZ 22)	FJ860040.1	673/677	(99)
	S. feltiae (SCM)	JF728857.1	673/678	(99)
52-60	*H. bacteriophora* (N-Arg isolate)	HQ225906.1	702/706	(99)
	H. bacteriophora (N-Riwaka isolate)	HQ225892.1	702/706	(99)
	H. bacteriophora (N-RDS109 isolate)	HQ225889.1	702/706	(99)

Table 3. Comporative morphometrics (in µm) of male of *Steinernema sp., Steinernema carpocapsae, Steinernema kraussei, Heterorhabditis bacteriophora*

Character	*Steinernema* sp.	*Steinernema carpocapsae*	Steinernema kraussei	*Heterorhabditis bacteriophora*	*Steinernema carpocapsae*	*Steinernema kraussei*	*Heterorhabditis bacteriophora*
	Isolate 52-26	Isolate 52-118	Isolate 52-126	Isolate 52-60	(Adam & Nguyen, 2002)	(Adam & Nguyen, 2002)	(Adam & Nguyen, 2002)
	n=15	n=15	n=15	n=15			
	male	male	male	male	Male	male	male
	Mean±SD (min-max)	Mean ±SD (min-max)	Mean±SD (min-max)	Mean±SD (min-max)	Mean±SD (min-max)	Mean±SD (min-max)	Mean±SD (min-max)
L	**1494.6±146.8** (1278.4-1667.0)	**1604.3±157.7** (1389.6-1812.5)	**1434.7±94.0** (1288.7-1545.8)	**855.8±47.6** (777.2-915.0)	-	-	-
W	**93.8±9.9** (80.2-107.4)	**128.1±13.2** (105.5-144.2)	**123.5±14.2** (102.0-140.4)	**48.2±4.8** (41.2-54.7)	**101** (77–130)	**128** (110–144)	**43** (38–46)
EP	**85.5±5.5** (77.3-92.5)	**71.0±7.8** (59.9-81.4)	**86.6±15.2** (65.4-107.7)	**97.8±2.7** (93.4-101.0)	-	-	-
NR	**105.0±6.2** (96.8-113.7)	**119.6±6.3** (111.3-128.0)	**108.2±13.8** (88.6-125.3)	**72.2±4.3** (65.6-77.2)	-	-	-
ES	**130.3±7.3** (118.5-138.7)	**159.2±7.4** (148.8-169.2)	**152.8±16.0** (128.4-170.1)	**105.3±3.2** (100.6-109.8)	-	-	-
T	**35.3±4.2** (29.0-40.4)	**27.1±4.1** (21.2-32.8)	**36.9±2.5** (33.1-40.1)	**33.0±2.0** (30.1-5.7)	-	-	-
ABW	**43.4±6.7** (34.7-52.6)	**48.2±5.1** (40.4-55.3)	**34.7±5.9** (26.5-43.2)	**16.8±1.5** (14.7-8.8)	-	-	-
a	**15.9±0.3** (15.5-16.6)	**12.9±0.9** (10.7-13.2)	**11.7±0.6** (11.0-12.6)	**17.8±0.8** (16.7-18.9)	-	-	-
b	**11.4±0.5** (10.8-12.0)	**10.1±0.5** (9.3-10.7)	**9.4±0.4** (9.0-10.0)	**8.1±0.2** (7.7-8.3)	-	-	-
c	**42.4±1.1** (41.0-44.1)	**59.6±3.4** (55.3-65.5)	**38.9±0.4** (38.3-39.4)	**26.0±0.3** (25.6-26.5)	-	-	-
D%	**65.6±0.8** (64.8-66.8)	**44.5±2.9** (40.3-48.1)	**56.3±4.3** (50.9-63.3)	**92.9±0.5** (92.0-93.5)	**41** (27–55)	**53**	**117**
E%	**243.2±14.2** (229.0-266.6)	**263.2±11.2** (248.2-282.5)	**233.0±25.5** (197.6-269.3)	**297.1±9.9** (282.9-310.3)	-	-	-
SL	**71.3±6.7** (62.8-80.6)	**68.9±4.2** (63.0-75.1)	**51.1±4.5** (44.3-57.8)	**43.7±2.8** (40.0-47.7)	**66** (58–77)	**55** (52–57)	**40** (36–44)
GL	**40.8±5.8** (33.0-49.2)	**46.0±3.2** (41.4-50.6)	**30.1±3.1** (26.0-34.2)	**18.3±1.2** (16.6-19.9)	**47** (39–55)	**33** (23–38)	**20** (18–25)

L: Body length **W:** Greatest diameter. **EP:** Anterior end to Excretory pore **NR:** Anterior end to Nerve ring **ES:** Pharynx length **T:**Tail length **ABW:**Anal body width **a:**L/W**. b:** L/ES **c:** L/T **D%:** EP/ES*100 **E%:** EP/T **x** 100. **SL:** Spicule length **GL:** Gubernaculum length Min: Minimum Max: Maksimum **SD:** Standart deviation.

Material and Methods

Soil sampling

A total of 156 soil samples were collected from 18 districts of Ordu during the period of 2009-2010. Site location, sampling date, elevation and associated vegetation were also recorded. In all cases, each soil sample (1.0 kg) was a composite of 8 random sub-samples taken distantly located from each other in an area of 10 m^2 and to a depth of 0-20 cm. Samples were taken with a hand shovel, placed in polyethylene bags to prevent water loss, and kept in coolers during transport to the laboratory.

Nematode isolation

Each soil sample was gently shaken so the particles were fairly uniform and friable and then 200 g of soil was transferred to 500 ml plastic containers (11 x 10 x 6 cm) and EPNs were isolated using the insect baiting method (Bedding and Akhurst, 1975). Five last-instar *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae were placed in each plastic container filled with the moistened soil obtained from each sample. Containers were covered with a lid, turned upside down and incubated for 15 days in the dark at 23± 1 ˚C. Water was added to the samples if they appeared dry at any point during the baiting. *Galleria mellonella* larvae were checked every three days and dead larvae were replaced with fresh ones. Dead *Galleria* larvae were collected and thoroughly rinsed in distilled water and placed in modified White traps (Kaya and Stock, 1997). All emerging nematodes were collected from single dead larvae and considered as one isolate. After that, each nematode isolate was cultured on *G*. *mellonella* larvae to produce nematodes for identification and establishment of cultures. These nematodes were stored at 10 ˚C in tissue culture flasks containing distilled water.

Morphometric characters of isolates

Ten *G*. *mellonella* larvae were exposed to about 300 infective juveniles (IJs) in a 9-cm-diameter petri dish lined with two moistened filter papers (Whatman No. 1). For *Heterorhabditis* sp., the first generation males were obtained by dissecting infected insects at four days and seven days, respectively, after the insects died. Third stage IJs were obtained during the first two days after emerging from insect cadavers.

For light microscope observations, fifteen individuals of IJs, and males were examined alive. Additional specimens of the different stages were killed and fixed in lactophenol. These nematodes were used when more observations were needed to confirm the morphology or variation of some structures. Fifteen IJs and males of each isolate were observed, and measured. Measurements were made using a drawing tube attached to an Olympus BX50 light microscope. The following characters were measured from males and IJs: Body length (L), greatest body diameter (W), distance from anterior end to excretory pore (EP), distance from anterior end to nerve ring (NR), distance from anterior end to end of pharynx (ES), tail length (T), anal body width (ABW),*a* (body length/greatest body diameter), *b* (body length/tail length), *c* (body length/ES (distance from anterior end to end of pharynx)), D% (EP/ES*100), E% (EP/T*100), spicule length, gubernaculum length. Microsoft Excel was used for analysis of the morphometrics variables of the males and IJs.

DNA extraction

Genomic DNA was extracted from a single female using a modification of method of Joyce *et al*. (1994). A single nematode female of each isolate was transferred into an eppendorf tube to which 10 µL of worm lysis buffer (500 mM of KCl, 100 mM of Tris-Cl [pH 8.3], 15 mM of MgCl2, 10 mM of DTT, 4.5% Tween 20, and 0.1% gelatin) and 2 µL of proteinase K (600 µg mL^{-1}) were added. The tubes were incubated at 80 ˚C 10 min. and then at 65 ˚C for 1 h and 95 ˚C for 10 min.The tubes were kept -20˚Cafter centrifugation (13,000 ×g for 1 min).

PCR amplification conditions

The entire internal transcribed spacer region (ITS) was PCR amplified using the primers 18S (5'-TTGATTACGTCCCTGCCCTTT-3' (forward) and 28S: 5'-TTTCACCGCCGTTACTAAGG-3' (reverse). In the PCR reaction for amplification, DNA suspension (5 µL) was added to a PCR reaction mixture that contained 5 µL of 10X PCR buffer, 2 µL of MgCl2 (25 mM), 1 µL of dNTP mixture (10 mM each), 0.3 µL (500 mM) of each primer, 1.5 U of *Taq* DNA polymerase, and 36 µL of double distilled water, to a final volume of 50 µL.All PCR reactions were conducted in a Thermocycler, PTC-100 (Biorad). The initial temperature was 94 ˚C for 6 minutes followed by 35 cycles of 94 ˚C for 1 minute, 55 ˚C for 2minute and 72 ˚C for 2minutes. The last step was 72 ˚C for 10 minutes. A portion (5 µl) of the amplification product was loaded on a 1% agarose gel containing0.5 µg/ml ethidium bromide. For direct sequencing, PCR products were purified with the Promega Wizard SV Gel and PCR Clean-UP System kit and sequenced at Macrogen (Korea) company. Sequences results were aligned by using Chromas v.1.45 and then compared with those in Genbank by means of a BLAST search.

Table 4. Comporative morphometrics (in µm) of infective-stage juveniles of *Steinernema* sp., *Steinernema carpocapsae*, *Steinernema kraussei*, *Heterorhabditis bacteriophora*

Character	*Steinernema* sp.	*Steinernema carpocapsae*	*Steinernema kraussei*	*Heterorhabditis bacteriophora*	*Steinernema carpocapsae*	*Steinernema kraussei*	*Heterorhabditis bacteriophora*
	Isolate 52-26	Isolate 52-118	*Isolate 52-126*	Isolate 52-60	(Adam and Nguyen, 2002)	(Adam and Nguyen, 2002)	(Adam and Nguyen, 2002)
	n=15 J2 Mean±SD (min-max)	n=15 J2 Mean ±SD (min-max)	n=15 J2 Mean±SD (min-max)	n=15 J2 Mean±SD (min-max)	J2 Mean±SD (min-max)	J2 Mean±SD (min-max)	J2 Mean±SD (min-max)
L	**845,4±58,0** 768,3-922,8	**557.8±42.5** (501.4-614.0)	**930.4±68.4** (821.0-1005.6)	**574.2±26.8** (534.6-609.8)	**558** (438–650)	**951** (797–1102)	**588** (512–671)
W	**27,1±2,8** 23,7-31,0	**26.8±3.4** (21.7-31.2)	**33.0±4.2** (27.7-38.6)	**25.3±2.1** (22.2-28.1)	**25** (20–30)	**33** (30–36)	**23** (18–31)
EP	**56,3±3,5** 51,1-61,0	**38.1±5.3** (30.5-45.1)	**56.4±3.1** (52.1-60.8)	**96.7±4.3** (90.3-102.0)	**38** (30–60)	**63** (50–66)	**103** (87–110)
NR	**91,7±7,6** 81,0-102,3	**84.4±6.7** (75.0-93.4)	**98.4±4.5** (92.6-105.5)	**86.3±3.9** (80.4-91.6)	**85** (76–99)	**105** (99–111)	**85** (72–93)
ES	**126,6±10,5** 110,0-138,0	**130.7±8.9** (117.3-141.8)	**127.5±8.4** (115.7-138.2)	**123.5±2.1** (120.5-126.0)	**120** (103–190)	**134** (119–145)	**125** (100–139)
T	**66,6±5,1** 59,6-73,2	**53.5±5.9** (45.6-62.0)	**76.4±6.7** (66.0-84.0)	**86.5±6.2** (78.8-95.1)	**53** (46–61)	**79** (63–86)	**98** (83–112)
ABW	**15,6±1,2** 13,8-17,0	**15.0±1.8** (12.2-17.3)	**18.5±1.0** (17.1-19.8)	**13.7±1.0** (12.3-15.1)	-	-	-
a	**31,3±1,1** 29,8-32,4	**20.9±1.2** (19.7-23.1)	**28.4±1.6** (26.1-29.9)	**22.7±0.9** (21.7-24.1)	**21** (19–24)	**29**	**25** (17–30)
b	**6,7±0,1** 6,6-7,0	**4.3±0.1** (4.2-4.3)	**7.3±0.1** (7.1-7.4)	**4.6± 0.1** (4.4-4.8)	**4.4** (4.0–4.8)	**7.1**	**4.5** (4.0–5.1)
c	**12,7±0,2** 12,5-13,0	**10.5±0.4** (9.9-11.0)	**12.2±0.2** (12.0-12.4)	**6.6±0.2** (6.4-6.9)	**10** (9.1–11.2)	**12.1**	**6.2** (5.5–7.0)
D%	**44,5±1,1** 43,3-46,5	**29.1±2.1** (26.0-31.8)	**44.3±0.8** (43.3-45.5)	**78.2±2.2** (74.9-81.0)	**26** (23–28)	**47**	**84** (76–92)
E%	**84,6±1,6** 82,6-87,2	**71.1±2.5** (66.9-74.3)	**74.1±2.9** (71.2-78.9)	**111.9±3.1** (107.3-115.6)	**60** (54–66)	**80**	**112** (103–130)

L: Body length. **W:** Greatest diameter. **EP:** Anterior end to Excretory pore. **NR:** Anterior end to Nerve ring. **ES:** Pharynx length. **T:** Tail length. **ABW:** Anal body width. **a:**L/W.**b:** L/ES.**c:** L/T. **D%:** EP/ES*100. **E%:** EP/T **x** 100. **SL:** Spicule length. **GL:** Gubernaculum length. Min: Minimum. Max: Maximum**. SD:** Standard deviation

Results and Discussion

The current study aimed to understand the presence of entomopathogenic nematodes in Ordu province and its vicinity during the period of 2009-2010. One hundred and fifty six soil samples obtained from 18 districts of Ordu were examined for entomopathogenic nematodes and 8 different isolates characterized. A total of 40 samples came from rangelands, with 3 (13.3%) samples testing positive for EPN's; 34 samples from forests, with 1(2.9%) testing positive; 32 samples from hazelnut orchards, with 1 (3.1%) testing positive; 27 samples from agricultural fields, with 3 (7.4%) testing positive. From all of the 156 samples tested, 5.1% were positive for EPNs. In the studies previously conducted in Turkey, entomopathogenic nematodes have been recovered as 4.72% (Özer *et al*., 1995), 2% (Hazır *et al*., 2003a) , 12.1% (Aydin, 2007) and 6.1% (Güneş, 2008). Those results of the authors overlap with our finding (5.1%). Some other investigations in worldwide achieved on entomopathogenic nematodes reported different recovery rates.

As a consequence of morphometric values and ITS region gene sequence of rRNA *S. feltiae* (4), *Steinernema* sp*., S. kraussei, S. carpocapsae, H. bacteriophora* species were identified from five different zones of Ordu (Figure 1). The morphometric and morphological examination of IJs and males of the isolates compared the original descriptions of the respective species (Tables 3, 4 and 5). All morphological and morphometric characters were in line with descriptions by (Nguyen and Smart, 1995; Hominick *et al*., 1997). A BLAST search of Gen Bank revealed that Ordu isolates were identical from different geographical regions. Sequence similarities of 8 isolates were identified as known species with previously published sequences of other populations of the same species varied between 95-100% (Table 2).

Steinernema species were present in hazelnut orchard, forest, potato field, rangeland and wheat field while *H. bacteriophora* was only found in the cornfield. Environmental factors such as pH and temperature can be considered as the important factors limiting EPN infectivity. For pH, we isolated entomopathogenic nematodes from slightly acidic (pH 6.3) to slightly alkaline (pH 7.9) soils (Table 1). Our study agrees with other studies where the pH of entomopathogenic nematode positive soil samples varied from 4.6 to 8 (Hara *et al*., 1991; Griffin *et al*., 1994). Soil temperatures ranged from 10 °C to 21 °C (Table 1). *Steinernema feltiae* can be infective from 2-30°C, whereas some heterorhabditids can infect host insects from 7 to 35°C and *Steinernema carpocapsae* is nearly inactive at 10°C (Kaya, 1990; Georgis *et al*., 2006; Lacey *et al*., 2006).

In this study, *S. feltiae* is found as the most common species in the research area. Out of 8 *Steinernema* isolates, 4 of this were detected as *S. feltiae* (Isolate 52-25; 52-100; 52-150; 52-153). In Turkey, the most common EPN species isolated has been *S. Feltiae* (Erbaş *et al*., 2014). The first identified entomopathogenic nematode from Turkey was also *S. feltiae* (Özer *et al*., 1995). Ten of 17 *Steinernema* isolates from all over Turkey were identified as *S. feltiae* by (Hazır *et al*., 2003c). In Aydın province of Turkey, out of 3 *Steinernema* isolates, 2 of this were detected as *S. feltiae* (Aydın, 2007) and in Marmara region of Turkey, 7 of 13 *Steinernema* isolates were detected as *S. feltiae* (Güneş, 2008). These results are the evidence of that *S. feltiae* is the most widespread EPN in Turkey. *Steinernema feltiae* is also considered the most widespread species in worlwide. It is distributed in almost every region of the world from Hawaii to the warm regions of Europe. This species was detected from the tropical regions to regions of cold climates. The possible cause of this, *S. feltiae* is an ancient species which was present before continents began breaking up and drifting or such a wide global distribution suggests that *S. feltiae* is an efficient disperser (Hominick *et al*., 1996). But it is mostly adapted to the cold climate regions and generally is a species occurring in off-shore locations (Wright, 1992; Hominick *et al*., 1995).

The isolate 52-26 obtained from spruce (ladin) forests in Ulubey district at an altitude of 1080 meters was supposed to be a new *Steinernema* species according to morphological and molecular data of isolate. Either this isolate showed the morphological similarities with *S. feltiae* or sequencing of rRNA ITS region showed the sequence similarity of 95% with other *S. feltiae.* This percentage of the similarity is considerably low. Therefore, it was suggested that the species might be a new species among the *S. feltiae* group. It will be studied to elucidate if this isolate is a new species or not according to sequences analysis of mitochondrial genes and the *D2-D3* expansion segments of the *28S rRNA* region using scanning electron microscopy and cross-breeding testing. The other *Steinernema* species isolate 52-118, isolated from potato field in Korgan district at an altitude of 1228 meters is *S. carpocapsae*. This species was firstly isolated from Mediterranean, Black-Sea and the Marmara regions of Turkey (Kepenekçi, 2002; Yilmaz, 2008; Güneş, 2008). Although *S. carpocapsae* was isolated from many countries such as Austria, Czech Republic, France, Germany, Great Britain, Italy, Norway, Poland,

Portugal, Slovakia, Spain, Sweden, Brazil, Mexico, Canada, USA, China, Taiwan, Australia. Natural distribution of *S. carpocapsae* varies from grasslands (Campbell *et al*., 1998) to desert habitats (Glazer *et al*., 1993). The reason of that might be the wide host range of the nematode. As a matter of fact, it was reported that *S. carpocapsae* can infect more than 250 insect species belonging to different orders (Poinar, 1979).

The other isolate, 52-126, with *S. kraussei* in this study was obtained from grassland in Aybastı district at an altitude of 1530 meters. The ITS region length of the this isolate was determined as 688 base pairs (bp). Sequence similarity of the isolate was found similar 99% with *S.kraussei* by BLAST search in all GenBank sequences (Table 2). The ITS Sequence of the isolate showed only 3 bases difference from those of referenced *S. kraussei* isolates. Spiridonov *et al*. (2004) showed that differences between the sequences of the ITS regions of *S. kraussei* isolates were generally between 1-11 bp (1%). But in some cases, the differences between the sequences can rise to 21 bp (2.8%). The reason of this might be some nucleotide changes in the secondary structure of RNA. Morphological data obtained from this isolate showed that it is more similar with the *S. kraussei* isolated from Europe. Stock *et al*. (2000) reported the morphological differences between populations of *S. kraussei* are the effect of geographical differences. Although the populations detected from Europe did not have important morphological differences, they found notable morphological differences between populations of Europe and North America.

This species was isolated before in Trabzon province of Turkey by Gokce *et al*. (2010). In Several studies conducted previously, this species was rarely found in open areas or alpines' (Steiner, 1996; Shishinov *et al*., 1998). The normal habitat of *S. kraussei* was pointed out as coniferous or mixed forests in many studies (Spiridonov and Moens,1999; Stock*et al*., 2000; Hominick, 2002; Mracek*et al*., 2005). *Steinernema kraussei* was first isolated from the body cavity of *Cephaleia abietis* (Hymenoptera) in Germany and was the first EPN (Steiner, 1923). This species was then isolated from different locations in Germany (Mracek, 1994). However, *S. kraussei* was also isolated from different countries such as Austria (Peters, 1996), Belgium (Spiridonov and Moens, 1999), Czech Republic (Mracek, 1977), the Netherlands (Hominick *et al*., 1995), Slovakia (Sturhan and Liskova, 1999), Switzerland (Steiner, 1996), UK (Hominick *et al*., 1995) and Spain (Garcia del Pino and Palomo, 1996). The other studies also showed the presence of the specie in America (Stock *et al*., 1999), Canada (Mracek and Webster, 1993) and Japan (Yoshida, 2003).

The isolate 52-60 obtained from the cornfield under sandy soil texture located on the riparian zone in Kabataş district was *H. bacteriophora*. Analysis of the entire ITS rDNA region composed of the partial 18S, ITS1, 5.8S, ITS2 and partial 28S was characterized by a sequence length of 705 base pairs (bp).This species was the most encountered EPN species in different geographical regions of Turkey (Susurluk *et al*., 2001; Kepenekçi, 2002; Hazır *et al*., 2003c; Güneş, 2008). *Heterorhabditis bacteriophora* was the species have been recovered by now from generally warm and cold regions, especially locations near the shore.

The obtained new isolates have contributed to Turkey's biological diversity. Some problems caused by harmful soil-dwelling insects e.g. *Melolontha melolontha* (Coleoptera, Scarabaeidae), *Polyphylla* spp. (Coleoptera, Scarabaeidae), *G. gryllotalpa* (Orthoptera, Gryllotalpidae) have been reported in the region. These pests are associated with many crops in the region including potatoes, strawberries, kiwi, various vegetables, fruits and hazelnut. Because of the natural structure of the soil, it is also natural barrier against applied insecticides. Therefore, it is very difficult to control the mentioned pests with the pesticides. However, soil is the natural habitat for soil entomopathogenic nematodes, so no barrier is an issue for them. Additionally, entomopathogenic nematodes do not cause any negative results on human health and environment as in the use of chemical insecticide. In the context of this study, nematodes isolated from the regions that have adapted to the environment over a period of millions of years would increase the success rate of biological control. For these reasons, the use of entomopathogenic nematode isolates obtained in studied regions against the economically significant pests in Ordu may be possible as an effective biological control agents in the future. The entomopathogenic nematodes species found in the study which are to serve as a successful microbial control agents are considered to be of possibilities against pests in the region.

This investigation showed the distribution and diversity of enthomopathogenic nematodes in Ordu province. The newly found isolates have contributed to Turkey's biological diversity. Some problems caused by harmful soil-dwelling insects e.g. *M. melolontha* (Coleoptera, Scarabaeidae), *Polyphylla* spp. (Coleoptera, Scarabaeidae), *G. gryllotalpa* (Orthoptera, Gryllotalpidae) have been reported in Ordu province.

Table 5. Comporative morphometrics (in µm) of infective-stage juveniles and male of Steinernema feltiae

Character	**Isolate 52-25**		**Isolate 52-100**		**Isolate 52-150**		**Isolate 52-153**		*Steinernema feltiae*	
	Steinernema feltiae		*Steinernema feltiae*		*Steinernema feltiae*		*Steinernema feltiae*		(Adam and Nguyen, 2002)	
	n=15 J2 Mean±SD (min-max)	n=15 Male Mean±SD (min-max)	n=15 J2 Mean ±SD (min-max)	n=15 Male Mean±SD (min-max)	n=15 J2 Mean±SD (min-max)	n=15 Male Mean±SD (min-max)	n=15 J2 Mean±SD (min-max)	n=15 Male Mean±SD (min-max)	J2 Mean±SD (min-max)	Male Mean±SD (min-max)
L	**859.6±38.5** (808.0-902.0)	**1673.5±225.2** (1355.0-1992.6)	**778.0±95.5** (669.0-878.0)	**1355.7±159.2** (1122.4-1558.6)	**793.7±114.1** (616.5-921.7)	**1531.1±155.1** (1308.7-1732.8	**840.3±37.7** (789.2-891.0)	**1351.7±171.2** (1040.3-1675.5)	**849** (736–950)	-
W	**27.1±2.8** (23.2-31.0)	**84.2±9.0** (72.7-98.9)	**28.0±3.4** (24.6-32.0)	**106.4±12.3** (89.2-122.3)	**34.6±6.9** (24.5-43.2)	**105.5±11.8** (88.3-121.3)	**30.2±7.0** (22.2-40.4)	**122.0±20.3** (92.3-147.6)	**26** (22–29)	**75** (60–90)
EP	**55.7±3.4** (51.5-60.3)	**89.7±7.5** (77.7-100.2)	**55.7±1.6** (53.5-57.4)	**89.3±9.3** (75.6-102.3)	**57.4±4.9** (50.2-64.5)	**93.7±10.3** (79.1-107.0)	**53.0±10.8** (39.1-68.0)	**113.1±21.7** (80.6-139.9)	**62** (53–67)	-
NR	**97.5±8.3** (87.4-110.6)	**113.5±9.2** (100.1-125.4)	**94.5±5.2** (88.4-101.3)	**124.1±7.0** (114.2-133.2)	**95.7±6.1** (87.2-104.6)	**125.8±8.4** (112.0-135.9)	**98.4±10.4** (83.4-112.5)	**130.2±30.8** (84.8-169.4)	**99** (88–112)	-
ES	**129.4±7.3** (117.4-138.5)	**148.1±10.2** (133.0-161.0)	**125.0±10.1** (111.7-135.3)	**148.6±5.3** (140.0-155.9)	**136.2±11.5** (119.3-150.3)	**158.7±7.3** (147.8-168.4)	**134.5±8.9** (121.0-145.7)	**164.1±28.3** (123.5-202.1)	**136** (115–150)	-
T	**78.5±3.3** (75.0-83.4)	**42.9±6.0** (33.2-49.6)	**71.1±8.6** (62.4-80.5)	**33.7±5.6** (25.1-40.3)	**72.1±9.8** (60.4-86.3)	**36.6±5.7** (28.6-44.5)	**60.5±8.2** (49.3-71.5)	**37.8±9.9** (23.3-49.0)	81 (70–92)	-
ABW	**15.7±1.3** (14.0-17.9)	**52.0±5.0** (44.4-57.6)	**16.1±1.8** (13.0-18.2)	**47.0±6.2** (38.2-56.2)	**17.7±2.1** (14.4-20.3)	**44.8±3.9** (39.4-50.3)	**18.1±1.8** (15.5-20.6)	**45.1±6.4** (35.5-53.7)	-	-
a	**31.9±1.9** (29.1-34.8)	**19.8±0.8** (18.6-21.0)	**27.8±0.5** (27.2-28.4)	**12.7±0.1** (12.6-12.8)	**23.1±1.4** (21.3-25.2)	**14.5±0.2** (14.3-14.8)	**28.8±5.2** (22.1-35.5)	**11.1±0.3** (10.5-11.3)	**31** (29–33)	-
b	**6.6±0.1** (6.5-6.9)	**11.3±0.8** (10.2-12.4)	**6.2±0.1** (6.0-6.5)	**9.1±0.8** (8.0-10.0)	**5.8±0.4** (5.2-6.1)	**9.6±0.5** (8.9-10.3)	**6.3±0.1** (6.1-6.5)	**8.2±0.2** (7.9-8.4)	**6** (5.3–6.4)	-
c	**11.0±0.3** (10.5-11.3	**39.1±1.3** (37.4-40.8)	**10.9±0.2** (10.7-11.3)	**40.5±2.3** (38.3-44.7)	**11.0±0.5** (10.2-11.7)	**42.2±2.4** (38.9-45.8)	**14.0±1.3** (12.5-16.0)	**36.7±4.6** (31.4-44.6)	**10.4** (9.2–12.6)	-
D%	**43.0±0.7** (42.3-43.9)	**60.4±1.7** (58.4-62.5)	**44.2±1.3** (42.4-47.9)	**60.1±4.0** (54.0-65.6)	**42.1±0.4** (41.6-42.9)	**58.9±3.8** (53.5-63.5)	**39.1±5.5** (32.3-46.7)	**68.7±2.1** (65.3-71.7)	**45** (42–51)	**60**
E%	**71.0±2.1** (68.7-73.0)	**210.6±12.4** (197.6-234.0	**78.8±6.7** (71.3-85.7)	**267.5±19.7** (248.3-301.2)	**80.1±4.2** (74.7-84.5)	**257.8±12.4** (240.4-276.6)	**86.8±6.1** (79.2-95.1)	**304.6±25.4** (276.8-345.9)	**78** (69–86)	-
SL		**78.7±6.0** (70.6-88.0)		**69.4±5.8** (61.2-77.4)		**69.0±5.1** (61.3-76.5)		**71.4±7.5** (60.2-79.8)		**70** (65–77)
GL		**39.7±3.7** (34.8-45.4)		**51.1±4.6** (44.1-57.2)		**40.7±5.0** (33.1-47.1)		**38.4±4.5** (31.5-44.2)		**41** (34–47)

L: Body length. **W:** Greatest diameter. **EP:** Anterior end to Excretory pore. **NR:** Anterior end to Nerve ring.**ES:** Pharynx length. **T:**Tail length. **ABW:**Anal body width. **a:**L/W.**b:** L/ES. **c:** L/T.**D%:** EP/ES*100.**E%:** EP/T **x** 100. **SL:** Spicule length.**GL:** Gubernaculum length. Min: Minimum. Max: Maximum**. SD:** Standard deviation

These pests are associated with many crops in the region including potatoes, strawberries, kiwi, various vegetables, fruits and hazelnut. Because of the natural structure of the soil, it is also natural barrier against applied insecticides. Therefore, it is very difficult to control the mentioned pests with the pesticides. However, soil is the natural habitat for soil entomopathogenic nematodes, so no barrier is an issue for them. In addition to those, entomopathogenic nematodes don't cause any known negative results on human health and environment as in the use of chemical insecticide. In the context of this study, nematodes isolated from the regions that have adapted to the environment over a period of millions of years would increase the success rate of biological control. For these reasons, the use of entomopathogenic nematode isolates obtained in studied regions against the economically significant pests in Ordu may be possible as effective biological control agents in the future.

References

Akhurst R.J. and Bedding, R.A., 1986. Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. *Journal of the Australian Entomological Society* 25: 241-244.

Ansari, M.A., Evans, M. and Butt, T.M., 2009. Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. *Crop Protection*28: 269-22.

Aydin, M.S., 2007. Entomopatojen nematodların (Steinernematidae ve Heterorhabditidae) Aydın ili ve çevresindeki topraklarda tür çesitliliği ve dağılımlarının belirlenmesi, Yüksek Lisans Tezi. Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü, Aydın, 55 s.

Bedding, R.A. and Akhurst, R.J., 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110.

Campbell, J.F., Orza, G., Yoder, F., Lewis, E. and Gaugler, R., 1998. Spatial and temporal distribution of endemic and released entomopathogenic nematode populations in turf grass. *Entomologia Experimentalis et Applicata* 86:1-11.

Ehlers, R.U., Deseo, K.V., Stackebrandt, E., 1991. Identification of *Steinernema* spp. (Nematoda) and theirsymbiotic bacteria *Xenorhabdus* spp. from Italian and German soils. *Nematologica* 37: 360-364.

Erbaş, Z.,Gökçe, C., Hazır, S., Demirbağ, Z. And Demir, İ., 2014. Isolation and identification of entomopathogenic nematodes (Nematoda: Rhabditida) from the Eastern Black Sea region and their biocontrol potential against *Melolontha melolontha* (Coleoptera: Scarabaeidae) larvae. *Turkish Journal of Agriculture and Forestry* 38: 187-197.

Garcia Del Pino F. And Palomo, A., 1996. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soils. *Journal of Invertebrate Pathology* 68:84–90.

Gaugler, R., 2007. Entomopathogenic Nematology. CABI Publishing. pp. 388.Wallingford, UK

Georgis, R., Koppenhofer, A.M., Lacey, L.A., Belair, G., Duncan, L.W., Grewal, P.S., Samish, M., Tan, L., Torr, P. and Van Tol, R.W.H.M., 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control* 38: 103-123.

Glazer, I., Liran, N., Poinar, G.O. and Smits, P.H., 1993. Identification and biological activity of newly isolated heterorhabditid populations from Israel. Fun. Appl. Nematol. 16: 467-472.

Gokce, C., Yilmaz, H., Erbas, Z., Demirbag, Z. and Demir, I., 2013. First Record of *Steinernema kraussei* (Rhabditida: Steinernematidae) from Turkey and Its Virulence against *Agrotis segetum* (Lepidoptera: Noctuidae). *Journal of Nematology* 45: 253–259.

Gökçe, C., Yilmaz, H., Demir, I. and Demirbağ, Z., 2010. A survey study on entomopathogenic nematodes in East Black Sea Region of Turkey, 43th Annual Meeting of the Society for Invertebrate Pathology, Karadeniz Technical University, 11-15 July 2010, Trabzon, p. 60

Griffin, C.T., Finnegan, M.M., Downes, M.J., 1994. Environmental tolerances and the dispersal of *Heterorhabditis* survival and infectivity of Europen *Heterorhabditis* following prolonged immersion in seawater. *Fundamental Applied Nematology,* 17, 415-421.

Grewal, P.S., Ehlers, R.U. and Shapiro-Ilan D.I. (Eds) 2005. Nematodes as biocontrol agents. CAB International, New York.

Güneş, C., 2008. Marmara Bölgesi'ndeki Entomopatojen Nematod Faunasının Belirlenmesi. Yüksek lisans Tezi, Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü, Çanakkale, 95 s

Hara, A.H., Gaugler, R., Kaya, H.K. and Lebeck, L.M., 1991. Natural populations of entomopathogenic Nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) from

Hawaiian Islands. *Environmental Entomology* 20: 211-216.

Haukeland, S., 1993. Entomopathogenic nematodes found in Norway. *Norwegian Journal of Agricultural Sciences* 7: 17-27.

Hazir, S., Stock, S.P. and Keskin, N., 2003a. A new entomopathogenic nematode, *Steinernema anatoliense* n. sp. (Rhabditida: Steinernematidae), from Turkey. *Systematic Parasitology* 55: 211-220.

Hazir, S., Keskin, N., Stock, P.S., Kaya, H.K. and Özcan, S., 2003b. Diversity and distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey. *Biodiversity and Conservation* 12: 375-386.

Hominick, W.M. and Briscoe, B.R., 1990. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soil. *Parasitology* 100: 295-302.

Hominick, W.M., 2002. Biogeography. Pp. 115-143. In: *Entomopathogenic Nematology* (R.GauglerEd.). CABI Publishing, Wallingford, UK.

Hominick, W.M.,Reid, A.P. and Briscoe, B.R., 1995. Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes isolated during soil surveys of the UK and The Netherlands. *Journal of Helminthology* 69: 27–32.

Hominick, W.M., Reid, A.P., Boham, A.P., Briscoe, B.R., 1996. Entomopathogenic nematodes: Biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Science and Technology. 6:317–331.

Hominick, W.M., Briscoe, B.R., Del-Pino, F.G., Heng, J., Hunt, D.J., Kozodoy, E., Mracek, Z., Nguyen, K.B., Reid, A.P., Spiridonov, S., Stock, P., Sturhan, D., Waturu, C. andYoshida, M., 1997. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *Journal of Helminthology* 71: 271-298.

Joyce, S.A., Reid, A., Driver, F. and Curran, J., 1994. Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. In: *Biotechnology: Genetics of entomopathogenic nematode bacteria complexes.*(A.M., Burnell, R.U. Ehlers,and J.P. Masson (Eds.).pp. 178-187. DG XII, Europen Commission, Luxembourg.

Kaya, H.K., 1990. Soil ecology. *In: Entomopathogenic nematodes in biological control.* (R., Gaugler, H.K. Kaya, Eds.).pp. 93–115. CRC Press, Boca Raton.

Kaya, H.K. and Stock, S.P., 1997. Techniques in insect nematology. In: *Manual of techniques in insect pathology* (Lacey, L.A. Ed.). pp. 281-324. Academic Press, London

Kepenekçi, I., Babaroğlu, N.E., Öztürk, G. and Halici, S., 1999. A new entomopathogenic nematode; *Heterorhabditis bacteriophora* Poinar, 1976: (Rhabditida: Heterorhabditidae) for Turkey. *Turkish Journal of Entomology* 9: 587-596.

Kepenekçi, I., Susurluk, I.A., 2000. A new entomopathogenic nematode species for Turkey; *Heterorhabditis marelatus* Luiand Berry, 1996 (Rhabditida: Heterorhabditidae). *Journal of Agricultural Sciences* 6: 59-64.

Kepenekçi, I., 2002. Entomopathogenic nematodes (Rhabditida) in the Mediterranean region of Turkey. *Nematologia Mediterranea* 30: 13-15.

Kepenekçi, I. and Susurluk, I.A., 2003. Three entomopathogenic nematodes (Rhabditida) from Turkey. *Pakistan Journal of Nematology* 21:19-23.

Lacey, L.A., Arthurs, S.P., Unruh, T.R., Headrick, H. and Fritts, R.Jr., 2006. Entomopathogenic nematodes for control of codling moth (Lepidoptera: Tortricidae) in apple and pear orchards: effect of nematode species and seasonal temperatures, adjuvant, application equipment and post-application irrigation. *Biological Control* 37:214–223.

Mracek, Z. and Webster, J.M., 1993. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada. *Journal of Nematology* 25: 710-717.

Mracek, Z., 1977. *Steinernema kraussei*, a parasite of the body cavity of the sawfly, *Cephaleia abietis*, in Czechoslovakia. *Journal of Invertebrate Pathology* 30:87-94.

Mracek, Z., 1994. *Steinernema kraussei* (Steiner, 1923) (Nematoda: Rhabditida: Steinernematidae): Redescription of its topotype from West phalia. *Folia Parasitologica* 41: 59–64.

Mracek, Z., Becvar, S., Kindlmann, P. and Jersakova, J., 2005. Habitat preference for entomopathogenic nematodes, their insect hosts and new faunistic records for the Czech Republic. *Biological Control* 34:27–37.

Mracek, Z. and Webster, J.M., 1993. Survey of Heterorhabditidae and Steinernematidae (Rhabditida: Nematoda) in western Canada. *Journal of Nematology* 25:710-717.

Nguyen, K.B. and Smart, Jr. G.C., 1995. Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida). *Journal of Nematology* 27: 206-212.

Özer, N., Keskin, N., Kirbaş, Z., 1995. Occurrence of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Turkey. *Nematologica* 41: 639-640.

Peters, A., 1996. The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Scienceand Technology* 6: 389-402.

Poinar, G.O. Jr., 1979. Nematodes for biological control of insects. pp. 277, CRS Press. Florida.

Shapiro-Ilan, D.I., Han, R. and Qiu, X., 2014. Production of entomopathogenic nematodes. *In: Mass production of beneficial organisms Invertebrates and entomopathogens* (J.Morales-Ramos, G. Rojas, and D.I. Shapiro-Ilan, Eds).pp. 321-356. Academic Press. San Diego, CA.

Spiridonov, S.E.,Reid, A.P., Podrucka, K., Subbotin, S.A. and Moens, M., 2004. Phylogenetic relationships within the genus Steinernema (Nematoda: Rhabditida) as inferred from analyses of sequences of the ITS1-5.8S-ITS2 region of rDNA and morphological features. *Nematology* 6: 547-566.

Spiridonov, S.E. and Moens, M., 1999. Two previously unreported species of steinernematids from woodlands in Belgium. *Russian Journal of Nematology*7:39-42.

Stock, S.P., Pryor, B.M. and Kaya, H.K., 1999. Distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in natural habitats in California. *Biodiversity and Conservation* 8:339-345.

Stock, S.P., Mracek, Z. and Webster, J.M., 2000. Morphological variation between allotropic populations of *Steinernema kraussei* (Steiner, 1923) (Rhabditida: Steinernematidae). *Nematology*2:143-152.

Sturhan, D. and Liskova, M., 1999. Occurrence and distribution of entomopathogenic nematodes in the Slovak Republic. *Nematology* 1:273-277.

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. *Nematology* 3: 833-841.

Susurluk, A., Hollmer, S., Mehta, U.K., Han, R., Tarasco, E., Triggiani, O., Peters, A. and Ehlers, R.U., 2003.Molecular identification of entomopathogenic nematodes from Turkey, India, China, Italy, Norway, Albania and Germany by PCR-RFLP. In: 9th European Meeting of the IOBC/WPRS Working Group, Schloss Salzau, Germany.

Steiner, G., 1923. *Aplectana kraussei* n. sp., eine in der Blattwespe *Lyda* sp. Parasitierende Nematoden Form, nebst Bemerkungenüber das Seitenorgan der parasitischen Nematoden. *Zentralblatt fur Bakteriologie Parasitenkunde Infektionskranheiten und Hygiene Abteilung* 59: 14–18.

Steiner, W.A., 1996. Distribution of entomophilic nematodes in the Swiss Alps. *Revue Suisse de Zoologie* 103: 439-452.

Ünlü, I.O., Ehlers, R.U. and Susurluk, A., 2007. Additional data and first record of entomopathogenic nematode *Steinernema weiseri* from Turkey. *Nematology* 9: 739-741.

Yilmaz, H., Waeyenberge, L., Demir, I., Demirbag, Z., Moens, M., 2008. Distribution of entomopathogenic nematodes from the Eastern Black Sea region of Turkey. Book of Abstracts of 60th International Symposium on Crop Protection, May 20, Gent, Belgium. pp 199.

Yılmaz, H., Waeyenberge, L., Demir, I., Moens, M., and Demirbag, Z., 2009. A new entomopathogenic nematode species for Turkey, *Heterorhabditis megidis* Poinar, Jackson and Klein 1987 (Rhabditida: Heterorhabditidae). *Turkish Journal of Agriculture and Forestry* 33: 385-391.

Yoshida, M., 2003. Intraspecific variation in RFLP patterns and morphological studies on *Steinernema feltiae* and *S. kraussei* (Rhabditida:Steinernematidae) from Hokkaido, Japan. *Nematology* 5: 735-746.

Vanninen, I., Husberg, G.B. and Hokkanen, H.M.T., 1989. Occurrence of entomopathogenic fungi and nematodes in cultivated soils in Finland. *Acta Entomologica Fennica* 53: 65-71.

Wright, P.J., 1992. Cool temperature reproduction of Steinernematid and Heterorhabditid. *Journal of Invertebrate Pathology* 60:148-15.