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Original article (Orijinal araştırma)

Molecular determination of root-knot nematode species, *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) infesting weeds in kiwifruit orchards in Türkiye[1]

Kivi bahçelerindeki yabancı otlarda görülen kök-ur nematodu türlerinin, *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) moleküler yöntemlerle tespiti

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Abstract

In this investigation, the species of root-knot nematodes (RKNs) infesting weeds in kiwifruit orchards were investigated in the Ordu Province, Türkiye. A survey was conducted in 2018 and roots of weeds with RKN infestations were found in kiwifruit orchards. The infested weed samples were collected from 27 kiwifruit fruit orchards located in the Ordu Province. Identification of RKNs was performed using the molecular method based on mitochondrial DNA (*mt*DNA). The *mtDNA* region between the cytochrome oxidase II and the large subunit ribosomal RNA was amplified using two pairs of primers TRNAH/MRH106 and MORF/MTHIS. Species-specific primers previously described were used to confirm *Meloidogyne* species as the last step. *Meloidogyne incognita* (Kofoid & White, 1919), *Meloidogyne arenaria* (Neal, 1889) and *Meloidogyne hapla* (Chitwood, 1949) (Tylenchida: Meloidogynidae) were identified from fifteen weed species (2 unidentified) in eight families. *Meloidogyne incognita* was the most frequent species with 74.1% of the samples infested, followed by *M. hapla* at 22.2% and *M. arenaria* at 3.7%. In this study found *Erigeron canadensis* L. (Asterales: Asteraceae), *Mercurialis annua* L. (Malpighiales: Euphorbiaceae), *Oxalis pes-caprae* L. (Oxalidales: Oxalidaceae), *Clinopodium nepeta* (L.) Kuntze (Lamiales: Lamiaceae), *Fumaria officinalis* L. (Ranunculales: Papaveraceae) and *Lycopus* spp. (Lamiales: Lamiaceae) to be previously unrecorded hosts of *M. incognita* and *Sigesbeckia orientalis* L. (Asterales: Asteraceae) and *Lythrum* spp. (Myrtales: Lythraceae) a host of *M. hapla*.

Keywords: Kiwifruit, *Meloidogyne*, mtDNA, SCAR, weeds, weed hosts

Öz

Bu araştırmada, Ordu ilinde kivi bahçelerinde yabancı otları enfekte eden kök-ur nematod türleri araştırılmıştır. 2018 yılında bir sürvey çalışması ile kivi bahçelerinde kök-ur nematodu ile bulaşık yabancı ot kökleri gözlemlenmiş ve 27 kivi bahçesinden yabancı ot örnekleri toplanmıştır. Tür teşhisleri mitokondriyal DNA'ya dayalı moleküler yöntem kullanılarak yapılmıştır. Sitokrom oksidaz II ve ribozomal RNA büyük alt birimi arasındaki *mtDNA* bölgesi, iki çift primer TRNAH/MRH106 ve MORF/MTHIS kullanılarak çoğaltılmıştır. Teşhislerin doğrulanması için türe özgü spesifik primerler kullanılmıştır. Sekiz familyaya ait 15 yabancı ot türünden (ikisi tanımlanamayan) *Meloidogyne incognita* (Kofoid & White, 1919), *Meloidogyne arenaria* (Neal, 1889) ve *Meloidogyne hapla* (Chitwood, 1949) (Tylenchida: Meloidogynidae) türleri tespit edilmiştir. Bulaşık bahçelerde *M. incognita* en sık görülen tür olup, örneklerin %74,1'i enfekteli bulunmuş, bunu %22.2 ile *M. hapla* ve %3.7 ile *M. arenaria* izlemiştir. Bu çalışmada *Erigeron canadensis* L. (Asterales: Asteraceae), *Mercurialis annua* L. (Malpighiales: Euphorbiaceae), *Oxalis pes-caprae* L. (Oxalidales: Oxalidaceae), *Clinopodium nepeta* (L.) Kuntze (Lamiales: Lamiaceae), *Fumaria officinalis* L. (Ranunculales: Papaveraceae) ve *Lycopus* spp. (Lamiales: Lamiaceae) türleri *M. incognita* için, *Sigesbeckia orientalis* L. (Asterales: Asteraceae) ve *Lythrum* spp. (Myrtales: Lythraceae) ise *M. hapla* için daha önce kaydedilmemiş konukçular olarak bulunmuştur.

Anahtar sözcükler: Kivi, *Meloidogyne*, mtDNA, SCAR, yabancı otlar, yabancı ot konukçuları

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Introduction

Kiwifruit (*Actinidia* spp.) is a globally important commercial fruit crop, currently being grown in more than 20 countries, including Türkiye. Ordu Province is the second largest producer of kiwifruit in Türkiye (TUIK, 2020). Root-knot nematodes (RKNs), *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae), having the wide range of host plants, is the most significant groups of the plant parasitic nematodes worldwide. The genus is known to infest more than 3,000 species of wild and cultivated plants (Hussey & Janssen, 2002; Moens & Perry, 2009; Abdellatif et al., 2016). In addition, RKNs are commonly found in kiwifruit orchards around the world. There are eight RKN species including *Meloidogyne incognita (*Kofoid & White, 1919)*, Meloidogyne arenaria* (Neal, 1889), *Meloidogyne hapla (*Chitwood, 1949), *Meloidogyne javanica* (Treub, 1885)*, Meloidogyne luci (*Carneiro et al., 2014) (as *M. ethiopica*) *Meloidogyne ethiopica (*Wheathead, 1968)*, Meloidogyne actinidiae* (Li & Yu, 1991) and *Meloidogyne aberrans* (Tao et al., 2017) (Tylenchida: Meloidogynidae) reported in kiwifruit producing areas around the world (Vovlas & Roca, 1976; Haygood et al., 1990; Le & Yu, 1991; Philippi et al., 1996; Nicotra et al., 2003; Corneiro et al., 2004; Corneiro et al., 2007; Ma et al., 2007; Ploetz, 2009; Tao et al., 2017; Shokoohi & Mashela, 2020). Eighty-six weed species in 32 families have been found in kiwifruit orchards in Ordu Province (Yonat & Kolören, 2017). Forty-nine weed species in 27 families were identified in kiwifruit orchards in Eastern Black Sea Region of Türkiye (Sezer & Kolören, 2019). Many weeds are excellent hosts of plant parasitic nematodes (Gharabadiyan et al., 2012). Such weeds can provide a reservoir of nematodes for the next season for the survival of plant parasitic nematodes in the absence of an annual crop (Rich et al., 2008). Quénéhervé et al. (2006) found 29 weed hosts of *Meloidogyne* spp. In Brazil, 24 weed species were determined to be hosts of RKNs (Belle et al., 2020). In addition, 226 weed species have been investigated for their suitability to different RKNs worldwide (Rich et al., 2009). Das et al. (1998) stated that many weeds are good hosts for RKNs and controlling weeds would be an excellent first step to reducing RKN populations. In agriculture, knowledge of the host status of weeds can be used to improve targeted weed management, especially to increase the effectiveness of the nematode management strategies used in organic farming.

When considering the high polyphagic potential of *Meloidogyne* spp., it is important to the range of host weeds in order to choose the appropriate management for these plant parasites. Therefore, the objective of this study was to determine the species RKN*s* in weeds in the kiwifruit orchards in Ordu Province, Türkiye.

Materials and Methods

Plant sampling

The survey was conducted in May-September 2018 in the Ordu Province, Türkiye. The weed samples were collected from 27 kiwifruit orchards located in the Altınordu, Fatsa, Gülyalı, İkizce, Kabadüz, Perşembe, Ulubey and Ünye districts of Ordu Province. Initially, roots of weeds were examined on site and plant samples including roots were collected from those with galls, labeled and placed in plastic bags for transport to the laboratory. Above and below parts of the samples were photographed and identified according to the Flora of Turkey (Davis, 1965-1988) and Ackerunkraeuter Europas (Hanf, 1990).

Nematode extraction and identification

Roots were examined under a stereo microscope (S8APO, Leica, Wetzlar, Germany) at magnifications of 10X after washing in tap water. Adult RKN females were carefully collected randomly from the infested roots by dissecting the roots with a needle.

Nematode Genomic DNA extraction and PCR amplification

Nematode genomic DNA was extracted from a single female using the procedure of Pagan et al. (2015). A single female was handpicked from infested roots and transferred into 10-µl AE buffer (10 mM Tris-Cl, 1 mM EDTA). Proteinase K (0.1 mg/ml) and Triton X (0.1%) were added. The females were macerated with a glass rod in a 1.5 ml tube. Samples in PCR tubes were frozen at -20°C overnight. Samples were then incubated at 56°C/1 h and 95°C/10 min, and used immediately for PCR or stored at -20°C.

To amplify the mitochondrial DNA fragments, the primers TRNAH/MRH106 or MORF/MTHIS developed by Stonton et al. (1997) were used. The primers set and sequences used for the identification of RKNs are presented in Table 1. A 25-µl PCR was performed containing 1.5 µl of DNA, 1.25 µl of each primer, 8.5 µl distilled water, and 12.5 µl DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, Massachusetts, USA). In order to determine the mitochondrial haplotype, the fragments amplified using the primer set TRNAH/MRH106 were digested with restriction enzymes, *Hin*fI and *Mnl*I, according to the manufacturer's instructions. Finally, the species of *Meloidogyne* was also determined using species-specific sequence-characterized amplified region (SCAR) primer sets Far/Rar developed for *M. arenaria* (Zijlstra et al., 2000), JMV1/JMV2/JMV *hapla* for *M. hapla* (Wishart et al., 2002) and Mi2F4/Mi1R1 for *M. incognita* (Kiewnick et al., 2013). The thermal cycler conditions for each primer set for PCR are given in Table 2.

Table 1. Primers used for *Meloidogyne* species determination for specimens collected from weeds in kiwifruit orchards

Primers	Sequence (5'-3')	Primer	Sequence (5'-3')	References
TRNAH	TGAATTTTTTATTGTGATTAA	MRH106	AATTTCTAAAGACTTTTCTTAGT	Stanton et al., 1997
MORF	ATCGGGGTTTAATAATGGG	MTHIS	AAATTCAATTGAAATTAATAGC	Stanton et al., 1997
Far	TCGGCGATAGAGGTAAATGAC	Rar	TCGGCGATAGACACTACAACT	Zijlstra et al., 2000
Mi2F4	ATGAAGCTAAGACTTTGGGCT	Mi1R1	TCCCGCTACACCCTCAACTTC	Kiewnick et al., 2013
JMV1 JMV	GGATGGCGTGCTTTCAAC	JMV2	TTTCCCCTTATGATGTTTACCC AAAAATCCCCTCGAAAAATCCACC	Wishart et al., 2002

Table 2. PCR conditions used for primer pairs in the identification of *Meloidogyne* species

Primers	Initial denaturation	Denaturation	Annealing (40 cycles)	Extension	Final extension
TRNAH/MRH106	3 min, 95°C	30 s, 95°C	30 s, 95°C	60 s, 68°C	7 min, 68°C
MORF/MTHIS	3 min, 95°C	30 s, 95°C	30 s, 95°C	60 s, 68°C	7 min, 68°C
Far/Rar	3 min, 95°C	30 s, 95°C	60 s, 95°C	60 s, 72°C	5 min, 72°C
Mi2F4/Mi1R1	3 min, 95°C	30 s, 95°C	30 s, 95°C	60 s, 72°C	1 min, 72°C
JMV1/JMV2/JMV hapla	3 min, 95°C	30 s, 95°C	30 s,95°C	2 min, 72°C	7 min, 72°C

Gel Electrophoresis

PCR products were separated using horizontal gel electrophoresis in 1.5% agarose gels containing ethidium bromide in 1X Tris-acetate EDTA (TAE) buffer. The gel was run for 25 min at 150 V then visualized and photographed under UV light using GEN-BOX imageER.

Results

In this study, the RKN species were investigated on the weeds occurring in kiwifruit orchards established in Ordu Province, Türkiye. Twenty-seven kiwifruit orchards were sampled and the 15 identified weed species (in 8 families) were found to be RKN hosts: *Solanum nigrum* L. (Solanales: Solanaceae), *Mercurialis annua* L. (Malpighiales: Euphorbiaceae), *Fumaria officinalis* L. (Ranunculales: Papaveraceae), *Clinopodium nepeta* (L.) Kuntze, *Melissa officinalis* L. (Lamiales: Lamiaceae), *Oxalis pes-caprae* L. (Oxalidales: Oxalidaceae), *Amaranthus retroflexus* L. (Caryophyllales: Amaranthaceae), *Erigeron canadensis* L., *Sonchus asper* (L.) Hill, *Artemisia absinthium* L., *Sigesbeckia orientalis* L., *Senecio vulgaris* L., *Taraxacum officinale* L. (Asterales: Asteraceae), and two unidentified weed species, *Lycopus* spp. (Lamiales: Lamiaceae) and *Lythrum* spp. (Myrtales: Lythraceae).

Twenty-seven populations of RKNs were obtained from the infested weed plants (Table 3). The weeds were found to be infested with *M. incognita, M. hapla* and *M. arenaria* at frequencies 74.1, 22.2 and 3.7%, respectively. *Meloidogyne incognita* was the most common RKN species corresponding to 74.1%, identified in *S. nigrum*, *M. annua*, *F. officinalis*, *M. officinalis*, *O. pes-caprae*, *A. retroflexus*, *E. canadensis*, *A. absinthium*, *C. nepeta*, *S. orientalis*, *S. vulgaris* and *Lythrum* spp. Secondly, *M. hapla* was the most frequent species encountered in five sampled locations. The host weeds infested by the nematode were detected as *E. canadensis, S. orientalis, S. asper*, *T. officinale* and unidentified weed species. Finally, *M. arenaria* was determined at one location in *A. retroflexus*.

Identification of RKN species were made using the molecular method based on mitochondrial DNA. The polymerase chain reactions amplification with the primers TRNAH/MRH106 produced a single fragment at 556 bp for *M. hapla*, 557 bp for *M. arenaria* and *M. incognita* (Figure 1). A fragment of 214 bp in *M. arenaria* and 742 bp in *M. incognita* were produced by PCR amplification using MORF/MTHIS primers whereas *M. hapla* did not give any fragment (Figure 2). The digestion assay of TRNAH/MRH106 using *Hinf*I produced 445 and 112 bp fragments for *M. arenaria*, 446 and 110 bp fragments for *M. hapla*, and 396, 112 and 49 bp for *M. incognita* (Figure 3). The *Mnl*I digestion assay produced a single fragment of 556 bp with *M. hapla*, three fragments of 340, 140 and 77 bp with *M. arenaria*, and 340 and 217 bp with *M. incognita* (Figure 4).

Figure 1. PCR amplification products of *Meloidogyne arenaria, M. hapla* and *M. incognita* mtDNA with TRNAH and MRH106 primers. M: 100 bp ladder marker; 557 bp for *M. arenaria (*Lane 6); 556 bp for *M. hapla* (Lanes 9-10, 13, 20 and 25-26); 557 bp for *M. incognita* (Lanes 1-5, 7-8, 11-12, 14-19, 21-24 and 27).

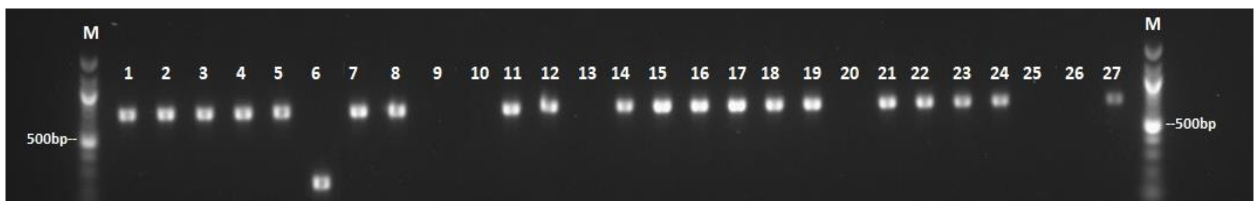

Figure 2. PCR amplification products of *Meloidogyne arenaria, M. hapla* and *M. incognita* mtDNA with MORF and MTHIS primers. M: 100 bp ladder marker; 214 bp for *M. arenaria (*Lane 6); No product for *M. hapla* (Lanes 9-10, 13, 20 and 25-26); 742 bp for *M. incognita* (Lanes 1-5, 7, 8, 11, 12, 14-19, 21-24 and 27).

Table 3. Weed species in kiwifruit orchards in Ordu Province, Türkiye that were found to be hosts of *Meloidogyne* spp.

District	Village		Common name	Family	Scientific name	*Meloidogyne*
Gülyalı	Eren	1	Black nightshade	Solanaceae	*Solanum nigrum*	*M. incognita*
Altınordu	Eyüplü	2	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*
	Şenocak	3	Common fumitory	Fumariaceae	*Fumaria officinalis*	*M. incognita*
	Karapınar	4	Lemon balm	Lamiaceae	*Melissa officinalis*	*M. incognita*
	Burhanettin	5	Sourgrass	Oxalidaceae	*Oxalis pes-caprae*	*M. incognita*
Fatsa	Eskiordu	6	Redroot pigweed	Amaranthaceae	*Amaranthus retroflexus*	*M. arenaria*
		7	Black nightshade	Solanaceae	*Solanum nigrum*	*M. incognita*
	Bozdağ	8	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*
	Kılıçlı	9	Horseweed	Asteraceae	*Erigeron canadensis*	*M. hapla*
		10	Gypyswort	Lamiaceae	*Lycopus* spp.	*M. hapla*
	Hıdırbeyli	11	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*
	Meşebükü	12	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*
Ünye	Cevizdere	13	Spiny sowthistle	Asteraceae	*Sonchus asper*	*M. hapla*
		14	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*
		15	Redroot pigweed	Amaranthaceae	*Amaranthus retroflexus*	*M. incognita*
	Yüceler	16	Wormwood	Asteraceae	*Artemisia absinthium*	*M. incognita*
		17	Nice mint	Lamiaceae	*Clinopodium nepeta*	*M. incognita*
		18	Black nightshade	Solanaceae	*Solanum nigrum*	*M. incognita*
Ulubey	Güven	19	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*
	Çatallı	20	Spiny sowthistle	Asteraceae	*Sonchus asper*	*M. hapla*
	Durak	21	Divine herb	Asteraceae	*Sigesbeckia orientalis*	*M. incognita*
		22	Horseweed	Asteraceae	*Erigeron canadensis*	*M. incognita*
		23	Common groundsel	Asteraceae	*Senecio vulgaris*	*M. incognita*
İkizce	Merkez	24	Loosestrife	Lythraceae	*Lythrum* spp.	*M. incognita*
Perşembe	Boğazcık	25	Divine herb	Asteraceae	*Sigesbeckia orientalis*	*M. hapla*
Kabadüz	Kabadüz	26	Common dandelion	Asteraceae	*Taraxacum officinale*	*M. hapla*
		27	Annual mercury	Euphorbiaceae	*Mercurialis annua*	*M. incognita*

Figure 3. PCR amplification products of *Meloidogyne arenaria, M. hapla* and *M. incognita* mtDNA with TRNAH and MRH106 primers after digestion with *Hinf* I restriction enzyme. M: 100 bp ladder marker; 445 and 112 bp for *M. arenaria* (Lane 6); 446 and 110 bp for *M. hapla* (Lanes 9-10, 13, 20 and 25-26); 396, 112 and 49 bp for *M. incognita* (Lanea 1-5, 7-8, 11-12, 14-19, 21-24 and 27).

Figure 4. PCR amplification products of *Meloidogyne arenaria, M. hapla* and *M. incognita* mtDNA with TRNAH and MRH106 primers after digestion with *Mnl* I restriction enzyme. M: 100 bp ladder marker; 340, 140 and 77 bp for *M. arenaria (*Lanes 1-4); 556 bp for *M. hapla* (Lanes 5-7); 340 and 217 bp for *M. incognita* (Lanes 8-11).

Using species-specific PCR primers, the specimens were determined as *M. arenaria, M. hapla* or *M. incognita* was also confirmed. SCAR primer set Far/Rar for *Meloidogyne arenaria* gave a 420-bp fragment, JMV1/JMV2/JMV PCR primers for *M. hapla* gave a 440-bp product and Mi2F4/Mi1R1 primers for *M. incognita* gave a 300-bp product (Figure 5).

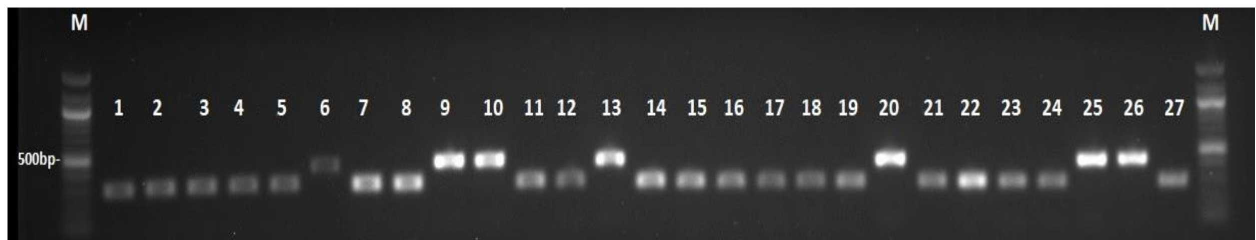

Figure 5. PCR amplification products of *Meloidogyne arenaria, M. hapla* and *M. incognita* in multiplex PCR assay using species-spesific primers Far/Rar, JMV, Mi2F4/Mi1R1 respectively. M: 100 bp ladder marker; 420 bp fragment for *M. arenaria (*Lane 6); 440 bp fragment for *M. hapla* (Lanes 9-10, 13, 20 and 25-26); 300 bp for *M. incognita* (Lane 1-5, 7, 8, 11-12, 14-19, 21-24 and 27).

Discussion

Weeds may impact the growth of the crops as competitors as well as sources of pests and pathogens, so it is important to identify both the host weed and any parasite nematodes they support. This investigation was detailed research about the identification of RKNs on the weeds occurring in kiwifruit orchards. In this study, some weeds were found to be infested by *M. arenaria, M. hapla* and *M. incognita*. Those three nematode species are considered the most common species worldwide (Taylor et al., 1982). In addition, even on a regional scale, the results were similar (Aydınlı & Mennan, 2016). In this context, the results of our investigation are consistent with the previous investigations. Fifteen weed species were found to be infested with RKNs. The most common weed host was *M. annua* at seven locations and followed by *S. nigrum* at three locations. The weed hosts, *A. retroflexus*, *E. canadensis*, *S. asper* and *S. orientalis* were found at two locations each and the least commonly detected weed host were *F. officinalis, M. officinalis*, *O. pes-caprae*, *A. absinthium*, *C. nepeta*, *S. vulgaris* and *T. officinale* at one location for each.

Mercurialis annua was infested at all sites but only with *M. incognita*. Bendixen (1986) listed this weed as a host of an unidentified *Meloidogyne* spp. Similarly, Philis & Siddiqi (1976) and Philis (1995) also listed this weed as associated with a *Meloidogyne* sp. In light of previous research, our result is the first to identify *M. incognita* in *M. annua*.

Amaranthus retroflexus was the host with the widest range of RKN species (Bendixen, 1988). In our investigation, the *M. arenaria* and *M. incognita* were found in this host. *Meloidogyne arenaria* was identified in *A. retroflexus* at all locations where it was sampled in the present study. In previous research on *A. retroflexus*, Amin (1994) reported *M. arenaria* in Hungary; Kornobis & Wolny (1997) *M. hapla* in Poland, Castillo et al. (2008) *M. incognita* in Spain, and Ercan & Elekcioğlu (2009) *M. incognita* and *M. javanica* in Türkiye. In addition, some investigations have examined the susceptibility of *A. retroflexus* to *Meloidogyne* spp. (Tedford & Fortnum, 1988; Belair & Benoit, 1996; Kaur et al., 2007; Kokalis-Burelle & Rosskopf, 2012).

In our investigation, the only RKN in *S. nigrum* was *M. incognita. Solanum nigrum* is a weed that has been a focus of previous work, and found to be as susceptible as susceptible crop cultivars (Zancada et al., 1998; Ehwaeti et al., 1999). In addition, as a reservoir of important RKN species such as *M. incognita*, the weed was considered as needing to be controlled (Ponce et al., 1995). The most frequently found RKN species in *S. nigrum* was *M. incognita* (Smit, 1978). In addition, Lindhardt (1963) and Whitehead (1969) found *M. hapla* and *M. javanica,* respectively, in this host, and Pajovic et al. (2007) found *M. arenaria*. In Spain (Castillo et al., 2008) and Türkiye (Ercan & Elekcioğlu, 2009), *M. incognita* and *M. javanica* have been found in *S. nigrum*. Also, *Meloidogyne exigua* (Goeldi, 1887) (Tylenchida: Meloidogynidae) and *M. ethiopica* have been found in *S. nigrum* (Curi, 1973; Aydınlı & Mennan, 2016), and *S. nigrum* and *S. vulgaris* have been reported as hosts of *Meloidogyne chitwoodi* (Golden, O'Bannon, Santo & Finley, 1980) (Tylenchida: Meloidogynidae) with galling and egg production (Kutywayo & Been, 2006).

Taraxacum officinale was found to be infested by *M. hapla* at one location in the present study. Kornobis & Wolny (1997) and Smiley et al. (2014) reported some other groups of nematodes associated with this weed, and Gaskin & Crittenden (1956) reported it as a host of *M. hapla*. Doucet et al. (2000) reported it as a good host that may enhance the spread of *M. hapla*. Mitkowski & Abawi (2002) reported successful reproduction of *M. hapla* in root culture system with *T. officinale*.

Erigeron canadensis was found to be infested with *M. incognita* and *M. hapla* at two separate locations in the present study. Bajwa et al. (2016) concluded that this weed as one of the most problematic, noxious, invasive and widespread weeds in modern-day agriculture. Kim et al. (1998) reported that *Erigeron canadensis* and *Erigeron annuus* L. (Asterales: Asteraceae) were infested by *M. hapla* under field conditions. Ijani et al. (2000) reported *M. javanica* infestion in *E. canadensis* and *Erigeron sumatrensis* Retz. (Astarales: Asteraceae).

Sonchus asper was found to be infested with *M. hapla* at two locations in the present study. In previous reports, Lindhardt (1963) identified *M. hapla* in heavily galled roots of this host. Mangat et al. (1985) obtained *M. javanica* eggs from the roots of *S. asper*. Amin (1994) reported *M. arenaria* in *S. asper* and additionally *M. incognita* and *M. arenaria* on both *Sonchus oleraceus* L. and *Sonchus arvensis* L. (Astarales: Asteraceae).

Artemisia absinthium was found to be infested with *M. incognita* at one location in the present study. Bendixen (1986) listed some species of *Artemisia* as hosts of *M. hapla,* but for *A. absinthium* the species of RKN was unidentified. In another study, Walker (1995) inoculated *A. absinthium* with *M. incognita* race 3 achieving a root galling rate between 26-51%.

Senecio vulgaris was found to be infested with *M. incognita* at one location in the present study. The host status of this weed has generally been determined in inoculation studies. Davidson & Townshend (1967) inoculated *S. vulgaris* with *M. incognita*, but no galls were observed. Townshend & Davidson (1962) conducted a similar investigation with *M. hapla* and even though they observed small galls in high numbers, no nematode reproduction was evident. Belair & Benoit (1996) inoculated *S. vulgaris* seedlings with *M. hapla* and they observed root galling but no eggs or juveniles. Thus, *S. vulgaris* has been reported as a potentially useful trap plant for RKN, especially *M. hapla* species.

Fumaria officinalis was found to be infested with *M. incognita* at one location in the present study. This weed has only previously been reported as a host of *M. javanica* (Philis & Siddiqi, 1976; Philis, 1995). Consequently, our finding indicates that *F. officinalis* can also host *M. incognita*.

Melissa officinalis was found to be infested with *M. incognita* at one location in the present study. The previous research has shown that this weed can be a host of major RKNs. Karl et al. (1997) inoculated *M. officinalis* with eggs of *M. javanica* and found that the weed as highly susceptible to this nematode. Tzortzakakis et al. (2011) reported *M. arenaria* and *M. javanica* in *M. officinalis* in Greece. Santos (2018)

reported the weed as the host of *M. hapla*. *Meloidogyne arenaria* was also found in *M. officinalis* in Greece by Karanastasi et al. (2008).

Sigesbeckia orientalis was found to be infested by *M. incognita* and *M. hapla* at separate locations in the present study. Silva et al. (2016) found *M. javanica* in *S. orientalis* in Brazil. *Sigesbeckia orientalis* is here first reported as a host of *M. hapla*.

Oxalis pes-caprae was found to be infested with *M. incognita* at one location in the present study. In previous studies, *Oxalis* spp. were found to be the hosts of the common RKNs (Martin, 1958; Oliveira & Kubo, 2006; Bellé et al., 2020). For *O. pes-caprae*, Ciancio et al. (1992) reported that *M. javanica* was the species parasitizing the weed in İtaly. Gonçalves et al. (2020) reported that *O. pes-caprae* is a potential host of *M. javanica* based on inoculation studies. Consequently, the in the present study is the first to report *O. pes-caprae* as a host of *M. incognita*.

Clinopodium nepeta was found to be infested with *M. incognita* at one location in the present study. being the first report of *M. incognita* in this weed.

In conclusion, our results showed that major RKN species, *M. arenaria, M. hapla* and *M. incognita,* can occur in many common weeds with these findings consistent with previous investigations, globally (Taylor et al., 1982) and regionally (Aydınlı & Mennan, 2016). In addition, *E. canadensis*, *M. annua*, *O. pes-caprae, C. nepeta*, *F. officinalis* and *Lythrum* spp. are reported for first time as hosts of *M. incognita*, and *S. orientalis* and *Lycopus* spp. as a host of *M. hapla*. Of the weed species, *S. nigrum*, *A. retroflexus, E. canadensis, M. annua,* and *T. officinale* were the most important species found in the present study as hosts of RKNs and as weeds in importance globally. Management practices for RKNs in the first three species must be given high priority, given they are common hosts for the major RKNs worldwide. The latter two species, *M. annua*, *T. officinale*, will be important regionally for managing *M. incognita* and *M. hapla*, respectively. Those considerations are also valid for specific management practices for both weeds and RKNs in Ordu Province. In the province, Yonat (2016) reported the weed status of kiwifruit plantations showing that *S. nigrum*, *A. retroflexus, E. canadensis, M. annua* and *T. officinale* occur in these plantations. Similarly, Sezer & Koloren (2019) determined the species and some parameters of the weeds in kiwifruit orchards in the Eastern Black Sea Region of Türkiye. They found that *S. nigrum*, *A. retroflexus, E. canadensis, M. annua* and *Taraxacum* sp. were present in the region, and that *E. canadensis* occurred frequently as at 75% in 2014 and 88% in 2015. Therefore, these five weeds must be specifically considered as nematode reservoirs in management practices in kiwifruit orchards. However, previous investigations have found that the RKN host status of weeds can be wide and variable. Although, the major RKNs were mostly reported in these investigations, some other RKNs species, including *M. ethiopica*, should also be considered (Aydınlı & Mennan, 2016). *Meloidogyne ethiopica* has been identified in Ordu Province and in other provinces to the west (Aydınlı & Mennan, 2016). Therefore, the weeds found in the surveys may affect the management practices used at a regional level in kiwifruit orchards. As a management option, trap plants can be used to reduce nematode numbers in the soil. The mechanism is that invasion and gall formation occur but the life cycle of the nematode apparently cannot be completed (Townshend & Davidson, 1962). Townshend & Davidson (1962) inoculated weeds with *M. hapla* and observed small galls in high numbers, but when inoculate with *M. incognita* (Davidson & Townshend, 1967), no galls were observed. Bélair & Benoit (1996) observed galling of the roots without eggs production with *M. hapla* inoculation. Similarly, they did not observe development of *M. hapla* on the root system of *A. retroflexus* grown in soil which when inoculated with around 18,000 juveniles. In this context, *S. vulgaris* may be the promising trap plant in kiwifruit orchards for especially *M. hapla*.

As result, there is value of knowing the host status of the weeds but it depends on many factors such as intra- and interspecies virulence of different races, different growing environments (open field or greenhouse), abiotic factors varying across regions which affect the performance of the host and nematode.

These all need to be investigated to help develop site-specific management approaches for the future. The results of the present study showed that weeds can potentially be reservoirs of RKNs and should be considered as factors affecting the success of integrated nematode management programs. Controlling the weeds would be a useful initial step in reducing RKN populations in the kiwifruit orchards.

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