

Plant parasitic nematodes associated with almond (*Prunus dulcis* Mill.) and walnut (*Juglans regia* L.) orchards in Adiyaman province, Turkey

Abstract

In this study, plant parasitic nematode species in almond (*Prunus dulcis* Mill.) and walnut (*Juglans regia* L.) growing areas in Adiyaman province were identified morphologically and taxonomically. On this purpose soil and root samples were collected from randomly selected fifteen almond and twentyone walnut orchards respectively during June and September in 2011-2012 from the upper rhizosphere under the canopy of each tree. As a result of this study plant parasitic nematodes from Tylenchoidea, Dolichodoroidea Anguinoidea, Aphelenchoidea, Hoplolaimoidea of Tylenchida, Aphelenchida and Dorylaimida orders were found in survey areas. The most encountered species in almond were determined as *Helicotylenchus digonicus*, *Ditylenchus myceliophagus* and *Aphelenchus avenae* whereas *Helicotylenchus digonicus*, *Pratylenchus neglectus* and *Merlinius brevidens* were most common in walnut plantations. No literature record of about the presence of plant parasitic nematodes has been determined in almond and walnut growing areas in Adiyaman province.

Keywords: plant parasitic nematodes, almond, walnut, Adiyaman, Turkey

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Introduction

Nuts is edible plants that have been recommended primarily for daily consumption due to their rich antioxidant, vitamin and mineral contents. Therefore they are an important component of the healthy diet. Walnut and almond have a big healthy fruits that keep the essential foods largely that people need to have healthy nourishment. Thus, consuming a handful of raw walnut or almond on a day largely provides the high-quality vegetable proteins, omega 3-fatty acids, phytosterols, tocopherols, fibre, antioxidants, phenolic compounds, cholesterol free, prevention and treatment of cardiovascular diseases, some vitamins and minerals that a person needs on a day.¹

Having favorable soil and climate conditions of walnut and almond trees can be grown in almost every region of Turkey. Walnut and almond have become popular among farmers because of its high marketing prices and leads to establishment of new production areas. Minimum labor and annual high operating cost also makes these plants most preferably agricultural fruit plants. Turkey have ranked sixth in almond (*Prunus dulcis* Mill.) production and fourth in walnut (*Juglans regia* L.) production in the world.²

Various factors are responsible for decrease of almond and walnut productivity in Turkey. Viruses, viroids, bacteria, fungi, weeds, insects and plant parasitic nematodes have been pests that some were reported as very problematic in various locations. One of the most important soil borne pests are plant parasitic nematodes. Nematodes are known to be among the most taxa rich organismal group on earth, having with an estimated 500 000 to 1 000 000 species. Only about 20 000 species have been described and the systematic literature is widely dispersed.³ Nematodes feed on roots and reduce their potential for uptake of water and nutrients.⁴ All plant parasitic nematodes use their stylet to puncture plant cells and to remove the contents. The major

symptoms have exhibited by plants affected by nematodes include retarded growth, wilting and predisposition to infection by other pathogens.⁵ Each nematode causes different damage due to secretions of the pharynx. Plant parasitic nematodes have caused significant yield losses worldwide. The damage caused by plant parasitic nematodes has been estimated at up to \$80 billion per year due to the 10-20% production loss of agricultural yield.⁶ Nevertheless, there is a little information on the occurrence of plant parasitic nematodes in Turkey. The plant parasitic nematode species have been determined in almond and walnut cultivation areas in different locations in Turkey.⁷⁻⁹

Adiyaman is located in Southeastern Anatolian Region of Turkey and is fifth rows almond producing province in Turkey. Walnut also has a good share in Adiyaman's fruit production. The Adiyaman province, which is located in the Southeastern Anatolia Project (GAP) area, is one of the city as agricultural, economic, social and cultural importance with its integrated regional development approach and sustainable human development philosophy.¹⁰

To date, no study about plant parasitic nematodes on almond and walnut cultivated areas had been recorded in Adiyaman province. In this study, plant parasitic nematode species were examined as faunistically and taxonomically in Adiyaman.

Materials and methods

A two year survey in June and September 2011 and 2012 was carried out in Adiyaman province. Almond (*P. dulcis* Mill.) soil samplings were conducted in randomly selected orchards located in six different locations of including Central, Besni, Gerger, Kahta, Sincik districts. Walnut (*J. regia* L.) soil samples were collected in randomly selected orchards located in five different locations of including Central, Besni, Gölbaşı, Kahta, Sincik districts (Figure 1).

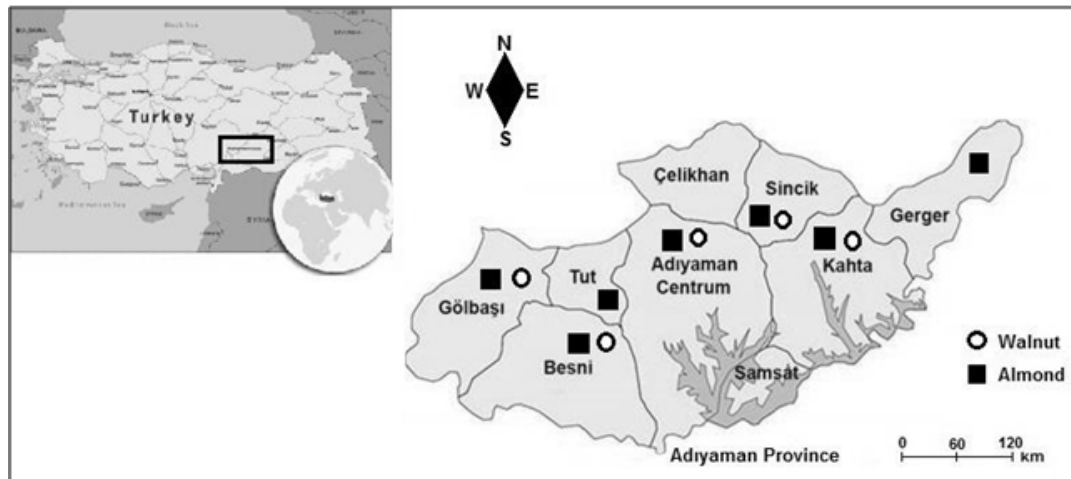


Figure 1 The sampling locations of almond and walnut cultivation areas in Adiyaman province in this study.

Approximately five soil samples (50 grams for each sample) were collected from each location. Soil samples were taken by soil auger from 0-30 cm the upper rhizosphere under the canopy of each tree. The sampling consists of a combination of soil samples taken from at least 10 locations of approximately 2.5 ha of each field.¹¹ The location, date, location of the soil samples, the name of the field owner, the plant species in the field and the development status of the plant are indicated. The samples were brought to the laboratory and stored in refrigerator at +4°C. In laboratory studies, active nematodes were extracted from soil with combination of sieving (850, 250, 150, 75 and 45µm opening sieves) and Baermann funnel methods.^{12,13} Each sample was thoroughly mixed and a 100 g subsample processed. To extract vermiform nematodes from the soil, a Petri sieving method, a modification of the enhanced Baermann funnel method, was used.^{14,15} Residues on the two sieves with the largest openings was discarded and material from the remaining sieves was extracted on Baermann funnels. For species specific identification, nematodes were fixed.¹⁶ Individuals nematode were put in a desiccator for the period of time required for all remaining water to evaporate and mounted in glycerol as described and the nematodes were kept in pure glycerin and were separated according to their genus then permanently mounted

on glass slides using the wax-ring method.^{17,18} The identification of species were carried out based on morphology and morphometrics of female individuals. Ten nematodes per sample were identified. If ten nematodes were not available, all specimens in the sample were identified. The taxonomical situations, synonyms of the genera and phylogenetic classification of Tylenchid and Dorylaimid nematode species that were determined at the end of the study.^{19,20}

Results

In this study, a total of twentytwo species of plant parasitic nematodes were identified as morphologically and taxonomically. Within these eleven species were found in association with almond and seventeen species were found in walnut orchards in Adiyaman province of Turkey. A total of twentytwo species within the subfamilies of Aphelenchoidinae, Tylenchoinae, Dolichodorinae, Hoplolaimoinea, Rotylenchoidinae, Neotylenchinae, Merlininae, Telotylenchoinae and Longidorinae were identified in almond and walnut plantations in Adiyaman province. The distribution of the plant parasitic nematode species in Adiyaman province and their host associations were given in Table 1.

Table 1 Plant parasitic nematode species in almond and walnut cultivation areas in Adiyaman province of Turkey in this study

Nematode classification (Order: Family: Subfamily)	Plant parasitic nematode species	Host
Aphelenchida:Aphelenchoididae:	<i>Aphelenchus avenae</i> Bastian	A,W
Aphelenchoidinae		
Tylenchida:Telotylenchidae: Merlininae	<i>Amplimerlinius viciae</i> Siddiqi	W
Tylenchida: Dolichodoroidae: Dolichodorinae	<i>Bitylenchus goffarti</i> Sturhan	W
Tylenchida:Tylenchidae: Neotylenchinae	<i>Boleodorus thyllactus</i> Thorne	A
Tylenchida:Anguinoidae:Anguinoinae	<i>Ditylenchus dipsaci</i> Kühn	A
	<i>D. myceliophagus</i> Goodey	A,W
Tylenchida:Tylenchoidae:Tylenchoinae	<i>Filenchus cylindricauda</i> Siddiqi	A
	<i>Helicotylenchus canadensis</i> Waseem	A
	<i>H. digonicus</i> Perry in Perry, Darling and Thorne	A,W
Tylenchida: Hoplolaimoidae: Hoplolaimoinea	<i>H. pseudorobustus</i> Golden	A,W
	<i>H. varicaudatus</i> Yuen	W

Table Continued....

Nematode classification (Order: Family: Subfamily)	Plant parasitic nematode species	Host
Tylenchida: Belonolaimoidae: Merlininae	<i>Merlinius brevidens</i> (Allen) Siddiqi	W
	<i>M. microdorus</i> (Geraert) Siddiqi	A, W
Tylenchida: Dolichodoroidae: Dolichodorinae	<i>Paratrophurus acristylus</i> Siddiqi and Siddiqi	W
Tylenchida; Hoplolaimidae: Rotylenchoidinae	<i>Pratylenchoides alkani</i> Yuksel	W
	<i>Pratylenchus crenatus</i> Loof	A, W
Tylenchida: Hoplolaimoidae: Hoplolaimoinae	<i>P. thornei</i> Sher and Allen	W
	<i>P. neglectus</i> Filipjev and Shuurmans	W
Tylenchida: Dolichodoroidae :Telotylenchoinae	<i>Trophurus imperialis</i> Loof	W
Tylenchida: Dolichodoroidae : Dolichodorinae	<i>Scutylenechus quadrifer</i> Andrassy	W
	<i>S. stegus</i> (Thorne and Malek) Siddiqi	A
Doryloimida: Longidoridae: Longidorinae	<i>Longidorus euonymus</i> Mali and Hooper	W

A, Almond; W, Walnut

Plant parasitic nematode species identified in this study belong to the genera, *Aphelenchus* (Bastian, 1865) (Tylenchida: Aphelenchidae), *Amplimerlinius* (Siddiqi, 1976) (Tylenchida: Telotylenchidae), *Bitylenchus* Filipjev, 1934 (Tylenchida: Dolichodoroidae), *Ditylenchus* Filipjev, 1936 (Tylenchida: Anguinidae), *Filenchus* Andrassy, 1954 (Tylenchida: Tylenchidae), *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae), *Merlinius* Siddiqi, 1970 (Tylenchida: Telotylenchidae), *Paratrophurus* Arias, 1970 (Tylenchida: Belonolaimidae), *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae), *Pratylenchoides* Winslow, 1958 (Tylenchida: Pratylenchidae), *Scutylenechus* Jairajpuri, 1971

(Tylenchida: Telotylenchidae) and *Trophurus* Loof, 1956 (Tylenchida: Belonolaimidae), *Boleodorus* Thorne, 1941 (Tylenchida: Tylenchidae) and *Longidorus* (Micoletzky, 1922) (Doryloimida: Longidoridae). The most common species in almond growing areas were determined as *Helicotylenchus digonicus*, *Ditylenchus myceliophagus* and *Aphelenchus avenae* while *H. digonicus*, *Pratylenchus neglectus* and *Merlinius brevidens* were major in walnut plantations. *H. digonicus* was the most abundant specimen in this study. Besides, *A. avenae*, *D. myceliophagus*, *H. digonicus*, *H. pseudorobustus*, *M. microdorus* and *P. crenatus* species were detected both almond and walnut plantation areas in this study in Adiyaman province (Figure 2).

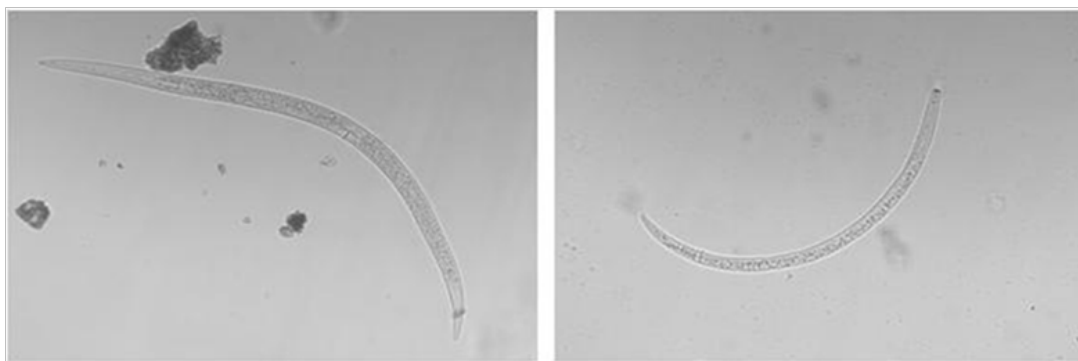


Figure 2 *Pratylenchus thornei* (right), *Merlinius brevidens* (left).

Several plant parasitic nematode species were found in almond cultivation areas in the world. *H. dihystra*, *P. neglectus*, *P. thornei* and *P. penetrans* were identified by several researchers in the world.^{21,22} Severe root damage due to heavy nematode feeding may lead to bacterial and fungal disease infestation of crop plants. There was a few literature record about plant parasitic nematodes on walnut (*J. regia* L.) in the world. Several species including *Longidorus proximus*, *L. caespiticola*, *L. elongatus*, *L. euonymus*, *L. goodeyi*, *L. juglandicola*, *L. leptcephalus*, *L. picensis*, *L. raskii*, *Paralongidorus maximus*, *Xiphinema diversicaudatum*, *X. simile*, *X. taylori*, *X. americanum*, *X. pachtaicum*, *X. italiae*, and *X. vuittenezi*. *Cacopaurus pestis*, *D. dipsaci*, *P. wulnus*, *Mesocriconema xenoplax*, *Meloidogyne* spp., *Tylenchorrhynchus* spp., *Gracilacus peratica*, *Pratylenchus*

paraperaticus sp. n were reported in France, Pakistan, USA, Italy, Iran, Slovakia and Spain.²³⁻²⁷

In this study *H. canadensis* Waseem, *H. digonicus* Perry in Perry, Darling and Thorne, *H. pseudorobustus* Golden and *H. varicaudatus* Yuen, 1964 were recorded in several locations in Adiyaman province. *H. canadensis* was firstly determined in *Lens esculenta* Moench growing areas in Nevşehir and Yozgat provinces of Turkey.²⁸ *H. digonicus* was never determined in Adiyaman province of Turkey. *H. digonicus* was confirmed as most prevalent and destructive nematode species in Spain and Jordan.²⁹ The most encountered specimen in almond and walnut plantation areas was determined as *H. digonicus* in this study. *H. digonicus* was firstly found in *Allium porrum* L. and grass areas in İstanbul province of Turkey.³⁰ Later the spiral nematodes

are observed most frequently in different plantations of Turkey.^{31–34} *H. digonicus* was recently found in grapevine and pistachio growing areas in Adiyaman province of Turkey. *H. pseudorobustus* was firstly determined in ornamental plants in İstanbul province of Turkey.³⁵ *H. varicaudatus* was firstly determined in ornamental plants in Izmir province of Turkey.³⁶ Both of them, *H. pseudorobustus* and *H. varicaudatus*, were not found in Adiyaman province of Turkey before. *H. digonicus* causes root necrosis and chlorosis.³⁷ The economic importance of the *H. canadensis*, *H. digonicus*, *H. pseudorobustus* and *H. varicaudatus* found in the Southeast Anatolian Region, is not known.

In this study, three different lesion nematode species *Pratylenchus crenatus*, *P. thornei* and *P. neglectus* were identified in walnut orchards in Adiyaman province. *P. crenatus* was identified in almond and walnut orchards in Adiyaman province in this study. Within these species *P. thornei* was one of the most prevalent species in Adiyaman walnut plantation areas. Approximately 70 species of *Pratylenchus* have been described in the world, Parasitising 400 crop plant species and seven including *P. brachyurus*, *P. coffeae*, *P. neglectus*, *P. penetrans*, *P. scribneri*, *P. thornei*, *P. vulnus* and *P. zaeae* were reported as most damaging species.³⁸ The feed of these nematodes inside root cortex results in formation of necrotic lesions. *P. crenatus* was firstly determined in *Solanum tuberosum* L. growing areas in Canakkale and Izmir provinces of Turkey.³⁹ *P. crenatus* recently was identified in melon growing areas in Adiyaman province of Turkey.³³ *P. thornei* Sher and Allen was firstly determined in *Triticum aestivum* L. growing areas in Eastern Anatolian Region and Southern Anatolian Region of Turkey.⁴⁰ *P. thornei* was recently found in cotton, grapevine, melon, tobacco, watermelon and wheat growing areas in Adiyaman province of Turkey.³³ The second encountered specimen in walnut plantation areas was determined as *P. neglectus* in this study. *P. neglectus* was firstly determined in cereals, corn and grass areas in Eastern Anatolian Region and Eastern Black Sea Region of Turkey.⁴⁰ *P. neglectus* was recently found in barley and tobacco growing areas in Adiyaman province of Turkey.³³ Plant parasitic nematodes in walnut was carried out only one researcher and the fourteen plant parasitic nematode species were determined in walnut orchards in Black Sea Region of Turkey.⁸ Also, *Helicotylenchus* spp. was determined in stone and pome fruits nurseries plantations in İzmir province of Turkey.⁹

The genus *Ditylenchus* Filipjev, 1936 has more than 80 valid species and grouped as mycophagous and phytophagous species.^{41,42} In this study, two species were identified with *D. myceliophagus* is being more important than *D. dipsaci*. The second encountered specimen in almond plantation areas was determined as *D. myceliophagus* in this study. *D. dipsaci* is one of the most economically important plant parasitic nematodes; mostly it infests onion and garlic, as well as many other crop plants and weeds in worldwide. *D. dipsaci* was firstly determined in onion growing areas in Central Anatolian Region of Turkey.⁴³ *D. dipsaci* has been recorded in several onion growing areas in Turkey.^{44,45} *D. dipsaci* was recently identified in wheat growing areas in Mardin province of Turkey.³³ *D. myceliophagus* was firstly determined in mushroom cultivated areas in Yalova province of Turkey.⁴⁶ *D. myceliophagus* was recently identified in barley, tobacco and wheat growing areas in Adiyaman province of Turkey.³³

Stunt nematodes feed on the roots of many kinds of plants some of which are of agricultural importance worldwide.⁴⁷ The taxonomy of this group of obligate ectoparasites is complex and in a state of transmutation.^{48–50} Species in the genera *Geocenamus*, *Merlinius*, and *Tylenchorhynchus* are reported most frequently.⁵¹ All stunt nematodes feed on epidermal cells and root hairs mostly in the cell elongation

region and may cause roots to thicken and elongate shape.⁵² This species has been associated with roots of many grass, cereal, vegetable, legume, brassica, fruit, and fiber crops.⁵³ *M. brevidens* was firstly reported in the United States on wheat in Texas.⁵⁴ *M. brevidens* was firstly determined in parsley growing areas in İstanbul of Turkey.³⁰ *M. brevidens* was recently determined in cotton, barley, melon, tobacco, watermelon, wheat growing areas in Adiyaman province of Turkey.³⁵ Also *M. brevidens* was recently determined in wheat growing areas in Kilis and Mardin province of Turkey. *M. brevidens* is the third encountered specimen in walnut plantation areas in this study. This specimen has been identified on different host plants in different locations in Turkey several researchers.^{7,55,33,34,56}

Aphelenchus avenae Bastian, 1865 is primarily fungivorous and has existed commonly in soil but also in leaf sheaths, plant crowns and the cortex of some roots.^{57,58} The pathogenicity of the species is insignificant but it might well be the carrier of other causative agents of diseases such as bacteria and fungi.⁵⁹ This nematode has been recorded in many countries worldwide in different soil types.⁶⁰ The fact that the nematode migrates and therefore may do mechanical damage, and that its waste products may interfere with the metabolism of the host cells. *A. avenae* should be considered a facultative plant parasite conclusion was that *A. avenae* is not attracted to living plant tissue and has a wide range of fungal host in both phytopathogenic and saprophytic species in different conditions.⁶¹ *A. avenae* is a mycophagous nematode which can't parasitize on higher plant and there is no record of real parasitism, damage or feeding of this nematode on the plant. Thus *A. avenae* is called fungal feeding nematode.⁶² Populations of *A. avenae* have been existed on a number of different fungi.^{63,64} *A. avenae* was firstly determined in parsley growing areas in İstanbul province of Turkey.³⁰ *A. avenae* was recently identified in barley, grapevine, melon, pistachio and wheat growing areas in Adiyaman province of Turkey.³³ Also, *A. avenae* was recently determined in wheat growing areas in Mardin province of Turkey.^{65,33} *A. avenae* was the third encountered specimen in almond plantation areas in this study. Also this specimen was determined in walnut plantation areas in this study, too.

The other nematode species which were not considered as economically important were *P. alkani* Yüksel, *F. thornei* Andrassy, *F. cylindricauda* (Wu) Siddiqi, *B. thylactus* Thorne, *B. goffarti* (Sturhan) Jairajpuri, *T. imperialis* Loof and *S. stegus* (Thorne and Malek) Siddiqi, *S. quadriifer* (Andrassy) Siddiqi, *P. acristylus* Siddiqi and Siddiqi, *A. viciae* Siddiqi and *L. euonymus* Mali and Hooper. Plant parasitic nematodes, such as *Meloidogyne* Goeldi species, *Heterodera* Schmidt species, *Rotylenchulus reniformis* Linford and Oliveira and *Tylenchulus semipenetrans* Cobb were not found in this study.

Conclusion

The Southeast Anatolian Region have covered an important place in the country's economy in terms of walnut and almond cultivated areas and production. Adiyaman province is important in terms of agricultural production in this region depending on climatic conditions, socioeconomic conditions and vegetation. The convenient environmental conditions of Adiyaman province is created suitable for plant parasitic nematodes, other pests and disease factors in agricultural production. Nematodes cannot be completely controlled in orchards. The chemical control is only possible before planting if nematodes are present. In addition fumigants may not be effective to longidorid nematodes which were mostly detected under 40 cm soil depths. The vast majority of applied fumigants have harmful effect on environment and the majority were banned due to related health concerns.

To prevent possible damage to these nematodes can be prevented by sowing, using certified seeds, developing durable varieties or using tolerant seedlings and saplings, solarization application and so on cultural measures need to be taken. First of all, alternative methods of controlling nematodes should be applied, especially legal measures. The most common species in almond growing areas were determined as *Helicotylenchus digonicus*, *Ditylenchus myceliophagus* and *Aphelenchus avenae* while *H. digonicus*, *Pratylenchus neglectus* and *Merlinius brevidens* were major in walnut plantations in Adiyaman province of Turkey in this study. These species which were identified in the study have a wide host range in the world. This situation has constituted a hazard for Turkey's agricultural product and yield. Also, climate and geography in Adiyaman have been suitable region for finding and living of many plant diseases and harmful species. Based on results of our study and previous studies, it is considered that plant parasitic nematodes could threaten the production of almond and walnut trees precautions must be taken before they reach the level of economic damage. More investigations are required to fully elucidate the damage potential of these nematodes in almond and walnut cultivation areas in Adiyaman province of Turkey. Besides more investigation should be done to provide information on the biology, population dynamics and pathogenicity of these nematodes. Therefore, more research on occurrence, biology, distributions and economic importance of these and other plant parasitic nematodes is needed to understand the role that these nematodes have in Adiyaman.

Acknowledgments

None.

Conflicts of interest

The author declares there is no conflicts of interest.

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