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First Report of Root-Knot Nematode *Meloidogyne hapla* (Chitwood, 1949) (Nematoda: Meloidogynidae) on *Petroselinum crispum* (Mill.) Nym. ex A.W. Hill) in Türkiye

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ABSTRACT
Parsley (*Petroselinum crispum*) is an important culinary herb originated from the Mediterranean basin, where it still can be found in wild forms, and is taxonomically positioned in the Apiaceae family. In Turkey, it is cultivated extensively in the Mediterranean and Aegean Regions, and in the Marmara Region. There are several diseases and pests affecting the yield and quality in the parsley cultivated areas. Root-knot nematodes are among the most important pests in winter crop production with the ability of a wide host plant range. Root-knot nematodes weaken the parsley plant, causing significant quality losses, and showing signs of stunting and yellowing of the leaves. In this study, parsley plants with typical symptoms of root-knot nematodes were detected in surveys conducted in Çanakkale province, Türkiye. Pure cultures of the root-knot nematode obtained from the roots of parsley were identified up to the species level. Species identification and diagnosis were made by morphological and morphometrical measurements from second-stage juveniles and female individuals obtained from pure cultures. As a result, *Meloidogyne hapla* was detected first time on parsley plants in Türkiye.

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Türkiye'de *Meloidogyne hapla* (Chitwood, 1949) (Nematoda: Meloidogynidae)'nın *Petroselinum crispum* (Mill.) Nym. ex A.W. Hill)'da İlk Konukçu Kaydı

ÖZET
Maydanoz (*Petroselinum crispum*), Akdeniz havzası orijinli, halen yabani formda bulunabilen ve taksonomik olarak Apiaceae familyasında yer alan önemli bir mutfak bitkisidir. Ülkemizde de ticari olarak Akdeniz ve Ege Bölgelerinde, geniş çaplı olarak Marmara Bölgesinde üreticiliği yapılmaktadır. Maydanoz ekim alanlarında verim ve kaliteyi etkileyen çeşitli hastalık ve zararlı bulunmaktadır. Kök-ur nematodları, geniş bir konukçu bitki yelpazesine sahip olma yetenekleri ile kışlık mahsul üretiminde en önemli zararlılar arasında yer almaktadır. Kök-ur nematodları maydanoz bitkisini zayıflatarak önemli kalite kayıplarına neden olup, bitkide bodurlaşma ve yapraklarda sararma belirtileri göstermektedir. Bu çalışmada Türkiye'nin Çanakkale ilinde yapılan surveylerde Kök-ur nematodlarının tipik simptomlarının gözlemlendiği maydanoz bitkileri tespit edilmiştir. Maydanoz köklerinde elde edilen kök-ur nematodunun saf kültürleri tür düzeyine kadar teşhis edilmiştir. Saf kültürlerden elde edilen 2. dönem larvalar ve dişi bireylerden morfometrik ölçümler ve morfolojik yöntemler kullanılarak tür teşhisi yapılmıştır. Çalışma sonucunda *Meloidogyne hapla* Türkiye'de ilk defa maydanoz bitkilerinde tespit edilmiştir.

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INTRODUCTION

Parsley (*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill), a popular leaf-consumed vegetable plant in the family Apiaceae, is commonly found in subtropical and temperate zones of the Mediterranean Climate zone. This family is the largest and most cosmopolitan family among angiosperms (Pimenov & Leonov, 1993; Hickey & King, 1997; Hançer & Uruşak, 2017). Parsley is a bright, green, pile-rooted plant with fragrant and segmented leaves, the length of leaves varies between 30-100 cm, the seeds are 2.5-3 mm long and with a special odour. It is produced and consumed as a cultural vegetable in all countries of the world (Vural et al., 2000). There are approximately 300 genera and 2500-3000 species of parsley, among which there can be found 97 genera and 400 species in Turkey, and many species are used as food and spicery, on the other hand, it is also used in the pharmaceutical and perfume industries (Seçmen et al., 1986). Parsley has an important place among the vegetables whose leaves are consumed in terms of health, nutrition and economy, and its production is increasing day by day (Ben-Amotz & Fishier, 1998).

There are many pests and diseases that negatively affect the yield and quality of the plant. Some of the pests can be counted as Carrot fly (*Psila rosae* F.), Carrot louse (*Cavariella aegopodii* Scop.), Gray worms (*Agrotis* spp.) and Root-knot nematodes (*Meloidogyne* spp.). Root-knot nematodes have symptoms such as weakening parsley, stunting and yellowing of leaves (Sikora & Fernández, 2005; Mennan et al., 2011). The damage caused by root-knot nematodes on the leaves of the consumed part of parsley is economically important and it is an undesirable situation for the growers. In the previous studies conducted around the world, it has been reported that parsley is a suitable host for Root-knot nematodes (*M. arenaria*, *M. enterolobii*, *M. floridensis*, *M. incognita*, *M. hapla*, *M. hispanica* and *M. javanica*) (Doucet & Pinochet 1992; Sikora & Fernández, 2005; Mennan et al., 2011; Quénéhervé et al., 2011; Maleita et al., 2012; Sasanelli et al., 2015). However, this is the first report of a parsley plant as a host for *M. hapla* species in Turkey.

MATERIAL and METHOD

A field survey was conducted in the areas where parsley is grown in Çanakkale in the winter period of 2021-2022. The roots of plant specimens with typical symptoms of root-knot nematodes were brought to the laboratory and examined under a binocular microscope (Figure 1). After the infected plants were detected, the female egg masses were taken from the root of each sample with forceps and alive second-stage infective juveniles were obtained after 48 hours on a modified Baermann funnel, (8 cm high, 10-12 cm wide Petri dishes were used with a sieve inside) for morphometric measurements. After that, pure cultures were formed by infecting tomato plants with one egg mass for each plant which are susceptible to Root-knot nematodes. After about 60 days, second-stage juveniles (J2s) were obtained from female individuals eggmasses reproduced on pure cultures for diagnostic studies.

Figure 1. Parsley roots infected with *Meloidogyne spp.* a: Sample 13; b: Sample 94
Şekil 1. Meloidogyne hapla ile infekteli maydanoz kökleri a: Örnek 13, b: Örnek 94

The obtained J2s were fixed in TAF solution (Courtney et al., 1955) and then taken into pure glycerin according to the Seinhorst (1959) method, fixed on the slide, and made ready for species identification. The standard measurements used in the morphological diagnosis of second-stage juveniles were made by Jepson (1987) and the species-level diagnosis was made using the Leica DM1000 light microscope according to Chitwood (1949) and Cliff & Hirschmann, (1985). For each infected plant population, 25 root-knot nematode second instar larvae were measured.

Obtained female individuals were cut after being kept in 45% lactic acid and their preparations were made between slide and coverslip in pure glycerin (Hooper, 1986). Morphological identifications of female individuals were made according to Jepson (1987) and Karssen (2002).

RESULTS and DISCUSSION

The results of the diagnosis performed with morphological and morphometric measurement methods showed that the species infecting the roots of the parsley plant was *M. hapla* (Table 1, Figure 2; 3).

In Turkey, as yet, *M. hapla* species host reports were detected on potato (Özarslandan et al., 2005), sugar beet (Alkan, 1962), pepper (Söğüt & Elekçioğlu, 2000), strawberry (Özarslandan et al., 2021) and kiwi (Akyazı et al., 2017).

Previous studies on the host plant of root-knot nematodes that infect parsley in different parts of the world were reported as susceptible to *M. arenaria* and *M. incognita* species (Ibrahim et al., 1983; Doucet & Pinochet 1992; Walker, 2002). In Turkey, so far only *M. arenaria* species were known on Parsley (Mennan et al. 2011).

Table 1. Morphometric measurements of Meloidogyne hapla J2s on Petroselinum crispum
Çizelge 1. Petroselinum crispum'dan elde edilen Meloidogyne hapla'ya ait J2s'lerin morfometrik ölçümleri

Diagnostic Characters	*This Study Sample 13*	*This Study Sample 94*	*Chitwood, 1949*	*Cliff & Hirschmann, 1985*
Body lenght	433.06±30.76 (382.52-496.92)	381.22 ±11.61 (362.17-403.40)	357-467	391.6-605.2
Greatest body width	15.43±1.16 (13.14-17.79)	15.56±0.28 (15.06-16.12)	-	12.8-17.8
Body width at stylet base	9.57±0.57 (8.44-10.95)	9.92±0.35 (9.19-10.40)	-	-
Body width at anus	11,46±1.09 (9.08-13.14)	9.87±0.28 (9.43-10.44)	-	-
Stylet length	15.23±1.01 (14.07-18.81)	14.74±0.44 (14.25-15.89)	10-12	10.1-11.9
DGO	3.64±0.37 (2.98-4.24)	2.70±0.34 (2.16-3.28)	3-4	2.7-4.7
Tail length	52.77±4.68 (42.04-64.36)	45.84±1.61 (42.81-48.33)	46-58	43.6-69.4
Excretory pore to head end	90.65±6.75 (80.88-106.36)	75.18±1.83 (72.14-78.15)	-	75-105.2
Body width at excretory pore	13.76±0.93 (12.09-16.18)	14.41±0.46 (13.10-14.99)	-	-
a	28.15±2.20 (24.27-35.07)	24.50±0.67 (23.16-25.56)	-	-
b	3.69±0.39 (3.00-4.42)	4.25±0.22 (3.77-4.66)	-	-
c	8.24±0.63 (7.25-9.57)	8.32±0.34 (7.49-8.97)	-	-
c'	4.62±0.45 (3.65-5.34)	4.64±0.18 (4.30-4.95)	-	-

Note: All mesurements are in µm (a: Body lenght/Body width, b: Body lenght/Intestine to head end, c: Body lenght/Tail lenght, c': Tail lenght/Body width at anus) and in form: mean ± s.d. (range)

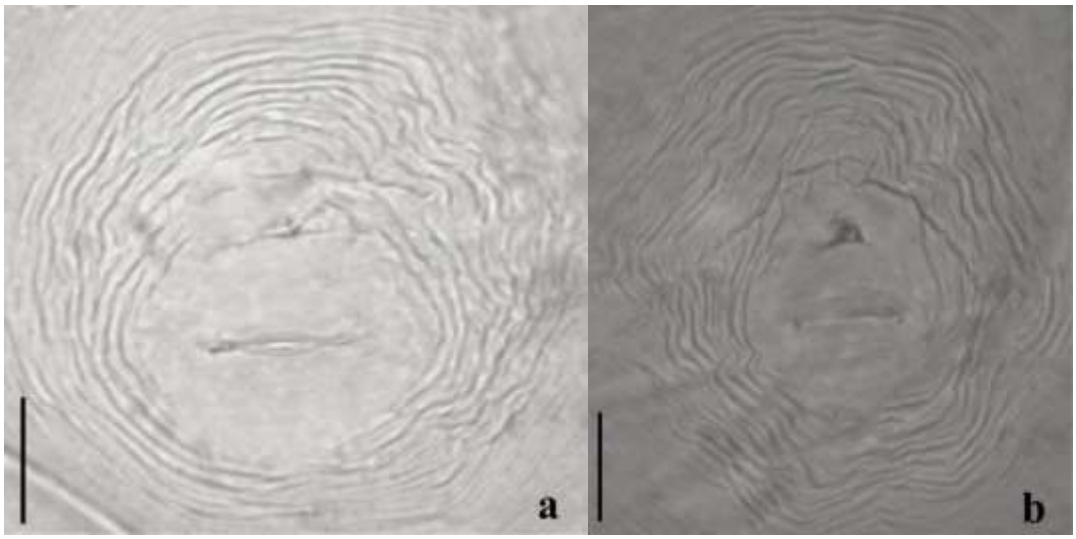

Figure 2. Perineal pattern of Meloidogyne hapla from Petroselinum crispum a: Sample 13; b: Sample 94 (Scale Bar: 20 µm)

Şekil 2. Petroselinum crispum'dan elde edilen Meloidogyne hapla'ya ait perineal pattern a: Örnek 13, b: Örnek 94 (Ölçek Çubuğu: 20 µm)

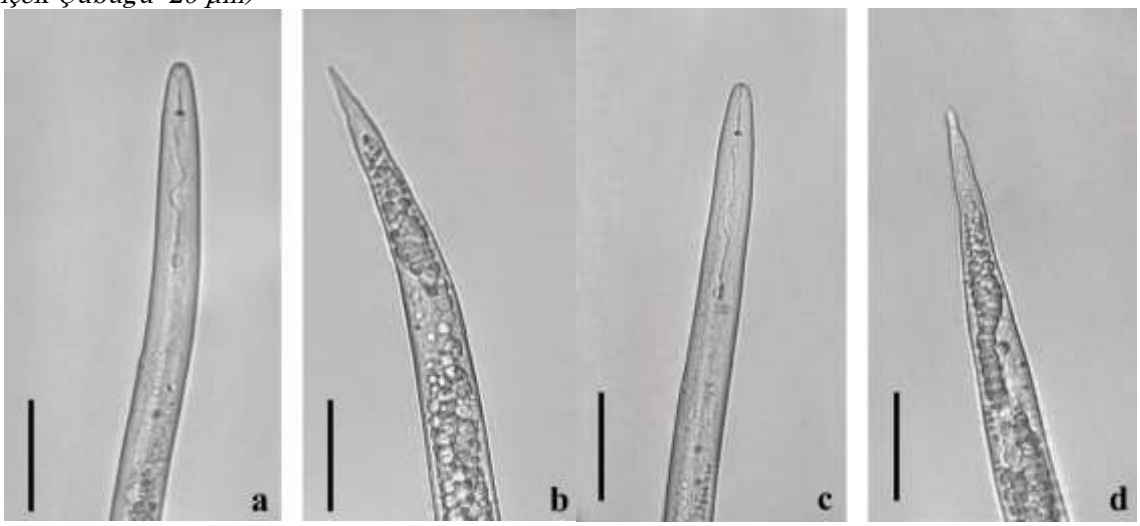

Figure 3. J2s of Meloidogyne hapla from Petroselinum crispum a: Anterior region of Sample 13; b: posterior region of Sample 13; c: anterior region of Sample 94; d: posterior region of Sample 94 (Scale Bar: 20 µm)

Şekil 3. Petroselinum crispum'dan elde edilen Meloidogyne hapla'ya ait J2s a: Örnek 13 anterior bölgesi, b: Örnek 13 posterior bölgesi, c: Örnek 94 anterior bölgesi, d: Örnek 94 posterior bölgesi (Ölçek Çubuğu: 20 µm)

From previous studies, it has been reported that *M. hapla* is rarely detected in Turkey (Yüksel, 1974; Elekçioğlu & Uygun, 1994; Mennan & Ecevit, 1996; Söğüt & Elekçioğlu, 2000), so far *M. hapla* was diagnosed on potato (Özarslandan et al. ., 2005), sugar beet (Alkan, 1962), pepper (Söğüt & Elekçioğlu, 2000; Akyazı et al., 2012), strawberry (Özarslandan et al., 2021), kiwi (Akyazı et al., 2017) plants.

It can be concluded that this study is the first record in Türkiye as a host for *M. hapla* on a parsley plant since the *M. hapla* that infects parsley has not been detected by previous studies. In the future it is recommended to carry out extensive survey studies on parsley growing areas and investigations on control strategies for this plant parasitic nematode using crop rotation, resistant varieties and solarization applications.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

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