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RESEARCH PAPER

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH *Citrus aurantiifolia* (Christm.) SWINGLE AND THEIR RELATIONSHIP WITH SOIL TYPE

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ABSTRACT

Plant-parasitic nematodes (PPNs) pose a significant challenge to citrus farming worldwide, but their distribution and impact in the Al-Ahsa Oasis, Saudi Arabia (KSA), remain poorly understood. This study investigates the prevalence, diversity, and ecological dynamics of PPNs associated with Hasawi Lumi (*Citrus aurantiifolia*) trees, a key crop in the region. During the summer of 2024, a survey was performed in ten major Hasawi Lumi-growing areas, with 250 soil and root samples collected. Four genera of PPNs were identified, with *Tylenchulus semipenetrans* (52%), *Helicotylenchus* (44.8%), *Pratylenchus* (42.8%), and *Xiphinema* (22%) being the most prevalent. The physicochemical properties of the soil (e.g., texture, pH, and minerals) were determined to assess their impact on nematode populations. Our results revealed that soil characteristics significantly affect the distribution of PPNs, with sandy soils and moderate organic matter favoring nematode diversity, while high salinity suppresses it. The current research constitutes the initial attempt to assess PPNs in Hasawi Lumi orchards and offers important recommendations that can be implemented to improve citrus fruit yield in Al-Ahsa Oasis. These results indicate that soil factors must be considered in any attempt to manage nematode infection, reflecting the necessity for adopting strategies to improve the productivity of citrus crops in the concerned area.

Keywords: citrus crops, diversity, Hasawi Lumi, plant-parasitic nematodes, soil properties, *Tylenchulus semipenetrans*

INTRODUCTION

Citrus is among the frequently produced fruit crops, especially in regions with tropical and subtropical climates [Maqbool et al. 2023]. Two-thirds of the global citrus production is concentrated in the US, China, Brazil, Mexico, India, Spain, and Egypt [Abd-Elgawad et al. 2016, Khan et al. 2021], with annual global output surpassing 100 million tons [Zou et al. 2016]. In Saudi Arabia (KSA), Hasawi Lumi (*Citrus aurantiifolia*) is a major fruit crop of Al-Ahsa Oasis. However, the Hasawi Lumi sector faces significant production constraints. Generally, the spread of plant-parasitic nematodes (PPNs) reduces citrus quality and yield in KSA [Al-Yahya 1988, Al-Hazmi 1997]. Citrus rhizospheres host several PPNs, including *Belonolaimus longicaudatus*,

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Helicotylenchus, *Hoplolaimus*, *Longidorus*, *Tylenchulus semipenetrans*, *Pratylenchus coffee*, *Radopholus similis*, and *Meloidogyne* spp., that inflict substantial economic damage on a global scale [Hammam et al. 2021]. Other nematodes, such as *Pratylenchus brachyurus*, *Pratylenchus vulnus*, *Hemicycliophora arenaria*, *Hemicycliophora nudata*, *Paratrichodorus lobatus*, *Paratrichodorus minor*, *Xiphinema brevicolle*, and *Xiphinema index*, are considered minor pests due to their limited impact or regional distribution [Badii et al. 2015]. The nematode *T. semipenetrans*, which infects citrus plants, is linked to many rootstocks in many citrus cultivation areas in KSA [Eissa 1979]. Its prevalence varies globally, with Egypt reporting 99.1%, northern Iran at 89%, California and Florida (USA) at 26%, Morocco at 88%, and Spain showing a range of 70% to 90% [Sorribas et al. 2008, Mokrini et al. 2018, Zoubi et al. 2022]. Drought conditions exacerbate the impact of nematode infestations, impairing the root system's capacity to uptake water and minerals [Duncan 2005, Maafi and Damadzadeh 2008].

The occurrence, density, and distribution of plant-parasitic nematodes in Hasawi Lumi trees in Al-Ahsa Oasis, along with their correlation with soil physicochemical characteristics, remain largely unexplored. This study aims to address this gap by:

1. Conducting an extensive survey of PPNs in the main Hasawi Lumi-growing zones of Al-Ahsa Oasis, where data is currently lacking.
2. Evaluating the PPNs' incidence, distribution, and diversity in the rhizosphere of Hasawi Lumi trees.
3. Investigating how soil variables influence PPN populations.

Additionally, this study aims to characterize the PPN communities infecting Hasawi Lumi trees in Al-Ahsa to improve management strategies.

MATERIALS AND METHODS

Survey and sample acquisition

Sampling was carried out during the summer of the 2024 growing season in Al-Ahsa Oasis, Saudi Arabia. The prevailing environmental conditions included high temperature (average: 38–45 °C), low humidity (20–30%), and minimal rainfall. A survey was performed in the ten primary Hasawi Lumi (*Citrus aurantiifolia*) cultivation regions, including Al-Bataliyah, Al-Bustan, Al-Halila, Al-Hofuf, Al-Jubail, Al-Omran, Al-Shaharin, Al-Shiraa, Al-Tarabil, and Briqa. From the rhizosphere of several Hasawi Lumi trees, 250 soil and root samples were collected at a depth of 20–40 cm using a stainless steel auger, as illustrated in Figure 1.

A 1 kg composite sample of soil and roots was gathered from each sampling location along a zigzag pattern. To avoid moisture evaporation, the samples were sealed in plastic bags and kept at 5 °C until further examination. Nematode-related investigations were conducted at the Nematology Laboratory within the Arid Land Agriculture Department, King Faisal University's College of Agricultural and Food Sciences (https://maps.app.goo.gl/4arHC1ZGM1Q2etcp9).

Extraction and identification of nematodes

The modified Baermann method was employed to isolate mobile nematodes from soil and root samples [Hooper et al. 2005]. Each sample's roots were rinsed under tap water and sectioned into 1 cm slices, using 25 g for nematode extraction. Nematodes were extracted from 250 g soil samples utilizing an adapted version of the Baermann technique. After extraction, nematodes were retained for processing. The number of individuals per 250 g of soil and 25 g of root fragments was counted. Nematodes were classified by genus based on morphological characteristics using stereomicroscopes, supplemented by light microscopy for detailed observations according to Mai et al. [1996]. For preservation, nematode specimens were soaked in 4% hot formalin–formaldehyde [Ryss 2017]. They were then put in a 7-centimeter square watch glass containing liquid I (99 parts 4% formaldehyde and 1 part pure glycerol). The latter was desiccated in one-tenth of its volume of 96% ethanol for 12 hours at 40 °C. The watch glass with nematodes was then detached from the desiccator and transferred to a 37 °C incubator. Nematodes were prepared using a dehydration solution of 95% ethanol and 5% pure glycerol, referred to as Dehydration Liquid II. Three milliliters of liquid II were added to the watch glass, which was partially covered to facilitate evaporation. Next, 2 mL of liquid III (a 1:1 mixture of pure glycerol and 96% ethanol) was introduced, and the watch glass was incubated overnight at 37 °C. Nematodes were generated on microscope slides for observation and identification using light microscopy.

Fig. 1. Survey Map of the ten regions of Hasawi Lumi-growing in Al-Ahsa Oasis, KSA (Ministry of Environment, Water and Agriculture, MEWA)

Nematode community assessment

The incidence and diversity of nematodes were calculated using prevalence, mean intensity, and maximum density according to Bello et al. [2020]:

% Prevalence =
= (Number of Infected Samples / Total Samples) × 100

Mean intensity =
= (Total Nematode Species in Infected Samples / / Number of Infected Samples) × 100

Maximum density =
= the Maximum Number of Certain Nematode Species Recovered

DNA extraction, PCR amplification, sequencing, and phylogenetic analysis

DNA was isolated from four individual worms for molecular identification, as previously outlined by Holterman et al. [2006]. The D2–D3 segment was amplified utilizing forward primer D2a (5′-ACA AGTACC GTG AGG GAA AGT TG-3′) and reverse primer D3b (5′-TCG GAA GGA ACCAGC TAC TA-3′) following De Ley et al. [1999]. One microliter (µL) of DNA was included in the PCR reaction mixture, which consisted of 22 µL of ddH2O, 25 µL of 2 × OnePCR™ (GeneDireX, Germany, Cat. No. MB203-0100), and 1 µM of each of the two primers. PCR was performed on the C1000 Touch PCR thermal cycler (Bio-Rad) using the following conditions: 5 minutes of denaturation at 95 °C, followed by 35 cycles of 1 minute at 94 °C, 45 seconds at 49 °C, and 1 minute at 72 °C. The final elongation was 8 minutes at 72 °C. Five µL of each PCR product with 1 µL of 6 × loading buffer (Fermentas Life Sciences, Germany) were electrophoresed on 1% Tris-Borate-EDTA (TBE) buffer.

After electrophoresis at 100 V for 40 minutes, the gel was stained with ethidium bromide (0.1 µg mL^{-1}) for 20 minutes and then examined and photographed under ultraviolet light. PCR product purification and sequencing were carried out at Macrogen, South Korea, in both directions.

The new sequences were blasted against the NCBI database for sequence identity, and three representative sequences were downloaded for each new sequence. Phylogenetic analysis was executed using MEGA11, with the Maximum Likelihood approach and the Kimura 2-parameter model. The tree was assessed using 1000 bootstrap replications.

Soil assessment

Surface and sub-surface soil samples were collected in triplicate using a stainless steel auger at two depths (20 cm and 40 cm) to account for variations in soil properties throughout the study region. Sampling locations were selected randomly within predefined areas to represent diverse soil types. The specimens underwent air drying, followed by pulverization and filtration through a 2 mm sieve to ensure they were ready for physicochemical evaluation. To determine the particle size distribution (including sand, silt, and clay amounts), we employed the hydrometer technique [Gee and Bauder 2018], which adheres to sedimentation principles. A calibrated pH meter was used to estimate the pH of the soil on a saturated soil paste, following the USDA Handbook [Sparks et al. 2020]. Electrical conductivity (EC) was assessed in a 1 : 5 soil-to-water extract utilizing an EC meter, providing a reliable estimate of soil salinity. The sodium adsorption ratio (SAR) was evaluated following Page et al. [1982] using the equation:

$$SAR = \frac{Na}{\sqrt{\frac{Ca + Mg}{2}}}$$

where: Ca, Na, and Mg refer to calcium, sodium, and magnesium concentrations in mmol L^{-1}.

Cation concentrations were measured using flame photometry or atomic absorption spectroscopy for accuracy and precision in chemical analysis. All physicochemical analyses were conducted at the Soil Laboratory, Environment and Natural Agricultural Resources Department, King Faisal University's College of Agricultural and Food Sciences.

Taxonomic diversity

The taxonomic diversity of PPNs was evaluated through two nematode indices. The Shannon-Wiener and Evenness indices are the two indices referenced (https://www.omnicalculator.com/ecology/shannon-index).

Data processing

Data were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance, as determined by SPSS software version 23 [SPSS 2016]. One-way ANOVA was employed to statistically evaluate the obtained data and determine whether there were any significant differences between the means of the analyzed parameters. A principal component analysis (PCA) was conducted to delineate the distribution of nematode taxa and soil properties based on their sampling locations. The FactoMineR package [Lê et al. 2008] was utilized for this purpose, while the factoextra package [Kassambara and Mundt 2020] was employed to generate the corresponding biplot.

RESULTS

Distribution and diversity of PPN populations

Four genera of PPNs were recognized through morphological analysis in soil and root samples obtained from the Hasawi Lumi orchards. Table 1 presents the prevalence, maximum density, and mean intensity for each analyzed region. Mean intensity and maximum density serve as quantitative metrics derived to assess the relative abundance of PPNs in soil and root systems and to gauge the potential distribution of nematodes within the plant environment. Citrus, spiral, lesion, and dagger nematodes were identified in all assessed Hasawi Lumi cultivation regions. The predominant PPNs identified in root and soil samples were citrus, spiral, and lesion nematodes, exhibiting prevalence rates reaching as high as 52%, 44.8%, and 42.8%, respectively. Dagger nematodes constituted 22% of the overall nematode population. Citrus nematode had significant prevalence in Briqa (72%), Al-Jubail and Al-Omran (64%), Al-Shiraa (60%), Al-Bataliyah (56%), Al-Bustan (52%), Al-Halila and Al-Shaharin (48%); however, it was the least widespread in Al-Hofuf (28%) and Al-Tarabil (24%). The citrus nematode exhibited the most significant overall density in Al-Jubail, reaching 533 individuals/soil sample (Figure 2). Soil and root analyses revealed the presence of several genera, including *Helicotylenchus*, *Pratylenchus*, *Tylenchulus*, and *Xiphinema*, with densities of 5 to 150 individuals per 250 g of soil.

The molecular identification results, as illustrated in Figure 3, confirmed the morphological classification of PPNs in Hasawi Lumi orchards. Phylogenetic analysis of the D2–D3 segment of the 28S rDNA revealed a high genetic similarity (≥99%) between the identified nematode species and their corresponding GenBank references. The predominant species, *Tylenchulus semipenetrans*, exhibited strong phylogenetic clustering, supporting its widespread prevalence across the surveyed regions. *Helicotylenchus*, *Pratylenchus*, and *Xiphinema* species were also confirmed through molecular sequencing, with discrete clades reflecting their genetic diversity. These findings validate the morphological identifications and highlight the reliability of combining molecular and morphological approaches for accurate nematode species charac-

Table 1. Plant-parasitic nematodes prevalence, intensity, and density from soil (250 g) and root (25 g) of Hasawi Lumi in the ten surveyed growing regions in Al-Ahsa Oasis, KSA

Nematode genera	*Helicotylenchus* (*He*)					*Pratylenchus* (*Pr*)					*Tylenchulus* (*Ty*)					*Xiphinema* (*Xi*)				
	Pr	In		De		Pr	In		De		Pr	In		De		Pr	In		De	
		Ro	So	Ro	So		Ro	So	Ro	So		Ro	So	Ro	So		Ro	So	Ro	So
Al-Bataliyah	36	6	18	14	28	44	8	20	20	68	56	31	123	73	423	20	6	20	–	33
Al-Bustan	36	4	15	14	25	40	5	17	20	65	52	28	120	73	420	20	4	17	–	30
Al-Halila	4	2	3	9	9	40	3	4	14	24	48	20	44	53	162	16	2	4	–	10
Al-Hofuf	76	1	8	3	13	48	3	8	4	20	28	3	10	6	43	32	–	5	–	5
Al-Jubail	48	5	15	14	35	44	8	8	21	20	64	18	390	34	533	16	–	13	–	28
Al-Omran	60	4	13	11	150	52	4	10	14	125	64	14	85	45	155	32	–	18	–	75
Al-Shaharin	32	6	16	12	25	36	7	18	18	62	52	28	113	64	389	16	6	18	–	30
Al-Shiraa	44	4	14	12	32	40	7	7	19	18	60	15	359	30	490	12	–	12	–	25
Al-Tarabil	72	1	7	2	12	44	2	7	3	18	24	2	9	6	39	28	–	5	–	5
Briqa	40	3	10	3	18	40	4	10	5	15	72	18	225	43	508	28	4	5	4	8

Pr: Prevalence (considering the nematodes' juvenile and adult stages) = % Prevalence = (Number of Infected Samples / Total Samples) × 100; In: Intensity = (Nematode Species in Infected Samples / Total Infected Samples) × 100; De: Density = the Maximum No. of Certain Nematode Species Recovered; Ro: Root; So: Soil

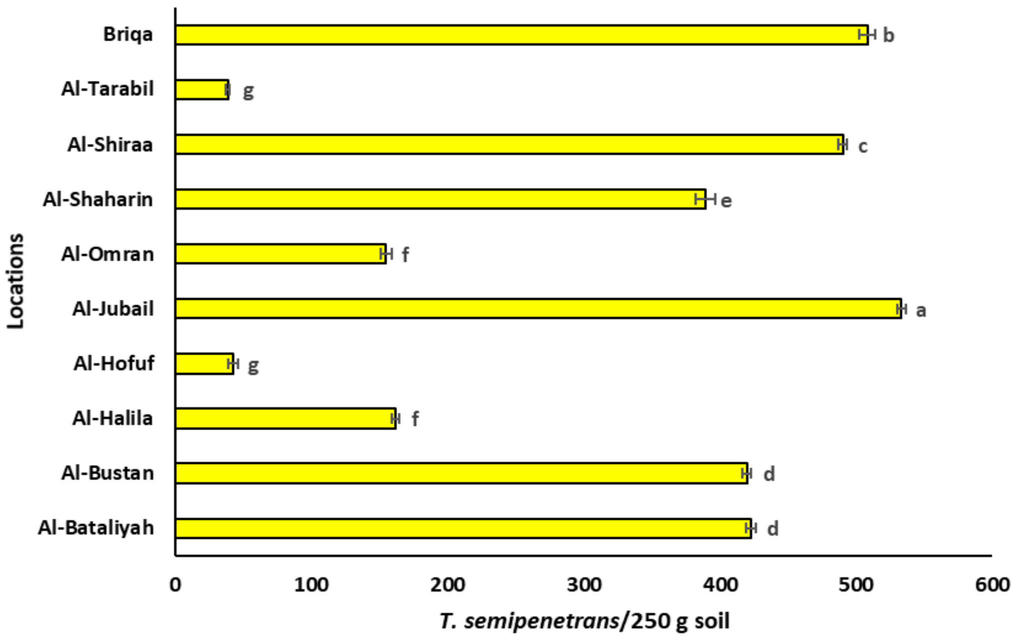

Fig. 2. Tylenchulus semipenetrans population densities per 250 g of soil in ten regions surveyed of Hasawi Lumi-growing in Al-Ahsa Oasis, KSA

terization. The sequences were submitted to GenBank with the accession numbers PQ843101, PQ843102, PQ843103, and PQ843104 for *Helicotylenchus digonicus*, *Tylenchulus semipenetrans*, *Pratylenchus loosi*, and *Xiphinema pachtaicum*, respectively. The phylogenetic tree further underscores the genetic relationships among the identified PPNs, providing a robust framework for understanding their distribution and ecological roles in Hasawi Lumi orchards.

The evolutionary history was reconstructed using the Maximum Likelihood approach with the Kimura 2-parameter model. The bootstrap values supporting the clustering were shown. The accession of newly sequenced data is shown in bold.

Interaction between physicochemical properties and nematode communities

The soil physicochemical properties across the Hasawi Lumi cultivation regions in Al-Ahsa Oasis are presented in Table 2. The data reveal significant variability in soil characteristics, likely influencing the distribution and abundance of PPNs in these regions.

Particle size distribution and soil texture. The distribution of the particle size varied considerably across the regions. Al-Halila had the highest sand content (90%), followed by Al-Bustan (88%) and Al-Jubail (84%). In contrast, Al-Omran and Briqa exhibited higher clay content (16% and 15%, respectively). Soil texture ranged from sandy in Al-Halila to sandy loam in Al-Bataliyah, Al-Hofuf, Al-Omran, and Al-Tarabil, and loamy sand in Al-Bustan and Al-Shaharin. These variations in texture likely affect water retention, aeration, and root penetration, which are critical factors for nematode survival and movement.

Bulk density and porosity. Bulk density values ranged from 1.44 g cm^{-3} in Al-Omran to 1.53 g cm^{-3} in Al-Halila, indicating relatively compact soils. Porosity, which reflects the soil's capability for holding water and air, ranged from 42.5% in Al-Omran to 47% in Al-Halila. Higher porosity in sandy soils, such as Al-Halila, may facilitate nematode movement, while lower porosity in clay-rich soils, like Al-Omran, could restrict nematode activity.

Cation exchange capacity (CEC) and pH. CEC values varied from 13.5 mmol 100 g^{-1} in Al-Halila to 19 mmol 100 g^{-1} in Al-Omran, indicating differences

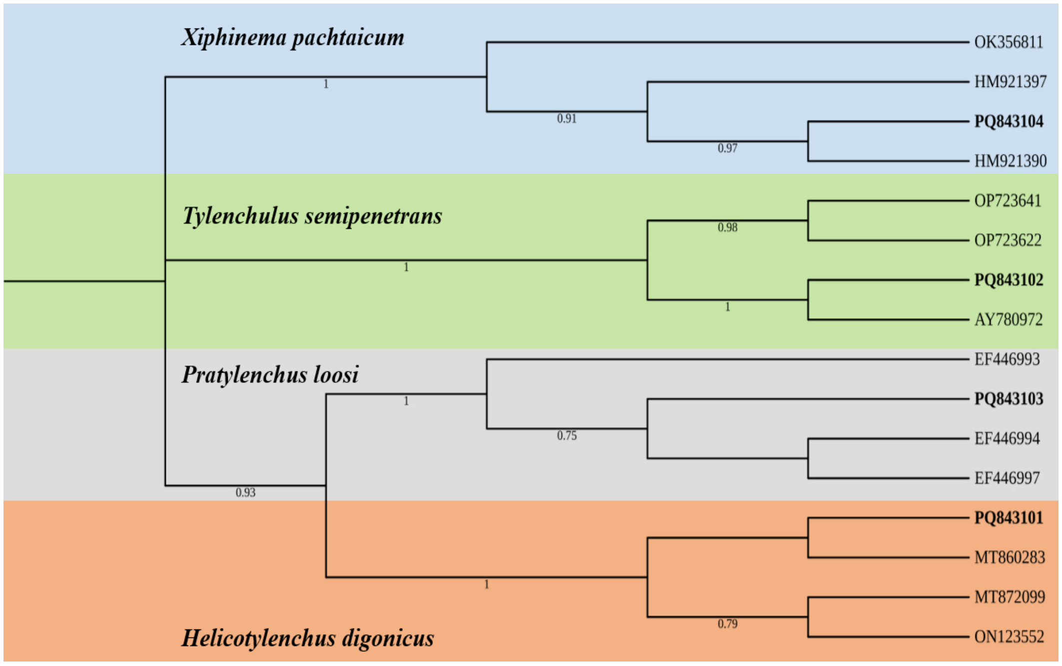

Fig. 3. Phylogenetic relationship of nematodes based on the D2D3 region of the 28S rDNA

in soil fertility and nutrient-holding capacity. The pH values were consistently alkaline, ranging from 8.1 in Al-Shaharin to 8.8 in Al-Tarabil. Specific nematodes, such as *Tylenchulus semipenetrans*, prefer alkaline soils and can tolerate an alkalinity range from neutral to slightly alkaline.

Electrical conductivity (EC) and total dissolved solids (TDS). EC values fluctuated considerably, with Al-Omran having the highest salinity (30 dS m^{-1}), followed by Briqa (25 dS m^{-1}). In contrast, Al-Hofuf had the lowest salinity (4.5 dS m^{-1}). High salinity in Al-Omran and Briqa may reduce nematode diversification, as many species are susceptible to salt content. TDS values range between 890 mg L^{-1} in Al-Hofuf and 970 mg L^{-1} in Al-Omran, which supports the salinity distributions inferred from EC values.

Organic matter (OM) and calcium carbonate ($CaCO_3$). Organic matter content ranged from 4.8% in Al-Halila and Al-Omran to 6.6% in Al-Bataliyah. Higher organic matter in Al-Bataliyah and Al-Jubail may support a more hospitable habitat and food resource, and induce more diverse nematode communities. The calcium carbonate content varied widely, ranging from 11.7% in Briqa to 24.8% in Al-Bustan. Nematodes like Xiphinema, which prefer calcareous soils, may develop well in soils with a moderate $CaCO_3$ content (e.g., Al-Bustan).

Sodium adsorption ratio (SAR) and major ions. SAR values ranged from 4.6 in Al-Hofuf to 5.1 in Al-Omran, indicating moderate sodium levels. Sodium (Na^+) concentrations were relatively consistent across regions, ranging from 11.2 mmol L^{-1} in Al-Hofuf to 12.5 mmol L^{-1} in Al-Omran. Potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) levels also showed minor variations, with Al-Omran having the highest Ca^{2+} (9.8 mmol L^{-1}) and Mg^{2+} (5.5 mmol L^{-1}) concentrations. These ions are involved in soil structure and nutrient availability, which can potentially influence nematode populations.

Anions (Cl^-, SO_4^{2-}, HCO_3^-). Chloride (Cl^-) and sulfate (SO_4^{2-}) concentrations were relatively uniform across regions, with Al-Omran having the highest Cl^- (13.0 mmol L^{-1}) and SO_4^{2-} (11.8 mmol L^{-1}) levels. Bicarbonate (HCO_3^-) levels ranged from 4.3 mmol L^{-1} in Al-Halila to 4.6 mmol L^{-1} in Al-Omran and Briqa. These anions are involved in soil salinity and may also affect nematode existence, particularly in areas of high salinity, such as Al-Omran.

Table 2. Hasawi Lumi samples soil type and physicochemical properties across locations

	Unit	Al-Bataliyah	Al-Bustan	Al-Halila	Al-Hofuf	Al-Jubail	Al-Omran	Al-Shaharin	Al-Shiraa	Al-Tarabil	Briqa
Particle size distribution	% sand	75.0	88.0	90.0	85.5	84.0	72.0	86.0	80.5	78.0	74.0
	% silt	10.0	7.0	5.0	8.0	9.0	12.0	8.5	10.5	12.0	11.0
	% clay	15.0	5.0	5.0	6.5	7.0	16.0	5.5	9.0	10.0	15.0
Texture		sandy loam	loamy sand	sandy	sandy loam	sandy	sandy loam	loamy sand	loamy	sandy loam	loamy
Bulk density	g cm^{-3}	1.45	1.5	1.53	1.48	1.49	1.44	1.47	1.46	1.45	1.48
Porosity	%	43.0	45.5	47.0	44.5	43.5	42.5	44.0	43.5	44.0	43.0
CEC	$cmol_{(+)}$/kg	17.0	15.0	13.5	15.5	16.0	19.0	15.5	17.5	18.0	17.5
pH		8.5	8.6	8.4	8.7	8.45	8.52	8.1	8.75	8.8	8.55
$EC_{1:5}$	dS m^{-1}	10.0	9.5	7.5	4.5	5.0	30.0	7.0	5.0	6.0	25.0
TDS	mg L^{-1}	940.0	950.0	920.0	890.0	910.0	970.0	900.0	940.0	930.0	960.0
Na^+	mmol L^{-1}	12.0	11.8	11.5	11.2	11.7	12.5	11.6	12.0	11.8	12.3
K^+	mmol L^{-1}	1.5	1.6	1.7	1.55	1.65	1.5	1.6	1.55	1.6	1.65
Ca^{2+}	mmol L^{-1}	9.5	9.4	9.3	9.6	9.7	9.8	9.5	9.5	9.6	9.8
Mg^{2+}	mmol L^{-1}	5.1	5.2	5.0	5.1	5.3	5.5	5.1	5.0	5.2	5.4
Cl^-	mmol L^{-1}	12.4	12.5	12.3	12.2	12.6	13.0	12.5	12.4	12.6	12.8
SO_4^{2-}	mmol L^{-1}	11.4	11.5	11.3	11.2	11.6	11.8	11.5	11.4	11.6	11.7
HCO_3^-	mmol L^{-1}	4.4	4.5	4.3	4.4	4.5	4.6	4.5	4.4	4.5	4.6
OM	%	6.6	5.2	4.8	5.0	6.5	4.8	6.2	5.5	6.4	5.7
SAR		4.8	4.9	4.7	4.6	4.9	5.1	4.8	4.7	4.9	5.0
$CaCO_3$	%	15.3	24.8	16.4	14.5	14.8	21.6	14.7	14.9	15.3	11.7

CEC: cation exchange capacity; EC: electrical conductivity; TDS: total dissolved solids (TDS); OM: organic matter; SAR: sodium adsorption ratio

Relationship between soil properties, nematode abundances, and location preference

The association between soil physicochemical characteristics and nematode genera was analyzed using PCA, which revealed that PC1 and PC2 explained 37% and 23.46% of the variability, respectively (Figure 4). The results showed that electrical conductivity (EC), CaCO3, and *Tylenchulus* are positively correlated. The nematode *Helicotylenchus* showed a positive correlation with pH, and a negative correlation with EC.

Organic matter (OM) was negatively correlated with EC, indicating that soils high in salinity may have lower organic matter content due to reduced fertility and microbial activity. Both nematodes, *Pratylenchus* and *Xiphinema*, were positively correlated with soil properties like $CaCO_3$ and, to a lesser extent, EC.

The variance explained percentage attained 37% and 23.46% for PC1 and PC2, respectively. Each location is represented by a unique color in the legend, with labels displayed near its corresponding point. Variables (red arrows) represent the contribution and direction of soil properties and nematode preferences. Variables aligned in the same direction demonstrate a positive correlation, whereas those pointing in opposite directions signify a negative relationship.

Variability in nematode diversity and abundance linked to soil physicochemical properties

The Shannon-Wiener diversity index (H′) and Evenness (E) values for plant-parasitic nematodes (PPNs) in the Hasawi Lumi cultivation regions of Al-Ahsa Oasis are presented in Table 3. The findings reveal significant variations in nematode diversity and community structure, which can be linked to the soil physicochemical properties of each region.

Nematode diversity and soil properties. The highest Shannon-Wiener diversity index (H′ = 1.33)

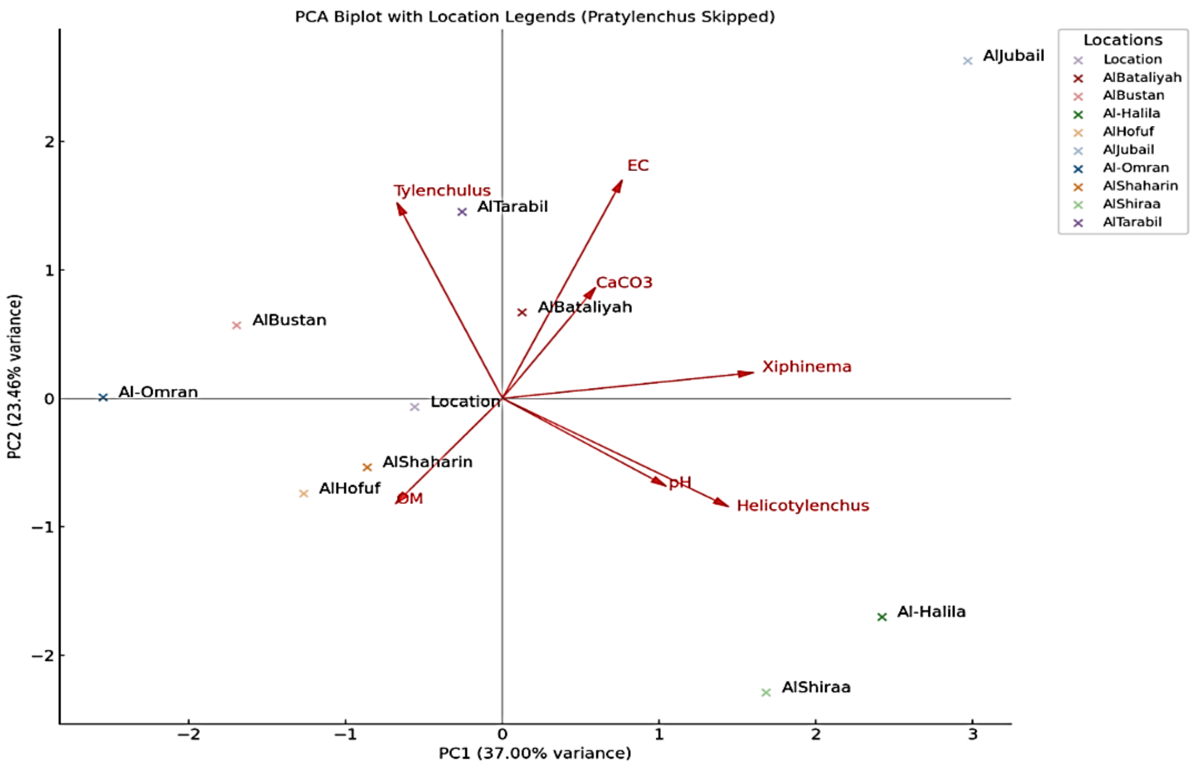

Fig. 4. Principal Component Analysis (PCA) of the association between soil physicochemical characteristics and nematode abundances in the ten regions surveyed in Al-Ahsa Oasis, KSA. EC: electrical conductivity; OM: organic matter

Table 3. The average number of nematode genera, Shannon-Wiener diversity (H′), and Evenness (E) in each of the ten locations surveyed in Hasawi Lumi

No.	Location	Average of PPNs*	Shannon Diversity Index (H′)*	Evenness (E)*
1	Al-Bataliyah	165 ab	0.81 c	0.53 d
2	Al-Bustan	162 ab	0.79 c	0.57 cd
3	Al-Halila	70 e	0.77 c	0.56 cd
4	Al-Hofuf	24 f	1.15 b	0.83 b
5	Al-Jubail	171 a	0.64 d	0.47 e
6	Al-Omran	144 d	1.33 a	0.96 a
7	Al-Shaharin	150 cd	0.80 c	0.58 c
8	Al-Shiraa	157 bc	0.64 d	0.46 e
9	Al-Tarabil	21 f	1.15 b	0.83 b
10	Briqa	151 cd	0.39 e	0.28 f
×	*P*	<0.05	<0.05	<0.05

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.
* https://www.omnicalculator.com/ecology/shannon-index

was observed in Al-Omran, followed by Al-Hofuf and Al-Tarabil (H′ = 1.15). These regions also exhibited higher organic matter (OM) content (4.8% in Al-Omran, 5.0% in Al-Hofuf, and 6.4% in Al-Tarabil) and moderate calcium carbonate ($CaCO_3$) levels (21.6% in Al-Omran, 14.5% in Al-Hofuf, and 15.3% in Al-Tarabil). Higher OM and moderate $CaCO_3$ may have created a suitable environment for various nematode communities, as the organic matter supports microbial activity and enhances nutrient availability. Additionally, moderate $CaCO_3$ helps stabilize the soil structure even more.

On the other hand, Briqa presented the lowest Shannon-Wiener index (H′ = 0.39), corresponding to its high salinity (EC = 25 dS m^{-1}) and low organic matter content (5.7%). High salinity is known to suppress nematode diversity, as many species are sensitive to saline conditions. Similarly, Al-Jubail and Al-Shiraa, with lower H′ values (0.64), also had lower OM content (6.5% and 5.5%, respectively) and higher salinity (EC = 5.0 dS m^{-1} in Al-Jubail and 5.0 dS m^{-1} in Al-Shiraa), further supporting the negative impact of salinity on nematode diversity.

Evenness and nematode distribution. The Evenness index (E), which measures the relative abundance of nematode species, was highest in Al-Omran (E = 0.96) and Al-Hofuf (E = 0.83), indicating a more balanced distribution of nematode species in these regions. It aligns with their higher OM and moderate $CaCO_3$ levels, which likely support a more stable and diverse nematode community. In contrast, Briqa had the lowest Evenness (E = 0.28), reflecting a less balanced nematode community, likely due to its high salinity and lower OM content.

Average number of PPNs. The average number of PPNs differs significantly across the regions, with the highest average (171) documented from Al-Jubail, followed by Al-Bataliyah (165) and Al-Bustan (162). In addition, moderate OM content (6.6% in Al-Bataliyah, 5.2% in Al-Bustan, and 6.5% in Al-Jubail) and lowered salinity (EC = 5.0 dS m^{-1} in Al-Jubail, 10.0 dS m^{-1} in Al-Bataliyah, and 9.5 dS m^{-1} in Al--Bustan) were reported, which designates moderate OM and lower salinity as attractive for higher nematode populations. Conversely, Al-Hofuf and Al-Tarabil had the lowest average number of PPNs (24 and 21, respectively), perhaps due to their lower OM content (5.0% and 6.4%) and higher salinity (EC = 4.5 dS m^{-1} in Al-Hofuf and 6.0 dS m^{-1} in Al-Tarabil).

A clear association has been demonstrated between soil physicochemical characteristics, nematode diversity, and abundance in Hasawi Lumi cultivation regions. Diverse and balanced nematode communities are associated with higher organic matter and moderate calcium carbonate levels in Al-Omran, Al-Hofuf, and Al-Tarabil. Conversely, high salinity and low organic matter, as seen in Briqa, induce a decrease in nematode diversity and abundance.

DISCUSSION

Extensive studies were conducted in the principal growth regions of Hasawi Lumi trees to determine the distribution of PPNs in Al-Ahsa Oasis. Based on the morphological and morphometric features, four genera of PPNs were identified, with *Tylenchulus semipenetrans* being the predominant PPN in Hasawi Lumi orchards. Comparable observations were reported in other regions, such as Iran, Egypt, and Spain [Maafi and Damadzadeh 2008, Abd-Elgawad et al. 2016, Sorribas et al. 2008]. This species stands out due to its strong connection with citrus and the Hasawi Lumi trees' ability to sustain high nematode populations until they reach senescence. The spread of the citrus nematode may have been facilitated by contaminated seedlings, crop debris, organic manure, irrigation, and machinery [Abd-Elgawad et al. 2016].

The spiral nematode, *Helicotylenchus* spp., accounted for the second most prevalent PPN, ranging from 76% in Al-Hofuf to 72% in Al-Tarabil, and 4% in Al-Halila. The Hasawi Lumi-growing zones of Al-Ahsa Oasis have more of this genus than Spain [Sorribas et al. 2008] and Egypt [Abu Habib 2020]. According to Kumar and Das [2019], 80% of *H. dihystera* were found in 80% of samples from Tinsukia. Population densities of lesion nematode (*Pratylenchus* spp.) spanned from 125 to 15 per 250 g of soil in Al-Omran and Briqa, respectively, and from 21 to 3 per 25 g of roots in Al-Jubail and Al-Tarabil, respectively. Numerous investigations have found these nematodes in citrus crops worldwide [Badii et al. 2015]. The coffee species infests 1% of Brazilian citrus nurseries and plantations, and is prevalent in Florida [Freitas et al. 2008]. These organisms can substantially decrease root mass, thereby reducing yield [Abd-Elgawad 2020].

The top 10 economically relevant PPNs include the dagger nematode *Xiphinema* spp. [Jones et al. 2013]. As migrating ectoparasites, these nematodes can transmit plant viruses, posing a significant threat to crops. Even in low populations, they can cause substantial harm to plants [MacFarlane and Robinson 2004]. This study found significant variances in *Xiphinema* spp. distribution across Hasawi Lumi cultivation areas. This nematode spreads the Arabis mosaic virus, which causes grapevine fan leaf degeneration [Mokrini et al. 2014]. *Xiphinema pachtaicum* was initially found with Hasawi Lumi in Al-Ahsa Oasis. This finding is particularly significant due to the nematode's economic impact, quarantine implications, and potential to devastate Hasawi Lumi orchards.

Al-Omran, Al-Hofuf, and Al-Tarabil, regions with long dry seasons, have the highest Shannon (H′) and Evenness (E) indexes. Freitas et al. [2008] found that the dry season and 0–10 cm soil depth increased Brazil's citrus plant PPN population.

The soil physicochemical properties in the Hasawi Lumi cultivation regions of Al-Ahsa Oasis exhibit significant variability, particularly in texture, salinity, organic matter, and calcium carbonate content. These factors likely influence the distribution and abundance of PPNs. For example, sandy soils with high porosity, such as those in Al-Halila, may facilitate nematode movement and proliferation. High salinity in Al-Omran and Briqa could limit nematode diversity, as many species are sensitive to saline conditions. Regions with higher organic matter, such as Al-Bataliyah, may support more diverse nematode communities due to improved soil fertility and microbial activity. Alkaline soils with moderate $CaCO_3$ content, as seen in Al-Bustan, may favor nematodes like *Xiphinema*. The higher salinity and carbonate content often favor the prevalence of *Tylenchulus* nematodes. The preference of Helicotylenchus nematodes for specific soil pH conditions suggests that higher salinity might suppress Helicotylenchus abundance, or that this nematode prefers less saline conditions. Both nematodes, *Pratylenchus* and *Xiphinema*, can coexist in soil environments with moderate salinity and sufficient carbonate levels. Grasping the relationship between soil physicochemical attributes and PPNs is crucial for efficient and sustainable management. The citrus nematode *T. semipenetrans* demonstrated a favorable association with soil mineral elements (Na, Ca, K, and C) and the concentration of organic matter. A significant association was observed between these traits and the occurrence of *T. semipenetrans* in citrus farming regions, including Spain [Sorribas et al. 2008]. Conversely, Benjlil et al. [2020] discovered an inverse relationship between the occurrence of PPNs affecting saffron and OM in the soil. Increasing soil organic matter may considerably decrease PPN amounts in wheat by compromising their fundamental characteristics [Hu and Qi 2013]. The presence of *Pratylenchus* spp., *Helicotylenchus* spp., and *Xiphinema* spp. was strongly associated with the mineral composition, particularly Ca, Fe, and Na [Yavuzaslanoglu et al. 2012, Karuri et al. 2017].

Francl [1993] also identified a direct link between the abundance of *Heterodera glycines* and magnesium (Mg) levels. In the current research, most detected PPNs exhibited an inverse association with nitrogen (N). The accumulation of nitrate during nitrification is regarded as harmful to PPNs [Rodríguez-Kábana 1986]. In the current study, the phosphorus concentration positively correlated with the *Xiphinema* spp. and *Pratylenchus* spp. populations. Similarly, Nisa et al. [2021] observed the same positive trend between the soil phosphorus content and nematode populations. The pH of the soil markedly influenced the occurrence of citrus nematodes – decreased pH enhanced nematode population and diversity [Al-Sayed et al. 1993]. However, according to Salahi Ardakani et al. [2014], the citrus nematode was most abundant in soils with a pH of 7. Additionally, Van Gundy and Martin [1962] observed that the population of *T. semipenetrans* in citrus was fourfold greater in neutral soils than in acidic conditions. Soil structure and texture have significant effects on the mobility of soil nematodes. Based on our findings, fine sandy soil texture enhances the movement and dispersion of nematodes. Clay soils exhibited the highest prevalence of citrus nematodes [Salahi Ardakani et al. 2014]. Recently, Laasli et al. [2022] demonstrated that sandy and silt types of soils found in wheat fields contained *Aphelenchoides* spp., *Merlinius* spp., and *Pratylenchus* spp. However, *Longidorus* spp. and *Xiphinema* spp. appeared to have a greater prevalence in soil samples with increased clay content. Mokrini et al. [2019] concluded that several PPNs impacting raspberries significantly correlated with soil granulometry. According to Kim et al. [2017], sandy soils facilitate the proliferation of nematodes like *M. incognita* by enabling their locomotion and feeding behaviors.

CONCLUSIONS

This study sheds light on the PPNs' diversity. Citrus nematodes were found to predominate in soil and root matrices, likely due to favorable local soil and environment in Hasawi Lumi farming areas. Other commercially essential nematodes, including *Pratylenchus*, *Helicotylenchus*, and *Xiphinema*, were initially described in *C. aurantiifolia* orchards within Al-Ahsa Oasis. Farmers may use the relationship between these nematodes and soil qualities to manage PPNs effectively. The findings of this study will help researchers and pest management authorities control and mitigate PPNs to increase Hasawi Lumi production in Al-Ahsa Oasis.

The relationship between soil properties such as OM, $CaCO_3$, and EC and nematode communities in Hasawi Lumi growing areas reveals that higher organic matter and moderate calcium carbonate levels increase nematode diversity, while high salinity suppresses it. Effective soil management strategies, such as increasing organic matter and regulating salinity, are crucial for mitigating nematode infestations and improving productivity. Additional research is needed to investigate the direct relationships between soil properties and nematode species, and to develop strategies for balancing soil health with sustainable agricultural practices.

AUTHORS' CONTRIBUTIONS

HHK was responsible for the conception and design, data acquisition, data analysis, data interpretation, and article drafting; MAA-S handled sample collection, nematode extraction, and enumeration. Molecular analysis and data interpretation and article drafting: SME-G; Soil analysis and data interpretation: LMH; Statistical analysis and data interpretation: BVC. All authors have reviewed and consented to the final version of the manuscript.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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