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ScienceDirect
Chemosphere 70 (2008) 1383–1389
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Saprobe fungi decreased the sensitivity to the toxic effect of dry olive mill residue on arbuscular mycorrhizal plants

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Received 25 June 2007; received in revised form 18 September 2007; accepted 18 September 2007
Available online 5 November 2007

Abstract

We studied the influence of olive mill dry residue (DOR) treated with saprobe fungi on growth of tomato and alfalfa colonized by *Glomus deserticola*. The application of 25 g kg^{-1} of dry DOR to soil decreased the shoot and root dry weight of tomato and alfalfa. Plants were more sensitive to the toxicity of DOR when colonized with the arbuscular mycorrhizal (AM) fungi. The sensitivity of both plants to the toxicity of DOR differed according to whether they were colonized by *G. deserticola* or by indigenous AM fungi. The phytotoxicity of DOR towards tomato and alfalfa was decreased by incubation the residue before planting with saprobe fungi for 20 wk. The beneficial effects of AM fungi on plant growth added with DOR incubated with saprobe fungi depend of the type of the plant and AM fungi. The contribution of AM fungi to the beneficial effect of DOR incubated with saprobe fungi varied according to the type of the plant and AM fungi. *G. deserticola* increased the shoot and root dry weight of plants when they were grown in the presence of DOR incubated with saprobe fungi for 20 wk. The beneficial effect of saprobe fungi on the dry weight and the level of AM mycorrhization of plants seem to be related to the decrease caused by these fungi in the phenol concentration in DOR. However, the toxicity of DOR due to substances other than phenols can not be ignored. The use of certain saprobe and AM fungi allows the possibility of using DOR as an organic fertilizer.
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Keywords: Arbuscular mycorrhizal; Biofertilizer; Dry olive mill residue; Phenols; Phytotoxicity; Saprobe fungi

1. Introduction

The importance of the olive mill industry in Mediterranean countries is well known and it is gaining importance in other countries outside the Mediterranean area. The two-phase extraction system is the most widely used technology to obtain oil and generates a semi-solid organic waste (alpeorujo) which is then dried and further extraction is carried out using solvents to obtain an extra yield of oil and a dry olive mill residue (DOR) (Vlyssides et al., 1998). The DOR, which has a firm, stackable consistency, is a lignocellulosic by-product with phytotoxic (Martín et al., 2002; Casa et al., 2003; Sampedro et al., 2005; Bonanomi et al., 2006) and antimicrobial properties (Moreno et al., 1987; Kotsou et al., 2004), and constitutes an environmental problem in the main olive growing regions.

Due to its content of organic matter and mineral nutrients, DOR might be employed for agronomic purposes (Paredes et al., 1999; Bonanomi et al., 2006). However, studies carried out with DOR have shown that it has notable antimicrobial and phytotoxic properties mainly due to its phenolic content (Moreno et al., 1987; Perez et al., 1992; Martín et al., 2002). Studies have shown that apart from monomeric phenols other substances can be implicated in the toxicity of DOR (Aranda et al., 2004). A decrease in the phytotoxic effect of DOR can be achieved by the use of saprobe fungi. Saprobe fungi are able to mobilize nutrients, degrade phytotoxic substances and to promote a more efficient use of the nutrients by the plant (Dix and Webster, 1995; Fracchia et al., 2000).

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0045-6535/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2007.09.033

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with higher plants in which the fungi receive carbon and, in return, transport phosphate to the host. Thus, the phosphorus status and the growth of the mycorrhizal plant are improved (Cooper and Tinker, 1978). Phosphorus fertilizers negatively affect the efficiency of the AM fungi colonization with a consequent negative effect on plant growth (Galvez et al., 2001; Grant et al., 2005). The presence of P in DOR has been observed (Sampedro et al., 2007).

Plants colonized by AM fungi show an enhanced resistance to plant against several abiotic factors such as drought, salt stress and heavy metals (Auge, 2001; Feng et al., 2002; Arriagada et al., 2005). However, AM fungi can increase the accumulation of toxic compounds such as heavy metals and pesticides in the plants (Ocampo, 1993; Arriagada et al., 2004; Gaur and Adholeya, 2004), and results from some experiments showed that AM fungi increase the phytotoxicity of DOR to plants (Martín et al., 2002). On the other hand, it has been described the presence of several heavy metals in DOR (Sampedro et al., 2007). Several experimental results confirm the existence of synergistic effects of saprobe fungi on plant root colonization by AM fungi and on the degree to which AM fungi improve plant resistance to heavy metals in soils (Arriagada et al., 2004, 2005; Fracchia et al., 2004; Martinez et al., 2004; Vogel-Mikus et al., 2005).

The objective of this work was to investigate whether the application of saprobe fungi to DOR (2 or 20 wk before planting) on its phenol, heavy metals, N and P content and the phytotoxicity of DOR. The influence of these compounds found in DOR on the colonization of tomato and alfalfa by AM fungi and on the efficiency of the symbiosis measured as its effect on shoot and root dry weight was also studied.

2. Materials and methods

2.1. Materials

DOR was collected from an olive oil manufacturer (Sierra Sur S.A., Granada, Spain) and stored at −20 °C until use. The main characteristics of DOR were as follows: total organic carbon, 58.5%; total nitrogen, 1.87%; total phosphorus, 0.21%; lignin, 24.7%; cellulose, 18%; hemicellulose, 11.4%; total phenols, 2.65%; total lipids, 0.2%; ashes, 9.2%. The most abundant elements, the concentration of which is reported in g kg^{-1} DOR were: potassium, 30.5; calcium, 13.6; magnesium, 3.8; iron, 1.1; sodium, 0.17; copper, 0.07; zinc, 0.06 and manganese, 0.04. The pH of the aqueous extract of DOR was 5.13. Deionized water was added to the solid residue to adjust its moisture content to 25% (w/w) prior to its sterilization (two cycles in autoclave at 120 °C for 20 min) and subsequent inoculation.

The saprobe fungi used were: *Fusarium lateritium* BAFC Cult. No. 2317, *Fusarium oxysporum* BAFC Cult. No. 738, *Paecylomices farinosus* BAFC Cult. No. F8846, *Coriolopsis rigida* (CECT 20449), *Poria subvermispora* (CBS 34763) and *Phanerochaete chrysosporium* IJFM A547 (ATCC 24725). Strains were stored in potato dextrose agar (PDA) and 2% malt extract agar (MEA) at 4 °C.

The soil used was a grey loam obtained from the field of the Estación Experimental del Zaidín (Granada, Spain). The soil had a pH of 8.1 in a 1:1 soil:water ratio. The P, N, K, Fe, Mn, Cu and Zn were determined by methods of Lachica et al. (1965) using H_2SO_4/H_2O_2 as extractants. $NaHCO_3$ extractable P was 6.2 mg kg^{-1}, N was 2.5 mg kg^{-1}, K was 132 mg kg^{-1}, Fe was 9.6 mg kg^{-1}, Mn was 110 mg kg^{-1}, Cu was 5.8 mg kg^{-1} and Zn was 5.7 mg kg^{-1}. The soil texture was 358 g kg^{-1} sand, 436 g kg^{-1} silt, 205 g kg^{-1} clay and 18 g kg^{-1} organic matter. Alfalfa (*Medicago sativa* L.) and tomato (*Solanum lycopersicum* L.) were used as tests plants.

Glomus deserticola Trappe, Bloss and Menge from the Instituto de Investigaciones Agrobiológicas de Galicia (CSIC) was used. The AM fungal inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores and colonized root fragments of *M. sativa* L. in 8 g per pot, which were predetermined to achieve high levels of root colonization. Uninoculated plants were given a filtrate (Watman No. 1 paper) of the inoculum containing the common soil microflora, but free of AM fungal propagules.

2.2. Experiments

Barleys seeds were inoculated with a thin slice of PAD (1 × 1 cm) with mycelia of a 14 d old culture of saprobe fungi grown at 28 °C. The incubation process was carried out in Erlenmeyer containing 125 g of DOR steam-sterilized three times, inoculates with three barleys seeds previously colonized by the mycelium for 7 d. Flasks were covered with sterile cotton plugs and incubated under static conditions at 28 °C for 2 and 20 wk. After the incubation with the saprobe fungi, the DOR was sterilized and added to soil in pots at concentrations of 0 and 25 g kg^{-1} soil.

The experiments were carried out in 0.3 l pots which contain soil which was either non-sterilized or steam-sterilized and mixed with sterilized quartz sand 1:1 by volume. Seeds were pregerminated and selected for uniformity prior to planting. Plants were grown in a greenhouse with natural light supplemented by Sylvania incandescent and cool-white lamps giving 400 nmol m^{-2} s^{-1} at 400–700 nm; there was a 16–8 h light–dark cycle at 25–19 °C and 50% relative humidity. Plants were watered from below, and fed with a nutrient solution at 10 ml per week (Hewit, 1952). Plants were harvested after 4 wk.

The experiment was designed as a complete randomised block with three factors. The factors were sterilized soil, sterilized soil inoculated with the AM fungi *G. deserticola* and non-sterilized soil. Each factor was designed considering two variables. Firstly, inoculation of DOR with saprobe fungi that contained eight treatments: plants without

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