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Entomopathogenic nematodes occurring in alfalfa fields, Tokat, Turkey

Tokat (Türkiye) ili yonca alanlarında bulunan entomopatojen nematodlar

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ABSTRACT

With this study, the presence of entomopathogenic nematodes (EPN) in 15 villages (Ulaş, Taşlıçiftlik, Söngüt, Büyükbağlar, Uğrak, Tahtoba, Dayılıhacı, Çördük, Kızılköy, Bakışlı, Ballıdere, Çöreğibüyük, Gaziosmanpaşa, Günevi, Akyamaç and 2 towns (Güryıldız, Emirseyit) of Tokat province where alfalfa was cultivated intensively was investigated between June-October 2016. For this purpose, 58 soil samples were taken and obtained 10 EPN isolates by trap-insect method. As a result of the morphological and molecular diagnosis, 8 of them were defined as *Steinernema carpocapsae*, one isolate as *S.feltiae* and one isolate as *Heterorhabditis bacteriophora*. This paper is the first report showing the occurrence of EPNs in alfalfa fields in Turkey.

INTRODUCTION

Recently, one of the most important issues discussed is the restriction of the use of pesticides. Efforts of biological control are an important tool to achieve this target in the world. *Entomopathogenic* nematodes (EPNs) belong to *Steinernematidae* and *Heterorhabditidae* families which are parasites of insects, killing them rapidly with the help of mutualistic bacteria. Therefore, they have great importance as biological control agents of many insect pests having economic importance (Hazır et al. 2003). In recent years, studies on the effectiveness of EPNs against important pest groups of economic importance have gained speed in Turkey.

In the world, a total of 104 EPN species have been identified. These are 84 species from the *Steinernema* genus, 1 species from the *Neosteinernema* genus and 19 species from the *Heterorhabditis* genus (Nguyen 2018). Studies on the EPNs in Turkey have begun in recent years (Kepenekci et al. 1999, Özer et al. 1995). When analyzed studies conducted to determine the EPNs in Turkey, it is seen that 7 species (*S. anatoliense*, *S. bicornutum*, *S. carpocapsae*, *S. feltiae*, *S. kraussei*, *S. websteri* and *S. weiseri*) belonging to the genus *Steinernema* and 3 species (*H. bacteriophora*, *H. marelatus* and *H. megidis*) belonging to the genus *Heterorhabditis* have been revealed (Kepenekci 2012).

To use EPNs in applied biological control studies, it is important to detect the species or isolates belonging to the species, determine their distribution and reveal the effectiveness of the identified EPNs. It was investigated effectiveness against different insect groups of different EPN isolates detected in Turkey and showed that there were significant differences between the activities of different isolates belonging to the same species (Esengül and Evlice 2020, Evlice et al. 2007, Kepenekci and Susurluk 2006, Kepenekci et al. 2013, Tülek et al. 2015, Yılmaz et al. 2010, Yüksel and Canhilal 2019).

Alfalfa, which is a perennial plant, has an average life span of 5 years, and the use of pesticides in alfalfa cultivation areas is either very limited or not. In this way, many different organisms can settle in these areas and create a living space for themselves. Thus, alfalfa cultivation contributes to the natural balance in agroecosystems. Some studies were conducted for to determine diversity of EPN in different cultivations areas in Turkey. However, no studies have been performed for the determination of EPNs in alfalfa fields, which are considered to be rich in fauna. In this work, a survey was carried out to isolate and identify EPNs from alfalfa fields in Tokat (Turkey) province.

MATERIALS AND METHODS

Field studies

Collecting soil samples

Soil sampling was carried out between June and October 2016 in order to obtain EPNs from the intensively alfalfa cultivated areas of Tokat province (Turkey). For this purpose, 58 soil samples were taken from the 15 villages (Ulaş, Taşlıçiftlik, Söngüt, Büyükbağlar, Uğrak, Tahtoba, Dayılıhacı, Çördük, Kızılköy, Bakışlı, Ballıdere, Çöreğibüyük, Gaziosmanpaşa, Günevi, Akyamaç) and 2 town (Güryıldız, Emirseyit) in Tokat province (Table 1). Soil samples which were approximately 1 kg and consisted of 10-15 subsamples were taken from a depth of 5-30 cm (Bulun 2011). While determining the areas to be sampled, it was considered that there was a certain distance between them, and they were in distribution to represent the study region of Tokat province (Sevim 2010). Soil samples were kept in ice boxes at 4 ^{O}C until they were brought from the field to the laboratory and kept in the refrigerator at the same temperature during the examination period.

Laboratory studies

Rearing of Galleria mellonella (L.) (Lepidoptera: Pyralidae)

The Greater wax moth, *Galleria mellonella*, was used for obtaining EPNs from soil and for their mass reproduction in the laboratory. For these reasons, an artificial diet was prepared for mass rearing of *G. mellonella* in laboratory. The diet ingredients consisted of wheat flour (890 g), dried yeast powder (222 g), glycerine (500 g), honey (500 ml), milk powder (445 g) and wheat bran (445 g) (Haydak 1936, Mohammed and Coppel 1983). The prepared diet was placed in 300 ml glass jars and then, the egg cluster was placed and closed with aluminium wire. The jars were placed in an insect rearing cabinet with 23-24 ^{O}C and illuminated for 16/8 hours. The last instar larvae of *G. mellonella* were obtained and used in the studies.

Isolation of nematodes

The nematodes were isolated from alfalfa field soils with *Galleria* trap methods. These soils were placed in 500 ml plastic containers. 10 last instar larvae of *G. mellonella* were placed in 10 cm diameter plastic wire cages and placed in the soil samples (Bedding and Akhurst 1975, Griffin et al. 2000). The plastic containers were placed in an incubator at 24 ^{O}C and checked every 3 days. Nematodes were obtained from the dead larvae by "White trap" method (White 1927). Collected larvae were checked by infectivity test (Koch's postulation) for confirming whether the obtained nematodes were EPN or not (Kaya and Stock 1997).

Morphology-based identification of EPNs

The genus level of identification of entomopathogenic nematodes was attempted by making temporary mounts of ten infective juveniles for each isolate. Infective juveniles were killed 60 ^{O}C and fixed in TAF (7 ml 40% formalin, 2 ml Triethamrolamine, 91 ml distilled water). Fixed nematodes were transferred to anhydrous glycerine according to Seinhorst's (1959) rapid method as modified by De Grisse (1969). Permanent slides were prepared according to ring method of Hooper (1986). All measurements were made using a drawing tube attached to Leica DM 3000 light microscope. Genus- level identification was made according to Poinar (1990) and Liu and Berry (1996).

Molecular characterization

For molecular characterization of entomopathogenic nematodes, the ITS (internal transcribed spacer) gene region, which is widely used all over the world and is a part of the rDNA gene region, was analyzed. DNA extraction was performed by following the protocol of DNA isolation kit (Thermo scientific). PCR amplification of DNA sample was prepared by following ingredients were added into a 0.2 ml specific PCR tubes; 37.3 µl ddH2O, 5 µl 10X buffer, 4 µl MgCl2, 1 µl dNTPs, 0.2 µl of forward primer 18S: 5-TTGATTACGTCCCTGCCCTTT-3, 0.2 µl of reverse primer 28S: 5-TTTCACTCGCCGTTACTAAGG-3, 0.3 µl Taq polymerase and 2 µl DNA (Vrain et al. 1992).

The prepared tubes were placed in the thermal cycler (Biorad) and subjected to a PCR cycle suitable for the primers used for the ITS region. The PCR conditions were applied as 94 ^{O}C for 7 minutes followed by 35 cycles of denaturation at 94 ^{O}C for 1 min, annealing at 50 ^{O}C for 1 min and elongation at 72 ^{O}C for 1 min and a final extension step at 72 ^{O}C for 7 min (Nguyen 2007). The amplification products obtained as a result of the PCR reaction were examined by subjecting them to 1% agarose gel electrophoresis.

The PCR products obtained were cleaned by using DNA purification kit (Macherey-Nagel) and sent to Medsantek

Table 1. Information of locations for the soil samples collected from alfalfa fields in Tokat province

Location	Coordinates		Altitude (m)
	N	E	
Ulaş	40 O 19 ' 37 "	36 O 26 ' 31 "	566
Ulaş	40 O 19 ' 37 "	36 O 25 ' 16 "	560
Ulaş	40 O 19 ' 33 "	36 O 25 ' 45 "	570
Güryıldız	40 O 20 ' 07 "	36 O 22 ' 11 "	575
Güryıldız	40 O 19 ' 57 "	36 O 22 ' 16 "	545
Güryıldız	40 O 19 ' 56 "	36 O 21 ' 31 "	567
Güryıldız	40 O 20 ' 03 "	36 O 23 ' 02 "	561
Emirseyit	40 O 20 ' 13 "	36 O 23 ' 59 "	554
Emirseyit	40 O 20 ' 13 "	36 O 25 ' 20 "	560
Emirseyit	40 O 20 ' 32"	36 O 24 ' 32 "	551
Söngüt	40 O 18 ' 58 "	36 O 24 ' 02 "	550
Söngüt	40 O 18 ' 53 "	36 O 23 ' 39 "	554
Söngüt	40 O 20 ' 52 "	36 O 28 ' 06 "	587
Taşlıçiftlik	40 O 19 ' 48 "	36 O 27 ' 29 "	572
Taşlıçiftlik	40 O 19 ' 49 "	36 O 28 ' 18 "	592
Taşlıçiftlik	40 O 20 ' 15 "	36 O 30 ' 03 "	576
Büyükbağlar	40 O 17 ' 30 "	36 O 21 ' 46 "	580
Büyükbağlar	40 O 17 ' 03 "	36 O 21 ' 41 "	616
Büyükbağlar	40 O 17 ' 15 "	36 O 21 ' 36 "	590
Büyükbağlar	40 O 17 ' 41 "	36 O 21 ' 31 "	559
Büyükbağlar	40 O 17 ' 38 "	36 O 21 ' 28 "	564
Uğrak	40 O 12 ' 32 "	36 O 29 ' 01 "	1119
Uğrak	40 O 11 ' 52 "	36 O 29 ' 07 "	1163
Uğrak	40 O 12 ' 11 "	36 O 28 ' 48 "	1135
Uğrak	40 O 11 ' 58 "	36 O 28 ' 03 "	1100
Tahtoba	40 O 11 ' 55 "	36 O 27 ' 36 "	1113
Tahtoba	40 O 12 ' 06 "	36 O 27 ' 37 "	1112
Tahtoba	40 O 12 ' 29 "	36 O 27 ' 45 "	1112
Tahtoba	40 O 12 ' 48 "	36 O 27 ' 56 "	1096
Dayılıhacı	40 O 12 ' 48 "	36 O 30 ' 54 "	1054
Dayılıhacı	40 O 12 ' 49 "	36 O 31 ' 01 "	1029
Dayılıhacı	40 O 12 ' 24 "	36 O 31 ' 08 "	1101
Dayılıhacı	40 O 12 ' 16 "	36 O 31 ' 06 "	1098
Dayılıhacı	40 O 12 ' 39 "	36 O 30 ' 02 "	1131
Çördük	40 O 14 ' 01 "	36 O 33 ' 06 "	821
Çördük	40 O 14 ' 16 "	36 O 33 ' 03 "	808
Çördük	40 O 14 ' 18 "	36 O 32 ' 35 "	844
Kızılköy	40 O 22 ' 50 "	36 O 40 ' 37 "	642
Kızılköy	40 O 22 ' 14 "	36 O 39 ' 50 "	624
Kızılköy	40 O 22 ' 29 "	36 O 40 ' 42 "	621
Bakışlı	40 O 20 ' 36 "	36 O 37 ' 43 "	607
Bakışlı	40 O 20 ' 26 "	36 O 37 ' 38 "	638
Bakışlı	40 O 20 ' 20 "	36 O 37 ' 17 "	625
Ballıdere	40 O 21 ' 03 "	36 O 38 ' 04 "	612
Ballıdere	40 O 21 ' 35 "	36 O 39 ' 18 "	615
Ballıdere	40 O 21 ' 28 "	36 O 38 ' 46 "	598
Çöreğibüyük	40 O 23 ' 38 "	36 O 42 ' 15 "	634
Çöreğibüyük	40 O 23 ' 35 "	36 O 42 ' 45 "	649
Çöreğibüyük	40 O 23 ' 02 "	36 O 42 ' 48 "	642
Gaziosmanpaşa	40 O 21 ' 43 "	36 O 41 ' 11 "	648
Gaziosmanpaşa	40 O 21 ' 56 "	36 O 41 ' 18 "	632
Gaziosmanpaşa	40 O 21 ' 54 "	36 O 41 ' 03 "	651
Günevi	40 O 21 ' 58 "	36 O 34 ' 22 "	861
Günevi	40 O 22 ' 21 "	36 O 35 ' 12 "	911
Günevi	40 O 21 ' 42 "	36 ° 34 ' 09 "	840
Akyamaç	40 O 20 ' 58 "	36 O 29 ' 38 "	591
Akyamaç	40 O 20 ' 54 "	36 O 28 ' 24 "	581
Akyamaç	40 O 20 ' 52 "	36 O 28 ' 06 "	587

Company (Turkey) for sequence analysis. Sequence results were corrected by using Bioedit (Hall 1999) and MEGA 6.0 (Tamura et al. 2013) software program, and the results were analyzed by entering the gene bank of the National Center for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION

As a result of the PCR study of the populations diagnosed on the basis of genus morphologically and morphometrically, a band of approximately 1000 bp was obtained (Figure 1). As a result of the sequence analysis of the PCR outputs, the nucleotide sequences obtained for 10 populations were separately blasted in NCBI and compared with other sequences and the species of each population was determined. As a result of this study, 10 samples out of 58 (17.2%) were found positive (infected with EPN). 8 of them were defined as *Steinernema carpocapsae* (Tokat-Ulas, Tokat-Baglar, Tokat-Bakıslı05, Tokat-Bakıslı60, Tokat-Yamac, Tokat-Ballı, Tokat-Corduk61, Tokat-Corduk02), the rest of them were found as *S.feltiae* (Tokat-Emir) and *Heterorhabditis bacteriophora* (Tokat-Songut) (Table 2, Figure 1).

H. bacteriophora and *S. carpocapsae*, which were revealed in the study, were previously known from the Tokat province (Kepenekci et al. 2018), while *S feltiae* is a new record for the EPN fauna of the Tokat province. *S. carpocapsae* was found for the first time by Kepenekci and Öztürk (2001) and *H. bacteriophora* by Kepenekci et al. (1999) in Turkey. Another species, *S. feltiae*, was determined in the soil samples taken from Rize (Turkey) by Özer et al. (1995). As a result of nematological surveys conducted later in Turkey, many isolates belonging to these species were revealed.

While the most common species in the world are found as *S. feltiae* and *H. bacteriophora* (Hominick et al. 1996), this situation was different in our study and a higher number of isolates belonging to *S. carpocapsae* were obtained.

Table 2. The obtained entomopathogenic nematode isolates from alfalfa fields in Tokat province

Isolates	Location Information			
	Location	Coordinates		Altitude (m)
		N	E	
Steinernema carpocapsae Tokat-Ulas	Ulaş	40 ° 19' 33 "	36 ° 25 ' 45 "	570
S. carpocapsae Tokat-Baglar	Büyükbağlar	40 ° 17 ' 03 "	36 ° 21 ' 41 "	616
S. carpocapsae Tokat-Bakıslı05	Bakışlı	40 ° 20 ' 36 "	36 ° 37 ' 43 "	607
S. carpocapsae Tokat-Bakıslı60	Bakışlı	40 ° 20 ' 20 "	36 ° 37 ' 17 "	625
S. carpocapsae Tokat-Yamac	Akyamaç	40 ° 20 ' 52 "	36 ° 28 ' 06 "	587
Heterorhabditis bacteriophora Tokat-Songut	Söngüt	40 ° 20 ' 52 "	36 ° 28 ' 06 "	587
S. carpocapsae Tokat-Ballı	Ballıdere	40 ° 21 ' 03 "	36 ° 38 ' 04 "	612
S. feltiae Tokat-Emir	Emirseyit	40 ° 20 ' 32 "	36 ° 24 ' 32 "	551
S. carpocapsae Tokat-Corduk61	Çördük	40 ° 14 ' 01 "	36 ° 33 ' 06 "	821
S. carpocapsae Tokat-Corduk02	Çördük	40 ° 14 ' 16 "	36 ° 33 ' 03 "	808

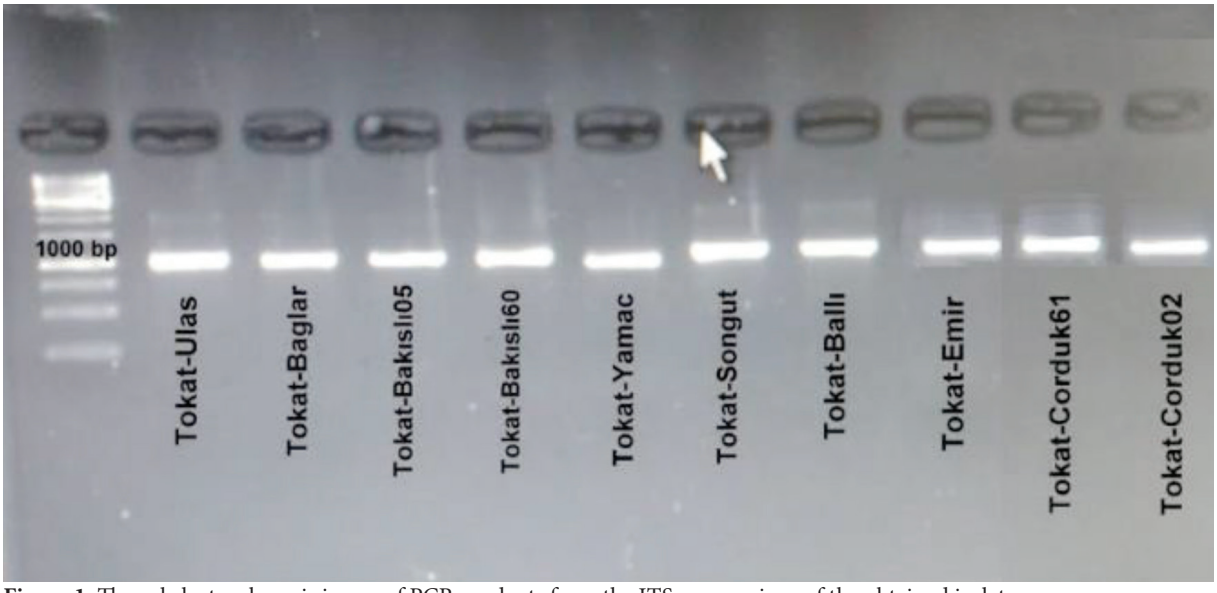

Figure 1. The gel electrophoresis image of PCR products from the ITS gene regions of the obtained isolates

When the number of EPNs obtained with the soil sample taken is compared, it is seen that a significant number of EPN isolates were obtained with a rate of 17.2%. In other studies, EPN prevalence was found 12.1% in Aydın (Aydın 2007), 8.3% in Trabzon (Gökçe 2010), 9.6% in Çanakkale (Bulun 2011), 6.1% in Marmara Region (Güneş and Gözel 2011), 9.09% in Trabzon (Erbaş 2012), 0.48% in İzmir (Ari 2014), %8.4 in Düzce (Gürel 2015), 3.3% in Kaz Mountains (Gürsoy 2017) and 6% in West Black Sea (Düzce, Bolu, Karabük and Zonguldak) (Gülcü 2018) and 3.6% in Tokat (Kepenekci et al. 2018).

Kepenekci et al. (2018) revealed, totally 5 EPNs isolates [2 isolates (TOK44, TOK20) belonging to *Heterorhabditis bacteriophora* and 3 isolates (TOK05, GOP81, GOP72) belonging to *Steinernema carpocapsae*] with 3.6% presence rate in Tokat province. Apart from this study, no other study has been done for the detection of EPNs in Tokat province.

In our study, the rate of EPN obtained from Tokat alfalfa fields was higher than those obtained in other studies (17.24%). It is thought that this is due to the following reasons: less soil cultivation since alfalfa generally has a 5-year life span, intense natural fauna and limited pesticide use or pesticide unused areas.

This was the first study to determination of entomopathogenic nematodes in alfalfa fields in Turkey. As a result of this study, it has been shown that alfalfa fields were rich in terms of EPNs. It is highly probable that new EPN isolates will be found with studies in alfalfa fields in different regions of Turkey in the future.

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ÖZET

Bu çalışma ile Tokat ilinin yoğun olarak yonca tarımı yapılan 15 köy (Ulaş, Taşlıçiftlik, Söngüt, Büyükbağlar, Uğrak, Tahtoba, Dayılıhacı, Çördük, Kızılköy, Bakışlı, Ballıdere, Çöreğibüyük, Gaziosmanpaşa, Günevi, Akyamaç) ve 2 beldesinde (Güryıldız, Emirseyit) 2016 yılı haziran-ekim ayları arasında entomopatojen nematod (EPN) varlığı araştırılmıştır. Bu amaçla 58 toprak örneği alınmış ve tuzak böcek yöntemiyle 10 EPN izolatı elde edilmiştir. Yapılan morfolojik ve moleküler teşhis çalışmaları sonucunda sekiz izolat Steinernema carpocapsae, bir izolat S. feltia ve bir izolat Heterorhabditis bacteriophora olarak belirlenmiştir. Bu çalışma Türkiye'de yonca alanlarında bulunan EPN'lerin tespitine yönelik ilk çalışmadır.

Anahtar kelimeler: Entomopatojen nematodlar, yonca alanları, Tokat, Türkiye

REFERENCES

Ari A., 2014. Entomopatojen nematodların (Steinernematidae ve Heterorhabditidae) Tire ilçesi (İzmir) topraklarındaki tür çeşitliliği ve dağılımlarının belirlenmesi. (Yüksek Lisans), Aydın Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 66 s., Aydın.

Aydın M.S., 2007. Entomopatojenik nematodların (Steinernematidae ve Heterorhabditidae) Aydın ili ve çevresindeki topraklarda tür çeşitliliği ve dağılımlarının belirlenmesi. Aydın Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 53 s., Aydın.

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

Bulun N., 2011. Çanakkale ili elma bahçelerindeki entomopatojen nematod faunasının belirlenmesi ve Elma iç kurdu (*Cydia pomonella*, Linnaeus) (Lepidoptera: Tortricidae)'na karşı laboratuvarda etkinliklerinin araştırılması. Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 142 s., Çanakkale.

De Grisse A., 1969. Redescription et modifications de quelque tecniques utilisees dans l' etude des neematodes phytoparasitaires. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent, 34, 351-359.

Erbaş Z., 2012. Trabzon yöresinden entomopatojen nematodların izolasyonu, karakterizasyonu ve *Melolontha melolontha* (Coleoptera: Scarabaeidae) üzerindeki etkilerinin araştırılması. Trabzon Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 110 s., Trabzon.

Esengül Ö., Evlice E., 2020. Assessment of the susceptibility of the Turkestan cockroach, *Blatta lateralis* to Turkish isolates of entomopathogenic nematodes. Türkiye Biyolojik Mücadele Dergisi, 11 (1), 129-137.

Evlice E., Kepenekci İ., Zeki C., 2007. İki entomopatojen nematodun Elma içkurdu *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) üzerindeki etkileri. Entomopatojenler & Mikrobiyal Mücadele Sempozyumu, Trabzon, 53 s.

Gökçe C., 2010. Çeşitli toprak örneklerinden entomopatojen nematodların izolasyonu, tanımlanması ve patojenitelerinin araştırılması. Trabzon Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 117 s., Trabzon.

Griffin C.T., Chaerani R., Fallen D., Reid A.P., Downes M.J. 2000. Occurrence and distribution of the entomopathogenic nematodes Steinernema spp. and *Heterorhabditis indica* in Indonesia. Journal of Helminthology, 74 (2), 143-150.

Gülcü B., 2018. Batı Karadeniz Bölgesi'ndeki entomopatojen nematodların (*Steinernematidae* ve *Heterorhabditidae*) tür çeşitliliği ve dağılımı. Anadolu Tarım Bilimleri Dergisi, 33 (1), 6-13.

Güneş Ç., Gözel U., 2011. Marmara Bölgesi'ndeki entomopatojen nematod faunasının belirlenmesi. Türkiye Biyolojik Mücadele Dergisi, 2 (2), 93-102.

Gürel S., 2015. Düzce ili fındık bahçelerindeki entomopatojen nematod faunasının belirlenmesi ve Fındık kurduna (*Curculio nucum* L.) (Col: Curculionidae) karşı laboratuvarda etkinliklerinin araştırılması. Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 62 s., Çanakkale.

Gürsoy S., 2017. Kaz dağı entomopatojen faunasının belirlenmesi. Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 58 s., Çanakkale.

Hall T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.

Haydak M.H., 1936. A food for rearing laboratory insect. Journal of Economic Entomology, 29 (5), 1026.

Hazır S., Kaya H.K., Stock S.P., Keskin N., 2003. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. Turkish Journal of Biology, 27 (4), 181-202.

Hooper D.J., 1986. Handling fixing, staining and mounting nematodes. Extraction of free living stages from soil. In: Laboratory methods for work with plant and soil nematodes. Southey J.F., (Ed.). London, 59-80.

Hominick W.M., Reid A.P., Bohan D.A., Briscoe B.R., 1996. Entomopathogenic nematodes biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Science and Technology, 6 (3), 317-332.

Kaya H.K., Stock S.P., 1997. Techniques in insect nematology. In: Manuel of techniques in insect pathology. Lacey L.A., (Ed.). Academic press, London, 281-324 p.

Kepenekci İ., Babaroğlu N., Öztürk G., Halıcı S., 1999. Türkiye için yeni bir entomopatojen nematod *Heterorhabditis bacteriophora* Poinar 1976 (Rhabditida: Heterorhabditidae), 4. Biyolojik Mücadele Kongresi, Adana, 587-596.

Kepenekci İ., Öztürk G., 2001. Türkiye için yeni bir entomopatojen nematod; *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae). Türkiye Entomoloji Dergisi, 25 (1), 23-31.

Kepenekci İ., Susurluk A., 2006. Susceptibility of the Mealy plum aphid, *Hyalopterus pruni* (Homoptera: Aphididae) of two isolates of *Steinernema feltiae* (Rhabditida: Steinernematidae) under laboratory conditions. Pakistan Journal of Nematology, 24 (1), 49-55.

Kepenekci İ., 2012. Nematology (plant parasitic and entomopathogenic nematodes) (General Nematology, volume-I) (Taxonomic Nematology, volume-II) pp.1155.] Eğitim, Yayım ve Yayımlar Dairesi Başkanlığı, Ankara, 1155 s.

Kepenekci İ., Evlice E., Özer N., 2013. Entomopatojen nematodların ağ kurdu [*Yponomeuta mallinellus* Zell. ve *Yponomeuta padella* L. (Lepidoptera: Yponomeutidae)] larvalarına etkileri. Bitki Koruma Bülteni, 53 (4), 199-206.

Kepenekci İ., Atay T., Çimen H., Akın A., Keleş G., Hazır S., 2018. Entomopathogenic nematodes (Nematoda) in Tokat province (Turkey). 6th ASM International Congress of Agriculture and Environment, Antalya, 273-285.

Liu J., Berry R.E., 1996. *Heterorhabditis marelatus* n.sp. (Rhabditida: Steinernematidae) from Oregon, USA. Fundamental and Journal of Invertebrate Pathology, 67, 48-54.

Mohammed M.A., Coppel H.C., 1983. Mass rearing of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) for small-scale laboratory studies. Great Lakes Entomology, 16, 139-141.

Nguyen K.B., 2007. Methodology, morphology and identification. In: Entomopathogenic nematodes: systematics, phylogeny and bacterial symbionts, Nguyen, K.B., Hunt, D.J. (Eds.). Nematology Monographs and Perspectives 5, Hunt, D.J., Perry, R.N. (Eds.). Leiden, The Netherlands, Brill, 59-119 p.

Nguyen K.B., 2018. Morphology and taxonomy of entomopathogenic nematodes. http://entnemdept.ufl.edu/nguyen/morph/kbnstein.htm (accessed date: 27.05.2020).

Özer N., Keskin N., Kırbaş Z., 1995. Occurence of entomopathogenic nematodes (Steinernematidae: Heterorhabditidae) in Turkey. Nematologica, 41, 639-640.

Poinar G.O. Jr., 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Entomopathogenic nematodes in biological control. Gaugler R., Kaya H.K., (Eds.). Boca Raton, CRC Press, USA, 23-58 p.

Seinhorst J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica, 4, 67-69.

Sevim A., 2010. Doğu Karadeniz Bölgesi'nden entomopatojenik fungusların izolasyonu, karakterizasyonu ve virülanslarının belirlenmesi. Trabzon Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Doktora Tezi, 166 s., Trabzon.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30 (12), 2725-2729.

Tülek A., Ertürk S., Kepenekci İ., Atay T., 2015. Efficacy of native entomopathogenic nematodes against the stored grain insect pest, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) adults. Egyptian Journal of Biological Pest Control, 25 (1), 251-254.

Vrain T.C., Wakarchuk D.A., Levesque A.C., Hamiltin R.I., 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology, 15 (6), 563-573.

White G.F., 1927. A method for obtaining infective nematode larvae from cultures. Science, 66 (1709), 302-303.

Yılmaz H., Gokce C., Demir İ., Demirbağ Z., 2010. Laboratory screening of the pathogenicity of some local entomopathogenic nematode isolates against the European cockchafer, *Melolontha melolontha* (Col.: Scarabaeidae). 43th Annual Meeting of Society for Invertebrate Pathology, Trabzon, p. 61.

Yüksel E., Canhilal R., 2019. Isolation, identification, and pathogenicity of entomopathogenic nematodes occurring in Cappadocia Region, Central Turkey. Egyptian Journal of Biological Pest Control (2019), 29-40

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