

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

بسم الله الرحمن الرحيم





MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY

USE OF SOME STREPTOMYCES SPECIES AS BIO-AGENTS TO CONTROL BEAN WHITE ROT DISEASE CAUSED BY Sclerotinia sclerotiorum

By

DOHA ALAAELDIN SAAD SOUFI ESMAIL GEBILY B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Fayoum Univ., 2010 M.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Fayoum Univ., 2015

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences (Plant Pathology)

Department of Plant Pathology
Faculty of Agriculture
Cairo University
EGYPT

2021

Format Reviewer

Vice-Dean for Graduate Students and Research

APROVAL SHEET

USE OF SOME STREPTOMYCES SPECIES AS BIO-AGENTS TO CONTROL BEAN WHITE ROT DISEASE CAUSED BY Sclerotinia sclerotiorum

Ph.D. Thesis In Agric. Sci. (Plant Pathology)

By

DOHA ALAAELDIN SAAD SOUFI ESMAIL GEBILY

B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Fayoum Univ., 2010 M.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Fayoum Univ., 2015

APPROVAL COMMITTEE

Dr. MOHAMED EL- KHALEELY BARAKATProfessor of Plant Pathology, Fac. Agric., Fayoum Univ.	
Dr. KAMEL KAMAL ALI SABET	
Professor of Plant Pathology, Fac. Agric., Cairo Univ.	
Dr. GAMAL AMIN MOHAMED GHANEM	
Professor of Plant Pathology, Fac. Agric., Cairo Univ.	
Dr. MONA MAHMOUD MAHER RAGAB	
Professor of Plant Pathology, Fac. Agric., Cairo Univ.	

Date: 27 / 5 /2021

SUPERVITION SHEET

USE OF SOME STREPTOMYCES SPECIES AS BIO-AGENTS TO CONTROL BEAN WHITE ROT DISEASE CAUSED BY Sclerotinia sclerotiorum

DOCTOR OF PHILOSOPHY

In Agric. Sci. (Plant Pathology)

BY

DOHA ALAAELDIN SAAD SOUFI ESMAIL GEBILY

B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Fayoum Univ., 2010 M.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Fayoum Univ., 2015

SUPERVITION COMMETEE

Dr. GAMAL AMIN MOHAMED GHANEM Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. MONA MAHMOUD MAHER RAGAB Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. NOUR EL-DEEN KAMEL SOLIMAN (Late) Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. AYAT MAHMUOD EL-SAYED Researcher, Organic Agriculture Lab., Agric.Res. Center, Giza

Dr. TAWFIK HAFEZ ABD EL-MOITY (Late) Cheif Researcher, Organic Agriculture Lab., Agric. Res. Center, Giza Name of Candidate: Doha Alaa Eldin Saad Gebily Degree: Ph.D.

Title of Thesis: Use of some Streptomyces species as bio-agents to control

bean white rot disease caused by Sclerotinia sclerotiorum

Supervisors: Dr. Gamal Amin Mohamed Ghanem

Dr. Mona Mahmoud Maher Ragab

Dr. Ayat Mahmuod El-Sayed

Dr.Nour El-Deen Kamel Soliman (Late) & Dr. Tawfik Hafez Abd El-Moity (Late)

Department: Plant Pathology Approval: 27 /5/2021

ABSTRACT

White mold disease, caused by Sclerotinia sclerotiorum the devastating pathogen, attacks green bean (Phaseolus vulgaris L.) and several crops worldwide. The present investigation was conducted to introduce some antagonistic microorganisms as novel antifungal substances to be an alternative and secure method to effectively control the disease. Out of 24 isolates, three Streptomyces isolates were molecularly characterized. PCR amplification of the fungus pathogen and Streptomyces isolates 16S rDNA gene sequences exhibited amplicons of around 535bp and 1300bp, respectively. The characterized *Streptomyces* isolates were sequenced and submitted into Genebank under accession numbers i.e., S. griseus (MT210913 "DG5"), S. rochei (MN700192 "DG4") and S. sampsonii (MN700191 "DG1"). Phylogenetic tree of the nucleotide sequence analysis of the three *Streptomyces* spp. indicated that S. griseus MT210913 was closely related to S. sampsonii MN700191 (96%), secondly ranked by S.rochei MN700192 (93.1%). Afterward, the antifungal activity of Streptomyces spp. against S. sclerotiorum was evaluated in vitro and in vivo (in the greenhouse and field). In vitro tests, proved that the reduction percentages in mycelial growth of pathogen ranged between 31.4-60.17%, indicating that S. rochie gave the highest inhibition percent. Incorporations of Streptomyces spp culture filtrate components into culture media proved that S. sampsonii was more efficient as a bioagent in reducing mycelial growth pathogen by 84.50%. When the effectiveness of the bioagent volatile compounds was evaluated, the inhibition of the pathogen growth ranged between 54.50-72.54%, respectively, revealing that S. rochei was the highest inhibitor followed by S. griseus. Results of GC-Mass analysis revealed the presence of 44, 54 and 47 diverse secondary metabolites compounds produced by S. sampsonii DG1, S. rochie DG4, and S. griseus DG5, respectively. Examining parasitic activity of Streptomyces spp upon S. sclerotiorum was demonstrated by light and scanning electron (SEM) micrographs exhibited the interaction as deformation, contraction, and collapse in the mycelium of the pathogen. Viability and germination of pathogen sclerotia were reduced when they dipped into the Streptomyces culture broth for 10, 20, and 30 days. Application of the 3 Streptomyces spp. in the field proved a great potential to control the disease. The results suggested that the 3 Streptomyces spp. and their secondary metabolites can be biofertilizers as enhancers in plant growth and potential biocontrol agents for controlling bean white rot disease. Finally, the author suggests that the phenomenon of the mycoparsitism in case of Streptomyces could be named actinoparasitism.

Key words: S. sclerotiorum, Streptomyces spp., antifungal activity, green bean

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my parents, my sister for their patience and help, as well as my family and friends for all the support they lovely offered along the period of my post-graduation.

ACNOWLEDGMENT

First of all and foremost, the unlimited thanks to (God), I wish to express my sincere thanks, deepest gratitude and appreciation to **Dr. GAMAL AMIN MOHAMED GHANEM**, Professor of Plant Pathology, Faculty of Agriculture, Cairo University, for his supervision, great assistance, valuable criticism, guidance encouragement, kind supervision and excellent advices throughout this study.

Many thanks and valuable gratitude is due to **Dr. MONA MAHMOUD MAHER RAGAB** Professor of Plant Pathology,
Faculty of Agriculture, Cairo University, for her cooperation,
patience, and kind supervision.

Many thanks and valuable gratitude is due to **Dr. AYAT MAHMUOD EL-SAYED** Researcher of Plant Pathology, Agric.
research center, for her cooperation and patience.

Many thanks and valuable gratitude is due to Dr. NOUR EL-DEEN KAMEL SOLIMAN Prof. of Plant Pathology, Fac. of Agric., Cairo University, and Dr. TAWFIK HAFEZ ABD EL-MOITY Prof. of Plant Pathology Biological control, Agric. Research Center, for their cooperation, patience, suggesting the problem and kind supervision may God have mercy on them.

Grateful appreciation is also extended to all staff members of Central Lab of organic agriculture, Agriculture research center, for their help and providing facilities needed throughout executing this investigation.

CONTENTS

INTRODUCTION
REVIEW OF LITERATURE
1. White rot disease and its economic importance
a. Survey of white rot disease
b . Isolation and identification of the causal organism
c . Molecular identification of the pathogen (S. sclerotiorum)
2. Actinomycetes (Actinobacteria)
a. Difination, classification and characteristics
b . Prevalence of Actinobacteria
c. Isolation and identification of Actinobacteria
d. Molecular characterization of Actinobacteria
e. Characterization of Streptomyces spp. bioactive secondary
metabolites utilizing Gas chromatography-mass spectrometry
(GC-Mass) analysis
f. Application of Actinobacteria to control plant diseases
1. Potential activities of Actinobacteria <i>in vitro</i>
2. Application of Actinobacteria <i>in vivo</i>
3. Postharvest studies
MATERIALS AND METHODS
1. Isolation and purification of the causal pathogen
2. Molecular identification of the pathogen
3. Isolation and purification of Streptomyces isolates
4. Molecular characterization of Streptomyces isolates
5. GC-Mass analysis of <i>Streptomyces</i> spp. secondary metabolites
a. Extraction of the secondary metabolites
b . Gas chromatography-mass spectrometry (GC-Mass analysis)
6. Antifungal bio-assays of Streptomyces spp in vitro
a. Dual culture technique
b . Mechanism of parasitism

	Page
c. Impact of light and darkness conditions on the efficiency of <i>Streptomyces</i> spp. antifungal compounds on the pathogen mycelial growth suppression	56
d. Impact of filtration and heat treatment on the efficacy of <i>Streptomyces</i> spp. antifungal compounds on the pathogen mycelial growth suppression	57
e. Antifungal activity of volatile compounds produced by <i>Streptomyces</i> spp.	57
f. Impact of soaking pathogen sclerotia into culture broth of <i>Streptomyces</i> spp. on germination and mycelium growth	58
7. Greenhouse experiments	59
a. Reactions of green bean cultivars to the infection by <i>S. sclerotiorum</i> under greenhouse conditions	60
b. Impact of inoculum potentiality of <i>S. sclerotiorum</i> on green bean cv. Paulista white mold diseases incidence	60
c. Determine the efficacy of Streptomyces spp. in vivo	61
8. Field experiments	61
a. Influence of spraying <i>Streptomyces</i> spp. at different times on the percentages of disease incidence and plant parameters & components	63
b. Effect of spraying <i>Streptomyces</i> spp. at different concentrations on the percentages of disease incidence and plant parameters & components	64
c. Impactof spraying <i>Streptomyces</i> spp. at different numbers on the percentages of disease incidence and plant parameters & components	64
9. Post-harvest and Shelf-life experiments	64
a . Influence of applying <i>Streptomyces</i> spp. at different times on controlling the disease and extending shelf-life period of bean pods	66
b. Effect of applying <i>Streptomyces</i> spp. at different concentrations on controlling the disease and extending shelf-life period of bean pods	66

	Pag
c . Influence of spraying <i>Streptomyces</i> spp. at different numbers on controlling the disease and extending shelf-life period of bean pods	67
Statistical Analysis	67
RESULTS AND DISCUSSION	69
1. Isolation, purification and molecular characterization of	0,
the pathogen	70
2. Molecular characterization of the <i>Streptomyces</i> spp	76
3. GC-Mass analysis of <i>Streptomyces</i> spp. secondary metabolites	80
4. <i>In vitro</i> evaluation of antifungal activity of <i>Streptomyces</i> spp.	101
a. In vitro inhibitory potential of Streptomyces spp. against	10.
S. sclerotiorum	10
b . Examination of the parasitic mechanisms of <i>S. rochei</i> using	
slide technique	10
c. Impact of light and darkness conditions on antifungal	
activity in suppression of pathogen mycelium growth	10
d. Impact of filtration and heat treatment on antifungal	
activity of <i>Streptomyces</i> spp. in suppression of pathogen mycelium growth	11
e. Inhibitory effects of VOCs produced by <i>Streptomyces</i> spp.	
on mycelium growth of <i>S. sclerotiorum</i>	11
f. Impact of soaking pathogen sclerotia into culture broth of	
Streptomyces spp. on sclerotia germination and mycelium growth	11
5. Greenhouse experiments	12
a. Reactions of green bean cultivars to the infection by	12
S. sclerotiorum under greenhouse conditions	12
b. Impact of inoculum potentiality of <i>S. sclerotiorum</i> on green	
bean cv. Paulista white mold diseases incidence	12
c. Evaluation of <i>Streptomyces</i> spp. antifungal activity against	
S. sclerotiorum under greenhouse conditions	12
6. Biological control of white mold of green bean disease by	
Streptomyces spp. under field conditions (in vivo)	12
	12
a. Times of application experiments	

	Page
a.1. Influence of applying <i>Streptomyces</i> spp. at different times on the percentages of disease incidence under field conditions	127
a.2. Impact of applying <i>Streptomyces</i> spp. at different times on green bean plant vigour under field conditions.	130
a.3. Influence of applying <i>Streptomyces</i> spp. at various times on chemical components of the treated plants	132
b. Impact of culture broth concentrations	136
b.1. Impact of applying <i>Streptomyces</i> spp. at different concentrations on bean white rot disease incidence	136
b.2. Impact of applying <i>Streptomyces</i> spp. at various concentrations of on green bean plant vigour	139
b.3. Effect of applying <i>Streptomyces</i> spp. at different concentrations on chemical components of the treated plants.	141
c. Number of applications	144
c.1. Impact of applying <i>Streptomyces</i> spp. at different numbers on the percentage of disease incidence	144
c.2. Influence of applying <i>Streptomyces</i> spp. at different numbers on green bean plant vigour	146
c.3. Effect of applying <i>Streptomyces</i> spp. at different numbers on chemical components of the treated plants	149
7. Post-harvest and shelf life.	152
a . Influence of applying <i>Streptomyces</i> spp. at different times on shelf-life of bean pods at 5°C and 20±2°C	152
b. Impact of applying <i>Streptomyces</i> spp. at different concentrations on shelf life of bean pods at 5°C and 20±2°C	157
c . Effect of applying <i>Streptomyces</i> spp. at different numbers on shelf- life of bean pods at 5°C and 20±2°C	159
CONCLUSION	164
RECOMMENDATIONS	165
SUMMARY	166
REFERENCES	173
ARABIC SUMMARY	

LIST OF TABLES

No.	Title	Page
1.	Sources and locations of the Streptomyces isolates	48
2.	Composition of the used media to grow the pathogen and actinobacteria.	49
3.	Sharing (%) of nucleotide sequences between the three Streptomyces spp. and others isolates from different geographical regions available in the GenBank	78
4.	GC-Mass analysis of the ethyl acetate extracts of Streptomyces spp.	87
5.	Inhibitory potential of <i>Streptomyces</i> spp. against fungus <i>S. sclerotiorum in vitro</i>	104
6.	Impact of light and darkness conditions on antifungal activity efficiency in the suppression of pathogen mycelium growth	108
7.	Suppression of pathogen mycelial growth by antifungal activity of <i>Streptomyces</i> spp. culture filtrate using Millipore membrane or heat treatment.	113
8.	Inhibitory effects of VOCs produced by <i>Streptomyces</i> spp. on mycelium growth of <i>S. sclerotiorum in vitro</i>	117
9.	Impact of soaking pathogen sclerotia into culture broth of <i>Streptomyces</i> spp. on sclerotia germination and mycelium growth	
10.	Reactions of green bean cultivars to the infection by <i>S. sclerotiorum</i> under greenhouse conditions	122
11.	Impact of inoculum potentiality of <i>S. sclerotiorum</i> on green bean cv. Paulista white mold diseases incidence	123
12.	Evaluation of <i>Streptomyces</i> spp. on controlling <i>S. sclerotiorum</i> under greenhouse onditions	125
13.	Influence of applying <i>Streptomyces</i> spp. at different times on the percentages of disease incidence under field conditions	129

No.	Title	Page
14.	Impact of applying <i>Streptomyces</i> spp. at different times on green bean plant vigour under field conditions	133
15.	Influence of applying <i>Streptomyces</i> spp. at various times on chemical components of the treated plants	134
16.	Impact of applying <i>Streptomyces</i> spp. at different concentrations on bean white rot disease incidence	140
17.	Impact of applying <i>Streptomyces</i> spp. at various concentrations of on green bean plant vigour	143
18.	Effect of applying <i>Streptomyces</i> spp. at different concentrations on chemical components in the treated plants	145
19.	Impact of applying <i>Streptomyces</i> spp. at different numbers on the percentage of disease incidence	148
20.	Influence of applying <i>Streptomyces</i> spp. at different numbers on green bean plant vigour	150
21.	Effect of applying <i>Streptomyces</i> spp. at different numbers on chemical components of the treated plants	153
22.	Influence of applying <i>Streptomyces</i> spp. at different times on shelf life of bean pods at 5°C and 20±2°C	158
23.	Impact of applying <i>Streptomyces</i> spp. at different concentrations on shelf life of bean pods at 5°C and 20±2°C	159
24.	Effect of applying <i>Streptomyces</i> spp. at different numbers on shelf life of bean pods at 5°C and 20±2°C	162