



بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة / سامية زكى يوسف

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد



**ROLE OF ECTOMYCORRHIZAE IN IMPROVING  
THE GROWTH OF WOODEN TREES UNDER  
ENVIRONMENTAL STRESS  
CONDITIONS**

By

**AYA GABER ALI AHMED**

B.Sc. Agric. Sci. (General Agricultural Production), Fac. of Agric., Sohag Univ., 2015

**A Thesis Submitted in Partial Fulfillment  
Of  
The Requirement for the Degree of**

**MASTER OF SCIENCE  
in  
Agricultural Sciences  
(Agricultural Microbiology)**

**Department of Agric. Microbiology  
Faculty of Agriculture  
Ain Shams University**

**2022**



**Approval Sheet**

**ROLE OF ECTOMYCORRHIZAE IN IMPROVING  
THE GROWTH OF WOODEN TREES UNDER  
ENVIRONMENTAL STRESS  
CONDITIONS**

By

**AYA GABER ALI AHMED**

B.Sc. Agric. Sci. (General Agricultural Production), Fac. of Agric., Sohag Univ., 2015

**This thesis for M.Sc. degree has been approved by :**

**Dr. Mohamed Fayez Foaad** .....

Prof. Emeritus of Agric. Microbiology, Faculty of Agriculture, Cairo University.

**Dr. Enas Abd El-Tawab Hassan** .....

Prof. of Agric. Microbiology, Faculty of Agriculture, Ain Shams University.

**Dr. Mona Mohammed Saied Zayed** .....

Prof. of Agric. Microbiology, Faculty of Agriculture, Ain Shams University.

**Date of examination:**    /    /2022



# **ROLE OF ECTOMYCORRHIZAE IN IMPROVING THE GROWTH OF WOODEN TREES UNDER ENVIRONMENTAL STRESS CONDITIONS**

By

**AYA GABER ALI AHMED**

B.Sc. Agric. Sci. (General Agricultural Production), Fac. of Agric., Sohag Univ., 2015

**Under the supervision of:**

**Dr. Shawky Mahmoud Selim**

Prof. Emeritus of Agric. Microbiology, Department of Microbiology,  
Faculty of Agriculture, Ain Shams University (Principal Supervisor)  
(late).

**Dr. Mona Mohammed Saied Zayed**

Prof. of Agric. Microbiology, Department of Microbiology, Faculty of  
Agriculture, Ain Shams University.

**Dr. Dalia Ahmed Abd El-Fattah**

Researcher of Agric. Microbiology, Biological Agricultural  
Department, Central Laboratory for Agricultural Climate, Agricultural  
Research Centre.



## ABSTRACT

**Aya Gaber Ali Ahmed "Role of Ectomycorrhizae in Improving the Growth of Wooden Trees Under Environmental Stress Conditions". Unpublished M.Sc. Thesis, University of Ain Shams, Faculty of Agriculture, Department of Microbiology, 2022.**

Twenty-three sporocarps (ECM fungal-like) grown near their host trees were obtained from various locations in Egypt. All Isolates were purified according to their morphological characteristics. Only seven isolates were purified isolates (D4, K10, M11, P13, S16, T17, and Pt23) were evaluated for ectomycorrhizal formation with *Eucalyptus* sp., *Azadirachta* sp., and *Pinus* sp. The isolates were identified using 18S rRNA and only three of them (K10, M11, and Pt23) were found to be *Suillus collinitus*, *Protuberata* sp., and *Pisolithus tinctorius*, respectively. A comparative evaluation was performed on the effects of some environmental parameters such as type of carbon and nitrogen sources, pH, salinity, and incubation temperature on the mycelial growth of the strains. The growth patterns of the strains showed the maximum growth weight after 24–33 days. Moreover, the effect of different carbon and nitrogen sources revealed the maximum growth weight with glucose, soluble starch, diammonium phosphate, and asparagine. In addition, different pH levels in the form of dry weight and the strains' growth effects on the medium pH at the end of the incubation period revealed the optimum levels ranging from pH 5 to 7 for the tested strains, which were also able to change the used medium pH. The tested strains showed tolerance to a range of 0.5%–4% NaCl, and the optimum temperature was proven to be 20°C–30°C. Finally, two greenhouse experiments were conducted to estimate the effect of ECM strains on the growth performance of *Eucalyptus globulus* and *Eucalyptus citriodora* under salinity stress conditions (6, 8, 10 dS/m) using two types of soils (sand & clay soil).

**Keywords:** Ectomycorrhizae, *Suillus collinitus*, *Protuberata* sp., *Pisolithus tinctorius* and Environmental stresses.





## ACKNOWLEDGMENT

There are few opportunities in most people's lives to demonstrate formally one's gratitude to people who have been mentors and supporters at different steps of our lives. Even though, we do not forget to stamp those feeling on paper.

I would like to express my deep indention to my supervisor (late) **Prof. Dr. Shawky Mahmoud Selim** (May Allah have mercy upon him), Professor Emeritus of Agric. Microbiology, Faculty of Agriculture., Ain Shams University, for his parental, scientific support, suggesting the problem and drawing the plan, valuable advice, expert guidance, and keen interest throughout this study.

I am highly grateful to **Prof. Dr. Mona Mohammed Saied Zayed**, Professor of Agric. Microbiology, Faculty of Agriculture., Ain Shams University, for her direct supervision, suggesting the problem and drawing the plan of work as well as her continuous advice and criticism during all the study period, as well as writing up the manuscript.

Special thanks are extended to **Dr. Dalia Ahmed Abd El-Fattah**, Researcher of Agric. Microbiology, Biological Agricultural Department, Central Laboratory for Agricultural Climate, Agricultural Research Centre for beneficial discussion and faithful help throughout this work as well as writing up the manuscript.

Sincere thanks are also extended to all my **Colleagues** and staff members of the **Microbial Inoculants Center**, Fac. Agric., Ain Shams Univ. for providing facilities and encouragement.

Sincere thanks to **Dr. Ola Hussein Abd Elbar** for her sincere help in sectioning and staining *Pinus* sp. root.

Finally, I have deep gratitude to all my family especially my parents for their continuous help and encouragement through this work.



## CONTENTS

	Page
<b>1. INTRODUCTION</b>	1
<b>2. REVIEW OF LITERATURE</b>	3
2.1. Classification of ectomycorrhizal fungi	3
2.2. Ectomycorrhizal association	3
2.3. Ectomycorrhizal host	4
2.4. Features of ECM roots	5
2.4.1. Mantle	5
2.4.2. Hartig net	5
2.4.3. The extraradical mycelium	6
2.5. Growth of ectomycorrhizal fungi <i>in-vitro</i>	6
2.5.1. Isolation of ectomycorrhizal fungi	6
2.5.2. Maintenance of ECM fungi	6
2.5.3. The nutrient requirements for ectomycorrhizal fungi	8
2.5.3.1. Carbon source	8
2.5.3.2. Nitrogen sources	9
2.5.3.2.1. Inorganic nitrogen sources	9
2.5.3.2.2. Organic nitrogen sources	9
2.5.3.3. Effect of pH on the fungal growth and mass	10
2.5.4. Identification of ECM fungi	11
2.5.5. Inoculation of plants with ectomycorrhizae	11
2.5.5.1. Inoculation by soil	11
2.5.5.2. Inoculation by fungal spores	12
2.5.5.3. Inoculation by vegetative mycelium	12
2.5.5.4. The selection of ectomycorrhizal inoculant	13
2.6. Formation ectomycorrhizal symbiosis	13
2.6.1. Precolonization	13
2.6.2. Development of symbiotic structure	15
2.6.3. Factors affecting ectomycorrhizal morphology	15
2.6.3.1. Tree genera	16

## II

2.6.3.2.Fungal type	16
2.7. The mutual beneficial effects between ectomycorrhizal fungi and their host plant	16
2.8. Mechanisms of nutrients exchanges between ECM fungi and their host plants	17
2.8.1. Movement of carbohydrates from plant to mycosymbionts	17
2.8.2. Nutrients uptake by mycosymbionts	18
2.8.3. Water uptake by mycosymbionts	20
2.9. Role of ECM fungi in reducing the effects of biotic and abiotic stresses on plants	21
2.9.1. Abiotic stresses	21
2.9.1.1.Drought stress	21
2.9.1.2.Salt stress	22
2.9.1.3.The soil pH	23
2.9.1.4.Heavy metals	24
2.9.1.5.Climate change	25
2.9.1.5.1. Atmospheric CO <sub>2</sub> enrichment	25
2.9.1.5.2. Temperature	26
2.9.2. Biotic stresses	27
2.10. The beneficial effect of ectomycorrhizal symbiosis on soil	28
2.10.1. Change the chemistry of the mycorrhizosphere	28
2.10.2. Increase physical access <i>via</i> extra metrical hyphae	29
2.10.3. Influence on rhizospheric bacterial population	29
2.11. Ectomycorrhizal helper bacteria	29
<b>3. MATERIALS AND METHODS</b>	32
3.1. Material	32
3.1.1. Sources of isolates	32
3.1.2. Plants used	32
3.1.3. Soil used	32
3.1.4. Media and solution used	32

### III

3.2. Methods	35
3.2.1. Sporocarps characterization	35
3.2.2. Isolation of ectomycorrhizal fungi from the sporocarps	35
3.2.3. Making spore prints	35
3.2.4. Formation of ectomycorrhizal associations in non-aseptic condition	36
3.2.4.1. Determination of the mycorrhizal colonization using a light microscope	37
3.2.4.2. Scanning electron microscope of ectomycorrhizal roots	37
3.2.5. Ectomycorrhizal synthesis in sterile culture	38
3.2.5.1. Semithin sections of ectomycorrhizal root	38
3.2.6. Molecular identification of ectomycorrhizal isolates	39
3.2.6.1. DNA extraction	39
3.2.6.2. Selection of specific couples of primers	39
3.2.6.3. PCR amplification conditions	39
3.2.7. Assessment of some ectomycorrhizal physiological activities	40
3.2.7.1. Growth pattern of ectomycorrhizal strains	40
3.2.7.2. Influence of different carbon sources on mycelium growth	40
3.2.7.3. Influence of different nitrogen sources on mycelium growth	41
3.2.7.4. Influence of different pH levels on mycelium growth	41
3.2.7.5. Influence of different temperatures on mycelium growth	41
3.2.7.6. Influence of different salinity stress levels on mycelium growth	42
3.2.8. Pot experiment	42
3.2.8.1. Plant growth parameters	42
3.2.8.2. Biochemical analysis	43
3.2.8.2.1. NPK and Na contents	43

3.2.8.2.2. Determination of chlorophylls and carotenoids	43
3.2.8.2.3. Determination of proline	44
3.2.8.2.4. Determination of soil EC and pH	44
3.2.9. Statistical analysis	44
<b>4. RESULTS AND DISCUSSIONS</b>	45
4.1. Morphological and microscopic characteristics of collected sporocarps	45
4.2. Formation of ectomycorrhizal associations in non-aseptic conditions	50
4.3. Aseptic synthesis of ectomycorrhizae ( <i>In vitro</i> mycorrhization)	56
4.4. Molecular identification of the ectomycorrhizal fungi	60
4.5. Growth pattern ectomycorrhizal strains	63
4.6. Effect of different carbon sources on mycelial growth	63
4.7. Effect of different nitrogen sources on mycelial growth	65
4.8. Effect of different pH level on mycelial growth	67
4.9. Effect of different temperatures on mycelial growth	70
4.10. Effect of different salinity stress levels on mycelial growth	71
4.11. Pot experiment	73
4.11.1. The growth performance, chlorophylls, carotenoids, proline, NPK, and Na of <i>Eucalyptus globulus</i> cultivated in salty soil as affected by irrigation with salted water and ECM strains	73
4.11.1.1. pH and EC of sandy soil cultivated by <i>Eucalyptus globulus</i>	77
4.11.2. Growth performance, chlorophylls, carotenoids, proline, NPK, and Na of <i>Eucalyptus citriodora</i> cultivated on clay soil as affected by salted irrigation water and ECM strains.	81
4.11.2.1. pH and EC (dS/m) of clay soil cultivated by <i>Eucalyptus citriodora</i>	84