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Original article (Orijinal araştırma)

Current occurrence and prevalence of root-knot nematodes species, *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) in ware potato fields of Turkey

Yemeklik patates alanlarında kök-ur nematodu türlerinin, *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) mevcut durumu ve yaygınlığı

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Abstract

The study was conducted to determine the occurrence, frequency and density of species of *Meloidogyne* Goeldi, 1892 (Tylenchida: Meloidogynidae) in ware potato cultivation areas of Turkey in Plant Protection Central Research Institute. Soil samples were taken from Afyonkarahisar (149), Aksaray (69), Bolu (94), Kayseri (94), Konya (127), Nevşehir (91), Niğde (226) and Sivas (77) Provinces in 2018-2019. *Meloidogyne* juveniles were extracted by modified Baarmenn funnel and counted under inverted microscope. *Meloidogyne* populations were identified by species-specific primers. *Meloidogyne* spp. were detected in 84 of 927 soil samples. *Meloidogyne* spp. was detected in Aksaray, Kayseri, Nevşehir, and Niğde while was not found Afyonkarahisar, Bolu, Konya, and Sivas Provinces. In survey areas, the occurrence of *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 and *Meloidogyne hapla* (Chitwood, 1949) (Tylenchida: Meloidogynidae) was 8.7 and 1.5%, respectively. Also, *M. chitwoodi* and *M. hapla* mixed populations were found in 1.2% of samples. Mean density of *Meloidogyne* spp. J2s was determined as 182, 175, 162 and 90 J2s/100 ml of soil in Niğde, Nevşehir, Aksaray and Kayseri Provinces, respectively. In conclusion, important ware potato cultivation areas of Turkey were found to be infested with *Meloidogyne* spp., but the important seed potato-growing areas were found to be free of *Meloidogyne* spp.

Keywords: *Meloidogyne chitwoodi, Meloidogyne hapla*, seed potato, survey, ware potato

Öz

Bu çalışma Türkiye'deki yemeklik patates ekiliş alanlarında *Meloidogyne* Goeldi, 1892 (Tylenchida: Meloidogynidae) türlerinin bulunuş, yaygınlık ve yoğunluklarının belirlenmesi amacıyla Zirai Mücadele Merkez Araştırma Enstitüsü'nde yapılmıştır. Toprak örnekleri 2018-2019 yıllarında Afyonkarahisar (149), Aksaray (69), Bolu (94), Kayseri (94), Konya (127), Nevşehir (91), Niğde (226) ve Sivas (77) illerinden alınmıştır. *Meloidogyne* ikinci dönem larvaları modifiye Baarmenn huni tekniği ile elde edilmiş ve inverted mikroskopta sayılmıştır. *Meloidogyne* spp. popülasyonları türe spesifik primerler kullanılarak teşhis edilmiştir. Çalışma sonucunda 927 toprak örneğinin 84'ünün *Meloidogyne* spp. ile bulaşık olduğu belirlenmiştir. Aksaray, Kayseri, Nevşehir ve Niğde illerinde *Meloidogyne* spp. tespit edilirken Afyonkarahisar, Bolu, Konya ve Sivas illerinde bulunmamıştır. Sürvey alanında *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 ve *Meloidogyne hapla* (Chitwood, 1949) (Tylenchida: Meloidogynidae) yaygınlığı sırasıyla %8.7 ve %1.5 olarak belirlenmiştir. Ayrıca *M. chitwoodi* ve *M. hapla* karışık popülasyonlarının yaygınlığı ise %1.2 olarak belirlenmiştir. *Meloidogyne* spp. J2 ortalama yoğunluğu Niğde, Nevşehir, Aksaray ve Kayseri illerinde sırasıyla 182, 175, 162 ve 90 J2/100 ml toprak/J2 olarak belirlenmiştir. Çalışma sonucunda, önemli yemeklik patates ekiliş alanlarının yoğun olarak *Meloidogyne* spp. ile bulaşık olduğu ancak Türkiye'deki önemli tohumluk patates ekiliş alanlarının ise *Meloidogyne* spp. açısından temiz olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Meloidogyne chitwoodi*, *Meloidogyne hapla*, tohumluk patates, sürvey, yemeklik patates

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Introduction

Potato, *Solanum tuberosum* L. (Solanaceae) is the most important non-grain crop in the world and the fourth major food crop after maize, rice and wheat with an annual production of 370 Mt (FAOSTAT, 2019). Considered one of the most promising products in the fight against hunger and poverty, potatoes are highly recommended by FAO as a food security crop due to the growing population and food demand (Thomas & Sansonetti, 2009). Potato crops are damaged by numerous pests and pathogens, including nematodes (Niere & Karuri, 2018). Plant-parasitic nematodes are one of the crucial factors restricting potato yield and quality in many potato-growing areas (Lima et al., 2018). Potato cyst nematodes, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *Globodera pallida* (Stone, 1973) Behrens, 1975 (Tylenchida: Heteroderidae) and root-knot nematodes, *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) (RKN) are the major parasites of potato (Lima et al., 2018). Estimated annual yield losses in potato due to plant-parasitic nematodes is over 13% worth about US$6 billion worldwide (Ravichandra, 2014). While cyst nematodes have been more troublesome in the past, there have been drastic rises in root-knot nematodes due to the ban of some effective nematicides and soil fumigants such as 1,3-D, cadusafos and ethoprophos and also lacking resistant cultivars (Wesemael et al., 2011).

Root-knot nematodes include the most economically damaging plant-parasitic nematodes to crops worldwide (Karssen & Moens, 2006). Although the genus *Meloidogyne* includes about 100 species, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949*, Meloidogyne javanica* (Treub, 1885) Chitwood, 1949*, Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949*, Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980*, Meloidogyne fallax* Karssen, 1996 and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) are the most common species. These species account for over 95% of the *Meloidogyne* occurrences in the world (Adam et al., 2007; Jones et al., 2013). The impact of these species is increasing due to their wide host ranges and it is estimated that they can affect more than 5,500 plant species (Trudgill & Blok, 2001) with crop losses of about 5% worldwide (Agrios, 2005).

Potato is one of the main hosts of *Meloidogyne* spp., and the prevalence and damage of these species have been increasing in recent decades. The damage of RKN to potatoes has been determined in all continents except Antarctica, including the major potato producing countries including China (Mao et al., 2019), India (Singh & Kumar, 2015), the USA (Golden et al., 1980), Germany (Müller et al., 1996), France (Djian-Caporalino, 2012), Holland (Keidel et al., 2007), South Africa (Fourie et al., 2001), and Australia (Nobbs et al., 2001). In potato production, *M. arenaria*, *Meloidogyne enterolobii* Yang & Eisenback, 1983, *M. incognita* and *M. javanica* and are present in warmer climates, while *M. chitwoodi*, *M. fallax, M. hapla* and *Meloidogyne minor* Karssen et al., 2004 are encountered in relatively colder climates (Nyczepir et al., 1982; Nobbs et al., 2001; Thoden et al., 2012; Onkendi & Moleleki, 2013; Medina et al., 2017). Of these, *M. arenaria, M. chitwoodi*, *M. fallax*, *M. hapla, M. incognita* and *M. javanica* are significant for potato production (Lima et al., 2018). In Turkey, *M. chitwoodi*, *M. hapla, M. incognita*, and *M. javanica* have been determined in potato-growing areas, however *M. chitwoodi* is the most common one among them (Kepenekci et al., 2006; Özarslandan & Elekcioğlu, 2010; Erdoğus et al., 2011; Evlice & Bayram, 2016; Demirbaş Pehlivan et al., 2020).

There are no reports of specific aboveground symptoms in potato associated with root-knot nematode infestation, however varying degrees of stunting, yellowing, and wilting under moisture stress can be seen in some of the infested plants. Root-knot nematodes reproduce on potato roots and tubers, but the size and shape of galls or knots could vary depending on the species and density of nematodes (Niere & Karuri, 2018). The economic threshold on potato cultivation in the USA and the Netherlands are 1 and 10 J2s/100 ml soil for *M. chitwoodi*, respectively (Brodie et al., 1993; Norshie et al., 2011) and 50 eggs/250 ml soil for *M. hapla* (Brodie et al., 1993).

Galls of temperate species, *M. chitwoodi*, *M. fallax*, *M. hapla* and *M. minor*, are usually smaller than those caused by other species and cause extensive lateral root formation. However, these species cause numerous small pimple-like swelling on the tuber surface (Elling, 2013). Even 5% necrotic spotting in the flesh of tubers make them inappropriate for the fresh market, and if 5-15% of the tubers have defects when harvested, the whole field crop can be discarded (King & Taberna, 2013).

For management, in most countries, at least a 2-year rotation is used in potato production. There are no commercially available potato cultivars resistant to root-knot nematodes. Under such conditions, *Meloidogyne* spp. populations can increase rapidly. For this reason, the most important and widely used management strategy is to apply nematicides.

This study has been conducted to determine (1) occurrence of the root-knot nematodes and species, (2) frequency and density of root-knot nematode species in ware potato-growing areas in Turkey.

Materials and Methods

Sampling strategy and sample processing

Surveys were conducted in 8 potato-growing provinces (Afyonkarahisar, Aksaray, Bolu, Kayseri, Konya, Nevşehir, Niğde and Sivas) between 2018 and 2019. A total of 927 soil samples were taken from fields immediately after potato harvest, 0.1% of the annual ware potato production area in each province (Figure 1). Soil sample of 2-3 kg consisting of 50 subsamples according to a grid pattern from 0-30 cm depth was randomly obtained from each field using an auger. The soil samples and symptomatic potato tubers (Figure 2) were placed in a plastic container. For each sampled area, GPS coordinates of sampling sites were recorded.

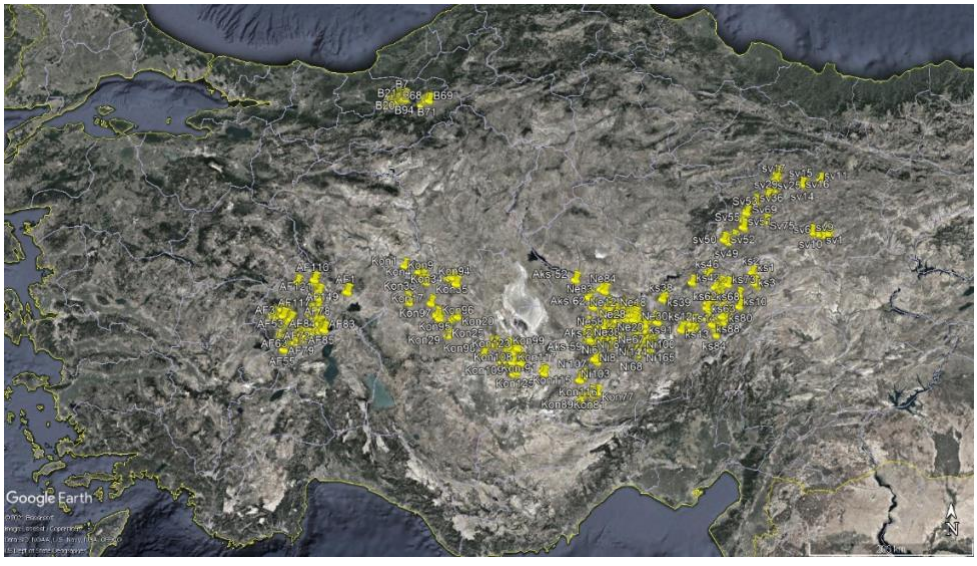

Figure 1. Soil samples taken from potato-growing areas in this study (mapped using www.earth.google.com).

Soil samples were mixed thoroughly and 200 ml subsamples were processed for nematode extraction for each field. Root-knot nematodes were extracted by the modified Baermann funnel technique (Hooper, 1986). After 24 h, nematode suspension was transferred to 100 ml graduated cylinder and collected in a 50 ml sample bottle for examination. The whole suspension was checked for *Meloidogyne* spp. and counted under an invert microscope (Leica DMI 400B).

DNA extraction

DNA were extracted from second-stage juveniles (J2s) for each isolate according to Waeyenberge et al. (2000). For this purpose, 10 *Meloidogyne* spp. J2s was picked from extracted soil samples using a small needle and cut into two pieces with a sterile scalpel under a stereomicroscope (Leica M165C) in 20 µl of molecular grade water on a glass microscope slide, then 10 µl with the nematode pieces was transferred by pipette into a 0.2 ml centrifuge tube containing 8 µl of worm lysis buffer (WLB; 500 mM KCl, 100 mM Tris-Cl pH 8.3, 15 mM MgCl2, 10 mM DTT, 4.5% Tween 20, 0.1% gelatin). After adding 2 µl of proteinase K (600 g/ml; Thermo Fisher Scientific, Waltham, MS, USA) the suspension was centrifuged at 13,500 rpm for 2 min and frozen to -80ºC for 10 min. After freezing, the contents were thawed rapidly. The lysis mix was incubated at 65ºC for 1 h followed by 95ºC for 10 min for a proteinase K inactivation. After incubation, the tubes were centrifuged for 1 min at 14,000 rpm and stored at -20ºC until used.

Figure 2. Potato tuber infested with *Meloidogyne chitwoodi*, pimple-like galls and necrotic spots on their surface.

DNA Amplification protocols

The primers used in PCR amplification for the identification of root-knot nematodes are given in Table 1. All multiplex PCR amplifications were conducted in a total volume of 25 µl consisting of 10x PCR Buffer, 200 µM dNTPs 0.2 µM of each primer, 1 unit Taq DNA polymerase (Thermo Fisher Scientific) and 2 µl template DNA. All DNA amplifications were conducted with a thermocycler (Techne, TC-5000).

Table 1. Primers used in the multiplex PCR to identify the species of *Meloidogyne* spp.

Species	Fragments	Primer	Primer sequences (5'-3')	Reference
Meloidogyne chitwoodi	540 bp	JMV1	GGATGGCGTGCTTTCAAC	Wishart et al., 2002
Meloidogyne fallax	670 bp	JMV2	TTTCCCCTTATGATGTTTACCC	
Meloidogyne hapla	440 bp	JMVhapla	AAAAATCCCCTCGAAAAATCCACC	
Meloidogyne incognita	150 bp	MincF1	GCACCTCTTTCATAGCCACG	Devran et al., 2018
		MincR1	GGTGCGCGATTGAACTGAGC	
Meloidogyne javanica	670 bp	Fjav	GGTGCGCGATTGAACTGAGC	Zijlstra et al., 2000
		Rjav	CAGGCCCTTCAGTGGAACTATAC	
Meloidogyne arenaria	420 bp	Far	TCGGCGATAGAGGTAAATGAC	Zijlstra et al., 2000
		Rar	TCGGCGATAGACACTACAACT	

The cycling parameters were as follows for *M. chitwoodi*, *M. fallax*, and *M. hapla*: an initial denaturation step at 94ºC for 3 min followed by 35 cycles at 94ºC for 30 s, 55ºC for 30 s, then 72ºC for 90 s and final extension step at 72ºC for 10 min; and for *M. incognita*, *M, javanica*, *M. arenaria*: initial denaturing step for 3 min at 95ºC followed by 35 cycles at 95ºC for 30 s, 56ºC for 30 s, then 72ºC for 60 s and a final extension step at 72ºC for 7 min. PCR products were separated by electrophoresis in Tris-EDTA buffer with 1.7% agarose gel stained with Pronosafe (Condalab, Spain) at 80 V for 1 h and then visualized with an image acquisition system (Vilber QUANTUM ST4 1000, Collégien, France).

***Meloidogyne* spp. community analyses**

The incidence, occurrence, and frequency of root-knot nematodes determined in the study at the genus and species level in potato-growing areas were calculated according to the formulas below (Norton, 1978).

$$Occurence\ of genus\ or\ species = \frac{Number\ of\ sample\ with\ root-knot\ nematodes\ infection}{Total\ number\ of\ sample\ surveyed} X100$$

$$Absolute\ frequency = \frac{Number\ of\ sample\ containing\ species}{Number\ of\ sample\ collected} X100$$

$$Relative\ frequency = \frac{Frequency\ of\ occurence\ of\ species}{Sum\ of\ frequency\ of\ all\ Meloidogyne\ spp.} X100$$

Results

Species identification

Multiplex PCR analysis by using JMV1/JMV2/JMVhapla primers produced a DNA fragment size of 540 bp specific to *M. chitwoodi* for 70 populations, 440 bp specific to *M. hapla* for three populations, and also both 540 bp and 440 bp for 11 populations. However, there was no 670-bp amplification product which was characteristic for the *M. fallax*. In addition, primer sets Far/Rar, MincF1/MincR1 and Fjav/Rjav, specific for *M. arenaria, M. incognita* and *M. javanica*, respectively, did not yield any amplification products. These results showed that 70 populations were *M. chitwoodi*, three populations were *M. hapla*, and 11 populations were mixed *M. chitwoodi* and *M. hapla.* There was no evidence of the existence of *M. arenaria*, *M. fallax, M. incognita* or *M. javanica* as previously reported in potato areas in Central Anatolia (Table 2).

***Meloidogyne* spp. community analyses**

J2s of *Meloidogyne* spp. were detected in 84 of 927 soil samples in all surveyed areas. *Meloidogyne* spp. was detected in Aksaray, Kayseri, Nevşehir and Niğde Provinces, but not in Afyonkarahisar, Bolu, Konya and Sivas Provinces (Tables 2 & 3). The occurrence, frequency and population density of *Meloidogyne* genus and species differed according to the location sampled. The occurrence of RKNs in all surveyed areas was 9.1% with a mean density of 176 J2s/100 ml of soil. *Meloidogyne chitwoodi* was the most abundant species in all provinces infested with *Meloidogyne* spp. The occurrence of *M. chitwoodi* and *M. hapla* in the field surveys were 8.7 and 1.5%, respectively, and *M. chitwoodi* and *M. hapla* mixed populations were 1.2%. *Meloidogyne chitwoodi* was the most prevalent species in all surveyed areas, with 96.4 and 85.3% absolute and relative frequency, respectively. This was followed by *M. hapla* with 16.7 and 14.7% absolute and relative frequency, respectively.

The highest occurrence of RKNs was found in Nevşehir with 25.3%, followed by Niğde, Aksaray, and Kayseri with 23.5, 8.7, and 2.1%, respectively. The occurrence and absolute frequencies were 25.3, 22.1%, 8.7 and 2.1%, and 100, 94.3, 100 and 100% in Nevşehir, Niğde, Aksaray, and Kayseri Provinces, respectively. *Meloidogyne hapla* was only found as the sole species in Niğde, but it was found in mixed populations with *M. chitwoodi* in Aksaray and Nevşehir Provinces. Additionally, *M. hapla* was not detected in any samples from Kayseri Province. Its occurrence and absolute frequencies were found to be 4.4, 3.5

and 2.9%, and 17.4, 15.1 and 33.3% in Nevşehir, Niğde and Aksaray Provinces, respectively. Mean density of *Meloidogyne* spp. J2s were similar in Nevşehir (175 J2s/100 ml of soil), Niğde (182 J2s/100 ml of soil) and Aksaray (162 J2s/100 ml of soil) Provinces, but lower in Kayseri (90 J2s/100 ml of soil).

Table 2. Occurrence of *Meloidogyne* spp. on potatoes collected from ware potato fields in Turkey

Province	District	Village	Altitude (m)	Coordinate	Species	J2s/100 ml soil
Aksaray	Gülağaç	-	1192	38º21'8.15"N 34º20'49.16"E	*M. chitwoodi*	44
		Demirci	1190	38º20'42.49"N 34º18'40.94"E	*M. chitwoodi*	108
	Güzelyurt	Alanyurt	1204	38º20'1.76"N 34º19'7.08"E	*M. chitwoodi, M. hapla*	237
			1212	38º20'40.34"N 34º20'16.93"E	*M. chitwoodi, M. hapla*	194
		Bozcayurt	1200	38º21'30.02"N 34º22'23.10"E	*M. chitwoodi*	83
	Merkez	Yenipınar	1412	38º12'37.95"N 34º16'50.61"E	*M. chitwoodi*	305
Kayseri	İncesu	Örenşehir	1046	38º41'42.26"N 35º14'46.92"E	*M. chitwoodi*	83
	Melikgazi	Yeşilyurt	1086	38º48'43.61"N 35º36'49.44"E	*M. chitwoodi*	97
Nevşehir	Acıgöl	Kurugöl	1288	38º22'32.75"N 34º32'27.47"E	*M. chitwoodi*	124
	Derinkuyu	Çakıllı	1342	38º24'28.98"N 34º39'13.37"E	*M. chitwoodi*	149
		Doğala	1387	38º25'37.96"N 34º36'26.69"E	*M. chitwoodi*	53
		Kuyulutatlar	1305	38º22'2.92"N 34º32'4.62"E	*M. chitwoodi*	196
			1292	38º23'20.81"N 34º31'48.68"E	*M. chitwoodi*	79
		Til	1500	38º25'29.67"N 34º47'23.46"E	*M. chitwoodi*	148
		Yazıhüyük	1287	38º21'22.85"N 34º36'42.45"E	*M. chitwoodi*	207
		-	1389	38º20'50.37"N 34º46'51.10"E	*M. chitwoodi, M. hapla*	417
		-	1320	38º20'37.51"N 34º44'35.28"E	*M. chitwoodi*	202
		-	1336	38º21'23.18"N 34º44'23.79"E	*M. chitwoodi, M. hapla*	581
		-	1363	38º23'6.14"N 34º44'52.66"E	*M. chitwoodi*	299
		-	1323	38º20'34.63"N 34º44'36.57"E	*M. chitwoodi, M. hapla*	45
		-	1336	38º21'23.28"N 34º44'23.78"E	*M. chitwoodi*	217
		-	1361	38º22'51.59"N 34º43'22.27"E	*M. chitwoodi*	102
		-	1449	38º27'26.94"N 34º42'11.31"E	*M. chitwoodi, M. hapla*	52
		-	1392	38º21'26.63"K 34º45'39.54"D	*M. chitwoodi*	74
	Merkez	Çardak	1373	38º32'43.71"N 34º46'8.02"E	*M. chitwoodi*	48
			1409	38º32'0.91"N 34º45'33.11"E	*M. chitwoodi*	294
		İcik	1384	38º29'56.68"N 34º35'42.48"E	*M. chitwoodi*	61
			1363	38º30'51.49"N 34º35'55.92"E	*M. chitwoodi*	121
		Kaymaklı	1423	38º28'56.74"N 34º45'10.41"E	*M. chitwoodi*	56
			1406	38º27'13.72"N 34º44'38.68"E	*M. chitwoodi*	81
	Ürgüp	Bahçeli	1474	38º31'0.78"N 34º47'23.01"E	*M. chitwoodi*	427
Niğde	Altunhisar	-	1212	38º0'30.00"N 34º19'39.00"E	*M. chitwoodi*	95
	Çiftlik	Azatlı	1572	38º12'39.63"N 34º31'2.85"E	*M. hapla*	61
			1582	38º13'0.93"N 34º31'22.52"E	*M. chitwoodi*	349
			1541	38º12'9.00"N 34º28'35.00"E	*M. chitwoodi*	57
			1580	38º11'51.02"K 34º29'9.67"D	*M. chitwoodi*	134
		Bozköy	1575	38º13'10.00"N 34º29'10.00"E	*M. chitwoodi, M. hapla*	167
			1591	38º13'43.00"N 34º29'10.00"E	*M. chitwoodi*	69
			1569	38º13'4.00"N 34º29'18.00"E	*M. chitwoodi*	60
		Divarlı	1546	38º12'15.00"N 34º28'51.00"E	*M. chitwoodi*	45
			1542	38º12'49.93"N 34º27'55.76"E	*M. hapla*	301
			1643	38º13'45.00"N 34º28'16.00"E	*M. chitwoodi*	59
		Kitreli	1422	38º11'48.28"N 34º21'31.36"E	*M. chitwoodi*	74

Table 2 Continued

Province	District	Village	Altitude (m)	Coordinate	Species	J2s/100 ml soil
		Ağcaşar	1320	38º18'35.03"K 34º45'15.70"D	*M. chitwoodi, M. hapla*	1159
		Alay	1302	38º17'5.84"N 34º41'32.33"E	*M. chitwoodi*	34
			1311	38º16'14.72"N 34º40'44.60"E	*M. chitwoodi*	56
		Bağlama	1308	38º16'2.60"N 34º40'35.93"E	*M. chitwoodi*	67
			1330	38º16'29.16"N 34º39'25.84"E	*M. chitwoodi*	295
			1325	38º16'16.63"N 34º39'32.55"E	*M. chitwoodi*	86
			1318	38º15'23.97"N 34º39'40.18"E	*M. chitwoodi*	45
			1315	38º16'8.33"N 34º40'25.80"E	*M. chitwoodi*	413
		Edikli	1368	38º14'7.20"N 34º55'10.86"E	*M. chitwoodi*	102
			1363	38º14'2.05"N 34º55'10.63"E	*M. chitwoodi*	147
			1428	38º17'0.66"N 34º53'35.87"E	*M. chitwoodi*	309
			1429	38º17'43.11"N 34º53'18.59"E	*M. chitwoodi*	416
		Kiledere	1306	38º16'36.40"N 34º40'45.45"E	*M. chitwoodi*	74
			1297	38º17'11.49"N 34º41'13.01"E	*M. chitwoodi*	56
			1300	38º17'34.37"N 34º41'11.16"E	*M. chitwoodi*	85
		Konaklı	1343	38º7'31.52"N 34º49'52.42"E	*M. chitwoodi*	167
			1339	38º8'40.37"N 34º49'41.42"E	*M. chitwoodi*	191
			1335	38º10'48.69"N 34º50'5.69"E	*M. chitwoodi*	368
			1343	38º11'37.52"N 34º49'23.86"E	*M. chitwoodi*	152
			1366	38º13'33.99"N 34º51'5.63"E	*M. chitwoodi*	202
Niğde	Merkez		1426	38º16'39.74"N 34º52'28.59"E	*M. chitwoodi*	64
		Orhanlı	1452	38º19'29.20"N 34º50'21.20"E	*M. chitwoodi*	445
			1426	38º17'16.40"N 34º52'58.64"E	*M. chitwoodi*	83
			1425	38º16'16.69"N 34º52'27.42"E	*M. chitwoodi*	367
			1452	38º18'27.21"N 34º52'48.99"E	*M. chitwoodi, M. hapla*	281
			1459	38º18'56.21"N 34º51'54.59"E	*M. chitwoodi, M. hapla*	384
			1474	38º19'38.19"N 34º56'26.21"E	*M. chitwoodi*	194
			1465	38º19'1.57"N 34º55'27.45"E	*M. chitwoodi*	226
			1529	38º20'43.58"K 34º48'48.41"D	*M. chitwoodi*	416
		Ovacık	1332	38º4'35.66"N 34º48'1.81"E	*M. chitwoodi*	281
			1383	38º8'33.71"K 34º46'39.37"D	*M. chitwoodi*	67
		Tırhan	1304	38º14'35.99"N 34º42'25.47"E	*M. chitwoodi*	81
			1296	38º15'0.00"N 34º43'50.54"E	*M. hapla*	194
			1293	38º15'9.37"N 34º43'28.00"E	*M. chitwoodi*	84
			1296	38º14'41.51"N 34º42'54.20"E	*M. chitwoodi, M. hapla*	106
			1297	38º14'17.73"N 34º43'4.81"E	*M. chitwoodi*	73
		Yeşilgölcük	1307	38º16'27.08"N 34º45'37.98"E	*M. chitwoodi*	124
			1308	38º15'56.28"N 34º45'41.84"E	*M. chitwoodi*	64
			1308	38º15'17.14"N 34º46'0.56"E	*M. chitwoodi*	81
			1304	38º14'57.90"N 34º45'51.97"E	*M. chitwoodi*	50
			1305	38º13'59.33"N 34º46'13.14"E	*M. chitwoodi*	63

Table 3. Community analyses of *Meloidogyne* spp. on potato in the survey areas

	Survey area	Afyonkarahisar	Aksaray	Bolu	Kayseri	Konya	Nevşehir	Niğde	Sivas
Number of samples	928	149	69	94	94	127	91	226	77
Meloidogyne spp. infestation	84	0	6	0	2	0	23	53	0
M. chitwoodi infestation	81	0	6	0	2	0	23	50	0
M. hapla infestation	14	0	2	0	0	0	4	8	0
M. chitwoodi+*M. hapla* infestation	11	0	2	0	0	0	4	5	0
Occurence of *Meloidogyne* spp.	9.1%	-	8.7%	-	2.1%	-	25.3%	23.5%	-
Occurence of *M. cihtwoodi*	8.7%	-	8.7%	-	2.1%	-	25.3%	22.1%	-
Occurence of *M. hapla*	1.5%	-	2.9%	-	-	-	4.4%	3.5%	-
Occurence of *M. chitwoodi*+*M. hapla*	1.2%	-	-	-	-	-	4.4%	2.2%	-
Absolute frequency of *M. chitwoodi*	96.4%	-	100%	-	100%	-	100%	94.3%	-
Absolute frequency of *M. hapla*	16.7%	-	33.3%	-	-	-	17.4%	15.1%	-
Relative frequency of *M. chitwoodi*	85.3%	-	75%	-	100%	-	85.2%	86.2%	-
Relative frequency of *M. hapla*	14.7%	-	25%	-	-	-	14.8%	14.8%	-

Discussion

The survey area covered about 58% of the potato production and harvested area of Turkey (TUIK, 2019). The findings showed that a total of 927 soil samples were obtained from potato-growing areas in eight provinces of Turkey, of which 9.1% were infested with *Meloidogyne* spp. *Meloidogyne chitwoodi* and *M. hapla* were detected at 8.7 and 1.5%, respectively, and *M. chitwoodi* and *M. hapla* were found in mixed populations in 1.2% of the samples. Nine root-knot species, *M. arenaria*, *Meloidogyne artiellia* Franklin, 1961, *M. chitwoodi*, *Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne exigua* Goeldi, 1887, *M. hapla*, *M. incognita*, *M. javanica* and *Meloidogyne thamesi* Chitwood, 1952 have been identified in different regions and different crops in Turkey (Yüksel, 1966; 1967; Elekçioğlu, 1992; Özarslandan et al., 2009; Aydınlı et al., 2013; Kepenekci et al., 2014; İmren et al., 2014). However, four root-knot species, *M. chitwoodi*, *M. hapla*, *M. incognita* and *M. javanica*, have been found in association with potato in Turkey (Kepenekci et al, 2006; Özarslandan et al., 2009; Erdoğus et al., 2011; Demirbaş Pehlivan et al., 2020). The first report of root-knot nematode in potatoes appears to have been made by Kepenekci et al (2006) as *M. hapla* from potato tubers collected from Aksaray, Nevşehir and Niğde Provinces. Following that, *M. chitwoodi* was found in Niğde potato-growing areas by Özarslandan et al (2009). This finding has been supported by other studies in which only *M. chitwoodi* was detected and *M. chitwoodi* has since be found in Aksaray, Balıkesir, Bitlis, Isparta, İzmir, Kayseri, Konya, Kütahya, Manisa and Nevşehir Provinces (Özarslandan & Elekcioğlu, 2010; Ulutaş et al., 2012; Özarslandan et al., 2013; Evlice & Bayram, 2016). The present findings are similar to previous studies, and the relative frequency of *M. hapla* (14.7%) was quite low compared to *M. chitwoodi* (85.3%). *Meloidogyne incognita* was first identified in the potato-growing areas of Edirne, then in İzmir (Erdoğus et al., 2011; Demirbaş Pehlivan et al., 2020). Additionally, *M. javanica*, *M. hapla* and *M. chitwoodi* was determined in potato fields in İzmir (Yıldız et al., 2009; Demirbaş Pehlivan et al., 2020). The occurrence of *Meloidogyne* spp. in potato cultivation areas was higher in İzmir (18.4%) than found overall in the present survey (9.1%) but lower than Nevşehir (25.3%) and Niğde (23.5%). Differing from the present findings, the dominant species in the potato cultivation areas of İzmir Province was determined as *M. incognita* and the relative frequency of *M. incognita*, *M. javanica*, *M. hapla* and *M. chitwoodi* was found 61, 24.4, 12.2 and 2.4%, respectively (Demirbaş Pehlivan et al., 2020). This was an expected result due to the widespread cultivation of good hosts of root-knot nematodes in the Aegean Region and the prevalence of tropical root-

knot nematodes species in these areas (Kaşkavalcı & Öncüer, 1999; Yağcı & Kaşkavalcı, 2018). Similar results were obtained in other surveys conducted in warmer regions worldwide (Okendi & Moleleki, 2013; Medina et al., 2017). Temperate species such as *M. chitwoodi* and *M. hapla* are cold temperature nematodes whereas tropical species such as *M. incognita* and *M. javanica* prefer at higher temperatures and cannot tolerate cold temperatures (Evans & Perry, 2009). The base threshold temperature is 4ºC and 8ºC for *M. chitwoodi* and *M. hapla* while 10ºC and 13ºC for *M. incognita* and *M. javanica*, respectively (Inserra et al., 1983; Lahtinen et al., 1988; Madulu & Trudgill, 1994; Ploeg & Maris, 1999).

The results presented here show that the mean density of *Meloidogyne* spp. ranged from 34 to 1159 J2s/100 ml of soil. *Meloidogyne chitwoodi* and *M. hapla* are the most important species within *Meloidogyne* for potatoes (Brodie et al., 1993). The economic threshold of *M. chitwoodi* in potatoes is reported to be 1 and 10 J2s/100 ml soil in the USA and Holland, respectively, whereas it is as 50 eggs/250 ml soil for *M. hapla* (Brodie et al., 1993; Norshie et al., 2011). Therefore, in the present study, the population density of RKN was above the economic damage threshold in all areas where it was determined. Crop loss from RKN infestation can be about 25%, depending on cultivars, environmental factors and population density, but may reach 100% in potato fields (Mai et al., 1981; Lima et al., 2018). The economic loss of cultivated potatoes caused by *M. chitwoodi* can reach US$ 9 900/ha (Ingham et al., 2007). Potato roots and tubers can be invaded by root-knot nematodes. However, the first generation resides mostly on the root system, with subsequent generations entering the tubers (Pinkerton et al., 1991). Depending on the nematode density and species, infested roots and tubers may have galls of varying sizes and shapes. *Meloidogyne chitwoodi* and *M. hapla* produced galls that are normally smaller than those induced by other species (Niere & Karuri, 2018). Even 5% necrotic spotting in the flesh of tubers makes them commercially unacceptable for the fresh market. The entire crop can be rejected if 5 to 15% of the field tubers have visual defects when processed (King & Taberna, 2013). While potato genotypes resistant to *M. chitwoodi* have been identified, there is currently no commercially available resistant cultivars (Brown et al., 2006; Brown et al., 2009; Norshie et al., 2011). For this reason, the application of nematicides is the most commonly used control measure (Jones et al., 2017). The approved rates in the USA for some nematicides for control of *M. chitwoodi* are higher than for other *Meloidogyne* spp. (Lima et al., 2018). Crop rotation or succession with non- or poor hosts, shortening the growing period, planting certified seed and destruction of volunteer potato plants are some of the other control methods (Jones et al., 2017).

This study demonstrated that important ware potato cultivation areas, such as in Nevşehir and Niğde, where seed potato cultivation has been prohibited, are heavily infested with *M. chitwoodi*. However, the most important seed potato-growing areas in Turkey including Afyonkarahisar, Konya and Sivas were free from *Meloidogyne* spp. Therefore, quarantine measures should be applied meticulously to prevent infestations in these areas. Turkey's annual requirement is about 510 kt of seed potatoes and the use of seed potatoes is about 340 kt (BÜGEM, 2019; TUİK, 2019). The difference of about 45% between production and use is due to the use of non-seed potatoes for planting. Although *Meloidogyne* spp. causes pimple-like swellings on the surface of potato tubers, these symptoms depend on the population density and the duration of vegetative growth. For this reason, asymptomatically infested tubers are observed in most cases, and the use of these tubers as seeds must be avoided to prevent the spread of *Meloidogyne* spp. to uninfested areas.

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