



Impact of rhizobia and vesicular arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *vicia faba* plant

By

Doaa El Sayed Mosaad Gaafar

B.Sc. Microbiology, Microbiology Dept., Faculty of Science, Suez Canal University, 2002

Thesis

Submitted in partial fulfillment of the requirements for the master science degree in Microbiology

Under supervision of

Prof. Zakaria Awad Mohamed Baka

Professor of microbiology, Botany Department, Faculty of Science, Damietta University.

Dr. Mohamed Ismail Abou-Dobara

Associate professor of microbiology, Botany Department, Faculty of Science, Damietta University.

Dr. Osama Nagdi Mohamed Massoud

Associate professor of microbiology, Soil, Water and Environment Institute, Agricultural Research Centre, Giza, Egypt.

2014

Supervisors

Title of Thesis:

Impact of rhizobia and vesicular arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *vicia faba* plant.

Name of the candidate:

Doaa El Sayed Mosaad Gaafar

Supervisors:

No.	Name	Position	Signature
1	Prof. Dr. Zakaria Awad Mohamed Baka	Professor of Microbiology, Botany Department, Faculty of Science, Damietta University.	
2	Dr. Mohamed Ismail Abou-Dobara	Associate professor of Microbiology, Botany Department, Faculty of Science, Damietta University.	
3	Dr. Osama Nagdi Mohamed Masood	Associate professor of Microbiology at Soils, Water and Environment Institute (SWERI), Agricultural Research Centre, Giza, Egypt.	

*Head of Botany
Department*

***Prof. Dr. Neamt
Mohamed Hassan***

Vice Dean

***Prof. Dr. Mamdouh
Mohamed Ahmed
Neamt Allah***

Dean of Faculty

***Prof. Dr. Ahmed El
Sayed Abdel Ghani
AlGhobashy***

REFREE COMMITTEE DECISION

Title of thesis: Impact of rhizobia and vesicular arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *vicia faba* plant

Name of candidate: Doaa El Sayed Mosaad Gaafar

Supervisors:

No.	Name	Position
1	Prof. Dr. Zakaria Awad Mohamed Baka	Professor of microbiology, Botany Department, Faculty of Science, Damietta University.
2	Dr. Mohamed Ismail Abou-Dobara	Associate professor of microbiology, Botany Department, Faculty of Science, Damietta University.
3	Dr. Osama Nagdi Mohamed Massoud	Associate professor of microbiology, Soils, Water and Environment Institute, Agricultural Research Centre, Giza, Egypt.

Refree committee:

No.	Name	Position
1	Prof. Dr. Yehia Ahmed Maher El-Zawahry	Professor of microbiology, Botany Department, Faculty of Science, Zagazig University.
2	Pr. Dr. Ahmed Ismail Abdel-Kader	Professor of microbiology, Environmental Science Department, Faculty of Science, Port Said University.
3	Prof.Dr. Zakaria Awad Mohamed Baka (common report)	Professor of microbiology, Botany Department, Faculty of Science, Damietta University.
4	Dr. Mohamed Ismail Abou-Dobara (common report)	Associate professor of microbiology, Botany Department, Faculty of Science, Damietta University.

*Head of Botany
Department*

**Prof. Dr. Neamt
Mohamed Hassan**

Vice Dean

**Prof. Dr. Mamdouh
Mohamed Ahmed
Neamt Allah**

Dean of Faculty

**Prof. Dr. Ahmed El
Sayed Abdel Ghani
AlGhobashy**

ACKNOWLEDGMENTS

First and foremost, great thankful, sincere and humble gratitude to "Allah", the most merciful for giving me the strength, determination and patience to complete this work.

*I am deeply grateful and wish to express my sincere appreciation and gratitude to my supervisor, **Prof. Dr. Zakaria Awad Mohamed Baka**, Professor of microbiology, Botany Department, Faculty of Science, Damietta University, for his valuable supervision, encouragement, endless help and support from the initial to the final level enabled me to develop an understanding of the subject.*

*It gives me great pleasure to acknowledge **Dr. Mohamed Ismaïl Abou-Dobara**, Associate professor of microbiology, Botany Department, Faculty of Science, Damietta University for his advices, fruitful guidance and continuous interest during the performance of this work.*

*Words are not enough to express my gratitude to **Dr. Osama Nagdi Mohamed Massoud**, Associate professor of microbiology, Soil, Water and Environment Institute, Agricultural Research Centre. His overwhelming work load have never been able to decrease his availability and accessibility by even an iota. I remain eternally indebted to his patient guidance, constant encouragement, support and invaluable suggestions that made this work successful. And without his help this work not have been accomplished.*

Dedication

I would like to dedicate this thesis to

My family,

My husband,

My children,

Hana and Karim

Contents

	page
Introduction	1
Review of literature	
1- Legumes.....	4
1.1. Nutritional, medical and economical importance of legumes.....	5
1.2. The relationship among the legume, N and CO ₂	6
1.3. The relationship between the legume and P supply.....	6
1.4. Faba bean.....	7
2- Biofertilization.....	9
2.1. The importance of biofertilization.....	9
3- Compost.....	10
4- Nitrogen fixation.....	11
5- Mechanism of plant invading by rhizobia.....	13
5.1. Detection and response to host-released signals by members of rhizobiaceae.....	13
5.2. Host detection during nodule formation.....	13
5.3. Early signals from legume to rhizobia.....	15
5.4. Structure and function of flavonoids.....	15
5.5. Reverse signals from rhizobia to legume roots – the chitolipooligosaccharide <i>nod</i> Factors.....	16
5.6. Transcriptional regulators of <i>nod</i> genes.....	17
5.7. Infection and nodule organogenesis.....	17
5.7.1 Root hair curling.....	17
5.7.2 Bacteroid formation.....	19
5.7.3 Symbiosome.....	20
6. Mycorrhizae.....	21
6.1. A short overview of mycorrhizas.....	21
6.2. Ectomycorrhizas.....	21
6.3. Endomycorrhizas.....	23
6.4. Ericoid mycorrhizas.....	23
6.5. Orchid mycorrhizas.....	23
7. Arbuscular mycorrhizas.....	23
7.1. Mechanism of arbuscular mycorrhizal fungi association with the host plant	24
7.2. Root hairs and arbuscular mycorrhizas.....	27
8. The legume- <i>Rhizobium</i> -AM fungi relationship.....	31
Materials and methods	
1. Part I: <i>In vitro</i> work.....	32
1.1. Isolation, characterization and identification of <i>Rhizobium</i> species from roots of Faba bean.....	32

1.1.1. Sample collection.....	32
1.1.2. Isolation.....	32
1.1.3. Rhizobia characteristics.....	33
1.1.4. Identification.....	33
1.2. Isolation and characterization of ArbuscularMycorrhizal (AM) fungi.....	34
1.2.1. Isolation of Arbuscularmycorrhizal spores.....	34
1.2.1.1. Sampling procedure.....	34
1.2.1.2. Extraction and estimation of AM spores.....	34
1.2.2. Propagation of AMF.....	35
1.2.3. AM characterization and identification.....	35
1.2.4. Estimation of mycorrhizal root infection.....	35
2. Part II: Effect of AM-mycorrhizae, N ₂ -fixing bacteria and compost on the growth and yield of Faba bean plant.....	36
2. 1. Field experiment.....	36
2.1.1. Layout of field experiment.....	36
2. 2. Seeds.....	38
2. 2.1. Preparation of seeds for cultivation.....	38
2. 2.2. <i>Rhizobium leguminosarum</i> application.....	38
2. 2.3. AM fungi application.....	38
2. 3. Organic matter (compost).....	38
2. 4. Fertilization.....	40
2. 5. The experiment.....	41
2. 6. Measured parameters.....	41
2. 7. Microbial determinations.....	42
2. 7.1. Dehydrogenase activity.....	42
2. 7. 2. Estimation of nitrogenease activity.....	42
2. 7. 3. Determination of photosynthetic pigments.....	44
2.8. Chemical analysis.....	44
2.8.1. Total phosphorus in plant.....	44
2.8.2. Total nitrogen in plant.....	45
2.8.3. Total potassium in plant.....	45
3. Statistical analysis.....	45
Experimental results.....	
Part I: <i>In vitro</i> work.....	
1. Isolation, characterization and identification of <i>Rhizobium</i> species from roots of faba bean.....	46
2. Isolation, characterization and identification of arbuscularmycorrhizalfungi from different cultivated plants.....	50
2.1.Number of AM spores.....	50
2.2.Root infection.....	51

2.3. Identification of the extracted AM spores.....	
Part II: Effect of AM-mycorrhizae, N₂-fixing bacteria and compost on the growth and yield of faba bean plant...	
1. Effect of arbuscular mycorrhizal fungi, <i>Rhizobium leguminosarum</i> and compost on shoot parameters.....	54
2. Effect of arbuscular mycorrhizal fungi, <i>Rhizobium leguminosarum</i> and compost application on some growth parameters.....	57
3. Effect of mycorrhizal colonization, <i>Rhizobium leguminosarum</i> and compost application on some root parameters.....	57
4. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on mycorrhizal infection percentage after 60, 90 and 120 days from sowing of faba bean plant.....	58
5. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on nitrogenase activity in rhizosphere region after 60, 90 and 120 days from sowing of Faba bean plant.....	64
6. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on dehydrogenase (DHA) activity in the rhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.....	64
7. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on total nitrogen percent of faba bean plant after 60, 90 and 120 days from sowing.....	67
8. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on total phosphorus percent of faba bean plant after 60, 90 and 120 days from sowing.....	67
9. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on total potassium percent of faba bean plant after 60, 90 and 120 days from sowing.....	70
10. Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> on some physiological parameters of faba bean plant after 60 and 90 days from sowing.....	70
Discussion	75
• Isolation.....	76
• Nodulation.....	77
• Growth parameters.....	78
• Yield and its components.....	79
• Chemical composition.....	80
Summary.....	83
References.....	85
Arabic summary.....	

List of tables

Table No.	Table title	Page No.
Table (1)	Mechanical and physico-chemical properties of the used soil.....	37
Table (2)	Chemical analysis of the used compost.....	39
Table (3)	Locations of the collected samples from <i>Vicia faba</i> plant and their nodules number and characteristics.....	48
Table (4)	Identification of the isolated bacterial samples.....	49
Table (5)	Survey and isolation and of arbuscularmycorrhizal fungi from a number of cultivated plant species.....	52
Table (6)	Characterization and identification of the isolated mycorrhizal fungi.....	53
Table (7)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> shoot length of faba bean plant after 60, 90 and 120 days from sowing.....	59
Table (8)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> shoot dry weight of faba bean plant after 60, 90 and 120 days from sowing.....	56
Table (9)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> shoot parameters of faba beanplant..... ..	59
Table (10)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> number of root nodules of faba bean plant after 60, 90 and 120 days from sowing.....	60

Table (11)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> nodules dry weight of faba bean plant after 60, 90 and 120 days from sowing.....	61
Table (12)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> microbial root colonization of faba bean plant after 60, 90 and 120 days from sowing.....	62
Table (13)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> mycorrhizal infection percentage after 60, 90 and 120 days from sowing of faba bean plant.....	63
Table (14)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> nitrogenase activity in rhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.....	65
Table (15)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> dehydrogenase activity in rhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.....	66
Table (16)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> total nitrogen percent of faba bean plant after 60, 90 and 120 days from sowing.....	68
Table (17)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> total phosphorus percent of faba bean plant after 60, 90 and 120 days from sowing.....	69
Table (18)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> total potassium percent of faba bean plant after 60, 90 and 120 days from sowing.....	71

Table (19)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> chlorophyll a content of faba bean plant after 60 and 90 days from sowing.....	72
Table (20)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> chlorophyll b content of faba bean plant after 60 and 90 days from sowing.....	73
Table (21)	Effect of inoculation with AM-fungi and <i>Rhizobium leguminosarum</i> carotenoids content of faba bean plant after 60 and 90 days from sowing.....	74

List of figures

Fig. No.	Figure title	Page No.
Fig. 1	Mechanism of plant invading by Rhizobia.....	14
Fig. 2	The different michorrhizal types.....	22
Fig. 3	Root hairs during the development of an ectomycorrhiza..	28
Fig. 4	Nodules of faba bean samples separated from the roots....	47
Fig. 5	Colony of the isolated <i>Rhizobium leguminosarum</i>	47

Introduction

Faba bean (*Vicia faba* L., broad bean, horse bean) is grown worldwide in cropping systems as a grain (pulse) and green-manure legume. The faba bean contributes to the sustainability of cropping systems via: (1) its ability to contribute nitrogen (N) to the system via biological N₂ fixation, (2) diversification of systems leading to decreased disease, pest and weed build-up and potentially increased biodiversity, (3) reduced fossil energy consumption in plant production, and (4) providing food and feed rich in protein (Erik *et al.*, 2010).

A large number of different interactions between fungi and bacteria occur in association with plants, depending on the nature of species involved and the plant can be positively or negatively affected. Microbial activity in rhizosphere soil affects plant health and growth (Ali, 2013).

As a consequence of inappropriate applications of chemical fertilizers during continuous crop cultivation, many countries suffer from problems such as pollution of agricultural lands, water resources, and soil salinization. In an attempt to reduce these chemical inputs and raise soil quality as well as improve crop production, biotechnological practices such as application of biofertilizers have been investigated (Talaat and Abdallah, 2008).

Biofertilizers, particularly those of diazotrophs, are well documented as a great part of plant N demand. Plant growth promoting Rhizobacteria (PGPR) have been used as biofertilizers for economically important crops and shown to increase crop growth and productivity (Desai *et al.*, 2012). The nitrogen-fixing interaction between Rhizobia and legumes and the mycorrhizal association between fungi and most of land plants are the most two commonly studied symbioses (Talaat and Abdallah, 2008).

Most agricultural crops are colonized by arbuscularmycorrhizal fungi (AMF). In this symbiotic association, host plants provide the fungi with carbohydrates and in return receive mineral nutrient. AMF can enhance growth of crop plants through increasing nutrient uptake, particularly P (Ryan and Angus, 2003).

Rhizobia stimulate plant growth mainly by modifying root development, which improved macro and micronutrients and water uptake, particularly in the early stages of plant development (Antounet *al.*, 1998).

Biological nitrogen fixation (BNF) can act as a renewable and environmentally sustainable source of N and can complement or replace fertilizer inputs. Its use can mitigate the need for fertilizer nitrogen, with concomitant benefits accruing in terms of effects on the global nitrogen cycle, global warming, and ground- and surface-water contamination. Intercropping legumes and other species capable of symbiotic N₂fixation offer an economically attractive and ecologically sound means of reducing external inputs and improving the quality and quantity of internal resources. Nitrogen from this source (biologically fixed N₂) is used directly by the plant, and so is less susceptible to volatilization, denitrification and leaching. BNF is a kind of beneficial plant–microbe interaction that provides a restricted range of plants with the often-limiting macronutrient-nitrogen (Neera and Geetanjali, 2007).

Compost is a highly diverse group of organic soil amendments which provides substantial nutritive fertility to soils. Dairy manure compost supplies not only the major nutrients (N, P, and K), but also a broad range of secondary nutrients, micronutrients and organic matter. Dairy manure can also improve water and nutrient holding capacity of the soil, reduce erosion, and reduce fluctuations in

soil pH. Nutrients in compost products are more stable and are typically released gradually over three or more years; whereas inorganic fertilizers are generally formulated to release nutrients within a year of application (David et al., 2011).

Review of literature

1- Legumes

Legumes belong to the family of Fabaceae, once called Leguminosae, which is the third largest family of angiosperm plants. This plant family contains approximately 17,000 to 19,000 species throughout the world; nearly 3,000 species have been established as potential symbiotically nitrogen-fixing plants with Rhizobia. Legumes form a fundamental component of our diet. Their use was first documented in Ancient Egypt followed by Southwestern Asia and Ancient Europe civilizations to pre-Columbian America. Legumes are now used commonly in intensively managed agricultural situations to substitute for nitrogen fertilizers and many are artificially inoculated with various strains of rhizobia (Bird, 2009).

Legumes are plants that bear seeds in pods. They markedly differ from non-legume crops because much of the nitrogen they require is produced through fixation of atmospheric nitrogen by bacteria inhabiting nodules borne on their roots. World-wide, more than 16,000 species of legumes are known, including herbs, shrubs, and trees, but only about 200 are cultivated. Historically, legumes have been part of dietary system throughout the world. They are second only to the cereals improvising food crops for world agriculture. The total world value for leguminous crops is thought to be approximately two billion US dollars per annum. Peanut (*Arachis hypogaea*) and lima bean (*Phaseolus lunatus*) have been used for centuries in South America. Among others, soybeans and mung beans have been a key part of Asian dishes throughout the history. The Middle East is the origin of broad beans(*Vicia faba*), chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*) (Javaid, 2010).

1.1.Nutritional, medical and economical importance of legumes

Seed legumes occupy an important place in human nutrition, especially in the dietary pattern of low-income groups of people in developing countries. They are a valuable source of food proteins. Proteins in legume seeds range from about 20% (dry weight) in pea and beans, up to 38–40% in soybean and lupin. Quite often, they represent a necessary supplement to other protein sources(Duranti, 2006).

Legumes are typically low in fat, contain no cholesterol, and are high in folate, potassium, iron, and magnesium (Andersen *et al.*, 1984; Grusak, 2002). Being a good source of protein, they could serve as a substitute for meat, which has more fat and cholesterol. Several reports claim that inclusion of legumes in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as, diabetes mellitus, coronary heart disease, and colon cancer (Tharanathan and Mahadevamma, 2003).

The use of legumes in rotation with cereal and oilseed crops is a well-established practice to increase soil fertility and crop yields (Lupwayi and Kennedy, 2007; Ncube *etal.*, 2009). Many legumes, such as *Lupinus* spp., *Medicago* spp., and *Trifolium* spp., are used as fodders, green manures, and forages. Legumes are also utilized for a variety of other purposes including timber, medicine, tannins, and gums. Various species of *Lonchocarpus* and *Derris* are the source of rotenone, which is used as an insecticide, fish poison, or molluscicide. Some legume trees yield valuable resins, used in varnishes, paints, and lacquers. In addition to the direct benefits from nitrogen fixation, legumes have long been known to have a longer-term effect on soils, via such means as reduction of pathogen load (Mourad *et al.*, 2009). Moreover, in regard to greenhouse gases, legumes can have a very

positive effect, not only by reducing emission of nitrous oxide from excess fertilizer nitrogen, but also by sequestering carbon in soils (Javaid, 2010).

1.2. The relationship among the legume, N and CO₂

The major elemental constituents of plant biomass are carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), sulphur (S), calcium (Ca), potassium (K) and magnesium (Mg). Most of the plant biomass is formed from the first three elements (C represents about 45%, O represents about 45% and H represents about 6%). The remaining six elements constitute the macronutrients. Of these, it is generally the supply of N, P and K which often limits plant growth. Nitrogen is a major constituent of proteins, nucleic acids and chlorophyll and its elemental content on a dry mass basis when N supply is not limiting and can be as high as 1.5 %. Nitrogen is a major component of the cellular catalytic machinery and is thus the most fundamental element limiting biomass production (Ågren, 1985).

1.3. The relationship between the legume and P supply

Phosphorous (P) is the other key element involved in biomass production. High specific rates of crop growth require available P in the form of HPO_4^- and H_2PO_4^- at soil contents greater than 10.0g P g^{-1} soil. Phosphorus requirement for plant growth ranges from 4 % to 10 % of the N accumulated by the plant. Its elemental content on a dry mass basis when P supply is not limiting approaches 0.2 %. Phosphate is a major component of sugar phosphates, nucleic acids, cell membranes and adenosine triphosphate (ATP), and the latter constituting the energy currency of the cell. Many biosynthetic reactions have a high demand for P in the form of ATP (Miller and Donahue, 1990).

Egyptian soils are typically containing high levels of CaCO_3 and high pH. This type of soil requires high amounts of P fertilizer to support adequate plant growth. Moreover, sources of P fertilizer are in short supply, which may result in a crisis in plant nutrition in the 21st century. Thus, understanding the means by which arbuscular mycorrhizal (AM) fungi enable P acquisition and finding ways to employ this symbiosis for world-wide agriculture are of critical importance. Arbuscular mycorrhizae (AM) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality (Talaat and Abdallah, 2008).

1.4.Faba bean:

Faba bean, *Vicia faba*, is a winter growing pulse, or food legume crop. It originated in the Middle East in the pre-historic period, and has since spread throughout Europe, North Africa, and Central Asia. It spread to China over 2,000 years ago via traders along the Silk Road, to South America in the Columbian period, and more recently to Canada and Australia.

Faba bean plants may grow to a height of 2 m at maturity. The plant is erect, and produces stems from its base. Leaves are compound, having 2-7 leaflets. First leaves have only 2 leaflets, but there are 7 in the last formed leaves. The taproot bears a profusion of fibrous roots in the top 30 cm of soil.

Flowering in early varieties begins from about the 5-7th leaf bearing stem node (joint), and up to the 15th or higher node in late varieties. Flowers are borne in clusters (inflorescences) comprising 3 to 8 flowers (varying with variety) in the

angle between leaf and stem (axil) at each node. Inflorescences form in succession up the stem as each new node is produced, over a period of 6–10 weeks, or about 15 flowering nodes.

Like many legumes, excess flowers are produced and fewer than 15% will develop to produce pods. Honeybees seek nectar from the flowers and in the process pick up pollen, transferring it between plants, and causing cross-pollination to occur at rates commonly in the range 25-30%. Flowering finishes when daytime temperatures approach 30°C, after which an extra few leaf bearing nodes are produced.

Pods in a well grown crop are borne from about 20 cm above ground to maybe 30 cm below crop height. Each pod contains 2 to 4 seeds. As pods mature they turn black, as eventually do the stems and leaves of the plant. Seeds vary in size depending on variety, from large flattened beans (also known as broadbeans) through medium sizes to smaller, rounded seeds like field peas (Matthews and Marcellos, 2003).

Faba bean is one of the most important legume crops worldwide because the nitrogen-fixing interaction between Rhizobia and the plant, offering high quality protein, capable of returning atmospheric nitrogen to the soil. The crop area in Egypt was about 250000 feddan (Talaat and Abdallah, 2008).

2- Biofertilization

As a consequence of inappropriate applications of chemical fertilizers during continuous crop cultivation, many countries suffer from problems such as pollution of agricultural lands, water resources, and soil salinization. In an attempt to reduce these chemical inputs and raise soil quality as well as improve crop production, biotechnological practices such as application of biofertilizers have been investigated. Increasing crop production is one of the major targets of agricultural policy, which could be achieved by several ways. Increasing the efficiency of atmospheric nitrogen fixation as well as the more organic phosphorus availability is considered as important ways to increase crop productivity (Talaat and Abdallah, 2008).

2.1.The importance of biofertilization

The nitrogen-fixing interaction between Rhizobia and legumes and the mycorrhizal association between fungi and most of land plants are the most two commonly studied symbioses. Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes.

The modern day intensive crop cultivation requires the use of chemical fertilizers but the relatively high cost of producing N fertilizer, as well as some of the problems associated with its application, such as runoff into soils and water sources, provide incentive for increased exploitation of biological nitrogen fixation(BNF). Rhizobia stimulate plant growth mainly by modifying root development, which improved macro and micronutrients and water uptake, particularly in the early stages of plant development. The plant host organisms may

be affected by one or more mechanisms such as nitrogen fixation, enhancing nutrient uptake, production of plant growth promoting substances, phytohormones, and organic acids, as well as protection of plant from pathogens and the organism benefits by acquiring photosynthates from the plant (Talaat and Abdallah, 2008).

3- Compost

Compost is a highly diverse group of organic soil amendments which provides substantial nutritive fertility to soils. The benefits of compost addition to soils are vast and have been well documented by a growing body of research. A variety of soil amendment products and potential nutrient sources provide flexibility for agricultural and horticultural systems.

Dairy manure compost, for example, supplies not only the major nutrients (N, P, and K), but also a broad range of secondary nutrients, micronutrients and organic matter. These plant nutrients have economic value, which can be used to estimate compost value for comparisons with traditional fertilizer materials. Organic matter applications, such as dairy manure, can also improve water and nutrient holding capacity of the soil, reduce erosion, and reduce fluctuations in soil pH.

Nutrients in compost products are more stable and are typically released gradually over three or more years; whereas inorganic fertilizers are generally formulated to release nutrients within a year of application. Thus, a realistic assessment of compost value requires at least a 3-year time frame. Also, since compost nutrient ratios and release rate may not be optimal for crop needs, some supplemental inorganic fertilizer (particularly N) may be necessary.

Currently, the need for reducing environmental impact requires diverse processes that permit an integrated reuse of solid residues, from which products or commodities of industrial importance can be obtained. The waste generated by communities is composed of diverse materials that vary according to climate, urbanization, and socio-economic stratum. Approximately 38% of all trash produced is biodegradable organic matter that does not have a market. This organic trash generates a serious environmental problem, even when it can be used for the production of compost or other uses(David *et al.*, 2011).

4- Nitrogen fixation

The Green Revolution was accompanied by a huge increase in the application of fertilizers, particularly nitrogen. Recent studies indicate that a sizeable proportion of the human population depends on synthetic nitrogen (N) fertilizers to provide the 53 million tons that is harvested globally in food crops each year (Neera and Geetanjali, 2007).

Nitrogen fertilizers affect the balance of the global nitrogen cycle, pollute ground water and increase atmospheric nitrous oxide (N₂O), a potent “greenhouse” gas. The production of nitrogen fertilizer by industrial nitrogen fixation not only depletes our finite reserves of fossil fuels, but also generates large quantities of carbon dioxide, contributing to global warming. The continued and unabated use of N fertilizers would accelerate the depletion of stocks of nonrenewable energy resources used in fertilizer production. The removal of large quantities of crop from the land depletes soil of its native N reserves. There are vast areas of the developing world where N fertilizers are neither available nor affordable due to weak infrastructure, poor transportation, and high cost(Peoples and Crasswell, 1992).

Biological nitrogen fixation (BNF) can act as a renewable and environmentally sustainable source of N and can complement or replace fertilizer inputs. Its use can mitigate the need for fertilizer nitrogen, with concomitant benefits accruing in terms of effects on the global nitrogen cycle, global warming, and ground- and surface-water contamination. Intercropping legumes and other species capable of symbiotic N₂ fixation offer an economically attractive and ecologically sound means of reducing external inputs and improving the quality and quantity of internal resources. Nitrogen from this source (biologically fixed N₂) is used directly by the plant, and so is less susceptible to volatilization, denitrification and leaching. BNF is a kind of beneficial plant–microbe interaction that provides a restricted range of plants with the often-limiting macronutrient-nitrogen (Neera and Geetanjali, 2007).

Biological nitrogen fixation is done by both free-living organisms (e.g., *Azotobacter*, *Beijerinckia*, *Clostridium*, *Bacillus*, *Klebsiella*, *Chromatium*, *Rhodospirillum*) and those that form symbiotic associations with other organisms. In agricultural settings, perhaps 80% of this biologically fixed N₂ comes from symbiosis involving leguminous plants and α -proteobacteria, order Rhizobiales, family Rhizobiaceae, including species of *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium* (Willems and Collins, 1993; Farrand *et al.*, 2003).

5- Mechanism of plant invading by rhizobia

5.1.Detection and response to host-released signals by members of rhizobiaceae.

Plants exude high levels of nutrients, and many of these act as chemoattractants for the bacteria. Binding of rhizobia to plant surfaces is essential

for establishing a long-term interaction of the bacteria with their hosts. Plant lectins (proteins that possess at least one non catalytic domain that binds reversibly to mono- or oligosaccharides) could serve as receptors for bacterial exopolysaccharides (EPS). Lectins might mediate specificity in the *Rhizobium*–legume symbiosis (Heeb and Haas, 2001; Rudiger and Gabius, 2001). Binding of rhizobia to plant surfaces is thought to take place in two steps. The first is a rather weak and reversible binding step that may involve a variety of bacterial polysaccharides. The second binding step requires the synthesis of bacterial **cellulose**, which causes a tight, irreversible binding and formation of bacterial aggregates on the host surface (Fig. 1) (Robertson *et al.*, 1988).

5.2. Host detection during nodule formation.

Nitrogen fixation can only occur when the plants are in the symbiotic state and the Rhizobia invade the root or stem cortex (Cooper, 2004). The progression to the symbiotic state by two initially independent, freeliving partners is governed by reciprocal signal generation and perception, which has been described as “molecular dialogue”. This leads to a gradual and coordinated differentiation and adjustment of physiology and metabolism in both partners (Broughton *et al.*, 2000; Perret *et al.*, 2000; Spaink, 2000).

5.3. Early signals from legume to rhizobia.

Symbiotic interaction is initiated by micromolar or nanomolar concentrations of flavonoids or isoflavonoids in legume root or seed exudates. These compounds may initially assist rhizosphere colonization by acting as chemoattractants or less likely, as growth enhancers for rhizobia (Copper, 2004).

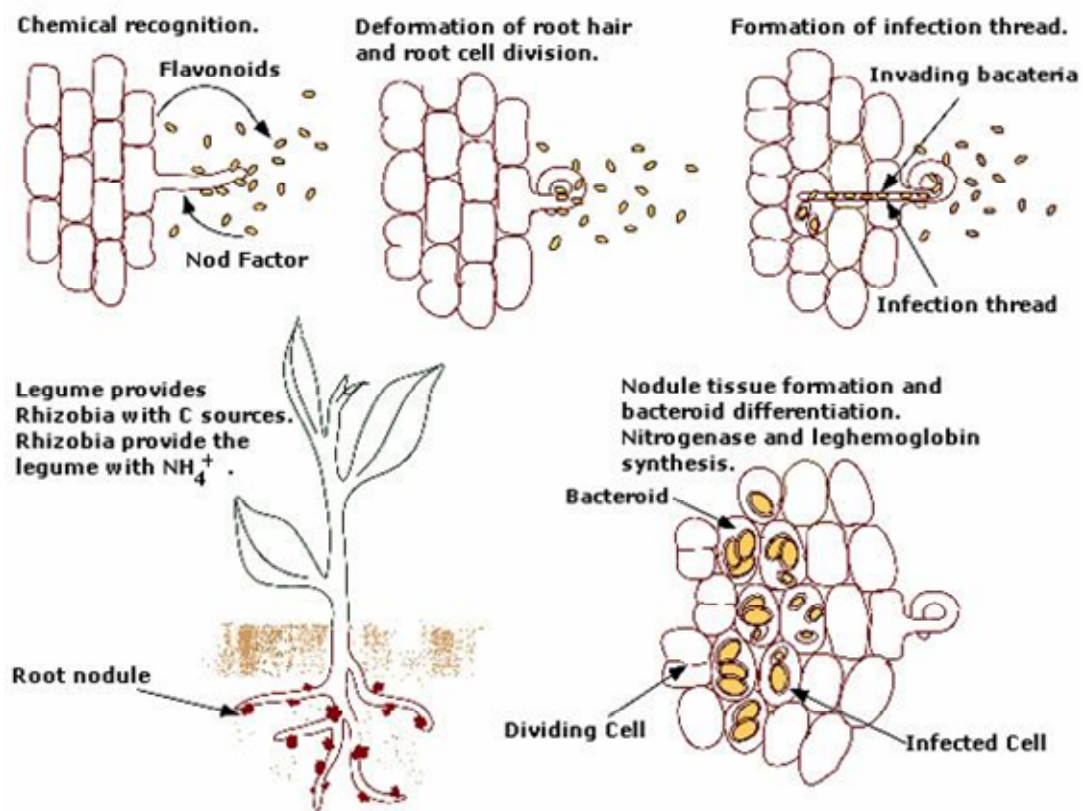


Fig. (1): Mechanism of plant invading by rhizobia.

5.4. Structure and function of flavonoids.

Flavonoids are released in their greatest amounts near root tips, and optimal concentrations occur near the emerging root hair zone, which is most favorable site for *Rhizobium* infection. Their main role in the initiation of a rhizobial symbiosis is an interaction with the constitutively expressed *nodD* gene product(s) of the microsymbiont to form a protein–phenolic complex – a transcriptional regulator of other rhizobial nodulation *nod* genes that are responsible for synthesis of reciprocal signals to the plant root. The combination of Nod D proteins with appropriate plant flavonoids triggers the production of highly specific reverse signal molecules by rhizobia – the chitolipooligosaccharide (CLOS) Nod factors – by means of the transcriptional activation of common and host specific *nod* genes (Cooper, 2004).

In addition to the flavonoids, several nonflavonoid nod inducers have been identified. Stachydrin (N-methylproline methylbetaine) and trigonelline (nictotinic acid N-methylbetaine) were identified as inducers of *nod* genes (Phillips *et al.* 1992).

These molecules are quaternary ammonium compounds collectively known as betaines (Chen and Murata, 2002). The concentrations (in low millimolar range) of betaines required for *nod* genes induction are much higher than those of flavonoids (low micromolar range). Two aldonic acids (tetronic acid and erytronic acid), as well as some simple phenolics (vanillin, coniferyl alcohol, chlorogenic acid, and ferulic acid) were also identified as natural inducers of *nod* genes in certain rhizobial species. The concentrations required for their activity are similar to those of betaines (Neera and Geetanjali, 2007).

5.5.Reverse signals from rhizobial to legume roots – the chitolipooligosaccharide *nod* Factors.

The key event in nodule formation is the synthesis and release by the bacteria of small molecules that are detected by the plant and that trigger the formation of the nodule. These molecules are called *Nod* factors. Detection of *Nod* factors by a legume host induces major developmental changes in the plant, which are required for entry of the rhizobia into the host (Geurts and Bisseling, 2002). The tip of a root hair, to which rhizobia are bound, curls back on itself, trapping the bacteria within a pocket, from which they are taken up into a plant made intracellular infection thread. *Nod* factors also induce cell division and gene expression in the root cortex and pericycle, where they initiate development of the nodule (Cullimore *et al.* 2001).

5.6.Transcriptional regulators of *nod* genes.

Plant-released flavonoids are detected by rhizobia through a variety of *Nod D* proteins. The *Nod D* proteins of several species are believed to be membrane associated. Many species of rhizobia have more than one copy of the *nod D* gene, and the properties of different *nod* genes vary within the same strain as well as from one *Rhizobium* species to another. Some strains possess two to five copies of *nod D*, and may in addition possess one or two copies of another LySR-type regulator gene called *syrM* (symbiotic regulator). *SyrM* is a *Nod D* homolog and also acts as an activator of *nod* genes (Brencic and Winans, 2005).

Chitolipooligosaccharide *Nod* factors are vital signals for rhizobial entry into legume roots and the success or otherwise of the infection process is in large part determined by their structural features. Application of nanomolar or femtomolar concentrations of purified rhizobial *Nod* factor to the roots of an appropriate legume host elicits responses like deformation of root hairs accompanied by root

hair plasma membrane depolarization; rapid increases then oscillations in intracellular free calcium in root hairs, referred to as calcium spiking (Walker *et al.*, 2000); change in the root hair cytoskeleton; preinfection thread formation in deformed root hairs; and localized cortical cell division at the sites of root nodule primordial. *Nod* factors alone can induce some of the plant genes (nodulins) that are expressed in the preinfection, infection, nodule development, and nodule function phases of symbiotic interaction, some examples of the more rapidly expressed genes being *enod12*, *enod40*, *rip1* and *dd23b* (Neera and Geetanjali, 2007).

Nod factors also control the number of nodules formed on a root system by inducing an autoregulation response in the host plant. A symbiosis receptor-like kinase (SYMRK) gene in *Lotus* and a nodulation receptor kinase (NORK) in *Medicago* that is required for early signal transduction in both rhizobial and mycorrhizal symbioses have recently been discovered. More recently, two genes that encode LysM receptor-like kinases that function upstream of SYMRK and could be direct receptors for rhizobial *Nod* factors were discovered in *Lotus japonicas* (Radutoiu *et al.*, 2003).

5.7.Infection and nodule organogenesis

5.7.1Root hair curling

The symbiotic interaction starts when the bacteria colonize the root surface and induce curling of the root hair tips. Root hair curling is thought to be caused by a gradual and constant reorientation of the growth direction of the root hair. The bacteria become entrapped within the pocket of the curl, where the plant cell wall is locally degraded, the cell membrane invaginated and new material deposited by both plant and bacteria. Simultaneously, pericycle and cortical cells are activated

for division, usually in front of a xylem pole, close to the infection point. The cortical cells actively divide to form the nodule primordium wherein large amounts of amyloplasts accumulate.

Root-hair curling only occurs in a few hairs in the root zone that is susceptible to rhizobia, whereas most root hairs within this zone show altered behavior, resulting in so called root hair deformations. These deformations are the result of isotopic growth, by a reinitiation of tip growth in an altered growth direction. Although many bacteria can be attached to a single hair, it is probable that one or only a few bacteria induce the curling. The growth direction of the hair needs to be constantly redirected towards the bacteria in order for them to become entrapped.

At the root surface, rhizobia caught in the root hairs, locally degrade the plant cell walls, and infection develops that grows within the root hair. Before the infection thread reaches the base of the root hair cell, the root cortical cells are induced to **dedifferentiate**, activating their cell – cycle and causing them to divide to form the nodule primordium. In addition to the cortical cells, pericycle cells are also activated and undergo some cell divisions (Stacey *et al.*, 2006). The infection thread, (only 1–5%),traverses the outer cell layers to reach the nodule primordium.

The infection threads penetrate and ramify into primordium cells traversing their walls, they then enter cortical cells, initiating a differentiation process that is heralded by cell enlargement (Crespi and Galvez, 2000). Within the infection thread, the rhizobia multiply, but remain confined by the plant cell wall. As the primordia develops into a nodule, bacteria are released from the tip of the infection thread by endocytosis and differentiate into bacteroids surrounded by peribacteroid membrane (Neera and Geetanjali, 2007).

5.7.2 Bacteroid formation

The release of bacteria into plant cells is initiated by the formation of an infection droplet. Infection droplets can form at the tip of short intracellular infection threads or, more usually at positions in the infection threads where the cell wall gets disrupted and rhizobial cells come into direct contact with the host cell plasma membrane (Brewin, 2004).

The plant cell membrane then outgrows and bacteria are taken up into the plant cell lumen by endocytosis. The newly formed structure, which consists of bacteria that are differentiating in bacteroids enclosed in a plant cell membrane, is called a symbiosome. Differentiated bacteroids present an important physiologic adaptation with respect to their enzymatic capacity, notably the production of nitrogenase (Crespi and Galvez, 2000). In determinate nodules, individual symbiosomes fuse and/or bacteroids further divide within the symbiosome, which results in symbiosomes that typically contain several bacteroids. However, in indeterminate nodules, individual symbiosomes further divide, together with the bacteroid, which mostly result in single bacteroids within a symbiosome (Prell and Poole, 2006). The bacterial carbon storage compound polyhydroxybutyrate (PHB) accumulates in rhizobial cells in infection threads (Lodwig *et al.*, 2005). This suggests that a plentiful carbon supply is available for bacteria during growth in the infection threads (Neera and Geetanjali, 2007).

5.7.3 Symbiosome

The symbiosome is critical for biological nitrogen fixation. While being released from the infection thread into the plant cytoplasm, a plasmalemma-derived symbiosome membrane forms an uninterrupted envelope around each bacterium

and delineates the symbiosome space between the symbiosome membrane and the bacterial outer membrane.

The symbiosome membrane, bacteroid, and symbiosome space form the basis of the symbiosome. Specifically, the symbiosome membrane serves both as a physical interface and as a mediator of metabolite exchange between the symbionts, both functions being essential for nodule formation.

In mature root nodule cells, the symbiosome membrane represents a mixture of proteins that resemble most closely the protein constituents of the plasma membrane and the tonoplast. Proteins are involved in transport, energy, metabolic processes, nodule formation and function, signaling, pathogen response, and protein destination have been identified from the symbiosome membrane. Also, channels and bacterial proteins have been identified for this membrane. The symbiosome membrane must proliferate in enlarged infected root nodule cells to accommodate bacteroid growth and division. During this process of symbiosome membrane proliferation, a large amount of lipid and protein synthesis is required since infected root nodule cells typically consist of hundreds of bacteroids, each enclosed by their own symbiosome membrane. Symbiosome membrane biogenesis and demand in infected plant cells is 30 times greater than that required for plasma membrane synthesis (Neera and Geetanjali, 2007).

6. Mycorrhizae

6.1. A short overview of mycorrhizas

Different kinds of mycorrhizas exist; they differ according to their anatomy and the involved fungi. Broadly speaking, mycorrhizas can be assigned to one of

the following groups: ectomycorrhizas or endomycorrhizas, depending on whether the fungus colonizes the root intercellular spaces or develops inside plant cells (Fig. 2) (Novero *et al.*, 2008).

6.2. Ectomycorrhizas

In this kind of association, the fungus does not enter the host cell. Two features are usually recognized: a mantle of fungal hyphae covering thin secondary roots, which gives them the characteristic shape similar to a finger of a glove and a hyphal network that develops between the root cells, called the Hartig net. The involved plants in ectomycorrhiza are usually trees (i.e., members of Fagaceae, Tiliaceae, Salicaceae, Pinaceae, etc.) and very rarely shrubs and herbaceous species (Peterson *et al.*, 2004). Most ectomycorrhizal fungi belong to the Basidiomycete and Ascomycete classes (Novero *et al.*, 2008).

6.3. Endomycorrhizas

This kind of association groups together very diverse fungi (Glomero-, Asco-, and Basidiomycota) and plants (Brundrett, 2002). Irrespective of this huge biodiversity, the fungus always enters into the epidermal and cortical cells of the host, where it develops hyphae or specialized structures such as arbuscules, coils, or vesicles. Such intracellular structures are always surrounded by an invagination of the host plasma membrane (Bonfante, 2001). Unlike ectomycorrhizas, morphological changes in the gross anatomy of the colonized roots are not easy to note. Different types of endomycorrhizas are currently listed, depending on the type of host – fungus association (Peterson *et al.*, 2004).

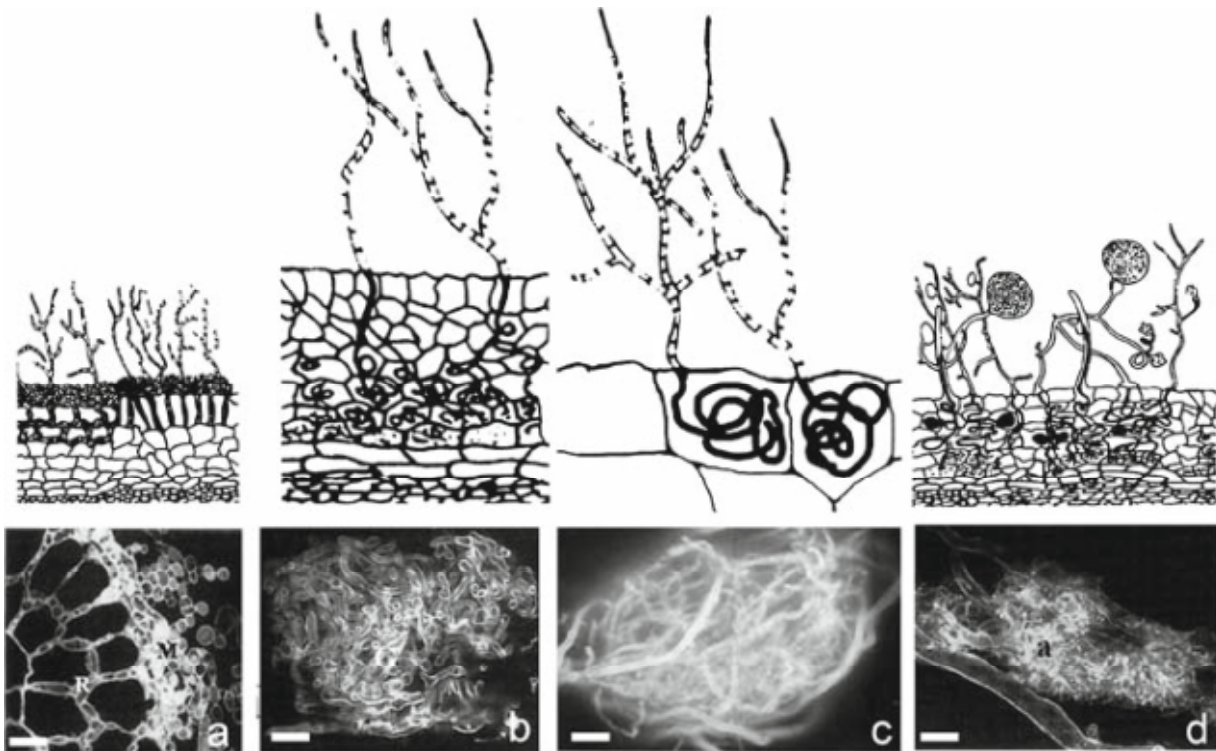


Fig. 2:Upper part: the different mycorrhizal types and the main colonization structures. From the left are ectomycorrhizas, arbuscular mycorrhizas, orchid mycorrhizas, and ericoid mycorrhizas.

Lower part: the fungal mantle and the Hartig net in *Quercus* ectomycorrhiza (**a**); a coil produced by an ericoid fungus in a hair root of *Calluna vulgaris* (**b**); a coil produced by an endophytic fungus inside an orchid root (**c**); an arbuscule in the cortical cell of a leek root (**d**). Bars correspond to 30 μ m (**a**) and to 15 μ m (**b – d**) (Bonfante, 2001).

6.4. Ericoid mycorrhizas

This type of mycorrhiza form specialized lateral roots, called hair roots. These are narrow (about 100 μ m in diameter), short, with a strikingly simple anatomy, and never undergo secondary growth. Transverse sections of such roots show a central vascular cylinder surrounded by one or two layers of cortical cells and an epidermal layer, which is usually the preferential niche for mycorrhizal fungi (Bonfante and Perotto, 1995). Interestingly, such hair roots do not possess root hairs, since, possibly due to their miniaturized structure, the roots themselves play a direct role in nutrient absorption, along with mycorrhizal fungi (Novero *et al.*, 2008).

6.5. Orchid mycorrhizas

The fungal partners are Basidio- and Ascomycota, which form hyphal coils (pelotons) usually within cortical cells. Interestingly, an increasing number of reports have shown how these fungi may act as a functional bridge between ectomycorrhizal plants and orchids that have low photosynthetic activity. The fungus can transfer the sugars acquired by the photosynthetic tree to the orchid, which is therefore fully dependent on the fungal partner for its carbohydrate nutrition. Such ecological and functional studies have led to a new research field, called mycoheterotrophy, and confirmed the ecological importance of mycorrhizal fungi in nutrient cycles (Selosse *et al.*, 2006).

7. Arbuscular mycorrhizas

One noteworthy aspect of arbuscular mycorrhizas (AM) is their ecological success. They are present in the roots of 80% of vascular plants (both angiosperms and gymnosperms), and also in ferns and bryophytes. It is quite exceptional that one small group of fungi, Glomeromycota (Schüßler *et al.*, 2001), can colonize

such diverse plant species irrespective of tissue ploidy, as both gametophytic and sporophytic tissues are involved in the symbiosis. Several features are common to this extremely successful group of mycorrhizas:

1. Land plants and AM fungi share a long coevolutionary history (Rémy *et al.*, 1994); the symbiosis gives benefits to both partners (Smith and Read, 1997).
2. Reciprocal nutrient exchange requires close physical contact between the partner cells (Genre and Bonfante, 2005).
3. Functional symbiosis requires profound readjustments in plant and fungal cells (Novero *et al.*, 2008).

7.1. Mechanism of arbuscular mycorrhizal fungi association with the host plant

Glomeromycota are highly dependent on their hosts. Hyphae germinating from their large asexual spores grow for only a few days in the absence of the plant. Upon the recognition of the host plant, these presymbiotic hyphae develop infection units that colonize the root epidermis and cortex. The sequence of events leading to AM symbiosis is largely conserved among different combinations of fungal and plant species, suggesting the presence of common molecular and genetic determinants across different plant taxa (Novero *et al.*, 2008).

Broadly speaking, three stages can be identified during the colonization process: (1) the presymbiotic phase; (2) the plant – fungal contact stage, followed by penetration; and (3) the intraradical fungal proliferation stage, leading to the development of arbuscules, which are the preferential sites of nutrient transfer (Paszkowski, 2006).

However, since root colonization is an asynchronous process, all of these steps can occur concurrently. During the presymbiotic phase, AM spores germinate spontaneously (asymbiotic stage) in the soil, developing a germ tube that is fed by the stored carbon in the spore (Bianciotto *et al.*, 1995). In the absence of a host plant, hyphal growth stops before the complete depletion of carbon resources (Bago *et al.*, 2000). On the other hand, when a host plant is present, fungal growth is enhanced and hyphal branching is induced, thereby increasing the probability of contact between the two partners (Novero *et al.*, 2008).

A recent breakthrough discovery demonstrated that plants produce a “branching factor” which was isolated and identified as a strigolactone (Akiyama *et al.*, 2005). The chemical nature of AM fungal signals (myc factor) remains elusive, even though indirect evidence of their existence has been presented (Kosuta *et al.*, 2003; Olah *et al.*, 2005 and Navazio *et al.*, 2007). Once the hypha contacts the root epidermis, appressorium-like hyphopodia (i.e., large adhesion structures) develop on its surface (Genre and Bonfante, 2007). Following this event, the host plant responds by forming a prepenetration apparatus (Genre *et al.*, 2005), which prepares the epidermal cell for fungal entry by assembling a tunnel-like structure. Fungal hyphae enter and cross the epidermal cell using the preformed tunnel and avoiding a direct contact between the fungal wall and the host cytoplasm, which remain separated by a thin apoplastic compartment called the interface (Bonfante, 2001).

Once the fungus has passed the epidermal layer, it grows inter- and intracellularly within the root. During this stage, some fungi (e.g., *Glomus* species) may differentiate particular structures, called vesicles, which occur within exodermal and cortical cells. These circular structures completely fill up the cell

lumen and are believed to act as storage sites. Only when the fungus has penetrated a cortical cell does a specialized branching process initiate, leading to the formation of arbuscules. These highly branched structures, formed by recursive dichotomous branching of a hyphal trunk (Bonfante, 1984), are the key elements of the symbiosis, since they are considered to be the main site of nutrient exchanges. The development of such a massive intracellular structure changes the architecture of the host cell to a great extent: the nucleus moves from the periphery to the center of the cell, the vacuole is fragmented, plastids change their morphology, and a new apoplastic space, based on membrane proliferation, is built around all the arbuscule branches (Bonfante and Perotto, 1995). The construction of this interface compartment, which mediates reciprocal nutrient exchange between the symbiont cells, results from an intense reorganization of the plant cell components and metabolic activity, ranging from specific gene activation (Gianinazzi-Pearson and Brechenmacher, 2004) to localized cell wall and membrane deposition (Balestrini and Bonfante, 2005), cytoskeleton remodelling (Genre and Bonfante, 1998), organelle mobilization (Lohse *et al.*, 2005) and phosphate transport (Balestrini *et al.*, 2007). Arbuscules are ephemeral structures with a life cycle of only a few days (Toth and Miller, 1984) : after 4 – 5 days, the arbuscule branches collapse, and the host cell gradually regains its original preinfection form.

To conclude this schematic overview on mycorrhizal types, it should be mentioned that root hairs are apparently not involved in ericoid mycorrhizas, since these tiny hair roots never produce root hairs; nor are they involved in the mycorrhizas of the large, hairless, orchid roots (Novero *et al.*, 2008).

7.2. Root hairs and arbuscular mycorrhizas

The first contact between an AM fungus and its host plant occurs at the root surface: hyphae adhere to epidermal cells and develop a specialized swollen structure called appressorium or *hyphopodium*. Penetrating hyphae originate from hyphopodial branches. Even though direct evidence is not available, some morphological and biochemical data suggest that penetrating hyphae may produce hydrolases or release small molecules that cause the epidermal wall to relax and allow hyphal penetration (Peretto *et al.*, 1995). AM fungi usually enter the root tissue through epidermal cells, crossing their radial or tangential walls (Bonfante *et al.*, 2000; Demchenko *et al.*, 2004; Genre *et al.*, 2005). Penetration through root hairs (Guinel and Hirsch, 2000) without the formation of a clear appressorium-like structure is less frequent. By contrast, nonvascular plants seem to follow the “root hair pathway” to start the colonization process. In natural conditions, liverwort gametophytes are usually colonized by Glomeromycota, which often use the rhizoids as the preferential access to the plant. Recent observations on Marchantiopsida and Metzgeriidae indicate that the fungus penetrated the rhizoids at any point, forming large intracellular hyphae running in both directions (Fig. 3) (Ligrone *et al.*, 2007).

The main role of AM fungi in symbiosis is to acquire mineral nutrients by exploring the soil. Their hyphae are more extended and thinner than roots or root hairs: for this reason, they are more efficient in reaching soil interstices (Novero *et al.*, 2008).

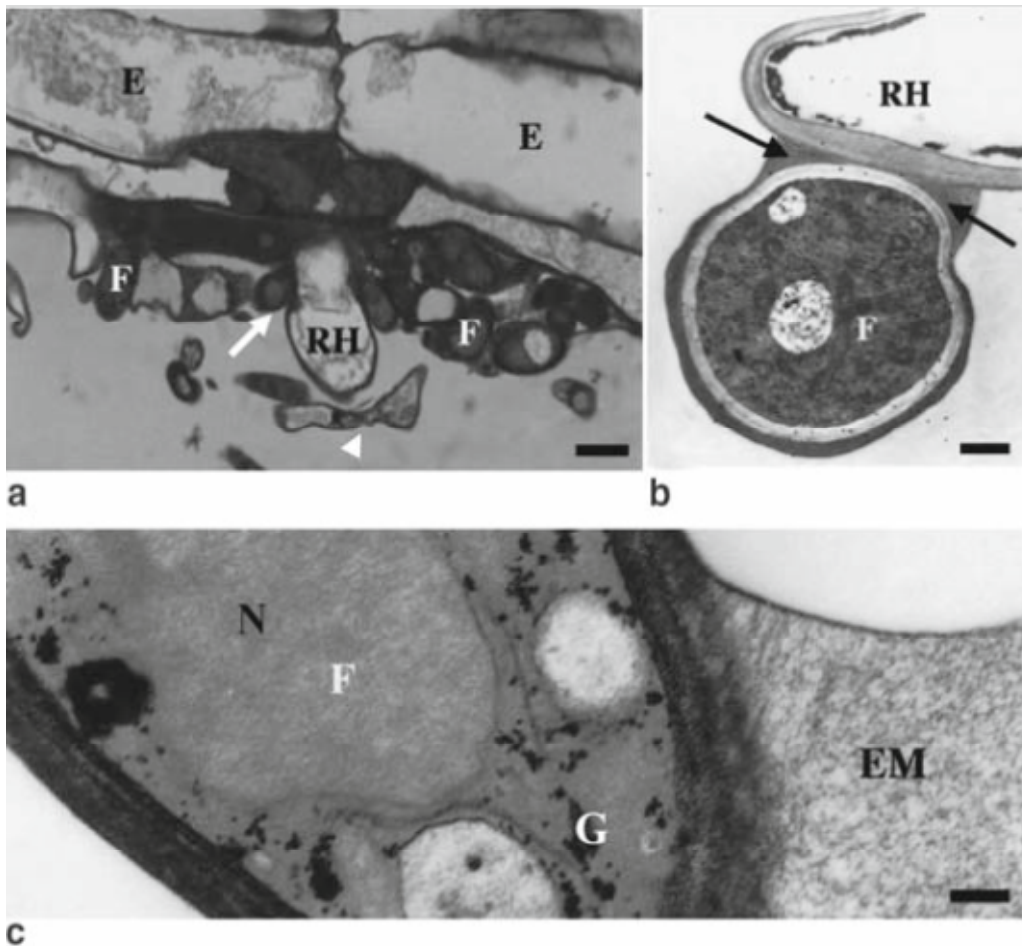


Fig. 3: Root hairs during the development of an ectomycorrhiza: hyphae of *Tuber borchii* grow in the presence of *Tilia platyphyllos* roots. (a) Light micrographs of a longitudinal section from a colonized root after 30 days. Hyphae closely surround a root hair, both at the base (*arrow*) and at the very tip (*arrowhead*). *E* epidermal cell, *F* hypha, *RH* root hair. Bar = to 8.8 μm . (b) Under-electron microscope, abundant extracellular material is seen at the contact point (*arrows*) between a root hair and a hypha. The section is labelled with an antibody against β 1 – 3 glucans. *RH* root hair, *F* hypha. Bar = to 0.5 μm . (c) At higher magnification the extracellular material shows a fibrillar structure that reacts to the silver reaction performed according to the method of Thiéry (1967): carbohydrates were oxidized with periodic acid, and visualized by incubation in thiocarbohydrazide and silver proteinate. *EM* extracellular material, *F* hypha, *N* nucleus, *G* glycogen. Bar = to 0.3 μm .

The AM fungi expand their filaments in soil and plant roots. This filamentous network promote bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network. Arbuscular Mycorrhizal symbiosis may benefit the host plant primarily by increasing the ability of the root system to absorb and translocate phosphorus through an extensive network of external hyphae. AM fungi contribute to biological control when they interact with plant pathogens and reduce disease incidence. Indeed, utilization of *Rhizobium*-AM association is a vital area for practical studies dealing with maximizing fertilizer efficiency to conserve energy, material resources, and reduce costs of food production. (Talaat and Abdallah, 2008)

An agronomic practice associated with increased yield is inoculation with *Rhizobium*. This type of inoculation improves not only the nutritional status of nodulated plants, derived from the biological nitrogen fixation (BNF) process, but recently it has been recognized that this inoculation may also increase drought or osmotic tolerance of nodulated legumes such as acacia, common bean, lotus, and medicago, through the biosynthesis of trehalose (α -D-glucopyranosyl- α -D-glucopyranoside). The accumulation of trehalose by different organisms (bacteria, yeast, fungi, nematodes, etc.) has been related to survival under different environmental stresses, like osmotic, low pH, high and low temperatures and dehydration. In the nodule, disaccharide is synthesized by the bacteroid, and its accumulation depends on the rhizobial strain, legume genotype and environmental conditions. It was reported that under well-watered conditions, there is a basal amount of trehalose in the nodules, having a negative correlation with the BNF, but under drought conditions, nodule trehalose levels increase significantly and may contribute to maintain BNF under these circumstances and to improve the plant tolerance to drought. Under field conditions, it was observed that trehalose is

detected in seeds from nodulated plants, with a positive correlation with seed longevity.

It has been reported that mycorrhizal inoculation increases the plant's tolerance to drought. The most accepted mechanism for this effect is the improvement of water-use efficiency of the mycorrhized plant. Interestingly, it is also known that ecto-mycorrhizae and endo-mycorrhizae synthesize trehalose. Nevertheless, in this case, it was demonstrated through the use of ^{14}C that there is no carbohydrate flux from the fungi to the plant, and fungi trehalose is mostly localized at the spores where it may serve, as in *Trichoderma* and *Aspergillus*, to increase the survival of the spores. In a recent *in vitro* study, it was observed that mycorrhizal roots under osmotic stress mobilized trehalose from the mycorrhizal root to the external mycelia (Farias-Rodriguez *et al.*, 1998), supporting the idea that trehalose biosynthesis in mycorrhizal symbiosis is only localized in fungal tissues and does not contribute to plant stress tolerance as in *Rhizobium*. In the case of co-inoculation (rhizobia and mycorrhizae) experiments on legumes, most of the reports argue for a synergistic effect of both microsymbionts on plant development (Ballesteros-Almanza *et al.*, 2010).

8. The legume-*Rhizobium*-AM fungi relationship

The value of microbiological symbiont inoculum as bio-fertilizer aid for augmenting or enhancing a legume crop positive growth response to chemical crop fertilization needs to be quantitatively evaluated. The ecology of symbiotic micro-organisms and their impact on crop productivity have been reviewed over the past decade (Friesse and Allen, 1991).

Murray (2011) pointed out that nodulation of legume roots typically begins with rhizobia attaching to the tip of a growing root-hair cell. The attached rhizobia

secrete Nod factors (NF), which are perceived by the plant. This initiates a series of preinfection events that include cytoskeletal rearrangements, curling at the root-hair tip, and formation of radially aligned cytoplasmic bridges called preinfection threads (PIT) in outer cortical cells. Within the root-hair curl, an infection pocket filled with bacteria forms, from which originates a tubular invagination of cell wall and membrane called an infection thread (IT). IT formation is coordinated with nodule development in the underlying root cortex tissues. The IT extends from the infection pocket down through the root hair and into the root cortex, where it passes through PIT and eventually reaches the nascent nodule. As the IT grows, it is colonized by rhizobia that are eventually released into cells within the nodule, where they fix nitrogen. NF can also induce cortical root hairs that appear to originate from PIT and can become infected like normal root hairs. Several genes involved in NF signaling and some of the downstream transcription factors required for infection have been characterized. More recently, several genes with direct roles in infection have been identified, some with roles in actin rearrangement and others with possible roles in protein turnover and secretion. Also, the same author provided an overview of the infection process, including the roles of NF signaling, actin, and calcium and the influence of the hormones ethylene and cytokinin.

Aim of the work

The aim of this study was to investigate the effects of the interactions between the microbial symbionts, *Rhizobiumleguminosarum* and arbuscular mycorrhizal fungi (AMF) in presence of compost on nitrogen and phosphorus accumulation by broad bean (*Vicia faba*) plants and how increased nitrogen and phosphorus content influence yield production and net photosynthetic rate.

Materials and methods

1. Part I: *In vitro* work:

1.1. Isolation, characterization and identification of *Rhizobium* species from roots of faba bean.

1.1.1. Samples collection:

Samples were collected from the roots of faba bean plants cultivated at Ismailia Agricultural Research Station. These include the rhizosphere of some faba bean plants at different sites.

1.1.2. Isolation:

Nodules of faba bean samples were separated from the roots, then first washed with tap water to remove associated soil contamination. The nodules were then immersed in 95% ethanol and then in a sterilant – 10% hydrogen peroxide (H_2O_2) solution. The immersion time averaged about 10 seconds but varied depending upon the size of the nodule. The nodules were removed from the sterilant and rinsed at least 5 times in sterile water. Each nodule was then crushed in a small amount of sterile water to give a turbid suspension. A drop of this was transferred to an agar plate with a flamed loop. The drop of this bacteroid suspension was streaked into the agar plate in a way that progressively dilutes the suspension. The used growth medium was yeast extract mannitol agar medium (0.5), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), NaCl (0.1), Mannitol (10), yeast extract (1), 1000 ml distilled water and pH 6.8.

The plates were incubated in an inverted position at about 28°C and checked for growth typical of rhizobia along the streak lines. The plates were left in the incubator until the colonies developed; that usually took about 3-5 days. Well isolated single colonies were picked and restreaked into fresh plates to obtain pure culture of the presumptive strain of rhizobia. It is possible that more than one “typical” colony type may appear on a plate streaked from a single nodule and each of these types were taken to pure culture and held for characterization (Date and Halliday, 1987).

1.1.3. Rhizobia characteristics:

Rhizobium is the generic name for the bacteria forming nitrogen-fixing nodules on leguminosae. The taxonomy of the genus *Rhizobium* is under review currently it is subdivided according to the ability of its members to nodulate particular host plants. This system of specification is inadequate and exceptions to the rule are many.

Rhizobia which nodulate Vicia faba belong to R. leguminosarum.

After several re-streakings, single and pure colonies of rhizobia were usually successfully obtained. The colony morphology was examined by noting colony characteristics like colony size, color, shape and growth rate. Each isolated organism was gram stained and examined under microscope. The shape of cells, gram reaction, and size of cells were all recorded.

1.1.4. Identification:

The tested isolates were kindly identified at the Rhizobia Unit, Microbiological Department, Soils, Water and Environmental Institute (SWERI),

Agricultural Research Center (ARC), Giza, Egypt according to the morphological, characteristics and microscopical examination of the nodules.

1.2. Isolation, characterization and identification of Arbuscular Mycorrhizal (AM) fungi:

1.2.1. Isolation of Arbuscular Mycorrhizal spores:

1.2.1.1. Sampling procedure:

Rhizospheric soil and root samples of the most cultivated plants were collected from different location located at Ismailia Agriculture Research Station, Ismailia, Egypt.

1.2.1.2. Extraction and estimation of AM spores:

The rhizosphere soil was gently removed from the root system. Spores were isolated from their substrate using a mixture of wet-sieving and sucrose gradient techniques (Brundrett *et al.*, 1996).

A 250 gm soil sample was diluted to 1 litre tap water and rinsed in through 400, 250, 150 and 75 μm sieves; and the soil material was recovered from each sieve, suspended in water, and centrifuged at 3000 rpm for 3 minutes. The supernatant was removed and the soil material was re-suspended in a sucrose solution (60 %) and centrifuged at 1000 rpm for 2 minutes. The supernatant containing spores were then filtered under vacuum on a filter paper. The spores were recovered one by one under binocular microscope.

1.2.2. Propagation of AMF:

The sieved spores were diluted with sterilized sandy soil and were sown with onion seeds to propagate the VAM. The plants were grown for 4 months, then the green parts of the plants were cut off and the pots left to dry. The culture substrate was sieved through 0.5 mm and 0.075 mm sieving to extract the spores. Then the spores were stored on sterilized peatmoss (20 % moisture) to be ready for use.

1.2.3. AM characterization and identification:

Some mycorrhizal spores were picked up from the extracted spores. The morphological characteristics of the spores were determined according to the key proceed by Trappe(1982). These characteristics include shape, size, color, distinct wall layer, surface configuration of spores, attached hyphae and sporocarps.

1.2.4. Estimation of mycorrhizal root infection:

The root samples were washed several times with tap water to remove remaining soil particles. Samples were cut to small pieces (1 cm long) and covered with 10 % KOH in test tubes or bottles and heated in a water bath (80 – 90 °C) for 10-40 minutes. Kormaniket *al.*(1980) modified this method by using the autoclave at pressure “1 bar” and temperature 121° C for about 20 minutes depending on the age and size of the roots, to remove the host cytoplasm and most of the cell nucleic acid to allow stains penetration. The rootswere then washed with tap water and acidified with 1% HCl. The dilute acid was then poured off. The trypan blue (0.05 %) stain in pure lactic acid was added to cover the root and then heated in water bath at 80 – 90 °C for 10 – 15 minutes (Phillips and Hayman, 1970).

2. Part II: Effect of AM-mycorrhizae, N₂-fixing bacteria and compost on the growth and yield of Faba bean plant:

2. 1. Field experiment:

A field experiment was conducted in sandy soil at Ismailia Agricultural Research Station to evaluate the effect of AM-mycorrhizae and N₂-fixing bacteria in the presence of compost and their impact on the nodulation, growth, yield, yield components and nutritional value of faba bean plants as an economic crop and at the same time for minimizing pollution caused by continuous application of nitrogen fertilizers. The physicochemical characteristics of the soil illustrated in Table 1.

2.1.1. Layout of field experiment:

Experimental area was divided into equal plots of 2 x 2 m. Plots were arranged in split plot design in three replicates for each treatment. The experimental unit consisted five ridges two meter in length and 60 cm apart (plot area = 2x2= 4m²). The distance between plants was 20 cm.

2. 2. Seeds:

Giza 3 cultivar of faba bean seeds were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt was used.

Table (1): Mechanical and physico-chemical properties of the used soil.

Mechanical properties	%
Sand	70.7
Slit	2.30
Clay	27.00
Chemical properties	
Organic carbon %	0.143
Total nitrogen %	0.020
Total phosphorus (ppm)	0.020
Available phosphorus (ppm)	0.025
Water holding capacity %	25.00
pH	7.75
Electric conductivity (EC) (ds/m)	1.150
CaCO ₃ %	0.600
Anions and Cations (meq/L)	
Carbonate	Traces
Bicarbonate	1.13
Chloride	0.20
Calcium	0.64
Magnesium	0.11
Sodium	0.29
Potassium	1.13
Sulphate	0.07

2. 2.1. Preparation of seeds for cultivation:

Seeds were surface sterilized with Clorox solution (0.05%), washed several times then coated with *Rhizobium leguminosarum* and / or mycorrhizal fungi (Massoud, 2005).

2. 2.2. *Rhizobium leguminosarum* application:

One ml of pure culture of *Rhizobium leguminosarum* strain was diluted on yeast extract mannitol broth several times to reach about 10^8 cfu / ml then mixed with peat moss (Difco, 1985). The formula was mixed well with faba bean seeds to reach complete coating using Arabic gum as a sticker, then left to dry in shade for 2 hours before sowing. Seeds were sown according to the treatments.

2. 2.3. AM fungi application:

The AM fungal spores (500 spores/ gm soil) involved the mixed genera of *Acaulospora*, *Gigaspora* and *Glomus* were prepared after propagation and mixed with sterilized Peat as a carrier (500 spore / gm soil). The spores were coated on Faba bean seeds (50 spore/ seed) using Arabic gum (40 %) as a sticker, then air dried for 2 hours before sowing. The coated seeds were sown according to the treatments (Massoud, 2005).

2. 3. Organic matter (compost):

Compost was analyzed at the research and studies laboratory of Soils, Water and Environmental Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. Chemical analysis of the used compost was summarized in Table 2.

Table (2): Chemical analysis of the used compost:

pH	7.31
EC	3.8 ds/m
Bulk density (gm / cm ³)	0.370
Organic carbon (%)	31.0
Organic matter (%)	54.0
Total nitrogen (%)	1.30
Carbon/Nitrogen ratio	23 : 1

Compost was added to soil at a rate of ten cubic meter/feddan (Massoud, 2005). Each plot (4 m²) needed 0.0096 cubic meter of compost (nearly 7.4 kg compost). Compost was added and mixed with the soil before planting during soil preparation according to the following treatments.

Treatments:

Seven treatments were laid in the order of:

1. Control(the recommended doses of nitrogen, phosphorus and potassium fertilizers) (full NPK).
2. Application of arbuscularmycorrhizal fungi, 33.3 % of the recommended dose of phosphorus fertilizer and the full dose of nitrogen and potassium fertilizers(AMF+ $\frac{1}{3}$ phosphorus + NK).
3. Application of *Rhizobium leguminosarum*, 50 % of the recommended dose of nitrogen fertilizer and the full dose of phosphorus and potassium fertilizers (*Rh.* + $\frac{1}{2}$ nitrogen + PK).

4. Application of compost, 50 % of the recommended dose of nitrogen fertilizer, phosphorus and potassium fertilizers (Compt. + $\frac{1}{2}$ NPK).
5. Application of Arbuscularmycorrhizal fungi, *Rhizobium leguminosarum*, 33.3 % of the recommended dose of phosphorus fertilizer, 50 % of the recommended dose of nitrogen fertilizer and the full dose of potassium fertilizers (AMF + *Rh.* + $\frac{1}{3}$ ph. + $\frac{1}{2}$ N + K).
6. Application of Arbuscularmycorrhizal fungi, compost, 33.3 % of the recommended dose of phosphorus fertilizer, 50 % of the recommended dose of nitrogen fertilizer and the full dose of potassium fertilizers (AMF + compt. + $\frac{1}{3}$ ph + $\frac{1}{2}$ N + K).
7. Application of Arbuscularmycorrhizal fungi, *Rhizobium leguminosarum*, compost, 33.3 % of the recommended dose of phosphorus fertilizer, 50 % of the recommended dose of nitrogen fertilizer and the full dose of potassium fertilizers (AMF + *Rh.* + compt. + $\frac{1}{3}$ ph + $\frac{1}{2}$ N + K).

2. 4. Fertilization:

Before faba bean seeds sowing, phosphorus was added as superphosphate (15.5 % P_2O_5) at a rate of 200 kg/feddan during soil preparation in the absence of mycorrhiza, while one-third of recommended dose was added for mycorrhizal treatments. Potassium was added in the form of potassium sulphate (48 % K_2O) at a rate of 50 Kg/ feddan once after 60 days of sowing. Nitrogen was added as urea (46.5 %) at a rate of 15 Kg/feddan in three equal doses at 15, 30 and 45 days of planting. NPK were added according the treatments.

2. 5. The experiment:

A field experiment was designed to evaluate the effect of compost with N₂-fixers and \ or AM-mycorrhizae and their interaction on plant growth and yield. The experiment was carried out in the field at Ismailia Research Station during winter season of 2011/ 2012.

2. 6. Measured parameters:

Plant samples were taken after 60, 90 and 120 days from sowing for evaluating growth parameters of faba bean (shoot length (cm) and shoot dry weight(g)). Number of pods / plant, number of branches / plant, weight of 100-seeds and total yield per feddan were determined as yield parameters. Nodules number and nodules dry weight were determined after 60, 90 and 120 days of sowing. The photosynthetic pigments content were determined using the spectrophotometric method described by Metzner *et al.* (1965). Nitrogenase activity in root nodules was also determined according to the method described by Somasegaran (1985), and soil dehydrogenase activity was recorded according to Skujins (1976), both enzymes were measured after 60, 90 and 120 days from sowing. AM infection percentage after 60, 90 and 120 days from sowing was determined. NPK content of shoots after 60, 90 and 120 days from sowing and in seeds (after harvest) were determined according to Jackson (1973). The crude protein content in seeds was calculated by multiplying N % by the factor (6.25).

2. 7. Microbial determinations:

2. 7.1. Dehydrogenase activity:

Dehydrogenase activity was determined according to Skujins(1976) based on the use of triphenyltetrazolium chloride (TTC) as an artificial electron acceptor. Nearly all microorganisms reduce TTC to tri-phenylformasan (TPF) which can be estimated after incubation at 30°C for 24 h.

Because of light sensitivity of TTC and TPF, all procedures should not be performed under diffused light; 2 g dry soil placed in test tubes (60ml volume and 2.5 cm diameter) were mixed with 5 ml of TTC solution, and the tubes were sealed with rubber stoppers and incubated for 24 h at 30 °C. The control contains only 5 ml tris buffer (without TTC). After the incubation, 40 ml acetone was added to each tube and the tubes were shaken at interval thoroughly and further incubated at room temperature for 2 hours in the dark. The soil suspension (15 ml) was filtrated and optical density of the clear supernatant was measured against the blank at 545 nm (red colour).

The enzyme activity was determined using a standard curve of TPF according to the following equation:

$$\text{Dehydrogenase activity TPF } (\mu\text{g/D.Wt./g soil}) = \frac{\text{O.D/K}}{\text{D.Wt}}$$

Where: O.D.= optical density

K = Theobtained factor from the standard curve

2. 7. 2. Estimation of nitrogenase activity:

Nitrogenase activity was estimated using the acetylene reduction technique according to Somasegaran (1985) in the rhizospheric samples. Pure acetylene

(C₂H₂) was injected into test tubes to give acetylene concentration 10% (v/v) of the atmospheric air. The samples were incubated at 30 °C for 2-4 hrs before measuring. Then 1 – 2 ml of gas samples was withdrawn for the determination of C₂H₄ formed by gas chromatography using Hewlett Packard chromatography model HP (6890 GC) fitted with dual flame detector and 150 cm x 0.4 cm diameter stainless steel column fitted with Propack-N x R 100 – 120 mesh. Nitrogen was used at a flow rate of 30 ml min⁻¹ as a carrier gas. The detector temperature was 250° C and the oven and injector part temperatures were 80 °C and 100 °C, respectively.

Acetylene gas used for the standard curve was purified by passing through concentrated sulphuric acid followed dry distilled water for 3 times before using. To calculate the ethylene amount in the gas sample, the peak height was measured in cm, and converted to the equivalent $\mu\text{mol}/\text{C}_2\text{H}_4$ using multiplication factors derived from the standard curve.

The concentration of ethylene in the samples ($\mu\text{mol}/\text{C}_2\text{H}_4/\text{h}$) was then converted to μmoles by dividing this values by the volume of the molecular weight of gas (22.4 L). Nitrogenase activity was then calculated by using the following formula:

$$\frac{R \times \text{container volume (tube)}}{\text{Time of incubation}} \times \frac{1}{\text{Inj. Vol.}} \times \frac{1}{D} \times \frac{1}{22.4} \times 10^6$$

Where: Inj. Vol. = injection volume / gm

R = reading of peak

D = volume of the medium (ml).

The results were presented as $\mu\text{mole C}_2\text{H}_4 / \text{g nodule} / \text{h}$.

2. 7. 3. Determination of photosynthetic pigments:

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined quantitatively as described by Metzner *et al.* (1965). A known fresh weight of leaves (samples of the same fresh weight were dried at 50°C till constant weight to be used for final calculation), was homogenized in 85 % aqueous acetone for 5 minutes (the experiment should be carried out in the dark); the homogenate was centrifuged at 4000 rpm. The supernatant was made up to known volume with 85 % acetone. The absorbance was measured calorimetrically against a blank of pure acetone at wavelengths; 452.5, 644 and 663 nm, taking into consideration the dilution made. The concentrations of pigment fractions (chlorophyll a, chlorophyll b and carotenoids) were calculated as µg/ml by using the following equations:

$$\text{Chlorophyll a} = 10.3 E_{663} - 0.918 E_{644}$$

$$\text{Chlorophyll b} = 19.7 E_{664} - 3.87 E_{643}$$

$$\text{Carotenoids} = 42. E_{452.5} - (0.0264 \text{ chlorophyll a} + 0.426 \text{ chlorophyll b})$$

Where E = absorbance

The results were expressed as mg/g fresh weight

2.8. Chemical analysis:

2.8.1. Total phosphorus in plant:

Total phosphorus in plant was determined spectrophotometrically at 660 nm wavelength as described by Jackson (1973) using spectrophotometer (spectronic 20 D Milton Roy Company). A standard curve was constructed using a serial concentration of KH_2PO_4 .

2.8.2. Total nitrogen in plant:

Total nitrogen was determined by Kjeldahl method as described by Jackson (1973). Plant samples were dried and ground. A 0.2 gm sample is weighed into a *digestion tube* and then digested by heating it in the presence of sulfuric acid. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide. The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The amount of ammonia present (hence the amount of nitrogen present in the sample) is determined by **back titration**. The end of the **condenser** is dipped into a solution of **boric acid**. The ammonia reacts with the acid and the remainder of the acid is then titrated with a sulphuric acid solution with a **methyl orange pH indicator**.

2.8.3. Total potassium in plant:

The potassium was determined by the flame photometer as described by Jackson (1973).

3. Statistical analysis:

The presented data represent the mean values of three replicates. Data obtained were subjected to 2-way ANOVA analysis to determine the significance of treatments difference using Costat. Comparison of the mean effects was performed using the least significant differences (L.S.D.) from the control.

Experimental results

Part I: *In vitro* work:

1. Isolation, characterization and identification of *Rhizobium* species from roots of faba bean.

Samples were collected from faba bean plants cultivated at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt. Nodules of faba bean samples were separated from the roots, washed, surface sterilized with 0.05% chlorox and then collected on Petri dish Fig. (4).

Data in Table (3) revealed that the nodules number and their characteristics differ according to location of collection. Location 4 showed the highest nodules number (130), followed by location 8 (115) whereas, location 1 showed the smallest nodules number. The nodules in location 4 were pink and many joined together, while in section 8 they were large and bifurcated, and finally in section 1 the nodules were small and round.

The nodules were blended then filtered. The internal sap was diluted with sterile water to isolate *Rhizobium leguminosarium* where the diluted sap was streaked several times to obtain pure colonies as shown in Fig. (5). Identification of the isolated colonies was kindly identified at the Rhizobia Unit, Microbiological Department, Soils, Water and Environmental Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt according to the morphological, characteristics and microscopical examination of the nodules and listed in Table (4). Results indicated that *Rhizobium leguminosarium* found in sections 3, 7, 8, 11 and 4. Whereas, the other sections contained other *Rhizobium* spp.



Fig (4): Nodules of faba bean samples separated from the roots.



Fig (5): Colony of the isolated *Rhizobium leguminosarum*.

Table (3): Locations of the collected samples from *Vicia faba* plants and their nodules number and characteristics:

Sample number	Locations (Ismailia Research Station)	Nodules number	Nodule characteristics
1	1	75	small, round
2	3	87	small, rod
3	5	67	small, bifurcated
4	6	99	small, lobed
5	7	101	small, rod
6	2	103	small, single lobed
7	8	115	large, bifurcated
8	10	110	pink, round, bifurcated
9	11	100	large rod
10	4	130	pink, many joined together

Table (4): Identification of the isolated bacterial samples:

Isolation	Locations (Ismailia Research Station)	Identification
1	1	<i>Rhizobium</i> spp.
2	3	<i>Rhizobium leguminosarum</i>
3	5	<i>Rhizobium</i> spp.
4	6	<i>Rhizobium</i> spp.
5	7	<i>Rhizobium leguminosarum</i>
6	2	<i>Rhizobiumleguminosarum.</i>
7	8	<i>Rhizobium leguminosarum</i>
8	10	<i>Rhizobium</i> spp.
9	11	<i>Rhizobium leguminosarum</i>
10	4	<i>Rhizobium leguminosarum</i>

2. Isolation, characterization and identification of arbuscularmycorrhizalfungi from different cultivated plants.

Plants collected from different locations at Ismailia Agricultural Research Station in Egypt were found to belong to the following three families.

Three species were identified as a member of Leguminosae, namely: *Glycine max*, *Medicago sativa* and *Vicia faba*. *Allium cepa* was identified as a member of Liliaceae. Two species represented family Gramineae: *Zea mays* and *Hordeum vulgare*.

2.1. Number of AM spores:

Results in Table (5) showed the density of arbuscularmycorrhizal spores extracted from 10 soil samples. The number of spores was markedly affected by plant species and sites. Samples 4, 3, 6, 9, 5 and 8 which their plants were *Zea mays*, *Medicago sativa*, *Allium cepa* and *Vicia faba* gave the highest number of spores compared to other plants. In contrast, samples number 1, 7, 10 and 2 which their plants were *Allium cepa*, *Hordeum vulgare*, *Vicia faba* and *Glycine max* showed the lowest number of spores.

2.2. Root infection:

With respect to the root infection levels, Table (5) showed that the root infection percentage highly varied from site to another. Samples 2, 7, 4, 1, and 9 which their plants were *Glycine max*, *Hordeum vulgare*, *Zea mays* and *Allium cepa* showed the highest infection being 50 %, 45 %, 42 %, 25 % and 25 % respectively. Lowest infection percentage was recorded in samples 6 and 10 which their plants are *Medicago sativa* and *Vicia faba*.

2.3. Identification of the extracted AM spores:

Results in Table (6) indicated that spores varied in their colour, some were honey, honey blackish others were brown. According to the spore wall, some have rigid others were smooth. Results showed that *Glomus* spp. was extracted from plants: *Allium cepa*, *Glycine max*, *Medicago sativa* and *Vicia faba*. While, *Gigaspora* spp. was found in *Zea mays* and in *Hordeum vulgare*. And *Acaulospora* spp. was found in *Allium cepa*.

Table (5): Survey and isolation and of arbuscularmycorrhizal fungi from a number of cultivated plant species.

Location	Soil texture	Family	Scientific name	Total count / gm soil	Mycorrhizal infection percent
1	Sandy	Liliaceae	<i>Allium cepa</i>	10	25
2	Sandy	Leguminosae	<i>Glycine max</i>	8	50
3	Sandy	Gramineae	<i>Zea mays</i>	15	27
4	Sandy	Gramineae	<i>Zea mays</i>	17	42
5	Sandy	Liliaceae	<i>Allium cepa</i>	12	23
6	Sandy	Leguminosae	<i>Medicago sativa</i>	14	17
7	Sandy	Gramineae	<i>Hordeumvulgare</i>	10	45
8	Sandy	Leguminosae	<i>Viciafaba</i>	12	22
9	Sandy	Liliaceae	<i>Allium cepa</i>	13	25
10	Sandy	Leguminosae	<i>Viciafaba</i>	9	17

Table (6): Characterization and identification of the isolated myccorrhizal fungi.

Location	Characteristics of spores	Probable identified name
1	Honey – rigid	Glomus spp.
2	Honey circular rigid	Glomus spp.
3	Honey circular smooth	Gigaspora spp.
4	Honey – blackish circular	Gigaspora spp.
5	Honey – blackish rigid wall	Glomus spp.
6	Brown rigid	Glomus spp.
7	Honey circular smooth	Gigaspora spp.
8	Honey – rigid	Glomus spp.
9	Brown circular small and big	Acualospora spp.
10	Honey – rigid	Glomus spp.

Part II: Effect of AM-mycorrhizae, N₂-fixing bacteria and compost on the growth and yield of faba bean plant:

1. Effect of arbuscularmycorrhizalfungi, *Rhizobiumleguminosarium* and compost on shoot parameters.

Results in Table (7) revealed that the application of 50% nitrogen, 33.3% phosphorus and 100% potassium of the recommended dose along with AM-fungi, *Rhizobiumleguminosarium* inoculants and compost (treatment 7) resulted in a significant increase in shoot length at 60 days from sowing (28.3 cm), 90 days from sowing (78.7 cm) and also at 120 days from sowing (120 cm) compared to the control (full NPK) (26, 70 and 115 cm) respectively. While, the application of AMF, 33.3 % phosphorus and 100 % nitrogen and phosphorus (treatment 2) caused reduction in shoot length at 60, 90 and 120 days from planting (25, 70.03 and 113 cm respectively).

Although, there was no significant increase in shoot length when using treatments 3,4 and 6 after 60 days from planting, results showed that there was a significant increase after 90 and 120 days. Finally, the results also showed that treatment 5 has no significant effect at 60, 90 and 120 days after planting.

Data in Table (8) showed that, at 60 days there is no significant difference between all the treatments compared with the control, except for treatment 7 where the shoot dry weight was 6.76gm compared to the control which was 5 gm. Whereas, after 90 days there is no significant differences among the treatments in shoot dry weight, but there is a significant difference between all the treatments and treatments 6 and 7.

Table(7): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* shoot length of faba bean plant after 60, 90 and 120 days from sowing.

Treatment number	Treatment*	Shoot length(cm / plant) (mean \pm S.E.)		
		60 days	90 days	120 days
1	Full NPK	26 \pm 0.58bc	70.03 \pm 0.75d	115 \pm 0.9d
2	AMF + $\frac{1}{3}$ P+ NK	25 \pm 0.29c	70.03 \pm 0.69d	113 \pm 0.26e
3	Rh + $\frac{1}{2}$ N + PK	26.5 \pm 0.35bc	73.3 \pm 0.72 bc	117 \pm 0.35bc
4	Comp + $\frac{1}{2}$ NPK	27 \pm 0.26ab	75 \pm 0.95 b	118 \pm 0.58 b
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	26.5 \pm 0.76bc	71.4 \pm 0.21cd	115.9 \pm 0.36cd
6	AMF + comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	27.2 \pm 0.69ab	75 \pm 0.81b	117 \pm 0.51bc
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	28.3 \pm 0.57a	78.7 \pm 0.84a	120 \pm 0.57 a
	L.S.D	1.62	2.25	1.63

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

Table(8): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* shoot dry weight of faba bean plant after 60, 90 and 120 days from sowing.

Treatment number	Treatment*	Shoot dry weight (gm / plant) (mean \pm S.E.)		
		60 days	90 days	120 days
1	Full NPK	5 \pm 0.4bc	25.66 \pm 1.16abc	119.5 \pm 0.83e
2	AMF + $\frac{1}{3}$ P+ NK	4.55 \pm 0.29bc	23.76 \pm 1.21c	122 \pm 0.46d
3	Rh + $\frac{1}{2}$ N + PK	5.33 \pm 0.42bc	25 \pm 0.23bc	135 \pm 0.32 c
4	Comp + $\frac{1}{2}$ NPK	4.91 \pm 0.22bc	24.31 \pm 0.96bc	95 \pm 0.95 f
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N +K	4.35 \pm 0.39c	23.99 \pm 0.89 bc	96 \pm 0.4 f
6	AMF + comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	5.66 \pm 0.33b	27 \pm 0.54 ab	140 \pm 0.57 b
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	6.76 \pm 0.42a	28.33 \pm 0.95 a	158.6 \pm 0.72a
	L.S.D	1.09	2.75	1.96

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

After 120 days from sowing data indicated obvious significant differences among treatments. Treatment 7 showed the greatest differences (158.6 gm) followed by treatments 6, 3 and 2 (140, 135 and 122 gm respectively). While, treatments 4 and 5 caused reduction in the shoot dry weight compared to the control (95 and 96gm respectively).

2. Effect of arbuscular mycorrhizal fungi, *Rhizobium leguminosarium* and compost application on some growth parameters.

Data presented in Table (9) indicated that the effect of inoculation of AM-fungi and *Rhizobium leguminosarum* was reflected on the number of branches, number of pods, weight of the seeds and total yield. Data showed that the highest values of branches number / plant (6), pods number / plant (34.67), 100 seed weight (73gm) and total yield (2.6 ton) were observed with treatment 7. While, the lowest values of branches number / plant (3.33), pods number / plant (23), 100 seed weight (56gm) and total yield (1.6 ton) was recorded in treatment 2. Table (9) also showed that there was no significant differences among treatments in crude protein in the seeds. Treatment 7 showed the highest value (22.56). While, treatment 1 showed the least value (18.13).

3. Effect of mycorrhizal colonization, *Rhizobium leguminosarium* and compost application on some root parameters.

Results in Table (10) show a markedly significant increase in nodules number. Treatment 7 was a superior for the mean of nodules number (99.67, 154.33 and 33) after 60, 90 and 120 days from sowing respectively. After 60 days there was no

differences in nodules number between treatment 3 and treatment 6 (55), but there is significant difference with the control (full NPK) (7), while after 90 and 120 days there was a significant differences between them. Although, treatment 2 didn't show a significant difference in nodules number (9) with the control after 60 days, there was a difference after 90 and 120 days of sowing (25 and 22.33 respectively).

Data in Table (11) indicate that after 60 days of planting treatment 2, treatment 4 and treatment 6 didn't show any significant difference in nodules dry weight compared to control. Whereas, treatment 3, treatment 5 and treatment 7 show a significant differences (0.3, 0.5 and 0.7 gm). After 90 days, treatment 7 recorded the highest value in nodules dry weight (1.1 gm), followed by treatment 5, treatment 3, treatment 6, treatment 4 and treatment 2 (1, 0.7, 0.66, 0.5, 0.11 gm respectively). All treatments showed a significant differences when compared to the control except for treatment 2. Data also show that after 120 days, treatment 7 led to the best nodule dry weight (1 gm), while treatment 2 gave the lowest one (0.1 gm).

4. Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* on microbial root colonization percent and mycorrhizal infection percentage after 60, 90 and 120 days from sowing of faba bean plant:

Results in Table (12) showed that microbial root colonization percent differ among treatments and in the three intervals. Where, treatment 7 showed the most effective followed by treatment 6. It was clear that the microbial colonization reach its optimum at the 90 days interval. Results in Table (13) revealed that all the treatments led to increase the severity of mycorrhizal colonization with great

Table(9):Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* on shoot parameters of faba bean plant.

Treatment number	Treatment*	Branches number / plant (mean)	Pods number / plant (mean)	100 seed weight (mean)	Total yield (ton /feddan)	Crude protein
1	Full NPK	3.67 b	25 d	60 d	1.73 bc	18.44 a
2	AMF + $\frac{1}{3}$ P+ NK	3.33 b	23 e	58 e	1.6 c	18.13 a
3	Rh + $\frac{1}{2}$ N + PK	4 ab	25.33 cd	60 d	1.81 bc	20.69 a
4	Comp + $\frac{1}{2}$ NPK	5 ab	27 c	62.1 c	1.97 abc	20.19 a
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	5.33 ab	31 b	65.6 b	2.1 abc	21.31 a
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	6 a	30 b	67 b	2.4 ab	22.19 a
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	6 a	34.67 a	73 a	2.6 a	22.56 a
	L.S.D	1.99	1.83	1.83	0.69	7.79

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

Table(10): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* number of root nodules of faba bean plant after 60, 90 and 120 days from sowing.

Treatment number	Treatment	Nodules number (mean \pm S.E.)		
		60 days	90 days	120 days
1	Full NPK	7 \pm 0.58 e	15.67 \pm 1.45 g	15 \pm 0.58 g
2	AMF + $\frac{1}{3}$ P+ NK	9 \pm 1.15 e	25 \pm 0.58 f	22.33 \pm 1.2 f
3	Rh + $\frac{1}{2}$ N + PK	55 \pm 1.15 c	130 \pm 1.15 c	121 \pm 0.58 c
4	Comp + $\frac{1}{2}$ NPK	44.33 \pm 1.21 d	77 \pm 0.58 e	59.67 \pm 0.88 e
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	85.67 \pm 1.45 b	145.33 \pm 1.2 b	135 \pm 1.15 b
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	55 \pm 1.0 c	90.33 \pm 0.88 d	77 \pm 1.15 d
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	99.67 \pm 1.2 a	154.33 \pm 0.88 a	143 \pm 0.58 a
	LSD	3.44	3.06	2.78

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$), each value represents the mean of 3 replicates.

Table(11): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* nodules dry weight of faba bean plant after 60, 90 and 120 days from sowing.

Treatment number	Treatment*	Nodules dry weight (gm) (mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	0.05 \pm 0.006 d	0.1 \pm 0.006 d	0.08 \pm 0.006 d
2	AMF + $\frac{1}{3}$ P+ NK	0.07 \pm 0.01d	0.11 \pm 0.01 d	0.1 \pm 0.01 d
3	Rh + $\frac{1}{2}$ N + PK	0.3 \pm 0.06 c	0.7 \pm 0.04 bc	0.56 \pm 0.24 bc
4	Comp + $\frac{1}{2}$ NPK	0.1 \pm 0.01 d	0.5 \pm 0.09 c	0.4 \pm 0.06 cd
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N +K	0.5 \pm 0.06 b	1 \pm 0.21 ab	0.95 \pm 0.08 ab
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	0.15 \pm 0.03 d	0.66 \pm 0.1 c	0.6 \pm 0.12 abc
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	0.7 \pm 0.03 a	1.1 \pm 0.08 a	1 \pm 0.17 a
	L.S.D	0.104	0.302	0.38

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$), each value represents the mean of 3 replicates.

Table (12): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* microbial root colonization of faba bean plant after 60, 90 and 120 days from sowing.

Treatments	Microbial root colonization percent (mean)		
	60 days	90 days	120 days
Full NPK	15e	20e	12e
AMF + $\frac{1}{3}$ P+ NK	20d	27d	18d
Rh + $\frac{1}{2}$ N + PK	26.67 c	35c	24.33 c
Comp + $\frac{1}{2}$ NPK	25c	33c	23c
AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N +K	40b	43b	36.67 b
AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	41b	44.67 b	37b
AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	44a	50a	42a
L.S.D	2.59	2.76	2.06

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$), each value represents the mean of 3 replicates.

Table(13): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* mycorrhizal infection percentage after 60, 90 and 120 days from sowing of faba bean plant.

Treatment number	Treatment*	Infection percentage of AMF(colonization of AMF)(mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	9.67 \pm 0.33 f	25 \pm 0.58 e	15 \pm 0.58 f
2	AMF + $\frac{1}{3}$ P+ NK	60 \pm 0.58 c	75 \pm 1.53 c	59.33 \pm 0.88 d
3	Rh + $\frac{1}{2}$ N + PK	30 \pm 0.58 e	40.33 \pm 0.88 d	30 \pm 1.73 e
4	Comp + $\frac{1}{2}$ NPK	40 \pm 1.15 d	39.67 \pm 0.88 d	29 \pm 1.15 e
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	84.33 \pm 0.33 b	95 \pm 0.58 b	75.33 \pm 0.33c
6	AMF + comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	85.67 \pm 0.33 b	97 \pm 1.0 b	80 \pm 0.58 b
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	99.67 \pm 0.33 a	100 \pm 0.0 a	85 \pm 1.15a
	L.S.D	1.76	2.7	3.08

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

significant differences. The highest values was recorded by the application of AMF, *Rhizobiumleguminosarum*, compost, 33.3 % phousphorus, 50 % nitrogen and 100% potassium (treatment 7) at 60, 90 and 120 days from planting (99.67, 100 and 85% respectively), followed by treatments 6 and 5 {(85.67, 97 and 80 % respectively) and (84.33, 95 and 75.33% respectively)}.

5. Effect of inoculation with AM-fungi and *Rhizobium leguminosarumon* nitrogenease activity in rhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.

Concerning to nitrogenase activity in response to inoculation with AMF and *Rhizobiumleguminosarum* data in Table (14) show that the most effective treatment after 60, 90 and 120 days of planting was treatment 7 (5.32, 18.55 and 12.31 μ mole respectively), followed by treatment 5 (4.66, 15.66 and 4.61 μ mole respectively). While, treatment 2 recorded the lowest activity (1.75, 10 and 6.51 μ mole respectively).

6. Effect of inoculation with AM-fungi and *Rhizobium leguminosarumon* dehydrogenase (DHA) activity in therhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.

Data presented in Table (15) show the ability of beneficial microorganisms to exist in a plenty populations in the rhizospheric area and in soil. The existence of these microorganisms increased the activity of dehydrogenaseespecially with the treatment 7 followed by treatment 5. The control recorded the least value of dehydrogenase. Treatment 7 led to the highest values 272.94, 295.32 and 255 μ g TPF/g dry soil after 60, 90 and 120 days respectively. The recorded highest activityat the interval 90 days where the maximum microbial activity occurred at this period.

Table(14): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* nitrogenase activity in rhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.

Treatment number	Treatment*	Nitrogenase (μ mole C_2H_4 / g dry nod./ day)(mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	1.48 \pm 0.32 d	5.31 \pm 0.28 e	3.13 \pm 0.28 f
2	AMF + $\frac{1}{3}$ P+ NK	1.75 \pm 0.67cd	10 \pm 0.24d	6.51 \pm 0.25 e
3	Rh + $\frac{1}{2}$ N + PK	2.45 \pm 0.28 c	11.31 \pm 0.16 c	7.21 \pm 0.24 d
4	Comp + $\frac{1}{2}$ NPK	2.4 \pm 0.19 c	11.23 \pm 0.31 c	7.11 \pm 0.07 de
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	4.66 \pm 0.28ab	15.66 \pm 0.46 b	9.61 \pm 0.21 b
6	AMF + comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	4.00 \pm 0.27 b	15.06 \pm 0.22 b	8.37 \pm 0.2 c
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	5.23 \pm 0.29a	18.55 \pm 0.23 a	12.31 \pm 0.17 a
	L.S.D	0.78	0.86	0.64

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$), each value represents the mean of 3 replicates.

Table(15): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* dehydrogenase activity in rhizosphere region after 60, 90 and 120 days from sowing of faba bean plant.

Treatment number	Treatments*	Dehydrogenase ($\mu\text{g TPF/g dry soil}$)(mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	144.43 \pm 0.58f	145 \pm 0.54f	140.31 \pm 0.44f
2	AMF + $\frac{1}{3}$ P+ NK	166 \pm 0.75 e	165.24 \pm 0.51e	153 \pm 0.59e
3	Rh + $\frac{1}{2}$ N + PK	170.65 \pm 0.84d	180.95 \pm 0.55d	165.54 \pm 0.64c
4	Comp + $\frac{1}{2}$ NPK	169.41 \pm 0.47d	195.64 \pm 0.86c	163.11 \pm 0.99d
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	181 \pm 0.29b	200 \pm 0.85b	169.82 \pm 0.27b
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	177.19 \pm 0.25c	196.99 \pm 0.5c	164.64 \pm 1.03cd
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	272.94 \pm 0.31a	295.32 \pm 0.54 a	255 \pm 0.97a
	L.S.D	1.64	1.94	2.29

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$), each value represents the mean of 3 replicates.

7. Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* on total nitrogen percent of faba bean plant after 60, 90 and 120 days from sowing.

The ability of *Rhizobium* to fix atmospheric nitrogen in their nodules reflected positively on the percentage of total nitrogen in faba bean plants and the increase in the nitrogen percent reached the optimum value at 90 days from sowing. Table (16) show that treatment 7 led to the highest percent of total nitrogen at the three intervals 60, 90 and 120 days respectively where their values of the total nitrogen are 1.90, 2.35 and 1.41 % at the three periods respectively. The highest significant difference at 60 days recorded with treatment number 7 followed by treatment 5. At 90 days, treatment 7 led to the highest nitrogen percent followed by treatment 5 (the values are 2.35 % and 2.31 % respectively). Other treatments including the control one (full NPK) exhibited the least nitrogen percent. The same trend also recorded at 120 days from planting.

8. Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* on total phosphorus percent of faba bean plant after 60, 90 and 120 days from sowing.

Data in Table (17) pointed out that treatment 7 show the highest total phosphorus percent after 60 days (0.3 %), 90 days (0.5 %) and 120 days (0.18 %) from sowing, followed by treatment 6 where it was 0.28 % after 60 days, 0.47 % after 90 days and 0.17% after 120 days from planting. Data also pointed out that the total phosphorus percent increased after 90 days from planting, while it decreased after 120 days among all treatments.

Table(16): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* total nitrogen percent of faba bean plant after 60, 90 and 120 days from sowing.

Treatment number	Treatments*	Total nitrogen (%)(mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	1.15 \pm 0.08 d	1.75 \pm 0.03 cd	1.00 \pm 0.02 e
2	AMF + $\frac{1}{3}$ P+ NK	1.45 \pm 0.06 c	1.88 \pm 0.01 bc	1.10 \pm 0.02 d
3	Rh + $\frac{1}{2}$ N + PK	1.66 \pm 0.05 b	2.10 \pm 0.15 ab	1.31 \pm 0.03 b
4	Comp + $\frac{1}{2}$ NPK	1.31 \pm 0.06 c	1.55 \pm 0.03 d	0.91 \pm 0.04 f
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	1.75 \pm 0.04 ab	2.31 \pm 0.1 a	1.33 \pm 0.02 b
6	AMF + comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	1.67 \pm 0.02 b	2.21 \pm 0.11 a	1.23 \pm 0.02 c
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	1.90 \pm 0.03 a	2.35 \pm 0.03 a	1.41 \pm 0.03 a
	L.S.D	1.16	0.25	0.08

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

Table(17): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* total phosphorus percent of faba beanplant after 60, 90 and 120 days from sowing.

Treatment number	Treatments*	Total phosphorus (%)(mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	0.21 \pm 0.17 b	0.37 \pm 0.006 f	0.13 \pm 0.006 c
2	AMF + $\frac{1}{3}$ P+ NK	0.27 \pm 0.26 a	0.41 \pm 0.006 de	0.14 \pm 0.01 b
3	Rh + $\frac{1}{2}$ N + PK	0.24 \pm 0.25 ab	0.40 \pm 0.01 e	0.11 \pm 0.01 c
4	Comp + $\frac{1}{2}$ NPK	0.25 \pm 0.15 ab	0.43 \pm 0.01 cd	0.12 \pm 0.006 c
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	0.27 \pm 0.006 a	0.45 \pm 0.006 bc	0.14 \pm 0.006 b
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	0.28 \pm 0.01 a	0.47 \pm 0.01 b	0.17 \pm 0.006 a
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	0.30 \pm 0.02 a	0.50 \pm 0.006 a	0.18 \pm 0.01 a
	L.S.D	0.05	0.03	0.02

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

9- Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* on total potassium percent of faba bean plant after 60, 90 and 120 days from sowing.

Results in Table (18) showed that after 60, 90 and 120 days of planting there was a significant differences among all treatments. Treatment 7 showed the highest potassium percentage (2.31, 1.81 and 0.9 % at 60, 90 and 120 days of planting respectively) compared to the control (full NPK) (1.3, 0.8 and 0.5% at 60, 90 and 120 days of planting respectively). Results showed also that there was a decrease in potassium percent on plant aging along all treatments.

10- Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* on some physiological parameters of faba bean plant after 60 and 90 days from sowing.

Results in Table (19) indicated that after 60 days of planting, there was a significant increase in chlorophyll (a) content of faba bean leaves with applying treatment 7 (11.17 mg). While after 90 days of planting there was a significant increase in chlorophyll (a) content of faba bean leaves with applying treatment 7 (12.46 mg), treatment 6 (12.13 mg) and treatment 4 (10.78 mg).

Regarding to chlorophyll (b) content in faba bean leaves, Table (20) showed that after 60 days of planting there was no any significant differences among treatments, whereas, after 90 days of planting there was a significant differences with treatment 7 (1.23 mg) and treatment 6 (1.19 mg) in comparable with the control (treatment 1) (0.87 mg). Also, data in Table (21) showed that after 60 days of planting there was no any significant differences among treatments in carotenoids content in faba bean leaves, whereas, after 90 days of planting there was a significant differences with treatment 7 (4.59 mg), treatment 6 (4.39 mg) and treatment 4 (4.36 mg) in comparable with the control (treatment 1) (3.12 mg).

Table(18): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* total potassium percent of faba bean plant after 60, 90 and 120 days from sowing.

Treatment number	Treatments *	Total Potassium (%)(mean \pm SE)		
		60 days	90 days	120 days
1	Full NPK	1.3 \pm 0.06 e	0.8 \pm 0.006 e	0.5 \pm 0.006 e
2	AMF + $\frac{1}{3}$ P+ NK	1.7 \pm 0.06 d	1.3 \pm 0.06 c	0.81 \pm 0.006 c
3	Rh + $\frac{1}{2}$ N + PK	1.9 \pm 0.01 c	1.3 \pm 0.01 c	0.81 \pm 0.01 c
4	Comp + $\frac{1}{2}$ NPK	1.61 \pm 0.01 d	1.15 \pm 0.02 d	0.7 \pm 0.006 d
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	2.1 \pm 0.006 b	2.0 \pm 0.02 a	1.0 \pm 0.03 a
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	2.31 \pm 0.01 a	1.77 \pm 0.03 b	0.85 \pm 0.02 c
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	2.31 \pm 0.06 a	1.81 \pm 0.01 b	0.9 \pm 0.02 b
	L.S.D	0.09	0.08	0.04

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

Table(19): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* chlorophyll a content of faba bean plant after 60 and 90 days from sowing.

Treatment number	Treatments*	Chlorophyll a (mg/ g fresh weight)(mean \pm SE)	
		60 days	90 days
1	Full NPK	8.47 \pm 0.39 bc	8.53 \pm 0.46 cd
2	AMF + $\frac{1}{3}$ P+ NK	7.53 \pm 0.84 c	10.04 \pm 0.26 bc
3	Rh + $\frac{1}{2}$ N + PK	8.42 \pm 0.81 bc	8.02 \pm 0.73 d
4	Comp + $\frac{1}{2}$ NPK	7.87 \pm 0.51 bc	10.78 \pm 0.54 ab
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	8.58 \pm 0.64 bc	9.77 \pm 0.25 bc
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	9.89 \pm 0.71ab	12.13 \pm 0.87 a
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	11.17 \pm 0.51 a	12.46 \pm 0.41 a
	L.S.D	1.97	1.67

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p=0.05$), each value represents the mean of 3 replicates.

Table(20): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* chlorophyll b content of faba bean plant after 60 and 90 days from sowing.

Treatment number	Treatments*	Chlorophyll b (mg/ g fresh weight)(mean \pm SE)	
		60 days	90 days
1	Full NPK	2.63 \pm 0.28 a	0.87 \pm 0.11 b
2	AMF + $\frac{1}{3}$ P+ NK	2.47 \pm 0.25 a	0.98 \pm 0.05 ab
3	Rh + $\frac{1}{2}$ N + PK	3.14 \pm 0.36 a	1.12 \pm 0.1 ab
4	Comp + $\frac{1}{2}$ NPK	2.87 \pm 0.23 a	1.06 \pm 0.07 ab
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	3.27 \pm 0.55 a	1.16 \pm 0.05 ab
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	3.51 \pm 0.53 a	1.19 \pm 0.1 a
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	3.53 \pm 0.22 a	1.23 \pm 0.14 a
	L.S.D	1.12	0.29

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

Table(21): Effect of inoculation with AM-fungi and *Rhizobium leguminosarum* carotenoids content of faba bean plant after 60 and 90 days from sowing.

Treatment number	Treatments*	Carotenoids (mg/ g fresh weight)(mean \pm SE)	
		60 days	90 days
1	Full NPK	1.97 \pm 0.15a	3.12 \pm 0.08b
2	AMF + $\frac{1}{3}$ P+ NK	2.52 \pm 0.46a	3.12 \pm 0.02b
3	Rh + $\frac{1}{2}$ N + PK	2.68 \pm 0.38a	4.24 \pm 0.36ab
4	Comp + $\frac{1}{2}$ NPK	2.69 \pm 0.2a	4.36 \pm 0.15a
5	AMF + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ N + K	2.54 \pm 0.1a	3.97 \pm 0.23ab
6	AMF + Comp + $\frac{1}{3}$ P + $\frac{1}{2}$ NK	2.71 \pm 0.42a	4.39 \pm 0.6a
7	AMF + comp + Rh + $\frac{1}{3}$ P + $\frac{1}{2}$ NK	2.98 \pm 0.47a	4.59 \pm 0.54a
	L.S.D	1.04	1.07

*N= nitrogen, P= phosphorus, K= potassium, AMF= arbuscularmycorrhizal fungi, Rh= *Rhizobium leguminosarum*, Comp= compost.

**Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p=0.05), each value represents the mean of 3 replicates.

Discussion

Current public concerns about the side effects of agrochemicals on human health have led to research efforts being aimed at developing alternative agricultural strategies that would both optimize the rate of turnover and recycling of organic matter and plant nutrients and encourage the use of biological control agents, so reducing the use of chemical fertilizers and biocides. These efforts then directly lead to so-called sustainable developments in agriculture (Barea *et al.*, 2005). Certain bacteria and fungi are known to play pre-eminent roles in sustainable agriculture.

Beneficial microorganisms are known to play fundamental roles in the sustainability of both agro-ecosystems and natural ecosystems, and some of them can be used as inoculants to benefit plant growth and health through either activating nutrient cycling or by controlling plant pathogens (Bowen and Rovira, 1999; Barea *et al.*, 2002a and 2002b). Arbuscular mycorrhizal (AM) fungi and some nitrogen fixing (NF) bacteria, such as *Rhizobium* spp. and *Frankia* spp., are the main mutualistic microbial symbionts. AM fungi are known to play key roles in plant nutrition and health and in soil quality, whereas NF symbiotic bacteria, by cycling N to the biosphere from the atmosphere, represent a key input of fixed-N into plant productivity (Vance, 2001). Very important crop plants, *i.e.*, legumes species, are able to form dual symbiosis with both AM fungi and *Rhizobium* spp., a fact of great ecological and/or agricultural importance with regard to sustainability (Jefferies and Barea, 2001).

Isolation:

The isolated Rhizobia from the root nodules of faba bean plants belong to the group of Rhizobia which are the fast-growing acid producers which develop pronounced turbidity in liquid media within 2-3 days and have a mean doubling time of 2-4 hours. The cells are rod shaped to pleomorphic, motile, they can grow on a wide range of carbohydrates, but usually grow best on glucose, mannitol or sucrose. Rhizobia of this group are generally infective on temperate legumes. Most of our isolates belonged to this group and identified as *Rhizobium leguminosarum*. They are gram negative bacteria don't form endospores. Uneven gram staining is frequently encountered with rhizobia depending on the age of the culture. Cells from a young culture and nodules bacteroids usually show even gram staining while older cells and longer cells show unstained areas along the cell giving a banded appearance. These unstained areas have been identified to be large granules of polymeric-hydroxybutyric acid (PHBA) (Somasegaran and Hoben, 1985).

In our results, the isolated mycorrhizal spores were identified as *Glomus*, *Gigaspora* and *Acalospora*. Most of the identified isolates belonged to the genus *Glomus*. Stahl and Christensen (1982) revealed that *Glomus* has been reported to be the most common Arbuscular mycorrhizal fungi. It is clear that the density of AM spores was markedly affected by cultivars as well as the age of plant and sites.

When *Vicia faba* plants inoculated with *Rhizobium leguminosarum* the uptake of nitrogen increased due to the activity of nitrogenase enzyme. The fixation of atmospheric nitrogen inside the nodules of faba bean plants enhanced plant growth. Addition of organic matter represented in compost and *Rhizobium leguminosarum* increased mycorrhizal colonization inside the plant roots. Thus, led to increase of phosphorus uptake by the plants. Severity of mycorrhizal infection was affected by

age of plants. The present study showed an increase in mycorrhizal infection during 60 and 90 days after planting while it decreased after 120 days of planting.

Jia *et al.* (2004) reported that inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation. The positive effect of AM fungi on phosphate uptake has been attributed to: (i) an exploration of a larger soil volume by the extraradical mycelium; (ii) the small hyphal diameter leading to an increased phosphate absorbing area and, compared to non-mycorrhizal roots, higher phosphate influx rates per surface unit; (iii) the formation of polyphosphates by mycorrhizal fungi and thus lower internal inorganic phosphate concentrations; and (iv) the production of organic acids and phosphate that catalyze the release of phosphate from organic complexes (Bucher, 2007). In addition to phosphate, AM fungi enhance uptake of nitrogen, potassium, calcium, copper, magnesium, iron and zinc (Clark and Zeto, 2000).

Nodulation:

Our data illustrated that the application of 50% of the recommended dose of N fertilizer with *Rhizobium leguminosarum* inoculant resulted in obvious increases on the number and dry weight of nodules compared to uninoculated ones, which only received the recommended dose of N and P. A similar positive effect was observed by Ahmed *et al.* (2000) and Talaat and Abdallah (2008). Inoculation with *Rhizobium leguminosarum* ensures the presence of a high density of these organisms in close to the root systems of the seedlings causing the development of high numbers of nodules. This is in agreement with Dileep-Kumar *et al.* (2001), who found that rhizobia are capable of colonizing the root of legumes and produce plant growth-promoting substances of phytohormonal nature and exhibit antagonistic effects against many plant pathogenic fungi. Seed inoculation with

Rhizobiumleguminosarum in presence of 50% of the recommended N induced more significant increase in nodulation.

Application of 50% of the recommended dose of mineral N and 33.3% of P fertilization in presence with both *Rhizobiumleguminosarum* and AM fungi along with compost was a superior practice for nodules formation compared to other treatments. This is in harmony with the results of El-Hassanin and Lynd (1985) and Rabie (1998) who mentioned that the main effect of AM fungi on *Rhizobium leguminosarum* activity and N fixation to be phosphate mediated mechanism and the synergism between both symbionts.

Growth parameters:

It is evident from our results that *Rhizobiumleguminosarum* inoculation with 50% of recommended N and AM fungi inoculation with 33.3% of recommended P improved plant growth expressed as plant height and shoot dry weight /plant at 60, 90 and 120 days after sowing compared with the control plants. These results are in harmony with those obtained by Badr El-Din and Moawad, (1998) and Ahmed and Elsheikh, (1998). This enhancement of plant growth could be referred to that rhizobia may induce plant growth by providing products of dinitrogen fixation, either by a direct bacterium-plant transport of fixed nitrogen or by a slow transfer due to a gradual death of the bacteria and their subsequent mineralization. In addition, the promotive effect of *Rhizobiumleguminosarum* or AM fungi on plant growth might be attributed to one or more of the following mechanisms, which include mobilization of insoluble nutrients followed by enhancement of uptake by the plant, production of toxic substances to soil-borne pathogens, and production of plant growth regulators that stimulate plant growth (Talaat and Abdallah, 2008).

The inoculation with both symbionts under 50% of recommended dose of N and 33.3 % of P fertilization induced more significant increases in all parameters than inoculation with either *Rhizobium leguminosarum* or AM fungi alone. These results are matching with those obtained by Mott and Zuberer (1987), El-Ghandouret *al.* (1996), Ahmed and Elsheikh (1998) and Rabie (1998) who found a significant increase in plant dry weight wherever the tripartite association of rhizobia, mycorrhizal fungi and legumes were present. It is quite apparent that the combination of mycorrhizae and *Rhizobium* was the most effective combination for plant growth, which could be due to the stimulative effect of arbuscular mycorrhizal fungus on nitrogen fixation through its effect on phosphorus uptake.

Yield and its components:

There were significant increases in number of pods and seeds /plant, number of branches / plant, 100 seed weight as well as total yield as compared to the control. These favorable results of *Rhizobium leguminosarum* or AM fungal inoculation on faba bean yield run parallel with the findings of El-Ghandouret *al.* (1996). Moawad *et al.* (1991) showed that rhizobial inoculation increased seed yield of faba bean plant, which could be attributed to the positive effect of *Rhizobium leguminosarum* on enhancement of nodulation, nitrogen content, and plant dry weight.

Arbuscular mycorrhizal fungi can assist the plant in obtaining mineral nutrients, primarily P, by using their extensive network of external hyphae, which branch and invade regions of the rhizosphere, i.e., AM fungi contribute to increasing availability and uptake of P and micronutrients (Krishna and Bagyaraj, 1991).

It is clear that the dual inoculation plus added 50% of recommended dose of N and 33.3 % of recommended P fertilization showed the maximum significant increases in number of pods/plant, number of branches/plant as well as 100 seed weight as compared with the uninoculated plants or those inoculated with *Rhizobiumleguminosarum* or AM fungi alone.

These positive effects of the dual inoculation on yield coincide with those of El- Ghandouret *al.* (1996). Hence, the maximum seed yield was observed in treatments inoculated with the both symbionts. This supports previous results indicating that *Rhizobiumleguminosarum* enhances the mycorrhizal establishment by producing polysaccharides that leading to increase in the synthesis of polygalacturonase enzyme at the infection site, which facilitate the permeability of the root cell to the fungus (Bagyaraj *et al.*, 1979). Moreover, inoculation with both symbionts increase the capacity of plants in utilizing light, water, mineral nutrients, and carbon dioxide in building great amount of metabolites easily translocated from source to sink and finally accumulated in pods and seeds of faba bean plant.

Faba bean plants treated with *Rhizobiumleguminosarum* in combination of AM fungi and compost gave the highest value of healthy plants as indicated by the increased plant shoot height, shoot dry weight, root nodulation, root colonization and seeds yield.

Chemical composition:

Rhizobial inoculation act as an economical importance, it improves N concentration as a result of increasing N₂ fixation capacity. Moreover, inoculation with *Rhizobiumleguminosarum* increased the nodulation of faba bean plant

causing more N_2 fixation and consequently the nutrients (nitrogenous materials) secreted into the soil, it is also increased rhizospheric microflora especially the acid producers and phosphate solubilizers causing more available phosphorus, as the findings of Lipman and Conybeare (1936). Similarly, Abd-Alla and Omar (2001) found that *Rhizobium leguminosarum* inoculation significantly increased N and P contents of faba bean plant.

The mechanism of increasing plant P concentration by AM fungi inoculation is based on the relatively high activity of AM fungal hyphae in absorbing soil P and then translocate onto the host root through a specific efficient active translocation as mentioned by Cooper and Tinker (1978). AM fungi may also lower the pH value of the soil, which increases the solubility of phosphorus.

Seguin *et al.* (2003) reported that the AM fungi expand their hyphae in soil and plant roots, and this hyphal network promotes bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network.

AM fungi increased protein percentage, this is quite logical since phosphorus is an essential element in the synthesis of protein in the leguminous plants, which are well known to be rich in this ingredient. AM fungi also increased total carbohydrate percentage, this could be explained by Hayman (1983) who revealed that AM fungi have been shown to increase the level of chlorophyll. The possibility of AM fungi to increase chlorophyll levels could be highly significant in terms of the consequent effect on photosynthesis. Moreover, Sivake and Walker, (1986) indicated that AM fungi have a role in the regulation of CO_2 fixation. Bethlenfalavay *et al.*, (1987) noted that the stimulation of CO_2 , N_2 fixation and the improvement of water

and nutrient use efficiency may be induced by modification of plant water status and leaf gas exchange.

These results also revealed that the inoculation of faba bean plants with AM fungi, *Rhizobium* along with compost under low fertility conditions increased the photosynthetic pigments compared to the uninoculated ones.

In conclusion, the application of *Rhizobium leguminosarum* with AM enhanced nodulation, growth, yield and nutritional values of faba bean plants under low-fertility conditions. Moreover, AM fungi was more effective when plants were inoculated with *Rhizobium leguminosarum* than when they were fertilized with nitrogen, this indicated the compatibility between *Rhizobium leguminosarum* and Mycorrhiza.

Increasing fertilizer costs and the increasing demand for food have emphasized the need for full exploitation of biosymbionts. Therefore, it is essential to evolve and adopt a strategy of integrated nutrient supply by using a judicious combination of chemical fertilizers and biofertilizers. The benefit to mankind and the economic potential makes it a worthwhile task (Talaat and Abdallah, 2008).

Summary

The present investigation was carried out under laboratory and field conditions to study the effect of dual inoculation with AM fungi and *Rhizobium leguminosarum* on the growth of faba bean in the presence of compost under low level of N and P fertilizers (50 and 33.3 % respectively). The results might be summarized as follows:

1- *Rhizobium leguminosarum* was isolated and identified from faba bean plant roots. AM fungi were isolated and identified from rhizospheric soil.

2- Shoot length and shoot dry weight at 60, 90 and 120 days from sowing were studied. The application of 50% nitrogen, 33.3% phosphorus and 100% potassium of the recommended dose along with AM-fungi, *Rhizobium leguminosarium* inoculants and compost resulted in a significance increase in shoot length and shoot dry weight at 60, 90 and 120 days from sowing compared to the control (full NPK).

3- Studying some growth parameters showed that the number of branches, number of pods, weight of the seeds and total yield were significantly increased with the dual inoculation of *Rhizobium leguminosarum* and AM fungi along with compost under low phosphorus and nitrogen contents.

4- Studying some root parameters showed a markedly significant increase in nodules number. The dual inoculation of *Rhizobium leguminosarum* and AM fungi along with compost under low phosphorus and nitrogen contents was a superior for the mean of nodules number and nodules dry weight after 60, 90 and 120 days from sowing.

5- Severity of mycorrhizal colonization was studied. The highest values was recorded by the application of AMF, *Rhizobiumleguminosarum*, compost, 33.3 %

phosphorus, 50 % nitrogen and 100% potassium at 60, 90 and 120 days from planting.

6- Enzymes activities were also investigated. Concerning to nitrogenase and dehydrogenase activities, the most effective treatment after 60, 90 and 120 days of planting was that inoculated with both *Rhizobium leguminosarum* and AM fungi along with compost under low phosphorus and nitrogen contents.

7- Estimation of NPK contents in faba bean plants were determined. Data pointed out that the application of 50% nitrogen, 33.3% phosphorus and 100% potassium of the recommended dose along with AM-fungi, *Rhizobium leguminosarium* inoculants and compost led to the highest percent of total nitrogen, total phosphorus and total potassium at the three intervals 60, 90 and 120 days. It was evident that the total phosphorus percent increased after 90 days from planting, while it decreased after 120 days among all treatments. While, there was a decrease in potassium percent on plant aging along all treatments.

8- Studying some physiological parameters showed that there was a significant increase in chlorophyll (a), chlorophyll (b) and carotenoids contents of faba bean leaves after 90 and 120 days of sowing.

9- Regarding total nitrogen percent and crude protein in the seeds, there was no significant differences among treatments.

References

- Abd-Alla M.H. and Omar S.A. (2001).** Survival of Rhizobia/Bradyrhizobia and a rock-phosphatesolubilizing fungus *Aspergillus niger* on various carriers from some agro-industrial wastes and their effects on nodulation and growth of faba bean and soybean. J. Plant. Nutr., 24: 261-272.
- Ågren G.I. (1985).** Theory for growth of plants derived from the nitrogen productivity concept. Physiol. Plant., 64: 17-28.
- Ahmed A.E. and Elsheikh E.A.E. (1998).** Effects of biological and chemical fertilizers on growth and symbiotic properties of faba bean under salt stress. J. Agric. Sci., 6: 150-164.
- Ahmed F.E., Yagoub S.O. and Elsheikh E.A.E. (2000).** Effects of mycorrhizal inoculation and phosphorus application on the nodulation, mycorrhizal infection and yield components of faba bean grown under two different watering regimes. U.K.J. Agric. Sci., 8: 107-116
- Akiyama K., Matsuzaki K. and Hayashi H. (2005).** Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature, 435: 824 – 827.
- Ali M.M. (2013).** Using some biofertilizers in biological control. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Cairo University, Egypt.
- Andersen J. W., Story L., Sieling B., Chen W. J. L., Petro M. S. and Story J. (1984).** Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. Am. J. Clin. Nut., 40:1146–1155.
- Antoun H., Beauchamp C.J., Goussard N., Chabat R. and Lalande R. (1998).** Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes; Effect on radishes (*Raphanus sativus* L.). Plant and Soil, 204: 57-67.
- Badr El-Din S.M.S. and Moawad H. (1988).** Enhancement of nitrogen fixation in lentil, faba bean, and soybean by dual inoculation with Rhizobia and mycorrhizae. Plant and Soil, 108: 117-124.

Bago B., Pfeffer P.E. and Shachar-Hill Y. (2000). Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiol.*, 124: 949 - 958.

Bagyaraj D.J., Manjunath A. and Patil R.B. (1979). Interaction between a vesicular arbuscular mycorrhiza and *Rhizobium* and their effect on soybean in the field. *New Phytol.*, 82: 141-145.

Balestrini R. and Bonfante P. (2005). The interface compartment in arbuscular mycorrhizae: a special type of plant cell wall? *Plant Biosyst.*, 139: 8-15.

Balestrini R., Gómez-Ariza J., Lanfranco L. and Bonfante P. (2007). Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. *Mol. Plant Microbe Interact.*, 20:1055–1062.

Ballesteros-Almanza L., Altamirano-Hernandez J., Peña-Cabriaes J.J., SantoyoG., Sanchez-Yañez J.M., Valencia-Cantero E., Macias-Rodriguez L., Lopez-Bucio J., Cardenas-Navarro R. and Farias-Rodriguez R. (2010). Effect of co-inoculation with mycorrhiza and rhizobia on the nodule trehalose content of different bean genotypes. *Open Microbiol. J.*, 17:83-92

Barea J.M. and Azcon-Aguilar C. (1983). Mycorrhizas and their significance in nodulating nitrogen-fixing plants. *Adv. Agron.*, 36: 1-54.

Barea J.M., Werner D., Azcon-Aguilar C. and Azcon R. (2005). Interactions of arbuscular mycorrhiza and nitrogen fixing symbiosis in sustainable agriculture. In: *Agriculture, Forestry, Ecology, and the Environment*. (D. Werner, W.E. Newton Eds.). Kluwer Academic Publishers, Netherlands: 199-222

Barea J.M., Azcón R., Azcón-Aguilar C. (2002a). Mycorrhizosphere interactions to improve plant fitness and soil quality. *Antonie van Leeuwenhoek*, 81:343–351.

Barea J.M., Gryndler M., Lemanceau Ph., Schüepp H. and Azcón R. (2002b). The rhizosphere of mycorrhizal plants. In: *Gianinazzi S, Schüepp H, Barea JM, Haselwandter K, eds. Mycorrhiza technology in agriculture: from genes to bioproducts*. Basel, Switzerland: Birkhäuser Verlag, 1–18.

Bethlenfalavy G.J., Brown M.S. and Newton W.E. (1987). Phytosynthetic water- and nutrient-use efficiency in mycorrhizal legume, In: D.M. Sylvia, L.L. Hung and G.H. Graham (Ed). “Mycorrhiza in the Next Decade”, U.S.A.

Bianciotto V., Barbiero G. and Bonfante P. (1995). Analysis of the cell cycle in an arbuscular mycorrhizal fungus by flow cytometry and bromodeoxyuridine labelling. *Protoplasma*, 188:161- 169.

Bird N. A. (2009). Development and identification of the rhizobiaceae nitrogen fixing symbiosis with fabaceae as a nitrogen source in reclamation of disturbed lands in Wyoming. M. Sc. Thesis, University of Wyoming, Laramie, Wyoming, USA.

Bonfante P., Genre A., Faccio A., Martini I., Schauser L., Stougaard J., Webb J. and Parniske M. (2000). The *Lotus japonicus* *LjSym4* gene is required for the successful symbiotic infection of root epidermal cells. *Mol. Plant Microbe Interact.*, 13: 1109–1120.

Bonfante P. (1984). Anatomy and morphology of VA Mycorrhizae. In: *Mycorrhiza VA*, Powell CL, Bagyaraj DJ (eds) CRC Press, Boca Raton, pp 5 – 33.

Bonfante P. (2001). At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Hock B (ed) *The Mycota IX: fungal associations*. Springer, Berlin Heidelberg New York, pp 45 – 61.

Bonfante P. and Perotto S. (1995). Strategies of arbuscular mycorrhizal fungi when infecting host plants. *New Phytol.*, 130: 3 – 21.

Bowen G.D. and Rovira A.D. (1999). The rhizosphere and its management to improve plant growth. *Adv. Agron.*, 66:1–102.

Brencic A. and Winans S.C. (2005). Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. *Microbiol. Mol. Biol. Rev.*, 69: 155–194.

Brewin N.J. (2004). Plant cell wall remodelling in the rhizobium legume symbiosis. *Crit. Rev. Plant Sci.*, 23: 293–316.

Broughton W.J., Jabbouri S. and Perret X. (2000). Keys to symbiotic harmony. *J. Bacteriol.*, 182:5641– 5652

Brundrett M. C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytol.*, 154: 275 – 304.

Brundrett M., Bougher N., Dell B., Grove T. and Malajczuk N. (1996). Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra

Bucher M. (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol.*, 173: 11-26.

Chen T.H.H., and Murata N. (2002). Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol.*, 5: 250–257.

Clark R.B. and Zeto S.K. (2000). Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nutr.*, 23:867-902.

Cooper J. (2004). Multiple responses of rhizobia to flavonoids during legume root infection. In: Callow J.A. (ed.), *Advances in Botanical Research: Incorporating Advances in Plant Pathology*. Academic, London, pp. 1–62.

Cooper, K.M. and Tinker P.B. (1978). Translocation and transfer of nutrients in vesicular arbuscular mycorrhizas. IV. Effect of environmental variables on movement of phosphorus. *New Phytol.*, 88: 327-339.

Crespi M. and Galvez S. (2000). Molecular mechanisms in root nodule development. *J. Plant Growth Regul.*, 19: 155–166.

Cullimore J.V., Ranjeva R. and Bono J.J. (2001). Perception of lipochitooligosaccharidic Nod factors in legumes. *Trends Plant Sci.*, 6: 24–30.

Date R.A. and halliday J. (1987). Collection, isolation, cultivation and maintenance of Rhizobia. In: Symbiotic nitrogen fixation technology. H Elken (ed). Marcel Dekker, Inc. New York

David C. Weindorf, James P. Muir, and Cesáreo Landeros-Sánchez, (2011). Organic Compost and Manufactured Fertilizers: Economics and Ecology. Issues in Agroecology – Present Status and Future Prospectus 1, DOI 10.1007/978-94-007-1309-3_2, © Springer Science+Business Media B.V. 2011

Demchenko K., Winzer T., Stougaard J., Parniske M. and Pawlowska K. (2004). Distinct roles of *Lotus japonicus* SYMRK and SYM15 in root colonization and arbuscule formation. New Phytol., 163: 381 – 392

Desai S., Minaksh D., Amalray E.L.D, Kumar G.P. and Ahmed S.K.M.H. (2012). Microorganisms in sustainable agriculture and biotechnology. Springer Sci., Business Media B.V., 227-241.

Difco Manual (1985). Dehydrated culture media and reagents for microbiology 10^{ul} Ed. Difco laboratories Defroit Michigan, USA. 4823 : 487 – 623.

Dileep-Kumar B.S., Berggren I. and Martensson A.M., (2001). Potential for improving pea production by co-inoculation with fluorescent *Pseudomonas* and *Rhizobium*. Plant and Soil, 229: 25-34.

Duranti M. (2006). Grain legume proteins and nutraceutical properties. Fitoterapia, 77:67–82

El-Ghandour I.A., El-Sharaawy M.A.O. and Abdel-Moniem E.M. (1996). Impact of vesicular arbuscular mycorrhizal fungi and *Rhizobium* on the growth and P, N and Fe uptake by faba-bean. Fertilizer Research, 43: 43-48.

El-Hassanin A.S. and Lynd J.Q. (1985). Soil fertility affects with tripartite symbiosis for growth, nodulation and nitrogenase activity of *Vicia faba*, L. J. Plant Nutr., 8: 491-504.

Erik S. Jensen a, Mark B. Peoples and Henrik Hauggaard-Nielsen (2010). Faba bean in cropping systems. Field Crops Research, 115: 203–216.

Fariao-Rodriguez R., Mellor R. B., Arias C. and Cabriaes J. J. (1998). The accumulation of trehalose in nodules of several cultivars of common bean (*Phaseolus vulgaris*) and its correlation with resistance to drought stress. *Physiologia plant.*, 102: 353-359.

Farrand S.K., Van Berkum P.B. and Oger P. (2003). *Agrobacterium* is a definable genus of the family *Rhizobiaceae*. *Int. J. Syst. Evol. Microbiol.*, 53: 1681–1687.

Friese C.F. and Allen M.F. (1991). The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphae architecture. *Mycologia*, 83:409-418

Genre A. and Bonfante P. (1998). Actin versus tubulin configuration in arbuscule-containing cells *New Phytol.*, 140: 745-752

Genre A. and Bonfante P. (2005). Building a mycorrhizal cell: how to reach compatibility between plants and arbuscular mycorrhizal fungi. *J. Plant Interact.*, 1: 3 -13

Genre A. and Bonfante P. (2007). Check-in procedures for plant cell entry by biotrophic microbes. *Mol. Plant Microbe Interact.*, 20:1023–1030

Genre A., Chabaud M., Timmers T., Bonfante P. and Baker G.D. (2005). Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell*, 17: 3489–3499.

Geurts R. and Bisseling T. (2002). *Rhizobium* Nod factor perception and signalling. *Plant Cell*, 14: S239–S249.

Gianinazzi-Pearson V. and Brechenmacher L. (2004). Functional genomics of arbuscular mycorrhiza: decoding the symbiotic cell programme. *Can. J. Bot.*, 82: 1228 – 1234

Grusak M.A. (2002). Enhancing mineral content in plant food products. *J. Am. Coll. Nutr.*, 21: 178–183

Guinel F. and Hirsch A.M. (2000). The involvement of root hairs in mycorrhizal associations. In: Ridge RW and Emos AMC (eds) *Cell and molecular biology of plant root hairs*. Springer, Berlin Heidelberg New York , 285 – 310

Hayman D.S. (1983). The physiology of Vesicular arbuscular endomycorrhizal symbiosis. *Can. J. Bot.*, 61: 944-963.

Heeb S. and Haas D. (2001). Regulatory roles of the GacS/GacA two-component system in plant-associated and other gram-negative bacteria. *Mol. Plant Microbe Int.*, 14: 1351–1363.

Jackson M.L. (1973). Soil Chemical Analysis. Pentice Hall of India Pvt. Ltd., New Delhi.

Javaid A. (2010). Role of arbuscular mycorrhizal fungi in nitrogen fixation in legumes. In: M.S. Khan et al. (eds.), *Microbes for Legume Improvement*, Springer-Verlag/Wien, Germany, pp. 409-426.

Jeffries P. and Barea J.M. (2001). Arbuscular mycorrhiza: a key component of sustainable plant–soil ecosystems. In: Hock B, ed. *The Mycota: fungal associations*, Vol. IX. Berlin, Heidelberg, New York: Springer, 95–113.

Jia J., Tong C., Wang B., Luo L. and Jiang J. (2004). Hedgehog signaling activity of Smoothened requires phosphorylation by protein kinase A and casein kinase I. *Nature*, 432: 1045-1050.

Kormanik P.P., Bryan W.C. and Schultz R.C. (1980). Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. *Can. J. Microbiol.*, 26: 536-538.

Kosuta S., Chabaud M., Loughon G., Gough C., Denarie J., Barker D.G. and Becard G. (2003). A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. *Plant Physiol.*, 131: 952 – 962

Krishna K.R. and Bagyaraj D.J. (1991). Role of vesicular arbuscular mycorrhiza in the uptake of micronutrient by groundnut plants. *Curr. Res.*, 20: 173-175.

Ligrone R., Carafa A., Lumini E., Bianciotto V., Bonfante P. and Duckett J.G. (2007). Glomeromycotean associations in liverworts: a molecular cellular and taxonomic analysis. *Am. J. Bot.*, 94:1756–1777

Lipman J.G. and Conybeare A.B. (1936). Preliminary note on the inventory and balance sheet of plant nutrients in the United States. N. J. Agri. Expt. Sta. Bull., 607.

Lodwig E.M., Leonard M., Marroqui S., Wheeler T.R., Findlay K., Downie J.A. and Poole P.S. (2005). Role of polyhydroxybutyrate and glycogen as carbon storage compounds in pea and bean bacteroids. Mol. Plant Microbe Int., 18: 67–74.

Lohse S., Schliemann W., Ammer C., Kopka J., Stracke D. and Fester T. (2005). Organization and metabolism of plastids and mitochondria in arbuscular mycorrhizal roots of *Medicago truncatula*. Plant Physiol., 139: 329 – 340

Lupwayi N.Z. and Kennedy A.C. (2007). Grain legumes in northern great plains: impacts on selected biological soil processes. Agron. J., 99:1700–1709

Massoud O.N. (2005). Microbiological and chemical evaluation of compost and its application in organic farming. Ph.D. Thesis, Department of Botany, Faculty of Science, Menoufiya University, Egypt.

Matthews P. and Marcellos H. (2003). *Faba bean* (2nd edition). Agfact P4.2.7, Division of Plant Industries, NSW, Australia.

Metzner H., Rau H. and Senger H. (1965). Untersuchungen Zur Synchronisierung der Zellteilung bei *Chlorella*. Planta, 65: 186.

Miller R.W. and Donahue R.L. (1990). Soils: An introduction to soils and plant growth. Sixth edition. Prentice Hall, Englewood Cliffs.

Moawad H., Badr El-Din S.M.S. and Khalafallah M.A. (1991). Assessment of faba bean (*Vicia faba*) response to inoculation with *Rhizobium leguminosarum* in clay loam Nile Delta soil. World J. Microbiol. Biotechnol., 7: 191-195.

Mott J.B. and Zuberer D.A. (1987). Effect of symbiotic association on clover grown in mine spoil. In: D.M. Sylvia, L.L. Hung and G.H. Graharm (Ed). "Mycorrhiza in the Next Decade". U.S.A.

Mourad K., Fadhila K., Chahinez M., Meriem R., De Lajudie P. and Abdelkader B. (2009). Antimicrobial activities of *Rhizobium* sp strains against

Pseudomonas savastanoi, the agent responsible for the olive knot disease in Algeria. *Grasas Aceites*, 60:139–146

Murray J.D. (2011). Invasion by invitation: rhizobial infection in legumes. *Mol. Plant Microbe Interact.*, 24: 631–639.

Navazio N., Moscatiello R., Genre A., Novero M., Baldan B., Bonfante P. and Mariani P. (2007). Diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. *Plant Physiol.*, 144: 673 – 681

Ncube B., Dimes J.P., Van Wijk M.T., Twomlow S.J. and Giller K.E. (2009). Productivity and residual benefits of grain legumes to sorghum under semi-arid conditions in south-western Zimbabwe: unravelling the effects of water and nitrogen using a simulation model. *Field Crops Res.*, 110:173–184

Neera G. and Geetanjali (2007). Symbiotic Nitrogen Fixation in Legume Nodules: Process and Signaling: A Review. *Agron. Sust. Devel.*, 27, 59-68

Novero M., Faccio A., Genre A., Stougaard J., Webb K.J., Mulder L., Parniske M. and Bonfante P. (2002). Dual requirement of the *LjSym4* gene for the mycorrhizal development in epidermal cells and cortical cells of *Lotus japonicus* roots. *New Phytol.*, 154: 741 – 749

Novero M., Genre A., Szczygłowski K., and Bonfante P. (2008). Root hair colonization by mycorrhizal fungi. *Plant Cell Monogr.* 12: 315-338

Olah B., Briere C., Becard G., Denarie J. and Gough C. (2005). Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. *Plant J.*, 44: 195 – 207

Paszkowski U. (2006). A journey through signaling in arbuscular mycorrhizal symbioses 2006. *New phytol.* 172: 35-46

Peoples M.B. and Crasswell E.T. (1992). Biological nitrogen fixation: investments, expectations and actual contributions to agriculture. *Plant Soil*, 141: 13–39.

Peretto R., Bettini V., Favaron F., Alghisi P. and Bonfante P. (1995). Polygalacturonase activity and location in arbuscular mycorrhizal roots of *Allium porrum* L. *Mycorrhiza*, 5: 157 – 163

Perret X., Staehelin C. and Broughton W.J. (2000). Molecular basis of symbiotic promiscuity. *Microbiol. Mol. Biol. Rev.*, 64:180–201

Peterson L., Massicotte H.B. and Melville L. H. (2004). *Mycorrhizas: anatomy and cell biology*. NRC Press, Ottawa

Phillips D.A., Joseph C.M. and Maxwell C.A. (1992). Trigonelline and stachydrine released from alfalfa seeds activate NodD2 protein in *Rhizobium meliloti*. *Plant Physiol.*, 99: 1526–1531.

Phillips J.M. and Hayman D.S. (1970). Improved procedures for clearing roots and staining vesicular arbuscular mycorrhizal fungi for rapid assessments of infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.

Prell J. and Poole P. (2006). Metabolic changes of rhizobia in legume nodules. *Trends Microbiol.*, 14: 161–168.

Rabie G.H. (1998). Induction of fungal disease resistance in *Vicia faba* by dual inoculation with *Rhizobium leguminosarum* and vesicular-arbuscular mycorrhizal fungi. *Mycopathologia*, 141: 159-166.

Radutoiu S., Madsen L.H., Madsen E.B., Felle H.H., Umehara Y., Gronlund M., Sato S., Nakamura Y., Tabata S., Sandal N. and Stougaard J. (2003). Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature*, 425:585–592

Rémy W., Taylor T.N., Hass H. and Kerp H. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc. Natl. Acad. Sci. USA.*, 91: 11841 – 11843

Robertson J.L., Holliday T. and Matthysse A.G. (1988). Mapping of *Agrobacterium tumefaciens* chromosomal genes affecting cellulose synthesis and bacterial attachment to host cells. *J. Bacteriol.*, 170: 1408–1411.

Rudiger H. and Gabius H.J. (2001). Plant lectins: occurrence, biochemistry, functions and applications. *Glycoconjugate J.*, 18: 589–613.

Ryan M.H. and Angus J.F. (2003). Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. *Plant Soil.*, 250: 225–239

Schüßler A., Schwarzott D. and Walker C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol. Res.*, 105: 1413 – 1421

Seguin S., Desaulniers N., Dalpé Y. and Levesque C.A. (2003). Development of AMF strains specific primers for detection in field-grown colonized roots. 4th Int. Conf. Mycorrhizae. Montreal Quebec Canada, August 10-15 2003, No. 444.

Selosse M. A., Faccio A., Scappaticci G. and Bonfante P. (2004). Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. *Microb. Ecol.*, 47: 416 – 426

Selosse M.A., Richard F., He X. and Simard S. (2006). Mycorrhizal networks: les liaisons dangereuses. *Trends Ecol. Evol.*, 21: 621 – 628

Sivake M.N. and Walker D.A. (1986). Photosynthesis in vivo can be limited by phosphate supply. *New Phytologist*, 102: 499-512.

Skujins J. (1976). Extracellular enzymes in soil. *CRC Crit. Rev. Microbiol.*, 4: 383-421.

Smith S.E. and Read D.J. (1997). Mycorrhizal symbiosis. Academic, London, pp 1 – 605

Somasegaran P. (1985). Inoculant production with diluted liquid cultures of *Rhizobium* spp. and autoclaved peat: evaluation of diluents, *Rhizobium* spp., peats, sterility requirements, storage, and plant effectiveness. *Appl. Env. Micro.*, 50:398-405.

Spaink H.P. (2000). Root nodulation and infection factors produced by rhizobial bacteria. *Annu. Rev. Microbiol.*, 54: 257–288.

Stahl P. D. and Christensen M. (1982). Mycorrhizal fungi associated with *Bouteloua* and *Agropyron* in Wyoming sagebrush-grasslands. *Mycologia.*, 74: 877-885.

Talaat Neveen B. and Abdallah Amany M. (2008). Response of Faba Bean (*Vicia faba* L.) to Dual Inoculation with *Rhizobium* and VA Mycorrhiza under Different Levels of N and P Fertilization. *J. Appl. Sci. Res.*, 4: 1092-1102.

Tharanathan R.N. and Mahadevamma S. (2003). Grain legumes – a boon to human nutrition. *Trends Food Sci. Technol.*, 14:507–518

Toth R. and Miller R.M. (1984). Dynamics of arbuscule development and degeneration in a *Zea mays* mycorrhiza. *Am. J. Bot.*, 71: 449 – 460

Trappe J. M. (1982). Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. *Phytopathology*, 72:1102-1108.

Vance C. (2001). Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiology*, 127: 391-397.

Willems A. and Collins M.D. (1993). Phylogenetic analysis of rhizobia and agrobacteria based on 16S rRNA gene sequences, *Int. J. Syst. Bacteriol.*, 43: 305–313.

الملخص العربي

قد أدت المخاوف العامة الحالية حول الآثار الجانبية للمواد الكيميائية الزراعية على صحة الإنسان إلى الجهود البحثية التي تهدف إلى تطوير استراتيجيات زراعية بديلة من شأنها تحسين كل من معدل دوران وإعادة تدوير المواد العضوية والمغذيات النباتية وتشجيع استخدام عوامل مكافحة البيولوجية وذلك للحد من استخدام الأسمدة الكيماوية والمبيدات الحيوية. تؤدي هذه الجهود إلى ما يسمى التطورات المستدامة في الزراعة. و يعتبر فطر الميكوريزا و بعض البكتيريا المثبتة للنيتروجين مثل *Rhizobium spp.* and *Frankiaspp.* من أهم الميكروبات التكافلية. تلعب فطريات الميكوريزا دورا أساسيا في صحة و تغذية النبات و في جودة التربة. بينما تقوم البكتيريا التكافلية بتثبيت النيتروجين الجوي للنبات. و للنباتات البقولية القدرة علي عمل علاقة تكافلية مع كل من فطر الميكوريزا و *Rhizobium spp.*

يهدف هذا البحث لدراسة تأثير العلاقة التكافلية مع كل من فطر الميكوريزا و *Rhizobium spp.* علي نمو نبات الفول البلدي في وجود السماد العضوي و نسب قليلة من المبيدات النيتروجينية و الفوسفورية. و قد تم عزل كل من فطر الميكوريزا و *Rhizobium leguminosarum* و التعرف عليهما. و قد ادت التجارب المختلفة الي العديد من النتائج و يمكن تلخيصها كالآتي:

١- إن إضافة كل من فطر الميكوريزا و *Rhizobium leguminosarum* في وجود السماد العضوي و نسبة قليلة من المبيدات النيتروجينية و الفوسفورية لنبات الفول إلي أفضل النتائج في طول و وزن الساق بعد ٦٠، ٩٠ و ١٢٠ يوم من الزراعة. و كان افضلهم بعد ١٢٠ يوم.

٢- إن إضافة كل من فطر الميكوريزا و *Rhizobium leguminosarum* في وجود السماد العضوي و ٥٠ % من النيتروجين و ٣٣ % من الفوسفور لنبات الفولالي أفضل النتائج في عدد الأفرع، وزن المائة بذرة و عدد القرون.

٣- إن إضافة كل من فطر الميكوريزا و *Rhizobium leguminosarum* في وجود السماد العضوي و ٥٠ % من النيتروجين و ٣٣ % من الفوسفور لنبات الفول الي أفضل النتائج في بعض الصفات الجذرية (عدد و وزن العقد البكتيرية) بعد ٦٠، ٩٠ و ١٢٠ يوم من الزراعة.

٤- إن إضافة كل من فطر الميكوريزا و *Rhizobium leguminosarum* في وجود السماد العضوي و ٥٠ % من النيتروجين و ٣٣ % من الفوسفور لنبات الفول الي أفضل النتائج في نسبة اصابة بفطر الميكوريزا .

٥- إن إضافة كل من فطر الميكوريزا و *Rhizobium leguminosarum* في وجود السماد العضوي و ٥٠ % من النيتروجين و ٣٣ % من الفوسفور لنبات الفول إلى أفضل النتائج في نشاط الانزيم Nitrogenase, dehydrogenase. و كان أفضلهم بعد ٩٠ يوم.

٦- ان اضافة كل من فطر الميكوريزا و *Rhizobium spp.* في وجود السماد العضوي و ٥٠ % من النيتروجين و ٣٣ % من الفوسفور ادت الي زيادة نسب النيتروجين و الفوسفور و البوتاسيوم الكلي بعد ٦٠، ٩٠ و ١٢٠ يوم من الزراعة.

٧- إن إضافة كل من فطر الميكوريزا و *Rhizobium leguminosarum* في وجود السماد العضوي و نسبة قليلة من المبيدات النيتروجينية و الفوسفورية لنبات الفول إلى أفضل النتائج في صبغات الكلوروفيل a, b و صبغة الكاروتينويد.

٨- و قد اظهرت النتائج انه لا يوجد اي تأثير معنوي علي نسبة النيتروجين الكلي و البروتين في الحبوب.



تأثير الريزوبيوم والفطريات الجذرية علي سحب النيتروجين والفسفور بواسطة نبات الفول

رسالة مقدمة من الطالبة

دعاء السيد مسعد جعفر

قسم الميكروبيولوجي – كلية العلوم – جامعة دمياط

وذلك كجزء من المتطلبات للحصول علي درجة الماجستير في الميكروبيولوجي

تحت اشراف

الدكتور / محمد اسماعيل ابو دبارة

الاستاذ الدكتور / زكريا عوض محمد بقا

استاذ مساعد الميكروبيولوجي، قسم النبات

استاذ الميكروبيولوجي، قسم النبات

كلية العلوم، جامعة دمياط

كلية العلوم، جامعة دمياط

الدكتور / اسامة نجدي محمد مسعود

استاذ مساعد الميكروبيولوجي،

بمعهد الأراضي و المياه والبيئة،

مركز البحوث الزراعية، القاهرة.

السادة أعضاء لجنة الاشراف

عنوان الرسالة: تأثير الريزوبيوم والفطريات الجذرية علي سحب النيتروجين والفسفور بواسطة نبات الفول

اسم الباحث: دعاء السيد مسعد جعفر

لجنة الاشراف:

الاسم	الوظيفة	التوقيع
١ الأستاذ الدكتور / زكريا عوض محمد بقا	استاذ الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة دمياط.	
٢ الدكتور / محمد اسماعيل ابو دبارة	استاذ مساعد الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة دمياط	
٣ الدكتور / اسامة نجدي محمد مسعود	استاذ مساعد الميكروبيولوجي، بمعهد الأراضي و المياه والبيئة، مركز البحوث الزراعية، القاهرة.	

عميد الكلية
أ.د./ أحمد السيد عبد الغني
الغباشي

وكيل الكلية
أ.د./ ممدوح محمد أحمد نعمة
الله

رئيس قسم النبات
أ.د./ نعمت محمد حسن

السادة أعضاء لجنة الحكم والمناقشة

عنوان الرسالة: : تأثير الريزوبيوم والفطريات الجذرية علي سحب النيتروجين والفسفور بواسطة نبات الفول

اسم الباحث: دعاء السيد مسعد جعفر

لجنة الاشراف:

الاسم	الوظيفة	التوقيع
أ.د. / زكريا عوض محمد بقا	استاذ الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة دمياط.	
د / محمد اسماعيل ابو ديارة	استاذ مساعد الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة دمياط	
د / اسامة نجدي محمد مسعود	استاذ مساعد الميكروبيولوجي، بمعهد الأراضي و المياه والبيئة، مركز البحوث الزراعية، القاهرة.	

لجنة المناقشة و الحكم:

الاسم	الوظيفة
أ.د./يحيى أحمد ماهر الطواهري	استاذ الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة الزقازيق.
أ.د./أحمد اسماعيل عبد القادر	استاذ الميكروبيولوجي، قسم معلوم البيئة، كلية العلوم، جامعة بورسعيد.
أ.د./ زكريا عوض محمد بقا(تقرير مشترك)	استاذ الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة دمياط.
د / محمد اسماعيل ابو ديارة(تقرير مشترك)	استاذ مساعد الميكروبيولوجي، قسم النبات، كلية العلوم، جامعة دمياط.

عميد الكلية
أ.د./ أحمد السيد عبد الغني
الغباشي

وكيل الكلية
أ.د./ ممدوح محمد أحمد نعمة
الله

رئيس قسم النبات
أ.د./ نعمت محمد حسن

