Research Note

Plant-parasitic nematodes associated with almond (*Prunus dulcis* Mill.) orchards

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Plant-parasitic nematodes are one of the main causes of yield loss in agricultural products. During a survey of almond (*Prunus dulcis* Mill.) orchards in the central part of Iran, seven species of plant-parasitic nematodes were identified from the soil and root samples in five orchards; ChamAli, Chelvan, Saman, Kharaji and Teshneez. The most common nematodes observed were *Scutylenchus rugosus* (168/100 g soil), *Boleodorus thyllactus* (58/100 g soil), *Helicotylenchus pseudorobustus* (36/100 g soil), and *Helicotylenchus digonicus* (34/100 g soil). We analysed pH, Zn, Mn, Fe and physical properties of the soils including percentages of sand, silt and clay. At Kharaji and Teshneez (soil texture sand 72%; silt 8%; clay 20% and low Fe), *Pratylenchus thornei*, *Merlinius brevidens*, and *H. digonicus* were observed more than at other localities, although levels were not as high at Teshneez, where Mn was lower, as they were at Kharaji. *S. rugosus* was dominant in soils with high levels of Fe in Saman, Chelvan, and ChamAli orchards, but this species was not observed at Kharaji and Teshneez. In conclusion, the almond-producing regions had various types of plant-parasitic nematodes, with six and five species found in Kharaji and Teshneez orchards, respectively. However, further studies are required to evaluate the tree damage caused by plant-parasitic nematodes on the almond orchards.

Keywords: Plant-parasitic nematodes, almond, soil variables, canonical correspondence analysis (CCA), biodiversity

Nuts are edible fruits of plants recommended primarily for daily consumption due to their rich antioxidant, vitamin, and mineral contents. Therefore, they are an essential component of a healthy diet. Almond (*Prunus dulcis* Mill.) has a big healthy fruit that includes food ingredients primary to healthy nourishment (Nur Tan et al. 2018). Consuming a handful of raw almonds provides high-quality vegetable proteins, omega 3-fatty acids, phytosterols, tocopherols, fibre, antioxidants, and phenolic compounds. Almond fruit are cholesterol-free and assist in the prevention and treatment of cardiovascular diseases. Almonds also contain many of the vitamins and minerals that a person needs daily. The economic value of the almond worldwide reaches billions of dollars (Nur Tan et al. 2018), and almond is a significant nut tree in Iran (Torki-Harchegani et al. 2015). Almond is becoming a favoured plant for farmers as they can earn more income because of its high market prices and new production areas are being developed (Nur Tan et al. 2018). Because of favourable soil and climate conditions, almond trees can be grown in the central part of Iran.

Soil nematodes are divided into free-living bacterivores and fungivores (Shokoohi and Abolafia 2019), predators and plant-parasitic nematodes (Ahmad and Jairajpuri 2010). Plant-parasitic nematodes are distributed worldwide and have effects on a wide range of crops (Moens et al. 2009). Almond productivity in the central part of Iran has been declining due to various factors including viruses, viroids, bacteria, fungi, weeds, and insects. Also, plant-parasitic nematodes have been detected in various locations and some have been reported as very problematic. However, there is limited study on plant-parasitic nematode affecting almond. Recently, *Meloidogyne floridensis* has been reported from almond trees in the USA

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(Westphal et al. 2019). Root lesion (*Pratylenchus* spp), ring nematodes (*Mesocriconema* spp) also have been reported with almond trees in USA (Anon 2004). So far, the most important plant-parasitic nematodes including *Pratylenchus thornei* and *Zygotylenchus guevarai* have been reported from Iran (Aliramaji et al. 2006).

Therefore, this study aimed to investigate the diversity of plant-parasitic nematodes associated with almond orchards and soil properties which are associated with the diversity of nematodes in the central part of Iran.

Materials and methods

Almond soil and root samplings were conducted in five randomly selected five orchards located in Chaharmahal va Bakhtiari Province, Central Iran. In total, 15 soil samples (100 grams for each sample) were collected from the five orchards (three per orchard) during 2017 (Saman: 32°29' N; 50°54' E, Chelvan: 32°28' N; 50°57' E, ChamAli: 32°31 N; 50°52 E, Kharaji: 32°04' N; 50°49' E, Teshneez: 32°04' N; 50°50' E) during 2017. Cores of soil samples were taken with a soil auger at depths of 0 - 30 cm at various spots within the rhizosphere region of each tree. The samples were brought to the Shahrekord Laboratory, Iran and stored in a refrigerator at 4°C. In the laboratory studies, nematodes were extracted from the soil and roots using a tray and sieve (25 µm pore size) (Shokoohi 2021).

Nematodes were fixed using hot formalin 37% and mounted in glycerin for species-specific identification. An Olympus CH-2 microscope equipped with a drawing tube was used to measure and draw different parts of nematode bodies. To identify nematodes, their morphological properties and morphometry were examined microscopically. Species were identified using available resources and keys. The nematodes were counted from the suspension extracted from 100 g of soil under a stereomicroscope. The identification of species was carried out based on the morphology and morphometrics (Geraert 2010).

Chemical properties of soil pH (KCl), Zn (mg/kg), Mn (mg/kg), Fe (mg/kg), and physical properties of soil including percentages of sand, silt and clay were evaluated (Table 1). The relationships between the nematode community and the soil chemical properties and textures were analysed using canonical correspondence analysis (CCA) implemented in XLSTAT software (Addinsoft 2007).

Prevalence was obtained as the percentage of samples with well-defined nematode taxon using the following equation:

$$\text{Prevalence (\%)} = Sn/St \times 100$$

Where Sn considers samples having the desired nematode taxon, and St is the total of samples processed (Laasli et al. 2022).

Table 1: Soil physicochemical analysis in the rhizosphere soil of almond trees in Chaharmahal va Bakhtiari Province, Iran

Sample	Sand%	Silt%	Clay%	pH(KCI)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
ChamAli	87	4.3	8	6.5	12	285	422
Chelvan	88	5.6	6.3	6.2	6	268	310
Saman	86	6	7.3	6.3	9	274	582
Kharaji	72	8	20	6.5	10	287	69
Teshneez	72	7.8	19.8	6.5	7.5	203	46

Results

A total of seven species of plant-parasitic nematodes from five families were identified morphologically and taxonomically; these were *Boleodorus thyllactus, Filenchus cylindricauda, Helicotylenchus digonicus, Helicotylenchus pseudorobustus, Merlinius brevidens, Pratylenchus thornei,* and *Scutylenchus rugosus* within the families of Tylenchidae, Hoplolaimoidae, Belonolaimoidae, Pratylenchidae, and Dolichodoroidae. The distribution of the plant-parasitic nematode species is shown in Table 2. *B. thylactus* was found in four of the five orchards sampled, it was not detected in Chelvan. *P. thornei* was found only in Kharaji and Teshneez. *S. rugosus* was found in three orchards, but not in Kharaji and Teshneez. The prevalence of plant-parasitic nematodes in the soil within the rhizosphere of almond trees showed that *S. rugosus* was more prevalent than the other plant-parasitic nematodes. The CCA (Figure 1) explained 99.55% of the distribution, being 78.75% explained by canonical variable 1 and 20.79% explained by canonical variable 2.

H. digonicus, *M. brevidens*, and *P. thornei* were dominant in substrates characterised by high levels of Zn and Mn found in Kharaji orchard. In Kharaji and Teshneez (soil texture - sand 72%; silt 8%; clay 20%, Table 1) *Pratylenchus thornei*, *Merlinius brevidens* and *H. digonicus* populations were higher than in other localities where sand percentages were higher. However levels were not as high at Teshneez, where Mn was lower, as they were at Kharaji. *S. rugosus* was dominant in soils with high levels of Fe and sand over 85% in Saman, Chelvan, and ChamAli, but this species was not observed at Kharaji and Teshneez. The CCA plot showed that almond orchards of Saman, Chelvan and ChamAli were in one group, indicating they have the same diversity of nematode. The almond orchards of Kharaji and Teshneez, which were more affected by plant-parasitic nematodes, formed the other group.

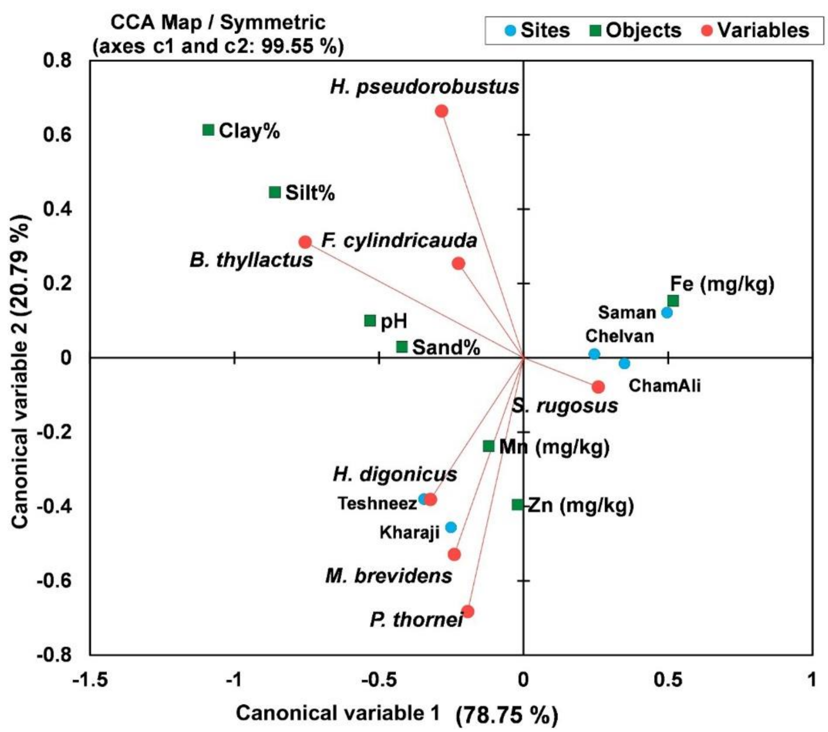

Figure 1: Scatter plot of Canonical Correspondence Analysis (CCA) indicating the relationship of the plant-parasitic nematode, selective soil parameters, and localities of the almond orchards

Table 2: Prevalence and mean papulation density (MPD) for plant-parasitic nematodes in the rhizosphere soil (100 g) of almond trees in Chaharmahal va Bakhtiari Province, Iran

Plant-parasitic nematode species	**ChamAli**		**Chelvan**		**Saman**		**Kharaji**		**Teshneez**	
	MPD	**Prevalence**	**MPD**	**Prevalence**	**MPD**	**Prevalence**	**MPD**	**Prevalence**	**MPD**	**Prevalence**
Boleodorus thyllactus	20	33	0	0	120	100	120	67	30	67
Filenchus cylindricauda	20	33	0	0	0	0	60	67	75	67
Helicotylenchus digonicus	0	0	0	0	0	0	130	100	40	33
Helicotylenchus pseudorobustus	10	33	0	0	80	67	90	67	0	0
Merlinius brevidens	0	0	0	0	10	33	100	33	50	66
Pratylenchus thornei	0	0	0	0	0	0	130	100	30	33
Scutylenchus rugosus	280	100	250	100	310	100	0	0	0	0

Discussion

Worldwide, various plant-parasitic nematode species have been found in almond cultivation areas; *H. dihystera* and *P. thornei* were identified by several researchers (Khan and Sharma 1992). Severe root damage due to heavy nematode feeding may lead to bacterial and fungal disease infestation (Nur Tan et al. 2018). In Iran, only *P. thornei* and *Z. guevarai* have been detected with almond, but the ecological effect and the yield loss have not been studied (Aliramaji et al. 2006).

The environmental conditions of Chaharmahal va Bakhtiari Province have helped create a suitable habitat for plant-parasitic nematodes (Torki-Harchegani et al. 2015), other pests and other agents of diseases in agricultural production. Chemical control before planting has been shown to be a useful method to control the presence of nematodes in the soil (Barnes et al. 2017). Silva et al. (2020) observed that soils under native vegetation generally show higher diversity, higher density and abundance of nematodes than those under cultivation; these findings were explained by the changes in soil physical properties, reduced organic material input and reduced deposition of roots. Additionally, geographic location affects plant-parasitic and free-living nematodes (Barnes et al. 2017). Ngeno et al. (2019) indicated that P and N have a positive and significant correlation with plant parasitic nematodes such as meloidogyne. Ngeno et al. (2019) indicated that Zn and Mn had a weak negative correlation with plant-parasitic nematode populations; in addition, pH showed a negative correlation with plant-parasitic nematode populations. In this study sandy soil texture was shown to affect the nematode dynamic and population density. Bai et al. (2020) indicated that the presence of the soil organisms such as bacterial and fungal genera that have been associated with nutrient mobilisation and plant growth was likely related to the higher soil organic matter (OM), total nitrogen (TN), nitrate-nitrogen ($NO3-N$), and total phosphorus (TP) contents in the non-fertilised plots of almond trees. In the presence of the higher bacteria and fungi the nematode number will change, in which the free-living nematodes will increase. However, the plant-parasitic nematode will decrease in the above-mentioned circumstances. Jagdale et al. (2021) indicated that in pecan orchards in USA, several plant-parasitic nematode genera are more common in the coastal plain locations, suggesting that the well-drained sandy soil of the coastal plain is favourable for movement/reproduction of plant-parasitic nematodes. The same result was obtained in the present work in which soil texture (sandy soil) appeared to affect the movement and population density of plant-parasitic nematode associated with almond trees.

Conclusion

As a habitat for wild almonds, Iran is one of the most important almond-producing countries. Given the economic importance and benefits of the almond tree species, it is necessary to study the factors limiting its growth. The almond tree is economically important for the people of the central part of Iran. The environmental conditions of Chaharmahal va Bakhtiari Province are favourable for plant-parasitic nematodes and for other pests and disease factors in agricultural production. Therefore, the first step was to ascertain which nematodes are in the almond orchards. The result indicated that in Kharaji and Teshneez orchards, plant-parasitic nematodes need to be investigated regarding their ecological role and their effects on the trees. The result indicated that *S. rugosus* was dominant in soil with high levels of Fe in Saman, Chelvan, and ChamAli orchards. Additionally, *P. thornei*, *M. brevidens* and *H. digonicus* populations were higher than in other localities. This suggests the need to assess the damage potential of the identified nematode species on almond trees. Furthermore, management strategies for further studies are required.

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