Türk. entomol. derg., 2019, 43 (4): 357-366
DOI: http://dx.doi.org/10.16970/entoted.578081

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Determination of plant parasitic nematodes associated with chickpea in Turkey[1]

Türkiye'de nohut alanlarındaki bitki paraziti nematodların belirlenmesi

Tohid BEHMAND[2*] **Naime Zülal ELEKCİOĞLU[3]** **Jens BERGER[4]**
Canan CAN[5] **İ. Halil ELEKCİOĞLU[2]**

Abstract

A survey of plant parasitic nematodes associated with chickpea was conducted in the chickpea growing areas of Turkey including 37 districts in 17 provinces during spring and summer of 2014-2016. A total of 211 soil and root samples were collected. Nematodes were extracted from soil by different extraction methods to ensure all kinds of nematode groups. Nematodes were identified using morphological and morphometric features. In addition, *Pratylenchus* spp. Filipjev, 1936 were determined using species-specific primers. *Ditylenchus dipsaci* (Kühn, 1857), *Pratylenchus neglectus* (Rensch, 1924) and *Pratylenchus thornei* Sher & Allen, 1953 were the most common of the plant parasitic nematodes associated with chickpea in the areas surveyed. *Pratylenchus neglectus*, *P. penetrans* (Cobb, 1917) and *P. thornei* were present in almost all samples. In descending order, *P. thornei*, *P. neglectus* and *D. dipsaci* were detected in 179, 138 and 95 in samples (84, 65 and 45% of samples, respectively). Other nematodes found at lower frequency were species of *Aphelenchus* Bastian, 1965, *Criconemoides* Taylor, 1936, Dorylaimida species, *Helicotylenchus* Steiner, 1945, *Merlinius* Siddiqi, 1970, *Paratrophurus* Arias, 1970, *Paratylenchus* Micoletzky, 1922, *Trophurus* Loof, 1957, *Tylenchorhynchus* Cobb, 1930, *Tylenchus* Bastian, 1865 and *Xiphinema* Cobb, 1913.

Keywords: Chickpea, plant parasitic nematodes, molecular identification

Öz

Türkiye nohut üretim alanlarında nematod türlerini belirlemek amacıyla 17 ile bağlı 37 ilçede 2014-2016 yılları arasında yürütülen sürvey çalışmasında toplam 211 toprak ve kök örnekler toplanmıştır. Elde edilen örneklerde tüm nematod gruplarını elde etmek amacıyla, topraktan farklı ekstraksiyon yöntemleriyle elde edilmiştir. Nematod türlerinin teşhisi, morfolojik ve morfometrik özellikler kullanılarak klasik teşhis yöntemlerine göre yapılmıştır. Ayrıca, *Pratylenchus* Filipjev, 1936 türlerinin teşhisi için türe özgü primer yardımıyla moleküler yöntemleri kullanılmıştır. *Ditylenchus dipsaci* (Kühn, 1857), *Pratylenchus neglectus* (Rensch, 1924) ve *Pratylenchus thornei* Sher & Allen, 1953, sürvey yapılan nohut alanlarda en yaygın bitki paraziti nematodları tespit edilmiştir. *Pratylenchus neglectus, Pratylenchus penetrans* (Cobb, 1917) ve *P. thornei* tüm örneklerde tespit edilmiştir. *Pratylenchus thornei*, *P. neglectus* ve *D. dipsaci* incelenen toprak ve köklerde sırasıyla 179, 138 ve 95 örnekte (toplam örneklerin sırasıyla %84, 65 ve 45'inde) tespit edilmiştir. Toprak örneklerinde daha düşük *Aphelenchus* Bastian, 1965, *Criconemoides* Taylor, 1936, Dorylaimida species, *Helicotylenchus* Steiner, 1945, *Merlinius* Siddiqi, 1970, *Paratrophurus* Arias, 1970, *Paratylenchus* Micoletzky, 1922, *Trophurus* Loof, 1957, *Tylenchorhynchus* Cobb, 1930, *Tylenchus* Bastian, 1865 ve *Xiphinema* Cobb, 1913 cinslerine bağlı türler belirlenmiştir.

Anahtar sözcükler: Nohut, bitki paraziti nematodlar, moleküler teşhis

[1] This study was financially supported by the Grains Research and Development Corporation (GRDC) as part of the Australian Coordinated Chickpea Improvement Program (ACCIP).
[2] Cukurova University, Faculty of Agriculture, Department of Plant Protection, 01330, Saricam, Adana, Turkey
[3] Cukurova University, Vocational School of Karaisalı, 01170, Karaisalı, Adana, Turkey
[4] Ecophysiologist, CSIRO Agriculture Flagship, Centre for Environment and Life Sciences, Australia
[5] Gaziantep University, Faculty of Agriculture, Department of Biology, 27310, Gaziantep, Turkey
* Corresponding author (Sorumlu yazar) e-mail: tohid.behmand63@gmail.com
Received (Alınış): 14.06.2019 Accepted (Kabul ediliş): 08.07.2019 Published Online (Çevrimiçi Yayın Tarihi): 02.09.2019

Introduction

Chickpea (*Cicer arietinum* L.) has a prominent place in total legume production in the world. Turkey is ranked fifth in the world for chickpea production (FAO, 2017). The most important chickpea producing countries in the world are India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, the USA and Canada (FAO, 2017). Chickpea originated in the Fertile Crescent, which borders the southeastern regions of Turkey, and spread west and south via the historically called Silk Route. The average global chickpea yield is changing due to the effect of many biotic and abiotic limitations that can cause an important reduction in grain quantity and quality of chickpea (Singh & Sharma, 1994; Sudupak et al., 2002). Plant parasitic nematodes have been reported an economically important pest affecting chickpea as the biotic factors (Castillo & Vovlas, 2007). Plant parasitic nematodes generally feed on different parts of the plant, especially on roots and other subterranean plant structure such as rhizomes of some legumes. Many researchers have shown that plant parasitic nematodes cause damage to food legumes (Greco, 1985; Greco & Vitro, 1988; Greco & Sharma, 1990; Sikora & Greco, 1990).

The root lesion nematodes (RLNs), *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae), are the most widespread nematodes in legume crops, such as alfalfa (*Medicago sativa* L.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medikus) in Mediterranean regions (Greco et al., 1984). Similarly, Hollaway et al. (2000) reported that chickpea is generally considered as more susceptible to RLNs than faba bean, field pea and lupin but less so than wheat. Vanstone et al. (1998) also reported that *Pratylenchus crenatus* Loof, 1960, *Pratylenchus neglectus* (Rensch, 1924), *Pratylenchus penetrans* (Cobb, 1917) and *Pratylenchus thornei* Sher & Allen, 1953 are the most important *Pratylenchus* species worldwide. In addition, chickpea crops infested with RLNs show symptoms of stunted growth and may have some yellowing of foliage, but often have no obvious foliar symptoms of the disease. When many nematodes attack chickpea roots, the affected tissues can turn dark brown-black, have a reduction in root hairs or nodules, and discolored root tissue. Discoloration often appears as brown or black stripes along the roots. However, diagnosis of root symptoms is usually difficult in the chickpea and are normally not observed until plants are older than 8 weeks (Pulse Australia, 2013). In a survey of chickpea in Turkey (Di Vito et al., 1994), the other plant parasitic nematodes species found were *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae), *Longidorus* Micoletzky, 1922 (Dorylaimida: Longidoridae), *Paratylenchus* Micoletzky, 1922 (Tylenchida: Paratylenchinae), *Trichodorus* Cobb, 1913 (Tylenchida: Trichodoridae), *Trophurus* Loof, 1956 (Tylenchida: Dolichodoridae), *Tylenchus* Bastian, 1865 (Tylenchida: Tylenchidae), *Xiphinema index* Thorne & Allen, 1950 and *Xiphinema pachtaicum* (Tulaganov, 1938) (Dorylaimida: Longidoridae).

The detection of new or potentially harmful species of nematode in the chickpea is important for in success of agriculture, and aids in the improvement and evaluation of quarantine or regulatory operation to minimize their spread. Correct identification of nematode species is basic to effective nematode control and successful plant quarantine procedure. Also, surveys in southern Spain chickpea fields showed that the legume and cereal root lesion nematodes such as *P. neglectus and P. thornei* were the most important and widespread plant parasitic nematodes (Castillo et al., 1996). RLNs are microscopic organisms and cannot be detected with the naked eye in the soil or in plants. Coolen (2013) reported that DNA analysis or direct counting (under a microscope) are the best ways to determine the presence of RLNs in the soil. Additionally, identification of *Pratylenchus* species is difficult because of the high degree of morphological similarity within the genus. Recently, Subbotin et al. (2008) stated that the different molecular techniques are needed to identify nematode species that have a close morphological similarity together.

Species of *Pratylenchus* Filipjev, 1936 infest a wide range of crops and causes important economic damage in global grain production. These nematodes have been found widely distributed in wheat field in Turkey. Toktay et al. (2006) reported that *P. thornei* is responsible for up to 19% of total losses in wheat fields in Turkey. Information on the species of plant parasitic nematodes infesting chickpea crops in Turkey is limited. A comprehensive study was done by Behmand (2018) on resistance of chickpea genotypes from Turkey against *P. neglectus*, *P. thornei* and *Ditylenchus dipsaci* (Kühn, 1857). The present study was undertaken to identify the most important plant parasitic nematode species potentially causing damage and yield loss in chickpea growing areas of Turkey.

Materials and Methods

Survey

A survey was conducted in 37 districts in 17 provinces in the Aegean, Central Anatolia, Central East Anatolia, East Marmara, Eastern Anatolia, Mediterranean, Southeastern Anatolia, Trace and West Marmara Regions of Turkey, during spring and summer 2014-2016 (Figure 1). A total of 211 soil and root samples (74 in 2014, 69 in 2015 and 68 in 2016) were collected using the sampling method of Bora & Karaca, (1970). Five to ten composite subsamples were taken from one location.

A soil auger was used to sample soil to 20 cm and combined to give 500-ml composite samples. Then, samples were individually packed in sealed plastic bags and brought to the laboratory as quickly as possible.

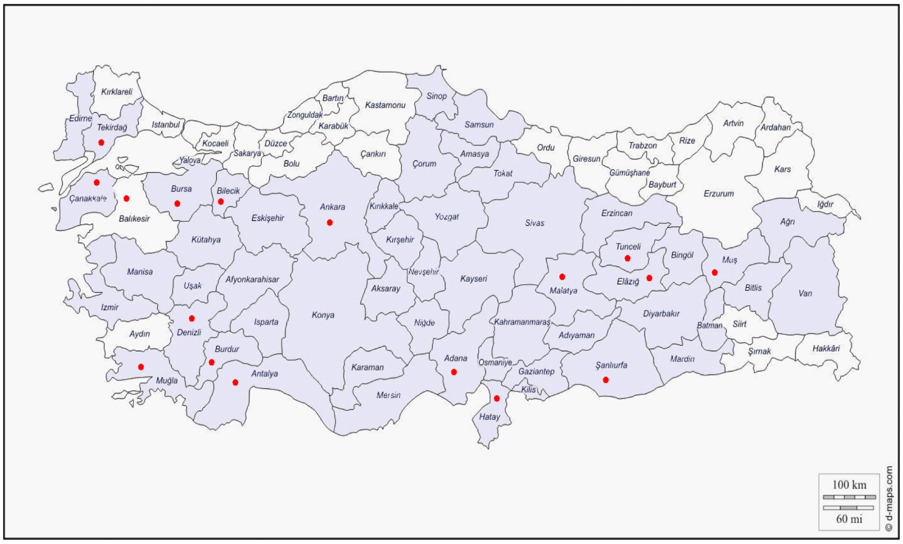

Figure 1. Location of sampling sites in 17 provinces of Turkey. Provinces in with over 10 ha of chickpea production are shown in gray.

Laboratory assessments

In the laboratory, plant shoots were removed and nematodes were extracted from the 500-ml soil samples by Cobb's sieving, centrifugal flotation (Jenkins, 1964) and modified Baermann funnels (Hooper, 1986), and extracted from roots by using an incubation technique (Young, 1954; Coolen, 1979). Then nematodes were killed at 60ºC for 1 min, fixed in a TAF solution and mounted on slides by wax-ring method (Seinhorst, 1959). The permanent slides were examined under a light microscope to identify specimens to species when possible. Also, for molecular confirmation, *P. neglectus* and *P. thornei* were identified by morphology (Handoo & Golden, 1989) and individually transferred in a small tube using a bamboo sliver under a light microscope, then placed onto surfaced sterilized carrot disk and incubated at 23±1°C for several generations to make a pure culture.

DNA was extracted from each nematode culture according to Waeyenberge et al. (2000), with some modification. From each *Pratylenchus* culture, five to ten second-stage juveniles were transferred with 25 µl sterile distilled water into an Eppendorf tube. Then, 10 µl of a suspension containing nematodes was pipetted into a 0.2-ml sterile Eppendorf tube with 8 µl of lysis buffer (500 mM KCl; 100 mM Tris-Cl, pH 8.3; 15 mM MgCl2; 10 mM dithiothreitol; 4.5% Tween 20; and 0.1% gelatin). The tube contents were frozen at -20°C for at least 20 min, then thawed, and 2 µl of proteinase K at 600 µg/ml added. The tubes were incubated for 60 min at 65°C and finally transferred to the thermocycler for 10 min at 95°C to inactivate proteinase. The tubes were then centrifuged at 16,000 rpm for 5 min and stored at -20°C until use as the DNA template.

A species-specific polymerase chain reaction (PCR) was used to identify the RLNs. The common reverse primer D3B5 and the primers PTHO D3B PNEG-F1 were used to identify *P. neglectus* and *P. thornei*, respectively (Table 1).

Table 1. Primer sequences and expected band sizes for *Pratylenchus neglectus* and *P. thornei*

Species Primer	Primer name*	Sequence (5′-3′)	Band size (bp)	Reference
P. neglectus	F: PNEG-F1	CGCAATGAAAGTGAACAATGTC	144	Yan et al. (2008)
	R: D3B5	AGTTCACCATCTTTCGGGTC		
P. thornei	F: PTHO	GAAAGTGAAGGTATCCCTCG	288	Al-Banna et al. (2004)
	R: D3B	TCGGAAGGAACCAGCTACTA		

* F, forward primer; R, reverse primer.

Results

From the 211 soil and root samples were collected from chickpea production areas surveyed, RLNs were determined in the Aegean, Central Anatolia, Central East Anatolia, East Marmara, Eastern Anatolia, Mediterranean, Southeastern Anatolia, Trace and West Marmara Regions of Turkey. *Pratylenchus* were observed in all samples in locations that were collected on chickpea growing areas. Of the *Pratylenchus* species, *P. thornei* and *P. neglectus* were identified by molecular methods in 179 (84%) and 138 (65%) samples, respectively. Chickpea plants infested with root lesion nematode had stunted growth, fewer leaves and branching. Symptoms of nematode infestation in roots were included loss of root hairs or nodules and poor root structure. Where the high population densities of nematodes attack chickpea roots, often show symptoms such as dark brown-black and discolored root tissue. Higher population densities of the RLNs was found in the Mediterranean and Aegean Provinces when compared with other regions of Turkey. A lower population density was determined in the West Marmara and Central Anatolia Regions (Figure 2). PCR with PNEG-F1/D3B5 primers and PTHO/D3B produced products of 144 and 288 bp for all the *P. neglectus and P. thornei* populations, respectively. (Figures 3 & 4). In addition, *D. dipsaci* was found in 95 soil samples (45% of the total samples). Chickpea fields infested with *D. dipsaci* showed symptoms of leaf and stem necrosis and pod deformity. Other plant parasitic nematodes found in the samples included species of *Aphelenchus* Bastian, 1965 (Aphelenchida: Aphelenchidae) (59%), *Helicotylenchus* (38%), *Merlinius* Siddiqi, 1970 (Tylenchida: Telotylenchidae) (37%), Dorylaimida (35%), *Tylenchus* (42%), *Tylenchorhynchus* Cobb, 1930 (Tylenchida: Dolichodoridae) (20%), *Paratylenchus* (10%), *Trophurus* (7%), *Paratrophurus* Arias, 1970 (Tylenchida: Dolichodoridae) (6%), *Paratylenchoides* Raski, 1973 (Tylenchida: Paratylenchidae) (8%), *X. pachtaicum* (3%), *X. index* (2%) and *Criconemoides* Taylor, 1936 (Tylenchida: Criconematidea) (2%). Generally, chickpea crops infested with these nematodes showed no symptoms and plant damage (Table 2).

Pratylenchus neglectus, P. thornei and *D. dipsaci* were observed in most samples and found to be causing damage to chickpea plants in the field. Geographical distribution of the most important plant parasitic nematodes in chickpea growing fields is shown in Figure 5. The four most common species were *P. thornei* (85% of samples), *P. neglectus* (65%), *D. dipsaci* (45%) and *P. penetrans* (18%) (Table 2).

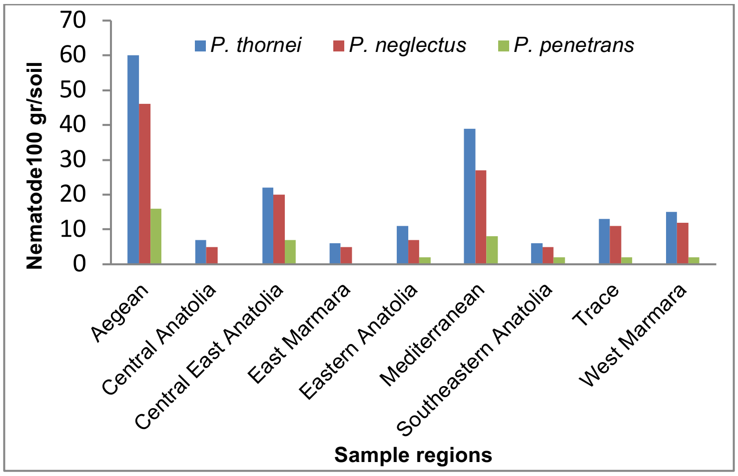

Figure 2. Frequency of RLNs (*Pratylenchus neglectus, P. penetrans* and *P. thornei*) in different chickpea production regions in Turkey.

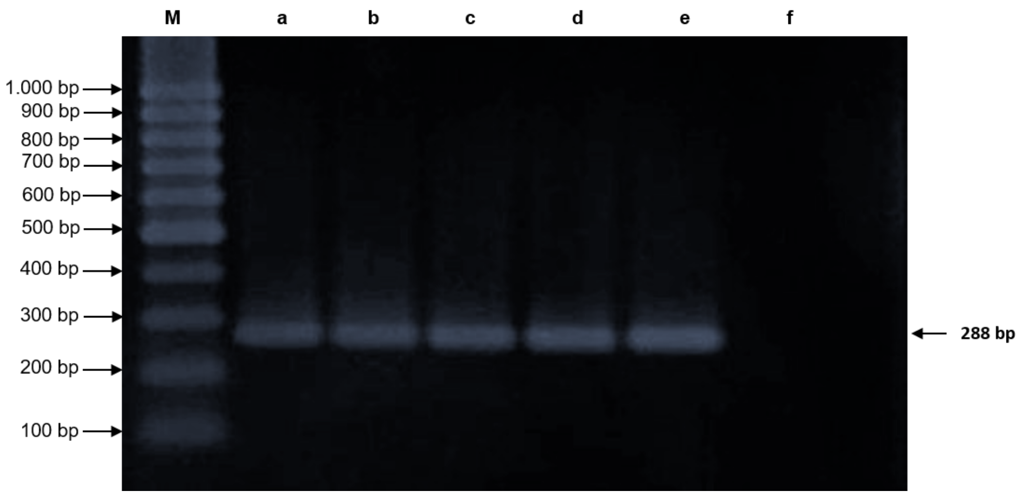

Figure 3. PCR patterns of *Pratylenchus thornei* amplified (288 bp) with specific primer set PTHO/D3B M: DNA molecular weight ladder (100 bp), a-e: samples, f: negative control.

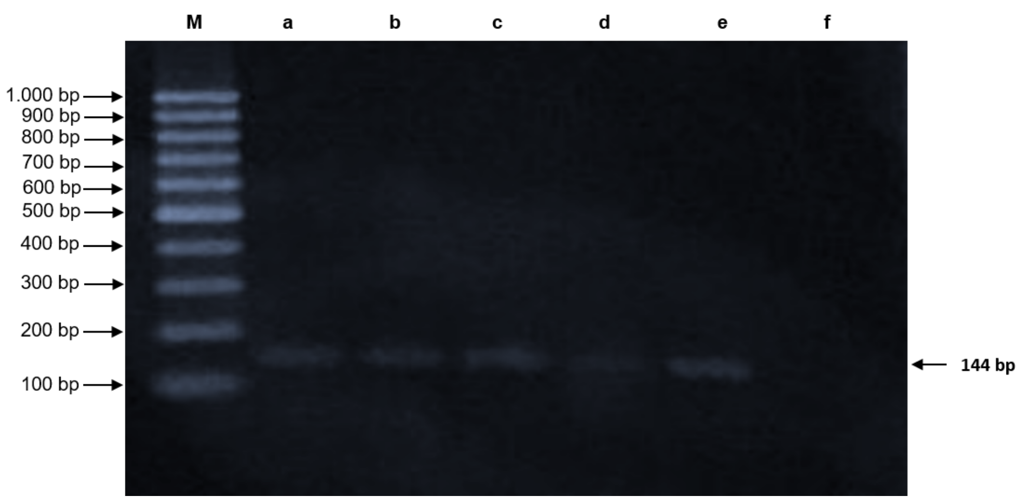

Figure 4. PCR patterns of *Pratylenchus neglectus* amplified (144 bp) with specific primer set PTHO/D3B M: DNA molecular weight ladder (100-bp), a-e: samples, f: negative control.

Table 2. Details of sampling locations and occurrence (number of positive samples per province) of identified nematodes

No	Region	Province	The number of samples collected	Latitude (N)	Longitude (E)	*D. dipsaci*	*P. thornei*	*P. neglectus*	*P. penetrans*	*Aphelenchus* spp	*Helicotylenchus* spp	Dorylaimida species	*Merlinius* spp	Other plant-parasitic nematodes*
1		Balıkesir	7	40º15'21"	27º50'14"	2	4	3	2	1	-	2	-	*Tylenchorhynchus* spp (5), *Trophurus* spp (3)
2		Balıkesir	10	40º12'56"	27º45'33"	3	5	3	1	10	5	3	2	*Paratylenchus* spp (4), *Pratylenchoides* spp
3		Balıkesir	4	40º12'56"	27º46'2"	2	6	4	1	1	-	-	1	*Criconemoides* spp, *Tylenchus* spp (6)
4		Bursa	3	40º12'47"	28º41'13"	0	6	0	0	5	2	1	4	*Tylenchus* spp (9), *Trophurus* spp (2)
5		Denizli	5	37º34'13"	29º19'36"	2	5	5	2	1	-	-	1	*Tylenchus* spp (5), *Xiphinema pachtaicum* (2)
6		Denizli	4	37º37'5"	29º14'53"	3	6	4	1	4	5	2	-	*Paratylenchus* spp (5), *Criconemoides* spp
7	Aegean	Denizli	10	37º50'0"	29º6'39"	0	6	5	1	5	4	1	3	*Tylenchorhynchus* spp (3), *Tylenchus* spp (5)
8		Denizli	8	37º34'54"	29º17'46"	2	5	6	2	6	10	5	1	*Paratylenchus* spp (3), *Xiphinema index*
9		Denizli	4	37º37'38"	29º12'37"	4	4	5	2	2	4	-	7	*Tylenchorhynchus* spp (4), *Paratylenchoides* spp (2)
10		Denizli	7	37º34'34"	28º59'24"	3	4	4	1	1	1	1	1	*Paratylenchoides* spp, *Tylenchus* spp (3)
11		Mugla	8	36º35'53"	29º35'53"	4	5	4	1	2	1	3	-	*Xiphinema index*, *Tylenchus* spp (2)
12		Mugla	5	36º51'19"	29º43'26"	3	4	3	2	1	-	-	2	*Paratrophurus* spp (2), *Trophurus* spp (3)
13	Central Anatolia	Ankara	4	39º55'32"	32º51'256"	5	7	5	0	2	1	3	2	*Paratylenchus* spp (3), *Tylenchus* spp (5)
14		Malatya	4	38º41'36"	37º33'12.8	0	4	3	1	1	1	5	12	*Tylenchus* spp (8), *Trophurus* spp (2)
15		Malatya	8	38º20'59.7	37º40'56.5	2	4	2	2	1	1	1	2	*Tylenchus* spp (8), *Criconemoides* spp (2)
16	Central East Anatolia	Malatya	3	38º16'29"	38º4'13"	2	5	5	1	1	2	-	-	*Paratylenchus* spp (3), *Tylenchus* spp (3)
17		Mus	5	38º52'52"	41º14'12"	3	4	3	2	1	2	-	-	*Tylenchus* spp (4), *Tylenchorhynchus* spp (3)
18		Mus	4	38º53'31"	41º26'5"	4	5	5	1	7	5	2		*Paratrophurus* spp (5), *Tylenchorhynchus* spp (5)
19		Tunceli	3	39º21'26"	39º30'55"	0	0	2	0	2	12	-	-	*Tylenchus* spp (6)
20	East Marmara	Bilecik	7	39º52'0"	30,º6'9"	0	6	5	0	10	2	3	4	*Paratylenchus* spp (3), *Pratylenchoides* spp (2)
21	Eastern Anatolia	Elazıg	4	38º34'22"	38º44'4"	3	5	3	1	1	1	-	-	*Paratrophurus* spp (2), *Tylenchorhynchus* spp (2)
22		Elazıg	5	38º38'50"	39º10'56"	4	6	4	1	5	2	5	7	*Tylenchorhynchus* spp (3)
23		Adana	8	37º0'6"	35º19'44"	4	6	6	0	4	3	1	2	*X. pachtaicum*, *Tylenchus* spp (3)
24		Antalya	3	37º13'3"	30º30'23"	3	5	4	0	3	-	3	-	*X. pachtaicum*, *X. index*
25		Antalya	5	36º53'34"	30º21'94"	0	5	3	1	5	-	2	-	*Paratrophurus* spp (2)
26		Antalya	8	37º17'7"	30º19'39"	2	5	4	1	1	1	-	2	-
27	Mediterranean	Burdur	3	37º26'11"	30º33'19"	3	4	3	1	10	4	2	3	*Tylenchorhynchus* spp (6)
28		Burdur	6	37º21'55"	30º30'41"	4	5	4	2	8	1	1	-	*Tylenchus* spp (4), *Tylenchorhynchus* spp (5)
29		Burdur	6	37º18'20"	30º28'6"	3	4	3	1	10	2	5	-	*Paratylenchus* spp (2)
30		Hatay	11	36º28'36"	36º17'3"	4	5	0	2	2	-	5	2	*Tylenchus* spp (3), *Tylenchorhynchus* spp (4)
31	Southeastern Anatolia	Sanliurfa	13	37º08'29"	38º46'30"	3	6	5	2	4	1	2	-	*Tylenchus* spp (4), *Heterodera ciceri*
32		Tekirdag	8	40º38'41"	26º59'8"	4	4	3	1	1	1	3	-	*Xiphinema index*, *Tylenchus* spp (2)
33	Trace	Tekirdag	2	40º49'48"	27º2'52"	3	4	4	0	-		-	-	*Paratylenchoides* spp (2), *Tylenchus* spp (3)
34		Tekirdag	5	40º38'37"	26º59'53"	2	5	4	1	2	4	8	4	*Paratrophurus* spp (2), *Xiphinema pachtaicum* (2)
35		Canakkale	2	39º42'27"	26º29'56"	4	4	5	2	2	-	4	7	*Trophurus* spp (2), *Tylenchus* spp (4)
36	West Marmara	Canakkale	7	40º16'30"	27º25'47"	3	6	4	0	1	-	2	-	*Xiphinema index*, *Tylenchorhynchus* spp (3)
37		Canakkale	2	39º41'32"	26º25'26"	2	5	3	0	2	2	-	9	*Trophurus* spp (5), *Tylenchus* spp (3)
Total			211			95	179	138	39	125	80	75	78	-
Percentage (%)						45	84	65	18	59	38	35	37	-

* Number nematodes found for each genus is given in parentheses.

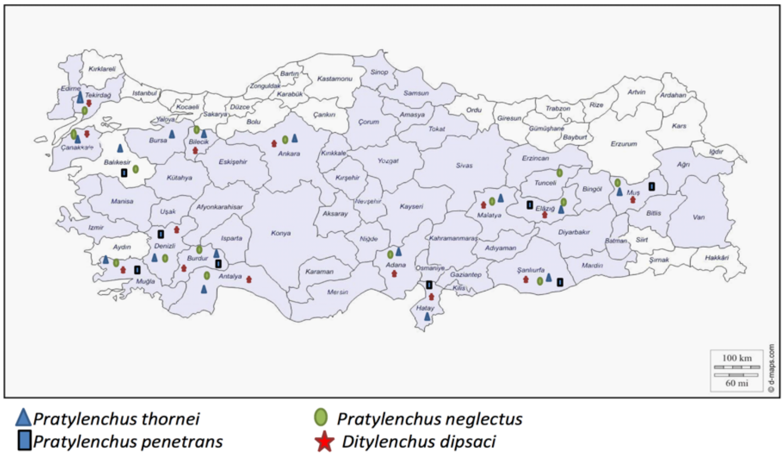

Figure 5. Geographical distribution of the four most important plant parasitic nematodes in chickpea growing areas of Turkey.

Discussion

Chickpea is a component of many Mediterranean and semiarid subtropical crop rotation systems (Whish et al., 2007; Chattopadhyay & Mohapatra, 2015). It is susceptibility to diseases and environmental conditions remains a challenge for optimizing productivity (Ghosh et al., 2013; Rubiales et al., 2015). Plant parasitic nematodes cause important damage to legumes (including chickpea) in different Mediterranean countries (Greco, 1985; Greco & Di Vito, 1988; Sikora & Greco, 1990; Greco et al., 1992; Di Vito et al., 1994). Sharma et al. (1992) reported that plant parasitic nematodes caused 14% yield loss in chickpea worldwide, but there is no information on crop losses in chickpea caused by nematodes in Turkey.

Pratylenchus spp. are found worldwide and infest a wide range of plant species. This study determined the distribution of RLNs in 17 chickpea growing provinces of Turkey. *Pratylenchus neglectus*, *P. penetrans* and *D. dipsaci* were the most important plant parasitic nematodes after *P. thornei* in all sampling sites in Turkey. Similarly, Di Vito et al. (1994) indicated that although different species of RLNs were found in different part of Turkey, *P. thornei* was dominant in Central Anatolia. Survey of plant parasitic nematodes in chickpea and lentil production areas in Syria and North Africa indicated that *P. neglectus*, *P. penetrans* and *P. thornei* were the most common nematodes and *P. thornei* the most common (Greco et al., 1992 & Di Vito et al., 1994). Consistent with those findings, *P. penetrans* was detected in 39 soil and root samples (18% of samples) in the present study. GRDC research on chickpea also reported that chickpea was susceptible to *P. neglectus*, *P. thornei* and *P. penetrans* (Grain Research Chickpea, 2015). Similarly, Greco & Di Vito (1988) reported that all these nematodes caused damage to chickpea around the world. Castillo et al. (1998) indicated that infestation of chickpea by *P. thornei* caused increases in the severity of root necrosis and enhances the root colonization by *Fusarium*. Similarly, Castillo & Vovlas (2007) indicated that these nematodes caused lesions on the roots that affect the growth and development of the crop and lead to significant yield loss. Di Vito et al. (1992) showed that among RLNs, *P. thornei* could cause yield loss of 50% in chickpea in Syria. *Pratylenchus* species ranked second after root-knot nematodes among the nematodes which cause damage to crops and chickpea (Barker & Noe, 1987; Jatala & Bridge, 1990; Castillo & Vovlas, 2007). Also, about 70 species of *Pratylenchus* have been described globally (Castillo & Vovlas, 2007). These species nematode reduce of the resistance of plants and damage by feeding roots (Orion et al., 1982). Similarly, Riley & Wouts (2001), Riley & Kelly (2002), Hollaway et al. (2008) and Thompson et al. (2010) showed that *P. thornei* and *P. neglectus* were a significant problem in chickpea production regions of Australia.

Di Vito et al. (1994) reported *Heterodera ciceri* Vovlas et al., 1986 (Tylenchoidea: Heteroderidae) as the first cyst nematode recorded in Siverek Province in Southeastern of Turkey. Similarly, *H. ciceri* was the first cyst nematode found in two samples collected at Şanliurfa Province in Southeastern Anatolia Region. Imren et al. (2012) reported *H. ciceri* was found as the first record in Adıyaman Province of the Southeastern Anatolia Region.

In the present survey, *D. dipsaci* was found in nearly half of root and soil samples. Similarly, it was reported *D. dipsaci* is one of the most detrimental pests of chickpea after root lesion, root-knot and cyst nematodes*i* (Barker & Noe, 1987; Jatala & Bridge, 1990). Chitwood & Krusberg (1977) indicated that the population densities of *D. dipsaci* can cause a gall formation in seedlings of a resistant cultivars of legumes.

Identification of *P. neglectus* and *P. thornei* based on morphological characteristics requires detailed microscopic measurements by an experienced nematologist. The genetic similarity between *P. neglectus* and *P. thornei* is reflected in their morphological similarities. Also, *P. neglectus* and *P. thornei* share some important morphological characters. Waeyenberge et al. (2000) reported that a PCR technique is rapid, efficient and can be used as a rapid identification tool for *Pratylenchus* species. Subbotin et al. (2008) reported that PCR methods can be used for identifying species of *Pratylenchus*. Whereas, Loof (1991) reported that the identification of *Pratylenchus* genus based on morphology and morphometric methods takes considerable time, requires skill and training in the observer and it is frequently ineffective because individual specimens often vary considerably within a population (Loof,1991). In the current study, *P. neglectus* and *P. thornei* were identified using molecular markers. Correct identification of important species of nematodes is critical to the success of chickpea production and integrated pest management strategies. Results of the present study will be helpful for setting priorities for further studies on of plant parasitic nematodes in chickpea production in Turkey.

References

Al-Banna, L., A. T. Ploeg, V. M. Williamson & I. Kaloshian, 2004. Discrimination of six *Pratylenchus* species using PCR and species- specific primers. Journal of Nematology, 36: 142-146.

Barker, K. R. & J. P. Noe, 1987. Establishing and using threshold population levels. Vistas on Nematology, 75-81.

Behmand, T., 2018. Screening of chickpea genotypes collected from Turkey against to the root lesion nematodes, *Pratylenchus neglectus*, *P. thornei* and *Ditylenchus dipsaci* for resistance. Çukurova University, Faculty of Agriculture, Department of Plant Protection, (Unpublished) PhD Thesis, Adana, Turkey, 163 pp.

Bora, T. & İ. Karaca, 1970. Kültür Bitkilerinde Hastalığın ve Zararın Ölçülmesi. Ege Üniversitesi Yardımcı Ders Kitabı, Yayın No: 167, E.Ü. Matbaası, Bornova-İzmir, 8s.

Castillo, P., A. Gomez-Barcina & R. M. Jimenez-Diaz, 1996. Plant parasitic nematodes associated with chickpea in southern Spain and effect of soil temperature on reproduction of *Pratylenchus thornei*. Nematologica, 42 (2): 211-219.

Castillo, P. & N. Vovlas, 2007. “*Pratylenchus* (Nematoda: *Pratylenchidae*): Diagnosis, Biology, Pathogenicity and Management, 305-324”. In: Biology and Ecology of *Pratylenchus* (Eds. D. J. Hunt & R. N. Perry). Nematology Monographs Perspectives 6, Brill, Leden-Boston, MA, 529 pp.

Castillo, P., N. Vovlas & R. M. Jimenez-Diaz, 1998. Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. Plant Pathology, 47: 370-376.

Chattopadhyay, C. & S. D. Mohapatra, 2015. Perception of constraints in chickpea production in India. Indian Journal of Agricultural Sciences, 85: 287-289.

Chitwood, D. J. & L. R. Krusberg, 1977. Pectolytic enzymes in three populations of *Ditylenchus dipsaci*. Journal of Nematology, 9: 187-192.

Coolen, W. A., 1979. “Methods for the Extraction of *Meloidogyne* spp. and Other Nematodes from Roots and Soil, 317-329". In: Root-Knot Nematodes (*Meloidogyne* Species) Systematics, Biology and Control (Eds. F. Lamberti & C. E. Taylor). Academic Press, London, 802 pp.

Di Vito, M., N. Greco, H. M. Haula Mabsout, M. Labdi, S. P. S. Beniwal, M. C. Saxena, K. B. Singh & M. B. Solh, 1994. Nematodes of winter season legumes in North Africa. Nematologia Mediterranea, 22: 3-10.

Di Vito, M., N. Greco, G. Ores, M. C. Saxena, K. B. Singh & I. Kusmenoglu, 1994. Plant parasitic nematodes of legumes in Turkey. Nematologia Mediterranea, 22: 245-251.

Di Vito, M., N. Greco & M. C. Saxena, 1992. Patogenicity of *Pratylenchus thornei* on chickpea in Syria. Nematologia Mediterranea, 20: 71-73.

FAO, 2017. Food and Agriculture Organization of the United Nations Statistical Data. (Web page: www.fao.org/faostat/en) (Date accessed: 02.11.2017).

Ghosh, R., M. Sharma, R. Telangre & S. Pande, 2013. Occurrence and distribution of chickpea diseases in central and southern parts of India. American Journal of Plant Sciences, 4: 940-944.

GRDC, 2015. Tips and tactics: Root lesion nematodes Western region. (Web page: grdc.com.au/resources-and-publications/all-publications/factsheets/2015/03/tt-rootlesionnematodes) (Date accessed: 03.03.2015).

Greco, N., 1985. “Nematodes of faba beans, chickpeas, and lentils in the Mediterranean region and their control, 179-187”. In: Proceedings of International Workshop on Faba Beans, Kabuli Chickpeas, and Lentils in the 1980s (Eds. M. C. Saxena & S. Varma), (16-20 May 1983, ICARDA, Aleppo, Syria), 457 pp.

Greco, N. & M. Di Vito, 1988. “The importance of plant parasitic nematodes in food legume production in the Mediterranean region, 28-45”. In: Proceedings of Workshop on nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions (1-5 March 1987, Larnaca, Cyprus), 217 pp.

Greco, N., M. Di Vito, M. V. Reddy & M. C. Saxena, 1984. A preliminary report of the survey of plant parasitic nematodes of leguminous crops in Syria. Nematologia Mediterranea, 12: 87-93.

Greco, N., M. Di Vito & C. Saxena, 1992. Plant parasitic nematodes of cool season food legumes in Syria. Nematologia Mediterranea, 20: 37-46.

Greco, N. & S. B. Sharma, 1990. “Progress and problems in the management of nematode diseases, 135-137”. In: Chickpea in the Ninetie, Proceedings of the Second International Workshop on Chickpea Improvement (4-8 December 1989, ICRISAT Center, India. Patancheru, Andhra Pradesh, India) (Eds. H. A. van Rheenen, M. C. Saxena, B. J. Walby & S.D. Hall), 403 pp.

Handoo, Z. A. & M. A. Golden, 1989. A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). Journal of Nematology, 21: 202-218.

Hollaway, G. J., P. T Sharyn, F. E Russeli & H. H Colleen, 2000. Effect of field crops on density of *Pratylenchus* in South Eastern Australia; Part 2: *P. thornei*. Journal of Nematology, 32 (4S): 600-608.

Hollaway, G. J., V. A. Vanstone, J. Nobbs, J. G. Smith & J. S. Brown, 2008. Pathogenic nematodes of cereal crops in south-west Victoria, Australia. Australia Plant Pathology, 37: 505-510.

Hooper, D. J., 1986. “Extraction of Free-Living Stages from Soil, 5-30”. In: Laboratory Methods for Work with Plant and Soil Nematodes (Ed. J. F. Southey), CAB International, London, UK, 629 pp.

Imren, M., L. Waeyenberge, N. Viaene, H. Toktay, A. Dababat & I. H. Elekcioğlu, 2012.Molecular characterization of cereal cyst nematodes from South Anatolian Region in Turkey using ITS-rDNA sequences. Turkish Journal of Entomology 36 (4): 491-499.

Jatala, P. & J. Bridge, 1990. “Nematode Parasites of Root and Tuber Crops,137-180”. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, A. Sikora & J. Bridge). CAB International, Wallingford, UK, 221 pp.

Jenkins, W. R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reports, 48: 692.

Loof, P. A. A., 1991. “The Family *Pratylenchidae* Thorne, 1949, 363-421”. In: Manual of Agricultural Nematology (Ed. W. R. Nickle). Marcel Dekker, New York NY, USA, 503 pp.

Orion, D., J. Krikun & J. Amir, 1982. “Population dynamics of *Pratylenchus thornei* and its effect on wheat in a semi-arid region, 48”. Abstracts of the 16th International Symposium of the European Society of Nematologists (30 August- 3 September 1982, St. Andrews, Scotland, UK), 160 pp.

Pulse Australia, 2013. Northern chickpea best management practices training course manual 2013. Pulse.

Riley, I. T. & S. J. Kelly, 2002. Endoparasitic nematodes in cropping soils of Western Australia. Australian Journal of Experimental Agriculture, 42: 49-56.

Riley, I. T. & W. M. Wouts, 2001. *Pratylenchus* and *Radopholus* species in agricultural soils and native vegetation in southern Australia. Transactions and Proceedings of the Royal Society of South Australia, 125: 147-153.

Rubiales, D., S. Fondevilla, W. Chen, L. Gentzbittel, T. J. Higgins, M. A. Castillejo & N. Rispail, 2015. Achievements and challenges in legume breeding for pest and disease resistance. Critical Reviews in Plant Sciences, 34: 195-236.

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

Sharma, S. B., D. H. Smith & D. I. McDonald, 1992. Nematode constraints of chickpea and pigeon pea production in the semi-arid tropics. Plant Disease, 76 (9): 868-874.

Sikora, A. & N. Greco, 1990. “Nematode Parasites of Food Legumes, 181-235”. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, R. A. Sikora & J. Bridge). CAB International, Wallingford, UK, 261 pp.

Singh, M. & S. B. Sharma, 1994. Temperature effects on development and reproduction of *Heterodera cajani* on pigeon pea. Journal of Nematology, 26: 241-248.

Subbotin, S. A., E. J. Ragsdale, T. Mullens, P. A. Roberts, M. Mundo-Ocampo & J. G. Baldwin, 2008. A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18s and D2-D3 expansion segments of 28s ribosomal RNA genes and morphological characters. Molecular Phylogenetics and Evolution, 48: 491-505.

Sudupak, A., M. S. Akkaya & A. Kence, 2002. Analysis of genetic relationships among perennial and annual *Cicer* species growing in Turkey using RAPD markers. Theoretical and Applied Genetics, 105: 1220-1228.

Thompson, J. P., T. G. Clewett, J. G. Sheedy, 2010. Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. Australasian Plant Pathology, 39: 254-264.

Toktay, H., L. McIntyre, J. M. Nicol, H. Ozkan & H. I. Elekcioglu, 2006. Identification of common root lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. Genome, 49: 1319-1323.

Vanstone, V. A., A. J. Rathjen, A. H. Ware & R. D. Wheeler, 1998. Relationship between root lesion nematodes (*Pratylenchus negleclus* and *P. thornei*) and performance of wheat varieties. Australian Journal of Experimental Agriculture, 38: 18-188.

Waeyenberge, L., A. Ryss, M. Moens, J. Pinochet & T. C. Vrain, 2000. Molecular characterization of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. Nematology, 2: 135-142.

Whish, J. P. M., P. Castor & P. S. Carberry, 2007. Managing production constraints to the reliability of chickpea (*Cicer arietinum* L.) within marginal areas of the northern grains region of Australia. Crop and Pasture Science, 58: 396-405.

Yan, G., S. Richard, A. Patricia, P. A. Okubara, A. Skantar, A. E Sandra, G. S. Jason & T. Alison, 2008. Detection and Discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA Extracts from Soil, Plant Disease, 92: 1480-1487.

Young, T. W., 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Disease Reporter, 38: 794-795.