Botany Department
Faculty of Science
Cairo University
Egypt

Assessment of Effective Microorganisms Technology for Treatment of Mycotoxins in Municipal Sewage Water and Its Impact on *Eruca sativa*

Thesis
Submitted to the Faculty of Science
Cairo University
For the M Sc. Degree

In

Microbiology

By

SAMAH ABDEL RAZIK MOHAMED MOHAMED

B.Sc.1998

Supervisors

Prof. Dr. TAHANY MOHMED ALI ABDEL RAHMANN

Prof. Dr. AHMED KAMEL HEGAZY

Prof. Dr. RABAB MOSTAFA KADRY

2009

جامعة القاهرة كلية العلوم قسم النبات

تقييم تكنولوجيا الميكروبات المؤثرة في معالجة السموم الفطرية بمياة الصرف الصحى و اثرها على نبات الجرجير

رسالة مقدمة الي كلية العلوم- جامعة القاهرة للحصول على درجة الماجستير في (نبات- ميكروبيولوجي)

مقدمة من سماح عبدالرازق محمد محمد بكالوريوس العلوم - كمياء ميكروبيولوجى كلية البنات للاداب و العلوم و التربية -جامعة عين شمس ١٩٩٨

السادة المشرفون الاستاذة الدكتورة / تهانى محمد على عبدالرحمن الاستاذ الدكتور/ احمد كامل حجازى الاستاذة الدكتورة / رباب مصطفى قدرى

Acknowledgement

I wish to express my deep gratitude and thanks to **Prof.** Dr. Tahany Mohamed Ali Abdel Rahman, Professor of Microbiology, Botany Department, Faculty of Science, Cairo University for direct supervision, Valuable discussion, Continuous encouragement and generous help throughout the present work, I am greatly indebted to **Prof. Dr. Ahmed Kamel Hegazy**, Professor of Ecology, Faculty of Science, Cairo University for his valuable help supervision and giving support complete this work. Also my deep appreciation to **Prof** Dr.Rabab Mostafa Kadry Professor of Mycotoxins in Agricultural Research Center, Giza, Dokki for her valuable share in Supervision, Valuable Support in Practical part, Guidance and Valuable efforts during the present investigation.

I express my sincere gratitude and appreciation to my husband **Dr. Amr Hassan** to whom I always indebted for his support and guidance throughout the investigation from the initial planning and proposing the research problem to the manuscript revision. I appreciate his encouragement, advice, constructive criticism and patience.

To my parents and sons.

Contents

1. Abstract	1
2. Introduction.	2
3. Literature Review	5
4. Ecological study	27
4.1 Study area.	27
4.2. Study plant species (<i>Eruca sativa</i>)	32
4.3. Climate	35
4.3.1. Temperature	35
4.3.2. Relative Humidity	36
4.3.3. Wind	37
4.3.4. Rain Fall.	37
5. Materials and Methods 5. I Samples & Analysis	39
5.1Samples& Analysis	39
5.2.a. Hydrogen Ion Concentration.	39
5.2.b. Chemical Oxygen Demand	39
5.2.c. Biological Oxygen Demand	40
5.2.d. Electric Conductivity	42
5.2.e. Ammonia nitrogen	42
5.2.f. Nitrate nitrogen	43
5.2.g. Total soluble phosphorus	44
5.2.h Total Phosphates	46
5.2.i Total Suspended Solids	46
5.3. Mycobiological surveys	46

5.3.a. Preparation of samples	46
5.3.b. Plating	46
5.3.c. Isolation and Identification of molds isolates	47
5.4 Mycotoxins Estimation	47
5.4. a. Extraction of Mycotoxins in <i>Eruca sativa</i> using VICAM	47
5.4.b. Aflatoxin estimation in <i>Eruca sativa</i>	48
5.4.c. Ochratoxin estimation in <i>Eruca sativa</i>	49
5.4.d. Zearalenone estimation in <i>Eruca sativa</i>	50
5.5. Dry Biomass.	51
5.6. Greenhouse design.	51
5.7 Statistical analysis	52
6. Experimental Results	53
Experiment (1): Physicochemical analyses, mycological surveys and mycotoxins estimations in <i>E.sativa</i> , soil and wastewater collected from six	53
drains Experiment (2): Green house experiment. Effect of primary treatment and EM ₁	
treatment to wastewater on physicochemical characters, Mycocount and mycotoxins accumulation in <i>E.sativa</i> Experiment (3): Effect of different concentrations of active EM ₁ on the total	69
fungal count of soil (cfu/mg) and <i>E.sativa</i> (cfu/cm ²) and mycotoxins accumulation in the plant	84
Experiment (4): Effect of different concentrations of EM ₁ on the growth (dry biomass) of the fungal species isolated allover the study	91
7. Discussion.	96
8. Summary and Conclusions	107
9. References.	109
10. Arabic Summary	120

APPROVAL SHEET

Title of the Thesis:

Assessment of Effective Microorganisms Technology for Treatment of Mycotoxins in Municipal Sewage Water and Its Impact on Eruca sativa

Name of the Candidate
Samah Abdel Razik Mohamed

Submitted to
Botany Department, Faculty of Science, Cairo
University
Supervision Committee:

Prof. Dr. Tahany Mohamed Ali Abdel Rahman

Professor of Microbiology, Botany Department, Faculty of Science, Cairo University

Prof. Dr. Ahmed Kamel Hegazy

Professor of Ecology, Faculty of Science, Cairo University

Prof .Dr.Rabab Mostafa Kadry

Professor of Mycotoxins in Agricultural Research Center, Giza, Dokki

Prof.Dr.E.F.Shabana.

Head of Botany Department faculty of Science, Cairo University

List of Tables

List of tables

Table 1. Physico chemical characters of untreated wastewater collected from the six study site	56
Table 2. Physico chemical characters of soils samples collected from the six study sites.	57
Table 3. The total fungal count and Relative density percent (R.D %) of species isolated from wastewater samples collected from the six study sites	60
Table 4. The total fungal count and Relative density percent (R.D %) of species isolated from soil samples collected from the six study sites	63
Table 5. The total fungal count and Relative density percent (R.D %) of species isolated from <i>E.sativa</i> plant samples collected from the six study sites	64
Table 6. Physico- chemical analyses of pretreated and primary treated wastewater collected from Al Rahawy and station site before and after addition of EM ₁ (4ml/L) River Nile water used as a control.	74
Table 7. Effect of EM ₁ addition on the Total fungal count and relative density percent (R.D) of species isolated from primary treated and pretreated wastewater from Al Rahawy station site. River Nile water used as a control.	75
Table 8. Effect of EM ₁ on the total fungal count and relative density percent (R.D) of species isolated from soil potted in green house cultivated with <i>E.sativa</i> and irrigated by primarily treated and pretreated wastewater from Al Rahawy station site of primary and Al Rahawy sites in comparison to control (River Nile)	76
Table 9. Effect of EM ₁ on the total fungal count and relative density percent (R.D) of species isolated from <i>E.sativa</i> plant in green house irrigated by primarily treated and pretreated wastewater from Al Rahawy station site	78

addition of different concentrations of EM ₁	88
Table 11. Total fungal count and relative density (%) of isolated species from <i>E.sativa</i>	
planted in green house and irrigated with pretreated and primarily treated wastewater from	
Al Rahawy station after addition of different concentrations of EM ₁	89
Table 12. Climate characterististics of Giza. Data from Environmental profile of Giza 2007	
and CLAC 2008. Temperature (°C), Relative Humidity (%), wind currents (%), wind speed	
(Km/hour) and Rainfall (mm per month)	121

List of Abbreviations

- BOD: Biological Oxygen Demand.
- CFU: Colony forming unit.
- COD: Chemical Oxygen Demand.
- DO: Dissolved oxygen.
- EC: Electric Conductivity.
- EM₁: Effective Microorganisms.
- EPA: Environmental Protection Agency.
- OD: Optical density.
- SS: Suspended Solids.
- TDS: Total dissolved solids.
- TSS: Total Suspended Solid.
- WWTP: Waste Water Treatment Plant.

List of figures

List of Figures

Fig. 4.3.	1 Monthly rates for Temperatures in meteorological station of	
Giz	za	3
Fig. 4.3.	2 Monthly rates for relative humidity in meteorological station of	
Giz	za	3
Fig. 4.3.	3 Average annual wind speed in the meteorological station of	
Giz	za	3
Fig. 4.3.	4 Average annual rainfall in the meteorological station of	
Giz	za	3
Fig. 6.1	the total fungal count isolated from wastewater, soil and <i>E.sativa</i> collected	
	from the six study sites. Bars represent standard deviation of the mean	
	and the different letters indicate a significant difference at $P \!> 0.05$	5
Fig. 6.2	Relative Density variation in the total fungal species isolated from	
	wastewater, soil and <i>E.sativa</i> in relation to six study sites. Bars represent	
	standard deviation of the mean and the different letters indicate a	
	significant difference at P > 0.05	6
Fig. 6.3	Relative density variation between the fungal species in isolated from	
	wastewater, soil and E.sativa relation to the species diversity. Bars	
	represent standard deviation of the mean and the different letters indicate	
	a significant difference at P>0.05	6
Fig. 6.4	Estimation of the residual mycotoxins (Aflatoxin, Ochratoxin and	
	zearalenone) in E. sativa plant samples collected from the six study	
	sites	6
Fig. 6.5	Effect of EM addition on the Relative density variation between the	
	isolated fungal species in relation to three study sites, in the green house	
	experiment	7
Fig. 6.6	Effect of EM addition on the species diversity of the isolated fungi in	
	green house experiment	8

Fig. 6.7 Effect of EM addition on the species diversity of the isolated fungi in	81
green house experiment	
Fig. 6.8 The residual mycotoxins accumulation in <i>E.sativa</i> irrigated with pretreated	
wastewater after addition of different EM concentrations	90
Fig. 6.9 The residual mycotoxins accumulation in <i>E.sativa</i> irrigated with primarily	
treated wastewater after addition of different EM concentrations	90
Fig. 6.10Effect of different EM concentrations on the growth (dry biomass) of	
Aspergilli isolated allover the study	93
Fig. 6.11 Effect of different EM concentrations on the growth (dry biomass) of the	
unicellular fungi isolated allover the study	94
Fig. 6.12 Effect of different EM concentrations on the growth (Dry Biomass) of	
phycomycetous fungi isolated allover the study	94
Fig. 6.13 Effect of different EM concentrations on the growth (dry biomass) of the	
deuateromycetous fungi isolated allover the study	95