Journal of Information Systems Engineering and Management
2025, 1 0 (62s)
e-ISSN: 2468-4376
https://jisem-journal.com/ Research Article

Occurrence and characterization of plant-parasitic nematodes in Algerian vineyards: The case of Médéa region

Nadia Boughelit[1], Djamel Smaha[2], Souhail Amini[3], Abderrahmane Sellami[3], Wahiba Aous[4], Karima Abdellaoui[4], Nassima Messaoud[3], Kahina Belmadani[3], Samia Sadaoui Yahia Cherif[5], Sonia Hamid[6], Fadila Neffah[3], El Mehdi Bouchtaoui[7], Marniche Faiza[8].

[1]Laboratory of Soft Technologies, Valorization, and Physico-Chemistry of Biological Materials, Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, Algeria.

[2]Department of Agricultural and Forest Zoology, Higher National School of Agronomy (ENSA), Algiers, Algeria

[3]Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, Algeria.
[4]Laboratory of Valorization and Conservation of Biological Resources, Department of Agronomic Sciences, University of M'hamed Bougara, Boumerdes, Algeria.

[5]Laboratory of Biological Sciences, Dynamics and Biodiversity, University of Science and Technology, Houari Boumediene, BP39 El Alia, Algiers, Algeria.

[6]Faculty of Natural and Life Sciences and Earth Sciences, University of Bouira, Algeria.

[7]Nematology Laboratory, Biotechnology Research Unit, National Institute of Agricultural Research, INRA-Rabat, Avenue Ennasr, BP 415 Rabat Principale, Rabat 10090, Morocco

[8]National Higher Veterinary School El Alia, Algiers, Algeria.

Corresponding author: E-mail: n.boughelit@univ-boumerdes.dz

ARTICLEINFO

Received: 12 Mar 2025
Revised: 07 Sept 2025
Accepted: 15 Sept 2025
Published: 30Nov2025

Abstract

In Algeria, surveys conducted in vineyards by Bounaceur in 2011 across different regions revealed the presence of several genera of nematodes, including Xiphinema, Longidorus, Helicotylenchus, Pratylenchus, Tylenchus, Tylenchorhynchus, Paratylenchus, Pratylenchoides, Ditylenchus, and Aphelenchus. The species Xiphinema index and Xiphinema pachtaicum were specifically reported in soil samples collected from vineyards in the Had S'hari region by Smaha in 2023.

The aim of the present study was to deepen the understanding of the distribution of phytoparasitic nematodes in Algerian vineyards, with a particular focus on the wine-growing region of Médéa. Two sampling campaigns were carried out: the first in December 2023, and the second during the period from March to April 2024. At each investigated soil depth (40 cm and 80 cm), a one-

Journal of Information Systems Engineering and Management
2025, 1 0 (62s)
e-ISSN: 2468-4376
https://jisem-journal.com/ Research Article

kilogram soil sample was collected at a distance of fifty centimeters from the vine trunk, using a shovel-type tool.

Nematological examination of the samples from the different sites allowed the identification of 21 nematode genera, with abundances varying significantly from one locality to another. The generic richness index, which measures taxonomic diversity, ranged from 13.61 to 18.61,

illustrating differences in species richness between stations. Data on average densities also highlighted inter-site variations, likely due to soil characteristics or the presence of weed flora.

The analyses revealed the presence of harmful genera such as Xiphinema and Longidorus, known for their role as vectors of major viral diseases, including fanleaf degeneration (Court-Noué), which negatively affects crop yield. These phytoparasitic nematodes represented the most dominant group within the biological communities associated with vineyards, outnumbering bacteriophagous nematodes and predators.

These findings highlight the importance of continuing investigations on nematodes and other plant pathogens in order to identify disease-tolerant grapevine varieties, improve production quality, and reduce economic losses.

Keywords: Vineyards, *Xiphinema* spp., *Longidorus* spp., distribution, Algeria.

1 Introduction

The vine, like the olive tree, is a symbol of the Mediterranean landscape and civilization. Having spread throughout the world, it became established wherever it found suitable physical and human conditions for its development. As in all ancient Mediterranean civilizations, the current distribution and essential characteristics of Algeria's vineyards are the result of a long history (Belhout, 1990). As with most plants, the majority of diseases affecting grapevines result from the interaction between a susceptible host and a living pathogenic organism. These causal agents, known as biotic pathogens, are highly diverse in nature and include primarily viruses, bacteria, insects, and fungi (Blanc, 2012).

In grapevines, the nematodes identified so far attack only the roots. Indeed, nematodes are among the most harmful bio-aggressors of grapevine, and they have been the subject of numerous studies and publications. However, understanding the dynamics, distribution, and composition of nematode populations is essential to better grasp the role of phytoparasitic

nematodes in agricultural ecosystems. Among these harmful nematodes, *Xiphinema* spp. (the main vector of grapevine fanleaf degeneration), which is distributed across the main wine-growing regions of Algeria and causes considerable damage to vineyards, is particularly important and may be responsible for soil decline in some of the country's vineyards (Hoceini et al., 2020).

In Algeria, studies conducted in vineyards in different regions have revealed the presence of several nematode genera, including *Xiphinema*, *Longidorus*, *Helicotylenchus*, *Pratylenchus*, *Tylenchus*, *Tylenchorhynchus*, *Paratylenchus*, *Pratylenchoides*, *Heterodera*, *Ditylenchus*, *Aphelenchoides*, and *Aphelenchus* (Bounaceur et al., 2011). The species *Xiphinema index* and *Xiphinema pachtaicum* were specifically reported in samples collected in the Had S'hari region of Djelfa (Smaha et al., 2023).

The objective of this study is to complement the existing data on the distribution of grapevine phytoparasitic nematodes in Algeria by conducting a nematological survey in the Médéa region.

2. Materials and Methods

2.1. Studied Wine-Growing Regions

This study was carried out in an area characterized by a Mediterranean climate, within the sub-humid bioclimatic zone, specifically in the district of Ouzra. This locality stands out as one of the largest agricultural communes of Médéa. Three stations were selected for the study: Benchicao, Bayasse, and Draa Salah (Fig. 1).

A 


B

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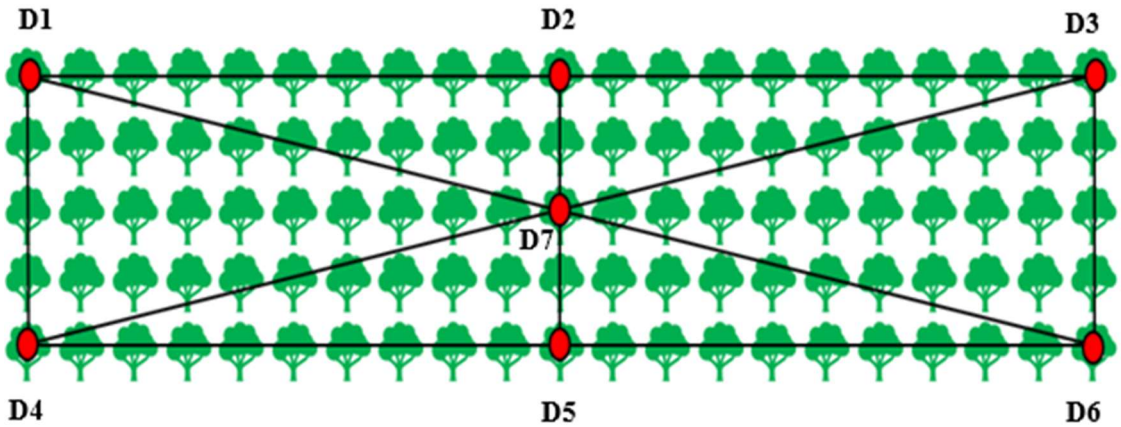
C

Fig. 1. Study stations: A: Benchicao., B: Bayasse., C: Draa Salah

2.2. Sampling Methods

For each site, soil sampling was carried out using a combination of two different approaches—diagonal and zigzag sampling—in order to improve the representativeness of the collected samples. This combined method is the one most commonly used in nematological studies (Hooper, 1993).

D



Z

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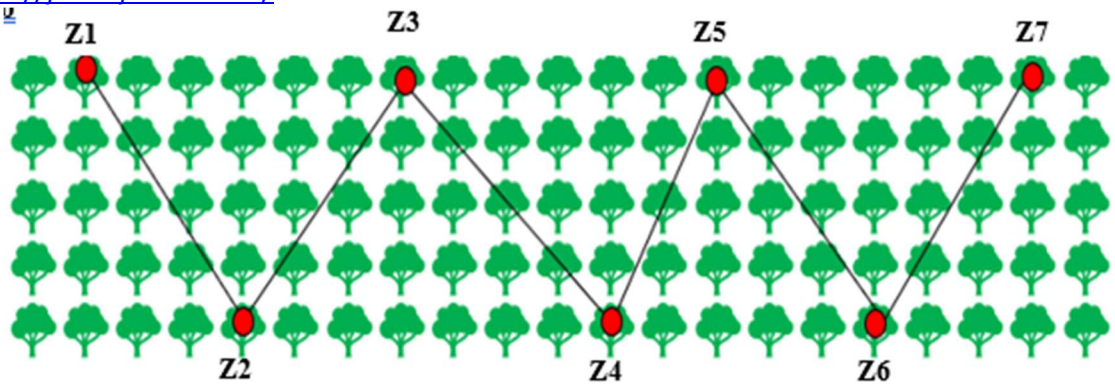

Fig.2 Experimental setups of the study stations.
D : Diagonale - Z : Zigzag.

a. Diagonal Method

In this method, the red sampling points are selected along the diagonal lines that connect the corners and edges of the rectangular site. As illustrated in Figure 2 (D), six red points (D1, D2, D3, D4, D5, D6) were identified along these diagonals. In addition, a central point (D7) is included, where the diagonals may intersect or represent the center of the site, and soil samples are collected separately from each designated red point (D1 to D7). The objective is to provide balanced coverage of the entire site by sampling areas located along the diagonal lines and the central zone.

b. Zigzag Method

In this second method, soil samples are collected along a zigzag or winding path that covers the entire surface area of the site. As illustrated in Figure 2 (Z), seven red points (Z1, Z2, Z3, Z4, Z5, Z6, Z7) were identified along this zigzag trajectory. The sampling route begins on one side of the site and meanders regularly across the area until most or all of the site is covered. Soil samples are collected separately from each designated red point (Z1 to Z7) along the zigzag path. The objective is to provide more comprehensive site coverage, especially when significant variability in soil properties occurs over short distances, thereby reducing potential bias that could arise if samples were taken only along straight lines.

The first sampling campaign was conducted in December 2023 (winter), and the second in spring (March–April 2024), in order to allow for seasonal comparison (Hoceini et al., 2020). Samples were taken at two depths (40 cm and 80 cm) with the aim of analyzing the vertical distribution of nematodes (Barker, 1985). For each grapevine plant, one kilogram of soil was collected approximately 50 cm from the trunk using a hand hoe. The samples were then stored in labeled, sealed plastic bags to prevent desiccation.

2.3. Sampling Record

Table 1 details the soil sample collection process at the studied sites. For each method and at each depth in the three sites, 7 samples were collected, bringing the total number of samples per site and per depth to 14 (7 samples using the diagonal method + 7 samples using the zigzag method). Because the sampling procedure was repeated for two different seasons at each site (Table 1), the total number of samples per season amounts to 56 samples per site**.**

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Tableau.1: Sampling data collected at the studied stations**.**

Station	Depth	Diagonale	Zigzag	Winter	Spring	Total
Draa Salah	40 cm	7 samples	7 samples	28 samples	28 samples	56 samples
	80 cm	7 samples	7 samples			
Bayasse	40 cm	7 samples	7 samples	28 samples	28 samples	56 samples
	80 cm	7 samples	7 samples			
Benchicao	40 cm	7 samples	7 samples	28 samples	28 samples	56 samples
	80 cm	7 samples	7 samples			

2.4. Extraction of Nematodes from Soil

The extraction of phytoparasitic nematodes from grapevine soil is carried out in the laboratory using the Baermann technique (Dalmasso, 1966) (Fig. 3). This method is based on the following steps:

- Each soil sample is mixed in a bucket (A) with 3 liters of water and stirred vigorously (the nematodes remain suspended while heavier soil particles settle to the bottom).
- The supernatant from bucket A is poured into bucket B through a sieve that retains large particles while allowing nematodes to pass through (heavy particles remain at the bottom of the bucket).
- The water from bucket B is then poured over a **40 µm sieve** (most nematodes are retained on this sieve).

- The residue retained on the sieve is collected and placed on filter paper inside a Petri dish filled with water. After 24 hours, the mobile nematodes migrate into the water, which is then transferred into a beaker. The solution is then ready for examination under a binocular stereomicroscope.



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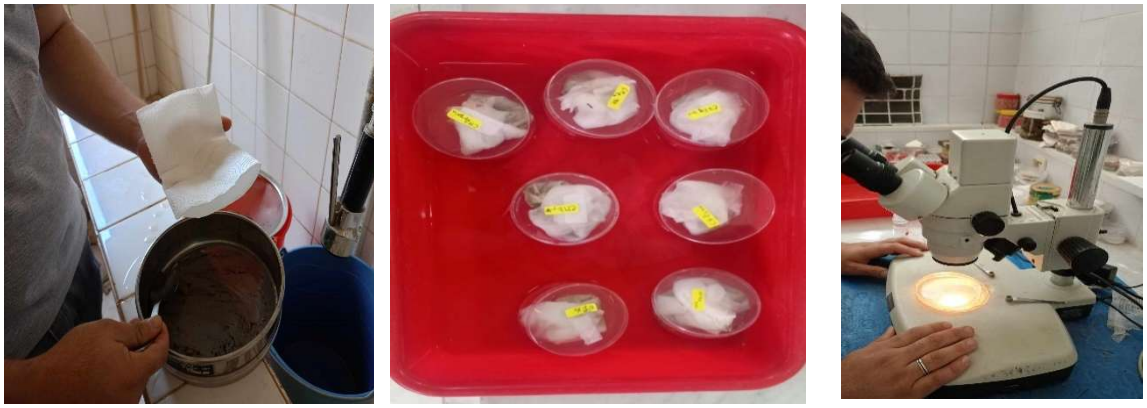
A B C



D E F

Fig.3 : Extraction of filiform nematodes.

A : Soil sample in the bucket ; B : Passing through a sieve into another bucket;
C : Filtering on a 40µm sieve ; D : Recovery of the residue ;
E : Leaching for 24 hours ; F : Observation under a binocular magnifying glass.

2.5. Counting and Identification of Nematodes

The extracted nematodes can be observed and counted using a binocular stereomicroscope with transmitted light. Nematodes that cannot be identified directly in the counting dish must be manually picked and mounted on a glass slide for identification at higher magnification under the microscope. Morphological identification is based on the observation of certain discriminating characters (the length and shape of the stylet, the shape of the head and tail, body length, and the position of the esophageal gland relative to the intestine), using the identification keys of Yeates et al. (1993).

Soil nematode populations are expressed as the number of nematodes per cubic decimeter (N/dm^3) (Merny & Luc, 1969).

3 Results

3.1 Nematode Inventory

The results of the nematological analysis revealed a total richness of 19 nematode genera, whose densities vary depending on the study sites. They are categorized according to their feeding habits into three trophic groups:

- Bacterivorous nematodes ;
- Predatory nematodes ;
- Phytophagous nematodes, which are:

Aphelenchus sp., Cephalenchus sp. (Fig.5), *Xiphinema sp., Ditylenchus sp., Dolichodorus sp., Helicotylenchus sp., Hemicriconemoides sp. (*Fig.5)., *Hemicycliophora sp., Heterodera sp., Hoplolaimus sp., Longidorus sp., Meloidogyne sp., Paratrichodorus sp., Paratylenchus sp., Rotylenchulus sp., Trichodorus sp., Tylenchulus sp., Tylenchorhynchus sp., and Criconemoides sp.*

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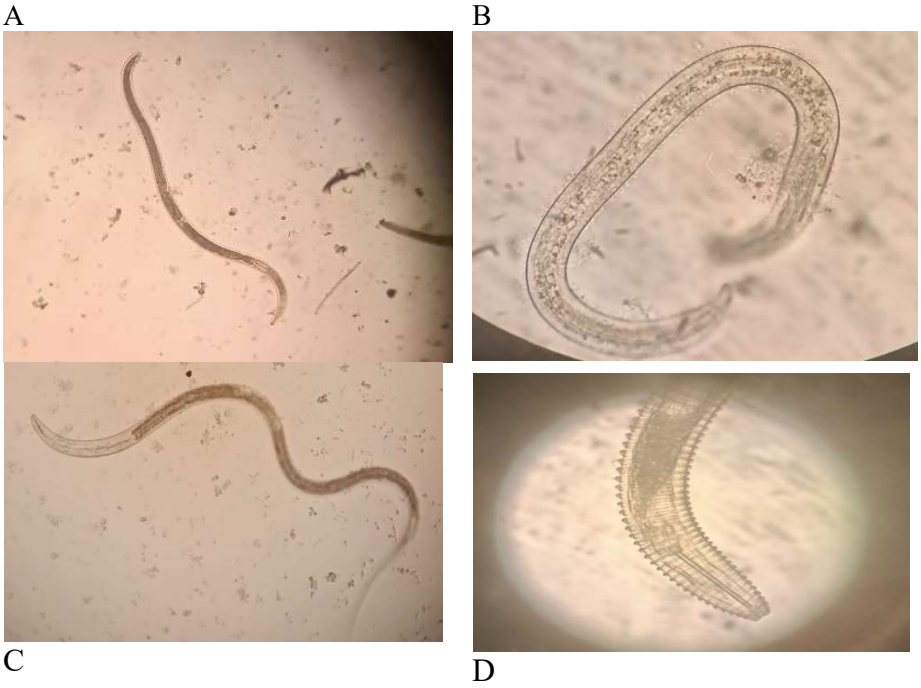

Fig. 5. Nematodes counted in the viticultural stations.

A : *Xiphinema spp.* B : *Hemicriconemoides spp.* C : *Cephalenchus spp.* D : *Criconemoides spp.*

3.2 Variations in mean abundances (N/dm^3) of nematodes

The analysis of the distribution of average soil nematode abundances revealed notable differences between the orchards. In particular, the Bayasse orchard stands out with a significantly higher nematode density compared to the other sites, making it the site with the greatest abundance. In contrast, the Draa Salah site exhibited the lowest densities, while Benchicao showed intermediate levels (Fig. 6).

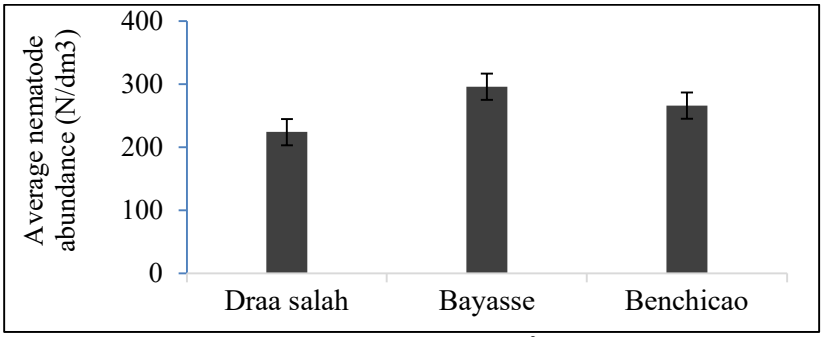

Fig. 6. Variation in mean abundances (N/dm^3) of nematodes

3.3 Seasonal variations in mean abundances (N/dm^3) of nematodes populations

The Médéa sites exhibit interesting seasonal variations in nematode abundance. At the Bayasse station, a notable increase in abundance was observed during the winter season compared to spring. In contrast, the Benchicao station showed relative stability in nematode

abundance between winter and spring, with very similar values. As for the Draa Salah station, the data indicate that nematode abundance was higher in spring than in winter (Fig. 7).

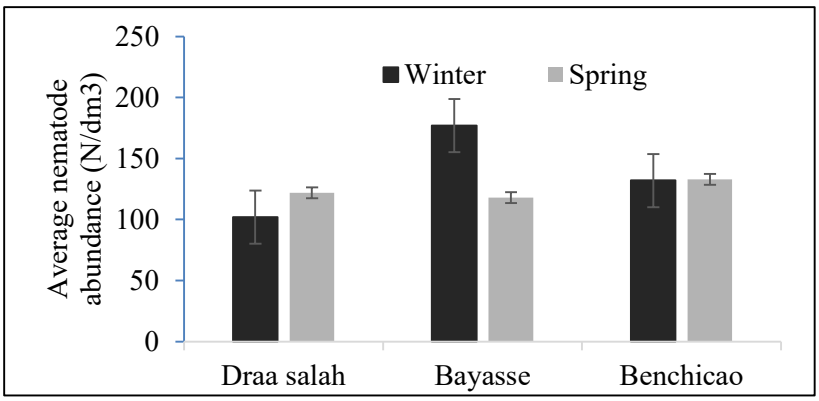

Fig. 7. Seasonal variations in mean abundances (N/dm^3) of nematodes populations

3.4 Variations in mean abundances (N/dm³) of nematodes populations as a function of soil depth

The results of the nematode analysis revealed a distinct variation in abundance according to soil depth. At the Draa Salah and Bayasse stations, nematode abundance at a depth of 40 cm was significantly higher in winter, with values decreasing considerably in spring. Conversely, at a greater depth of 80 cm, nematode abundance was higher in spring at both stations.

However, the Benchicao station exhibited a different pattern compared to Draa Salah and Bayasse. Although some variations in nematode abundance between winter and spring were observed at both studied depths, the general trend seen at the other two stations (higher abundance in winter at 40 cm and higher abundance in spring at 80 cm) was not observed at Benchicao or was less pronounced (Fig. 8).

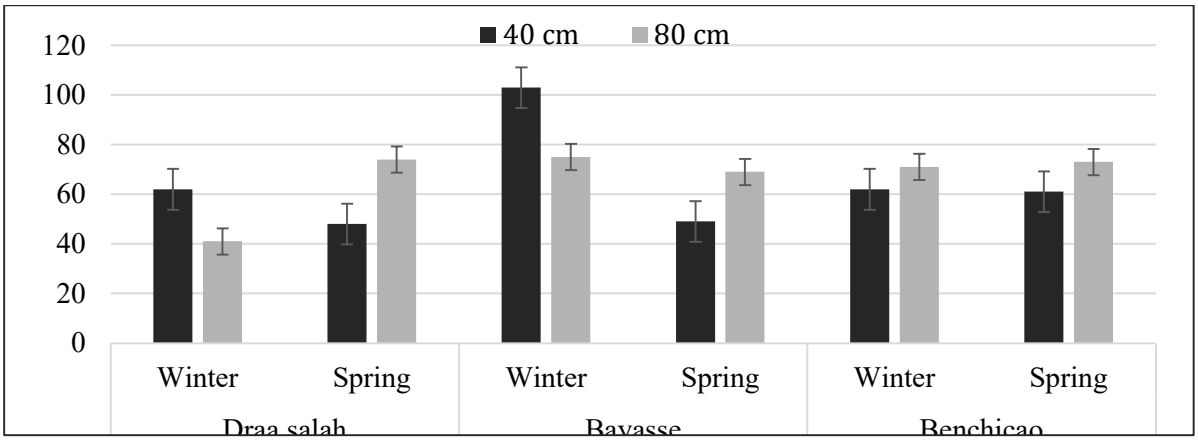

Fig. 8. Seasonal variations in mean abundances (N/dm^3) of nematodes populations

3.5 Ecological diagnosis of nematode communities in vineyard stations

3.5.1 Frequency (F%)

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A detailed analysis of species frequency across the different study stations highlighted significantly high concentrations of certain specific species at these sites. Indeed, the surveys indicated a notable presence of Longidorus spp., with a frequency reaching 21.84%, followed by Xiphinema spp. at 12.92%, and Paratylenchus spp., which accounted for 12.58% of occurrences at these same stations. These observations suggest substantial species richness and diversity within these geographic locations.

In contrast, other species exhibited a distinct distribution pattern. This was particularly the case for species belonging to the genera Dolichodorus spp. and Cephalenchus spp., whose occurrence was limited to a small number of stations or characterized by very low presence percentages.

3.5.2 Absolute Abundance

Regarding the absolute abundance of the recorded nematodes, the data reveal significant disparities among the different study stations. The Bayasse station stands out with the highest abundance, reaching 415. In comparison, the Benchicao station shows an intermediate value of 373, while the Draa Salah station is characterized by a relatively low abundance of only 314 compared to the other investigated sites.

3.5.3 Generic Richness (G)

To evaluate the diversity of taxa present within each study station, we used the generic richness index (G) which allows revealing a notable variability across the different analyzed sites. The values obtained for this index range between 13.61 and 18.61, depending on the stations.

Table 2. Spatial variation of ecological indices in the study stations

Station	Absolute abundance	Generic Richness (G)
Benchicao	373	13.61
Bayasse	415	18.61
Draa Salah	314	14.61

° Absolute abundance: individuals found at the study sites. This is used to estimate the total number of nematodes at each study station. This indicates the severity of the infestation.

° Generic richness (G), which measures the genetic diversity of nematodes:

$$G = (S - 1) / \log N$$

S: the number of genera.
N: the total number of individuals identified.

3.5.4. Total Richness (S)

These results reveal a notable variability in the total richness of nematodes depending on the study stations analyzed. The station of Bayasse is clearly distinguished by the highest wealth with a total of 19 taxa identified.

In comparison, the station of Draa Salah has a total richness of 15 taxa, which represents a decrease of 4 taxa compared to Bayasse. The Benchicao station has a wealth of 14 taxa, a difference of 5 taxa less than the richest station and one taxon less than Draa Salah (Fig.9).

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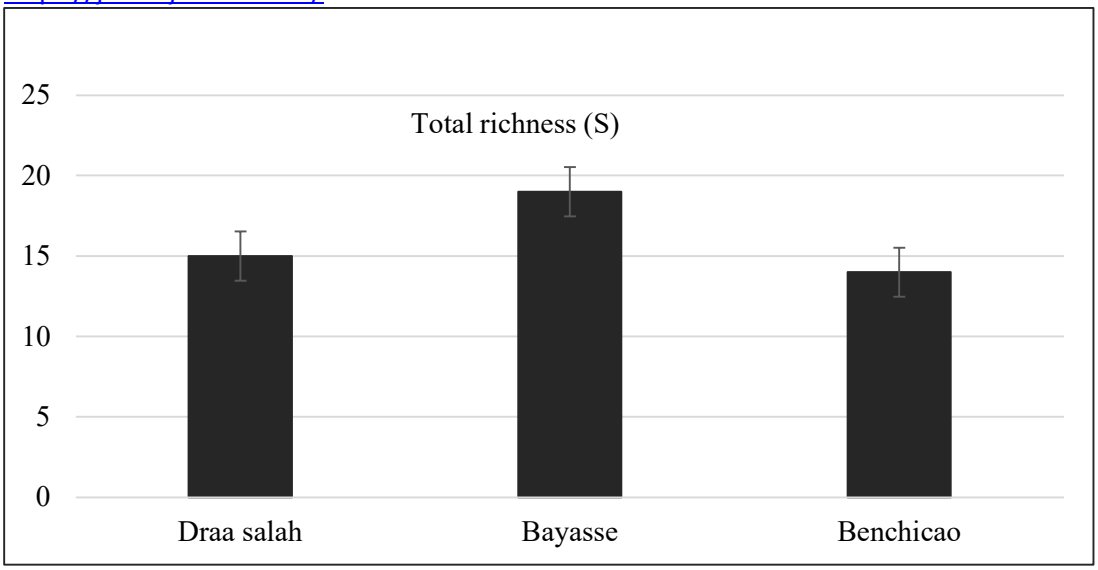

Fig. 9. Spatial variation of total richness in the study stations

4 Discussion

Our results on the inventory of nematodes present in the Médéa vineyards are consistent with those obtained in various countries, both in terms of the taxa encountered and the methodological approach. Indeed, the work of Hoceini *et al.* (2020) in Algeria revealed the presence of *Xiphinema* spp. mainly in the Médéa plots. The same is true of the research of Bounaceur *et al.* (2011), who identified *Tylenchus* spp. and *Aphelenchus* spp. in northern Algeria. In Morocco, investigations conducted by Mokrini (2019) highlighted *Paratylenchus* spp. and *Helicotylenchus* spp., while in Spain, the work of Weiland (2001) identified *Longidorus* spp.

Ecological indices show variation in nematological diversity between sites. The generic richness index, which quantifies taxon diversity, has values that vary between 13.61 and 18.61, reflecting differences in taxon richness from one station to another, and the mean density results show variations from one station to another, probably due to differences in the soil or the amount of weeds present. The presence of weeds seems to be associated with an increased number of roots in the soil, which could explain why nematode density is higher in these areas. This observation was confirmed by the work of Villenave *et al.*, (2001) who showed that simply removing weeds from the soil reduces the number of roots available for plant-parasitic nematodes.

The frequency index on the distribution of nematode species in the studied sites reveals that *Xiphinema* spp. and *Longidorus* spp. are among the most frequently observed in these vineyards. This observation is confirmed by Arias and Navacerrada (1973) who identified the genus *Xiphinema* spp. in 70% of the samples collected in Spanish vineyards, as well as *Longidorus* spp., which can also transmit grapevine viruses and are present in Spain in the samples collected. Our results show that the average densities in the sites were almost similar in spring as in winter, with few differences. This is consistent with the results of Hosseini *et al.* (2020), indicating that the soil moisture level during these periods likely plays a role. This idea is also supported by Sarah (1995) who showed that a long dry season reduces populations, while they increase sharply after the rains.

The results also show that the density of nematodes varies according to the depth of the soil. We observed a much higher abundance at 40 cm than at 80 cm. Previous work (Esmenjaud et al., 1992) has already reported a maximum concentration between 40 and 70 cm, linked to better stability in humidity, temperature, and food availability. Other authors (McSorley, 2003 and

Neher, 2010) confirm that the deep layers maintain an ecological microstability favorable to nematode survival, unlike the superficial layers more exposed to disturbances. Similarly, Howland et al., (2014) showed in the vineyards of Vitis vinifera a net decrease in the density of the species studied with the increase in depth, confirming that more than 80 cm is much less favorable than more than 40 cm. Finally, the pioneering study by Brodie (1976), having examined the profile up to 105 cm, highlighted that if certain species could persist at depth, their maximum density remained between 45 and 75 cm, falling sharply to-beyond 80 cm. All these results support the idea that the depth of about 40 cm up to 60 cm constitutes an optimal horizon for the survival and activity of plant parasitic nematodes.

5 Conclusion

Our study, conducted on three sites within the wilaya of Médéa in Algeria, has demonstrated the existence of significant biological diversity among phytoparasitic nematodes associated with the viticultural agricultural system of the region. The analyses identified the prevalence of nematode species considered as major pests, such as Xiphinema and Longidorus, which play a crucial role as vectors of viral diseases that significantly threaten the health and productivity of vineyards.

In this ecosystem, plant-parasitic nematodes were found to be the dominant group of microorganisms associated with roots, followed in terms of abundance by bacteriophages and nematode predator organisms. The study also revealed that the spatial and temporal distribution of these groups is clearly influenced by various environmental factors, including geographical location, seasonal variations, and soil depth.

These results encourage a deeper understanding of the complex ecological interactions taking place in the rhizosphere of the vine, with particular emphasis on the central role of phytoparasitic nematodes and their impact on the sustainability of viticulture. They also highlight the need for further scientific research to explore disease resistance mechanisms in local grape varieties and develop innovative agricultural strategies aimed at promoting plant health and reducing reliance on interventions expensive and unsustainable chemicals.

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