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RESEARCH ARTICLE

Spatial and molecular characterization of root-knot nematodes (*Meloidogyne*) in Algerian vegetables: impact of soil and organic matter

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Abstract

In Algeria, several species of root-knot nematodes belonging to the genus *Meloidogyne*, including *M. incognita*, *M. arenaria* and *M. javanica*, have been identified. These parasites are widespread in coastal market gardening areas and Saharan regions, where they constitute a major phytosanitary constraint, particularly for tomato crops, due to the economic losses they cause. In this survey of 369 greenhouses *Meloidogyne* infestation was detected in 168 greenhouses , with infestation rates varying by region: 100 % in Beni Saf (Ain Temouchent) and 70 % in Tipaza, while some areas of Mostaganem, Oran and Biskra showed no infestation. Furthermore, a molecular analysis using the ITS rDNA marker identified the species *Meloidogyne incognita* with a similarity of 99.54 % compared to the NeMITG7 reference sequence deposited in the GenBank database. These results are reinforced by a statistical analysis revealing a highly significant negative correlation between the organic matter content and the gall index (Spearman's Rho = -0.651; $P < 0.01$). suggesting that soils rich in organic matter are less conducive to infestation. Moreover, soil type appears to be a determining factor: clay soils showed no infestation, unlike sandy soils which recorded the highest gall indices, with an average of 6.98. These results highlight the importance of soil type and its organic matter content in root-knot nematode management and provide a basis for developing control strategies adapted to local conditions.

Keywords: Algeria; *Meloidogyne incognita*; molecular analysis; organic matter; soil type

Introduction

In the world, China is the leading producer of fruits and vegetables, its production was 506634000 tons in 2004, representing 36.62 % of world production. India is in second position, with a production of 127560000 tons, or 9.22 % of world production in fruits and vegetables (1). In the Maghreb, Algeria is in second position, after Morocco, with a production of fruits and vegetables of 5151000 tons or 0.37 % of world production (1). Vegetables are one of the most important components of the daily diet all over the world and high economic value for both small and large farmers (2).

In Algeria, market gardening under greenhouses is mainly spread in the coastal regions of the country represented by the wilayas of Tipaza, Mostaghanem, Chelif, Algiers, Boumerdes and Skikda where the area covered in 2014 reached 40 % of the country's plastic areas and ensured more than 33 % of the total production (3) (Fig. 1). However, these crops are subject to numerous bioaggressors which cause them significant losses in yield in quantity and quality (4). These pests include plant-parasitic nematodes and root-knot nematodes.

Plant-parasitic nematodes PPN are among the most widespread and damaging pests worldwide, affecting economically important crops and forests and are responsible for an estimated 12.3 % yield losses (5, 6). In the South of Russia, Root-Knot Nematoda (RKN) actively parasitize vegetable crops, reducing yields from 10 to 25 % (7). During the process of parasitism, RKNs deliver secretions through their stylet into host cells that ultimately develop into typical feeding structures, called giant cells (8). Root-knot nematodes Meloidogyne *spp.* are among the most economically damaging genera of herbivorous nematodes causing serious losses to vegetable

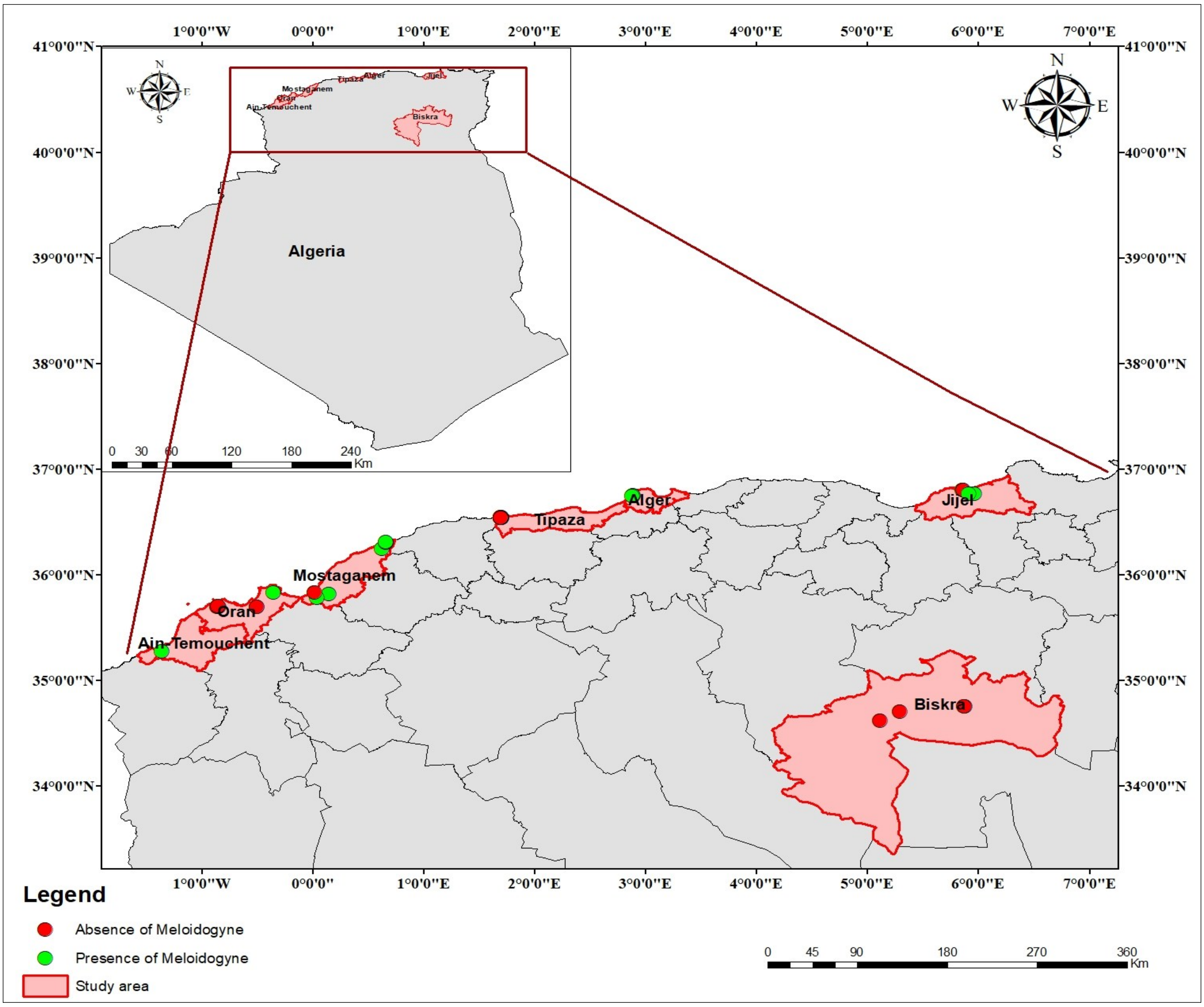

Fig. 1. Geographical distribution of *Meloidogyne sp* in different regions of Algeria.

crops on a global scale (9). The development of root galls disrupts plant nutrition and thus decreases the quality and yield of crops and may interfere with plant disease resistance (10).

Plants are infected by RKNs in the soil (11). Warm and humid regions provide ideal conditions for RKNs. These pests are highly prolific at elevated temperatures. Additionally, RKNs require humid conditions for their life cycle to progress and survive. Greenhouses can create an optimal microclimate in which the pests proliferate rapidly (12, 13). In 1928, the presence of Meloidogyne in Algeria was first reported (2). Subsequently, *Meloidogyne* species are widespread in almost all market gardening areas, where they represent a significant threat to these crops in Algeria (14, 15). Areas with high vegetable production are affected by the distribution of Meloidogyne nematodes (Algiers, Constantine, Biskra, Ouargla, Adrar) (16). In the MENA region, *Meloidogyne spp*. exceed economic nuisance thresholds by 8 to 14 times and have a frequency of occurrence of 100 % in soil samples, representing a catastrophic threat to agricultural productivity (17). According to previous study conducted on the coast of Algiers, root-knot nematodes (*Meloidogyne spp*.) are widely distributed, with infestation levels ranging from low to severe, the maximum being recorded in Aïn El Kahla 2 (18). Molecular identification confirmed the predominance of tropical species and the absence of *M. javanica* in the studied areas. In Algeria, several recent studies have highlighted the presence of antagonistic microbial agents, including *Trichoderma harzianum* and *T. afroharzianum*, some isolates of which were found to be toxic to juvenile *M. incognita* under in vitro conditions, paving the way for local biocontrol strategies (19). In neighboring countries, such as Morocco, surveys have shown a high prevalence of plant-parasitic nematodes in citrus orchards (*Tylenchulus semipenetrans*, *Helicotylenchus spp*., *Pratylenchus spp*.), with distributions closely correlated with soil physicochemical properties, confirming the importance of edaphic factors in PPN population dynamics (20).

Several factors can influence the degree of aggressiveness of *Meloidogyne* root-knot nematodes (14). These factors include the age of the greenhouse, previous cultivation, the origin of the plants, the varieties used and the nematicide products used. This study aims to conduct survey in the regions of high production in greenhouse market gardening in Algeria in order to establish a distribution map and to identify the different *Meloidogyne* species present in each region, which provides a clearer basis for developing targeted management strategies aimed at controlling populations effectively, while preserving environmental integrity and safeguarding human health.

Materials and Methods

Survey and soil sample collection

Root-knot nematode survey were carried out on 369 greenhouse farms grown at different sites in the wilayas, Oran, Mostaghanem, Ain Temouchent, Alger, Tipaza, Jijel, Biskra during two periods: from May to December 2023 and from March to November 2024.

A random zigzag sampling was carried out in the vegetable growing stations at the end of the cycle, when *Meloidogyne* infestation is generally at its highest. In each greenhouse, whole root samples were taken at different points along the zigzag line, ensuring good representation of the entire greenhouse. The number of greenhouses sampled in each region was adjusted according to the total number of greenhouses available in the region, to ensure a balanced representation of the study area.

Twenty roots from each greenhouse were collected to estimate the degree of infestation based on a gall index scale from 0 to 10 (21); these roots were kept separately in a plastic bag along with information on the sampling site and the cultivated variety and soil type.

Organic matter analysis

Soil samples weighing between 200 and 300 g were collected from the rhizosphere of plants at a depth of 10 to 30 cm to study some edaphic parameters: rate of organic matter rate for each greenhouse, three soil samples were taken as replicates. The analysis of organic matter is based on the method of calcination (loss on ignition) of a soil sample in a muffle furnace at high temperature. 1 g of soil, air-dried and ground to 0.200 mm, is weighed and placed in the crucible. The sample is then dried at 105 °C for 24 hrs to remove residual moisture. After cooling in a desiccator, the weight of the assembly (crucible + sample) is measured precisely. The crucible containing the sample is then placed in a muffle furnace and put through a gradual heating program, reaching 400 °C in 1 hr, then continuing to 550 °C, where it is held for 3 hrs. After calcination, the crucible is removed from the oven and allowed to cool in a desiccator to room temperature before being weighed again (22). The loss on ignition is then calculated using the following formula:

$$OM\,\% = ((Cn-CC) / ((Ca-CC)) * 100$$

OM: Organic matter

Cc: represents the mass of the empty crucible,

Ca: represents the mass of the crucible with the sample dried at 105°C

Cn: represents the mass of the crucible with the sample after ignition.

Molecular identification

Females of *Meloidogyne* were isolated from the roots of infested tomatoes from the Damous region of Tipaza. The nematodes were placed in sterile distilled water for DNA extraction. Nematode DNA was extracted using the nucleospin kit (Marcherey Nagel Germany). In order to identify the different species of Meloidogyne, the amplification was done by using the following conditions: initial denaturation step at 95 °C for 5 min followed by 35 cycles of 95 °C for 30s, 48 to 57 °C (as described in Table 1) for 45s, 72 °C for 45s and final elongation step at 72 °C for 7 min. The amplification products were revealed by electrophoresis on a 1.5 % agarose gel, by depositing 10 µL of PCR product followed by staining with RedGel. After migration, the DNA is visualized and photographed under UV light and purified using the Clean-up kit from Macherey-Nagel (MN, Germany). The isolated and purified PCR products were sequenced by the Sanger technique using the BigDye v3.1 kit from Applied Biosystems and by the use of primers which were used for PCR amplification (23). The sequences obtained are analyzed and cleaned using the software CHROMAS PRO. The final sequences are compared with those of GenBank data using the BLAST Program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) from NCBI for the identification of isolates. The studies were based on the percentage homology with the reference strains. Primer codes used for identification of Meloidogyne (Table 1).

Statistical analysis

The normality was verified by the Shapiro-Wilk test appropriate for small samples across tested parameters: gall index and organic matter rate. A one factor ANOVA was applied to compare the values of gall index between soil types. The means of different modalities of the factor soil type were classified according to the Tukey test's at the significance of $P \leq 0.05$ after checking the homogeneity of variances by the Levene test's. All statistical analyzes and graphical representations were processed by SPSS statistical software version 26 (IBM SPSS, 2019).

Results and Discussion

According to the results of the Survey A total of 369 market garden greenhouses were studied in the seven wilayas targeted by the survey. Among them, 168 presented confirmed infestation caused by root-knot nematodes belonging the genus *Meloidogyne*, representing an overall infestation rate of 45.5 %. The percentage of infested greenhouses is different from one wilaya to another, the most infested wilaya is Ain Temouchent, with an infestation rate of 100 % in Beni Saf (100 greenhouses out of 100 affected). It is followed by certain municipalities of Mostaganem (Ain Nouissy, Ain Sidi Cherif and Ouled Boughalem), as well as Arzew (Oran), which also display 100 % infestation. Then, Tipaza (Damous) records a high rate of

Table 1. List of primers used to identify *Meloidogyne* species

Region	Primer	Sequences 5'- 3'	Annealing temperature
ITS-rRNA	TW81	GTT TCC GTA GGT GAA CCT GC	
	AB28	ATA TGC TTA AGT TCA GCG GGT	55°C
D2-D3 of 28S rRNA	D2A	ACA AGT ACC GTG AGG GAA AGT TG	
	D3B	TCG GAA GGA ACC AGC TAC TA	48°C
18S rRNA	G18SU	GCT TGT CTC AAA GAT TAA GCC	
	R18Tyl1	GGT CCA AGA ATT TCA CCT CTC	56°C
COI	JB3	TTT TTT GGG CAT CCT GAG GTT TAT	57°C
	JB5	AGCACCTAAACTTAAAACATAATGAAAATG	

70 %, followed by Jijel (Chekfa) with 43.75 %, Jijel (Tassoust) and Algiers (Staouali) with 40 %, as well as Jijel (Tahir) with 32 % and Mostaganem (Achaacha) with 33.33 %. On the other hand, several municipalities, such as Hassi Bounif and Al Onçor (Oran), Stidia (Mostaganem), Laghrous and Sidi Okba (Biskra), show a zero infestation rate (0 %). Several localities did not benefit from any nematicide treatment. This is the case in Al onçor and Hassi Bounif in the Oran region, Achacha, Ain Nouissy and Ouled Boughalem in the Mostaganem wilaya, as well as all the municipalities of Jijel mentioned above, namely Tasoust, Chekfa and Taher. Similarly, in Ain Temouchent and Sidi Okba in the Biskra wilaya, no nematicide treatment was carried out. However, some localities were treated. In Arzew (Oran) and Ain Sidi Cherif (Mostaganem), the product Nema-dead was used, while in Stidia (Mostaganem), Mocap was applied. Finally, in the commune of El Ghrous, in the wilaya of Biskra, treatment was carried out using Vydate and Velum Prime.

Molecular identification

The molecular identification of root-knot nematodes isolated from tomato roots in the Damous region Tipaza named HFT23 was carried out using the ITS marker of rDNA. PCR amplification was performed using the primers ITS1 (TW81) and ITS2 (AB28), with an annealing temperature of 55 °C. The purified PCR products were then sequenced using the Sanger sequencing method (Fig. 2). The analysis of the obtained sequences, compared with those in the GenBank database via the BLAST program, showed 99.54 % homology with the *Meloidogyne incognita* strain (accession number NeMITG7). These results confirm the presence of this species in the Tipaza region. In addition, the sequences obtained from the Tipaza sample show a high similarity to the reference sequences of *M. incognita* available in the database, as illustrated in the nucleotide alignment (Table 2).

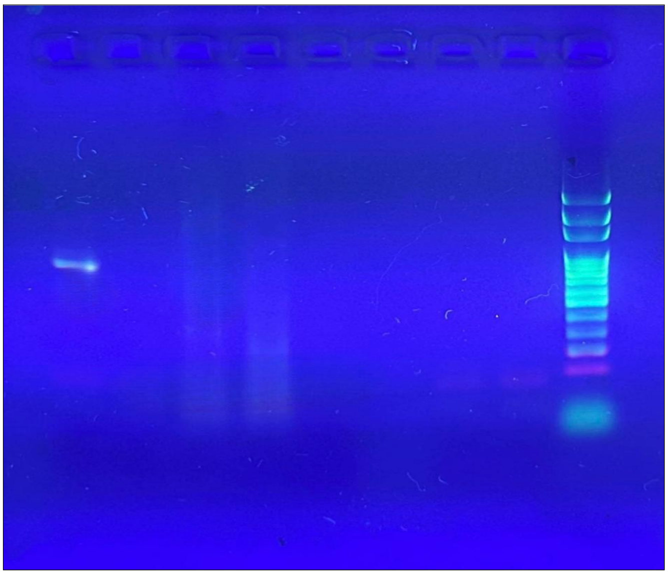

Fig. 2. Agarose gel electrophosis profile showing PCR amplification products of ITS region of *Meloidogyne* spp.

Phylogenetic tree of the genus Meloidogyne

A maximum likelihood phylogenetic analysis of the ITS rRNA region was performed using the newly generated HFT23 sequence deposited in the GenBank database under accession number PV838139 along with 15 reference sequences representing the major Meloidogyne species, as well as two outgroup taxa, *Zygotylenchus guevarae* (AF442189) and *Subanguina radicicola* (AF202164), which formed a strongly supported basal group (bootstrap = 100). The HFT23 isolate clustered within the genus *Meloidogyne* and formed a distinct and well-supported clade (bootstrap = 100) with two *M. incognita* reference isolates (AY438556 and AF516723), confirming its identification as *M. incognita*. The overall phylogenetic topology clearly separated all *Meloidogyne* species from outgroup taxa, confirming the monophyly of the genus and demonstrating the robustness of the analysis (Fig. 3).

Organic matter analysis

In both regions, the wilaya of oran had the highest organic matter content, which vary from 15.32 % in the Al onçor region, the maximum is obtained in the region of hassi bounif 17.37 %., with a gall index of 0 followed by the wilaya of jijel which shows a high rate reaching 10.1 without any infestation in the region of tassout as all other regions seem infested by Meloidogyne.The lowest rates of organic matter are recorded in several wilayas Mostaganem, Ain Temouchent, Algiers, Jijel and Tipaza in different regions vary from 2 % to 6.36 % with higher Wales indices the maximum is recorded in the Damouss region 8.6 followed by indices of 7.45, 7.5, 7.14 and 7.01 in the Ain Sidi Cherif region Mostaganem, Ain Temouchent, Ouled Boughalem Mostaganem and Tipaza respectively. According to the linear correlation results, there was a highly significant negative correlation (Rho of Spearman = -0,651 ; $P < 0.01$) between galls index and organic matter rate (Fig. 4), effect size analysis revealed a strong negative correlation $\rho^2 = 0.42$ indicating that 42 % of the variability in gall index is explained by organic matter. the linear regression model confirmed this trend $R^2 = 0.423$, showing that lower organic matter content is associated with a higher gall index

Soil type

According to the information obtained during surveys, there are several types of soil, namely clayey, clay-loamous, loamy-sandy, clayey-sandy and sandy. The ANOVA test showed a significant difference $p < 0.05$ concerning the effect of soil type on gall index (Fig. 5), the effect size was high ($\eta^2 = 0.241$), suggesting a strong influence of soil type on gall severity. The clay type soil reported the total absence of galls in three different crops (tomato, cucumber and zucchini). On the other hand, the sandy

Table 2. Nucleotide sequence of a genomic fragment from *Meloidogyne incognita*

Species	Sequence
Meloidogyne incognita	TTCGAGAAATTTGGGGACCGTTGATTTAATTTTTCTAAATTACTTTGATGGAAGCCAATTTAATCGCAGTGGCTTGAACCGGGCA AAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCTGCTGGATCATTACTTTATGTGATGTTCAAATTTGAATTCGCAATGAAATG ATCGTTGTGAAACGGCTGTCGCTGGTGTCTAAGTGTTGCTGATACGGTTGTGAACGTCCGTGGCTGTATATGTGGCGACATGTT AGGACTCTAATGAGTTTAAGACCTAATGAGCCTCTTAAGTGAGGCCGCCAGCAACCTTTTTTTTCTCTACATTTTAAAAAAAAAAC TAAAATTCTATCCTTATCGGTGGATCACTAGGCTCGTGGATCGATGAAGAACGCAGCAAACTGCGATAATTATTGCGAACTGCAG AAGTATTGAGCAACTAAAGTTTTGAACGCAAATGGCCGCATTGAGGTCAAACTCTTTGCAACGTCTGGTTCAGGGTCATTTTCTC TTATAGCGGAAGCTTTAATTTCTATAATGATGTTGTTGCTTTATATTTTAAAAGGATTTTTGTTTATTCATGTATTAAATCTAACTG TGAAAATCAAACAATTTTGACCTGAACTCAGTCGAGAGCACCCGCTGA ACTT

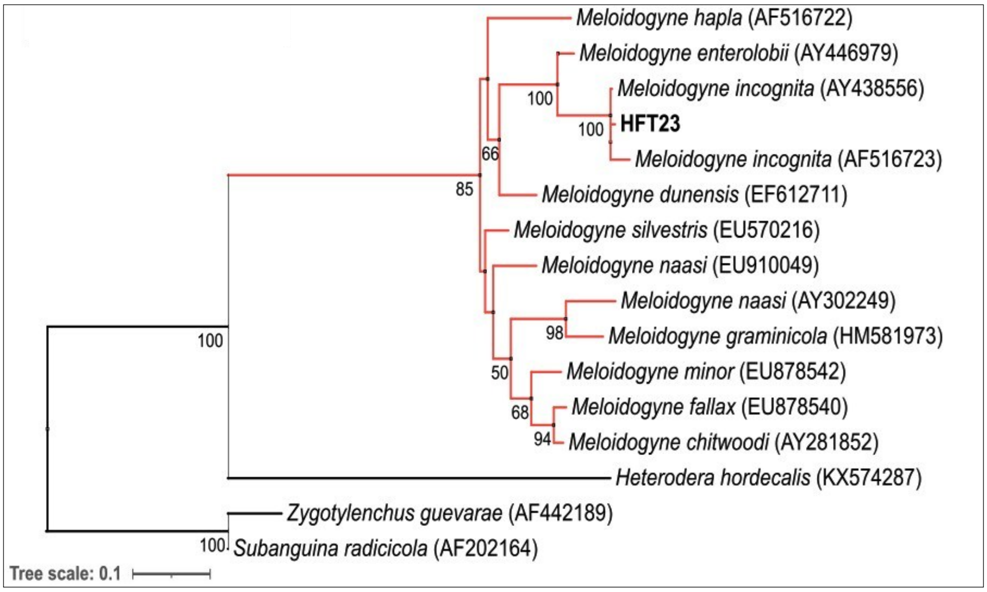

Fig.3 . Phylogenetic tree of the genus *Meloidogyne* based on the ITS of rRNA under maximum likelihood. Numbers above branches represent bootstrap support values > 50 %. GenBank numbers are shown next to the taxon names.

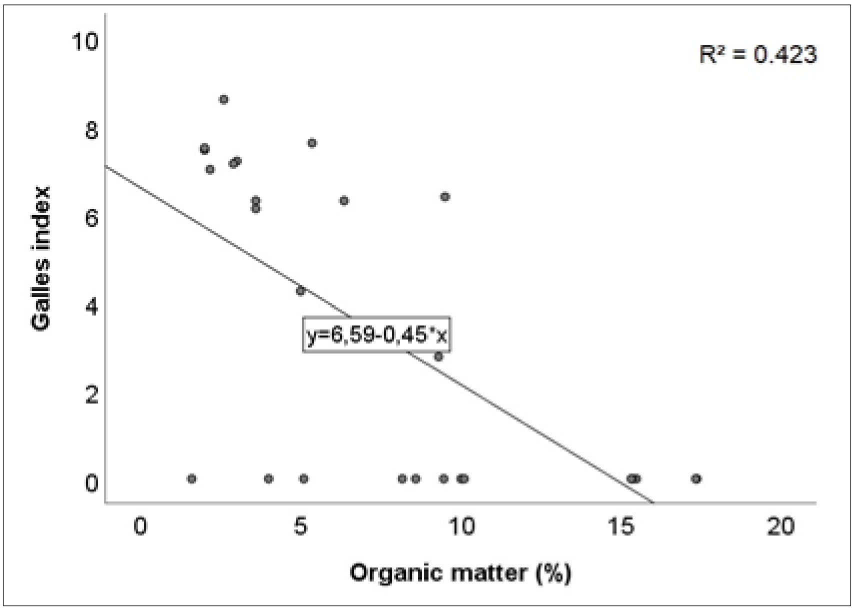

Fig.4. Correlation between soil organic matter content and gall index.

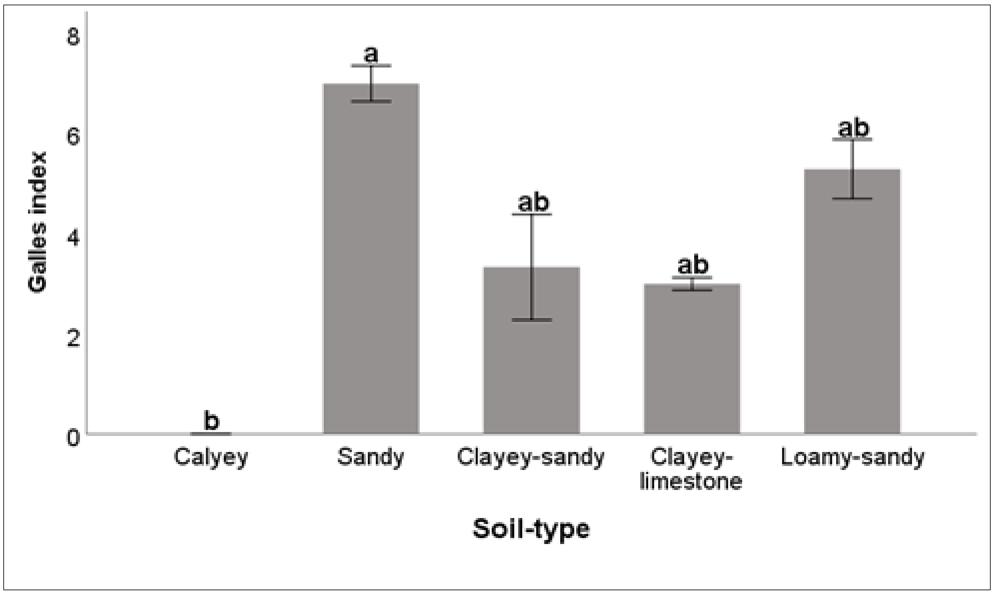

Fig 5. Gall index in different soil types (Means ± SEM).

type recorded the highest index with a average of 6.98 in two crops: tomato and cucumber. The rest of soil types as clayey-loamy, clayey-sandy and loamy-sandy indicated intermediate indices varied from 2.98 to 5.27 in several crops such as: tomato, cucumber, chili pepper, eggplant, melon and zucchini.

The study conducted on a total of 369 greenhouses revealed that 168 were infested with nematodes of the genus Meloidogyne. This relatively high infestation rate indicates that these pests are widespread in greenhouse crops in the regions studied. The absence of root-knot nematode infestation in some greenhouses could be related to the use of a few nematicide products.. These chemical treatments are known to reduce or even eliminate nematode populations in the soil, particularly when applied preventively or at the early stages of infestation. In addition to nematicides, good agricultural practices such as soil disinfection, crop rotation and the use of resistant varieties can also help limit the spread and establishment of root-knot nematodes. Chemical nematicides are widely used in conventional agriculture to effectively control nematodes, although their use is now increasingly regulated due to environmental concerns (24). *Meloidogyne incognita* species is known for its wide geographic distribution and significant economic impact on greenhouse crops. Our results confirm trends reported in other studies conducted in Algeria. Indeed, according to Hammache, *M. incognita* represents approximately 65 % of Meloidogyne species identified in greenhouse market gardening areas in Algeria, followed by *M. arenaria* (25 %) and *M. javanica* (10 %) (25). The high prevalence of these nematodes in greenhouses could be linked to several factors, such as the repeated use of the same soils, the absence of crop rotation, or even conditions favorable to their development (humidity, temperature, type of soil) (26).

Our study shows a total absence of infestation by *Meloidogyne* root-knot nematodes in regions with clay soil, which is in agreement with early studies, which found a negative correlation between nematode reproduction and the clay texture of the soil (18, 27). In contrast sandy soils, which seem infested by nematodes as observed in Babaali (2). Sandy soils favor nematode movement and dispersal (28). The effect of soil on nematodes is a determining factor in their development and propagation (26). Indeed, these parasites favor light and well-aerated soils, where their movements are facilitated, thus allowing them to infest crops more quickly. Conversely, heavy soils, rich in clay or organic matter, create less favorable environment for their proliferation due to the difficulty of movement that they impose on nematodes. The influence of some types of Algerian soils on the development of root-knot nematodes was studied (4). Three types of soil were tested: sandy, clayey and clay-loamy soil whose rate of organic matter differs from one soil to another. Their studies show that nematodes can be present in any type of soil but with different populations: 2680 for sandy soils, 2272 for clay soils and 2327 for intermediate soils with an almost similar multiplication rate (respectively 4.12, 3.49 and 3.58) (4). This same author reported a positive effect of organic matter on nematode activity through a balanced clay-humic complex favorable to any activity of microorganisms (4). The use of organic amendments not only improves soil fertility but also contributes to the biological regulation of pests through microbial-nematode interactions. This highlights the potential of organic amendments as a sustainable soil management strategy, improving both nutrient cycling and soil suppression capacity, thus promoting a more resilient agroecosystem. Soils rich in organic matter are generally colonized by some biocontrol agents such as *Trichoderma harzianum* that improve biocontrol activity (29).

The increase in soil organic matter can be attributed to the addition of organic amendments such as compost and manure. The application of organic amendments leads to a significant increase in total microbial biomass, including bacterial biomass, fungal biomass and the biomass of gram positive and gram negative bacteria (30). This stimulation of microbial activity creates a more competitive and functionally diverse soil environment, which is consistent with our observation of reduced root-knot nematode incidence. Microbial communities suppress RKNs and the genus *Bacillus*, as a member of these communities, acts as a biological agent, significantly reducing the number of galls and egg masses of RKNs (31). Beyond direct antagonism, increased microbial biomass can alter nutrient cycling and soil physicochemical conditions, making the rhizosphere less favorable for nematode infection. For example, during decomposition, organic matter releases toxic products such as butyric acid, leading to a decrease in the nematode population. Increased decomposition of organic matter can increase nutrient availability to plants, potentially improving their vigor and resistance to nematode attack. Previous work reported that in vitro, greenhouse and field experiments have shown that development of ecto- and endoparasitic nematodes can be modulated by bacteria through various mechanisms, such as colonization, parasitism and the synthesis of antimicrobial compounds, bacteria can directly impact nematode development (32). These mechanisms include lytic enzymes, antibiotics, toxins and volatile compounds, which play a key role in the regulating nematode populations and maintaining the soil microbiological balance (32). Soil nematodes are widely used as bioindicators due to their essential role in the food web and soil fertility. Saprophytic nematodes, in particular, participate in nutrient recycling by increasing the availability of nitrogen and promoting the decomposition and dispersal of microorganisms in organic residues. Thus, their diversity and ecological functions make them valuable indicators of soil health and biological quality (33). Our results, showing a significant reduction in the gall index in the presence of organic matter, are consistent with studies reporting the role of beneficial microorganisms such as *Trichoderma spp*. in the suppression of plant-parasitic nematodes. The most studied species (*T. harzianum, T. longibrachiatum, T. virens, T. viride*) mainly target *Meloidogyne, Pratylenchus, Globodera* and *Heterodera. Trichoderma* acts by mycoparasitism, antibiosis, competition in the rhizosphere, production of lytic enzymes and toxic metabolites and induces systemic resistance in plants. The integration of *Trichoderma* into an integrated nematode management strategy, combined with crop rotation, organic amendments and resistant varieties, allows for a reduction in the use of chemical nematicides and promotes more sustainable agriculture (34).

The integration of *Trichoderma harzianum* with organic matter and the fumigant (1,3-dichloropropene) gives the best results in reducing *Meloidogyne incognita* populations. The effect of the fumigant alone remains limited in time, whereas the combination with *T. harzianum* and organic amendment also promotes plant growth and yield. These results validate our interpretation that an integrated management strategy, combining biological agents, organic amendments and appropriate cultural practices, can reduce the use of chemical nematicides and contribute to a more sustainable agriculture (35).

Conclusion

A study conducted across seven wilayas in Algeria, Oran, Mostaganem, Ain Temouchent, Algiers, Tipaza, Jijel and Biskra covering 369 greenhouses in different agroecological zones, revealed variable levels vegetable crop infestation, influenced by multiple factors. The species *Meloidogyne incognita* was identified in the Damous region (Tipaza wilaya) through molecular identification techniques, based primarily on DNA extraction followed by amplification of specific sequences via PCR, confirming its presence in certain vegetable-producing areas of the country. Furthermore, the analysis of edaphic factors highlighted the significant influence of soil type and organic matter content on the development of root-knot nematodes. Clay soils, due to their compact structure, limit nematode mobility and slow their spread, whereas sandy soils, being lighter and well-aerated, favor their development. Organic matter plays a key role in regulating nematode populations. Its enrichment enhances the biological activity of soils, notably by stimulating antagonistic microorganisms (bacteria and fungi) capable of naturally inhibiting nematodes. Therefore, the rational management of organic matter emerges as a promising agroecological strategy to reduce infestations while enhancing the sustainability of vegetable cropping systems. The results of this study confirm the decisive influence of organic matter and soil type on the dynamics of root-knot nematode infestation in vegetable farming systems in Algerian farms. Based on these observations, it is recommended to promote the regular application of well-decomposed organic amendments to improve soil structure and biological activity, thus contributing to the reduction of nematode populations. Promote cultivation in well-drained sandy loam soils. The adoption of crop rotations integrating non-host species, as well as improving the texture and drainage of clay soils by adding organic matter or sand, are also effective practices to limit infestations. Finally, the establishment of systematic monitoring of nematode populations and raising farmers' awareness of integrated pest management approaches appear essential for the sustainable management of these pests in local agroecological conditions.

Acknowledgements

HFZ initiated the literature review, field trips to collect samples and laboratory work and analysis of the results, writing a manuscript, RAF developed the research methodology, outlined the different sections of the research area and participated in analyzing the results. RK also developed the research methodology, outlined the different sections of the research area and participated in analyzing the results. ZSAE contributed to discussing the results and editing the article, BA contributed to contribute to prospecting and collecting samples, MM helped shape the scientific idea of this manuscript and have contributed to writing and correcting it. All authors read and approved your final manuscript.

Authors' contributions

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Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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