Field Crops Research 125 (2012) 35–48

Contents lists available at SciVerse ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions

Ibrahim Ortas*

Department of Soil Science and Plant Nutrition, University of Çukurova, Faculty of Agriculture, Balcali, 01330 Adana, Turkey

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 11 August 2011
Accepted 12 August 2011

Keywords:
Plant species
Mycorrhizal colonization
Inoculation effectiveness
Fumigation
Phosphorus
Zinc uptake

ABSTRACT

The potential effect of indigenous and selected mycorrhizal fungal inoculation and phosphorus (P) treatment on plant growth, yield, root infection and inoculation effectiveness (IE) were tested with and without methyl bromide (MBr) for three successive years under field conditions. In 1997–1999, twelve plant species were used as host plants in a Menzilat soil series (Typic Xerofluvents) in the Mediterranean coastal region of Turkey. Compared to non-inoculated control plants, mycorrhizal inoculation increased yield in some years, but not in others. The mycorrhizal inoculum increased the root colonization of garlic, horsebean, soybean, chickpea, melon, watermelon, cucumber, maize, cotton, pepper, eggplant and tomato plants compared with the non-inoculated treatments. Compared to fumigation, plant roots grown in non-fumigated soil and successfully infected by indigenous mycorrhiza, resulted with better plant growth. Plant species belonging to the Solanaceae, Leguminosae, and Cucurbitaceae showed high responses to the mycorrhizal inoculation effectiveness under both fumigated and non-fumigated soil conditions. In general, IE was higher under low P supply than under high P supply. The effects of mycorrhizal inoculation on plant P and Zn concentrations were determined: mycorrhiza-inoculated plants had a higher nutrient content than non-inoculated plants, and this was most pronounced under fumigated soil conditions. After 3 years of field experiments, it has been concluded that for (seeded) field crops, soil and plant management systems make a great contribution to indigenous mycorrhiza to improve plant development. Whereas for horticultural plants, on the other hand, (plants transplanted into the field as seedlings), mycorrhizal inoculation makes it easy to use for large agricultural areas compared with the non-inoculated plants. It can be suggested to the farmers that arbuscular mycorrhizal fungus inoculated seedlings can be used under field conditions for high yield and quality.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with most economically important cash crops (Ortas, 2008a,b). The symbiotic root-fungal association is postulated to increase plant growth and the uptake of relatively immobile nutrients such as zinc (Zn) and phosphorus (P). Ortas et al. (2003a) and Ortas (2010) showed that under field conditions, mycorrhizal inoculation increased plant P and Zn concentrations. AMF also provide biological protection against certain soil-borne pathogens of tomato, onion, and watermelon (Caron et al., 1986; Torres-Barragan et al., 1996; Li et al., 2000, 2004) and from salinity (Copeman et al., 1996; Al-Karaki and Hammad, 2001). Mycorrhizal inoculation usually increases the growth of tomato, pepper, and eggplant (Ortas et al., 2003a), watermelon, capsicum, cucumber (Ortas, 2010) and green beans (Yang et al., 1994; Olsen et al., 1999a,b; Liu et al., 2003; Li et al., 2004; Ortas, 2008a) especially under conditions of low P availability.

The mycorrhizal effect on plant growth is quantified by measuring the host's growth response, termed "mycorrhizal dependency" (MD) It was identified by Gerdemann (1975) as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility". Menge et al. (1978) defined MD by expressing the dry mass of a mycorrhizal plant as a percentage of the dry mass of a non-mycorrhizal plant at a given level of soil fertility. Plenchette et al. (1983) proposed a calculation and established "relative mycorrhizal dependency (RMD), which expresses the MD of the plant in a particular experimental condition". Gemma et al. (2002) developed the new terms "ecological mycorrhizal dependency" (EMD) and "agricultural mycorrhizal dependency" (AMD) to refine the concept of MD. Tawaraya et al. (2001) showed that plant shoot P concentrations and biomass increase with mycorrhizal colonization and that the MD of shoot growth ranges from 73 to 95%.

* Tel.: +90 322 3386643/102; fax: +90 322 3386643.
E-mail address: iortas@cu.edu.tr

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doi:10.1016/j.fcr.2011.08.005

Regarding these statements, very recently Janos (2007) and Smith et al. (2009) have suggested "responsiveness instead of mycorrhizal dependency. Janos (2007) has defined "mycorrhiza dependency" as the inability of the non-mycorrhizal plants to grow or survive without appropriate levels of available P.

In the case of a plant's response to mycorrhizal inoculations under field conditions, it is very difficult to determine the exact contribution of indigenous and selected mycorrhizal inoculation on plant growth. Under field conditions, despite fumigation with methyl bromide, the procedure is not effective in removing all of the indigenous mycorrhizal fungi. In many experiments, after soil fumigation and other partial sterilization methods root colonization has still been high (Hetrick et al., 1986; Wilson et al., 1989; Ortas et al., 2003a; Ortaş and Sari, 2003). Thus, the calculation of MD on the plant's response to inoculation was based on a control that was highly colonized by indigenous AMF.

The term of MD is conceptually wrong and does not convey the results and the concept of "mycorrhizal dependency – MD" which must be considered on an absolutely non-mycorrhizal control. Since plants grown in fumigated soil have a high root infection percentage, there is a need to find a "new term" instead of "mycorrhizal dependency", it may be much more suitable to use the term "inoculation effectiveness (IE)".

Inoculation effectiveness can be influenced by soil type (Gerdemann, 1971; Daft and Hacskaylo, 1977), cultivar (Khalil et al., 1994, 1999), ecotype (Kormanick et al., 1977), soil P (Mosse et al., 1973; Plenchette et al., 1981; Ortas, 2003) and mycorrhizal species (Mosse et al., 1973; Menge et al., 1978; Ortas, 2008a; Kafkas and Ortas, 2009; Ortas, 2010; Ortas and Akpinar, 2011). Response to mycorrhizal inoculation is linked with the level of soil fertility and it is well known that P is the most influential element in mycorrhizal development and efficiency. In P-deficient soils, the yields of horticultural and field crops were found to be largely dependent on their mycorrhizal status under field (Ortas, 2008a,b) and greenhouse conditions (Ortas, 2003)

Differences in responses to mycorrhizal colonization among plant species were calculated in 20 crops (Plenchette et al., 1983), 80 woody species (Zangaro et al., 2003), 5 forage species (Schweiger et al., 1995) and 4 endemic species of Hawaiian plants (Gemma et al., 2002). Differential responses to AMF colonization among cultivars have also been reported in wheat (Azcon and Ocampo, 1981), barley (Baon et al., 1993) and tomato (Bryla and Koide, 1990), improved and unimproved corn and soybean cultivars (Khalil et al., 1994, 1999), linseed (Thompson, 1996), citrus (Ortas et al., 2002), maize (Ortas, 2003; Ortas and Akpinar, 2011), taro plants (Li et al., 2000), kidney bean (Ortas and Akpinar, 2006) and pistachio (Kafkas and Ortas, 2009). Ortas (2008a) indicated that onion, garlic, chickpea, horsebean, clover and lentil are highly response to mycorrhizal inoculation under field conditions. Edathil et al. (1999) grew tomato seedlings in sterile, P-deficient soil and inoculated them with four species of AMF and they found that tomato plants depend on mycorrhizal inoculation.

Plenchette et al. (2005) indicated that although mycorrhizal symbiosis holds great potential to improve crop production, there is an urgent need to improve and widely apply analytical methods to evaluate characteristics such as relative field RMD, and soil mycorrhizal infectivity. The objectives of this study were to determine the inoculation effectiveness (IE) of several plants growing in the Mediterranean coastal region and to verify the contribution of indigenous and inoculated mycorrhizal fungi under field conditions. The study was based on the hypothesis that under field conditions plant species growth and nutrient uptake depends on mycorrhizal inoculation and that indigenous mycorrhiza also has an effect on plant growth and nutrient uptake.

Table 1
Selected physical, chemical and biological properties of Menzilat soil series with and without soil fumigation at the research site in Adana, Turkey.

Properties	Unit	Non-fumigated	Fumigated
Clay	$g\,kg^{-1}$	318.8 ± 30.6	–*
Silt		360.9 ± 87	–
Sand		320.3 ± 23.0	–
Soil organic carbon – 1997	$g\,kg^{-1}$ soil	0.88 ± 0.08	0.90 ± 0.09
Soil organic carbon – 1998		0.89 ± 0.07	0.92 ± 0.08
Soil organic carbon – 1999		0.96 ± 0.05	0.98 ± 0.09
Inorganic carbon		3.77 ± 0.35	3.87 ± 0.22
Total nitrogen		0.08 ± 0.01	0.09 ± 0.01
CEC[a]	$Cmol^{+}\,kg^{-1}$	20.50 ± 2.00	–
pH	H_2O	7.56 ± 0.66	7.45 ± 0.70
Salt	%	0.05 ± 0.00	0.05 ± 0.00
P	$mg\,kg^{-1}$	14.50 ± 1.96	19.20 ± 2.20
Fe		2.48 ± 0.80	2.46 ± 0.42
Mn		3.84 ± 0.32	13.81 ± 0.91
Zn		0.19 ± 0.02	0.24 ± 0.05
Cu		1.16 ± 0.05	1.18 ± 0.11
Number of AMF spores 1997	$10\,g^{-1}$ soil	108 ± 12	105 ± 9
Number of AMF spores 1998		98 ± 10	101 ± 15
Number of AMF spores 1999		120 ± 11	111 ± 17

Values are the averages of three samples ± standard deviation. *Not measured.
% denotes the mass in percentage, the notation.
[a] CEC, cation exchange capacity.

2. Materials and methods

2.1. Site description and soil fumigation

The experiment was carried out from 1997 to 1999 in the Menzilat soil series (Typic Xerofluvents Fluvents, Entisols) located at the Research Farm of the Çukurova University (37°00′ 54.31″ N, and 35° 21′ 21.56″ E and 31 m above mean sea level) in eastern part of the Mediterranean region of Adana–Turkey. The regional climate is typical Mediterranean with long-term average annual air temperature of 19.1 °C (ranging from 14.2 °C in January–February to 25.5 °C in July–August), and precipitation of 670.8 mm. As much as 80% of the annual precipitation is received between November and April, with a mean annual humidity of 66% (Anonymous, 2008).

Immediately before sowing, the site was ploughed and wheat residue was incorporated into the surface at 10–15 cm with a disc harrow. Half the experimental area was not fumigated and the other half was sealed under a clear polythene sheet and fumigated with methyl bromide (MBr; $60\,g\,m^{-2}$). The experimental area was ploughed just before sowing the soil and wheat residue incorporated into the surface in a 10–15 cm layer with a disc harrow and divided into experiment blocks. One half was used as such (non-fumigated) and the other half was sealed under a clear polythene sheet and subjected to methyl bromide fumigation (fumigated) (MBr; $60\,g\,m^{-2}$). After 5 days, the polythene sheet was removed and the area was left to aerate. The soil was analyzed 10 days after fumigation (5 days before sowing). Every year, from 1997 to 1999, before each culture, fumigation was repeated.

Some soil properties were analyzed by Page et al. (1982) and data are presented in Table 1. Indigenous spores were extracted from the soil samples taken in early autumn using the wet-sieving technique (Gerdemann and Nicolson, 1963). The non-fumigated soil in the year of 1997, 1998 and 1999 had a wild arbuscular mycorrhizal community in Table 1.

2.2. Experimental design

A complete randomized block design with three replications was used with each block containing two treatments (fumigated and non-fumigated soil). In each block, in the main treatments, P0 (0 kg P) and P1 (100 kg P_2O_5/ha) were applied with and without

mycorrhizal inoculation. Under field conditions in MeBr-fumigated and non-fumigated soils, the growth of 12 plant species (Cotton (*Gossypium hirsutum*) Çukurova-1518 cultivar, maize (*Zea mays* L.) Darva genotypes, chickpea (*Cicer arietinum* L.) local Inci, cultivar, soybean (*Glycine max* [L.] Cultivar Sa88), Local horsebean, (*Vicia faba* L.), local garlic cultivar (*Allium sativum* L.) "Urfa local", eggplant [aubergine] (*Solanum melongena* L.) (CV. Pala), tomato (*Lycopersicon esculentum* Mill), (CV. SC2121) pepper (*Capsicum annuum* L.), (CV. Kahramanmaras), cucumber (*Cucumis sativus*), (Yayla F1 local variety) watermelon (*Citrullus lanatus* Thunb.), Madera Fı and melon (*Cucumis melo* L.) were compared with and without P application. Each crop species was the subject of a separate experiment according to ecological growth period: some plants were seeded in the autumn, whereas others were planted as seedlings in the spring. The plots size for field crops were $2 \times 5 = 10\,m^2$ and for horticulture plants were $3 \times 5 = 15\,m^2$.

Since soil organic matter content is low, every year, from 1997 to 1999, each culture received the same amount of nitrogen. The field soil was amended with a base fertilization of 200 kg N-NH_4NO_3 ha^{-1} for both fumigated and non-fumigated plots. The nitrogen (N) was supplied in two applications, consisting of equal portions. Half of N was applied at the beginning of sowing and the remaining half was applied before the flowering stage.

2.3. Seedling production and transplantation to field conditions

In Turkey, most horticultural seedlings are transplanted into the field at the five-leaf stage. The transplanted seedlings need a period of time to recover their growth, and mycorrhiza can assist with better seedling establishment. Tomato, eggplant, pepper, cucumber, watermelon and muskmelon were used as horticultural plant material. Horticultural plant seeds were sown in a sand: soil: animal manure (7:2:1, v/v/v) growth medium. Horticultural seedlings (plantlets) were produced under greenhouse conditions and then seedlings were inoculated during transplantation.

Glomus mosseae inoculum isolated from Rothamsted (UK), consisted of 1000 spores/plant potted in mix in the form of chopped roots and mycorrhizal spores. The inoculum was placed approximately 50 mm below the seedlings. The inoculum was calculated based on the number of spores present in 10 g inoculum. In non mycorrhizal treatments, each tray was filled with the same amount of mycorrhiza fungi free substrate (autoclaved inoculum medium (growth medium, root, hyphae and spore). Water was added daily to maintain moisture to 80% of field capacity. The seedlings were grown in a greenhouse for 5–6 weeks, depending on the weather and growth conditions, then transferred to the main field plots.

For field crops cotton, maize, chickpea, soybean, horsebean, and garlic were used as plant material. Field-crop seeds were sown in the field and mycorrhizal spores (*G. mosseae*) were calculated based on the number of spores present in 10 g inoculum (mix of spores, hyphae, root and growth medium) under 1000 spores which were placed approximately 50 mm below the seeds. In non-mycorrhizal treatments, the same amount of mycorrhiza free substrate (autoclaved growth medium) was used. Every year the freshly produced inoculum's number of spores was re-counted.

2.4. Measurements

2.4.1. Determination of leaf P and Zn concentrations

Before each crop flowered upper mature plant leaves were taken for nutrient analysis. Plants leaves were taken accordingly (Jones, 1998). Plant leaves were oven-dried at 65 °C for 48 h. The dry material was ground using a Tema mill, and 0.2 g of the ground plant material was ashed at 550 °C, then dissolved in 3.3% HCl. Leaf P concentration was determined with the vanadate–molybdate yellow colorimetric method using a spectrophotometer and Zn concentration was determined by atomic absorption spectrometry (Chapman and Pratt, 1961).

2.4.2. Mycorrhizal colonization

Before plant flowering, the roots of the two plants that had been removed were carefully washed for an assessment of mycorrhizal colonization. The root clearing and staining procedure followed the method described by Koske and Gemma (1989). The percentage of AMF colonization was calculated as the number of 10-mm-long root segments identified as colonized under a stereo microscope at 20× magnification out of 100 root segments (Giovanetti and Mosse, 1980).

2.5. Inoculation effectiveness

At harvest, for field crops, dry weight and for horticulture crops, fresh weight yields were recorded. Inoculation effectiveness of plant yield on AMF was calculated for each species based on the following formula:

$$\text{Inoculation effectiveness (IE)} \frac{\text{yield}\,(+\text{M}) - \text{yield}\,(-\text{M})}{\text{yield}\,(+\text{M})} \times 100$$

+M, inoculated plants; −M, non-inoculated plants.

2.6. Statistical analysis

All data were statistically analyzed using the analysis of variance (ANOVA) procedure in SAS (2009) program to assess the effects of block, fumigation, fertilizer treatments and mycorrhizal treatment. Last significance differences (LSD) at $P = 0.05$ were tested to determine the significant differences between treatment means.

3. Results

3.1. Total yield

In the experiments, field crops and horticultural plants were screened for three successive years for IE. Twelve plant species were divided into five groups according to family and response to mycorrhiza.

3.1.1. Pepper–tomato–eggplant

Fruits were harvested weekly and total yield was recorded (Table 2). Mycorrhizal inoculation increased fruit yield of the three plants significantly compared to their non-inoculated counterparts. The effect of mycorrhizal inoculation on the yield of plants in control plots (P0) was higher than that on the yield of plants grown with additional P application (P1). The results showed that in 1997 and 1999 mycorrhizal inoculation significantly ($P < 0.05$, 0.04 respectively) increased pepper plant yield under non-fumigated soil conditions. Mycorrhizal inoculation in fumigated and non-fumigated soil conditions significantly ($P < 0.01$) increased tomato yield. However mycorrhizal inoculation has a weak effect on eggplant yield increase.

In 1997, all three plants showed high IE, especially green pepper and eggplant. IE was higher in the P0 vs. P1 treatment. Of these solanaceae plants, pepper exhibited the highest IE in all 3 years. Plants inoculated with mycorrhiza and grown in fumigated plots showed higher IE than their counterparts grown in non-fumigated plots.

In 1998, overall IE was lower than that in 1997 and 1999. The reasons for this lack of response to mycorrhizal inoculation, and even a negative response (Table 2), in that year are not clear, but it might have been due to the efficiency of the mycorrhizal spores.

Mycorrhizal inoculation increased P and Zn concentrations in pepper, tomato and eggplant (Table 3). In general, P addition

Table 2
Effect of mycorrhizal inoculation and P addition on green pepper, tomato and eggplant yield and inoculation effectiveness under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	Yield (kg/1000 m^2)			Inoculation effectiveness (%)		
	1997[a]	1998	1999	1997	1998	1999
Pepper						
+Fumigated						
P0−M	2924 ± 483 c[!!]	1022 ± 308 a–c	1004 ± 258 d			
P0+M	3965 ± 658 a	1045 ± 9 a–c	2148 ± 263 ab	26.3	2.2	53.2
P1−M	3255 ± 28 a–c	1141 ± 349 ab	2246 ± 628 ab			
P1+M	3923 ± 583 ab	1239 ± 70 a	2448 ± 125 a	17.0	7.9	8.2
−Fumigated						
P0−M	2978 ± 120 b–c	691 ± 15 c	1689 ± 144 b–d			
P0+M	3785 ± 488 a–c	762 ± 48 b-c	2116 ± 237 a–c	21.3	9.2	20.2
P1−M	2918 ± 131 c	715 ± 112 c	1399 ± 58 c–d			
P1+M	3525 ± 417 a–c	716 ± 94 c	2046 ± 441 a–c	17.2	0.2	31.7
Tomato						
+Fumigated						
P0−M	4635 ± 987 e	2861 ± 301 ab	4271 ± 223 ab			
P0+M	6219 ± 339 d	3216 ± 765 ab	5198 ± 1850 a	25.5	11.1	17.8
P1−M	4860 ± 50 e	2514 ± 129 b	4150 ± 1738 ab			
P1+M	6792 ± 265 c-d	3165 ± 100 ab	5476 ± 1508 a	28.4	20.6	24.2
−Fumigated						
P0−M	7906 ± 15 b	2831 ± 165 b	2161 ± 161 b			
P0+M	9485 ± 551 a	2851 ± 228 ab	2226 ± 412 b	16.6	0.7	2.9
P1−M	7646 ± 147 b–c	3820 ± 479 a	2206 ± 1089 b			
P1+M	10018 ± 147 a	3336 ± 644 ab	3557 ± 21 ab	23.5	−14.5	38.0
Eggplant						
+Fumigated						
P0−M	6499 ± 823 b	6938 ± 835 a	3736 ± 1312 a			
P0+M	11153 ± 4117 ab	7704 ± 123 a	4725 ± 1445 a	41.7	9.9	20.9
P1−M	12202 ± 4413 ab	7065 ± 3464 a	4490 ± 497 a			
P1+M	15404 ± 1432 a	8983 ± 594 a	5233 ± 2661 a	20.8	21.4	14.2
−Fumigated						
P0−M	6809 ± 191 b	7812 ± 209 a	6020 ± 374 a			
P0+M	12909 ± 4217 ab	8598 ± 427 a	6215 ± 424 a	47.3	9.1	3.1
P1−M	10566 ± 3452 ab	7881 ± 1277 a	6288 ± 449 a			
P1+M	11011 ± 1583 ab	8724 ± 91 a	6043 ± 160 a	4.0	9.7	−4.1

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

increased plant P concentration, but the difference in year 1998 was not statistically significant. Mycorrhizal inoculation in years 1997 and 1998, under fumigated soil conditions, significantly ($P < 0.02$ and 0.03 respectively) increased plant P concentration. The tomato plant P concentration in year 1999 was significantly ($P < 0.04$) affected under in fumigated soil conditions.

The root colonization was significantly affected by the disinfection and mycorrhizal inoculation. The extent of AM colonization differed among the plant species. In general pepper and tomato plants have higher root colonization than eggplant (Fig. 1). In both fumigated and non-fumigated soil conditions mycorrhizal inoculation significantly affected root colonization ($P < 0.01$–0.05).

3.1.2. Melon–watermelon–cucumber

Plants of the cucurbitaceae are mainly mycorrhizal plants. Plants were harvested several times, their yields were recorded and their IE was calculated (Table 4). In 1997, mycorrhiza-inoculated watermelon and cucumber plants grew less in non-fumigated soil than in fumigated soil. For melon plants, in 1999, plant yields were not harvested and IE was not calculated due to the presence of infection by soil-borne pathogens. The results showed that in 1997, mycorrhizal inoculation significantly ($P < 0.05$) increased melon yield in non-fumigated plots. Although mycorrhizal inoculation increased watermelon yield, statistically there is a weak effect of mycorrhizal inoculation on yield. In the years 1997, 1998 and 1999, when cucumber plants were grown in non-fumigated plots, mycorrhizal inoculation had a significant ($P < 0.03$–0.05 respectively) effect on yield. It seems phosphorus addition had less effect on yield. For watermelon plants, P addition increased yield ($P < 0.03$) in for the year 1998 and for cucumber plants in 1997 and 1998; in non-fumigated soils, P addition statistically and significantly increased yield ($P < 0.03$).

Calculated IE was higher in 1997 than in 1998. In general, under P0 conditions, IE was higher than under the P1 treatment. In 1997, watermelons showed a strong negative response to IE, but in 1999 their IE was positive and high. The yield reduction for watermelon and cucumber was attributed to the influence of the soil-borne pathogens.

These results confirm the importance of mycorrhizal symbiosis for the development of horticultural plants under field conditions. In the case of seedling-grown plants, the production of mycorrhiza-inoculated seedlings is particularly important for better establishment and healthy production.

As can be seen in Table 5, mycorrhiza-inoculated melon, watermelon and cucumber plants had high P and Zn concentrations. The statistical significance of the impact of mycorrhizal inoculation differed among years. In 1998 watermelon and cucumber plants' Zn contents were not statistically significant. Mycorrhizal inoculation significantly increased melon plant P% ($P < 0.01$–0.06) concentration. Although mycorrhizal inoculation increased the root colonization in both fumigated and non-fumigated soils, statistically there is a weak effect of mycorrhizal inoculation on plants of the Cucurbitaceae family. In general, the extent of AM colonization is higher in non-fumigated treatments than in the fumigated ones (Fig. 2).

Table 3
Effect of mycorrhizal inoculation and P addition on green pepper, tomato and eggplant P and Zn content under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	P (%)			Zn (%)		
	1997[a]	1998	1999	1997	1998	1999
Pepper						
+Fumigated						
P0−M	0.23 ± 0.00c[!!]	0.27 ± 0.04a	0.21 ± 0.01b	15.9 ± 0.1a	25.1 ± 8.6a	21.2 ± 3.6b
P0+M	0.24 ± 0.01c	0.26 ± 0.04a	0.30 ± 0.01a	16.4 ± 4.2a	26.7 ± 9.8a	28.6 ± 2.6b
P1−M	0.25 ± 0.00ab	0.25 ± 0.01a	0.22 ± 0.00b	21.3 ± 7.8a	24.3 ± 15.0a	23.5 ± 1.2b
P1+M	0.27 ± 0.00a	0.27 ± 0.02a	0.31 ± 0.01a	21.1 ± 1.6a	28.0 ± 16.9a	27.7 ± 2.5b
−Fumigated						
P0−M	0.23 ± 0.01c	0.28 ± 0.01a	0.21 ± 0.02b	15.8 ± 1.4a	28.7 ± 13.2a	25.9 ± 1.2b
P0+M	0.26 ± 0.00c	0.26 ± 0.01a	0.29 ± 0.01a	17.0 ± 4.5a	28.8 ± 14.8a	39.9 ± 14.1a
P1−M	0.27 ± 0.01bc	0.27 ± 0.03a	0.22 ± 0.03b	19.7 ± 1.6a	29.5 ± 6.6a	25.4 ± 0.4b
P1+M	0.28 ± 0.00a–c	0.28 ± 0.00a	0.28 ± 0.01a	20.4 ± 2.0a	27.8 ± 6.9a	24.0 ± 0.3b
Tomato						
+Fumigated						
P0−M	0.23 ± 0.01b	0.23 ± 0.02a	0.26 ± 0.03ab	15.5 ± 0.5d	33.5 ± 5.9a	15.1 ± 1.4b
P0+M	0.24 ± 0.02b	0.23 ± 0.03a	0.28 ± 0.04a	16.4 ± 1.4cd	24.1 ± 3.5a	16.0 ± 1.1ab
P1−M	0.24 ± 0.02b	0.25 ± 0.00a	0.25 ± 0.01b	16.6 ± 0.6b–d	30.2 ± 3.9a	15.5 ± 0.5ab
P1+M	0.27 ± 0.09a	0.31 ± 0.05a	0.27 ± 0.00a	18.1 ± 0.7a–d	31.4 ± 1.5a	15.5 ± 0.2ab
−Fumigated						
P0−M	0.22 ± 0.01c	0.22 ± 0.00a	0.28 ± 0.05a	18.0 ± 3.1a–d	26.1 ± 10.9a	17.4 ± 1.2ab
P0+M	0.27 ± 0.02a	0.24 ± 0.01a	0.2 ± 0.08a	21.1 ± 2.4a–c	24.6 ± 7.7a	17.2 ± 1.4ab
P1−M	0.27 ± 0.06a	0.25 ± 0.01a	0.25 ± 0.03b	21.7 ± 2.7ab	29.2 ± 7.9a	15.8 ± 0.2ab
P1+M	0.28 ± 0.02a	0.24 ± 0.00a	0.27 ± 0.02a	23.2 ± 4.0a	24.7 ± 11.8a	17.9 ± 1.5s
Eggplant						
+Fumigated						
P0−M	0.19 ± 0.00e	0.29 ± 0.01a	0.43 ± 0.02a	12.4 ± 0.1d	22.5 ± 1.1ab	24.7 ± 2.4c
P0+M	0.21 ± 0.02d–e	0.28 ± 0.01a	0.37 ± 0.04a	12.9 ± 1.3cd	21.2 ± 0.3b	31.4 ± 0.6ab
P1−M	0.23 ± 0.02cd	0.30 ± 0.01a	0.40 ± 0.04a	13.8 ± 0.5cd	21.8 ± 1.5b	31.7 ± 3.7ab
P1+M	0.25 ± 0.00bc	0.33 ± 0.01a	0.43 ± 0.02a	14.5 ± 0.4bc	23.8 ± 1.2ab	35.2 ± 2.1ab
−Fumigated						
P0−M	0.24 ± 0.00cd	0.28 ± 0.02a	0.34 ± 0.04a	13.9 ± 3.8b–d	24.2 ± 0.1ab	36.8 ± 3.9a
P0+M	0.25 ± 0.00bc	0.24 ± 0.10a	0.26 ± 0.21a	16.7 ± 2.7a–c	23.1 ± 2.5ab	31.6 ± 2.5ab
P1−M	0.27 ± 0.01ab	0.32 ± 0.04a	0.41 ± 0.07a	18.1 ± 1.3ab	21.6 ± 1.5b	27.1 ± 4.4ab
P1+M	0.28 ± 0.02a	0.32 ± 0.02a	0.38 ± 0.02a	19.1 ± 0.6a	25.9 ± 3.2a	35.0 ± 7.3ab

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

3.1.3. Maize–cotton

Maize and cotton are common plants in the region and are grown in large plantations. In general, both plants are highly responsive to mycorrhizal inoculation. The growth of maize and cotton was similar in both fumigated and non-fumigated treatments (Table 6). They were both relatively less stunted in fumigated vs. non-fumigated soils than the horticultural plant group. In 1997, in fumigated and non-infected soils, mycorrhizal inoculation

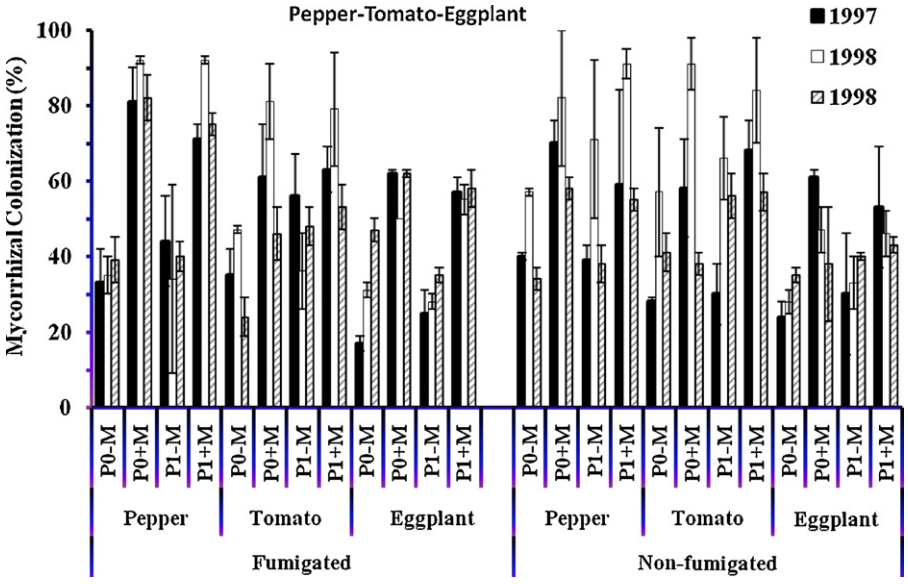

Fig. 1. Colonization of pepper, tomato and eggplant plants under fumigated with and without methyl bromide in three years. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.

Table 4
Effect of mycorrhizal inoculation and P addition on melon, watermelon and cucumber yield and inoculation effectiveness under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	Yield (kg/1000 m^2)			Inoculation effectiveness (%)		
	1997[a]	1998	1999	1997	1998	1999
Melon						
+Fumigated						
P0−M	1685 ± 1500ab[!!]	2657 ± 400ab	–			
P0+M	3444 ± 566a	3748 ± 1550a	–	51.1	29.1	–
P1−M	2348 ± 50ab	1705 ± 961b	–			
P1+M	3318 ± 1233a	1700 ± 262b	–	29.2	−0.3	–
−Fumigated						
P0−M	1106 ± 118b	1582 ± 501b	–			
P0+M	1874 ± 968ab	2904 ± 767ab	–	41.0	45.5	–
P1−M	864 ± 149b	2150 ± 380ab	–			
P1+M	2013 ± 138ab	2869 ± 641	–	57.1	25.1	–
Watermelon						
+Fumigated						
P0−M	174 ± 25b	3206 ± 2054a	1284 ± 821a	64.9	7.7	39.7
P0+M	496 ± 457ab	3475 ± 671a	2129 ± 312a			
P1−M	603 ± 488ab	1957 ± 677ab	1202 ± 865a	37.9	42.8	42.4
P1+M	971 ± 325a	3419 ± 405a	2086 ± 599a			
−Fumigated						
P0−M	1754 ± 1338ab	594 ± 159b	871 ± 964a	8.9	1.4	30.2
P0+M	1926 ± 1304a	603 ± 236b	1248 ± 201a			
P1−M	1840 ± 554ab	3048 ± 1699a	772 ± 497a	−54.9	−44.2	31.9
P1+M	1188 ± 59ab	2114 ± 311ab	1134 ± 315a			
Cucumber						
+Fumigated						
P0−M	1529 ± 213d	1485 ± 133c	1321 ± 257b			
P0+M	1641 ± 515c–d	1729 ± 636c	1401 ± 141b	6.9	14.1	5.7
P1−M	1676 ± 289c–d	1720 ± 364c	1355 ± 166ab			
P1+M	1835 ± 439b–d	1996 ± 829c	1370 ± 25ab	8.7	13.8	1.1
−Fumigated						
P0−M	2062 ± 200b–c	2440 ± 417a–c	1345 ± 50b			
P0+M	2511 ± 111b	2861 ± 164ab	1746 ± 40a	17.9	14.7	23.0
P1−M	2605 ± 140a	3349 ± 430a	1431 ± 173ab			
P1+M	2596 ± 103a	3396 ± 107a	1367 ± 82ab	−0.4	1.4	−4.7

Values are the averages of three samples ± standard deviation.
−M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

significantly ($P < 0.03$) increased maize yield. However, for 1998 and 1999 the increase was not significant.

Maize and cotton exhibited the lowest IE, with a maximum of 27.5% and a minimum of 13.5% for all 3 years. In 1997, mycorrhizal inoculation significantly increased plant growth, but in 1998 and 1999, the increase was not significant.

In general, mycorrhiza-inoculated maize and cotton plants had higher P and Zn concentrations than their non-inoculated

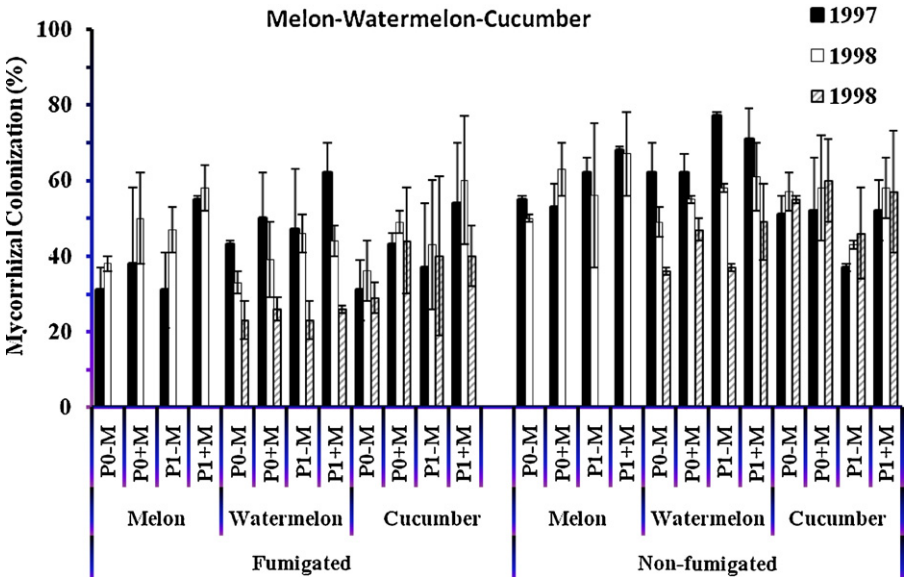

Fig. 2. Colonization of melon, watermelon and cucumber plants under fumigated with and without methyl bromide in three years. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.

Table 5
Effect of mycorrhizal inoculation and P addition on melon, watermelon and cucumber P and Zn content under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	P (%)			Zn (%)		
	1997[a]	1998	1999	1997	1998	1999
Melon						
+Fumigated						
P0−M	0.20 ± 0.01c[!!]	0.20 ± 0.04c	–	20.4 ± 2.5e	21.0 ± 0.1c	–
P0+M	0.35 ± 0.00ab	0.30 ± 0.03a	–	26.9 ± 2.4cb	24.9 ± 2.2cb	–
P1−M	0.23 ± 0.06c	0.29 ± 0.01ab	–	22.6 ± 2.3de	21.7 ± 1.0c	–
P1+M	0.36 ± 0.01a	0.33 ± 0.01a	–	26.9 ± 2.7bc	24.1 ± 1.3cb	–
−Fumigated						
P0−M	0.25 ± 0.06c	0.24 ± 0.05bc	–	29.5 ± 0.1b	21.1 ± 4.4c	–
P0+M	0.31 ± 0.01b	0.28 ± 0.01ab	–	33.8 ± 1.4a	31.3 ± 1.3a	–
P1−M	0.21 ± 0.02c	0.28 ± 0.01bc	–	24.5 ± 0.1cd	23.4 ± 0.1cb	–
P1+M	0.35 ± 0.00a	0.32 ± 0.00a	–	28.7 ± 0.4b	27.6 ± 2.0b	–
Water melon						
+Fumigated						
P0−M	0.26 ± 0.03b	0.26 ± 0.00c	0.25 ± 0.03b	26.9 ± 2.6a	30.0 ± 3.2a	32.0 ± 5.3a
P0+M	0.29 ± 0.00ab	0.32 ± 0.03ab	0.33 ± 0.05ab	27.0 ± 2.7a	30.3 ± 3.3a	32.4 ± 4.2a
P1−M	0.29 ± 0.03ab	0.31 ± 0.05a-c	0.32 ± 0.08ab	25.2 ± 1.4a	28.5 ± 2.7a	30.7 ± 3.8a
P1+M	0.31 ± 0.01ab	0.34 ± 0.02ab	0.36 ± 0.03a	31.2 ± 8.2a	31.2 ± 4.8a	30.0 ± 10.2a
−Fumigated						
P0−M	0.26 ± 0.03b	0.29 ± 0.02a-c	0.31 ± 0.01ab	29.7 ± 8.3a	29.2 ± 0.2a	27.6 ± 4.5a
P0+M	0.30 ± 0.01ab	0.31 ± 0.02a-c	0.32 ± 0.05ab	37.8 ± 14a	34.4 ± 7.5a	29.7 ± 10.2a
P1−M	0.28 ± 0.01ab	0.29 ± 0.00bc	0.30 ± 0.01ab	27.1 ± 4.6a	28.8 ± 2.1a	29.4 ± 3.1a
P1+M	0.31 ± 0.03a	0.35 ± 0.02a	0.38 ± 0.01a	28.9 ± 5.4a	29.6 ± 1.4a	29.2 ± 2.5a
Cucumber						
+Fumigated						
P0−M	0.33 ± 0.01a	0.27 ± 0.02b	0.32 ± 0.02ab	35.6 ± 6.7a	36.1 ± 3.5a	21.8 ± 5.1e
P0+M	0.35 ± 0.04a	0.35 ± 0.02ab	0.35 ± 0.03ab	36.0 ± 3.2a	34.1 ± 0.5a	35.0 ± 1.8ab
P1−M	0.36 ± 0.03a	0.36 ± 0.00ab	0.36 ± 0.02ab	34.5 ± 6.4a	34.5 ± 0.9a	29.5 ± 3.6cd
P1+M	0.37 ± 0.12a	0.38 ± 0.08ab	0.37 ± 0.10ab	41.6 ± 3.7a	34.2 ± 1.2a	37.9 ± 2.5a
−Fumigated						
P0−M	0.41 ± 0.02a	0.43 ± 0.00a	0.42 ± 0.01a	40.8 ± 4.5a	20.3 ± 23.9a	25.6 ± 2.1de
P0+M	0.31 ± 0.10a	0.26 ± 0.09b	0.29 ± 0.10b	31.0 ± 9.2a	31.1 ± 9.1a	31.1 ± 0.1bc
P1−M	0.36 ± 0.00a	0.35 ± 0.02ab	0.36 ± 0.01ab	32.3 ± 7.1a	32.0 ± 7.8a	28.2 ± 7.4cd
P1+M	0.36 ± 0.07a	0.26 ± 0.08b	0.31 ± 0.00ab	33.2 ± 2.4a	34.6 ± 0.6a	32.4 ± 24.7bc

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

Table 6
Effect of mycorrhizal inoculation and P addition on maize and cotton yield and inoculation effectiveness under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	Yield (kg/1000 m^2)			Inoculation effectiveness (%)		
	1997[a]	1998	1999	1997	1998	1999
Maize						
+Fumigated						
P0−M	1218 ± 75 c–d[!!]	847 ± 76 a	891 ± 244 a			
P0+M	1461 ± 76 b	842 ± 12 a	963 ± 5 a	16.6	−0.6	7.5
P1−M	1329 ± 76 b–c	829 ± 106 a	1113 ± 327 a			
P1+M	1652 ± 5 a	903 ± 147 a	1024 ± 241 a	19.5	8.2	−8.7
−Fumigated						
P0−M	1080 ± 6 d	1006 ± 440 a	803 ± 208 a			
P0+M	1227 ± 76 c	1230 ± 38 a	910 ± 94 a	12.0	18.2	11.8
P1−M	1326 ± 77 b–c	1143 ± 175 a	809 ± 130 a			
P1+M	1644 ± 6 a	1026 ± 223 a	835 ± 74 a	19.3	−11.4	3.2
Cotton						
+Fumigated						
P0−M	304 ± 68 e	391 ± 39 a	335 ± 11 a			
P0+M	387 ± 21 c–e	414 ± 6 a	345 ± 28 a	21.4	5.6	2.9
P1−M	423 ± 17 c–e	364 ± 1 a	311 ± 12 a			
P1+M	457 ± 7 a–c	426 ± 61 a	356 ± 33 a	7.5	14.6	12.6
−Fumigated						
P0−M	341 ± 40 d–e	405 ± 38 a	339 ± 2 a			
P0+M	470 ± 26 a–c	448 ± 55 a	341 ± 28 a	27.5	9.4	0.5
P1−M	477 ± 33 ab	477 ± 91 a	363 ± 38 a			
P1+M	515 ± 42 a	460 ± 62 a	321 ± 15 a	7.5	−3.7	−13.3

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

Table 7
Effect of mycorrhizal inoculation and P addition on maize and cotton P and Zn content under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	P (%)			Zn (%)		
	1997[a]	1998	1999	1997	1998	1999
Maize						
+Fumigated						
P0−M	0.34 ± 0.01c[!!]	0.20 ± 0.01b	0.28 ± 0.02a	21.0 ± 2.8a	17.7 ± 3.1c	16.2 ± 4.0a
P0+M	0.48 ± 0.06a	0.35 ± 0.04a	0.26 ± 0.05a	25.0 ± 1.4a	25.2 ± 1.5ab	26.8 ± 8.1a
P1−M	0.49 ± 0.01a	0.36 ± 0.02a	0.26 ± 0.06a	31.0 ± 2.1a	16.7 ± 3.2c	18.7 ± 12.7a
P1+M	0.44 ± 0.03ab	0.31 ± 0.04ab	0.27 ± 0.00a	24.5 ± 0.7a	25.6 ± 0.3a	27.7 ± 3.3a
−Fumigated						
P0−M	0.39 ± 0.05a–c	0.27 ± 0.08ab	0.24 ± 0.05a	19.0 ± 2.8a	18.7 ± 0.7bc	17.0 ± 7.2a
P0+M	0.37 ± 0.07bc	0.26 ± 0.04ab	0.22 ± 0.07a	21.3 ± 2.4a	21.6 ± 2.8a–c	19.0 ± 4.1a
P1−M	0.43 ± 0.02ab	0.31 ± 0.02ab	0.24 ± 0.00a	21.3 ± 3.3a	17.2 ± 2.5c	19.0 ± 0.2a
P1+M	0.43 ± 0.01ab	0.32 ± 0.01ab	0.23 ± 0.01a	22.5 ± 2.2a	21.9 ± 2.2a–c	15.2 ± 3.3a
Cotton						
+Fumigated						
P0−M	0.24 ± 0.02c	0.23 ± 0.02b	0.21 ± 0.01b	28.5 ± 0.7a	27.8 ± 1.2bc	34.5 ± 4.9ab
P0+M	0.25 ± 0.01bc	0.23 ± 0.01b	0.33 ± 0.05a	30.0 ± 1.4a	28.8 ± 1.2bc	31.3 ± 2.6ab
P1−M	0.24 ± 0.02c	0.23 ± 0.02b	0.29 ± 0.08ab	33.0 ± 8.5a	26.8 ± 1.2c	28.0 ± 0.2b
P1+M	0.33 ± 0.04a–c	0.31 ± 0.04ab	0.29 ± 0.01ab	32.4 ± 2.2a	32.5 ± 2.1a	32.6 ± 6.3ab
−Fumigated						
P0−M	0.29 ± 0.08a–c	0.27 ± 0.08ab	0.21 ± 0.01b	27.5 ± 0.7a	28.1 ± 0.7bc	36.6 ± 2.7a
P0+M	0.28 ± 0.04a–c	0.26 ± 0.04ab	0.28 ± 0.02ab	32.9 ± 0.4a	30.0 ± 1.4ab	30.0 ± 0.4ab
P1−M	0.33 ± 0.03ab	0.31 ± 0.02a	0.36 ± 0.02a	31.5 ± 0.7a	27.5 ± 0.7bc	31.9 ± 0.6sb
P1+M	0.34 ± 0.01a	0.32 ± 0.01a	0.32 ± 0.04a	32.9 ± 1.3a	30.0 ± 1.4ab	34.3 ± 5.0ab

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

counterparts (Table 7). However, for cotton plants phosphorus application had a significant ($P < 0.02$) effect on yield. As can be seen in Fig. 3, higher root colonization occurred in non-fumigated soil than in fumigated soils even in non-inoculated soil, possibly due to the indigenous spores. Accordingly, there were fewer differences between inoculated and non-inoculated maize and cotton plants.

3.1.4. Horsebean–chickpea–soybean

Yield and IE data are presented in Table 8. Mycorrhizal inoculation from 1997 to 1999 significantly ($P < 0.01$–0.05) increased the horsebean yield. The yield of chickpea was significantly ($P < 0.01$–0.05) affected by mycorrhizal inoculation in the years of 1997 and 1999. However, soybean yield was weakly affected by the mycorrhizal inoculation.

The yields of chickpea and horsebean grown in soil that was not fumigated were much higher than they were in the fumigated soil. The yield of plants grown in the latter was much higher than that of those grown in the fumigated soil. The differences in yield production in non-fumigated plots were considered to be related to infection by indigenous mycorrhizae. Mycorrhizal inoculation significantly increased horsebean yield ($P < 0.001$–0.05). The growth of horsebean and chickpea was strongly responsive to mycorrhizal inoculation in fumigated plots, where mycorrhizal inoculation significantly increased the growth and P uptake of horsebean and chickpea. Horsebean showed the highest IE in all 3 years, especially in P0 plots. Chickpea and soybean plants also have high IE, but less than the horsebean. Soybean plants showed high IE in 1997, but in 1998, even in non-fumigated soil, they showed a negative response. For legumes, N2 fixation implies that the supply of other nutrients, particularly P, is adequate for optimum growth.

The plants' P and Zn concentrations increased with mycorrhizal inoculation (Table 9). P addition also increased the P content. In general, soybean plants had the highest P and Zn concentrations of the three legumes. Mycorrhizal inoculation significantly increased the horsebean P and Zn concentrations in 1997 and 1999.

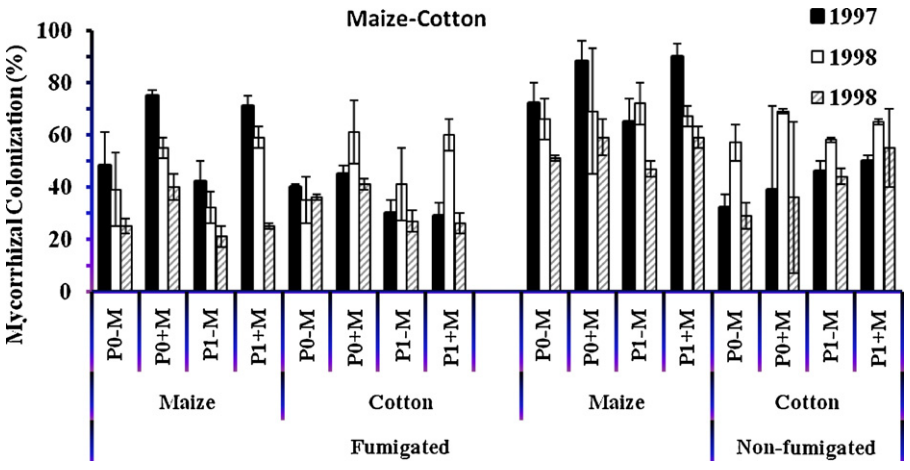

Fig. 3. Colonization of maize and cotton plants under fumigated with and without methyl bromide in three years. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.

Table 8
Effect of mycorrhizal inoculation and P addition on horsebean, chickpea and soybean yield and inoculation effectiveness under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	Yield (kg/1000 m^2)			Inoculation effectiveness (%)		
	1997[a]	1998	1999	1997	1998	1999
Horsebean						
+Fumigated						
P0−M	52 ± 10c[!!]	38 ± 34d	44 ± 13b			
P0+M	110 ± 16c	90 ± 12c–d	48 ± 2b	52.5	57.5	8.3
P1−M	83 ± 6c	88 ± 2c–d	16 ± 9b			
P1+M	121 ± 28c	101 ± 1c	25 ± 3b	31.7	12.5	24.3
−Fumigated						
P0−M	307 ± 6b	317 ± 19b	298 ± 70a			
P0+M	405 ± 21a	415 ± 14a	433 ± 23a	24.2	21.7	31.3
P1−M	304 ± 47b	306 ± 44b	307 ± 78a			
P1+M	351 ± 70ab	331 ± 31b	436 ± 13a	12.8	7.6	29.5
Chickpea						
+Fumigated						
P0−M	363 ± 22 b	363 ± 22 b	154 ± 35 d			
P0+M	457 ± 83 ab	404 ± 157b	281 ± 184 b–d	20.5	10.2	45.4
P1−M	363 ± 35 b	363 ± 36 b	297 ± 50 b–d			
P1+M	430 ± 60 b	430 ± 61 ab	380 ± 73 c–b	15.7	15.7	21.7
−Fumigated						
P0−M	472 ± 149 ab	485 ± 168ab	209 ± 16 c–d			
P0+M	551 ± 14 ab	491 ± 99 ab	434 ± 31 b	14.3	1.2	51.8
P1−M	551 ± 259 ab	551 ± 259 ab	412 ± 66 b			
P1+M	755 ± 207 a	755 ± 206 a	526 ± 96 a	27.1	27.1	21.7
Soybean						
+Fumigated						
P0−M	199 ± 21 d	400 ± 40 a	401 ± 4 a			
P0+M	240 ± 32 c–d	436 ± 5a	404 ± 20a	17.0	8.4	0.8
P1−M	320 ± 54 a–c	373 ± 51 a	435 ± 90 a			
P1+M	392 ± 9 a	463 ± 38 a	453 ± 111 a	18.5	19.4	4.1
−Fumigated						
P0−M	267 ± 40 b–d	510 ± 71 a	447 ± 61 a			
P0+M	331 ± 52 ab	494 ± 81 a	453 ± 4 a	19.4	−3.3	1.3
P1−M	329 ± 38 ab	493 ± 56 a	466 ± 54 a			
P1+M	392 ± 29 a	465 ± 100 a	475 ± 68 a	16.0	−6.0	1.9

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

The results of root colonization showed that mycorrhizal colonization increased with mycorrhizal inoculation. In general, soybean plants have high root colonization. Also root colonization in non-fumigated soil is higher than in fumigated soils (Fig. 4).

3.1.5. Garlic

Garlic was significantly influenced by MBr application. Plants grown in fumigated plots were all stunted but mycorrhiza-inoculated plants grew much better than non-inoculated ones

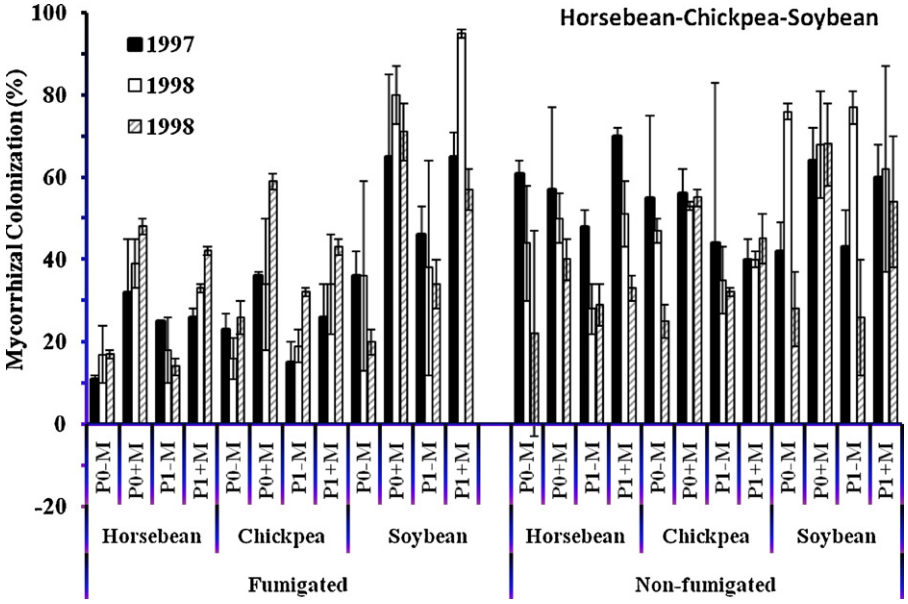

Fig. 4. Colonization of horsebean, chickpea and soybean plants under fumigated with and without methyl bromide in three years. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.

Table 9
Effect of mycorrhizal inoculation and P addition on horsebean, chickpea and soybean P and Zn content under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	P (%)			Zn (%)		
	1997[a]	1998	1999	1997	1998	1999
Horsebean						
+Fumigated						
P0−M	0.16 ± 0.01f[!!]	0.23 ± 0.04ab	0.13 ± 0.07c	17.7 ± 0.7e	20.5 ± 6.4b	17.5 ± 0.4b
P0+M	0.20 ± 0.01d–f	0.25 ± 0.01ab	0.22 ± 0.02ab	19.5 ± 1.4cd	27.1 ± 1.5ab	20.3 ± 1.8ab
P1−M	0.18 ± 0.02ef	0.25 ± 0.00ab	0.13 ± 0.03c	18.5 ± 0.4d–e	18.8 ± 5.9b	16.4 ± 3.3b
P1+M	0.23 ± 0.02c–e	0.27 ± 0.05a	0.28 ± 0.05a	19.4 ± 0.8cd	30.0 ± 11.0ab	19.4 ± 1.1ab
−Fumigated						
P0−M	0.28 ± 0.01bc	0.18 ± 0.03b	0.24 ± 0.05ab	20.8 ± 0.1b	17.3 ± 4.4b	14.8 ± 4.3ab
P0+M	0.34 ± 0.02a	0.24 ± 0.06ab	0.18 ± 0.02bc	22.4 ± 0.8a	41.3 ± 11.7a	16.7 ± 3.5b
P1−M	0.24 ± 0.05cd	0.19 ± 0.03ab	0.21 ± 0.02a–c	20.3 ± 0.3bc	25.1 ± 12.1ab	20.1 ± 1.9ab
P1+M	0.32 ± 0.01ab	0.23 ± 0.02ab	0.26 ± 0.01ab	22.4 ± 1.4a	41.2 ± 5.3a	25.6 ± 0.4a
Chickpea						
+Fumigated						
P0−M	0.18 ± 0.11b	0.21 ± 0.00b	0.12 ± 0.07a	14.0 ± 1.6c	39.2 ± 0.1ab	12.4 ± 2.2b
P0+M	0.21 ± 0.01b	0.26 ± 0.06a	0.25 ± 0.02a	18.9 ± 2.1a–c	27.7 ± 1.8ab	16.0 ± 5.1ab
P1−M	0.21 ± 0.05b	0.21 ± 0.01b	0.21 ± 0.04a	19.0 ± 2.3a–c	28.5 ± 4.7ab	12.4 ± 0.6b
P1+M	0.24 ± 0.09ab	0.25 ± 0.04a	0.24 ± 0.03a	23.4 ± 1.7a	59.4 ± 9.5a	14.0 ± 0.0b
−Fumigated						
P0−M	0.29 ± 0.02ab	0.26 ± 0.03a	0.27 ± 0.04a	17.1 ± 2.0bc	21.4 ± 5.4b	22.4 ± 5.3a
P0+M	0.31 ± 0.02a	0.20 ± 0.08b	0.26 ± 0.00a	22.8 ± 0.3a	39.3 ± 1.7ab	17.4 ± 4.4ab
P1−M	0.30 ± 0.02a	0.22 ± 0.01ab	0.25 ± 0.02a	21.4 ± 1.1ab	28.0 ± 3.1ab	17.0 ± 0.5ab
P1+M	0.32 ± 0.09a	0.21 ± 0.02b	0.29 ± 0.03a	22.7 ± 1.8ab	34.8 ± 12.7ab	21.4 ± 0.5a
Soybean						
+Fumigated						
P0−M	0.20 ± 0.00cd	0.28 ± 0.01b	0.25 ± 0.01d	50.2 ± 1.4a	58.4 ± 3.1a–c	40.4 ± 1.9b
P0+M	0.31 ± 0.01bc	0.34 ± 0.03a	0.31 ± 0.01ab	52.1 ± 0.6a	61.7 ± 3.7a	50.0 ± 8.3ab
P1−M	0.28 ± 0.00de	0.32 ± 0.03ab	0.26 ± 0.02cd	28.5 ± 39.5a	53.9 ± 5.2c	48.6 ± 5.1ab
P1+M	0.29 ± 0.01de	0.34 ± 0.01a	0.29 ± 0.01a–c	54.9 ± 6.6a	61.1 ± 1.2ab	56.2 ± 10.4a
−Fumigated						
P0−M	0.27 ± 0.00e	0.29 ± 0.01ab	0.26 ± 0.00cd	48.7 ± 0.2a	54.6 ± 3.0bc	41.1 ± 1.7b
P0+M	0.32 ± 0.00ab	0.31 ± 0.04ab	0.33 ± 0.02a	48.0 ± 1.2a	57.5 ± 1.0a–c	39.1 ± 2.3b
P1−M	0.31 ± 0.01bc	0.31 ± 0.04ab	0.28 ± 0.04b–d	33.8 ± 21.3a	57.1 ± 1.2a–c	39.8 ± 2.8b
P1+M	0.34 ± 0.01a	0.32 ± 0.01ab	0.30 ± 0.03ac	42.3 ± 0.4a	57.4 ± 0.6a–c	45.8 ± 1.8ab

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

(Table 10). Mycorrhizal inoculation significantly increased yield for all three years ($P < 0.01$–0.05). Garlic was calculated to depend strongly on mycorrhiza for its growth in both fumigated and non-fumigated plots. In the P0 treatment, IE was higher than in the P1 treatment.

Mycorrhizal inoculation increased garlic P and Zn concentrations (Table 11). The addition of P had no influence on tissue P content, but soils fumigated did have an influence on mycorrhizal colonization and nutrient contents. In 1997, mycorrhizal inoculation significantly ($P < 0.01$–0.03) increased plant tissue P and Zn concentrations. The mycorrhizal inoculum increased the level of colonization of garlic plants compared with those in non-inoculated treatments. Root colonization in non-fumigated soils is higher than in fumigated soils (Fig. 5).

4. Discussion

This study shows the importance of mycorrhizal infection for the growth of several plant species under field conditions. On the basis of yield differences between plants growing in

Table 10
Effect of mycorrhizal inoculation and P addition on garlic yield and inoculation effectiveness under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	Yield (kg/1000 m^2)			Inoculation effectiveness (%)		
	1997[a]	1998	1999	1997	1998	1999
Garlic						
+Fumigated						
P0−M	63 ± 14 d[!!]	23 ± 5 d	219 ± 44 d			
P0+M	217 ± 141 c–d	79 ± 52 b–d	294 ± 41 b–c	70.8	70.8	25.6
P1−M	63 ± 5d	23 ± 2 d	208 ± 36 d			
P1+M	155 ± 23c–d	57 ± 9 d–c	302 ± 15 b–c	59.5	59.5	31.0
−Fumigated						
P0−M	235 ± 67 b–c	186 ± 110 b	229 ± 57 c–d			
P0+M	395 ± 119 b	228 ± 69	352 ± 41 b	40.6	18.7	35.0
P1−M	239 ± 24 b–c	138 ± 14 c	241 ± 71 b–c			
P1+M	406 ± 6	235 ± 4	393 ± 18	41.0	41.0	38.8

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

Table 11
Effect of mycorrhizal inoculation and P addition on garlic P and Zn content under methyl-bromide-treated (+Fumigated) and non-treated (−Fumigated) field conditions.

Treatments	P (%)			Zn (%)		
	1997[a]	1998	1999	1997	1998	1999
Garlic						
+Fumigated						
P0−M	0.23 ± 0.01e[!!]	0.17 ± 0.00ab	0.33 ± 0.01ab	18.7 ± 1.0d	16.7 ± 1.1b	21.8 ± 1.4ab
P0+M	0.29 ± 0.01bd	0.19 ± 0.01ab	0.37 ± 0.05ab	20.2 ± 0.2cd	21.9 ± 2.8ab	24.0 ± 0.5a
P1−M	0.29 ± 0.01bd	0.19 ± 0.01ab	0.30 ± 0.13ab	21.6 ± 0.7bd	23.9 ± 2.9ab	22.5 ± 1.7ab
P1+M	0.33 ± 0.01a–c	0.23 ± 0.02ab	0.42 ± 0.04a	21.5 ± 0.8bd	27.7 ± 11.1a	25.5 ± 2.8ab
−Fumigated						
P0−M	0.23 ± 0.02e	0.16 ± 0.04b	0.27 ± 0.01b	18.2 ± 1.8d	16.7 ± 4.5b	21.4 ± 6.8ab
P0+M	0.35 ± 0.01ab	0.21 ± 0.00ab	0.31 ± 0.06ab	23.7 ± 0.7bc	24.1 ± 2.8ab	16.2 ± 2.2b
P1−M	0.27 ± 0.01de	0.23 ± 0.06ab	0.34 ± 0.01b	24.6 ± 0.7b	24.6 ± 3.9ab	23.8 ± 1.4ab
P1+M	0.37 ± 0.01a	0.24 ± 0.07a	0.28 ± 0.05b	29.5 ± 0.8a	25.0 ± 0.2ab	20.9 ± 4.9ab

Values are the averages of three samples ± standard deviation. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.
[a] Comparison of means of LSD were calculated for each year separately.
[!!] Values in a column followed by the same latter are not significantly different (LSD, $P \leq 0.05$).

fumigated and non-fumigated soils, the plant species used in this study showed better growth with mycorrhizal inoculation. However, the plants' responses to fumigated and mycorrhizal inoculation differed. The most important growth stimulation was recorded with horticultural and legume plant species, groups which are strongly mycorrhizal. These results are supported by Ortas (2008a) and (Ortas and Akpinar, 2011) early works. Almaca and Ortas (2010) reported that under field and greenhouse conditions, previous crops had a significant influence on the growth of maize that could be related to differences in the indigenous mycorrhiza inoculum potential.

Although under non-fumigated soil conditions, during the vegetative growth period, mycorrhiza-inoculated eggplants were larger and grew more rapidly, this was not reflected in an expected yield increase. Al-Raddad (1987) also studied eggplant, tomato and pepper plants inoculated with *G. fasciculatum*, *G. monosporum* and *G. mossea* under greenhouse conditions and found a significant increase in eggplant dry weight. In Japan, under greenhouse conditions, the effects of AMF inoculation on seedlings of 17 species of vegetable crops were investigated and growth was reported to be noticeably enhanced by the inoculation (Matsubara et al., 1994).

4.1. The effect of AMF and P fertilizer under fumigated and non-fumigated soils conditions on inoculation effectiveness

The field crop plants showed lower IE than the horticultural crops. Strongly mycorrhiza-responsive plants exhibited better growth under non-fumigated conditions as well. The IE of all plants studied here was higher in the P0 vs. P1 treatments. It appears that the differential effects of mycorrhiza are masked at higher rates of P application. Liu et al. (2003) showed that the growth of the most mycorrhiza-dependent hybrid in their study was more suppressed by fumigation than that of the other hybrids. They also found that fumigation has a significant, but smaller influence on soil-extractable *P* level than on plant P uptake and growth. The potential value and importance of mycorrhiza in natural ecosystems seem to diminish under high rates of fertilizer application. Khalil et al. (1999) showed that soybean cultivars differ in their response to mycorrhiza and also indicated that under low P conditions, mycorrhized Soja has 7.8 times greater total shoot P than its non-mycorrhized counterparts. Plenchette et al. (1983) tested RMD in 20 plant species grown in MBr-fumigated and non-fumigated soils under field conditions to understand the role of mycorrhizal inoculation. They distinguished three groups in terms of the plants' responses to mycorrhiza in fumigated and non-fumigated soil plots. Plenchette et al. (1983) indicated that plants with high P requirements may have very different relative field mycorrhizal dependency (RFMD) indices.

Our results also confirm the importance of the mycorrhizal symbiosis for the development of horticultural plants in Mediterranean soils. In the case of horticultural plants from fallow areas, this is particularly important for healthy food production. Since horticultural plant species are highly responsive to mycorrhizal inoculation, it is important to produce mycorrhiza-inoculated seedlings (Ortas

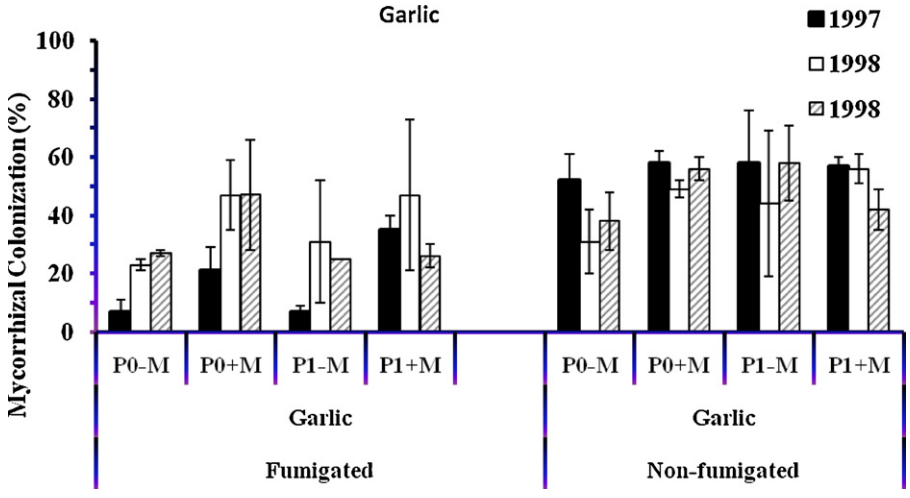

Fig. 5. Colonization of garlic under fumigated with and without methyl bromide in three years. −M no AMF inoculum, +M AMF inoculum used, P0 = 0 kg P_2O_5/ha, P1 = 100 kg P_2O_5/ha.

et al., 2003b; Ortas and Varma, 2007). Li et al. (2004) indicated that seedlings of vegetable crops are usually cultured on seedling beds or in containers and then transplanted into the field, providing a convenient sowing stage during which inoculation with AMF can be applied. Although it is known that the cucurbitaceae family is given to a high response to inoculation, in the present experiment, cucurbitaceae plants gave a lower response to mycorrhizal inoculation. Previously Ortas (2010) showed that under field conditions mycorrhiza inoculation significantly increased cucumber seedling survival, fruit yield, P and Zn shoot concentrations. Also it has been indicated that indigenous mycorrhiza inoculum was also successful in colonized cucumber roots and resulted in better plant growth and yield.

The horticultural plants are highly sensitive to soil-borne organisms, and therefore showed a high response to mycorrhiza under fumigated soil conditions. On the other hand, under the same soil conditions, the N-fixing legumes were stunted. In this study, compared to non-fumigated soils, all the legume species grown in fumigated soils were severely stunted, especially horsebean. Plants grown in fumigated soil are exposed to only a minimum level of mycorrhizal infection; in non-fumigated control plots, they are exposed to a higher level of mycorrhizal infection. Horticultural plants were less stunted in fumigated soils, and in fact the plants grew better in fumigated vs. non-fumigated soil.

Since the legumes studied here exhibited high IE, it is likely that mycorrhiza is as important for their P nutrition as *Rhizobium* is for their N requirements. Duponnois et al. (2001) tested the response to mycorrhizal inoculation of 12 tropical legumes and found it to vary between 92.7 and 26.2%. Leguminous species, especially horsebean, are used as green-manure crops in the coastal Mediterranean region, as well as for ecological (organic) farming Ortas (2008a). Leguminous plants, being mycorrhizal, showed better growth in non-fumigated vs. fumigated soil. Stunting of these plants following soil fumigation was mainly attributable to the elimination of indigenous mycorrhiza and N-fixing bacteria and other rhizospheric micro-organisms, leading to poor plant growth.

Plant species belonging to the solanaceae, leguminosae, and cucurbitaceae families showed high responses to the mycorrhiza under both fumigated and non-fumigated soil conditions. Moreover, the importance of inoculum viability should be noted: sometimes the plants did not respond to the mycorrhiza. In the present experiment, seeds were sown in beds and inoculated, and then placed directly into the field, resulting in less labor and time investment in facilitating post-transplant seedling recovery. Since horticultural plants have high IE, mycorrhizal inoculation can help plant growth and increase yields. Russo and Perkins-Veazie (2010) showed that average pod fresh weight for bell pepper plants developed from seedlings inoculated with beneficial AMF was greater than that from plants developed from conventionally grown seedlings.

4.2. The effect of mycorrhizal inoculation and P fertilization on P and Zn uptake

Since there is a weak effect of phosphorus addition on plant growth and mycorrhizal development, the level of P application for soil conditions such as these needs to be reconsidered. Also it seems that AMF application was very effective under high P1 levels treatment, relative to low P levels. It may be suggested that the high P levels used in the study were not high enough to suppress AMF colonization and contribution to the plant or inoculated or/and the indigenous mycorrhiza already in the soil are efficient in 100 kg P_2O_5/ha application. Under given field conditions, it has been estimated that a reduction of 80% of the recommended phosphate fertilizer could be supplemented by inoculation with AM fungi (Jakobsen, 1995).

In general, mycorrhizal inoculation had higher P and Zn concentrations under non-fumigated conditions than under fumigated ones. Since methyl bromide eliminated other beneficial soil organisms they may also have some effect on plant development.

4.3. The effect of soil fumigation and P fertilization on root colonization

Since non-inoculated plants grown in fumigated and non-fumigated plots have high root colonization, it seems indigenous mycorrhizas are efficient. Although there is a high root colonization ratio of non inoculated plants grown in fumigated soil, root colonization quality such as external hyphae, arbuscular-vesicular and the number of infection units are important points. It is a general problem in working with mycorrhiza to quantify the exact effect of root colonization ratio on plant growth. When plant roots grow through a soil profile, roots are infected with indigenous mycorrhiza. Results showed that non-inoculated plants grown in fumigated soils have up to 68% root colonization. It looks like the methyl-bromide treatment did not reduce the indigenous mycorrhizal propagules enough. As can be seen in Tables 8 and 10, horsebean and garlic plants grown in fumigation (disinfection) plots did not grow as well as in the non-fumigated plots. MBr is efficient in the surface layers, however is not effective in the deeper layer. Unfortunately this was not under the scope of the present study.

Since standardized guidelines are difficult, if not impossible to establish, it would be useful to thoroughly investigate mycorrhizal development on seedlings before and after transplanting in the field. Moreover, further studies are needed to provide standard practices for commercial production from greenhouse to field conditions. The results show that it is sounder to use mycorrhiza for horticultural plants than for field crops.

5. Conclusions

The 3-year experiment showed that under field conditions, mycorrhizal spores effectively infected garlic, horsebean, soybean, chickpea, melon, watermelon, cucumber, maize, cotton, pepper, eggplant and tomato plants. It seems that indigenous mycorrhizas can successfully infect plant roots, resulting in better plant growth and nutrient (P and Zn) uptake. However, the effect of mycorrhizal inoculation on plant growth changes with the effectiveness of the inoculum and with time. In general, horticultural plants such as melon, green pepper and eggplant showed higher IE than the field crops maize and cotton. The highly mycotrophic leguminous field crops, horsebean, chickpea and soybean also showed higher dependency for their growth on mycorrhizal colonization. Also garlic had high IE. Under field conditions, plants depend not only on mycorrhizal inoculation but also on P supply. IE was found to decrease to some extent with high P application.

The overall results revealed that in non-inoculated plots, yields were lower in fumigated plots than in non-fumigated ones. Although mycorrhizal inoculation increased some vegetable yield, this increase is not easily explained by better nutrient uptake by the AMF-colonized vs. non-colonized plants. Mycorrhizal inoculation may provide the plants with other benefits, such as protection against and control of disease and increasing plant resistance to soil-borne pathogens and environmental stress. After 3 years of study, it was concluded that for field crops, soil and plant management systems are advised, but for horticultural plants, mycorrhizal inoculation is the more beneficial practice.

Acknowledgements

This study was supported by the State Planning Organization (DPT). The author thanks Dr. Hinanit Koltai and Tamara Cosby Ortas for critical reading of the manuscript, Dr. Nebahat Sarı for supplying seeds and the valuable assistance of Derya Ortakci, Server Ercan, Okkes Kosse and Betul Ergun are gratefully acknowledged. Particular thanks are due to the unknown reviewer for suggesting replacement of the term Mycorrhizal Dependency with Inoculation Effectiveness.

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