

إقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

**Evaluation the Effect of Local Endomycorrhizal Fungi on Growth of
Solanum melongena and *Capsicum annuum* Plants in Gaza Strip**

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The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification

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**Evaluation the Effect of Local Endomycorrhizal Fungi on
Growth of *Solanum melongena* and *Capsicum annum* Plants in
Gaza Strip.**

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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/ اسماعيل محمد اسماعيل الجمل لنيل درجة الماجستير في كلية العلوم قسم العلوم الحياتية - نبات وفطريات وموضوعها:

تقييم أثر الفطريات المحلية على نمو نباتي الباذنجان والفلفل في قطاع غزة Evaluation the Effect of Local Endomycorrhizal Fungi on Growth of *Solanum melongena* and *Capsicum annum* Plants in Gaza Strip

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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


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سورة النحل

قال تعالى ﴿هُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ

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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 (Fatma, Mawda, Basmah and Yasmeen)

To my brothers and sisters

To my university IUG

To all of them I dedicate this work

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Evaluation the Effect of Local Endomycorrhizal Fungi on Growth of *Solanum melongena* and *Capsicum annum* Plants in Gaza Strip.

Abstract

The main objective of this work is to comparing the influence of local endomycorrhizae fungus (*Glomus sp*) isolated from the roots of some plants, then comparing the influence of local endomycorrhizae fungus and nutrition substances on the growth of two important seasonal plants in Gaza Strip.

The fungus isolated from eggplant and pepper roots in PDA media, and obtaining pure cultures.

160 plants were grown of each species describe as:

- ❖ "40" divided as 20 mycorrhized and 20 non mycorrhized plants in sterilized soil.
- ❖ "40" divided as 20 nutritive and 20 non -nutritive plants sterilized soil.
- ❖ "40" divided as 20 mycorrhized and 20 non mycorrhized plants in non sterilized soil.
- ❖ "40" divided as 20 mycorrhized plants and 20 nutritive plants sterilized soil.

Our results show a positive influence of the AMF and NS on the growth of Eggplant and pepper seedling compared with control in sterilized and non-sterilized soil in all growth parameters SL, WW, DW, RL, NL, RW and SW after incubated in the green house for two months. We conclude that the use of AMF gives positive influence on the growth of plants especially compared with control, and similar compared with plants grown on NS. According to these results we strongly recommend the use of symbiotic fungi as total or partial substitute of other fertilizer.

Key words : Endomycorrhization, Growth, Glomus, Eggplant, Pepper.

تقييم أثر الفطريات المحلية على نمو نباتى الباذنجان و الفلفل فى قطاع غزة

المستخلص

الهدف الأساسى من دراستنا كان مقارنة تأثير احد الفطريات المتكافلة داخليا و المعزول من جذور بعض النباتات مع نباتات زرعت بدون فطريات " نباتات الضابط " ومقارنة الفطريات التكافلية مع المواد المغذية على نوعين من النباتات المهمة فى قطاع غزة وهما نباتى الباذنجان و الفلفل . و قد تم استخراج الفطر و عزله من جذور اشثال الباذنجان و الفلفل فى وسط غذائى مناسب وتم زراعة 160 شتلة من كل نوع موزعة كالاتى:
40 شتلة قسمت الى 20 تم تلقيحها بالفطر المعزول و20 أخرى تم زراعتها بدون فطر فى تربة معقمة.
40 شتلة قسمت الى 20 تم تلقيحها بالفطر فى عينة تربة غير معقمة و20 أخرى زرعت فى تربة غير معقمة بدون فطر.

40 شتلة قسمت الى 20 تم تلقيحها بالمواد الغذائية و20 أخرى زرعت بدون مواد غذائية فى تربة معقمة.
40 شتلة قسمت الى 20 شتلة تم تلقيحها بالمواد الغذائية و20 أخرى تم تلقيحها بالفطر فى تربة معقمة.
أظهرت النتائج تأثير ايجابي للفطر المعزول والمواد المغذية على نمو نباتات الباذنجان و الفلفل مع نباتات الضابط سواء فى التربة المعقمة وغير المعقمة فى جميع القياسات (طول الساق، طول الجذر، الوزن الجاف، الوزن الرطب، الوزن الرطب للساق، الوزن الرطب للجذر و عدد الأوراق) وذلك بعد شهرين من تاريخ الزراعة داخل الدفيئة الزراعية.

نستطيع الاستخلاص هنا أن استخدام الفطر التكافلي الداخلى يعطى تأثيرا ايجابيا على نمو نباتى الباذنجان و الفلفل مقارنة مع نباتات الضابط، وعند مقارنة استخدام الفطر التكافلي الداخلى مع المواد المغذية لوحظ التأثير الايجابى للفطر على نمو النباتات يساوي تأثير المغذيات تقريبا، ومن خلال هذه النتائج يمكننا أن ندعم و بشكل قوى استخدام هذه الفطريات كبديل كلي أو جزئي مع الأسمدة الاخرى .

الكلمات المفتاحية : التكافل الفطري الداخلى ، النمو ، الفطر الداخلى، نبات الباذنجان، نبات الفلفل.

Table of content

content	page
DEDICATION	IV
Acknowledgements	V
Abstract	VI
المستخلص	VII
List of Figures	XI
List of abbreviation	XIII
Chapter 1	1
Introduction	1
1.1 Overview.....	1
1.2 Main types of mycorrhizae:	3
1) Arbuscular mycorrhizae (AM).....	4
2) Ectomycorrhizas Fungi (ECM)	4
1.3 Aim of the study.....	6
1.3.1 General objective	6
1.3.2 Specific objectives	6
1.4 Significance.....	7
Chapter 2	8
Literature Review	8
2.1 Fungi and Mycorrhizal Fungi.....	8
2.2 Mycorrhizal Fungi.....	9
2.2.1 Ectomycorrhizal Fungi (ECM).	9
2.2.2 Arbuscular Mycorrhizal Fungi (AMF).	10
2.2.2. 1 Arbuscular mycorrhizal interactions	11
2.2.2.2 Arbuscular Mycorrhizal Physiology	12
2.2.2. 3 Structural characteristics and Development of AMF.....	13
2.2.2.4 AMF and Agriculture	14

2.2.2.5	AMF Taxonomy	15
2.2.2.6	Form and functions of AMF.....	16
2.2.2.7	Factors affecting arbuscular mycorrhizal fungi	18
2.3	Importance of mycorrhizae	18
2.3.1	Mineral nutrition.....	19
2.3.1.1	Phosphorus up-take	19
2.3.1.2	Uptake of other nutrients	20
2.3.1.3	Uptake of the immobile nutrients	20
2.3.2	AMF accelerates decomposition and acquires nitrogen directly from organic material.....	21
2.3.3	AMF and Alleviation of Soil Heavy Metal Stress.....	21
2.3.4	AMF and Plant Disease Control.....	22
2.3.5	The Biocontrol Effect of AMF on Soilborne Fungal Pathogen.....	22
2.3.6	Effects of AMF on Drought and Salinity Stress	23
2.4	Plants used in this study	23
2.4.1	Eggplant (<i>Solanum melongena</i> L) Brinjal plant	23
2.4.1.1	Classification	24
2.4.2	Pepper (<i>Capsicum annuum</i>).....	27
2.4.2.1	Classification	28
2.5	Previous Studies	31
Chapter 3	33
Materials and Methods	33
3.1	Materials	33
3.1.1	Chemicals	33
3.1.2	Equipments	33
3.2	Organisms	34
3.2.1	Fungi.....	34
Taxonomy	34
3.2.2	Plants	34
3.3	Methods	35
3.3.1	Isolation of Fungus	35
3.3.2	Identification of Fungus.....	35
3.3.3	Preparation of inoculum.....	36
3.3.4	Application of Plant Fungi Symbiosis in pots	36

3.3.5 Statistical Analysis.....	37
Chapter4.....	38
Results	38
4.1 Isolation and Identification of Fungus	38
4.2 Plants fungi symbiosis	39
4.2.1 Measurements	39
4.2.2 The Occurrences of Roots Colonization of AMF in plants.....	40
4.2.3 Growth of Eggplant and Pepper plants	41
4.3 Results of Preparation of inoculum	42
Eggplant.....	42
Dry weight of Eggplant.....	46
Pepper.....	48
Dry weight of Pepper.....	52
Chapter 5	54
Discussion	54
5.1 Isolation and Identification of AMF.....	55
5.2 Use of Arbuscular Mycorrhizal Fungul and Nutrition Substances	55
5.2.1 Plants (Eggplant and Pepper).....	56
Chapter 6.....	58
Conclusion & Recommendations.....	58
6.1 Conclusions	58
6.2 Recommendations	60
References.....	61
Appendix	86

List of Figures

Figure	page
Figure 2.1 Eggplant plant shape and morphology	27
Figure 2.2 Pepper plants shape and morphology	31
Figure 4.1 Colonies morphology of <i>Glomus sp</i> on PDA media	38
Figure 4.2 <i>Glomus sp</i> hyphae and spores under light microscope.....	39
Figure 4.3 Roots colonization of eggplant treated with fungus spore suspension	40
Figure 4.4 Roots colonization of Eggplant treated with fungus spore suspension	40
Figure 4.5 The relationship between mycorrhized and non mycorrhized plants	42
Figure 4.6 The relationship between nutritive and non -nutritive plants.....	43
Figure 4.7 The relationship between mycorrhized and non mycorrhized plants in NSS	44
Figure 4.8 The relationship between the mycorrhized plants and nutritive plants	45
Figure 4.9 The relationship between MP and NMP in dry weight	46
Figure 4.10 Growth of Eggplant plant treated with AMF comparing with non AMF	47
Figure 4.11 Growth of Eggplant roots treated with AMF comparing with non AMF	47
Figure 4.12 The relationship between mycorrhized and non mycorrhized plants	48
Figure 4.13 The relationship between nutritive and non -nutritive plants.....	49
Figure 4.14 The relationship between mycorrhized and non mycorrhized plants in NSS	50
Figure 4.15 The relationship between mycorrhized plants and nutritive plants	51
Figure 4.16 The relationship between MP and NMP in dry weight	52
Figure 4.17 Growth of Pepper plant treated with AMF comparing with non AMF	53
Figure 4.18 Growth of Pepper roots treated with AMF comparing with non AMF	53

List of Tables

Table	page
Table 1.1 kinds of mycorrhiza.	5
Table.2.1 Classification of Eggplant.....	24
Table 2.2 Classification of Pepper plant	28
Table 3.1 A list of the chemicals used in this work	33
Table 3.2 A list of the main equipment's used in this work.....	33
Table 3.3 Taxonomy of <i>Glomus sp</i>	34
Table 4.1 The relationship between mycorrhized and non mycorrhized plants in SS.....	42
Table 4.2 The relationship between nutritive and non-nutritive plants in SS.	43
Table 4.3 The relationship between mycorrhized and non mycorrhized plants in NSS.....	44
Table 4.4 The relationship between mycorrhized plants and nutritive plants in SS.....	45
Table 4.5 The relationship between MP and NMP in dry weight	46
Table 4.6 The relationship between mycorrhized and non mycorrhized plants in SS	48
Table 4.7 The relationship between nutritive and non -nutritive plants in SS	49
Table 4.8 The relationship between mycorrhized and non mycorrhized plants in NSS.....	50
Table 4.9 The relationship between mycorrhized plants and nutritive plants in SS.....	51
Table 4.10 The relationship between MP and NMP in dry weight	52

List of abbreviation

Arbuscular Mycorrhizae	AM
Arubuscular Mycorrhizal Fungi	AMF
Dry Weight	DW
Ectomycorrhizae	ECM
Least significant difference	LSD
Mycorrhized Plants	MP
Sodium hypochlorite	NaOCl
Number of Leaves	NL
Non Mycorrhized Plants	NMP
Non-Nutritive Plants	NNP
Nutritive Plants	NP
Nutrition Substances	NS
Non Sterilized Soil	NSS
Potato Dextrose Agar	PDA
Root Length	RL
Root Weight	RW
Sterilized distilled water	SDW
Stem Length	SL
Sterilized Soil	SS
Stem Weight	SW
Vesicular arbuscular mycorrhiza	VAM
Vesicular mycorrhiza fungi	VMF
Wet Weight	WW

Chapter 1

Introduction

1.1 Overview

The mycorrhizal symbiosis is arguably the most important symbiosis on earth. Fossil records indicate that arbuscular mycorrhizal interactions evolved 400 to 450 million years ago (Smith and Read, 2008) and that they played a critical role in the colonization of land by plants. Approximately 80 % of all known land plant species form mycorrhizal interactions with ubiquitous soil fungi (Wang and Qiu, 2006).

Roots of most terrestrial plants form symbiotic associations with fungi. These ubiquitous symbioses, called mycorrhizas, function as conduits for the flow of energy and matter between plants and soils (Cardon and Whitbeck, 2007).

Arbuscular Mycorrhizal Fungi (AMF) determine physical, chemical and biological processes in soil. The AMF symbiosis plays a critical role in plant nutrition. The AMF external mycelium develops around the host plant roots and efficiently exploits a larger volume of soil than roots thereby enhancing mineral acquisition by the plant (Smith and Read, 2008). AMF are particularly important in phosphorus uptake (Koide, 1991; Ortas *et al.*, 1996; Liu *et al.*, 2000; Ortas *et al.*, 2002). The external hyphae of AMF can extend >10 cm away from the root surface in the soil beyond the phosphorus (P) depletion zone and access a greater volume of un-depleted soil than the root alone (Jakobsen *et al.*, 1992). The small diameter of hyphae (2–5 μ m) of the fungal network allows the fungus to access soil pores that cannot be exploited by roots, enabling the Arbuscular Mycorrhizal plant to explore a greater volume of soil than non-mycorrhizal roots. AMF can also enhance (P) supply

of the soil in acidic soils where phosphorus is mainly bound with Iron (Fe) or Aluminium (Al) (Cardoso and Kuyper, 2006).

Unlike (P), nitrogen (N), especially in its anionic form nitrate (NO_3^-) is mobile in the soil solution and therefore subject to leaching. Because of nitrate mobility the mycorrhizal symbiosis may not be important for the uptake of mineral (N) by the host plant. However, under N-deficient conditions, growth of fungal hyphae in organic patches may be an effective way of supplying (N) to both the fungus and the host plant (Hodge *et al.*, 2001; Leigh *et al.*, 2009; Hodge and Fitter, 2010). AMF hyphae likely penetrate into the organic material and compete for mineralized NO_3^- or NH_4^+ with other microbes, resulting in increased (N) acquisition by the plant (Hodge *et al.*, 2001). Extraradical mycelia of AMF convert acquired inorganic (N) (NO_3^- or NH_4^+) to arginine before transporting it to intraradical fungal structures where the amino acid is broken down and transported to the plant and assimilated into plant proteins (Govindarajulu *et al.*, 2005; Jin *et al.*, 2005). The AMF symbiosis is also associated with increased uptake of other macro- and micro-nutrients and enhanced water uptake (Liu *et al.*, 2000; Augé, 2004; Birhane *et al.*, 2012).

AMF are among the most important biological factors influencing soil structure (Jastrow *et al.*, 1998; Rillig and Steinberg, 2002; Rillig and Mummey, 2006 ; Smith and Read, 2008). Extraradical hyphae of AMF create a skeletal structure that holds soil particles together initiating formation of macro-aggregates, and create conducive conditions for formation of micro-aggregates within macro-aggregates (Six *et al.*, 2002). Due to its long residence time in soil and low palatability to fungivorous soil fauna, the AMF network is a major component of soil microbial biomass allowing for a more permanent contribution to soil aggregate stabilization than hyphae of saprobic fungi (Rillig and Steinberg, 2002; Purin and Rillig, 2007).

The symbiosis between plants and mycorrhizal fungi is extremely widespread and ancient in the plant kingdom, they have been observed in fossils dating back 400 million years ago (Remy and Taylor, 1994).

Root colonization with mycorrhizal fungi generally has positive effects on plant growth (Chalk *et al.*, 2006), increases in height (Hayman, 1986; Hoeksema *et al.*, 2010; Safapour *et al.*, 2011), biomass (Vejsadova *et al.*, 1993; Mathur and Vyas, 2000; Ramana *et al.*, 2010), shoot :root ratio (Gavito *et al.*, 2000; Veresoglou *et al.*, 2012), production of flowers (Dodd *et al.*, 1983; Carey *et al.*, 1992), and yield in crop plants (Vejsadova *et al.*, 1993; Bethlenfalvay *et al.*, 1997; Abdel-Fattah, 1997; Li *et al.*, 2005; Ramana *et al.*, 2010; Safapour *et al.*, 2011).

1.2 Main types of mycorrhizae:

There are seven types of mycorrhizae described (arbuscular "endomycorrhizae", ectomycorrhizae, ectendomycorrhizae, arbutoid mycorrhizae, monotropoid mycorrhizae, ericoidmycorrhizae and orchidaceous mycorrhizae), arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread (Smith and Read, 1997; Allen *et al.*, 2003).

Mycorrhizae are generally classified according to the arrangement (anatomy) of hyphae (the individual filament of a fungus) in the root cortex in to three major group: Endomycorrhizae that have hyphae inside cortex cells (intracellular), ectomycorrhizae that have hyphae only between cortex cells (intercellular), and ectendomycorrhizae with booth inter and intracellular colonization. Because each major type contain varlation in structure that are given separate name (Harley and smith, 1983).

1) Arbuscular mycorrhizae (AM)

The name 'arbuscular' is derived from characteristic structures, the arbuscule which occur within the cortical cells of many plant roots colonized by AMF (Smith *et al.*, 2008). Together with storage vesicles located within or between the cells, these structures have been considered diagnostic for AM symbioses. The fungal partners in AM associations are remarkably abundant, accounting from 5 to 50 percent of the microbial biomass in agricultural soils (Smith *et al.*, 2008 and Mohammadi 2011).

AMF, as obligate symbionts, also depend for their growth and activity on the supply of carbon compounds from the photosynthetic partner (Ocampo and Azcon, 1985; Amijee *et al.*, 1990; Schwab *et al.*, 1991; Jennings 1995).

2) Ectomycorrhizas Fungi (ECM)

Ectomycorrhizal (ECM) fungi are a diverse group of mutualistic root symbionts that receive carbon from their host plants and in return provide enhanced nutrient uptake and resistance to stress and disease (Smith & Read, 2008). Although the ECM symbiosis has been known for > 100 years (Frank, 1885), most studies on ECM ecology and biodiversity have focused on northern temperate forests and a narrow range of host plant families (e.g. *Pinaceae*, *Fagaceae*) (Alexander, 2006; Dickie & Moyersoen, 2008).

Table 1.1 kinds of mycorrhiza (Boris Börstler, 2010).

Kinds of mycorrhiza	Arbuscular Mycorrhiza	Ectomycorrhiza	Ectendomycorrhiza	Arbutoid mycorrhiza	Monotropoid mycorrhiza	Ericoid mycorrhiza	Orchid mycorrhiza
Fungi septate	-	+	+	+	+	+	+
septate	+	-	-	-	-	-	-
Intracellular colonization	+	-	+	+	+	+	+
Fungal mantle	-	+	+ or-	+or-	+	-	-
Hartig net	-	+	+	+	+	-	-
Achlorophylly	-(+)	-	-	-	+	-	+
Fungal taxa	Glomero	Basidio /Bsc (Glomero)	Basidio /Asco	Basidio	Basidio	Asco	Basidio
Plant taxa	Bryo	Gymno	Gymno	Ericales	Monotro-poideae	Ericales	Orchid-ales
	Pterido	Angio	Angio			Bryo	
	Gymno						
	Angio						
<p>All orchids are achlorophyllous in the early seedling stages .Most orchid species are green as adults. The fungal taxa are abbreviated form Glomeromycota ,Ascomycota and Basidiomycota; the plant taxa from Bryophyta, Pteridophyta , Gymnospermae and Angiospermae.</p>							

1.3 Aim of the study

1.3.1 General objective

Evaluation the effect of Local endomycorrhizal fungi on growth of some importance seasonal plant (Eggplant and Pepper) in Gaza Strip.

1.3.2 Specific objectives

- ❖ To isolate and identify some endomycorrhizal fungi from roots of (Eggplant and Pepper).
- ❖ In *vitro* application of symbiosis between plant and the isolated fungi.
- ❖ Study the effect of symbiosis on the growth of plants in non-sterilized soil, and comparing with non-symbiotic plants in the same soil.
- ❖ Study the effect of symbiosis in comparing with plants supplied with nutrition substance.

1.4 Significance

Gaza Strip is an agricultural land. It depends heavily on agriculture sector, because the area is small.

Because of this limited area and siege caused by the Israeli army against Gaza Strip, we need modicultural system which reduces the environmental pollution, especially because of the use of chemical fertilizers. So, we suggest mycorrhizal symbiosis for these purposes:

- 1) The main importance of this work is the utilization of local fungi; isolated from the same agricultural environment used in Gaza Strip.
- 2) Ecologically, mycorrhizal symbiosis on this side reduces the use of chemical fertilizers, and this affects the environment positively.
- 3) On the healthy side, the use of mycorrhizal fungi has great effects because it limits the health hazards resulted from the chemical fertilizers. These hazards form a bad effect on the underground water and human health.
- 4) Economically, the use of symbiosis process (endomycorrhizal fungi) as fertilizer have much less costs than the chemical fertilizers.
- 5) Most of scientific agricultural institutions encourage the use of mycorrhizal fungi and mycorrhization as fertilizer.

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, Zygomycota, 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).

2.2 Mycorrhizal Fungi

There are two major groups of Mycorrhizal Fungi 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 Ectomycorrhizal Fungi (ECM).

ECM fungi are associated with many forest trees throughout the world, including the important families Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae and Pinaceae (Newman and Reddell, 1987). One estimate suggests that there are 5000-6000 species of mycorrhizal fungi with the majority being ectomycorrhizal (Molina *et al.*, 1992).

ECM fungi are a diverse group of mutualistic root symbionts that receive carbon from their host plants and in return provide enhanced nutrient uptake and resistance to stress and disease (Smith, 2008).

ECM play a critical role in tree nutrition and carbon balance, supplying soil resources to their plant hosts in exchange for sugars (Smith *et al.*, 1997).

Although the ECM symbiosis has been known for > 100 years (Frank, 1885). Over 5000 species of EM fungi have been described (Molina *et al.*, 1992).

ECM fungi can enhance the ability of forest plants to grow in unfavourable environmental and soil conditions (Jones and Hutchinson, 1988).

The extraradical mycelia of ECM fungi exploit the greater soil volume and can reach micropore areas and absorb nutrients that may otherwise be inaccessible both physically and chemically (Perez-Moreno and Read, 2000).

The ECM fungi have the ability to provide buffering capacity to plant species against various environmental stresses (Malajczuk *et al.*, 1994).

ECM fungi are ecologically important in some tropical systems because they mitigate plant stress (Bandou *et al.*, 2006) and enhance seedling establishment and growth (Newbery *et al.*, 2002; Henkel *et al.*, 2005a; McGuire, 2007).

2.2.2 Arbuscular Mycorrhizal Fungi (AMF).

AMF 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 (Schüßler *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 AMF 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).

AM 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*).

2.2.2. 1 Arbuscular mycorrhizal interactions

AM formed by a wide variety of host plants (approximately 65% of all known land plant species) (Smith *et al.*, 2008). AMF is composed of approximately 150 fungal species (Smith *et al.*, 2008), with a high genetic and functional diversity within each species.

AM fungi are classified into three classes Archaeosporomycetes, Glomeromycetes, and Paraglomeromycetes, and the five orders: Archaeosporales (e.g. *Geosiphon pyriformes*, *Archaeospora trappei*), Diversisporales (e.g. *Scutellospora calospora*, *Acaulospora laevis*, *Entrophospora infrequens*), Gigasporales (e.g. *Gigaspora margarita*, *G. rosea*), Glomerales (e.g. *Glomus intraradices*, *G. mosseae*, *G. geosporum*) and Paraglomerales (e.g. *Paraglomus occultum*, *P. laccatum*). This group of fungi is unique due to its age, lifestyle and genetic make-up. AM fungi may have evolved over 1000 million years ago and can be seen as living fossils

because they co-exist relatively morphologically unaltered with plants (Parniske, 2008).

The symbiosis is frequent in all early diverging lineages of the major plant clades. Non-mycorrhizal species or other mycorrhizal types developed in plant lineages of more recent origin. This suggests that this symbiosis is the ancestral form of mycorrhizal interactions and that it played a critical role in the evolution of land plants (Smith *et al.*, 2008).

In comparison, the symbiosis with nitrogen-fixing Rhizobia bacteria evolved much later (approximately 60 million years ago), and this symbiosis is restricted to only one plant clade. AM fungi hyphae and spores contain hundreds of nuclei (Hosny *et al.*, 1998).

The polymorphic nature of these nuclei and the relatively large genome of these fungi has made genome sequencing and annotation of this important group of fungi particularly challenging (Parniske, 2008 and Martin *et al.*, 2008), but recently the first transcriptome of the AM fungus *Glomus intraradices* became available (Tisserant *et al.*, 2001).

They are asexual, but an exchange of genetic material between closely related fungi via anastomosis has been observed.

2.2.2.2 Arbuscular Mycorrhizal Physiology

AMF, members of the Glomeromycota, are by far the most widespread of the mycorrhizal fungi (Brundrett, 1991), occurring in 80% of all plant species (Smith, 1997). Morphologically, these fungi are a network of hyphae that grow within the roots of plants and extend out into the soil. Unlike the ectomycorrhizal fungi, AMF actually penetrate the walls of root cells and form intracellular structures. They produce two distinct structures: the sac-like vesicles, which are thought to act as storage structures for lipids (Morton and Benny, 1990), and densely branched or coiled hyphal masses called arbuscles, which act as the site of nutrient

exchange between the plant and the fungus. These fungi were previously known as VAM. However, it has been shown that vesicles can be produced by non-mycorrhizal fungi and only arbuscles are unique to this group of fungi (McGonigle *et al.*, 1990). There is evidence that the proportion of arbuscles to vesicles can be influenced by ambient nutrient levels and can act as an indicator of the level of benefit received by each partner of the symbiosis (Johnson *et al.*, 2003). While these fungi are generally considered to be obligate symbionts they have been shown to also have saprophytic capabilities (Hodge *et al.*, 2001), and have limited spread and viability in the absence of a live host (Warner and Mosse, 1980).

2.2.2. 3 Structural characteristics and Development of AMF

AM fungi are obligate biotrophs and rely on their autotrophic host to complete their life cycle and to produce the next generation of spores. The spores are able to germinate without the presence of a host, but the spores respond with an increase in hyphal branching and metabolic activity to root exudates (Tamasloukht *et al.*, 2003 and Gachomo *et al.*, 2009). Plant roots release for example strigolactones that are able to induce pre-symbiotic growth of AM fungal spores (Akiyama *et al.*, 2006).

The fungus begins symbiotic growth and produces longitudinal hyphae from which extend the highly branched, tree-like arbuscules (Smith and Read, 1997). Arbuscules are formed by dichotomous branching of intra-radical fungal hyphae and the wall structure becomes more open (Bonfante- Fasolo *et al.*, 1990).

During arbuscule development, the plant vacuole fragments, organelles multiply and move to surround the arbuscule (Lohse *et al.*, 2005) and the nucleus expands and moves into the centre of the cell (Balestrini *et al.*, 1992). Although the arbuscule eventually expands to largely fill the cortical cell, the fungus never penetrates the host cell plasma membrane. Increased

biosynthetic activity within the host cell allows the production of additional membrane components to keep pace with arbuscule growth and, as the arbuscule expands and branches, it is enveloped by newly synthesized host membrane. In the mature arbusculated cell, the host membrane will have increased in surface area several times to completely surround the fungal structure (Alexander *et al.*, 1988). Subsequently, the fungus develops an extensive network of extraradical hyphae that extends beyond the plant root system and provides an increased soil volume for nutrient acquisition (Smith and Read, 1997). In certain mycorrhizal interactions the pattern of fungal growth is somewhat different with the production of coiled structures taking the place of the arbuscules (Dickson *et al.*, 2007).

2.2.2.4 AMF and Agriculture

Agricultural practices have dramatic impacts on soil and soil organisms, and AMF are no exception. A number of studies have shown that agriculture reduces the diversity of the AMF community (Helgason *et al.* 1998; Daniell *et al.*, 2001; Oehl *et al.*, 2003). This has been attributed to physical disturbance from tilling (Kabir *et al.*, 1997; Jansa *et al.*, 2003), the effects of supplemental fertilizers (Linderman and Davis, 2004), and the use of fungicides and soil fumigants (Menge, 1982), all of which reduce the abundance and or diversity of AMF. Interestingly, the use of some pesticides has been shown to increase the diversity of AMF, possibly due to a decline in mycophageous insects after treatment (Vandenkoornhuyse *et al.*, 2003). Some alternative agriculture methods have less of an impact on AMF communities. Generally low input and low till agricultural systems have a higher abundance and diversity of AMF than their traditional counterparts (Douds and Millner 1999; Galvez *et al.*, 2001).

Organic fertilizers can be less damaging to AMF functioning than chemical fertilizers (Linderman and Davis, 2004), and one study found that an

organically farmed system had a similar AMF diversity to a nearby native grassland (Oehl *et al.*, 2003). It has also been shown that the presence of agricultural weeds can increase the abundance of beneficial AMF in fields (Vatovec *et al.*, 2005). Agricultural practices such as tilling and fertilizing not only cause a decline in AMF diversity, there is substantial evidence that they produce a shift in the AMF community composition (Boddington and Dodd 2000; Warburton and Allen 2000; Jansa *et al.*, 2003). These agriculturally adapted AMF have been shown to be slower to infect, faster to sporulate and to produce fewer arbuscles (Johnson 1993; Scullion *et al.*, 1998; Oehl *et al.*, 2003).

2.2.2.5 AMF Taxonomy

Taxonomy within the AMF is difficult. There is a lack of morphological structures upon which to base taxonomic classification. Historically spore morphology has been used to develop this classification and spore abundance has been used to survey for communities of AMF; however, this method has some major drawbacks. There is a seasonality to spore production, field collected spores can be difficult to identify, not all AMF have been found to sporulate, the density of spores is not necessarily related to the abundance of hyphae and there is a lack of relation between functional diversity and spore morphological diversity (Douds and Millner, 1999). Often in order to facilitate identification of spores, field collected AMF samples are grown in pot culture and the spores are collected and identified. This allows adequate quantities of fresh spores for identification (Stutz and Morton, 1996).

These techniques, however, only allow identification of a subset of AMF that perform well with disturbed mycelial networks (Rillig, 2004), and the diversity of spores changes as the pot culture is grown for successive generations (Stutz and Morton, 1996). Molecular techniques have been

employed and show promise but as of yet a comprehensive library of taxon specific probes does not exist and PCR products from field samples are often variable and unpredictable (Douds and Millner, 1999). Given these limitations it has been suggested that there is currently no method of AMF identification that provides a useful representation of diversity and abundance in the field (Douds and Millner, 1999) and that the true diversity of AMF is likely highly underestimated (Vandenkoornhuyse *et al.*, 2002). Despite the difficulties in understanding the complexity of natural communities of AMF there are about 155 identified species of AMF (Douds and Millner, 1999). While it has been shown that different species of AMF have varying interactions with plants under identical conditions (Sanders *et al.*, 1977; StreitwolfEngel *et al.*, 1997; Stampe and Daehler, 2003).

2.2.2.6 Form and functions of AMF

The function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and/or organic nutrients available in soil (Marschner *et al.*, 1994).

AMF are abundant in soil. They account for about 25% of agricultural soils' microbial biomass and live in symbiosis with about 80% of land plant species, including the most economically important ones (Hamel *et al.*, 1991; Olsson *et al.*, 1999).

AMF association was so successful that in the course of evolution, AMF became obligate biotrophs i.e., they can not live without connection to, and carbon supply from, a living host plant. This feature has important implications on the life of AMF in cultivated soils. AMF are found close to plant roots and most of their biomass is in the top 0-20 cm of the soil (Kabir *et al.*, 1998b).

AMF appear as networks of fine tubes of a few micrometers in diameter, filled with cytoplasm, and producing spores, These networks are extensive, often with tens of meters per gram of soil (Leake *et al.*, 2004).

It is important to keep in mind that AMF isolates are not all the same, and vary functionally and morphologically. These networks, enmeshing the soil matrix, connect to plant roots, and penetrating cell walls of the root cortex area without disrupting plant cells (plasma membrane) where they acquire carbon- and energy-rich photosynthesis products (Hamel, 2007).

In turn, plants tap in on the mineral nutrients contained in these networks. AMF networks were shown to provide plants with all essential nutrients, but they are particularly important as a source of P, Cu and Zn, these nutrients have low solubility in soil and are often found in low concentrations in the soil solution. Thus, they are more difficult to extract from the soil matrix than highly soluble nutrients such as nitrate-N, for example.

AMF are useful to insure the adequate nutrition of their host plant, but they also are a very important component of soil quality (Jeffries *et al.*, 2003; Six *et al.*, 2004). Their (sticky) hyphae and soil enmeshing hyphal networks contribute importantly to soil aggregate stabilization (Six *et al.*, 2004), enhancing soil aeration and water infiltration, and reducing the erodibility of soils. AMF's abundant mycelium, which is supplied by plant photosynthesis, distributes carbon compounds in soil. Carbon availability is the factor generally limiting the activity of soil microorganisms. Carbon distribution in soil may be the major mechanism explaining the relationship between AMF and soil microbial diversity, and the impact of these fungi on the structure of soil microbial communities (Marschner and Baumann, 2003). Soils abundant in AMF are healthier and have been associated with reduced population of soilborne pathogens and disease incidence (Dehne 1982; St-Arnaud *et al.*, 1995).

2.2.2.7 Factors affecting arbuscular mycorrhizal fungi

1) Climatic and Environmental factors

Such as temperature, rainfall, light, atmospheric CO₂, soil pH, moisture content, fertility level and density of inoculums have significant influence on VAM and root colonization. The influence of climatic and soil factors vary with plant species and can be positive or negative (Muthukumar and Udaiyan, 2002).

2) Soil condition

Such as Soil pH, soil moisture, nutrient status affected of AMF (Miller and Jackson, 1998 ; Klinomoros *et al.*, 2001), and other factors like salinity, temperature (Abbott *et al.*, 1991).

3) Agriculture, tillage and phosphorus fertilizer agriculture practices

Such as tillage, heavy fertilizers and fungicides, poor crop rotations and selection for plants which survive these conditions, hinder the ability of plants to form symbiosis with AMF.

2.3 Importance of mycorrhizae

Mycorrhiza is a mutualistic association between fungi and higher plants (Menge, 1983).

VAF are associated with improved growth of many plant species due to increased nutrients uptake, production of growth promoting substances, tolerance to drought, salinity and transplant shock and synergistic interaction with other beneficial soil microorganisms such as N-fixers and P-solubilizer (Sreenivasa *et al.*, 1989). Symbiotic association of plant roots with VAF often result in enhanced growth and other low mobile mineral nutrients (Kwapata *et al.*, 1985; Augé *et al.*, 2001). Effective nutrient acquisition by VAF is generally attributed to the extensive hyphal growth beyond the nutrient depletion zone surrounding the root (Sanders, *et al.*, 1971; Tisdale *et al.*, 1995).

They can also interfere with pathogens (Newsham *et al.*, 1995), increase micronutrient uptake and alter drought resistance (Smith *et al.*, 1996). It is thought that these fungi have little ability to increase plant uptake of more mobile ions (for example, NO_3^-) as these diffuse rapidly to roots in soil (Tinker *et al.*, 2000), although they do transport the less mobile NH_4^+ (Tobar *et al.*, 1994).

AMF are often implicated in functions which may or may not be related to enhanced nutrient uptake. For example, they have been associated with enhanced chlorophyll levels in leaves and improved plant tolerance of diseases, parasites, water stress, salinity, and heavy metal toxicity (Bethlenfalvay, 1992). Moreover, there is increasing evidence that hyphal networks of AM fungi contribute significantly to the development of soil aggregates, and hence to soil conservation (Miller *et al.*, 1992).

Most agricultural crops can perform better and are more productive when well colonized by VAM fungi due to increases the micronutrient uptake and growth of their plant host (George *et al.*, 1992).

2.3.1 Mineral nutrition

2.3.1.1 Phosphorus up-take

Phosphorus (P) is required in relatively large amounts but is often poorly available in soil. It is controlled mainly by soil chemical reactions and, to a lesser extent, by biological processes (Schachtman *et al.*, 1998).

The major role of VAF is to supply infected plant roots with (P), because it is an extremely immobile element in soils.

AM fungi may have biochemical capabilities for increasing the supply of available (P) and other immobile nutrients (Podila *et al.*, 2001).

These capabilities may involve increases in root phosphatase activity, excretion of chelating agents, and rhizosphere acidification. (Habte *et al.*, 1993).

Even if (P) was added to soil in soluble form soon, it becomes immobilized as organic (P), calcium phosphates, or other fixed forms (Jones *et al.*, 1992 and Chapin *et al.*, 1993).

2.3.1.2 Uptake of other nutrients

Copper, Zinc, Potassium and other micronutrients

The efficiency of uptake of both Zn and Cu is increased in AM 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* (Daft *et al.*, 1975).

Increased Cu uptake in AM plants has also been confirmed for a number of plant fungus combinations (Killham and firestone, 1983; Manjunath and Habte, 1988).

AM colonization increased uptake of Zn by *Araucaria* roots (Bowen *et al.*, 1974). Analyses of K concentrations in plant tissues have occasionally indicated increases. In K uptake in AM plants, which might be expected considering the relative immobility of this ion in soil (Mosse, 1957; Holevas, 1966; Possingham and Groot Obbink, 1971; Huang *et al.*, 1985).

2.3.1.3 Uptake of the immobile nutrients

Results of experiments suggest that AM fungi absorb N, P, K, Ca, S, Cu, and Zn from the soil and translocate them to associated plants (Tinker *et al.*, 1983). AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn (Pacovsky 1986, Manjunath *et al.*, 1988). The fungi enhance immobile nutrient uptake by increasing the absorptive surfaces of the root.

2.3.2 AMF accelerates decomposition and acquires nitrogen directly from organic material

Nitrogen (N) is a critical limiting nutrient in many ecosystems (Vitousek *et al.*, 1991). Plants capture (N) largely in inorganic form, relying on microbes to release inorganic (N) as NH_4^+ during decomposition of organic material. However, most (N) in soils is in organic form, often occurring in complex molecules. Some plants can take up simple, soluble organic (N) compounds (Jones *et al.*, 1992 and Chapin *et al.*, 1993), and others can use organic (N) sources directly by association with specialist mycorrhizal fungi (Read, 1991). Mobile ions such as NO_3^- or NH_4^+ are being produced in decomposing patches of organic material, the ability of the one hyphae of AMF to penetrate into the material and to compete with other microbes could lead to increased N acquisition by the plant.

2.3.3 AMF 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 redox reactions, in electron transfer, and have structural function in nucleic acid metabolism (Gohre and Paszkowski, 2006). AM fungi are significant in the remediation of contaminated soil as accumulation (Jamal *et al.*, 2002). The external mycelium of AMF 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).

2.3.4. AMF and Plant Disease Control

AMF 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, AMF may provide a more suitable and environmentally acceptable alternative for sustainable agriculture and forestry.

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 AMF in the host plant generally reduced 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.

2.3.5 The Biocontrol Effect of AMF on Soilborne Fungal Pathogen

Most data about bioprotection of mycorrhization are available for soilborne fungal pathogens. Numerous studies show a clear localized protective effect (reviewed by Singh *et al.*, 2000; Azcon-Aguilar *et al.*, 2002; Xavier and Boyetchko 2004; St- Arnaud and Vujanovic, 2007), while recently a systemic protective effect with different soilborne fungal pathogens has also been reported (Cordier *et al.*, 1998a; Pozo *et al.* 2002; Khaosaad *et al.*, 2007).

2.3.6 Effects of AMF 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).

Several mechanisms 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 Azcon, 1996), enhanced water uptake through increasing leaf conductance and photosynthetic activity (Dell-Amico *et al.*, 2002), osmotic adjustment and changes in cell-wall elasticity (Sanchez-Diaz and Honrubia, 1994).

AMF can also reduce the impact of environmental stresses such as salinity (Ruiz-Lozano *et al.*, 1996a).

2.4 Plants used in this study

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, 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 (Aljabi, 1995).

2.4.1 Eggplant (*Solanum melongena* L) Brinjal plant

Eggplant is the third most important crop in the *Solanaceae* family after potato and tomato (Faostat, 2000).

2.4.1.1 Classification

Table.2.1 Classification of Eggplant (www.greenpharmacy.info/article. 2014)

Kingdom	Plantae
Subkingdom	Viridaeplantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Solanales
Family	<i>Solanaceae</i>
Genus	<i>Solanum</i>
Species	<i>Solanum melongena</i>

Importance

Eggplant is considered one of the important crops in Palestine and the farmers prefer to plant it because of the local increasing on it and its possible profit. Eggplant is considered one of the crops which need a lot of water, potassium fertilizer which gain the fruit forest black color. Eggplant and Pepper are one of the crops which tolerate simple ratio salinity. According to (Usda, 2009), eggplant contains nutrients such as dietary fiber, ascorbic acid, vitamin K, vitamin B6, pantothenic acid, potassium, iron, magnesium, manganese, phosphorus, and copper. Eggplants fruits have nutritional value and can be compared with the value of tomato (Sutarni *et al.*, 1993).

The agriculture time

Its planted on three stages :

In Spring specially, in March and April

In Outumn specially, in August and Septemper

In Winter specially, in October (Minstry of Agriculture, 2013).

Number of Donems grown in Gaza Strip

650 donems are grown in Septemper inside the greenhouses every years and its grown approximately 2900 donems in open land specially in March, April and May. The Eggplant plants are grown on distances 50 cm between plants and 160cm between one line to another to have approximately 1500 plants for one doneme (Minstry of Agriculture, 2013).

Irrigation

It is irrigated by water net through drops with rate 2 m^3 every day for one doneme and the amount of water increases gradually to reach to $6-8 \text{ m}^3$ every day for one doneme, then the amount increases according to the atomesferic condition, large of plants and the needs of the plants especially in Jule and August (Minstry of Agriculture, 2013).

Fertilization

Before agriculture the chicken and cows manure is added on the rate of 1:2 or chemical fertilizers are added such as super phosphate then , tilling the land and sterilized by methyl promide gas. After growing specially on the third week approximatly, the fertilzer 20-20-20 is added on the rate of 0.5kg/doneme every day and this process continus for three weeks, then increasing this amount to reach to 2.5 kg/doneme in production stage. (Minstry of Agriculture, 2013).

Eggplant Sorts

Classic, its dominated sort in Gaza Strip which have around, long shape and florescent color in addition to its abundant production. There are other sorts like Black Bell, Bonica and Apic (Minstry of Agriculture, 2013).

Diseases

- 1) Powdery mildo, we control it by using offer on rate of 100 cm³/doneme.
- 2) Aphids, we control it by using marshal on rate of 2 cm/litter
- 3) White Mold (Sclerotinia), we control it by using dolsan on the rate of 1g/litter.
- 4) Gray Mold (Botrytis Blight), we control it by using rovrал on the rate of 100g/doneme..
- 5) Alternaria leaf blight, we control it by using daconil on the rate of 300g/doneme.
- 6) Red spider, we control it by using vertimec on the rate of 100 cm³/doneme (Minstry of Agriculture, 2013).



Figure 2.1 Eggplant plant shape and morphology

2.4.2 Pepper (*Capsicum annuum*)

The genus *Capsicum* is a member of the *Solanaceae* family that includes tomato, potato, tobacco, and eggplant.

The agriculture time

Its planted on three stages :

In Spring specially, in March and April

In Outumn specially, in August and Septemper

In Winter specially, in October (Minstry of Agriculture, 2013).

2.4.2.1 Classification

Table 2.2 Classification of Pepper plant (<http://www.chipotlechiles.com> 2014)

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta - Vascular plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons (two seed leaves)
Order	Solanales
Family	<i>Solanaceae</i>
Genus	<i>Capsicum</i> L
Species	<i>Capsicum annuum</i>

Importance

Pepper is considered one of the important crops in Palestine and the farmers prefer to plant it because of the local increasing on it and its possible profit (Minstry of Agriculture, 2013).

According to (Bosland andVotava, 2000), sweet pepper and hot pepper, like tomato and eggplant are rich in Vitamins A and C and a good source of B2, potassium, phosphorus and calcium (Anonymous, 1998). It has been found that as hot peppers mature, the Pro-vitamin A (B Carotene) and ascorbic acid increase.

Pepper is planted through the seeds in the greenhouse or in the open land .

Number of Donems grown in Gaza Strip

Eight handerd donems are grown in Septemper inside the greenhouses every years and its grown approximately 2050 donems in open land specially in March, April and May.

The Pepper plants are grown on distances 50 cm between plants and 80-100 cm between one line to another to have 2000-2500 plants for one doneme. (Minstry of Agriculture, 2013).

Irrigation

It is irrigated by water net through drops with rate 2 to 3 cubic for one doneme and the amount of water increases gradually to reach to 5 to 6 cubic for one doneme, then the amount increases according to the atomesferic condition and the needs of the plants (Minstry of Agriculture, 2013).

Fertilization

Before agriculture the chicken or the cows manure is added on the rate of 5 cubic for one doneme or chemical fertilizers are added such as super phosphate then, tilling the land and sterilized by methyl promide gas. After growing specially on the third week approximatlly, the fertilzer 20-20-20 is added on the rate of 0.5kg/doneme and this process continus for three weeks. (Minstry of Agriculture, 2013).

Medicinal uses

Medicinal use of Capsicum has a long history, dating back to the Mayas who used them to treat asthma, coughs, and sore throats. A survey of the Mayan pharmacopoeia revealed that tissue of capsicum species is included in a number of herbal remedies for a variety of ailments of probable microbial origin (I-San Lin, 1994). According to (Bosland and Votava, 2000), pepper is the most recommended tropical medication for arthritis. The pharmaceutical industry uses capsaicin as a counter-irritant balm (cream), for external application of sore muscles (Thakur, 1993).

Diseases

- 1) Powdery mildew, we control it by using offer on rate of 100cm³/doneme.
- 2) Aphids, It activates in spring , and we control it by using marshal on rate of 2cm/litter
- 3) Red Spider, we control it by using vertimec on the rate of 70-100cm³/doneme.
- 4) White Spider, we control it by using vertmic on the rate of 1cm/ litter.
- 5) Vertmic worm, we control it by using vertimec on the rate of 70-100cm³/doneme.
- 6) Gray Mold (Botrytis Blight), we control it by using rovril on the rate of 100cm³/doneme.
- 7) Bacterial diseases, we control it by using kocide on the rate of 3g/litter (Minstry of Agriculture, 2013).

Currently, it is produced in many parts in Gaza Strip because, for most Palestinians food is tasteless without hot pepper. That is, it is the main parts of the daily diet of most Palestinians society.



Figure 2.2 Pepper plants shape and morphology

2.5 Previous Studies

The effect of AMF on some of the growth parameters of pepper plants was investigated and the physiological activity of Mycorrhizal plants. It was found to be better than that of Non Mycorrhizal plants, also it was found that the physiological activity, chlorophyll content of plants, in Mycorrhizal plants were be higher than those in Non-Mycorrhizal plants, photosynthetic rate was improved by AM fungus, mycorrhizae often leads to increases in the leaf area ratio and to leaf hydration, the effect of mycorrhizae on leaf morphology is also probably partly caused by the enhanced P nutrition, dry matter contents, chlorophyll concentrations and amounts of some reducing sugars (fructose, a glucose, b glucose), sucrose and total sugar. All parameters was found to be increased in Mycorrhizal pepper plants by 12%-47% compared with those of the Non-Mycorrhizal plants (**Semra DEMÜR,2004**).

The effects of (*Glomus intraradices*), soil salinity and P availability on growth (leaf area and dry weight), nutrient absorption, chlorophyll, soluble sugar and proline content and alkaline phosphatase activity of pepper plants. Plants were grown at four levels of salinity (NaCl), and two P levels. Mycorrhizal plants maintained greater root and shoot biomass at all salinity levels comparing with non-mycorrhizal plants, regardless the P level. Interactions between salinity, phosphorous and mycorrhizae were significant for leaf area, root and shoot dry mass. AMF alleviate detrimental effects of salinity on growth, improve nutrition and alleviate salinity impacts on cell membrane stability, at high P concentration and high saline conditions. Thus, use of AMF provides a sustainable and environmentally safe treatment to improve salinity tolerance **(Beltrano et al., 2013)**.

The influence of AMF inoculation on growth, nutrient uptake, arsenic toxicity and chlorophyll content of eggplant grown in arsenic amended pot soil. Three levels of arsenic concentrations (10ppm, 100ppm and 500ppm) were used in pot soil and eggplant was grown in arsenic amended soils with or without mycorrhizal inoculation. Root length, shoot height, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight were higher in AMF inoculated plants in comparison to their respective treatments and decreased significantly with the increase of rate of arsenic concentrations. Less arsenic content and higher chlorophyll and nutrient uptake were recorded in mycorrhiza inoculated plants in compare to noninoculated plants. The findings of the study indicated that AMF inoculation not only reduce arsenic toxicity but also can increase growth and nutrient uptake of eggplant shoot **(Elahi et al., 2010)**.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 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
Trypan Blue	Biological industries – Israel
Glycerol	Frutarom – Israel
Sheavah 11as organic fertilizer	Westren blank -Palestine
PDA Media	Mumbai -India
Ethyl Alcohol	Frutarom – Israel
Miphenicol(Antibiotic)	Egypt
HCl	Israel

3.1.2 Equipments

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	China

3.2 Organisms

3.2.1 Fungi

Glomus sp is the fungi isolated from locally grown Eggplant and Pepper, this fungus can be found in almost all soils, this fungus isolated from locally environment in Gaza Strip from Eggplant and Pepper plants roots.

Taxonomy

Table 3.3 Taxonomy of *Glomus sp*

Kingdom	Fungi
Division	Glomeromycota
Class	Glomeromycetes
Order	Glomerales
Family	<i>Glomeraceae</i>
Genus	<i>Glomus</i>
Species	<i>Glomus .sp</i>

3.2.2 Plants

Two types of plants of the same family (*Solanaceae*) were selected, Eggplant and Pepper. These seasonal plants are grown widely in Gaza Strip, which rely on chemical fertilizers and other fertilizers like organic manure. 90 seedlings of these plants with age about two weeks of each species were obtained from modern agriculture arboretum (Harris Moran Seed Company) planted inside a mini-green house.

3.3 Methods

3.3.1 Isolation of Fungus

After planting in normal soil (sandy and loamy) with little quantity of organic matters, and without any chemical fertilizers. After 30 days of growing, roots were uprooted and prepared for the isolation of the fungus from its.

In this isolation we used standard medium like a potato dextrose agar (PDA) media, supplemented with Miphenicol (Antibiotic).

To obtain a pure culture of fungus from mycorrhizal roots of our seedlings Eggplant and Pepper, and make sure it is endomycorrhizal symbiotic with the target plants we proceed as following:

Roots of Eggplant and Pepper were grown in normal soil with little quantity of organic matters and without any chemical fertilizers at least for two weeks. Then we separated roots from shoots and washed with running water.

The roots disinfected by different concentrations of Sodium hypochlorite (NaOCl) ranging from 2 to 10% during 1 to 5 minute, and then washed again with sterilized distilled water (SDW).

All these steps took place in an a xenic conditions, the roots then cultured in (PDA media), after 7 days of culture at room temperature, 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.

3.3.2 Identification of Fungus

Then we depended on culture and growth of fungus in Petri dishes and observing its colony characteristics and morphological features.

For the identification of fungus the following method was used:

Identification and classification of AMF were derived from the similarities and differences in morphological characteristics of AMF. This involves a detailed description of the hyphae, spores, and vesicles (Manoharachary *et al.*, 2002).

The morphological characteristics and features of the *Glomus sp* such as shape and color of fungus used for their taxonomy and classification.

It was found that the isolated fungus belong to genus *Glomus*.

3.3.3 Preparation of inoculum

The entire fungus were used hyphae and spores, for preparation the solution.

- 1) 5 Litter sterilized distailed water with 10 petri dishes.
- 2) Mixation together.
- 3) The roots of both plants incubated by this solution one-time only, then the seedlings were treated after transplanting by 100 ml\ seedling also one-time only.
- 4) On the other hand in this study, we used nutrition substances (Sheavah 11 fertilizer). The seedlings were treated after transplanting by 100 ml\ seedling and then once every two weeks during the study period, after Prepared the solution by 1% of (Sheavah 11 fertilizer).

3.3.4 Application of Plant Fungi Symbiosis in pots

The experimental soils were prepared by sandy soil from local habitat then sterilized by autoclaving. 800 ml soil samples were taken in each pot. 320 pots of soil were used for the culture of Eggplant and Pepper plants, 160 pots for each type. Four sets of experiments were conducted, each set consist of 40 pots with 40 seedlings.

- 1) The 1st set was the sterilized soil plus AMF (SS+AMF) and control (sterilized soil without any kind of fertilizer) (SS-AMF).
- 2) The 2nd set, sterilized soil plus nutrition substances (Sheavah 11fertilizer) (SS+NS) and sterilized soil without nutrition substances (SS-NS).
- 3)The 3rd set normal soil (non sterilized) plus AMF (NSS+AMF) and normal soil without AMF (NSS-AMF).
- 4) The 4th set sterilized soil plus AMF (SS+AMF) and sterilized soil plus nutrition substances (SS+NS).

Eggplant and pepper plants were incubated after the application of previous conditions in the green house for two months (from November 2013 to January 2014).

At the end of the incubation period, we compared between these sets of plants growth via plant height after 8 weeks, the Stem length (cm), Root length (cm),Wet Weight (g), Number of leaves, Dry Weight (g), Root Weight (g), and Stem Weight (g), per plant at maturity of all plants .

3.3.5 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.

Chapter4

Results

4.1 Isolation and Identification of Fungus

After culturing at room temperature, the fungus mycelia were multiplied and sub cultured in many other dishes up to obtaining pure cultures. The compound light microscope were used to observe the diagnostic characteristics of the isolated fungus, such as spore, colour, size and type of hyphal attachment according to (Schenck and Prez, 1990). Observing these characteristics may be helpful to identify the genus of the target fungus accurately.



Figure 4.1 Colonies morphology of *Glomus sp* on PDA media

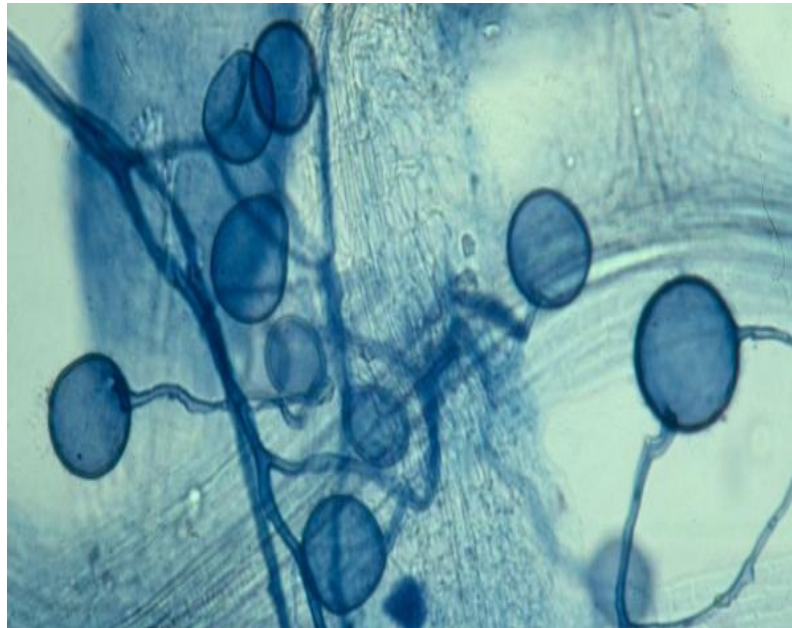


Figure 4.2 *Glomus sp* hyphae and spores under light microscope

4.2 Plants fungi symbiosis

4.2.1 Measurements

Analysis of variance (ANOVA) was performed to assess the effects of AMF, NS, on growth of two seasonal plants Eggplant and Pepper. Differences between treatment means were analyzed by multiple range comparison based on least significant difference (LSD) at $P < 0.05$ (sig).

The measurements criteria of plant growth is the Stem length (SL), Root length (RL) ,Wet Weight (WW) , Number of leaves (NL) , Dry Weight (DW), Root Weight (RW) and Stem Weight (SW), and we divided the groups of two plants (Eggplant and Pepper) into two kinds :

The first is Mycorrhized Plants (MP), and Non-Mycorrhized Plants (NMP).

The second is Nutritive Plants (NP) and Non-Nutritive Plants (NNP).

There is no AMF colonization was noted in roots of control plants, but roots of infected eggplant and pepper plants were highly colonized by *Glomus sp*.

4.2.2 The Occurrences of Roots Colonization of AMF in plants

Segments of plant roots treated by fungus spore suspension, were cleaned, stained according to the method described by (Phillips and Hayman, 1970), and examined under light microscope. Eggplant plant was 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.

Pepper plant roots also colonized by AMF, (see Figure 4.3 and Figure 4.4). The roots of Eggplant and Pepper plants were colonized by AMF, the presence of vesicles and hyphae is very clear.

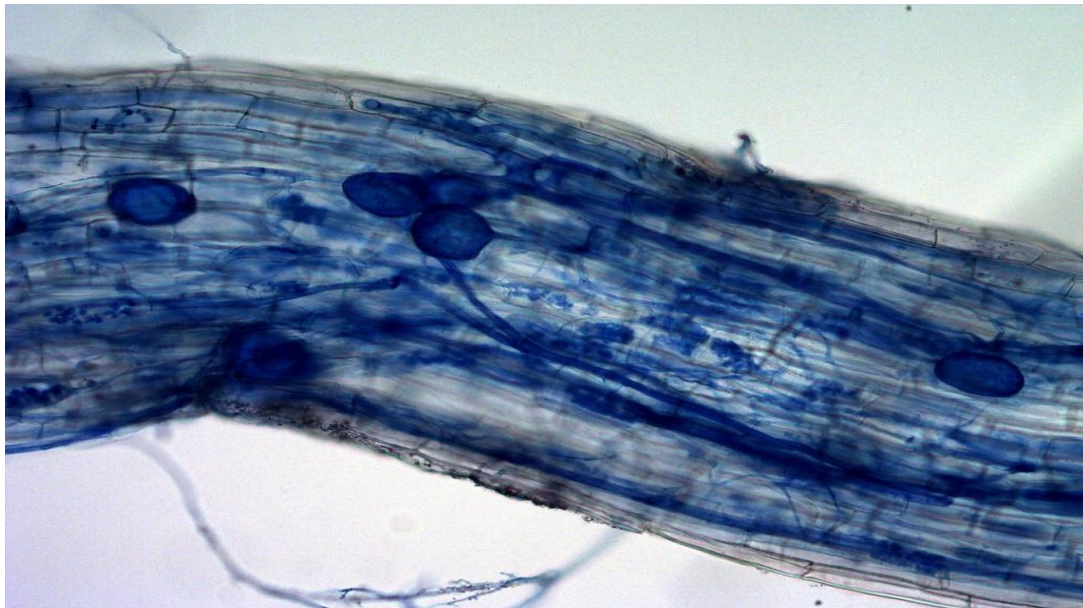


Figure 4.3 Roots colonization of eggplant treated with fungus spore suspension



Figure 4.4 Roots colonization of Pepper treated with fungus spore suspension

4.2. 3 Growth of Eggplant and Pepper plants

The study included Eggplant and Pepper plants treated with fungus spores suspension (MP), and control plants without AMF (NMP), and treated with nutrition substances (NP), and control plants without nutrition substances (NNP). All measurements of the growth parameter for both plants were higher in presence of fungal spores suspension treated plants and nutrition substances ones, than the control plants, these results supports the role of these fungi on increasing the growth of the small seasonal plants (table 4.1-10 and fig 4.5-16).

4.3 Results of Preparation of inoculum

Eggplant

Table 4.1 The relationship between mycorrhized and non mycorrhized plants in SS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	MP	10	34.63	2.21	3.98	0.001
	NMP	10	30.30	2.63		
Root Length	MP	10	64.03	7.44	4.406	0.000
	NMP	10	53.45	1.49		
Wet Weight	MP	10	39.01	3.32	5.27	0.000
	NMP	10	31.59	2.96		
Number Leaf	MP	10	13.20	1.32	3.20	0.005
	NMP	10	11.30	1.33		
Dry Weight	MP	10	22.02	1.91	5.07	0.000
	NMP	10	18.44	1.15		
Root weight	MP	10	8.90	0.81	8.731	0.000
	NMP	10	6.44	0.37		
Stem weight	MP	10	13.12	1.14	2.55	0.020
	NMP	10	12.00	0.79		

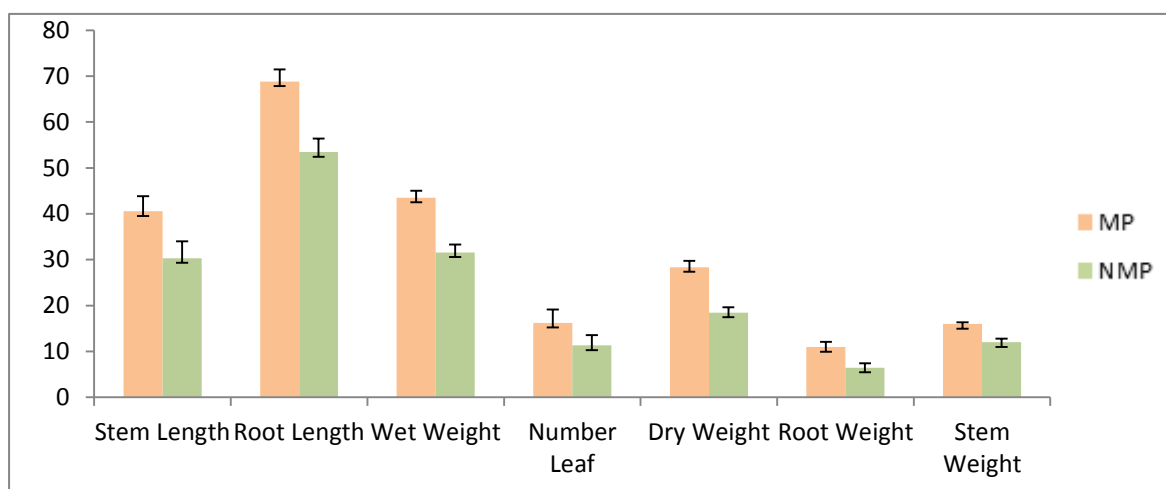


Figure 4.5 The relationship between mycorrhized and non mycorrhized plants

Table 4.1 and fig. 4.5 show that the growth of mycorrhized plant is better than those non -mycorrhized plants in all measures , and with statistical clear significance, this supports the role of these fungi on increasing the growth of the small seasonal plants, and this means that the presence of fungus has a positive influence on plant growth.

Table 4.2 The relationship between nutritive and non-nutritive plants in SS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	NP	10	40.54	3.29	7.68	0.000
	NNP	10	30.30	2.63		
Root Length	NP	10	68.85	3.70	12.19	0.000
	NNP	10	53.45	1.49		
Wet Weight	NP	10	43.51	2.95	9.01	0.000
	NNP	10	31.59	2.95		
Number Leaf	NP	10	16.20	1.68	7.21	0.000
	NNP	10	11.30	1.34		
Dry Weight	NP	10	28.37	2.28	12.26	0.000
	NNP	10	18.44	1.15		
Root weight	NP	10	10.94	1.15	11.77	0.000
	NNP	10	6.44	0.37		
Stem weight	NP	10	15.96	0.97	9.97	0.000
	NNP	10	12.00	0.79		

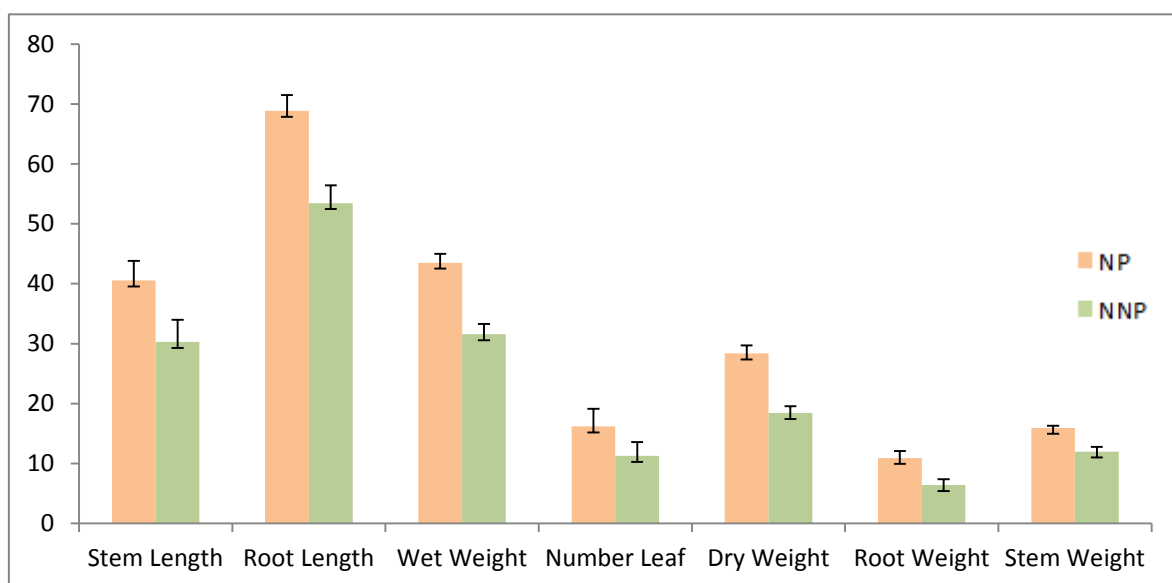


Figure 4.6 The relationship between nutritive and non -nutritive plants

Table 4.2 and fig. 4.6 demonstrate the growth of nutritive plant is better than those of non- nutritive plants in all measures , and with statistical clear significance.

Table 4.3 The relationship between mycorrhizized and non mycorrhizized plants in NSS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	MP	10	49.70	3.77	14.94	0.000
	NMP	10	30.84	1.29		
Root Length	MP	10	71.68	5.20	6.93	0.000
	NMP	10	58.86	2.67		
Wet Weight	MP	10	48.03	3.52	5.80	0.000
	NMP	10	34.31	6.59		
Number Leaf	MP	10	18.60	2.75	6.89	0.000
	NMP	10	11.60	1.64		
Dry Weight	MP	10	33.71	2.73	12.92	0.000
	NMP	10	18.96	2.36		
Root weight	MP	10	11.84	1.13	10.58	0.000
	NMP	10	7.08	0.86		
Stem weight	MP	10	19.50	1.88	10.31	0.000
	NMP	10	11.86	1.39		

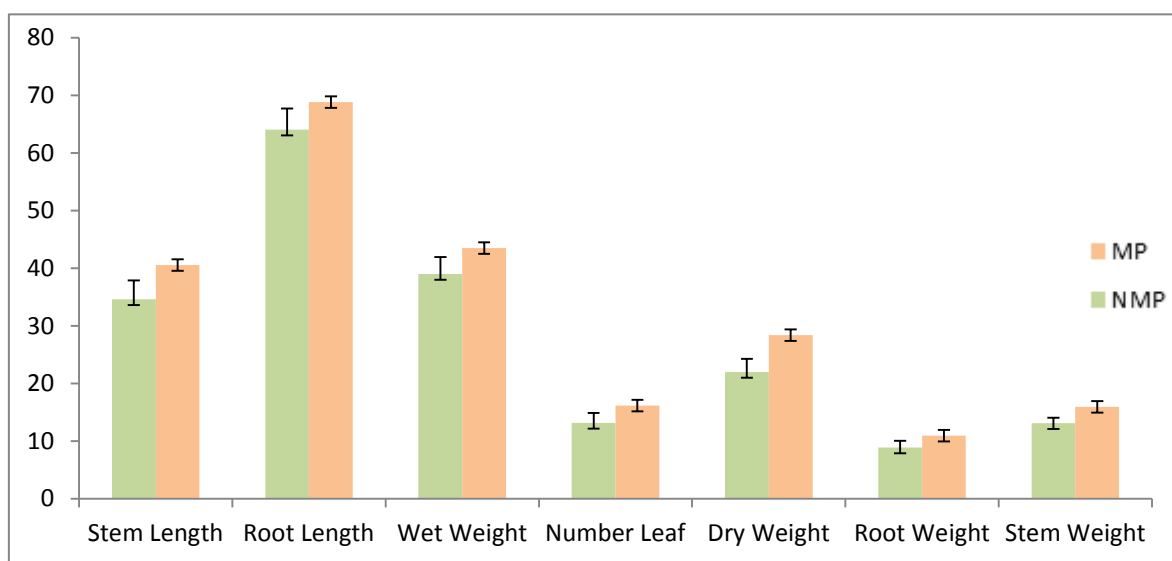


Figure 4.7 The relationship between mycorrhizized and non mycorrhizized plants in NSS

As shown in table 4.3 and fig. 4.7 the growth of mycorrhizized plant is better than those of non- mycorrhizized plants in all measures in non -sterilized soil , and with statistical clear significance, this supports the role of these fungi on increasing the growth of the small seasonal plants .

Table 4.4 The relationship between mycorrhizal plants and nutritive plants in SS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	MP	10	34.63	2.21	4.71	0.000
	NP	10	40.54	3.29		
Root Length	MP	10	64.03	7.44	1.83	0.083
	NP	10	68.85	3.70		
Wet Weight	MP	10	39.01	3.32	3.19	0.005
	NP	10	43.51	2.95		
Number Leaf	MP	10	13.20	1.32	4.43	0.000
	NP	10	16.20	1.68		
Dry Weight	MP	10	22.02	1.91	6.73	0.000
	NP	10	28.37	2.28		
Root weight	MP	10	8.90	0.81	4.58	0.000
	NP	10	10.94	1.15		
Stem weight	MP	10	13.12	1.13	6.00	0.000
	NP	10	15.96	0.97		

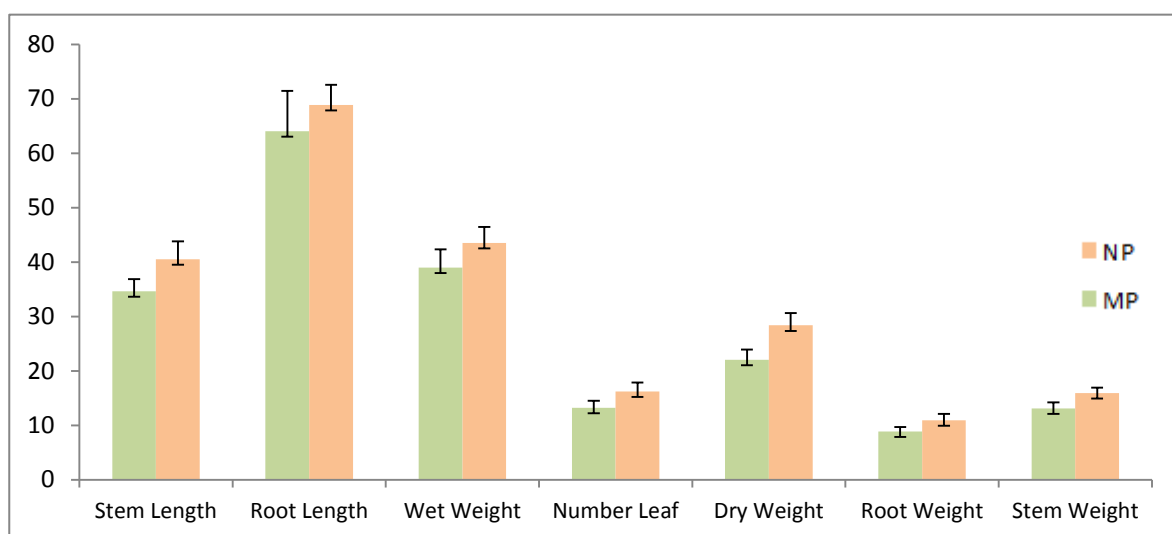


Figure 4.8 The relationship between the mycorrhizal plants and nutritive plants

As shown in table 4.4 and fig. 4.8 the growth of nutritive plant is better than those of mycorrhizal plants in all measures, with statistical clear significance, we observed that there was no significances between mycorrhizal and nutritive plants in the measure of RL.

Because dry weight is the most important measurement in our study, we put a special table for both of the plants.

Dry weight of Eggplant

Table 4.5 The relationship between MP and NMP in dry weight

	Group	No.	Means	Standard Deviation	T-test	Sig.
Dry Weight	MP	10	22.02	1.91	5.07	0.000
	NMP	10	18.44	1.15		
Dry Weight	NP	10	28.37	2.28	12.26	0.000
	NNP	10	18.44	1.15		
Dry Weight	MP	10	33.71	2.73	12.92	0.000
	NMP	10	18.96	2.36		
Dry Weight	AMF	10	22.02	1.91	6.73	0.000
	NS	10	28.37	2.28		

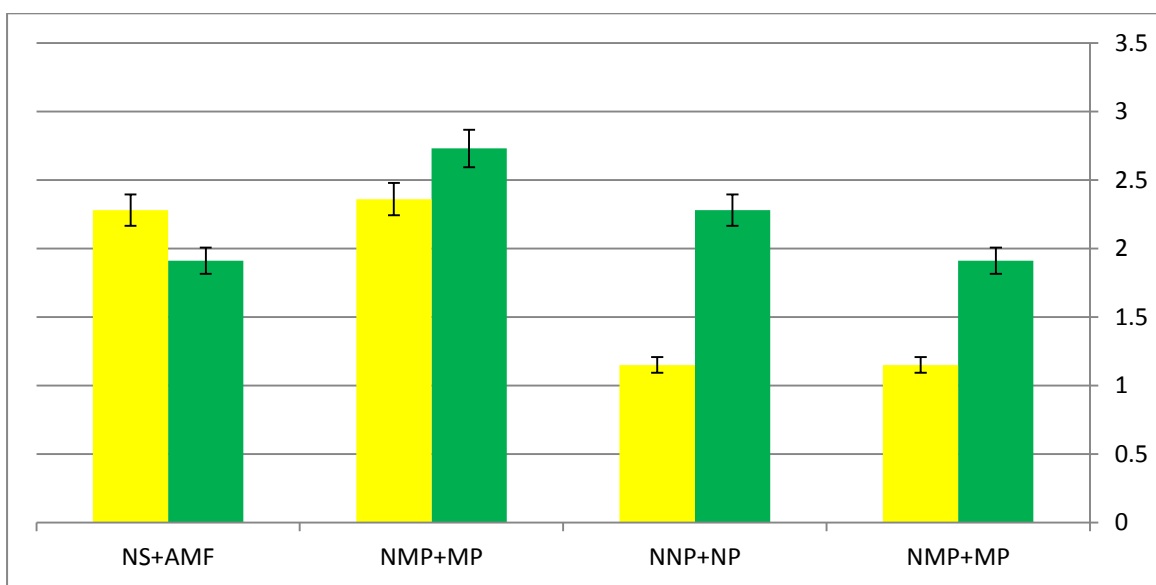


Figure 4.9 The relationship between MP and NMP in dry weight

Table 4.5 and fig. 4.9 illustrate relationship between MP and NMP in dry weight, and the MP is better than those of NMP in the measurement of dry weight, with statistical clear significance.



Figure 4.10 Growth of Eggplant plant treated with AMF comparing with non AMF



Figure 4.11 Growth of Eggplant roots treated with AMF comparing with non AMF

Pepper

Table 4.6 The relationship between mycorrhizized and non mycorrhizized plants in SS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	MP	10	20.70	21.29	0.56	0.582
	NMP	10	24.49	2.05		
Root Length	MP	10	42.22	5.39	2.57	0.019
	NMP	10	37.21	2.96		
Wet Weight	MP	10	30.00	2.81	5.28	0.000
	NMP	10	24.54	1.65		
Number Leaf	MP	10	44.00	6.41	5.89	0.000
	NMP	10	31.30	2.31		
Dry Weight	MP	10	17.19	1.66	6.94	0.000
	NMP	10	12.33	1.46		
Root weight	MP	10	6.89	0.66	10.11	0.000
	NMP	10	4.27	0.48		
Stem weight	MP	10	10.30	0.99	5.04	0.000
	NMP	10	8.06	0.99		

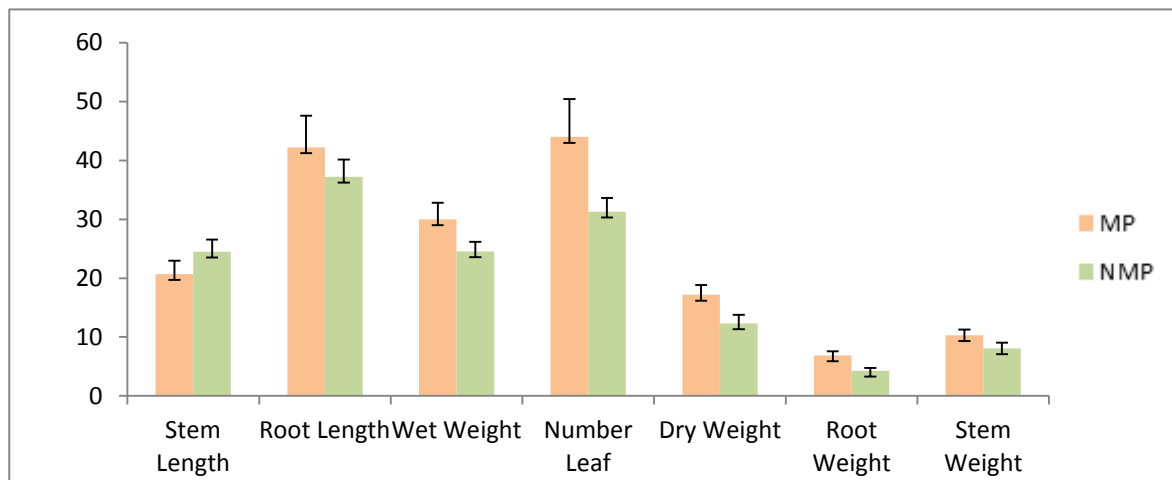


Figure 4.12 The relationship between mycorrhizized and non mycorrhizized plants

Table 4.6 Fig. 4.12 show that the growth of mycorrhizized plant is better than those of non- mycorrhizized plants in all measures , with statistical clear significance, this supports the role of these fungi on increasing the growth of the small seasonal plants, and this means that the presence of fungus has a positive influence plant growth.

Table 4.7 The relationship between nutritive and non-nutritive plants in SS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	NP	10	28.62	2.81	3.75	0.001
	NNP	10	24.49	2.05		
Root Length	NP	10	48.00	3.69	7.19	0.000
	NNP	10	37.21	2.97		
Wet Weight	NP	10	33.81	2.45	9.91	0.000
	NNP	10	24.54	1.66		
Number Leaf	NP	10	41.70	7.24	4.33	0.000
	NNP	10	31.30	2.31		
Dry Weight	NP	10	19.84	1.86	10.03	0.000
	NNP	10	12.33	1.46		
Root weight	NP	10	8.40	0.69	15.51	0.000
	NNP	10	4.27	0.48		
Stem weight	NP	10	11.61	0.85	8.57	0.000
	NNP	10	8.06	0.99		

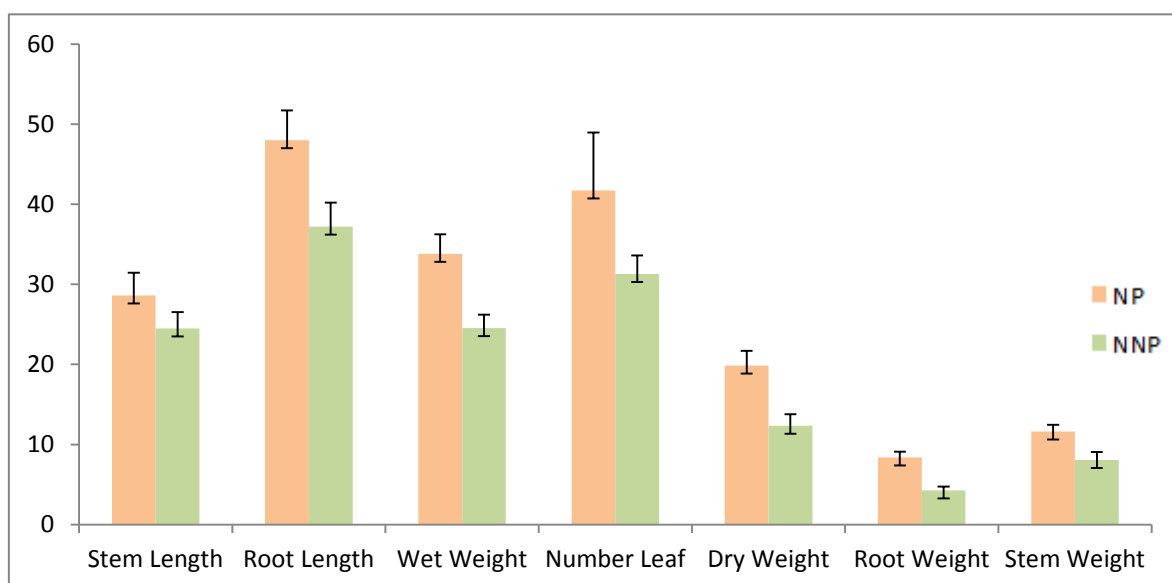


Figure 4.13 The relationship between nutritive and non-nutritive plants

As shown in table 4.7 fig. 4.13 the growth of nutritive plant is better than those of non-nutritive plants in all measures, with statistical clear significance.

Table 4.8 The relationship between mycorrhizized and non mycorrhizized plants in NSS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	MP	10	34.08	2.46	7.56	0.000
	NMP	10	26.45	2.03		
Root Length	MP	10	54.42	4.62	6.75	0.000
	NMP	10	41.53	3.87		
Wet Weight	MP	10	38.73	5.90	6.12	0.000
	NMP	10	26.74	2.21		
Number Leaf	MP	10	46.30	7.60	3.25	0.004
	NMP	10	36.70	2.21		
Dry Weight	MP	10	28.02	7.90	8.25	0.000
	NMP	10	13.85	4.96		
Root weight	MP	10	10.09	5.31	11.39	0.000
	NMP	10	5.18	1.11		
Stem weight	MP	10	17.26	4.68	5.34	0.000
	NMP	10	8.67	0.71		

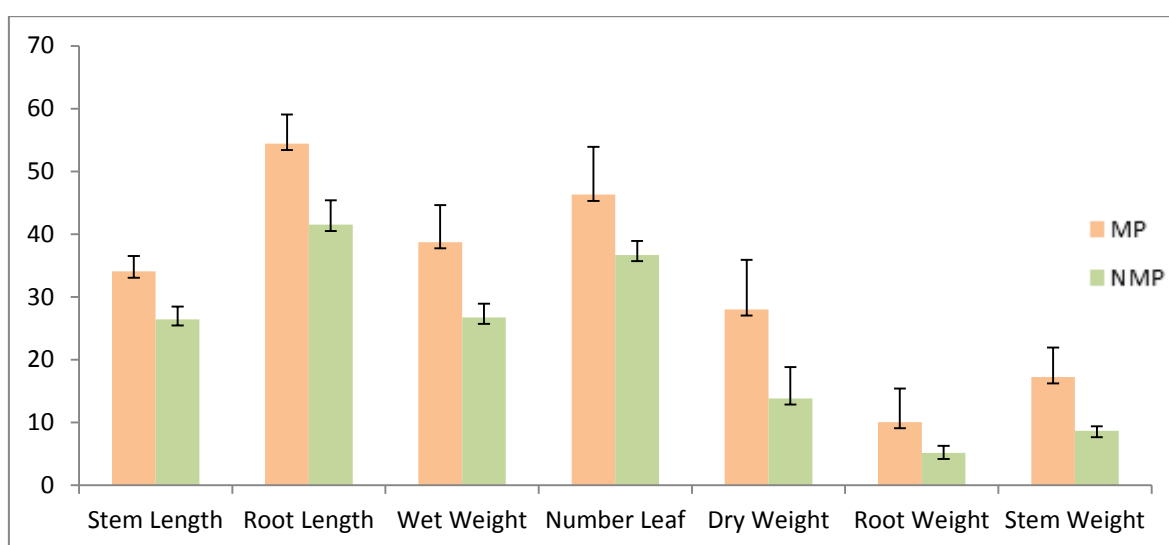


Figure 4.14 The relationship between mycorrhizized and non mycorrhizized plants in NSS

Table 4.8 fig. 4.14 demonstrate that the growth of mycorrhizized plant is better than those of non- mycorrhizized plants in all measures in non -sterilized soil , with statistical clear significance, this supports the role of these fungi on increasing the growth of the small seasonal plants.

Table 4.9 The relationship between mycorrhizal plants and nutritive plants in SS

	Group	No.	Means	Standard Deviation	T-test	Sig.
Stem Length	MP	10	20.70	21.29	1.12	0.051
	NP	10	28.62	2.81		
Root Length	MP	10	42.22	5.39	2.79	0.012
	NP	10	48.00	3.69		
Wet Weight	MP	10	30.00	2.81	3.22	0.005
	NP	10	33.81	2.45		
Number Leaf	MP	10	44.00	6.41	0.75	0.462
	NP	10	41.70	7.24		
Dry Weight	MP	10	17.19	1.66	3.36	0.003
	NP	10	19.84	1.86		
Root weight	MP	10	6.89	0.66	4.97	0.000
	NP	10	8.40	0.69		
Stem weight	MP	10	10.30	0.99	3.16	0.005
	NP	10	11.61	0.855		

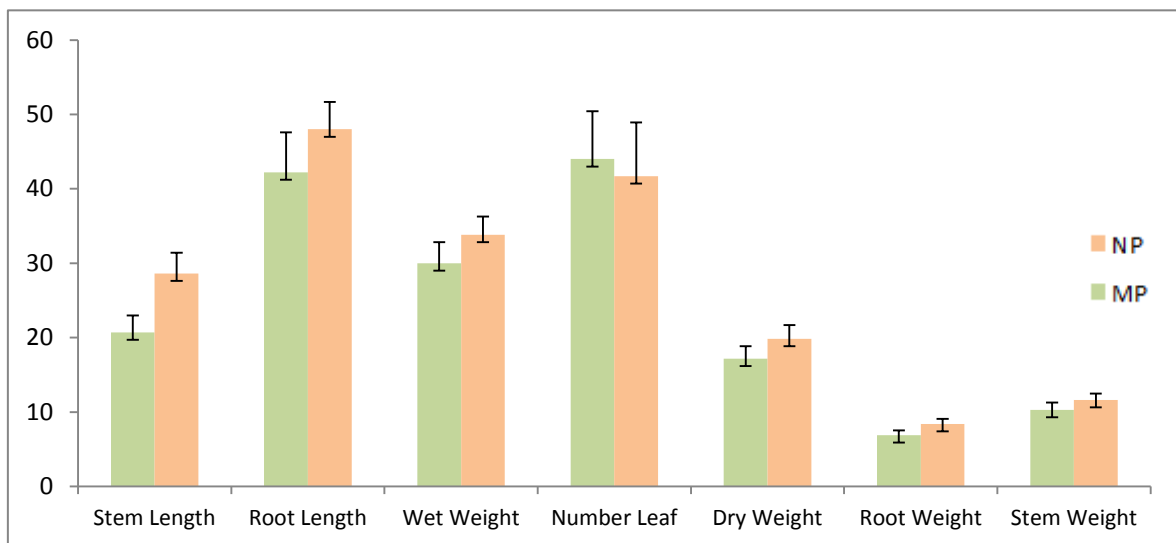


Figure 4.15 The relationship between mycorrhizal plants and nutritive plants

Table 4.9 and fig. 4.15 illustrate that the growth of nutritive plant is better than those of mycorrhizal plants in all measures , with statistical clear significance,we note that there is no difference between mycorrhizal plants and nutritive plants in the measure of NL.

Dry weight of Pepper

Table 4.10 The relationship between MP and NMP in dry weight

	Group	No.	Means	Standard Deviation	T-test	Sig.
Dry Weight	MP	10	17.19	1.66	6.94	0.000
	NMP	10	12.33	1.46		
Dry Weight	NP	10	19.84	1.86	10.03	0.000
	NNP	10	12.33	1.46		
Dry Weight	MP	10	28.02	7.90	8.25	0.000
	NMP	10	13.85	4.96		
Dry Weight	AMF	10	17.19	1.66	3.36	0.003
	NS	10	19.84	1.86		

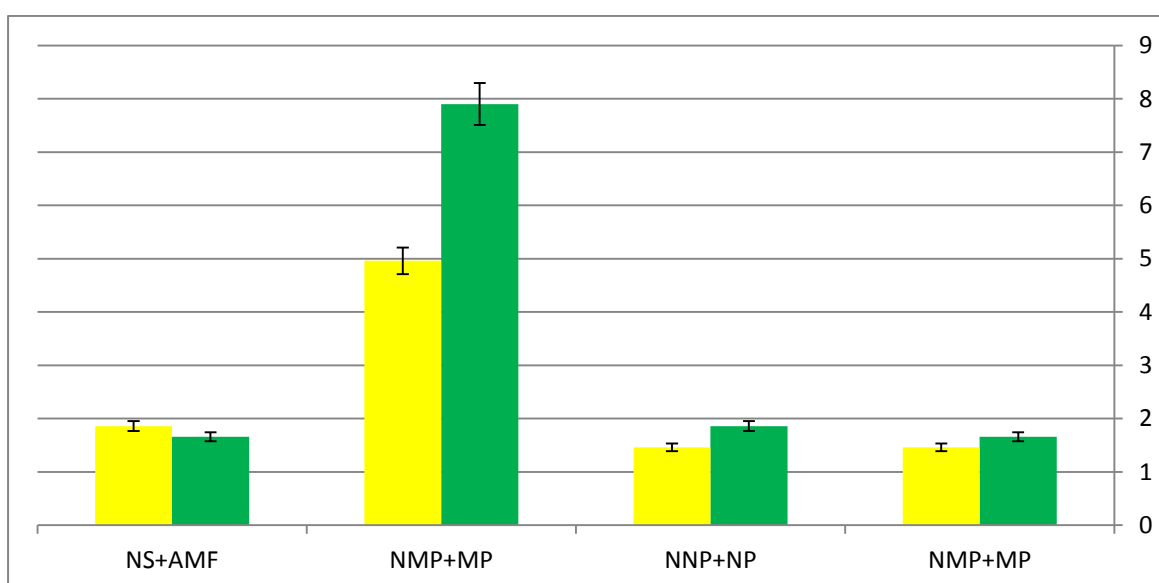


Figure 4.16 The relationship between MP and NMP in dry weight

As shown in table 4.10 and fig. 4.16 the relationship between MP and NMP in dry weight, and the MP is better than those of NMP in the measurement of dry weight, with statistical clear significance.



Figure 4.17 Growth of Pepper plant treated with AMF comparing with non AMF



Figure 4.18 Growth of Pepper roots treated with AMF comparing with non AMF

Chapter 5

Discussion

The main aim of our study was to investigate the influence of a locally isolated AMF (*Glomus sp*) on the growth of local plants (Eggplant and Pepper) by measuring different growth parameters like (SL, WW, RL, NL, RW, SW and specially DW).

The second objective of our study was to show the influence of *Glomus sp* on the growth of local plants, and the role of soil in both sides; sterilize and non-sterilize on the mycorrhization process by measuring the growth of plants, and the existances and not existances of the NS.

The main objective was to compare between the growth of mycorrhized plant and nutritive plants.

Other comparisons was made for fulfillment our main objective like SL, WW, DW, RL, NL, RW and SW between all two groups of plants to investigate the goal of our study.

The problem of chemical fertilizers is a global problem, 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 chemicals and salts into the groundwater, which increases the salinity. 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. Where high rainfall caused the descent of chemicals into the groundwater.

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 some time not available for farmers (Al- Khiat, 2006).

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.

Biofertilizers have several advantages over chemical fertilizers, they are non pollutant, in-expensive, utilize renewable resources. In addition they also supply other nutrients such as vitamins and growth substances (Contra costa, 2003).

For these reasons and other reasons we carried out this research on two important plants (Eggplant and Pepper), for nutrition specially in Gaza Strip in a reticence attempt from chemical fertilizers, and get alternative ways for agriculture fertilizers in Gaza Strip.

5.1 Isolation and Identification of AMF

Glomus is a genus of arbusculamycorrhizal (AM) fungi, and all species form symbiotic relationships (mycorrhizas) with plant roots. *Glomus* is the largest genus of AM fungi, with 85 species described.

5.2 Use of Arbuscular Mycorrhizal Fungul and Nutrition Substances

According to the statistical analysis , the results of the Eggplant and Pepper were close together (similar). Thus, we are going to discuss them together except for some limited points.

After the statistical analysis, a clear difference comes out in growth between the nutritive plants and non- nutritive plants.

An obvious difference also shows the growth of the mycorrhized plants and non- mycorrhized ones.

The outcomes of our study showed that endomycorrhization and nutrition substances plays clear role in positively on eggplant and pepper growth when AMF add to plants as suspension or add the nutrition substances.

A greater growth was always evident in the presence of fungi and nutrition substances in comparison with control plants.

Our study has the same results with previous studies . These studies are the following:

- 1) Wael Shehadeh study in 2010, The Islamic University (Palestine) in two different plants, Watermelon and Summer Squash.
- 2) DEMÜR *et al.*, 2004 study, in Turkey in Pepper plants.
- 3) Beltrano *et al.*, 2013 study, in Argentina in Pepper plants.
- 4) Elahi *et al.*, 2010 study, in Bangladeshi in Eggplant plants.

In general, we can say that the positive influence of AMF on plant growth is very clear compared to those of NS plants. So we can confirmed that the use of AMF as fertilizer is very beneficial to plant growth and environmental healthy.

5.2.1 Plants (Eggplant and Pepper)

The results of the study showed that growth of roots and shoots is increased in the presence of AMF and NS when compared to the control plants.

1- The table 4.1 in Eggplants and table 4.6 in Pepper illustrate the relationship between the mycorrhized plants and non-mycorrhized ones cultured in sterilized soil.

Results shown clear difference between the measures, and there is clear significance for all measures in Eggplant and Pepper, this indicates that the fungus plays a basic role in growth of the plants.

2- Table 4.2 in Eggplants and table 4.7 in Pepper illustrate the relationship between nutritive plants and non- nutritive plants in sterilized soil.

The outcomes data shown similar results in mycorrhizal plants and non-mycorrhizal for all parameters.

3-The table 4.3 in Eggplants and table 4.8 in Pepper illustrate the relationship between the mycorrhizal plants and non-mycorrhizal ones in non-sterilized soil.

Our statistically analysis of the data shown similar results in mycorrhizal plants and non-mycorrhizal for all parameters in sterilized soil.

This indicates that, the normal soil doesn't contain beneficial organisms, and this also shows the bad use of fungicides and pesticides, as methyl bromide, which destroys every thing in the soil specially beneficial living organisms.

4-The table 4.4 in Eggplants and table 4.9 in Pepper shows the relationship between nutritive plants and mycorrhizal ones.

Our statistically analysis illustrate the clear difference for nutritive plants for all measures except:

A)The measure of RL in Eggplant there is no difference between the nutritive plants and mycorrhizal ones.

B)The measure of NL in Pepper there is no difference between nutritive and mycorrhizal plants

Chapter 6

Conclusion & Recommendations

6.1 Conclusions

The present study investigated the influence of endomycorrhizal fungus like *Glomus sp* isolated from local soil on the growth of two seasonal plants in Gaza Strip (Eggplant and Pepper).

In our study, we noticed that in both plants, inoculated with AMF and NS there were a very remarkable growth in comparison with control ones.

According to our statistical analysis, we also noticed that there was a slight difference in growth between the plants inoculated with AMF and NS in favour of the NS.

On the other hand we measured the effect of two different soil (sterilized and non-sterilized) on two plant growth.

The information's that can be concluded from this study are:

1. We obtained a net increasing of growth of two plants Eggplant and Pepper in the presence of AMF suspension and NS when compared to the control plants in sterilized soil.
2. Our statistical analysis illustrate the clear difference in Eggplant and Pepper plants on the growth in the presence of AMF suspension than control plants in non-sterilized soil.
3. Our statistical analysis illustrate the clear difference in Eggplant and Pepper plants on the growth in the presence of nutrition substances than control plants in sterilized soil.
4. The comparison between the growth of the Eggplant and Pepper plants treated with AMF suspension and NS.

In this case our statistical analysis illustrate the difference in Eggplant and Pepper plants on the growth of nutritive plants than mycorrhized in all measures except:

The measurement of RL in Eggplant there is no differences between nutritive plants and mycorrhized ones, because the fungus investigated the growth of eggplant shoot, then the root length increase water uptake and immobile elements.

The measurement of NL in Pepper there is no differences between nutritive plants than mycorrhized, because the plants which fertilized chemically and organically in a good way doesn't need forming leaves in a huge amount owing to availability of nutrition in the soil.

5. In Pepper plants, table 4.6 shown a better growth of mycorrhized plants, than control ones except the measurement of SL.

We shown no differences between mycorrhized and non-mycorrhized plant, may be measurement of SL doesn't affect the productivity of the plant.

6. There is no differences in growth of Eggplant and Pepper in sterilized and non-sterilized soil when added AMF to two the plants comparing with control plants.

6.2 Recommendations

1. It is recommended to isolate other local fungi in agricultural areas and determine the species accurately.
2. The experiments of this study may be repeated using a wider range of plants including vegetables particularly those useful to the human diet as Tomato.
3. The experiments of this study may be repeated using another *Glomus sp* or using mixture of different *Glomus sp*.
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. Our study repeated in the presence of the fungus and chemical fertilizers by different quantities to measure the synergistic effects of fungus and chemical fertilizers.
6. We recommend to perform this experiment in the field.

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Appendix

Components of nutrition substances (Sheavah 11 fertilizer) used in this study

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)

Eggplant and pepper plants (0.5 litter)

Every two weeks (cm \ 3 acres)

Eggplant and pepper plants (0.5 – 1 litter)

In this study, we used nutrition substances (Sheavah 11 fertilizer) . The seedlings were treated after transplanting by 100 ml\ seedling and then once every two weeks during the study period.

Components of PDA media used in this study:

Ingredients	Gms/litre
Potatoes infusion form	200
Dextrose	20
Agar	15
Final PH at 25C ⁰	5.6 +-0.2.

How to use :

Suspend 39 grams in 1000ml distilled water .

Heat to boiling to dissolve the medium completely.

Sterilize by autoclaving at 15 lbs pressure 121 C⁰ for 15 minutes.

Mix well before dispensing.

Techniques to Observe AMF.

Most observations of AMF 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 cutting roots of into small pieces(1-5cm), and clean water quietly.

Then treated in hot 10% KOH for 90 minutes to removes the host cytoplasm and then the nuclei.

Acid(HCl 1%) has been added to reduce the alkaline solution.

After the roots are neutralized in a weak acid wash, they are stained in Trypan blue(0.05%). 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.