

CITRUS NEMATODE TYLENCHULUS SEMIPENETRANS AND ITS PATHOGENIC RELATION TO CITRUS TREES

A THESIS

Presented to the Graduate School Faculty of Agriculture, Alexandria University in partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in

"PLANT PATHOLOGY" Nematology

by

Alaa Habib Abd Elsalam Abu Habib

Alexandria University

2020

CONTENTS

ACKNOWLEDGEMENT	Ι
TABLE OF CONTENTS	II & III
LIST OF TABLES	IV
LIST OF FIGURES	\mathbf{V}
LIST OF ABBREVIATIONS	VI
INTRODUCTION	1
REVIEW OF LITERATURE	3
MATERIALS AND METHODS	15
1- Isolation and Identification of Plant-Parasitic Nematodes	15
2- Seasonal Population Dynamic of the Citrus Nematode	15
3- Citrus Nematode inoculum:	16
4- Reactions of Some Citrus Species to T. semipenetrans	18
5- Reaction of Two Grape Cultivars to <i>T. semipenetrans</i>	18
6- Reaction of Three Olive Cultivars to <i>T.semipenetrans</i>	19
7- Reaction of Loquat to T. semipenetrans	20
8- Reaction of Persimmon to T. semipenetrans	20
Nematode Control Study	21
9. Bioassay Tests	21
a. Effect of Two Bacillus Isolates on Juvenile Mortality of T. semipenetrans	21
b. Effect of Three Plant Extractas on Juvenile Mortality of <i>T. semipenetrans</i>	22
10. Effects Some Biocontrol Agents and Three Nematicides on the Infections	
of Sour Orange with T. semipenetrans	23
11.Effect of Some Biocontrol Agents and Nematicides on Controlling T.	
semipenetrans Infecting citrus trees navel orange (citrus Sinensisl	
<i>L</i>)	24
Statistical Analysis	27

EXPER	EMENTAL RESULTS	28
I. Is	solation and Identification of Plant-Parasitic Nematodes	28
II. S	easonal Population Dynamic of the Citrus Nematode	30
Host ra	nge Studies	33
i.	Reaction of Some Citrus Rootstocks to Some Isolates of <i>T. semipenetrans</i>	33
а) Reaction of sour orange to Some Isolates of <i>T. semipenetrans</i>	33
b) Reaction of mandarine to Some Isolates of <i>T. semipenetrans</i>	35
С) Reaction of lime to the Some Isolates of <i>T. semipenetrans</i>	38
ii.	Reactions of Two Grape Cultivars to T. semipenetrans	40
iii.	Reaction of Three Olive Cultivars to <i>T.semipenetrans</i>	40
iv.	Reaction of Loquat and Persimmon to T. semipenetrans	44
Control	studies	47
1	. Effect of two Bacillus Isolates on T. semipenetrans Juvenile Mortality	47
2	. Effect of Three Plant Extracts on T. semipenetrans Juvenile Mortality	48
3	• Effects of Some Biocontrol Agents on <i>T. semipenetrans</i> on Citrus Seedlings	50
4	•. Effect of some Nematicides and Biocontrol Agents on Controlling <i>T. semipenetrans</i> Infecting Citrus Trees under Field Conditions	55
DISCUS	SION	55
SUMMA	ARY	57
LITERATURE CITED		
ARABI	C SUMMARY	

LIST OF TABLES

Table	Title	Page
No.		No.
1	Rates and application methods of nematicides used in controlling <i>T</i> . <i>semipenetrans</i> infecting citrus trees under field condition	26
2	Frequency of occurrence (FO) and population density (PD) of plant- parasitic nematode genera in citrus soil samples collected from	
	Alexandria, El-Behera and Kafr El-Sheikh governorates	31
3	Reactions of sour orange seedlings to the six isolates of the citrus	
	nematode T. semipenetrans	34
4	Reactions mandarine seedlings to six isolates of the citrus nematode T.	
	semipenetrans	36
5	Reaction lime seedlings to six isolates of the citrus nematode T.	
	semipenetrans	38
6	Reactions of the grape cultivars Crimson and Tompson to citrus	
	nematode T. semipenetrans	41
7	Reaction of the olive cultivars Spanish, Ogeizei and Pikwal to citrus	
	nematode T.semipenetrans	42
8	Reactions of loquat and persimmon to citrus nematode T.	
	semipenetrans	45
9	Effect of two isolates Bacillus spp. on 2nd stage juveniles (J2) mortality	
	of the citrus nematode T. semipenetrans	47
10	Effect of three plants extract on 2nd stage juvenile Mortality of T.	
	semipenetrans	49
11	Effects of the biocontrol agents and three nematicides on the infections	
	of T. semipenetrans on sour orange seedlings	51
12	Effect of some nematicides and biocontrol on controlling citrus	
	nematode T. semipenetrans infecting sour orange trees under field	
	conditions	56

LIST OF FIGURES

Figure	Title	Page
No.		No.
1.	Citrus trees naturally infected with citrus nematode T. semipenetrans,	
	at Kafr El-sheikh governorate	17
2.	Chart 1: Seasonal population Dynamics of T. semipenetrans associated	
	with citrus orchards	32
3.	Effect of artificial infection of six isolates of the T. semipenetrans on	
	the seedlings of sour orange under greenhouse conditions	35
4.	Effect of artificial infection of six isolates of the T. semipenetrans on the	
	seedlings of mandarine under greenhouse conditions	37
5.	Fig. 4. Effect artificially infection of six isolates of the T. semipenetrans	
	on the seedlings of lime under greenhouse condition	39
6.	Effect of infection of citrus nematode T. semipenetrans on seedlings	
	olive of cvs Spanish, Ogeizei and Pikwal under greenhouse conditions.	43
7.	Effect of infection of citrus nematode T. semipenetrans on seedlings	
	loquat under greenhouse conditions	44
8.	Effect artificially infection of citrus nematode T. semipenetrans on	
	seedlings persimmon under greenhouse conditions	46
9.	Effects of the tested nematicides Fibermax, Nemacur and Abamectin	
	on the infections of T. semipenetrans on sour orange seedlings under	
	greenhouse conditions	52
10.	Effects of the biocontrol agents on the infections Bacillus sp. and	
	Bacilus subtilus of T. semipenetrans on sour orange seedlings under	
	greenhouse conditions	53
11.	Effects of some plant extracts and nematicides on the infections of T.	
	semipenetrans on sour orange seedlings under greenhouse conditions	54

Summary

The present research work aimed to study the occurrence, distribution of plant parasitic nematodes in three governorates (Alexandria, El-Behera and Kafr El-Siekh), pathogencity and control of the citrus nematode *Tylenchuulus semipenetrans* study aimed to identificate plant-parasitic nematodes associated with citrus trees orchards, particularly specially and to eliminate the damages induced by this complex through applications of some bioagents, fungicides and nematicides. Summary of the present work showed the followings:

1. A survey study was conducted to determine the plant-parasitic nematodes associated with citrus trees in Alexandria, EL-Behera and Kafer-Elshiekh governorates. The results indicated that 9 nematode genera were present in the surveyed citrus trees orchards i.e., *Tylenchuulus, Trichodorus, Rotylenchus, Helicotylenchus, Aphelenchoides, Tylenchus, Pratylenchus, Tylenchorhynchus* and *Meloidogyne*.

In soil samples of the surveyed governorates, *Tylenchuulus semipenetrans* was the most common with 100% FO, and the highest population density $(1105 - 7121 \text{ J}_{28} / 250 \text{ g}$ soil). The other nematode genera and their respective percentage of occurrence were; Trichodorus (16.4%), *Pratylenchus* (14.6%), *Helicotylenchus* (13.3%), *Aphelenchoides* (10.9%), *Tylenchorhynchus* (8.4%), *Meloidogyne* (3.6%), *Rotylenchus* (2%) and *Tylenchus* (0.23%).

2. The reaction of the citrus species sour orange rootstock (*C. aurantium*), mandarine (*Citrus deliciosa*) and lime (*Citrus aurantifolia*) to *T. semipenetrans* indicated that all the tested citrus species were susceptible to *T. semipenetrans*. Seedlings of the tested citrus species showed symptoms of slow decline disease such as smaller leaves, stunt growth and di-eback branchs.

3. The reaction of the grapevine cultivars Crimson and Thompson to the citrus nematode, *T. semipenetrans* indicated that the tested cultivars were susceptible to *T. semipenetrans*. Infection with *T. semipenetrans* reduced the shoot and root dry weights of the tested grape cvs Crimson and Thompson seedless.

4. The reaction of the olive cultivars Spanish, Ogeizei and Pikwal to the citrus nematode, *T. semipenetrans* indicated that all the tested olive cultivars were susceptible to *T. semipenetrans*. Infection with *T. semipenetrans* reduced the shoot and root dry weights of the tested olive cultivars.

5. The reaction of the loquat seedlings to citrus nematode, *T. semipenetrans* indicated that all the tested seedlings were susceptible to *T. semipenetrans*. Infection with *T. semipenetrans* reduced the shoot and root dry weights of loquat seedless.

- 6. The reaction of the Kaki seedlings *T. semipenetrans* indicated that the tested Kaki seedlings were susceptible to *T. semipenetrans*. Infection with *T. semipenetrans* reduced the shoot and root dry weights of Kaki seedlings.
- 7. The effect of *Bacillus subtilis* and *Bacillus* sp. against 2nd stage juveniles of *T*. *semipenetrans* was studied *in vitro*. The results showed that *B. subtilis* and *Bacillus* sp. had a high mortality effect (58-100%) on *T. semipenetrans* 2nd stage juveniles.
- 8. The effect of the plants extractsi.e., moringa (*Moringa oleifera*), dodonia (*Dodonnea angustfolia*) and basil (*Ocimumb asilicum*) on the mortality of *T. semipenetrans* 2nd stage juveniles was studied, *in vitro*. The results showed that the highest mortality effects (85-100%) were induced with moringa and basil treatments, whereas dodonia treatment gave a lesser mortality percentage of nematode juveniles.
- 9. The effects of the biocontrol agents i.e., (*Bacillus subtilis* and *Bacillus* sp., moringa (*Moringa oleifera*) and abamectin) and nematicides i.e., Nemacur[®]10G and Fibermax[®] 10% GR on the infections of *T. semipenetrans* on citrus seedlings indicated that the highest reductions of citrus nematode (99-100%) were recorded with treatments of moringa and abamectin. *Bacillus subtilis* & Nemacur[®]10G gave 78.4-89.6% reductions of *T. semipenetrans* and enhanced growth of shoot and roots system of seedlings.
- The effects of soil treatments with the biocontrol agents abamectin and six nematicides, i.e.,Nemacur[®]L, Rugby[®] L, Fluopyram[®], Krop card[®], Dinto[®] and Fibermax[®] on the

infection citrus trees cv navel orange (*citrus sinensis L.*) grafted on sour orange rootstock with *T. semipenetrans* indicated that the highest reductions (99- 97 -92%) of *T. semipenetrans* 2^{nd} stage juveniles *T. semipenetrans* were obtained with Rugby[®], abamectin and Nemacur[®] respectively[®], followed by Fluopyram[®] treatment whish showed (76%) reductions in *T. semipenetrans* J₂s. Fibermax[®] and Krop card[®] showed (50- 52%) reductions, but the lowest reductions was obtained with Dinto[®] (0%). All treatments enhanced growth of shoots, branched and roots of the treated trees, compared to untreated trees.