

## Diversity and Structure of Plant-Parasitic Nematode Communities in Some Olive Nurseries Along the Coastal Region of Syria

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### Abstract

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Olive is one of the most important economic crops in Syria. Nurseries are often the main source for pathogens dissemination in olive orchards, especially soil-borne organisms such as *Verticillium* and plant-parasitic nematodes (PPN), and investigating their occurrence and distribution in olive nurseries seems of great importance. No scientific data on the distribution of PPN in olive nurseries along the Syrian coastal region is available. Therefore, the present study was conducted in order to: (i) explore for the first time the occurrence and diversity of PPN communities distributed in some olive nurseries along the Syrian coastal region, and (ii) compare the nematode diversity and their community structure between two olive varieties. One hundred eight soil samples were collected from different nurseries in Latakia and Tartous governorates, from two common olive varieties Khdiry and Qaissy. Taxonomical and functional indices were calculated and compared between olive varieties and soil mixture used. The community structure was defined by using principal component analysis (PCA). The results revealed the wide distribution of PPN in olive nurseries with a total of 17 genera identified. *Aphelenchoides*, *Aphelenchus*, *Ditylenchus* and *Tylenchorhynchus* were the most common. Impact of olive varieties or soil mixture on functional diversity was observed, but not on the taxonomical indices. PCA also revealed a distinct structure of communities in each of the two olive varieties as well as in the soil mixture. Economically important genera such as *Meloidogyne* and *Pratylenchus* were also recorded with high population densities in some nurseries, suggesting the inevitable introduction of such nematodes to olive orchards. Certification programs for plant propagation materials in nurseries seem extremely important to be adopted in Syrian nurseries to ensure the production and distribution of "healthy" seedlings to growers.

**Keywords:** Diversity, nurseries, olive varieties; plant-parasitic nematodes, soil mixture, Syria.

### Introduction

Olive tree *Olea europaea* L. subsp. *europaea* (Oleaceae) has always been considered as the typical tree and one of the best biological indicators of the Mediterranean region, from where, its cultivation was widely expanded through the exchanges that accompanied the expansion of the Mediterranean civilizations (Blondel *et al.*, 2010; Zohary & Hopf, 2000). According to archaeological and molecular studies, Syria is considered among the main location for olive domestication (Besnard *et al.*, 2013). Olive crop in Syria is considered a strategic crop; it comes in the third place after cereals and cotton and first among fruit trees (Abdine *et al.*, 2007). In addition, it is a source of livelihood for more than 25% of the Syrian population, for food and a source of inputs for industry (e.g. soap and cosmetics) (Mansour, 2007). Cultivated olive areas are mainly located in the northern, western and central regions of the country: Aleppo, Idleb, Latakia, Tartous, Hama and Homs. Syria was ranked 10<sup>th</sup> worldwide in olive production estimated at 358,666 metric tons in 2019 (NationMaster, 2022).

Olive trees are host to a large number of plant-parasitic nematodes (PPN). A high diversity of PPNs with 153 species and 56 genera has been reported on olive trees worldwide (Ali *et al.*, 2014; Castillo *et al.*, 2010). More recently, 60 other species were added for the first time in association with olive (Ali *et al.*, 2017). The most common and important genera were: *Criconemoides*, *Helicotylenchus*, *Longidorus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Rotylenchus*, *Tylenchorhynchus*, *Tylenchulus*, *Tylenchus* and *Xiphinema*.

Some of them, such as the root-knot nematodes (*Meloidogyne* spp.) reduce tree growth (Lamberti & Baines, 1969; Sasanelli *et al.*, 2002) and may be responsible for 5 to 10% crop losses (Koenning *et al.*, 1999).

However, the impact of PPN is more important in nurseries due to the availability of suitable conditions, especially irrigation, which improves roots development and consequently, the reproduction and multiplication of nematode populations (Castillo *et al.*, 2010). In addition, seedling roots are usually more sensitive to nematode infection compared to trees (Nico *et al.*, 2003). In Morocco, 63 species belonging to 26 genera were identified in olive nurseries (Ait Hamza *et al.*, 2018). Two species of root-knot nematodes *M. javanica* Treub, and *M. incognita* Kofoid & White were identified in association with olive seedlings in Iraq (Hanoon *et al.*, 2018). *M. arenaria* Neal was also detected in addition to *M. javanica* and *M. incognita* in olive nurseries in Morocco (Ait Hamza *et al.*, 2017), and eight nematode species in Iran (Sanei & Okhovvat, 2011). Sultan (2007) stated that *Tylenchorhynchus* was the most frequent nematode in olive nurseries in the West-Bank. In southern Spain, Nico *et al.* (2003) reported 34 PPN species.

Nurseries are often the main source for pathogens dissemination in olive orchards, especially soil-borne organisms including the plant-parasitic nematodes (PPN), consequently, investigating their occurrence and distribution in olive nurseries seems of great importance. Because of the economic importance of olive cultivation and the lack of information on the plant-parasitic nematodes (PPN) associated with olive nurseries in Syria, the present study

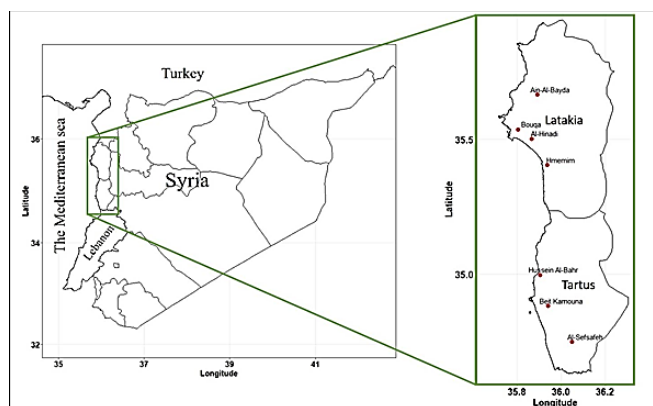
was carried out in order to: (i) explore, for the first time, the occurrence and diversity of PPN communities in some olive nurseries along the Syrian coastal region, (ii) compare the nematode diversity and their community structure between two olive varieties and the used soil mixture.

## Materials and Methods

### Study sites and olive nurseries surveyed

The study focused on the extraction, identification and enumeration of the plant-parasitic nematode community associated with two olive varieties (Khdiry and Qaissy) as well as with the soil mixture used from some olive plantation nurseries along the Syrian coastal region. These two cultivars were chosen because they are among the most common olive varieties in Syria (Jbara *et al.*, 2010). Khdiry is one of the most desirable and widespread varieties in the Syrian coastal region due to its importance as a source of oil and table olives. Qaissy is also very desirable as a source of table olive and because of its resistance to the peacock eye disease *Spilocoaea oleagina* (Castagne) Hughes (Abdine *et al.*, 2007; Al-Chaabi *et al.*, 2012; Jbara *et al.*, 2010).

Olive plants were grown in 2–3 liters plastic bags filled with solid soil mixtures containing either sandy alluvial riverbank soil, organic forest soil, or loamy soil. Eight commercial nurseries from four sites in Latakia (Ain Al-Bayda, Boukka, Al-Hinadi, Hmeimim), and four nurseries from three sites in Tartous (Beit Kamouni, Al-Sefsafah, Hussein Al-Bahr) were surveyed (Figure 1).



**Figure 1.** Sampling sites of olive nurseries along the Syrian coast.

### Sampling

Sampling took place during 2019-2020. Considering that PPNs spend all or a part of their life in the soil (Cadet & Thioulouse, 1998), the nematode sampling was based on soil sampling. A total of 108 soil samples were collected using a small shovel. From each nursery and for each variety of olive, three soil samples were taken from three olive plantlets (selected randomly). Three samples were also taken from the used soil mixture. Each soil sample (about 300 cm<sup>3</sup>) was labelled (site and the surveyed variety). Agricultural practices, such as irrigation, fertilization, nematode treatments (if applicable) were also recorded. Soil samples were then transferred to the Plant Protection Research Laboratory in the Faculty of Agricultural engineering at

Tishreen University for nematode extraction and identification.

### Nematode extraction, identification, and quantification

A 250 cm<sup>3</sup> wet soil was taken from each sample for nematode extraction using the modified Bermann funnel method (Walker & Wilson, 1960). PPN belonging to the Aphelenchida, Dorylaimida, Triplonchida and Tylenchida orders were counted in 5 cm<sup>3</sup> counting chambers (Merny & Luc, 1969) under a microscope (OPTIKA B-195, Italy) at  $\geq 40\times$  magnification. Mobile stages of nematodes (juveniles, males, and females) were identified to the genus level based on dichotomous keys (Mai & Mullin, 1996). The population density of each genus was expressed as the number of individuals per 250 cm<sup>3</sup> of fresh soil.

The number of samples in which the genus was detected (frequency), the prevalence (%), the mean, minimum and maximum of population density for each genus/site, as well as the occurrence of the genus on olive varieties and/or in soil mixture were also recorded.

### Analyses of nematode diversity

Several biodiversity indices were used:

**Taxonomical indices** - the total number of PPN in a community ( $N$ ); the genus richness ( $G$ ) (number of genera in a community); the Shannon-Wiener diversity index

$$H' = -\sum p_i \ln p_i$$

where  $p_i$  is the proportion of individuals in each genus, which quantifies the local diversity or the heterogeneity of diversity ( $H'$  ranges from 0 to  $\ln G$ ); and the evenness

$$E = H' / \ln G$$

that quantifies the regularity of species distribution within the community ( $E$  varies between 0 and 1).

**Functional indices** - PPN genera were distributed into life-strategy groups according to their colonizer/persister value ( $cp$ -value) of the family to which they belong as described by Bongers (1990). The feeding type for each genus as herbivore or fungivore was also recorded. Functional diversity was described by calculating the plant-parasitic index PPI which quantifies the plant-feeding diversity of the communities as follows:

$$PPI = \sum c_{pi} n_i / N$$

where  $c_{pi}$  is the  $cp$  value of the genus ( $i$ ) and  $n_i$  is the number of individuals of ( $i$ ).

The relative mean abundance (%) of each  $cp$ -value group in a community was calculated as follows:

$$R_{cpi} = c_{pi} n_i / N$$

The dominance of each PPN genus was estimated by the modelling of its abundance ( $A$ ), the total population density of the genus in the whole samples (expressed in log) and its frequency  $F$  (% of number of samples containing the genus), based on the model developed by Fortuner & Merny (1973). According to the model, thresholds were set. A genus was considered as not abundant when  $A < 2.30$  (that means average population was  $< 200$  individuals/cm<sup>3</sup> (Log 200 = 2.30), and as abundant when  $A \geq 2.30$ . On the other hand, the

genus was considered frequent if detected in at least 30% of the samples ( $F \geq 30\%$ ), and occasional or less frequent when  $F < 30\%$ .

### Data analyses

R version 3.3.2 was used for statistical analyses (R Core Team, 2016). Data were log-transformed before analysis to improve normality. The diversity indices were calculated using the Vegan Library (Oksanen *et al.*, 2016). Mean values were analysed by one-way ANOVA. Tukey test was then used for pair-wise multiple comparisons to determine significance based on ANOVA results at  $P$ -value = 0.05. Multivariate statistical analyses (principal component analysis (PCA)) were used to describe the PPN community structure between the two olive varieties and used soil mixture. The multivariate analyses and graphs were performed using the *ade4* library (Chessel *et al.*, 2004; Dray & Dufour, 2007).

## Results

### Description of PPN communities distributed in olive nurseries along the Syrian coast:

A total of 17 genera of plant-parasitic nematodes were identified associated with olive nurseries along the Syrian coast, and they are members of two families of Aphelenchida, twelve families of Tylenchida and one family of Triplonchida (Table 1).

Nematode genera identified in each nursery site are listed in Table 2, and they are distributed as follows: in Latakia governorate nurseries, 14 genera were detected in Ain Al-Bayda and in Al-Hinadi, 11 genera in Boukka and 7 genera in Hmeimim; in Tartus governorate nurseries, 15 genera were detected in Al-Safsafah and 12 genera in each of Beit Kamouni and Hussein Al-Bahr. The genera

*Aphelenchoides*, *Aphelenchus*, *Ditylenchus*, *Helicotylenchus*, *Tylenchorhynchus* and *Tylenchus* were found widely distributed, as they were detected in all nurseries. In contrast, other genera such as *Tylenchulus* seem to be limited in distribution, as it was only recorded in two samples in Al-Hinadi. Some economically important genera such as the root-knot nematodes (RKN) *Meloidogyne* spp. and the root-lesion nematodes (RLN) *Pratylenchus* spp. were also reported in all sites except in Boukka (absence of RKN) and in Hmeimim (absence of RLN), with high population density levels (e.g. in Al-Hinadi). Other important genera such as the cyst nematodes *Heterodera* spp. (in Ain Al-Bayda, Boukka, Al-Hinadi and in Hussein Al-Bahr) and the reniform nematodes *Rotylenchulus* spp. (in Ain Al-Bayda, Al-Hinadi, Al-Safsafah and in Hussein Al-Bahr) were also recorded.

### Diversity of PPN communities in nurseries according to olive varieties and soil mixture

The diversity of PPN communities was described using some taxonomical and functional indices. Mean value of each index for the two olive varieties (Khdiry and Qaissy) was compared, in addition to the used soil mixture (Table 3). The taxonomical indices ( $N$ ,  $G$ ,  $H'$ ,  $E$ ) were homogeneous across these three modalities because no significant difference was observed ( $P$ -value=0.05). The highest number of nematodes ( $N$ ) was recorded for the Khdiry variety (426 individuals/250 cm<sup>3</sup> of soil), and the lowest was in the soil mixture (233 individuals/250 cm<sup>3</sup> of soil), contrary to what was observed for the richness index ( $G$ ), where more genera were recorded in the soil mixture and less for the Khdiry variety. The  $H'$  and  $E$  indices were higher in the used soil mixture and lower for the Qaissy variety.

**Table 1.** Order, family, common name,  $cp$ -value and feeding type of plant-parasitic nematode genera identified in olive nurseries along the Syrian coast.

Nematode Order	Nematode family	Nematode genus	Nematode common name	$Cp$ -value	Feeding type
Aphelenchida	Aphelenchidae	<i>Aphelenchus</i> spp.	Fungal Nematode	2	Fungivore
	Aphelenchoididae	<i>Aphelenchoides</i> spp.	Foliar Nematode	2	Fungivore
Tylenchida	Anguinidae	<i>Ditylenchus</i> spp.	Stem and Bulb Nematode	2	Fungivore
	Dolichodoridae	<i>Brachydorus</i> spp.	Awl Nematode	3	Herbivore
	Heterodoridae	<i>Heterodera</i> spp.	Cyst Nematode	3	Herbivore
	Hopolaimidae	<i>Helicotylenchus</i> spp.	Spiral Nematode	3	Herbivore
		<i>Rotylenchus</i> spp.		3	Herbivore
	Meloidogynidae	<i>Meloidogyne</i> spp.	Root-knot Nematode	3	Herbivore
	Paratylenchidae	<i>Paratylenchus</i> spp.	Pin Nematode	2	Herbivore
	Pratylenchidae	<i>Pratylenchus</i> spp.	Root-lesion Nematode	3	Herbivore
	Psilenchidae	<i>Psilenchus</i> spp.	-	2	Herbivore
	Rotylenchulidae	<i>Rotylenchulus</i> spp.	Reniform Nematode	3	Herbivore
	Telotylenchidae	<i>Tylenchorhynchus</i> spp.	Stunt Nematode	3	Herbivore
Tylenchidae	<i>Boleodorus</i> spp.	Tylenchids	2	Herbivore	
	<i>Tylenchus</i> spp.		2	Herbivore	
	Tylenchulidae	<i>Tylenchulus</i> spp.	Citrus Nematode	3	Herbivore
Triplonchida	Trichodoridae	<i>Trichodorus</i> spp.	Stubby-root Nematode	4	Herbivore

Regarding the functional indices, the PPN genera identified in the study were allocated into *cp-2*, *cp-3* and *cp-4* groups (Table 1). The comparison between soil mixture and olive varieties revealed significant differences for functional indices (*PPI*, *cp-2*, *cp-3*) (Table 3). The Khdiry and Qaissy varieties revealed nematode communities with significantly higher plant-parasitic indices (*PPI*) than those

in soil mixture. *Cp-2* nematodes were significantly dominant in the soil mixture, whereas *cp-3* seemed to be more frequent and abundant on olive varieties, especially on the Khdiry compared to the Qaissy. A very low abundance of *cp-4* was recorded in the used soil mixture and on the Qaissy but not on the Khdiry variety.

**Table 2.** Prevalence, and population density (expressed as mean, minimum and maximum number of individuals in 250 ml of soil) of plant-parasitic nematodes identified in olive nurseries along the Syrian coast.

Nematode's genus identified (No. of samples/site)	No. of samples were positive	Prevalence (%)	Mean	Min	Max	Occurrence in nurseries		
						Khdiry	Qaissy	Soil Mix
<b>Latakia/Ain-Al-Bayda (9)</b>								
<i>Aphelenchus</i>	5	55.56	24.4	4.0	48	+	+	+
<i>Aphelenchoides</i>	1	11.11	16.0	16	16			+
<i>Boleodorus</i>	1	11.11	36.0	36	36			+
<i>Ditylenchus</i>	5	55.56	17.6	4.0	36	+	+	+
<i>Helicotylenchus</i>	2	22.22	14.0	8.0	20	+		+
<i>Heterodera</i>	1	11.11	12.0	12	12	+		
<i>Meloidogyne</i>	3	33.33	136	8.0	216	+		+
<i>Paratylenchus</i>	1	11.11	28.0	28	28		+	
<i>Pratylenchus</i>	2	22.22	25.0	4.0	46		+	
<i>Psilenchus</i>	2	22.22	15.0	4.0	26			+
<i>Rotylenchulus</i>	2	22.22	88.0	64	112	+		
<i>Rotylenchus</i>	1	11.11	16.0	16	16	+		
<i>Tylenchorhynchus</i>	3	33.33	16.0	8.0	24	+	+	+
<i>Tylenchus</i>	2	22.22	44.0	12	76			+
<b>Latakia/Boukka (18)</b>								
<i>Aphelenchus</i>	16	88.89	138.1	4	704	+	+	+
<i>Aphelenchoides</i>	10	55.56	26.2	8	96	+	+	+
<i>Boleodorus</i>	5	27.78	29.2	4	104	+	+	+
<i>Brachydorus</i>	4	22.22	122	56	248			+
<i>Ditylenchus</i>	8	44.44	27.8	4.0	96		+	+
<i>Helicotylenchus</i>	7	38.89	14.6	4.0	48	+		+
<i>Heterodera</i>	5	27.78	10	4.0	16	+		+
<i>Paratylenchus</i>	8	44.44	14	4.0	56		+	+
<i>Pratylenchus</i>	10	55.56	26	4.0	80	+	+	+
<i>Tylenchorhynchus</i>	10	55.56	24	4.0	136	+	+	+
<i>Tylenchus</i>	10	55.56	16.4	4.0	68	+	+	+
<b>Latakia/Al-Hinadi (36)</b>								
<i>Aphelenchus</i>	32	88.89	127.8	4.0	408	+	+	+
<i>Aphelenchoides</i>	19	52.78	18.2	4.0	48.0		+	+
<i>Brachydorus</i>	7	19.44	6.3	4.0	8.0	+	+	+
<i>Ditylenchus</i>	19	52.78	17.5	4.0	64.0			+
<i>Helicotylenchus</i>	4	11.11	1002	4.0	223.6		+	
<i>Heterodera</i>	4	11.11	7.0	4.0	8.0	+		
<i>Meloidogyne</i>	16	44.44	583.1	4.0	7440	+	+	
<i>Pratylenchus</i>	11	30.56	126.5	4.0	600	+	+	+
<i>Rotylenchulus</i>	2	5.56	158	140	176		+	
<i>Rotylenchus</i>	12	33.33	138.2	4.0	440	+	+	+
<i>Trichodorus</i>	2	5.56	6.0	4.0	8.0		+	
<i>Tylenchulus</i>	2	5.56	24.0	12	36.0			+
<i>Tylenchorhynchus</i>	23	63.89	52.4	4.0	352.0	+	+	+
<i>Tylenchus</i>	8	22.22	7.0	4.0	16.0		+	+

Nematode's genus identified (No. of samples/site)	No. of samples were positive	Prevalence (%)	Mean	Min	Max	Occurrence in nurseries		
						Khdiry	Qaissy	Soil Mix
<b>Latakia/Hmeimim (9)</b>								
<i>Aphelenchus</i>	3	33.33	45.3	4	124	+		+
<i>Aphelenchoides</i>	1	11.11	12.0	12	12			+
<i>Ditylenchus</i>	2	22.22	13.0	8.0	18		+	
<i>Helicotylenchus</i>	3	33.33	20.0	4.0	32	+	+	
<i>Meloidogyne</i>	2	22.22	34.0	12	56	+		
<i>Tylenchorhynchus</i>	1	11.11	16.0	16	16		+	
<i>Tylenchus</i>	2	22.22	30.0	18	42			+
<b>Tartus/Al-Sefsafeh (9)</b>								
<i>Aphelenchus</i>	4	44.44	18.0	4.0	26		+	+
<i>Aphelenchoides</i>	4	44.44	15.0	4.0	44		+	+
<i>Boleodorus</i>	1	11.11	4.0	4.0	4			+
<i>Brachydorus</i>	1	11.11	24.0	24	24			+
<i>Ditylenchus</i>	5	55.56	19.6	4.0	58	+	+	+
<i>Helicotylenchus</i>	4	44.44	13.5	4.0	26	+		+
<i>Meloidogyne</i>	3	33.33	60.0	4.0	164	+		+
<i>Paratylenchus</i>	4	44.44	25.5	4.0	78		+	+
<i>Pratylenchus</i>	2	22.22	80.0	44	116		+	
<i>Psilenchus</i>	2	22.22	15.0	4.0	26		+	+
<i>Rotylenchulus</i>	2	22.22	29.0	12	46	+		
<i>Rotylenchus</i>	2	22.22	4.0	4.0	4.0	+		+
<i>Trichodorus</i>	1	11.11	4.0	4.0	4.0			+
<i>Tylenchorhynchus</i>	1	11.11	8.0	8.0	8.0	+	+	+
<i>Tylenchus</i>	1	11.11	36	36	36			+
<b>Tartus/Beit Kamouni (9)</b>								
<i>Aphelenchus</i>	9	100	110.7	12	304	+	+	+
<i>Aphelenchoides</i>	7	77.78	71.4	4.0	200	+	+	+
<i>Brachydorus</i>	1	11.11	8.0	8.0	8.0	+		
<i>Ditylenchus</i>	6	66.67	24.0	4.0	56	+	+	+
<i>Helicotylenchus</i>	6	66.67	15.3	8.0	24	+	+	+
<i>Meloidogyne</i>	1	11.11	8.0	8.0	8.0			+
<i>Paratylenchus</i>	3	33.33	5.3	4.0	8.0	+		+
<i>Pratylenchus</i>	3	33.33	25.3	4.0	56			+
<i>Psilenchus</i>	2	22.22	40	8.0	72		+	
<i>Trichodorus</i>	1	11.11	4.0	4.0	4.0		+	
<i>Tylenchorhynchus</i>	7	77.78	92	4.0	472	+	+	
<i>Tylenchus</i>	5	55.56	19.2	4.0	68	+	+	+
<b>Tartus/Hussein Al-Bahr (18)</b>								
<i>Aphelenchus</i>	14	77.78	47.3	4.0	312	+	+	+
<i>Aphelenchoides</i>	12	66.67	22.3	4.0	64	+	+	+
<i>Brachydorus</i>	4	22.22	12.0	4.0	28	+		+
<i>Ditylenchus</i>	9	0.5	9.8	4.0	32	+	+	+
<i>Helicotylenchus</i>	3	16.67	6.7	4.0	8.0	+	+	
<i>Heterodera</i>	2	11.11	6	4.0	8.0			+
<i>Meloidogyne</i>	3	16.67	6.7	4.0	12	+		
<i>Paratylenchus</i>	2	11.11	4.0	4.0	4.0			+
<i>Pratylenchus</i>	5	27.78	9.6	4.0	20		+	+
<i>Rotylenchulus</i>	2	11.11	4.0	4.0	4.0	+		
<i>Tylenchorhynchus</i>	6	33.33	78.7	4.0	280	+	+	+
<i>Tylenchus</i>	5	27.78	15.2	4.0	40		+	+

**Table 3.** Diversity indices (mean values) in PPN communities in olive nurseries soil mixture compared with olive varieties.

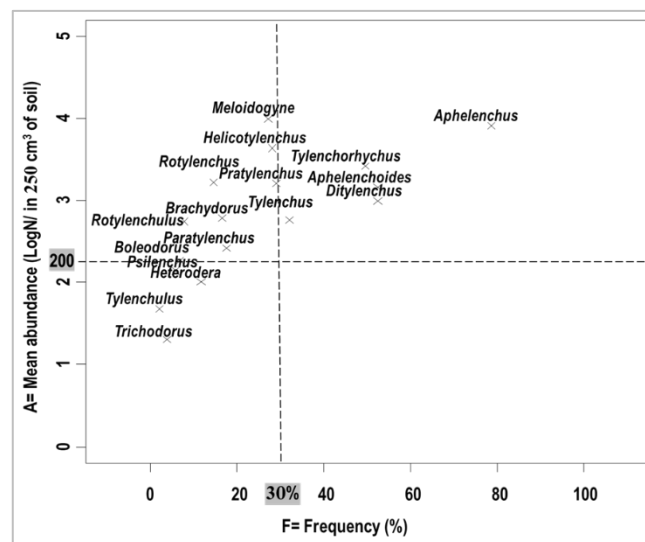
Treatment	Measured parameters								No of samples
	<i>N</i>	<i>G</i>	<i>H'</i>	<i>E</i>	<i>PPI</i>	<i>Rcp-2</i>	<i>Rcp-3</i>	<i>Rcp-4</i>	
Soil mixture	232.72 a	4.81 a	0.94 a	0.61 a	2.24 b	79.46 a	17.76 b	2.78a	36
Khdiry	425.65 a	4.00 a	0.82 a	0.60 a	2.57 a	42.84 b	57.16 a	0.00 a	34
Qaissy	321.64 a	4.24 a	0.79 a	0.56 a	2.45 a	54.86 ab	44.85 a	0.29 a	33

*N* = Total number of PPN, *G* = Generic richness, *H'* = Shannon Index, *E* = Evenness index, *PPI* = Plant Parasitic Index, *Rcp* = Relative mean abundance (%) of each *cp*-value (*cp-2*, *cp-3*, *cp-4*).

Values followed by the same letters in the same column are not significantly different at  $P=0.05$ .

### Nematode genera dominance in the samples of olive nurseries

The dominance of each genus in the samples was described by modelling the abundance (*A*) and the frequency (*F*) of the genus in the whole sample set. According to the thresholds defined by the model for each genus, the seventeen identified genera were distributed into three groups (Figure 2). The first group included the most frequent and abundant genera ( $F \geq 30\%$  and  $A \geq 200$  nematodes/250 cm<sup>3</sup> of soil). This group included five genera: *Aphelenchus*, *Ditylenchus*, *Aphelenchoides*, *Tylenchorhynchus* and *Tylenchus*. The second group included eight abundant genera ( $A \geq 200$  individuals/250 cm<sup>3</sup> of soil) but less frequent ( $F < 30\%$ ), and those were: *Pratylenchus*, *Helicotylenchus*, *Meloidogyne*, *Rotylenchus*, *Brachyidorus*, *Rotylenchulus*, *Paratylenchus* and *Boleodoris*. The last four genera can be considered occasional ( $A < 200$  individuals/250 cm<sup>3</sup> of soil and  $F < 30\%$ ), and those were: *Psilenchus*, *Heterodera*, *Tylenchulus* and *Trichodoris*.

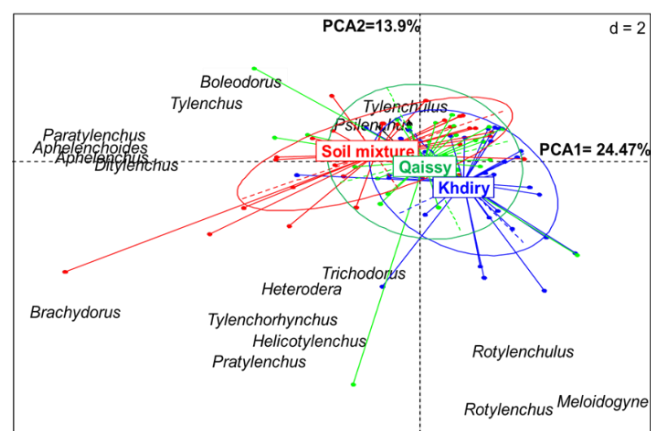


**Figure 2.** Dominance diagram of the nematode genera. Dotted lines indicate delineation between low and high abundances and frequencies as described by (Fortuner & Merny, 1973).

### PPN community structure in nurseries of the two olive varieties and the used soil mixture

The structure of PPN communities was described using PCA (Figure 3). The analysis indicated that the two first axes represented 38.37% of the total inertia of the dataset. The

loading plot indicated a distinct structure of PPN communities in the soil mixture compared to the two olive varieties. PPN communities in the soil mixture were structured of *Brachyidorus* on one hand, and *Boleodoris*, *Tylenchus*, *Tylenchulus* and *Psilenchus* on the other hand, and of the group *Paratylenchus*, *Aphelenchoides*, *Aphelenchus* and *Ditylenchus*. The community structure also differed between the two studied varieties: on the Khdiry variety, the communities essentially included *Meloidogyne*, *Rotylenchus* and *Rotylenchulus*, whereas the nematode communities on the Qaissy variety were mainly *Pratylenchus*, *Helicotylenchus* and *Tylenchorhynchus* and to a lesser extent *Heterodera* and *Trichodoris*.



**Figure 3.** PPN community structure between the soil mixture and the two olive varieties according to PCA.

### Discussion

The current study provides the first report from Syria on the PPN communities associated with olive nurseries along the coastal region of Syria. It showed the wide distribution of PPN in nurseries (95.37% of samples were infected by at least one genus of nematodes), and only five samples were free of nematodes. Seventeen genera were identified and most of them are herbivore nematodes (Table 1). Some genera (*Helicotylenchus*, *Meloidogyne*, *Rotylenchus*, *Rotylenchulus*, *Pratylenchus* and *Tylenchulus*) correspond to the most widespread taxa on olive trees worldwide, especially in the Mediterranean Basin (Ali *et al.*, 2014; Castillo *et al.*, 2010). These genera were previously reported in nurseries (Ait Hamza *et al.*, 2018; Nico *et al.*, 2003; Sanei & Okhovvat, 2011; Sultan, 2007). This study confirmed that

*Aphelenchus*, *Aphelenchoides*, *Tylenchorhynchus*, *Ditylenchus*, *Tylenchus* were the most common genera, which may suggest that they are well adapted to the olive tree microenvironment, especially under nursery conditions (Aït Hamza *et al.*, 2018).

The mean values of the taxonomical indices of nematodes diversity ( $N$ ,  $G$ ,  $H'$ ,  $E$ ) were close, indicating that these indices were homogeneous on olive varieties and in soil mixture. The local diversity ( $H'$ ) was low and the evenness ( $E$ ) indicated that species were quite uniformly distributed in communities. The mean genus richness ( $G$ ) was rather low with less than five genera, but the high value of  $G$  was reported in the soil mixture. Most nurseries along the Syrian coast were generally infested, thus the soil mixtures used to propagate olive plantlets were the main source of PPN spread, which is in agreement to what has been reported earlier (Aït Hamza *et al.*, 2018).

Most nurseries along the Syrian coast are generally non-sanitized; that could explain that more genera were reported in the soil mixture, which is in agreement to what has been reported earlier (Aït Hamza *et al.*, 2018) that showed the non-sanitized nurseries can encompass a high PPN richness.

In contrast to taxonomic diversity indices, a significant impact was revealed on functional indices, especially the  $PPI$  and the composition of  $cp-2$  and  $cp-3$  nematodes. The  $cp-2$  nematodes were most abundant in the soil mixture, in contrast to the  $cp-3$  group that seems to reproduce more on olive varieties. This high amount of  $cp-3$  nematodes could explain the high values of  $PPI$  recorded on the Khdiry and on the Qaissy varieties. According to Aït Hamza *et al.* (2018), nursery conditions favors the selection and the multiplication of the most competitive and harmful PPN species such as *Meloidogyne* spp. and *Pratylenchus* spp. ( $cp-3$ ). This dominance of  $cp-2$  and  $cp-3$  leads to scarcity [e.g., *Trichodorus* ( $cp-4$ ) recorded in a very low abundance] or the absence of other groups [e.g., *Xiphinema* and *Longidorus* ( $cp-5$ ), not recorded in this study]. The  $cp-4$  and  $cp-5$  groups are very sensitive to environmental disturbances (as in nurseries by agricultural practices). This observation is in agreement with previous studies (Bongers & Ferris, 1999; Van Eekeren *et al.*, 2008) revealing that the higher  $cp$ -value nematodes is usually associated with low stress and undisturbed environment.

Another significant impact of soil mixture and olive varieties in the PPN community structure. The results obtained showed that the structure was compatible with the composition of  $cp$ -value groups. The genera *Aphelenchoides*, *Aphelenchus*, *Boleodorus*, *Ditylenchus*, *Paratylenchus* and *Tylenchus* ( $cp-2$ ) were essentially detected in the soil mixture, whereas the  $cp-3$  nematodes (e.g. *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Rotylenchus* and *Tylenchorhynchus*) were most abundant on olive varieties. This dominance could be explained by fertilization amendments and irrigation applied to the olive seedlings. Such practices appeared to enhance the populations of PPN such as *Meloidogyne* spp. (Aït Hamza *et al.*, 2018). The olive variety is among the main factors driving PPN community composition (Palomares-Rius *et al.*, 2015). However, other factors could also affect the PPN community composition such as the physicochemical properties (e.g. pH, sand

content and exchangeable K) and the climatic characteristics (e.g. minimum average temperature of the sampled locations) as already concluded by Palomares-Rius *et al.* (2015).

The present study also revealed high population levels of *Meloidogyne* (RKN) and *Pratylenchus* (RLN) especially on the Khdiry and on the Qaissy varieties, respectively (Table 2). Although damaging population thresholds for these nematodes to olive planting stocks are unknown (Nico *et al.*, 2003), this high density should be underlined and attract attention, especially because they are classified among the top 10 economically important and harmful nematodes worldwide (Jones *et al.*, 2013). Additionally, damaging levels to olive seedlings of these genera were reported in southern Spain (Nico *et al.*, 2003) and in northern Iran (Sanei & Okhovvat, 2011). The parasitism of olive roots by RKN and RLN may potentially contribute to the decline of olive planting stocks in olive nurseries or in new orchards where a high occurrence is usually noticed (Hashim, 1982; Nico *et al.*, 2003). As endoparasitic nematodes, the detection of these two nematodes on olive plantlets leads to expect their inevitable spread into olive orchards through contaminated seedlings or soil.

RKN nematodes were detected in about 20% of the samples (in 22 samples only), compared to 52.1% of nurseries samples reported earlier by Aït Hamza *et al.* (2017). Our results corroborate other reports which revealed only scarce spread of RKN in olive-producing areas (Ali *et al.*, 2016).

Although citrus nematodes were recorded in low density/abundance and frequency (they were detected in two samples of soil mixture in Al-Hinadi only), this detection should be also underlined because these nematodes are potentially a threatening factor to olive production (McKenry, 1994). The identification of *Tylenchulus* spp. in Syrian olive nurseries at a low frequency is in line with the results of Aït Hamza *et al.* (2018), but contrary to what has been reported by Nico *et al.* (2003) and Sanei & Okhovvat (2011), where no detection of this nematode was reported.

Similar to other soil-borne pathogens, the use of infested propagating materials and crop residues could be an important source for nematodes spread. To highlight the role of soil mixture in nematode dissemination, Aït Hamza *et al.* (2017) indicated that in Morocco, nursery soil mixtures are often prepared with soil from cropped fields potentially infested with soil-borne pathogens such as plant-parasitic nematodes. Nico *et al.* (2003) also indicated that the occurrence of PPN is directly related to the origin of the soil mixtures that are often not sterilized. Thus, management of plant-parasitic nematodes in olive nurseries mainly involves the use of pathogen-free material for propagation and the certification of olive propagation plants in sanitized substrates. Soil solarization should be also encouraged, both in orchards and in nurseries in order to reduce the soil inoculum density (Abdine *et al.*, 2007). This method was applied to control *M. incognita* in olive nurseries in southern Spain (Nico *et al.*, 2003). These measures are important not only for avoiding the transmission or the multiplication of PPN but also for other soil-borne diseases.

Certification programs for nursery materials have been implemented in some countries such as Italy and Spain.

According to the Italian certification law, certified nursery materials must be free from the olive knot bacterium (*Pseudomonas savastanoi*), Verticillium wilt (*V. dahliae*), nematodes (*Meloidogyne* spp.) and viruses such as SLRSV, ArMV, CLRV, OlyvV and OIV-1 (Abdine *et al.*, 2007). Such programs seem important to adopt in Syrian nurseries to ensure the production and distribution of "healthy" seedlings for growers. Additionally, monitoring the sanitary status of olive orchards, especially in the new orchards, seems to be essential.

It can be concluded from the present study that there was a wide distribution of the plant-parasitic nematodes in olive nurseries along the coastal region of Syria. Functional diversity indices and PPN community structure were influenced by olive variety and used soil mixture. However other factors such as soil physicochemical properties and climatic conditions could also affect the diversity and the structure of nematode communities and should be taken into consideration in further studies. Economically important nematodes genera such as *Meloidogyne*, *Pratylenchus* and *Rotylenchulus* were recorded to be present.

High population densities of *Meloidogyne* and *Pratylenchus* were reported on the cvs. Khdiry and Qaissy seedlings, suggesting their inevitable introduction into orchards and can consequently cause damage to olive roots depending on the olive variety and their abundance. Further analysis to determine the resistance response of most common Syrian olive varieties to the prevalent nematodes genera is also essential to select the most appropriate ones. Certification programs for nursery materials seem important to adopt in Syrian nurseries to ensure the production and distribution of "healthy" seedlings to growers.

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## المخلص

علي، نادين. 2024. تنوع وتركيب مجتمعات النيماطودا الممرضة للنبات في مشاتل الزيتون في الساحل السوري. مجلة وقاية النبات العربية، 42(3):

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تعدّ المشاتل المصدر الرئيس لانتشار مسببات الأمراض في بساتين الزيتون، وبخاصة تلك المحمولة بالتربة مثل النيماطودا الممرضة للنبات، وبالتالي فإن التحري عن وجودها وتوزعها في مشاتل الزيتون يبدو ذو أهمية كبيرة. لا توجد بيانات علمية حول توزع النيماطودا الممرضة للنبات في مشاتل الزيتون في الساحل السوري، وبالتالي، تم إجراء هذا البحث بغرض الكشف عن وجود وتنوع مجتمعات النيماطودا الممرضة للنبات في بعض مشاتل الزيتون في الساحل السوري، ومقارنة تنوع وتركيب مجتمعات النيماطودا ما بين صنفين من أصناف الزيتون وخليط التربة المستخدم. جُمعت 108 عينة تربة من مشاتل مختلفة في اللاذقية وطرطوس، من صنفين شائعين (الخشري والقيسي) ومن خليط التربة المستخدم. فُورنت بعض المؤشرات التصنيفية والوظيفية بين أصناف الزيتون وخليط التربة. حُدّد تركيب المجتمع باستخدام تحليل المكونات الرئيسية. أظهرت النتائج الانتشار الواسع للنيماطودا الممرضة للنبات في مشاتل الزيتون بإجمالي 17 جنساً تمّ تحديدها. كانت الأجناس *Aphelenchoides*، *Aphelenchus*، *Ditylenchus* و *Tylenchorhynchus* هي السائدة. لوحظ وجود تأثير للأصناف أو خليط التربة في مؤشرات التنوع الوظيفي، كذلك كشف تحليل المكونات الرئيسية عن بنية متميزة للمجتمعات في صنف الزيتون وفي خليط التربة. سُجّلت أجناس مهمة اقتصادياً، مثل: *Meloidogyne* و *Pratylenchus* بكثافة عالية في بعض المشاتل، مما يشير إلى الإدخال الحتمي لهذه النيماطودا في بساتين الزيتون لاحقاً، وبالتالي من الأهمية بمكان اعتماد برامج شهادات صحية في المشاتل السورية لضمان إنتاج وتوزيع غراس "سليمة" للمزارعين.

**كلمات مفتاحية:** أصناف زيتون، تنوع، خليط تربة، سورية، مشاتل زيتون، نيماطودا ممرضة للنبات.

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