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Diversity of Phytonematodes Associated with some Fruit Trees in Northwestern Coast, Egypt

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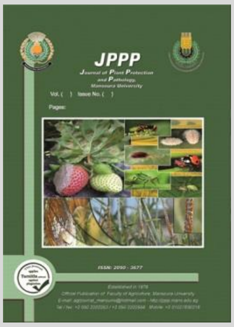

ABSTRACT

A survey study was conducted to identify the current status of the plant nematode community associated with growing trees in Northwestern of Egypt. About 1839 soil and root samples were collected, during the period from 2021 to 2022 at the entire costal districts besides the Siwa district of Marsa Matrouh governorate. Root- knot nematode, *Meloidogyne* spp. was the most abundant and dominant genus in all nine surveyed regions viz., Al Hammam, Al Alameen, El Moghra, Al Daba, Matrouh, Al Nigela, Sidi Barrani, Al Salloum, and Siwa. Results showed that 12 plant parasitic nematodes (PPNs) genera were recovered from all locations and identified as; *Criconema, Hoplolaimus, Helicotylenchus, Meloidogyne, Rotylenchulus, Pratylenchus, Pratylenchus, Tetylenchus, Trichodorus, Tylenchorhynchus, Tylenchus* and *Xiphinema*. Nematode genera were more diverse in Siwa district than others, since it possessed 10 genera (all recorded genera except *Pratylenchus, Pratylenchus*). The density of PPNs was affected with soil type as the fine sand texture favored nematode population, soil pH not greatly influenced nematode reproduction, but the host type significantly affects nematode population. Also, this study will pay the attention to consider nematode occurrence to prevent contamination of new reclaimed farms as well as manage their population in infested fields in these desert areas.

***Keywords*:** Plant parasitic nematodes, diversity, survey, fruits, Northwestern coast, Egypt

INTRODUCTION

Plant parasitic nematodes (PPNs) are microscopic animals; these worms consider the more abundant group in comparing with all other animal groups. PPNs can attacks many crops and causing losses range from mild and severe according to their population, plant species and nematode genus. *Meloidogyne* is the most destructive genus in phytonematodes, species and can infect over than five thousands plant belonging to many families.

Diversity of nematode groups are one of the aims of nematologists research, knowing the nematode genera and trophic groups are indicators in soil fertility while the detection of parasitic forms help suggesting the combating strategies as well as avoiding the translocation of phytonematodes from field to another and also restrict their transportation from area to another in the same field. Many nematological surveys were carried out in many Egyptian governorates including desert areas of Egypt (korayem et al., 2014 El-Nubby et al., 2019-Bakr et al., 2020), while in northwestern area of Egypt a limited surveys were accomplished (Ibrahim et al. 2000; (Youssef and Korayem, 2015; Basyony et al. 2020). These studies were confined in Burg Al Arab- Al Hamam - Matrouh not extended to Al Salloum. The diversity data aid the decision makers (farms, extension sectors, investors) in operating their farms in right ways to monitor these pests. Out of all phytonematodes, root-knot nematode, *Meloidogyne* has been reported as the most highly distributed nematode genus worldwide as it was found in various soils in all ecosystems characterized by temperature above than 3°C. *Meloidogyne* spp. are considered the most important group of phytonematodes due to their considerable damage occurred for plants (Ibrahim et al., 2010; Anwar, 2006; Anwar and Mckenry, 2010; Shakeel et al., 2012; Adamou et al., 2013; Korayem et al., 2014; Korayem and Youssef, 2015; Singh and Khanna, 2015; Kumar et al., 2017; Tariq-Khan et al., 2017 and Vindhyarani, 2017; El-Nuby et al., 2019; Basyony et al., 2020). The biodiversity of indigenous plant parasitic nematode communities can play an important role in the adaption and success of a new species in specific area, since native nematodes could be potential antagonists to the invasive one. For example, competition for roots between PPN species among communities has been observed (Garcia et al., 2018). As a result, information concerns the biodiversity and the factors affecting native phytonematodes communities in root crop fields are needed (Garcia et al., 2022). The current study was aimed to study the phytoparasitic nematodes diversity associated with some fruit trees in northwestern Egypt (Marsa Matrouh governorate), also studying the relationship between some soil properties, host species and nematode population density and their abundance.

MATERIALS AND METHODS

1. Sampling protocol

Nematological surveys were conducted in Maras Matrouh governorate (Northwestern coast besides Siwa district in western desert) during 2020 to 2021. A total of 1839 soil and root samples were collected from various fruit trees viz., Apple, Apricot, Citrus, Date palm, Fig, Grapes, Guava, Olive and Plum. Samples were collected from Rhizosphere zone at a depth of 30-60 cm. Samples were randomly collected for each crop by making a zigzag pattern across each field with soil auger, each sample composite form 4 cores and 4 sides of tree trunk that mixed together to form a one sample then bulked in plastic bags and labeled. About 1 kg for each sample was placed

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in an ice box during collection and was transported to laboratory, then stored at 4°C until processed. All samples were taken during the cropping growth season, March to October.

2. Extraction of nematodes from soil samples

Nematodes were extracted from soil samples no later than 48 h after sampling, using a modification of Cobb's decanting and sieving method (Van Bezooijen, 2006). After extensive mixing, a 250 cm^3 subsample of the soil was taken and washed through a sieve (1 cm aperture), to remove large debris. The soil was then mixed with 1.8 L water in a 2L beaker and stirred, until a homogeneous suspension was obtained. The suspension was then left to settle for 20 s and the supernatant passed sequentially through 250, 90 and 45µm sieves. Sieve residues were poured into a surface of plastic plate contains sieve covered with tissue paper. Samples were left for 24 h to allow any nematodes present to move into the collecting plates. Nematodes were subsequently examined under microscopes 100x. 3ml of nematode suspension was separately examined and the mean was calculated. Identification was done based on morphological characters to the genus level.

3. Nematode Identification

Morphological identification of *phytoparasitic* nematode

The genera of PPNs were initially identified to the genus level using microscopic examination of semi-permanent mounts. First, morphometric measurements were taken of individual nematodes using a Mejia compound microscope at 200x magnification. Morphological characters of adult and juvenile forms were detected and compared with pictorial key of Mai and Lyon, (1975) and illustrated key of Merete et al. (2012).

Molecular identification of root-knot nematode (RKN)

DNA extraction was done using nematode eggs that extracted from infected roots and purified by sucrose gradient centrifugation. Eggs were re-suspended in DNA lies buffer (100 mM NaCl, 100 mM Tris-HCl pH 8.5, 50 mM EDTA, 1% SDS, 1%β mercaptoethanol, and 100µg/ml Proteinase K), and incubated at 65°C for 1 hour. DNA was extracted with chloroform / isogamies alcohol 24:1 and precipitated in isopropanol at room temperature, and the DNA pellet was then washed twice with 70% ice-cold ethanol, re-suspended in H_2O and stored at -80°C. In the PCR reactions using species-specific primers, the following conditions were used: Denaturation 96°C for 1 min, annealing 50°C for 1 minute and extension 72°C for 2 min, repeated for 35 cycles. A 10-min incubation period at 72°C 5-min followed the last cycle to complete any partially synthesized strands. The primers sequence used in this process was 1-*M. incognita* TAGGCAGTAGGTTGTCGGG & 2-*M. incognita* CAGATATCTCTGCATTGGTGC. Details of RKN and primers used samples collected for this study illustrated in Table (1) and Figs.1&2 showed PCR products on Gel of RKN isolated from EL Moghra and Siwa locations. The PCR products were screened on a 1.0% Aganose gel, stained with ethidium bromide, and visualized on a midrange UV Tran's illuminator.

4. Nematode Estimation

Obtained data were based on two parameters viz., frequencies of occurrence (FO) and population densities (PD) per 250 cm^3 soils. The FO% of the PPNs was determined from the relationship among the numbers of samples (e) in which the genus was found divided by the total number of samples taken (E) from that location or crop, multiplied by 100 to express as a percentage [(FO = e/E) X100] according to (Seadog et al. (2009). Percentage of total frequency was calculated by submission all positive samples (that containing specific genus) in all locations and divided by the total numbers of samples collected with the locations containing the specific genus then multiplied with 100. PD of nematode species was calculated as the averages of the total number of nematodes recorded for those samples in which a nematode species was found (summation of individuals of specific nematode genus in all samples/total number of samples containing the same genus) as described by Norton (1978).

Table 1. Details of RKN and primers used samples collected for this study.

Sample Code	Species of RKN	Latitude (N)	Longitude (E)	Fragment size (bop)	location
RKN-01	*M. incognita*	30 29 076	029 02 326	TAGGCAGTAGGTTGTCGGG	EL Moghra
RKN-02	*M. incognita*	029 12 923	025 32 864	CAGATATCTCTGCATTGGTGC	Siwa

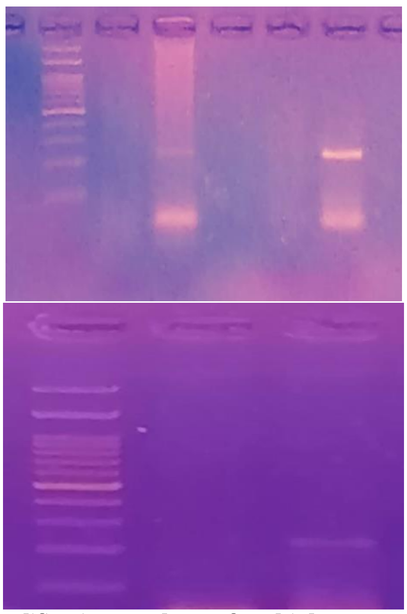

Fig. 1. Amplification products of multiplex PCR, Reactions using forward primers H- 18S, I — ITS,
EF — ITS and the reverse primer in a single PCR. (*M. incognita* of Fig host in EL Moghra location).

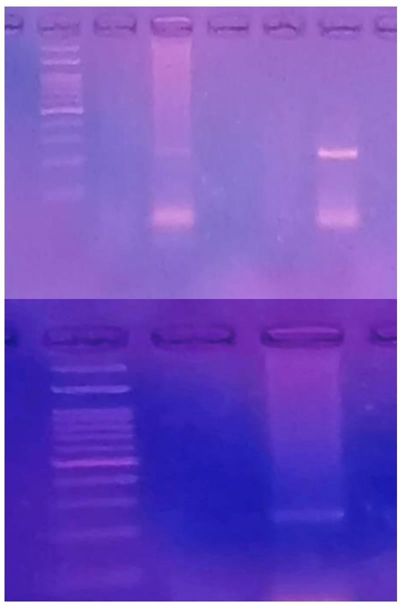

Fig. 2. Amplification products of multiplex PCR, Reactions using forward primers H- 18S, I — ITS,
EF — ITS and the reverse primer in a single PCR. (*M. incognita* of Fig host in Siwa location).

RESULTS AND DISCUSSION

Results:

Nematological survey carried out in Mersa Matrouh governorate in northwestern Egypt showed the presence of twelve PPN genera (*Criconema*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Tetylanchus*, *Trichodorus*, *Tylenchorynchus*, *Telemachus* and *Xiphinema*) that identified across the 1839 soil samples collected from this governorate. Data in table (2) and Fig.3 reveal that *Meloidogyne* was more frequent in all districts than other genera, followed by *Rotylenchulus*, which was found in nine districts. FO of *Meloidogyne* peaked in Al Salloum (61.2%) and Siwa (48.2 %). The maximum PD of *Meloidogyne* was present in Matrouh and Siwa (5370 and 5216 individual /250 cm3 soil, respectively). Root-knot nematodes isolated from El-Moghra and Siwa districts were identified as *Incognita*.

The diversity of PPNs was greater in Siwa district compared with others. *Criconema* and *Xiphinema* were confined to Siwa district only and were not observed in any fruit orchards. EL Daba district was the lowest one in the nematode diversity, since it contains only five nematode genera. Collectively, data of Mersa Matrouh governorate revealed that *Meloidogyne* spp. was the most prevalent genus (47.9%) and also possessed the maximum population density (3545.4 individuals), followed by *Rotylenchulus* (7%) with average density of 666.5 individuals. While the lowest frequent genera were *Xiphinema* (2.2%) and *Criconema* (5.8%), respectively.

Table 2. Population densities and frequencies of occurrence of phytonematodes associated with some fruit trees as influenced by locations in Northwestern coast, Egypt

Locations Nematode genera	Al Salloum N=181		Matrouh N= 214		Siwa N= 213		Al Hammam N=299		Sidi Barrani N=244		AL Nigela N=288		EL Mohra N=128		EL Daba N=173		AL Alameen N=99		Total N= 1839	
	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O	P.D	F.O
Criconema	-	-	-	-	800	5.8	-	-	-	-	-	-	-	-	-	-	-	-	800	12.0
Hoplolaimus	-	-	200	0.5	400	4.4	400	0.6	-	-	400	1.2	-	-	-	-	200	1.0	1400	22.3
Helicotylenchus	200	2.2	-	-	400	4.4	400	2.5	200	0.4	200	0.3	233	2.3	200	0.6	400	4.0	2233	20.4
Meloidogyne	4435	6.2	5370	29.9	5216	40.2	1186	16.7	3437	28.8	4733	17.4	2549	39.1	4343	28.9	640	50.0	31909	47.9
Pratylenchus	1300	6.8	1300	5.1	-	-	-	-	600	1.6	200	0.7	967	5.5	-	-	300	2.0	4667	25.2
Pratylenchus	-	-	400	1	-	-	1000	7	-	-	200	0.3	200	0.6	-	-	333	3.0	2133	16.7
Rotylenchulus	800	3.3	700	1.9	1033	9.8	1000	3.8	967	5.6	400	2.4	-	-	200	1.2	200	4.0	5300	19.5
Tetylanchus	300	3.3	600	1.4	600	5.3	-	-	200	0.4	560	1.7	500	4.7	-	-	200	1.0	2960	19.3
Trichodorus	-	-	500	1.8	1050	6.9	500	1.3	600	1.6	-	-	433	4.6	732	5.5	246	7.1	4061	18.6
Tylenchorynchus	825	3.4	200	0.5	600	5	-	-	-	-	300	0.7	700	5.5	200	1.2	-	-	2825	13.6
Tylenchus	200	0.6	-	-	1433	6.3	486	3	200	0.8	-	-	200	1.6	-	-	267	3.0	2786	14.1
Xiphinema	-	-	-	-	200	2.2	-	-	-	-	-	-	-	-	-	-	-	-	200	11.3

P. D = Population Density/250 cm^3 soil, F.O = Frequency of Occurrence %, Number between parentheses () represented samples

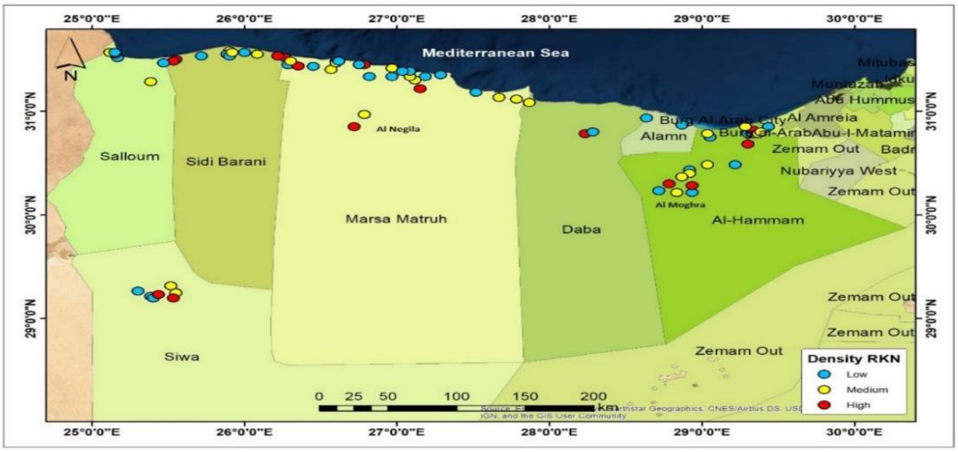

Fig. 3. Population densities of root-knot nematode associated with some fruit trees grown in different districts in Northwestern coast, Egypt

Results presented in Table (3) show that PPNs community in Mersa Matrouh was found to be varied according to the plant hosts. It was observed that favorable hosts were fig, olive and date palm according to their association with five nematode genera. It was noticed that olive plants harbored the maximum number of nematode genera (12) followed by fig and date palm (11 genera for both). On the other hand, apricot was a poor host as it restricted the diversity of PPNs in their Rhizosphere (only associated with root knot nematode), followed by plum and apple that found to be associated with two genera (*Meloidogyne* & *Pratylenchus* and *Meloidogyne* & *Hoplolaimus*, consequently).

Physiochemical properties of soil samples represent the surveyed locations were listed in Table (4) and illustrated in Fig. 4&5. It was noticed that RKN density not typically related with changes in pH and electrical conductivity (EC) values. The population density in samples 3,4 and 5 in Al Hammam location were matched with high values of pH and varied EC values. The fine sand (? % sand) in par with medium sand (? % sand) are favorable soil textures that increased nematode density. Organic matter (O.M %) or calcium carbonate (CaCo3%) content were found to have a poor effect on the nematode density. In El Mogra the highest density was closed to high pH and fine sand texture and not correlated with EC., O.M% and CaCo3% values, also low density was correlated to loamy sand structure. The similar finding was found in EL Daba, Matrouh, AL Nigala and Al Salloum as positive relation between texture and pH and density and diminished in loamy sand (? % sand) structure. Some samples of Sidi Barrani and Siwa showed some exceptions as low density observed to associate with fine or very fine sand structure. Collectively moderate to high organic matter content are favored the root-knot nematode population.

Table 3. Population densities and frequencies of occurrence of phytonematodes associated with some fruit tree as influenced with various hosts in Northwestern coast, Egypt

Host	Fig		Palm		Olive		Grapes		Apricot		Plum		Citrus		Apple		Guava		Total	
Nematode genera	P.D	FO	P.D	FO	P.D	FO	P. D	FO	P.D	FO	P.D	FO	P.D	FO	P.D	FO	P.D	FO	P.D	FO
Criconema	200	12.0	200	10.0	200	11.3	200	10.0	-	-	-	-	-	-	-	-	-	-	800	18.0
Hoplolaimus	200	10.0	200	10.4	200	8.5	-	-	-	-	-	-	-	-	400	12.5	200	10.2	1200	20.6
Helicotylenchus	550	16.7	467	30.6	633	14.3	-	-	-	-	-	-	400	21.3	-	-	-	-	2050	23.2
Meloidogyne	4074	36.9	4820	44.4	4695	57.4	4149	43.2	2580	43.2	3326	57.4	4199	47.4	2722	36.7	3492	48.3	34057	57.4
Pratylenchus	1100	17.4	1827	38.2	1300	26.2	440	12.0	-	-	-	-	-	-	-	-	-	-	4667	16.3
Pratylenchus	200	8.3	400	13.2	400	14.5	200	10.5	-	-	200	10.3	-	-	-	-	-	-	1400	13.5
Rotylenchulus	1433	9.8	1100	15.3	1367	12.6	400	13.2	-	-	-	-	200	10.3	-	-	-	-	4500	21.3
Tetylanchus	980	16.7	867	8.2	1300	17.3	-	-	-	-	-	-	200	11.5	-	-	200	14.7	3547	25.5
Trichodorus	1672	10.7	1083	11.3	850	11.8	-	-	-	-	-	-	-		-	-	200	18.6	3805	24.3
Tylenchorynchus	1025	8.6	900	7.9	900	10.4	-	-	-	-	-	-	-		-	-	-	-	2825	12.1
Tylenchus	300	18.5	200	10.2	200	8.3	200	12.4	-	-	-	-	1400	26.5	-	-	-	-	2300	17.8
Xiphinema	-	-	-	-	200	12.0	-	-	-	-	-	-	-	-	-	-	-	-	200	12.0

P.D = Population Density/250 cm^3 soil, F O = Frequency of Occurrence %, Number between parentheses () represented samples

Table 4. Impact of soil texture, pH, EC, calcium carbonate and organic matter content of samples represented all districts of Matrouh governorate on root-knot nematode density.

No	Location	N	E	pH	EC	Soil Texture	RKN Density	O.M%	CaCo3%
1	Al Hammam	30 47 175	029 18 925	5.82	2.75	M.S	400	0.98	36.32
2		30 47 587	029 20 118	7.76	1.86	F.S	400	0.32	3.71
3		30 49 876	029 20 591	8.05	1.08	M.S	1186	1.23	35.49
4		30 47 559	028 14 190	7.33	2.43	F.S	1000	0.68	42.79
5		30 41 100	029 18 878	7.60	3.15	M.S	1000	1.02	1.38
6		30 47 673	029 22 146	6.77	3.76	M.S	500	0.23	38.65
7		30 51 279	029 26 960	8.73	0.19	F.S	486	0.92	35.89
8		30 51 075	029 17 148	8.04	0.07	F.S	800	1.02	3.22
9		30 27 962	030 40 802	7.65	1.17	F.S	200	1.23	2.30
10		30 48 523	029 23 241	8.5	0.19	F.S	600	0.98	5.52
1	AL Alameen	30 47 578	029 02 533	8.03	0.82	F.S	200	1.02	2.30
2		30 45 297	029 03 244	8.93	0.13	F.S	400	0.43	30.18
3		30 47 297	029 02 578	8.39	0.48	F.S	640	1.18	4.14
4		30 52 344	028 52 740	8.38	0.96	F.S	300	0.62	40.49
5		31 20 062	027 11 613	8.77	0.25	V.F.S	333	0.23	42.79
6		30 56 768	028 38 566	8.86	0.3	F.S	200	0.91	2.76
7		31 18 430	027 07 764	8.67	0.94	F.S	200	1.42	31.29
8		31 18 430	027 07 764	7.76	0.821	F.S	246	0.63	3.40
9		31 18 430	027 07 764	7.93	0.647	F.S	267	0.98	2.30
10		31 18 430	027 07 764	7.32	2	V.F.S	800	0.32	3.22
1	EL Moghra	30 13 403	028 56 335	8.4	0.95	F.S	233	1.23	3.22
2		30 17 517	028 56 032	8.21	1.73	F.S	2549	0.58	28.07
3		30 13 562	028 50 393	7.96	5.79	F.S	967	1.02	21.63
4		30 29 054	029 13 253	8.1	6.22	F.S	200	0.23	42.79
5		30 29 076	029 02 326	7.82	7.9	L.S	500	1.18	1.38
6		30 26 592	028 57 421	7.95	8.15	F.S	433	0.52	38.65
7		30 24 472	028 55 301	7.92	4.62	F.S	700	0.23	35.89
8		30 14 556	028 43 123	7.27	0.936	V.F.S	200	0.98	43.61
9		30 22 509	028 52 014	7.88	0.836	F.S	600	0.22	12.22
10		30 18 704	028 47 136	7.71	2.92	F.S	1000	1.43	15.23

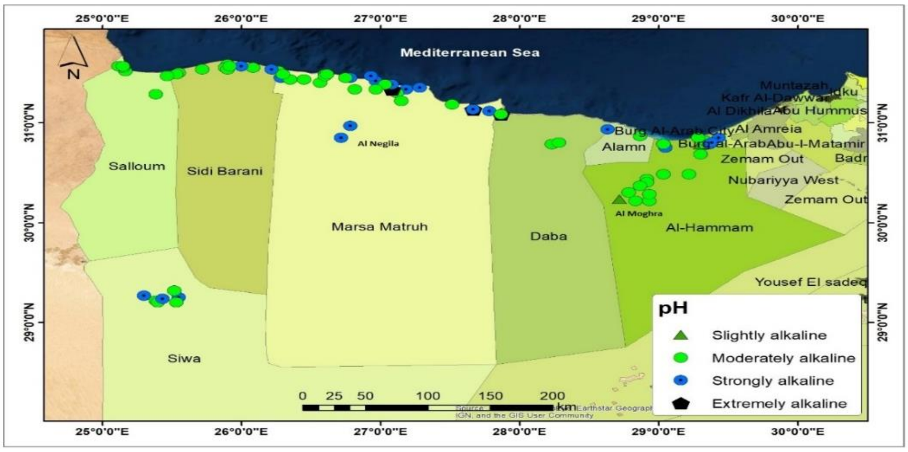

Fig. 4. Population densities of root-knot nematodes associated with some fruit trees as influenced with different pH values in Northwestern coast, Egypt

Table 4. cont.

No	Location	N	E	pH	EC	Soil Texture	RKN Density	O.M%	CaCo3%
1	EL Daba	30 51 584	029 51 571	8.63	0.22	F.S	200	0.98	36.51
2		31 05 272	027 52 232	9.29	0.22	F.S	4343	1.02	40.61
3		31 21 566	027 03 036	8.5	1.45	F.S	200	0.29	36.51
4		31 27 233	026 17 334	8.15	2.4	F.S	400	1.98	46.71
5		31 27 464	026 45 586	8.12	2.44	F.S	732	0.92	34.51
6		31 29 083	026 36 098	7.96	2.73	V.F.S	200	1.23	48.71
7		31 20 203	027 05 019	9.19	0.16	L.S	600	0.98	44.71
8		31 11 049	027 31 378	7.33	2.43	L.S	400	1.02	40.61
9		30 09 432	027 37 279	7.6	3.15	F.S	200	0.23	38.61
10		31 08 242	027 40 401	9.02	0.16	F.S	800	1.98	56.81
1	Matrouh	31 23 326	027 05 227	8.51	1.01	F.S	200	1.02	27.42
2		31 25 408	026 58 708	8.49	0.96	F.S	600	1.23	32.51
3		31 27 463	026 47 555	8.45	2.38	F.S	5370	0.28	30.40
4		31 28 358	026 56 188	8.43	2	F.S	1300	0.32	60.90
5		31 23 029	027 02 225	8.37	0.75	F.S	400	1.23	24.40
6		31 11 049	027 31 378	8.66	0.68	M.S	700	1.28	12.20
7		31 08 242	027 40 401	8.79	0.66	F.S	600	1.02	26.40
8		31 07 023	027 47 335	6.77	3.76	F.S	500	1.23	19.30
9		31 27 233	026 17 334	8.73	0.19	F.S	200	0.94	33.50
10		31 05 272	027 52 332	8.05	2.49	F.S	500	0.32	12.20
1	AL Nigala	31 35 541	028 35 541	8.28	2.23	L.S	400	0.29	42.60
2		31 20 081	026 49 426	7.33	1.78	F.S	200	1.92	62.90
3		31 31 033	026 16 688	8.07	3.49	F.S	4733	1.02	50.7
4		31 35 541	026 35 541	8.28	3.19	L.S	200	1.23	54.8
5		31 20 083	026 58 727	8.31	2.94	F.S	200	1.18	7.1
6		30 48 925	028 17 766	8.27	1.06	F.S	400	0.36	3.0
7		31 24 498	026 34 683	8.16	1.37	F.S	560	1.23	31.5
8		31 28 815	026 36 090	7.95	8.15	L.S	800	0.94	13.2
9		31 29 304	026 37 227	7.92	4.62	M.S	300	1.02	31.5
10		31 27 852	026 45 189	8.12	1.22	F.S	400	0.23	14.2

Table 4 cont.

No	Location	N	E	pH	EC	Soil Texture	RKN Density	O.M%	CaCo3%
1	Sidi Barrani	31 26 671	026 27 382	8.32	0.91	L.S	200	0.93	56.8
2		31 26 856	026 21 092	8.4	1.18	F.S	3437	1.02	64.9
3		31 29 127	026 18 806	8.3	1.56	V.F.S	600	0.23	54.8
4		31 32 123	026 13 588	8.5	1.18	F.S	3000	0.91	34.5
5		31 33 729	026 05 755	8.3	2.28	L.S	967	1.02	56.8
6		31 34 730	026 00 532	8.52	1.79	M.S	200	1.23	67.0
7		31 35 365	025 54 131	7.79	8.24	F.S	600	1.92	36.5
8		31 33 105	025 53 614	7.33	1.78	M.S	400	1.02	50.7
9		31 32 330	025 54 380	8.07	3.49	M.S	200	1.23	52.8
10		31 34 663	025 55 188	7.94	7.55	V.F.S	850	0.78	38.6
1	Al Salloum	31 32 876	025 43 695	7.72	4.45	F.S	200	1.02	56.8
2		31 30 299	025 33 169	7.96	1.73	F.S	4435	1.23	43.6
3		31 29 179	025 32 059	8.07	2.73	M.S	1300	0.95	44.7
4		31 28 080	025 28 370	8.18	1.7	F.S	400	1.02	36.5
5		31 27 717	025 22 434	5.82	2.75	M.S	800	1.25	54.8
6		31 31 385	025 10 188	7.76	1.86	F.S	300	1.28	36.5
7		31 33 212	025 09 859	8.05	1.08	M.S	1000	1.02	35.8
8		31 34 079	025 07 602	7.79	8.24	M.S	825	0.73	8.9
9		31 34 312	025 09 311	7.94	7.55	F.S	200	1.88	40.2
10		31 17 106	025 23 444	7.33	2.43	F.S	600	0.72	26.8
1	Siwa	31 13 845	027 09 687	7.6	3.15	F.S	1033	1.23	40.2
2		30 58 929	026 47 368	6.77	3.76	F.S	600	1.90	40.2
3		30 51 511	026 43 430	8.73	0.19	F.S	1050	1.02	40.2
4		029 15 314	025 33 567	8.5	0.19	F.S	600	1.23	44.7
5		029 12 923	025 32 864	8.03	0.82	F.S	1433	0.98	26.8
6		029 16 822	025 18 070	8.93	0.13	F.S	200	1.02	22.4
7		029 13 582	025 23 617	8.39	0.48	V.F.S	400	1.23	44.7
8		029 12 653	025 24 277	7.6	3.15	F.S	400	0.98	13.4
9		029 14 088	025 26 153	6.77	3.76	F.S	5216	1.02	22.4
10		029 19 019	025 31 162	8.38	0.96	F.S	800	0.23	8.9

EC: Electrical conductivity, M.S: Medium Sand, F.S: Fine Sand, V.F.S: Very Fine Sand, L.S: Loamy Sand, RKN= Root knot nematode, O.M: organic matter content

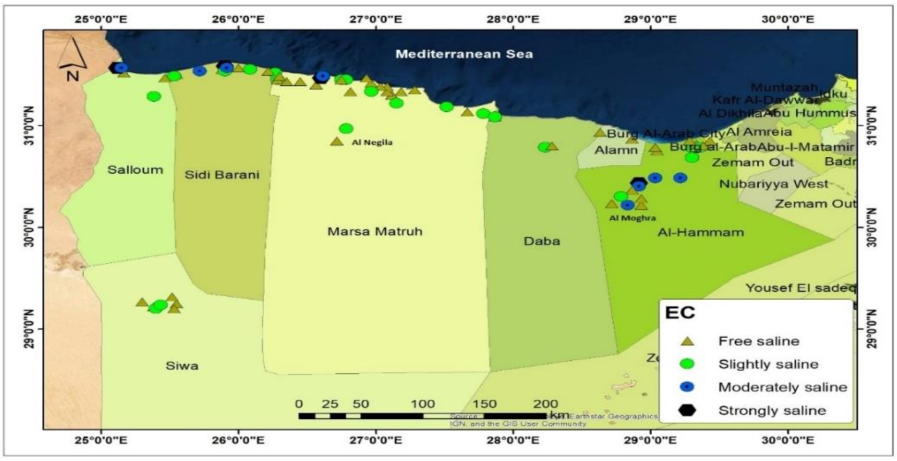

Fig. 5. Population densities of root-knot nematodes associated with some fruit trees as influenced with different soil salinity in Northwestern coast, Egypt

Discussion

Plant parasitic nematodes infect all plants of economic crops; also they consider a major challenge to the production of various crops, particularly in sandy soil and newly cultivated desert lands (Ibrahim et al., 2000 and El-Nubby et al., 2019). The current study describes the PPNs communities in agro systems of various districts in Marsa Matrouh governorate associated with various fruit trees (apple, apricot, citrus, date palm, fig, grapes, guava, olive and plum). Based on the covered sample area and the number of nematode taxa assessed, the first extensive investigation of PPNs in Northwestern coast, accomplished few surveys confined to Borg Al Arab location, starting from El Hammam to Al Salloum district in northern Egypt either under rain conditions in all districts or under irrigation conditions fields in Al Hamam and Siwa districts. Sampling in 9 geographical areas or districts highlighted differences between communities due to some factors including; agricultural practices, soil properties, physical and chemical and host species. The overall biodiversity revealed 12 PPN taxa (*Criconema*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Tetylanchus*, *Trichodorus*, *Tylenchorynchus*, *Tylenchus* and *Xiphinema*) that identified across the various examined fields. Meloidogyne spp. were identified as *M.incognita* in El-Moghra and Siwa districts

Presence of these PPNs on fruit plantations must be taken seriously by farmers; especially they live in tropical and subtropical areas including Egypt. The association of these nematodes with fruit trees has been reported to diminish yields (Abd-El Gawad et al., 2009, Kepenekci et al., 2014). Many investigations were carried out to analyze the parasitic nematode community in several fruit trees; Mohamed et al. (2017) carried out a survey in three governorates of Egypt, to determine the density and frequency of phytonematodes occurring in grape orchards. They reported the presence of 10 PPN genera viz., *Criconemoides*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus*, *Tylenchulus* and *Xiphinema*. Frequency and density of each nematode genus was varied due to plant cultivars and soil type. The root-knot nematode (RKN) was the predominant nematode in all surveyed locations as well as it was abundant in sandy soil in conversely to citrus nematode that was found in soil contains more clay percent with high occurrence value.

Taha (2018) studied the abundance and distribution of PPNs associated with some different plant hosts including some host similar to our study (grapes, apples and lemon), they found that most frequent nematodes were *Meloidogyne*., *Helicotylenchus*, *Pratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Hoplolaimus*, *Tylenchorhynchus*, *Tylenchulus* and *Xiphinema.*

An investigation was carried out by Ibrahim et al. (2000) in northwestern coast of Egypt. They found that 9 genera of PPNs collected from Matrouh governorate (El Hammam district) namely *Helicotylenchus*, *Meloidogyne*, *Tylenchus*, *Ditylenchus*, *Pratylenchus*, *Boleodorus*, *Aphelenchoides*, *Aphelenchus* and *Tylenchorhynchus* were the most common nematodes in the soil from this desert governorate. The RKN *M. incognita* and *M. javanica* were common in most samples. They also recovered nematodes associated with olive viz., *Ditylenchus*, *Meloidogyne* and *Tylenchorhynchus.* While the PPNs isolated form date palm Rhizosphere were *Helicotylenchus*, *Meloidogyne*, *Tylenchus*, *Ditylenchus*, *Pratylenchus*, *Boleodorus* and *Tylenchorhynchus.*

In northern Egypt a few nematological surveys were conducted, El-Nuby et al. (2019) carried out the faunistic survey in Sinai Peninsula, Egypt. They recorded 13 genera of PPNs (*Belonolaimus*, *Criconema*, *Criconemoides*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Tetylenchus*, *Trichodorus*, *Tylenchorhynchus*, *Tylenchus* and *Xiphinema*) in 9 families, belonging to 3 orders of phylum Nematoda *Meloidogyne* was the most abundant and dominant genus in all surveyed districts. Nematodes in North Sinai were more diverse than South Sinai, where Korayem et al. (2014**)** surveyed some villages of Rommna district, North Sinai for PPNs associated with various plants, they recorded 14 genera of PPNs (*Criconema*, *Criconemoides*, *Ditylenchus*, *Hemicriconemoides*, *Heterodera*, *Hoplolaimus*, *Longidorus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus*, *Tylenchulus s*, *Tylenchus* and *Xiphinema*). They found that olive trees harbored 6 genera of PPNs (*Ditylenchus*, *Meloidogyne*, *Rotylenchulus*, *Tylenchorhynchus*, *Tylenchus* and *Criconemoides*), date palm harbored 5 PPNs genera (*Tylenchorhynchus*, *Meloidogyne*, *Pratylenchus*, *Tylenchus* and *Hoplolaimus*), guava harbored 7 PPNs genera (*Hemicriconemoides*, *Meloidogyne*, *Tylenchorhynchus*, *Tylenchus*, *Rotylenchulus*, *Helicotylenchus*, *Ditylenchus*), grapes harbored 3 PPNs genera (*Criconema*, *Helicotylenchus* and *Meloidogyne*), citrus harbored 3 PPNs genera (*Rotylenchulus* and *Tylenchulus semipenetrans*) and only one genus (*Pratylenchus*) was found associated with apple Recently, Zoubi et al., (2022) reported that the most predominant PPN species were *Tylenchulus semipenetrans* (88%), *Helicotylenchus* spp. (75%), *Pratylenchus* spp. (47%), *Tylenchus* spp. (51%), and

Xiphinema spp. (31%) in some Moroccan citrus orchards. Similarly, Abu Habib et al. (2020) found 9 nematode genera (*Aphelenchoides*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchus*, *Trichodorus*, *Tylenchorhynchus*, *Tylenchus*, *Tylenchulus)* associated with citrus trees (which district?) and citrus nematode was the dominant species.

Another investigation carried out by Bakr et al. (2020) in Beheira and Menoufia governorates, Egypt including various crops), showed that RKN was found associated with all investigated plants. They also reported that P.D and F.O of RKN were varied in the different crops and different areas. Furthermore, fruit trees viz., grapes and citrus possessed 100% F.O of *Meloidogyne* spp. In 2021, *Sweelam et al.*, conducted a nematological survey in newly reclaimed areas belonging to El-Sadat Province. The survey included some fruit trees viz., orange, mango, grapes and peach. There were 4 PPNs genera associated with orange trees, *Tylenchulus*, *Pratylenchus*, *Hemicycliophora* and *Xiphinema*. The most important nematode genera or species in the soil of grape variety were *Meloidogyne*, *Pratylenchus*, *Criconemoides* and *Xiphinema*. Grapes vineyards were infested with many phytonematodes, the survey accomplished by Mario Fajardo et al. (2011) who found 6 PPN genera (*Xiphinema*, *Mesocriconema*, *Meloidogyne*, *Tylenchulus semipenetrans* and *Helicotylenchus)* associated with grapes in three districts in Chile. Al-Halabi and Al-Assas (2021) recovered eight phytoparasitic nematodes viz., *Xiphinema*, *Meloidogyne*, *Pratylenchus*, *Paratylenchus*, *Helicotylenchus*, *Tylenchorynchus*, *Tylenchus* and *Ditylenchus* from grapevine roots in Sweida Governorate, Syria. A survey of phytoparasitic nematodes associated with apple trees in Homs governorate, Syria, carried out by Khaleel et al. (2010) revealed the presence of 15 PPNs genera and their frequencies were; *Helicotylenchus* (26.35), *Paratylenchus* (34.46%), *Pratylenchoides* (60.14%), *Pratylenchus* (47.97%), *Rotylenchulus* (15.54%), *Tylenchorhynchus* (9.46%) and *Xiphinema* (16.22%), while *Meloidogyne* spp. was the less frequent one (7.43%).

A survey for PPNs in citrus plantations was conducted by Marshall and Opoku-Asiama (2009) in Ghana. They recorded seven PPN genera (*Helicotylenchus*, *Meloidogyne*, *Pratylenchus, Rotylenchulus*, *Tylenchorhynchus, Tylenchus* and *Xiphinema)* associated with citrus trees. Abrantes et al. (2008) conducted a survey in fig orchards in Portugal, they found 8 PPN genera. The most widely distributed genera were *Helicotylenchus*, *Heterodera*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, and *Xiphinema*. They reported that RKN consider the most damaging to fig trees. Also, date palm was surveyed for detecting phytonematodes; Mani et al. (2005) surveyed the date palm plantation in some districts of Sultanate of Oman. They identified 17 PPN genera associated with date palm trees. The important parasitic nematodes found in date palm rhizosphere included populations of *Helicotylenchus*, *Meloidogyne*, *Rotylenchulus* which were found in high densities. The predominant *Meloidogyne* spp. in current investigation was supported by many findings; Hamza et al. (2017) stated that RKNs were found in 159 soil samples out of 305 from olive nurseries, in Morocco. Past reports also in harmony with these findings, Hashim (1982) reported that *Meloidogyne* spp. are major pest of olive trees as high occurrence is usually noticed.

The impact of soil properties either physical or chemical and soil type was variably affected the nematode distribution and abundance, population density, and also the variation of the PPN communities or biodiversity. These soil physicochemical properties are known to be important factors for PPNs as they may modify their habitat, metabolisms, or movement etc. In current study, the relationship between soil structure and population density was observed in many cases. Sandy soil texture or light texture enhanced the root knot nematode population density. This observation is in accordance with other investigations; RKNs prefer light soils with low clay content and its reproduction was greater than in heavy soil. Its population density is higher in sandy soils than in silt and clay soils (Jaraba-Navas et al., 2014). The results of Mario Fajardo et al. (2011) declared that the density of *Meloidogyne* spp. was negatively related with the sand content but positively correlated with the more structured soil. This may be due to RKN which travels and aerates easily in light sandy soil, causing more damage to host plants (Feyisa, 2022). Root penetration rates and the number of galls, egg masses, of *M. incognita* increased in sandy soil compared to other soil textures (Kim et al., 2017 & Anwar and Mckenry, 2010). Zoubi et al., (2022) showed that PPN distributions were correlated with soil physicochemical properties such as soil texture, pH levels, and mineral content. Based on their obtained results, it was concluded that besides the direct effects of the host plant, physicochemical factors of the soil could greatly affect PPN communities in citrus growing orchards. In the opposite, Hamza et al. (2017) stated that physiochemical soil factors did not greatly contribute to the structuring of *Meloidogyne* diversity, also this concept are matched with our results in some cases.

Nematode population appeared to decrease with decreasing soil ph. This observation was confirmed by positive correlation between soil pH and nematode, there was no obvious effect of pH on nematode population under each crop during the study (Marshall and Opoku-Asiama, 2009). These results relatively matched with our study the pH was positively correlated with high nematode population in many cases. Talwana et al. (2008) found relationships between densities of some PPNs and soil physiochemical characterizes besides the cropping history. Also it was stated that either increase or decrease in soil pH negatively affected nematode population (Salahi Ardakani et al., 2014). Generally, nematode root infections were favoured by reduced silt and clay content, like *Meloidogyne* and *Pratylenchus* that *tended* to increase with the raise in sand content in the soil and the reduction of clay percent in the soil. Also soil pH was correlated with diminishing *Pratylenchus* populations while increasing in N content was associated with a decline in nematode population densities.

Studying the nematode diversity is an important issue because well knowing of various trophic groups of soil nematode are greatly beneficial for good governing the parasitic forms, in particular PPNs, also they improve the understating the food web in soil. Our investigation was carried out to study the biodiversity of pant parasitic nematodes associated with some fruit trees in rarely explored areas belonging to Marsa Matrouh governorate. The knowledge of the nematode community' structure provides information related to the various processes occurred in the soil, the food web in the soil, and the state of stability of agro-ecosystems and soil biodiversity (Laasli, 2022).

CONCLUSION

The current study represents one of the rare surveys carried out in Northwestern coast of Egypt. The nematological survey was conducted in nine districts of Matrouh governorate, (Al Hamam, It was found that 12 phytopathogenic nematode genera variably distributed in different surveyed locations of fruit cultivations in Matrouh governorate. Root- knot nematode was the most predominant parasitic nematode, as it was recovered from all districts under this extensive work, the frequency of this nematode varied from district to another but still topped other genera. The diversity of phytonematodes fauna was found to be affected by many factors including; plant host, cultural practices, cultivation intensity, and soil type and irrigation system. Current and future studies should put the objective of mapping out of phytophages nematodes incidence and the impact of cropping sequence on survival, diversity and dynamics of PPNs. Also, this study will make alarm to growers and investors to avoid infestation with PPNs especially root- knot nematode in new reclaimed lands and focusing on managing phytonematodes in their farms as well as in fruit orchards either under rain fed or irrigated conditions in north coast. Finally, any agricultural extension project should consider these data in cultivation or selection crop composition in these infested locations. Also special procedures must be included in new cultivated land to prevent the introduction of serious nematode pests. In this concern, this study provides a useful design of plant parasitic nematode management strategy in cultivated area in particular fruit orchards.

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تنوع النيماتودا المتطفلة نباتياً والمصاحبة لبعض أشجار الفاكهة في الساحل الشمالي الغربي ، مصر

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الملخص

أجري هذه المسح لتحديد الوضع الراهن لمجتمع النيماتودا المتطفلة نباتياً والمرافقة لبعض أشجار الفاكهة في محافظة مرسي مطروح- شمال غرب مصر. تم جمع حوالي ١٨٣٩ عينة من التربة والجذور للأشجار خلال الفترة من ٢٠٢١ إلى ٢٠٢٢ بكامل المراكز الساحلية بجانب مركز سيوة بجنوب محافظة مرسى مطروح. إتضح من النتائج أن نيماتودا تعقد الجذور *Meloidogyne*كان الجنس الأكثر إنتشارًا وسيادة في جميع المناطق التسع التي شملها المسح وهي: الحمام، العلمين، المغرة، الضبعة، مطروح، النجيلة، سيدي براني، السلوم وسيوة. أظهرت النتائج وجود ١٢ جنساً من النيماتودا المتطفلة علي النبات (PPN) تم عزلها من جميع المواقع وتم تعريفها كالتالي: *Helicotylenchus, Hoplolaimus, Rotylenchulus, Tylenchorhynchus, Criconema, Meloidogyne, Paratylenchus, Pratylenchus, Tetylenchus, Trichodorus, Tylenchus, Xiphinema* . وكان مركز سيوة هو أكثر تنوعًا من غيره، حيث تم عزل ١٠ أجناس نيماتودية منه (جميع الأجناس المسجلة، بإستثناء *Pratylenchus, Paratylenchus*). تأثرت كثافة النيماتودا النباتية بنوع التربة حيث أن قوام الرمل الناعم عزز تعداد النيماتودا، ولم يؤثر الرقم الهيدروجيني (الحموضة) للتربة بشكل كبير على تكاثر الديدان، بينما يؤثر نوع العائل بشكل كبير على تعداد النيماتودا. أيضًا، ستولي هذه الدراسة الإهتمام للنظر في تواجد النيماتودا في أماكن معينة مما يساعد في منع أوتقليل تلوث البساتين المستصلحة حديثاً بالنيماتودا وكذلك إدارة أعدادها في الحقول الموبوءة في هذه المناطق الصحراوية.