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# Diversity and Management Strategies of Plant Parasitic Nematodes in Moroccan Organic Farming and Their Relationship with Soil Physico-Chemical Properties

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**Abstract:** Organic farming has been increasing steadily over the last decade and is expected to grow drastically in the future. Plant parasitic nematodes (PPNs) are known as one of the most important pests attacking various plants in conventional and organic farming systems. A survey was conducted in January 2019 to determine the occurrence and diversity of PPNS, their associations with soil properties, and to assess their management methods in organically farmed fields in Southern Morocco. Twelve genera of PPNS were identified in soil and root samples collected from 53 organic fields, including *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Tylenchus*, *Tylenchorynchus*, *Criconemoides*, *Trichodorus*, and *Xiphinema*. The root-knot nematodes (*Meloidogyne* spp.) and the root-lesion nematode (*Pratylenchus* spp.) were the most prevalent PPNS. Vegetable crops (bean, onion, and tomato) had high nematode diversity indices compared to some aromatic and medicinal crops, including the Shannon, Evenness, and plant parasitic index (PPI). Our study underlined that several PPN genera were significantly correlated with soil physico-chemical properties, in particular, soil structure and organic matter. Therefore, it was concluded that soil properties have a considerable impact on PPN communities in organic farming systems located in Southern Morocco. There are numerous strategies for the control of PPNS in organic farming systems.

**Keywords:** organic; Souss-Massa; control; diversity; nematodes; vegetables

## 1. Introduction

Organic farming can be defined as an ecological and economical way of farming, playing a major role in preserving biodiversity, promoting animal and plant health, and contributing to sustainable development [1]. This type of farming system relies on eco-friendly practices such as sanitation practices and crop rotation instead of using agrochemical inputs [2]. Moreover, in recent years, the consumer

demand for organic foods and the amount of production in organic farming have increased rapidly [3]. Currently, organic agriculture is practiced in approximately 181 countries worldwide [4]. According to the last statistical report, 2.9 million farms worldwide are currently managed with organic practices in a total estimated area of 71.5 million hectares [5]. Australia has the largest organic agricultural area (35.7 million hectares), followed by Argentina and China with areas of 3.6 and 3.1 million hectares, respectively. In Africa, there are slightly more than 2 million hectares of certified organic land, with only two countries, that is, Morocco and Tunisia, having legislation rules for organic agriculture [6]. Organic arming in Morocco started in 1986 with citrus groves planted in the Marrakech region [7]. Organic practices then expanded to include fresh vegetables such as tomatoes, potatoes, onions, eggplant, medicinal and aromatic plants, and other exotic products [8]. The geographical situation and weather conditions of Morocco allowed a significant production of organic crops with respect to competitive countries. In 2000, more than 1500 tons of eight commodities were produced in three different areas: Agadir, Taroudant, and El-Jadida [9]. Furthermore, these regions harbor 90% of Moroccan organic production [10]. The Souss-Massa region (south of Morocco) has developed into an important producer of organic crops in this country in the past five years due to favorable pedoclimatic conditions such as high soil temperature and water content [9]. However, organic crops may have high pest pressures compared to conventional farming due to both the regulations of organic farming and few management practices [11].

Plant parasitic nematodes, impacting both quantity and quality of crop yields, are one of the most important and dangerous groups of pests for many organic crops. The annual yield loss due to PPN damage was estimated at \$100–157 billion on a worldwide basis [12]. However, the overall losses due to PPNs in organic farms are not fully estimated yet. Several PPN genera have been reported to be associated with organic crops including *Meloidogyne*, *Pratylenchus*, *Tylenchorhynchus*, *Helicotylenchus*, *Criconeimoides*, *Tylenchus*, *Xiphinema*, *Ditylenchus*, *Paratylenchus*, *Aphelenchoides*, and *Aphelenchus* [13–15]. In Morocco, a relatively large number of PPNs were identified on various vegetables in conventional farming [16–19]. Particularly, *M. javanica* and *M. incognita* are known as the most common and destructive nematodes infecting different crops in the agricultural areas of Morocco [20]. Recently, *M. arenaria* was detected for the first time in a conventionally cultivated eggplant farm in Southern Morocco [18].

However, the success of organic production systems relies primarily on effective disease management. To control PPNs associated with organic farming systems, farmers employ diverse practices such as crop rotation, use of cover crops, resistant crop cultivars, soil amendments as well as other beneficial practices to promote diversity and activity of soil microorganisms [11]. These nematodes react differently to changes in diverse practices and are therefore good indicators for evaluating farming systems [21].

In many cases, methods applied in organic farming may not be sufficient to effectively control the nematode population [22]. Management of nematodes in organic fields, especially in organic vegetable fields is quite challenging compared to conventional farming [23,24]. In Morocco, there is limited information on organic farmers' management approaches and situations that effectively lead to the suppression of PPNs that are economical and can easily be adopted by most farmers.

To date, the scientific data on PPNs associated with organic farms is still limited and largely absent in Morocco, although nematode damage is known to occur. Such information is valuable to gain insights into their potential contribution to the loss of crop yield and in the development of reliable and effective management strategies.

Given this lack of information and to orientate further nematological research, this study represents an extensive overview of the incidence of PPNs associated with organic farms in Southern Morocco. Therefore, the main objectives of this work are (i) to study and characterize morphologically PPN taxa associated with organic crops in Southern Morocco; (ii) to determine the frequency of occurrence, abundance, and densities of each genus of nematodes; (iii) to investigate the effect of soil

physico-chemical properties on PPN community structure; and (iv) to assess farmers' control measures for PPNs in Southern Morocco.

## 2. Materials and Methods

### 2.1. Nematode Survey

A comprehensive survey was conducted in January 2019 to determine the occurrence and distribution of PPNs associated with organic farms in Southern Morocco. This region has the largest producers of vegetables and medicinal plants in Morocco. Samples were taken from fields located in Belfaa, Biogra, and Oulad dahou. A total of 53 representative fields were sampled representing the organic farming areas in those studied regions. From each field, soil and root samples were collected from the rhizosphere of seven surveyed crops, viz. bean (*Phaseolus vulgaris* L.), onion (*Allium cepa* L.), tomato (*Solanum lycopersicum* L.), calendula (*Calendula officinalis* L.), chive (*Allium schoenoprasum* L.), rosemary (*Salvia rosmarinus* L.), and thyme (*Thymus vulgaris* L.). For each field, 10 subsamples were collected from the top 25 cm layer of soil in a zigzag pattern across an area of 0.5 to 1.5 hectares in a field using an auger (25 mm diameter) then mixed thoroughly to form a representative sample of 1 kg including soil and root. All samples were placed in polyethylene bags to prevent water loss and immediately delivered to the Nematology Laboratory at the National Institute of Agronomic Research (INRA-Agadir) for nematode extraction and identification. Additionally, a simple questionnaire was administered in the studied area to obtain baseline data concerning common farming practices among biological farmers.

### 2.2. Nematode Extraction and Identification

Samples from each surveyed field were thoroughly mixed and processed for extraction in 48 h. For each sample, nematodes were extracted separately from soil and root. Nematodes were extracted from 100 cm<sup>3</sup> of soil using a modified Baermann technique for each soil sample [25]. Roots of each sample were gently washed in tap water to free adhered soil particles, cut into pieces (ca 0.5 cm), and then nematodes were extracted from a sub-sample of 20 g using a modified Baermann technique [25]. After 48 h of incubation, nematode suspensions from both soil and roots were collected and examined under a stereomicroscope (Olympus CH-2, Tokyo, Japan). PPNs in each sample were identified to genus level based on morphological features including body shape, body size, stylet length, stylet type, lip region, pharyngeal overlap, tail type, spermatheca shape, and vulva position using dichotomous keys [26,27]. The population number of each genus was expressed as the total number in 100 cm<sup>3</sup> soil and 20 g roots. Nematodes were killed and fixed by adding 4% hot (60–80 °C) formaldehyde to a small drop of water in a glass cavity vessel that contained the nematodes. The nematodes were transferred to solution I (99 parts 4% formaldehyde + 1-part pure glycerin) in a square 7 cm diameter watch glass. This square watch glass dish was placed in a desiccator containing about 1/10th of its volume of 96% ethanol. The next day, the watch glass containing the nematodes was removed from the desiccator and placed in an incubator at 37 °C. Then 3 mL of solution II (95 parts 96% ethanol + 5 parts pure glycerin) were added to the watch glass. This was repeated three times at intervals of 3 h, while the watch glass was partially covered by a glass slide to allow evaporation. Finally, 2 mL of solution III (50 parts 96% ethanol + 50 parts pure glycerin) were added and the watch glass was left overnight at 37 °C in the incubator [28]. Specimens were subsequently prepared using the glycerin-ethanol method for identification to species level under a light microscope (Nikon Eclipse E200, Tokyo, Japan) using the available identification keys [29–35]. For the *Criconemoides* genus, nematodes were morphologically and morphometrically identified using data collected by Geraert [36]. Species of root-knot nematodes were identified according to perineal patterns [37] supported by the observation of juveniles. The infected crops of each surveyed crops were collected and washed. Adult females, for perineal patterns, were carefully removed from root tissues and teased apart with needle and tweezers and kept in a solution of 0.9% sodium chloride. Neck and lip regions were excised, and the

posterior end was cleared. The perineal pattern was trimmed and transferred to a drop of glycerin for microscopic observations. For each infected crop, five perineal patterns were prepared and mounted in glycerin for microscopic observation ( $\times 100$  magnification).

### 2.3. Assessment of Nematode Population Densities

Nematode diversity and incidence were assessed by calculating prevalence, mean intensity, and maximum density [38]. Prevalence was measured as the number of samples containing a particular nematode taxon (expressed as a percentage). The proportion of the sample comprising PPNs was derived by the following equation:

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

where Sn is the number of samples having a particular nematode species, and St is the number of samples examined. The mean intensity was calculated for each nematode genus.

$$\text{Mean intensity} = \text{Ps/Pt}$$

where Ps is the number of individuals of a particular nematode species in the positive samples, and Pt is the number of positive samples. Maximum density (i.e., the maximum number of individuals of a particular nematode species recovered from a sample) was also measured for each crop. The root damage with galls, necrosis, lesions, or with no symptoms was determined and expressed as a percentage of total roots collected.

### 2.4. Physico-Chemical Analysis of Soil

The soil analyses were carried out at the INRA soil laboratory in Agadir, using standard methods [39]. The following soil properties were analyzed including soil texture: proportions of clay (0–2  $\mu\text{m}$ ), silt (2–50  $\mu\text{m}$ ) and sand (50 to >200  $\mu\text{m}$ ); pH and electrical conductivity EC ( $\mu\text{S/cm}$ ) using the 1:2.5 soil:water ratio methodology described by Richards [40]; exchangeable cations: potassium, manganese, and magnesium and exchangeable acidity were determined by atomic absorption spectrometry [41]; total (TOM) and humic (HOM) soil organic matter were estimated using the method described by Allison [42]; soil carbon and nitrogen contents were obtained using Walkley–Black [43] and Kjeldahl methods [44], respectively to determine carbon to nitrogen (C/N) ratio; soil solution including iron, copper, zinc, sodium, and phosphorus was determined by colorimetry (Shimadzu UV-1205; Shimadzu Scientific Instruments, Columbia, MD, USA).

### 2.5. Diversity of Plant Parasitic Nematodes

The diversity of PPNs was established using the Shannon–Wiener index [45].

$$H' = - \sum_{i=1}^s pi \ln pi$$

where  $s$  is the number of genera,  $pi$  is the proportion of characters belonging to the corresponding number of genera, and  $H'$  is commonly used to characterize species diversity in a community.  $H'$  accounts for both the number of species and the evenness  $J$ . A separate measure of the evenness is usually given.

$$J = \frac{H}{H_{max}}, H_{max} = \log_2 s$$

The taxon dominance parameter was calculated for each nematode genus in prospected localities alongside the frequency. This parameter represents the regression between abundance and frequency for each sampling genera [46]. The distribution diagram of nematode communities was applied as abundance variables were transformed to  $\log_{10}(X + 1)$  before analysis. In addition, spatial nematode

distribution for whole crop systems was predicted via bubble-PCA analysis using PRIMER-E (ver. 6.1.7) (Plymouth Routines in Multivariate Ecological Research, Auckland, New Zealand) using Bayesian clustering algorithms. In addition, the plant parasitic index (PPI) was calculated for each herbivorous nematode according to Bongers [47]

$$PPI = \sum vi \times fi$$

where  $vi$  is the  $c-p$  value of taxon  $i$  in each organic crop, and  $fi$  is the frequency of the taxon.

## 2.6. Statistical Analyses

Principal component analyses (PCA) were applied to explore PPN community patterns, crops, and physico-chemical soil patterns from organic farming systems surveyed. After data normalization using the Anderson–Darling normality test [48], PPN and soil variables associated with the PCA were subjected to a two-way ANOVA performed using XLSTAT 2016.02.28451 software (Addinsoft, New York, NY, USA) and based on a randomized factorial design with five replications ( $n = 5$ ) in each cropping experimental units. Univariate notched Box and Whisker plots were established from main PPN genera variables. Significant differences among variables were performed using Fisher’s protected least significant difference (LSD) and Tukey test at  $P < 0.05$ . Differences obtained at levels of  $P < 0.05$  were considered to be significant. Hierarchical cluster analysis was conducted based on the Bray–Curtis similarity index to reveal similarities between crop systems in terms of PPN abundance. Canonical correspondence analyses in R software [49] were used to visualize relationships between PPN and soil physico-chemical properties and further enhanced by an advanced heatmap analysis (heatmap-2) based on Ward’s clustering algorithm of R software. To meet conditions of normality, PPN patterns were square-root transformed prior to CCA and cluster analyses. All multivariate analyses were performed using R.

## 3. Results

### 3.1. Density and Diversity of PPNs Associated with Organically Grown Vegetables, Medicinal, and Aromatic Plants

A total of 12 PPN genera were found to be associated with organically grown vegetables and medicinal and aromatic plants in Southern Morocco (Table 1). The number of samples collected per crop during the survey varied depending on the cultivated crop (Table 2). The root-knot nematode (RKN) *Meloidogyne* spp., was the most frequent (72.6%) followed by the root-lesion nematodes (RLN) *Pratylenchus* spp., (68.8%) (Figure 1).

**Table 1.** Grouping of plant parasitic nematode genera found on organic fields in Southern Morocco (survey January 2019).

Phylum	Class	Order	Family	Genus	Common Name	Code
Nematoda	Secernentea	Tylenchida	Heteroderidae	<i>Meloidogyne</i>	Root-knot nematode	MEL
			Hoplolaimidae	<i>Helicotylenchus</i>	Spiral nematode	HEL
			Pratylenchidae	<i>Pratylenchus</i>	Lesion nematode	PRA
			Tylenchidae	<i>Tylenchus</i>	Tylenchids	TYL
			Tylenchulidae	<i>Paratylenchus</i>	Pin nematode	PAR
			Dolichodoridae	<i>Tylenchorhynchus</i>	Stunt nematode	TYLE
			Hoplolaimidae	<i>Rotylenchulus</i>	Reniform nematode	ROT
			Criconematidae	<i>Criconemoides</i>	Ring nematode	CRI
			Anguinidae	<i>Ditylenchus</i>	Stem Nematode	DIT
			Enoplea	Dorylaimida	Longidoridae	<i>Xiphinema</i>
	<i>Longidorus</i>	Needle nematode				LON
				<i>Trichodorus</i>	Stubby-root nematode	TRI

**Table 2.** The occurrence of plant parasitic nematodes in samples from fields with organically grown crops in Southern Morocco (survey January 2019).

Host Plant	Location	Number of Fields Sampled	Control	Number of PPN Genera
Bean ( <i>Phaseolus vulgaris</i> L.)	Belfaa	24	Crop rotation (Alfalfa/Corn/Maize)	9
Onion ( <i>Allium cepa</i> L.)	Belfaa	30	Crop rotation ( <i>Tagetes</i> spp./ <i>Ricinus communis</i> L.)	11
Tomato ( <i>Solanum lycopersicum</i> L.)	Biogra	6	Cover crop ( <i>Tagetes</i> spp./ <i>R. communis</i> L.)	8
Chive ( <i>Allium schoenoprasum</i> L.)	Oulad dahou	26	Seyland + Nemguard granules	9
Rosemary ( <i>Salvia rosmarinus</i> L.)	Oulad dahou	12	None	10
Thyme ( <i>Thymus vulgaris</i> L.)	Oulad dahou	2	None	7
Calendula ( <i>Calendula officinalis</i> L.)	Oulad dahou	6	None	5

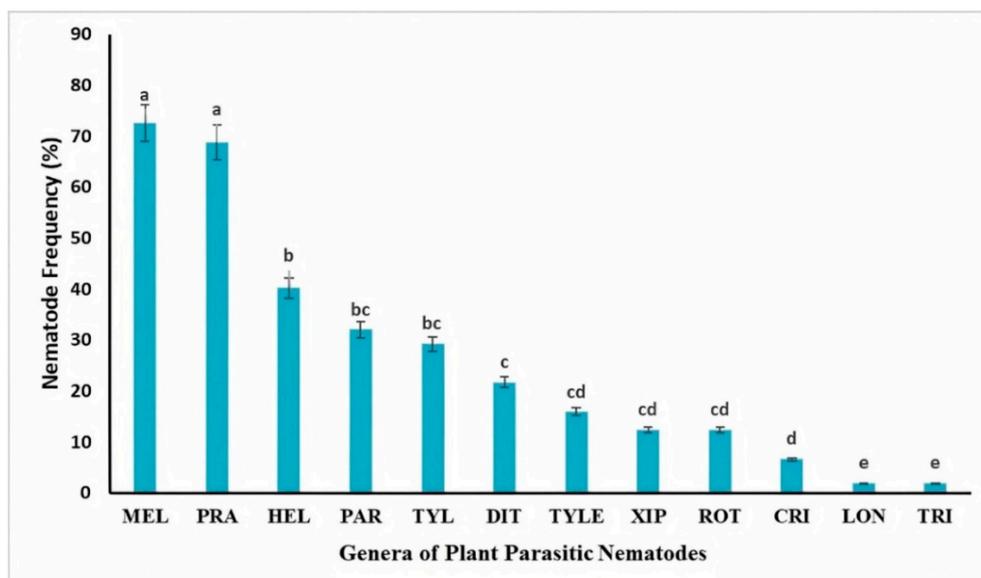
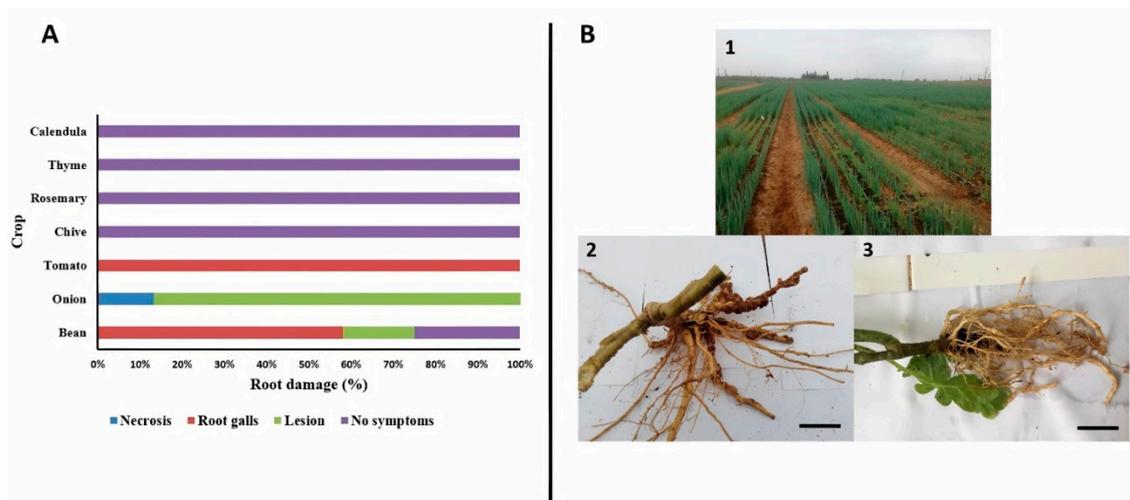
**Figure 1.** Total frequencies of plant parasitic nematodes obtained in all organic farming systems surveyed. Letters represent homogeneous groups based on protected least significant difference test (LSD) for each variable at ( $P < 0.001$ ).

Table 3 summarizes the prevalence of PPNs, mean intensity, and maximum densities. Accordingly, 11 PPN genera were recorded on onion, 9 genera on bean, and 8 genera on tomato (Table 3). The most prevalent nematodes on organically grown tomato, bean, and onion in both localities (Belfaa and Biogra) were RKN and RLN, which occurred in 82% and 73% of samples, respectively. *Meloidogyne* spp. and *Pratylenchus* spp. were found in both soil and root from all three surveyed crops and were associated mainly with sandy soils. Maximum nematode densities were achieved by *Meloidogyne*, *Pratylenchus*, and *Tylenchus* genera with 29, 13, and 11 nematodes ( $100 \text{ cm}^3 \text{ soil}^{-1}$ ), respectively. Other PPNs detected in soil and root samples were *Paratylenchus* spp., *Helicotylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Criconea* spp., *Tylenchorhynchus* spp., *Rotylenchulus* spp., and *Trichodorus* spp., and their population levels were generally very low. The following species were identified from randomly selected

specimens: *P. penetrans*, *P. thornei*, *P. crenatus*, *P. neglectus*, *M. javanica*, *M. incognita*, *Ditylenchus dipsaci*, *X. diversicaudatum*, *Rotylenchulus reniformis*, and *Helicotylenchus vulgaris*.

In soil samples from fields with organically grown medicinal and aromatic plants, a total of 10 genera were found to be associated with rosemary (*Salvia rosmarinus* L.), 9 genera with chive (*Allium schoenoprasum* L.), 7 with thyme (*Thymus vulgaris* L.), and 5 with calendula (*Calendula officinalis* L.) (Table 2). When considering all organic medicinal and aromatic plants surveyed in this study, the spiral nematode *Helicotylenchus* was the most prevalent genus (62.6%) followed by *Meloidogyne* (62.5%), *Pratylenchus* (57.2%), *Tylenchus* (51.5%), *Paratylenchus* (28.6%), and the remaining genera (<20%) (Table 4). Maximum densities of the genera *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, and *Tylenchus* were relatively higher in chive (22, 10, 13, 11 nematodes (100 cm<sup>3</sup> soil)<sup>-1</sup>) compared to other crops (Table 4). In addition, the number of PPNs was more variable and higher in fields with organically grown vegetables than at those with medicinal and aromatic plants (Table 3). Generally, disease symptoms including galls and lesions were observed in the roots of organically grown vegetables (tomato, onion, and bean), whereas, no disease symptoms were observed on the roots of medicinal and aromatic plants (Figure 2A). Root galling caused by RKN was observed on 100% and 58% of the root samples (Figure 2B) collected from tomato and bean, respectively. Furthermore, the effect of this genus in the vegetable fields was manifested *Thymus* visually through the yellowing of leaves, stunted growth, and root galling. Root lesions were seen on 87% and 17% of the root samples collected from onion and bean, respectively. Whilst, root necrosis was detected only on 13% onion roots.



**Figure 2.** Root damage in samples collected from organic vegetable and medicinal crops in Southern Morocco. **(A)** Percentages of root damages recorded for each organic cropping systems. **(B)** Onion field with symptoms of stunting of growth and yellowing of leaves caused by *Pratylenchus* spp. **(1)**, damage (galls) caused by *Meloidogyne javanica* **(2)**, and *M. incognita* **(3)** on tomato roots (*Solanum lycopersicum*).

**Table 3.** Prevalence, mean, and maximum density of plant parasitic nematodes from soil (100 cm<sup>3</sup>) and root (20 g) of organic vegetable crops in Southern Morocco (survey January 2019).

Nematode Taxa/Genus and Species	cp-Value <sup>a</sup>	Prevalence (%)	Bean				Prevalence (%)	Onion				Prevalence (%)	Tomato			
			Mean Intensity		Maximum Density			Mean Intensity		Maximum Density			Mean Intensity		Maximum Density	
			Root	Soil	Root	Soil		Root	Soil	Root	Soil		Root	Soil	Root	Soil
<i>Meloidogyne</i>	3	100	5	11	13	29	63	2	3	8	9	83	1	6	2	7
<i>Pratylenchus</i>	3	80	3	3	5	7	70	2	3	6	13	68	1	6	3	9
<i>Ditylenchus</i>	2	29	1	1	3	3	30	1	1	3	4	17	-	1	-	4
<i>Helicotylenchus</i>	3	37.5	1	2	3	7	40	1	2	2	7	50	-	3	-	6
<i>Paratylenchus</i>	2	54	1	2	5	5	30	1	1	5	6	34	-	2	-	4
<i>Tylenchus</i>	2	33	-	2	-	9	26.6	-	2	-	11	33.7	-	1	-	3
<i>Tylenchorhynchus</i>	3	12.5	-	1	-	5	10	-	1	-	4	17	-	1	-	4
<i>Longidorus</i>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Xiphinema</i>	5	4	-	1	-	2	26.6	-	3	-	7	34	-	1	-	3
<i>Rotylenchus</i>	3	-	-	1	-	4	20	-	2	-	8	-	-	-	-	-
<i>Criconemoides</i>	3	-	-	-	-	-	10	-	1	-	3	-	-	-	-	-
<i>Trichororus</i>	4	-	-	-	-	-	6.6	-	1	-	2	-	-	-	-	-

- Absence of nematodes, <sup>a</sup> Colonizer-persister value according to Bongers [47].

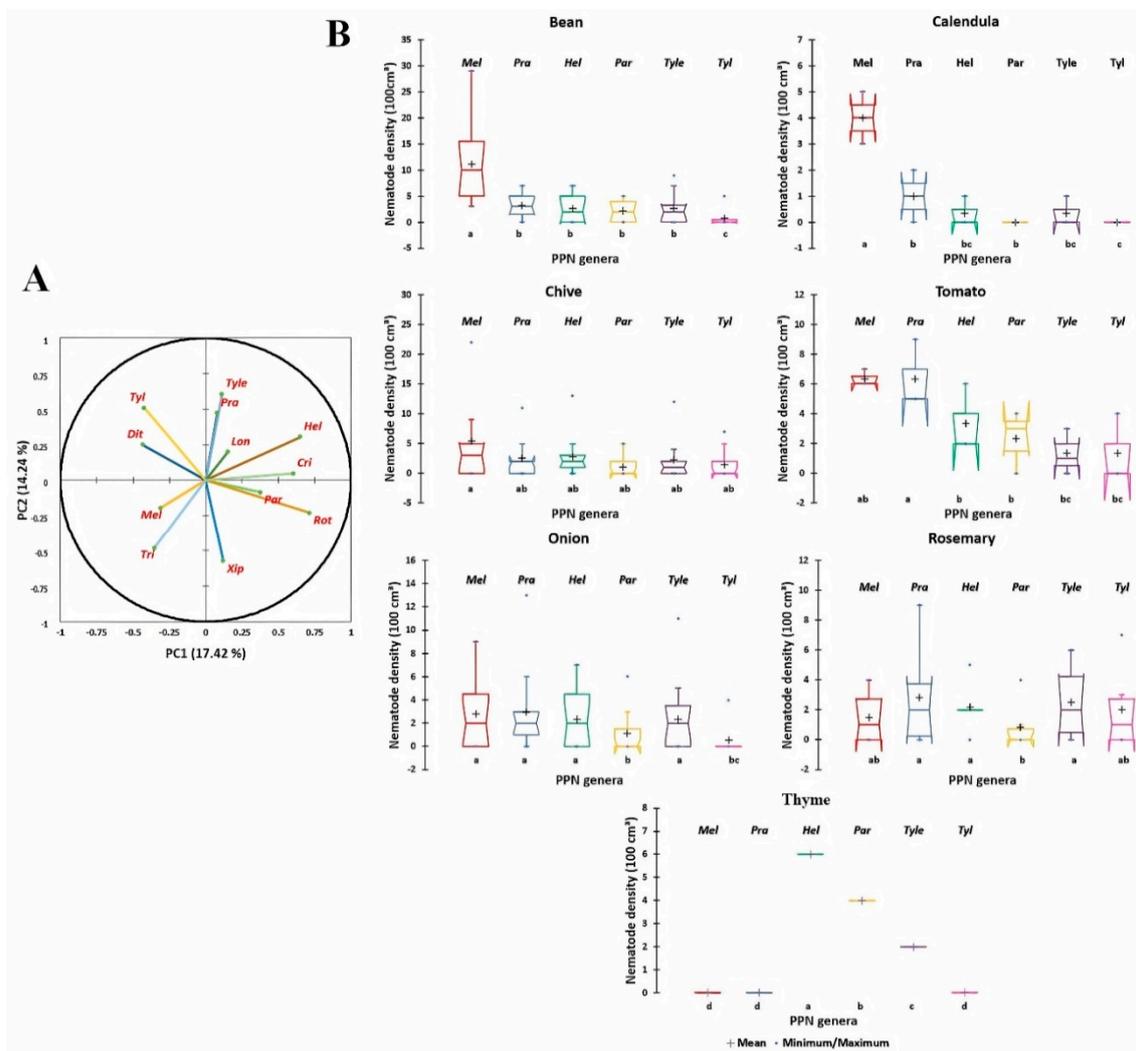
**Table 4.** Prevalence, mean, and maximum density of plant parasitic nematodes from soil (100 cm<sup>3</sup>) and root (20 g) of medicinal crops and aromatic plants in Southern Morocco (survey January 2019).

Nematode Taxa/Genus and Species	cp-Value <sup>a</sup>	Prevalence (%)	Calendula				Prevalence (%)	Rosemary				Prevalence (%)	Thyme				Prevalence (%)	Chive			
			Mean Intensity		Maximum Density			Mean Intensity		Maximum Density			Mean Intensity		Maximum Density			Mean Intensity		Maximum Density	
			Root	Soil	Root	Soil		Root	Soil	Root	Soil		Root	Soil	Root	Soil		Root	Soil	Root	Soil
<i>Meloidogyne</i>	3	83	4	4	5	5	67	2	1	5	4	50	1	-	3	-	50	3	8	7	22
<i>Pratylenchus</i>	3	50	2	2	2	2	75	2	3	5	9	50	1	-	2	-	54	3	4	5	10
<i>Ditylenchus</i>	2	-	-	-	-	-	17	1	-	2	-	-	-	-	-	-	11.5	3	3	5	3
<i>Helicotylenchus</i>	3	33.5	-	1	-	1	67	1	2	3	5	100	1	3	1	6	50	3	4	5	13
<i>Paratylenchus</i>	2	16.7	2	-	2	-	16.7	-	1	-	4	50	-	2	-	4	31	2	3	2	5
<i>Tylenchus</i>	2	50	-	1	-	1	33	-	2	-	6	100	-	1	-	2	23	-	4	-	12
<i>Tylenchorhynchus</i>	3	-	-	-	-	-	25	-	2	-	7	-	-	-	-	-	15.3	-	4	-	7
<i>Longidorus</i>	5	-	-	-	-	-	16.7	-	1	-	2	-	-	-	-	-	-	-	-	-	-
<i>Xiphinema</i>	5	16	-	-	-	-	8	-	1	-	1	-	-	-	-	-	-	-	-	-	-
<i>Rotylenchus</i>	3	-	-	-	-	-	25	-	1	-	2	50	-	1	-	2	4	-	2	-	2
<i>Criconemoides</i>	3	-	-	-	-	-	17	-	1	-	1	50	-	1	-	1	4	-	2	-	2
<i>Trichodorus</i>	4	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-

- Absence of nematodes, <sup>a</sup> Colozier-persister value according to Bongers [47].

### 3.2. Community Patterns of PPNs in Organic Farming Systems

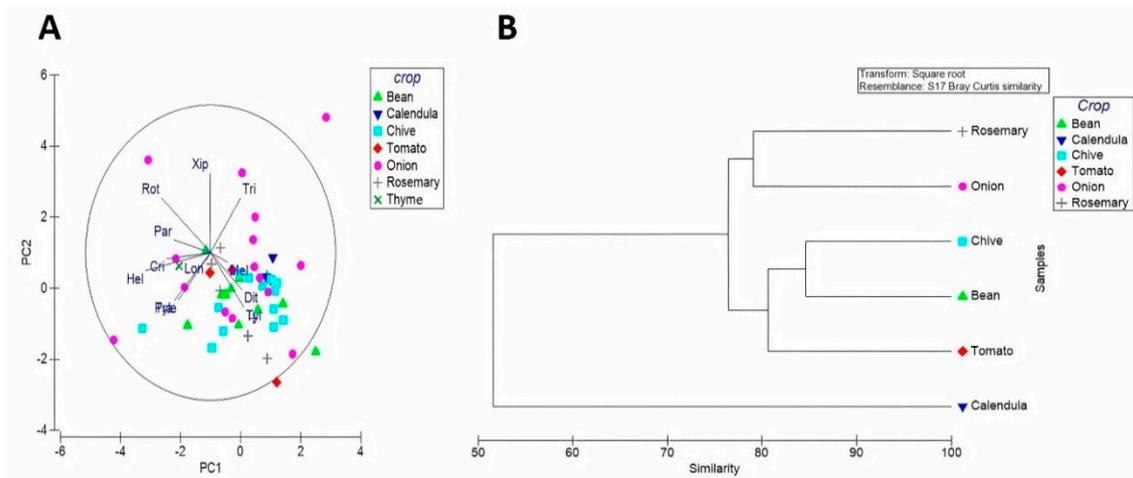
Principal component analyses of the distribution of nematode genera across total localities of the surveyed organic farming fields showed that the fraction of variance accounted for by the first two PC axes is 17.42% and 14.24% (eigenvalues), respectively (Figure 3A). The PC1 axis is related to *Xiphinema*, *Rotylenchulus*, and *Paratylenchus* species (positive PC values), and to *Meloidogyne* and *Trichodorus* species (negative PC values). The PC2 axis is related to *Tylenchus* and *Ditylenchus* species (positive PC values), and *Meloidogyne* and *Trichodorus* species (negative PC values). The projection of the sample eigenvalues on the PC axes using notched box and whisker plots (Figure 3B) indicated that *Meloidogyne* and *Pratylenchus* species were significantly abundant in all organic crops, except for thyme, which had *Helicotylenchus* spp. as the main PPN. Moreover, *Paratylenchus* spp., *Tylenchorhynchus* spp. and *Tylenchus* spp. were more abundant in tomato compared to the other crops.



**Figure 3.** Plant-associated nematode community patterns in organic farming systems in Morocco. (A) PCA loading plot (Spearman type) for all nematode genera obtained in surveyed farming areas. (B) Notched box and whisker plots for the main PPNs identified for each organic crop. Nematode genera codes are given in Table 1. Letters represent homogeneous groups based on protected least significant difference test (LSD) for each variable at ( $P < 0.001$ ). Error lines on the bars represent the standard error ( $n = 5$ ).

Organic crops assemblages were analyzed thoroughly (Figure 4). The Pearson-type Bi-plot was used to visualize crop patterns in interaction with the PPNs obtained (Figure 4A). The similarity among

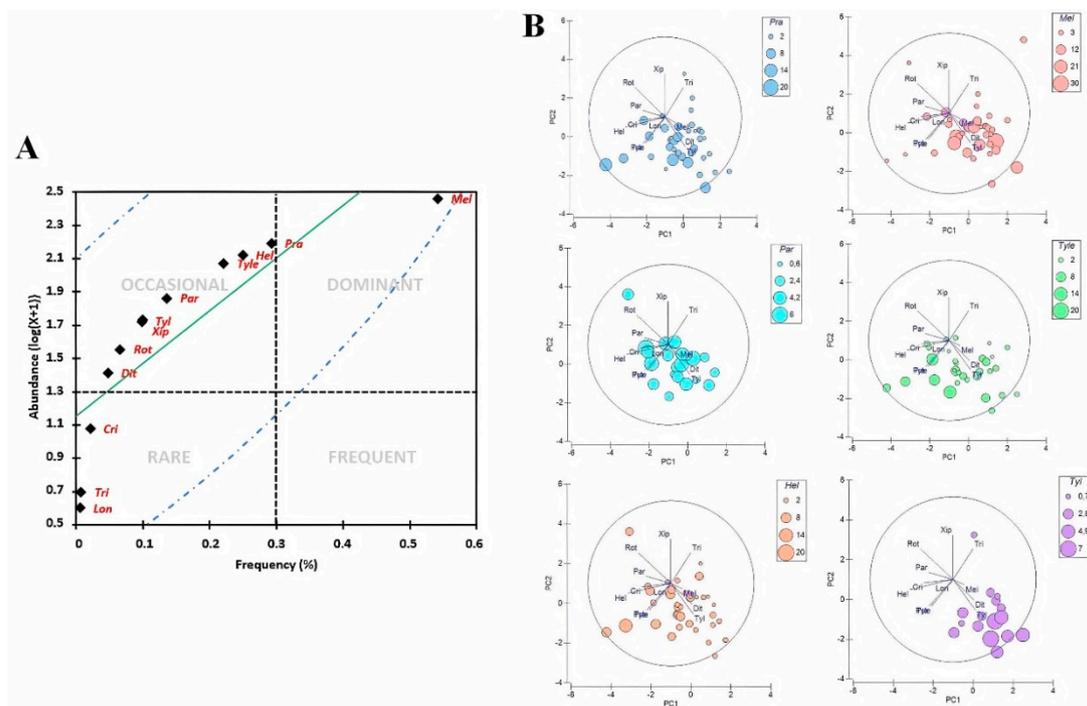
them in terms of nematode agro-ecological characteristics was established via hierarchical cluster analysis (Figure 4B). Rosemary and onion crops showed high similarity followed by chive and bean crops which were similar to tomato. On the other hand, calendula was completely different in terms of nematode communities.



**Figure 4.** Organic crop patterns and their affiliation with species' agro-ecological characteristics. (A) Bi-plot (Pearson type) of organic crops surveyed in interaction with PPN genera. (B) Hierarchical cluster analysis showing similarities between crops in terms of PPNs (square-root transformed) based on the Bray-Curtis similarity index. Nematode genera codes are given in Table 1.

### 3.3. Distribution and Diversity of PPNs Associated with Organic Crops

The abundance and frequency of PPNs in organic farming systems are shown in Figure 5. Of the 12 PPN genera identified, RKN and RLN were shown to be more abundant and more frequent. Indeed, *Helicotylenchus*, *Tylenchorhynchus*, *Paratylenchus*, and *Xiphinema* genera were less frequent with a high abundance (Figure 5A). In contrast, three genera (e.g., *Criconemodes*, *Trichodorus*, and *Longidorus*) were considered rare due to their low abundance and frequency rates. The spatial distribution of the obtained PPN genera was visualized using a bubble-type PCA spatial plot (Figure 5B). Six PPN genera (*Meloidogyne*, *Pratylenchus*, *Paratylenchus*, *Tylenchorhynchus*, *Helicotylenchus*, and *Tylenchus*), whose occurrence was measured earlier, were assessed with density as the main input. Root-knot nematodes (*Meloidogyne* spp.) displayed large bubbles explained by their high field coverage with multiple PPN genera. The RLN (*Pratylenchus* spp.) showed considerable multiple spots indicating its significant presence even though it did not have large bubble densities. The same applies to *Tylenchorhynchus* spp. and *Helicotylenchus* spp., while *Paratylenchus* spp. and *Tylenchus* spp. also occurred in the field. However, their coverage seems to be limited. Table 5 shows the number of genera and the Shannon–Weaver diversity index ( $H'$ ) and evenness ( $J$ ) values for each organic crop studied. The Shannon–Weaver diversity index was significantly ( $P < 0.05$ ) higher in onion, tomato, and rosemary (2.03, 1.87, and 1.84 respectively), compared to the other crops. The lowest index value was reported with calendula (1.73). There was no significant difference in evenness between all the studied crops.



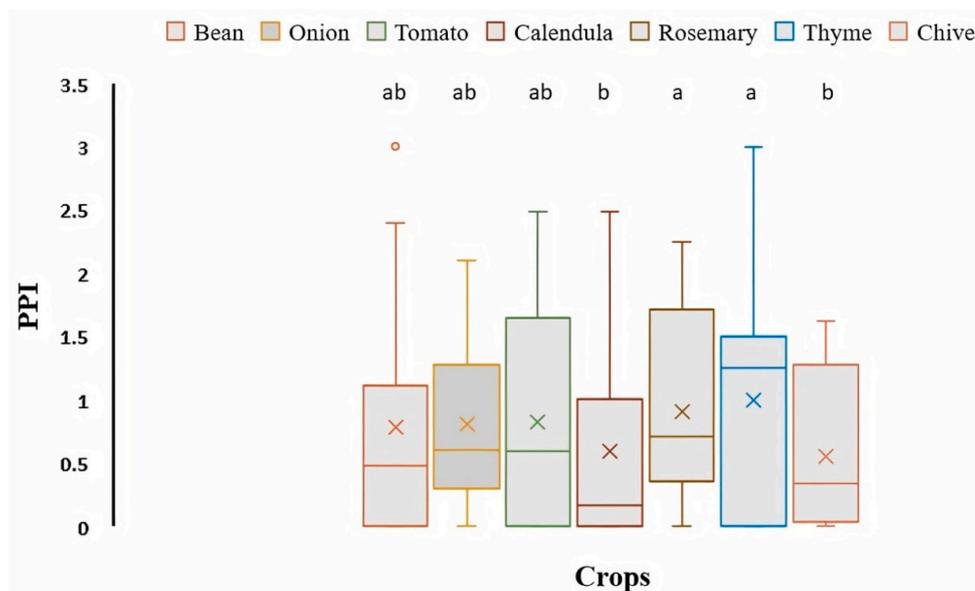
**Figure 5.** Plant parasitic nematode taxon dominance patterns and their spatial population dynamics. (A) Distribution diagram (Abundance-Frequency) of nematode communities. The green line represents the linear regression obtained between calculated parameters. Dotted lines represent confidence intervals at a 95% probability rate. (B) Bubble-PCA spatial plot of main PPN populations associated with organic crops. Bubbles represent predicted spatial densities with various ranges. Nematode genera codes are given in Table 1.

**Table 5.** Shannon–Weaver diversity ( $H'$ ), evenness ( $J$ ), and the number of nematode genera in each of the six surveyed crops.

Vegetable Crop	Number of PPN Genera	Diversity Parameters	
		Shannon Diversity Index ( $H'$ )	Evenness ( $J$ )
Bean	23.6 a	1.64 ab	0.58 a
Onion	22.16 a	2.03 a	0.72 a
Chive	17.3 ab	1.73 ab	0.61 a
Rosemary	6.83 b	1.84 a	0.65 a
Tomato	6 b	1.87 a	0.66 a
Calendula	1.58 c	1.13 c	0.4 a
Thyme	1.25 c	1.26 c	0.44 a
<i>P</i>	<0.001	0.0129	0.488

Means in the same column followed by different letters are significantly different by Tukey’s test.

The plant parasitic index (PPI) was calculated for each organic crop surveyed. Overall, aromatic crops (mainly rosemary and thyme) were shown to have a significant index ( $P < 0.05$ ) with high values up to 3 (Figure 6). The same trend was more or less observed with vegetable crops (bean, onion, and tomato). The lowest values were recorded in calendula and chive crops with less than 1 PPI values.



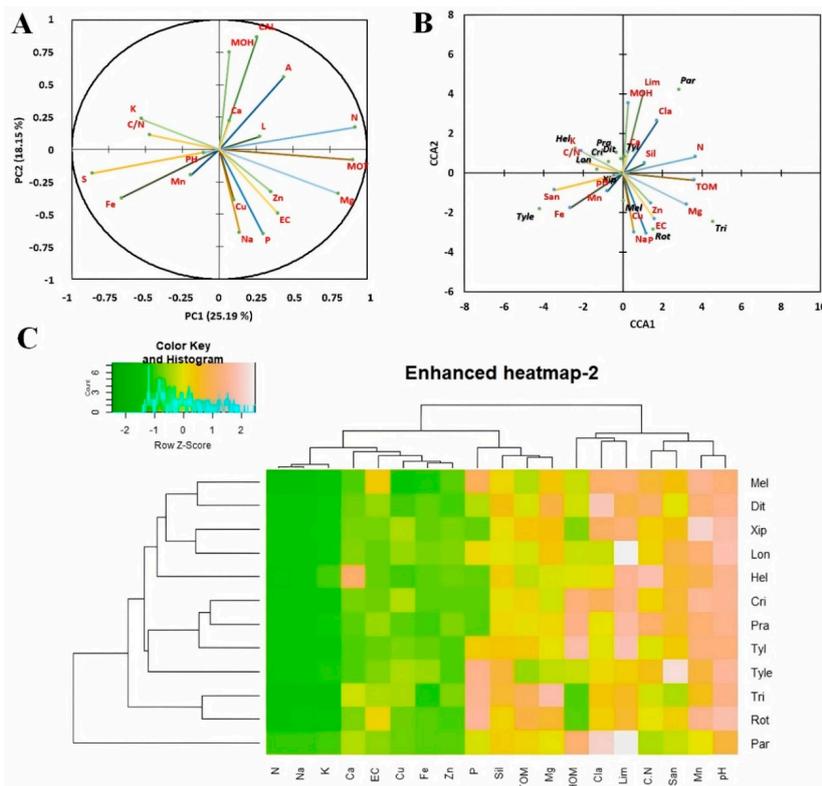
**Figure 6.** Plant parasitic index (PPI) of organic vegetable and medicinal crops surveyed in Southern Morocco. Letters represent homogeneous groups based on protected least significant difference test (LSD) for each variable at ( $P < 0.05$ ) ( $n = 5$ ). The middle boxplot line represents the median (Q2) while the (x) spots represent the mean. Quartiles Q1 and Q3 were calculated accordingly for each treatment. Error lines represent the maximum and minimum values, respectively.

#### 3.4. Physico-Chemical Soil Characteristics and Their Interaction with Organic Crops Nematode Communities

Principal component analysis of the soil characteristics (Table 6) across the surveyed organic farming systems (Figure 7) showed that the fraction of variance accounted for by the first two PC axes is 25.19% and 18.15% (eigenvalues), respectively. A loading plot of the soil factors (Figure 7A) indicated that the PC1 axis was related to mineral content (mainly Mn and Fe) alongside with sand content (S) in negative PC values and to phosphorus (P), total organic matter (TOM), and electrical conductivity (EC) in positive PC values. The PC2 axis was related to potassium, C/N ratio and, to a lesser extent, pH and Mn content. A canonical correspondence analysis (Figure 7B) was used to visualize the relationship between soil factors and PPN abundance. The first axis explains 48.5% of the variance while the second axis explains 33.7%. *Trichodorus* spp., *Rotylenchulus* spp., and *Meloidogyne* spp. were linked to seven edaphic factors (TOM, Mg, Zn, Cu, EC, P, and Na). Meanwhile, *Helicotylenchus* spp., *Paratylenchus* spp., *Ditylenchus* spp., *Longidorus* spp. and *Criconimoides* spp. were seemingly connected to potassium (K) and C/N ratio. The significance of this relationship was assessed using an enhanced heatmap (heatmap-2) (Figure 7C). It was clearly indicated by the analysis that all PPN genera identified were significantly correlated with pH, Mn content, and limestone (Lim). Interestingly, soil texture (sand, clay, and silt content) was also positively correlated with many PPN genera. For instance, *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Longidorus* spp. and *Criconemoides* spp. significantly occurred in sandy soils, whereas, *Paratylenchus* spp., *Xiphinema* spp. and *Meloidogyne* spp. were more abundant in clay soils. With the exception of *Ditylenchus* spp. and *Trichodorus* spp., most of the nematodes were not attracted or slightly attracted to silt content. Regarding mineral contents, few (Mn and Mg) were shown to have a significant positive relationship with all PPNs, thus promoting their occurrence, while the rest (K, N, Na, P, Cu, and Fe) were not affiliated very closely with those pests. Unlike most of them, *Helicotylenchus* spp. showed decent activity represented by high abundance in the presence of high calcium (Ca) concentrations in soil. Electrical conductivity slightly affected two genera (*Meloidogyne* spp. and *Rotylenchulus* spp.) while organic matter (OM) (humic (HOM) and total (TOM)) had a positive impact on some nematode communities (*Paratylenchus* spp., *Tylenchus* spp. and *Criconemoides* spp.).

**Table 6.** Physico-chemical soil characteristics analyzed in the CCA and heatmap analyses, and their corresponding codes.

Soil Characteristics	Code
<i>Granulometry</i>	
Clay	Cl
Sand	San
Silt	Sil
<i>Organic matter</i>	
Organic matter (Total, Humic)	OM (TOM, HOM)
Nitrogen	N
pH H <sub>2</sub> O	pH
Limestone	Lim
<i>Soil solution</i>	
Calcium	Ca
Phosphorus	P
Iron	Fe
Magnesium	Mg
Manganese	Mn
Potassium	K
Sodium	Na
Zinc	Zn
Copper	Cu
Electrical Conductivity	EC
Carbon to Nitrogen Ratio	C/N



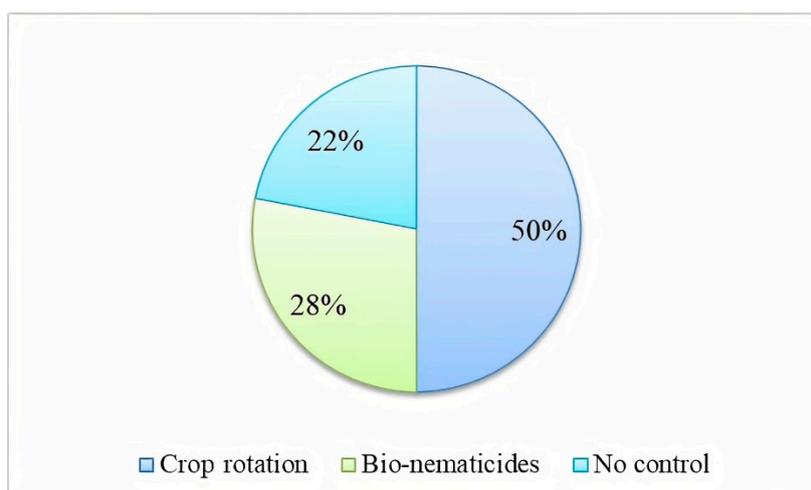
**Figure 7.** Physico-chemical soil characteristics in all organic farming systems surveyed. (A) PCA loading plot for the soil characteristics. (B) Canonical correspondence analysis (CCA) showing the relationship between nematode communities of organic crops and soil properties. (C) Advanced heatmap (enhanced heatmap-2) showing the population structure of nematode genera in organic crop fields in different farming systems surveyed. Ward’s clustering algorithm was applied to the Spearman dissimilarity matrix of nematode distribution in organic crop fields in interaction with physico-chemical soil characteristics. The upper dendrogram represents soil patterns. The left dendrogram represents the nematode genera. The color key scale represents the normalized Row Z-score of nematode abundance (per 100 cm<sup>3</sup> of soil). Genus and soil codes are given in Table 1; Table 6.

### 3.5. Control Measures of Plant Parasitic Nematodes Adopted by Organic Farmers in Southern Morocco

In Southern Morocco, the most important PPNs associated with organic farming of vegetables and medicinal and aromatic plants were *Meloidogyne* spp. and *Pratylenchus* spp. To reduce their population densities in the soil, organic farms used several strategies (Table 7 and Figure 8). The results of our questionnaire revealed that 50.9% of the organic vegetable farmers adopted crop rotation to reduce the densities of PPNs below the economic threshold. Specifically, these farmers grew alfalfa, corn, and sorghum in three-year crop rotations. Additionally, 23% of farmers who cultivated medicinal and aromatic used bio-nematicides (Table 7). Moreover, 5.8% of organic tomato farmers grew cover crops (*Tagetes* spp. and *Ricinus communis* L.) in attempts to reduce the population of *Meloidogyne* spp. However, 20.3% of the surveyed farmers did not use any of the above strategies.

**Table 7.** Product name, active ingredient, mode of treatment, and application rate of biological nematicides used in Morocco.

Product Name	Formulation	Active Ingredient	Product Category	Dose	Treatment Mode	Crops	Max. Appli.
SESAMIN EC	EC	Sesame Oil (70%)	Nematicide	10l/ha	Drip irrigation systems	Bean, Eggplant, Lentil, Pea, Pepper, Tomato, Watermelon, Melon, Zucchini	4
SEYLAND	WP	<i>Bacillus subtilis</i> (strain IAB/BS03) $7.5 \times 10^6$ CFU	Nematicide	5 kg/ha $\times 2$	Soil treatment	Bean	-
NEMGUARD GRANULES	GR	Garlic extract 450 g/kg	Nematicide	25 kg/ha	Soil treatment	Tomato	1
OIKOS	EC	Azadirachtine (31.95 g/L)	Insecticide	1L/ha	Drip irrigation systems	Tomato	-
BIOACT WG	WG	<i>Paecilomyces lilacinus</i> strain 251 ( $6\% (1 \times 10^{10})$ spores/g)	Nematicide	4 kg/ha	Soil treatment	Banana, Strawberry, Tomato	-



**Figure 8.** Control measures for nematodes adopted by organic farmers in most organic farming systems in Souss-Massa Morocco.

## 4. Discussion

### 4.1. Distribution and Diversity of PPN Communities in Some Moroccan Organic Farming Systems

This study gives initial baseline information on the level of PPN diversity and abundance related to organic farming systems in Morocco, including vegetables, medicinal, and aromatic plants. Among the identified 12 PPN genera and 10 species, *Meloidogyne* and *Pratylenchus* were the predominant genera, which have also been reported in organic crops cultivated in other countries, including Egypt [14,50–52], Germany [13], and USA (Minnesota) [11]. This agrees with Kranti et al. [53] and Kranti and Nishi [54] who reported that *Meloidogyne* spp., and *Pratylenchus* spp. were the most damaging PPNs in the organic farming system. The variability of PPN genera detected in this study could be attributed to higher crop diversity grown in organic farms. Moreover, their distribution could be affected by environmental factors, production systems, and cultural practices employed in, particularly, the practice of vegetable intercropping with other crops, mainly maize. *Meloidogyne* spp. as the most predominant genus occurring in 72.6% of sampled organic crops with a mean density of 24 nematodes (100 cm<sup>3</sup> soil)<sup>-1</sup> in the current study, was reported as the most abundant in conventionally grown vegetables in Southern Morocco [17,18]. Although, our results indicate that the maximum densities of *Meloidogyne* spp. detected in infested vegetable fields, including bean (29 nematodes per 100 cm<sup>3</sup>), and tomato fields (9 nematodes per 100 cm<sup>3</sup>) were below the damage thresholds defined by Di Vitto et al. [55] and Di Vito and Zaccheo [56] (0.55 eggs or juveniles/cm<sup>3</sup> soil). Hallmann et al. [13] reported a 51% occurrence for *Meloidogyne* spp. in organic farming in Germany, which was lower than the level detected in the current study. In contrast, the lower densities found in organic farms in Southern Morocco indicated that rotation with non-host crops, including alfalfa (*Medicago sativa*), corn (*Zea mays*), and sorghum (*Sorghum bicolor*), and the use of bio-nematicide might reduce recoverable densities of *Meloidogyne* spp. to near undetectable levels. In another study, *Meloidogyne* spp. was detected in 47% and 57% of the samples collected in 2009 and 2011, respectively, in organic farming in Egypt [14]. In our study, the population density of *Meloidogyne* spp. was generally higher in vegetables than in medicinal plant farms. Furthermore, bean was the preferable host for *Meloidogyne* spp. and the lowest density was recorded on thyme. This low susceptibility of thyme to *Meloidogyne* spp. appears to be logical due to its nematicidal effect [57]. The abundance of *Meloidogyne* spp. on organic crops has been reported by numerous authors [14,15,58]. Van Bruggen and Termorshuizen [58] showed that over the past years, RKN populations increased in organic tomato farms in the Netherlands. In our study, two RKN species, namely *M. javanica* and *M. incognita*, were found on organically grown vegetables, either separately or in mixed populations. Both of these species generally occur in warmer climates and were previously recorded in conventionally grown vegetables in Southern Morocco [16,17]. Recently, *M. arenaria* was detected for the first time in conventional eggplant (*Solanum melongena* cv. Black beauty) production farms in Southern Morocco [18].

The next most predominant PPN was *Pratylenchus* spp., occurring in 68.8% of all sampled organic crops, which was also the most dominant genus with 90% occurrence in organic farming in Germany [13]. The incidence of *Pratylenchus* spp. in organic farming in Southern Morocco can be considered higher when compared to those previously reported from organic farming in Egypt (20%) [14], and in the Philippines (60%) [15], whereas Hallmann et al. [13] reported a higher percentage of *Pratylenchus* spp. (89%) in organic farming in Germany. In addition, in the current study, the occurrence of this genus in all samples varied between crop types. As expected, *Pratylenchus* spp. was present in significantly more bean and onion sites than tomato and other aromatic and medicinal sites, given that both organic bean and onion growers practiced rotation with cereals, which are considered as preferred hosts of this genus [13]. Moreover, this genus poses a serious threat to cereal and saffron production in Morocco [59,60] and species of *Pratylenchus* have been reported to form disease complexes with a large number of soil-borne fungi, thus causing root disease [61]. Population densities of *Pratylenchus* spp. ranged from 2 to 13 vermiform stages in soil (100 cm<sup>3</sup> soil)<sup>-1</sup> and 2 to 6 vermiform stages in roots (20 g root)<sup>-1</sup>. Unfortunately, there is no information in the literature regarding the damaging population

threshold for *Pratylenchus* spp. in organic farming systems. Moreover, our results indicate that the population densities encountered may not present a potential risk to organically grown crops when compared with economic threshold densities reported on other crops (e.g., cereals) [62]. In organically grown vegetables in Germany, Hallmann et al. [13] reported that the population density of *Pratylenchus* spp. detected in soil samples (572 nematodes (100 cm<sup>3</sup> soil)<sup>-1</sup>) was higher compared to our results. This can be explained by the different rotation practices such as with cereals, which promote the multiplication of this genus [13]. In this current study, four species of *Pratylenchus*, viz. *P. penetrans*, *P. thornei*, *P. neglectus*, and *P. crenatus* were found, which was in agreement with what has been previously reported in organic farming in Germany [13], the United States [63,64], as well as in conventional wheat and saffron cultivations in Morocco [16,60]. *Pratylenchus penetrans* has a broad host range of more than 400 different plants, as the most damaging species [65]. A previous survey in four cereal-growing areas of Morocco revealed that *P. penetrans* was the most dominant species [59]. Berkelmans et al. [23] showed that the density of *Pratylenchus* spp. was higher in organic fields than in conventional fields. *Helicotylenchus vulgaris* was also an important nematode in Southern Morocco, affecting 40.2% of the sampled organic farms with a population density of up to 7 nematodes per 100 cm<sup>3</sup> of soil. In previous studies, *H. vulgaris* was recorded in around 59% and 30% of the sampled Moroccan saffron fields of Taliouine and Taznakht, respectively [60], and 6.5% of the organically grown vegetable fields in Germany [13]. In another study, Adam et al. [14] reported the presence of *Helicotylenchus* spp. in 40.7% of organic farming fields in Egypt. Species of this genus can increase susceptibility to plant-pathogenic fungi enabling them to gain access to the host cells [66]. *Tylenchus* spp. are root-hair feeders [67], which were detected in different crops, affecting 29% of the sampled organic farms. However, this genus mostly feeds on fungi and lower plants in the soil [68]. Ectoparasitic nematodes, such as *Xiphinema* spp., *Longidorus* spp., and *Trichodorus* spp. were the least abundant nematodes, affecting 12.4%, 2%, and 2% of sampled organic crops, respectively. Hallmann et al. [13] reported only the presence of both *Longidorus* spp. and *Trichodorus* spp. in organically grown vegetables in Germany. Adam et al. [14] reported the presence of *Xiphinema* spp. in organic farming in Egypt. In our study, the average density of *Xiphinema* spp. was lower than that recorded by the same genus on vegetable organic farming [14]. Recently, *X. diversicaudatum* was declared for the first time in Morocco in conventional onion production farms with an average of nine nematodes per 100 cm<sup>3</sup> of soil [19]. Several diversity indexes were determined in our study (nematode abundance, Shannon and Evenness diversity indexes, and PPI. Plant Parasitic Index can be useful as a bioindicator to assess the dynamics of PPNs in soil [69]. The value found was similar to the results reported by Thoden et al. [70] who indicated that crop fields in association with organic soil amendments had increased the plant-feeding nematode biomass. Cultural practices could accurately limit the natural succession of agroecosystems and promote the normal growth and development of organic crops, which leads to high values of PPI as observed with rosemary and thyme in our study.

#### 4.2. Effect of Soil Characteristics on PPN Abundance in Moroccan Organic Farming

Understanding the correlation between abiotic (soil physico-chemical) patterns and PPNs is essential to investigate whether their distribution, diversity, and behavior are influenced and to implement appropriate management approaches [71]. Soil can directly or indirectly influence the PPN competition aspects (e.g., PPN soil occupation and competition for root infection) within nematode communities and plays a crucial part in the nematode habitat [72]. The canonical correspondence and advanced heatmap analyses indicated a significant relationship between some soil traits and PPN abundance. Our study underlined that soil structure positively affected nematode distribution. Van Diepeningen et al. [73] demonstrated the effect of soil type and texture on the PPN populations in both conventional and organic farming systems and they found a strong influence. Similarly, clay, sand, and silt contents were significantly ( $P < 0.05$ ) associated with the PPN communities in this study and could separately define PPN population structures [74,75]. Previous studies [76–78] revealed the same results with loamy and clay soils. The sand content effect on PPN occurrence on organic crops is due,

probably, to its irreversible impact on soil temperature and pH as well. The thermal conductivity in sandy soils is considerably higher than soils with high clay contents, which may be the reason behind their significant relationship with PPN patterns. In a recent study, Mokrini et al. [79] indicated that several identified PPNs (*Helicotylenchus* spp., *Rotylenchulus* spp., *Longidorus* spp., *Criconemoides* spp., *Paratylenchus* spp., and *Xiphinema* spp.) have a strong association with some soil granulometry (soil textures) in raspberry (*Rubus idaeus*) based on their abundance. Prot and Van Gundy [80] found that sandy soils seemed to be the preferred ecosystem for nematodes such as *Meloidogyne* spp. and *Pratylenchus* spp., as it can promote their mobility capacity. A similar result was observed by Prasad and Rao [81] with *Tylenchorhynchus* spp. In contrast, high reproduction rates of *Pratylenchus* [82,83] and *Ditylenchus* [84] were obtained in clay soils. Moreover, many PPNs (e.g., *Aphelenchoides*, *Pratylenchus*, *Rotylenchidae*, and *Tylenchidae*) were shown to have tolerance in Southern Morocco coarse soils [85]. Soil pH had also a major role and its effect on all identified PPNs in organic crops was relevant. An acidic pH might be one of the reasons for increased PPN abundance, as the highest performance of RKN was observed for pH ranging from 4.5 to 5.4 [86]. Similar studies confirmed this phenomenon [87–92]. As for mineral content, several inputs were not related significantly to the PPN community, except for Mn, Mg, and P. The spiral nematode (*Helicotylenchus* spp.) was significantly attributed to calcium content. Mokrini et al. [60] highlighted a strong affiliation between PPNs and soil mineral contents (Ca, Cu, K, Mn, and Zn) in saffron. Moreover, numerous studies have demonstrated the strong relationship between soil minerals (particularly Fe, P, and Mg) and nematode species distribution in some organic crops [76,93]. Francl [94] showed a positive relationship between the density of *Heterodera glycines* and the level of magnesium. Positive correlations were obtained between nematode densities and soil exchangeable bases (Mg and K) for RKN in tobacco [95] and tomato [96], and for *Scutellonema* spp., in beans, *Crotalaria*, *Tephrosia*, and *Sesbania* spp. cropping systems [97]. Georgieva et al. [98] noticed that soil minerals (Cu and Zn) had a negative impact on the nematode community structure, decreasing genus richness and maturity indices of free-living nematodes. On the other hand, according to our findings, nitrogen does not appear to have any attraction or depletion effect on nematodes. Benjlil et al. [99] indicated that most of the genera identified in saffron (*Crocus sativus* L.) showed a negative association with total nitrogen (N), which can be explained by the accumulation of nitrate through nitrification that is considered to be harmful to PPNs [100,101]. Similarly, Oteifa [102] showed that the input of nitrogen to the soil had decreased drastically the population of *M. incognita* in beans crops. However, in another study P was found to be positively correlated with *M. incognita* while an increase of *Pratylenchus* spp. abundance was observed likely due to an increase in superphosphate application [103]. The total and humic soil organic matter tended to reduce significantly the nematode community structure and decrease their vital activities. Organic matter also can affect the reproduction rate of nematodes [104], which probably can explain our results as defined by the significant decrease of PPNs in organic farming systems. Recently, Mokrini et al. [60] emphasized that organic matter was negatively correlated with PPN patterns in Saffron. This trend was recorded in the same cropping system by Benjlil et al. [99]. Organic matter accumulation in soils causes a significant decrease in nematode abundance [105,106]. Alternatively, soil organic matter contents were positively correlated with free-living nematodes [107], probably due to microbial community (bacteria and fungi) influence, which could increase significantly these nematode population abundances and contribute to plant growth [108,109]. The ratio C/N, which monitors the contribution of carbon and nitrogen in agricultural soils, was positively related to our identified PPNs. Nematode community changes were reported to show a significant correlation with high organic carbon rates [110]. These results were similar to ours as the majority of PPNs identified were significantly abundant in soils with high carbon levels.

#### 4.3. PPN Management in Organic Farming Systems of Southern Morocco

Organic farming has the same PPN issues as conventional farms [22]. Nonetheless, their control is more difficult in organic farming than conventional farming due in particularly harsh restrictions and rules about the use of curative synthetic chemicals such as fumigants. Generally, the small organic

farmers in Morocco have a rudimentary understanding of PPNs associated with various crops and therefore, lack the knowledge to manage infestation caused by PPNs. However, the results of our questionnaire showed that 81% of organic growers in Southern Morocco, had practiced different control methods to reduce nematode numbers, such as crop rotation, soil amendments, and biological nematicides. Crop rotation was applied by 50.9% of organic vegetable farmers to reduce the densities of PPNs below the economic threshold. However, the effectiveness of this approach in reducing PPNs depends on several factors, in particular, the type of nematode species and the duration time for which nematode species can survive in the field in the absence of their respective hosts [111]. In the current study, onion and bean organic growers grew alfalfa, corn, and sorghum in three-year crop rotations. Our results showed that the mean density of *Meloidogyne* spp. was very low in onion fields (3 nematodes (100 cm<sup>3</sup> soil)<sup>-1</sup>) and bean fields (11 nematodes (100 cm<sup>3</sup> soil)<sup>-1</sup>), compared to conventionally grown vegetables in Southern Morocco [17]. Crop rotation practiced in the organic farms with non-host crops is certainly responsible for lowering the density of *Meloidogyne* in the soil. However, crop rotation alone will not be sufficient to control the PPN population. It must also be accompanied by other agronomic practices [112]. Furthermore, Hallmann et al. [13] found that rotations with a high percentage of organic vegetables promote *Meloidogyne* spp. and *Pratylenchus* spp. populations.

Due to increased adverse effects of chemical nematicides and growing concerns for human health and environment, interest in biological nematicides has attracted more attention by both researchers and the agro-industry and has become an important component of eco-friendly management strategies [113–115]. The results of our questionnaire showed that 23% of organic farmers applied bio-nematicides for managing PPNs, specifically, *Meloidogyne* spp. in Southern Morocco, including Sesamin (derived from extracts of specific cultivars of hybrid sesame plant) and NemGuard granules, which are formulated as a micro granule containing 45% garlic extract, and contains fingerprinted polysulfides protected by four patent families with nematicidal action [116]. Makunde et al. [117] reported that Sesamin EC was effective in controlling *Meloidogyne* spp. on tobacco crops. More recently, D'Addabbo et al. [118] found that soil treatment with sesame oil effectively controlled *M. incognita* on tomato crops. Furthermore, several studies underlined the efficacy of garlic extracts against insects [119,120] and PPNs, including the pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle [121], *M. incognita* [122]. Abd-Elgawad et al. [123] underscored that soil treatment with a commercial product containing aqueous garlic extract reduced the nematode root gall index. Ladurner et al. [116] confirmed the effectiveness of NemGuard granules as a nematicide on horticultural crops. Alternatively, cover crops can be used accurately as a management method to suppress PPN population densities. The results of our questionnaire showed that both marigolds (*Tagetes* spp.) and *Ricinus communis* were frequently used by several farmers in Southern Morocco to control *Meloidogyne* species. These trap crops released  $\alpha$ -terthienyl, and cyanogenic, or ricinin compounds, respectively, which are allelopathic to many species of PPNs [124]. Ferji et al. [125] concluded that the use of *R. communis* as an organic amendment reduced the population of *M. javanica* on tomato under greenhouse conditions in Southern Morocco. Moreover, several studies have reported the efficiency of different botanical plant extracts against PPNs, including *Meloidogyne* spp. [126]. Fourie et al. [127] reported that the Brassicaceae-based cover-crop/rotation practice decreased populations of the three most economically damaging genera of PPNs, including *Meloidogyne*, *Pratylenchus*, and *Heterodera*. Oduor-Owino et al. [128] and Sturz and Kimpinski [129] showed that *Paecilomyces lilacinus* and endophytic root bacteria from *Tagetes minuta* and *T. erecta*, respectively, can play a crucial role in controlling both the eggs of *M. javanica* in tomato and *P. penetrans* in the potato root zone respectively. In our study, the maximum density of *Meloidogyne* spp. detected on tomato fields was very low, (7 nematodes per 100 cm<sup>3</sup> of soil), probably due to the association with *Tagetes* spp. and *R. communis* as a cover crop. However, as small galls were detected on the roots of tomato, a downward shift in the *Meloidogyne* population may occur with the practice of crop rotation including less susceptible crops such as cereals and maize. Regardless of their effect in controlling PPNs, both practices (rotation

and cover crop) maintain soil fertility and improve the productivity of organic farms [130]. Finally, our questionnaire revealed that 20.3% of the surveyed farmers did not use any of the above strategies.

## 5. Conclusions

Undoubtedly, PPNs, in particular *Meloidogyne* spp., *Pratylenchus* spp., and *Helicotylenchus* spp. are a serious threat to organic farming fields in Southern Morocco. The results obtained from the present study may help organic farmers to develop appropriate nematode management strategies and therefore reduce nematode populations below their threshold levels. Moreover, the analysis of the relationship between genera abundance and soil properties demonstrated that sand and pH are important soil parameters in describing nematode population variation. However, further efforts are needed to study the dynamics and community structure of PPNs in several organic farms located in different regions of Morocco and to identify nematode species with greater accuracy in establishing appropriate integrated management strategies.

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