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Study on plant parasitic nematodes (*Pratylenchoides* Winslow, 1958, *Ditylenchus* Filipjev, 1936 and *Longidorus* Micoletzky, 1922) infecting durum wheat (*Triticum durum* Desf., Poales Poaceae) in Beni-Slimane (Algeria)

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ABSTRACT The study on plant parasitic nematodes (*Pratylenchoides* Winslow, 1958, *Ditylenchus* Filipjev, 1936 and *Longidorus* Micoletzky, 1922) infecting durum wheat (*Triticum durum* Desf.) like a waha variety in the pilot farm of Si Achour (Medea) revealed that three taxa of phytoparasite nematodes belong to two orders (Tylenchida and Dorylaimida). The degree of infestation by these parasites varies from plot to plot and within the same plot. Variation in infestation is the result of several factors related to the growing system and environmental conditions. The study was conducted in two fields of durum wheat that have different textures throughout the vegetative cycle. The objective was to study the dynamics and density of nematodes under the influence of a few various physical-chemical factors. Besides, nematodes' soil and root were extracted respectively by the Cobb (1918) method (so-called "bucket" method) and the Baermann (1917) method that are modified using Coyne et al. (2010). At both experimental sites, 3 species of phytoparasitic nematodes were recorded during the maturation stage. These species include *Pratylenchoides* sp., *Ditylenchus* sp., *Longidorus* sp. and some saprophagous nematodes. Among them, *Ditylenchus* sp. and saprophagenenes represent the vast majority of individuals found in the soil. Alternatively, durum root nematodes are represented mainly by two species: *Pratylenccoides* sp. and *Ditylenchus* sp.

KEY WORDS Algeria; Hard wheat; Plant parasitic nematodes; soil types.

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INTRODUCTION

In 2021, the cereal production in Algeria was estimated at 3.5 million tons. According to the statistical services of the Algerian Directorate of Agricultural Services, this production is spread over an area of 3,385,560 ha, which is below the five-year average and about 38% less than the year 2020 and has no longer covered the needs of the population since 1970 (Smadhi & Zella, 2009).

Plants are exposed to many stressors during their life cycle and this has a significant effect on plant growth. Biotic (pathogens, competition with other organisms) and abiotic (drought, salinity, radiation, high or freezing temperatures, ...) stress cause changes in the normal physiological functions of all plants. The water deficit is undoubtedly the most hostile of all natural hazards because it affects the growth and development of plants and causes a significant reduction in agricultural production worldwide. The water deficit is revealed in the plant by a series of changes that affect morphological, physiological, biochemical, and gene expression levels (Gerszberg & Hnatuszko-Konka, 2017).

The degradation of the yield of durum wheat has reached a threshold economically unbearable to the Algeria state. The region of Medea is part of the cereal zones of Algeria hit by the drought and the manifestation of several bio-aggressors that contributed to a decrease of the output of the wheat as recorded here. Among these, the current state of infestations and distribution of plant parasitic nematodes in this region of Algeria is the subject of this study.

Nematodes are among the most important faunal groups in soil food webs and serve as important bioindicators in evaluating subsurface ecological processes and ecosystem functioning (Liu et al., 2022).

In Algeria, several researchers have revealed the presence of cyst nematodes in several cereal growing areas (Mokabli et al., 2016; Righi et al., 2017; Rahim et al., 2020). On the other hand, studies on the presence of filiform plant parasitic nematodes (NPP) in soil and roots in the region of Medea are null. The overall objective of the present study is to evaluate the infestation status of the two experimental sites of different textures, throughout the vegetative cycle of the Waha variety of durum wheat by the so-called plant-parasitic nematodes (free-living) and other saprophagous nematodes that feed on the organic matter during their decomposition, to study the evolution of the population density according to the vegetative stages. Hence the necessity of a nematological analysis which constitutes the only reliable diagnosis since it allows a quantitative estimation of the nematode populations present in the soil and the roots and their identification for a good phytosanitary management of these territories.

MATERIAL AND METHODS

Study area

The study was conducted during 2017–2018, in the E.U.R.L. pilot farm of Si Achour. It is located in the plain of Béni-Slimane, commune of Béni-Slimane at approximately 5 km from the commune of Bouskène and 65 Km South-East of the chief town of the wilaya of Médéa at an average altitude of 600 m.

The average annual rainfall is 420 mm with a poor interannual and inter-seasonal distribution. The farm is inserted between the following Lambert coordinates: X= 551,600 m at 555,040 m - Y= 323,250 m at 324,800 m

Material

The plot is cultivated with durum wheat (*Triticum durum* Desf., Poales Poaceae) type of Waha variety with a surface area of 10 ha from which we delimited our experimental plot. It was estimated at two hectares (2 ha) for each experimental site.

We sampled the soil to conduct some physico-chemical analyses in order to see the difference in the edaphic factors found between the two sites.

The experimental design is a total randomization with 2 experimental sites. The first is in a clay loam soil and the second in a clayey limestone soil. Each of the two experimental sites has 6 replicates, including 18 sampling points. These were collected in 36 samples of 5.4 Kg (for each site, and for each phenological stage).

Soil samples were taken near the plants with a trowel where the roots were located at a depth of 0 to 30 cm zigzagging to cover an area of approximately 2 ha or the part of a field that appeared to be affected. These sub-samples were collected and transferred in a sealed plastic bag clearly identified with the producer's name, address of the sampling site and date of collection. The crop history of the field and soil texture were also recorded for each vegetative stage. These soil samples were then sent to the nematology laboratory of the University of Khemis-Miliana in Ain Defla. Sampling was conducted from the emergence stage until harvest had started. Populations of most plant parasitic nematodes in the soil tend to peak in this period once the aerial part of the crops has wilted or died (Celetti & Potter, 2006).

Many nematode species multiply abundantly during the plant growth period and decrease during the bad season (dry season) (Coyne et al., 2010). For this reason, sampling is conducted in five growth phases (emergence, tillering, bolting, heading and maturation).

However, a second sample was taken concerning the root system. Three root samples were taken one meter apart (108 samples for each experimental site and morphological stage). Such distributions make it necessary to sample a large number of plants and measure the infestation of a plot. Roots were collected at the same time and location as the soil samples (Coyne et al., 2010).

After sampling, samples for extracting nematodes with roots were stored in a refrigerator (approximately 10 °C) to keep them in good conditions and to ensure the survival of the parasite. We consider 300 g per sample and ½ g per root nematode extraction.

Nematode extraction, identification and data processing and analysis

The extraction of nematodes from the soil was performed using Cobb (1918) method (buckets). A soil fraction of 300 cm^3 was taken per sample and subjected to the three phases of this extraction method: the elutriation phase, the sieving phase and the active filtration phase. This method separates the nematodes from soil impurities based on their mobility. After extraction, nematode identification was conducted using biological criteria such as body shape, stylet shape and head bristles. The nematode count was conducted using the counting plate under the binocular magnifying glass. Population counting was expressed in several nematodes/dm^3 of soil (N/dm^3). The extraction of nematodes from the roots was performed using the method of Baerman (1917) modified by Coyne et al. (2010). The root sample was washed, cut into small pieces, weighed and fixed the weighing on ½ g of fresh roots. Then, the cut pieces were placed in the bottom of a Petri dish filled with water. This method is suitable for sedentary endoparasitic nematodes.

Migratory endoparasitic nematodes migrate out of the roots into water. We can count them using a counting plate under a binocular magnifying glass (G 1.6 x 10). Nematode population numbers are expressed as the number of nematodes / ½ g of fresh roots (N/ ½-g roots).

The identification of the species was conducted at the Laboratory Institute of Nematology for Sustainable Plant Protection (CNR), Bari (Italy).

For accurate identification of nematodes, we took approximately 10 samples of soil and root nematodes individually. The population of nematodes fixed for morphological identification coincided with the maturation stage of durum wheat at the two sites.

Species identification was based on the slides of the permanent supports according to the Hooper (1970) standard. The nematodes were fixed in formaldehyde at 4% + 1% propionic acid and transformed into glycerin. Specimens were examined under an optical microscope.

Three groups of variables were considered in our study: host plant variables, pest variables (infestation status) and variables related to edaphic parameters to investigate the link between them and their impact; especially on the distribution of nematodes.

The data obtained were entered into the Excel spreadsheet and subjected to analysis of variance (ANOVA). The means were compared using the Tukey test at the 5% probability level. Pearson correlation analyses were performed to explore relationships between total trophic group abundance, edaphic parameters, and site infestation levels. Analyses of variance were performed using the software statistix 9.0 (Analytical, Software, Talahassee, FL, USA).

RESULTS

The results related to texture and chemical characteristics are reported in Tables 1−3, and Figs. 1, 2.

The abundance of trophic groups of nematodes changes with environmental soil conditions (Solhenius et al., 1987). Several factors favor the development of plant parasitic nematode populations like climate, cropping system and soil. Algerian soils offer favorable characteristics to the development of nematodes. These factors include texture, pH and organic matter (Hammache, 2010).

- The characteristic profile of these soils (A) is of type C (granular soils) and LC (labile carbon). The horizon appears brown to dark brown. The structure is lumpy with many small limestone pebbles and crust debris following upwelling by plowing.

- Profile (B), iron sesquioxide (or manganese) soils - red soils formed under a Mediterranean climate - red soils with a calcium reserve and little leached - on alluvium - deep - clayey. The average abundances of nematodes and trophic groups found on durum wheat Waha vary, according to the vegetative stages, explain the large increases in nematode population density. At the two wheat sites, 3 species of plant parasitic nematodes were recorded in the soil, as well as some saprophagous nematodes: *Pratylenchoides* sp., *Ditylenchus* sp. and *Longidorus* sp. Among them, *Longidorus* sp. with saprophagous nematodes represent the vast majority of individuals with a population density exceeding 4N/dm^3 in both sites. After this first group, two nematode species *Pratylenchoides* sp. and *Ditylenchus* sp. were second in importance, with population densities not exceeding 2 N/dm^3 at the maturation stage of the two sites (Fig. 3).

Horizon	Granulometry %				
	C	FS	CS	FS	CS
0–40	37	23.6	18	18.9	2.6
40–100	Gravel + rolled stones				
>100	72	23	10	8	2.9

Table 1. The percentage grain size of the profile (A). C: Clay, FS: Fine Silt, CS: Coarse Silt, FS: Fine Sand, CS: Coarse sand.

Horizon	Granulometry %				
	C	FS	CS	FS	CS
0 – 50	62	8.7	13.3	14	1.6
50 – 100	77	4.7	10.9	6.5	0.6

Table 2. The percentage grain size of the profile (B). C: Clay, FS: Fine Silt, CS: Coarse Silt, FS: Fine Sand, CS: Coarse sand.

Parameters	Soil type	
Chemical components	**CC Soil**	**SC Soil**
pH	8.09–8.13	8.02–7.80
Ec (µs/cm)	540	412
Limestone (%)	24	2.6
Carbon (%)	0.26	1.09
Organic matter (%)	0.45	1.87

Table 3. Chemical analysis results for both soil types. CC: Calcareous Clayey, SC: Silty Clayey.

The population density of plant nematodes, recorded at the upstream stage, ranged from 9 to 70 N/ ½-g roots at site I (Fig. 8).

In addition, from 11 to 53 N/ ½ g of the root in site II was registered at the tillering stage. However, at the maturation stage, *Pratylenchoides* sp. and *Ditylenchus* sp. were the two nematode species found in roots with an infestation rate ranging from 2 to 10 N/ ½ g of the root in site I whereas in site II it ranged between 4 and 16 N/ ½ g (Figs. 4−7).

The results of the variance analysis studied at the two sites show that the difference in the number of root plant nematode parasites in the two sites has a very highly significant difference. Therefore, the Waha variety is similar to this variance analysis in calcareous clayey and silty clayey soil. So, the texture variable affects this parameter.

The analysis of variance concerning the variable of the number of nematodes found in the soil shows us a very highly significant difference between the two sites. Similarly, between vegetative stages (Table 5).

Results of the analysis of the variance analysis of the variable concerning the degree of infestation (L2/g soil) reveal a very highly significant difference at the threshold ($p<0.001$). Therefore, the host variety influences this parameter (Table 6).

There are some positive relationships between the variables in the 1st site. Concerning the Waha variety of durum wheat. However, others are negative like the number of soil filiform nematodes (SNF) with %CaCO3 and electrical conductivity (EC). The only positive correlation is that of NFS with the degree of soil filiform nematode infestation

Figure 1. Soil profile (A). Figure 2. Soil profile (B).

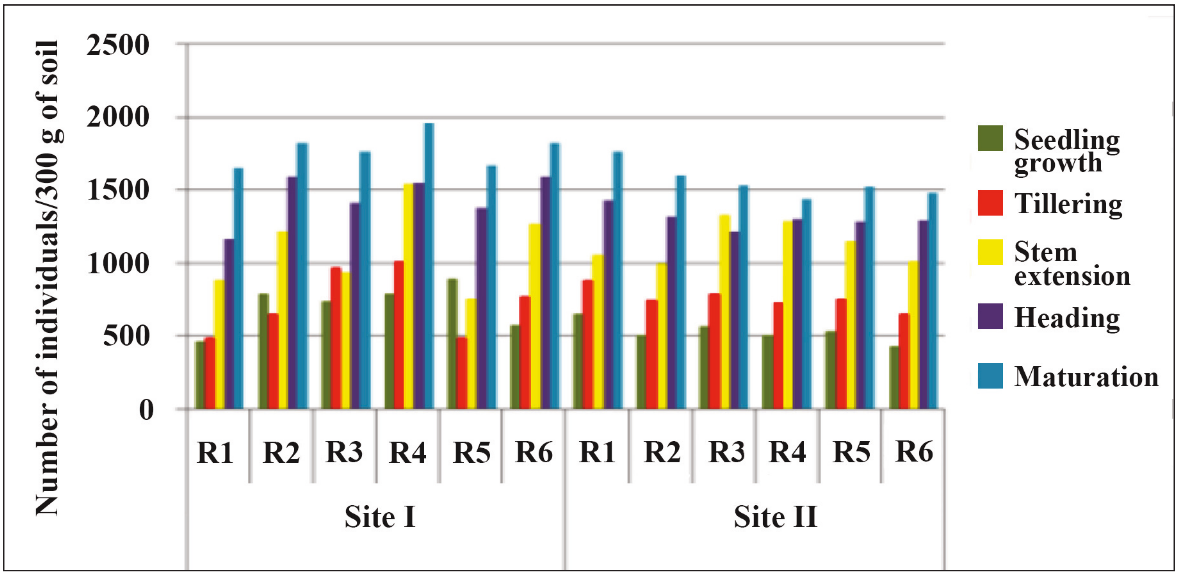


Figura 3. Variation of the average densities of the trophic group of nematodes in soil.

(1.000). It is highly significant. However, the other correlations are non-significant. The correlation matrix also shows a negative relationship with the number of NFS and DINF for the variable limestone content (CaCO3). Concerning the number of root phytoparasitic nematodes, the organic carbon rate and the organic matter content, the correlation matrix shows no correlation between the other parameters studied. Nevertheless, in the second site the correlation matrix shows the existence of correlations, some positive and very highly significant at the 0.001 threshold with the different parameters studied, between the number of SFN and DINF (1.000) and between the OM rate and the CO rate (0.998). The other correlations are negative and significant at the threshold $p<0.05$, such as those between the number of NFRs and the organic carbon rate (−0.587); organic matter and the number of FRs (−0.563) and those negative but not significant.

DISCUSSION

Soil nematodes occupy a vital position in soil food webs and belowground ecosystems (Becerra et al., 2014; Sun et al., 2019). Considerable evidences showed that soil nematodes participate directly or indirectly in various soil ecological processes, such as decomposition of organic substances and mineralization of nutrients (Barrett et al., 2008; Wang et al., 2017; Ranoarisoa et al., 2018).

Variable	SC Effect	DL Effect	MC Effect	SC Error	DL Error	MC Error	F	P	CV
Root parasitic nematodes (RPN) (site I)	11982.0	4	2995.50	2741.0	20	137.05	21.86	0.0000***	33.20
Root parasitic nematodes (RPN) (site II)	6762.69	4	1690.67	1406.63	20	70.33	24.04	0.0000***	24.81

Table 4. Effect of vegetative stages on the evolution of the density of plant nematodes in roots. ***: Very Highly Significant.

Variable	SC Effect	DL Effect	MC Effect	SC Error	DL Error	MC Error	F	P	Cv
Soil filiform nematodes (SFN) (site I)	4693837.00	4	1173459.00	53845.00	20	326923.00	43.59	0.0000***	14.38
Soil filiform nematodes (SFN) (site II)	4365699.00	4	1091425.00	314847.00	20	15742.00	69.33	0.0000***	11.71

Table 5. Effect of vegetative stages on the evolution of the density of phytoparasitic and free nematodes in the soil. ***: Very Highly Significant.

Variable	SC Effect	DL Effect	MC Effect	SC Error	DL Error	MC Error	F	P	cv
Degree of infestation (Eggs and J2/g soil) (site I)	50.340	4	12.5851	6.1534	20	0.3077	40.90	0.0000***	14.51
Degree of infestation (Eggs and J2/g soil) (site II)	47.8315	4	11.9579	3.1528	20	0.1576	75.86	0.0000***	11.14

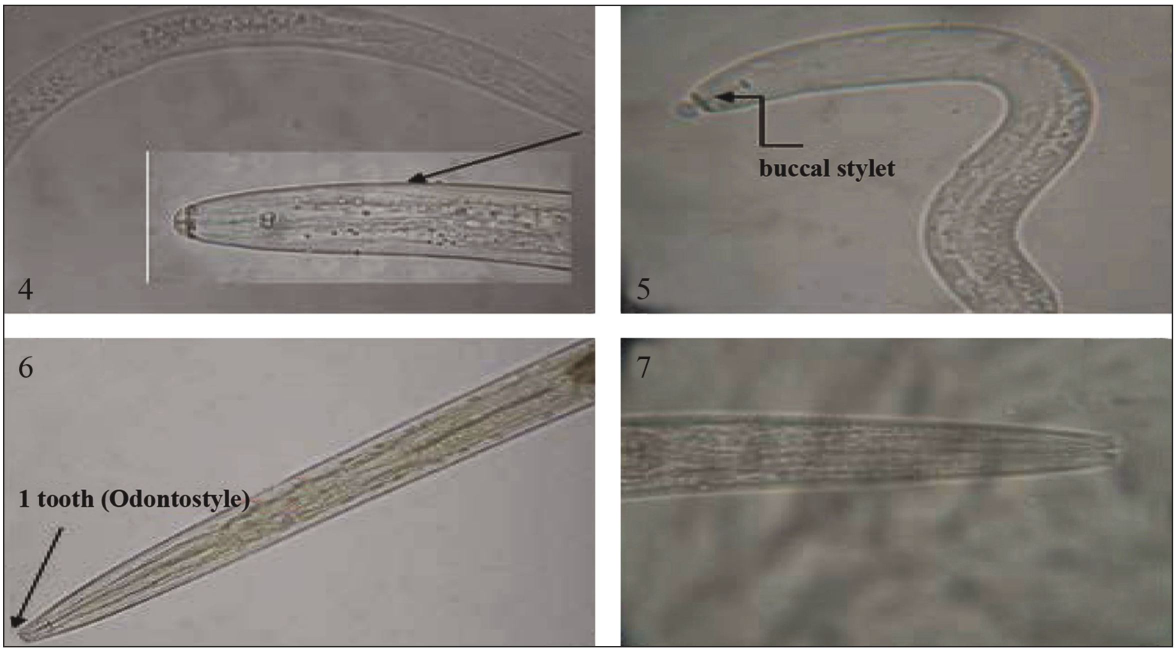
Table 6. Effect of vegetative stages on the evolution of infested samples in the soil. ***: Very Highly Significant.

Previous studies have reported that different vegetation types have significant influences on the abundance of soil nematodes (Veen et al., 2017; Kooch & Noghre, 2020). However, it remains uncertain how nematode abundance would change in the roots of a durum wheat plant based on these vegetative stages.

The second experimental site has a clay rate exceeding 62% clay while the first experimental site has a silty clay texture which means a very highly significant difference in soil nematodes between the two sites. The average number of individuals varies from 708.11 individuals/300 g of soil at the emerged stage to 1. So, the density of plant parasitic nematodes varies from 3 to 6 nematodes/dm^3. In clay soils, the average number of individuals is between 540 and 1613.30 individuals/300 g of soil. This means a density between 2 and 5 nematodes/g of soil. The difference between these two sites is insignificant compared to the results obtained by (Cadet et al., 2003, 2005). The latter noted that the densities of phytoparasitic and free nematodes vary between 0 and 2480 nematodes/dm^3 depending on species, samples and sub-parcel. These values are low compared to those found in cultivated or fallow land in the Sudano-Sahelian zone.

In our experiment, we found that the organic matter content is higher in silty clay soils than in calcareous clay soils. It varies from 0.70 to 1.87%, it is less than 0.45% in clayey soil. According to (Sohlenuis 1980), despite the nematodes high density, they contribute little to the direct mineralization of organic matter. These results disagree with several authors who have shown that nematodes are not favored by the presence of organic matter in carrot soils (Ferris & Bongers, 2006). This explains the low densities found in clayey soil. All the nematodes counted are phytophagous. They need the plant to grow.

The sampling analysis of the same durum wheat's variety in relation to its locations and stages of its development provided us with information on the spatial variation of nematodes associated with this crop. These variations are explained by changes in their ecosystems related to different cultural practices and to the bioclimatic conditions prevailing in the sites surveyed as shown by (Ritz & Trudgill, 1999). In addition, soil nematodes have been widely used as bioindicators in various ecosystems due to their extreme sensitivity to environmental disturbances, including fertilization, land use change and warming (Thakur et al., 2014; Xiao et al., 2014; Zhao et al., 2014).



Figures 4–7. Anterior parts of nematodes identified; Fig. 4: *Pratylenchoides* sp. (Pratylenchidae); Fig. 5: *Ditylenchus* sp. (Anguinidae); Fig. 6: *Longidorus* sp. (Dorylaimida); Fig. 7: A saprophagous nematode.

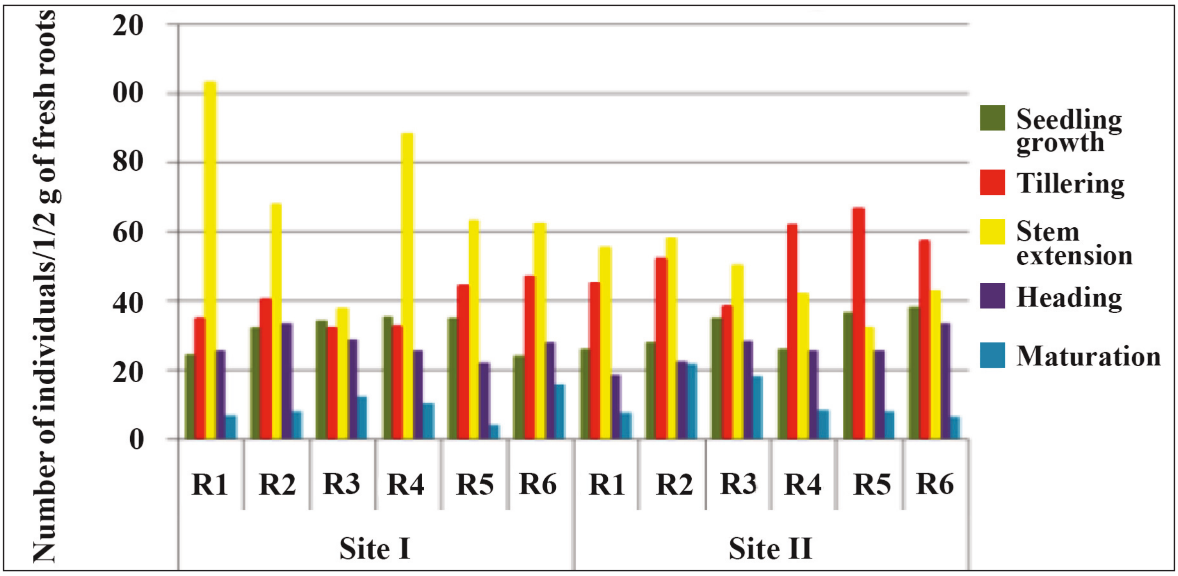


Figure 8. Evolution of densities of the trophic group of plant nematodes in the roots.

Sites	Variable	**RFN**	**SFN**	**DINF**	**%CaCO₃**	**CE**	**%CO**	**%MO**	**pH**
	RFN	1							
Site I	SFN	Ns	1						
	DINF	Ns	**1.000****	1					
	%CaCO₃	Ns	**-0.499***	**-0.499***	1				
	CE	Ns	**-0.497***	**-0.497***	ns	1			
	%CO	Ns	Ns	Ns	ns	ns	1		
	%MO	Ns	Ns	Ns	ns	ns	Ns	1	
	pH	Ns	Ns	Ns	ns	**0.586***	Ns	ns	1
Site II	RNF	1	Ns	Ns	ns	ns	**-0.587***		
	SNF	Ns	1						
	DINF	Ns	**1.000****	1					
	%CaCO₃	Ns	**-0.489***	**-0.489***	1				
	CE	Ns	Ns	Ns	ns	1			
	%CO	**-0.587***	Ns	Ns	ns	ns	1		
	%MO	**-0.563***	ns	Ns	ns	ns	**0.998****	1	
	pH	Ns	ns	Ns	**0.487***	**-0.533***	Ns	Ns	1

Table 7. Correlation matrix obtained from the main parameters studied at the two sites.
ns : non-significant; *: significant ; **; highly significant.

Chabrier (2008) noted that females reportedly used part of their reserves to search for food, develop and lay eggs (hence the reappearance of young juveniles around 70 days and sporadically around 3 and 4 months). Conversely, males who would not feed, would not have used their reserves either to search for food or to lay eggs. Their metabolic activity would have been lower.

The filiform nematode's multiplication and density in soil and roots during the growing cycle of the durum wheat waha variety is in relation to the soil type. It is called the mesological relationship which has been known for a long time. Many authors have observed that the distribution of plant parasitic nematodes is related to soil. Soil nematode communities are often not directly associated with plants, except herbivorous nematodes that feed on or parasitize plant roots (Brinkman et al., 2015). In most cases, the effects of plant communities on soil nematode communities are mediated by soil properties and microbial communities (Lv et al., 2014; Liu et al., 2017). For instance, increasing amounts of soil organic carbon generally promoted nematode abundance (Pan et al., 2020), while the abundance of bacterivorous nematodes was reported to be positively correlated with bacterial biomass (Jiang et al., 2017). The difference in the quantity and quality of nutrients from specific plants that are inputted into the soil environment might affect soil microbial communities and further affect the abundance and community composition of soil nematodes (Scharroba et al., 2012).

CONCLUSIONS

This work led to the identification of 3 taxa of plant parasitic nematodes, which belong to the following: Tylenchida, Dorylaimida and Anguinidae. The fight of these nematodes requires a perfect knowledge of these parasites, in particular their distribution and the factors determining their development. In this context, the results of this study reveal these parasites in the two experimental sites of the Si-Achour pilot farm for cultivating durum wheat which contains heavy soils and a rotation system unfavorable to the development of this parasites.

Monoculture and unsustainable rotation are contributing factors to the development of these parasites. Therefore, non-host crops with durum wheat nematode and long rotation should be introduced to limit their spread in healthy plots and their development in infested ones. Effective integrated pest management can be applied to these pests. However, the rotation remains the best way to fight these pests because they are economical, nonpolluting and environmentally friendly methods.

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