BACTERIAL SYMBIONTS OF ENTOMOPATHOGENIC NEMATODES AS BIOCONTROL AGENTS

By

JIHAN MUHAMMAD SAYED AHMED

B. Sc. Agric. Sci., (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2013

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2019

Format Reviewer **Vice Dean of Graduate Studies**

SUPERVISION SHEET

BACTERIAL SYMBIONTS OF ENTOMOPATHOGENIC NEMATODES AS BIOCONTROL AGENTS

Ph.D. Thesis
In
Agricultural Sci. (Agricultural Microbiology)

By

JIHAN MUHAMMAD SAYED AHMED

B. Sc. Agric. Sci., (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2013

SUPERVISION COMMITTEE

Dr. MOHAMED ABDEL-ALIM ALI
Professor of Agricultural Microbiology, Fac. Agric., Cairo University

Dr. WALEED DIAEDDEEN SALEH
Professor of Agricultural Microbiology, Fac. Agric., Cairo University

Dr. AHMED MOHAMMED AZAZY
Head Researcher of Pest Physiology Department. ARC, Giza

Name of Candidate: Jihan Muhammad Sayed Ahmed **Degree:** Ph.D.
Title of Thesis: Bacterial Symbionts of Entomopathogenic Nematodes as Biocontrol Agents
Supervisors : Dr. Mohamed Abdelalim Ali
Dr. Waleed Diaeddeen Saleh
Dr. Ahmed Mohammed Azazy
Department: Agricultural Microbiology **Approval**: 9 /1 /2019

ABSTRACT

In this work, five strains of *Photorhabdus luminescence* isolated from a local nematode strains namely *Heterorhabditis indica* (EGAZ1), *H. indica* (EGAZ2), *H. indica* (EGAZ3), *H. indica* (EGAZ4), *H. indica* (EGAZ5). In addition to a second bacterial symbiont *Providencia rettgeri* of *H indica* (EGAZ3), *Photorhabdus luminescens* subsp. *laumondii* HP88 which was isolated from *Heterorhabditis bacteriophora* (HP88), and *Xenorhabdus nematophilus* isolated from the hemolymph of *Galleria mellonella* infected with *Steinernema carpocapsae* (BA2). The identity of the isolated bacteria were authenticated referring to their cultural, morphological and biochemical traits as well as to their entomopathogenicity against *Galleria mellonella*. The 16S r RNA gene sequence technique was adopted for conclusive identification of the five isolates. Lab and pots experiments conducted to examine antimycotic activities of cell-free culture filtrates with different concentrations against plant pathogenic fungi *Fusarium oxysporum*, *Fusraium solani*, and *Schloritium rolfsii*, also the nematicidal activities of the bacterial cell suspensions and cell-free culture filtrates with different concentrations against *Meloidogyne incognit were studied* and larvae toxicity of the bacterial cell suspensions and cell-free culture filtrates with different concentrations pointed out to moderate susceptibility of *T. absoluta* larvae to all bacterial cell suspensions as well as their toxins. However, the degree of susceptibility varied according to the strain genotype and toxin concentration. Higher densities cell suspensions up to 4 x 10^7cells.ml^{-1} and undiluted cell- free supernatant (100%) were the most effective larvicidal fluids. Percentage of *T. absoluta* mortality increased with increasing the supernatant concentration. Larvae mortality of up to 70 % was achieved when *T. absoluta* larvae were treated with either a cell suspension or the cell-free culture filtrates of *P. luminescence* (EGAP2). Histopathological effect of *P. luminescence* (EGAP2) on the midgut of *Tuta absoluta* was examined.

Key words: *Photorhabdus*, *Xenorhabdus*, Fungicidal, Nematicidal and Insecticidal.

DEDICATION

This work is dedicated to my father, because I owe it all to you. Many Thanks, my mother, my brothers (Mahmoud, Khaled, Ahmed, Amr and Hussien), my lovely sister (Shymaa) for their emotional support, my nephews, my nieces (Gody, Gana , Gehan, Malk, Gomana, Shrouk, Rodina) for always making me see the bright side of life, and finally to my dear friends (Heba Adel , Mai Mahrous and Amal Samir)

ACKNOWLEDGEMENT

Praise and thanks be to ***ALLAH****, for assisting and directing me to the right way.*

I wish to express my sincere thanks, and appreciation to ***Dr. Mohamed. A. Ali; Dr. Waleed Diaeddeen Saleh ,*** *Professors of Agricultural Microbiology, Faculty of Agriculture, Cairo University and* ***Dr. Ahmed Mohammed Azazy*** *, Head Researcher of Pest Physiology. ARC, Giza, for suggesting the problems, supervision, continued assistance and their guidance through the course of study and revising the manuscript.*

My infinity gratitude to my ideal ***Dr. Olfat S. Barakat****, Professor of Agricultural Microbiology, Faculty of Agriculture, Cairo University whose guidance, support and advice has been invaluable in my journey.*

My grateful also goes to ***Dr. Aziza El Sayed Abdel Aal, Dr. Tarek Afifi A. El Sheikh, Dr. Tarek Raies Amin, Dr. Sami Sayed El Badawy, Dr. Heba Abdel Gelil El Ghanam, Dr. Salem Mohamed Salem, Hagar Mohsen Hassan****, Plant Protection, Res. Inst., ARC. and* ***Dr. Manal Farouk M. Abdelall,*** *AGERI, ARC. for their help in this work.*

Grateful appreciation is also extended to all staff members and colleagues in the Department of Agricultural Microbiology, Faculty of Agriculture, Cairo University and all staff members in the Department of pest physiology, Plant Protection, Res. Inst., ARC.

CONTENTS