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Original article (Orijinal araştırma)

Distribution and identification of important plant parasitic nematodes in anise growing areas[1]

Anason yetiştirilen alanlarda önemli bitki paraziti nematodlarının dağılımı ve tanımlanması

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Abstract

Anise, *Pimpinella anisum* L. (Apiales: Apiaceae) is an important medicinal aromatic plant and can be attacked by different pests and pathogens. Plant parasitic nematodes are important pests that can be confused with nutrient deficiency or symptoms of various diseases or pests. Therefore, rapid and accurate identification of these pests is essential for integrated nematode management and rotation. In 2021, a survey was conducted in Bolvadin District of Afyonkarahisar Province, which is one of the most important anise production areas of Türkiye. Forty-two soil samples were collected from the anise growing areas in the district and 16 species-specific primers were used for molecular identification of plant parasitic nematodes. In the samples, *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Heteroderidae), *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) and *Aphelenchoides besseyi* Christie, 1942 (Aphelenchida: Aphelenchoididae), were detected at the rates of 57% (24), 52% (22), 36% (15) and 7% (3), respectively. Plant parasitic nematodes were found in both single and mixed populations. In addition, *A. besseyi* was found for the first time in anise growing areas.

Keywords: Anise, identification, PCR, plant parasitic nematodes

Öz

Anason, *Pimpinella anisum* L. (Apiales: Apiaceae) önemli bir tıbbi aromatik bitkidir ve farklı zararlılar ve patojenler tarafından saldırıya uğrayabilmektedir. Bitki paraziti nematodlar, zararları besin eksikliği veya çeşitli hastalık veya zararlıların semptomları ile karıştırılabilen önemli zararlılardır. Bu nedenle, entegre nematod mücadele programları ve ürün rotasyonu için bu organizmaların hızlı ve doğru tanımlanması esastır. 2021 yılında Türkiye'nin en önemli anason üretim alanlarından biri olan Afyonkarahisar İli Bolvadin İlçesi'nde sürvey çalışması yapılmıştır. Bölgedeki anason alanlarından 42 toprak örneği alınmış ve bitki paraziti nematod türlerinin moleküler tanımlanmasında türe özgü 16 primer kullanılmıştır. Örneklerde *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Heteroderidae), *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) ve *Aphelenchoides besseyi* Christie, 1942 (Aphelenchida: Aphelenchoididae) sırasıyla %57 (24), %52 (22), %36 (15) ve %7 (3) oranlarında belirlenmiştir. Bitki paraziti nematodlar hem tek hemde karışık popülasyonlar halinde bulunmuştur. Ayrıca *A. besseyi*, anason üretim alanlarında ilk kez belirlenmiştir.

Anahtar sözcükler: Anason, tanımlama, PCR, bitki paraziti nematodlar

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Introduction

Anise, *Pimpinella anisum* L. (Apiales: Apiaceae) is an annual and aromatic herb belong to the Apiaceae family (Ghorbanpour et al., 2017; ITIS, 2021). The flowers of the plant are umbrella-shaped and white color, while the fruits are greenish yellow and hairy (Orav et al., 2008; Shojaii & Abdollahi, 2012; Karık, 2020). Plants belonging to the order Apiales are generally rich in essential oil. In addition, this oil is extremely valuable in terms of vitamins and minerals (Keskin & Baydar, 2016). Many are of pharmaceutical importance in making herbal medicines. Anise which is one of the most important plants in this family as an example cumin [*Carum carvi* L. (Apiales: Apiaceae)], coriander [*Coriandrum sativum* L. (Apiales: Apiaceae)] and fennel [*Foeniculum vulgare* Miller (Apiales: Apiaceae)], is an ancient cultivar originated from the eastern Mediterranean Basin. Anise is cultivated in countries such as China, Egypt, Greece, India, Russia, Spain, Syria and Türkiye (Demirayak, 2002). In 2019, about 2 Mt of fennel, star anise and coriander was harvested from about 2 Mha globally, with an average yield of 0.95 t ha^{-1} (FAOSTAT, 2019). The countries with the highest production areas were India, Syria, Türkiye, Russia and China (in decreasing order of production) (FAOSTAT, 2019). In Türkiye, 10.7 kt of anise was produced on 15 kha, according to 2020 data, and the average yield is 0.69 t ha^{-1} (TUIK, 2021). Afyonkarahisar is one of the main anise growing provinces of Türkiye (TUIK, 2021).

In the production of anise, different diseases, organisms, nematodes and various weeds can cause significant losses (Anonymous, 2008). However, among these factors plant parasitic nematodes (PPNs) are very important pests but their damage is mostly confused with the symptoms of other factors or can be misidentified as nutrient deficiencies (Singh & Phulera, 2015). Globally, studies on anise have been limited. *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Heteroderidae) has only reported in anise production areas in Nepal (Bhardwaj & Hogger, 1984). There are various studies on other medicinal aromatic plants such as cumin, fennel and coriander. In the cumin production areas of India, it was found that these areas were infested with *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae), *Hoplolaimus indicus* Sher, 1963 (Tylenchida: Hoplolaimidae) and *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Heteroderidae) (Kant et al., 2017). In another study, *M. incognita* was found in fennel production areas in Egypt (Ibrahim & Mokbel, 2009). Similarly, fennel production areas in Iran, PPNs in the genera *Meloidogyne*, *Helicotylenchus*, *Tylenchorhynchus*, *Tylenchus* and *Xiphinema* was reported (Nasresfahani et al., 2015). In a study conducted on the coriander in Pakistan, *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971 (Tylenchida: Belonolaimidae), *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963 (Tylenchida: Hoplolaimidae) and *Xiphinema* sp. were detected (Khan et al., 2021).

In Türkiye there is only one report of PPNs in anise production areas and 15 plant parasitic nematode species, including *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae), *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 (Tylenchida: Hoplolaimidae), *M. arenaria* and *Pratylenchus zeae* Graham, 1951 (Tylenchida: Pratylenchidae) were identified (Kepenekci, 2003). Also, in fennel, another important plant of the Apiaceae family, 10 PPNs have been identified, including *P. thornei*, *P. zeae* and *H. dihystera* (Evlice & Kepenekci, 2006). Crop rotation is extensively practiced in anise production in Türkiye and cereals are generally used in the rotational crop. *Pratylenchoides alkani* Yüksel, 1977, *Pratylenchus crenatus* Loof (1960) (Tylenchida: Pratylenchidae), *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 (Tylenchida: Pratylenchidae), *P. thornei* and *Pratylenchus vulnus* Allen & Jensen, 1951 (Tylenchida: Pratylenchidae) have been reported in cereal growing fields of Türkiye (Söğüt et al., 2011; Yavuzaslanoğlu et al., 2012; Kasapoğlu Uludamar et al., 2018; Dababat et al., 2019; Yavuzaslanoğlu et al., 2020; Göze Özdemir et al., 2021). Therefore, identification of PPNs is needed for anise fields. This study was conducted in Bolvadin District of Afyonkarahisar Province which has an important province in anise growing in Türkiye.

Materials and Methods

Sampling

To detect harmful PPNs in anise, sampling was done from anise areas in Bolvadin District, where production is made within the scope of organic or good agriculture in June 2021 (Figures 1 & 2). The samples were taken with a shovel along zigzag transects in the fields. Each sample consisted of 5-10 spade slices (3 cm thick, 15-20 cm deep and 15 cm wide). Forty-two samples were taken in total. Global positioning system coordinates of the sampled sites are given in Table 1.

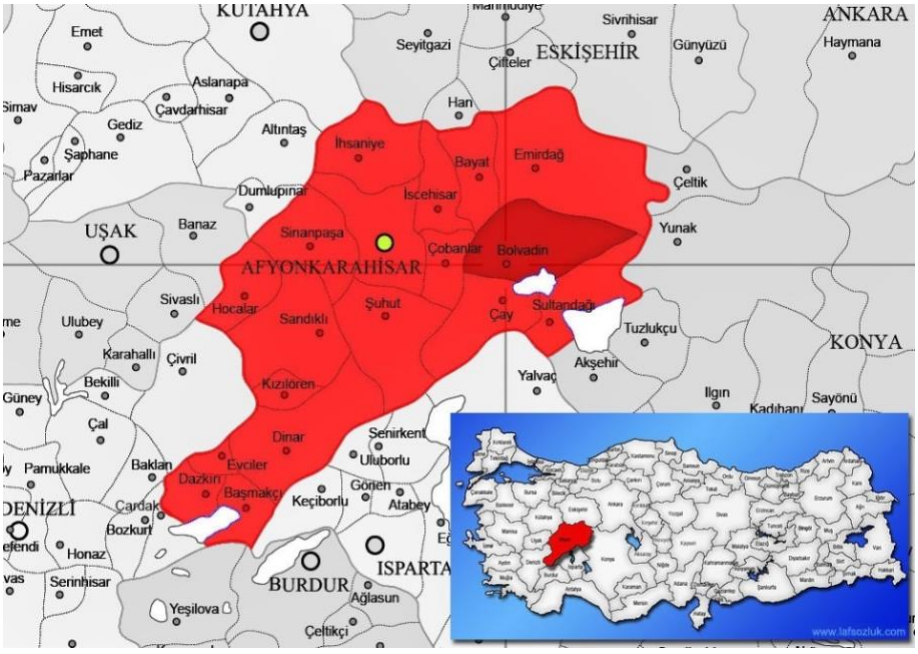

Figure 1. Location of the Bolvadin District where the samples were collected for this study (Anonymous, 2021).

Figure 2. A typical anise crop in Bolvadin District.

Table 1. Morphological-molecular analysis results and location information of samples obtained from anise growing areas in Bolvadin, Türkiye

Sample codes	Coordinates	Morphologic results *	Mi **(MincF/R)	Mi** (INK14F/R)	Mj** (Fjav/Rjav)	Ma** (Far/Rar)	Mh** (JMV1/JMV2/JMVHapla)	Pn**(PNEG/D3B)	Pn** (PNEGF1/D3B5)	Pp** (PPENA/A28)	Pt** (PTHO/D3B)	Pt** (18sInt/26sInt)	Hf**(HfITS-F1HfITS-R1)	Ha** (HalTS-F6/ R4)	Hl** (Hlat-actF/R)	Ab** (AbF5/AbR5)	Af** (AfragF1/R1)	Ar** (BSF/ArtR)
A-1	38º45'50" / 31º16'01"	M, P, A	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
A-2	38º45'47" / 31º16'02"	M, P, A	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
A-3	38º45'46" / 31º16'00"	M, P	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-
A-5	38º47'39" / 31º16'27"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-6	38º47'52" / 31º17'21"	M, P, A	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
A-7	38º47'53" / 31º17'19"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-8	38º47'52" / 31º17'16"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-9	38º47'54" / 31º17'15"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-10	38º47'56" / 31º17'15"	M, P	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-
A-11	38º44'29" / 31º13'33"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-12	38º44'28" / 31º13'32"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-13	38º47'34" / 31º16'43"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-14	38º47'38" / 31º16'44"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-15	38º45'46" / 31º16'00"	M, P	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
A-16	38º47'41" / 31º18'02"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-17	38º47'46" / 31º18'04"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-18	38º47'44" / 31º18'07"	M, A	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
A-19	38º48'08" / 31º20'00"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-20	38º48'05" / 31º20'03"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-21	38º48'03" / 31º20'05"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-22	38º47'08" / 31º16'26"	P	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
A-23	38º48'43" / 31º20'59"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-24	38º48'41" / 31º20'56"	M, P	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-
A-25	38º48'43" / 31º20'55"	P	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
A-26	38º48'44" / 31º21'00"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-27	38º49'00" / 31º21'17"	M, P	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-
A-28	38º48'57" / 31º21'20"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-30	38º49'24" / 31º21'43"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-31	38º49'21" / 31º21'47"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-32	38º49'16" / 31º21'50"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-33	38º47'52" / 31º17'16"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-34	38º49'14" / 31º21'49"	M, P	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-
A-35	38º49'19" / 31º21'55"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-36	38º49'21" / 31º21'57"	M, P	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-
A-37	38º49'23" / 31º21'53"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-38	38º49'45" / 31º22'06"	P	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-

Table 1. Continued

Sample codes	Coordinates	Morphologic results *	Mi **(MincF/R)	Mi** (INK14F/R)	Mj** (Fjav/Rjav)	Ma** (Far/Rar)	Mh** (JMV1/JMV2/JMVHapla)	Pn**(PNEG/D3B)	Pn** (PNEGF1/D3B5)	Pp** (PPENA/A28)	Pt** (PTHO/D3B)	Pt** (18sInt/26sInt)	Hf**(HfITS-F1HfITS-R1)	Ha** (HaITS-F6/ R4)	Hl** (Hlat-actF/R)	Ab** (AbF5/AbR5)	Af** (AfragF1/R1)	Ar** (BSF/ArtR)
A-41	38º49'58" / 31º22'14"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-42	38º50'00" / 31º22'17"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-43	38º50'00" / 31º22'19"	P	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
A-46	38º49'55" / 31º22'22"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-47	38º49'54" / 31º22'25"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-50	38º49'52" / 31º22'32"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mi[1]	-	*M. incognita*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mj[1]	-	*M. javanica*	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Ma[1]	-	*M. arenaria*	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Mh[2]	-	*M. hapla*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Pn[2]	-	*P. neglectus*	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Pp[2]	-	*P. penetrans*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Pt[2]	-	*P. thornei*	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Hf[3]	-	*H. filipjevi*	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Ha[3]	-	*H. avenae*	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Hl[3]	-	*H. latipons*	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ab[4]	-	*A. besseyi*	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Af[5]	-	*A. fragariae*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Ar[5]	-	*A. ritzemabosi*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

* Morphological identification indicates M: *Meloidogyne* spp. P: *Pratylenchus* spp. A: *Aphelenchoides* spp., PPN: Plant parasitic nematodes;

** Mi: *Meloidogyne incognita*; Mj: *M. javanica;* Ma: *M. arenaria*; Mh: *M. hapla*; Pn: *Pratylenchus neglectus*; Pp: *P. penetrans*; Pt: *P. thornei*; Hf: *Heterodera filipjevi*; Ha: *H. avenae*; Hl: *H. latipons*; Ab: *Aphelenchoides besseyi*; Af: *A. fragariae*; Ar: *A. ritzemabosi*;

[1] Positive control from laboratory culture (Devran & Söğüt, 2009);

[2] Positive control from previous study (Sert Celik et al., 2019);

[3] Positive control from Bolu Abant Izzet Baysal University, Türkiye (by Mustafa Imren);

[4] Positive control from previous study (Devran et al., 2017);

[5] Positive control from National Plant Protection Organization, Netherlands (by Gerrit Karssen).

Nematode extraction

Nematodes in the soil samples were extracted by using the modified Baermann funnel technique (Hooper, 1986).

Morphological identification

Plant parasitic nematode species were checked as morphologically in genus level with the stereo binocular microscope according to Jepson (1987), Handoo & Golden (1989) and EPPO (2021).

Molecular identification

DNA isolation

Isolation of total genomic DNAs from nematodes was performed using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions.

PCR amplification

PPNs were determined using species-specific primers (Table 2). The PCR reactions were carried out on the SimpliAmp™ (Applied Biosystems, San Francisco, CA, USA) using the reaction conditions specified in former studies for different plant parasitic nematode species (Waeyenberge et al., 2000; Zijlstra et al., 2000; Wishart et al., 2002; Al-Banna et al., 2004; Gleason et al., 2008; Troccoli et al., 2008; Yan et al., 2008; Devran et al., 2018). PCR outcomes were analyzed on a 1.5% agarose gel in 1X TAE and visualized with Xpert Green DNA Stain using the Gel iX Imager (Intas Science, Göttingen, Germany).

Results

Morphological identification

Morphological analysis indicated PPNs belonging to the genera *Aphelenchoides, Meloidogyne* and *Pratylenchus* were present in the samples. PPNs were not found in nine samples (Table 1).

Molecular identification

Molecular analysis of the samples were performed with 16 species-specific primer sets that could identify PPNs belonging to *P. thornei*, *P. neglectus*, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 (Tylenchida: Pratylenchidae), *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* (Madzhidov, 1981), *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae), *Aphelenchoides besseyi* Christie, 1942, *Aphelenchoides fragariae* (Ritzema-Bos, 1891) Christie, 1932, *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932 (Aphelenchida: Aphelenchoididae), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *M. incognita*, *M. arenaria* and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Heteroderidae) (Table 2).

Both PTHO/D3B and 18sInt/26sInt primer sets were used to identify *P. thornei*. DNA fragments were obtained of about 288 bp and 828 bp, respectively (Table 2). *Pratylenchus thornei* was detected in 15 samples in total (Table 1). DNA fragments of about 150 bp and 300 bp were obtained with primer sets PNEGF1/D3B5 and PNEG/D3B, respectively, which were used for identification of *P. neglectus,* which was found in 22 samples (Table 1). The PPENA/AB28 primer sets (Table 2) were used for the detection of *P. penetrans*, however, no DNA band was obtained in the samples analyzed.

In all identification of samples species specific primer sets were used, HaITS-F6/R4, Hlat-act F/R, Hf ITS-F1/R1 that identified *H. avenae*, *H. latipons* and *H. filipjevi* positive controls respectively; however, primers did not give any DNA fragments from the samples assayed (Table 1).

To detect foliar nematodes, primer sets AbF5/AbR5, BSF/ArtR and AfragF1/AfragR1 were used to identify *A. besseyi*, *A. ritzemabosi* and *A. fragariae*, respectively (Table 2), with only *A. besseyi* detected in three samples (Table 1).

Species-specific primers MincF/MincR-Inck14F/Inck14R, Far/Rar and Fjav/Rjav were used for identification of *M. incognita*, *M. arenaria* and *M. javanica,* respectively. However, these root-knot nematodes (RKNs) were not detected in the samples assayed (Table 1). Samples were assayed with JMV1, JMV2 and JMV hapla primer sets (Table 2). *Meloidogyne hapla* was detected in 24 of the 42 samples (Tables 1 & 2).

Distribution of PPNs

Meloidogyne hapla, *P. neglectus*, *P. thornei* and *A. besseyi* were detected at the rates of 57, 52, 36 and 7%, respectively, in the anise production areas in Bolvadin District. *Meloidogyne hapla* was the most prevalent species and *A. besseyi* was the least common in sampled areas. Similarly, *P. thornei* and *P. neglectus* were also determined a moderate number of sampling sites. In addition, these species were found as mixed populations in the fields. PPNs were mixed in 50% of the samples analyzed (Table 1).

Table 2. Species-specific primer pairs used in molecular analyses of samples obtained from anise production areas in Bolvadin, Türkiye

Species	Primer name	Amplicon (s) size (bp)	References
Meloidogyne incognita	IncK14-F / IncK14-R	~399	Randig et al., 2002
	MincF / MincR	~150	Devran et al., 2018
Meloidogyne javanica	Fjav / Rjav	~670	Zijlstra et al., 2000
Meloidogyne arenaria	Far / Rar	~420	Zijlstra et al., 2000
Meloidogyne hapla	JMV1 / JMV2 / JMVhapla	~440	Wishart et al., 2002
Aphelenchoides besseyi	AbF5 / AbR5	~340	Devran et al., 2017
Aphelenchoides fragariae	AFragF1 / AFragR1	~169	McCuiston et al., 2007
Aphelenchoides ritzemabosi	BSF / ArtR	~208	Cui et al., 2010
Heterodera filipjevi	HfITS-F1 / HfITS-R1	~170	Yan et al., 2013
Heterodera avenae	HaITS-F6 / HaITS-R4	~242	Yan et al., 2013
Heterodera latipons	Hlat-actF / Hlat-actR	~204	Toumi et al., 2013
Pratylenchus neglectus	PNEG / D3B	~290	Al-Banna et al., 2004
	PNEGF1 / D3B5	~144	Yan et al., 2008
Pratylenchus penetrans	PPENA / AB28	~660	Waeyenberge et al., 2000
Pratylenchus thornei	PTHO / D3B	~288	Al-Banna et al., 2004
	18sInt / 26sInt	~828	Troccoli et al., 2008

Discussion

Medicinal aromatic plants are unique plants that have many uses such as food, spice, medicine and cosmetics and are known to have been used for similar purposes since the beginning of humanity (Ullah et al., 2015). Some of these plants can be collected directly from nature, while others are cultivated professionally. Anise is known as the most important medicinal and aromatic plants cultivated for using agricultural and cosmetic industries (Demirayak, 2002). However, many pests and diseases can cause yield losses in anise including PPNs, the damage of which can be confused with nutrient deficiency (Anonymous, 2008; Singh & Phulera, 2015). Morphological and morphometric identifications of PPNs are time-consuming and require expertise. In addition, mixed plant parasitic nematode species can be found in agricultural production areas. Therefore, rapid, correct and easier identification of plant parasitic nematode species in production areas is important for the management of these pests. For these reasons, molecular identification techniques can be used intensively to identify the pests in question. In this study, species-specific primer sets were used to detect economically important RKNs, only *M. hapla* was detected in anise production areas. Adam et al. (2007) reported that *M. incognita*, *M. arenaria* and *M. javanica* are common in tropical regions, while *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 (Tylenchida: Heteroderidae), *M. hapla* and *Meloidogyne fallax* Karssen, 1996 (Tylenchida: Heteroderidae) are mostly distributed in cooler areas. Our sampling area has an elevation of 995-1100 m and is a relatively cooler area. Therefore, our results are consistent with previous reports on the geographical distribution of *Meloidogyne* spp. However, *M. arenaria* was reported in anise production areas in Nepal (Bhardwaj & Hogger, 1984). Also, in a study conducted in Pakistan, *M. javanica* and *M. incognita* were determined in

about 12% of the production areas of coriander from the same family as anise (Anwar & McKenry, 2010). Similarly, Singh & Gupta (2011) show that RKNs (*M. incognita* and *M. javanica*) were detected in coriander and ginger production areas in India. The reason for the differences may be climatic conditions.

In Türkiye, no previous work has been done on the identification of PPNs in the areas surveyed. However, a study was conducted in anise production areas in Burdur, which is also located in the Lakes Region of Türkiye, 15 PPN species were morphologically determined (Kepenekci, 2003). However, cereals are used for rotation in anise fields in Türkiye. Some studies were conducted to identify PPNs in cereal growing areas. *Pratylenchus thornei* and *P. neglectus* were reported as mixed populations (Sahin et al., 2008; Yavuzaslanoğlu et al., 2012; Göze Özdemir et al., 2021). In the present study, *P. neglectus* and *P. thornei* were found as mix populations in the area sampled. Our results were consistent with previous studies. The population densities and prevalence of PPNs may be due to variations in crop rotation and soil conditions (such as humidity and temperature) (Wallace et al., 1993; Yavuzaslanoğlu et al., 2020). In cereal production fields of Türkiye, *H. filipjevi*, *H. latipons* and *H. avenae* can cause significant crop damage (Imren et al., 2012; Yavuzaslanoğlu et al., 2012, Dababat et al., 2015). However, these species were not found in the anise growing areas surveyed. It is thought that the reason why *Heterodera* spp. could not be found in the samples examined may be due to the sampling time. *Aphelenchoides besseyi* is reported to occur in rice fields of Türkiye (Oztürk & Enneli, 1997; Tülek & Cobanoğlu, 2010). Recently, studies were conducted on molecular identification of *A. besseyi* and estimation of the number of it in paddy rice (Devran et al. 2017; Sert Celik & Devran, 2019; Sert Celik et al., 2020). In this study, *A. besseyi* was identified for the first time in anise production areas of Türkiye. However, there is no published information about the suitability of anise as a host for foliar nematodes (*Aphelenchoides* spp.) (CABI, 2021; Nemaplex, 2021; EPPO, 2021). However, *Avena sativa* L. (Poales: Poaceae) is a known host for *A. besseyi* (Nemaplex, 2021). In Türkiye, oats can be grown in rotation with anise in the area surveyed. Therefore, this nematode may have originated from oats.

In conclusion, this is the first study on the identity of PPNs in anise growing areas in Afyonkarahisar Province of Türkiye. These results could prove useful for integrated pest management practices and crop rotation to decrease the yield loses and increase the quality in anise growing areas.

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