DECREASE OF TOMATO TOXICITY CAUSED BY OLIVE MILL DRY RESIDUES USING ARBUSCULAR AND SAPROBE FUNGI

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ABSTRACT

We studied the influence of the olive mill dry residue (DOR) on the dry matter of tomato (*Lycopersicum esculentum*). The application of 60 g Kg^{-1} of DOR inhibited the dry weight of tomato plants. The inoculation of *C. rigida* decrease the phytotoxic effect of DOR after two weeks of incubation. The application of DOR incubated during 20 weeks with *C. rigida* to plant colonized with *G. deserticola,* increased the shoot dry weight of tomato compared with plants grown in the absence of DOR.

C. rigida was capable to eliminate the toxicity of DOR. The arbuscular mycorrhizal fungus *G. deserticola* was resistant to the actions of DOR and improve plant growth in presence of the DOR transformed by *C. rigida*. These results open the possibility of the use of DOR as organic fertilizer.
Keywords**:** *Arbuscular mycorrhizal fungi, Coriolopsis rigida, olive mill dry residue, phytotoxicity.*

INTRODUCTION

In the two stage centrifugation process to extract olive oil, a wet residue (80% of the weight of the olive) is obtained along with the oil, which is dried and extracted with solvents to obtain a dry residue (DOR). DOR might be used as fertilizer due to their high organic content, but as the majority of the by-products, DOR need to be treated to reduced or eliminate their phenolic compounds. Studies carried out with the olive mill wastewater obtained by the former process of extraction of olive oil have showed that the phenols contained in it were the principal causers of his great antimicrobial activity (Perez et al., 1992). These phenolic compounds can also have phytotoxic effect (Capasso et al., 1992).

Although contamination of soils with DOR can be a serious problem, their remediation may be possible using biological methods such as bioremediation with saprobe fungi. Saprobe fungi are important since they take part in the mobilization of nutrients and degradation of phytotoxics substances, they produce substances that promote or inhibit the growth of other rhizosphere microorganisms, add great amount of microbial biomass to the soil and also they contribute to the best use of the nutrients by the plant (Fracchia et al., 2000). It is known that these saprobe fungi use lignin and cellulose, secrete substances of industrial interest and are able to degrade toxic substances, including those of phenolic type (Madrid et al., 1996).

Arbuscular mycorrhizal (AM) fungi can improve plant growth by taking up relatively immobile nutrients suchs as phosphate (Barea and Jeffries, 1995). There is increasing evidence that phenolics are able to influence plant growth and root colonization. However, the role of phenolic in the AM symbiosis is not well established.

Addition of DOR to soil might result in increased of their colonization of root by AM fungi. However, these residues contain phenolics that may have negative effect on plant growth directly and/or through the decrease of AM symbiosis. The aim of this work was to study the influence of saprobe fungi on the effect that has the dry residues from olive on AM symbiosis and plant growth.

MATERIALS AND METHODS

Olive mill dry residue (DOR) was collected from an orujo manufacturer (Sierra Sur S.L., Granada, Spain).

Coriolopsis rigida was isolated from soil and transferred to tubes of 2% malt extract. Thins agar slices of MEA (1x1 cm^2) containing cells of *C. rigida* grown for 1 week at 28°C were used for DOR inoculation. The incubation process was carried out in glass jar containing 500 g of DOR sterilized three times, inoculated or not with the saprobe fungus. Static incubation was performed at 28°C for 0, 10 and 20 weeks. After the incubation with the saprobe fungus, the DOR was sterilized and added to soil pots at concentrations of 0, and 60 g Kg^{-1} soil. Sterilized DOR noninoculated with saprobe fungus was used as control.

The experiments were carried out in 0.3 l pots of soil from the Estación Experimental del Zaidín (Granada, Spain) which was steam-sterilized and mixed with sterilized quartz sand 1:1 by volume. Tomato (*Lycopersicum esculentum* L) plant were grown in a greenhouse under controlled conditions, watered and fed with a nutrient solution at 10 ml per week (Hewitt, 1952). Plants were harvested after 4 weeks and dry matter weight was determined. Part of the root were cleared and stained (Phillips and Hayman, 1970), and the percentage of root length colonization was measured (Giovannetti and Mosse, 1980).

RESULTS

The application of DOR decreased the shoot and root dry weight of tomate inoculated or not with *G. deserticola*. (Tables 1 and 2). The inoculation of the saprobe fungus decreased the phytotoxic effect of DOR in all the times of inoculation tested. *C. rigida* increased the shoot dry weight of tomato inoculated or not with *G. deserticola* after 2 weeks of incubation. (Table 1). Shoot dry weight of mycorrhizal and non mycorrhizal plants increase as the time of incubation with *C. rigida* increase. The application of DOR incubated with *C. rigida* during 20 weeks increased the shoot dry weight of tomato inoculated with *G. deserticola* compared with plants grown in the absence of DOR (Table 1). As table 2 shows the root dry weight of tomato also increased when DOR was incubated 2 weeks with *C. rigida*. Root dry weight increased compared with plants grown in the absence of DOR either in plants inoculated or not with *G. deserticola* (Table 2). The percentage of AM root length colonization of tomato inoculated with *G. deserticola* decreased in presence of DOR (Table 3). However the application of DOR incubated with *C. rigida* incubated during 10 or 20 weeks increase the percentage of AM root length colonization compared with plant growth with DOR.

***Table 1**. Shoot dry weights (mg) of tomato (Lycopersicum esculentum L.) inoculated or not with G. deserticola in presence of olive mill dry residue (DOR) incubated at different times with Coriolopsis rigida.*

Olive mill dry residue (DOR) treatments	Soil treatments					
	Sterilized soil			Sterilized soil plus *G. deserticola*		
	Time of incubation (Weeks)			Time of incubation (Weeks)		
	2	10	20	2	10	20
Without DOR	412	417	417	766	736	734
DOR non inoculated	10	11	10	24	21	23
DOR + *Coriolopsis rigida*	43	134	250	81	124	1000

LSD (DOR treatments) 16.27; LSD (soil treatments) 12.60; LSD (weeks) 12.60

Table 2. *Percentage of root length colonization in tomato (Lycopersicum esculentum L.) inoculated with G. deserticola in presence of olive mill dry residue (DOR) incubated at different times with Coriolopsis rigida.*

Olive mill dry residue (DOR) treatments	Soil treatments					
	Sterilized soil			Sterilized soil plus *G. deserticola*		
	Time of incubation (Weeks)			Time of incubation (Weeks)		
	2	10	20	2	10	20
Without DOR	158	154	141	216	218	207
DOR non inoculated	9	9	9	17	14	13
DOR + *Coriolopsis rigida*	19	93	205	34	63	254

LSD (DOR treatments) 5,63; LSD (soil treatments) 4,36; LSD (weeks) 4,36

Table 3. *Percentage of root length colonization in tomato (Lycopersicum esculentum L.) inoculated with G. deserticola in presence of olive mill dry residue (DOR) incubated at different times with Coriolopsis rigida.*

Olive mill dry residue (DOR) treatments	Times of incubation of DOR with *Coriolopsis rigida* (Weeks)		
	2	10	20
Without DOR	61	58	59
DOR non inoculated	44	49	41
DOR + *Coriolopsis rigida*	36	52	52

LSD (DOR treatments) 3.42; LSD (weeks) 2.64

DISCUSSION

Our results shows that the olive mill dry residues has phytotoxic properties. The application of 60 g Kg^{-1} of DOR inhibited the dry weight of tomato plants. Phenols are considered one of the main responsible of the toxicant effect of wastes on plant health (Wang et al., 2002). It is possible that the phenolic content of DOR could be the responsible of their phytotoxicity. Most of phenolic acids began to manifest their phytotoxicity at a concentration of 50 mg Kg^{-1} (Wang et al., 1967). The DOR has 6 mg Kg^{-1} of soluble phenolic compounds, thus the application to the soil of 60 g Kg^{-1} of DOR (360 mg Kg^{-1} of phenolic content) was expected to decrease the dry weight of plants.

It is known the role of microorganisms in the decrease of soil contamination by toxic residues from plants (Moreno et al., 1990). One of the main ways of the detoxification effects of microorganisms have been attributed to their capacity to metabolise phenols compounds (Wang et al., 2002). It is know that *C. rigida* was able to decrease the phenol content of DOR (Sampedro et al., 2004a). A correlation has been found between the decrease in phenol content of DOR by *C. rigida* and the increase of the dry weight of tomato plants as has been described with other fungi (Sampedro et al., 2004b). These results suggests that the decrease of phenolic content of DOR caused by *C. rigida* is important in the decrease of the phytotoxicity of DOR.

Arbuscular mycorrhizal fungi increase the sensitivity of plants to the phytotoxicity caused by the application of DOR (Martin et al., 2002). However, the addition of DOR to soil without incubation with *C. rigida* did not inhibited the shoot and root dry weight of tomato inoculated with *G. deserticola* compared with the noninoculated controls. As have been observed with other phytotoxic substances, the best application of DOR may explain their resistance to the phytotoxic action of this residue (Ocampo, 1993). The establishment of the AM symbiosis seems to depend in great measure on the concentration of phenolic compounds present in the soil. There is the possibility that *C. rigida* degrades certain phenols that are toxic for the arbuscular fungus and plants. Thus, DOR inoculated with *C. rigida* may decrease the phenolic compounds and may improve plant growth by taking up nutrient.

CONCLUSION

C. rigida was capable of transforming the DOR after its incubation for 2 weeks, so that the plants of tomato to which this transformed DOR was applied increased significantly its shoot dry weight compared with the plants of tomato to which did not apply DOR. These results open the possibility of the use of DOR as organic fertilizer.

Future studies will be carried out to optimise the transformation of DOR by saprobe fungi in order to obtain organic fertilizer.

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