UTILIZATION OF SOME SOIL MICRO-ORGANISMS FOR MANAGEMENT OF ROOT-KNOT NEMATODES ON CUCUMBER PLANTS

By HANAN MOHAMED ZAKARIA

B.Sc. Agric. Sc. (General Branch), Zagazig University, 1996 M. Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2006

A thesis submitted in partial fulfillment of the requirements for the degree of

in
Agricultural Science
(Plant Pathology)

Department of Plant Pathology
Faculty of Agriculture
Ain Sham University

Approval Sheet

UTILIZATION OF SOME SOIL MICRO-ORGANISMS FOR MANAGEMENT OF ROOT-KNOT NEMATODES ON CUCUMBER PLANTS

By HANAN MOHAMED ZAKARIA

B.Sc. Agric. Sc. (General Branch) , Zagazig University , 1996M. Sc. Agric. Sc. (Plant Pathology) , Ain Shams University , 2006

The thesis for Ph. D. degree has been approved by:

Date of Examination: 25/6/2012

	Tr the tr
Dr.	Samia Ibrahem Massoud Prof. Emeritus of Plant Pathology and Nematology , Faculty of Agriculture , Suez Canal University
Dr.	Mohamed Nagy Shatla Prof. Emeritus of Plant Pathology , Faculty of Agriculture , Air Shams University
Dr.	Abdalla Shehata Mohammed Kassab Prof. Emeritus of Agricultural Zoology, Faculty of Agriculture Ain Shams University
Dr.	Madih Mohamed Aly Prof. Emeritus of Plant Pathology , Faculty of Agriculture , Air Shams University

استخدام بعض ميكروبات التربة في مكافحة نيماتودا تعقد الجذور على نباتات الخيار

رسالة مقدمة من

حنان محمد زكريا

بكالوريوس علوم زراعية (شعبة عامة) ، جامعة الزقازيق ، 1996 ماجستير علوم زراعية (أمراض نبات) ، جامعة عين شمس ، 2006

للحصول على

درجة دكتور فلسفه في العلوم الزراعية (أمراض نبات)

قسم أمراض النبات كلية الزراعة جامعة عين شمس

صفحة الموافقة

إستخدام بعض ميكروبات التربه في مكافحة نيماتودا تعقد الجذور على نباتات الخيار

رسالة مقدمة من حنان محمد زكريا

بكالوريوس علوم زراعية (شعبة عامة) ، جامعة الزقازيق ، 1996 ماجستير العلوم الزراعيه (أمراض نبات) ، جامعة عين شمس ، 2006

الحصول على درجة دكتور فلسفة في العلوم الزراعية (أمراض نبات)

وقد تمت مناقشة الرسالة والموافقة عليها

اللجنسسة:	
	د. سامية إبراهيم مسعود
يماتودا المتفرغ، كلية الزارعة، جامعة قناة السويس	أستاذ أمراض النبات والن
	د. محمد ناجي شتلة
ِ المتفرغ، كلية الزراعة، جامعة عين شمس	أستاذ أمراض النبات غير
	د. عبد الله شحاته محمد ك
لمتفرغ، كلية الزارعة، جامعة عين شمس	أستاذ الحيوان الزراعي ا
	د. مديح محمد علي
فرغ، كلية الزارعة، جامعة عين شمس	أستاذ أمراض النبات المت

تاريخ المناقشة 2012/6/25

UTILIZATION OF SOME SOIL MICRO-ORGANISMS FOR MANAGEMENT OF ROOT-KNOT NEMATODES ON CUCUMBER PLANTS

By HANAN MOHAMED ZAKARIA

B.Sc. Agric. Sc. (General Branch) , Zagazig University , 1996M. Sc. Agric. Sc. (Plant Pathology) , Ain Shams University , 2006

Under the supervision of:

Dr. Madih Mohamed Aly

Prof. Emeritus of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Abdalla Shehata Mohammed Kassab

Prof. Emeritus of Agricultural Zoology, Department of plant protection Faculty of Agriculture, Ain Shams University

Dr. Muhammad Shamseldean Mostafa Shamseldean

Prof. of Entomology, Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University

Dr. Mona Mansour Mahmoud Oraby

Associate Prof. of Microbiology, Department of Microbiology, Faculty of Agriculture, Ain Shams University

ABSTRACT

Hanan Mohamed Zakaria: Utilization of Some Soil Microorganisms for Management of Root-knot Nematodes on Cucumber plants Unpublished Ph. D. Thesis, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, 2012.

Isolation and identification trials of fungal bio-agents resulted in five isolates of *Trichoderma* spp. and two isolates of *Verticillium* spp. The most effective isolates on percentage mortality and hatching of Meloidogyne incognita were identified as T. harzianum and V. chlamydosporium. Effects of all forms of propagules suspension and culture filtrate were increased by increasing the concentration and incubation period. Two obtained isolates of entomopathogenic nematodes-symbiotic bacteria, *Photorhabdus* luminescens Xenorhabdus hominickii showed high efficiency on mortality and hatching of *M. incognita*. Temperatures regimes and storage periods had a great effect on the shelf life of culture filtrates of *P. luminescens*. Longevity of the chlamydospores of *V. chlamydosporium* was able to persist for 6 months in two forms of formulations, alginate granules and vermiculite paste, which were the most suitable forms. The effect of stored formulated products of V. chlamydosporium on mortality and hatching of M. incognita was gradually decline by increasing the period of storage, especially under room temperature.

Results from pot experiments revealed that, V. $chlamydosporium(10^7)$ + CF of P. luminescens caused a decreasing number of galls, females, egg-masses and immature stages per root system and number of IJ_2 in soil and total number of nematodes. Moreover, the same treatment caused a significant positive effect on the high vegetative growth of cucumber plants. All treatments with the various formulated products of V. chlamydosporium mixed with kaolin powder, alginate

granule or vermiculite paste resulted a significant reduction in nematode infection parameters compared with control. Also, all treatments resulted an improvement in the high vegetative growth.

Simulated field studies showed the greatest role of the soil treatment with *V. chlamydosporium* and *P. luminescens* (alone or in combination) compared to nematicide treatment with Oxamyl 24% SL, in controlling of root-knot nematode *M. incognita* in cucumber plants. When the soil was applied with *V. chlamydosporium* + *P. luminescens* joining with animal compost (AC), caused the highest reduction effect on all nematode infection criteria followed by the treatment with *P. luminescens* + AC compared with control. All soil treatments with *V. chlamydosporium*, *P. luminescens* and soil different soil amendments (alone or in combination) caused an improvement in top vegetative growth compared with control.

Key Words: Biological control, Nematophagous fungi, *Trichoderma harzianum*, *Verticillium chlamydosporium*, *Photorhabdus luminescense*, *Xenorhabdus* hominickii, Soil amendments *Meloidogyne incognita*.

ACKNOWLEDGMENT

I am highly thankful to Prof. Dr. Madih M. Aly, for his supervision, valuable scientific help, kind advice, valuable discussions during the tenure of investigation and for his assistance in finalizing data, preparing and writing the manuscript.

Deeply thanks to Prof. Dr. Abdalla Shehata Kassab, for his supervision, valuable scientific help, valuable discussions and encouragement during the tenure of investigation and for his assistance in finalizing data, preparing and writing the manuscript.

Whole thanks to Prof. Dr. Muhammad. M. Shamseldean, for his supervision, valuable scientific help, gracious help, useful suggestion and encouragement during the progress of this study and writing the manuscript.

Thanks also due to Dr. Mohamed Youssef Banora, for his valuable scientific help during finalizing data, preparing and writing the manuscript.

I am also thankful Dr. Mona Mansour Oraby, for her supervision and valuable scientific help.

I am also thankful Dr. Emad EL dien Y. Mahmoud, Associate Professor of Plant Pathology, Institute of Plant Pathology, Agriculture Research Center, Giza, Cairo, for his valuable scientific help during the subjection of experimental data to statistical analysis.

Whole hearted thanks for my family for their gracious help and encouragement. Really I am feeling fully indebted to them.

Thanks are also extended to all friends and colleagues in Department of Plant Pathology, Faculty of Agriculture, Ain Shams University and in Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University for their valuable assistance.

CONTENTS

LIST OF TABLES
LIST OF FIGURES
I. INTRODUCTION
II. REVIEW OF LITERATURE
2.1. Root-knot nematodes (<i>Meloidogyne</i> spp.)
2.2. Management of root-knot nematodes
2.3. Biological control.
2.4. Nematophagous fungi
2.4.1. Nematode-endoparasitic fungi
2.4.2. Egg-parasitic fungi.
2.4.3. Toxin-producing fungi.
2.5. Bacteria associated with insect entomopathogenic nematodes
2.6. The symbiotic bacteria of entomopathogenic nematodes
2.7. The role of symbiotic bacteria as a bio-agent against plan
parasitic nematodes
2.8. Soil amendments
2.9. Propagation and formulation of biological control agents
III. MATERIALS AND METHODS
3.1. General procedures
3.1.1. Collection of samples
3.1.2. Isolation, propagation and identification of root-known
nematodes
3.1.3. Preparation of root-knot nematode inoculum
3.1.4. Isolation and identification of bio-agent fungi
3.1.4.1. Isolation of <i>Trichoderma</i> spp
3.1.4.2. Isolation of <i>Verticillium</i> sp.
3.2. Laboratory experiments
3.2.1. Evaluate the effect of bio-agents propagules on infective
stages and eggs of <i>M. incognita</i>

3.2.1.1. Preparation of bio-agent fungi propagule
3.2.1.2. Preparation of bio-agent bacteria
3.2.1.3. Testing the effect of fungal and bacterial bio-agents
propagules on juvenile mortalities of M. incognita
3.2.1.4. Testing the effect of fungal and bacterial bio-agents
propagules on egg hatching of M. incognita
3.2.2. Testing the effect of culture filtrates of fungal and bacterial
bio-agents on J_2 s and eggs of M . incognita
3.2.2.1. Fungal bio-agents filtrates
3.2.2.2. Bacterial Bio-agents filtrates
3.2.2.3. Testing the effect of culture filtrate of fungal and bacterial
bio-agents on juvenile mortalities of M. incognita
3.2.2.4. Testing the effect of culture filtrate of fungal and bacterial
bio-agents on egg hatching of M. incognita
3.2.3. Effect of temperature and storage time on shelf life of the
culture filtrate of Photorhabdus luminescens
3.2.3.1. Evaluate the effect of the storage culture filtrate of P .
<i>luminescens</i> on juvenile mortalities of <i>M. incognita</i>
3.2.3.2. Testing the effect of the storage culture filtrate of P .
luminescens on egg hatching of M. incognita
3.2.4. Testing the antagonistic effect between the selected bio-
agents
3.2.5. Formulation of <i>V. chlamydosporium</i> on different carriers
3.2.5.1. Kaolin clay powder formula
3.2.5.2. Kaoline-vermiculite mixture
3.2.5.3. Alginate granules
3.2.6. Viability tests of <i>V. chlamydosporium</i> on differet media
3.2.7. Testing the formulations of <i>V. chlamydosporium</i> on the
viability of root-knot nematodes
3.2.7.1. Testing the formulations of <i>V. chlamydosporium</i> on the
viability of juveniles

3.2.7.2. Testing the formulations of <i>V. chlamydosporium</i> on the eggs	
hatching	
3.3. Pot experiments	
3.3.1. General procedures to achive pot experiments	
3.3.1.1. Soil sterilization and transplanting of cucumber seedlings	
3.3.1.2. Preparation of fungal biocontrol agents	
3.3.1.3. Preparation of bacterial culture	
3.3.1.4. Soil treatment with bio-agents	
3.3.1.5. Soil treatment with formulated products of Verticillium	
chlamydosporium	
3.3.1.6. Soil infestation with nematode	
3.3.1.7. Experimental design	
3.3.1.8. Assessment of nematode parameters	
3.3.1.8.1. Assessment of the average number of egg-masses	
3.3.1.8.2. Assessment of the average numbers of females and	
developing stages	
3.3.1.8.3. Assessment of final nematode popultion in soil	
3.3.1.9. Evaluation of vegetative growth characters	
3.3.2. Evaluation of the different bio-agents against M. incognita on	
cucumber plants	
3.3.3. Evaluation the different combinations of the selected bio-	
agents on M. incognita infectivity on cucumber plants	
3.3.4. Testing the effect of bio-agent filtrates on infection of	•
cucumber plants with root-knot nematode	
3.3.5. Evaluation of various formulations of V. chlamydosporium on	
the <i>M. incognita</i> infection on cucumber plants	
3.4. Simulated field experiments	
3.4.1. General procedures to achive simulated field experiments	
3.4.1.1. Soil sterilization	
3.4.1.2. Treatment with soil amendments	
3.4.1.3. Treatment with the bio-agents	

3.4.1.4. Treatment with nematicides	3
3.4.1.5. Experimental design	3
3.4.2. Evaluation of the selected fungal and bacterial bio-agents on	
cucumber infected plants with M. incognita	3
3.4.3. Evaluation the influence of animal compost and\ or plant	
compost with some bio-agents on the infection of cucumber	
plants with <i>M. incognita</i>	3
3.5. Statistical Analysis	3
IV. RESULTS	2
4.1. Identification of root-knot nematode	2
4.2. Isolation and identification of bio-agent fungi	2
4.3. Results laboratory experiments	2
4.3.1. Evaluating the effect of bio-agents propagules on infective	
stages and eggs of M. incognita	2
4.3.1.1. Preparation of bio-agent bacteria	2
4.3.1.2. Effect of fungal and bacterial bio-agents propagules on	
juvenile mortalities and egg hatching of <i>M. incognita</i>	2
4.3.2. Effect of culture filtrate of fungal and bacterial bio-agents on	
juvenile mortalities and egg hatching of M. incognita	2
4.3.3. Effect of temperature and storage time on effectiveness of the	
culture filtrate of Photorhabdus luminescens	4
4.3.4. Testing the antagonistic effect between the selected bioagents	4
4.3.5. Viability tests of <i>V. chlamydosporium</i> on differet media	4
4.3.6. Evaluation of <i>V. chlamydosporium</i> formulations on juveniles	
viability and egg hatching of root-knot nematodes	4
4.4. Pot experiments	6
4.4.1. Evaluation of the efficacy of different bioagents in reducing	
root-knot nematode on cucumber plants	(
4.4.2. Influence of different bio-agents on vegetative growth of	
cucumber plants	6

4.4.3. Efficacy of different combinations of the selected bio-agents	
on reducing root-knot nematode in cucumber plants	65
4.4.4. Evaluatin of the bioagent filtrates on infection of cucumber	
plants with root-knot nematode	65
4.4.5. Evaluation of the various formulations of <i>V. chlamydosporium</i>	
on infection of cucumber plants with root-knot nematode	72
4.5. Simulated field experiments	75
4.5.1. Efficacy of selected fungal and bacterial bioagents in reducing	
infection of cucumber plants with root-knot nematode	75
4.5.2. Efficacy of animal compost and\ or plant compost with some	
bioagents in reducing infection of cucumber plants with root-	
knot nematode	78
V. DISCUSSION	82
VI. SUMMARY AND CONCLUSION	90
VII. REFERENCES	93
VIII. ARABIC SUMMARY	1

LIST OF TABLES

Table No.	
1. Isolates of the fungal bio-agents	41
2. Percentage mortality of IJ ₂ Meloidogyne incognita affected	
by propagules of the fungal and bacterial bio-agents at 25	
°C	44
3. percentage of egg hatching per egg-mass of <i>Meloidogyne</i>	
incognita affected by propagules of the fungal and bacterial	
bio-agents at 25 °C	46
4. Percentage mortality of IJ ₂ Meloidogyne incognita as	
affected by the culture filtrate of fungal and bacterial bio-	
agents at 25 °C	49
5. Percentage of egg hatching per egg-mass of Meloidogyne	
incognita as affected by of the culture filtrate of fungal and	
bacterial bio-agents at 25 °C	51
6. Efficacy of different bio-agents at different concentrations	
on reducing root-knot nematode in cucumber plants in pot	
experiment	62
7. Influence of different concentrations of bio-agents on shoot	
growth of cucumber plants infected with root-knot	
nematode in pot experiment	63
8. Efficacy of different combinations of bio-agents in reducing	
root-knot nematode on cucumber plants in pot	
experiment	66
9. Influence of different combinations of bio-agents on shoot	
growth of cucumber plants infected with root-knot	
nematode in pot experiment	67

10. Efficacy of culture filtrates produced by selected bioagents	
in reducing root-knot nematode on cucumber plants in pot	
experiment	69
11. Influence of culture filtrates produced by selected	
bioagents on vegetative growth of cucumber plants infected	
with root-knot nematode in pot experiment	71
12. Efficacy of various formulated products of <i>Verticillium</i>	
chlamydosporium in reducing root-knot nematode on	
cucumber plants in pot experiment	73
13. Influence of various formulated products of <i>Verticillium</i>	
chlamydosporium on vegetative growth of cucumber plants	
infected with root-knot nematode on cucumber plants in pot	
experiment	74
14. Efficacy of selected bio-agents in reducing root-knot	
nematode infection on cucumber plants under simulated	
field conditions	76
15. Influence of selected bio-agents on vegetative growth of	
cucumber plants during root-knot nematode infection under	
simulated field conditions	77
16. Efficacy of different combinations between selected bio-	
agents with plant or animal compost on reducing root-knot	
nematode infection in cucumber plants under simulated	
field conditions	79
17. Influence of different combinations between selected bio-	
agents with plant or animal compost on vegetative growth	
of cucumber plants during root-nematode infection under	
simulated field conditions	80