RESEARCH/INVESTIGACIÓN

PATHOGENICITY AND CONTROL OF THE CITRUS NEMATODE *TYLENCHULUS SEMIPENETRANS* ON CITRUS, GRAPE, OLIVE, LOQUAT, AND PERSIMMON SPECIES AND CULTIVARS

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ABSTRACT

Ibrahim, I. K. A., A. H. A. Abu-Habib, M. Kantor, and Z. A. Handoo. 2022. Pathogenicity and control of the citrus nematode *Tylenchulus semipenetrans* on citrus, grape, olive, loquat, and persimmon species and cultivars. Nematropica 52:79-84.

The pathogenicity of the citrus nematode, *Tylenchulus semipenetrans,* on citrus, grape, olive, loquat, and persimmon species and cultivars was determined in several greenhouse tests. The results showed that *T. semipenetrans* infected and reproduced successfully on all the tested fruit species and cultivars. The citrus (lime, mandarin, and sour orange), red seedless grape ('Crimson'), olive ('Pikwal' and 'Spanish'), loquat ('Premier'), and persimmon ('Balady) trees were highly susceptible to *T. semipenetrans*, while green seedless grape ('Thompson') vines and olive ('Ogeizi') trees were susceptible to *T. semipenetrans*. Soil amendments with dried plant materials of horseradish tree, blue gum tree, lime, crude culture suspensions of *Bacillus subtilis* and *B. thuringiensis*, Vertimec® (abamectin), and Nemacur® (fenamiphos) were effective in suppressing *T. semipenetrans* infection and reproduction on sour orange. The highest reductions (90-94%) in nematode reproduction (numbers of second-stage juveniles [J2]) were recorded with treatments of horseradish tree dried leaves, Vertemic, and Nemacur. Treatments with lime peel, *S. subtilis*, and *B. thuringiensis* showed 77-86% reduction in *T. semipenetrans* J2 densities. Amendment with blue gum tree leaves resulted in 62-62% reduction in *T. semipenetrans* J2 densities. This represents the first study in Egypt on the pathogenicity of *T. semipenetrans* on loquat.

Key words: Citrus, control, Egypt, grape, loquat, olive, pathogenicity, persimmon, *Tylenchulus semipenetrans*

RESUMEN

Ibrahim, I. K. A., A. H. A. Abu-Habib, M. Kantor, and Z. A. Handoo. 2022. Patogenicidad y control del nematodo de los cítricos Tylenchulus semipenetrans en algunas variedades y especies de cítricos, uva, oliva, níspero y caqui. Nematropica 52:79-84.

La patogenicidad del nematodo de los cítricos *Tylenchulus semipenetrans* en algunas variedades y especies de cítricos, uva, oliva, níspero y caqui fue determinada en varios ensayos de invernadero. Los resultados mostraron que *T. semipenetrans* infecta y se reproduce exitosamente en todas las variedades y especies de frutales evaluados. Los cítricos (lima, mandarina, naranja agria), uva roja sin semilla

('Crimson'), oliva ('Pikwal', 'Spanish'), níspero ('Premier'), y árboles de caqui ('Balady') fueron altamente susceptibles a *T. semipenetrans*, mientras que la uva verde sin semilla ("Thompson") y los árboles de oliva ('Ogeizi') fueron susceptibles a esta especie de nematodo. Las enmiendas al suelo con materiales secos de plantas de moringa, eucalipto, lima, suspenciones de cultivos crudos de *Bacillus subtilis* y *B. thuringiensis*, Vertimec® (abamectina), y Nemacur® (fenamifos) fueron efectivos en suprimir la infección y reproducción de *T. semipenetrans* en raíces de naranja agria. Las reducciones más altas (90.2-94.2%) de la reproducción del nematodo (número de juveniles en segundo estado, J2) se registraron con los tratamientos de hojas secas de moringa, Vertemic® and Nemacur®. Los tratamientos con cáscara de frutos de lima, *S. subtilis* y *B. thuringiensis* mostraron una reducción del 76.5-86.4% de J2 de *T. semipenetrans*. Por otro lado, enmiendas con hojas de eucalipto resultaron en una reducción del 61.6-62.4% de J2 de T. semipenetrans. Esto representa el primer estudio en Egipto sobre la patogenicidad de *T. semipenetrans* en níspero.

Palabras clave: Cítricos, control, Egipto, uva, níspero, oliva, patogenicidad, caqui, *Tylenchulus semipenetrans*

INTRODUCTION

In Egypt, plant-parasitic nematodes have been recognized as important pests on citrus trees since 1955 when Oteifa reported the citrus nematode *Tylenchulus semipenetrans* on citrus trees in northern Egypt (Oteifa, 1955). Information concerning the occurrence and pathogenicity of plant-parasitic nematodes on citrus and other fruit trees in Egypt is very important, since many nematode genera such as *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Meloidogyne*, *Mesocriconema*, *Pratylenchus*, *Tylenchorhynchus*, *Tylenchulus*, and *Xiphinema* may occur at high densities and cause economic damage to infected fruit trees (Oteifa, 1955; Tarjan, 1964; Oteifa and Tarjan, 1965; Ibrahim *et al.*, 1989, 1992, 2010; Lamberti *et al.*, 1996; Radwan and Fatma, 2003; Handoo *et al.*, 2015).

Several authors have shown that the citrus nematode *T. semipenetrans* is among the most important plant pathogens causing growth problem and yield losses to citrus, grape, olive, and persimmon fruit trees (Inomoto *et al.*, 1991; Lamberti and Vovlas, 1993; Radwan and Fatima, 2003; Korayem and Hasabo, 2005). The main objectives of this study were to determine the pathogenicity of *T. semipenetrans* on citrus, grape, olive, loquat, and persimmon species and cultivars as well as evaluating treatments to control *T. semipenetrans* on sour orange.

MATERIALS AND METHODS

Inoculum of *T. semipenetrans*, was isolated from infected sour orange (*Citrus aurantium* L.) trees grown in the greenhouse of Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Second-stage juveniles (J2) of *T. semipenetrans* were extracted from infested soil using Cobb's wet-sieving and centrifugal sugar flotation techniques (Ayoub, 1980).

In two separate experiments, the reaction of the citrus rootstocks lime (*Citrus aurantifolia*), mandarin (*C. nobilis*), and sour orange (*C. aurantium*); grape (*Vitis vinifera*) cv. Crimson, Thompson; olive (*Olea europea*) cv. Spanish, Ogeizi, Pikwal; Loquat (*Eriobotrya japonica*) cv. Premier; and persimmon (*Diospyros kaki*.) cv. Baladi to *T. semipenetrans* were determined in several greenhouse tests. One 4-month-old seedling of each species and/or cultivar was transplanted into plastic pots (30-cm diam) filled with a mixture of equal volumes of steam-sterilized sand and clay soil. Seven days after planting, the pots were inoculated by making holes near the plant roots and then adding an initial population (Pi) of 5,000 *T. semipenetrans* J2/pot. Pots were arranged in randomized complete block design in a greenhouse at 22-28°C. The experiments were conducted twice in 2020 and 2021 with each species/cultivar replicated five times.

The experiments were terminated 60 days after nematode inoculation. Roots were washed free of soil, and *T. semipenetrans* J2 were extracted from the soil by wet-sieving and centrifugal sugar flotation techniques (Cobb, 1918). *Tylenchulus semipentrnas* J2 densities (final population, Pf) were counted and the reproduction factors (Rf) = Pf/initial population density (Pi) were determined.

The fruit species and varieties were rated on a 0 to 2 scale for Rf. Fruit species and varieties with Rf = 0 to 0.1 were considered resistant, Rf = 0.2 to 1.0 moderately susceptible, Rf = 1.1 to 2.0 susceptible and >2 highly susceptible.

In two separate experiments (conducted in 2020 and 2021), the effect of dried plant materials of leaves of blue gum tree (*Eucalyptus globules*) and horseradish tree (*Moringa oleifera*), lime peels (*Citrus aurantifolia*) Swingle), the biocontrol agents, *Bacillus subtilis* and *B. thuringiensis*, the bionematicide, Vertimec® (abamectin), and the nematicide, Nemacur® (fenamiphos) on infection of *T. semipenetrans* on sour orange were determined in greenhouse tests.

One 4-month-old seedling of sour orange (*C. aurantium*) was transplanted into 30-cm-diam plastic pots filled with a mixture of equal portions of autoclave-sterilized sand and clay soil. Seven days after transplanting, the soil was inoculated with 5,000 *T. semipenetrans* J2/pot, and 3 days later the control materials were incorporated into the upper part of the soil of treated pots. The amount of dried plant materials and peels added to each pot was 60 g/pot. Inocula of *B. subtilis* and *B. thuringiensis* were obtained from the Faculty of Agriculture, Alexandria University, Alexandria, Egypt. and cultured on T3 broth liquid medium for 72 hr at 30°C (Travers *et al.*, 1987). Culture fluid suspensions of *B. subtilis* and *B. thuringiensis* were used and added to the soil at the rate of 20 ml/pot. Vertimec® at concentration of 200 ug/ml and a rate of 20 ml/pot and Nemacur® 10 G at the rate of 0.5g/pot were applied to the soil. Treatments were replicated five times. Pots were arranged in a randomized complete block design in a greenhouse at 22-28°C.

Plants were harvested 60 days after nematode inoculation. Roots were washed free of soil. Numbers of J2 (Pf) in the soil were counted, and the dry weights of shoots and roots were determined.

Data of the number of J2 and the dry weights of the shoots and roots of the citrus plants were analyzed with analysis of variance (ANOVA) and means separated with least significant difference (LSD, SAS Institute, 1997).

RESULTS

Results obtained from experiments of 2020 were almost similar to those from 2021 (Tables 1 and 2). *Tylenchulus semipenetrans* infected and reproduced successfully on all the tested fruit trees. Lime, mandarin, sour orange, grape (Crimson), olive (Pikwal, Spanish), loquat (Premier), and persimmon (Balady) trees were highly susceptible to *T. semipenetrans* with Rf values of 2.1 to 2.6. Grape (Thompson) and olive (Ogeizi) were susceptible to *T. semipenetrans* with Rf values of 1.6 to 1.7 (Table 1).

The effects of soil amendments on the pathogenicity and reproduction of *T. semipenetrans* on sour orange are presented in Table 2. The soil treatments suppressed nematode reproduction and reduced *T. semipenetrans* J2 density by 61.6 to 94.2%, depending upon the treatment. The highest reductions (90.2 to 94.2%) of *T. semipenetrans* J2 were recorded with treatments of horseradish tree dried leaves, Vertemic®, and Nemacur®. Treatments with lime peels, *S. subtilis* and *B. thuringiensis* resulted in 76.5 to 86.4% reduction in *T. semipenetrans* J2 densities. Treatment with blue gum tree leaves resulted in 61.6 to 62.4% reduction in *T. semipenetrans* J2 density. Treatment with the tested control materials caused significant increase in shoot and root dry weights of treated sour orange plants compared to the control (Table 2).

DISCUSSION

The present research demonstrated that citrus, grape, olive, loquat, and persimmon species and cultivars were susceptible and good hosts for *T. semipenetrans,* which infected and reproduced successfully on the tested fruit trees. The results support earlier studies in Egypt (Ibrahim *et al.*, 1985, 1989; Korayem and Hasabo, 2005; Salem, 1980) indicating that *T. semipenetrans* can infect and reproduce on grape, lime, mandarin, grapefruit, rough lemon, sour orange, and Troyer citrange. Recently, several authors (Radwan and Fatma, 2003; El-Banhawy *et al.*, 2006; Korayem and Hasabo, 2006) showed that *T. semipenetrans* is of widespread occurrence on citrus trees in the Nile Delta and Middle Egypt.

In conclusion, our study shows that determining the host status of citrus, grape, olive, loquat, and persimmon species and cultivars to *T. semipenetrans* is useful and that knowledge of reistance/susceptibility is of significance and can be incorporated into breeding programs as a control measure. More research is needed for finding and

Table 1. Reproduction of *Tylenchulus semipenetrans* on citrus, grape, olive, loquat, and persimmon.

	2020			2021		
Host plant	No. of J2/pot[wx]	Rf[y]	Reaction[z]	No. of J2/pot[wx]	Rf[y]	Reaction[z]
Citrus:						
Lime	11,189 b	2.24	HS	11,310 b	2.22	HS
Mandarin	10,499 c	2.10	HS	10,860 b	2.17	HS
Sour orange	11,433 b	2.29	HS	12,108 b	2.42	HS
Grape:						
Crimson	12,867 a	2.57	HS	12,680	2.53	HS
Thompson	8,344 d	1.67	S	8,546 d	1.74	S
Olive:						
Ogeizi	8,202 d	1.64	S	8,438 d	1.68	S
Pikwal	10,800 c	2.16	HS	11,048 b	2.21	HS
Spanish	12,802 a	2.56	HS	12,678 a	2.53	HS
Loquat:						
Premier	10,750 c	2.15	HS	10.864 c	2.17	HS
Persimmon:						
Balady	12,948 a	2.59	HS	13,036 a	2.61	HS

[w]Means are the average of five replicates.
[x]Means with the same letter in each column are not significantly different at $P = 0.05$.
[y]Rf = Final population (*Pf*)/Initial population (*Pi*). *Pi* = 5,000 J2/pot.
[z]HS = Highly susceptible, S = Susceptible.

Table 2. Effects of soil amendment with dried plant materials of blue gum, horseradish, and lime trees, *Bacillus subtilis*, *B. thuringiensis*, Vertemic®, and Nemacur® on *Tylenchulus semipenetrans* on sour orange.

				Dry weight (g)	
Test	Treatment	No. J2/pot[yz]	Reduction %	Shoot	Root
1-2020	Blue gum tree	4,085 b	62.4	4.32 c	4.81 c
	Horseradish tree	782 g	92.8	5.64 a	5.10 a
	Lime	1,725 d	84.1	4.90 b	5.28 b
	B. subtilis	2,548 c	76.5	4.56 c	4.96 c
	B. thuringiensis	1,478 e	86.4	4.88 b	5.70 b
	Vertemic®	680 g	93.7	5.75 a	6.18 a
	Nemacur®	1,065 f	90.2	5.14 b	5.82 b
	T. semipenetrans	10,864 a	--	3.86 d	3.74 d
2-2021	Blue gum tree	4,285 b	61.6	4.61 c	5.04 c
	Horseradish tree	759 f	93.2	5.78 a	6.18 a
	Lime	1,641d	85.3	4.84 c	5.36 c
	B. subtilis	2,522 c	77.4	4.66 c	5.10 c
	B. thuringiensis	1,473 d	85.8	5.02 b	5.81 b
	Vertemic®	647 g	94.2	5.84 a	6.28 a
	Nemacur®	971 e	91.3	5.20 b	5.76 b
	T. semipenetrans	11,160 a	--	3.74 d	3.88 d

[y]Means are the average of five replicates.
[z]Means with the same letter(s) in each column are not significantly different at $P = 0.05$.

developing resistant or tolerant cultivars of these fruit crops to *T. semipenetrans*.

Soil treatments with the tested plant materials, the biocontrol agents *B. subtilis* and *B. thuringiensis*, Vertemic®, and the nematicide, Nemacur®, greatly reduced the reproduction of *T. semipenetrans* and J2 densities on infected sour orange trees. The present results agree, to a certain extent, with those of other authors (Timmer and French, 1979; Ibrahim *et al.*, 1992, 2019) who indicated the effectiveness of certain biocontrol agents and chemical nematicides against *T. semipenetrans*. Also, the results support earlier studies (Ibrahim *et al.*, 2013, 2014) indicating that

soil treatment with plant materials, marine algae, *B. thuringiensis*, Vertemic®, or Nemacur® were effective against the *M. incognita,* and *Heterodera goldeni* and *H. schachtii*. To our knowledge, this is the first study on loquat in Egypt regarding the pathogenicity of *T. semipenetrans*. The study showed that loquat is a good host for *T. semipenetrans*.

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