

Original article (Orijinal araştırma)

Identification and prevalence of potato cyst nematodes and root-knot nematodes in the potato production areas of İzmir Province, Turkey¹

İzmir İli (Türkiye) patates üretim alanlarında patates kist nematodları ve kök-ur nematodlarının teşhisi ve yaygınlığı

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Abstract

Globodera spp. and *Meloidogyne* spp. were identified using morphological and molecular methods. Also, the distribution and population densities of these nematodes were determined in potato cultivation areas of İzmir (Turkey) in 2015. Two hundred and twenty-three soil samples were collected during the survey and 32 samples were found to be infested with *Globodera* spp. and 41 samples with *Meloidogyne* spp. The identification of nematodes was made morphologically using perennial patterns of cyst/female individuals and morphometrics of second stage juveniles. Also, species specific primers were used for molecular identification. Only *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) were found in the samples that contained cyst nematodes. Also, the root-knot nematodes, *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *Meloidogyne hapla* (Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) were found. The prevalence rates of *Meloidogyne* species were determined as 2.4, 12.2, 61.0 and 24.4%, respectively. In terms of the number of individuals in soil, all *G. rostochiensis* (10 eggs/g soil) and *M. chitwoodi* (1 juvenile/250 cm³ soil) population levels were detected above the economic damage thresholds for potato production. Also, two populations of *M. incognita* (0.5-2 juvenile/250 cm³ soil) were found above the specified threshold levels.

Keywords: *Globodera* spp., *Meloidogyne* spp., molecular identification, potato, Turkey

Öz

Globodera spp. ve *Meloidogyne* spp. morfolojik ve moleküler yöntemler kullanılarak tanımlanmıştır. Ayrıca, 2015 yılında İzmir (Türkiye) patates üretim alanlarında bu nematodların dağılımı ve popülasyon yoğunlukları tespit edilmiştir. Sürvey sırasında toplam 223 toprak örneği alınmış ve 32 örneğin *Globodera* spp. ile 41 örneğin *Meloidogyne* spp. ile bulaşık olduğu tespit edilmiştir. Nematodların tür teşhisi, kist/dişi bireylerin anal kesitleri ve ikinci dönem larvaların morfolojik ölçümleri kullanılarak morfolojik olarak yapılmıştır. Aynı zamanda moleküler tür teşhislerinde türe özgü spesifik primerleri kullanılmıştır. Kist nematodları ile bulaşık olan tüm örneklerde sadece *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) türü bulunmuştur. Ayrıca, kök-ur nematodlarından *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *Meloidogyne hapla* (Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 ve *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) türleri tespit edilmiştir. *Meloidogyne* türlerinin yaygınlık oranları sırasıyla %2.4, 12.2, 61.0 ve 24.4 olarak belirlenmiştir. Topraktaki birey sayıları açısından, tüm *G. rostochiensis* (10 yumurta/g toprak) ve *M. chitwoodi* (1 larva/250 cm³ toprak) popülasyon yoğunlukları patates üretimi için ekonomik zarar eşiklerinin üzerinde tespit edilmiştir. *Meloidogyne incognita*'ya ait iki popülasyon (0.5-2 larva/250 cm³ toprak) da belirtilen eşik seviyelerinin üzerinde bulunmuştur.

Anahtar sözcükler: *Globodera* spp., *Meloidogyne* spp., moleküler tanılama, patates, Türkiye

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Introduction

Potato (*Solanum tuberosum* L.) is a tuberous plant in the Solanaceae family that has been spread to various climatic regions around the world from its native origin in South America. It is one of the most produced crops after corn, rice and wheat (Günel et al., 2005). World potato production was about 390 Mt in 2016 (FAO, 2017). In Turkey, annual production is around 5 Mt from an area of 142 Kha. İzmir is one of the most important provinces for potato production in Turkey (TÜİK, 2017). However, there are many pests and diseases that negatively affect potato production. Among these, plant parasitic nematodes are important and some of them are on the quarantine list. In particular, cyst nematodes, *Globodera* spp. Skarbilovich, 1959 (Tylenchida: Heteroderidae) and root-knot nematodes, *Meloidogyne* spp. Göeldi, 1892 (Tylenchida: Meloidogynidae) are on the top of the quarantine list in Europe (EPPO, 2020a, b).

One of the most important plant parasitic nematode genera that causes economic losses in potato is *Globodera* spp. (Van Riel & Mulder, 1998). Although tomato, eggplant, other *Solanum* plants and hybrids are important hosts of *Globodera* spp., potato is a crucial host for these nematodes (Anonymous, 2019). Typical symptoms of *Globodera* spp. especially aboveground are weakly developed plants and yellowing, which is similar to water and nutrient deficiencies, growth retardation, wilting under heat stress, and also reduced size and number of tubers (EPPO, 2017).

Globodera spp. was recorded for the first time in potato cultivation areas of Bolu Province in Turkey and was reported to be quarantined in the area (Enneli & Öztürk, 1996). Also, Özarıslandan et al. (2009) found that potato production areas of Niğde Province were infested with *Meloidogyne chitwoodi* Golden, O'Bannon, Santos & Finley, 1980. Yıldız et al. (2009) reported that the infestation rate of *M. chitwoodi* was 37.9% in the potato areas of the Ödemiş District of İzmir. Moreover, Ulutaş (2010) reported that *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 infestation in Aegean Region potato production areas was 17.5 and 61.7% in Ödemiş District of İzmir.

Meloidogyne spp., which has more than 90 described species, is the most destructive, damaging and economically important plant parasitic nematode genera that affects most of the agricultural crops around the world (Hunt & Handoo, 2009; Jones et al., 2013). *Meloidogyne* spp. can cause yellowing, stunting, wilting and brown spots in potato plants. Also, these nematodes can lead to physical and chemical deformations in the tubers which can reduce their market value (Vovlas et al., 2005). *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne chitwoodi* Golden, O'Bannon, Santos & Finley, 1980, *Meloidogyne fallax* (Karssen, 1996), *Meloidogyne hapla* (Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae), which cause economic crop losses, have been identified in potato cultivation areas in tropical and subtropical regions (Brodie et al., 1993; Molendijk & Mulder, 1996; Vovlas et al., 2005; Moens et al., 2009; Wesemael et al., 2011; Jones et al., 2013; Okendi & Moleleki, 2013). Although there are many studies conducted with plant parasitic nematodes on different crops in Turkey (Yüksel, 1966, 1967, Elekcioğlu & Uygun, 1994; Kaşkavalcı & Öncüer, 1999; Kepenekci et al., 2002; Özarıslandan et al., 2009; Aydınlı et al., 2013; İmren et al., 2014; Aydınlı, 2018), only a few recent studies have examined potato cultivation areas in İzmir Province. Therefore, this survey was performed to identify *Globodera* and *Meloidogyne* spp. in İzmir Province using the morphological and molecular methods. Reliable and quick identification of nematode species is crucial for choosing appropriate integrated management strategies.

Materials and Methods

Materials

The main material of the study consisted of soil samples that were collected from potato production areas in İzmir Province, and *Meloidogyne* and *Globodera* spp. individuals obtained from the soil. In addition,

primers, consumables, chemicals and equipment were used in molecular studies. Besides, the GPS was used to obtain the coordinates was used.

Survey

The survey studies were conducted during the potato production season of 2015, in two cultivation periods (May-June and September-November) in potato production areas in İzmir Province. One hundred and fifteen soil samples were collected from Bayındır, Dikili, Kiraz, Ödemiş and Tire Districts of İzmir Province during the spring potato production period (May-June 2015) and 108 soil samples from Ödemiş during the autumn potato production season (September-November 2015).

Fenwick can (Fenwick, 1940) was used to extract *Globodera* spp. from soil samples, and motile plant parasitic nematodes were extracted using a modified Baermann funnel method (Hooper, 1986), specifically for the second stage juveniles of *Meloidogyne* spp. The prevalence of nematodes in these districts were determined as proportion of *Globodera* and *Meloidogyne* spp. in infested soil samples to the total number of the samples. Moreover, *Globodera* and *Meloidogyne* spp., the number of cysts and juveniles were counted under microscope in order to investigate the density of each population. The soil samples infested with *Meloidogyne* spp. were placed separately in plastic pots and one susceptible tomato (SC2121) seedling was planted in each pot in a greenhouse at 25°C and 60% RH. After 3 months, the infected tomato plants were harvested and single egg masses were inoculated into a hole 2-3 cm deep near sterile tomato seedlings within 7 d after transplanting. These pure cultures form *Meloidogyne* spp. populations were used for identification.

Morphological identification

Perineal patterns

For identification of *Globodera* spp., the perineal regions of vulva-anus parts of cysts were examined. The vulva region of the 8-10 cysts was cut with the help of a scalpel and kept in 15% H₂O₂ to ensure decoloration. The vulva parts of the cysts were placed into glycerin on a slide, covered with coverslip and sealed with nail-polish (Hooper, 1986). For identification of *Meloidogyne* spp., 5-6 female individuals were taken and permanent preparations were made using the perineal specimens as described by Taylor & Netscher (1974) and developed by Hartman & Sasser (1985). For the permanent preparations, vulval regions of mature females were cut in 45% lactic acid, then transferred into glycerin and were mounted on slides under a Leica DM4000B stereo microscope.

Morphometric measurements of second stage juveniles

Fixation and permanent preparation of second stage juvenile of *Globodera* and *Meloidogyne* spp. were performed. Fixation was performed according to the method developed by Hooper (1986). The fixed nematodes were taken into pure glycerin according to the Seinhorst (1959) method. Wax ring method was used in making the preparation (Hooper, 1986). *Globodera* and *Meloidogyne* spp. were identified by morphological and morphometric characters according to Siddiqi (2000).

Molecular identification

Nematode DNA extraction

For molecular identification of *Globodera* spp. (32 populations) and *Meloidogyne* spp. (41 populations), DNA isolation was performed using five cysts and five egg masses from each population, respectively, using a Qiagen DNA isolation kit (DNeasy Blood & Tissue Kit).

Polymerase chain reaction

Globodera and *Meloidogyne* specimens, specific primers were identified using primers from White et al. (1990), Bulman & Marshall (1997), Zijlstra et al. (2000), Wishart et al. (2002) and Tesarova et al. (2003) (Table 1).

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

Species	Primers	Primer sequences (5'-3')	Fragments (bp)	Reference
<i>G. rostochiensis</i> , <i>G. pallida</i>	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	-	White et al., 1990
	PITSp4	5'-ACAACAGCAATCGTCGAG -3'	264	Bulman & Marshall, 1997
	PITSr3	5'-AGCGCAGACATGCCGCAA -3'	434	Bulman & Marshall, 1997
<i>M. chitwoodi</i> , <i>M. fallax</i> , <i>M. hapla</i>	JMV1	5'-GGATGGCGTGCTTCAAC-3'	540	Wishart et al., 2002
	JMV2	5'-TTTCCCCTTATGATGTTTACCC-3'	540	Wishart et al., 2002
	JMV _{hapla}	5'-AAAAATCCCCTCGAAAAATCCACC-3'	440	Wishart et al., 2002
<i>M. incognita</i>	Sec-1f	5'-GGGCAAGTAAGGATGCTCTG-3'	502	Tesarova et al., 2003
	Sec-1r	GCACCTCTTTCATAGCCACG	502	Tesarova et al., 2003
<i>M. javanica</i>	Fjav	5'-GGTGC GCGATTGAACTGAGC-3'	720	Zijlstra et al., 2000
	Rjav	5'-CAGGCCCTTCAGTGGAACTATAC-3'	720	Zijlstra et al., 2000
<i>M. arenaria</i>	Far	5'-TCGGCGATAGAGGTAATGAC-3'	420	Zijlstra et al., 2000
	Rar	5'-TCGGCGATAGACACTACAAACT-3'	420	Zijlstra et al., 2000

The PCR mixture was prepared in 25 µl (2 µl DNA sample, 12.5 µl PCR master mix, 7.5 µl water and 1 µl specific primers) for each sample. For the PCR cycle, the parameters specified in Tables 2 and 3 were applied.

Table 2. Parameters used in the PCR study to determine the species of *Meloidogyne* spp.

Time & Degree	Time & Degree	Time & Degree	Time & Degree	Time & Degree	Time & Degree
		45 cycles			
		50°C (194/195)			
		61°C (Far/Rar)			
		64°C (Fjav/Rjav)			
94°C	94°C	50°C (JMV primers)	72°C	4°C	
2 min	30 s	30 s	7 min	∞	
		35 cycles			
94°C	94°C	50°C (Sec-1f/Sec-1r)	72°C	72°C	4°C
3 min	30 s	30 s	90 s	10 min	∞

Table 3. Parameters used in the PCR study to determine the species of *Globodera* spp.

Time & Degree	Time & Degree	Time & Degree	Time & Degree	Time & Degree	Time & Degree
		35 cycles			
94°C	94°C	60°C (ITS5/PITSp4/PITSr3)	72°C	72°C	4°C
2 min	30 s	30 s	30 s	10 min	∞

PCR products were processed with 1.5% agarose gel prepared with tris-borate-EDT buffer and then stained with ethidium bromide and imaged under UV.

Results and Discussion

Morphological identification

As a result of the surveys conducted in potato fields of İzmir Province, only *G. rostochiensis* was found in the samples infested with cyst nematodes. Also, *Meloidogyne* spp. were detected in 41 soil samples and all populations were cultured. From the morphological identification, the specimens were identified as *M. chitwoodi* (1 sample), *M. hapla* (5 samples), *M. incognita* (25 samples) and *M. javanica* (10 samples).

Tail length, hyaline terminus, stylet length, body diameter at anus, greatest body diameter, body length (L), a, c and c' of juvenile are the main morphometric properties for identification of *Globodera* and *Meloidogyne* spp. (Whitehead, 1968; Stone, 1973; Hesling, 1978; Eisenback et al., 1981; Golden, 1986; Jepson, 1987; Karssen, 2002; EPPO, 2017). The mean morphometric measurements of the second stage juveniles of *G. rostochiensis*, and *M. chitwoodi*, *M. hapla*, *M. incognita* and *M. javanica* are shown in Table 4.

Table 4. Morphometric data for second stage juveniles of potato cyst and root-knot nematode specimens collected from potato fields in Izmir Province, Turkey

Morphometric characters	J2 measurements (µm) mean±standard error (min-max)		
<i>Globodera rostochiensis</i>	This study (n=96)	Salgut (2017)	Tirchi et al. (2016)
Stylet length	22.1±0.8 (16.2-25.8)	21.4±0.4 (19.5-23.6)	21.5±0.7 (20.0-22.8)
Tail length	46.0±2.7 (23.9-74.3)	54.1±1.6 (42.0-60.0)	45.8±4.9 (37.0-54.7)
Body diameter at anus	12.1±0.6 (8.8-14.4)	15.2±0.6 (12.0-17.0)	12.0±0.6 (10.5-12.5)
Body diameter	18.4±1.1 (11.1-23.3)	22.8±1.1 (18.0-28.0)	19.4±1.4 (16.2-21.7)
Hyaline terminus	22.8±1.7 (10.2-35.1)	27.5±1.2 (22.8-33.2)	27.1±2.8 (22.8-31.9)
Body length (L)	415.7±10.2 (334.7-464.1)	496.8±14.0 (445.0-585.0)	422.5±40.9 (371.1-502.5)
a	22.9±1.5 (16.7-36.3)	-	21.9±2.4 (18.6-26.0)
c	9.2±0.5 (4.9-12.0)	-	9.3±1.0 (8.1-11.4)
c'	3.8±0.2 (2.9-5.4)	-	3.8±0.3 (3.2-4.4)
<i>Meloidogyne chitwoodi</i>	This study (n=3)	Evlice & Bayram (2016)	Golden et al. (1980)
Stylet length	15.7±1.5 (13.4-18.5)	10.0±0.4 (9.3-10.8)	9.9±0.3 (9.0-10.3)
Dorsal oesophageal gland (DGO)	2.8±0.1 (2.7-3.0)	3.0±0.2 (2.6-3.3)	3.2±0.2 (2.6-3.9)
Body diameter at S-E pore	14.0±0.7 (12.6-15.0)	12.4±0.4 (11.5-13.4)	-
S-E pore/Stylet length	2.8±0.7 (1.5-3.9)	-	-
Tail length	65.7±12.3 (43.9-86.3)	43.0±2.1 (40.2-47.9)	43.0±1.8 (39.0-47.0)
Body diameter at anus	12.7±2.0 (9.2-16.1)	9.6±0.5 (8.8-10.8)	-
Anus primordium	88.8±20.4 (66.0-129.6)	89.2±6.3 (80.4-97.8)	-
Body diameter at vulva	14.5±1.4 (11.8-16.5)	-	-
Greatest body diameter	15.5±1.7 (12.3-18.0)	13.1±0.4 (12.5-13.9)	-
Hyaline terminus	11.5±0.7 (10.3-12.6)	10.4±0.9 (8.8-12.2)	11.0±1.0 (8.6-13.8)
Body length (L)	356.4±16.3 (324.3-377.6)	366.8±11.3 (352.3-390.0)	390.0±16.0 (336.0-417.0)
a	23.6±3.1 (20.0-29.9)	28.1±0.8 (26.0-29.1)	27.5±1.2 (24.5-29.8)
b'	5.8±1.1 (3.8-7.1)	7.6±0.4 (7.0-8.3)	-
c	5.9±1.3 (4.4-8.4)	8.5±0.4 (7.4-9.0)	8.9±0.4 (7.9-9.6)
c'	5.1±0.2 (4.8-5.4)	4.5±0.2 (4.1-4.7)	-
(S-E pore/L) X100	11.1±3.1 (5.9-16.7)	18.3±0.6 (17.5-19.3)	-
<i>Meloidogyne hapla</i>	This study (n=15)	Uysal et al. (2017)	Whitehead (1968)
Stylet length	15.1±1.2 (11.5-18.4)	12.4±0.8 (11.2-13.6)	9.7±0.92 (7.9-10.9)
Dorsal oesophageal gland (DGO)	3.1±0.4 (2.1-4.7)	4.8±1.7 (3.2-6.4)	-
Body diameter at S-E pore	14.6±0.8 (11.2-17.1)	-	-
S-E pore/Stylet length	3.3±0.3 (2.0-4.2)	-	-
Tail length	53.6±3.5 (31.2-67.8)	49.5±2.9 (44.8-56.0)	43.0±4.0 (33.0-48.0)
Body diameter at anus	11.3±0.7 (7.2-14.4)	-	-
Anus primordium	68.7±4.9 (54.2-92.1)	-	-
Body diameter at vulva	14.3±0.7 (10.1-17.6)	-	-
Greatest body diameter	15.3±0.8 (11.3-18.9)	-	-
Hyaline terminus	11.4±1.7 (4.8-17.4)	13.1±2.1 (11.2-17.6)	-
Body length (L)	358.1±7.9 (319.1-420.6)	380.5±23.7 (328-412.8)	337.0±11.4 (312.0-355.0)
a	23.8±1.1 (19.5-30.9)	29.2	23.9±1.7 (20.1-26.6)
b'	4.9±0.4 (4.0-6.7)	-	-
c	6.9±0.6 (4.8-8.0)	7.7	7.9±0.2 (7.3-10.2)
c'	4.8±0.3 (3.6-6.0)	-	-
(S-E pore/L) X100	13.8±1.4 (8.0-17.3)	-	-

Table 4. Continued

Morphometric characters	J2 measurements (µm) mean±standard error (min-max)		
<i>Meloidogyne incognita</i>	This study (n=75)	Uysal et al. (2017)	Whitehead (1968)
Stylet length	14.4±0.6 (11.6-22.0)	13.3±0.7 (12-14.4)	10.5±0.59 (9.6-11.7)
Dorsal oesophageal gland (DGO)	2.9±0.3 (1.5-4.7)	3.4±0.5 (3.2-4.8)	-
Body diameter at S-E pore	13.7±0.8 (10.6-18.4)	-	-
S-E pore/Stylet length	4.4±0.3 (3.3-6.3)	-	-
Tail length	52.6±3.5 (37.3-105.0)	57.6±5.5 (50.4-68.8)	46.0±3.0 (38.0-55.0)
Body diameter at anus	10.8±0.7 (6.7-14.7)	-	-
Anus primordium	100.1±7.4 (69.7-181.0)	-	-
Body diameter at vulva	14.1±0.7 (10.6-18.2)	-	-
Greatest body diameter	15.2±0.8 (11.4-21.2)	-	-
Hyaline terminus	10.6±1.1 (4.50-18.90)	11.6±2.2 (6.4-16.0)	-
Body length (L)	361.9±5.6 (308.3-403.0)	409.7±22.4 (360.0-441.6)	371.0±16.0 (337.0-403.0)
a	24.2±1.3 (17.1-30.1)	29.5	28.3±1.7 (24.9-31.5)
b	-	-	2.4±0.3 (2.0-3.1)
b'	4.5±0.2 (2.9-6.2)	-	7.1±0.4 (6.4-8.4)
c	7.1±0.5 (3.5-10.2)	7.1	8.1±0.7 (6.9-10.6)
c'	4.9±0.3 (3.7-7.8)	-	-
(S-E pore/L) X100	17.5±1.2 (12.3-22.7)	-	-
<i>Meloidogyne javanica</i>	This study (n=30)	Uysal et al. (2017)	Whitehead (1968)
Stylet length	15.3±0.8 (11.7-18.7)	14.0±0.5 (13.6-14.4)	10.4±0.5 (9.4-11.4)
Dorsal oesophageal gland (DGO)	3.1±0.3 (2.1-4.4)	3.4±0.4 (3.2-4.0)	-
Body diameter at S-E pore	13.8±0.7 (10.4-17.1)	-	-
S-E pore/Stylet length	3.7±0.3 (2.5-4.8)	-	-
Tail length	50.3±2.8 (27.9-62.3)	55.2±3.8 (52.8-60.8)	49.0±4.0 (36.0-56.0)
Body diameter at anus	10.2±0.5 (8.0-11.9)	-	-
Anus primordium	90.1±2.6 (59.8-112.2)	-	-
Body diameter at vulva	13.6±0.8 (8.8-16.4)	-	-
Greatest body diameter	14.7±0.7 (10.4-18.4)	-	-
Hyaline terminus	11.7±1.6 (4.7-23.8)	14.4±2.3 (12.8-17.6)	-
Body length (L)	387.9±10.5 (326.5-439.3)	448.0±16.3 (427.2-465.6)	417.0±22.0 (387.0-459.0)
a	26.7±1.7 (21.0-37.7)	32.0	30.6±2.0 (27.1-35.9)
b'	5.4±0.4 (3.9-7.8)	-	7.5±0.2 (7.1-8.0)
c	7.9±0.5 (6.2-10.8)	8.1	8.5±0.7 (7.3-11.1)
c'	4.9±0.2 (3.5-5.8)	-	-
(S-E pore/L) X100	14.6±0.9 (11.6-19.5)	-	-

The morphological and morphometric data of populations identified as *G. rostochiensis* (Figure 1), and *M. chitwoodi*, *M. hapla*, *M. incognita* and *M. javanica* (Figure 2) were confirmed according to the original definition (Siddiqi, 2000).

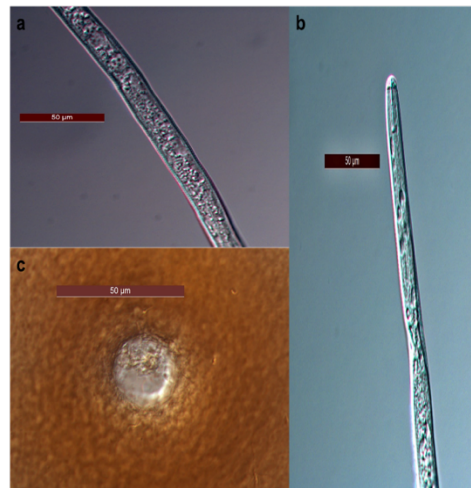


Figure 1. *Globodera rostochiensis* isolates collected from İzmir Province: a) midbody region view of second stage juvenile; b) anterior view of second stage juvenile; and c) perineal pattern.

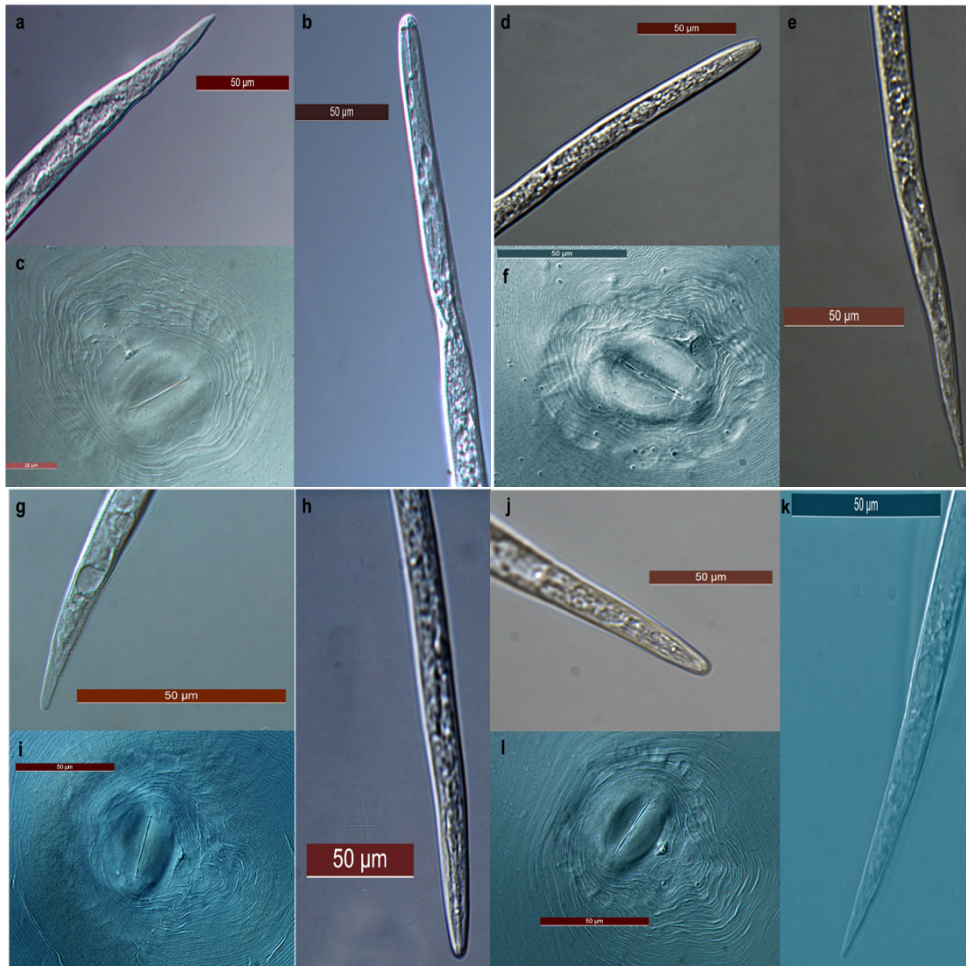


Figure 2. *Meloidogyne* spp. isolates collected from İzmir Province: a) tail view of second stage juvenile of *M. incognita*; b) anterior view of second stage juvenile of *M. incognita*; c) perineal pattern of *M. incognita*; d) anterior view of second stage juvenile of *M. javanica*; e) tail view of second stage juvenile of *M. javanica*; f) perineal pattern of *M. javanica*; g) tail view of second stage juvenile of *M. hapla*; h) anterior view of second stage juvenile of *M. hapla*; i) perineal pattern of *M. hapla*; j) anterior view of second stage juvenile of *M. chitwoodi*; k) tail view of second stage juvenile of *M. chitwoodi*; and l) perineal pattern of *M. chitwoodi*.

Molecular identification

PCR studies were conducted using primer set PITSr3/PITSp4/ITS5 (Bulman & Marshall, 1997) for the determination of *G. rostochiensis* species and a band was obtained at 434 bp for all *G. rostochiensis* specimens (Figure 3). Ulutaş et al. (2012) had determined *G. rostochiensis* with the same primers in potato cultivation areas of Aegean Region of Turkey.

All *M. chitwoodi* and *M. hapla* specimens were diagnosed using JMV1/JMV2/JMV_{hapla} primer set, with 440 and 540 bp DNA bands, respectively (Figure 3), as described by Wishart et al. (2002) and these results are compatible with the results of earlier studies in Turkey (Devran et al., 2009; Akyazı et al., 2012; Evlice & Bayram, 2016; Uysal et al., 2017). For *M. javanica*, specific SCAR primers (Zijlstra et al., 2000) produced 720 bp DNA bands (Figure 3), and this finding is in agreement with previous studies which conducted on different crops in Turkey (Devran & Söğüt, 2009; Devran, 2013; Özarlıandan & Elekcioğlu, 2010; Devran et al., 2017; Uysal et al., 2017). SEC-1F/SEC-1R primers (Tesarova et al., 2003) were used and a DNA band was obtained at 502 bp for all *M. incognita* populations (Figure 3). These results are similar to previous studies in Turkey (Devran & Söğüt, 2009; Akyazı & Felek, 2013; Devran, 2013; Uysal et al., 2017). The perineal patterns, measurements of the second stage juveniles and molecular definitions overlapped for all nematode species.

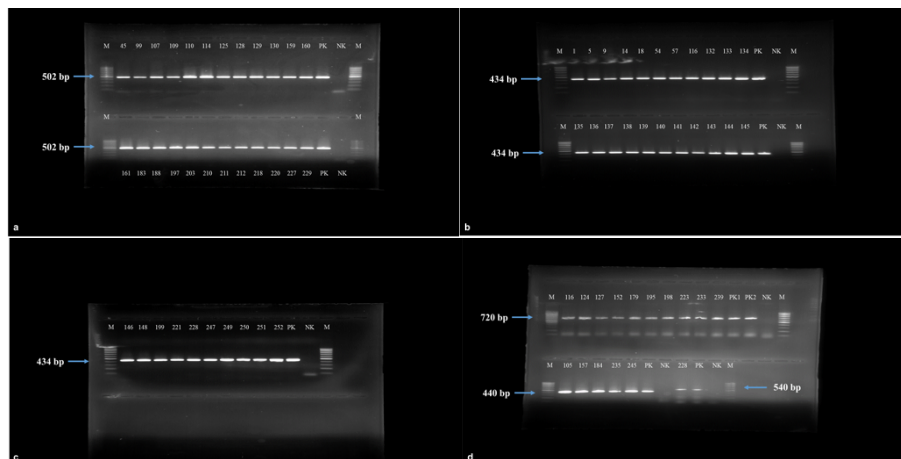


Figure 3. Amplification products with the *Globodera* and *Meloidogyne* spp. collected from İzmir Province: a) 502 bp PCR product obtained from *M. incognita* samples using SEC 1-F/SEC 1-R primers; b-c) 434 bp PCR product obtained from *G. rostochiensis* samples using PITSr3/PITSp4/ITS5 primers; d) 720 bp PCR product obtained from *M. javanica* samples using Fjav/Rjav, 440 bp PCR product obtained from *M. hapla* samples and 540 bp *M. chitwoodi* samples using JMV1/JMV2/JMV_{hapla} primers (PK, positive control; NK, negative control; M, molecular marker (100-1000 bp Thermo)).

Occurrence of *Globodera* spp. and *Meloidogyne* spp. in districts of İzmir

Meloidogyne spp. were detected in 41 soil samples from Bayındır (3 samples), Dikili (3 samples), Kiraz (1 sample) and Ödemiş (34 samples) Districts. No plant parasitic nematodes were found in potato cultivation areas Tire District. *Globodera* sp. was detected only in soil samples taken from 32 different fields in Ödemiş District. As a result, *Meloidogyne* spp. (18.4%) were found more widely distributed than *Globodera* spp. (14.4%) in İzmir potato cultivation areas in 2015 (Figure 4).

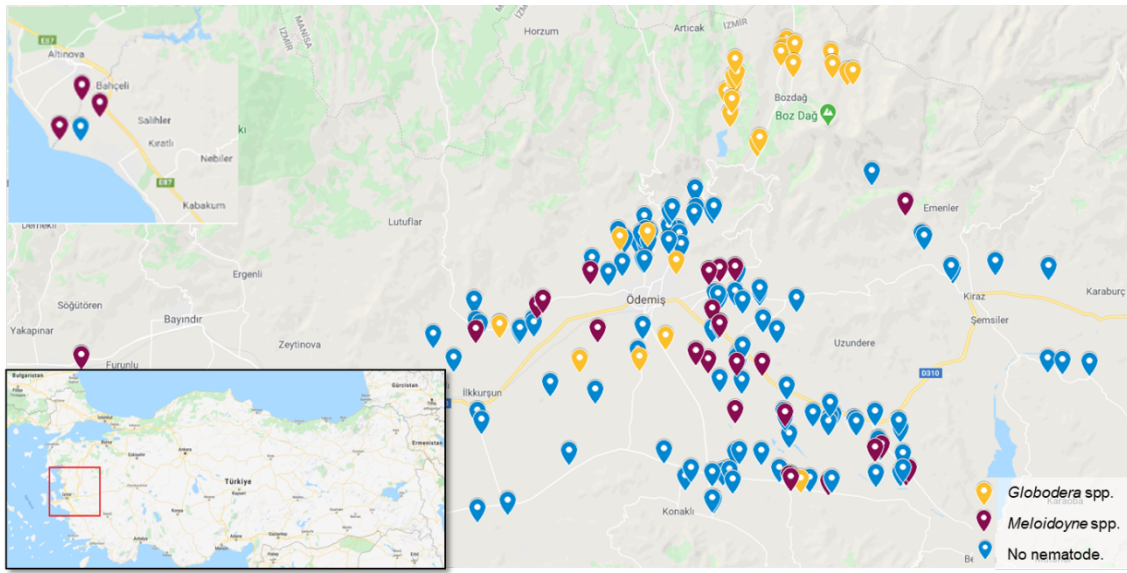


Figure 4. Surveyed area and the locations of nematode species found in potato fields in İzmir Province (Anonymous, 2020).

From the morphological and molecular identification studies, only *G. rostochiensis* was found in samples infested with cysts. Also, 61.0% of samples contained *M. incognita*, 24.4% *M. javanica*, 12.2% *M. hapla* and 2.4% *M. chitwoodi*.

Several nematode species that cause significant yield losses are found in potato cultivation areas. The main nematode species associated with potatoes are the yellow potato cyst nematode *G. rostochiensis*, the white potato cyst nematode, *Globodera pallida* (Stone, 1973) Behrens, 1975 and also the root-knot nematodes *Meloidogyne* spp. (Vovlas et al., 2005; Medina et al., 2016). In this study, *G. rostochiensis*, *M. chitwoodi*, *M. hapla*, *M. incognita*, and *M. javanica* have been found in different parts of İzmir potato cultivation areas. In contrast, *G. pallida* was not detected in the surveyed areas of İzmir Province. In Turkey, many studies had been carried out to determine the nematode species on different crops. *Globodera* spp. was determined for the first time in Turkey in potato cultivation areas of Bolu Province (Enneli & Öztürk, 1996) and in the following decade, this nematode was found in Afyon, İzmir (Ödemiş-Bozdağ), Konya and Sivas Provinces (Anonymous, 2008). Ulutaş (2010) reported that *G. rostochiensis* prevalence in the Aegean Region potato production areas at 17.5% and also in Ödemiş District of İzmir at 61.7%. In İzmir, potato is cultivated both in high lands and low lands in two different growing seasons. In this study, while *G. rostochiensis* was found in all potato growing areas of the high lands, in the low lands infestation rate was only 6.82%.

Unfavorable environmental conditions cause the death of the eggs in the cyst and annual mortality rates are more than 50% for temperate regions and 75% in warmer climates (Marks & Brodie, 1998). Due to the high egg mortality of *Globodera* spp. in warmer climates, these species may better adapt to subtropical regions and its cycle is interrupted at temperatures above 28°C (Lima et al., 2018). Moreover, *G. rostochiensis* can develop well between 15-25°C (EPPO, 2017).

In addition, *M. incognita* and *M. javanica* have been found to be the predominant species and also *M. arenaria*, *Meloidogyne artiella* Franklin, 1961, *Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne exiqa* Goeldi, 1892, *M. hapla*, *Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno & Karssen, 2014, and *Meloidogyne thamesi* (Chitwood, 1952) were identified in different cultivation areas of Turkey (Yüksel, 1966, 1967, Elekcioğlu & Uygun, 1994; Kaşkavalcı & Öncüer, 1999; Kepenekçi et al., 2002; Özarslandan et al., 2009; Aydınli et al., 2013; İmren et al., 2014; Aydınli, 2018). Also, Yıldız et

al. (2009) reported that *M. chitwoodi* was found in 37.9% of potato areas of İzmir. In Central and East Anatolia Region, only *M. chitwoodi* has been detected in potato cultivation areas in different studies (Özarslandan & Elekçioğlu, 2010; Özarslandan et al., 2013; Evlice & Bayram, 2016). With this study, *M. incognita* and *M. javanica* were found to be the widely distributed nematodes, but only one sample was infested with *M. chitwoodi*. These results may be explained due to the fact some nematode species have been found with low population levels for years. Furthermore, climate change could be affecting population density of plant parasitic nematode species in a way that the cool climate species such as *M. chitwoodi* and *M. hapla* are being replaced by species with high adaptability to higher temperatures such as *M. incognita* and *M. javanica* (Gözel & Elekçioğlu, 1996; Da Conceição et al., 2009).

Distribution of *Globodera* and *Meloidogyne* spp. in İzmir

Globodera and *Meloidogyne* spp. were detected in 14.4% and 18.4% of samples. When grouped in terms of the number of individuals in 100 cm³ soil, for *Globodera* species, 1-50 individuals were detected in 37.5%, 51-250 individuals in 46.9% and 251-500 individuals in 15.6% of the samples. For *Meloidogyne* species 1-50 individuals were found in 85.4% of the samples 51-250 individuals were detected in 14.6% of the samples (Table 5).

There are many factors such as initial population density, host plants, crop rotation, season and soil type that can affect the economic damage caused by *Globodera* spp. and *Meloidogyne* spp. (Greco et al., 1992; Greco, 1993; Potter & Olthof, 1993). The economic damage threshold levels of potato cyst nematodes are considered as 10 eggs/g soil (Phillips et al., 1991). In this study, all the samples which were infested with *G. rostochiensis* were determined above the economic damage levels for potato production. Damage thresholds have been established for several crops, where the average is about 0.5-2 juveniles/g of soil for *Meloidogyne* species. Also, it is reported that the economic damage threshold of *M. chitwoodi* is 1 juvenile/250cm³ of soil (Brodie et al., 1993). Although *M. chitwoodi* detected in this study was above the economic damage threshold, it should be noted that this species was found only in one sample. Considering the population densities of the 41 infested samples, only three populations (*M. chitwoodi*, 1 population; *M. incognita*, 2 populations) were found above the specified threshold levels.

Table 5. Rate of nematode detections in a survey in the potato production areas in İzmir in 2015 by population density (%)

Nematode species	Positive samples	The presence of nematodes according to the number of individuals (%)					
		Proportion positive samples by genera (%)*	Total availability rate (%)	Population density (individuals/100 cm ³ soil) (%)			
				1-50	51-250	251-500	≥ 500
<i>Globodera rostochiensis</i>	223	32	100.00	37.50	46.88	15.62	-
<i>Meloidogyne incognita</i>	223	25	60.97	80.00	20.00	-	-
<i>Meloidogyne javanica</i>	223	10	24.39	100.00	-	-	-
<i>Meloidogyne hapla</i>	223	5	12.20	100.00	-	-	-
<i>Meloidogyne chitwoodi</i>	223	1	2.44	-	100.00	-	-

* 32 and 41 positive samples for *Globodera* spp. and *Meloidogyne* spp., respectively, of a total of 223 samples.

The main factors affecting the degree of damage caused by nematodes on potato tubers include the population density of the existing species and the duration of the plant in the soil. During planting time, if there is a high *Globodera* spp. or *Meloidogyne* spp. population present in the soil, these plants are affected more quickly (Lima et al., 2018).

The importance of these nematode species varies depending on their adaptation to the geographical region (local climate) and host plant species. Although nematode species have their own biology and behavior, it is often difficult to control or eliminate when they infect an area. Also, morphological similarities make it difficult to identify the nematodes.

Reliable identification of *Globodera* spp. and *Meloidogyne* spp. and determination of the population densities in potato cultivation areas are crucial to selection of effective control methods and to apply quarantine regulations (Lima et al., 2018). The differences between temperate and tropical *Meloidogyne* spp. and their distribution in Turkey indicate that different strategies are needed in various geographical regions.

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