شبكة المعلومات الجامعية

بسم اللّه الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم باللّه 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

شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة

بالرسالة صفحات
لم ترد بالأصل

BIOTECHNOLOGICAL POTENTIAL OF BACTERIAL METABOLITES IMMOBILIZED ON IRRADIATED NANO CLAY PARTICLES IN RELATION TO CROP PRODUCTION

By

HEBA HAMED BAIOUMY MAGHRAWY

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2011

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2020

Format Reviewer **Vice Dean of Graduate Studies**

APPROVAL SHEET

BIOTECHNOLOGICAL POTENTIAL OF BACTERIAL METABOLITES IMMOBILIZED ON IRRADIATED NANO CLAY PARTICLES IN RELATION TO CROP PRODUCTION

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

By

HEBA HAMED BAIOUMY MAGHRAWY

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2011

APPROVAL COMMITTEE

Dr. FATMA IBRAHIM EL ZAMIK ……
Professor of Agric. Microbiology, Fac. Agric., Zagazig University

Dr. MOHAMED FAYEZ FOUAD……...
Professor of Agric. Microbiology, Fac. Agric., Cairo University

Dr. SAMIR ABD ELWAHED ELGIZAWY ……..
Professor of Agric. Microbiology, Fac. Agric., Cairo University

Date: 2/11/2020

SUPERVISION SHEET

BIOTECHNOLOGICAL POTENTIAL OF BACTERIAL METABOLITES IMMOBILIZED ON IRRADIATED NANOCLAY PARTICLES IN RELATION TO CROP PRODUCTION

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

By

HEBA HAMED BAIOUMY MAGHRAWY

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2011

SUPERVISION COMMITTEE

Dr. SAMIR ABD ELWAHED ELGIZAWY
Professor of Agric. Microbiology, Fac. Agric., Cairo University

Dr. REFAIE IBRAHIM REFAIE
Professor of Agric. Microbiology, Fac. Agric., Cairo University

Dr. HUSSEIN ABD EL KAREEM AHMED
Researcher Professor of Microbiology, NC for Radiation Research and Technology

Name of Candidate: Heba Hamed Baioumy Maghrawy **Degree:** Ph.D.
Title of Thesis: Biotechnological Potential of Bacterial Metabolites Immobilized on Irradiated Nanoclay Particles in Relation to Crop Production
Supervisors: Dr. SAMIR EL GIZAWY
Dr. REFAE IBRAHIM REFAE
Dr. HUSSEIN ABD EL KAREEM
Department: Agricultural Microbiology **Date: 2/11/2020**

ABSTRACT

Potato is one of the major food crops belonging to family Solanaceae. Black scurf and stem canker, caused by *Rhizoctonia solani* are destructive diseases of potato causing serious damage in most potato-producing areas throughout the world. Chemical pesticides are widely used to fungal pathogens control. However, the application of chemical pesticides is harmful to both the environment and human health. Therefore, the use of biocontrol agents (BCAs) is considered to be potentially sustainable, cost-effective and eco-friendly ways to control *R. solani.* Accordingly, 84 isolates of PGPR were recovered and screened for their ability to produce antifungal compound(s), from rhizosphere of healthy potato plant collected from different areas. One strain was selected based on its ability to suppress the growth of *R. solani*. Phylogenetic analysis of this strain based on 16S rRNA gene sequences showed highest similarity (99%) with *Bacillus* sp. GRP (GenBank accession number: MK030136)**.**

The *Bacillus* sp. GRP culture filtrate contained protease, diffusible antibiotic, hydrogen cyanide and siderophore, it was capable of inhibiting growth of the pathogen up to 15 days compared to 7 and 10 days for other *Bacillus* isolates. It also produced indole acetic acid which promoted plant growth. Morphological and structural changes that took place as a result of *Bacillus* sp. and *R. solani* interaction were evaluated using light, SEM and TEM.

The results showed that *Bacillus* sp. caused loss of structural integrity, abnormal coiling, shriveling and lysis of the *R. solani* hyphae, in addition to complete cytoplasm and internal organelles depletion. Among four media tested, the maximum growth and antifungal activities were found in tryptone soya broth medium under static condition at 30°C, pH7, inoculum density of 1% and 72 h incubation. Antifungal substances were extracted and identified by Thin Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FT-IR), Ultraviolet-visible spectroscopy (UV-Vis) and Liquid Chromatography Mass Spectrometry (LC-MS) that revealed the presence of two compounds which were assigned as lipopeptides (fengycin and surfactin). Significant inhibitory effect of lipopeptides was observed at concentration as low as 200 µg/ml. No changes in viability of human cell line were observed even at the highest concentration tested (200 µg/mL) as indicated by inverted microscope. Lipopeptides showed an increased stability to high temperature, pH, UV and sunlight. Nanoclay particles exposed to different doses of gamma irradiation (0, 25, 50 and 100 KGy) were studied. *Bacillus* sp. GRP and lipopeptides were immobilized on nanoclay to form a bionanocomposite and bio-nano-fungicide. Bionanocomposite and bio-nano-fungicide were characterized by Dynamic light Scattering Measurments (DLS), FTIR spectroscopy, X- ray diffraction (XRD), TEM and SEM. Bionanocomposite exhibited biocontrol efficiency along 8 months storage, while bio-nano-fungicide maintained higher activity during 12 months at room temperature in comparison with the free lipopeptide. Both *in vitro* and greenhouse experiments for both bioformulations showed high inhibition of *R. solani* radial growth. Results indicate that the prepared bionanocomposite and bio-nano-fungicide are a promising alternative to the commercial products.

Key Words: *Bacillus* sp. GRP, *Rhizoctonia solani*, optimization, lipopeptides, bionanocomposite, bio-nano-fungicide, Biocontrol.

DEDICATION

I dedicate this work to whom my heartfelt thanks; to the soul of my father; my dear mother for her deep prayers, continuous care and love, as well as to my dear husband and my children for their patience, love and help.

Also I dedicate this work to my dear brother for his love and deep prayers. Finally, I would thank my dear colleague Marwa Gamal for her support and love.