

Original article (Orijinal araştırma)

Determination of plant-parasitic nematode fauna and evaluation of soil quality in apple orchards of Çanakkale province (Türkiye)¹

Çanakkale ili (Türkiye) elma bahçelerinde bitki paraziti nematod faunasının belirlenmesi ve toprak kalitesinin değerlendirilmesi

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Abstract

In September 2023, a total of 130 soil samples were collected from five different districts in the province of Çanakkale (Türkiye) and its surroundings to identify plant-parasitic nematode communities in apple orchards and to create distribution maps of these species. The density and distribution of nematode communities were examined. In total, 14.090 nematode individuals were analyzed, and 30 genera were identified. The nematodes were classified into six different orders, with the order Rhabditida standing out as the most dominant (53.87%). The order Tylenchida ranked second (23.22%). Among the most common plant-parasitic nematodes identified in the study were *Paratylenchus* spp. Micoletzky, 1922 (Tylenchida: Tylenchulidae) (3.46%), *Merlinius* spp. Siddiqi, 1970 (Tylenchida: Dolichodoridae) (3.36%), and *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae) (3.02%). The results of the study indicate that the c-p 2 group is prevalent under disturbed soil conditions, and the dominance of the p-p 2 and p-p 3 groups poses a significant threat to apple orchards. These findings highlight that nematode c-p series are important bioindicators in the assessment of soil quality, and such analyses should be expanded through regional studies.

Keywords: Apple orchards, biodiversity, bioindicators, plant-parasitic nematodes, soil quality

Öz

Çanakkale (Türkiye) ili ve ilçelerindeki elma bahçelerinde bitki paraziti nematod topluluklarının tespiti ve bu türlerin dağılım haritalarının oluşturulması için 2023 yılı Eylül ayında beş farklı ilçeden toplam 130 toprak örneği toplanmış ve nematod topluluklarının yoğunluğu ve yayılımı incelenmiştir. Çalışmada toplam 14.090 nematod bireyi incelenmiş ve 30 cins tanımlanmıştır. Nematodlar altı farklı takımda yer almakta olup, Rhabditida takımı (53.87%) ile en baskın takım olarak öne çıkmıştır. Tylenchida takımı (23.22%) ile ikinci sırada yer almıştır. Araştırmada en fazla bulunan bitki paraziti nematodlar arasında *Paratylenchus* spp. Micoletzky, 1922 (Tylenchida: Tylenchulidae) (3.46%), *Merlinius* spp. Siddiqi, 1970 (Tylenchida: Dolichodoridae) (3.36%), ve *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae) (3.02%) yer almaktadır. Çalışmada elde edilen sonuçlar c-p 2 grubunun bozulmuş toprak koşullarında yaygın olduğunu ve p-p 2 ile p-p 3 gruplarının baskınlığı ile elma bahçelerinde önemli bir tehdit oluşturduğunu göstermektedir. Bu bulgular nematodların c-p serilerinin toprak kalitesinin değerlendirilmesinde önemli bir biyoindikatör olduğunu ve bölgesel çalışmalarla bu tür analizlerin yaygınlaştırılması gerektiğini ortaya koymaktadır.

Anahtar sözcükler: Elma bahçeleri, biyoçeşitlilik, biyoindikatörler, bitki paraziti nematodlar, toprak kalitesi

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Introduction

Nematodes represent a large group of invertebrates and are organisms belonging to the phylum Nematoda. This phylum is divided into three main classes, which include approximately 32 orders, 3.030 genera, and 28.537 species (Hodda, 2022). Nematodes are unsegmented roundworms, most of which are microscopic in size. They have adapted to various habitats (soil, water, marine, freshwater). There are both free-living and parasitic species. Parasitic species can cause significant damage to both plants and animals (Bhat et al., 2022). Globally, approximately 4.100 plant-parasitic nematode species have been identified (Decraemer & Hunt, 2006).

Plant-parasitic nematodes are microscopic organisms, typically ranging in size from 0.5 mm to 3 mm, that are commonly found worldwide and cause significant economic losses in agricultural production. These nematodes penetrate plant parts such as roots, stems, and leaves, feeding and causing damage to plant tissues. Additionally, the damage they inflict weakens the plant's defense mechanisms, making it more susceptible to infections by other pathogens (Sato et al., 2019). *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae), *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae), *Paratylenchus* spp. Micoletzky, 1922 (Tylenchida: Tylenchulidae) and *Helicotylenchus* spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) are the primary nematodes causing damage in apple orchards (Askary et al., 2012). The economic losses caused by these nematodes are substantial. Globally, the economic damage to agricultural production caused by plant-parasitic nematodes is estimated to be \$215 billion annually (Abu-Elgawad & Askary, 2015).

Türkiye holds a significant position in fruit cultivation due to its diverse climate and fertile soils. The fruit-growing sector not only plays an important role in human nutrition but also adds economic value to Türkiye (Niyaz & Demirbaş, 2011). Among the most widely cultivated fruits in Türkiye are figs, grapes, apricots, apples, hazelnuts, pomegranates, and cherries. In 2022, the total fruit production in Türkiye amounted to 26.794.706 tons, with apple orchards covering an area of 1.709.408 decares and total production reaching 4.817.500 tons (TUİK, 2022).

Türkiye plays a crucial role in apple cultivation, and apples hold a significant share in the country's agricultural production. Globally, Türkiye ranks second among the top apple-producing countries with an annual production of approximately 4.8 million tons (FAO, 2024). Apple cultivation provides an important economic contribution both for the domestic market and for export.

Nematodes stand out as important microorganisms for assessing the health of soil ecosystems. The diversity and distribution of nematode populations play a critical role in determining soil health and ecosystem functions (Bongers & Ferris, 1999). The use of nematodes, particularly for monitoring the impacts of agricultural practices and pollution, serves as a valuable method for measuring soil health (Neher, 2001). For these reasons, the commercial potential of using nematodes as bioindicators is noteworthy, highlighting the significance of their role in environmental assessment programs (Trett et al., 2009).

The primary objective of this study is to identify the plant-parasitic nematodes in apple orchards within the province of Çanakkale (Türkiye) and its districts, as well as to determine their distribution maps. Additionally, the study aims to understand the ecological roles of nematodes in apple orchards and to examine the effects of different environmental and climatic conditions on nematode populations.

Materials and Methods

Survey

Soil sampling was conducted in apple orchards within the province of Çanakkale and its districts during the period leading up to harvest with the aim of identifying plant-parasitic nematodes and determining their densities. Since nematodes generally exhibit clustered distributions, samples were collected in a

manner that best represents the region (Southey, 1986). Soil samples were taken from a depth of 15-30 cm under the canopy projection of the trees. The samples were placed in polyethylene bags with label information and stored under appropriate conditions at the Nematology Laboratory of Çanakkale Onsekiz Mart University. A total of 130 soil samples were collected from five different districts throughout September 2023 (Figure 1).



Figure 1. Map of sampling locations from five different districts in Çanakkale.

Nematode extraction from soil

The Modified Baermann Funnel Method, which is used for the extraction of mobile nematodes from the soil through separation using water, was employed (Hooper, 1986). In the Modified Baermann funnel method, plastic Petri dishes with a diameter of 12 cm and a height of 2 cm were used. In each Petri dish, 100 g of soil was placed with filter paper, allowing the active nematodes to migrate into the water. After keeping the Petri dishes in this state for 48 hours, the water they contained was transferred into 100 ml glass cylinders, and after 24 hours, it was transferred into 10 ml glass tubes. Subsequently, these glass tubes were stored under appropriate conditions at the Çanakkale Onsekiz Mart University Nematology Laboratory.

Diagnosis at the genus level under light microscopy

The water in the preserved glass tubes was allowed to settle for 6-8 hours and then diluted to 1 ml. After the dilution process, the glass tubes were mixed using a vortex device, and 100 µl of water was taken with a micropipette and placed between a slide and a coverslip. To immobilize individuals and ensure accurate identification, the specimens were processed on a heated plate operating at a specific temperature. Samples were identified at the genus level under a Leica DM 1000 light microscope using the Leica Application Suite v4 software.

Analysis of nematode communities

Taxonomic keys were used as the basis for the classification of nematodes. For the classification of plant-parasitic nematodes in particular, the book *Plant-parasitic Nematodes: A Pictorial Key to Genera* was used (Mai et al., 1996). The Coloniser-persister (c-p) classification categorizes nematodes on a scale of 1 to 5 based on their life cycles (Bongers, 1990). Nematodes were evaluated according to this classification

system by Yeates et al. (1993) and Du Preez et al. (2022). The Maturity Index (MI) was used to assess ecosystem disturbances in nematode communities (Ferris & Bongers, 2009). The online calculation tool Nematode Indicator Joint Analysis (NINJA) was used to perform the specified analyses (Sieriebriennikov et al., 2014).

Result

Among the total number of 14,090 nematodes examined, six different orders and 30 genera were identified (Table 1). The order Rhabditida, with 7.590 individuals, constituted the majority of the population (53.87%), making it the most dominant order. This was followed by the order Tylenchida, with 3.273 individuals (23.22%). The Aphelenchida order represented 2.888 individuals, accounting for 20.50 % of the total population. Orders represented in lower proportions included Mononchida with 215 individuals (1.53%), Dorylaimida with 100 individuals (0.71%), and Triplonchida with 24 individuals (0.17%).

Table 1. The prevalence rates, cp series, and feeding types of nematode communities

Genus Name	Order: Family	Prevalence Rate (%)	C-p Class	P-p Class	Feeding Type
<i>Aglenchus</i> Andrassy, 1954	Tylenchida: Tylenchidae	0.92	0	2	Herbivores
<i>Aphelenchoides</i> Fischer, 1894	Aphelenchida: Aphelenchoididae	13.61	2	0	Fungivores
<i>Aphelenchus</i> Bastian, 1865	Aphelenchida: Aphelenchidae	6.89	2	0	Fungivores
<i>Boleodorus</i> Thorne, 1941	Tylenchida: Tylenchidae	0.09	0	2	Herbivores
<i>Criconema</i> Hofmann & Menzel, 1914	Tylenchida: Criconematidae	0.01	0	3	Herbivores
<i>Discocriconemella</i> De Grisse & Loof, 1965	Tylenchida: Criconematidae	0.04	0	3	Herbivores
<i>Ditylenchus</i> Filipjev, 1936	Tylenchida: Anguinidae	2.78	2	0	Fungivores
<i>Dorylaimus</i> Dujardin, 1845	Dorylaimida: Dorylaimidae	0.33	4	0	Omnivores
<i>Eucephalobus</i> Steiner, 1936	Rhabditida: Cephalobidae	46.83	2	0	Bacterivores
<i>Filenchus</i> Andrassy, 1954	Tylenchida: Tylenchidae	2.16	2	0	Fungivores
<i>Helicotylenchus</i> Steiner, 1945	Tylenchida: Hoplolaimidae	1.21	0	3	Herbivores
<i>Hoplolaimus</i> von Daday, 1905	Tylenchida: Hoplolaimidae	0.14	0	3	Herbivores
<i>Longidorus</i> Micoletzky, 1922	Dorylaimida: Longidoridae	0.03	0	5	Herbivores
<i>Malenchus</i> Andrassy, 1968	Tylenchida: Tylenchidae	0.25	0	2	Herbivores
<i>Meloidogyne</i> Goeldi, 1892	Tylenchida: Meloidogynidae	0.20	0	3	Herbivores
<i>Merlinius</i> Siddiqi, 1970	Tylenchida: Dolichodoridae	3.36	0	3	Herbivores
<i>Mononchus</i> Bastian, 1865	Mononchida: Mononchoidea	1.53	4	0	Predators
<i>Paratylenchus</i> Micoletzky, 1922	Tylenchida: Tylenchulidae	3.46	0	2	Herbivores
<i>Pratylenchoides</i> Winslow, 1958	Tylenchida: Pratylenchidae	0.47	0	3	Herbivores
<i>Pratylenchus</i> Filipjev, 1936	Tylenchida: Pratylenchidae	3.02	0	3	Herbivores
<i>Psilenchus</i> de Man, 1921	Tylenchida: Psilenchidae	0.84	0	2	Herbivores
<i>Rhabditis</i> Dujardin, 1844	Rhabditida: Rhabditidae	7.04	1	0	Bacterivores
<i>Rotylenchus</i> Filipjev, 1936	Tylenchida: Hoplolaimidae	0.06	0	3	Herbivores
<i>Scutellonema</i> (Steiner, 1937) Andrassy, 1958	Tylenchida: Hoplolaimidae	0.31	0	3	Herbivores
<i>Trichodorus</i> Cobb, 1913	Triplonchida: Trichodoridae	0.17	0	4	Herbivores
<i>Trophurus</i> Loof, 1956	Tylenchida: Telotylenchidae	0.34	0	3	Herbivores
<i>Tylencholaimus</i> De Man, 1876	Dorylaimida: Tylencholaimoidea	0.04	4	0	Fungivores
<i>Tylenchorhynchus</i> Cobb, 1913	Tylenchida: Telotylenchidae	0.81	0	3	Herbivores
<i>Tylenchus</i> Bastian, 1865	Tylenchida: Tylenchidae	2.75	0	2	Herbivores
<i>Xiphinema</i> Cobb, 1913	Dorylaimida: Longidoridae	0.31	0	5	Herbivores

Classification of nematodes according to their feeding type

The distribution of nematode communities based on feeding types showed significant differences between districts (Figure 2). Generally, bacterivores (nematodes feeding on bacteria) were the dominant group in all districts, with the highest rate observed in the center district (62.90%). Herbivores (plant-parasitic nematodes) were more prominent in Lapseki (27.80% and Bayramiç (20.90%), while they were absent in Biga. Fungivores (fungus-feeding nematodes) stand out with a high proportion in Biga (45.70%) and were more evenly distributed in other districts. Predator nematodes were found in low proportions in some districts, but slightly higher in Lapseki and Biga (around 5.00%). These results suggest that organic matter cycling in the soils of these districts was more related to bacteria and fungi, with variations in biodiversity.

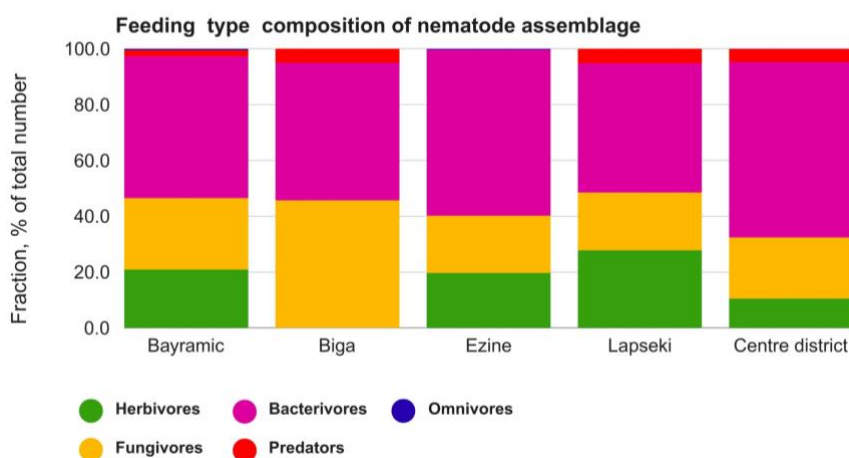


Figure 2. Distribution of nematode communities based on feeding types.

The distribution of free-living nematodes based on feeding types indicates that bacterivores were dominant in all districts (Figure 3). The highest rate was found in Ezine (74.10%), while the lowest was in Biga (49.30%). Fungivores were especially prominent in Biga (45.70%). Predator nematodes had higher rates in Lapseki (7.20%) and Biga (5.00%), whereas omnivores generally had low levels, with the highest rate observed in Bayramiç at 0.70%. Unicellular eukaryote feeders were recorded as 0% in all districts. These distributions indicate that bacterial and fungal activity varies between districts, with possibly greater biodiversity in Lapseki and Biga.

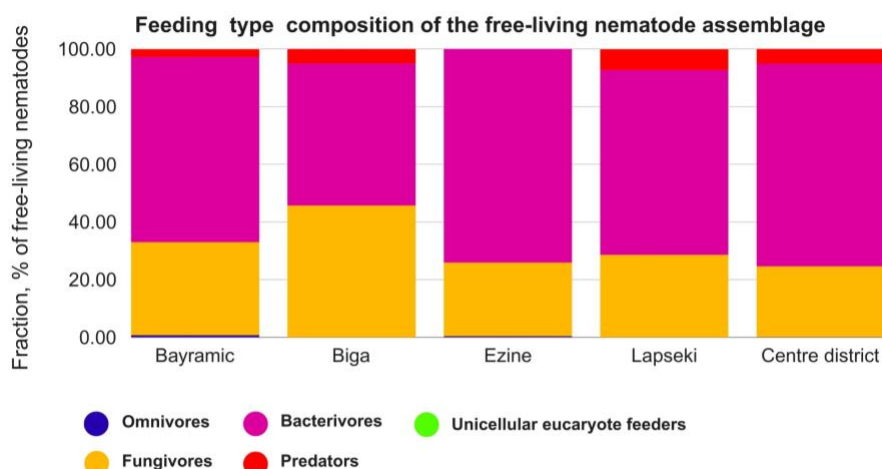


Figure 3. Distribution of free-living nematode communities based on feeding types.

The distribution of plant-parasitic nematodes based on feeding types varies between districts (Figure 4). In Bayramiç, the most dominant group was epidermal/root hair feeders at 32.50%, while in Ezine, it was the ectoparasites group at 43.30%. In Lapseki, ectoparasites and semi-endoparasites were almost equally present (38.80% and 38.09%), whereas in the center district, ectoparasites had the highest proportion at 63.70%. Sedentary parasites were either absent or found in very low proportions in most districts. These results indicate that the feeding strategies of plant-parasitic nematodes vary according to the ecological conditions of each district.

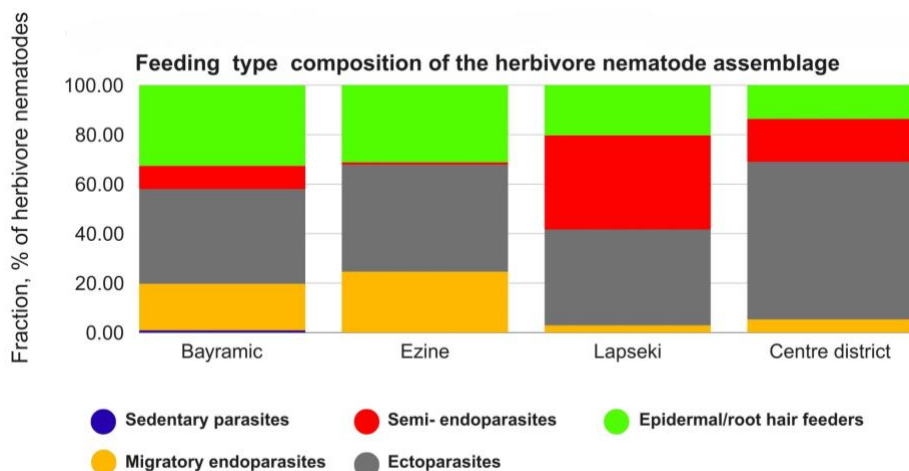


Figure 4. Distribution of plant-parasitic nematode communities based on feeding types.

In the food web analysis, soil samples from different regions (Bayramiç, Biga, Ezine, Lapseki, Center district) were evaluated based on enrichment and structure indices (Ferris et al., 2001). Most points had low enrichment and low structure indices, indicating that the soils in these regions were depleted or degraded in terms of organic matter (Figure 5). However, in some regions, particularly in the center district, Bayramiç, and Lapseki, the points shifted towards the middle and upper right of the graph, indicating more structured and enriched soils, which mean that these soils were more balanced and fertile.

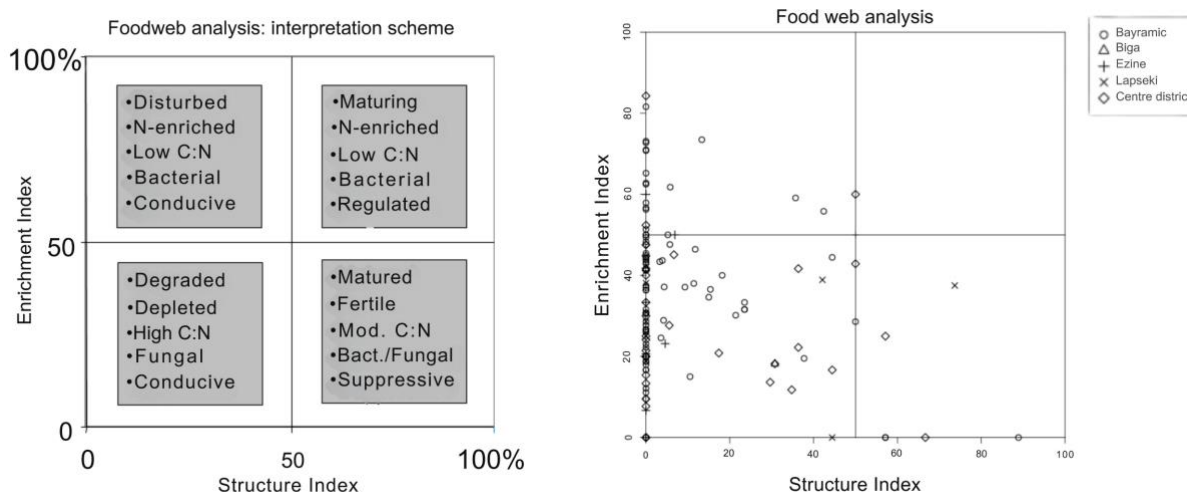


Figure 5. Overall food web analysis of the districts.

Index analysis and classification according to c-p series

According to the Maturity Index (MI) analysis, values showed significant variation between districts (Figure 6). The MI values were found to be 1.98 for Bayramiç, 2.10 for Biga, 1.96 for Ezine, 2.20 for Lapseki, and 2.06 for the center district. MI values between 2-5 reflected the maturity levels of free-living nematode communities more specifically and exclude c-p 1 nematodes (Bongers and Korthals, 1993). The values for Bayramiç were recorded as 2.06, Biga as 2.10, Ezine as 2.01, Lapseki as 2.21, and the center district as 2.11. Sigma MI values were also analyzed, with 2.11 for Bayramiç, 2.10 for Biga, 2.10 for Ezine, 2.32 for Lapseki, and 2.15 for the center district. These data suggest that Lapseki had a more mature ecosystem compared to the other regions. Plant-parasitic Index (PPI) values were also evaluated, with 2.59 for Bayramiç, 2.73 for Ezine, 2.64 for Lapseki, and 2.94 for the center district. These results reflected the plant-parasitic potential of nematode communities, with a particularly higher pressure in the center district (Ferris & Bongers, 2009).

According to the Enrichment Index (EI) analysis results, nutrient enrichment levels varied between districts (Figure 5). Bayramiç had the highest EI value at 37.10, while Biga had 29.92, Ezine 26.73, Lapseki 26.69, and the center district 27.38, indicating lower enrichment levels. These results showed that ecosystems in Bayramiç were characterized by a higher accumulation of organic matter and nutrients compared to other regions. In the Structure Index (SI) analysis, the structural complexity of nematode communities was assessed (Figure 5). The SI value for Bayramiç was relatively low at 7.98, while it was 15.38 for Biga, 1.44 for Ezine, 22.89 for Lapseki, and 14.56 for the center district. Lapseki stood out as the region with the highest structural diversity in this context. These differences could be interpreted as indicators of the stability of regional ecosystems and the complexity of nematode communities.

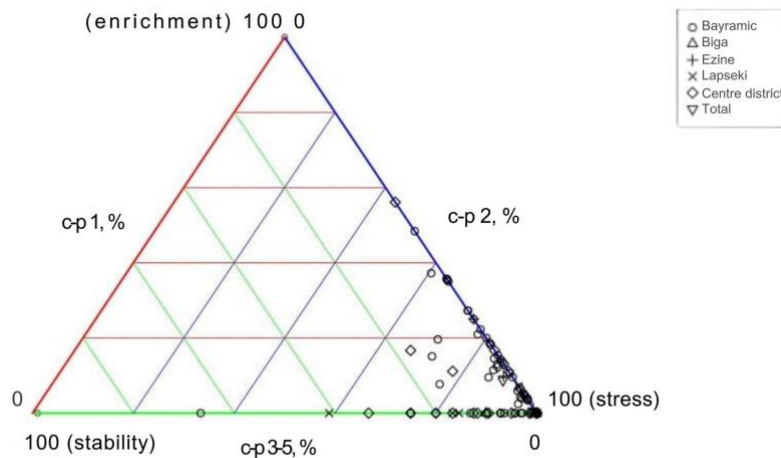


Figure 6. Triangular diagram representing the structure of nematode communities.

This ternary diagram represents the responses of nematodes to environmental conditions (enrichment, stress, stability) according to their life strategies, displayed as a maturity index graph (Figure 6). It also shows the distribution of samples from different regions (Bayramiç, Biga, Ezine, Lapseki, Center district) based on their c-p (colonizer-persister) groups. Most samples were located between c-p 2 and c-p 3-5 groups. Nematodes in the c-p 2 group represent species that thrive in nutrient-rich but more stressful conditions, while the c-p 3-5 group represents species found in soils with lower nutrient levels but lower environmental stress, indicating more stable conditions. Overall, these results reveal that the examined nematode communities had adapted to higher stress conditions and unstable environments, reflecting these characteristics in the soils of these regions.

In the analysis of free-living nematodes based on the colonizer-persister (c-p) structure, the dominance of the c-p 2 group was observed in all districts (Figure 7). The dominance of colonizer species was evident in Bayramiç (88.60%), Biga (95.00%), Ezine (95.30%), Lapseki (91.00%), and the center district (90.00%). Resilient species (c-p 4) were detected at the highest rates in Lapseki (7.80%) and the center district (5.20%). Additionally, c-p 1 species were found above 4% in Ezine and the center district, while in Bayramiç, they were recorded at a higher level of 8% but remained at lower levels in other districts. C-p 3 species were not detected in any district. These results indicate that c-p 2 species dominate ecosystems, although resilient species were also present in some regions.

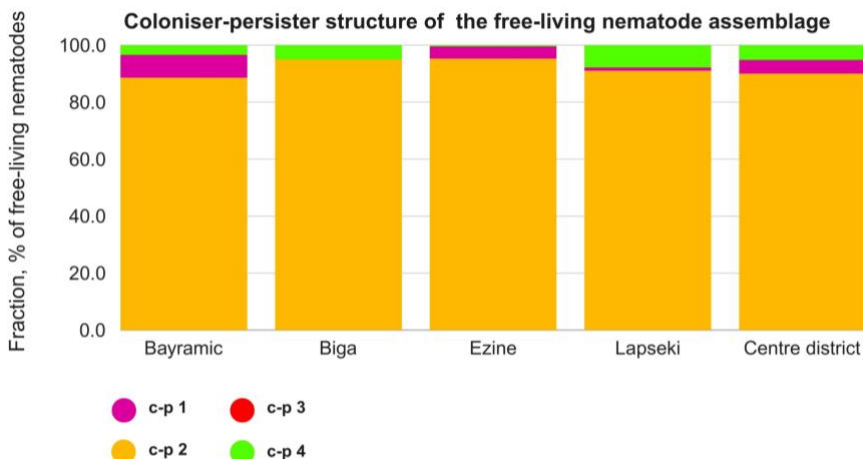


Figure 7. C-p structure of free-living nematode communities.

In the analysis of plant-parasitic nematodes based on their life strategies, the p-p 2 and p-p 3 groups predominantly distributed among the districts (Figure 8). In Bayramiç, the p-p 2 ratio was recorded as 47.00%, p-p 3 as 51.10%, p-p 4 as 0.60%, and p-p 5 as 1.40%. In Ezine, the p-p 2 ratio was 36.40% and the p-p 3 ratio was 63.60%, with no observation of p-p 4 and p-p 5 groups. In Lapseki, the p-p 2 ratio was 23.3% and p-p 3 was 76.70%, with no p-p 4 and p-p 5 groups observed here either. In the center district, the p-p 2 ratio was 27.80%, p-p 3 was 59.10%, p-p 4 was 5.40%, and p-p 5 was 7.70%. Generally, the p-p 3 group was dominant across all districts, with particularly higher proportions of p-p 4 and p-p 5 groups in the center district.

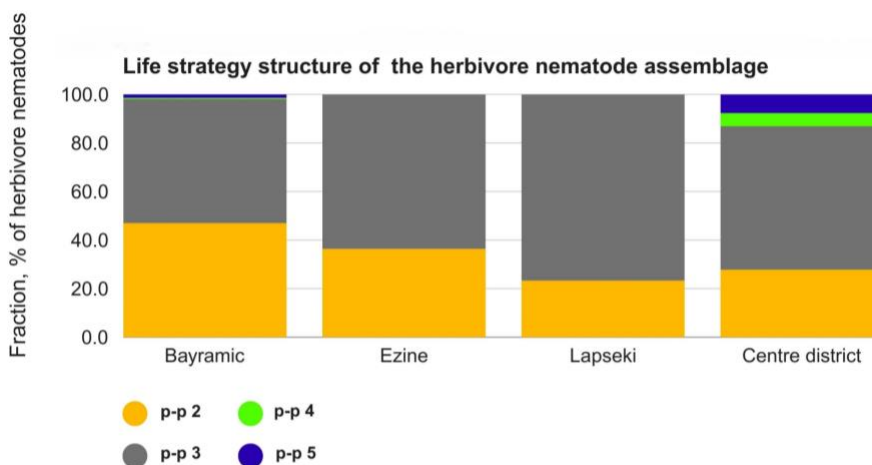


Figure 8. P-p structure of plant-parasitic nematode communities.

Discussion

In this study, a total of 30 nematode genera were identified, with *Pratylenchus* species being predominant, and root-knot nematodes were detected in three samples. In a study by Yüksel et al. (2023), 8 nematode genera were identified in samples from two provinces, with root-knot nematodes found in only one sample. This difference may be attributed to regional environmental conditions, variations in sampling methods, or differences in the number of samples. In the first study conducted in apple orchards in Türkiye by Kepenekçi & Öztürk (2002), 13 genera belonging to the order Tylenchida were identified, while Gözel & Yıldız (2015) reported that root-knot nematodes were found at low levels in apple nurseries in Ödemiş. In contrast, the identification of a greater number of nematode genera in this study suggests that it could provide more information about the distribution of nematode populations. The dominance of the *Pratylenchus* genus in this and other studies emphasizes the need to develop sustainable management strategies against this harmful species in apple orchards.

There are no studies in Türkiye evaluating soil quality through the examination of c-p series in apple orchards. However, a study by Pokharel et al. (2015) in apple orchards in the United States found that the c-p 2 group was predominant among free-living nematodes. This finding aligns with the results obtained in this study, confirming that the c-p 2 group is prevalent in soil ecosystems under stress or degraded soil conditions. Additionally, plant-parasitic nematodes (herbivores) were predominantly of the p-p 3 type. In present study, similar findings of predominant p-p 2 and p-p 3 groups indicate that these groups pose a significant threat to plant health and should be considered in soil management strategies. These findings highlight the importance of using c-p series as bioindicators for evaluating soil quality and suggest that such analyses should be extended through regional studies.

The Plant-parasitic Index (PPI) values recorded in the districts indicate the impact of plant-parasitic nematodes on the soil ecosystem. The moderate PPI values in these regions suggest that soil health is threatened by plant-parasitic nematodes. A similar study conducted in China by Yin et al. (2014) examined the effects of nematode communities on soil health and found that low PPI values did not pose a serious threat to apple orchards. However, the PPI values obtained in this study, especially in the center district (PPI: 2.94), are higher, indicating that regional differences should be considered when developing management strategies for apple orchards (Bongers et al., 1997).

The differences in nematode communities' feeding types among the districts suggest that nematodes contribute differently to bacterial and fungal cycles. Studies examining the contributions of nematodes to bacterial and fungal cycles emphasize that in soils dominated by bacterivores, bacteria play a central role in organic matter decomposition (Ferris et al., 2001). In this study, bacterivores are observed to be dominant. The high proportions of plant-parasitic nematodes in Lapseki and Bayramiç suggest that they could pose a threat to agricultural areas. The high proportions of fungivores in Biga support studies suggesting that fungus-based organic matter cycles are more dominant in soil ecosystems in this region (Neher, 1999). These findings underscore that soil ecosystems vary in biological diversity across districts and that management strategies should be adapted to these differences.

The study reveals the distribution of nematode populations in soil ecosystems and the impact of plant-parasitic nematodes on ecosystem health. The observed differences in nematode communities' feeding types and various index values among districts highlight how regional environmental conditions influence the distribution of nematodes and soil health. The dominance of *Pratylenchus* species and moderate PPI values emphasize the need to develop sustainable management strategies against these harmful species in apple orchards. Since studies on soil quality evaluation based on c-p series in Türkiye are limited, the impact of c-p and p-p groups on nematode fauna should be used as bioindicators. It is recommended to expand agricultural soil management and nematode population monitoring programs, considering regional differences. In regions like the center district, where PPI is high, adopting integrated management strategies specific to the region against harmful plant-parasitic nematodes will contribute to the preservation of soil health.

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