



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

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## 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.

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## 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. Tawarayama 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%.

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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 Hacskeylo, 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 <sup>-1</sup>	318.8 ± 30.6	–*
Silt		360.9 ± 87	–
Sand		320.3 ± 23.0	–
Soil organic carbon – 1997	g kg <sup>-1</sup> 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 <sup>a</sup>	Cmol <sup>+</sup> kg <sup>-1</sup>	20.50 ± 2.00	–
pH	H <sub>2</sub> O	7.56 ± 0.66	7.45 ± 0.70
Salt	%	0.05 ± 0.00	0.05 ± 0.00
P	mg kg <sup>-1</sup>	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 <sup>-1</sup> 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.

<sup>a</sup> 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 (MB; 60 g m<sup>-2</sup>). 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) (MB; 60 g m<sup>-2</sup>). 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<sub>2</sub>O<sub>5</sub>/ha) were applied with and without

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