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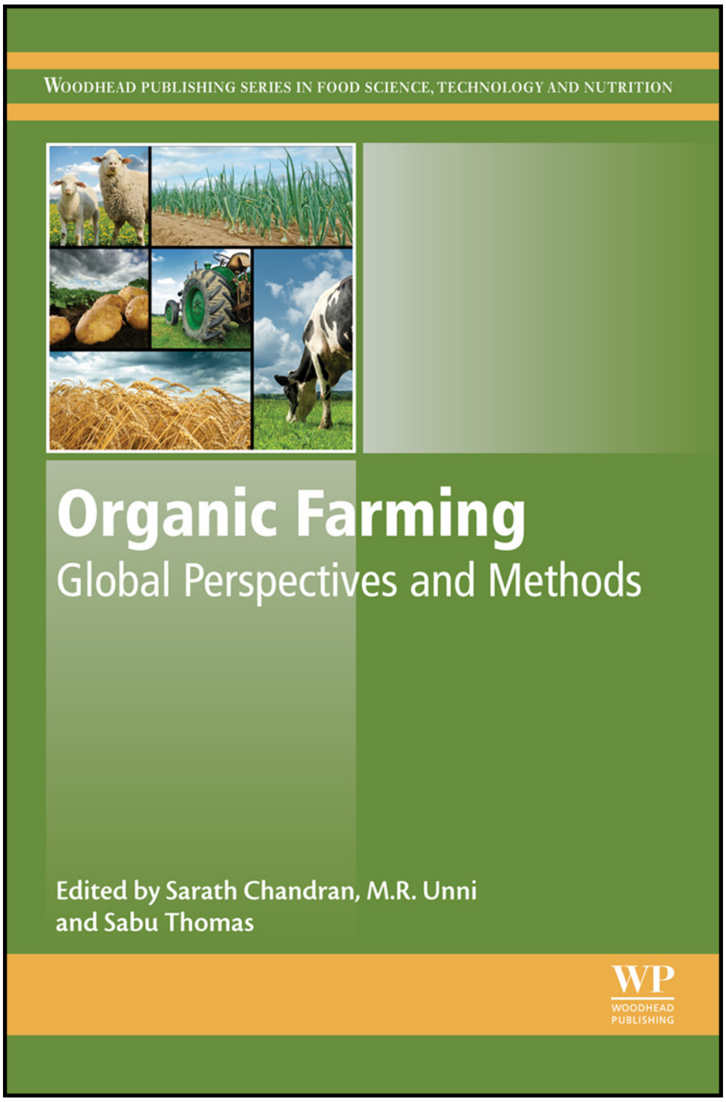

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From Ibrahim Ortas, Role of Microorganisms (Mycorrhizae) in Organic Farming. In: Sarath Chandran, M.R. Unni and Sabu Thomas, editors, Organic Farming. Chennai: Woodhead Publishing, 2019, pp. 181-212.

ISBN: 978-0-12-813272-2
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Chapter 6

Role of Microorganisms (Mycorrhizae) in Organic Farming

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6.1 INTRODUCTION

Agricultural production at present is much more dependent on inputs such as fertilizer, irrigation, soil management, and seed quality than ever before. For better and proper plant growth it is necessary to have fertilizer added to the soil. World fertilizer sources are becoming depleted and in the near future it will be difficult to find rock phosphate sources for phosphorus fertilizer manufacturing. Increasing fertilizer use has recently affected both human health and natural life. Since fertilizers are expensive t has become increasingly difficult for developing countries to afford. Also some countries, especially some in Asia and Africa, have insufficient income to buy fertilizer to attain greater yields. Because of their high clay and lime contents, most Mediterranean soils are poor in availability of macro- and micronutrients, such as P, Zn, and Fe, which are diffusion-limited in soils. Also, in semiarid areas, high calcium carbonate content, high pH, and low organic matter content influence soil fertility and nutrient uptake. In Mediterranean coastal areas there is a serious problem with soilborne diseases, such as plant parasitic nematodes, soilborne plant pathogens, root-rot, and some weed pests. Nearly 25% of yield reduction is occurring year to year for wheat, banana, citrus, and vegetable crops. Farmers are also seriously challenged by parasitism. It is imperative to find an ecological approach for food security and safety.

Mycorrhizal symbiosis is a widespread, natural biological occurrence. Ecologically important, it invariably has benefits for both the plant host and fungal symbiont. Carbohydrates from the plant are utilized by the microorganism for its energy requirements, whilst the plant takes up mineral ions more effectively via the fungal hyphae.

Organic Farming. DOI: https://doi.org/10.1016/B978-0-12-813272-2.00006-9
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Mycorrhiza-inoculated plants achieve a balance in the uptake of nutrients and water. Mycorrhizae can be a very important and useful biofertilizer source for organic agriculture and production. Therefore, mycorrhizal inoculation is a good strategy for sustainable agriculture. Mycorrhizae uptake of nutrients and water for plants may be a major factor for ecological and organic farming. Also, since mycorrhizae directly influence plant physiology and the plant produces safe food, this is beneficial for humans as well. Recently there has been a growth in the demand for ecologic or organic production, encouraging the use of organic fertilizers, such as mycorrhizae.

Mycorrhizal fungi that penetrate root cortical cells, and hence usually lack a mycelial sheath, are *endomycorrhizas*. Arbuscular mycorrhizal fungi (AMF) can be integrated into soil management systems to achieve low-cost sustainable agricultural systems (Hooker and Black 1995). Arbuscules are formed inside infected tissue. The former are for storage purposes; the latter probably for nutrient transfer. Specifically, mycorrhizae are extensive within the existing root system, thereby exploiting a greater soil volume for nutrient uptake, simultaneously increasing the effective root surface area capable of absorption. The ability of different mycorrhizae to colonize rapidly and extensively leads to differences in nutrient uptake levels—more so than absorbance rates or the amount of external mycelium (Abbott and Robson, 1982). Additionally, more efficient uptake and translocation of ions may take place by way of hyphae rather than through a reliance on the soil–root relationship alone.

Mycorrhizal fungi occur in most soils, and colonize the roots of many plant species. Mycorrhiza are structures resulting from the symbiosis between these fungi and plant roots, and are directly involved in plant mineral nutrition. The symbiotic root–fungal association increases the uptake of less mobile nutrients, essentially phosphorus (P) but also micronutrients like zinc (Zn) and copper (Cu); the symbiosis has also been reported as influencing water uptake. AMF are generally known to benefit established symbioses with the majority of plant roots. There is much current interest in the legume–*Rhizobium*–AM triumvirate, as this also has agricultural significance. The best-known advantage of AM fungi inoculation is increasing plant growth. Mycorrhizal fungi increase the uptake of plant macro- and micronutrients, especially those elements with low soil mobility, such as phosphorus, zinc, and copper (Marschner, 1993; George et al., 1994, 1995; Ortaş et al., 2000; Ortaş, 2002a,b, 2003). It has been reported that N, K, Fe, Mg, Ca, and S can be taken up my mycorrhzae in soil of low fertility (Kothari et al., 1990; Sieverding, 1991; Frey and Schüepp, 1993; Smith and Read, 1997) and ecological condition. Mycorrhizae also contribute to improving water use. Recently, it was reported that mycorrhizae increased plant tolerance against various stress factors, such as drought and water deficiency (Drüge and Schönbeck 1992; Goicoechea et al. 1996; Bowen and Rovira 1999). Also it has been shown that plant root mycorrhizal inoculation is very important for soil structure development, which is a key factor for plant health (Ortas, 2002) and soil quality (Celik et al., 2004).

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In greenhouse conditions, it has been extensively shown that AMF can increase plant growth and nutrient uptake. However, there is still a lack of information concerning increases in plant growth and nutrient uptake under field conditions. Thus, there is a tendency to use natural sources, such as mycorrhizal fungi, to reduce fertilizer applications, and hence to promote better plant growth in nutrient-deficient soils. Also mycorrhizal inoculation reduces the quantity of P fertilizer normally required (Charron et al., 2001).

Therefore, it is very important to manage indigenous mycorrhizae when soil nutrients, especially P, are limited under field conditions. Since chemical and biological factors in the soil strongly affect nutrient management, mycorrhizal inoculation is important for sustainable agriculture. For sustainable nutrient and water management, soil and crop management can help to get maximum benefit from indigenous mycorrhizae using selected mycorrhizal spore (Ortaş 2003) or producing mycorrhiza-inoculated seedlings (Ortas et al., 2011). Also under field conditions, rhizosphere management is possible using crop rotation, compost and other organic fertilizers, and using beneficial soil organisms. Bowen and Rovira (1999) suggested that a well-managed rhizosphere would increase soil and plant quality. Rhizosphere management can increase the number of useful microorganisms in the plant−soil system. It is also very important to manage the indigenous mycorrhizae when the soil nutrients, especially phosphorus, are limited under field conditions.

For several reasons, especially for future water deficiency, global climate change, and soil and water pollution, management of indigenous mycorrhizae and selected microbial inoculums, such as with mycorrhizal application, is very important for food chain security, food safety, and agricultural sustainability. In the near future, the effect of mycorrhizae on plant physiology is going to be a key mechanism for healthy food for all living organisms. Using these agricultural adaptation mechanisms is also environmentally sound for the sustainability of agriculture and reducing fertilizer usage. The long-term benefits of using mycorrhizal inoculation in the field as an alternative strategy for plant nutrition, include reducing agrochemical applications.

6.2 WHY MYCORRHIZAL INOCULATION IS IMPORT FOR HORTICULTURE PLANTS IN THE MEDITERRANEAN COAST

Horticultural cultivation is widespread in countries along the Mediterranean coast. Horticultural products are very important in the agricultural sector and have a significant position in the economy of Mediterranean coast countries. Since the soils in the Eastern Mediterranean region are less fertile and the climate is semiarid, plants have a high response to mycorrhizal inoculation. Soils in the region have high levels of clay and lime, causing P, N, Zn, Fe, and Mn deficiencies (Ortaş, 2003). Since horticultural plants are cultivated through seedling production, it is important to have mycorrhiza-inoculated seedling production and transplanting to the field conditions. As most commercially important crops are horticultural plants which are raised under

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nursery conditions before being transplanted to the main field (Ortas et al., 2011), the inoculation of soil in the nursery would not only result in a saving of the cost of production of the inoculums but would also help in the better establishment of the transplanted horticultural seedling.

6.3 MYCORRHIZAL WORK IN THE MEDITERRANEAN

Horticulture has an ancient tradition in the Mediterranean region, and the origins of many important fruit and vegetable crops, including pear, quince, plum, sweet and sour cherry, hazelnut, pistachio, almond, walnut, chestnut, olive, fig, pomegranate, rose, grape, lettuce, carrot, melon, and leek are in this region. The region is rich in cash horticultural crops, such as citrus plants and vegetables. Under field conditions, the mycorrhizal dependency of several plants and the effect of indigenous mycorrhizae on plant growth and root infection have been tested with and without methyl bromide application for several years (Ortas, 2010).

During the years 1995–2010 several experiments were carried out at field conditions with mycorrhiza spores effectively infecting several plant roots (Ortas and Varma, 2007). Their early results showed that mycorrhiza-infected seedlings are highly resistant to environmental stress factors. Under field conditions, the effect of mycorrhizal inoculation on mortality of seedling was tested (Ortas and Varma 2007). So far, the results have also shown that soil indigenous mycorrhiza successfully infected plant roots, resulting in better plant growth. The effect of mycorrhizal inoculation on plant growth is related to the effectiveness of inoculums and time (Ortas et al., 2011).

6.4 MYCORRHIZA-INOCULATED SEEDLING PRODUCTION

Since AM fungi cannot be grown on laboratory media, the production of a large quantity of inocula is difficult, as is the inoculation of soil under field conditions (Ortas, 1996). It is very important to produce mycorrhiza-inoculated seedling in vegetables before transplanting to the field (Ortaş et al., 2004, 2011). Most horticultural crops are grown under controlled nursery conditions before being transplanted to the greenhouse or open field.

After using mycorrhizal fungi under field condition for a long time, it became clear that it is better to produce mycorrhiza-inoculated horticultural seedlings rather than using mycorrhizal fungi.

Horticulture plants which are grown as seedlings give a high response to mycorrhizae. It is better to produce mycorrhiza-inoculated seedlings before transplanting to field conditions. Also, it is more economical to produce mycorrhiza-inoculated seedlings than producing and using a large quantity of mycorrhizal inocula. For farmers and producers buying inoculated seedlings is safe and guaranteed. They will also lead to a reduction in cost of inocula for farmers.

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Very recently producers and farmers, instead of directly using seed, have tried to produce seedlings for safe plant production. Therefore, seedling production is a large industry and market. However, there are still several problems during production and also during transplantation stages. Also, very recently several biotechnology laboratories have been directly produce in vitro seedlings. So far greenhouse and in vitro produced seedlings have not been successfully transplanted to the field. There remain several problems to find a proper growth medium for each plant species. Also, since plants are grown in controlled conditions, after transplanting to the field there is usually a high mortality rate. The major difficulty is the growth medium and host plant mycorrhizal species interaction. The experiment was done in two stages; in the first stage, seeds are cleaned and sown in nests (Fig. 6.1). After 4 weeks growth, seedlings are transplanted into bigger pots. As can be seen in Fig. 6.2, several mycorrhizal species were used for pepper and eggplant seedlings produced with different growth mediums.

FIGURE 6.1 Seedling production system. Inocula were incorporated into the growth medium.

FIGURE 6.2 Effect of several mycorrhizal species on pepper and eggplant seedling production.

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6.5 GROWTH MEDIUM

Growth medium is very important for better seedling production. For seed germination, good aeration and root development growth medium are need. Seed germination is very delicate and the chemical, biological, and physical properties of growth medium directly affect seedling quality. High nutrient content, salt content, high pH, bicarbonate ions, fresh organic matter, or compost directly affect seedling root development. Most of the time, as seedling roots do not grow, consequently the plant cannot grow under field condition.

Several pot experiments were conducted to develop an easy, reliable, and applicable seedling production system.

Most of the time the seed dormancy level is not high. At the same time the quality of mycorrhizal inocula in the market is not guaranteed against high infection levels. Two separate works producing seedlings and using mycorrhizal inocula during transplanting seedlings can be expensive and time-consuming. Consequently, it is more economical to have inoculated seedlings before transplanting to the main field.

The goal of our work was to screen the mycorrhizal species for better horticultural seedling production, te enable better establishment and reduce the costs and plant mortality. This is a general problem for horticultural seedling transplantation to field conditions. Most of the time seedlings did not survive longer, however mycorrhizae can help seedlings to survive (Ortaş et al., 2004)

6.6 MASS SEEDLING PRODUCTION

Usually, the horticultural seedling production market uses a nested system: for each seedling producing one nest. This is easy and practical. However, it is expensive and often there are shortages of nesting trailers. Producers and farmers living in the countryside can use mass seedling production, as can be seen in Fig. 6.3.

We used large containers made from wood. Several growth media were used in these experiments and every year we the produced seedlings were of better quality. Also, several mycorrhizal inoculum techniques were tested for better seed and mycorrhizal spore application. Inocula were incorporated with the growth medium (Fig. 6.1). A mix of growth medium and mycorrhiza medium (root, hyphen, soil, sand) gave a better response. Seedling roots are easily attached to mycorrhizal spores in any direction. Water was added daily to maintain moisture to near field levels. The seedlings were grown in a greenhouse or in an open area (Fig. 6.4).

Pepper, tomato, and tomato seedlings were inoculated with mycorrhizae for three successive years. Root colonization was determined before the seedlings were transplanted to the field. It is important to keep seedling roots

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FIGURE 6.3 Effect of several mycorrhizal species on tomato, pepper, and eggplant seedling mass production.

moist during the transplantation stages. If seedlings are to be kept dry, it may be difficult for them to become established.

Seeding root colonization percentages were determined before seedlings were transplanted to the field. Seedling roots were highly infected by mycorrhizal species. *G. etinicatinium*-inoculated seedlings were infected at a higher level than *G. mossea*-inoculated seedlings. It is important to know the seedling root colonization before transplanting to the field. If seedlings are not well infected they may need to be reinoculated. In several experiments under field conditions, reinoculation during transplantation was necessary, and

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FIGURE 6.4 Transplanting of seedlings to field. Under field conditions with mycorrhizal and nonmycorrhizal plant growth.

TABLE 6.1 Effect of Mycorrhizal Inoculation on Seedling Root Colonization (%)

Treatments	**Root colonization (%)**		
	Control	***G. mossea***	***G. etinicatinium***
Tomato	4 ± 2	58 ± 6	76 ± 8
Eggplant	8 ± 2	65 ± 5	79 ± 9
Pepper	5 ± 3	72 ± 9	90 ± 9

gave better results. After several experiments were done over several years it was concluded that root colonization depends on the host plant, mycorrhizal species, and most importantly the number of spores (Table 6.1).

6.7 SEEDLING INOCULATION TECHNIQUES UNDER FIELD CONDITIONS

Since most commercially important crops are horticultural plants and are raised under nursery conditions before being transplanted to the main field, the inoculation of soil in the nursery would not only result in a saving in the cost of production of the inocula but would also help in better establishment of the transplanted horticultural seedling. Horticulture plants that are grown as seedlings give a high response to mycorrhizae. It is sound to produce mycorrhiza-inoculated seedlings before transplanting to field conditions. Inocula strategies are very important; Ortaş et al. (2004) tested several techniques to develop suitable inocula strategies.

Sometimes, if mycorrhiza-inoculated seedlings are not well infected with mycorrhizae spores, may need to be reinoculated during transplanting to the

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soil. Usually reinoculation seems to give a better response, however it is important to produce well-inoculated seedlings. If seedlings are not infected with mycorrhiza there is usually high mortality. Also, it is very important to know the mycorrhizal inoculation potential before using mycorrhizal inocula.

Our early results showed that mycorrhiza-infected seedlings are highly resistant to environmental stress factors. Under greenhouse and field experiments it has been observed that nonmycorrhizal horticultural seedlings had a high mortality but that mycorrhizal seedlings had lower mortality. Inocula strategies are very important for horticultural growth.

Very recently, biotechnological techniques were applied before transplanting mycorrhiza-inoculated seedlings to the field conditions. Several techniques were developed to transplant seedlings to the field, dependent on the production system, expectations, and land size.

Horticultural production under field and greenhouse conditions starts with seedling production and later transplanting to field conditions. Seedling production is very common in this region. Several field experiments were done with several mycorrhizal species to determine the possibility of inoculation techniques under field conditions. Also, a large number of studies have expanded our understanding of the potential contribution of mycorrhizae to nutrient uptake under field conditions.

One field experiment was conducted to see the role of mycorrhizal inoculation on seedling mortality ratio. Tomato, pepper, eggplant, bell pepper, marrow, cucumber, melon, and watermelon seedlings were sown to the field conditions. It has been found that mycorrhiza-inoculated seedlings can successfully survive, but noninoculated plants sometimes died. In general, cucumber, melon, and watermelon seedlings are more sensitive than other plants species (Ortas and Varma, 2007).

Fig. 6.5 shows the effect of different mycorrhizae inoculations on tomato, pepper, and eggplant yields under +/− P addition researched under field conditions.

6.8 FIELD INOCULATION

Field responses to mycorrhizal inoculation were often disappointing, especially in high-input agricultural systems. Inocula potential can be adversely affected by management practices such as fertilizer application, pesticide use, crop rotation, fallowing, and tillage and topsoil removal. Soil moisture content and irrigation are also important factors for successful seedling transplantation, as are soil biological properties.

Competition occurs between microorganisms, such as native mycorrhizae spores, soilborne fungi, and nematodes. Mycorrhizal inoculation significantly applied to the field conditions and inoculation increased plant yield compared with noninoculated plants. Mycorrhizal inoculation also increased the phosphorus, zinc, and copper uptake of plants.

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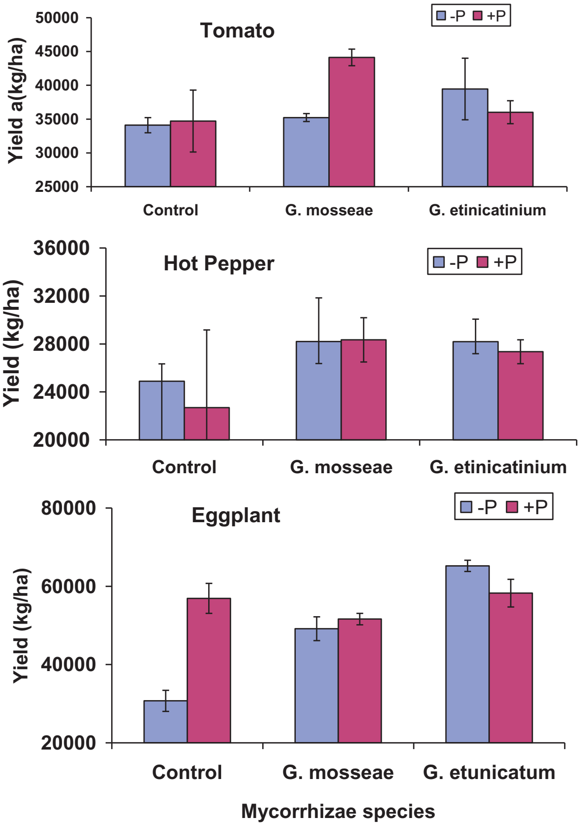

FIGURE 6.5 Effect of different mycorrhizae inoculation on tomato, pepper and eggplant yield under +/− P addition were searched under field condition.

It was found that mycorrhiza-inoculated tomato, pepper, and eggplant had significantly increased yield compared to noninoculated plants. The effect of mycorrhizal inoculation on plant yield was higher in P0 than yield increased with P100 application. When no P was applied, the contribution of mycorrhize on yield was high in *G. etinicatinum*, however P100 application with *G. mossea* was high. This shows that mycorrhizal inoculation is necessary at field conditions to obtain healthy and well-grown horticultural plants.

Pepper is a vegetable with shallow roots that is in need of abundant nutrient elements. The weak absorption ability of roots does not allow for the absorption of nutrient elements in the deep parts of the soil (Simsek et al., 1998).

Lee and George (2005) showed that *G. mosseae*-inoculated cucumber (*Cucumis sativus* L.) plants had increased P, Zn, and Cu concentrations, and mycorrhizal hyphae transported those nutrients to the plants. Wang et al. (2008) indicated that mycorrhiza-inoculated cucumber plants have high N, P,

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Cu, and Zn. Previously it was reported that mycorrhizal treatments gave greater growth and Zn concentration than nonmycorrhizal treatments with no supplemental Zn (Ortas et al., 2001).

The mycorrhizae-inoculated seedlings would not only result in the saving the cost of production of inocula but also help in better establishment of the transplanted horticultural seedlings, especially under semiarid Mediterranean soil conditions. However the most suitable growth medium for mycorrhizal inoculation is not clear; the suitable medium for host plants and seedlings should be determined.

AM fungi can also benefit plants by stimulating the production of growth-regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses, and increasing resistance to pests and soilborne diseases (Al-Karaki, 2006).

Plenchette et al. (2005) reported that the stimulation in plant growth shown in many studies dealing with mycorrhiza is mainly attributed to improved phosphorus nutrition. Estaun et al. (2002) showed beneficial effects of these fungi in agriculture, horticulture, and land reclamation.

In the same area, one year later, the effect of mycorrhizal species and P application was tested for cucumber plants. Mycorrhizal inoculation increased cucumber yield under field conditions. Compared to control plants, *G. mossea*-inoculated plants increased yields by 17% and *G. etinicatinum*-inoculated plants by 13% (Fig. 6.6). There were no significant differences between phosphorus applications in terms of yield. Cucumber is a mycorrhizal-dependent plant. In this experiment there were no differences in P application.

Another experiment, again under field conditions, tested the role of mycorrhizal inoculation with three different P levels, with and without mycorrhizal inoculation. It is clear that for tomato and eggplant mycorrhizal inoculation increased the yield and plant nutrients. The results show that colonization by AM fungus resulted in higher yield compared to NM

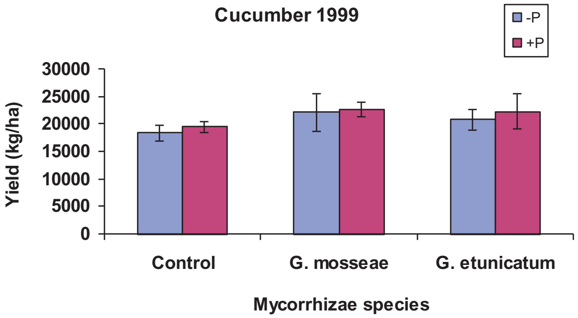

FIGURE 6.6 Effect of several mycorrhizal species on cucumber yield under field conditions.

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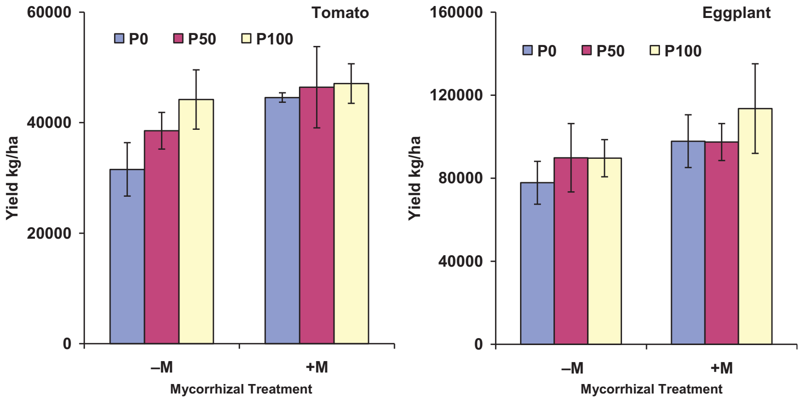

Mean (three replicates) -M no AMF inoculum +M AMF inoculum used

FIGURE 6.7 Effect of mycorrhizae on tomato and eggplant yield with several P level under field condition.

treatments, suggesting that the AM fungus significantly contributed to tomato and eggplant growth under field condition (Fig. 6.7).

As can be seen from Fig. 6.7, without mycorrhizal inoculation but increasing phosphorus application, tomato and eggplant yield were increased. However, with mycorrhizal inoculation the increase with P addition was smaller. The results showed that at higher rates of P application, any differential effect of mycorrhizae is masked. Also, it seems that mycorrhizal inoculation reduces the quantity of P fertilizer normally required for noninoculated plant conditions, which has been reported several times (Ortaş et al., 1996, 2003, 2004; Charron et al., 2001). Sylvia and Chellemi (2001) conclude that reduced P application may allow tomato plants to take advantage of their inherent responsiveness to mycorrhizae in a low to moderate soil-P environment. When available soil P concentration is high, the fresh weight of tomato is significantly reduced (Waterer and Coltman, 1988).

In 1998, cucumber plants with several mycorrhizal species, including indigenous and cocktail mycorrhizal applications, were used under field conditions. Fruits were harvested 12 times, and at the end of harvest the total fruit yield was counted. As can be seen from Fig. 6.8, selected mycorrhizal inoculation and cocktail compared to the control plot gave an increased cucumber yield. *G. etinicatinum* and *G. caledonium* produced more fruits than other mycorrhizal application. In general, inoculation produces a greater yield than control plot treatments.

6.9 POTATO–COMPOST–MYCORRHIZAE INTERACTION

Potato is one of the most consumed vegetables all over the world. The plant has a core root system and is highly mycorrhizal inoculated. The effect of

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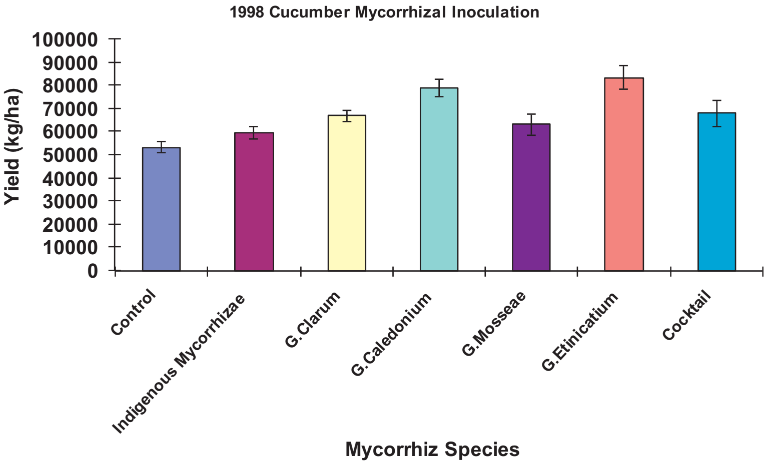

FIGURE 6.8 Effect of several mycorrhizal species on cucumber yield under field condition.

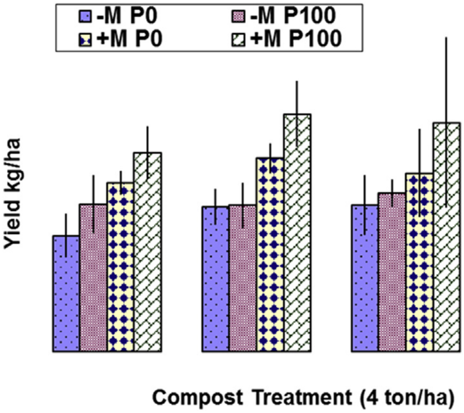

FIGURE 6.9 Effect of mycorrhizal inoculation and compost application on potato yield under field condition.

mycorrhizal inoculation and compost application on potato yield was researched under field conditions. Two different composts were compared to the control treatment. Compost one (K1) was made from wheat and maize straw, while compost two (K2) was made from maize straw and animal manure. It was found that mycorrhizal inoculation significantly increased potato yield in all treatments (Fig. 6.9).

There were small differences between compost applications, however the mycorrhizal inoculation effect was higher than that for the compost

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application. Compost and mycorrhiza inoculation technology need to be worked on together for further potato growth.

The only problem with the potato plant is that potato seeds are not uniform. A total of 70,000 potato seeds were sown in the field. Consequently, large seeds are early growing and the results are expected to be different. Also, there is a need for potato seed producers to produce uniform seeds, or plant biotechnology or plant breeding researchers could work on uniform potato seedling production.

Watermelon is one of the major horticultural plants largely cultivated in the plain of Çukurova. Under field conditions in the Çukurova region of the Mediterranean, watermelon was grown with five different mycorrhizal species (including *G. mossea* and *G. etinicatinium*) and treated with 0 and 100 kg P_2O_5/ha phosphorus application. Water melons were harvested and weighed several times in the season. The results showed that mycorrhizal inoculation nearly doubled yield increases compared to control treatments (Fig. 6.10).

In another of our previous works, the effect of mycorrhizal species (*Glomus mossea, G. etinicatinium, G. caledonium*, cocktail, and control) was treated with 0, 50, and 100 kg P_2O_5/ha phosphorus application on watermelon yield (Sari et al., 2006). The results showed that *G. etinicatinum*-inoculated land increased watermelon yield by 24% in the first location and 26% in the second location (Sari et al., 2006). Watermelon plants yield increased as a result of mycorrhizal inoculation. At low-level P applications, mycorrhizal treatment resulted in a greater yield than with nonmycorrhizal treatment. Although there were no statistical differences between treatments, compared to control treatment mycorrhiza-inoculated seedlings had nearly double the watermelon production. In P0 treatment, the control plot produced 2.39 kg/m^2 watermelon, however *G. etinicatinum*-inoculated seedlings produced 5.19 kg/m^2 watermelon (Table 6.2).

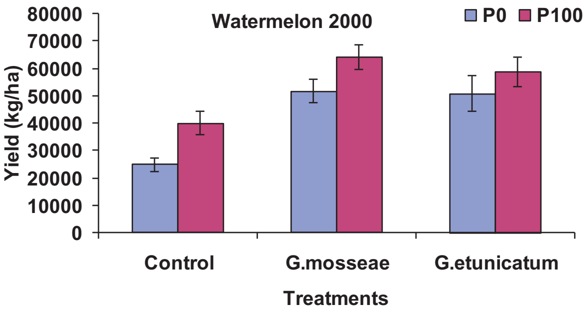

FIGURE 6.10 Effect of several mycorrhizal species on watermelon yield under field condition.

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TABLE 6.2 Effect of AM on Total Yield of Watermelon (kg/m^2) was Done Under Field Conditions (Sari et al., 2006)

Mycorrhizae	Phosphorus Doses			Mean of Mycorrhizae
	P0	P50	P100	
G. mosseae	3.99	5.86	6.49	5.45
G. etunicatunum	5.19	5.66	5.18	5.35
G. caledonium	5.19	4.53	5.10	4.94
Cocktail	4.11	6.41	3.88	4.80
Control	2.39	6.31	4.02	4.24
Mean of P	4.18	5.75	4.94	—

D 5%(myco.): ns D 5% (P): ns- not significant.

6.10 MYCORRHIZAL INOCULATION UNDER SOILLESS CONDITIONS

Recently there has been growing demand from growers to produce vegetables under soilless greenhouse conditions, with the use of several sources as a fertilizer and growth medium. Usually heavy fertilizer is used. As a result of heavy fertilizer use there is a food quality problem, and excess fertilizer also causes greenhouse disease problems. Some previous studies in soil and open soilless systems (Cigsar et al., 2000) have shown that the AM fungi colonization is essentially accompanied with plant growth increases. Uses of AM fungi on soilless-grown melon (Rehber, 2004) plants in soilless open substrate systems have been previously reported. The results suggested that mycorrhiza could promote plant growth and increase fruit yield.

Mycorrhizal inoculation reduces the quantity of fertilizer application compared with that required for noninoculated plant conditions (Charron et al., 2001).

Ikiz et al. (2008) reported that under greenhouse conditions, Effects of two different mycorrhiza species (*Glomus caledonium* and *Glomus clarum*), three different inoculation treatments (sowing, transplanting, and sowing + transplanting) which were investigated on plant growth of soilless grown pepper. They found that the plants inoculated in Experiment 1 with AM fungi at sowing, transplanting, and sowing + transplanting had increases of 24, 17, and 36% for plant dry weight, in comparison to control plants in spring. The increases in Experiment 2 were 28, 26, and 41%, respectively (Ikiz et al., 2008).

The result indicated that double inoculation was differentiated from the others to a considerable degree, and the total dry weight of *G. clarum* increased more than the others. At the fourth measurement date the best application was found to be the double inoculation again and *G. clarum* was found to be better than the others (Table 6.3).

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TABLE 6.3 Plant Dry Weight (root + shoot) in Experiments 1 and 2 (g plant^{-1}) Under Greenhouse Conditions. Data 84 Days After Sowing (Ikiz et al., 2008)

Treatments	Experiment 1			Experiment 2 (g plant^{-1})		
	G. cale	*G. clar.*	Mean	*G. cale.*	*G. clar.*	Mean
Sowing (S)	93.44	113.57	103.51	35.33	47.3	41.32
Transplanting (T)	86.9	104.87	95.89	37.34	43.43	40.39
S + T	123.56	121.85	122.71	46.6	54.44	50.52
Control	79.11	79.11	79.11	29.92	26.92	29.92
Mean	95.76	104.85	100.31	36.55	43.02	39.78

Among the treatments, double inoculation, sowing + transplanting, was the most effective method for promoting plant growth. Previously. Al-Raddad (1987) also grew pepper plants inoculated with *G. fasciculatum*, *G. monosporum*, and *G. mossea* under greenhouse conditions and found that the dry weight of plants increased significantly. Dasgan et al. (2008) carried out a greenhouse experiment hydroponically, under open and recycling close prelate substrate system to see the effect of mycorrhizal inoculation with *Glomus fasciculatum* on tomato growth, yield, fruit properties, nutrient uptake, and substrate ion accumulation. Moreover, in recycling soilless systems, mycorrhizal response was also investigated by Dasgan et al. (2008). They found that mycorrhizal colonization in open or closed systems significantly affected the tomato yield. Higher fruit production was found for the mycorrhizal versus the nonmycorrhizal plants in both closed and open systems.

6.11 METHODOLOGY OF SOIL STERILIZATION OR FUMIGATION

In order to prepare a safe seedbed and healthy yield, farmers use high amounts of chemical. Therefore, it is sound practice to use partial soil sterilization for the control of nematodes and soilborne diseases. In the coastal plains of the Mediterranean region there are serious plant parasitic nematodes, soilborne plant pathogens, and some weed pests. Farmers are seriously challenged with parasitism, however pesticides are not always able to control these destructive pests. It is possible to find alternative ways. Soil solarization and using organic sources, such as compost application, are very important for alternative methods of controlling damaging nematodes, soilborne fungi, and bacterial diseases.

Methyl bromide (60 g/m^2) was applied under a polyethylene sheet, which was laid on the surface of the soil. One week after the application the plastic sheet was removed from the surface. Following this, the soil was aerated for a 5-day period before sowing.

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FIGURE 6.11 The way of soil sterilization by using methyl bromide and mycorrhiza application to the filed.

Mycorrhizal inoculation is done under field conditions using fumigation or solarization. After soil sterilization, sterilized land has less indigenous plant species and nonsterilized areas have more and diverse plant species (Fig. 6.11).

6.12 SOIL SOLARIZATION

Pesticides and other methods do not always control these destructive pests and soilborne diseases. Very recently alternative methods have been suggested, such as soil solarization. Soil solarization and using organic sources together, such as compost application, are very important for alternative methods of controlling damaging nematodes, soilborne fungi, and bacterial diseases. Recently soil solarization has been concentrated on, using mycorrhizal inoculation for better plant nutrition and healthy plant growth. Because of the undesirable effects of MBr on other soil organisms, new alternatives have been searched for. Soil solarization if especially useful for the Mediterranean coastal area, as there are over 250–270 sunshine days. Solarization is important as an alternative solution to agrochemical application.

The methodology is simple. After soil was plowed and irrigated, the soil surface is covered with a polyethylene sheet. Over 3 and 4 weeks the time soil temperature in the surface reaches up to 50–55°C. Under this condition, soil organisms are partially eliminated. After the sheet is removed from the soil surface, the soil is ready for seed and seedling sowing.

6.13 EFFECT OF PARTIAL SOIL STERILIZATION AND MYCORRHIZAL INOCULATION ON PLANT GROWTH UNDER FIELD CONDITIONS

Until recently, due to a combination of soilborne pathogens, nematodes, and weeds, the use of soil fumigation with products such as methyl bromide (MBr) has been essential for horticultural practice in this area and all over the world. Although MBr is now banned, since there are no strong alternative methods, it is still used extensively. Since MBr eliminates both desirable

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organisms, such as arbuscular mycorrhizal fungi (AMF), and undesirable soil organisms, plant growth and nutrient uptake, especially P and Zn, have significantly declined (Haas et al., 1987; Ellis et al., 1995; Bendavid-Val et al., 1997). Since soil-borne pathogens significantly reducing the yield, soils were partially sterilized by using fumigation with MBr.

Since Mediterranean soils are less fertile in term of nutrients, soil sterilization is a serious problem, but at the same time, because of soilborne pathogens, partial soil sterilization is necessary for some areas. It is especially vital for mycorrhizal inoculation.

In order to see the effect of soil sterilization and phosphorus application on mycorrhizal development and yield, several field experiments were set up over 3 years. Ortaş et al. (2003) used MBr as a sterilization material under field conditions. They found that mycorrhizal inoculation increased plant yield compared with noninoculated plants.

Under experimental field conditions, mycorrhizal inoculation increased tomato and pepper plant fruit yields, especially with low levels of P. During vegetative growth, mycorrhizae-inoculated eggplants were larger and developed earlier than the nonmycorrhizal plants; this was not reflected in a yield increase. The yield of mycorrhizal plants grown with no P was higher than that of plants to which P was applied. When no P was applied, mycorrhizal inoculation increased tomato yields by up to 52%, eggplant by up to 28%, and pepper by up to 36%, but with P addition, mycorrhizal inoculation increased yields by 28%, 14%, and 21% for these crop species, respectively (Fig. 6.12).

Controversially, methyl bromide application reduced tomato yield compared to nonfumigated plants, whether mycorrhiza-inoculated or not. Moreover, it has been concluded that soil sterilization (not necessarily to use methyl bromide) in same cases is needed for healthy horticultural plants as well.

However, growth responses are erratic and sometimes occur when AMF are added to nonsterile soil (Ortaş et al., 1996). In some cases, root colonization is less in nonsterile soil than in sterilized soil (Hetrick et al., 1986; Ortaş, 2003).

The overall results revealed that yields were lower in fumigated plots than in nonfumigated plots, when not inoculateds. Conversely, MBr application reduced yield compared with nonfumigated, whether or not the plants were inoculated. Mycorhizal inoculation may have had some other benefits to the plant such as protecting.

Al-Raddad (1987) also grew eggplant, tomato, and pepper inoculated plants with *G. fasciculatum, G. monosporum*, and *G. mossea* under greenhouse conditions and found that the dry weight of eggplant increased significantly. It seems that plant yield associated with mycorrhizal inoculation cannot be explained only by the effect of mycorrhizal inoculation on nutrient uptake.

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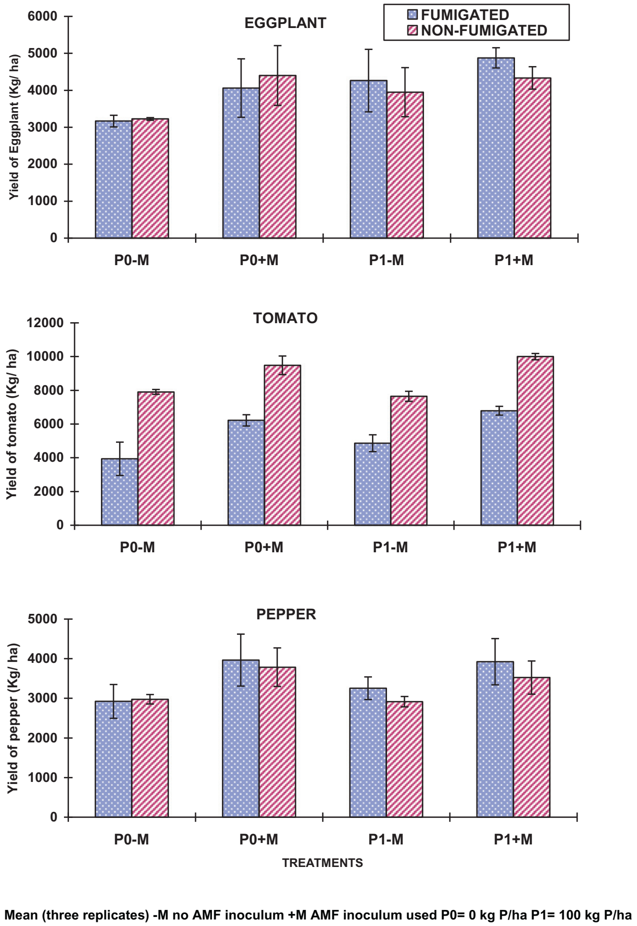

FIGURE 6.12 Effect of MBr and P application on tomato, pepper and eggplant yield under field condition.

In the present study, since soil P level was average and plant P concentration was not affected by mycorrhizal inoculation, soil P level must have been sufficient for both mycorrhizal and nonmycorrhizal plants.

The potential value of mycorrhizae in natural ecosystems and their importance is diminished under high rates of fertilizer application. Also, it has been concluded that soil indigenous mycorrhiza function efficiently. In order to manage the indigenous mycorrhiza spores, soil and crop management are important. Since MBr kills nearly all useful organisms, it is necessary to find safe and less harmful partial sterilization methods.

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FIGURE 6.13 Effect of indigenous and selected mycorrhizae species on soure orange seedling growth.

6.14 CITRUS PLANT DEPENDENT ON MYCORRHIZAL INOCULATION

Citrus cultivation is expected to expand along the Mediterranean coast. Since citrus plants depend on mycorrhizae infection, arbuscular mycorrhizal (AM) fungi may improve plant growth and nutrient uptake (Graham and Syvertsen, 1985). Several pot experiments were done in greenhouses. It has been discovered that *G. clarium* is one of the most effective spores for further inoculation of sour orange citrus (Fig. 6.13) (Ortaş et al., 2002, 2003).

Since native indigenous mycorrhizal spores were eliminated due to soil sterilization, strongly mycorrhiza-dependent sour orange (*Citrus aurantium* L.) seedlings were stunned and did not respond to P and Zn supply in nonmycorrhiza-inoculated soils. The results revealed that *G. clarum* inoculation significantly increased plant P, Zn, and Cu uptake (Ortaş et al., 2002).

The selection of the most effective AM fungi for growth enhancement of citrus cultivars used as rootstocks was the first step toward development of an AM inoculation system in citrus nurseries in the east coast of the Mediterranean. Sour oranges (*Citrus aurantium* L.) are the most common rootstocks presently used in several experiments (Ortaş et al., 2004). The results obtained showed that sour orange is strongly mycorrhizal-dependent (MD). Nevertheless, with increasing P and Zn supply, mycorrhizal dependency was gradually decreased (Ortaş, 2002). The decrease in mycorrhizal dependency was more pronounced for P requirement than Zn. It is quite clear that mycorrhizal inoculation significantly reduced the amount of fertilizer use.

6.15 MYCORRHIZAE INTERACTIONS WITHIN THE RHIZOSPHERE

The presence of AM and other soil microorganisms in the rhizosphere is liable to have beneficial consequences for plants. Plants are also capable of regulating the microbial population, including mycorrhizal fungi, through the nature of root exudates which can change the rhizosphere. According to Rovira et al. (1983), the rhizosphere is “that zone of soil extending from the root–soil interface to the point in the soil where the microflora is unaffected

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by the root." It affects plant nutrition by being an area of altered nutrient availability and nutrient uptake processes. Plant physiology and root characteristics are also different for this area.

In the rhizosphere and mycorhizosphere, microorganisms involved in N or P cycling are mainly centered on N2-fixers, P-mobilizers, and AM fungi. Barea and Richardson (2015) indicated that the capacity of some microorganisms to mobilize P from poorly available sources of this nutrient can help plant P nutrition. Barea (2015) indicated that bacteria belonging to diverse genera, collectively termed "rhizobia," can fix N_2 in mutualistic symbiosis with legume plants, while others (actinomycetes), belonging to the genus Frankia, form N_2-fixing nodules on the root of the so-called "actinorrhizal" plant species. In rhizospheres of the legume group of plants there is a significant relationship between bacteria and mycorrhizae. It seems likely that, depending on the species involved, AM may have either a synergistic or antagonistic effect on other soil microbes, and vice versa. Most research has centered on the AM–*Rhizobium*–N_2-fixation interaction in legumes because they are widely cultivated. Yet little is known about the interaction of AM and other rhizosphere inhabitants (Azcon, 1989). An experiment relating rhizosphere bacteria with AM found that bacterial inoculation increased the growth of plants with AM, but this did not always happened (Azcon, 1989); some of the mycorrhizal tomato plants used showed no growth response, and in those that did, a gradation of effectiveness of bacterial introduction was seen. It would appear that the whole issue may be quite complex. Azcon-Aguilar and Barea (2015) reported that saprophytic microorganisms, such as mycorrhizae ,are recognized for their abilities to propel nitrogen (N) fixation and/or phosphorus (P) mobilization. Also, they indicated that the mycorrhizosphere of legume plants involves N_2-fixing nodulating rhizobial bacterial symbiosis. Azcon (1989) also noted that bacterial inoculation improved AM germination, sporulation, and hyphal growth, implying that the bacteria were imposing on the AM in some way. In the mycorrhizosphere of legume plants, interactions between mycorrhizae and N_2-fixing bacteria have a significant influence on nutrient mobilization and cycling.

Agronomic management also may have an effect on microorganisms' interactions and nutrient cycling. Fertilizer forms and application form, irrigation and soil tillage also have effects on rhizosphere dynamics and nutrient availability.

6.15.1 Nitrogen Fixation and Related Microfauna

Nitrogen (N) is the element to which agricultural crops are most responsive, hence it is usually the most limiting to plant growth. Deficiencies need to be alleviated by the addition of fertilizers, or else they are eliminated by appropriate cropping practices or plant nutrient supply mechanisms. The effects of a deficiency or excess of nitrogen on the plant and the resulting alterations in both morphology and response of a crop have been highlighted by Wild (1988).

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AM were associated with N_2-fixing microorganisms; it was suspected that N increases were not due to uptake from soil but essentially due to these specialist N_2-fixers. Barea et al. (1989) have recently shown that AM definitely improves symbiotic N_2 fixation, almost certainly through improving plant phosphorus uptake as well.

Raven et al. (1978) concluded that N-nutrition was affected by mycorrhizas, and Ortas (1994),Ortaş and Harris (1996), and Ortas et al. (1996) showed that nitrogen forms have a significant effect on mycorrhizae–rhizosphere dynamics.

It has already been observed that different forms of N are used by plants, and that this involves not only uptake, but also storage, and incorporation processes. NH_4^+ and NO_3^- are quite different ions in many respects for plant and soil organisms. NH_4^+ is a cation and NO_3^- an anion, so their chemical processes are facilitated by different sets of reactions, especially rhizosphere pH changes (Ortas, 1996). The N_2-fixing rhizobial bacteria and mycorrhizae fungi are the most relevant representatives of beneficial plant symbionts (Barea, 2015), which are directly related to nutrient uptake and cycling. The N_2-fixation cycle itself is a separate study however, as the uptake of gaseous N_2 would provide an extra source to plants for their N-nutrition, as well as relieving some pressure on the soil organic N pool, it needs to be reconsidered for soil and crop quality.

Hayman (1986) reported that the *Rhizobium*–AM–leguminous plant interaction exist and that some legumes grew so poorly without mycorrhizae as to be ecologically obligate to mycorrhizae. In an experiment, clover plant roots mycorrhizae densely colonized $>70\%$ of its root system.

A more efficient nutrient cycling by mycorrhiza fungi may relieve some plant stress, thereby leading to improved nodulation and N_2-fixation by *Rhizobium*. In our long-term observations some plants, such as soybeans, under sterile soil conditions without mycorrhizal inoculation plant nodulation were not well developed. Also, under field conditions with soil fumigation, nitrogen-fixing plants did not grown properly (Ortas, 2012). Ames and Bethlenfalvay (1987) reported that pre-establishment of mycorrhizae improved cowpea nodule activity and root dry weight, but showed no competitive interaction with *Rhizobium* for nodulation sites.

AM may also interact with other N_2-fixing organisms. Pacovsky et al. (1985) searched an AM–*Azospirillum*–sorghum mixture and found that in the presence of *Azospirillum*, free-living, N_2-fixing bacteria, AM colonization of sorghum roots increased.

6.15.2 Role of Mycorrhizae on Soil Organic Carbon and Soil Aggregation

The green revolution enhanced agricultural production through inputs such as chemical fertilizers, pesticides, tillage, irrigation, and improved seeds.

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Over time, however, an increase in agricultural inputs does not necessarily increase the yield. In some soils, yields are extremely low without fertilizer input. Because of supraoptimal temperatures, high concentrations of $CaCO_3$ and clay content and excessive heavy tillage, soil organic matter (SOM) decomposes rapidly and productivity of soil is low in the eastern part of the Mediterranean regions. Depletion of SOM is exacerbated on continually cropped arable land, because of burning of crop residues and excessive tillage. These practices adversely affect soil biological and physical properties and the arbuscular mycorrhizal fungal (AMF) hyphal network, the microbial biomass, and consequently soil structure. Total organic C storage in soil is a principal attribute of soil biological and physical fertility.

Soil aggregation is also an important process that mediates numerous chemical, physical, and biological properties, and improves soil quality and sustainability. The AMF are key organisms in the soil/plant system because of their positive effects on soil aggregation and stability by the combined action of extraradical hyphae and of an insoluble, hydrophobic proteinaceous

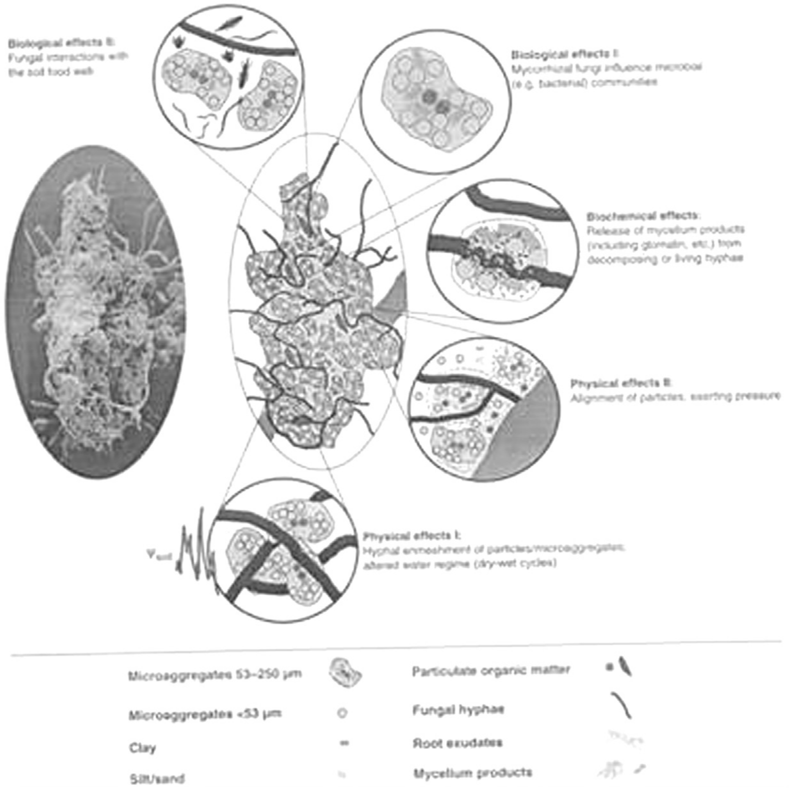

FIGURE 6.14 Effect of hyphae and roots on aggregation (after Rillig and Mummey, 2006).

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substance named glomalin-related soil protein (Bedini et al., 2009). The relationship between aggregation and soil development depends on mycorrhizae and plant roots (Fig. 6.14).

The mycorrhizae mycelia network can bind particles and improve soil structure (Tisdall et al., 1997;Caravaca et al., 2006; Rillig et al., 2010). Andrade et al. (1998) and Bedini et al. (2009) observed that secretion by AM fungi glomalin contributes to soil stability, quality, and water retention.

There is a need for an understanding of soil biological systems' effect of aggregation related to mycorrhizae under real field conditions. The root growth rate is of importance for the availability of the nutrient and water as well aggregation. The growth rate of root is greatly affected by soil physical conditions, soil strength, and water content in particular. The root extends more rapidly in wet than dry soils. Also, it is possible to increase the efficiency of growth of agricultural and horticultural crops by either inoculation of effective isolates of mycorrhiza or the management of indigenous populations of mycorrhiza by agronomic practices on soil aggregation and other soil physical properties, including fertility.

6.16 CONCLUSION

Under field conditions, several experiments were performance to understand the potential contribution of mycorrhizae on horticultural plant growth and nutrient uptake. These facts show that mycorrhizal inoculation is necessary for healthy, effective, and well-grown seedling production. Also, mycorrhizal inoculation depends on soil, plant species, inocula, and the method of inoculation and other ecological factors. The results of experiments have revealed that under field conditions selected mycorrhizal spores and also indigenous mycorrhiza successfully infected plant roots resulting in better yields. Under field conditions plants depend on mycorrhizal inoculation but also depend on P supply and their annual differences. With high P application, mycorrhizal dependence significantly reduces.

It has been concluded that for field crops, soil and plant management systems, but for horticultural plants mycorrhizal inoculation or/and mycorrhiza-inoculated seedlings are more practical and recommended to be used. It appears that there are some other benefits of mycorrhizae on horticultural plants, such as controlling disease and increasing plant resistance. We conclude that although mycorrhizal inoculation increased some vegetable yields, this increase is not easily explained through better nutrient uptake by AMF plants than by uncolonized plants. Mycorhizal inoculation may have some other benefits to plants such as protection against soilborne pathogens and environmental stress and also the role of mycorrhizal inoculation on soil quality and aggregation.

In order to manage indigenous mycorrhizae under long-term field conditions, soil management and crop rotation have a positive effect on

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mycorrhizal spore development. Because of soilborne disease, soil solarization and compost technology can also be part of the soil and crop management system by using mycorrhizal inoculation for better plant nutrition and healthy plant growth. Many horticultural plants are mycorrhizal-dependent, however, under field conditions; plants depend on mycorrhizal inoculation but also depend on P supply and climatic differences. The effects of other factors involved in plant growth, such as soil and weather conditions, nutrient status of soil, seedling growth medium, number of mycorrhizal spores, spore effectiveness, and transplanting technology need to be researched further in order to effectively integrate mycorrhiza technology into horticulture production. The soil and crop management system and using mycorrhizal inoculates in horticultural seedlings for large agricultural practices is very important for soil quality.

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