PHYTOTOXICITY DECREASE OF WATER-SOLUBLE SUBSTANCES FROM OLIVE MILL DRY RESIDUE BY HYDROLYTIC ENZYMES PRODUCED BY SAPROBE FUNGI

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ABSTRACT

We study the influence of saprobe fungi on the toxic effect that has the water-soluble substances of dry residues from olive (ADOR) in the growth of the plant. All the saprobe fungi were able to decrease the phytotoxicity of ADOR, however the toxicity of this residue did not decrease in the same way. *Penicillium chrysogenum* was capable to reduce the toxicity of ADOR when this residue was applied at the highest dose of 15%. *Fusarium lateritum* was able to reduce the toxicity of ADOR when this residue was applied at the intermediate doses, however *F. oxysporum* decrease the phytotoxicity of ADOR only when the residue was applied at the lowest dose of 2.5%. All saprobe fungi tested produce endoglucanase and endopolymetylgalacturonase when were grown in presence of ADOR. A close relationship between the decrease of phytotoxicity of ADOR and the amount of hydrolytic enzymes by saprobe fungi were found. These results shows that hydrolytic enzymes can be important in the process of degradation of phytotoxic substances present in the dry mill oil residues.
***Keywords*:** *Hydrolytic enzymes, olive mill dry residue, phytotoxicity, saprobe fungi.*

INTRODUCTION

The agroindustrial residues derived from the extraction of oil from the olive constitutes an environmental problem due to its polluting capacity. The dry mill oil residue might be used as fertilizer due to their high organic content (Nogales et al., 1999), but as the majority of the by-products, the dry mill oil residue can be phytotoxic (Martín et al., 2002). The water-soluble substances of the dry mill olive residues can be responsible of their phytotoxicity. It is known that water-soluble materials liberated from plant residues are highly phytotoxic. Although contamination of soils with dry mill oil residue can be a serious problem, their remediation may be possible using biological methods such as bioremediation with saprobe fungi. These soil fungi are important since in general take part in the degradation of phytotoxics substances and also they contribute to the best use of the nutrients by the plant (Fracchia et al., 2000). Phenolic compounds seem to be the main causer of the phytotoxic effect of plant residues (Capasso et al., 1992). However, other substances such as cell wall fragments (oligosaccharides) and glycoproteins have toxic effects on plant health (Bucheli et al., 1990). Some hydrolytic enzymes degradatives of plant cell wall have been shown that can be involved in the detoxification of dry mill oil residue, however, no studies on the role of cellulase and pectinase have been carried out. Some saprobe fungi are able to produce cellulases and pectinases (Tribak et al., 2002). Thus the oil mill dry residue might contain water-soluble substances that may have negative effect on plant growth. The aim of this work was to study the influence of saprobe fungi on the effect that has the water-soluble substances of dry residues from olive in the growth of the plants.

MATERIAL AND METHODS

The saprobe fungi *Fusarium oxysporum*, *F. lateritium*, and *Penicillium chrysogenum* were

maintained in tubes of 2% malt extract at 4 ºC as stock cultives.

Olive mill dry residue was collected from an orujo manufacturer (Sierra Sur S.L., Granada, Spain). The aqueous extract of olive mill dry residue (ADOR) obtained as described Aranda et al., (2004) was used as grown media. The fungi were grown in ADOR at 25 ºC during 15 days. The culture liquid was separated from the mycelium by centrifugation (8.000 x g) and the supernatant was used to analyze the effect of the ADOR in the growth of the plants. Total phenolic contents of aqueous extracts of olive mill dry residue was estimated according to Riberau-Gayon (1968) using tannic acid as standard and expressed as g kg^{-1} of olive mill dry residue.

This experiment was carried out in 20 x 200 mm glass tubes with 15 ml of a Hewitt nutrient solution (Hewitt, 1952). Aqueous extract of olive mill dry residue after the incubation with the saprobe fungi were added to the rooting medium at final concentration of 0%, 2%, 5%, 10% and 15%. Tubes with tomato plants (*Lycopersicum esculentum* L.) after 15 days growth were harvested and shoot dry matter was determined. The aqueous extract of olive mill dry residue extracted as described above, after the incubation with the saprobe fungi, were assayed to determine endoglucanase (endo-GN) (EC 3.2.1.4) and endopolymethylgalacturonase (endo-PMG) (EC 3.2.1.15) activities. The hydrolytic activities were assayed by the viscosity method (Rejón-Palomares et al., 1996) using carboxymethylcellulose (CMC) and citrus pectin. Total proteins contents were determined by the method of Bradford (1976) using a Biorad kit with BSA as the standard.

RESULTS AND DISCUSSION

The application of ADOR, even at the lower dose of 2%, decreased the shoot dry weight of tomato (Fig. 1). None of the saprobe fungi tested eliminated the phytotoxicity caused by ADOR. Nevertheless, all saprobe fungi were able to decrease the phytotoxicity of ADOR when the inoculated residue was applied at dose of 2%. The incubation of *F. oxysporum* in ADOR did not affect its inhibitory effect on the shoot dry weight of tomato when was applied at the dose of 5% to the plant growth medium, however the incubation of the other saprobe fungi decrease the phytotoxic effect of ADOR at this dose. The incubation of ADOR with *F. lateritium* and *P. chrysogenum* decreased the phytotoxicity caused by the application of dose of 10%. Whereas only *P. chrysogenum* was able to decrease the phytotoxicity of ADOR when it residue was applied at the dose of 15%. Phenols are considered one of the main responsible of the toxicant effect of wastes on plant health (Wang et al., 2002). It is known that some microorganisms decreased soil contamination produced by toxic residues from plants (Moreno et al., 1990). One of the main ways of the detoxification effects of microorganisms has been attributed to their capacity to metabolise phenols compounds (Wang et al., 2002). The phenol content of ADOR was 22.2 ± 2.2 mg phenol per gr ADOR dry weight and 19.8 ± 1.8 after 14 days fungal incubation. Non significant decrease of phenol content of ADOR by any of the saprobe fungi tested after fortnight of fungal culture in presence of ADOR was observed.

In spite of the importance of hydrolytic enzymes on detoxification of plant residues (Benitez et al., 2002), there are not information available about the role of endoglucanases (endo-GN) and endopolymethylgalacturonases (endo-PMG) on waste phytotoxicity. All saprobe fungi tested produced endo-GN and endo-PMG when were grown in presence of ADOR (Fig. 2). *F. oxysporum* produced the lower quantity of hydrolytic enzymes specially endo-PMG, which did not reach 1 unit/mg of protein. *F. lateritium* produced higher endo-GN and endo-PMG activity than *F. oxysporum*. Finally *P. chrysogenum* produced the highest activity of all the enzymes. These results indicate that there was correlation ($r = 0.92$; $P = 0.008$) between the decrease of phyto-

toxicity of ADOR (Fig. 1) and the production of hydrolytic enzymes by the saprobe fungi (Fig. 2). In fact, *F. oxysporum*, which produced the lowest quantity of hydrolytic enzymes, was no able to decrease the phytotoxicity of doses of 5% of ADOR. Incubation with *F. lateritium* with more hydrolytic enzymes production than *F. oxysporum*, decreased the phytotoxicity of ADOR when was applied at the dose of 5% and 10% to the plant growth medium. *Penicillium chrysogenum*, which produced the highest quantity of hydrolytic enzymes, was able to decrease the phytotoxicity of 15% of ADOR.

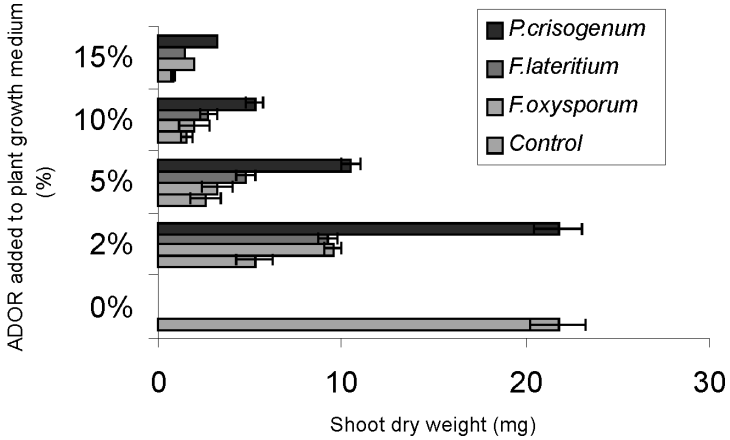

Figure 1. *Shoot dry weight (mg) of tomato (Lycopersicum esculetum L) cultivated in the presence of aqueous extracts from olive mill dry residues (ADOR) incubated with different saprophytic fungi.*

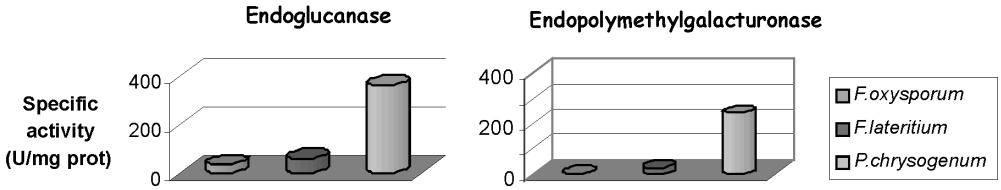

Figure 2. *Endoglucanase and endopolymethylgalacturonase activities produced by different saprophytic fungi grown on aqueous extracts from ADOR.*

CONCLUSIONS

Our results shows that hydrolytic enzymes can be important in the process of degradation of phytotoxic substances present in the dry mill oil residues. However we can not excluded that the fungi grown in ADOR dot not produce secondary metabolites that in a way favour plant growth independent of the ADOR effect. A detailed study under way in our laboratory will shed further light on the process of degradation of phytotoxic substances in the dry mill oil residues.

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