BIOTREATMENT OF OLIVE MILL WASTEWATER FOR AGRICULTURAL USES

Submitted By

Ghada Amin Zaki Ibrahim

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A thesis submitted in Partial Fulfillment
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The Requirement for the Doctor of Philosophy Degree
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2016


Abstract

ABSTRACT

Olive mill waste water (OMWW) is a liquid by-product generated during olive oil production and widely known for its fertilizing value in sustainable agricultural systems. OMWW samples collected from different localities in Egypt were subjected to different chemical, physical and microbiological analysis to determine its quality as fertilizer. The presence of high content of phenolic compounds, COD and BOD were the great challenge to convert this waste rich with nutrients into safe and highly efficient biofertilizer. Aerobic OMWW fermentation processes were conducted to eliminate its phytotoxicity by removal of phenolic compounds present.

From twenty nine microbial isolates, the most efficient two bacterial isolates which degraded phenols to a greater extent within a relatively short time were selected for fermentation process and identified by partial 16S rRNA gene sequence analysis as *Enterobacter asburiae*, and *Pseudomonas aeruginosa*. Factors affecting the fermentation process and their impact on phenolic compounds degradation were optimized and the results revealed that the optimum pH values were (6 and 7), incubation temperature (25^0C and 30^0C), inoculum size (2ml\ 100ml OMWW), nitrogen sources (ammonium sulphate 1gm\L for both microbes), incubation period (27 and 30 days) anaerobic static cultures with 25% OMWW for *E. asburiae* and *Ps. aeruginosa*, respectively. Under these conditions of fermentation, phenolic compounds degrading ability was 82.6% for *Pseudomonas aeruginosa* and 79.9% for *Enterobacter asburiae*.

At the end of fermentation process, high energy components as total phenolic compounds phytohormones, amino acids, and antioxidants were determined using HPLC, amino acid analyzer and spectrophotometer, respectively. HPLC analysis revealed that the degradation rate of phenolic compounds ranged from 75 to 98.5 and 57.5 to 99.16% for *Ps. aeruginosa* or *E. asburiae*, respectively. Qurectin, catechol and caffiec acid were subjected to the maximum degradation rate of phenolic compounds while tyrosol, gallic acid and catchin showed less degradation levels by either *Ps. aeruginosa* or *E.*

asburiae. The phytohormones in the form of gebrillic acid increased in fermented OMWW by *Ps. aeruginosa* and *E. asburiae* to 15687.06 ppm and 12915.68 ppm, compared to11815.89 ppm for unfermented control. A remarkable increase in total amino acids in fermented OMWW was detected reaching 459.19 mg\ml and 559.81 mg\ml for OMWW fermented with *Ps. aeruginosa* and *E. asburiae*, respectively comparing with unfermented control (1.844 mg\ml). The average concentration of antioxidants (Butylated hydroxyanisole "BHA" and tertiary butylhydroquinone "TBHQ", respectively as standards) using in fermented OMWW by *Ps. aeruginosa* were (0.869 mol\ l) and (0.831 mol\ l), respectively, while in fermented OMWW by *E. asburiae* were (0.675 mol\l) and (0.641 mol\l) comparing to unfermented OMWW in which they were (0.862 mol\l) and (0.587 mol\l), respectively.

A Pot experiment was conducted at Desert Research Center - Egypt during summer 2012, to evaluate using of raw OMWW and different types of fermented ones as fertilizers using different types of application methods on *Sorghum bicolor* (L). The data indicated that application of OMWW fermented with *Ps. aeruginosa* or *E. asburiae* applied as soil drench application recorded the maximum positive effects on different traits as height of shoot and length of root, fresh and dry weight of shoot and root and leaf area index while unfermented OMWW recorded the minimum results comparing to control. Also application of fermented OMWW by *Ps. aeruginosa* and *E. asburiae* gave the maximum results of NPK, chlorophyll and carbohydrate contents followed by that of unfermented one compared to control (chemically fertilized plants).

A field experiment was conducted at Banger El-Sokkar area – Alexandria, Egypt during summer 2013 to study the effect of fermented OMWW applied as soil fertilizer on the growth and productivity of *Sorghum bicolor*. The experiment revealed that the maximum growth and yield parameters of plant were recorded with OMWW fermented by *Ps. aeruginosa* (80%), followed by *E. asburiae* (68%) as soil drench. This was also true for all plant chemical constituents measured.

Microbiological analysis of *Sorghum* rhizosphere revealed that application of OMWW fermented with *Ps. aeruginosa* or *E. asburiae* had positive effects on counts of total microbes, nitrogen fixers count and *Pseudomonads* comparing with control while unfermented OMWW had negative effects.

The present study deals with the potential of natural chemicals present in OMWW extracts with sequential concentrations as bio-herbicides. OMWW extracts investigated for the inhibitory effects at 0, 25, 50, and 100 % (v/v) dilutions under laboratory conditions while, higher concentrations of OMWW completely suppressed the weeds: *Portulaca oleracea, Echinochloa crus-galli* and *Corchorus olitorius*) as well as *Sorghum bicolor* seed germination and growth parameters by 100 %, comparing with control. The results showed that OMWW ethyl acetate crude extract at 500, 1000 and 2000 ppm inhibited the growth of *P. oleracea, E. crus-galli* and *C. olitorius* and slightly reduced *S. bicolor* growth parameters under laboratory conditions, which confirmed the herbicidal activity of sterilized OMWW water extract under laboratory and greenhouse conditions. For weed applications, unfermented OMWW can be used as bioherbicides for their markedly broadcast phytotoxicity, while the fermented OMWW with *E. asburiae* and *Ps. aeruginosa* can be used as biofertilizers without any negative impact on soil microbial activity. The LC-ESI-tandem MS analysis of OMWW ethyl acetate extract confirmed the presence of the common phenolic compounds; phenyl propionic acids, quinic acids, caffeoylquinic acids, apigenin -7-rutinoside, benzoic acid, apigenin, vanillic acid, sinapayl alcohol 4-glycoside, tyrosol, hydroxytyrsol acetate, hydroxyltyrsol rhamoside, hydroxytyrosol glycoside and luteolin-7-glucoside. Some unusual compounds such as ligstroside isomers, pinoresinol, oleoside and oleuropein were also detected. Their presence in the extract may be responsible of the biological and bio-pesticides activities a matter that should need further investigation for use in sustainable agricultural systems.

Key words: Olive mill waste water, fermentation, *Enterobacter asburiae, Pseudomonas aeruginosa*, Sorghum

ACKNOWLEDGEMENT

Foremost, I would like to express my sincere gratitude to **Prof. Dr. Elshahat Mohamed Ramadan**, Professor of Microbiology, Faculty of Agriculture, Ain Shams University, and **Prof. Dr. Tarek Said El-Tayeb,** Professor of Microbiology, Faculty Agriculture, Ain Shams University. For supervision, continuous support of my PhD study and research, for their patience, motivation, enthusiasm and immense knowledge. I could not have imagined having better advisors and mentors for my PhD study.

I would like to thank **Dr. Amal Mohammed Omar Salem**, Assoc. Professor of Microbiology in soil microbiology unit – Desert Research Center and **Dr. Mohammed Abd El Aziz Abo El-khair,** Assoc. Professor of pestside in Plant Protection Division – Desert Research Center**.** For supervision, their encouragement, insightful comments and their essential assistance in reviewing the patient files of this study.

Special thanks to **Prof. Dr. Gabriele Berg** and **Dr. Martina Koberl**, Institute of Environmental Biotechnology, Graz University of Technology, Graz, for Identification of bacterial isolates.

My sincere thanks also to **Dr. Sherif Ibrahim**, Researcher at Fertility and Microbiology of Soil, **Dr. Rabaa Yasien,** Researcher at soil microbiological unit, **Mrs. Eman Abd El Lateef**, Assistant Researcher - Desert Research Center for giving me untiring help during my difficult moments.

During this work I have collaborated with coworkers for whom I have great regard, and I wish to extend my warmest thanks to all those who have helped me with my work.

Last but not the least; I would like to thank my family: my parents for supporting me spiritually throughout my life, and I owe my loving thanks to my son **Amr**, he has lost a lot due to my research. Without his encouragement and understanding it would have been impossible for me to finish this work, to him I dedicate this thesis.

CONTENTS