

# Determination of Plant Parasitic Nematode Fauna and Evaluation of Soil Quality in Olive Orchards of Çanakkale Province, Türkiye

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#### ABSTRACT

In September 2023, a total of 185 soil samples were collected from the olive orchards in Çanakkale province's Centre district and the districts of Ayvacık, Bayramiç, Biga, Ezine, and Lapseki to identify the plant-parasitic nematode communities present, determine their distribution maps, and evaluate soil quality by demonstrating the use of nematodes as bioindicators. A total of 22.257 nematode individuals were examined, and 33 genera belonging to 19 families were identified. The Rhabditida order constituted 47.62% of the population, followed by the Tylenchida order with 23.18% and the Aphelenchida order with 22.44%. The most prevalent plant-parasitic nematodes were identified as Merlinius spp. Siddiqi, 1970 (Tylenchida: Dolichodoridae) (10.41%), Tylenchus spp. Bastian, 1865 (Tylenchida: Tylenchidae) (3.47%) and Helicotylenchus spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) (1.77%). The dominance of the p-p 3 group indicates that this group has a common life strategy among herbivorous nematodes and poses a potential threat in agricultural ecosystems. The prevalence of the c-p 2 group among free-living nematodes highlights the critical role of this group in ecosystem processes, particularly in organic matter cycling and soil health.

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# Türkiye, Çanakkale İli Zeytin Bahçelerindeki Bitki Paraziti Nematod Faunasının Belirlenmesi ve Toprak Kalitesinin Değerlendirilmesi

### ÖZET

Eylül ayı 2023'te Çanakkale ilinin Merkez ilçesi ile Ayvacık, Bayramiç, Biga, Ezine ve Lapseki ilçelerindeki zeytin bahçelerinden toplam 185 toprak örneği toplanmıştır. Bu örnekler bölgede bulunan bitki-paraziti nematod topluluklarını tanımlamak, dağılım haritalarını belirlemek ve nematodların biyoindikatör olarak kullanımını göstererek toprak kalitesini değerlendirmek amacıyla incelenmiştir. Toplamda 22.257 nematod bireyi incelenmiş ve 19 familyaya ait 33 cins tanımlanmıştır. Popülasyonun %47.62'sini Rhabditida takımı oluştururken bunu %23.18 ile Tylenchida ve %22.44 ile Aphelenchida takip etmiştir. En yaygın bitki-paraziti nematodlar Merlinius spp. Siddiqi, 1970 (Tylenchida: Dolichodoridae) (%10.41), *Tylenchus* spp. Bastian, 1865 (Tylenchida: Tylenchidae) (%3.47) ve Helicotylenchus spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) (%1.77) olarak tespit edilmiştir. Herbivor nematodlar arasında p-p 3 grubunun baskınlığı, bu grubun ortak bir yaşam stratejisine sahip olduğunu ve tarımsal ekosistemlerde potansiyel bir tehdit oluşturabileceğini göstermektedir. Serbest yaşayan nematodlar arasında c-p 2 grubunun yaygınlığı ise bu grubun ekosistem süreçlerinde, özellikle organik madde döngüsü ve toprak sağlığında oynadığı kritik rolü vurgulamaktadır.

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### INTRODUCTION

The olive tree stands out as an agricultural activity of both economic and cultural importance (Kocadağlı, 2011; Pilak & Ülger, 2021). Olive trees have a history spanning thousands of years and are an essential part of dietary habits and traditional culinary culture (Liphschitz et al., 1991). Due to their richness in phenolic compounds and vitamins, olives have become a valuable food in terms of nutrition and health (Ozturk et al., 2021). The health benefits and high nutritional value of olives, combined with olive oil production, have led to increasing global demand (Özata & Cömert, 2016). Furthermore, olive cultivation plays a significant role in sustainable agriculture, particularly in arid regions, by protecting soil health and combating desertification (Pleguezuelo et al., 2018).

Most of the global olive production takes place in regions with a Mediterranean climate (Sakar & Ünver, 2014). Türkiye, located in the Mediterranean basin, ranks among the top countries worldwide with a total production area of 889.000 hectares and a production volume of 1 million tons (FAO, 2024). In Türkiye, olives and olive oil are products with high income potential and hold a significant position in exports (Tunç & Yilmaz, 2023). Olive cultivation is concentrated mainly in the Aegean, Marmara, and Mediterranean regions of the country. Çanakkale, located in the Marmara Region, is one of Türkiye's leading provinces in terms of olive production area and volume (TUİK, 2022).

Plant-parasitic nematodes are generally cylindrical, thread-like microscopic organisms. A significant portion of important species of these nematodes belong to the order Tylenchida within the phylum Nematoda (Kepenekçi & Ökten, 1999). Approximately 4,100 species of plant-parasitic nematodes have been identified globally, many of which cause significant economic losses in agricultural production (Kornobis, 2023). These economic losses are estimated to be around 157 billion dollars annually (Chariou & Steinmetz, 2017). As one of the major pests in agricultural areas, these nematodes cause serious damage to olive trees, especially olive seedlings, posing a significant threat to olive production worldwide (Ali et al., 2014). By damaging the root systems of plants and agricultural crops, they threaten the health of the plants (Göze Özdemir, 2022). *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae) and *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) are nematodes with the greatest potential to harm olive trees (Belahmar et al., 2015). Particularly, finding and implementing alternative control methods against root-knot nematodes is of great importance (Çetintaş et al., 2018).

Nematodes are widely used as effective bioindicators to monitor ecosystem health and environmental changes. They are regarded as key components of biodiversity and nutrient cycling in soil ecosystems. Therefore, the diversity of nematodes provides important information about soil health and biodiversity. The abundance of fungivorous and bacterivorous nematodes, in particular, is evaluated as a reflection of sustainable agricultural practices (Yeates & Bongers, 1999). These organisms are critical for maintaining the balance of soil microflora and fauna, and they are considered potential bioindicators for sustainable soil management practices (Moura & Franzener, 2017).

This study aims to determine the distribution maps and densities of nematodes using soil samples collected from olive orchards in Çanakkale province and its districts. By mapping the distribution of plant-parasitic nematodes commonly found in olive orchards, the areas with high concentrations of these pests will be identified. If areas with a high concentration of *Meloidogyne* spp. and *Pratylenchus* spp., which cause significant economic losses in olive trees, are identified, this will contribute to the development of effective nematode control programs in these areas.

### MATERIALS and METHODS

### Survey

To determine the genera and densities of plant-parasitic nematodes a total of 185 soil samples were collected between 2023 and 2024 from olive orchards in Çanakkale's the centre district as well as the districts of Ayvacık, Bayramiç, Biga, Ezine, and Lapseki. The soil samples were collected in amounts of 1 kg from a depth of 10-30 cm beneath the canopy drip line of trees, ensuring the representation of the region. The samples were placed in polyethylene bags, labeled, and stored in coolers. After the sampling process was completed the soil samples were transported to the Nematology Laboratory at Çanakkale Onsekiz Mart University's Faculty of Agriculture and stored at + 4 °C in a refrigerator until the analysis phase (Figure 1).

### Nematode Extraction from Soil

The Baermann Funnel Method, which facilitates the migration of mobile nematodes from the soil medium to a water medium was used to extract nematodes from the soil (Hooper, 1986). This method was carried out using 12

cm diameter and 2 cm height plastic petri dishes with plastic sieves placed at a height of 0.5 cm. Filter paper was laid on the sieves, and 100 g of homogeneous soil samples were added. After the soil was moistened with water the petri dish was sealed and left for 48 hours. At the end of the period the water in the petri dish was transferred to 100 ml glass measuring cylinders and left for 24 hours to allow the nematodes to concentrate. The water was then carefully reduced to 10 ml, placed in 10 ml glass tubes, and stored under appropriate conditions.



Figure 1. Map of olive sampling areas from districts Sekil 1. İlçelerden alınan zeytin örnekleme alanlarının haritası.

# Light Microscopy Diagnosis at Genus Level

The water in the glass tube was diluted to 1 ml and homogenized using a vortex mixer. Subsequently a 100  $\mu l$  water sample was taken with a micropipette, placed on a microscope slide and covered with a coverslip. To immobilize the nematodes and ensure accurate identification the samples were prepared on a heated plate set to a specific temperature. The counting and identification of the samples at the genus level were performed using a Leica DM 1000 light microscope and Leica Application Suite v4 software.

# Nematode Communities and Analyses

Taxonomic keys were primarily used for the classification of nematodes. For the identification of plant-parasitic nematodes the book "Plant-Parasitic Nematodes: A Pictorial Key to Genera" by Mai et al. (1996) was particularly useful. The life cycle traits of nematodes were ranked from 1 to 5 based on the colonizer-persister classification proposed by Bongers (1990; 1999). The feeding types of nematodes were determined in accordance with the classification criteria presented by Yeates et al. (1993) and Du Preez et al. (2022). To evaluate the maturity of nematode community composition in the ecosystem, structure and enrichment indices were calculated (Ferris et al., 2001; Ferris & Bongers, 2009). The Nematode Indicator Joint Analysis (NINJA) software, an online tool was used to analyze the data (Sieriebriennikov et al., 2014).

NINJA is an R-based automated calculation system designed to facilitate the computation of nematode-based biological monitoring metrics. The program was developed to automate statistical and analytical processes for calculating various metrics. As a parametric tool, NINJA primarily employs ANOVA-based parametric approaches in analyses. ANOVA is a fundamental statistical method used to evaluate whether there are significant differences among sampling areas, providing results supported by mean values, standard deviations, and p-values. Additionally, it calculates the mean and standard deviation values for sampling areas and presents them to users in summary tables. By implementing these parametric analysis methods quickly and accurately, NINJA simplifies data analysis processes for users and offers additional tools for visualizing results. This system stands out as a reliable and accessible solution for nematode-based ecological studies.

### RESULTS

In the present study a total of 22.257 nematode individuals were identified. The Tylenchida order comprised

23.18% of the nematode population with 5,159 individuals while the Aphelenchida order represented 22.44% with 4.994 individuals. The Dorylaimida order accounted for 2.80% with 624 individuals, and the Mononchida order made up 3.96% with 882 individuals. Lastly the Rhabditida order formed the largest portion of the population with 47.62% amounting to 10.598 individuals (Table 1).

Genus Name	Order: Family	Prevalence Rate (%)	C-p Class	P-p Class	Feeding Type
Aglenchus Andrassy, 1954	Tylenchida: Tylenchidae	0.14	0	2	Herbivores
Anguina Scopoli, 1777	Tylenchida: Anguinidae	0.02	0	2	Herbivores
Aphelenchoides Fischer, 1894	Aphelenchida: Aphelenchoididae	13.96	2	0	Fungivores
Aphelenchus Bastian, 1865	Aphelenchida:Aphelenchidae	8.47	2	0	Fungivores
Belonolaimus Steiner, 1949	Tylenchida: Belonolaimidae	0.02	0	3	Herbivores
Boleodorus Thorne, 1941	Tylenchida: Tylenchidae	0.02	0	2	Herbivores
Coslenchus Siddiqi, 1978	Tylenchida: Tylenchidae	0.17	0	2	Herbivores
<i>Discocriconemella</i> De Grisse & Loof, 1965	Tylenchida: Criconematidae	0.02	0	3	Herbivores
<i>Ditylenchus</i> Filipjev, 1936	Tylenchida: Anguinidae	0.94	<b>2</b>	0	Fungivores
<i>Dorylaimus</i> Dujardin, 1845	Dorylaimida: Dorylaimidae	2.56	4	0	Omnivores
Eucephalobus Steiner, 1936	Rhabditida: Cephalobidae	46.53	2	0	Bacterivores
Filenchus Andrassy, 1954	Tylenchida: Tylenchidae	1.22	2	0	Fungivores
Gracilacus Raski, 1962	Tylenchida: Tylenchulidae	0.04	0	2	Herbivores
Heterodera Schmidt, 1871	Tylenchida: Heteroderidae	0.01	0	3	Herbivores
Helicotylenchus Steiner, 1945	Tylenchida: Hoplolaimidae	1.77	0	3	Herbivores
Hoplolaimus von Daday, 1905	Tylenchida: Hoplolaimidae	0.36	0	3	Herbivores
Malenchus Andrassy, 1968	Tylenchida: Tylenchidae	0.36	0	2	Herbivores
Meloidogyne Goeldi, 1892	Tylenchida: Meloidogynidae	0.11	0	3	Herbivores
Merlinius Siddiqi, 1970	Tylenchida: Dolichodoridae	10.41	0	3	Herbivores
Mononchus Bastian, 1865	Mononchida: Mononchoidea	3.96	4	0	Predators
Paratylenchus Micoletzky, 1922	Tylenchida: Tylenchulidae	0.49	0	2	Herbivores
Pratylenchoides Winslow, 1958	Tylenchida: Pratylenchidae	0.35	0	3	Herbivores
Pratylenchus Filipjev, 1936	Tylenchida: Pratylenchidae	1.18	0	3	Herbivores
<i>Psilenchus</i> de Man, 1921	Tylenchida: Psilenchidae	0.28	0	2	Herbivores
Rhabditis Dujardin, 1844	Rhabditida: Rhabditidae	1.09	1	0	Bacterivores
<i>Rotylenchulus</i> Linford & Oliveira, 1940	Tylenchida: Hoplolaimidae	0.02	0	3	Herbivores
Rotylenchus Filipjev, 1936	Tylenchida: Hoplolaimidae	0.08	0	3	Herbivores
<i>Scutellonema</i> (Steiner, 1937) Andrassy, 1958	Tylenchida: Hoplolaimidae	0.13	0	3	Herbivores
Trophurus Loof, 1956	Tylenchida Telotylenchidae	0.17	0	3	Herbivores
<i>Tylencholaimus</i> De Man, 1876	Dorylaimida: Tylencholaimoidea	0.16	4	0	Fungivores
Tylenchorhynchus Cobb, 1913	Tylenchida: Telotylenchidae	1.41	0	3	Herbivores
Tylenchus Bastian, 1865	Tylenchida: Tylenchidae	3.47	0	2	Herbivores
Xiphinema Cobb, 1913	Dorylaimida: Longidoridae	0.08	0	<b>5</b>	Herbivores

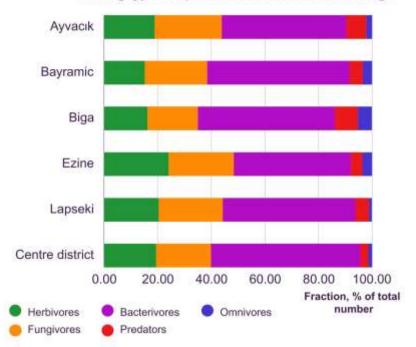
Table 1 The prevalence rates, cp series, and feeding types of nematode communities *Cizelge 1. Nematod toplulukların bulunma oranları, cp serileri ve beslenme tipleri.* 

These findings indicate that the Rhabditida order was dominant in the population and the Tylenchida and Aphelenchida orders also constitute significant proportions. The Dorylaimida and Mononchida orders were represented at lower rates in the population.

### Classification of Nematodes According to Their Feeding Type

When analyzing the feeding type composition of nematode communities in districts of Çanakkale, bacterivorous nematodes were found to be the most common. Bacterivorous nematodes were the dominant group in Ayvacık (46.5%), Bayramiç (53.1%), Biga (51.1%), Ezine (43.6%), Lapseki (49.4%), and the (55.7%). Fungivorous nematodes

also had significant proportions in Ayvacık (25.0%), Bayramiç (23.3%), Biga (18.8%), Ezine (24.3%), Lapseki (23.9%), and the centre district (20.5%). Herbivorous nematodes were present in all districts at rates ranging from 15.0% to 25.0%, while predator and omnivorous nematodes were found at lower levels compared to other groups. These results showed that bacterivorous and fungivorous nematodes were dominant across Çanakkale although differences in feeding types were observed among the districts (Figure 2).



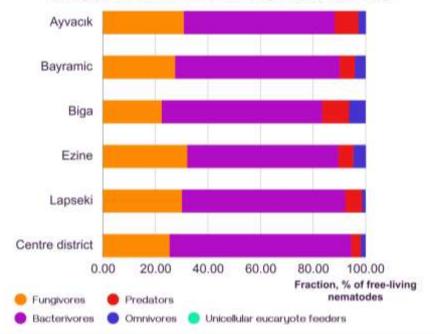
Feeding type composition of nematode assemblage

Figure 2. Distribution of nematode communities by feeding types *Şekil 2. Nematod topluluklarının beslenme tiplerine göre dağılımı.* 

According to the feeding type composition results of free-living nematode communities, bacterivores were the most dominant group. Bacterivores reached the highest rates in all districts, particularly in the the centre district (69.1%). In other districts, bacterivore rates were 62.6% in Bayramiç, 61.0% in Biga, 62.1% in Lapseki, 57.4% in Ayvacık, and 57.5% in Ezine. Fungivores were the second most prevalent group after bacterivores, with higher proportions in Ayvacık (30.8%) and Ezine (32.1%). Predatory and omnivorous nematodes were detected at lower rates in all districts, with predator rates ranging from 3.7% (the centre district) to 10.3% (Biga) and omnivore rates between 1.4% (Lapseki) and 6.3% (Biga). These results indicated that bacterial decomposition is dominant in soil ecosystems and that environmental conditions influence the distribution of nematode feeding types (Figure 3).

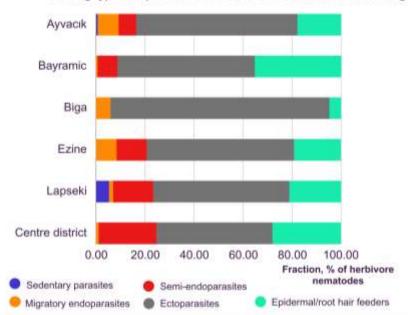
In the composition of herbivorous nematode communities ectoparasites were the dominant group in all districts. The highest rate was observed in Biga (89.3%) and the lowest in the centre district (47.3%). Epidermal/root hair feeders were detected at rates of 17.8% in Ayvacık, 35.3% in Bayramiç, 4.8% in Biga, 19.1% in Ezine, 21.1% in Lapseki, and 27.9% in the centre district. Migratory endoparasites were found at 5.9% in Biga, 8.4% in Ezine, and 8.5% in Ayvacık, with rates below 1.7% in other districts. Semi-endoparasites were prominent in Lapseki (16.3%) and the centre district (23.6%), while sedentary parasites were found in low proportions only in Ayvacık (0.7%) and Lapseki (5.3%). These results showed that ectoparasites were dominant in all regions, while other feeding groups vary depending on the district (Figure 4).

The food web analysis values for each district indicate variability in the soil ecosystems (Figure 5). Ayvacık and Ezine, with high Enrichment Index (EI) and moderate Structure Index (SI) values, possess nutrient-rich and structurally balanced ecosystems, while Biga showed the highest structural development with the highest SI value despite its low EI. Lapseki, with both a high EI and low SI, indicates a nutrient-rich but structurally weaker ecosystem. Bayramic and the centre district with both low EI and SI values, reveal that their ecosystems were weaker in both nutrient and structural aspects (Ferris et al., 2001). These differences between the districts were clearly seen in the graph.



Feeding type composition of the free-living nematode assemblage

Figure 3. Distribution of free-living nematode communities based on feeding types *Şekil 3. Serbest yaşayan nematod topluluklarının beslenme tiplerine göre dağılımı.* 



Feeding type composition of the herbivore nematode assemblage

Figure 4. Distribution of plant-parasitic nematode communities by feeding types Şekil 4. Bitki paraziti nematod topluluklarının beslenme tiplerine göre dağılımı.

#### Index Analysis and Classification According to C-P Series

In the colonizer-persister structure of free-living nematode communities, c-p 2 was the most common group in all districts, with the highest rate in the centre district (93.2%) and the lowest in Biga (83.5%). The c-p 4 group had the highest proportion in Biga (16.5%) and the lowest in the centre district (5.7%). The c-p 1 group reached the highest value in Lapseki (2.7%) and the lowest in Ayvacık and Bayramiç (0.9%). As a result, the c-p 2 group emerged as the most prevalent in all districts, while the c-p 4 and c-p 1 groups showed less prevalence (Figure 6).

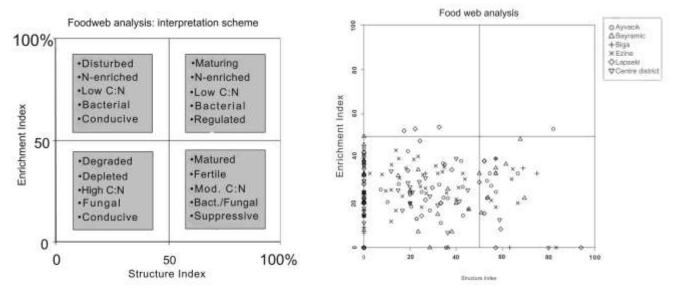
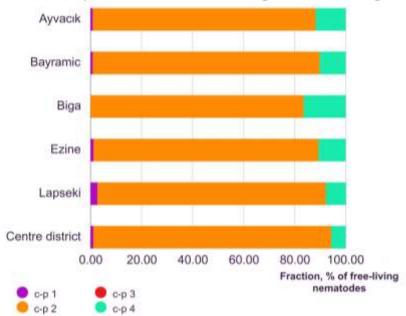
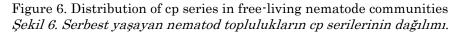


Figure 5. Food web analysis of total nematode communities across all districts *Sekil 5. Tüm ilçelerdeki toplam nematod topluluklarının besin ağı analizi.* 







The results of the life strategy analysis of herbivorous nematodes indicated that the p-p 3 group was dominant in all districts, with proportions of 80.8% in Ayvacık, 61.0% in Bayramiç, 95.2% in Biga, 77.8% in Ezine, 77.2% in Lapseki, and 69.2% in the centre district. The p-p 2 group typically ranked second, with its highest rate observed in Bayramiç (39.0%). The p-p 5 group was found only in low proportions in Ezine (1.0%) and the centre district (0.3%). These findings showed that the p-p 3 group were dominant in the life strategy of herbivorous nematodes across all regions (Figure 7).

The maturity and plant-parasitic index analyses of nematodes provide important insights into the ecosystem status and the effects of agricultural activities in the studied districts. The Maturity Index (MI) values reflect the impacts of environmental degradation and enrichment (Bongers, 1990). Low MI values in Ayvacık (2.22), Bayramiç (2.20), Biga (2.35), Ezine (2.20), Lapseki (2.15), and the centre district (2.10) suggest higher environmental degradation and nutrient enrichment. The MI2-5 index follows a similar trend with low values in Ayvacık (2.23), Bayramiç (2.21), Biga (2.35), Ezine (2.22), Lapseki (2.18) and the centre district (2.12) indicating that pollution-related stress was more pronounced particularly in Lapseki and the centre district (Bongers & Korthals, 1993).

Sigma MI values also show that nematode community structures were less mature in Ayvacık (2.34), Bayramiç (2.26), Biga (2.43), Ezine (2.35), Lapseki (2.26) and the centre district (2.22). The Plant-Parasitic Index (PPI) for plant-parasitic nematodes was determined as Ayvacık (PPI: 2.80), Bayramiç (2.65), Biga (2.86), Ezine (2.80), Lapseki (2.73), and the centre district (2.68), indicating that plant health was more affected by plant-parasitic nematodes, particularly in Biga and Ayvacık.

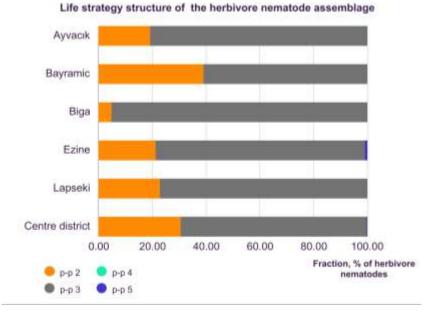


Figure 7. Distribution of pp series in plant-parasitic nematode communities *Şekil 7. Bitki paraziti nematod toplulukların pp serilerinin dağılımı.* 

Based on the Enrichment Index (EI) and Structure Index (SI) values, we can compare the ecological statuses of nematode communities in Ayvacık, Bayramiç, Biga, Ezine, Lapseki, and the centre district (Ferris et al., 2001). Ayvacık (EI 26.99, SI 28.51), Ezine (EI 27.68, SI 27.84), and Lapseki (EI 27.72, SI 20.41) were nutrient-rich areas, while Biga (EI 21.85, SI 36.37) stands out in terms of ecosystem complexity and biodiversity. Bayramiç (EI 23.01, SI 25.65) showed moderate levels of both nutrient richness and ecosystem complexity, whereas the centre district (EI 23.35, SI 16.90) drew attention with both the lowest EI and SI values, indicating that it has the lowest organic matter richness and ecosystem complexity, and it may be more vulnerable to environmental pressures.

The Basal Index (BI) and Channel Index (CI) values exhibited notable and similar variations. The BI values were calculated as 55.29 for Ayvacık, 60.14 for Bayramiç, 55.01 for Biga, 55.12 for Ezine, 58.55 for Lapseki, and 65.75 for the Centre district. These results indicated that the BI values in all regions were high (>50), suggesting that the soil food web was depleted or damaged. The higher BI value observed in the centre district suggested that this area was one of the most affected by soil degradation.

The CI values were determined as 92.88 in Ayvacık, 92.04 in Bayramiç, 100 in Biga, 88.84 in Ezine, 82.89 in Lapseki, and 89.26 in the centre district. It was observed that the CI values were high (>50) in all regions, indicating that decomposition processes were predominantly carried out by fungi and that complex organic matter was being broken down slowly. Particularly, the CI value of 100 in Biga indicated that fungal-dominated decomposition was at its maximum level, and the transformation of organic matter occurred more slowly in this region compared to others. These findings revealed that the biological functionality of nematode communities and their effects on ecological processes varied across different regions.

The triangular diagram reflected the ecological structure and strategies of nematode communities, balancing between enrichment, stress tolerance, and stability (de Goede et al., 1993). Most district points were close to the cp 2 region indicating that nematode communities in the districts could quickly respond to nutrient increases, although ecosystem complexity was limited. Ayvacık, Lapseki, and Ezine were located closer to stability suggesting that the nematode communities in these areas were more resilient to environmental changes. Ayvacık and some other districts showed a balanced structure between enrichment and stress tolerance, but most points indicate that nematodes preferred more stressful and less balanced soils (Figure 8).

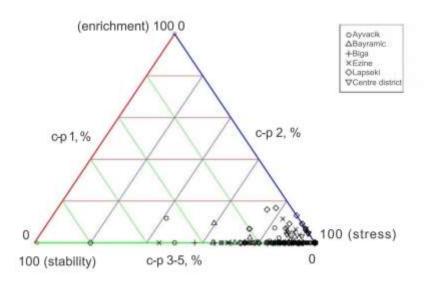


Figure 8. Triangular diagram illustrating the structure of nematode communities *Şekil 8. Nematod topluluklarının yapısını gösteren üçgen diyagram.* 

# Metabolic Footprints

The results for the Composite footprint, Enrichment footprint, and Structure footprint values were as follows: The Composite footprint values were calculated as 29.52 for Ayvacık, 31.56 for Bayramiç, 31.39 for Biga, 60.31 for Ezine, 32.26 for Lapseki, and 32.53 for the centre district. These results indicated that Ezine had the highest value in terms of ecosystem functionality and biological activity compared to other regions. The Enrichment footprint values were determined as 2.82 in Ayvacık, 3.55 in Bayramiç, 1.29 in Biga, 8.41 in Ezine, 6.97 in Lapseki, and 4.88 in the centre district. These values suggested that Ezine and Lapseki exhibited a decomposition process more sensitive to nutrient inputs. The Structure footprint values were identified as 19.67 in Ayvacık, 21.66 in Bayramiç, 25.86 in Biga, 36.27 in Ezine, 9.61 in Lapseki, and 18.17 in the centre district. These findings indicated that Ezine and Biga had complex and stable food webs, while Lapseki likely had a less developed ecosystem structure. Overall, the variation in these metabolic footprint values across different regions revealed that the contributions of nematode communities to ecological processes depended on local conditions.

The Herbivore footprint, Fungivore footprint, Bacterivore footprint, Predator footprint, and Omnivore footprint values exhibited distinct differences. The Herbivore footprint values were determined as 2.16 in Ayvacık, 1.46 in Bayramiç, 1.11 in Biga, 6.57 in Ezine, 11.26 in Lapseki, and 3.22 in the centre district. These results showed that Lapseki and Ezine had a greater impact from plant-parasitic nematodes on the ecosystem compared to other regions. The Fungivore footprint values were calculated as 1.93 in Ayvacık, 1.68 in Bayramiç, 1.29 in Biga, 4.51 in Ezine, 2.25 in Lapseki, and 2.40 in the centre district. These values revealed that Ezine had higher biological activity in fungus-based energy transformation processes compared to other regions. The Bacterivore footprint values were identified as 5.76 for Ayvacık, 6.81 for Bayramiç, 3.12 for Biga, 13.03 for Ezine, 9.15 for Lapseki, and 8.77 for the centre district. This indicated that bacterial decomposition processes mediated by bacterivorous nematodes were dominant in Ezine. The Predator footprint values were 4.14 in Ayvacık, 3.17 in Bayramiç, 3.33 in Biga, 7.18 in Ezine, 3.27 in Lapseki, and 4.79 in the centre district. The high value in Ezine emphasized the significance of predator species in the food web of this region. The Omnivore footprint values were recorded as 15.53 for Ayvacık, 18.44 for Bayramiç, 22.54 for Biga, 29.02 for Ezine, 6.34 for Lapseki, and 13.36 for the centre district. Notably, Ezine and Biga showed high values, indicating complex food webs dominated by omnivorous nematodes. These findings demonstrated that nematode communities contributed differently to ecosystem processes across trophic levels, depending on the region.

### DISCUSSION

Although Çanakkale is a significant region for olive cultivation in Türkiye, plant-parasitic nematodes cause substantial damage to olive trees (Castillo et al., 1999; Nico et al., 2002). Among them, *Pratylenchus* spp. (root lesion nematodes) and *Meloidogyne* spp. (root-knot nematodes) stand out as nematodes with the potential to cause severe damage to olive trees (Belahmar et al., 2015). In this study, a total of 33 nematode genera were identified, and key harmful nematodes, such as *Meloidogyne* and *Pratylenchus*, were found in olive plants. Similar taxa have also been reported in global studies (Belahmar et al., 2015; Hamza et al., 2015; Guesmi-Mzoughi et al., 2022). In

previous studies conducted in Türkiye, Kepenekci (2001) identified 23 different plant-parasitic nematode genera in research carried out in the Mediterranean and Black Sea regions, but *Meloidogyne* was not detected. In another study conducted in Ödemiş, Izmir, the species *Helicotylenchus multicinctus* was identified in olive orchards (Yıldız & Gözel, 2015). Literature review revealed only one study focused on determining the plant-parasitic nematode fauna in olive orchards in the Çanakkale region. This study, conducted by Öztürk (2023), identified 12 different nematode genera from 15 samples taken from olive orchards in Ayvacık, Bayramiç, Bozcaada, the centre district and Ezine. However, *Meloidogyne* and *Pratylenchus*, known to cause significant damage to olive plants, were not detected. This could be explained by the limited number of sampling sites or the inadequacy of the soil samples. Therefore, more comprehensive research covering a wider range of areas is necessary to fully determine the plantparasitic nematode fauna in the region.

When plant-parasitic nematode groups (p-p) and free-living nematode groups (c-p) from different districts of Türkiye are evaluated, it is observed that the p-p 3 group is dominant among plant-parasitic nematodes in all regions. In agreement with this study Palomares-Rius et al. (2015), Ali et al. (2017) and Hamza et al. (2018) found that the p-p 3 group was followed by the p-p 2 group, while the p-p 5 group was present at lower rates. A similar finding was observed in Öztürk (2023) study on free-living nematodes in Çanakkale, where the c-p 2 group was the most common, with no detection of the c-p 5 group. The consistency between this study and Öztürk's study supports the widespread presence of the c-p 2 group and suggests that free-living nematodes exhibit similar strategies under Mediterranean climatic conditions. These results generally indicate that the p-p 3 group dominates among plant-parasitic nematodes, while the c-p 2 group is dominant among free-living nematodes in olive-growing Mediterranean ecosystems.

Significant differences in nematode community feeding types across districts of Çanakkale are also observed. Bacterivorous nematodes were generally the dominant group, indicating that bacterial decomposition processes play an essential role in organic matter cycling within the districts. Fungivorous nematodes were also widespread, underscoring the contribution of fungi to soil ecosystems (Ferris et al., 2001). Herbivorous nematodes showed variability among the districts, suggesting that they may have agricultural significance as plant-parasites in some areas. Predator and omnivorous nematodes were found at lower rates, suggesting that these groups play more limited roles in ecosystem balance. In a similar study conducted by Cakmak (2024) on olive trees, bacterivorous nematodes were also dominant (70.7%), and plant-parasitic nematodes (20.9%) were observed at significant rates. These findings highlight that bacterivorous and fungivorous nematodes play central roles in organic matter cycling throughout Çanakkale, although there are some differences in feeding types between districts.

Plant-Parasitic Index (PPI) values in various districts of Çanakkale generally range between 2.65 and 2.86, aligning with similar international studies conducted in the Mediterranean region, where PPI values in olivegrowing areas typically range from 2.0 to 3.0. In a study by Ali et al. (2017) in Morocco, PPI values varied according to irrigation methods, with an average of 2.60. This value closely matches the PPI values in Bayramic and the centre district, suggesting that regional differences and agricultural practices directly affect PPI values. The PPI value of 2.85 detected in Ragusa, Italy by Landi et al. (2022) is consistent with the 2.86 value observed in Biga, indicating that similar ecological conditions in olive-growing soils may contribute to higher PPI values. However, the lower PPI value of 2.03 found in Foggia suggests that this region may have different environmental conditions and soil composition (Bongers et al., 1997). In conclusion, the PPI values of this study largely correspond with those from various regions of the Mediterranean.

Metabolic footprints have provided valuable insights into ecosystem functions and services within the soil food web, highlighting the roles of nematode communities in carbon transfer and nutrient cycling. Additionally ANOVA results, with p values ranging between 0.01 and the threshold of 0.05, confirmed statistically significant differences between regions. This finding indicates that local conditions have a strong influence on the metabolic activities and ecological roles of nematode communities. The usability of metabolic footprints as sensitive indicators of soil ecosystem functions and services supports a detailed understanding of regional ecological dynamics (Ferris, 2010).

Composite footprint values revealed the highest value in Ezine (60.31), emphasizing this region's superior ecosystem functionality and biological activity. The composite footprint serves as a comprehensive indicator measuring energy flow within the soil food web regardless of the trophic roles of nematode communities. Enrichment footprint values were particularly high in Ezine (8.41) and Lapseki (6.97), reflecting increased carbon utilization by lower trophic levels (cp-1 and cp-2) in response to nutrient inputs and dynamic resource enrichment processes. Structure footprint values were notably higher in Ezine (36.27) and Biga (25.86), indicating the presence of complex and stable food webs dominated by higher trophic groups (cp-3 to cp-5), which play a regulatory role in suppressing opportunistic organisms. Additionally herbivore footprint values were highest in Lapseki (11.26), suggesting more intense energy flow through plant-parasitic nematodes in this region. Fungivore and bacterivore footprint values showed regional variation, with Ezine standing out in fungal (4.51) and bacterial (13.03)

decomposition pathways, highlighting dominant energy transformations in these processes. Predator and omnivore footprint values were particularly prominent in Ezine and Biga, underscoring strong trophic interactions and regulatory functions in these regions. This finding suggests potential contributions to pest suppression and maintaining food web balance.

The metabolic footprint data are not only limited to understanding ecosystem functions but also provide critical information for strategies aimed at improving soil health. For instance, the high enrichment footprint values in Ezine and Lapseki emphasize the sensitivity of these regions to organic matter inputs, which could be optimized to enhance decomposition processes. Furthermore, the dominance of predator and omnivore nematodes in regions like Ezine and Biga highlights the regulatory roles of these communities in ecosystem stability. These findings support the applicability of metabolic footprints in improving agricultural lands, restoring ecosystems, and managing soil health (Ferris et al., 2012). Overall, these results demonstrate that metabolic footprints are effective tools for evaluating soil ecosystem status through a multidimensional approach.

This study provides valuable insights into the distribution of plant-parasitic nematodes in olive-growing areas of Çanakkale and their impact on plant health, offering important information about the region's ecosystem. The PPI values observed align with international studies conducted in various regions of the Mediterranean. This highlights the need for more extensive sampling and research across diverse areas to gain a more comprehensive understanding of the nematode fauna in the region. Additionally, using nematodes as bioindicators can offer valuable information about environmental conditions and soil health. It is known that different nematode groups serve as indicators of soil quality and ecosystem health. Therefore, increasing research on the use of both plant-parasitic and free-living nematodes as bioindicators to understand the ecological balance in olive-growing areas would be beneficial for preserving soil health and promoting sustainable agricultural practices. Such studies can help olive growers develop environmentally friendly farming practices and enhance the role of nematodes in ecosystems.

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### Summary of Researchers' Contribution Rate Declaration

The authors declare that they have contributed equally to the article.

### Conflict of Interest Statement

The authors declare no conflict of interest.

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