



**Faculty of Science**

# **USING SOME MICROORGANISMS IN WEED CONTROL AND THEIR DEVELOPMENT AS BIOHERBICIDES**

Thesis

Submitted for Master Degree in Microbiology

**By**

**Merhan Mohammed Galal Eldine Tawfik**

B. Sc. (Microbiology/Chemistry), Ain Shams University, 2013

**Under Supervision of**

**Prof. Dr. Mohamed A. Abouzeid**

Professor of Microbiology  
Department of Microbiology  
Faculty of Science  
Ain Shams University

**Prof. Dr. Mohamed A. Balah**

Professor of Pesticides  
Plant Protection Department  
Desert Research Center

**Dr. Nevin A. Ibrahim**

Lecturer of Microbiology  
Department of Microbiology  
Faculty of Sciences  
Ain Shams University

**(2019)**



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**Approval sheet**

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**Supervisors**

**Prof. Dr. Mohamed A. Abouzeid**

Professor of Microbiology, Department of Microbiology, Faculty of  
Science, Ain Shams University

**Prof. Dr. Mohamed A. Balah**

Prof. of Pesticides, Plant Protection Department, Desert Research Center

**Dr. Nevin A. Ibrahim**

Lecturer of Microbiology, Department of Microbiology, Faculty of  
Sciences, Ain Shams University

**Examination committee**

**Prof. Dr. Tarek A. El-shahawy**

Professor of weed biology and control, Botany department, National  
Research Center

**Prof. Dr. Nasr E. El-Bordeny**

Professor of Animal Nutrition, Animal production department, Faculty of  
Agriculture, Ain Shams University

**Prof. Dr. Mohamed A. Abouzeid**

Professor of Microbiology, Head of Microbiology department, Faculty of  
Science, Ain Shams University

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## Table of Abbreviations

| Abbreviation     | Meaning   |
|------------------|---|
| ANOVA            | Analysis of Variance                              |
| EC <sub>50</sub> | Half maximal effective concentration              |
| LC-MS/MS         | Liquid chromatography-tandem mass spectrometry    |
| HPLC             | High performance liquid chromatography            |
| M + H            | Molecular ion+ protons                            |
| MS               | Murashige and Skoog media                         |
| M W              | Molecular weight                                  |
| m/z              | Mass-to-charge                                    |
| NA               | Nutrient agar medium                              |
| PDA              | Potato dextrose agar                              |
| DCPA             | Dichloran-chloramphenicol-peptone agar            |
| CYA              | Czapek yeast extract agar                         |
| MEA              | Malt extract agar                                 |
| KB               | King's B medium                                   |
| MR&VP            | Methyl red test and Vogues- proskauer             |
| MIO              | Motility Indole Ornithine medium                  |
| TSI              | Triple Sugar Iron medium                          |
| LSD              | Least significant difference                      |
| NCBI             | National Center for Biotechnology Information     |
| BLAST            | Basic Local Alignment Search Tool                 |
| MEGA             | Molecular Evolutionary Genetics Analysis          |
| TRFLP            | Terminal Restriction Fragment Length Polymorphism |
| AFLP             | Amplified Fragment Polymorphism Technique         |

## ABSTRACT

Microbial weed control represents an alternative ecofriendly solution in suppressing weed plants, reducing weed risks, and overcoming the deleterious effect of synthetic herbicides. In this study two noxious weeds (*Portulaca oleracea* and *Convolvulus arvensis*) were chosen to biocontrol as their severity in the newly reclaimed lands of Egypt. Screening of 36 fungal and 40 bacterial isolates culture filtrates against target weeds led to the discovery of four active bacterial isolates (two of them under the genera *Pseudomonas* and the other two under genera *Bacillus* and *Xanthomonas*) and four active fungal isolates (*Myrothecium verrucaria* FS80, *Cladosporium cladosporioides* FS73, *Aspergillus flavus* FS76, and *Aspergillus terreus* FS67). Through bioassaying ethyl acetate crude extracts of these isolates against seedling stage of *P. oleracea* and *C. arvensis*, three isolates (*Pseudomonas* sp. isolate 1 FS15, *M. verrucaria* FS80, and *C.*

*cladosporioides* FS73) showed the highest herbicidal activity. The aqueous culture filtrate of *Pseudomonas* sp. isolate 1 FS15 gave 100% reduction in seed germination, shoot and root length of *P. oleracea* and *C. arvensis* while, at 40 mg/ml crude extract of the same isolate the reduction percentage in total biomass fresh weight of *P. oleracea* and *C. arvensis* reached 71% and 39%, respectively. The second active isolate which was *C. cladosporioides* FS73 gave 100% reduction in seed germination, shoot length, and root length of *P. oleracea* and its extract gave 77.69% reduction in total biomass fresh weight of *P. oleracea* at the highest concentration (40 mg/ml). The third isolate *M. verrucaria* FS80 gave 72% reduction in seed germination, 92% reduction in shoot length, and 80% reduction in root length of *P. oleracea* while, in case of *C. arvensis*, it gave 63% significant reduction in root length and 65.3% reduction in shoot length. At 40 mg/ml of *M. verrucaria* crude extract, the highest reduction percentage in total seedling biomass fresh weight of *P. oleracea* and *C. arvensis* reached 84.92 and 58.79%, respectively. Foliar application of  $5 \times 10^7$  conidia/ml

of *M. verrucaria* plus 0.2% silwet L-77 in pots containing weeds led to 74% significant reduction in chlorophyll a of *P. oleracea* and 36% reduction in chlorophyll b of *C. arvensis*. Through identifying *Pseudomonas* sp. isolate 1 by sequencing, it showed 99% similarity with dozens of *Pseudomonas aeruginosa*. LC-MS/MS analysis of the crude extracts of the most active isolates led to the presence of 13 compounds from *C. cladosporioides* (FS73) which identified as (Mollicelin A, P-methyl benzoic acid, P-hydroxy benzoic acid, Cladosporin, Isocladosporin, Diacetyl cladosporin, Cladosporinone, Cladosporol, Cladosporol C, 3,7-dimethyl-8-hydroxy-6-methoxy isochroman, Cinamic acid, Chlorogenic acid, and Viriditoxin), while in case of *M. verrucaria* (FS80) seven compounds were detected most of them belonging to macrocyclic trichothecene like (Verrucar-L-acetate, Verrucar A, Verrucar M, Verrucarol, Myrothecol A, Roridin E, and Roridin A) and finally 11 compounds were deduced from *P. aeruginosa* (FS15) including (Pyoluteorin, Phenazine-1-carboxylic acid, Phenazine-1-sulfate, Phenazine-1-carboxamide, Pyocyanin, Pyrrolnitrin,