# Complementary technical information about compost inoculated with *Trichoderma harzianum*

The use of specific compost can be an attractive approach to reduce plant pathogens. They can be used as organic amendment that further to increase soil fertility, they can also control some pests (Pascual et al., 2002; Ros et al., 2005; Alabouvette et al., 2006;) or as a growing medium in horticulture (Bustamante et al., 2008; López-Mondéjar et al., 2010). The main advantages of compost use are the: 1) high content in organic matter and nutrients, 2) pathogens free, and 3) suppressive effect against phytopathogens (Ros et al., 2005; Segarra et al., 2007). Most of the compost posses some capacity to increase disease suppression in horticultural crops and improve plant health (Deepak et al., 2008; Ros et al., 2004; Borrero et al., 2004), but they have showed different efficacy depending on their specific biotic and abiotic characteristics (Ros et al., 2004; Albouvette et al., 2006). For this reason, if they are inoculated with suppressive microorganisms such *Trichoderma sp*. to improve its suppressive efficacy, facilitating microorganism survival and reusing waste materials (Lopez-Mondejar et al., 2010).

Species within the genus *Trichoderma* are some of the most widely-utilized fungal biological control agents in agriculture (Papavizas, 1985; Chet, 1987). The antagonistic effect by *Trichoderma sp.* has been demonstrated against a range of agriculturally-devastating phytopathogenic microorganisms, including those within the genus *Fusarium* (pathogen used in this study)*, Phytophthora, Sclerotinia, Rhizoctonia* and *Pythium* (Inbar et al., 1996*;* Schoeman et al., 1999; Aerts et al., 2002; Rojo et al., 2007; Almeida et al., 2007).

These fungi act as mycoparasites against competing fungi by secreting hydrolytic enzymes such as chitinase and glucanase, which break down cell walls (Kubicek et al., 2001). *Trichoderma sp.* also produces antibiotic compounds which influence the biocontrol capacity (Howell, 2003). Their rapid growth allows these species to directly compete for space and nutrients with phytopathogens (Sivan et al., 1989; Hjeljord & Tronsmo, 1998) while also indirectly fighting infection through stimulating plant growth and inducing acquired resistance mechanisms in the plant (Bailey and Lumsden, 1998).

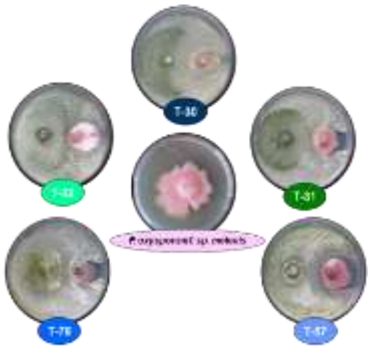


Figure 4. Mycoparasitism of five selected isolates of T. harzianum on F. oxysporum f. sp. melonis

*Trichoderma* sp. (T) Mycoparasiting *Rhizoctonia solani (R)* (source: Harman *et al*, 2004).

The mycoparasitic capacity of *Trichoderma* genera depends on the specie and in particular for the specific isolate (Markovich & Kononova, 2003). Isolates of *T. harzianum* with a high potential for the secretion of hydrolytic enzymes can be obtained naturally from compost and/or agricultural soil (Rincon et al., 2008) as it was selected the natural strain Trichoderma harzianum T78, that was isolated from a compost from a huge amount of different locations. It was characterized by its high capacity to survive in different organic materials (BernalVicente et al., 2009) and further its high recognized mycoparasitism action than permits to use it as biopesticide in a huge range of plant pathogens. Trichoderma suppress various disease-causing fungal pathogens, such as Fusarium sp. (e.g. Martinez-Medina et al. 2009, Martinez-Medina et al. 2010, Srivastava et al. 2010), Rhizoctonia solani (e.g. Chandanie et al. 2009), Colletotrichum (e.g. Zivkovic et al. 2010) Botrytis (e.g. Mehofer et al. 2009, rev. By Kohl 2009).

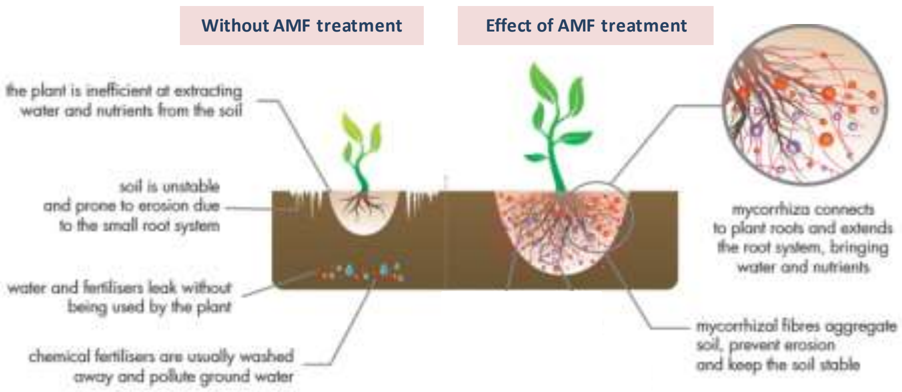

Plants of melón plants that have been infected with *F. oxysporum through rhizosphere, 1. treated with a commercial liquid Trichoderma strain and* **2.** treated with Trichoderma

Furthermore, it is known that plants have evolved a sophisticated immune system to "perceive" attack by alien organisms, and to translate this "perception" into an appropriate level of defense. The plant immune system is based on a complex signaling network that is highly flexible in its capacity to recognize and respond to the invader encountered, which involve a number of signaling molecules, including the action of the phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (Kunkel and Brooks 2002). Their signaling pathways differentially regulate defenses that are effective against specific types of attackers. Pathogens that require a living host (biotrophs) are commonly more sensitive to SA-dependent defense responses, whereas pathogens that kill the host and feed on the content (necrotrophs) and herbivorous insects are generally affected by JA- and ET-dependent defences. Interestingly, hormone-regulated defense pathways intimately cross-communicate in an antagonistic or synergistic manner, providing plants with a powerful regulatory capacity to quickly adapt to their biotic and abiotic environment and to utilize their resources in a cost-efficient manner (10). In some of the last results recently published by Dr Pascual research group at CEBAS-CSIC (MartinezMedina et al., 2009, 2010). it has been demonstrate the effect of Trichoderma harzianum T78 or the different AMFs used, and their interactions, on the plant different immune system, where different signalling compounds have been detected depending on the presence of a specific fungi or the interactions.

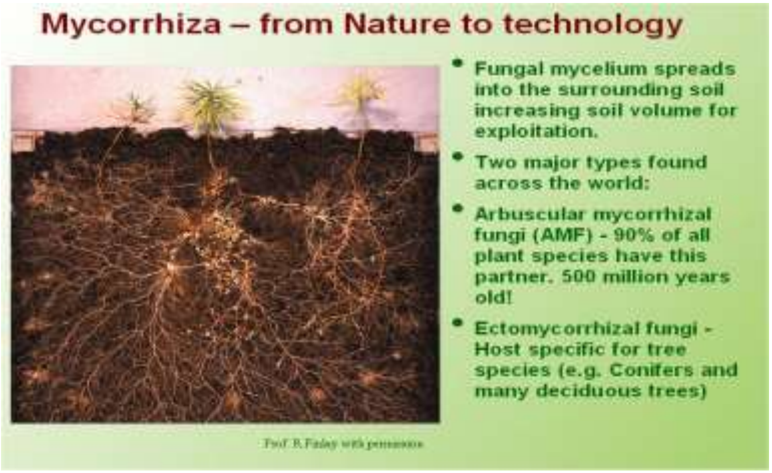
# Complementary technical information about *Mycorrhizal*

### *What are effects of ARBUSCULAR MYCORRHIZAL FUNGI (AMF)?*

### ARBUSCULAR MYCORRHIZAL FUNGI:



Some fungi naturally present in soil form a mutualistic relationship with the roots of the plants, called symbiosis. This partnership was invented by nature itself, to help plants grow better and be more resistant to environmental stress. Thanks to their incredible ability to connect to plant roots, the microscopic fungal fibres vastly extend the root system. They extract water and nutrients from a large volume of surrounding soil, and bring them to the plant, improving nutrition and growth. One of the most important abilities of mycorrhizal fungi is that they stay attached to the roots and support the plant **for its entire life**. Moreover, plants with mycorriza are often more resistant to diseases, such as those caused by microbial soil-borne pathogens, and are also more resistant to the effects of drought.



## Mycorrhiza effects on plants and current unique selling points

Mycorrhiza form a beneficial soil microbe–plant interaction that affects the physiological traits of many crop plants, including yield and food-quality. Recently, mycorrhiza have become a prospective tool for sustainable, low input crop produc systems. Mycorrhizas inhabit the ecosystems of terrestrial plants as a natural way to acquire nutrients from soils (Pirozynski and Dalpe 1992). The majority of crop plants form relationships with arbuscular mycorrhizal (AM) fungi, and thein responsiveness to mycorrhiza depends on numerous factors including genotype, soil-related and climate properties, and agrochemical inputs into the cultivation system. Extensive mycorrhizal research conducted during the last few decades has focused on the mechanisms of the symbiosis and its role in the management of a sustainable crop production where mycorrhiza could play an important role in combating these problems. (rev. by Vosátka and Albrechtová 2008).

Mycorrhiza's problem-solving potential applies to (1) increasing agronomic production, (2) enhancing carbon sequestration in terrestrial ecosystems to stabilize the atmospheric CO2 concentration, (3) converting degraded, polluted or desertified soils to restored land or sustainable agroecosystems, and (4) developing sustainable farming/cropping systems aimed at improving water use efficiency and soil properties to vombat increasing erosion and minimize risks of water pollution and eutrophication. Thus, AM fungi are regarded as essential components of sustainable soil–plant systems (Schreiner et al. 2003). As an ecological biofertilizer, a bioprotectant against environmental stresses, an agent controlling root pathogens and a soil improver acting as an anti-erosion agent, mycorrhiza possesses great potential in sustainable or organic agriculture. Moreover, it appears that its contribution to carbon sequestration is substantial; mycorrhizal fungi can be an important soil carbon sink and often constitute 20–30% of total soil microbial biomass (Leake et al. 2004). Under conditions of continuously increasing ambient CO2 concentrations, AM fungi are expected to increase their role.

## CHANGES IN FOOD QUALITY PROPERTIES OF PLANTS and TOLERANCE AGAINST ROOT PATHOGENS INDUCED BY MYCORRHIZA

One of the main potentials of mycorrhizas for sustainable agriculture is that they induce plant physiological changes that affect the quality and safety of food crops, including a higher production of antioxidants or essential oils, or reduced uptake of pollutants such as heavy metals to plant tissues (Toussaint 2007; Toussaint et al. 2007; Vosa´tka and Albrechtova´ 2008, Geneva et al. 2010). Although no clear mechanism other than an improvement in the nutritional status (mainly P) has been proposed (Toussaint 2007), yet the beneficial fungus–plant interactions has shown enhancement in productivity of crops by synthesizing an increased level of active compounds (Rai et al.

2001). For example, the suitable selection of host plant–fungus genotype led to an altered accumulation of essential oil levels in AM-colonized plants of Mentha arvensis (Freitas et al. 2004) and sweet basil Ocimum basilicum L. (Copetta et al. 2006, 2007; Toussaint et al. 2007). Thus, the use of mycorrhiza is being extended to herbal plants in order to get maximum benefits by the steadily increasing herbal medicine industry (Ernst 2000).

Recent research shows also increase in antioxidant properties of harvested plant parts what increases biological value of mycorrhiza in agricultural and horticulatural production.  For exapmle, It was found that the application of microbial consortia (Pseudomonas + Azotobacter + Azosprillum + AMF) treatment had the most effect on lycopene, antioxidant activity and potassium contents on tomato (Ordookhani et al. 2010). Tolerance to Fusarium wilt and anthracnose and the changes in antioxidative abilities in mycorrhizal strawberry plants were observed in strawberry (Fragaria x ananassa Duch., 'Nohime') plants inoculated with AMF (Li et al., 2010). Recently Symbio-m presented results from our ongoing research at conference of ongoing COST project in Evora (see the poster below, Latr et al. 2010).

Arbuscular mycorrhizal fungi (AMF) can act also as bioprotector agents against different pathogens, as illustrated below on experiments conducted in Spain by professor Barea. For example, AMF acts as bioprotector agents against wilt induced by Verticillium spp. in pepper (e.g. Goicoechea et al. 2010,


# Organic substrate by composting vineyard residues plus Th78 and implemented with AMFs

During previous research projects, the consortium of microorganisms Trichoderma-AMF was selected and optimised, at laboratory and field level, through a factorial analysis of the various consortia Th78-AMF under controlled conditions and was developed the organic substrate, and was jointly demonstrated the efficacy of the different products by the proposed trials.

The obtained microbial consortium Th-AMF, inoculated into the compost, proved a clear benefit on the crops tested, providing further growth of the plants due to a synergistic effect of the product. This effect is based in the higher mycorrhizal colonization of the consortium Th-AMF than the AMF alone, and in the strong bio-stimulating effect provided by the Trichoderma strain T78.