Distribution and occurrence of plant-parasitic nematodes associated with raspberry (Rubus idaeus) in Souss-Massa region of Morocco: relationship with soil physico-chemical factors

Article *in* Russian Journal of Nematology · March 2020

DOI: 10.24411/0869-6918-2019-10011

Russian Journal of Nematology, 2019, 27 (2), 107 – 121

Distribution and occurrence of plant-parasitic nematodes associated with raspberry (*Rubus idaeus*) in Souss-Massa region of Morocco: relationship with soil physico-chemical factors

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Accepted for publication 2 August 2019

Summary. Raspberry (*Rubus idaeus*) polytunnels in Morocco's Souss-Massa region were surveyed between February and April 2018. The study was aimed to investigate the diversity and incidence of plant-parasitic nematodes affecting raspberry crop and to assess the effects of soil physico-chemical properties on the nematodes. Twelve nematode genera were identified form the soil and root samples collected from 41 raspberry polytunnels across the three provinces (Belfaa, Biougra and Khmis Ait Aimra). The most common plant-parasitic nematodes (PPN) were *Pratylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp. In terms of their abundance and frequency, four PPN (*Pratylenchus* spp., *Meloidogyne* spp., *Helicotylenchus* spp. and *Tylenchus* spp.) were abundant and frequent throughout the region. Several genera of nematodes were significantly associated with soil texture, organic matter and pH, which indicate that soil properties play an important role in PPN communities. This description of PPN assemblages associated with red raspberry polytunnels in Souss-Massa region provides a starting point from which further studies will be implemented for the other regions of Morocco cultivating raspberries, and to develop efficient management strategies.

Key words: *Helicotylenchus*, management, *Meloidogyne*, nematode survey, *Pratylenchus*, soil characteristics, *Tylenchus*.

Red raspberry (*Rubus idaeus* L.) is cultivated more or less intensively in different countries of the world such as Russia, USA, Poland, Mexico, Morocco and Serbia (McGregor, 1976; Jennings, 1988; Trager, 1995; Hummer, 2010). The total world production of raspberries is estimated to be 812.735 t year^{-1} (FAO, 2017), with Russia supplying 18% (146.377 t). Other major producers were Mexico (14.8%), Serbia (13.5%), the USA (13.0%) and Poland (12.8%). In Morocco, the main red raspberry production areas are the Loukkos and Souss-Massa regions, which cover approximately more than 2000 ha and produce 319 t year^{-1} (MAPM, 2017). During recent years, interest is increasing in red raspberry cultivation as an alternative cash crop for big farms in Souss-Massa region of Morocco.

Raspberry production decreased drastically due to biotic and abiotic factors. Among the biotic stresses, plant-parasitic nematodes (PPN) play an important role in decreasing crop yield (Gigot *et al.*, 2013). Several PPN were associated with red raspberry disease throughout the world (McElroy, 1972, 1977; Tsolova & Koleva, 2015; Kroese *et al.*, 2016), including *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Criconemoides xenoplax* and *Ditylenchus dipsaci* (Coev *et al.*, 1971; Kroese *et al.*, 2016; Pinkerton *et al.*, 2008; Poiras *et al.*, 2014). Species of dagger nematodes (*Xiphinema bakeri*, *X. diversicaudatum* and *X. americanum*) and needle nematodes (*Longidorus elegantus*, *L. macrosoma* and *L. attenuatus*) are ectoparasitic nematodes significantly associated with this crop since they transmit some devastating viruses such as Raspberry ringspot virus (RRSV) and Strawberry latent ringspot (McElroy, 1972). Due to the intensive and continuous cultivation system, soil borne diseases, especially nematodes, become an important constraint in red raspberry production.

In Morocco, red raspberry is cultivated in the South of the country, which is characterised by a wide diversity of soil types (silty clay and clay loam) and climates (arid and semi-arid). Several reports highlighted the diversity, distribution and density of PPN on various crops in Morocco (Mokrini *et al.*, 2009, 2016; Janati *et al.*, 2018) but there is currently no literature available on the occurrence and distribution of PPN associated with raspberry and their relationship with soil physico-chemical properties in Morocco. In order to guide further research, we present an extensive overview on the incidence of PPN associated with raspberry in Souss-Massa region of Morocco. This study aimed: i) to study which PPN taxa are associated with raspberry in Souss-Massa region; ii) to determine PPN occurrence and abundance in Southern Morocco raspberry growing region; and iii) to investigate the effect of soil physico-chemical properties on PPN community structure.

MATERIAL AND METHODS

Nematode survey. An intensive survey was conducted between February and April 2018 to determine the occurrence and distribution of PPN in raspberry in polytunnels located in Khmis Ait Amira (30°10'36.546" N, –9°29'20.901" E), Biogra (30°12′52″ N, 9°22′15″ O) and Belfaa (29°58'55.344" N, –9°30'19.476" E) provinces of Souss-Massa region, Morocco (Fig. 1). The number of polytunnels sampled per province was determined as a function of the importance (surface planted) of the raspberry crop. A total of 41 representative polytunnels were visited and sampled (Fig. 1), representing three provinces of the raspberry production area. In each province, eight to thirteen raspberry polytunnels were randomly selected for sampling. From each polytunnel, 10 subsamples were arbitrarily collected then mixed thoroughly to form a representative sample of 2 kg soil. Samples were kept in plastic bags and stored at 4°C before analysis to minimise changes in nematode populations (Berker *et al.*, 1969). Analyses of soil texture and composition from each surveyed polytunnel were carried out at the Regional Centre of Agricultural Research (INRA) in Agadir, Morocco, and are summarised in Table 1.

Nematode extraction and identification. Representative samples from each surveyed polytunnel were carefully mixed and processed for extraction no later than 48 h after storage. For each sample, nematodes were extracted separately from roots and soil. Roots of each sample were gently washed in tap water to free adhered soil particles, chopped into pieces (*ca* 0.5 cm) and then nematodes were extracted from a sub-sample of 20 g using a modified Baermann technique (Hooper, 1986). Nematodes were also extracted from 100 cm^3 of soil using the same modified Baermann technique used for extraction of nematodes from roots. After 48 h, nematode suspensions from both soil and roots were collected in beakers, allowed to settle for 2 h and concentrated to about 20 ml by removing excess water (supernatant) using the settling-siphon method (Caveness, 1975). Nematodes were identified to genus level using dichotomous keys (Mai & Lyon, 1975; Mai & Mullin, 1996). The Seinhorst (1959) method as modified by De Grisse (1969) was used to kill and fix the nematodes 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 diam. watch glass. This square watch glass dish was placed in a desiccator containing about one-tenth 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) was 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) was added and the watch glass was left overnight at 37°C in the incubator. The nematodes in pure glycerin from each sample were mounted on glass slides for identification to species level under a light microscope. Species of root-lesion nematodes were identified using the keys of Castillo & Vovlas (2007) and Ryss (1988).

Assessment of nematode population densities. Nematode diversity and incidence were assessed by calculating the prevalence, mean abundance and maximum density (Boag, 1993). Prevalence was defined as the number of samples having a particular nematode species, divided by the total number of samples examined, expressed as a percentage. Mean abundance was defined as the number of individuals of a particular nematode species per 100 cm^3 soil in the positive samples, divided by the number of positive samples, and maximum density was defined determined as the maximum number of individuals of a particular nematode species per 100 cm^3 soil recovered from a sample.

Physico-chemical analyses of soil. The soil analyses were carried out at the INRA soil laboratory in Agadir, using standard methods (Anderson & Ingram, 1993). The following soil properties were analysed including soil texture: proportions of clay (0-2 μm), silt (2-50 μm) and sand (50 to > 200 μm); pH and electrical conductivity EC (μS cm^{-1}) using 1:2.5 soil: water ratio methodology described by Richards (1954); exchangeable cations: potassium, manganese and magnesium; exchangeable acidity; total soil organic matter; nitrogen content; soil solution including iron, copper, zinc, sodium, and phosphorus.

Diversity of plant-parasitic nematodes. The taxon dominance parameter was calculated for each nematode genus in prospected localities together with the frequency. This parameter represents the regression between abundance and frequency for each sampling genera (Fortuner & Merny, 1973). The distribution diagram of nematodes communities was applied as abundance variables were transformed to $\log_{10}(X + 1)$ before analysis.

Statistical analysis. Principal component analyses (PCA) were applied to explore PPN community patterns and physico-chemical soil factor patterns in relation to the surveyed localities. After data normalisation using the Shapiro-Wilk normality test (Shapiro & Wilk, 1965), PPN and soil variables associated with the principal component analyses were subjected to a one-way ANOVA performed using XLSTAT 2016.02.28451 software (Addinsoft, USA). Significant differences among variables were tested by the protected least significant difference (LSD) at probability level ($P < 0.001$). An advanced Heat map analysis was used to investigate relationships between PPN and soil physico-chemical properties using R package.

RESULTS

Density and diversity of PPN associated with raspberry. PPN were detected in all polytunnels surveyed in Biougra, Khmis Ait Amira and Belfaa provinces. Twelve genera were morphologically identified from the 41 soil samples collected from the representative polytunnels (Table 2). Biougra possessed 12 genera, Khmis Ait Amira 11 genera, Belfaa possessed 9 genera, while 8 genera were common to the three regions (Table 2). Data concerning their prevalence, mean abundance and maximum density of nematodes in each province surveyed are presented in Table 2. Among the most prevalent nematodes on raspberry in Biougra province were *Meloidogyne* spp. (65%), *Pratylenchus* spp. (60%), and *Tylenchus* spp. (55%). In Khmis Ait Aimera province, the most prevalent genera were *Helicotylenchus* spp. (84.6%), *Pratylenchus* spp. (53.8%), and *Tylenchus* spp. (54%). For Belfaa province, *Helicotylenchus* spp. (75%), *Meloidogyne* spp. (62.5%), and *Pratylenchus* spp. (62%), were the most prevalent genera. The following species were identified from the randomly selected specimens: *Pratylenchus penetrans*, *P. thornei*, *Meloidogyne incognita*, *M. javanica* and *Ditylenchus dipsaci*. The density of PPN associated

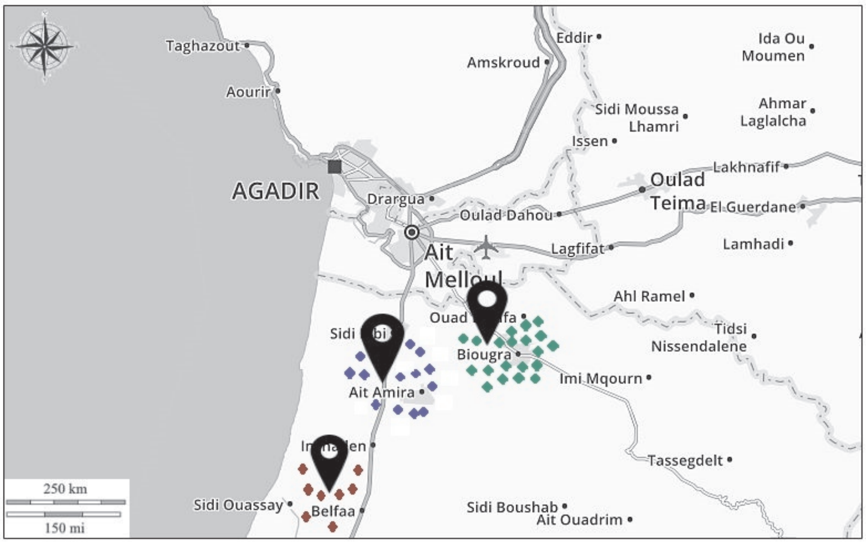

Fig. 1. Map of the agro-ecological locations in Souss-Massa region of Morocco where samples were collected from raspberry polytunnels.

Table 1. Major ecological characteristics of the surveyed eco-regions of red raspberry.

Locality (Province)	Altitude (m a.s.l.)	Soil texture	Temperature (◦C)	Rainfall (m /year)	Samples per site	Letter code
Biougra	133	Sandy clay loam	19.3	214	20	BIO
Khmis Ait Amira	97	Sandy clay loam	24.1	212	13	KAM
Belfaa	137	Sandy loam	20.0	250	8	BEL

Table 2. Prevalence, mean, and maximum density of plant-parasitic nematodes from soil (100 cm^3) sampled from red raspberry polytunnels in Souss-Massa region of Morocco.

Nematodes	Biougra			Khmis Ait Amira			Belfaa		
	Prevalence (%)	Mean abundance	Max density	Prevalence (%)	Mean abundance	Max density	Prevalence (%)	Mean abundance	Max density
Meloidogyne spp. *M. javanica* *M. incognita*	65	4	16	38	2	12	62.5	3	12
Pratylenchus spp. *P. penetrans* *P. thornei*	60	3	15	53.8	4	23	62	2	7
Ditylenchus spp.	30	1	6	15.3	1	4	50	1	2
Helicotylenchus spp.	50	2	11	84.6	2	7	75	2	5
Paratylenchus spp.	35	1	8	46.1	1	7	12.5	1	2
Tylenchus spp.	55	2	9	54	4	30	50	1	3
Tylenchorhynchus spp.	45	2	8	23	1	8	0	0	0
Longidorus spp.	5	1	1	7		3	0	0	0
Xiphinema spp.	20	1	5	0	0	0	37.5	2	7
Rotylenchus spp.	10	1	3	23	1	3	0	0	0
Criconemoides spp.	20	1	3	23	1	4	37.5	1	3
Trichodorus spp.	10	1	3	15.3	1	2	25	1	2

with red raspberry differed between the sampled regions. The total PPN densities were generally higher in Khmis Ait Amira province (45 PPN (100 cm^3 soil)$^{-1}$). The lowest density were found in Biougra (4 PPN (100 cm^3 soil)$^{-1}$) followed by Belfaa province (5 PPN (100 cm^3 soil)$^{-1}$) (Table 3). The genera with the highest mean abundance and maximum densities were *Meloidogyne* spp. (4 and 16 in Biougra, respectively; 3 and 12 in Belfaa), *Pratylenchus* spp. (4 and 23 in Khmis Ait Amira; 3 and 15 in Biougra; 2 and 7 in Belfaa), *Tylenchus* spp. (4 and 30 in Khmis Ait Amira), and *Xiphinema* spp. (2 and 7 in Belfaa) (Table 2).

Community patterns of plant associated nematodes. PCA analysis of the nematode genera distribution across total localities of the surveyed red raspberry fields showed that the fraction of variance accounted for by the first two PC axes is 19.24% and 16.72% (eigenvalues), respectively (Fig. 2A). The PC1 axis is related to *Meloidogyne*, *Longidorus*, *Ditylenchus* and *Tylenchus* species (positive PC values), and to *Pratylenchus* and *Rotylenchus* species (negative PC values). The PC2 axis is related to *Helicotylenchus*, *Paratylenchus*, *Tylenchorhynchus* and *Trichodorus* species (positive PC values), and to *Pratylenchus* and *Rotylenchus* species (negative PC values). The projection of the sample eigenvalues on the PC axes indicated that *Pratylenchus* spp., *Helicotylenchus* spp., *Paratylenchus* spp., and *Tylenchus* spp. were significantly abundant in Ait Amira region. *Meloidogyne* spp. and *Tylenchorhynchus* spp. were also more abundant in Biougra region. The Belfaa region had just two genera, *Meloidogyne* and *Helicotylenchus*, with moderate presence (Fig. 2B).

Physico-chemical soil patterns and their relationship between red raspberry nematodes communities. After soil assessment (Table 5), PCA analysis of the soil characteristics across total localities of the surveyed red raspberry polytunnels showed that the fraction of variance accounted for the first two PC axes is 30.36% and 23.05% (eigenvalues), respectively. The loading plot of the soil factors (Fig. 3A) indicated that the PC1 axis was related to sand (San), limestone (Cal), humic organic matter (HOM), electrical conductivity (EC) and mineral content (especially Na, K, Mg, N, P, Ca and Zn). Meanwhile, the PC2 axis was related to silt (Sil), clay (Cla), soil pH and to mineral content such as (Cu, Mn and Fe). The Bi-plot of soil factors in interaction with sampling regions indicated an important differentiation between all surveyed localities

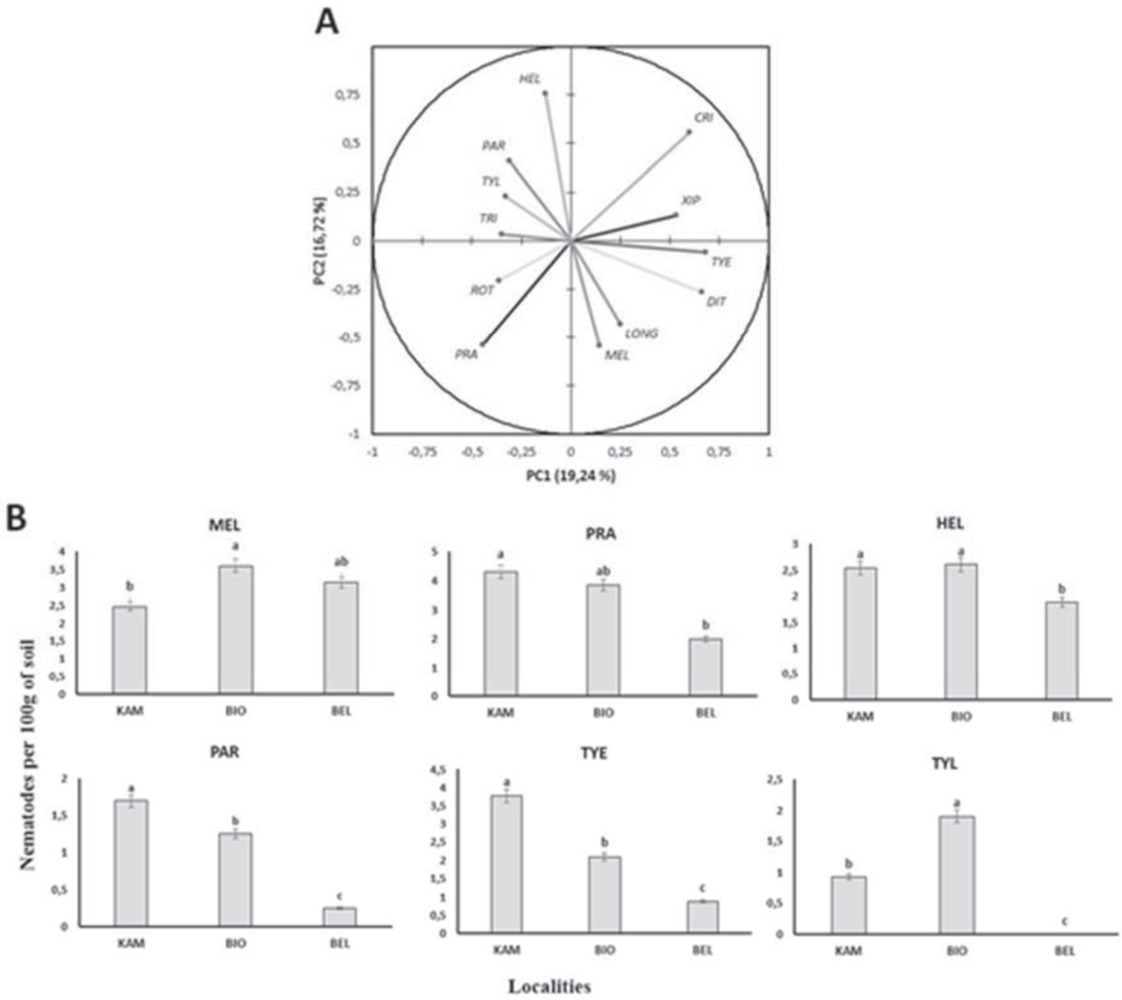

Fig. 2. Plant-associated nematode community patterns in all surveyed red raspberry fields. A: PCA loading plot for the nematode genera identified. B: Mean value of the discriminant nematode variables according to the PCA analysis. Letters represent homogeneous groups based on protected least significant difference test (LSD) for each variable at ($P < 0.001$). Error lines on the bars represent the standard error. Provinces and genus codes are given in Tables 1 and 4.

in terms of soil patterns (Fig. 3B). The Belfaa province was shown to be significantly associated with six soil characteristics, including electrical conductivity (EC), organic matter: humic (HOM) and total (TOM), and some mineral content such as zinc (Zn) and nitrogen (N). By contrast, the Biougra province was strongly associated with sand content (San) of soil together with some minerals (Mg, K and Na). The remaining province Khmis Ait Amira was especially associated with clay (Cla) and silt (Sil) content of soil and to its pH parameter. As several nematodes persisted with red raspberry crop in the sampled polytunnels, it is natural for them to have an affiliation with the physico-chemical characteristics of soil. For this reason, an advanced heat map analysis was conducted to determine if this interaction exists (Fig. 3C). The analysis clearly indicated that the majority of nematodes genera identified were positively correlated with soil texture (sand, clay or silt content); *Helicotylenchus*, *Rotylenchus*, *Longidorus* and *Criconemoides* genera were associated with clay content (Cla), whereas, *Paratylenchus* and *Xiphinema* were most likely associated with sandy soils. Except for the last two, all genera were moderately attracted to silt content. Concerning mineral characteristics, a few (Mn, Ca, Cu and Zn) were shown to have good association with some nematode genera such as *Criconemoides*, *Meloidogyne* and *Ditylenchus* while the others (K, N, Na, P and Mg) are not associated with those genera. Electrical conductivity, pH and total organic matter (TOM) had an impact on nematodes communities as they had a strong correlation with the majority of genera.

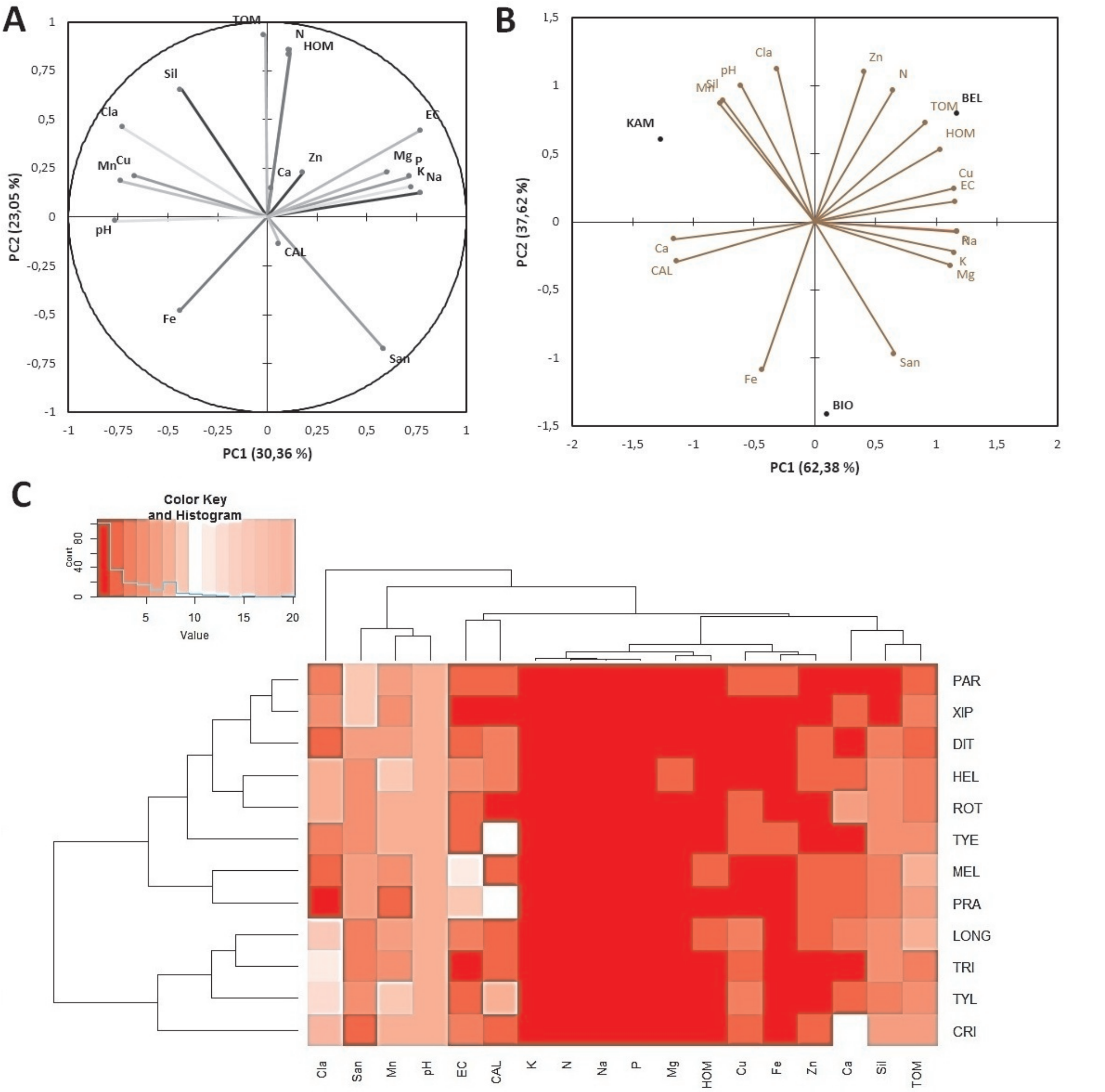

Fig. 3. Physico-chemical soil characteristics in all the surveyed provinces of red raspberry. A: PCA loading plot for the soil characteristics. B: Bi-plot of soil characteristics in interaction with the regions prospected. C: Advanced Heatmap of population structure of nematodes genera in red raspberry fields in different regions surveyed. Ward's clustering algorithm was applied to the Spearman dissimilarity matrix of nematode distribution in red raspberry fields in interaction with physico-chemical soil characteristics. Upper dendrogram represents soil patterns. Left dendrogram represents the nematode genera. The color key scale represents normalised nematode abundance (per 100 cm^3 of soil). Provinces, soil and genus codes are given in Tables 1 and 4.

Diversity of plant associated nematodes communities of red raspberry. The diversity of nematodes on red raspberry from the surveyed fields was described in term of abundance and frequency of those pests (Fig. 4). Among 12 genera identified, *Helicotylenchus*, *Pratylenchus*, *Meloidogyne* and *Tylenchus* were more abundant and frequent, while *Paratylenchus*, *Tylenchorhynchus*, *Ditylenchus*, *Criconemoides* and *Xiphinema* were shown to have a moderate abundance and frequency. *Trichodorus*, *Rotylenchus* and *Longidorus* had the lowest diversity.

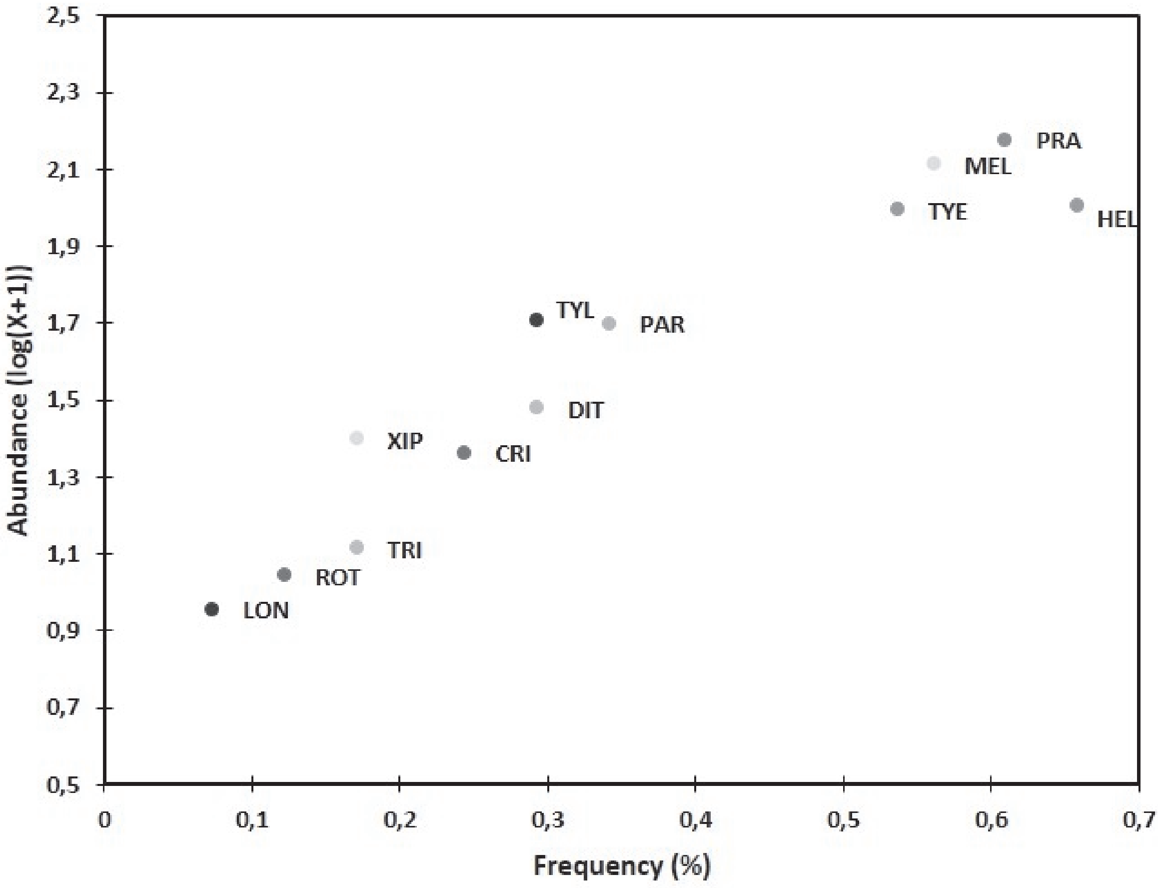

Fig. 4. Distribution diagram (Abundance-Frequency) of nematode communities in red raspberry fields from all surveyed provinces. Genus codes are given in Table 4.

DISCUSSION

This study is the first to demonstrate the widespread occurrence, distribution and frequency of PPN in raspberry polytunnels in Morocco. The results indicated that 12 genera of both endoparasitic and ectoparasitic plant nematodes were identified in raspberry polytunnels in Souss-Massa region. Several studies reported that PPN are major pests of raspberry reducing yield and cane growth, and leading to economic losses in eastern Scotland and Canada, respectively (Trudgill, 1986; Belair, 1991). From all genera identified, species of *Meloidogyne* and *Pratylenchus* are known to be among the top PPN for scientific and economic importance (Jones *et al.*, 2013). Other nematode genera reported in this study have been previously observed on raspberry fields from several regions of the world (Belair & Khanizadeh, 1994; Zasada *et al*., 2015; Mohamedova & Samaliev, 2018). Belair & Khanizadeh (1994) identified six genera in raspberry fields from seven agricultural regions of the Quebec province and they concluded that the most commonly encountered nematode genus was *Pratylenchu*s, with 46% occurrence. Poiras *et al.* (2014) reported 27 nematode species belonging to 19 genera associated with raspberry in Moldova. Similarly, Romanenko *et al.* (2014) described nine PPN genera in the rhizosphere of raspberries growing in Moscow region of Russia. The root-lesion nematode *Pratylenchus* spp. was one of the dominant nematode genera among PPN, found in 60, 53.8 and 62% of samples collected from Biougra, Khmis Ait Amira, Belfaa provinces, respectively. This widespread distribution of *Pratylenchus* spp. in all surveyed polytunnels is consistent with results from earlier surveys of cereal and saffron fields in Morocco (Mokrini *et al*., 2016, 2019). The impact of this genus on raspberry cultivation needs further study. Two species of *Pratylenchus*, *viz. P. penetrans* and *P. thornei*, were

Table 3. Densities of plant-parasitic nematodes associated with red raspberry in each polytunnel surveyed representing three provinces of Souss-Massa region.

Provinces	Polytunnels surveyed	Plant-parasitic nematodes (PPN) (numbers per 100 cm^3 of soil)												Total
		***Meloidogyne* spp.**	***Pratylenchus* spp.**	***Ditylenchus* spp.**	***Helicotylenchus* spp.**	***Paratylenchus* spp.**	***Tylenchus* spp.**	***Tylenchorhynchus* spp.**	***Longidorus* spp.**	***Xiphinema* spp.**	***Rotylenchus* spp.**	***Criconemoides* spp.**	***Trichodorus* spp.**	
Khmis Ait Amira	P1	0	0	0	4	0	2	0	0	0	0	0	0	6
	P2	0	0	0	2	6	0	0	0	0	0	0	0	8
	P3	0	0	0	1	2	0	0	0	0	0	0	0	3
	P4	0	0	0	3	1	0	0	0	0	0	0	1	5
	P5	5	0	0	4	3	3	0	0	0	0	0	0	15
	P6	4	0	1	2	0	2	0	0	0	0	0	0	9
	P7	6	2	0	7	0	30	0	0	0	0		0	45
	P8	0	4	0	2	3	9	2	0	0	3	2	0	25
	P9	5	17	0	2	0	1	0	0	0	0	0	2	27
	P10	12	23	0	0	0	0	0	0	0	2	0	0	37
	P11	0	2	4	1	7	2	2	3	0	1	1	1	24
	P12	0	7	0	5	0	0	8	0	0	0	0	0	20
	P13	0	1	0	0	0	0	0	0	0	0	4	0	5
Biougra	P14	4	3	0	0	0	0	0	0	0	0	0	0	7
	P15	2	2	0	0	2	1	0	0	0	0	0	0	7
	P16	0	0	0	11	0	0	0	0	5	0	3	0	19
	P17	0	0	0	0	0	8	0	0	0	0	0	0	8
	P18	2	0	0	0	0	2	0	0	0	0	0	0	4
	P19	1	0	0	9	0	3	6	0	0	0	0	0	19
	P20	0	0	0	5	7	0	4	0	0	0	2	0	18
	P21	5	7	0	2	0	0	0	0	0	0	0	2	16
	P22	0	12	2	0	0	0	3	4	3	0	0	0	24
	P23	16	15	0	2	3	0	3	0	0	1	0	0	40
	P24	4	0	3	0	0	0	8	0	0	0	0	0	15
	P25	12	2	0	1	0	6	0	0	1	0	0	0	22
	P26	6	0	3	1	2	9	1	0	0	0	3	0	25
	P27	0	3	2	5	0	2	0	0	0	0	0	0	12
	P28	0	3	0	0	0	0	4	0	0	3	0	0	10
	P29	2	8	0	9	1	1	0	0	0	0	0	3	24
	P30	1	7	1	7	8	2	8	0	0	0	0	0	34
	P31	11	3	6	0	0	7	0	1	2	0	1	0	31
	P32	6	9	0	0	2	0	0	0	0	0	0	0	17
	P33	0	3	0	0	0	1	1	0	0	0	0	0	5
Belfaa	P34	0	2	0	3	0	2	0	0	1	0	0	0	8
	P35	3	7	0	2	0	1	0	0	0	0	1	0	14
	P36	2	0	2	2	0	1	0	0	5	0	0	1	13
	P37	0	0	2	5	0	0	0	0	0	0	2	0	9
	P38	7	0	1	1	0	0	0	0	0	0	0	2	11
	P39	12	1	0	2	0	0	0	0	0	0	0	0	15
	P40	1	3	0	0	2	3	0	0	7	0	3	0	19
	P41	0	3	2	0	0	0	0	0	0	0	0	0	5

Table 4. Physico-chemical soil characteristics and plant-parasitic nematode genera analysed in the PCA and heatmap analyses, and their corresponding codes.

Soil characteristics	Code	Nematode genera	Code
Granulometry			
Clay	Cla	*Criconemoides*	*CRI*
Sand	San	*Ditylenchus*	*DIT*
Silt	Sil	*Helicotylenchus*	*HEL*
Organic matter	OM	*Longidorus*	*LONG*
Total organic matter	TOM	*Meloidogyne*	*MEL*
Humic organic matter	HOM	*Paratylenchus*	*PAR*
Nitrogen	N	*Pratylenchus*	*PRA*
pH H_2O	pH	*Rotylenchus*	*ROT*
Limestone	Lim	*Trichodorus*	*TRI*
Soil solution		*Tylenchus*	*TYE*
Calcium	CAL	*Tylenchorhynchus*	*TYL*
Phosphorus	P	*Xiphinema*	*XIP*
Iron	Fe		
Magnesium	Mg		
Manganese	Mn		
Potassium	K		
Sodium	Na		
Zinc	Zn		
Copper	Cu		
Electrical Conductivity	EC		

detected in all surveyed provinces. Several studies have reported the presence of *Pratylenchus* spp. in the raspberry rhizosphere (Romanenko *et al.*, 2014; Zasada *et al.*, 2015). In the USA, according to McElroy (1977) and Kroese *et al.* (2016), one of the most important factors limiting the raspberry production is the presence of *P. penetrans*. Similarly, Zasada & Walters (2016) and Zasada *et al.* (2015) reported the presence of *P. penetrans* in non-bearing raspberry fields in the Pacific Northwest region of North America. In our study, population densities of *Pratylenchus* spp. ranged from seven (Belfaa province) to 23 (Khmit Ait Amira province) per 100 cm^3 of soil. Other species of this genus are known to parasitise raspberry, such as *P. crenatus* and *P. scribneri* (Mohamedova & Samaliev, 2018), and *P. pratensis*, *P. sonvallatiae* and *P. neglectus* (Tsolova & Koleva, 2015). The root-knot nematodes *Meloidogyne*, particularly *M. javanica* and *M. incognita*, were encountered in 65%, 38% and 62.5% of the polytunnels sampled in Biougra, Khmis Ait Amira and Belfaa provinces, respectively. In Morocco, both species were reported recently in several crops including saffron (Mokrini *et al.*, 2019) and vegetables (Janati *et al.*, 2018). Mohamedova & Samaliev (2018) and Belair & Khanizadeh (1994) reported both *M. hapla* and *M. arenaria* as most abundant species of genus *Meloidogyne* in the raspberry fields in Bulgaria and Quebec, respectively. In our study, the absence of both species *M. hapla* and *M. arenaria* is related to the warm climate that characterises the Souss-Massa region of Morocco. Several studies reported that the northern root-knot nematode *M. hapla* occurs in cold region of crop production (Taylor *et al.*, 1978; Wu *et al.*, 2018). Nematode vectors of plant viruses belonging to genera *Xiphinema* and *Longidorus* have been considered as the most damaging PPN to raspberry (Poiras *et al.*, 2014; Romanenko *et al.*, 2014). In the current study, both *Xiphinema* spp. and *Longidorus* spp. were detected in low densities, which varied in different provinces surveyed between 1 and 7 nematodes (100 cm^3 soil)$^{-1}$.

In addition, this study highlighted the possible interaction between nematode communities and soil physico-chemical proprieties of soil where the red raspberry is cultivated in Souss-Massa region of Morocco. This interaction led to an understanding of the effect of edaphic proprieties on PPN distribution in raspberry growing localities and it is a crucial step in their control, since this relationship can be complex and is influenced by environmental factors (Nielsen *et al.*, 2014). The advanced heat map analysis showed strong correlations between nematodes and soil characteristics. Such relationships were indicated in previous studies (Robinson *et al.*, 1987; Zoon *et al.*, 1993). Our findings indicated that several PPN (*Helicotylenchus* spp., *Rotylenchus* spp., *Longidorus* spp., *Criconemoides* spp., *Paratylenchus* spp. and *Xiphinema* spp.) were identified have a strong relationship with some soil granulometry (total organic matter, silt, clay and sand) content based on

Table 5. Physico-chemical analyses of each red raspberry polytunnel surveyed.

Location Sand	(%)	Silt (%)	Clay (%)	Total Organic Matter (%)	Humic Organic Matter (%)	N (%)	CAL (%)	P (%)	Na (%)	K (%)	Mg (%)	Ca (%)	Fe (%)	Mn (%)	Cu (%)	Zn (%)	pH (H_2O)	EC (µs/cm)
1	59.21	38.89	1.9	7.56	1.81	0.14	21.11	0.3	0.248	0.556	0.953	1.916	0.8	4.6	0.7	1.4	7.5	1172
2	62.23	37.62	0.15	5.58	1.1	0.05	9.7	0.259	0.178	0.463	1.054	2.505	1.2	2.4	0.8	1.6	7.6	909
3	60.42	39.48	0.1	3.19	0.68	0.07	10.77	0.102	0.149	0.44	0.577	1.474	0.6	3.7	0.7	2	7.93	251
4	70.19	29.66	0.15	3.87	0.71	0.08	9.75	0.075	0.188	0.973	0.758	7.516	0.4	3	1.2	1.2	7.53	738
5	46.57	37.33	16.1	4.33	1.49	0.07	6.48	0.102	0.053	0.181	0.447	1.326	1	8.8	2.2	1.3	7.81	125
6	48.01	37.94	14.05	5.55	1.3	0.09	5.11	0.075	0.056	0.042	0.52	1.768	1.3	7.2	1.6	1.1	7.96	393
7	52.63	34.77	12.6	4.16	0.73	0.06	7.96	0.123	0.062	0.025	0.318	1.621	1.2	8.2	2.7	1.2	8.06	150
8	27.94	56.81	15.25	6.86	1.45	0.11	15.62	0.082	0.007	0.044	0.888	3.537	1	5.5	2.8	2.1	7.98	289
9	82.42	12.98	4.6	2.73	0.57	0.05	11.74	0.136	0.059	0.019	0.491	1.474	1.2	4.3	1.1	0.8	7.78	131
10	62.77	31.13	6.1	3.93	0.88	0.07	7.37	0.286	0.03	0.209	1.472	2.8	0.8	3.6	1.1	1.4	7.41	330
11	61.02	37.48	1.5	2.39	0.26	0.05	18.43	0.075	0.069	0.139	0.419	2.505	1.2	4.3	1.2	1.5	7.67	124
12	46.50	51.6	1.9	3.04	0.66	0.07	19.2	0.082	0.089	0.116	0.548	1.326	0.6	5.1	1.5	1.2	8.01	173
13	66.00	32.6	1.4	2.36	0.29	0.06	27.24	0.082	0.109	0.139	0.346	1.179	1.2	6	0.9	1.4	7.62	137
14	51.33	41.36	7.3	3.04	0.33	0.05	27.44	0.068	0.119	0.232	1.508	1.474	1	8.2	0.8	1.6	7.48	492
15	55.42	30.98	13.6	3.76	0.77	0.08	18.47	0.109	0.099	0.185	0.397	1.179	0.8	7.1	1.2	1.7	7.82	214
16	38.58	52.17	9.25	3.39	0.33	0.07	6.22	0.027	0.035	0.114	0.606	1.474	1.3	7.3	0.8	0.9	7.91	216
17	55.59	40.56	3.85	2.95	0.35	0.06	8.19	0.014	0.03	0.107	0.346	7.074	1.3	6.4	0.9	1.5	8.01	145
18	30.57	55.83	13.6	5.31	0.98	0.1	15	0.041	0.06	0.197	0.462	3.095	0.8	4.5	1.2	0.9	7.8	203
19	47.06	45.04	7.9	2.96	0.35	0.06	12.48	0.007	0.045	0.153	0.274	5.453	1.3	7.7	1.4	0.9	7.99	174
20	14.38	65.47	20.15	5.54	0.8	0.09	15.86	0.048	0.069	0.199	0.433	10.611	1.2	7.3	2	1.7	7.92	281

Table 5. (continued) Physico-chemical analyses of each red raspberry polytunnel surveyed.

21	36.11	52.49	11.4	3.75	0.26	0.04	26.61	0.02	0.056	0.167	0.404	1.326	1.3	4.2	1.5	0.9	7.95	114
22	75.45	16.4	8.15	3.59	0.75	0.06	10.05	0.034	0.048	0.158	0.296	1.768	1.2	4.8	1.8	1.2	7.71	173
23	90.62	5.63	3.75	2.11	0.29	0.02	14.18	0.075	0.056	0.211	0.274	1.032	2.1	5.6	2.4	0.9	7.94	164
24	43.93	52.27	3.8	5.07	0.93	0.08	9.73	0.048	0.06	0.218	0.397	1.179	1.5	8	1.8	1.1	7.93	146
25	66.42	33.53	0.05	3.97	0.89	0.09	9.65	0.164	0.119	0.371	0.996	1.326	0.2	3.8	0.8	1.2	7.45	863
26	83.09	16.81	0.1	2.33	0.13	0.05	9.4	0.136	0.169	0.533	0.52	1.326	1	2.5	0.5	2.5	7.97	319
27	63.14	36.71	0.15	3.9	0.77	0.1	10.38	0.075	0.149	0.301	0.852	7.958	1.2	4.6	1.6	1.3	7.47	266
28	72.24	27.51	0.25	2.69	0.56	0.05	42.7	0.17	0.109	0.209	0.505	7.074	1.5	3.4	0.7	1	7.49	467
29	83.77	16.18	0.05	2.16	0.38	0.07	6.8	0.15	0.119	0.209	1.364	5.158	1.5	3.4	0.9	0.7	7.43	366
30	62.77	31.13	6.1	3.93	0.88	0.07	7.37	0.286	0.03	0.209	1.472	2.8	0.8	3.6	1.1	1.4	7.41	330
31	62.00	34.6	2.4	3.36	0.31	0.05	28.24	0.072	0.106	0.132	0.34	1.18	1.4	5	0.7	1.5	7.52	135
32	52.34	42.33	8.31	4.08	0.32	0.06	25.44	0.069	0.12	0.237	1.504	1.47	1	8.3	0.8	1.7	7.47	482
33	52.42	32.98	14.6	2.76	0.7	0.08	17.47	0.106	0.098	0.176	0.387	1.18	0.9	8.1	1.5	1.6	7.95	224
34	48.53	53.17	8.24	4.39	0.31	0.07	6.41	0.029	0.045	0.115	0.806	1.475	1.5	7.4	0.9	0.6	7.97	213
35	72.66	32.14	5.12	3.86	0.82	0.09	7.3	0.291	0.041	0.309	1.622	2.5	0.7	4.6	2.1	2.4	7.61	432
36	62.01	36.48	1.4	3.33	0.25	0.06	17.43	0.065	0.079	0.134	0.422	2.605	1.2	5.3	1.3	1.3	7.7	134
37	45.50	52.6	1.91	3.06	0.56	0.07	18.2	0.083	0.09	0.115	0.54	1.325	0.7	7.1	1.5	1.4	8.15	178
38	55.60	42.56	2.85	3	0.36	0.06	9.19	0.015	0.031	0.108	0.335	8.072	1.5	5.4	1	1.5	6.01	155
39	32.54	45.83	14.6	7.31	0.95	0.13	15.2	0.061	0.05	0.196	0.46	4.075	0.7	6.5	1.3	0.9	8.82	210
40	46.05	45	8.9	2.92	0.34	0.07	13.45	0.085	0.055	0.154	0.276	6.451	1.3	7.8	1.6	0.7	7.79	178
41	34.38	66.47	22.15	5.56	0.89	0.09	13.86	0.068	0.089	0.2	0.485	8.611	1.2	7.5	2	1.6	7.95	285

their degree of abundance. Prot & Van Gundy (1981) found that sandy soils seem to be the favoured habitat for nematodes, such as *Meloidogyne* spp. and *Pratylenchus* spp., as it can enhance their mobility. The same result was observed by Prasad & Rao (1980) with *Tylenchorhynchus* spp. Our study also reported the correlation of these pests with soil mineral content (Cal, Cu, K, Mn and Zn). Many studies have demonstrated the strong relationship between soil minerals and nematode species distribution in bulbous crops (particularly Fe, K, and Mg) (Ardakani *et al.*, 2014; Fiscus & Neher, 2002; Yavuzaslanoglu *et al.*, 2012). Francl (1993) reported a positive relationship between the density of *Heterodera glycines* and the level of Mg. Moreover, Mateille *et al.* (2014) detected a strong correlation between soil minerals and nematode communities and concluded that nematodes such as *Hemicycliophora* spp., *Longidorus* spp., and *Merlinius* spp. colonise carbonated and mineralised soils and they are more commonly found in coarse textured soils under oligotrophic conditions. Gaidashova *et al.* (2009) observed that *P. goodeyi* population densities were significantly and positively correlated with soil K in banana eco-regions. Low pH can also enhance nematode abundance (Cadet *et al.*, 1994; Korthals *et al.*, 1996; Norton, 1989), which was confirmed in our study. However, negative correlations were observed between nematode population densities and soil pH on *P. brachyurus* in pineapple (Sarah *et al.*, 1991), and between Mn on *H. dihystera* and *X. elongatum* in sugarcane (Cadet *et al.*, 2004). By contrast, positive correlations were demonstrated between nematode abundance and soil exchangeable bases (Mg and K) for root-knot nematodes (*Meloidogyne* spp.) in tobacco (Kincaid *et al.*, 1970) and tomato (Dabiré *et al.*, 2007), and for *Scutellonema* spp. in maize/beans, *Crotalaria*, *Tephrosia* and *Sesbania* spp. cropping systems (Kandji *et al.*, 2001). Cadet *et al.* (2004) mentioned that soil factors had the strongest relationship with nematode abundance (particularly *Helicotylenchus* spp. and to a lesser extent *Xiphinema* spp.) in sugarcane. Previous studies demonstrated that relationships between soil factors and nematode species could vary depending on the corresponding habitat (Noe & Barker, 1985; Francl, 1993). In terms of surveyed provinces, we found lower nematodes densities in Belfaa compared to others. This can be explained by the soil components and texture (Badra & Yousif, 1979). Belfaa has mostly sandy loam soil texture, which may not be ideal to assemble an appropriate habitat for nematodes. Manlay *et al.* (2000) reported that bulk content associated with clay texture can led to enhanced nematodes densities. The strong presence of PPN in the Biougra and Khmiss Ait Amira provinces may be considered as indicative of some improvement in the chemical characteristics of the soil. Inorganic ions also were shown to have an impact on nematodes movement (Castro & Belser, 1990) and to influence the balance between species (Villenave & Cadet, 1999), which could explain why the variation of frequency and abundance of red raspberry nematodes on the surveyed provinces depended on the soil type. This suggests that soil properties play an important role in PPN communities.

In conclusion, this study provides general information on genera diversity, incidence and distribution of PPN in raspberry polytunnels of the Souss-Massa region in Morocco and reveals a high diversity of economically damaging PPN. This description of PPN assemblages and their relationship with soil physico-chemical properties provide a starting point from which appropriate nematode management strategies can be implemented. However, more intensive observation including yield loss estimate are required to have better understanding of the relative importance of PPN in Moroccan raspberry polytunnels.

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Fouad Mokrini, Salah-Eddine Laasli, Driss Iraqui, Ahmed Wifaya, Abdelaziz Mimouni, Gül Erginbas-Orakci, Mustafa Imren and Abdelfattah A. Dababat. Распространение и встречаемость паразитических нематод растений, связанных с малиной (*Rubus idaeus*) в регионе Сусс-Масса, Марокко: связь с физико-химическими свойствами почвы.

Резюме. Малина (*Rubus idaeus*), выращиваемая в парниках, была исследована с февраля по апрель 2018 года в регионе Соус-Масса, Марокко. Цель исследования – изучить разнообразие и частоту появления паразитических нематод, влияющих на урожай малины, и оценить влияние физико-химических свойств почвы на этих нематод. Двенадцать родов нематод были определены из образцов почвы и корней, собранных из 41 парника в трех провинциях (Belfaa, Biougra и Khmis Ait Aimra). Наиболее распространенными паразитическими нематодами были виды из родов *Pratylenchus*, *Meloidogyne* и *Helicotylenchus*. Что касается их численности и частоты встречаемости, то четыре рода (*Pratylenchus*, *Meloidogyne*, *Helicotylenchus* и *Tylenchus*) были многочисленными и частыми по всему региону. Несколько родов нематод были значительно связаны с текстурой почвы, органическим веществом и pH, что указывает на то, что свойства почвы играют важную роль в сообществах паразитических нематод растений. Это описание комплексов нематод, связанных с парниками, в которых возделывается красная малина в регионе Сусс-Масса, обеспечивает отправную точку, с которой будут проводиться дальнейшие исследования для других регионов выращивания малины в Марокко, а также для разработки эффективных стратегий управления.