



Determination of Plant Parasitic Nematode Species of Tylenchida (Nematoda) in Apple (*Pyrus malus* L.) and Walnut (*Juglans regia* L.) Orchards in Bingöl Province of Turkey

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ABSTRACT

Plant parasitic nematodes (PPNs) are the leading source of damage to crops in agricultural production and they can cause very serious yield reductions. The Tylenchida (Nematoda) constitutes the most important group of PPNs due to containing a large number of economically important plant parasitic species. This study was conducted in apple (*Pyrus malus* L.) and walnut (*Juglans regia* L.) orchards in Bingöl (Turkey) between 2016 and 2017. For this purpose, total of 52 soil/root samples were taken from 20-40 cm depth of related areas during summer months. In laboratory, active PPNs were extracted from soil and roots parts by modified Baermann Funnel method. Obtained nematodes were killed at 60°C, fixed in TAF in mounted slides. PPNs belonging to Tylenchida were identified morphometrically and morphologically under the light microscope. As result of the study; eighteen PPN species were determined and classified under the Tylenchida order as three suborders, three superfamilies, four families and seven genera in the apple and walnut growing areas. The most encountered PPN species in this study were *Filenchus filiformis*, *Helicotylenchus canadiensis*, *H. digonicus*, *H. vulgaris*, *Pratylenchoides alkani* and *Psilenchus hilarulus*. All species were determined for the first time in the nematoda fauna of Bingöl province in apple and walnut orchards.

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ÖZET

Bitki paraziti nematodlar (BPN'ler), tarımsal ürünlerde önemli zarar oluşturmakta ve çok ciddi verim kayıplarına neden olabilmektedirler. Tylenchida (Nematoda) takımı ekonomik açıdan önemli çok sayıda bitki paraziti nematod türünü barındırdığından en önemli BPN grubunu oluşturmaktadır. Bu çalışma 2016-2017 yılları arasında Bingöl (Türkiye) ili elma (*Pyrus malus* L.) ve ceviz (*Juglans regia* L.) bahçelerinde yürütülmüştür. Bu amaçla, yaz aylarında ilgili alanlardan 20-40 cm derinlikten toplam 52 toprak/kök örneği alınmıştır. Laboratuvarında, geliştirilmiş Baermann Huni yöntemi ile toprak ve kök kısımlarından aktif BPN'ler ekstrakte edilmiştir. Elde edilen nematodlar 60°C'de öldürüldükten sonra TAF çözeltilisinde fikse edilerek lam üzerinde sabitleştirilmiştir. Tylenchida takımına ait BPN'ler, ışık mikroskobu altında morfolojik ve morfolojik olarak tanımlanmıştır. Çalışma sonucunda; elma ve ceviz yetiştiriciliği yapılan alanlarda 18 BPN türü belirlenmiş ve bu türler Tylenchida takımını altında üç alttakım, üç üstfamilya, dört familya ve yedi cins olarak sınıflandırılmıştır. Bu çalışmada *Filenchus filiformis*, *Helicotylenchus canadiensis*, *H. digonicus*, *H. vulgaris*, *Pratylenchoides alkani* ve *Psilenchus hilarulus* en çok tespit edilen

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BPN türleri olmuştur. Tüm türler Bingöl ilindeki elma ve ceviz bahçeleri nematod faunasında ilk kez belirlenmiştir.

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INTRODUCTION

Turkey, due to its geographical location, has a very favorable climate for all fruits excluding some tropical plants. In this regard, Turkey, the origin of horticultural production, is the main homeland of many fruit species in the world (Ağaoğlu et al., 1997). A significant portion of fruit species being grown in Turkey is temperate zone fruits. Among these, grape, apple, hazelnut, pear, peach, apricot, plum, cherry, walnut, chestnut, quince, almond, pistachios are widely grown crops. Apple (*Pyrus malus* L.) is one of the fruits grown in almost every climatic region of this country. The annual apple production of Turkey is approximately 3.6 million ton. Apple cultivation has been concentrated in all provinces of the country recently. Walnut (*Juglans regia* L.) is the oldest cultivated fruits in the world grown spontaneously almost all across of the country (Sen, 1986). The annual walnut production of Turkey is approximately 225 thousand ton. Plant parasitic nematodes (PPNs) are the leading cause of damage to crops, and although actual size of the their damage to agriculture is too difficult to assess, they are able to cause a very serious reductions in crop yield. Nematodes can be primer pathogens by direct attacking plants or can be seconder parasites by creating the entrance to the other many soil-borne pathogens. The Tylenchida (Nematoda) constitutes of the most important group of PPNs due to its host range of economically important crops. There are various studies about PPN-host association and distribution in different localities of Turkey. A total of 240 PPNs belonging to 56 genera of Tylenchida were detected from 66 different host plants in 48 different localities of the country by second half of 2014 (Kepenekci, 2014). Pest and pathogen management are fairly important for the country's agriculture. Increasing agricultural production per unit are becoming even more vital due to the rapid population increase in Turkey. Within this perspective, the crops including apple and walnuts need to be protected from pests and pathogens including PPNs. Therefore, species of PPN species need to be identified correctly in order to develop effective management strategies. There is only one study available about identification of PPN species in forage areas in Bingöl (Yıldız et al., 2012). There is no any other study of PPN species in any crops in this province. Therefore, the aim of this study was to determine PPN species causing economic losses in apple and walnut orchard areas in Bingöl province.

MATERIAL and METHOD

A total of 52 soil-root samples were taken from apple and walnut orchards of Bingöl (Turkey) and its surroundings (Adaklı, Genç, Karlıova, Kiğı, Solhan, Yayladere) to determine PPN species. The samples were collected incidentally to represent the whole region. Samples were usually taken from orchards possessing five years or older trees. Each orchard was considered as one unit and samples were taken from five different spots of the same tree from a depth of 20-40 cm. The soil and root samples of each tree was collected into a polyethylene bag and labelled.

Laboratory studies

Constitution of permanent preparations of nematodes

A petri method, Modified Baermann Funnel Method, was used to extract free and mobile PPNs from the soil (Barker, 1985; Southey, 1986). In order to identify nematodes at the species level, constitution of permanent preparations processes were followed based on common preparation methods. For this purpose, nematodes precipitated in the centrifuge tubes were kept for 2 minutes in hot water bath at 60°C. The dead nematodes were fixed in TAF solution (7 ml formalin (40% formaldehyde) +2 ml triethanolamine +91 ml distilled water) (Hooper, 1986) and kept in solution I (1 part of glycerin and 79 parts of distilled water) and solution II (5 parts glycerin and 95 parts (96%) ethanol) (Seinhorst, 1959). In this way, the nematodes, taken in pure glycerin, were separated according to their genus and taken on the slide prepared by Wax-Ring method (Hooper, 1986). The nematodes later were fixed on the heater with coverslip and made ready for the diagnosis.

Identification of nematodes

PPNs were identified at the species level by morphological and morphometric characteristics. Synonyms, systematic position and phylogenetic classification of Tylenchid nematodes were determined by following Siddiqi (2000).

RESULTS and DISCUSSION

In this study, PPN species belong to the genera of *Coslenchus* Siddiqi (Tylenchida: Tylenchidae), *Filenchus* Andrassy (Tylenchida: Tylenchidae), *Helicotylenchus* Steiner (Tylenchida: Hoplolaimidae), *Pratylenchoides* Winslow (Tylenchida: Pratylenchidae), *Pratylenchus* Filipjev (Tylenchida: Pratylenchidae), *Psilenchus* DeMan (Tylenchida:

Belonolaimidae) and *Trophurus* Loof (Tylenchida: Belonolaimidae) were identified. PPN species determined in the survey are shown in Table 1. Among the species found, six PPN species were determined from apple orchards and classified in the Tylenchida order, three suborders, two superfamilies, three families and in five genera. These species were *Coslenchus turkeyensis* Siddiqi; *Filenchus cylindricauda* Wu, Siddiqi; *Helicotylenchus canadiensis* Waseem; *H. digonicus* Perry, in Perry, Darling & Thorne; *Pratylenchoides alkani* Yüksel; *Psilenchus hilarulus* De Man. Among the species found, sixteen PPN species were determined in the walnut orchards and classified under the Tylenchida order as three suborders, three superfamilies, four

families and six genera. These species were *F. filiformis* (Bütschli) Meyl; *H. canadiensis*; *H. crenecauda* Sher; *H. digonicus*; *H. striatus* Firoza & Maqbool; *H. tunisiensis* Siddiqi; *H. vulgaris* Yuen; *P. alkani*; *P. camachoi* Barcina, Castillo & Pais; *P. erzurumensis* Yüksel; *P. leiocauda* Sher; *P. ritteri* Sher; *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven; *P. thornei* (Sher & Allen), (Sher); *P. hilarulus* and *Trophurus sculptus* Loof. These species were determined for the first time in the nematoda fauna of apple and walnut orchards in Bingöl province (Turkey). The most encountered PPN species in this study were *F. filiformis*, *H. canadiensis*, *H. digonicus*, *H. vulgaris*, *P. alkani* and *P. hilarulus*.

Çizelge 1. Bingöl ilindeki elma ve ceviz bahçelerinde Tylenchida (Nematoda) takımında tespit edilen bitki paraziti nematod türleri

Table 1. The detected plant parasitic nematode species under the Tylenchida (Nematoda) order in apple and walnut orchards in Bingöl province.

Family	Nematode species	Host
Familya	Nematod türleri	Konukçu
Tylenchidae	<i>Coslenchus turkeyensis</i> Siddiqi	apple
	<i>Filenchus cylindricauda</i> Wu, Siddiqi	apple
	<i>F. filiformis</i> (Bütschli) Meyl	walnut
Hoplolaimidae	<i>Helicotylenchus canadiensis</i> Waseem	apple, walnut
	<i>H. crenecauda</i> Sher	walnut
	<i>H. digonicus</i> Perry, in Perry, Darling & Thorne	apple, walnut
	<i>H. striatus</i> Firoza & Maqbool	walnut
	<i>H. tunisiensis</i> Siddiqi	walnut
	<i>H. vulgaris</i> Yuen	walnut
Pratylenchidae	<i>Pratylenchoides alkani</i> Yüksel	apple, walnut
	<i>P. camachoi</i> Barcina, Castillo & Pais	walnut
	<i>P. erzurumensis</i> Yüksel	walnut
	<i>P. leiocauda</i> Sher	walnut
	<i>P. ritteri</i> Sher	walnut
	<i>Pratylenchus penetrans</i> (Cobb) Filipjev & Schuurmans Stekhoven	walnut
Belonolaimidae	<i>P. thornei</i> (Sher & Allen), (Sher)	walnut
	<i>Psilenchus hilarulus</i> DeMan	apple, walnut
	<i>Trophurus sculptus</i> Loof	walnut

In this study, a total of 18 PPN species were found in Bingöl province (Turkey). All species were determined for the first time in the nematoda fauna of apple and walnut orchards in this province. *Coslenchus turkeyensis*, *Filenchus filiformis*, *Helicotylenchus canadiensis*, *H. digonicus*, *H. tunisiensis*, *H. vulgaris*, *Pratylenchoides alkani*, *P. leiocauda*, *Pratylenchus penetrans* and *P. thornei* species were found in apple and walnut orchards of Bingöl province. Related to this study, Evlice and Ökten (2008) identified the same species in pear orchards in Ankara (Turkey). Yüksel (1977) studied on measurements and drawings of taxonomic character of some BPN and he found *P. alkani* and *P. erzurumensis* which were similarly detected in walnut of this current study. Kepenekci and Ökten (1996) studied in Ankara province and they

identified plant parasitic nematodes species belonging to *Helicotylenchus* genus from tomato cultivation areas. In this study, *H. digonicus* and *H. tunisiensis* species detected in apple and walnut orchards were similar. *Psilenchus hilarulus* and *Trophurus sculptus* were found in both apple and walnut orchards. Similar to this study, Kepenekci et al. (1998) reported *P. hilarulus* in Balıkesir and Ankara (Turkey), and Kepenekci et al. (1999) reported *T. sculptus* in Ankara on rice. Kepenekci and Zeki (2002) also reported *Helicotylenchus digonicus*, *Pratylenchoides erzurumensis* and *Psilenchus iranicus* in apple orchards of Isparta and Burdur. In current study, *H. digonicus* and *P. erzurumensis* species were detected in walnut orchards. *Helicotylenchus crenacauda* and *H. striatus* were found in walnut orchards. Similar to

in this study, Kepenekci and Ökten (1999) reported that *H. crenacauda* and *H. striatus* were detected in Samsun and Sinop (Turkey) from tobacco growing areas. Kepenekci and Ökten (1998) identified *Coslenchus turkeyensis*, *Filenchus filiformis*, *F. cylindricauda*, *P. alkani*, *P. leiocauda* in their study in

the fields of tomato cultivation which went into rotation with carrots in Beypazarı (Ankara). The nematode species detected in apple and walnut orchards in current study were similar of those of detected in some of other crops of previous studies (Table 2).

Çizelge 2. Türkiye'deki diğer çalışmalarda tespit edilen bu bitki paraziti nematod türlerinin konukçuları
 Table 2. Hosts of these plant parasitic nematode species detected in other studies in Turkey

Family	Nematode species	Host	Distribution	Reference
Familiya	Nematod türleri	Konukçu	Yayılım	Referans
Tylenchidae	<i>Coslenchus turkeyensis</i>	tomato	Ankara	Kepenekci and Ökten (1997a)
		pear	Ankara	Evlice and Ökten (2008)
		apple	Bingöl	In this study
	<i>Filenchus cylindricauda</i>	tomato	Ankara	Kepenekci and Ökten (1998)
		wheat	Adıyaman	Kasapoğlu Uludamar et al. (2018)
		apple	Bingöl	In this study
	<i>F. filiformis</i>	tomato	Ankara	Kepenekci and Ökten (1998)
		pear	Ankara	Evlice and Ökten (2008)
		walnut	Bingöl	In this study
	<i>Helicotylenchus canadiensis</i>	pear	Ankara	Evlice and Ökten (2008)
		apple, walnut	Bingöl	In this study
	<i>H. crenacauda</i>	tobacco	Samsun, Sinop	Kepenekci and Ökten (1999)
lentil		Yozgat	Kepenekci (1999)	
walnut		Zonguldak	Kepenekci (2001)	
<i>H. digonicus</i>	walnut	Bingöl	In this study	
	grapevine, pistachio	Adıyaman	Kasapoğlu Uludamar et al. (2018)	
	tomato	Ankara	Kepenekci and Ökten (1996)	
	pear	Ankara	Evlice and Ökten (2008)	
	apple	Burdur, Isparta	Kepenekci and Zeki (2002)	
Hoplolaimidae	<i>H. striatus</i>	walnut	Bingöl	In this study
		tobacco	Samsun, Sinop	Kepenekci and Ökten (1999)
		chestnut	Sinop, Giresun	Kepenekci (2001)
	<i>H. tunisiensis</i>	walnut	Bingöl	In this study
		tomato	Ankara	Kepenekci and Ökten (1996)
		chickpea	Ankara, Yozgat	Kepenekci (1999)
		bean	Burdur	Kepenekci (1999)
		lentil	Yozgat	Kepenekci (1999)
		kidney bean	Isparta	Kepenekci (1999)
		walnut	Ordu	Kepenekci (2001)
eggplant, grapevine, pepper, tomato	Diyarbakır	İmren (2007)		
<i>H. vulgaris</i>	pear	Ankara	Evlice and Ökten (2008)	
	walnut	Bingöl	In this study	
	pear	Ankara	Evlice and Ökten (2008)	
	walnut	Bingöl	In this study	
	bean	Erzurum	Yüksel (1977)	
	eggplant, grapevine, pepper,	Diyarbakır	İmren (2007)	

Pratylenchid ae	<i>Pratylenchoides alkani</i>	tomato		
		cotton, unidentified grass, wheat	Şanlıurfa	Yıldız (2007)
		wheat	Mardin	Kılıç (2011)
		barley, melon, watermelon, wheat, tobacco	Adıyaman	Kasapoğlu Uludamar et al. (2018)
		tomato	Ankara	Kepenekci and Ökten (1997b)
	<i>P. camachoi</i>	pear	Ankara	Evlice and Ökten (2008)
		apple, walnut	Bingöl	In this study
		kiwifruit	East Black Sea Region	Kepenekci and Öztürk (1999)
		walnut	Bingöl	In this study
		mulberry	Erzurum	Yüksel (1977)
<i>P. erzurumensis</i>	chickpea	Mardin	Di vito et al. (1994)	
	unidentified grass, wheat	Şanlıurfa	Yıldız (2007)	
	grapevine	Diyarbakır	İmren (2007)	
	plum	Antalya Burdur Isparta Trabzon Zonguldak	Kepenekci et al. (2001)	
	olive	Trabzon	Kepenekci (2001)	
	apple	Burdur Isparta	Kepenekci and Zeki (2002)	
	forage crops	Bingöl	Yıldız et al. (2012)	
	walnut	Bingöl	In this study	
	chickpea, lentil	Diyarbakır	Di vito et al. (1994)	
	chickpea	Mardin Şanlıurfa	Di vito et al. (1994)	
<i>P. leiocauda</i>	tomato	Ankara	Kepenekci and Ökten (1997b)	
	pear	Ankara	Evlice and Ökten (2008)	
	walnut	Bingöl	In this study	
	bean	Ankara Karaman Yozgat	Kepenekci (1999)	
	apple	Burdur Isparta	Kepenekci and Zeki (2002)	
<i>P. ritteri</i>	walnut	Ordu	Kepenekci (2001)	
	walnut	Bingöl	In this study	
	eggplant, grapevine, pepper, tomato, wheat	Diyarbakır	İmren (2007)	
	chickpea, lentil	Diyarbakır	Di vito et al. (1994)	
	corn, lentil, unidentified grass	Şanlıurfa	Yıldız (2007)	
	pear	Ankara	Evlice and Ökten (2008)	
	walnut	Bingöl	In this study	
	wheat	Diyarbakır	İmren (2007)	
	chickpea, lentil	DiyarbakırMardin	Di vito et al. (1994)	
	barley, cotton, unidentified grass, wheat	Şanlıurfa	Yıldız (2007)	
<i>Pratylenchus penetrans</i>	wheat	Mardin	Kılıç (2011)	
	cotton, grapevine, melon, tobacco, watermelon, wheat	Adıyaman	Kasapoğlu Uludamar et al.(2018)	
	forage crops	Bingöl	Yıldız et al. (2012)	
	Pratylenchidae			
	<i>Pratylenchus thornei</i>			

	apple	Burdur	Kepenekci and Zeki (2002)
	pear	Ankara	Evlice and Ökten (2008)
	walnut	Bingöl	In this study
	garlic, parsley, grassland, melon, sunflower	İstanbul	Saltukoğlu (1974)
<i>Psilenchus hilarulus</i>	grass	Ankara	Akgül (1991)
	rice	Balıkesir Ankara	Kepenekci et al. (1998)
Belonolaimidae	strawberry	Bursa	Akgül (2003)
	apple, walnut	Bingöl	In this study
<i>Trophurus sculptus</i>	rice	Ankara	Kepenekci et al. (1999)
	walnut	Bingöl	In this study

It can be concluded that, PPN fauna of apple and walnut orchards in Bingöl province was revealed for the first time by this study. Therefore, these first record of nematoda fauna of apple and walnut orchards could be the good source of future nematological studies. Due to relatively difficult management strategies of nematodes, the detection of these parasites in the region is becoming very important for developing new and proper control methods/tactics. In order to minimize the nematode damage, cultural practices such as developing resistant varieties, using tolerant grafted seedlings, and etc. should be applied. In addition to these applications, attentions should be paid to internal quarantine measures to prevent dissemination of plant parasitic nematodes to clean areas.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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