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In Vitro Mycorrhization of Some Seasonal Plants By Using Local Soil Fungi

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بسم الله الرحمن الرحيم

قال تعالى:

{إنَّ في خلْقِ السَّمواتِ والأرضِ واختلافِ اللَّيلِ والنَّهارِ لآياتٍ لأولي الألباب * الَّذين يَذْكُرونَ الله قياماً وقعوداً وعلى جُنوبهم ويتفَكَّرونَ في خلْقِ السَّمواتِ والأرضِ ربَّنا ما خلَقْت هذا باطلاً سبحانَك فَقِنَا عذابَ النَّار}

سورة آل عمران آية (191,190)

Declaration

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**
Shehadeh w	Wael Nizar Shehadeh	July 2010

DEDICATION

To my parents who are always supporting me

To my wife who helped me to accomplish this thesis

To my sons , Obeida , Alaa

To my brothers and sisters

To my university IUG

To all my students who helped me

To all of them I dedicate this work

Acknowledgements

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In Vitro Mycorrhization of Some Seasonal Plants By Using Local Soil Fungi

Abstract

The main objective of this work is to study the influence of local endo-symbiotic fungus on the growth of two important plants. So we achieved a bibliographic introduction that describes our knowledge about the phenomenon of mycorrhization in general and the endomycorrhization in particular. To serve this objective we summarized some works dealing with the effects of the mycorrhization on ecosystem, the important of this phenomenon on plant nutrition and growth and factors influencing mycorrhization. This first part is a preliminary study for our experimental work. The second part was an experimental study which shows the influence of a local fungus extracted from squash seedling on the growth of summer squash and watermelon plants. Theses two seedlings have been shown on vermiculite substrates containing little amount of organic matters. 20 days after planting 120 seedling of each species, we have inoculated part of seedling by the symbiotic fungus. The inoculation has been achieved in two ways, the first by injection of a suspension of fungus spores around the seedling roots and the second by surrounding the plant roots by the fungus mycelium. 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. Our results show a positive influence of the symbiotic fungus on the growth of summer squash seedling compared with control and with the seedling treated with chemical fertilizers especially root systems. Concerning watermelon plants the positive effect of the fungus was compared only with control plants. We also show that the plant roots inoculation with spore suspension was given the best results. We conclude that the use of arubuscular mycorrhizal fungus gives positive influence on the growth of plants especially compared with control and better or similar compared with plants treated with chemical fertilizer. According to these results we strongly recommend the use of symbiotic fungi as total or partial substitute of chemical fertilizer.

Key words : Mycorrhization, Arbuscular Mycorrhiza, Watermelon, Summer Squash.

إجراء التكافل مخبريا لبعض النباتات الموسمية باستخدام فطريات التربة المحلية

المستخلص

الهدف الأساسي من دراستنا كان إظهار تأثير احد الفطريات المتكافلة داخليا عل نمو نوعين من النباتات المهمة. من أجل ذلك قمنا بعمل دراسة نظرية تظهر معرفتنا بظاهرة التكافل بشكل عام والتكافل الداخلي بشكل خاص وذلك بتقديم عرض كامل عن تأثير التكافل على التوازن البيئي وكذلك على التغذية والنمو النباتي ودراسة العوامل التي تؤثر على هذه الظاهرة. كانت هذه الدراسة النظرية كمقدمة أولية تخدم دراستنا العملية المخبرية.

الجزء الثاني من هذا البحث كان عبارة عن دراسة عملية مخبريه لإظهار تأثير أحد الفطريات المتكافلة داخليا على نمو نوعين من النباتات وهما الكوسة والبطيخ وقد تم استخراج الفطر وعزلة من جذور أشتال الكوسة زرعت في منطقة قريبة من المناطق الزراعية لخدمة هدف هذه الدراسة.

بعد 20 يوما من زراعة 120 شتلة من كل نوع تم تلقيح عدد من هذه النباتات بالفطر المعزول وذلك بطريقتين الأولى باستخدام معلق من جراثيم الفطر والثانية بوضع خيوط الفطر مباشرة على جذور الأشتال.

دراسة التأثير الفطري على النباتات كان بمقارنة نمو الأشتال الملقحة مع نباتات الضابط وكذلك مع نباتات سمدت بمواد غذائية كيميائية.

أظهرت النتائج تأثير ايجابي للفطر على نمو نباتات الكوسة مقارنة مع نباتات الضابط ومع النباتات التي أضيف لها السماد الكيميائي وخصوصا نمو جذور تلك النباتات.

بالنسبة لنباتات البطيخ لوحظ أن التأثير الايجابي للفطر كان فقط مقارنة مع الضابط. أما بالنسبة للمقارنة بين طريقتي التلقيح أظهرت نتائجنا أن التلقيح باستخدام المعلق الجرثومي كان أفضل في كل الحالات.

نستطيع الاستخلاص هنا أن استخدام الفطر التكافلي يعطي تأثيرا ايجابيا على نمو النباتات مقارنه مع الضابط أما مقارنة النباتات الملقحة بالمعلق الجرثومي مع النباتات المسمدة كيميائيا فقد كان التأثير الفطري على نمو النباتات إما أفضل أو يساوي تأثير السماد الكيميائي. من خلال هذه النتائج يمكننا أن ندعم وبشكل قوي استخدام مثل هذه الفطريات كبديل كلي أو جزئي عن الأسمدة الكيميائية.

الكلمات المفتاحية : التكافل الفطري ، التكافل الفطري الداخلي ، نبات البطيخ ، نبات الكوسة .

Table of Contents

List o Figures	Page

List of Tables	Page

Abbreviations

AMF	**Arubuscular Mycorrhizal Fungi**
AM	**Arubuscular Mycorrhiza**
VAM	**Vesicular – Arubuscular Mycorrhiza**
EMF	**Ectomycorrhizal Fungi**
ECM	**Ectomycorrhiza**
MMN	**Modified . Media – Norkrans**

Chapter 1
Introduction

1.1. Overview

In 1885 Albert Bernard Frank (Frank, 1885), in his study of soil microbial-plant relationships, introduced the Greek term ‘mycorrhiza’, which literally means ‘fungus roots’. Mycorrhizal fungi form symbiotic relationships with plant roots in a fashion similar to that of root nodule bacteria within legumes. Of 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(Smith and Read, 1997; Allen *et al.*, 2003). Arbuscular mycorrhizal (AM) fungi comprise 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 Glomeromycota and have a ubiquitous distribution in global ecosystems (Redecker *et al.*, 2000). Ectomycorrhizal (ECM) fungi are also widespread in their distribution but associate with only 3% of vascular plant families (Smith and Read, 1997).These fungi are members of the phyla Ascomycota and Basidiomycota, and the ECM mutualism is thought to have been derived multiple times independently from saprophytic lineages (Hibbett *et al.*, 2000).

Ectendomycorrhizae possess characteristics of both ECM and AM fungi (Table 1.1). As with ECM, both a hartig net and mantle structures are produced in ectendomycorrhizae, although the mantle may be reduced compared with ECM . The hartig net is defined as an inward growth of hyphae which penetrates the root structure. Intracellular penetration of healthy plant cells by these fungi also occurs, a characteristic unlike that of ECM but consistent with AM. Ectendomycorrhizae can be formed with roots of many angiosperm and gymnosperm species; fungal symbionts include members of the Basidiomycota, Ascomycota, or Zygomycota. In fact, the same fungal species can form either ECM or ectendomycorrhizae depending upon the plant species with which it is associated. Similarly, arbutoid mycorrhizae possess characteristics of both ECM and AM fungi, i.e., there is a well developed mantle, a hartig net, and prolific

extrametrical mycelium. Additionally, intracellular penetration occurs and hyphal coils are produced in autotrophic cells.These mycorrhizae are associated with members of the Ericales; namely, Arbutus and Arctostaphylos species. The fungal symbionts are exclusively Basidiomycete species, which may form ECM with other autotrophic hosts.

Monotropoid and orchid mycorrhizae are formed between Basidiomycete fungi and achlorophyllous plant species. Monotropoid mycorrhizae are formed between plants of the Monotropaceae family and a specific subset of fungi in the Russulaceae or the Boletaceae family. Orchid mycorrhizae have only been found in association with Basidiomycete species. In the other mycorrhizal symbioses plants are usually generalists and associate with a wide array of fungal species. In contrast, plants that participate in monotropoid and orchid mycorrhizal associations are highly specific, associating with only a narrow range of fungal species. It had been thought that these mycorrhizal associations are formed exclusively with Basidiomycete fungal species; however, it has recently been discovered that several species of tropical achlorophyllous epiphytes form mycorrhizal associations with AM fungal species in the Glomeromycota (Bidartondo *et al.*, 2002). Ericoid mycorrhizae are known to form between autotrophs in the Ericaceae and fungi in the Ascomycota. Intracellular penetration of root cells occurs and there is no mantle or hartig net development.

Table 1.1. Major categories of mycorrhizae and their attributes. (Adapted from Smith and Read, 1997).

Mycorrhizal type	Arbuscu-lar	Ecto	Ectendo	Arbutoid	Monotro-poid	Ericoid	Orchid-aceous
Fungal taxa	Glomero	Basidio Asco Zygo	Basidio Asco	Basidio	Basidio	Asco	Basidio
Plant taxa	Bryo Pterido Gymno Angio	Gymno Angio	Gymno Angio	Ericales	Monotr-opoideae	Ericales Bryo	Orchid aceae
Intracellular colonization	+	–	+	+	+	+	+
Fungal sheath	–	+	+/–	+/–	+	–	–
Hartig net	–	+	+	+	+	–	–
Vesicles	+/–	–	–	–	–	–	–
Achlorophylly	–	–	–	–	+	–	+

+ = present; – = absent

AM fungi (AMF) help plants to capture nutrients such as phosphorus and micronutrients from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants (Brundrett, 2002). They induce greater resistance to soil pathogens, enhance tolerance to drought stress, and reduce sensitivity to toxic substances occurring in the soil. Despite the small area of Gaza Strip, the agricultural areas are widespread and abundant.The chemical fertilizers are over used to compensate for the small agricultural areas. Therefore the search for alternatives of the chemical fertilizer is of great importance, and this led us to use mycorrhization as biological fertilization system.

1.2. Objectives

1.2.1. General objective

Using mycorrhizal local fungi as fertilizers for some seasonal plants in order to decrease the utilization of chemical fertilizers.

1.2.2. Specific objectives

- Isolation and mycorrhizal identification of fungi from plant roots.
- In vitro application of symbiosis between plant and fungi.
-To evaluate the effect of mycorrhizal fungi isolated from the soil of local environment on summer squash and watermelon plant growth.

1.3. Significance

Our study is considered as the first study in Gaza Strip, the establishment of a model for plant mycorrhization techniques and determining the essential factors that influence the success of mycorrhization present is considered as a very important point of the view in the agriculture field in our country. The use of chemical fertilization represents a critical point on the ground water pollution, especially when it's present in a small area with intensive agriculture activities. In the other hand the permanent siege caused by the Israeli army against Gaza Strip limits the arrival of chemical fertilizers, caused real problems for the entire agriculture domain, so the use of biological fertilization like mycorrhizal fungi may be a solution for these problems.

Chapter 2

Literature Review

2.1. Fungi and Mycorrhizal 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 (Hawksworth et al., 1995).

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 and Zygomycota, and the Ascomycota and Basidiomycota (Berbee & 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. The Zygomycota and Chytridiomycota can produce septa but these are infrequent in vegetative hyphae. In contrast, vegetative hyphae in the Ascomycota produce perforate septa at regular intervals and this is also found in the Basidiomycota, suggesting that this trait was present in their common ancestor (Berbee & Taylor,2001). As hyphae grow they branch and fuse, eventually forming a multicellular network of interconnected cells (Glass et al., 2004).

2.1.1. 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).

2.1.1.1. Ectomycorrhizal Fungi

Ectomycorrhizal fungi (EMF) play a critical role in tree nutrition and carbon balance, supplying soil resources to their plant hosts in exchange for sugars (Smith and Read 1997). Ectomycorrhizal trees dominate nitrogen-limited forest ecosystems, and EMF vary in their nitrogen uptake physiology (Smith and Read 1997, Chalot and Brun 1998), so we might expect different species to dominate in soils with different levels and forms of nitrogen.

2.1.1.2. Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizas (AM) are the most common mycorrhizal type. They are formed in an enormously wide variety of host plants by obligately symbiotic fungi which have recently been reclassified on the basis of DNA sequences into a separate fungal phylum, the Glomeromycota (Schüβler *et al.*, 2001). The plants include angiosperms, gymnosperms and the sporophytes of pteridophytes, all of which have roots, as well as the gametophytes of some hepatics and pteridophytes which do not (Read *et al.,* 2000) It seems highly likely that the fungi had their origins possibly over 1000 million years ago (predating current estimates of colonization of land) and that arbuscular mycorrhizal (AM) symbioses are also extremely ancient. Through their roles in nutrient uptake, AM fungi were probably important in the colonization of land by plants (Heckman *et al.*, 2001) they remain major determinants of plant interactions in ecosystems to the present day. The name ‘arbuscular’ is derived from characteristic structures, the arbuscules which occur within the cortical cells of many plant roots and also some mycothalli colonized by AM fungi. Together with storage vesicles located within or between the cells, these structures have been considered diagnostic for AM symbioses. However, a rather wide range of intraradical structures formed by AM fungi is recognized , including well-developed intracellular hyphal coils, which sometimes occur in the absence of any arbuscules . The variations in developmental pattern are determined by both plant and fungal partners, adding to the complexities of identifying a symbiosis as ‘AM’ on the basis of intraradical fungal morphology. The term vesicular-arbuscular mycorrhiza (VAM), which was in use for many decades, has been dropped in recognition that vesicles are formed by only 80% of AM fungi,

but the name ‘arbuscular’ is currently retained, regardless of the structural diversity which is more and more widely appreciated. (Dickson, 2004).

Arbuscular mycorrhizas were first recognized and described in the last decades of the nineteenth century. Their widespread occurrence and common presence in plants of many phyla in most parts of the world, especially in the tropics, was realized very soon (Gallaud, 1905), but very little functional information was learnt about them until the mid-1950s. Almost all writings about the identity of the fungi until 1953 may be ignored, except for those of Peyronel (1923) who showed that hyphae of the endophyte could be traced to the sporocarps of species of fungi, then classified in the Endogonaceae, in the surrounding soil. Later, Butler (1939) in an influential review, agreed that the fungi called *Rhizophagus* were almost certainly imperfect members of the Endogonaceae, which then included the majority of fungi now transferred to the Glomeromycota. The work of Mosse (1953), which showed convincingly that mycorrhizal strawberry plants were colonized by a species of *Endogone* (later transferred to *Glomus*), may be said to have heralded the modern period. Soon Mosse, Baylis, Gerdemann, Nicolson and Daft and Nicolson greatly extended these early observations and demonstrated by inoculation that fungi in the Endogonaceae were symbiotic with many kinds of plants.

2.1.1.3. Climatic Specificity of Mycorrhizal Fungi

Environmental factors and soil conditions influence the occurrence of mycorrhizal associations in ecosystems, but it is hard to examine the direct impacts of these factors on mycorrhizal fungi because they rarely occur in nature without a host and members of the Glomales can not be grown axenically (Harley and Smith, 1983).

However, some evidence of the physiological diversity of mycorrhizal fungi has been provided by comparing experimental responses to soil pH, soil nutrient levels, soil moisture, salinity, temperature and other factors (Abbott and Robson, 1991). Most of this data has been collected using simplified experimental systems which allow the influence of one factor on one mycorrhizal fungus to be examined, but some field data are also available for comparison. Changes to soil properties occurring during succession or between sites with similar climates can be correlated with the predominance of different species or isolates of VAM fungi (Rose, 1988).

Ebbers *et al.,* (1987) discovered changes in predominate species of VAM fungi across a soil moisture (soil fertility) gradient in a prairie site, which had a much greater influence on plant populations. Henkel *et al.,* (1989) observed that isolates of four VAM fungi from adjacent ridgetop, mid-slope and basal sites in an arid plant community were most infective in the soil from which they were collected and less infective in soil from the other two sites.

2.2. Importance of Mycorrhizae

The driving force behind AM interactions is an exchange of nutrients between fungus and plant. Glomeromycotan fungi are obligate symbionts and rely on the carbon provided by their plant hosts to complete their life cycle. In return, the fungus provides nutritional benefits to the plant by delivering minerals, including the biologically essential nutrients phosphorus (P) and nitrogen (N). The majority of this nutrient exchange is believed to occur within root cortical cells containing highly-branched hyphal structures termed *arbuscules*. As arbuscules develop they become enveloped by newly synthesized host membrane tissue; the arbuscules never enter the host cytoplasm. The plant and fungal arbuscular membranes define a space, the interfacial apoplast, into which nutrients can be delivered and from which they can be extracted (Balestrini and Bonfante, 2005).

2.2.1. Arbuscular Mycorrhizal Phosphate Acquisition

Phosphate is an essential nutrient and is limiting for plant growth in many environments (Bucher, 2007). Phosphate is present in the soil in the form of inorganic orthophosphate (Pi) and is readily sequestered by cations, especially in acidic conditions, of which the most abundant are iron, aluminium and calcium. The mobility of sequestered phosphate is reduced and, as a consequence, plant uptake rapidly exhausts the phosphate available in the vicinity of the root system and creates a localised depletion zone (Bucher,2007). Furthermore, the efficiency of phosphate uptake may be as low as 20% (Zhu *et al.*, 2003) and much of the added phosphates will pass to adjacent water courses with detrimental environmental consequences. It has been demonstrated that, in wild ecosystems, plants derive much of their phosphate via mycorrhizal fungi (Smith *et al.*, 2004). Investigating the current importance, and potential future benefits, of mycorrhizal

colonization to crop phosphate uptake remains one of the major concerns of current mycorrhiza research.

2.2.2. Arbuscular Mycorrhizal Nitrogen Up-Take

Similarly to phosphate, nitrogen is a major limiting nutrient of plant growth, especially during the production of cereal crops. Consequently nitrogen additions also feature heavily in modern high-input agricultural systems. Nitrogen is available in the soil in the form of ammonium (NH4+) and nitrate (NO3–). Although the concentration of ammonium in the soil is 10–1,000 times lower than that of nitrate, ammonium is the preferential form of nitrogen absorbed when plants are subjected to nitrogen deficiency (Gazzarrini *et al.*, 1999) or grown in water-logged or acid soils (Marschner, 1995). Ammonium has low mobility in the soil and a depletion zone is formed in the vicinity of the roots in a fashion similar to that observed with phosphate. The extraradical mycelium of mycorrhizal fungi can absorb ammonium (Johnson *et al.*, 1997) nitrate and amino acids (Hodge *et al.*, 2001) and the role of mycorrhizal nitrogen delivery is becoming increasingly recognized (Cruz *et al.*, 2007). The majority of nitrogen is thought to be taken up in the form of ammonium (Toussaint *et al.*, 2004).

Arginine is by far the most abundant amino acid in the extraradical mycelium and is thought to be the major form of translocated nitrogen (Jin *et al.*, 2005). Within the extraradical mycelium, ammonium is thought to be first combined with glutamate to form glutamine by the enzymes of the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle (Breuninger *et al.*, 2004). Arginine can then be readily synthesized from glutamine by the enzyme argininosuccinate synthase (Cruz *et al.*, 2007). Having been translocated to the intraradical hyphae, arginine is broken acterized from *Glomus intraradices* (Lopez-Pedrosa *et al.*, 2006) down by ornithine aminotransferase and urease to release free ammonium. Both of these enzyme activities have been shown to be higher in the intraradical hyphae than in the extraradical mycelium (Cruz *et al.*, 2007). Additionally, putative mycorrhiza induced nitrate transporters have been identified in tomato, medic and rice that could play a role (Hohnjec *et al.*, 2005). There is also the possibility for passive ammonia uptake across the peri-arbuscular

membrane, perhaps facilitated by the presence of aquaporin proteins (Uehlein *et al.*, 2007).

2.2.3. AM Fungi and Alleviation of Soil Heavy Metal Stress

Some heavy metal elements such as Cu, Fe, Mn, Ni and Zn are essential for normal growth and development of plants. These metals are required in numerous enzyme-catalyzed or redox reactions, in electron transfer, and have structural function in nucleic acid metabolism (Gohre and Paszkowski,2006). In contrast, metals like Cd, Pb, Hg, and As are not essential (Mertz,1981) and may be toxic to plants at very low concentrations in soils. AM fungi are significant in the remediation of contaminated soil as accumulation (Jamal *et al.*, 2002). The external mycelium of AM fungi allows for wider exploration of soil volumes by spreading beyond the root exploration zone (Khan *et al.*, 2000), thus providing access to greater quantities of heavy metals present in the rhizosphere. Higher concentrations of metals are also stored in mycorrhizal structures in the root and in fungal spores. AM fungi can also increase plant establishment and growth despite high levels of soil heavy metals due to improved nutrition (Taylor and Harrier, 2001), water availability (Auge, 2001), and soil aggregation properties (Kabir and Koide, 2000) associated with this symbiosis. AM fungi occur in the soil of most ecosystems, including polluted soils. By acquiring phosphate, micronutrients and water and delivering a proportion to their hosts they enhance the host nutritional status. Similarly, heavy metals are taken up via the fungal hyphae and can be transported to the plant. Thus, in some cases mycorrhizal plants experience enhanced heavy metal uptake and root-to-shoot transport while in other cases AM fungi contribute to heavy metal immobilization within the soil. The result of mycorrhizal colonization on remediation of contaminated soils depends on the plant– fungus–heavy metal combination and is influenced by soil chemical and physical conditions. The significance of AM fungi in soil remediation has been recognized (Khan, 2005).

2.2.4. Plant Nutrition and Water Relations

A substantial biomass component in many ecosystems is resultant influence of mycorrhizal associations (Allen, 1991). Mycorrhizae tend to be the largest component in the ecosystem primarily because both the fungi and the associated roots are turned over rapidly. Mycorrhizae as well dictate nutrient cycling rates and patterns by altering host plant resource acquisition and plant production. Odum and Biever (1994) have catalogued six main pathways in ecosystems through which the nutrients are recycled from plants, viz. grazing, seed consumption, feeding on nector, loss of soluble exudates, active extraction by parasitic and mutualistic organisms, and decomposition of plant structures. In this background, mycorrhizae play a vital role in last three categories in capturing nutrients (Jalali, 2001). Mycorrhizae therefore link the biotic and geochemical parts of the ecosystem. Their contribution in sustainable ecosystem is well recognized. With this recognition, the management of symbiotic fungal populations would become a potential tool for overall crop health and resultant sustainability.

Table 2.1. Summary of some of the potential effects of agricultural management practices on AM fungi in the field. (Atkinson *et al.*, 2002).

Factors	Potential effect on			
	Effectivity of symbiosis	Host pressure	Spore populations and viability	Extra radical hyphae
Crop choice	–	✓	–	–
Variety choice	✓	–	–	✓
Sequence (rotation)	✓	✓	✓	✓
Tillage	–	✓	–	✓
Fallow period	–	✓	✓	–
Organic farming systems	✓	✓	✓	✓
Inoculants	✓	–	✓	–
Fumigants	✓	–	✓	–
pH changes	✓	–	✓	✓
Phosphorus	✓	–	–	–
Manures and crop residuse	✓	–	–	–

Mycorrhizal fungi may not only enhance soil-plant transfer of nutrient, but may also be instrumental in movement of nutrients between plants (Eason *et al.*, 1991). Read *et al.* (1989) demonstrated through the use of $^{14}CO2$ that carbon moves freely between plants connected by mycorrhizal mycelium. Arbuscular mycorrhizal fungi play an important role also in the water economy of plants. These associations improve the unsaturated hydraulic conductivity of the roots either by modifying root morphology and root anatomy or indirectly by hormonal and structural changes in the host plant. These improvements are the factors contributing towards better uptake of the water by the plants. It has been suggested that the AM fungi help the plants in better absorption of water by the roots resulting on a better performance (Kehri and Chandra, 1990) and by exploring water in wider zones of soil (Safir *et al.*, 1971, 1972). It has been noted that the mycorrhizal plants show a better survival than non-mycorrhizal ones in extremely dry condition (Allen *et al.*, 1981). It appears that the most established benefits from mycorrhizal fungus to the host plant is through the widespread mycelial network which penetrates deeper and wider in the soil in search of water and nutrients thereby widening the zone of activity.

2.2.5. AM Fungi and Plant Disease Control

Plant diseases can be controlled by manipulation of indigenous microbes or by introducing antagonists to reduce the disease-producing propagules (Linderman, 1992). AM fungi and their associated interactions with plants reduce the damage caused by plant pathogens (Harrier and Watson, 2004). With the increasing cost of pesticides and the environmental and public health hazards associated with pesticides and pathogens resistant to chemical pesticides, AM fungi may provide a more suitable and environmentally acceptable alternative for sustainable agriculture and forestry. The interactions between different AM fungi and plant pathogens vary with the host plant and the cultural system.Moreover, the protective effect of AM inoculation may be both systemic and localized. Plant parasitic nematodes occur in agricultural soils worldwide, and most crops are susceptible to damage by these parasites. Nematode parasitism on host plants may cause up to 50% yield losses, and these losses may be aggravated when the plant is predisposed to other pathogens.The physiological and biochemical changes caused by mycorrhizal fungi in the host plant generally reduce the

severity of nematode diseases(Dehne, 1982).An increase in lignin and phenols in mycorrhizal plants was observed and was associated with reduced nematode reproduction (Singh *et al.*, 1990). Suresh and Bagyaraj (1984) reported that AM inoculation increased the quantities of sugars and amino acids in plant tissue which may be responsible for the reduction of nematode infestation. However, inferences based on the absence of galling on segments of roots and split root experiments argue for a more localized effect (Fitter and Garbaye, 1994). Nematodes in mycorrhizal plants were smaller and took longer times to mature to the adult form (Suresh, 1980). AM fungi are dependent on the host as a carbon source and 4–20% of the host net photosynthate is transferred to the AM fungus (Smith and Read, 1997). There is much information to support the competition for host photosynthates and this phenomenon may have an important role in interactions with endoparasitic nematodes because of the obligate nature of both organisms for host-derived compounds (Azcon-Aguilar and Barea, 1996). AM fungal colonized plants differ from non-mycorrhizal roots in terms of microbial community composition of the rhizosphere (Marschner *et al*., 2001). These differences have been attributed to alterations in root respiration rate and quality and quantity of exudates. Plant root systems colonized by AM fungi differ in their effect on the bacterial community composition within the rhizosphere and rhizoplane. The number of facultative anaerobic bacteria, fluorescent pseudomonads, *Streptomyces* species and chitinase producing actinomycetes differ depending on the host plant and the isolate of AM fungus (Harrier and Watson, 2004).

2.2.6. Effects of AM Fungi on Drought and Salinity Stress

Drought stress is a major agricultural constraint in the semi-arid tropics. AM fungi symbiosis can protect host plants against detrimental effects caused by drought stress (Ruiz-Lozano et al., 1999).Quilambo (2000) reported that inoculation with an indigenous inoculant resulted in increased leaf and root growth and prevented the expected increase in root to shoot ratio and root-weight ratio that are normally observed under phosphorus deficient and drought stress conditions in peanut. In watermelon (*Citrullus lunatus* Thunb.) mycorrhizal colonization was found to improve not only the plant yield and water use efficiency, but also the quality of the fruit (Kaya et al., 2003). Several mechanims have been proposed to explain the

protection of AMF symbiosis, such as changes in plant hormones (Goicoechea et al,1995) increased leaf gas exchange and photosynthetic rate (Ruiz-Lozano et al, 1996a); direct hyphal water uptake from the soil and transfer to the host plant, enhanced activity of enzymes involved in anti-oxidant defence (Ruiz-Lozano et al, 1996b), nitrate assimilation (Ruiz-Lozano and Ázcón, 1996), enhanced water uptake through improved hydraulic conductivity and increasing leaf conductance and photosynthetic activity (Dell-Amico et al, 2002), osmotic adjustment and changes in cell-wall elasticity (Sanchez-Diaz and Honrubia,1994). Often mycorrhizal improvement of drought tolerance occurs via drought avoidance. It can be a function of the often observed improved acquisition of phosphorus, nitrogen and other growth promoting nutrients by AMF plants (Augé et al., 2001). According to Fitter (1988) the influence of AMF on water uptake and transport may be a secondary consequence of enhanced host phosphorus nutrition, although these effects are not consistent (Davies et al., 2002). AMF can also reduce the impact of environmental stresses such as salinity (Ruiz-Lozano et al., 1996a). In *Azadirachta indica* with increased salinity level, there was a decrease in percent of root infection by AMF (Pande and Tarafadar, 2002).

2.2.7. Indirect Contribution of AM Fungi in Soil Aggregation and Plant Growth

Mycorrhizal symbiosis has evolved to assist plants in colonizing the land. In the environment of early Earth, ecological pressures resulted in a highly efficient symbiotic relationship where plants traded photosynthetic carbon for fungally-acquired nutrients and water. The formation of a biomolecule such as glomalin would have served as an evolutionary advantage to the fungus. Glomalin is a glycoprotein produced on hyphae of AM in the soil. Originally, glomalin production might have arisen to protect fungal hyphae from losses of water or nutrients when being carried from microsites in the soil back to the plant, from fluctuations in turgor pressure due to wet/dry cycles, and from decomposition by microbial attack. The indirect or ‘secondary’ impacts of glomalin on the formation and stabilization of soil aggregates further improved the efficiency of the symbiotic relationship and the growth environment (Rillig and Mummey, 2006).Modern agricultural practices have placed new pressures on plant mycorrhizal symbiosis. Tillage practices physically disrupt soil aggregates and AM hyphal networks. This action

deteriorates soil structure, lessens fertility and nutrient cycling ability, and results in more carbon (C) allocation within the fungal hyphae to reestablishing these networks and less C to glomalin formation (Nichols and Wright, 2004). No-tillage practices along with continuous cropping systems (by eliminating fallow periods and/or growing cover crops), using mycorrhizal host crops, and reducing synthetic inputs (especially P), enhance the plant-mycorrhizal symbiotic relationship (Nichols and Wright, 2004; Roldan *et al.*, 2007). These practices also increase the percentages of water-stable aggregates within the soil by increasing hyphal length, root and microbial exudates in the mycorrhizosphere, and allocating more C to glomalin production. In addition, higher levels of C sequestration are possible in these systems, since not only is C being allocated below-ground to hyphal networks and formation of the highly stable glomalin molecule, but organic matter occluded within aggregates appears to have a turn over time double that of free organic matter (Nichols and Wright, 2004; Roldan *et al.*, 2007). Therefore, effective management of soil organisms and, as a consequence, agricultural systems, will maintain a consistent supply of plant-available nutrients to meet the demands of food, feed, fiber and biofuels production for a growing world population while maintaining optimal ecosystem function.

2.2.8. Mycorrhizal Fungi and Sustainable Agriculture

Mycorrhizal fungi, particularly AM, are ubiquitous in soil and create symbiotic associations with most terrestrial plants including agricultural crops, cereals, vegetables, and horticultural plants. In agriculture, several factors such as host crop dependency to mycorrhizal colonization, tillage system, fertilizer application, and the potential of mycorrhizal fungi inoculation affect plant response and plant benefits from mycorrhizae. Interest in AM fungi propagation for sustainable agriculture is increasing due to its role in the promotion of plant health, and improvements in soil fertility and soil aggregate stability. These fungi can be utilized effectively for increasing yields while minimizing use of pesticides and inorganic fertilizers.To improve crop production in infertile soils, chemical fertilizers have been intensively used, organic matter is incorporated and soil management technologies such as fallow or legume cultivation have also been to advance soil conditions, enhance soil biological activity and optimize nutrient cycling to minimize external inputs and maximize the efficiency of their use (Sanchez, 1994).

This approach has been developed for soil biota management using earthworms and microsymbionts (Woomer and Swift,1994).These soil organisms may represent more than 90% of soil biological activity and thus contribute to nutrient cycling, soil fertility and symbiotic processes in the rhizosphere. Soil fungal diversity and activity have not been adequately studied and understood (Hawksworth, 1991). Mycorrhizae represent an important group because they have a wide distribution, and may contribute significantly to microbial biomass and to soil nutrient cycling processes in plants (Harley and Smith, 1983). Mycorrhizal associations are used beneficial to plants and thus crop productivity for sustainable agriculture(Bethlenfalvay,1992). They improve nutrient uptake, especially P, and also uptake of micronutrients such as zinc or copper; they stimulate the production of growth substances and may reduce stresses, diseases or pest attack (Smith and Read, 1997). For appropriate use of this technology, it is necessary to select the best inoculation adapted to the specific limiting environmental factors for crop productivity.

2.2.9. AM Fungal Communities and Grain Production

With population increase, urban sprawl and the growing interest in the use of biofuels, significant pressures are occurring on some of the highest quality agricultural soils in many nations. Growth of grain and oilseed crops such as barley, corn, soybean and wheat have been an important part of the agricultural economy for years and the continuous increases in demand and prices have led farmers to apply highly intensive agricultural management practices, with the aim of increasing crop productivity. Tillage, crop rotation, fallows, changes in plant cultivars and pesticide application are often used with broad acre field crops, and all these practices influence the surrounding environment (Carter and Campbell, 2006). Fertilizer use represents a common agricultural management practice, but a growing body of evidence has demonstrated an array of negative impacts on ecosystems from their use. No matter which form of fertilizer is applied (organic or mineral), conventional farming generates large N and P surpluses, which can lead to N leaching through the soil profile and P losses in runoff (Brady and Weil, 2002). Not only is there a high financial cost to farmers associated with this loss, but the phenomenon also resulted in soil contamination. In addition, excess fertilizer inputs can be a major threat to aquatic ecosystems through surface and

groundwater degradation (Kirchmann and Thorvaldsson, 2000). Recently, fertilizer runoff from agricultural fields was emphasized among the causes of excessive cyanobacterial growth and increasing of potentially harmful blooms leading to restricted access to lakes. Low-input agricultural systems have gained attention in many Industrialized countries due to increased interest in the conservation of natural resources, reduction of environmental degradation, and the escalating costs. Conventional farming systems using lower application rates of fertilizers and pesticides have been developed, but are used only minimally in North American grain production, perhaps due to insufficient understanding of agricultural soils dynamics (Ryan and Graham, 2002). Numerous biological, chemical and physical factors influence soil quality. Among them, rhizosphere microbial communities have been shown to directly affect soil fertility by carrying out essential processes that contribute to nutrient cycling, and enhancing soil structure and plant growth and health (Miransari *et al.*, 2007). The extent to which these communities interact is thus of great importance and involves phenomena such as hormone production, enhancement of nutrient availability, and decrease of root diseases. Arbuscular mycorrhizal symbioses have been shown to benefit growth of many field crops in large part due to the extensive hyphal network development in soil, more efficient exploitation of nutrients, and enhanced plant uptake (Smith and Read, 1997). AM symbiosis also increases resistance to biotic and abiotic stresses and reduces disease incidence, representing a key component of sustainable agriculture (St-Arnaud and Vujanovic, 2007). Appropriate management of mycorrhizae in agriculture should ultimately result in a substantial reduction in chemical use and production costs. Soils generally contain indigenous AM fungi that colonize plant roots (Covacevich *et al.*, 1995). The growth enhancement and P uptake of plants colonized by AM fungi is a well-known process (Jeffries *et al.*, 2003). Not all plants are dependent on mycorrhizal associations (Hetrick *et al.*, 1993) however, most increase in yield following inoculation with AM fungi (Al-Karaki *et al.*,1998) particularly in low-P soils (Rubio *et al.*, 2003). With the current tendency for reduced use of agrochemicals, research is being directed at crop yield improvement and yield sustainability. The efficient use of AM fungi may allow for the attainment of acceptable yield levels with minimum fertilizer dose, while also reducing costs and environmental pollution risk (Covacevich *et al.*, 2007).

2.3. Limitations of AM Fungi Inoculums Production

AM fungi are rarely found in commercial nurseries due to the use of composted soil-free media, high rates of fertilizer application and regular application of fungicide drenches. The potential advantages of AM fungi in horticulture, agriculture, and forestry are not perceived by these industries as significant. This may be due in part to inadequate methods for large-scale inoculum production. Monoxenic root-organ in vitro culture methods for AMF inocula production have also been attempted by various workers (Mohammad and Khan, 2002) but these techniques, although useful for the study of physiological, biochemical, and genetic relationships, have limitations in terms of producing inoculums of AM fungi for commercial purposes. Pot cultures in pasteurized soils have been the most widely used method for producing AM fungi inoculum but are time consuming, bulky, and often not pathogen-free. To overcome these difficulties, soil-free methods such as soil-less growth media, aeroponics, hydroponics and axenic cultures of AM fungi have been used successfully to produce AMF-colonized root inoculation (Mohammad and Khan, 2002). Substrate-free colonized roots produced by these methods can be sheared and used for large-scale inoculation purposes. Cropping sequences, fertilization, and plant pathogen management practices affect both AM fungal propagules in soil and their effects on plants (Bethlenfalvay and Linderman, 1992). In order to apply AM fungi in sustainable agriculture, knowledge of factors such as fertilizer inputs, pesticide use, and soil management practices which influence AM fungi is essential (Bethlenfalvay and Linderman, 1992). In addition, efficient inoculants should be identified and employed as biofertilizers, bioprotectants, and biostimulants for sustainable agriculture.

2.4. In Vitro Mycorrhization

Large numbers of *in vitro* studies have been carried out to evaluate the factors that influence mycorrhization. Under natural conditions, interactions of biotic and abiotic factors make the interpretation of the results difficult. The methods of axenic synthesis are object of criticism because working under conditions where (1) interacting factors are eliminated, (2) carbon sources are provided to allow fungal growth before the infection sets in, and (3) substrates are sterilized, may change the efficiency and type of infection (Piché and Peterson, 1988).

In parallel with *in vitro* studies, non axenic studies have been made (Piché *et al.*, 1982). It was possible to demonstrate that there are no significant differences between mycorrhizae synthesized under axenic and non axenic conditions (Piché and Peterson, 1988) other than the time of infection (Duddridge and Read, 1984a). The axenic system studied had a time of infection starting at 3 weeks and completed by weeks 6 to 8, while in natural soils, the association was retarded until 11 to 19 weeks. Morphological differences between axenic and non axenic synthesized mycorrhizae exist only when high sucrose levels are used (Duddridge and Read, 1984b). Under these conditions the host-fungus interface is changed and there is callose deposition at the cells walls in response to host infection.

Non axenic systems allow detailed studies of the root colonization by the fungus (Fortin *et al.*, 1983). Fungus connection to the root epidermis is due to the root polysaccharides secretion (Nylund, 1980). The translocation of photosynthetic products to the root increases the concentration of carbon compounds in root exudates. These are mainly amino acids, proteins, carbon compounds, organic acids and plant growth regulators. Mineral balance and plant growth regulators concentrations, directly control cell permeability and the mechanism of fungus adhesion to the roots when mycorrhization takes place (Barea, 1986).

Axenic and non axenic mycorrhizal syntheses mainly differ in the time and degree of infection (Duddridge and Read, 1984a). These findings validated the use of *in vitro* mycorrhization techniques. Mycorrhizas obtained by different methods of *in vitro* synthesis had mantles and hartig nets hyphae penetrating between cortical cells may vary with substrate and the synthesis method used.

The difference in ratio between dry weights of roots and shoots is more related to plant dimension than to the colonization rate (Bougher *et al.*, 1990). The total number of short roots of mycorrhizal plants is higher than for nonmycorrhizal ones, exhibiting completely altered root morphology by the association with the mycorrhizal fungi. The number of roots per unit length and per unit weight was higher for mycorrhizal root systems (Brundrett *et al.*, 1996). Root colonization by mycorrhizal fungi can result in lower plant growth rates if fungus compatibility, nutrient availability, light intensity or temperature is not suitable for plant development (Conjeaud *et al.*, 1996; Smith and Read, 1997). Decrease of growth rates is expected when a symbiont depends on the others to obtain the carbon compounds for survival, and the other depends on the essential mineral nutrients provided by the former for its growth and photosynthesis. Decrease in growth is also expected under light conditions limiting photo-synthesis (Conjeaud *et al.*, 1996), nutrient availability in soil, conditioning plant growth but not colonization intensity (Colpaert *et al.*, 1992; Smith and Read, 1997).

Son and Smith (1988) observed an increase in plant growth after colonization of plants under high PAR (photosynthetic active radiation) and a decrease in growth of plants colonized under low PAR, independently of the levels of P availability. When nutrient availability allows fungal growth and there is no light or temperature limitation, fungal growth can require large amounts of carbon compounds conditioning plant growth (Colpaert *et al.*, 1992).

2.4.1. In Vitro Mycorrhization of Micropagated Plants

Micropropagated plants are adversely affected by water stress, either due to low absorption capacity of their roots or due to stomata deficient regulation of water loss (Flick *et al.*, 1983). Acclimation of micropropagated plants corresponds to a transition period when roots become adapted to a substrate with less available nutrients, and to an autotrophic condition. At this stage, the presence of mycorrhizae could increase the availability of limiting nutrients such as phosphorus (P) and nitrogen (N), facilitating the absorption. Water stress can be responsible for the low survival of many micropropagated woody plant species during the acclimation process and *C. sativa* is one of these species.

Micropropagated plants develop under high moisture and low lighting conditions, often with low lignifications levels and decreased functionality of the root systems that cause low survival rates to weaning. Mycorrhization of micropropagated plants before acclimation increases survival, enhancing the functionality of the root system and the mineral plant nutrition (Díez *et al.*, 2000). Similarly, *in vitro* mycorrhization of micropropagated plants can be used to increase survival and growth during *ex vitro* weaning (Nowak, 1998). Mycorrhization trials have been made with different micropropagated plant species: pine (Normand *et al.*, 1996), birch (Grellier *et al.*, 1984), poplar (Heslin and Douglas, 1986), eucalyptus (Tonkin *et al.*, 1989), oak (Herrmann *et al.*, 1998), chestnut (Pais, 2005), cork oak (Díez *et al.*, 2000). These trials were performed as an effort to make micropropagation a sustainable propagation method for plant species recalcitrant to conventional propagation, increasing *in vitro* plant performances.

Herrmann *et al.* (1998) used an *in vitro* mycorrhizal system of *Quercus robur* micropropagated plants, intending to develop a method to analyze the mycorrhization of forest species without the constraints of the methods using seedlings. Genetic heterogeneity of seedlings (reflected in different germination times), seedling vigour and asynchronous development are only some of these constraints. These trials were made to work with (1) genetically uniform plants deprived of cotyledons, to function as older plants, (2) with selected material, to warranty the uniformity of repetitions, and (3) with a mycorrhizal system that allows following the development along the trials, in order to characterize mycorrhizal effects on plant morphology.

Castanea sativa micropropagated plants were studied along 90 days of plant-fungus association *in vitro*, after preliminary studies on plant-fungus compatibility with four fungi species (Martins *et al.*, 1996).
The studies included: (1) development of mycorrhizal morphological structures (mantle and Hartig net) along 90 days; and (2) mycorrhizal influence on growth rates (heights, stem diameter, length of major root, total plant length, fresh weights and dry weights).

2.5. AM Fungi Management and Perspectives

The main areas in which the benefits of introducing inoculant AMF into a plant growth system will accrue, are those in which they are lacking indigenous inoculum of AMF. These include sterilised soils or post *in vitro* plant micro-propagation, buried, extremely fertilised, degraded areas (Dodd, 2000) or rooting of pepper cuttings (Thanuja et al., 2002). It is widely accepted that plants with highly branched root system (Gramineae) are less mycotrophic (less dependent on the fungi for normal growth) than those with coarser roots (e.g. cassava,onion). Root branching determines plant dependence on the symbiosis. Soils under low-input management show higher VAM fungus spore populations than soils under conventional management (Galvez et al, 2001).

Early colonizing sand dunes species are nonmycorrhizal, whereas the later seral grasses are colonized with AMF (Nicolson, 1960). Survival of AMF in soil may be affected by the presence or absence of crops and by the crop being grown (Troeh et al., 2003). The same author also reported differences related to crop succession. Fallow on fields had less spores than cultivation of corn followed by soybean, independently of the cultivars of corn or soybean. In cowpeas, inoculation and amendment with organic manure resulted in increased growth and yield (Muthukumar and Udayan, 2002). Inoculation with AMF and addition of composted grape pomace was beneficial to plants. This has been intrepreted as the result of mycorrhizal fungus enhancing P uptake through extraradical hyphae. Such uptake increases nutrient-use efficiency (Linderman and Davis, 2002).In some cases, composted municipal waste addition and mycorrhizal inoculation were effective tools in programmes for revegetation of shrub species in semiarid mediteranean areas (Caravaca et al., 2003a).

The use of native mycorrhizal as a potenntial source of AM inoculum was considered a preferential strategy for ensuring the successful re-establishment of native shrub species in semi-arid degraded soil (Caravaca et al., 2003b). Bell et al. (2003) found that the susceptibility of Acacia seedlings to colonization by AMF appeared to be seasonal. Colonization increased with increasing daytime temperatures and and daylength.Despite the beneficial effects of AMF, their

activity may be greatly limited by soil fumigation, non-responsive plant varieties, or rotations based primarily on non-mycorrhizal crops or crops of low AMF dependency. Salicylic acid contents in the plant reduced mycorrhization, suggesting that enhanced salicylic acid levels in plants delay AMF root colonisation. Although salicylic acid affect AMF root colonization, it has no effect on the potential of plants to be colonized by AMF (Medina et al., 2003). Manipulation of agricultural systems to favour AMF colonization must occur only if there is a clear evidence that AMF make a positive contribution to yield or are vital for maintenance of ecosystem health and sustainability (Ryan et al., 2002).

2.6. Techniques to Observe AM Fungi

Most observations of VA mycorrhizae are based on the use of Trypan blue (0.05%) to stain fungi in host roots (Phillips and Hayman, 1970). In this technique the mycorrhizal roots are treated in hot 10% KOH that first removes the host cytoplasm and then the nuclei. After the roots are neutralized in a weak acid wash, they are stained in Trypan blue. The stain penetrates deeply and usually stains the hyphae but does not deeply stain the plant tissue. This technique generally is satisfactory for agronomic crops and many other species. Kormanik et al. (1980) described an acid fuschin technique in which clearing and staining of many plant root samples for observation can be accomplished. This technique produces more satisfactory results in plants with heavy pigmented roots. In 1984 Brundrett et al. developed another technique in which chlorazol black E allowed the detection of the developmental stages of VAM fungi in the host roots with more clarity than other techniques. There are problems with all these techniques.

All the techniques are destructive to the sample and involve time-consuming procedures. Different taxa are stained with different intensities in the same roots. Many species of *Gigaspora* and *Scutellospora* stain intensely with Trypan blue, regardless of the host species (Morton, 1988). *Acaulospora trappei* exhibits intermediate staining in Trypan blue (Abbott, 1982). *Glomus dimorphism, G. fecundisporum, G. leptoticum, G. maculosum, G. occultum, G. tortuosum, Acaulospora myriocarpa*, and *Entrophospora schenckii* are not stained or are weakly stained in Trypan blue (Morton, 1985). The variation in staining may leave

regions unstained and cause inaccurate estimations of fungal colonization of a root. Ames et al. (1982) developed a nondestructive approach to estimate fungal metabolic activities in structures within and outside the host roots. This technique depends on using fluorescein diacetate (FDA) as a non-polar molecule that is taken up by the fungus. If the proper enzymes are present, FDA is hydrolyzed, and fluorescein accumulates in the cell. Fluorescein, when excited with ultraviolet (UV) light (450-490 nm), becomes fluorescent and emits at 520- 560 nm. The problem with this technique is that much of the hyphae, vesicles, and intraradical spores are not visible. A further problem is that suberized or lignified root tissue may occlude the fungal structures and autofluorescence.

2.7. Vegetables

Vegetables play an important role in providing 91% of domestic consumption food. The climate variability in Palestine (west bank and Gaza strip) allows production of vegetables all year round, also with the current use of greenhouses in the coastal and semi-coastal areas. Open field vegetables are the most common pattern of planting covering about 9 thousand hectares, which is 70% of the total area devoted for vegetable growing. The most common vegetable crops are tomatoes, cucumbers, watermelon and squash. (Aljabi,1995).

2.7.1. Summer Squash Plant

A summer squash (*Cucurbita pepo*) is an annual plant that trails for several feet and forms a compact plant of three feet (90 cm) spread. Summer squash fruit have a cylindrical shape about 12 inches (30 cm) long and 5 inches (13 cm) in diameter. They may be trailing or bushy with green, yellow, white or striped skin. Summer squash are best grown during warmer seasons with ideal temperatures between 64 and 81 degrees F (18 to 27 degrees C). (Badifu and Ogunsua ,1991).

2.7.1.1. Classification

Genus *Cucurbita* of the family *Cucurbitaceae* widely cultivated as vegetables and for livestock feed. The principal species are *C. maxima* and certain varieties of *C. pepo.* Taxonomy of Summer Squash plant (Berenyl, 1998):

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Dilleniidae*
Order	*Violales*
Family	*Cucurbitaceae*
Genus	*Cucurbita*
Species	*Cucurbita pepo*

2.7.1.2. Special Features

Summer squash is generally insect-pollinated; however, during colder seasons, if fruits are not setting, it may be necessary to hand pollinate. The female flower has a tiny bump (the embryonic fruit) behind the petals that the male flower lacks making it easy to distinguish between them. (Badifu and Ogunsua ,1991).

2.7.2. Watermelon Plant

Watermelon (*Citrullus lanatus*) is an annual herb with long (up to 10 m) stems lying or creeping on the ground, with curly tendrils. Leaves are 5-20 by 3-19 cm, and hairy, usually deeply palmate with 3-5 lobes, on 2-19 cm long petioles. Male flowers on 12-45 mm long pedicels. Flowers 1-2.5 cm long, pale green. Flowers monoecious, solitary, on pedicels up to 45 mm long; with 5 shortly united petals, pale green. Fruit of wild plants 1.5-20 cm in diameter, subglobose, greenish, mottled with darker green; of cultivated plants up to 30x60 cm, subglobose or ellipsoid, green or yellowish, evenly coloured or variously mottled or striped. Fruits vary considerably in morphology. Whereas the fruits of the wild Kalahari form are small and round, the cultivated forms are large oblong fruits. In addition, they vary from pale yellow or light green (wild form) to dark green (cultivars), and with or without stripes; the pulp varies from yellow or green (wild forms) to dark red (cultivars)(Jeffrey 1978).

2.7.2.1. Classification (Berenyl,1998):

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Dilleniidae*
Order	*Violales*
Family	*Cucurbitaceae*
Genus	*Citrullus*
Species	*Citrullus lanatus*

2.7.2.2. Growth Requirements

Citrullus lanatus grows on well drained soil and seeds require soil temperatures of 70-95 F to germinate. Root growth is impeded by compacted soil. *Citrullus lanatus* withstands drought better than most melons. (Jeffrey 1978).

Chapter 3

Materials and Methods

3.1. Materials

3.1.1. Fungi

In order to get the largest proportion of our target fungus and make sure it is symbiotic, 30 seedlings of summer squash plants were planted in sandy soil with little quantity of organic matters. The area where we planted the seedling is relatively distant from agricultural lands where the chemical fertilizers frequently used, so we can avoid the arrival of these materials to our seedling. After 30 days of culture, seedlings were uprooted and prepared for the isolation of the fungus.

3.1.2. Plants

Two types of plants of the same family were selected, watermelon (*Citrulus latanus*) and summer squash (*Cucurbita pepo*). These seasonal plants are grown widely in the Gaza Strip, which rely on chemical fertilizers. 120 seedlings of each species planted inside a mini-green house.

3.1.3. Chemicals

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**
• **HCL**	**Frutarom – Israel**
• **TRYPAN BLUE**	**Biological industries – Israel**
• **LACTIC ACID**	**Riedel – deHaen - Germany**
• **GLYCEROL**	**Frutarom – Israel**
• **MMN MEDIA**	**Prepared manually**
• **ETHYL ALCOHOL**	**Frutarom – Israel**
• **CEMICAL FERTILIZER SOLUTION**	**Fertilizers and chemicals _ Israel**

3.1.4. Equipments

The main equipments that were used are listed in table 3.2.

Table 3.2. A list of the main equipments used in this work.

Instruments	Manufactures
Analytical balance	**Napco - China**
Autoclave	**N- Bioteck – Korea**
Compound microscope	**LW- Scientific - USA**
Dissecting microscope	**LW- Scientific - USA**
Oven	**N- Bioteck – Korea**
Safety cabinets	**N- Bioteck – Korea**
Thermometer	**Hauhai - China**
Vortex	**LW- Scientific - USA**

3.2. Methods

3.2.1. Isolation and Multiplication of Fungus

In order to obtain a pure culture of fungus from mycorrhizal roots of our seedlings, we proceed as the following: Roots were separated from shoots and washed with running water and disinfected by different concentrations of Sodium hypochlorite ranging from 2 to 10% during 1 to 10 minutes and then washed again with sterile water. All these steps took place in an a xenic conditions. The roots then cultured in a MMN media, the media components as shown in the table 3.3.

Table 3.3. Culture Media (MMN) (Marx 1969)

$CaCL_2$	0.05 g	Thiamine HCL	100m
NaCL	0.025 g	Malt Extract	3g
KH_2PO_4	0.5 g	Sucrose	10g
$(NH_4)_2\ HPO_4$	0.25	Bacto – agar (optional)	15 g
$MgSO_4\ 7H_2O$	0.15	H_2O	1000ml
$FeCL_3$	1.2 ml		

After 7 days of culture, we obtained a heavy growth of fungus mycelia in Petri dishes, the fungus mycelia were multiplied and sub cultured in many other dishes up to obtaining pure cultures.

3.2.2. In Vitro Application of Plant Fungi Symbiosis

The experimental soils were prepared by mixing three parts of vermiculite with one part of compost. The soil was sterilized by autoclaving. 800 ml soil samples were taken in each pot. 240 pots of soil were used for the culture of Squash and watermelons plants, 120 pots for each type. Four sets of experiments were conducted, each set consist of 30 pots with 30 seedlings. The 1st set was the control (sterilized soil without any kind of fertilizer). The 2nd set, roots directly inoculated with fungus mycelia. The 3rd set, roots treated each 2 weeks with 100 ml of chemical fertilizer (Suspension of Shifah 11) (Annex 1). The 4th set, roots inoculated by injection of 10 ml/ pot (approx 200 spores) of fungus spores suspension. Fungus spores suspension (contains about 25-30 spores/ml) was prepared under sterile conditions by gently mixed 30 ml of sterile water with the mycelia taken from 1 or 2 Petri dishes (Limpens *et al*. 2004).

Watermelon and squash plants were incubated after the application of previous experiments in the green house for two months (from March to May 2010). After the end of the incubation, samples were taken from the roots and were dug very carefully to get most of the finer roots. Root samples were cleaned, cut into 1 cm segments (Hayman, 1974) and stained according to the method described by Phillips and Hayman (1970). The root segments were then observed under light microscope. The results were determined by comparing the difference between dry weights of mycorrhizal and non-mycorrhizal plants.

3.2.3. Statistical Analysis

Data were collected and computed by using version 17 of Statistical Package for Social Science, (SPSS). One way ANOVA was the main statistical test used in our study.

Chapter 4
Results

4.1. The Occurrences and Intensity of Roots Colonization of AMF in Species of *Cucurbitaceae* Family

Segments of plant roots treated by fungus spore suspension were cleaned and stained according to the method described by Phillips and Hayman (1970), examined under light microscope. Summer squash (*Curcurbita pepo*) and watermelon (*Citrulus lanatus*) plants were colonized by AMF as indicated by the presence of hyphae and vesicles. There were no signs of AMF colonization in the roots of control plants (Figure 4.1, Figure 4.2).

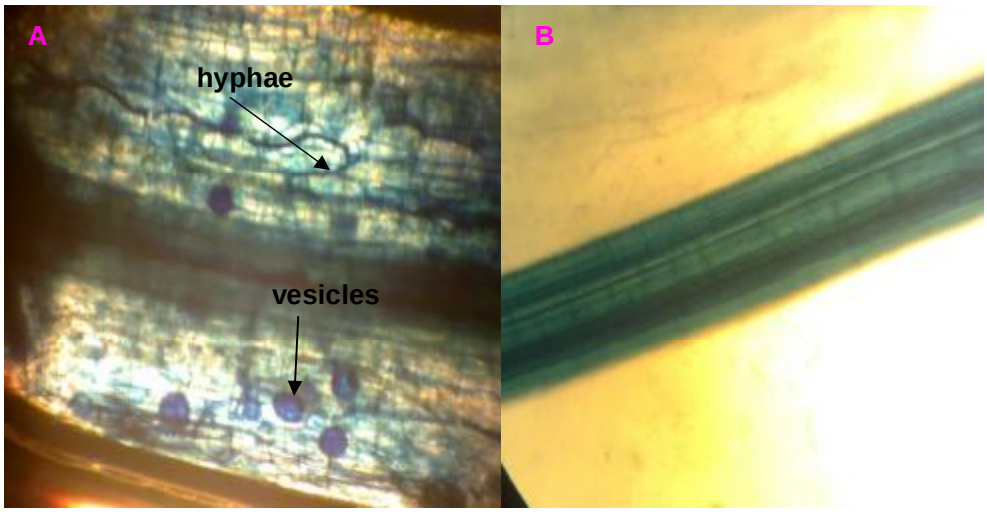

Figure 4.1. The occurrences and intensity of root colonization of AMF in summer squash plants. Roots colonization of plants treated with fungus spore suspension (A), roots of untreated control plants (B).

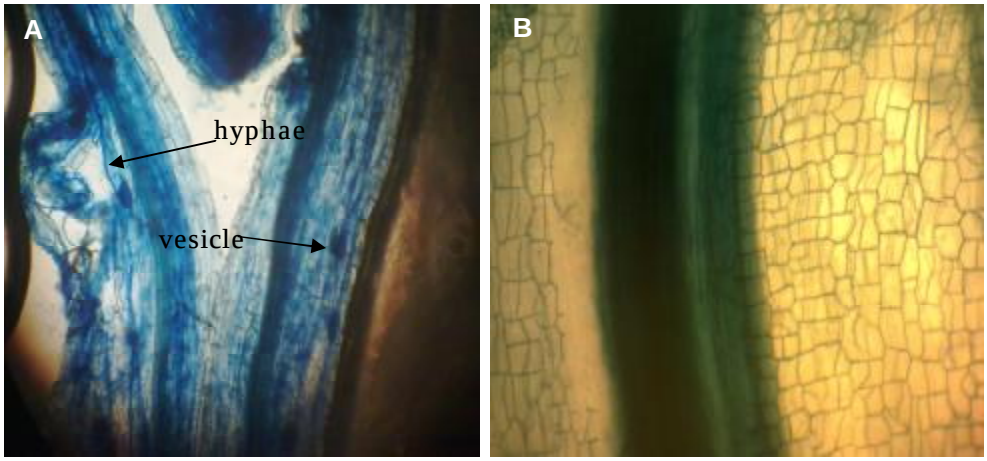

Figure 4.2. The occurrences and intensity of roots colonization of AMF in watermelon plants. Roots colonization of plants treated with fungus spore suspension (A), roots of untreated control plants (B).

4.2. Growth of Watermelon and Summer Squash Plants

The study included squash and watermelon plants treated with fungus spores suspension or without treatment as a control. As can be shown in (Figure 4.3, 4.4) respectively, the shoot growth was larger in fungus spores suspension treated plants than the control plants.

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Figure 4.3. Growth of summer squash plants. Experimental pots treatment with fungus spores suspension (k_1), control soil (k_c) for summer squash plants.

Figure 4.4. Growth of watermelon plants. Experimental pots treatment with fungus spores suspension (B_2), control soil (B_C) for watermelon plants.

4.3. Dry Weights of Summer Squash Plants

In order to be able to numerically compare the growth of fungus spores suspension treated plants versus the control and chemical fertilizers treated plants , we will consider the dry weight of the shoots or the roots together or independently. Table 4.1 shows the mean dry weight of summer squash shoots. The mean dry weight of chemically treated plants is higher than that of fungus spores suspension treated and both are higher than the control plants. The mean difference is statistically significant in the case of chemical fertilizer treatment (P value = 0.00) and fungus spores suspension (P value = 0.002) compared to control and not significant in the case of fungus mycelia directly (Table 4.2).

Table 4.1. Mean of shoot dry weight (summer squash)

Experiments	N	* Mean ± (S.D)
Fungus mycelia directly	27	2.296 **±** (0.399)
Fungus spores suspension	27	2.719 **±** (0.643)
Chemical fertilizer	27	3.112 **±** (0.399)
Control	27	2.319 **±** (0.379)

* The mean of 27 independent experiments

N: Number of samples

Table 4.2. Comparison of the shoot dry weight means for different experiments (summer squash)

(I) experiments	(J) experiments	Mean Difference (I-J)	P value
Fungus mycelia directly	Fungus spores suspension	- 0.422*	0.001
	Chemical fertilizer	- 0.816*	0.000
	control	- 0.022	0.862
Fungus spores suspension	Fungus mycelia	0.422*	0.001
	Chemical fertilizer	- 0.394*	0.003
	control	0.400*	0.002
Chemical fertilizer	Fungus mycelia	0.816*	0.000
	Fungus spores suspension	0.394*	0.003
	control	0.794*	0.000
Control	Fungus mycelia	0.022	0.862
	Fungus spores suspension	- 0.400*	0.002
	Chemical fertilizer	- 0.794*	0.000

* The mean difference is significant at the .05 level.

Table 4.3 shows the mean dry weight of summer squash roots. The mean dry weight of fungus spores suspension treated plants is higher than that of control plants and both are higher than the chemically fertilizers treated. The mean difference is statistically significant in the case of fungus spores suspension (P value = 0.003) compared to control and (P value = 0.000) compared to chemical fertilizer (Table 4.4).

Table 4.3. Mean of roots dry weight (summer squash)

Experiments	N	* Mean ± (S.D)
Fungus mycelia directly	27	0.194 ± (0.062)
Fungus spores suspension	27	0.333 ± (0.088)
Chemical fertilizer	27	0.244 ± (0.064)
Control	27	0.270 ± (0.087)

* The mean of 27 independent experiments.

N: Number of samples.

Table 4.4. Comparison of the roots dry weight means for different experiments (summer squash)

(I) experiments	(J) experiments	Mean Difference (I-J)	P value
Fungus mycelia directly	Fungus spores suspension	- 0.139*	0.000
	Chemical fertilizer	- 0.050*	0.016
	control	- 0.076*	0.000
Fungus spores suspension	Fungus mycelia	0.139*	0.000
	Chemical fertilizer	0.088*	0.000
	Control	0.063*	0.003
Chemical fertilizer	Fungus mycelia	0.050*	0.016
	Fungus spores suspension	- 0.088*	0.000
	Control	- 0.025	0.214
Control	Fungus mycelia	0.076*	0.000
	Fungus spores suspension	- 0.063*	0.003
	Chemical fertilizer	0.025	0.214

* The mean difference is significant at the .05 level.

Figure 4.5 shows the fold increase in the dry weight of shoots and roots

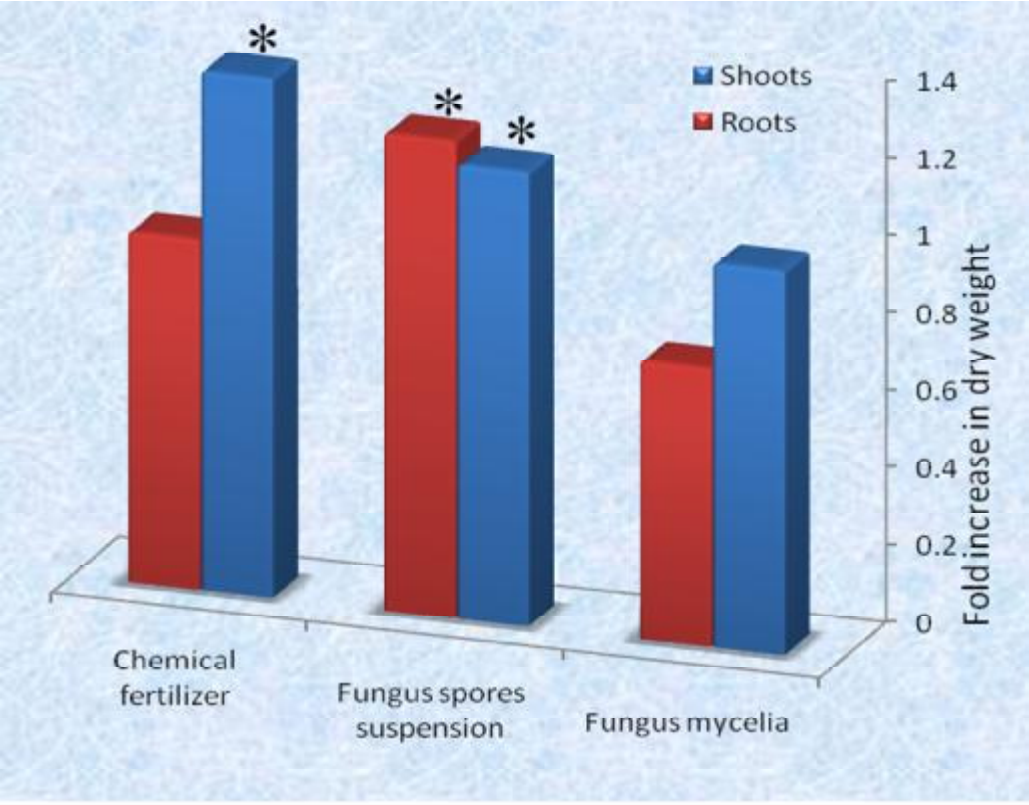

Figure 4.5. Fold increase in dry weight relative to control (summer squash). The fold increase was calculated as the ratio between the dry weight of shoots or roots of summer squash (g) of each treatment to that of the control. The star indicates statistically significant relation.

Table 4.5 shows the mean dry weight of the whole plant (shoot and root) for summer squash plant. The mean dry weight of chemically treated plants is higher than that of fungus spores suspension treated and both are higher than the control plants and fungus mycelia directly. The mean difference is statistically significant in the case of chemical treatment (P value = 0.00) and fungus spores suspension (P value = 0.005) compared to control and not significant in the case of fungus mycelia directly (P value = 0.467) (Table 4.6).

Table 4.5. Mean of dry weight for the whole plant (summer squash)

Experiments	N	* Mean ± (S.D)
Fungus mycelia directly	27	2.490 ± (0.413)
Fungus spores suspension	27	2.974 ± (0.663)
Chemical fertilizer	27	3.357 ± (0.427)
Control	27	2.589 ± (0.442)

* The mean of 27 independent experiments.

N: Number of samples.

Table 4.6. Comparison of the dry weight of the whole plant means for different experiments (summer squash)

(I) experiments	(J) experiments	Mean Difference (I-J)	P value .
Fungus mycelia directly	Fungus spores suspension	- 0.484*	0.001
	Chemical fertilizer	- 0.866*	0.000
	Control	- 0.098	0.467
Fungus spores suspension	Fungus mycelia	0.484*	0.001
	Chemical fertilizer	- 0.382*	0.006
	Control	0.385*	0.005
Chemical fertilizer	Fungus mycelia	0.866*	0.000
	Fungus spores suspension	0.382*	0.006
	Control	0.767*	0.000
Control	Fungus mycelia	0.098	0.467
	Fungus spores suspension	- 0.385*	0.005
	Chemical fertilizer	- 0.767*	0.000

* The mean difference is significant at the .05 level.

4.4. Dry Weights of Watermelon Plants.

Table 4.7 shows the mean dry weight of watermelon shoots. The mean dry weight of chemically treated plants is higher than that of fungus spores suspension treated and both are higher than the control plants. The mean difference is statistically significant in the case of chemical treatment (P value = 0.002) and fungus spores suspension (P value 0.008)compared to control (Table 4.8).

Table 4.7. Mean of shoot dry weight (watermelon)

Experiments	N	*Mean ± (S.D)
Fungus mycelia directly	28	2.826 ± (0.562)
Fungus spores suspension	28	3.004 ± (0.488)
Chemical fertilizer	28	3.068 ± (0.541)
Control	28	2.621 ± (0.508)

* The mean of 28 independent experiments.

N: Number of samples.

Table 4.8. Comparison of the shoot dry weight means for different experiments (watermelon)

(I) experiments	(J) experiments	Mean Difference (I-J)	P value
Fungus mycelia directly	Fungus spores suspension	- 0.177	0.210
	Chemical fertilizer	- 0.241	0.089
	Control	0.205	0.148
Fungus spores suspension	Fungus mycelia	0.177	0.210
	Chemical fertilizer	- 0.064	0.648
	Control	0.382*	0.008
Chemical fertilizer	Fungus mycelia	0.241	0.089
	Fungus spores suspension	0.064	0.648
	Control	0.446*	0.002
Control	Fungus mycelia	- 0.205	0.148
	Fungus spores suspension	- 0.382*	0.008
	Chemical fertilizer	- 0.446*	0.002

* The mean difference is significant at the .05 level.

Table 4.9 shows the mean dry weight of watermelon roots. The mean dry weight of chemically treated plants and fungus spores suspension is higher than that of control plants. The mean difference is statistically significant in the case of fungus spores suspension treatment (P value = 0.009) compared to control and the mean difference is not statistically significant in the case of fungus spores suspension (P value = 0.180) compared to chemical fertilizer (Table 4.10).

Table 4.9. Mean of roots dry weight (watermelon)

Experiments	N	* Mean ± (S.D)
Fungus mycelia directly	28	0.261 ± (0.087)
Fungus spores suspension	28	0.312 ± (0.068)
Chemical fertilizer	28	0.339 ± (0.078)
Control	28	0.257 ± (0.069)

* The mean of 28 independent experiments.

N: Number of samples.

Table 4.10. Comparison of the roots dry weight means for different experiments (watermelon)

(I) experiments	(J) experiments	Mean Difference (I-J)	P value
Fungus mycelia directly	Fungus spores suspension	- 0.051*	0.014
	Chemical fertilizer	- 0.078*	0.000
	Control	0.003	0.861
Fungus spores suspension	Fungus mycelia	0.051*	0.014
	Chemical fertilizer	- 0.027	0.180
	Control	0.054*	0.009
Chemical fertilizer	Fungus mycelia	0.078*	0.000
	Fungus spores suspension	0.027	0.180
	Control	0.082*	0.000
Control	Fungus mycelia	- 0.003	0.861
	Fungus spores suspension	- 0.054*	0.009
	Chemical fertilizer	- 0.082*	0.000

* The mean difference is significant at the .05 level.

Figure 4.6 shows the fold increase in the dry weight of shoots and roots.

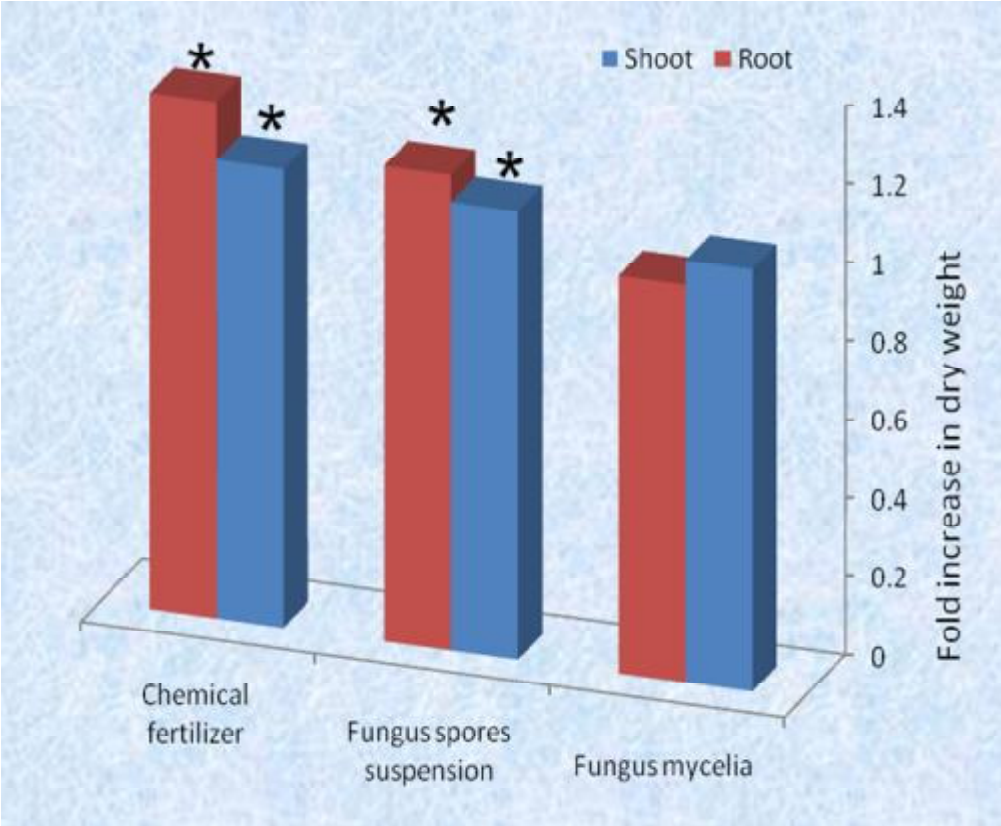

Figure 4.6. Fold increase in dry weight relative to control (watermelon).
The fold increase was calculated as the ratio between the dry weight of shoots or roots of watermelon (g) of each treatment to that of the control .The star indicates statistically significant relation.

Table 4.11 shows the mean dry weight of the whole plant (shoot and root) for watermelon plant. The mean dry weight of chemically treated plants is higher than that of fungus spores suspension treated and both are higher than the control plants and fungus mycelia directly. The mean difference is statistically significant in the case of chemical treatment (P value = 0.000) and fungus spores suspension (P value = 0.003) compared to control and the mean difference of fungus mycelia directly is not statistically significant (P value = 0.156) compared to control (Table 4.12).

Table 4.11. Mean of dry weight for the whole plant (watermelon)

Experiments	N	*Mean ± (S.D)
Fungus mycelia directly	28	3.087 ± (0.605)
Fungus spores suspension	28	3.318 ± (0.512)
Chemical fertilizer	28	3.407 ± (0.540)
Control	28	2.879 ± (0.520)

* The mean of 28 independent experiments.

N: Number of samples.

Table 4.12. Comparison of the dry weight of the whole plant means for different experiments (watermelon)

(I) experiments	(J) experiments	Mean Difference (I-J)	P value
Fungus mycelia directly	Fungus spores suspension	- 0.230	0.117
	Chemical fertilizer	- 0.320*	0.030
	Control	0.208	0.156
Fungus spores suspension	Fungus mycelia	0.230	0.117
	Chemical fertilizer	- 0.089	0.542
	Control	0.439*	0.003
Chemical fertilizer	Fungus mycelia	0.320*	0.030
	Fungus spores suspension	0.089	0.542
	Control	0.528*	0.000
Control	Fungus mycelia	- 0.208	0.156
	Fungus spores suspension	- 0.439*	0.003
	Chemical fertilizer	- 0.528*	0.000

* The mean difference is significant at the .05 level.

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, its positive effects are particularly important. It 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 as 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. This study is the first to tackle this issue in Gaza strip. Among the specific objectives of this study was the use of fungi isolated from the same natural soil of the agricultural areas. In this regard question may be raised about the benefit of this study especially that these fungi are coexisting side by side with the plants in the field, the answer to this is by highlighting the destructive effect of the intensively used chemical fertilizers on the symbiotic fungi that prevents the fungi from reaching the roots of the plants (Miranda *et al.,* 1989, Bougher *et al.,* 1990, El kichaoui, 1995). The intensive use of chemical fertilizers thus make the growing plants live independently from symbiotic fungi. According, this study will be of great benefit in establishing a role of mycorrhizal fungi in encouraging plant growth with similar efficiency as chemical fertilizers. The results of our study will encourage decision makers to adopt a strategies for isolating, growing and using mycorrhization as an efficient alternatives for chemical fertilizers.

5.1. Use of Fungus Spores Suspension

The outcomes of this study showed that endomycorrhization plays clear role in positively impacting on shoot and root growth when a fungus spores suspension injected. A greater growth was always evident in the presence of fungi in all forms in comparison with control plants.

5.1.1. Summer Squash Plants

The results of the study showed that growth of roots and shoots is increased in the presence of fungus spores suspension when compared to the control plants. Moreover, the roots growth was significantly higher in fungus spores suspension than chemically treated plants (P value = 0.000).This results is in concordance with most similar previous studies (Tisserant *et al.,* 1991; Berta *et al.,* 1995; Dalpe, 2005;Porras-Soriano *et al.*, 2009).

The presence of fungi in roots of the plant works an increasing their growth especially in the case of endomycorrhization as it causes the roots to enlarge in order to accommodate the fungal mycelia accumulating inside. In the case of shoots growth the effect of mycorrhization was significant in stimulating the growth compared with the control growth but not the chemically fertilized plants, which showed greater growth than all groups. This may be explained by the short study period that did not allow for establishing a clear positive role of mycorrhization on shoots like in the case of fertilizers. The effect of mycorrhization needs longer time to be visible in the case of shoots than roots .

5.1.2. Watermelon Plants

Like in summer squash plants, the result of the study showed a better growth both in shoots and roots in the fungus spores suspension injected plants than control plants . Although not statically significant, the growth of watermelon plants with chemical fertilizers was better than with fungus spores suspension injection. This may be explained by the fact that the fungi used in this case were isolated from summer squash seedlings and therefore they may be already specialized.

It may be argued that fungi isolated from summer squash must not symbiotically influence watermelon plants(van der Heijden *et al.,*1998). This is true in the field where the number and infiltration of mycorrhizal fungi is low, and chemical

fertilizers are extensively used. However, in our case, we isolated and concentrated the fungi from summer squash roots and injected them next to the watermelon roots (in vitro mycorrhization)(van der Heijden *et al.,*1998).

5.2. Use of Fungus Mycelia Directly

When the fungus was directly used by sticking the fungal mycelia to the roots in the presence of agar growth media the results came as follows.

The chemically fertilized plants showed significantly better growth in all cases than plants directly treated with fungal mycelia. The explanation for this relatively lower growth comes from the details of experimental procedure. In this study the agar with the growing fungal mycelia were cut into small cubes which intern were placed next to the plant root therefore we can claim that the fungus would preferentially grow on the agar cubes remains rather than symbiosis with the plant roots. This may be responsible for the weak influence on the shoot and root growth compared the chemical fertilizers.

The comparison between the growth of the plants directly treated with fungal mycelia and control plants showed an advantage of the control plants.

In the case of watermelon plants the fungal treatment gave slightly better growth than the control but with no statistical significance. In these cases a competition between the growing plant and fungal mycelia for the limited amount of nutrients in the pots may be responsible for this unexpected result. This is supported by the notably increased growth of the fungus in the pots. Similar result was obtained in summer squash plants like in watermelon plants therefore we may explain the decrease in root growth in fungal treated plants similarly. We found a better roots growth in control plants than in direct treatment supporting the explanation of fungal takeover of organic matter and nutrients in the soil, which resulted in weaker plant growth in general and in roots in particular.

Chapter 6

Conclusion & Recommendations

6.1. Conclusions

The present study investigated the influence of some fungi isolated from local soil on the growth of two seasonal plants which are watermelon and squash.

We have adopted to determine the effect of fungus on plant growth by comparing plants inoculated with fungi and plants treated with chemical fertilizer, as well as the control plants. On the other hand we measured the effect of two different methods of inoculation of fungus on the plant growth. The information's that can be concluding from this study are:

1- We obtained a net increasing of roots growth and shoots in the presence of fungus spores suspension when compared to the control plants.

2- The roots growth of the summer squash plants was significantly higher in fungus spores suspension than chemically treated plants**.** In the case of shoots growth the effect of mycorrhization was not significant in stimulating the growth compared with chemically fertilized plants.

3- In watermelon plants, the result of the study showed a better growth both in shoots and roots in the fungus spores suspension injected plants than control plants**.** Although not statically significant, the growth of watermelon plants with chemical fertilizers was better than with fungus spores suspension injection**.**

4- The chemically fertilized plants showed significantly better growth in all cases than plants directly treated with fungal mycelia**.**

5- The comparison between the growth of the plants directly treated with fungal mycelia and control plants showed an advantage of the control plants.

6- In the case of watermelon plants the fungal treatment gave slightly better growth than the control but with no statistical significance.

6.2. Recommendations

1. It is recommended to classify local fungi in agricultural areas and to specify the symbiotic strains among them.

2. The experiments of this study may be repeated using a wider range of plants including vegetables particularly those which demand extensive use of chemical fertilizers.

3. To sub-classify the isolated fungi in terms of plants that is best benefiting from symbiosis with them.

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 other parameters.

5. It is worthy to perform symbiosis experiments with the presence of the fungus and chemical fertilizers simultaneously.

6. Such experiments should be performed in the field rather than in the greenhouses.

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ANNEX 1

Components of chemical fertilizer used in this study (Shifah 11)

Compound	**Concentration (part per million)**
Nitrogen	**1500 ppm**
Iron	**1430 ppm**
Phosphorus	**330 ppm**
Zinc	**14 ppm**
Potassium	**3500 ppm**
Calcium	**165 ppm**
Magnesium	**238 ppm**

How to use :

Treatment after transplanting(cm \ 3 acres)

Summer squash and watermelon plants (0.5 litter)

Every two weeks (cm \ 3 acres)

Summer squash and watermelon plants (0.5 – 1 litter)

In this study, we used 1% of chemical fertilizer. The seedlings were treated after transplanting by 100 ml\ seedling and then once every two weeks during the study period.

ANNEX 2

Figure 1 (AMF on MMN Media , AMF Mycelium and spores , AMF inoculums and chemical fertilizer suspension)**.** A. AMF on MMN Media, B. AMF Mycelium and Spores, C. Fungus spores Suspension, D. Chemical Fertilizer Suspension.

ANNEX 3

Figure 2. watermelon and squash pots in green house. A. summer squash Plants**,** B. watermelon plants.

ANNEX 4

(The occurrences and intensity of root colonization of AMF in species of Cucurbitaceae family)