



جامعة عين شمس
كلية البنات للآداب و العلوم و التربية
قسم النبات

تأثير العلاقة التكافلية بين البقوليات – الريزوبيا و الكمبوست على خصوبة التربة من خلال الدورة الزراعية

رسالة مقدمة من الطالبة

سعاد يوسف سرى (ميكروبيولوجي) ١٩٩٨
ماجستير فى العلوم (ميكروبيولوجي) ٢٠٠٧

للحصول علي درجة نكتوراه /الفلسفة فى العلوم
(ميكروبيولوجي)

قسم النبات
كلية البنات للآداب و العلوم و التربية
جامعة عين شمس



**EFFECT OF LEGUME-*RHIZOBIUM* SYMBIOSIS AND
COMPOST ON SOIL FERTILITY THROUGH CROP
ROTATION SYSTEM**

**A Thesis
Submitted to Botany Department
Women's College, Ain Shams University**

For

The Degree of Ph. D. in Science (Microbiology)

By

Soaad Yousef Serry El-Sayed

B.Sc. Microbiology, Women's College, Ain Shams Univ., 1998

M.Sc. Microbiology Women's College, Ain Shams Univ., 2007

Botany Department

Women's College, for Arts, Science and Education

Ain Shams University

2013

**EFFECT OF LEGUME-*RHIZOBIUM* SYMBIOSIS AND
COMPOST ON SOIL FERTILITY THROUGH CROP
ROTATION SYSTEM**

A Thesis

**Submitted to Botany Department
Women's College, Ain Shams University**

For

The Degree of Ph. D. in Science (Microbiology)

By

Soaad Yousef Serry El-Sayed

B.Sc. Microbiology, Women's College, Ain Shams Univ., 1998

M.Sc. Microbiology Women's College, Ain Shams Univ., 2007

Supervised by

- **Prof. Dr. Fatma Abd El-Wahab Helemish.....**
Professor of microbiology, Botany Department Women's
College for Arts, Science and Education Ain shams university
- **Prof. Dr. Atef Fathalla Mohamad Abdel wahab.....**
Senior Researcher, Department of Agricultural Microbiology
Soil, Water and Environment Institute Agricultural Research
Center (ARC)
- **Dr. Mona Mohamad Abo El Nour.....**
Doctor of Microbiology, Botany Department Women's College
for Arts, Science and Education Ain Shams University

2013

ABSTRACT

Three rhizobial strains (ARC-201,202 and 203) were investigated in an *in vitro* experiment to evaluate their efficiency to some environmental limiting factors (pH, salinity, temperature and nitrogen concentration). The growth response was evaluated using plate count technique. A plant infection technique experiment was done to evaluate the symbiotic performance of the tested rhizobial strains to nodulate two *Pisum sativum* varieties (master pea and little marvel). Result revealed that ARC-201 and 202 strains performed good results with master pea variety.

Accordingly, field experiments were conducted at Ismailia Agricultural Experiments Research Station, El-Ismailia Governorate during the two successive seasons November 2008 to February 2009 and June 2009 to September 2009 to accommodate two rotation cycles (pea/maize – potato/maize). Addition of biofertilizers (*Rhizobium* and plant growth promoting rhizobacteria) and organic fertilizer (compost) were also investigated to study their integrated effects on improving of nutrients availability, chemical and biological activity of the tested sandy soil.

The two rotation cycles were established to investigate the effect of inclusion legume crops (pea) in the rotation compared to heavy feeder crops (potato), on plant growth and yield of the subsequent crop (maize), as well as soil organic matter level, microbial biomass and soil enzymes and microbial activities of the soil. Results demonstrated that the pea/corn rotation has been

shown to improve the investigated growth parameters, yield response and NPK accumulation significantly relative to potato/corn rotation. Significant soil fertility improvement was also observed. Balanced fertilization using both organic and chemical fertilizers in such legume rotation system was reported to reduce fertilizer N requirement.

Maximum values of all the investigated parameters were obtained when 120 and 90 kg N/ha were applied in combination with the enriched compost in the pea/corn rotation. At the end of the experiments the results also showed a significant improvement in chemical properties (organic C, total N and available P and K) and biological properties (total microbial count, CO₂ evolution and dehydrogenases activity) of the soil.

Key words: Compost, PGPR, *Rhizobium*, pea productivity and legume crop rotation, inorganic fertilizer, maize productivity, strain selection.

Dedication

*After God almighty, there are several people to whom I owe a great deal
of gratitude and thanks*

My dearest parents

Without them this work would never have been started.

My beloved husband

Who patiently supported this work

My daughters and son

For them this work has been done

My sisters and brother

Without them this work would never have been done

My closely friend

Dr. Hend M. El-Egami

ACKNOWLEDGEMENT

This is the time for a last and personal words .This is the time to say ,

Thanks for all of you

*I wish to express my sincere thanks and appreciation to my **Prof. Dr. Fatma Abdel Wahab Helemish** Doctor of Microbiology, Faculty of Women, Ain Shams University for her supervision, positive support during the study and guidance through the course of study and revision the manuscript of this thesis.*

*I am particularly indebted to my dearest **Dr.Mona Mohammad Abou ELNour**. Lecturer of Microbiology, Faculty of Women, Ain Shams University for her countless fruitful discussions and for giving me every possible help through the different stages of this work .special thanks are also due to her carefully reviewing the manuscript and providing invaluable comments and suggestion for its improvement .*

*I would like to express my worm thanks to **Prof. Dr. Atef Fathalla Mohamed Abdel-Wahab** Head of Researcher, Dep. Of Agric. Microbiology, Soils Water and Environ. Res. Institute, ARC, Giza. For his supervision, guidance, continual assistance and for supplying all facilities, help and providing the required need for this work and revision the manuscript of this thesis.*

*Grateful appreciation is also extended to **all staff** members of Unit Biofertilizers, Microbiology Department, Soil, Water and Environment Institute, Agriculture Research Center (ARC).*

Thanks are also to stuff members of Botany Department ,Faculty of Women, Ain Shams University.

CONTENTS

1. Introduction	1
2. Review of literature	7
2.1. Importance of legume inoculation	7
2.2. Symbiotic nitrogen fixation as renewable source of soil improvement.....	11
2.3. The key role of legumes in crop rotation system.....	15
2.3.1. Soil fertility and nutrients availability	15
2.3.2. Growth and yield (productivity) improvement.....	19
2.4. Beneficial effects of crop rotations system.....	25
2.5. The possible role of fertilizers on crop growth and soil fertility improvement.	28
2.5.1. Combined use of different fertilizers.	29
2.6 Soil enzyme activity as bio-indicator of soil fertility.....	39
3. Materials and Methods	44
3.1. Materials.....	44
3.1.1 Bacteria used.....	44
A- Rhizobial strains.....	44
B- Plant Growth Promoting Rhizobacteria (PGPR)	44
3.1.2. Media used	44
3.1.3. Seeds.....	46
3.1.4 Experimental site.....	47
3.1.5 Compost used.	47
3.2. Methods.....	50
3.2.1. Maintenance of stock cultures.....	50
3.2.2. Inoculants preparation.....	50
3.2.3. Assessment of growth and survival of the rhizobial strains under some limiting factors.....	50
3.2.3.1. Salt stress.....	51
3.2.3.2. Temperature	51
3.2.3.3. pH-value	51
3.2.3.4 Nitrogen concentration.....	52
3.2.4. Crop rotation experiment.....	52
3.2.4.1. Experimental design.....	53
3.2.4.2 The first phase of rotation cycle (<i>Pisum sativum</i>)	53
3.2.4.3. The second phase of the rotation cycle (<i>Zea maize</i>)...	54

3.3. Soil analysis	56
3.3.1. Soil chemical determinations	56
3.3.2. Evaluation of soil biological activity.....	57
3.4 Plant measurements	58
3.5. Statistical analyses	59
4. Results	60
4.1 <i>In vitro</i> growth of <i>Rhizobium leguminosarum</i> strains under some environmental stress conditions	60
4.2. Pea plants responses to inoculation in a pot experiment	63
4.3. The field experiments	66
4.3.1. The first phase of the rotation cycle	66
4.3.1.1. Response of <i>Pisum sativum</i> (master pea) to bio, organic, and reduced N-fertilizer.....	66
4.3.2. The second phase of the rotation cycle.	68
4.3.2.1. Growth aspects of maize plants and some macronutrients accumulation.....	69
4.3.2.2. Yield and its attributes of maize plants and some macronutrients accumulation.....	83
4.4 Chemical and biological properties assay of soil corn rhizosphere after harvesting.....	95
5. Discussion	102
5.1. Effect of different environmental stress conditions on <i>in vitro</i> growth of rhizobial strains.....	102
5.2. <i>Rhizobium</i> - legume compatibility.....	103
5.3. First phase of the rotation	104
5.3.1. Improvement of leguminous cultivation by integrated use of bio, organic and mineral fertilization.	104
5.4. The second phase of the rotation.	112
5.4.1. Growth response of maize to integrated nutrient management in different cropping systems.....	112
5.4.2. Yield response of maize to integrated nutrient management in different cropping systems.	121
5.4.3. Maize shoot, root and grain mineral accumulation	127
5.5. Impact of rotation program on soil chemical and biological properties amended with integrated nutrients management.....	133
6. Summary	146

7.	Conclusion	153
8.	Recommendation	155
9.	References	157

LIST OF TABLES

Table 1: Physical, chemical and microbiological analysis of the used soil.....	48
Table 2. Some physical, chemical and microbiological properties of the prepared compost.	49
Table 3. Effect of some environmental stresses on growth and survival of the tested <i>Rhizobium leguminosurum</i> strains... ..	61
Table 4. Response of pea varieties to the tested rhizobial strains (Plant infection technique).....	63
Table 5. Effect of co-inoculation, reduced nitrogen and compost application on growth, nodulation and yield of pea plants (60days)	68
Table 6a. Main effect of N-fertilizer levels, compost manuring and rotation systems on vegetative growth of maize after 70 days.	72
Table 7a. Main effect of N-fertilizer levels, compost manuring and rotation systems on nutrient status of maize after 70 days	73
Table 6b. Effect of compost manuring and N-fertilization on vegetative growth of maize plants grown in sandy soil previously cultivated with pea or potato after 70 days of planting.....	76
Table 7b. Effect of compost manuring and N-fertilization on nutrient status of maize plants grown in sandy soil previously cultivated with pea or potato after 70 days of planting.....	80
Table 8a. Main effect of compost manuring and N-fertilization on the yield and its attributes of maize plants grown in sandy soil previously cultivated with pea or potato...	83

Table 9a. Main effect of compost manuring and N-fertilization on some macronutrients of maize yield grown in sandy soil previously cultivated with pea or potato.....	84
Table 8b. Effect of compost manuring and N-fertilization on yield and its attribute of maize plants grown in sandy soil previously cultivated with pea or potato.....	87
Table 9b. Effect of compost manuring and N-fertilization on macronutrients of maize yield grown in sandy soil previously cultivated with pea or potato.....	92
Table 10. Some chemical and biological characteristic, soil before and after pea and potato harveste.....	100
Table 11. Effect of compost manuring and N-Fertilization on chemical and biological characteristic, soil after maize harvest.	101

LIST OF FIGURE

Figure 1. Effect of some environmental stresses on growth and survival of the tested <i>Rhizobium leguminosurum</i> strains	62
Figure 2a. Effect of rhizobial inoculation on the vegetative growth of master pea and little marvel cultivars	64
Figure 2b. Effect of rhizobial inoculation on the vegetative growth of master pea and little marvel cultivars	65
Figure 3a. Effect of combined application of organic-mineral fertilizer on vegetative growth of maize plants under crop rotation system.	77
Figure 3b. Effect of combined application of organic-mineral fertilizer on vegetative growth of maize plants under crop rotation system.....	78
Figure 4a. Effect of combined application of organic-mineral fertilizer on nutrient status of maize plants under crop rotation system.	81
Figure 4b. Effect of combined application of organic-mineral fertilizer on nutrient status of maize plants under crop rotation system.	82
Figure 5a. Effect of combined application of organic-mineral fertilizer on yield and its components of maize plants under crop rotation system.....	88
Figure 5b. Effect of combined application of organic-mineral fertilizer on yield and its components of maize plants under crop rotation.....	89
Figure 6a. Effect of combined application of organic-mineral fertilizer on macronutrients of maize yield under crop rotation system.	93
Figure 6b. Effect of combined application of organic-mineral fertilizer on macronutrients of maize yield under crop rotation system.	94

LIST OF ABBREVIATIONS

ATPase	Adenosine Triphosphatase
BNF	Biological Nitrogen Fixation
CFU	Colony Forming Units
FYM	Farm Yard Manure
IAA	Indole Acetic Acid
NA	Nutrient Agar
OM	Organic Matter
PGPB	Plant Growth Promoting Bacteria
PGPR	Plant Growth Promoting Rhizobacteria
Tg	Teragram
TPF	Triphenyl formazane
TTC	Tetrazolium Chloride
YEM	Yeast Extract Mannitol