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Occurrence and abundance of nematodes in almond growing areas in Karaman, Türkiye and their relationships with almond characteristics[1]

Karaman, Türkiye'de badem yetiştirme alanlarında nematodların dağılımı ve popülasyon yoğunluğu ile badem özellikleri ile ilişkileri

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Abstract

Almond, *Prunus dulcis* Rouchy (Rosales: Rosaceae) is an economically important plant species widely grown in Türkiye. Plant parasitic nematodes have been known to affect the economical production of almond. In this study, the occurrence and abundance of plant parasitic and free-living nematodes were determined in almond-growing areas in Karaman province in 2024. The soil samples were collected from 40 almond orchards and two wild almond production areas. Each orchard was represented by 20 subsamples collected in a zigzag pattern with 20-25 footsteps in between using a 2.5 cm diameter soil corer from the projection area of the trees. The nematodes were extracted from soil samples using petri dish technique for 72 hours. The most prevalent plant parasitic nematodes were *Ditylenchus* spp. Filipjev, 1936 (Rhabditida: Anguinidae) (97.6%), *Tylenchus* spp. Bastian, 1865 (Rhabditida: Tylenchidae) (88.1%) and *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae) (83.3%). The populations of most prevalent nematode species *Ditylenchus* spp. and *Pratylenchus* spp. ranged between 0-143.9 and 0-197.4 nematodes per 100 g dry soil, respectively. The population of bacterivorous nematode *Cephalobus* spp. Bastian, 1865 (Rhabditida: Cephalobidae) was positively correlated with 10-year-old, seed rootstocks almonds cultivated under rainfed conditions.

Keywords: Almond, distribution, plant parasitic nematodes, rootstock, variety

Öz

Badem, *Prunus dulcis* Rouchy (Rosales: Rosaceae) Türkiye'de yaygın olarak yetiştirilen ekonomik açıdan önemli bir bitki türüdür. Bitki paraziti nematodların bademin ekonomik üretimini etkilediği bilinmektedir. Bu çalışmada, Karaman ilindeki badem yetiştirme alanlarında bitki paraziti ve serbest yaşayan nematodların varlığı ve bolluğu 2024 yılında belirlenmiştir. Toprak örnekleri 40 badem bahçesi ve 2 yabani badem yetiştirme alnından toplanmıştır. Her bahçe, zikzak şeklinde 20-25 adım ara ile ağaçların izdüşüm alanından 2,5 cm çapında bir toprak örnekleme aleti kullanılarak toplanan 20 alt örnekle temsil edilmiştir. Nematodlar, 72 saat boyunca petri kabı tekniği kullanılarak toprak örneklerinden ayrılmıştır. En yaygın bitki paraziti nematodlar *Ditylenchus* spp. Filipjev, 1936 (Rhabditida: Anguinidae) (97.6%), *Tylenchus* spp. Bastian, 1865 (Rhabditida: Tylenchidae) (88.1%) ve *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae) (83.3%) olmuştur. En yaygın nematod türleri olan *Ditylenchus* spp. ve *Pratylenchus* spp.'nin popülasyonları sırasıyla 100 g kuru toprakta 0-143,9 ve 0-197,4 nematod arasında değişmiştir. Bakteriyel nematod *Cephalobus* spp. Bastian, 1865 (Rhabditida: Cephalobidae) popülasyonu, kıraç koşullarda yetiştirilen 10 yaşındaki badem ağaçlarının çöğür anaçlarıyla pozitif korelasyon göstermiştir.

Anahtar sözcükler: Badem, dağılım, bitki paraziti nematodlar, anaç, çeşit

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Introduction

Almond, *Prunus dulcis* Rouchy (Rosales: Rosaceae) is a perennial medicinal and aromatic plant with high economic value. It is widely cultivated in the Western Mediterranean, Western and Southeastern Anatolia regions of Türkiye. Almond planting has been increasing due to drought climate conditions and government supports in Karaman province, which is among the top 10 provinces in almond production in Türkiye (TÜİK, 2025). The most widely grown fruit is apples in the region. It is replaced by almonds in areas where water is limited. Wild almond species also occur naturally in the region.

Plant-parasitic nematodes in crop production areas cause crop losses. The plant-parasitic nematodes in almond production areas in Karaman province have not been previously investigated. Root knot, *Meloidogyne* spp. Göldi, 1892 (Rhabditida: Meloidogynidae), root lesion, *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae) and criconematid nematodes, *Criconema* spp. Hofmänner & Menzel, 1914 (Rhabditida: Criconematidae) have been reported as plant parasitic nematode species that cause significant losses in stone fruits and *Prunus* species L. (Rosales: Rosaceae) (Pinochet et al., 1996). *Helicotylenchus digonicus* Perry, 1959 (Rhabditida: Hoplolaimidae), *Ditylenchus myceliophagus* Goodey, 1958 (Rhabditida: Anguinidae) and *Aphelenchus avenae* Bastian, 1865 (Rhabditida: Aphelenchidae) were reported as the most common nematode species in almond production areas in Adıyaman in Türkiye (Tan et al., 2018). Similarly, *Helicotylenchus* Steiner, 1945 (Rhabditida: Hoplolaimidae) was found in almond sapling production areas in İzmir, Türkiye (Yıldız & Gözel, 2015). *Heterodera* Schmidt, 1871 (Rhabditida: Heteroderidae), *Meloidogyne* spp., *Pratylenchus* spp., *Tylenchulus* spp. Bastian, 1865 (Rhabditida: Tylenchidae), *Trichodorus* spp. Cobb, 1913 (Triplonchida: Trichodoridae), *Hemicycliophora* spp. de Man, 1921 (Rhabditida: Hemicycliophoridae), *Tylenchorhynchus* spp. Cobb, 1913 (Rhabditida: Dolichodoridae), *Paratylenchus* spp. Micoletzky, 1922 (Rhabditida: Tylenchulidae), *Criconema* spp., *Paratrichodorus* spp. Siddiqi, 1974 (Triplonchida: Trichodoridae), *Helicotylenchus* spp. with *Xiphinema americanum* Cobb, 1913 (Dorylaimidae: Longidoridae) have been recorded in almond plantation areas in California, USA (McKenry & Kretsch, 1987; Wauters et al., 2025). *Scutylenchus rugosus* (Siddiqi, 1963) Siddiqi, 1979 (Rhabditida: Dolichodoridae) (168/100 g soil), *Boleodorus thylactus* Thorne, 1941 (Rhabditida: Tylenchidae) (58/100 g soil), *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 (Rhabditida: Hoplolaimidae) (36/100 g soil) and *H. digonicus* (34/100 g soil) were in high abundance in almond orchards in Iran. Additionally, *Pratylenchus thornei* Sher & Allen, 1953 (Rhabditida: Pratylenchidae), *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970 (Rhabditida: Dolichodoridae) and *H. digonicus* were reported in lower population density (Aminisarteshnizi, 2022). *Scutylenchus rugosus, Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966 (Rhabdtida: Pratylenchidae) and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Rhabditida: Meloidogynidae) were present in 6 almond orchards which nematode management practices were conducted in Pakistan. Plant parasitic nematodes; *Helicotylenchus* spp., *Ditylenchus* spp. Filipjev, 1936 (Rhabditida: Anguinidae), *Paratylenchus* spp. and *Boleodorus pakistanensis* Siddiqi, 1963 (Rhabditida: Tylenchidae) with fungivores nematode; *Aphelenchoides* spp. Fischer, 1894 (Rhabditida: Aphelenchoididae) were also in high incidence in those almond orchards (Khan et al., 2015). Distribution of fungivores and bacterivorous nematode species which contribute to mineralization in soil has been shown to be closely related to organic fertilizer applications (Aminisarteshnizi, 2022) and diversity of cover crops (Wauters et al., 2025) in almond plantations.

Plant parasitic nematodes significantly damage the roots of almond saplings by inhibiting water and nutrient uptake. After uprootation of mature trees, the nematodes persist in the remaining capillary roots in soil and cause problems in subsequent crops (Anonymous, 2025). *Criconemella xenoplax* (Raski, 1952) Luc & Raski, 1981 (Rhabditida: Criconematidae) was found associated with bacterial canker in a rot complex in almond orchards in California (McKenry & Kretsch, 1987).

Some of plant parasitic nematodes cannot be differentiated in subspecies level (Subbotin et al., 2005). Because steps for morphological diagnosis takes time and requires expertise, the use of molecular methods in the nematode identification has been increasing (Ateş Sönmezoğlu et al., 2020).

Determining the risks posed by plant-parasitic nematodes in almond production provides the basis for taking management actions. For this purpose, this study determined the distribution of nematodes in almond orchards in Karaman province using morphological and molecular identification methods. Additionally, the relationships between nematode distributions and location, rootstock, cultivar and age of almond trees in the orchards were investigated.

Materials and Methods

Sampling locations

Almond orchards with an area of larger than 20 da were sampled in May 2024 in Karaman Central District. A 2 kg of soil sample from approximately 20 trees in 15-20 footsteps apart were combined for each orchard. Soil samples were taken 0-30 cm soil depth around the tree projection area using a 2.5 cm diameter soil corer for nematodes. In addition, two soil samples were taken in the same manner from the naturally distributed wild almond trees in Karaman. Totally, 42 samples were collected from almond orchards and wild almond growing areas (Table 1).

Table 1. Soil sampling locations and information related to almond orchards

Sample No	Location	Latitude	Longitude	Rootstock	Cultivar	Production type	Age of tree (year)	Planting distance
1	Paşabağı	37.1100	33.3918	GF677	Ferragnes Ferraduel	Rainfed	7	6x5
2	Gülkaya	37.0472	33.3529	Seed rootstock	Ferragnes Ferraduel	Watered	4	5x5
3	Gülkaya	37.04694	33.35556	Wild almond (*Amygdalus orientalis*)	-	-	-	-
4	Yeşildere	37.17306	33.41302	Seed rootstock	Ferragnes Ferraduel	Watered	2	.-
5	Yeşildere	37.17306	33.41302	Wild almond (*Amygdalus orientalis*)	-	-	-	-
6	Dereköy	37.1292	33.2915	Seed rootstock	Ferragnes Ferraduel	Rainfed	9	5x5
7	Dereköy	37.0712	33.1724	Nemaguard	Ferragnes Ferraduel	Watered	7	5x5
8	Dereköy	37.1222	33.3080	Garnem	Ferragnes Ferraduel	Watered	8	5x5
9	Gökçe	37.0665	33.2860	GF677	Ferragnes Ferraduel Texas Bertina Nonperial	Watered	7	5x5
10	Kozlubucak	36.9972	33.2780	-	-	Watered	3	6x5
11	Tavşanlı	37.0313	33.2492	Seed rootstock	Ferragnes Ferraduel Texas Nonperial	Rainfed	10	6x5
12	Tavşanlı	37.02521	33.26037	Seed rootstock	Ferragnes Ferraduel	-	8	6x5
13	Cerit	36.9549	33.1924	Seed rootstock	-	Watered	4	6x5
14	Elmadağı	36.91302	33.16864	-	-	Rainfed	-	-
15	Kılbasan	37.3585	33.2207	Seed rootstock	Ferragnes Ferraduel	Watered	4	4x4
16	Dinek	37.3994	33.2571	GF677	Ferragnes Ferraduel	Watered	8	6x5
17	Karacaören	37.4760	33.2437	Seed rootstock	Ferragnes Ferraduel	Watered	5	5x5
18	Eğilmez	37.5564	33.1972	GF677	Ferragnes Ferraduel	Watered	10	5x5
19	Eğilmez	37.4981	33.1585	GF677	Ferragnes Ferraduel	Watered	9	5x5
20	Eğilmez	37.4894	33.1462	Garnem	Mokako	Watered	1	6x6
21	Üçkuyu	37.4353	33.0890	GF677	Ferragnes Ferraduel	Watered	6	6x6
22	Süleymenhacı	37.4541	33.0834	GF677	Ferragnes Ferraduel	Watered	5	5x5
23	Ortoba	37.4416	33.0344	Seed rootstock	Ferragnes Ferraduel	Watered	3	6x6
24	Ortaoba	37.4623	33.0288	Seed rootstock	Ferragnes Ferraduel	Watered	3	6x6
25	Ortaoba	37.4461	33.0453	GF677	Ferragnes Ferraduel	Watered	3	6x6
26	Eminler	37.3320	33.1227	Seed rootstock	Ferragnes Ferraduel	Rainfed	3	6x6

Table 1. Continued

Sample No	Location	Latitude	Longitude	Rootstock	Cultivar	Production type	Age of tree (year)	Planting distance
27	Masara	37.1837	33.0927	Seed rootstock	Ferragnes Ferraduel	Watered	12	5x5
28	Yollarbaşı	37.2019	33.0486	Seed rootstock	Ferragnes Ferraduel	Watered	3	5x5
29	Yollarbaşı	37.1987	33.0126	Seed rootstock	Ferragnes Ferraduel	Watered	3	6x6
30	Yollarbaşı	37.2068	33.0007	Seed rootstock	Ferragnes Ferraduel	Watered	4	5x5
31	Pınarbaşı	37.1252	33.0491	Seed rootstock	Ferragnes Ferraduel	Rainfed	3	6x4
32	Pınarbaşı	37.1332	33.0753	Garnem	Ferragnes Ferraduel	Watered	5	6x6
33	Çavuşpınar	37.1365	33.1345	Seed rootstock	Ferragnes Ferraduel	Watered	8	6x5
34	Boyalı	37.0836	33.1601	Seed rootstock	Ferragnes Ferraduel	Watered	2	6x5
35	Boyalı	37.0423	33.1735	-	-	Rainfed	-	-
36	Boyalı	37.0756	33.1815	Garnem	Ferragnes Ferraduel	Watered	3	6x6
37	Pınarbaşı	37.06185	33.15756	Seed rootstock	Ferragnes Ferraduel	-	4	6x6
38	Pınarbaşı	37.09891	33.06993	Seed rootstock	Ferragnes Ferraduel	-	15	6x6
39	Burhan	37.0410	33.0550	Seed rootstock	Ferragnes Ferraduel	Watered	7	6x5
40	Çatak	37.0190	32.9749	Seed rootstock	Ferragnes Ferraduel	Rainfed	4	6x6
41	Aybastı	36.96895	33.07040	Seed rootstock	Ferragnes Ferraduel	-	10	7x7
42	Bucakkışla	36.96411	33.03435	Seed rootstock	Ferragnes Ferraduel	-	15	-

Nematode extraction, morphological identification and population estimation

Nematodes from soil samples were extracted using a 15 cm diameter petri dish from 100 gr of fresh soil (Hooper, 1986). Soil samples were placed on a sieve with a Kleenex tissue and incubated for 72 hours in tap water in petri dishes for nematode extraction. The nematode suspension from each petri dish was transferred to a 100 ml measuring cylinder and kept for 24 hours for settlement of the nematodes. The volume of the nematode suspension was reduced to 10 ml after settlement of the nematodes. Extracted nematodes examined under a light microscope at 40X magnification in 100 µl subsample of nematode suspension. Morphological identification was performed at genus level. Only Dorylaimida were identified at order level.

Dry weight of 10 g fresh soil from each sample was determined at 110℃ overnight in oven. Nematode numbers were presented as per 100 g of dry soil by calculating using the formula below (Eq. 1);

Number of nematodes per 100 g dry soil = Number of nematodes per sample/ Dry weight of 100 g fresh soil sample x 100 (Eq. 1)

Nematodes were grouped according to their feeding behaviors (Yeates et al., 1993). Morphometrical measurements were done on permanent slides of key plant parasitic nematodes and compared to literature (Hooper, 1986).

Nematode molecular identification

DNA extraction

DNA extraction procedure developed by Waeyenberge et al. (2000) and modified by Holterman et al. (2006) was used to isolate from all nematode samples with some minor modifications. Accordingly, 5-10 nematodes were placed in 30 µl of sterile water within a 1.5 ml Eppendorf tube. Subsequently, 30 µl of WLB+ was added. The WLB+ solution consisted of 950 µl WLB- buffer (2 ml 1 M NaCl, 2 ml 1 M Tris-HCl and 5.5 ml ddH2O), 10 µl beta-mercaptoethanol and 40 µl of 20 mg/ml Proteinase K. The mixture was first incubated at -20ºC for 90 min, followed by 65ºC for 90 min, and finally at 95ºC for 5 min in a heating block. After incubation, the mixture was centrifuged at 14,000 rpm for 1 min. The extracted DNA was stored at -20ºC until further use.

Molecular identification of *Ditylenchus dipsaci*

Due to the typically yield low DNA yields and short DNA fragments obtained from nematodes, direct visualization of DNA on agarose gels remains challenging (Kantor et al., 2021; Singh et al., 2021). Therefore, primer pairs PF1/PR1 and PF2/PR2 were used to accurately identify *Ditylenchus dipsaci* (Yavuzaslanoglu et al., 2018). These primers were designed based on the ITS rDNA sequence of *D. dipsaci*, targeting the 18S rRNA gene region and enabling efficient amplification (Marek et al., 2005). PCR amplification using these primers confirmed the identity of the target nematode species collected from almond orchards in Karaman province.

Primers used for the identification of the nematode *D. dipsaci* and root-lesion nematodes are given in Table 2. PCR reactions were performed in a total volume of 25 µL containing PCR buffer (final $MgCl_2$ concentration of 1.5 mM), 200 µM of each dNTP, 0.5 µL of each primer, and 1 U of Taq DNA polymerase (Thermo Scientific). For the PF1/PR1 and PF2/PR2 primers, the PCR cycling profile included an initial denaturation at 95ºC for 3 min, followed by 30 cycles denaturation at 94ºC for 2 min, annealing at 62ºC for 30 s, extension at 72ºC for 2 min, with a final extension at 72ºC for 10 min. For each PCR reaction, distilled water was used as the negative control (nc), and samples obtained from previously prepared cultures of *Ditylenchus dipsaci* were used as the positive control (pc) (Ateş Sönmezoğlu et al., 2020). PCR amplifications were carried out using a BIO-RAD C1000 Touch Thermal Cycler.

Table 2. The markers used for the detection of *D. dipsaci* and root-lesion nematodes

Primer	Target species/Region	Primer sequence (5’→3’)	Expected band size (bp)	Reference
PF1	*Ditylenchus dipsaci*	AAC GGC TCT GTT GGC TTC TAT	327	Marek et al. 2005
PR1	(flanking ITS regions)	ATT TAC GAC CCT GAG CCA GAT		
PF2	*Ditylenchus dipsaci*	TCG CGA GAA TCA ATG AGT ACC	396	Marek et al. 2005
PR2	(flanking ITS regions)	AAT AGC CAG TCG ATT CCG TCT		
PNEG	*Pratylenchus neglectus*	ATGAAAGTGAACATGTCCTC	290	Al-Banna et al. 2004
PPEN	*Pratylenchus penetrans*	TAAAGAATCCGCAAGGATAC	278	Al-Banna et al. 2004
PSCR	*Pratylenchus scribneri*	AAAGTGAACGTTTCCATTTC	286	Al-Banna et al. 2004
PTHO	*Pratylenchus thornei*	GAAAGTGAAGGTATCCCTCG	288	Al-Banna et al. 2004
PVUL	*Pratylenchus vulnus*	GAAAGTGAACGCATCCGCAA	287	Al-Banna et al. 2004

Molecular identification of root-lesion nematodes (*Pratylenchus* spp.)

Species-specific primers targeting the 26S rDNA D3 expansion region for species identification of root-lesion nematodes are listed in Table 2. Forward primer sequences were chosen to be compatible with the common reverse primer, D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Al-Banna et al., 2004). PCR conditions were optimized by testing different annealing temperatures for each primer set. PCR was carried out in a 25 µL reaction mixture containing DNA template, 2 U of Taq polymerase (Thermo Scientific), 200 µM each dNTP, 0.8 µM of each primer, 2.5 mM $MgCl_2$, and 1x buffer solution. PCR cycles were as follows: 3 min at 95ºC for the initial denaturation step, 35 cycles of denaturation at 95ºC for 1 min at, annealing at 62ºC for 1 min (PTHO, PVUL) or 52°C (PNEG, PSCR, PPEN) for 1 min, and extension at 72ºC for 1 min, with a final extension at 72ºC for 10 min.

In species-specific PCR screens for *Paratylenchus* species, genomic DNA from *Pratylenchus thornei* was used as a positive control. This sample yielded amplification only with the *P. thornei*-specific primer set (PTHO); band formation at the expected fragment size was not observed with the other species-specific primer sets. The positive control, although it only gave bands with its own primer set, was also used to rule out false positives or contamination with other primer sets. In addition, it served to assess the reliability of the PCR products.

To confirm whether the sizes of the PCR products were consistent with the fragment sizes predicted based on known nucleotide sequences, all PCR experiments were performed in triplicate. During the analysis, each well was manually marked in the gel imaging software; the band size and well positions of the DNA ladder were also recorded. This allowed for the detection of non-target, non-species-specific products that produced bands of similar fragment size, and careful comparisons were made.

The PCR products were separated by electrophoresis on a 2% agarose gel prepared in a total volume of 120 ml containing 24 ml 5x TBE, 96 ml dH_2O, 2.4 g agarose and 7 µl of ethidium bromide (10 mg/ml) and the results were visualized using a gel documentation system (Biorad ChemiDoc MP).

Statistical analysis

Number of plant parasitic, fungivores, bacterivorous, predator and omnivorous nematodes were determined in the samples. Occurrence frequency of nematode genera was calculated for each Location. Nematode numbers in Locations were analyzed by ANOVA and TUKEY HSD test for each nematode genus. Significantly higher nematode population for each nematode genus was determined. Mean and standard deviation of nematode numbers in each Location were calculated. Relationship of the nematode populations with rootstock and plant production type was determined by using ANOVA, TUKEY HSD and student t test, respectively. Multiple correspondence analyses of production type, rootstock and age of almond trees in sampled locations was conducted. Data was evaluated at significance level of $p<0.05$ at the statistical analysis. JMP® Pro16.0.0 statistical program was used for conduction of statistical analysis (JMP, 2025).

Results and Discussion

Occurrence of nematodes

Plant parasitic, fungivores and bacterivorous nematode feeding groups were found in all samples taken from almond orchards. Omnivorous nematodes were found in 28 samples (66.6%) in the order Dorylaimida, while predatory nematodes were found in only one sample in the genus *Mononchus* spp. Bastian, 1865.

Plant parasitic nematode species identified in the samples were *Ditylenchus* spp. Filipjev, 1936 (Rhabditida: Anguinidae), *Pratylenchus* spp. Filipjev, 1936 (Rhabditida: Pratylenchidae), *Pratylenchoides* spp. Winslow, 1958 (Rhabditida: Pratylenchidae), *Paratylenchus* spp. Micoletzky, 1922 (Rhabditida: Tylenchulidae), *Trophurus* spp. Loof, 1956 (Rhabditida: Dolichodoridae), *Psilenchus* spp. de Man, 1921 (Rhabditida: Tylenchidae), *Merlinius* spp. Siddiqi, 1979 (Rhabditida: Dolichodoridae), *Helicotylenchus* spp., *Tylenchus* spp. Bastian, 1865 (Rhabditida: Tylenchidae) and *Filenchus* spp. Andrássy, 1954 (Rhabditida: Tylenchidae).

The most common plant parasitic nematode species was *Ditylenchus*, which was found in all of the samples except one sample in Tavşanlı Location (occurrence frequency; 97.6%). Following, *Tylenchus* spp.was the most common genus at 88.1% occurrence frequency. Root lesion nematodes (*Pratylenchus* spp.), which are reported to cause economic losses in almonds (Pinochet et al., 1996), were also found to be widespread in almond growing areas in Karaman province (83.3%). It was determined that ectoparasitic plant parasitic nematode species, *Paratylenchus* spp. and *Merlinius* spp. were widely distributed in almond growing areas at 64.3% and 52.4%, respectively. *Helicotylenchus* spp. (35.7%), *Filenchus* spp. (19.0%), *Trophurus* spp. (16.6%) *Pratylenchoides* spp. (11.9%) and *Psilenchus* spp. (9.5%) were detected at low frequencies in the sampled areas (Table 3).

Fungivores nematodes of the genera *Aphelenchus* spp. Bastian, 1865 (Rhabditida: Aphelenchidae) and *Aphelenchoides* spp. were found at 95.2% and 97.6% of samples, respectively, in the sampled almond growing areas (Table 3).

Table 3. Occurrence of nematode genera and feeding groups from locations in Karaman province central district

Location	Number of samples collected	*Ditylenchus* spp.	*Pratylenchus* spp.	*Pratylenchoides* spp.	*Paratylenchus* spp.	*Trophurus* spp.	*Psilenchus* spp.	*Merlinius* spp.	*Helicotylenchus* spp.	*Tylenchus* spp.	*Filenchus* spp.	PLANT PARASİTİC NEMATODES	*Aphelenchus* spp.	*Aphelenchoides* spp.	FUNGAL-FEEDING NEMATODES	*Dorylaimida* order	*Cephalobus* spp.	*Eucephalobus* spp.	*Acrobeles* spp.	*Acrobeloides* spp.	*Panagrolaimus* spp.	*Wilsonema* spp.	*Ceratoplectus* spp.	BACTERIAL-FEEDING NEMATODES	*Mononchus* spp.
Aybastı	1	1	1	0	0	0	0	1	0	0	0	1	1	1	1	0	1	1	0	1	0	0	1	1	0
Boyalı	3	3	2	0	2	2	0	1	0	2	2	3	2	3	3	1	3	2	3	2	1	1	1	3	0
Bucakkışla	1	1	1	0	1	0	0	0	0	1	0	1	1	1	1	0	1	0	0	0	0	0	1	1	0
Burhan	1	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	0
Cerit	1	1	1	0	1	0	0	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	0	1	0
Çatak	1	1	1	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	0	1	0	0	0	1	0
Çavuşpınar	1	1	1	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0
Dereköy	3	3	3	0	3	0	0	3	3	3	0	3	3	3	3	3	2	0	1	3	0	0	1	3	0
Dinek	1	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	0	1	1	1	1	0	0	1	0
Eğilmez	3	3	3	0	1	0	0	0	0	3	0	3	2	3	3	2	1	3	2	3	1	0	0	3	0
Elmadağı	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	0
Eminler	1	1	1	0	0	0	1	1	0	1	0	1	1	1	1	0	1	1	1	1	0	0	0	1	0
Gökçe	1	1	1	0	1	0	0	1	1	0	0	1	1	1	1	1	1	1	0	1	0	1	1	1	0
Gülkaya	2	2	1	0	2	0	1	0	0	2	0	2	2	2	2	2	1	2	2	2	0	1	0	2	0
Karacaören	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1	0	0	1	0	1	0	0	0	1	0
Kılbasan	1	1	1	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0
Kozlubucak	1	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	0
Masara	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	0
Ortaoba	3	3	3	0	0	0	0	0	0	3	0	3	3	3	3	1	1	3	2	3	1	0	0	3	0
Paşabağı	1	1	1	0	1	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0
Pınarbaşı	4	4	2	1	1	2	0	2	0	3	2	4	4	4	4	3	2	4	3	4	0	1	1	4	1
Süleymanhacı	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0
Tavşanlı	2	1	1	0	2	0	0	1	2	2	0	2	2	2	2	2	2	2	0	2	0	0	0	2	0
Üçkuyu	1	1	0	0	1	0	0	1	0	1	0	1	1	1	1	0	0	1	1	1	0	0	0	1	0
Yeşildere	2	2	2	0	1	0	0	1	2	2	1	2	2	2	2	2	1	1	1	2	0	0	0	2	0
Yollarbaşı	3	3	2	3	3	3	1	2	0	2	1	3	3	3	3	2	2	3	3	3	2	1	1	3	0
Total number of samples	42	41	35	5	27	7	4	22	15	37	8	42	40	41	42	28	28	36	29	40	8	10	8	42	1
Occurrence frequency %	100	97,6	83,3	11,9	64,3	16,7	9,5	52,4	35,7	88,1	19,0	100,0	95,2	97,6	100,0	66,7	66,7	85,7	69,0	95,2	19,0	23,8	19,0	100,0	2,4

Bacterivorous nematode species identified in the samples were *Cephalobus* spp. Bastian, 1865 (Rhabditida: Cephalobidae), *Eucephalobus* spp. Steiner, 1936 (Rhabditida: Cephalobidae), *Acrobeles* spp. Linstow, 1877 (Rhabditida: Cephalobidae), *Acrobeloides* spp. (Cobb, 1924) Thorne, 1937 (Rhabditida: Cephalobidae), *Panagrolaimus* spp. Fuchs, 1930 (Rhabditida: Panagrolaimidae), *Wilsonema* spp. Cobb, 1913 (Plectida: Plectidae) and *Ceratoplectus* spp. Andrássy, 1984 (Plectida: Plectidae). The most common genera of these nematodes were *Acrobeloides* spp. (95.2%) and *Eucephalobus* spp. (85.7%). Following, the genera *Acrobeles* spp. (69.0%) and *Cephalobus* spp. (66.6%) have widespread distribution in the sampling area. Other bacterivorous nematode species; *Wilsonema* spp. (23.8%), *Panagrolaimus* spp. (19.0%) and *Ceratoplectus* spp. (19.0%) were detected at lower frequencies (Table 3).

The distribution of nematode species identified in the survey conducted in almond growing areas in Karaman province is similar to the distribution of species identified in previous surveys conducted on different plant species in Karaman province. Like almond orchards, *Ditylenchus*, *Tylenchus* and *Paratylenchus* species were common in the wheat and onion fields of Karaman province (Yavuzaslanoglu et al., 2015, 2020).

Karaman province has continental climate characteristics and plant production activities are carried out with limited water use. Therefore, since the fungal feeding channels of nematodes are wider in production areas, the *Ditylenchus* and *Tylenchus* genera, including species that feed on fungal hyphae, were widely distributed (Yeates, 1993). Despite the prevalence of the *Ditylenchus* genus in almond orchards, *Ditylenchus dipsaci* was detected in only five locations (11.9%) (Table 6).

Root lesion nematodes, which have a relatively broad host spectrum, are reported to cause significant economic losses in perennial fruit trees as well as in annual plants. Similar to the distribution rates determined in this study, they were reported to have a distribution rate of 67-72% in apple orchards in Niğde and Kayseri provinces in the Central Anatolia Region (Yüksel et al., 2023).

Among the plant parasitic nematode species identified in almond orchards in the United States, Pakistan and Iran, *Pratylenchus*, *Paratylenchus*, *Helicotylenchus*, *Merlinius* and *Ditylenchus* are common to the genera identified in our study (McKenry & Kretsch, 1987; Khan et al., 2015; Aminisarteshnizi, 2022; Wauters et al., 2025).

Helicotylenchus digonicus and *D. myceliophagus* were common in almond orchards in Adıyaman (Tan et al., 2018). As well as, the *Helicotylenchus* spp. has been found in almond sapling production areas in Izmir province, Türkiye (Yıldız & Gözel, 2015).

The fungivores nematode *Aphelencoides* has been reported to be dominant in almond orchards in Pakistan as well as and *A. avenae* in Adıyaman, Türkiye (Khan et al., 2015; Tan et al., 2018). Since almonds consume little water, it caused fungal-feeding nematode species to become dominant in their rhizosphere.

The distribution of fungivores and bacterivorous nematode species contributing to soil mineralization has been shown to be closely related to organic fertilizer applications (Aminisarteshnizi, 2022) and diversity of cover crops (Wauters et al., 2025) in almond plantations. In the Karaman province, no cover crops have been used in the sampled orchards and weeds have been controlled mechanically. However, a wide diversity of bacterivorous nematode species was found in the sampled orchards. This may be attributed to the lack of excessive chemical use in almond cultivation. Further studies will be useful in assessing the plant health risks in almond cultivation by ecologically investigating the free-living nematode fauna identified in almond production areas.

Abundance of nematode populations

Plant parasitic nematode populations totally ranged between 15.4 and 361.2 nematodes/ 100 g dry soil. The total populations were distributed through the locations. Exceptionally, the populations of *Ditylenchus* spp., *Pratylenchoides* spp., *Paratylenchus* spp., *Trophurus* spp. and *Helicotylenchus* spp. significantly differentiated (p<0.05). The highest nematode population of *Ditylenchus* were in Paşabağı Location (178.0 nematodes/ 100 g dry soil) while the lowest population level was recorded in Kozlubucak Location (5.7 nematodes/ 100 g dry soil). The highest nematode populations of *Pratylenchoides* spp., *Paratylenchus* spp., *Trophurus* spp. and *Helicotylenchus* spp. were in Masara (28.7 nematodes/ 100 g dry soil), Üçkuyu (83.0 nematodes/ 100 g dry soil), Yollarbaşı (11.3 nematodes/ 100 g dry soil) and Cerit (59.3 nematodes/ 100 g dry soil) Locations, respectively. The populations of *Pratylenchus* spp. ranged between 0-197.4 nematodes/ 100 g dry soil among locations. The populations of *Merlinius* spp. were relatively higher in some locations and ranged between 0-143.3 nematodes/ 100 g dry soil. *Tylenchus* spp. is widely distributed in the sampling area and the highest population reached up to 89.5 nematodes/ 100 g dry soil. The populations of *Psilenchus* spp. and *Filenchus* spp. were low frequency and also populations were lower; maximum number of nematodes per 100 g dry soil was recorded as 6.1 and 16.1, respectively (Table 4).

Table 4. Abundance of plant parasitic nematodes in locations in Karaman province central district (Data were presented in the form of mean±standard deviation (range), all values are per 100 g dry soil)

Location	*Ditylenchus* spp.	*Pratylenchus* spp.	*Pratylenchoides* spp.	*Paratylenchus* spp.	*Trophurus* spp.	*Psilenchus* spp.	*Merlinius* spp.	*Helicotylenchus* spp.	*Tylenchus* spp.	*Filenchus* spp.	PLANT PARASITIC NEMATODES
Aybastı	10.3	5.1	0	0	0	0	5.1	0	0	0	20.5
Boyalı	11.1±6.3 (5.1-17.6)	3.7±3.3 (0-5.9)	0±0 (0-0)	21.3±20.3 (0-40.5)	3.6±3.2 (0-5.9)	0±0 (0-0)	3.4±5.8 (0-10.1)	0±0 (0-0)	6.9±6.0 (0-10.7)	9.3±8.3 (0-16.1)	59.4±14.7 (42.9-70.9)
Bucakkışla	15.9	5.3	0	16.0	0	0	0	0	5.3	0	42.5
Burhan	10.0	10	0	10.0	0	0	5	5	10	0	50
Cerit	65.2	17.8	0	17.8	0	0	11.8	59.3	65.2	0	237.2
Çatak	10.6	5.3	0	0	0	0	0	0	10.7	0	26.6
Çavuşpınar	42.4	5.3	0	42.4	0	0	10.6	0	15.9	5.3	121.9
Dereköy	44.3±9.7 (38.6-55.5)	5.5±0.0 (5.5-5.5)	0±0 (0-0)	42.4±27.7 (16.6-71.7)	0±0 (0-0)	0±0 (0-0)	68.1±66.6 (16.6-143.3)	27.6±19.8 (11.1-49.6)	16.6±5.5 (11.1-22.1)	0±0 (0-0)	204.6±109.6 (133.2-330.8)
Dinek	17.1	5.7	0	0	0	0	0	5.7	5.7	0	34.2
Eğilmez	27.2±1.1 (26.4-28.4)	14.6±8.8 (5.3-22.7)	0±0 (0-0)	1.8±3.1 (0-5.3)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	12.5±8.0 (5.7-21.4)	0±0 (0-0)	56.1±2.4 (48.1-58.1)
Elmadağı	36.1	6.0	0	42.1	00	6.0	12.0	6.0	36.1	0	144.4
Eminler	50.8	62.1	0	0	0	5.6	5.6	0	5.6	0	129.8
Gökçe	11.5	5.8	0	37.9	0	0	23.0	11.5	0	0	89.7
Gülkaya	26.2±11.1 (18.3-34.1)	5.7±8.0 (0-11.4)	0±0 (0-0)	20.1±19.8 (6.1-34.1)	0±0 (0-0)	3.1±4.3 (0-6.1)	0±0 (0-0)	0±0 (0-0)	5.9±0.3 (5.7-6.1)	0±0 (0-0)	60.8±34.3 (36.6-85.1)
Karacaören	10.8	5.4	0	0	0	0	5.4	0	10.8	0	32.3
Kılbasan	21.7	5.4	0	16.3	0	0	5.4	0	32.5	0	81.3
Kozlubucak	5.7	5.7	0	17.0	0	0	11.3	5.7	5.7	0	51.1
Masara	40.1	120.4	28.7	28.7	0	0	103.2	34.4	5.7	0	361.2
Ortaoba	33.7±3.3 (31.7-37.5)	80.0±101.7 (21.1-197.4)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	10.6±5.2 (5.4-15.9)	0±0 (0-0)	124.4±100.3 (64.3-240.1)
Paşabağı	178.0	5.7	0	5.7	0	0	0	11.5	5.7	0	206.6
Pınarbaşı	27.2±15.2 (5.4-40.4)	3.8±4.8 (0-10.0)	1.3±2.8 (0-5.5)	2.6±3.0 (0-5.0)	2.6±3.0 (0-5.4)	0±0 (0-0)	5.6±7.8 (0-16.6)	0±0 (0-0)	6.6±6.2 (0-15.0)	2.8±3.3 (0-5.8)	51.3±23.7 (16.3-65.1)
Süleymanhacı	31.7	5.3	0	79.4	0	0	0	0	5.3	5.3	127.1
Tavşanlı	8.4±11.8 (0-16.8)	23.5±33.3 (0-47.1)	0±0 (0-0)	18.5±18.2 (5.6-31.4)	0±0 (0-0)	0±0 (0-0)	5.6±7.9 (0-11.2)	46.1±6.0 (41.8-50.3)	50.0±55.8 (10.5-89.5)	0±0 (0-0)	152.1±30.1 (130.8-173.4)
Üçkuyu	10.4	0	0	83	0	0	5.2	0	5.2	0	103.7
Yeşildere	29.4±33.7 (5.6-53.3)	17.4±8.9 (11.1-23.7)	0±0 (0-0)	5.9±8.4 (0-11.8)	0±0 (0-0)	0±0 (0-0)	2.9±4.2 (0-5.9)	17.4±9.0 (11.1-23.7)	14.4±4.7 (11.1-17.8)	2.8±3.9 (0-5.6)	90.3±64.8 (44.4-136.1)
Yollarbaşı	92.8±50.0 (43.9-143.9)	9.9±12.5 (0-24.0)	11.7±10.6 (5.5-24.0)	19.3±12.1 (5.5-28.3)	7.6±3.2 (5.5-11.3)	2.0±3.5 (0-6.0)	11.3±11.0 (0-21.9)	0±0 (0-0)	15.5±15.0 (0-30.0)	2.0±3.5 (0-6.0)	172.1±92.4 (98.7-275.8)

Free living nematode populations were only significant in *Cephalobus* and *Panagrolaimus* genera. Fungivores nematodes totally ranged from 21.3 to 413.7 nematodes/ 100 g of dry soil. *Aphelenchus* spp. (0-311.8 nematodes/ 100 g dry soil) and *Aphelencoides* spp. (0-230.9 nematodes/ 100 g dry soil) distributed in higher level of populations through the locations. The highest *Cephalobus* spp. population was recorded in Eminler Location as 118.5 nematodes/ 100 g dry soil. *Eucephalobus* spp. (0-136.1 nematodes/ 100 g dry soil), *Acrobeles* spp. (0-134.8 nematodes/ 100 g dry soil) and *Acrobeloides* spp. were other bacterivorous nematodes widely distributed at higher population levels through the locations. *Panagrolaimus* spp., *Wilsonema* spp. and *Ceratoplectus* spp. were rarely found in lower population levels in sampling area. Population levels ranged between 0-48.1, 0-24.1 and 0-5.8 nematodes/ 100 g dry soil, respectively. Predator nematode *Mononchus* spp. was found at 5.0 nematodes/ 100 g dry soil in one sample in Pınarbaşı. Population level of omnivorous nematodes in Order Dorylaimida was between 0-91.1 nematodes/ 100 g dry soil (Table 5).

Pratylenchus; the predominant plant-parasitic nematode in the present study, was recorded at low densities in almond orchards in Iran. Furthermore, in a survey conducted in almond orchards in California (McKenry et al., 1987), three species of root lesion nematodes were identified, as in the present study. *Pratylenchus thornei* was reported at higher population levels (280 nematodes/250 g soil) while, *P. neglectus* (averagely 2 nematodes/250 g soil), and *P. vulnus* (5-19 nematodes/250 g soil) were at lower population levels in almond orchards in California. In the present study, root lesion nematode populations exceeded 100 nematodes per 100 g of dry soil at two locations (Masara and Ortaoba) (Table 5). Similarly, in Iran, *P. thornei* species was determined at the level of 0-130 nematodes/100 g soil in almond orchards (Aminisarteshnizi, 2022).

Table 5. Abundance of free-living nematodes in locations in Karaman province central district (Data were presented in the form of mean±standard deviation (range), all values are per 100 g dry soil)

Location	*Aphelenchus* spp.	*Aphelenchoides* spp.	FUNGAL-FEEDING NEMATODES	*Cephalobus* spp.	*Eucephalobus* spp.	*Acrobeles* spp.	*Acrobeloides* spp.	*Panagrolaimus* spp.	*Wilsonema* spp.	*Ceratoplectus* spp.	BACTERIAL-FEEDING NEMATODES	*Dorylaimida* order	*Mononchus* spp.
Aybastı	25.6	30.8	56.4	5.1	25.6	0	5.1	0	0	5.1	41.0	0	0
Boyalı	9.1±11.1 (0-21.4)	56.6±23.3 (30.4-75.0)	65.7±33.3 (30.4-96.5)	14.6±3.9 (10.1-17.6)	28.5±29.3 (0-58.6)	62.1±63.7 (16.1-134.8)	92.3±109.0 (0-212.6)	3.9±6.8 (0-11.7)	1.7±2.9 (0-5.1)	1.7±2.9 (0-5.1)	204.7±74.1 (123.3-268.2)	30.3±52.6 (0-91.1)	0±0
Bucakkışla	10.6	37.2	47.8	31.8	0	0	0	0	0	5.3	37.2	0	0
Burhan	25.0	65.0	90.0	35.	15.0	15.0	135	0	0	0	200	1.0	0
Cerit	53.4	195.7	249.1	0	77.1	5.9	207.6	0	5.9	0	296.6	0	0
Çatak	21.3	0	21.3	10.7	26.7	0	32.0	0	0	0	69.3	0	0
Çavuşpınar	47.7	95.4	143.2	42.4	132.6	53.0	180.3	0	5.3	0	413.6	10.6	0
Dereköy	35.1±17.0 (16.5-49.9)	84.9±28.1 (55.1-111.0)	120.1±42.3 (71.7-149.8)	7.4±8.4 (0-16.5)	0±0 (0-0)	12.9±22.3 (0-38.6)	99.7±36.3 (61.0-133.2)	0±0 (0-0)	0±0 (0-0)	1.8±3.2 (0-5.5)	121.7±54.2 (61.0-165.4)	27.7±20.0 (5.5-44.4)	0±0 (0-0)
Dinek	11.4	39.9	51.3	0	39.9	11.4	108.3	5.7	0	0	165.3	28.5	0
Eğilmez	8.8±8.1 (0-15.9)	80.0±23.7 (52.9-96.5)	88.9±17.6 (68.7-101.5)	1.8±3.1 (0-5.3)	28.5±2.4 (5.7-48.1)	3.5±3.1 (0-5.3)	70.0±46.8 (34.1-122.9)	3.8±6.6 (0-11.4)	0±0 (0-0)	0±0 (0-0)	107.5±67.0 (51.1-181.6)	3.5±3.1 (0-5.3)	0±0 (0-0)
Elmadağı	162.4	228.6	391.1	0	90.3	6.0	240.7	48.1	24.1	0	409.1	36.1	0
Eminler	62.1	174.9	237.0	118.5	62.1	11.3	220.1	0	0	0	412.0	0	0
Gökçe	57.5	34.5	92.1	17.3	28.8	0	149.6	0	5.8	5.7	207.1	40.3	0
Gülkaya	20.3±11.4 (12.2-28.4)	56.2±15.3 (45.4-67.1)	76.5±3.9 (73.8-79.3)	6.1±8.6 (0-12.2)	56.2±15.3 (45.4-67.1)	5.9±0.3 (5.7-6.1)	93.8±11.9 (85.4-102.2)	0±0 (0-0)	6.1±8.6 (0-12.2)	0±0 (0-0)	168.1±21.0 (153.2-182.9)	8.9±4.6 (5.7-12.2)	0±0 (0-0)
Karacaören	21.5	32.3	53.8	0	21.5	0	123.8	0	0	0	145.3	0	0
Kılbasan	43.3	81.3	124.6	5.4	65.0	32.5	173.3	5.4	5.4	0	287.1	5.4	0
Kozlubucak	28.4	34.1	62.4	11.4	22.7	11.4	79.5	0	0	0	124.9	5.7	0
Masara	68.8	120.4	189.2	5.7	11.5	17.2	235.1	0	0	0	269.5	11.5	0
Ortaoba	62.1±22.3 (37.4-80.4)	42.6±5.0 (37.5-47.6)	104.7±21.4 (80.0-117.9)	1.8±3.1 (0-5.4)	56.8±54.8 (10.7-117.4)	23.1±20.2 (0-37.5)	55.2±43.6 (26.4-112.5)	3.5±6.2 (0-10.7)	0±0 (0-0)	0±0 (0-0)	140.4±35.1 (100.4-166.1)	1.8±3.1 (0-5.3)	0±0 (0-0)
Paşabağı	17.2	166.5	183.7	51.7	120.6	23.0	246.8	0	0	5.7	447.8	22.9	0
Pınarbaşı	41.4±34.8 (16.6-92.4)	102.5±87.9 (32.5-230.9)	143.9±122.2 (54.2-323.3)	7.1±8.6 (0-17.3)	100.6±149.2 (5.4-323.3)	23.0±18.4 (0-40.4)	129.7±63.8 (54.2-184.8)	0±0 (0-0)	5.7±11.5 (0-23.1)	1.4±2.9 (0-5.8)	267.6±231.6 (59.6-594.7)	17.1±21.0 (0-44.2)	1.3±2.5 (0-5.0)
Süleymanhacı	47.7	15.9	63.6	10.6	52.9	95.3	74.2	0	10.6	0	243.6	10.6	0
Tavşanlı	75.6±4.1 (72.7-78.5)	89.2±0.4 (88.9-89.5)	164.8±3.7 (162.2-167.4)	13.4±3.2 (11.2-15.7)	29.8±5.2 (26.2-33.6)	0±0 (0-0)	163.4±53.6 (125.5-201.3)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	206.7±55.7 (167.4-246.1)	10.6±7.1 (5.6-15.7)	0±0 (0-0)
Üçkuyu	15.6	36.3	51.9	0	129.7	10.4	124.5	0	0	0	264.5	0	0
Yeşildere	20.3±13.1 (11.1-29.6)	42.7±10.3 (35.5-50.0)	63.1±2.8 (61.1-65.1)	2.8±3.9 (0-5.6)	11.8±16.7 (0-23.7)	8.9±12.6 (0-18-7.8)	64.7±75.8 (11.1-118.3)	0±0 (0-0)	0±0 (0-0)	0±0 (0-0)	88.2±101.2 (16.7-159.8)	11.5±0.5 (11.1-11.8)	0±0 (0-0)
Yollarbaşı	115.1±170.3 (17.0-311.8)	93.7±30.2 (60.3-119.0)	208.8±179.9 (76.8-413.7)	15.4±14.3 (0-28.3)	109.7±29.4 (77.9-136.1)	15.4±9.2 (5.7-24.0)	152.7±28.7 (120.6-175.7)	3.7±3.2 (0-5.7)	1.9±3.3 (0-5.7)	1.9±3.3 (0-5.7)	300.8±55.1 (257.7-362.8)	13.2±18.3 (0-34.0)	0±0 (0-0)

Helicotylenchus spp., another plant parasitic nematode present in the study was determined to be 34-36 nematodes/100 g soil in Iran almond production (Aminisarteshnizi, 2022) which is similar to our findings in the current study. In California almond orchards, *Helicotylenchus* levels ranged from 3 to 131 nematodes per 250 g of soil (McKenry et al., 1987).

Merlinius spp. and *Filenchus* spp. populations in Iran (Aminisarteshnizi, 2022) were similar to our results. Another plant- parasitic nematode prevalent in this study; *Paratylenchus* spp. was also abundant at 35-367 nematodes/ 250 g soil in California almond production soils (McKenry et al., 1987) whilst population levels were not such high in present study.

Nematode distribution and abundance had similar profile in almond production soils in different geographical regions in the world as well as in Karaman Region in different plant cultivation conditions. As it is known, the soil physicochemical properties and plant production practices strongly influence the nematode fauna in soil, therefore both agroecological conditions and plant cultivated effected the nematode distribution and abundance in almond production (Aminisarteshnizi, 2022; Wauters et al., 2025).

Five different rootstocks were determined in the area surveyed. The prevalent rootstock was Seed rootstock was established in 24 orchards. The rootstock GF677 and Garnem were present in 8 and 4 orchards, respectively. Nemaguard rootstock was only established in one orchard. Two of the locations were wild almond areas. The rootstocks in 3 locations were not able to be determined. There was not any relationship between the rootstocks and the nematode distribution in the surveyed almond plantations. *Cephalobus* spp. populations significantly differentiated between production types of almond orchards. The number of *Cephalobus* spp. was mean of 29.6 nematodes/ 100 g dry soil of 8 rainfed orchards, while averagely 8.8 nematodes/ 100 g dry soil were recorded in 27 watered orchards. Seven of them could not determine. According to multiple correspondence analysis, *Cephalobus* spp. were clustered together with Seed rootstock, rainfed and 10 years old orchards in sampled areas. On the other hand, *Cephalobus* spp. was not found in relatively young orchards (5 years old) established with rootstocks of Garnem, Nemaguard and GF677 in watered conditions (Figure 1).

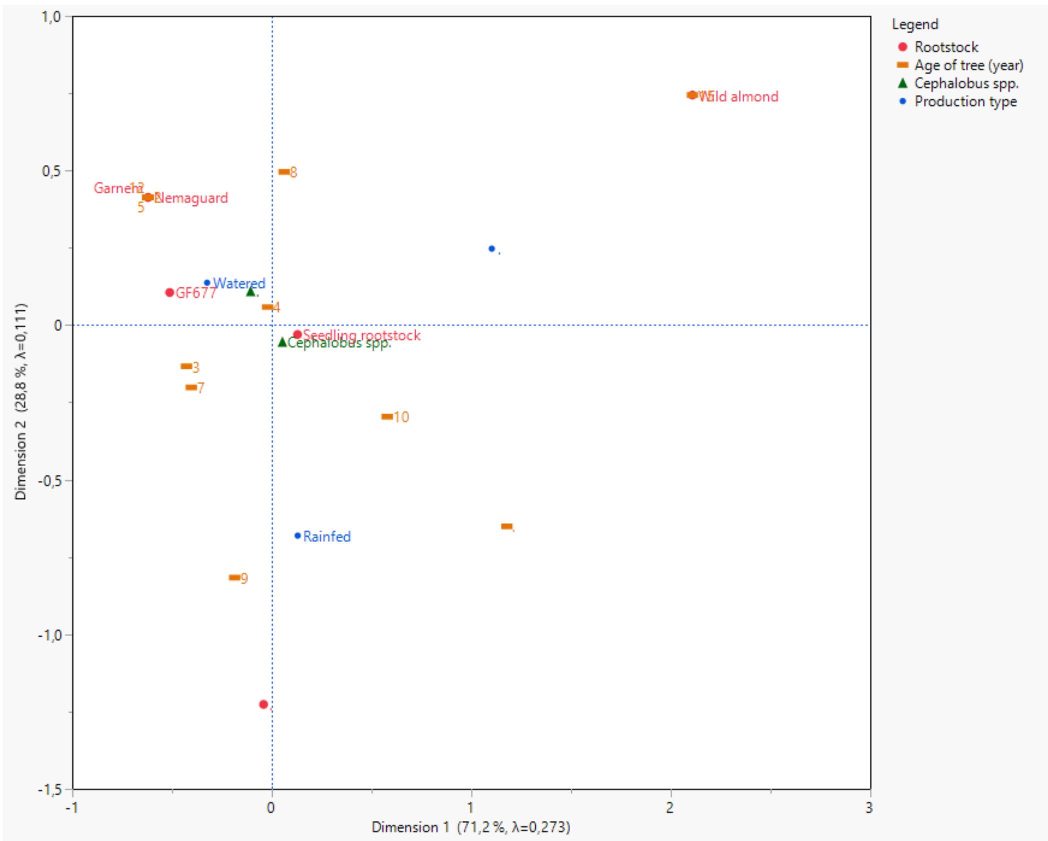

Figure 1. Multiple correspondence analyses of *Cephalobus* spp., production type, rootstock and age of almond trees in sampled locations.

Liang et al. (2005), in a study comparing bacterivorous nematodes in different growing areas, showed that bacterivorous nematodes were more common in forest areas than in annual plant growing areas. Another study reported that *Cephalobus* spp. were more prevalent in undisturbed forest ecosystems (Renčo et al., 2019). In current study, it is thought that older and relatively less maintained orchards established with seedling rootstocks that have strong root structures provided undisturbed soil ecosystem for development of bacterivorous nematodes especially *Cephalobus* spp. which had significant relationships with almond orchard characteristics. This situation was also clearly demonstrated in nematode community analyses conducted in pasture areas of different ages; species diversity and abundance were found to be significantly higher in areas that are 8-10 years old and over 20 years old (Wasilewska, 1997).

Species identification of key plant parasitic nematodes

Despite to prevalent distribution of *Ditylencus* spp. in the surveyed almond production areas, *D. dipsaci* was identified in 5 samples from Eğilmez, Gökçe, Karacaören and Paşabağı Locations in Karaman province.

Three species of root lesion nematodes were detected in the surveyed almond production area. *Pratylenchus thornei* was the most prevalent species identified in 19 locations in Aybastı, Bucakkışla, Cerit, Çatak, Çavuşpınar, Dereköy, Dinek, Eğilmez, Eminler, Gökçe, Gülkaya, Karacaören, Ortaoba, Pınarbaşı, Süleymanhacı, Tavşanlı and Yollarbaşı Locations. *Pratylenchus neglectus* was in two locations in Masara and Paşabağı Locations. *Pratylenchus vulnus* was in Aybastı, Eğilmez and Eminler Locations in three samples. Two root lesion nematodes (*P. penetrans* and *P. scribneri*) were not found in the samples (Table 6).

Table 6. Sampling locations of *D. dipsaci, P. thornei, P. neglectus* and *P. vulnus* identified using species specific PCR technique

Sample No	Location	*D. dipsaci*	*P. thornei*	*P. neglectus*	*P. penetrans*	*P. scribneri*	*P. vulnus*
41	Aybastı	-	+	-	-	-	+
42	Bucakkışla	-	+	-	-	-	-
13	Cerit	-	+	-	-	-	-
40	Çatak	-	+	-	-	-	-
33	Çavuşpınar	-	+	-	-	-	-
7	Dereköy	-	+	-	-	-	-
16	Dinek	-	+	-	-	-	-
18	Eğilmez	+	+	-	-	-	-
19	Eğilmez	-	-	-	-	-	-
20	Eğilmez	-	+	-	-	-	+
26	Eminler	-	+	-	-	-	+
9	Gökçe	+	+	-	-	-	-
2	Gülkaya	-	+	-	-	-	-
17	Karacaören	+	+	-	-	-	-
27	Masara	-	-	+	-	-	-
24	Ortaoba	-	+	-	-	-	-
25	Ortaoba	-	+	-	-	-	-
1	Paşabağı	+	-	+	-	-	-
31	Pınarbaşı	-	+	-	-	-	-
22	Süleymanhacı	-	+	-	-	-	-
12	Tavşanlı	-	+	-	-	-	-
28	Yollarbaşı	+	+	-	-	-	-

Morphometrical measurements of *D. dipsaci* and *P. thornei* were presented in Tables 7 and 8. Morphological and morphometric data from specimens agreed with those in the literature (Sher and Allen, 1953; Öztürk, 1990; Sturhan & Brzeski, 1991; Elekcioglu, 1992; Kepenekci, 1999; Mennan, 2001; Imren, 2007; Yavuzaslanoglu et al., 2019) (Tables 7 & 8).

Table 7. Morphometric measurements of *Ditylenchus dipsaci* identified in this study and in references (measurements in the present study are in the form: mean±std. error of mean (range); n: number of specimens)

Characteristics	Present Study	Özturk, 1990	Sturhan & Brzeski,	Kepenekci, 1999	Mennan, 2001	Yavuzaslanoglu et al., 2019
N	4	20		12	200	28
L (mm)	0.76±0.07 (0.67-0.98)	0.53-1.1	1.0-2.2	0.65-0.87	1.55 (1.25-1.71)	0.91±0.12 (0.78-1.28)
Stylet length (µm)	10.12±1.05 (8.15-13.12)	6.0-11.8	10-13	10-13	12-14	9.80±0.33 (9.37-10.51)
Tail length (µm)	56.32±5.63 (42.09-69.36)	48.0-66.2		45-53		60.42±6.50 (50.54-72.81)
A	42.37±3.65 (36.36-52.75)	34.7-44.2	36-64	39.5-52.2	39-51	44.04±3.36 (37.59-50.26)
B	6.84±0.84 (5.58-9.32)	4.8-6.8	6.5-12	5.6-7.2	8.41-8.70	6.17±0.37 (5.45-6.69)
C	13.80±0.90 (11.76-16.09)	9.9-12.6	11-20	11.2-17.3	11.42-21.60	15.13±1.50 (12.54-18.44)
c'	3.65±0.42 (3.05-4.90)	5.6-6.9	3-6	4.09-4.81		5.12±0.64 (4.13-6.48)
V (%)	80.33±1.69 (77.38-85.15)	73.4-81.2	76-86	80.2-81.1	76-73	79.49±1.80 (75.01-82.49)

Table 8. Morphometric measurements of *Pratylenchus thornei* identified in this study and in references (measurements in the present study are in the form: mean±std. error of mean (range); n: number of specimens)

Characteristics	Present study	Sher & Allen, 1953	Elekcioglu, 1992	Kepenekci, 1999	Imren, 2007	Yavuzaslanoglu et al., 2019
N	3		20	20	14	3
L (mm)	0.46±0.01 (0.44-0.49)	0.45-0.77	0.46-0.62	0.48-0.63	0.48-0.60	0.53±0.06 (0.49-0.60)
Stilet length (µm)	17.31±0.91 (15.82-18.96)	17-19	15-17	16-18	16.2-18.5	14.91±0.34 (14.58-15.27)
Tail length (µm)	22.80±1.10 (21.66-25.02)	-	21-24	25-36	22.5-30.0	28.90±0.93 (27.82-29.46)
a	28.39±1.41 (25.67-30.41)	26-39.6	24-30	26.9-34.4	29.9-36.6	29.10±0.99 (28.3-30.22)
b	5.09±0.03 (5.03-5.15)	5.5-8.0	6.4-6.9	4.7-5.9	4.8-6.2	5.56±0.06 (5.50-5.62)
C	20.41±1.42 (17.97-22.90)	18-22	18-24	16.6-21.0	16.0-26.6	18.32±1.81 (16.96-20.38)
c'	2.14±0.09 (2.03-2.33)		1.7-2.4	2.3-2.9	2.01-2.72	2.55±0.25 (2.27-2.76)
V (%)	75.74±1.86 (72.05-78.03)	73-80	76-80	73.8-79.2	71.6-79.0	77.15±1.51 (75.73-78.75)

PCR screening using the PF1/PR1 primer set produced the expected 327 bp band in six samples (1, 9, 17, 18 and 27) confirming the presence of *D. dipsaci* in these samples (Figure 2). Similarly, the PF2/PR2 primer, targeting the D2–D3 region of 28S rDNA gene, detected positive results in the same samples, supporting the reliability of the molecular assay (Marek et al., 2005; Pethybridge et al., 2016).

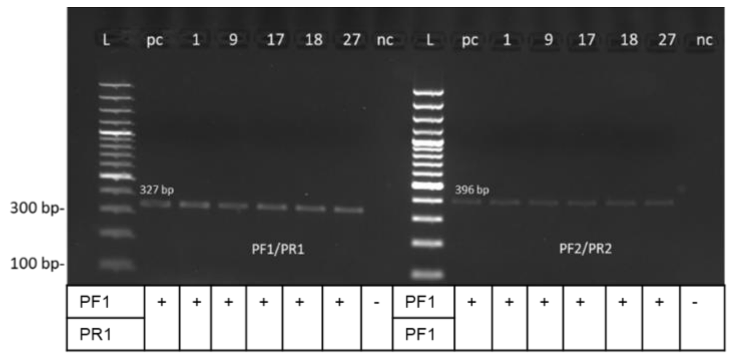

Figure 2. PCR amplifications of positive samples using PF1/PR1 and PF2/PR2 primers. L: 100 bp Ladder (Thermo Scientific), pc: positive control, nc: negative control (ddH_2O), numbers are survey samples numbers.

To identify *Pratylenchus* spp. using the D3 expansion region of the 26S rDNA gene, species-specific primers were designed to amplify DNA from the target *Pratylenchus* spp. (Al-Banna et al., 1997). A genomic DNA sample obtained from a previously cultivated *P. thornei* culture was used as a positive control (pc) for the PTHO primer. Molecular screening revealed an amplification product of the expected size (288 bp) in the positive control, while no positive bands were detected with the other species-specific primers examined (Figure 3). This result confirms the reliability of the PCR assay. No bands of the expected sizes were obtained from the samples for *P. penetrans* and *P. scriberi* using the PPEN and PSCR primers, which is consistent with the previously reported lack of amplification in similar regions (Yavuzaslanoglu et al., 2019).

Among the species-specific primers used, the PNEG primer yielded positive amplification only for samples 1 and 27, whereas the PTHO and PVUL primers produced positive bands with multiple species-specific primers from the same DNA samples (Table 6, Figure 3). The results for these samples (samples 20, 26, and 41) indicated the presence of multiple *Pratylenchus* species, suggesting that they may contain mixed populations. This finding is consistent with previous reports of mixed *Paratylenchus* populations in the literature. In support of this, a comprehensive study conducted across the vast agricultural lands of the "Great Plains" in North America found that approximately 20% of fields infested with *Pratylenchus* contained more than one species, indicating the occurrence of mixed populations (Özbayrak, 2019). In their study, Bucki et al. (2020) confirmed, through molecular analyses, the coexistence of *P. thornei* and *P. mediterraneus* within mixed infections in agricultural fields in the northern Negev region of Israel. The authors also reported that *P. thornei* able to survive across a wide range of soil types and frequently associated with mixed populations.

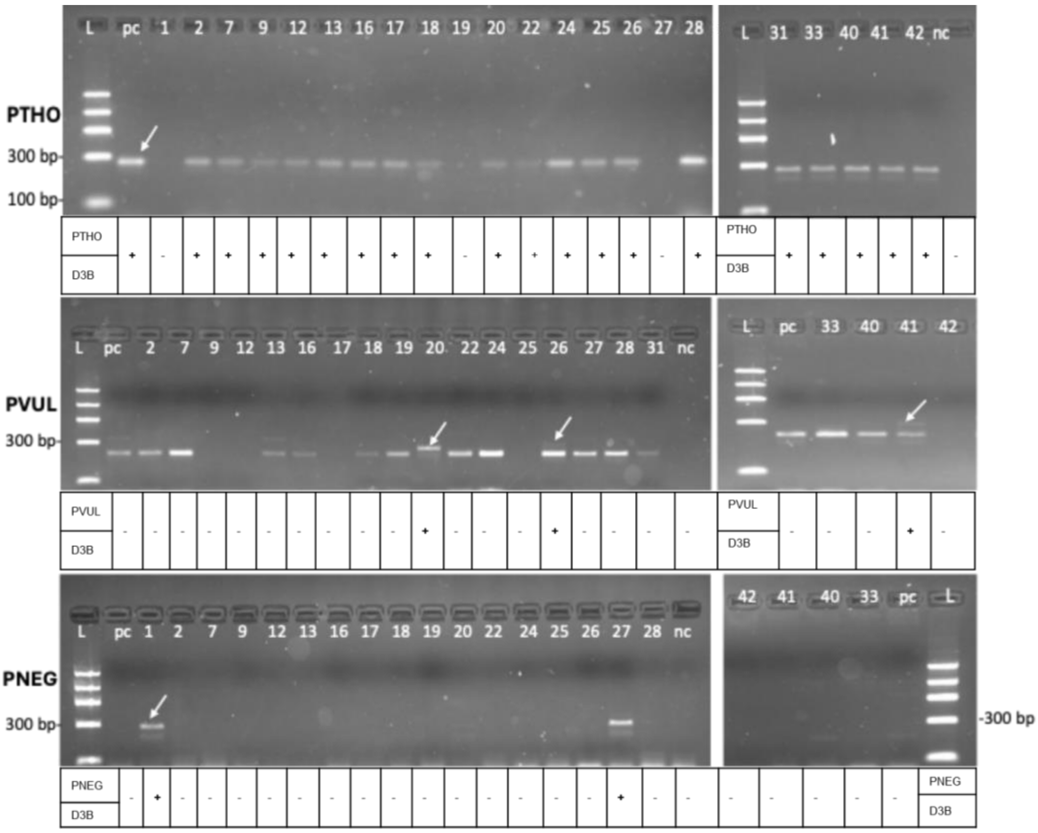

Figure 3. PCR amplifications of positive samples using PTHO, PVUL and PNEG primers. L: 100 bp Ladder (Thermo Scientific), pc: positive (*Pratylenchus thornei*) control, nc: negative control (ddH_2O), numbers are survey samples numbers.

PCR assays using species-specific primers can yield either positive or negative results directly based on the presence or absence of species-specific bands. This enables rapid and practical identification. These primers were developed to distinguish plant-parasitic nematode species and are widely used as diagnostic tools (Al-Banna et al., 2004; Yiğit & Akyazi, 2024). Subbotin et al. (2008) reported that the D2-D3 region of the 28S rDNA is an informative molecular marker for species-level identification within the genus *Pratylenchus*. The D3 region within this genetic region has been widely used for the identification of *Pratylenchus* species (Hodda et al., 2014). Given the high specificity and reliability demonstrated for the D3 region in species-level identification, morphological observations were supported by molecular analyses. The species-specific primers used were confirmed to be effective for the rapid and reliable diagnosis of plant-parasitic nematode species from almond orchards (Table 6).

Although molecular methods provide high sensitivity and specificity for species identification, confirming these results with morphological characteristics is essential to enhance diagnostic accuracy. Variation, particularly among populations obtained from different geographic regions or hosts, may increase the margin of error when interpretations rely solely on molecular data. Therefore, molecular identification should be performed in conjunction with traditional morphological analyses, especially in critical specimens.

The study provided useful information on the distribution of the nematodes on the expanding almond production areas in Karaman, Türkiye to assess the risks posed by plant parasitic nematodes and analyze the soil health status using nematode communities.

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