Faculty of Science

USING SOME MICROORGANISMS IN WEED CONTROL AND THEIR DEVELOPMENT AS BIOHERBICIDES

Thesis
Submitted for Master Degree in Microbiology

By

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

Under Supervision of

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Approval sheet

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Table of Abbreviations

Abbreviation	Meaning
ANOVA	Analysis of Variance
EC_{50}	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	Trible Sugar Iron medium
LSD	Least significant difference
NCBI	National Center for Biotechnology
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 5×10^7 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 (Verrucarin-L-acetate, Verrucarin A, Verrucarin 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,