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Evaluation the Effect of Ectomycorrhizal Fungi on Prunus cerasifera x salicina Growth Compared with Chemical and Organic Fertilizer

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Student's name: عبدالله حسين شاهين أبو عجوة اسم الطالب:

Signature التوقيع:

Date: 2015 -5 -24 التاريخ:

The Islamic University – Gaza
Deanery of Higher Education
Faculty of Science
Department of Biological Sciences & Biotecnology

الجامعـــــة الإسلاميــة- بغـزة
عمـــادة الدراســات العليــا
كليــــــــة العلـــــــوم
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Evaluation the Effect of Ectomycorrhizal Fungi on *Prunus cerasifera x salicina* Growth Compared with Chemical and Organic Fertilizer

Prepared by

AbedAllah Hussein Abu Ajwa

B. Sc. in Biology

Supervisors

Dr. Abboud El Kichaoui	**Dr. Tarek Elbashiti**
Assoc. Prof. in Botany & Mycology	**Assoc. Prof. of Biotecnology**

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ **عبدالله حسين شاهين أبو عجوة** لنيل درجة الماجستير في **كلية** ***العلوم*** قسم **<u>العلوم الحياتية –نبات وفطريات</u>** وموضوعها:

فحص تأثير الفطريات المتكافلة خارجياً على نمو نبات السنطروزه المركبة على الموربيلان مقارنة مع السماد الكيميائي والكمبوست

Evaluation the effect of Ectomycorrhizal fungi on *Prunus cerasifera x salicina* growth compared with Chemical and organic fertilizer

وبعد المناقشة التي تمت اليوم الاثنين 29 رجب 1436هـ، الموافق 2015/05/18م الساعة الحادية عشرة صباحاً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق ،،،

مساعد نائب الرئيس للبحث العلمي و للدراسات العليا

أ.د. فؤاد علي العاجز

بسم الله الرحمن الرحيم

قال تعالى: ﴿هُوَ الَّذِيَ أَنزَلَ مِنَ السَّمَاء مَاء فَأَخْرَجْنَا بِهِ نَبَاتَ
كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ النَّخْلِ
مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ
مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انظُرُواْ إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي
ذَلِكُمْ لآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ﴾ سورة الأنعام آية ﴿99﴾

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I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgment has been made in the text.

Signature	Name	Date
Abdullah	***Abdullah H Abu Ajwa***	***April 2015***

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DEDICATION

To my parents who are always

supporting me

To my wife who helped me to accomplish

this thesis

To my daughters and sons

To my brothers and sisters

To my university IUG

To all of them I dedicate this work

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A thesis is the result of a number of years of work. In the process, one meets many people that, in one way or another, influence your life at that time. These acknowledgments try to recognize most of them.

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Evaluation the Effect of Ectomycorrhizal Fungi on *Prunus cerasifera x salicina* Growth Compared with Chemical Fertilizer and Compost

Abstract

Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The use of biofertilizer is steadily increased in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. The main objective of this study is to evaluate the effect of local Ectomycorrhizal fungi isolated from the roots of some plants, on growth of *Prunus cerasifera x salicina* under greenhouse conditions. The impact of symbiotic fungus on the plant growth was measured by comparing the inoculated plants, with control plants and plants treated with chemical fertilizer and compost. The fungus isolated from *Prunus cerasifera* (myrobalan) roots in PDA media, and obtaining pure cultures.

50 plants were grown 10 for each parameter as follow:

- "10" seedlings planted in sterile soil without fungus and fertilizer (control).
- "10" seedlings planted in sterile soil was fertilized fungus isolated
- "10" seedlings planted in sterile soil was fertilized chemical fertilizers without fungus.
- "10" seedlings planted in sterile soil 50% and 50% compost.
- "10" seedlings planted in 100% compost.

Our results show a positive influence of the Ectomycorrhizal fungi on the growth of *Prunus cerasifera x salicina* seedling compared with control, chemical fertilizer and compost, in all growth parameters Number of Leaf (NL), Number of branch (NB), Stem Length (SL), Root Length (RL), Wet weight of Stem (WWS), Wet weight of Root (WWR), Dry weight of Stem (DWS), Dry weight of Root (DWR) and Root Length (RL), after incubated in the green house for four months. We conclude that the use of Ectomycorrhizal fungi gives positive influence on the growth of plant. According to these results we strongly recommend the use of symbiotic fungi as total or partial substitute of other fertilizer.

Key words: **Ectomycorrhization, Compost, chemical fertilizer, *Prunus cerasifera x salicina*.**

فحص تأثير الفطريات المتكافلة خارجيا على نمو نبات السنطروزه المركبة على الموربيلان مقارنة مع السماد الكيميائي والكمبوست

المستخلص

يعتبر التسميد الحيوي عنصر هام من عناصر تقليل الضرر الناتج عن استخدام الأسمدة الكيميائية، ويساعد علي تقليل التكلفة والطاقة المستخدمة في انتاجها. و الهدف الأساسي من هذه الدراسة فحص تأثير أحد الفطريات المتكافلة خارجيا على نمو نبات السنطروزه المركبة على الموربيلان ، وذلك بمقارنة نمو النباتات الملقحة بالفطر مع نباتات زرعت بدون فطر وبدون اسمدة " الضابط "، ونباتات سمدت بالأسمدة الكيميائية وأخرى بالأسمدة العضوية (الكمبوست). و قد تم استخراج الفطر و عزله من جذور أشجار الموربيلان في وسط غذائي مناسب وتم زراعة 50 شتلة موزعة كالآتي:

- 10 شتلات زرعت في تربة معقمة بدون فطر وبدون اسمدة (الضابط).
- 10 شتلات زرعت في تربة معقمة تم تلقيحها بالفطر المعزول.
- 10 شتلات زرعت في تربة معقمة بدون فطر وتم تسميدها بالأسمدة الكيميائية.
- 10 شتلات زرعت في 50% تربة معقمة و 50% كمبوست.
- 10 شتلات زرعت في 100%كمبوست.

أظهرت النتائج تأثير ايجابي للفطر المعزول على نمو نبات السنطروزه المركبة على الموربيلان بالمقارنة مع النباتات الأخرى في جميع القياسات (طول الساق، طول الجذر، الوزن الجاف للجذر، الوزن الرطب للساق، الوزن الرطب للجذر، عدد التفرعات و عدد الأوراق) وذلك بعد أربعة أشهر من تاريخ الزراعة داخل الدفيئة الزراعية. نستطيع الاستخلاص هنا أن استخدام الفطر التكافلي الخارجي يعطي تأثيرا ايجابيا على نمو نبات السنطروزه المركبة على الموربيلان. ومن خلال هذه النتائج يمكننا أن ندعم و بشكل قوي استخدام هذه الفطريات كبديل كلي أو جزئي للأسمدة الاخرى .

الكلمات المفتاحية : الفطريات المتكافلة خارجيا، كومبوست، سماد كيميائي، السنطروزه المركبة على الموربيلان.

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List of abbreviation

Arbuscular Mycorrhizae	**AM**
Arubuscular Mycorrhizal Fungi	**AMF**
Branch Number	**BN**
Control	**C**
Chemical Fertilizer	**CHF**
100% compost	**COM 100**
50% compost	**COM 50**
Ectomycorrhizae	**ECM**
Extraradical Mycelium	**ERM**
Ectomycorrhizal Plants	**SECM**
Intraradical Mycelium	**IRM**
Leaf Number	**LN**
Potato Dextrose Agar	**PDA**
Root Dry Weight	**RDW**
Root Length	**RL**
Root Wet Weight	**RWW**
Stem Dry Weight	**SDW**
Stem Length	**SL**
Stem Weight	**SW**
Vesicular Arbuscular Mycorrhizae	**VAM**

Chapter 1

Introduction

Mycorrhization is a mutualistic association (non-pathogenic association) between soil borne fungi and the root of the higher plants. The term mycorrhiza *(Gr. Mykes=Fungus or Mushroom; Rhiza=Root)* i.e. *“Fungal Root”* was coined by the German Pathologist (Frank, 1885) to describe the union of two different beings to form a single morphological organ in which the plant nourishes the fungus and fungus does the same for the plant. Mycorrhiza confers many attributes to plants such as growth stimulation due to increased nutrient uptake, tolerance of plants to adverse conditions and bio-control of root disease (Molina *et al.,* 1992). Between the seven types of mycorrhizae described (arbuscular, ecto, ectendo-, arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae), arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread in forest communities (Smith and Read, 1997; Allen *et al.*, 2003). Arbuscular mycorrhizal (AM) fungi are the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants (Brundrett, 2002). AM fungi are obligate mutualists belonging to the phylum Glomero-mycota and have a ubiquitous distribution in global ecosystems (Redecker *et al.*, 2000). Ectomycorrhizal (ECM) fungi are a more recently evolved association (approximately 125 million years ago) and despite their widespread distribution, associate with only 3% of vascular plant families (Smith and Read, 1997). Almost all ECM fungi belong to the Ascomycota and Basidiomycota phyla and the ECM mutualism is thought to have been derived several times independently from saprophytic lineages

(Hibbett *et al.*, 2000) The mycorrhizal symbiosis between plant roots and fungus is essential for the survival of both the partners (Harley and Smith, 1983). It has been suggested that this symbiosis was a prerequisite for the successful colonization of the terrestrial environment by plants some 400 million years ago (Pyronzinski and Mallock, 1975). The term mycorrhiza describes a range of symbiotic structures formed between the fine root and different fungi.

Being highly specialized in their nutritional requirement, the mycorrhizal fungi obtain simple sugars, amino acids and plant growth substances from the host for growth and development. Mycorrhizae also benefit the host directly by influencing important ecosystem properties such as soil structure (Finley and Söderström, 1992). The biological requirement of many forest tree species in respect of the ectomycorrhizal association was initially observed when attempts to establish plantation of exotic pines routinely failed, and this could only 36be overcome by the introduction of symbiotic fungal associates (Gibson, 1963; Madhu, 1967). Thus mycorrhizae should be regarded as an integral part of the natural and normally functioning root system of plants, taking part in symbiosis and function as dynamic biological linkages.

1.2 Study Objectives

1.2.1 General objective

Evaluation the effect of local Ectomycorrhizal fungi on growth of *Prunus cerasifera x salicina* in Gaza Strip.

1.2.2 Specific objectives

- Isolation, Identification and multiplication of ECM from different plant roots.
- In *vitro* (green house) application of symbiosis between plant and the isolated fungi.
- To compare between the mycorrhized plant with chemical and compost fertilizered plants.

1.3 Significance

1) Most of scientific agricultural institutions encourage the use of mycorrizal fungi and mycorrhization process as fertilizer and the importance of this research is the utilization of local fungi; isolated from the same agricultural environment used in Gaza Strip.
2) On the other hand, Gaza is a limited area and the height agriculture level obligates us to find an alternative solution in order to decrease the underground water pollution by the heavy use of chemical fertilizers.
3) Ecologically, mycorrizal symbiosis on this side reduces the use of chemical fertilizers, and this affects the environment positively.
4) On the healthily side, the use of mycorrizal fungal has great effects because it limits the health hazards resulted from the chemical fertilizers. These hazards form a bad effect on the spraying chemical fertilizer workers, underground water and human health.
5) Economically, the use of mycorrizal fungi has its value because it costs much less than chemical fertilizers.
6) Because of the Israelian siege, obtaining any kind of agriculture fertilizers is very complicated and difficult.

Chapter 2

Literature Review

2.1 Fungi

Fungi are one of three major clades of eukaryotic life that independently evolved multicellular organization. They have radiated into a large variety of terrestrial and aquatic niches, employing strategies ranging from symbiotic to saprobic to pathogenic, and are remarkable for their developmental diversity and ecological ubiquity, with the number of species estimated to exceed one million. The fungi are highly varied in their mode of growth, ranging from unicellular yeasts to multicellular hyphal forms that produce complex fruiting bodies (Hawksworth *et al.,* 1995). Hyphae grow through polarized tip extension of a tubular cell (hypha), which can be partitioned by the formation of cross-walls called septa. Phylogenetic analysis reveals four major groups of fungi: the early-diverging Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota (Berbee and Taylor, 2001; Lutzoni *et al.,* 2004), which are sister clades that evolved more recently and contain the majority of fungal species (Hawksworth *et al*., 1995). Hyphae are the predominant mode of vegetative cellular organization in the fungi and groups of fungi can be defined based on consistent differences in hyphal structure.

2.2 Mycorrhizal Fungi

The two groups are differentiated by the fact that the hyphae of ectomycorrhizal fungi do not penetrate individual cells within the root, while the hyphae of endomycorrhizal fungi penetrate the cell wall and invaginate the cell membrane (Allen, 1991).

2.2.1 Types of Mycorrhizae

In early days, mycorrhizae were classified into two main groups, based upon the structure of the root fungus association, namely endomycorrhizae and ectomycorrhizae (Payronel *et al.,* 1969); which were subsequently renamed as endotrophic for endomycorrhizae and ectotrophic for ectomycorrhizae. The latest classification of mycorrhizae has seven groups, namely *Vesicular-Arbuscular Mycorrhizae*, *Ectomycorrhizae*, *Ectendomycorrhizae, Arbutoid mycorrhizae, Monotropoid mycorrhizae, Ericoid mycorrhizae* and *Orchid mycorrhizae* (Harley and Smith, 1983).

2.2.1.1 Vesicular Arbuscular Mycorrhizae (VAM)

In the first group VAM belonging to the fungal order Endogonales of the Zygomycetes with aseptate hyphae (lower fungi) has been included. In the other six groups the endophytes are fungi with septate hyphae (higher fungi) belonging to Ascomycetes or Basidiomycetes. In all these mutualistic types, the mycorrhizal association contributes significantly to the host health, in exchange for photosynthates (Barker *et al.,* 1998); It is common VAM found in the most herbaceous and graminaceous species. These fungi belong to the family *Endogonaceae*. Vesicular arbuscular mycorrhizal association generally lacks specificity. Individual species of VAM fungi can associate with diverse plants groups from herbs to long-lived woody perennials. Similarly, VAM plants often associate with many VAM fungi. (Molina, 1979).

2.2.1.6 Ectendomycorrhizae

An intermediate type of mycorrhizal association is also found on coniferous and deciduous trees in nurseries and burned forest sites. The ectendomycorrhizae type forms a typical EM structure, except the mantle is slight or missing and hyphae in the *“Hartig net”* may penetrate root cortical cells. The ectendomycorrhiza is replaced by EM as the seedling matures. The fungi involved in the association were initially designated *"E-strain"* but were later shown to be ascomycetes and placed in the genus *Wilcoxina* (Sylvia *et al.,* 2005).

2.2.1.7 Ectomycorrhizae

Among several types of mycorrhizas, ectomycorrhiza are a specific group, most of which are formed by basidiomycetes. They are particularly important for the growth of plants of silvicultural interest, including species of Eucalyptus, *Fagus*, *Quercus* and *Pinus* (Wilcox, 1990). In case of *Pinus*, the association with ectomycorrhizal fungi is absolutely necessary for the survival and growth of the plants (Smith and Read, 1997).

Some also belong to Ascomycetes. These fungi occur naturally on the plants belonging to some families of angiosperms viz. Dipterocarpaceae (e.g. *Shorea robusta*) and *Fagaceae* (e.g. *Quercus leucotrichophora* and other spp.). Most of the conifers are also associated with these fungi including all species of the family Pinaceae (e.g. *Pinus roxburghii*). ECM contributes by increasing uptake of water and nutrients, particularly those nutrients presenting low mobility in the soil, such as phosphorus (Tacon, 1987). This increased uptake is the result of a significant increase in the plant-soil interface, which can be attributed to two factors.

Firstly, the hyphae of the ectomycorrhizal fungi grow out beyond the root zone and, secondly, colonized roots are, in general, more branched than the uncolonized roots (Castellano and Molina, 1989). In this mycorrhizal symbiosis the fungus grows into the root of the host plant and the hyphae penetrate between outer cortical cells, forming a typical structure called the “Hartig net”. On the root surface the fungus forms a mantle or sheath, a structure, typical of ectomycorrhizae. This structure is typically connected to the hyphae or hyphal aggregates, which penetrate the surrounding soil and often form extensive mycelium. In this way, the mycorrhizal roots acquire access to a much greater soil volume in contrast to uninfected ones and therefore the effective surface area for nutrient absorption is greatly increased.

The ectomycorrhizal roots can be distinguished from non-mycorrhizal roots. Ectomycorrhizas can also augment hydraulic conductivity inside the plant, the resistance of the plant to drought and soil-borne pathogens and can improve soil aggregation and structure (Bogeat *et al.,* 2004). Through these different mechanisms, ectomycorrhizas promote plant growth and productivity even in low fertility or disturbed soils (Marx *et al.,* 1977).

Besides being beneficial to plants, many ectomycorrhizal fungi are an important source of food for men and forest animals, both in temperate and in tropical regions, contributing to the economy of many human communities and to the maintenance and stability of forest ecosystems. As pointed out by Smith and Read (Smith and Read, 1997), there is a great potential to exploit fruiting bodies of ectomycorrhizal fungi as commercial foods.

Although ectomycorrhizal fungi are naturally present in many soils, their ability to colonize and benefit plants is variable (Marx and Cordell, 1989) therefore it is advantageous to inoculate the seedlings with specific strains that provide benefits to the plant in question, within the specific environment that it will experience (Garbaye, 1984). This so called ’mycorrhization control’ is usually done by planting seedlings that have been previously inoculated in the nursery with the chosen fungal strain. This practice improves not only the survival of the seedlings upon transplanting but also their subsequent growth (Marx, 1980). The economic benefits of this practice, in terms of increased productivity, have been demonstrated in plantations in the United States of America and in France (Selosse *et al.,* 2000).

- Categories of ectomycorrhizas

There are two basic morphological categories of ECM: (i) associations typical of angiosperms such as *Eucalyptus*, *Betula, Populus*, *Fagus* and *Shorea* with a Hartig net confined to epidermal cells, and (ii) those of gymnosperms such as members of the *Pinaceae* where the Hartig net occupies multiple layers of cells in the cortex (Ackerley and Peterson, 1987). There are a few exceptions to this rule such as the angiosperm *Dryas integrifolia*, which has a cortical Hartig net (Melville, and Peterson, 1987). These categories result from anatomical features of the host root and the same fungus can form both types with different hosts (Brundrett, 2002). It is proposed that these be designated as ‘epidermal’ and ‘cortical’ categories of ECM to reflect these fundamental differences. Some reports indicate that cortical Hartig nets in angiosperms result from errors of examining root cross sections, where slanting epidermal cells can appear multi-layered (Massicotte *et al*., 1993).

Observations of longitudinal sections of roots or cleared whole roots provide a clearer picture of Hartig net organization than do cross sections (Brundrett *et al*., 1996). Convergent evolution of plants with

ECM results in dimorphic root systems, where short roots have limited apical growth and high branching densities (Brundrett, 2002).

Plant growth regulators supplied by the ECM fungus influence root swelling, extension and branching, and, when applied experimentally, can induce similar root morphologies in the absence of fungi (Barker and Tagu, 2000). Roots with transfer cells in the Hartig net, such as occur in *Pisonia grandis* (Ashford and Allaway, 1982) and *Alnus spp*. (Massicotte *et al*., 1987), should probably be considered a separate subcategory of epidermal ECM. There are considerable variations in the structure and function of ECM formed by one host associating with different fungi (Agerer, 1995). The degree of short root branching and the structure of the mantle and Hartig net vary because of the presence of different mycorrhizal fungi (Godbout and Fortin, 1985; Newton, 1991; Agerer, 1995).

2.3 Ectomycorrhizal interactions

There are approximately 7000 to 10000 fungal species and 8000 plant species that form ECM associations (Taylor and Alexander, 2005). The number of plant species is relatively small (approximately 3%), but the group includes plants with high global and economic importance due to the disproportionate large terrestrial land surface that these plants cover, and as main producers of timber. The plant species include wooden perennials, trees or shrubs from cool, temperate boreal or Montana forests, but also species from arctic alpine shrub communities (Smith and Read, 2008). However, most of these plant species are not exclusively colonized by ECM fungi. Many species, such as *Populus* , *Salix*, *Betula* and *Fagus* also form AM interactions, and there are indications that the AM symbiosis is the common mycorrhizal form of this taxon (Smith and Read, 2008). ECM fungi are relatively closely related to saprotrophic fungi and mainly belong to the Basidiomycota (e.g. *Amanita muscaria*, *Hebeloma cylindrosporum*, *Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius*, *Suillus bovinus*, *Xerocomus badius*), but also include some Ascomycota (e.g. *Cenococcum geophilum*, *Tuber borchii*, *Scleroderma hypogaeum*) (Smith and Read, 2008). The switch from the presumably ancestral saprotrophic to the symbiotic behavior developed convergently in several fungal families during evolution. In contrast to AM fungi, many ECM fungi can be grown in axenic culture without a host, and this has allowed screening of their ability to use different carbon or nutrient sources (Salzer *et al*., 1996). ECM fungi have a dual life style and are considered to be facultative saprotophs. In the soil they are highly competitive in nutrient acquisition and secrete a number of hydrolytic enzymes that allow them to degrade litter polymers, and to use organic nutrient sources (Finlay, 2008). At the same time they live within plant roots as symbionts and this requires a set of adaptation mechanisms to avoid plant parasitism. ECM fungi have for example lost their ability to degrade plant cell wall polysaccharides (cellulose, pectins, and pectates), and this restricts their penetration into the root to the intercellular spaces (Martin *et al*., 2009).

2.4 Colonization of the root by ectomycorrhizal fungi

Typical for ECM roots are changes in the root morphology, such as the dichotomous branching of lateral roots, e.g. in Pines, the production of a large number of root meristems and as a result an extensive root branching, the inhibition of root hair formation, and the enlargement of cortical cells. Many of these morphological effects can be observed prior to colonization and can be interpreted as a preparation of the plant to increase root symbiosis. Prior to the establishment of a functional ECM root and similar to the processes during AM development, there is an exchange of signals and cross-talk between both partners. The fungal tryptophan betaine hypaphorine has been shown to trigger reduced root hair elongation and swelling of the root hair tip and a stimulation of short root formation (Ditengou *et al*., 2000). ECM fungi also produce phytohormones, including auxins, cytokinins, abscisic acid and ethylene, and it has been shown that the changes in the root morphology are caused by an overproduction of auxin in ECM fungal hyphae and changes in the endogenous hormone levels in the roots. The effect of ECM fungi on lateral root formation is independent from the plant´s ability to form ECM associations. The ECM fungus *Laccaria bicolor* can induce lateral root formation also in *Arabidopsis thaliana*, a non-mycorrhizal plant, and the effect is correlated to an accumulation of auxin in the root apices (Felten *et al*., 2009). The auxin accumulation in the root tips and/or other fungal signals could stimulate basipetal auxin transport and lateral root primordia formation by an induction of plant genes involved in auxin transport and signaling. The fungal partner responds to root exudate components, such as rutin and zeatin, with stimulation in hyphal growth and branching and growth towards the root and an accumulation of hypaphorine (Ditengou, 2000; Martin, 2001).

2.5 Structural characteristics of ectomycorrhizal roots

An established ECM symbiosis is characterized by three structural components: the hyphal sheath or mantle, the Hartig net (in later passages of this text sometimes also referred to as intraradical mycelium), and the extraradical mycelium. The hyphal sheath or mantle encloses the root completely. The structural composition of the mantle is very diverse and can range from relatively thin, loosely arranged assemblages of hyphae to very thick, multilayered and pseudoparenchymatous mantles. The surface of the mantle can be compact and smooth or rough with numerous emerging hyphae and hyphal strands or rhizomorphs. The fungal sheath is involved in nutrient storage and controls the nutrient transfer to the host. The fungal mantle can represent a significant apoplastic barrier (Bücking *et al*., 2002). The Hartig net plays the key role in the nutrient transfer between both partners. The Hartig net is formed by hyphae that penetrate into the root cortex intercellularly. The penetration depth of the Hartig net differs between angiosperms and gymnosperms. Most angiosperms develop an epidermal Hartig net and confine the penetration of the Hartig net to the outer epidermis, which is often radially elongated. By contrast, the Hartig net in gymnosperms normally encloses several layers of cortical cells and sometimes extends up to the endodermis (Smith and Read. 2008).

The extra radical mycelium (ERM) of the fungus acts as an extension of the root system and it has been estimated that the ERM of the fungus *Pisolithus tinctorius* can represent 99% of the nutrient-absorbing surface length of pine roots (Rousseau, 1992). The ERM of ECM fungi can account for 32% of the total microbial biomass and 700-900 kg ha-1 in forest soils (Bücking *et al*., 2002). The ERM can have a relatively simple organization with individual hyphae with similar structure that grow into the soil (mainly in *ascomycetes*) or can be differentiated into singular hyphae and rhizomorphs. Rhizomorphs are aggregates of hyphae which grow in parallel and whose organization level can range from simple assemblages of undifferentiated and loosely woven hyphae to complex aggregations of hyphae with structural and functional differentiations (Agerer, 2001).

2.6 Specificity and Diversity of Ectomycorrhizae

2.6.1 Specificity

In early specificity experiments Molina and Trappe (1982) examined the specificity of 27 fungi in ECM formation with seven Pacific Northwest conifers, and indicated that the fungi varied widely to form mycorrhizae with the various conifers. These can be classified into three groups:

- Fungi with wide ECM host potential, low specificity, and sporocarps usually associated with diverse hosts in the field
- Fungi with intermediate host potential yet specific or limited in sporocarp-host associations, and
- Fungi with narrow host potential, only form ECM with a specific host species or species within a genus and likewise limited in their sporocarp association

The fruiting body assessments and long-term fungal community collections suggest a range of specificity patterns from generalist to specialist for both fungal species and vascular plants. In mixed spruce and hardwood forest communities in the northeastern United States, hardwoods and spruce shared only 8 of 54 fungal species while 19 were associated only with spruce (Bills *et al.*, 1986). In greenhouse experiments, (Molina and Trappe, 1994) examined host specificity between fungal and plant partners, and also studied the influence of neighboring plants on ECM development using seedlings of 6 coniferous trees grown in monoculture and dual culture inoculated with spore slurries of 15 species of ECM hypogeous fungi (11 *Rhizopogon* species, and each of 4 other genera). None of the fungal species had broad host range affinities. A variety of specificity responses were exhibited by the different fungal taxa, ranging from genus-restricted to intermediate host range. In dual culture, 9 of the 11 *Rhizopogon* species examined formed abundant ECM on *Pinus ponderosa*, and formed some ECM on secondary hosts such as *Abies grandis, Tsuga heterophylla, Pseudotsuga menziesii* and *Picea sitchensis*. None of the fungi tested, however, developed ECM on these secondary hosts in monoculture, which suggests potential interplant linkages and community dynamics.

2.6.2 Diversity

In a field survey of a Swedish boreal forest, between 60,000 and 1.2 million ectomycorrhizae were found in one square meter of forest soil and 95% of the root tips examined formed ectomycorrhizae (Jonsson, 1998). (Bruns, 1995) reported that 13 to 35 species exist in about 0.1 ha and the ECM fungal diversity is very high. Individual ECM fungal species were reported to possess different physiological features (Abuzinadah and Read, 1986) and functional roles to their host trees (Koide *et al*., 2007). High ECM diversity suggests that there is a potential for significant community-level effects of these associations on host plant performance. (Jonsson *et al.*, 2001) reported that biomass production of birch seedlings (*Betula pendula*) was greater when inoculated with eight ECM fungal species than with single species under low fertility conditions, but not under high fertility. (Baxter and Dighton, 2001) reported that ECM diversity per seedling was a better determinant of improved nutrient status of birch (*B. populifolia*) than species composition or colonization rates. The ECM diversity increases plant productivity and improves nutrient uptake of the host plant to a greater degree under nutrient limiting conditions.

(Baxter and Dighton, 2005) examined the effect of ECM diversity on *Pinus rigida* in unsterilised field soils. After one growing season, growth and nutrient uptake of *Pinus rigida* seedlings increased with increasing ECM diversity on tree root systems, and this effect was not considered to be due to fungal species composition. This result suggests that multiple inoculations of ECM fungi into host plants may achieve a successful outcome in afforestation efforts. In order to use this multiple inoculation successfully, further trials to select the number and composition of ECM fungal species and to develop methods of multiple inoculation of ECM species are needed.

2.8 Factors influences Ectomycorrhizal fungal

Human-induced stress factors can affect ectomycorrhiza directly through reduced growth of fine roots and decreased uptake of nutrients, and/or indirectly through foliar damage to the host plant and, consequently, a changed Carbon allocation belowground (Kraigher *et al*., 2007; Cudlin *et al*., 2007). When natural stress factors (e.g., water shortage, elevated and low temperature, and pathogens) interact, they can supplement the effects of pollutants or induce antagonistic effects. The impact of anthropogenic stress factors is most pronounced in effects on biodiversity of below-ground ECM communities and, consequently, on the sustainability, productivity, and vitality of forests (Erland and Taylor 2002; Cudlin *et al*., 2007).

2.8.1 Acid Deposition

In general, acidification did not reduce the degree of mycorrhization of tree fine roots, which is close to 100% in most of the forests studied, but the total number of ECM root tips per soil volume and the number of morphotypes were reduced (Cudlin *et al*., 2007). A moderate reduction in pH does not drastically affect the diverse types of ectomycorrhizae, since ECM communities are mostly adapted to acid soil conditions (Read, 1991).

2.8.2 Nitrogen Deposition

Increased soil N concentrations are often correlated with changes in the number of ECM community attributes, such as decreased sporocarp production, lower community diversity, and shifts in the relative abundance of ECM community members. The first documented biological effect of nitrogen deposition on the diversity of ECM fungi was mainly limited to observations made in sporocarp surveys of ECM fungi (Arnolds, 1991).

2.8.3 Metal Deposition

Some fungal species tolerate increased metal levels in soil (Erland and Taylor, 2002). Mycorrhizal fungi can accumulate metals in their sporocarps (Cocchi *et al*., 2006; Al Sayegh Petkovsˇek, 2008). Moreover, they have the capacity to accumulate metals in the external mycelium (Berthelsen *et al*., 1995).

2.8.4 Elevated CO2

Ectomycorrhizal roots respond positively to elevated CO2 (Alberton *et al*., 2005). An increase in root tip abundance under elevated CO2 is consistent with reported changes in the root: shoot ratio, especially under conditions of severe nutrient limitation (Poorter and Nagel, 2000). It is important to note, however, that such an increased carbon allocation to ECM roots does not automatically translate into an increased uptake of limiting nutrients and hence to increased forest productivity.

2.8.5 Drought

In a meta-analysis of the effects of several stress factors, the clearest effect found was a decrease in the fine root biomass during drought (Cudlin *et al.,* 2007). A relative reallocation of growth to below-ground organs at the expense of aboveground ones during mild drought has often been found, and even absolute root growth may increase during mild drought (Becker *et al*., 1987). However, when water stress continues, the usual response is a reduction in root growth (Joslin *et al*., 2000). In contrast to fine root biomass, the ECM fractional colonization did not show a reduction but a slight (insignificant) increase. This may be due to a negative effect of drought on the total number of root tips. This kind of effect was shown in by (Feil *et al*., 1988).

2.9 Importance of Ectomycorrhizae

The improvement of mycorrhizal plants' nutrition by enhancing N, P and potassium (K) uptake, among other nutrients, has been known for decades (Smith and Read, 2008). Mycorrhizal fungi have a competitive advantage in harvesting nutrients, since they do not need to compete as much for primary carbon reserves. An important feature of mycorrhizal fungi is the diameter of the fungal mycelium (approximately 15 ìm) is significantly less than the diameter of the plant roots. (Hoffland *et al.,* 2004) some ECM fungi have the ability to access organic N and P pools by nutrient mobilization from natural organic substrate (Bending and Read, 1995, 1997; Perez-Moreno and Read, 2000). ECM fungi can also obtain P by inducing dissolution of phosphate rock (Finlay and Rosling, 2005; Liu *et al.,* 2005; Rosling, 2009). The often extensive below-ground ECM extrametrical mycelium networks can connect trees, of the same or different species (Newman, 1988; He *et al.,* 2006). ECM associations are important in plant water uptake, especially in areas with low water availability (Morte *et al.,* 2001; Marjanovic *et al.,* 2005; Smith and Read, 2008). Similarly, ECM associations can confer host plant tolerance to heavy metals and salt in areas exposed to salinization (Smith and Read, 2008). Mycorrhizas can protect host plants roots against pathogens (Fitter and Garbaye, 1994; Sen, 2001) and insect herbivory (Gehring and Whitham, 2002).

2.9.1 Mineral nutrition:

2.9.1.1 Nitrogen up-take:

The ability of ECM fungi to take up inorganic nitrogen and transport nitrogen containing solutes to their host plant is well established (Chalot and Brun 1998; Chalot *et al*., 2002). Mycorrhizal roots and external hyphae have been found to have more transporters and higher N uptake rates than nonmycorrhizal roots (Javelle *et al*., 1999; Selle *et al*., 2005). More specifically, ECM fungi can help increase ammonium (NH_4) uptake. Most of the tree species present in ECM dominated ecosystems, as well as most ECM fungi, prefer NH_4 to nitrate (NO^{3-}) as N source, presenting higher NH_4 uptake rates and growth than the ones measured for NO^{3-} (Eltrop and Marschner, 1996; Anderson *et al*., 1999; Plassard *et al*., 2000). The ECM fungus *Laccaria* bicolor was found to have a widely expanded NH_4 transporter family when compared with other basidiomycetes, indicating a higher potential for its uptake (Lucic *et al*., 2008). Correspondingly, the uptake of NH_4 is generally improved in ECM plants, whereas the same has less frequently been observed for NO^{3-} (Eltrop and Marschner 1996; Plassard *et al*., 2000). The importance of the external hyphae as NH_4 - absorbing a structure in ECM roots has been demonstrated. Hyphal NH_4 acquisition was observed to contribute with 45–73% of total plant N uptake under N deficiency (Brandes *et al*., 1998; Jentschke *et al*., 2001)

2.9.1.2 Phosphorus up-take

The positive effect of mycorrhizal fungi on phosphate (P) nutrition is long known and has been attributed to:

- The exploration of large soil volumes by the ERM in which orthophosphate is scavenged and delivered to plant cortical cells, bypassing the plant pathway for P uptake (Jakobsen *et al*., 1992,)
- The small hyphal diameter that allows the fungus to penetrate into small soil cores in search for P, and higher P influx rates per surface unit (Jakobsen *et al*., 1992; Li HY *et al*., 2008)
- The capability of mycorrhizal fungi to store P in form of polyphosphates, which allows the fungus to keep the internal orthophosphate concentration relatively low, and allows an efficient transfer of P from the ERM to the IRM (Hijikata *et al*., 2010).
- The production and secretion of acid phosphatases and organic acids that facilitate the release of P from organic complexes (Ezawa *et al*., 2005). AM and ECM fungi express high affinity P transporters in the ERM that are involved in the P uptake from the soil (Maldonado-Mendoza *et al.*, 2001). The expression of these transporters is regulated in response to the externally available P concentration, and to the P demand of the fungus. Under Pi starvation the transcript levels generally increase. Interestingly, in the ERM of the ECM fungus *Hebeloma cylindrosporum* two P transporters are expressed, one transporter is up-regulated under low, and one transporter is upregulated under high P supply conditions (Tatry *et al*., 2009).

2.9.1.3 Uptake of other nutrients: Copper, Zinc, Potassium and other micronutrients

The efficiency of uptake of both Zn and Cu is increased in ECM plants. Some of the earliest work showed an increase in concentration of Cu in AM apple seedlings (Mosse, 1957) and, subsequently, similar results were obtained in such diverse species as Zea mays (Deft *et al*., 1975).

2.9.2 Uptake of Water

Hypotheses to explain mycorrhizal enhancement of root hydraulic conductivity are based on work with AM, and water use has been found to be greater for AM plants than for non-mycorrhizal plants. AM and ectomycorrhizae are different in many respects, so they may alter host plant water uptake via different mechanisms. (Coleman *et al*., 1990) examined hydraulic conductivity of Douglas fir (*Pseudotsuga menziesii*) seedlings inoculated with *Laccaria* bicolor or *Hebeloma crustuliniforme*, and non-inoculated seedlings infected naturally with *Thelephora* that were grown under three low levels of P fertilization. Seedling morphology, tissue P levels, hydraulic conductivity and plant growth substance levels in xylem sap were measured after 9 months growth. Increased tissue P and decreased root/shoot ratio correlated with increased hydraulic conductivity in each of the mycorrhizal treatments. When adjusted for the effect of these two factors, hydraulic conductivity of *Laccaria* and *Hebeloma* seedlings was still lower than that for the *Thelephora* seedlings. In a subsequent experiment the hydraulic conductivity of seedlings with *Hebeloma* and *Rhizopogon vinicolor* mycorrhizae was compared to that of non-mycorrhizal seedlings (grown at 100 mM P) and no differences were found among treatments.

2.10 Chemical Fertilizers and it's Disadvantages

The use of chemical fertilizers alone has not been helpful under intensive agriculture because it aggravates soil degradation. The degradation is brought about by loss of organic matters which consequently results in soil acidity, nutrient imbalance and low crop yields, Due to its high solubility, up to 70% of inorganic fertilizer can be lost through leaching, denitrification and erosion and reducing their effectiveness. (Ayoola, and Makinde, 2007; Alimi *et al*., 2007). Over application can result in negative effects such as leaching, pollution of water resources, destruction of microorganisms and friendly insects, crop susceptibility to disease attack, acidification or alkalization of the soil or reduction in soil fertility, thus causing irreparable damage to the overall system (Jen-Hshuan, 2006).

The problem of chemical fertilizers is a global problem, and researchers are working all over the world to find a solution to this problem as it is in the last century, when the chemical fertilizers were first introduced into the agriculture field, most of the problems faced by farmers to increase yield of their plantation have been solved. However, chemical fertilizers slowly started to show their side effect on human and environment (Bin Zakaria, 2009).

The increased use of chemicals fertilizers have a negative impact on soil quality over time, leading to the accumulation of certain compounds and salts in the soil or transfer such chemicals and salts into the groundwater, which increases the salinity. Gaza Strip is an agricultural land, has a high population density with a small space, and lack of farm land. Farmers use chemical fertilizers in agriculture which caused negative impact on some plants and the environment contributed to the deterioration of biodiversity.

In addition, because of fluctuation of rainfall in our country, the effects of chemical fertilizer may be negative in often times, lack of rainfall caused chemicals to accumulate in the soil, lead to low productivity because of the high salinity of the soil due to add fertilizer, where high rainfall caused the descent of chemicals into the groundwater. So due to the fluctuation and irregular rains fall, the use of fertilizers have many risks. It should be noted that chemical fertilizers are sometimes difficult to obtain due to the siege as they are costly and have side effects and multiple damages. Moreover the price of chemical fertilizer is expensive and sometime not available for farmers (Al- Khiat, 2006).

2.11 Plants used in study

2.11.1 *Prunus cerasifera x salicina*

A- Classification

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Tracheophyta
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Rosales
Family	*Rosaceae* – Rose family
Genus	*Prunus* L. – plum
Species'	*Prunus cerasifera x salicina*

B- Productivity in Gaza strip

Sort name	Fruit full area/d	Fruit less area/d	Productivity d/kg	Total productivity /Ton
Almond	375	360	870	642
Peach	313	435	1280	1168
Apricot	318	230	1000	318
Plum	130	88	1000	125

C- The agriculture time

There are three seasons

1- Malash agriculture / in December and January
2- Spring agriculture / in March and April
3- Seed agriculture / in December and January
(Ministry of Agriculture, 2013).

D- Irrigation

This tree doesn't need too much water but it need complementary irrigation through water net by rate 600 m^3 / donmes annually , and the water must be fresh and with less insolation than 500 ppm with giving attention to the soil sorts (Ministry of Agriculture, 2013).

E- Fertilization

Using organic fertilizers in December on the rate 5 m^3/donmes in addition to using Super phosphate in December too. Fertilization must be a year after year or annually and using fertilizers Such as 14.14.14 (P.N.K) (Ministry of Agriculture, 2013).

F- Diseases

1- **Nematodes,** it limits the plants from growing and causes death, it control by Grafting through the origin tree resistant to the diseases or by sterilization by methyl Promide gas before agriculture or by using Nemacor to the seedlings

2- **Wdery mildo**, we control it by using Oveir on of 100 cm /d.

3- **Aphids,** it activates in spring, and it control by using Marshal on rate of 2 cm / L.

4- **Gumming diseases,** it is disease catching nut ,and it control by dusting Sebrol at the begging of flower and after two weak it need another dusting on the rate of 1cm/L Gumming appears in July , august and September.

5- **Insects**, especially *Dorsphella*, it control by using Lepasiad on rate of 1 cm/L and needs fifteen days as a safety period.

6- **Leaf spot,** it causes brown spots in the leaves and it control by using Coside on rate of 4 cm/L. (Ministry of Agriculture, 2013).

2.12 Previous Studies

Garbaye in 1990 reviewed 25 studies performed in experimental field plantations in different countries, in which plants inoculated in the nursery with selected ectomycorrhizal fungi were compared with uninoculated plants that were naturally colonized by native ectomycorrhizal fungi after transplanting. In the majority of these studies, the inoculated plants showed, after transplanting from the nursery to the field, an increase of 130 % in height, 40 % in terms of volume and 25 % in survival, when compared to controls. Therefore mycorrhization control programs may be considered as an alternative to conventional nursery practices to increase plant growth and productivity.

A study by Jones *et al* 1991 measured P uptake over 90 days for *S. viminalis* rooted cuttings, with and without ECM inoculation. They found that mycorrhizal colonization of willow roots caused a two-fold increase in growth due to substantially higher P uptake. The major increase occurred over the first 50 days, suggesting that the early stages of mycorrhizal infections are particularly effective in supplying P to the plant. This was due to higher inflow rates of P. Hypotheses accounting for the higher inflow are: ECM alter the soil chemistry so more exchangeable P comes into solution, being able to use different forms of P such as organic P, increasing the volume of the soil to which the plants have access via their external hyphae.

In order to assess the influence of ectomycorrhization on the growth of Pine (*Pinus sylvestris*) and Birch (*Betula verrucosa*) seedlings, and also the effect of decayed wood material on mycorrhizal formation, 900 seedling of pine and birch were inoculated with *ECM, Paxillus* *involutus* in substrates constituted by brown rot and white rot of pine and birch. The results show a significant influence of the origin of wood-rote (broad-leaved and resinous) on the growth and mycorrhizal formation rate of both pine and birch seedling. The nature of wood-rots (white and brown) influence only the seedling of birch. In addition, the growth of mycorrhized seedlings was significantly better than those of non-mycorrhized. Also the results showed that, under aseptic conditions the mycorrhizal formation rate of pine seedling is much better than under semi-aseptic conditions. (El kichaoui *et al* 1995)

Baum *et al* (2000) used *Populus trichocarpa* cv. Weser cuttings as the host plant in a pot experiment to determine the effect of inoculation by ECM on leaf nutrient concentrations, shoot lengths, root and shoot biomass production and N accumulation in the biomass. The result obtained was positively and significantly affected by the ectomycorrhizal fungi.

Several studies have shown the potential of using ECM fungi in conifer vegetative propagation. Inoculation with specific fungi can enhance root formation and/or subsequent root branching of in vivo cuttings and in vitro adventitious shoots. Germination of somatic embryos and subsequent root growth can also be improved by the use of ECM fungi. In addition, inoculation can increase the tree’s ability to overcome the stress related to ex vitro transfer (Karoliina *et al*., 2003).

Eucalyptus dunnii seedlings inoculated with isolates three ectomycorrhizal fungi – UFSC-Sc68 (Scleroderma sp.), UFSC-Ch163 *Chondrogaster angustisporus*), and UFSC-Pt188 (*Pisolithus* *microcarpus*) – during three months had a phosphorus shoot content and a shoot dry matter higher or equivalent to these supplemented with macro- and micro-nutrients which had been grown in the same of the conditions, (Luiz Afonso, 2008).

Used *Picea mariana* (black spruce) seedlings grown in field-collected soils under controlled conditions. The soils were dominated by a different ectomycorrhizal host shrub: *Betula glandulosa*, *Arctostaphylos alpine* or *Salix herbacaea*. Within each habitat, half of the soils collected contained roots of ectomycorrhizal shrubs and the other half were free of host plants. Forest and glacial moraine soils were also included for comparison. The results indicate that ECM capable of colonizing black spruce are widespread above the current tree line in Eastern Labrador and that the level of available inoculum has a significant influence on the growth of seedlings under controlled conditions (Reithmeier *et al.*, 2013).

In his study published in 2013 Pyasi. was proved that seed sowing was done with inoculation of ectomycorrhizal inocula prepared by isolating the fungi from surface sterilized young basidiocarp of *Lycoperdon compactum* and *Russula michiganensis*. The inocula of ectomycorrhizal fungus were prepared in wheat grains treated with gypsum. The synthesis of ectomycorrhiza was observed in the sapling planted in the experimental field at Jabalpur with production of *basidiocarp* of *Lycoperdon compactum* near saplings. The mycorrhized saplings also showed higher growth indices.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Chemical

The chemicals that were used are listed in table 3.1

Table 3.1 A list of the chemicals used in this work

Chemicals	Manufactures
KOH	Himedia - India
Trypan Blue	Biological industries - Israel
Glycerol	Frutarom - Israel
Chemical fertilizer	Fertilizers and chemicals - Israel
PDA Media	Mumbai - India
Ethyl Alcohol	Frutarom - Israel
Miphenicol " Antibiotic"	ALnaser - Egypt

3.1.2 Equipment's

The main equipment's that were used are listed in table 3.2.

Table 3.2 A list of the main equipment's used in this work.

Instruments	Manufactures
Autoclave	N- Bioteck – Korea
Compound microscope	LW- Scientific – USA
Dissecting microscope	LW- Scientific – USA
Oven	N- Bioteck – Korea
Safety cabinets	N- Bioteck – Korea
Microwave	Hauhai - China

3.2 Organisms

3.2.1 Fungi

The fungus used in this study is ***Hebeloma hiemale***.

3.2.2 Plants

One type of plants from the family (*Rosaceae*) was selected, *Prunus cerasifera x salicina*. This plant is grown widely in Gaza Strip.

3.3 Methods

3.3.1 Isolation, Identification, Culturing, and Multiplication of Fungus

A. Isolation of Fungus

The fungi used in this study were isolated from roots of the genus *Prunus* plants found in private area in Jabalia city–Gaza strip, these areas are relatively distant from agricultural area where the chemical fertilizer is frequently used. Roots were taken out and prepared for the isolation of the fungus as the following:

1. Roots were washed with running water.
2. Disinfected by different concentrations of Sodium hypochlorite ranging from 2 to 10% for 1 to 5 minutes.
3. Washed with distilled water.

B. Culturing and Multiplication of Fungus

The short roots obtained from plants were subjected to the following:

1. Cutting to short pieces (2-3 cm).
2. Washed again with sterile water.
3. The roots then cultured on PDA media.

After 7 days of culture, was obtained a heavy growth of fungus mycelia in petri dishes. The fungus mycelia were sub-cultured for multiplication.

All these steps took place in an axenic condition.

C. Identification of Fungus

Our research aim not to identify type of the fungus. But to evaluate its influence on plants growth, however we tried to identification of the fungus was carried out by types symbiosis ectomycorrhiza of fungi with the genus *Prunus* and compared it with our mycelia obtained from root.

3.3.2 Preparation of inoculum

1. Flasks containing fungal cultures grown in partially solidified nutrient solution (0.3% agar). These static cultures were periodically shaken to break up the mycelium into smaller segments.
2. Flasks containing ECM fungi in semi-liquid culture (as in 1), incubated with continuous agitation by a rotary shaker. Note that fungal mycelium has formed into large or small balls, which can be directly used as inoculum.
3. Homogenizing mycelium from a culture with sterile water to produce mycelial slurry inoculum.
4. Mycelial slurries, ready to inoculate eucalypt seedlings in the greenhouse or nursery.

These steps were performed according to Brundrett *et., al* (1996).

3.3.3 Preparation of Prunus cerasifera x salicin seedling groups

The seedlings of *Prunus cerasifera x salicina* obtained from Modern Agriculture Arboretum planted on sterilized normal soil inside pots (d=20 cm, h=30 cm).

5 groups of seedlings were prepared:-

1. Control (C) 10 seedlings in sterilized normal soil without fungus.
2. Ectomycorrhiza fungi (SECM) 10 seedlings in sterilized normal soil treated by the suspension of our Ectomycorrhizal fungi.
3. Chemical fertilizer (CHF), 10 seedlings in sterilized normal soil treated by chemical fertilizer (14-14-14) 10 ml/L every two weeks through the experimental study period.
4. 50% Compost (COM 50), 10 seedlings in 50% sterilized normal soil mixed by 50% compost.
5. 100% Compost (COM 100), 10 seedlings in 100% compost.

- **Inoculation with ECM:**

The seedlings in 2nd group (SECM) were inoculated with Ectomycorrhiza fungi suspension one-time only.

All seedlings were incubated in the green house for 3 months (from November 2013 to March 2014).

3.3.4 The measurements.

After 3 months of growth we take many measurements of growth parameters to evaluate their growth and comparing the influence of ECM fungi, chemical fertilizers and compost on plants growth.

The parameters are:

- Leaf number (LN).
- Branch number (BN).
- Stem length (SL).
- Root length (RL).
- Stem wet weight (SWW).
- Root wet weight (RWW).
- Stem dry weight (SDW).
- Root dry weight (RDW).

3.3.5 Statistical Analysis

Data was collected and computed by using version 18 of Statistical Package for Social Science, (SPSS). One way ANOVA was the main statistical test used in our study.

Chapter 4

Results

4.1 Isolation and Identification of Fungus.

We have confirm that the isolated fungi from plant's roots was *Hebeloma hiemale* used in our study. We had identifed it according to morphological features of the *Prunus* tree sympiotic fungi, as external hyphae, mantle and hartig net.

Figure 4.1 Colonies morphology of *Hebloma hiemale* on PDA media

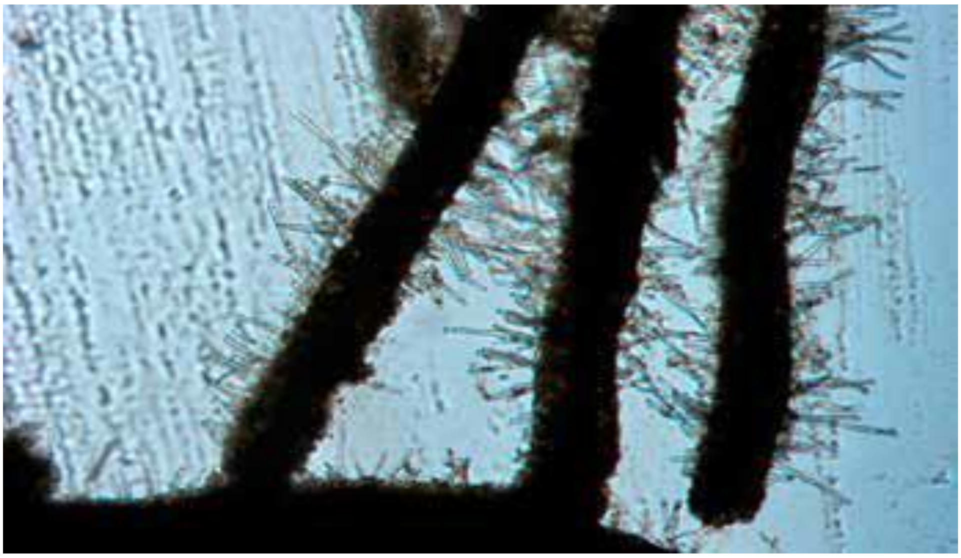


Figure 4.2 *Hebloma hiemale* hyphae under light microscope

4.2 Seedling Growth.

Table 4.2.1 Comparison between (C) and (SECM)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	C	10.80	8.22	12.50	0.000
	SECM	86.60	17.32		
Branch Number	C	3.30	1.06	7.82	0.000
	SECM	9.50	2.27		
Stem Length	C	47.10	9.71	6.22	0.000
	SECM	73.40	9.18		
Root Length	C	47.10	9.71	1.41	0.174
	SECM	52.90	8.61		
Stem Wet weight	C	24.64	2.94	4.82	0.000
	SECM	42.76	11.51		
Root Wet weight	C	25.24	1.51	7.93	0.000
	SECM	42.68	6.79		
Stem Dry weight	C	8.99	2.37	6.01	0.000
	SECM	19.52	4.93		
Root Dry weight	C	11.27	1.95	9.03	0.000
	SECM	22.22	3.30		

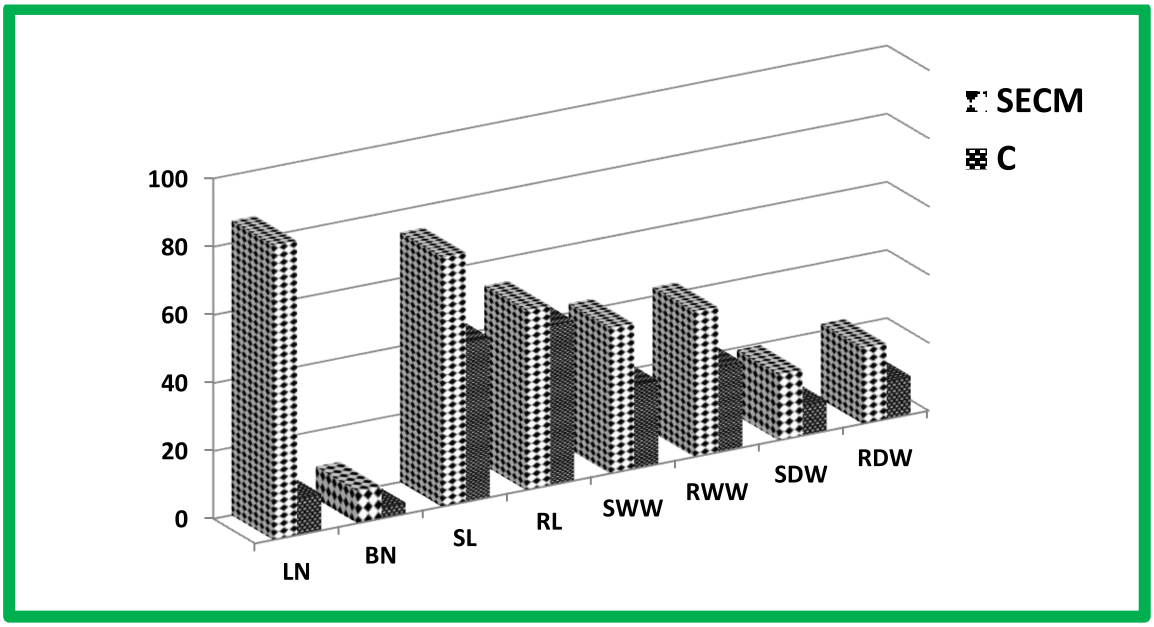


Figure 4.3 growth parameters of (SECM) compared with (C)

The results above show that the growth of mycorrihized plants is better than those of control plants in all measures, and with clear statistical significance at $P < 0.05$.

Table 4.2.2 Comparison between (C) and (CHF)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	C	10.80	8.22	15.89	0.000
	CHF	74.00	9.52		
Branch Number	C	3.30	1.06	7.73	0.000
	CHF	7.50	1.35		
Stem Length	C	47.10	9.71	2.73	0.014
	CHF	57.20	6.54		
Root Length	C	47.10	9.71	0.997	0.332
	CHF	43.80	3.91		
Stem Wet weight	C	24.64	2.94	8.63	0.000
	CHF	35.63	2.75		
Root Wet weight	C	25.24	1.49	3.41	0.003
	CHF	27.94	2.01		
Stem Dry weight	C	8.99	2.37	6.97	0.000
	CHF	19.39	4.15		
Root Dry weight	C	11.27	1.95	2.29	0.035
	CHF	14.02	3.26		

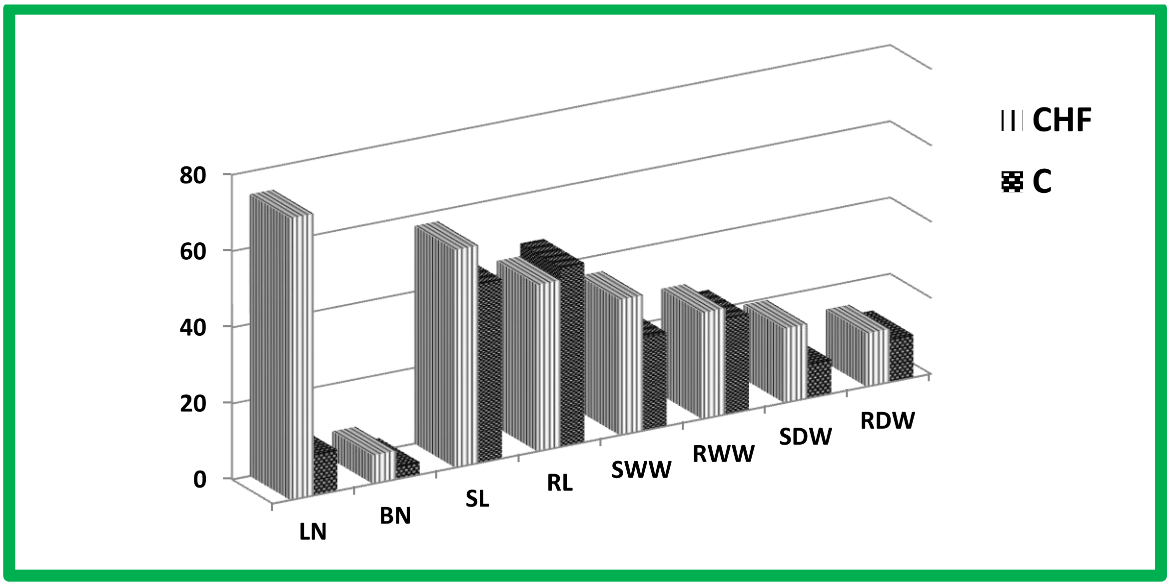


Figure 4.4 growth parameters of (CHF) compared with the (C).

The results demonstrate that the growth parameters of (CHF) plants are better than those of (C) plants in all measures except (RL), and with clear statistical significance.

Table 4.2.3 Comparison between (C) and (COM 50)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	C	10.80	8.23	4.15	**0.001**
	COM 50	0.00	0.00		
Branch Number	C	3.30	1.06	9.85	**0.000**
	COM 50	0.00	0.00		
Stem Length	C	47.10	9.71	0.024	**0.981**
	COM 50	47.00	8.59		
Root Length	C	47.10	9.71	3.56	**0.002**
	COM 50	32.80	8.12		
Stem Wet weight	C	24.64	2.94	6.92	**0.000**
	COM 50	12.72	4.58		
Root Wet weight	C	25.24	1.49	9.02	**0.000**
	COM 50	14.80	3.34		
Stem Dry weight	C	8.99	2.37	1.44	**0.166**
	COM 50	11.18	4.17		
Root Dry weight	C	11.27	1.95	0.12	**0.911**
	COM 50	11.42	3.43		

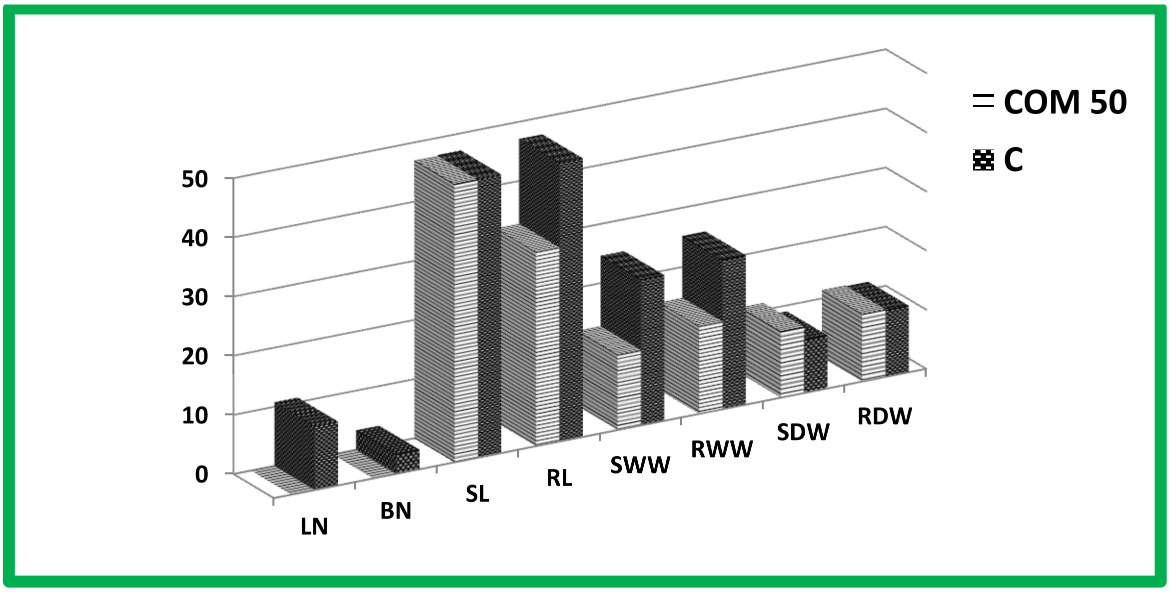


Figure 4.5 growth parameters of (C) compared with (COM 50).

As we shown above, the growth of (C) plants is significantly better than plants grown in (COM 50) in all measures except (SDW, RDW).

Table 4.2.4 Comparison between (C) and (COM 100)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	C	10.80	8.22	4.16	0.001
	COM 100	0.00	0.00		
Branch Number	C	3.30	1.06	9.85	0.000
	COM 100	0.00	0.00		
Stem Length	C	47.10	9.71	0.46	0.652
	COM 100	44.90	11.65		
Root Length	C	47.10	9.71	4.75	0.000
	COM 100	28.30	7.88		
Stem Wet weight	C	24.64	2.94	7.77	0.000
	COM 100	13.50	3.44		
Root Wet weight	C	25.24	1.49	16.47	0.000
	COM 100	11.35	2.21		
Stem Dry weight	C	8.99	2.37	2.28	0.035
	COM 100	11.74	2.97		
Root Dry weight	C	11.27	1.95	2.49	0.023
	COM 100	9.18	1.79		

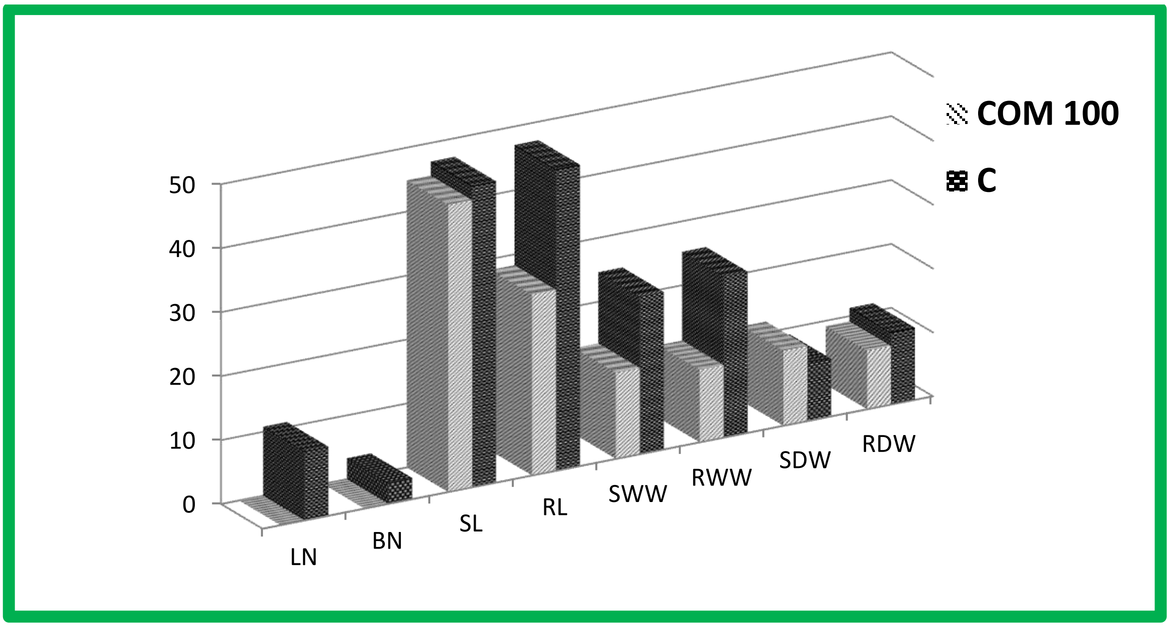


Figure 4.6 growth parameters of (C) compared with (COM 100).

The results above show that growth of (C) plants is significantly better than those of (COM 100) plants in all measures except (SDW).

Table 4.2.5 Comparison between (SECM) and (CHF)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	CHF	74.00	9.52	2.02	0.059
	SECM	86.60	17.32		
Branch Number	CHF	7.50	1.35	2.39	0.028
	SECM	9.50	2.27		
Stem Length	CHF	57.20	6.54	4.54	0.000
	SECM	73.40	9.18		
Root Length	CHF	43.80	3.91	3.05	0.007
	SECM	52.90	8.59		
Stem Wet weight	CHF	35.63	2.75	1.91	0.073
	SECM	42.76	11.51		
Root Wet weight	CHF	27.94	2.00	6.58	0.000
	SECM	42.68	6.79		
Stem Dry weight	CHF	19.39	4.15	0.064	0.950
	SECM	19.52	4.93		
Root Dry weight	CHF	14.02	3.26	5.58	0.000
	SECM	22.22	3.30		

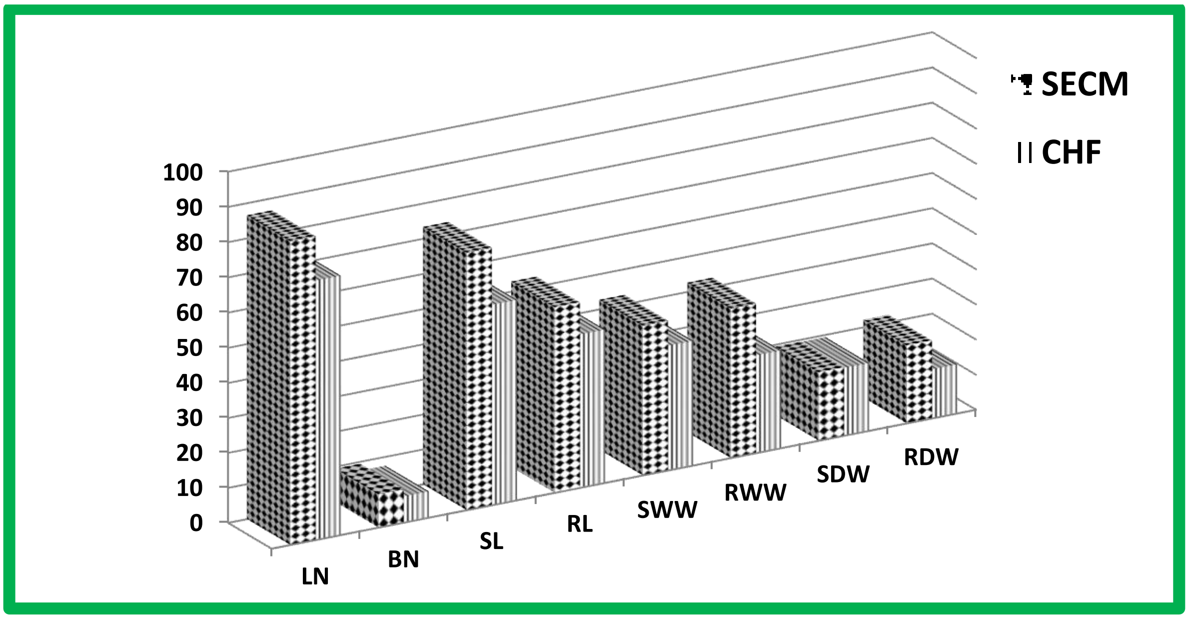


Figure 4.7 growth parameters of (SECM) compared with (CHF).

These results illustrate that growth parameters of (SECM) plants is better compared with the (CHF) treated plants. These results indicate that the mycorrihizal fungi may be more benefit for plants growth than chemical fertilizers.

Table 4.2.6 Comparison between (CHF) and (COM 50)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	CHF	74.00	9.52	24.57	0.000
	COM 50	0.00	0.00		
Branch Number	CHF	7.50	1.35	17.52	0.000
	COM 50	0.00	0.00		
Stem Length	CHF	57.20	6.54	2.99	0.008
	COM 50	47.00	8.59		
Root Length	CHF	43.80	3.91	3.833	0.001
	COM 50	32.80	8.18		
Stem Wet weight	CHF	35.63	2.75	13.56	0.000
	COM 50	12.72	4.58		
Root Wet weight	CHF	27.94	2.01	10.66	0.000
	COM 50	14.80	3.34		
Stem Dry weight	CHF	19.39	4.15	4.48	0.000
	COM 50	11.18	4.17		
Root Dry weight	CHF	14.02	3.26	1.74	0.100
	COM 50	11.42	3.44		

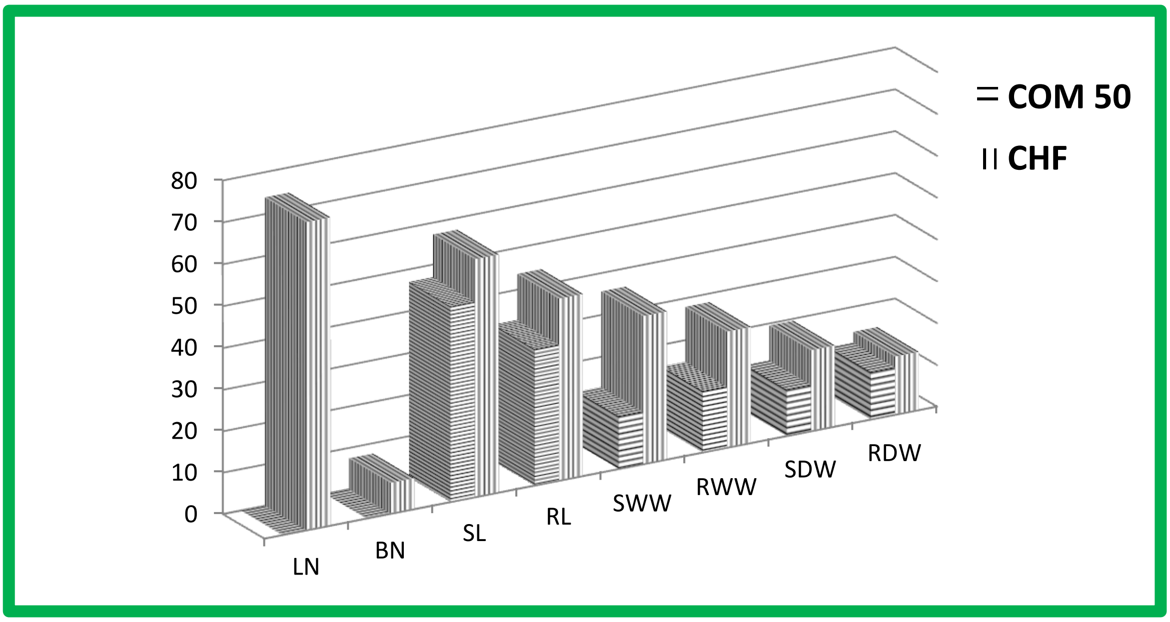


Figure 4.8 growth parameters of (CHF) compared with (COM 50).

The results above show that growth of (CHF) treated plants is significantly and better than those of (COM 50) plants in all measures.

Table 4.2.7 Comparison between (CHF) and (COM 100)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	CHF	74.00	9.52	24.57	0.000
	COM 100	0.00	0.00		
Branch Number	CHF	7.50	1.35	17.52	0.000
	COM 100	0.00	0.00		
Stem Length	CHF	57.20	6.54	2.91	0.009
	COM 100	44.90	11.65		
Root Length	CHF	43.80	3.91	5.57	0.000
	COM 100	28.30	7.88		
Stem Wet weight	CHF	35.63	2.75	15.88	0.000
	COM 100	13.50	3.44		
Root Wet weight	CHF	27.94	2.00	17.61	0.000
	COM 100	11.35	2.21		
Stem Dry weight	CHF	19.39	4.15	4.82	0.000
	COM 100	11.74	2.98		
Root Dry weight	CHF	14.02	3.26	4.11	0.001
	COM 100	9.18	1.79		

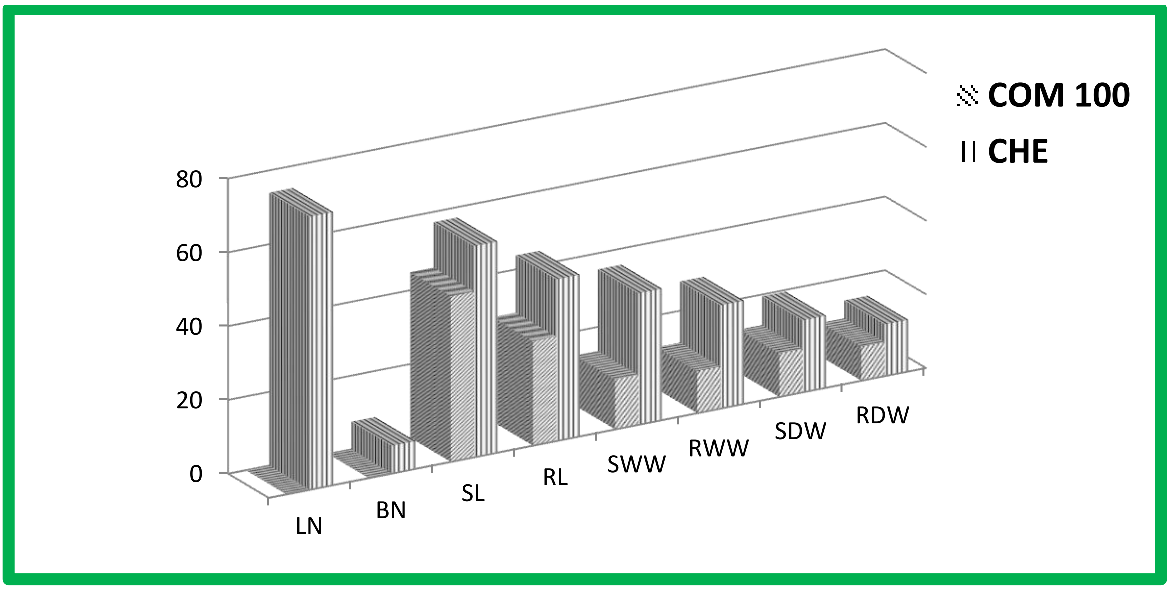


Figure 4.9 growth parameters of (CHF) compared with (COM 100).

These results demonstrate that growth of (CHF) plants is better than those of (COM 100) plants in all measures, with clear statistical significance.

Table 4.2.8 Comparison between (SECM) and (COM 50)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	SECM	86.60	17.32	15.81	0.000
	COM 50	0.00	0.00		
Branch Number	SECM	9.50	2.27	13.22	0.000
	COM 50	0.00	0.00		
Stem Length	SECM	73.40	9.18	6.64	0.000
	COM 50	47.00	8.59		
Root Length	SECM	52.90	8.59	5.35	0.000
	COM 50	32.80	8.18		
Stem Wet weight	SECM	42.76	11.51	7.67	0.000
	COM 50	12.72	4.58		
Root Wet weight	SECM	42.68	6.79	11.65	0.000
	COM 50	14.80	3.34		
Stem Dry weight	SECM	19.52	4.93	4.02	0.001
	COM 50	11.18	4.17		
Root Dry weight	SECM	22.22	3.30	7.16	0.000
	COM 50	11.42	3.35		

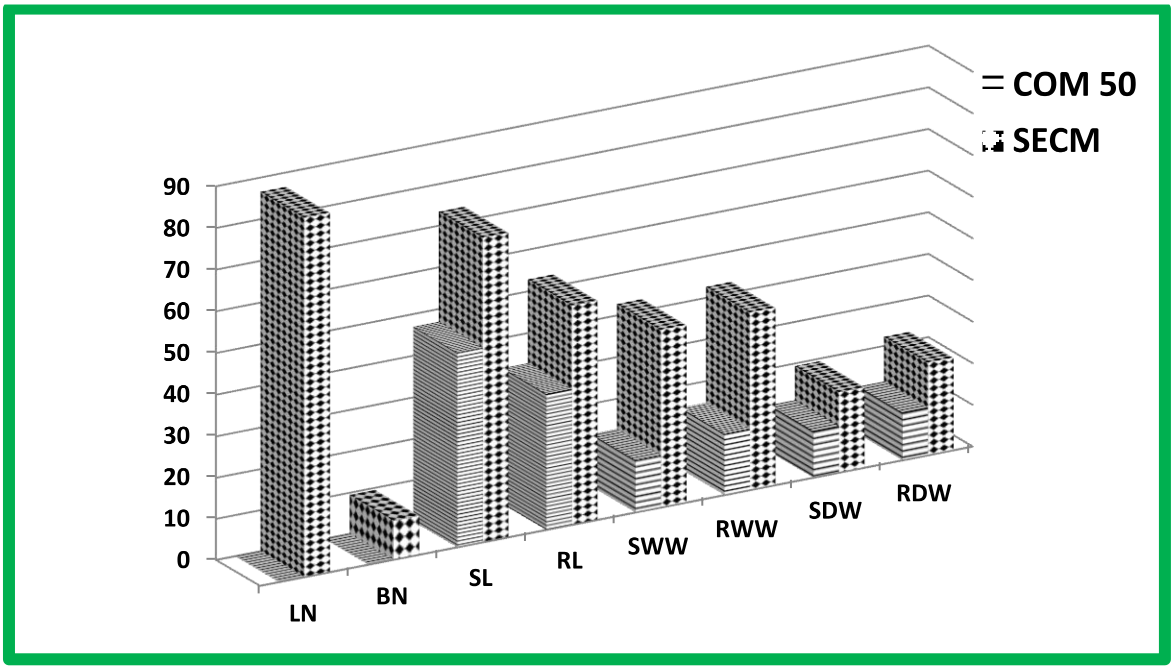


Figure 4.10 growth parameters of (SECM) compared with (COM 50).

The results above confirm that the growth (SECM) plants are better than those of (COM 50) plants in all measures, with clear statistical significance.

Table 4.2.9 Comparison between (SECM) and (COM 100)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	SECM	86.60	17.32	15.81	0.000
	COM 100	0.00	0.00		
branch Number	SECM	9.50	2.27	13.21	0.000
	COM 100	0.00	0.00		
Stem Length	SECM	73.40	9.18	6.07	0.000
	COM 100	44.90	11.65		
Root Length	SECM	52.90	8.59	6.67	0.000
	COM 100	28.30	7.88		
Stem Wet weight	SECM	42.76	11.51	7.70	0.000
	COM 100	13.50	3.44		
Root Wet weight	SECM	42.68	6.79	13.87	0.000
	COM 100	11.35	2.21		
Stem Dry weight	SECM	19.52	4.93	4.20	0.001
	COM 100	11.74	2.98		
Root Dry weight	SECM	22.22	3.30	10.96	0.000
	COM 100	9.18	1.79		

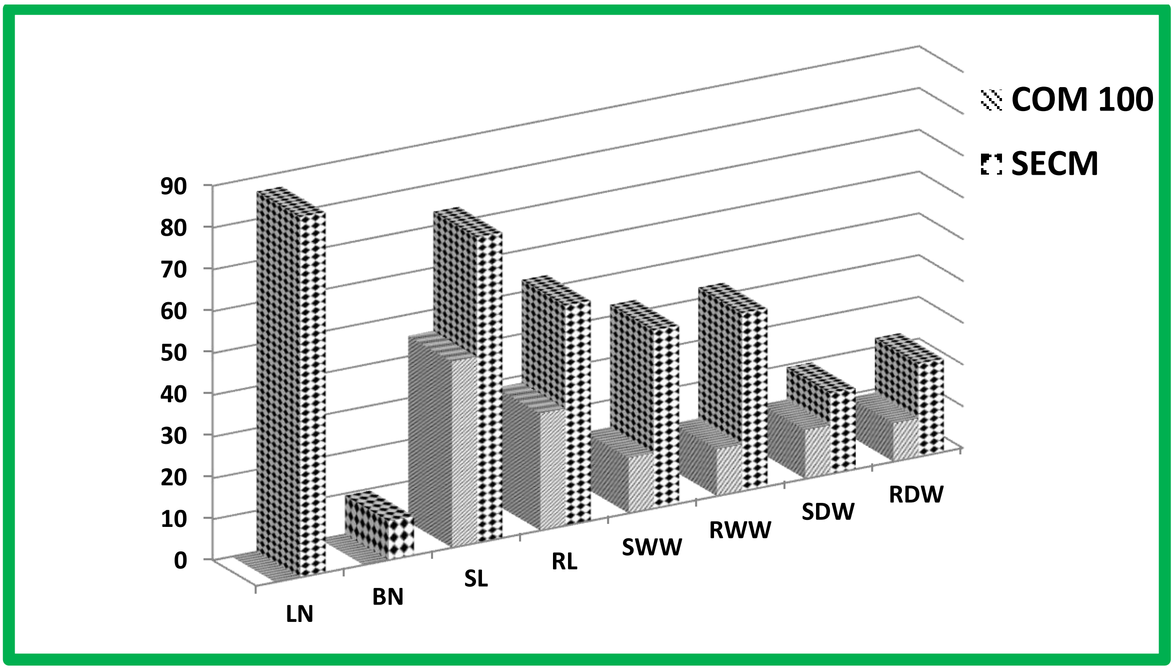
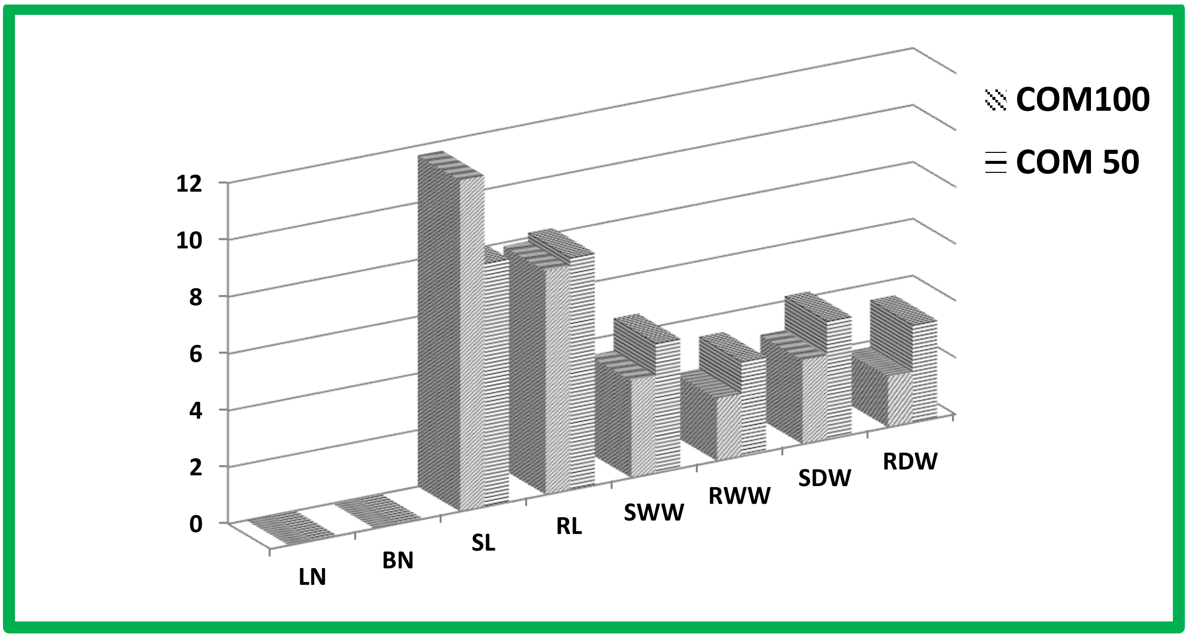


Figure 4.11 growth parameters of (SECM) compared with (COM 100).

The results above illustrate that (SECM) plants is better than those of (COM 100) plants in all measures, with clear statistical significance.

Table 4.2.10 Comparison between (COM 100) and (COM 50)

	Group	Means	Standard Deviation	T-test	Sig.
Leaf Number	COM 100	0.00	0.00	-	-
	COM 50	0.00	0.00		
branch Number	COM 100	0.00	0.00	-	-
	COM 50	0.00	0.00		
Stem Length	COM 100	47.00	8.58	0.46	0.652
	COM 50	44.90	11.66		
Root Length	COM 100	32.80	8.18	1.25	0.227
	COM 50	28.30	7.88		
Stem Wet weight	COM 100	12.72	4.58	0.430	0.672
	COM 50	13.50	3.44		
Root Wet weight	COM 100	14.80	3.34	2.72	0.014
	COM 50	11.35	2.21		
Stem Dry weight	COM 100	11.18	4.17	0.35	0.734
	COM 50	11.74	2.97		
Root Dry weight	COM 100	11.42	3.44	1.83	0.084
	COM 50	9.18	1.79		



Figures 4.12 growth parameters of (COM 100) compared with (COM 50).

These results confirm the results in table 4.8 and table 4.9 that the compost used is not benefit for plant growth, it showed poisonous effects on plants which led to death.

4.3 Dry weight of plant (shoot and root)

Due to the importance of the dry weight in determining plant growth we have decided to compare all the parameters using the dry weight as evidence.

Table 4.3 Comparison dry weights of stem and root between tested parameters

Group	Stem		Root	
	Mean	Standard Deviation	Mean	Standard Deviation
SECM	19.52	4.93	22.22	3.30
C	8.99	2.37	11.27	1.95
CHF	19.39	4.15	14.02	3.26
COM50	11.18	4.17	11.42	3.43
COM100	11.74	2.97	9.18	1.79

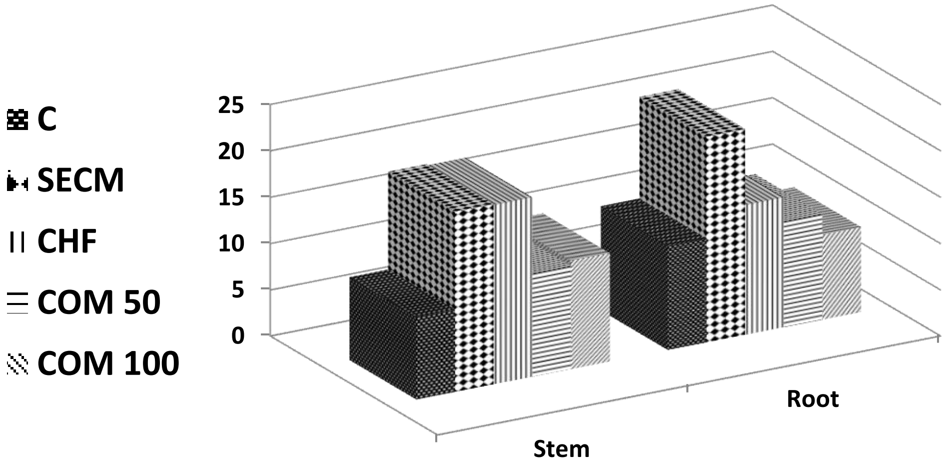


Figure 4.13 growth parameters of SECM compared with other parameters in dry weight

The results have shown that displayed symbiotic fungus (SECM) showed a clear positive effect on plant growth represented by dry weight, which is similar to the (CHF) in the shoot state and it's better than of all the other variables. It seems clear that symbiotic fungi (SECM) have a significant and positive impact on the root growth and better than all the other parameters, including the effect of (CHF) in the case of the roots.

Chapter 5

Discussion

Soil fungi are playing an essential role in equilibrium of ecosystem either by parasitic, symbiotic, saprophytic. Despite its negative role in causing a number of plant diseases, Fungi positive effects are particularly important. It's symbiotic effect is considered a main source of minerals nutrition for a number of plants and trees. It is noteworthy to mention that symbiotic plants represent more than 95% of all plants (Smith and Read, 1997). Moreover limited agricultural areas with intensive agriculture are particularly in need of such symbiotic organisms in order to limit the use of chemical fertilizers and reduce the ground water pollution. Gaza strip is a good example for such areas with agriculture representing a backbone for population life. In this regard this study focused on using fungi isolated from the environment as a partial or complete alternative for chemical fertilizers. It may aid in reducing the consumption of these fertilizers and thus minimize the environmental and health burden on human life. The main aim of our study was to show the influence of fungi locally isolated from soil in Gaza Strip on the growth of local plants *Prunus cerasifera x salicina* by measuring different growth parameter such as (LN, BN, SL, RL, SWW, RWW and specially RDW, SDW). The second objective of our study was to compare the mycorrhizal on plants growth and the role of compost and chemical fertilizer by measuring the growth of plants. Biofertilizers will be the best solution to replace chemical fertilizers to overcome the harmful effects of chemical fertilizers and to maintain soil fertility and groundwater. So we carried out this research on an important plant, *Prunus cerasifera x salicina*.

So by Comparison of different variables on plant growth. The statistical analysis of our results has shown a real positive role of ECT on *prunus* plant growth comparing to CHF, C, COM 50 and COM 100 plants. These results agree with the result obtained by (Abhishek *et al*., in 2013, Luiz Afonso, in 2008, Karoliina *et al*., in 2004, El Kichaoui *et al*., in 1995). This can explain the role of these fungi in the plants nutrition by increasing the uptake of N, P, K from soil, and other benefits of ECT is the increase water absorption and other hand Protection against pathogens. These results supported our main objective in this study, which motivates us to encourage the farmers and the agricultural officials to begin using the biological fertilizer instead of the chemical fertilizer. These results are in concordance with most similar previous studies (Karoliina Niemi *et al*., 2004)

The results have shown that the use of commercial compost were so bad on the growth of the plants and even dead. No doubt we have used high rate of compost (50%-100%), which may be the cause of the death of plants, but this doesn't prevent the chemical analysis for compost in order to know the accurate structure for it especially the rate of the different elements.

The influence of chemical fertilizer in all parameters is better than control, except root length, we can explain that the chemical fertilizer contains nutrient elements that limit the root growth because plant doesn't need that. These results are in concordance with most similar previous studies (Lyr, H. and G. Hoffman. 1967).

The control plants is higher than (50% ,100%) compost plants with statistical clear significance to control, except RDW, SDW in 50% compost and RDW in 100% compost, because the compost keeps a lot of water than soil. This allows the plant to absorp a lot quantity of water. These results are in agreement with studies made by El Kichaoui *et al*.1995, Karoliina *et al*., 2004. Abhishek *et al.*, 2013.

In general, we can say that used the biological fertilizer (fungi) is the better way for obtaining a good growth. So we can confirm that the use of ECM as fertilizer is very useful for plant growth and environment health.

Chapter 6

Conclusion & Recommendations

6.1 Conclusions

The present study investigated the influence of ECM fungus isolated from local soil on the growth of *Prunus cerasifera x salicina* plants in Gaza Strip. We have adopted to determine the effect of fungus on plant growth by comparing plants inoculated with C, CHF, COM 50 and COM 100 plants. The informations that can be concluded from this study are:

1. A net increasing of growth of *Prunus cerasifera x salicina* in the presence of ECM suspension when compared to CHF, C, COM 50 and COM 100 plants.

2. Our statistical analysis illustrate the clear difference in *Prunus cerasifera x salicina* growth in the presence of chemical fertilizer or SECM than control plants.

3. The comparison between the growths of the *Prunus cerasifera x salicina* plants treated with ECM suspension and C plants. In this case our statistically analysis illustrate that growth of ECM plants better than, C plants in all measures.

4. The chemically fertilized plants showed significantly better growth in all cases than control plants**,** except for root length.

5. There was increase in the wet weight of plants that are planted in compost.

6.2 Recommendations

1. It is recommended to isolate other fungi in our agricultural areas and determine the species accurately.

2. The experiments of this study may be repeated using a wider range of plants including trees particularly those useful to the human diet.

3. The experiments of this study may be repeated using another ECM and using mixture of different chemicals.

4. The experiments conducted in this study may be repeated with extended time in order to examine the effect of mycorrhization on fruiting, flowering and different vegetables, and to study the impact of environmental factors on plants growth.

5. We recommend to perform this experiment in the field.

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