Plant Parasitic Nematodes Associated with Citrus Trees and Reaction of Two Citrus Cultivars to *Tylenchulus semipenetrans* in Northern Egypt

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ABSTRACT: A survey of plant-parasitic nematodes (PPN) associating citrus trees in Alexandria, El-Behera and Kafr El-Shiekh governorates, northern Egypt was carried-out during 2017 – 2019 growing seasons. A total of 432 root and rhizosphere soil samples were collected from the surveyed citrus trees. Nematodes were extracted using sieving and Bearmann-pan techniques and, identified to the generic level, based on the morphological characters. Besides, the reaction of mandarine, *Citrus deliciosa* and lime, *C. aurantifolia* against the citrus nematode, *Tylenchulus semipenetrans*, was determined under greenhouse conditions. Results showed that 9 genera of PPN were found in association with the surveyed citrus trees in the three governorates. The citrus nematode, *Tylenchulus semipentrans* was found to be very common (frequency of occurrence (FO) = 100% in all the surveyed locations, followed by the stubby root nematodes, *Trichodorus* spp., and the lesion nematodes, *Pratylenchus* spp. The reaction of the two citrus cultivars; Mandarine, *Citrus deliciosa* and Lime, *Citrus aurantifolia* to *T. semipenetrans* showed that the two cultivars are susceptible to all the tested populations of *T. semipentrans* with a nematode reproduction factor (RF) was (1.67-2.6).The shoot and root dry weights of these cultivars were reduced as a result of the nematode infection.

Key words: citrus nematode, lime, mandarin, reaction, survey

INTRODUCTION

Citrus is one of the most important economic fruit crops in various regions of the world. So, it has received a great attention in agriculture and industry. In Egypt, citrus has great attention due to its importance for local consumption or exportation to the European countries. Plant diseases caused by soil borne pathogens such as nematode and /or fungi have been considered as major problems in agricultural production throughout the world (Abd-El Gawad *et al*., 2009). In Egypt, plant parasitic nematodes especially *Meloidogyne* spp., *Patylenchus* spp., *Tylenchulus semipenetrans*, and *Xiphinema* spp. constitute one of the most important nematode pest groups on many economic fruit crops (Ibrahim *et al*., 2010).

The nematode of the citric fruits probably infest more than 50% of the citrus production areas. The losses of the citrus yields caused by these nematodes were estimated to be ≈10% (van Gundy, 1984). Studies on the economic yield losses of citrus trees due to *T. semipenetrans* infection showed that these losses might reach up to 10% - 30%, depending on the level of nematode infection (Duncan and Cohn, 1990 and Verdego-Lucas and McKenry, 2004).

Nematode infection of the citrus feeder roots might increase the severity of the drought stress on the citrus trees, and decrease the ability of these roots to uptake water and minerals from the soil. Affected trees have an unthrifty appearance, fewer yields, and smaller fruits, compared to the non-infected trees (Duncan and Cohn, 1990).

The objective of the present work, survey study was to identify phytoparasitic nematodes associated with citrus trees in northern Egypt and estimate the frequency of occurrence and population densities of these nematodes in the collected rhizospheric soil samples and reaction of some citrus cultivars to *T. semipenetrans*.

MATERIALS AND METHODS

1. Survey study

A total of 432 soil and root samples were collected from the rhizosphere of citrus trees showing disease symptoms during the period from 2017 to 2019 from different localities at Alexandria (142 samples), El – Behera (65 samples) and Kafr El-Sheikh (225 samples) governorates, northern Egypt. Soil and root samples were placed in polyethylene bags, and kept in the refrigerator at 4°C until nematode extraction within 48 hrs. Whenever processed, each sample was thoroughly mixed and rendered for nematode extraction using wet-sieving and Baermann-pan technique (Goodey, 1957; Ayoub, 1980). Plant parasitic nematodes in aliquots of 1 ml of each nematode suspension were placed in Peter's 1 ml eelworm counting slide and the nematode genera were identified and counted with the aid of a compound microscope. The nematodes were identified to the generic level, based on the morphological characteristics of adult and larval forms following the descriptions of Thorne (1961), Goodey (1963) and Mai and Lyon (1975).

2. Reaction of some citrus species to Tylenchulus semipenetrans

The reaction of mandarine, *Citrus deliciosa* and lime, *Citrus aurantifolia* to *T. semipenetrans* was determined under greenhouse conditions. Citrus seedlings of five months old were planted in 20 cm diam. prickled polythene bags, containing 4 kg of steam-sterilized sandy clay soil (1:1, v/v) as one seedling/bag. Soil of each bag was infested with 5000 J_2s/ bag of *T. semipenetrans* the nematode inoculum was added in 10 ml water suspension into four holes around the seedling roots. Non inoculated plants served as control. Nematode treatments and control were replicated 5 times. Bags were arranged in a randomized complete block design on a greenhouse bench and irrigated and fertilized as needed.

Plants were harvested after 180 days of nematode inoculation and assessed for nematode infection. Root systems were carefully washed with a gentle stream of running tap water. Number of J_2s in each experimental unit (final nematode population =Pf) was determined and the nematode

reproduction factor (Rf = Pf / Pi*)* was calculated to determine the host suitability. The tested citrus plants were rated according to their nematode Rf values, as follows: Plants with Rf = 0 were considered as resistant; those with Rf = 0.1-0.5 moderately resistant; those with Rf = 0.6-1.0 moderately susceptible; those with Rf =1.1-5.0 susceptible; and those with Rf > 5 were considered as highly susceptible (Ali, 2005). The fresh and dry weights of the shoot and root systems of the harvested plants were also determined.

Data were statistically analyzed using a computer statistically analysis system (SAS institute Inc., 2000). and means were separated using least significant differences test (LSD)at P ≤ 0.05.

RESULTS AND DISCUSSION

Data presented in Table 1 showed the frequency of occurrence (FO) and population density (PD) of plant-parasitic nematode (PPN) genera associating the citrus trees in Alexandria, El-Behera and Kafr El-Sheikh governorates.

Nine PPN genera were found in association with soil and roots of the surveyed citrus trees i.e. *Tylenchulus semipenetrans, Trichodorus, Rotylenchus, Helicotylenchus*, *Aphelenchoides, Tylenchus, Pratylenchus*, *Tylenchorhynchus* and *Meloidogyne*. The nematode genera; *Helicotylenchus, Pratylenchus, Trichodorus* and *Tylenchulus semipenetrans* (Table1). were detected in all the soil samples collected from citrus trees fields.

Soil samples from Alexandria governorate (Abees location) showed that four nematode genera were associating the rhizosphere of citrus trees including *Tylenchulus semipenetrans* which was the most frequent nematode genus detected with 100% FO. The nematode genera *Tylenchorhynchus* and *Pratylenchus* detected 18-20% FO followed by *Trichodorus* (FO= 11%). *Tylenchulus semipenetrans* showed the highest population densities (PD)(2695 nematodes /250g soil). On the other hand, the lowest Pd was recorded for *Trichodorus* (PD= 11 nematodes/250g soil) Table 1).

Soil samples of Alexandria governorate (El-maamora location) showed the presence of eight nematode genera in the rhizosphere of citrus trees. *Tylenchulus semipenetrans* was also the most prevalent with (FO= 100% and PD=1105 nematodes /250g soil) (Table 1).

Five nematode genera were detected in the collected citrus soil samples from El-Behera governorate (Abu Homus location) *Tylenchulus semipenetrans* was the most prevalent with (FO= 100% and PD= 6810 nematodes /250g soil).The nematode genera *Helicotylenchus and Trichodorus* had 10-20% FO*.* and PD of 6 -23 nematodes /250g soil) (Table 1).

Six nematode genera were detected in the collected citrus soil samples collected from El-Behera governorate (Kafr El**-**Dawar location) *Tylenchulus semipenetrans* was also the most prevalent (FO= 100% and PD=3560 nematodes /250g soil), flowed by *Helicotylenchus* and *Pratylenchus* (FO = 32-36 % and PD= *45* -160 nematodes /250g soil) (Table 1).

Seven nematode genera were detected in the citrus soil samples collected from Kafr El-Sheikh governorate (Motobus location) *Tylenchorhynchus* was the most common (FO= 100% and PD= 7121 nematodes /250g soil). On the other hand, *Helicotylenchus, Tylenchorhynchus, Pratylenchus,* and *Aphelenchoides* were the least prevalent (FO= 1.3-8% and PD= 115-204 nematodes /250g soil) (Table 1).

Six nematode genera were recovered from the citrus soil samples collected from Kafr El-Sheikh governorate (Fuoa location) *T. semipenetrans* was the most common (FO= 100% and PD= 4021 nematodes /250g soil), followed by *Tylenchorhynchus* and *Trichodorus* showed (FO= 14% and PD = 15-92 nematodes /250g soil). However, *Pratylenchus, Helicotylenchus,* and *Aphelenchoides* were less common with 2-6% FO and PD of 13-34 nematodes /250g soil. (Table **1**).

The survey studies evident that Nine PPN genera were found in association with soil and roots of the surveyed citrus trees, the nematode genera *T. semipenetrans* was the most prevalent with (FO= 100%).

Table (1). Frequency of occurrence (FO) and population density (PD) of plant-parasitic nematode genera associated withcitrus trees in Alexandria, El-Behera and Kafr El-Sheikh governorates

Nematode genera	Alexandria				El-Behera				Kafr El-Sheikh			
	Abees 50[a]		El-maamora 92		Abu Homus 15		Kafr El-Dawar 50		Motobus 175		Fuoa 50[a]	
	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD
Aphelenchoides	-	-	11	37	22	36	-	-	8	126	6	34
Helicotylenchus	-	-	1	55	20	6	32	45	1.3	193	3	13
Meloidogyne	-	-	6.5	20	-	-	9	135	-	-	-	-
Pratylenchus	20	110	7	240	-	-	36	160	6	204	2	24
Rotylenchus	-	-	8.6	50	-	-	-	-	-	-	-	-
Trichodorus	11	6	7	160	10	23	16	273	12.6	289	14	92
Tylenchorhynchus	18	120	-	-	-	-	-	-	4	115	14	15
Tylenchus	-	-	1	10	-	-	-	-	-	-	-	-
Tylenchulussemipenetrans	100	2695	100	1105	100	6810	100	3560	100	7121	100	4021

a =Number of collected samples.
FO = (Number of positive samples/total no. of collected samples) × 100.
PD = Mean number of nematodes/250g soil in the positive samples.

Reaction of two citrus rootstocks to some populations of *Tylenchulus semipenetrans:*

1- Reaction of mandarine to some populations of *T. semipenetrans*:

The data presented in Table 2 revealed that mandarine *citrus aurantum* L. was susceptible to all populations of *T. semipenetrans,* with nematode reproduction factor Rf (1.67 - 2.1). Infection with *T. semipenetrans* reduced the shoot and root dry weights of mandarine the die back branch ends were noticed on the arial parts of tested seedlings of mandarine, *Citrus deliciosa*.

2- Reaction of lime to the some populations *of T. semipenetrans*:

Data presented in Table 3 showed that lime, *citrus aurantum* L. was susceptible to all the tested populations of *T. semipenetrans* where nematode reproduction factor Rf reached up to 2.1 - 2.29. Infection with *T. semipenetrans* reduced the shoot and root dry weights of Lime seedlings (Table 3).

Table (2). Reactions mandarine seedlings to six populations of the citrus nematode, *T. semipenetrans*, 180 days after inoculations

Nematode population	No. of J_2s/Bag (Pf)	Rf	Host Reaction	*Dry weight (g)*	
				Shoot	***Root***
1- Abeis	10499 a	2.10 a	S	1.35 d	0.65 b
2- El-mamora	8711 c	1.74 c	S	2.00 b	0.85 b
3- KafrEl-Dawar	9811 b	1.96 b	S	1.40 d	0.53 b
4- Abu Homus	9857 b	1.97 b	S	2.00 b	0.32 b
5- Motobus	9785 c	1.96 b	S	1.76 c	0.71 b
6- Fuoa	8795 b	1.67 d	S	1.44 d	0.55 b
Control	-	-	-	2.9 a	1.85 a

Data are average of 4 replicates each.
Means followed by the same letter (s) in each column are not significantly different at $P \leq 0.05$.
Rf = Final population (Pf) / Initial population (Pi= 5000 J_2s /bag).
S = Susceptible.

Table (3). Reaction lime seedlings to six populationsof the citrus nematode *T. semipenetrans,* 180 days after inoculation

Nematode population	No. of J2s/ Bag (Pf)	Rf	Host Reaction	Dry weight (g)	
				Shoot	Root
Abeis	11433 a	2,29 a	S	1.35 b	0.35 b
El-mamora	10286 a	2.10 a	S	1.45 b	0.58 b
KafrEl-Dawar	11189 a	2.24 a	S	1.47 b	0.58 b
Abu Homus	10425 a	2.10 a	S	1.49 b	0.47 b
Motobus	10913 a	2.18 a	S	1.43 b	0.65 b
Fuoa	10641 a	2.13 a	S	1.49 b	0.79 b
Control	-	-	-	2.67 a	2.5 a

Data are average of 5 replicates each.
Means followed by the same letter (s) in each column are not significantly different at $P \leq 0.05$.
Rf = Final population (Pf) /Initial population (Pi= 5000 J_2s /bag).
S = Susceptible.

Plant-parasitic nematodes especially, the citrus nematode *T. semipenetrans* and the burrowing nematode *Radopholus similis* are considered among the most important nematode pests in the citrus orchards. These nematodes and other fungus and virus pests play an important role in limiting the productivity of many citrus orchards in Egypt and other parts of the world (Ibrahim *et al.,* 2010).

Nine PPN genera were found in association with citrus trees in Alexandria, El-Behera and Kafr-Elshiekh governorates, Egypt. *T. semipenetrans, Trichodorus, Helicotylenchus* and *Pratylenchus* were the most prevalents in general. Most of the detected nematode genera were previously recorded on citrus trees in Egypt by other workers (Otiefa, 1955; Otiefa and Tarjan 1965; Ibrahim *et al*., 2010; Bakr, *et al*,. 2011).

The citrus nematode *T. semipenetrans* considers common in the orchards of citrus trees in the surveyed governorates. However, *Trichodorus, longidorus, Helicotylenchus*, *Aphelenchoides, Tylenchus, Pratylenchus*, *Tylenchorhynchus* and *Meloidogyne* showed variable FO and PD values throughout the surveyed locations. These variations could be attributed to the differences existed in the surveyed location, citrus orchards, soil type and time of collecting the soil samples. The detected nematode genera in this study and others were previously found in association with citrus trees in Egypt (Ibrahim, 1994, Radwan and Fatima, 2003; Korayem and Hasabo, 2005; El-Banhawy *et al*., 2006; Ibrahim, *et al.,* 2010). mandarine and lime seedlings showed a susceptible reaction to all the tested populations of *T. semipenetrans* in this study. (Yousif, 1984; Ibrahim *et al.* 1985; Haroon and Osman, 2003; Ahmad *et al.*, 2004; Korayem and Hasabo 2005; El-Banhawy *et al*., 2006; Ibrahim, *et al.,*

2010; Bakr, *et al,.* 2011). The infection of mandarine and lime with *T. semipenetrans* led to a significant reduction of shoot and root dry weight of the infected seedlings (Verdejo-Lucas and McKenry, 2004).

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الملخص العربي

النيماتودا المتطفلة نباتيًا المصاحبة لأشجار الموالح ورد فعل صنفين من الموالح لنيماتودا *Tylenchulus semipenetrans* فى شمال مصر

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تم إجراء حصر للنيماتودا المتطفلة نباتيًا المصاحبة لأشجار الموالح فى ثلاث محافظات بشمال مصر وهى الاسكندرية والبحيرة و كفر الشيخ خلال العامين (٢٠١٧ – ٢٠١٩). تم جمع ٤٣٢ عينة تربة وجذور من أشجار الموالح، وتم استخلاص النيماتودا منها بطريقة المناخل وأطباق بيرمان. أوضحت النتائج تواجد ٩ أجناس من النيماتودا المتطفلة نباتيًا مصاحبة لأشجار الموالح، وكان نوع نيماتودا الموالح *T. semipenetrans* هو الأكثر انتشاراً بنسبة تواجد ١٠٠%. يليه في ذلك نيماتودا التقرح .*Pratylenchus* spp ونيماتودا تقصف الجذور .*Trichodorus* spp متواجدة بنسب ١٤.٦ – ١٦.٥ %.

تم تقيم رد فعل صنفين من الموالح وهما اليوسفى والليمون لنيماتودا الموالح *Tylenchulus semipenetrans* واوضحت النتائج قابليتها جميعا للإصابة بالعشار الست المختبرة من النيماتودا (عامل تكاثر النيماتودا = ١.٦٧– ٢.٦). كما أظهرت النتائج أيضا أن إصابة الصنفين المختبرين بالنيماتودا قد أدت إلىً انخفاض الوزن الجاف للجذور والمجموع الخضري معنوياً مقارنة بنباتات المقارنة.

وفى ضوء النتائج المتحصل عليها يوصى بمكافحة نيماتودا الموالح *Tylenchulus semipenetrans* بإختيار العوامل الاكثر فاعلية فى مكافحة هذا المرض والموصى بها من وزارة الزراعة وإستصلاح الاراضى.