



Saprobic fungi decrease plant toxicity caused by olive mill residues

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Abstract

We studied the influence of dry olive mill residue (DOR), as affected by saprobic fungi on the growth of soybean (*Glycine max*) and tomato (*Lycopersicon esculentum*). The application of 60 g kg^{-1} of DOR decreased the dry weight of tomato and soybean plants mainly because of its phenolic content. The phytotoxicity of DOR decreased when it was incubated in the presence of almost all of the saprobic fungi tested. All saprobic fungi tested in our experiments were able to decrease the phenol content of DOR, and a correlation between the decrease in the phenol content of DOR by saprobic fungi and the increase in the dry weight of plants was found. However, the toxicity of these residues to either tomato or soybean plants did not decrease in the presence of all saprobic fungi in spite of a decrease in phenolic compounds. Some of the saprobic fungi tested eliminate completely the phytotoxicity of DOR. *F. oxysporum* 738 and *F. lateritum* were capable of transforming the DOR, after incubation for 20 weeks, so that the tomato plants to which this transformed DOR was applied increased significantly in shoot dry weight compared with the tomato plants which did not receive DOR. These results open the possibility of the use of DOR as organic fertilizer.

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1. Introduction

The expansion of agroindustry has led to major production of organic residues that constitute an environmental problem due to their polluting capacity. The disposal of olive mill wastewater, a by-product of olive oil extraction, is regarded as a serious environmental issue in the olive-growing regions (Ramos-Cormenzana et al., 1995). In recent years, a new process to extract olive oil has been developed that avoids the production of olive mill

wastewater (Alba et al., 1993). In this extraction process, 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 its high organic content (Nogales et al., 1999), but the majority of the by-products contain phenolic compounds (Martín-García et al., 1997). Phenolic compounds are widespread in the ecosystem and have a high potential for environmental pollution (Wang et al., 2002). Studies carried out with the olive mill wastewater obtained by the former process of extraction of olive oil have shown that the phenols contained in it can have phytotoxic effects (Capasso et al., 1992).

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Although contamination of soils with DOR can be a serious problem, their remediation may be possible using biological methods such as bioremediation with saprobic fungi. Saprobian fungi are important and common components of the rhizosphere soil from which they obtain nutritional benefits in the form of inorganic compounds and exudates from the root (Dix and Webster, 1995). These soil 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, they add great amounts of microbial biomass to the soil and they also contribute to the optimum use of nutrients by the plant (Dix and Webster, 1995; Fracchia et al., 2000). It is known that these saprobic fungi use lignin and cellulose, secrete substances of industrial interest and are able to degrade toxic substances, including those of the phenolic type (Madrid et al., 1996).

Thus, addition of DOR to soil might result in increased plant growth because of its nutrient content. However, these residues contain phenols that may have negative effect on plant growth. In this work, the influence of DOR on the growth of soybean and tomato and the influence of saprobic fungi on the decrease of the phytotoxicity caused by DOR will be examined.

2. Material and methods

2.1. Materials

Dry olive mill residue (DOR) was collected from an orujo manufacturer (COLGRA S.L., Granada, Spain). Total C, N, P, K, Fe, Mn, Cu and Zn content of the DOR were determined by Nogales et al. (1999). These were: 464 g kg⁻¹ C, 11.2 g kg⁻¹ N, 1 g kg⁻¹ P, 6.5 g kg⁻¹ K, 1.5 g kg⁻¹ Fe, 23 mg kg⁻¹ Mn, 14 mg kg⁻¹ Cu and 15 mg kg⁻¹ Zn.

The saprobic fungi used were: *Wardomyces inflatus* (Hennebert, 1968), *Trichoderma koningii* and *T. harzianum* (Rifai, 1969), *Paecilomyces farinosus* (Samsom et al., 1976), *Penicillium brevicompactum* and *P. chrysogenum* (Samsom et al., 1976), *Pleurotus ostreatus* (Domsch et al., 1980), *Fusarium oxysporum* 93, *F. oxysporum* 738, *F. graminearum*, *F. concolor* and *F. lateritum* (Booth, 1977). The fungi were isolated from soil by the particle washing method

(Widden and Bisset, 1972) using a multichamber washing apparatus. Thirty washings were necessary to remove sclerotia, spores, and other fungal structures from soil particles. Twenty soil particles (2 mm) were dried on sterilized filter paper and plated on 2% malt extract agar containing antibiotics (5 µg l⁻¹ streptomycin and 10 µg l⁻¹ tetracycline). The fungal isolates were transferred to tubes of potato dextrose agar (PDA) and 2% malt extract at 4 °C as stock cultures.

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 contents were determined by the methods of Lachica et al. (1965). These were 6.2 mg kg⁻¹ NaHCO₃-extractable P, 2.5 mg kg⁻¹ N, 132 mg kg⁻¹ K, 9.6 g kg⁻¹ Fe, 110 mg kg⁻¹ Mn, 5.8 mg kg⁻¹ Cu and 5.7 mg kg⁻¹ Zn. 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.

Soybean (*Glycine max*) and tomato (*Lycopersicon esculentum* L) were used as tests plants.

2.2. Determination of phenolic content

The phenolic content of the DOR was determined by extraction with distilled water (200 g l⁻¹) after agitation for 18 h. Total phenolic contents of extracts was estimated according to Ribereau-Gayon (1968) using syringic acid as standard and expressed as mg g⁻¹ of DOR.

2.3. Experiments

An aqueous suspension of fungal strains in sterile distilled water, containing approximately 7.5 × 10³ spores ml⁻¹ of each saprobic fungi, was prepared from cultures grown in PDA for 1 week at 28 °C. The incubation process was carried out in glass jars containing 500 g of DOR steam-sterilized three times, inoculated or not with 3 ml of the saprobic fungal spore suspension. Each saprobic fungus was inoculated separately. Static incubation was performed at 28 °C for 0, 2, 5, 10, 15 and 20 weeks. After incubation with saprobic fungi, the DOR was steam-sterilized and added to soil pots at concentrations of 0 and 60 g kg⁻¹ soil. Sterilized DOR uninoculated with saprobic fungi, and uninoculated plants or plants inoculated with each saprobic fungus in the absence of DOR were used as

controls. Plants were inoculated with 3 ml per pot of an aqueous suspension of 7.5×10^3 spores ml⁻¹ of each saprobic fungus prepared from PDA as described above. Before plant inoculation the fungal cultures were autoclaved at 115 °C for 25 min.

The experiments were carried out in 0.31 pots containing soil that was steam-sterilized and mixed with sterilized quartz sand 1:1 by volume. Plant 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 (Hewitt, 1952). Plants were harvested after 4 weeks and dry matter weight was determined.

2.4. Experimental design and statistical analysis

The experiment was designed as a 2 × 5 × 12 factorial with DOR application, incubation time and saprobic fungal inoculum type as factors. These data were subjected to factorial ANOVA followed by one way ANOVA to assess the effect on shoot and root dry weights of plants uninoculated or individually inoculated with sterilized suspensions of each saprobic fungus in the absence of DOR. Analysis by linear re-

gression between phenol content of DOR inoculated with saprobic fungi and dry matter of plants was also carried out. The mean values of five replicate pots were compared using the LSD test ($P = 0.05$).

3. Results

The application of 60 g kg⁻¹ of DOR to soil, without incubation with saprobic fungi, reduced the shoot ($F = 37.1$, $P = 0.003$) and root ($F = 29.3$, $P = 0.005$) dry weight of soybean plants (Tables 1 and 2). Inoculation with saprobic fungi decreased the phytotoxic effect of DOR on shoot ($F = 12.5$, $P = 0.005$) and root ($F = 19.3$, $P = 0.001$) dry weight of soybean. The reduction of phytotoxicity of DOR on shoot ($F = 5.1$, $P = 0.001$) and root ($F = 9.7$, $P = 0.005$) dry weight of soybean increased with the time of incubation. Inoculation of DOR with *T. koningii* and *P. farinosus* did not affect its inhibitory effect on shoot growth of soybean (Table 1). However, the phytotoxic effect of DOR on shoot dry weight of soybean was reduced by inoculation with *W. inflatus* ($F = 5.7$, $P = 0.009$), *T. harzianum* ($F = 9.9$, $P = 0.006$), *P. brevicompactum* ($F = 13.3$, $P = 0.001$), *P. chrysogenum* ($F = 18.2$, $P = 0.001$), *P. ostreatus* ($F = 9.0$, $P = 0.0009$), *F. oxysporum* 3 ($F = 4.9$, $P = 0.01$), *F. oxysporum* 738 ($F = 4.9$,

Table 1

Shoot dry weights (mg) of soybean (*Glycine max*) cultivated without and in the presence of 60 g kg⁻¹ of dry olive mill residue (DOR) incubated for different length of times with different saprobic fungi

| Saprobic fungi | Pots minus DOR | Time of incubation of DOR with saprobic fungi (weeks) | | | | | |
|--------------------------|----------------|---|-----|-----|-----|-----|-----|
| | | 0 | 2 | 5 | 10 | 15 | 20 |
| Not inoculated | 72b | 46a | 47a | 48a | 45a | 48a | 44a |
| <i>W. inflatus</i> | 87b | 45a | 47a | 83b | 89b | 86b | 90b |
| <i>T. koningii</i> | 77b | 47a | 32a | 51a | 46a | 47a | 50a |
| <i>T. harzianum</i> | 78b | 59a | 57a | 63a | 90b | 94b | 92b |
| <i>P. farinosus</i> | 78b | 55a | 60a | 53a | 47a | 55a | 60a |
| <i>P. brevicompactum</i> | 84b | 61a | 66a | 67a | 95b | 96b | 97b |
| <i>P. chrysogenum</i> | 86b | 44a | 47a | 47a | 98b | 97b | 95b |
| <i>P. ostreatus</i> | 78b | 50a | 43a | 48a | 76b | 86b | 78b |
| <i>F. oxysporum</i> 3 | 69b | 48a | 51a | 53a | 71b | 71b | 70b |
| <i>F. oxysporum</i> 738 | 69b | 49a | 53a | 64b | 67b | 70b | 72b |
| <i>F. graminearum</i> | 78b | 40a | 36a | 38a | 87b | 88b | 90b |
| <i>F. concolor</i> | 77b | 56a | 58a | 69b | 70b | 75b | 72b |
| <i>F. lateritium</i> | 76b | 53a | 55a | 67b | 68b | 70b | 70b |

Row values followed by the same letter are not significantly different as determined by one way ANOVA followed by the LSD test ($P = 0.05$).

Table 2

Root dry weights (mg) of soybean (*Glycine max*) cultivated without and in the presence of 60 g kg⁻¹ of dry olive mill residue (DOR) incubated for different length of times with different saprobic fungi

| Saprobic fungi | Pots minus DOR | Time of incubation of DOR with saprobic fungi (weeks) | | | | | |
|--------------------------|----------------|---|-----|-----|------|------|------|
| | | 0 | 2 | 5 | 10 | 15 | 20 |
| Not inoculated | 53b | 26a | 28a | 27a | 25a | 26a | 27a |
| <i>W. inflatus</i> | 58c | 27a | 28a | 44b | 45b | 46b | 40b |
| <i>T. koningii</i> | 60b | 28a | 28a | 25a | 27a | 28a | 27a |
| <i>T. harzianum</i> | 65c | 27a | 26a | 51b | 64c | 63c | 63c |
| <i>P. farinosus</i> | 60c | 23a | 24a | 25a | 29a | 30a | 28a |
| <i>P. brevicompactum</i> | 59b | 29a | 37a | 38a | 57b | 55b | 57b |
| <i>P. chrysogenum</i> | 55bc | 25a | 25a | 50b | 52b | 60c | 62c |
| <i>P. ostreatus</i> | 60c | 28a | 37a | 38a | 54b | 48b | 48b |
| <i>F. oxysporum</i> 3 | 65c | 24a | 26a | 44b | 55bc | 54bc | 65c |
| <i>F. oxysporum</i> 738 | 55c | 29a | 24a | 41b | 43b | 48b | 47b |
| <i>F. graminearum</i> | 53b | 25a | 25a | 53b | 51b | 52b | 54b |
| <i>F. concolor</i> | 60b | 26a | 32a | 65b | 62b | 68b | 60b |
| <i>F. lateritum</i> | 54c | 24a | 22a | 40b | 44b | 40b | 47bc |

Row values followed by the same letter are not significantly different as determined by one way ANOVA followed by the LSD test ($P = 0.05$).

$P = 0.01$), *F. graminearum* ($F = 7.3$, $P = 0.002$), *F. concolor* ($F = 4.9$, $P = 0.01$) and *F. lateritum* ($F = 5.3$, $P = 0.008$) but after different periods of incubation (Table 1). *W. inflatus*, *F. oxysporum* 738, *F. concolor* and *F. lateritum* reduced the phytotoxic effect of DOR on shoot dry weight of soybean after 5 weeks incubation whereas *T. harzianum*, *P. brevicompactum*, *P. chrysogenum*, *P. ostreatus*, *F. oxysporum* 3 and *F. graminearum* did so after 10 weeks incubation (Table 1). The phytotoxic effect of DOR on root dry weight of soybean (Table 2), was also reduced by inoculation with *W. inflatus* ($F = 14.2$, $P = 0.001$), *T. harzianum* ($F = 27$, $P = 0.0001$), *P. brevicompactum* ($F = 21$, $P = 0.0001$), *P. chrysogenum* ($F = 11.5$, $P = 0.003$), *P. ostreatus* ($F = 9.0$, $P = 0.0009$), *F. oxysporum* 3 ($F = 25$, $P = 0.0002$), *F. oxysporum* 738 ($F = 12$, $P = 0.004$), *F. graminearum* ($F = 14$, $P = 0.0001$), *F. concolor* ($F = 5.9$, $P = 0.005$) and *F. lateritum* ($F = 12$, $P = 0.006$) after different periods of incubation. As Table 2 shows the phytotoxic effect of DOR on root dry weight of soybean was reduced when it was incubated for 5 weeks with *W. inflatus*, *T. harzianum*, *P. chrysogenum*, *F. oxysporum* 3, *F. oxysporum* 738, *F. graminearum*, *F. concolor* and *F. lateritum* and when it was incubated for 10 weeks with *P. brevicompactum* and *P. ostreatus*. Incubation of DOR with *T. koningii* and *P. farinosus* did not decrease its phytotoxicity to the roots of soybean (Table 2).

There were no significant differences in shoot and root dry weight between uninoculated soybean and soybean inoculated with a sterilized suspension of saprobic fungi in the absence of DOR (Tables 1 and 2).

The shoot ($F = 79.7$, $P = 0.0009$) and root ($F = 94.8$, $P = 0.0006$) dry weight of tomato was decreased by the application of 60 g kg⁻¹ of untreated DOR to soil (Tables 3 and 4). Inoculation with saprobe fungi decreased the phytotoxic effect of DOR on tomato shoot ($F = 80.3$, $P = 0.001$) and root ($F = 44.1$, $P = 0.001$) dry weight. The reduction of phytotoxicity of DOR to shoot ($F = 39.2$, $P = 0.002$) and root ($F = 21.3$, $P = 0.001$) dry weight of tomato increased with the time of saprobic fungal incubation. Table 3 shows that inoculation of DOR with *P. brevicompactum* and *F. oxysporum* 3 did not affect its inhibitory effect on of tomato shoot growth. However, the phytotoxic effect of DOR on shoot dry weight of tomato was reduced by inoculation with *W. inflatus* ($F = 43.6$, $P = 0.007$), *T. koningii* ($F = 87.7$, $P = 0.005$), *T. harzianum* ($F = 64.3$, $P = 0.001$), *P. farinosus* ($F = 48.1$, $P = 0.001$), *P. chrysogenum* ($F = 89.0$, $P = 0.0009$), *P. ostreatus* ($F = 125.2$, $P = 0.001$), *F. oxysporum* 738 ($F = 120.4$, $P = 0.0001$), *F. graminearum* ($F = 66.2$, $P = 0.003$), *F. concolor* ($F = 48.4$, $P = 0.001$) and *F. lateritum* ($F = 37.3$, $P = 0.007$) but after different periods

Table 3

Shoot dry weights (mg) of tomato (*Lycopersicon esculentum* L.) cultivated without and in the presence of 60 g kg⁻¹ of dry olive mill residue (DOR) incubated for different length of times with different saprobic fungi

| Saprobic fungi | Pots minus DOR | Time of incubation of DOR with saprobic fungi (weeks) | | | | | |
|--------------------------|----------------|---|--------|---------|---------|--------|--------|
| | | 0 | 2 | 5 | 10 | 15 | 20 |
| Not inoculated | 139.3c | 8.1a | 8.3a | 8.9a | 8.4a | 7.9a | 8.0a |
| <i>W. inflatus</i> | 139.0d | 9.3a | 12.3a | 18.3ab | 23.9abc | 31.9bc | 39.6c |
| <i>T. koningii</i> | 138.3e | 8.0a | 32.0b | 45.3bc | 57.3c | 78.6d | 87.6d |
| <i>T. harzianum</i> | 138.3e | 8.9a | 17.3ab | 34.0bc | 50.6c | 81.3d | 123.3e |
| <i>P. chrysogenum</i> | 140.1e | 9.2a | 13.3a | 40.6b | 68.0c | 95.0d | 105.0d |
| <i>P. farinosus</i> | 139.0d | 9.0a | 15.0a | 25.3ab | 41.3bc | 56.0c | 137.0d |
| <i>P. brevicompactum</i> | 132.0b | 8.1a | 9.5a | 9.3a | 9.6a | 13.6a | 20.3a |
| <i>P. ostreatus</i> | 135.3e | 8.1a | 37.3b | 60.0c | 80.0d | 82.6d | 12.36e |
| <i>F. oxysporum</i> 3 | 128.9b | 9.1a | 14.3a | 15.3a | 20.0a | 21.5a | 22.3a |
| <i>F. oxysporum</i> 738 | 132.0c | 8.8a | 16.3a | 26.4a | 55.7b | 67.2b | 175.6d |
| <i>F. graminearum</i> | 139.5d | 8.8a | 21.3ab | 38.6bc | 56.0c | 58.6c | 133.0d |
| <i>F. concolor</i> | 138.9d | 8.0a | 6.8a | 8.4a | 25.6b | 39.6b | 55.3c |
| <i>F. lateritum</i> | 135.9d | 9.7a | 16.5ab | 25.3abc | 50.3bc | 62.5c | 196.0e |

Row values followed by the same letter are not significantly different as determined by one way ANOVA followed by the LSD test ($P = 0.05$).

of incubation. *T. koningii* and *P. ostreatus* reduced the phytotoxic effect of DOR on shoot dry weight of tomato after 2 weeks incubation, *T. harzianum*, *P. chrysogenum* and *F. graminearum* after 5 weeks, whereas *P. farinosus*, *F. oxysporum* 738, *F. concolor* and *F. lateritum* decreased the phytotoxic effect of DOR on shoot dry weight of tomato after 10 weeks incubation. *W. inflatus* reduced the phytotoxic effect

of DOR on shoot dry weight of tomato after 15 weeks (Table 3). No significant differences were observed between shoot dry weight of tomato grown in the absence of DOR and plants grown in the presence of DOR incubated with *T. harzianum*, *P. farinosus*, *P. ostreatus* and *F. graminearum* for 20 weeks. The application of DOR incubated with *F. oxysporum* 738 and *F. lateritum* for 20 weeks increased the shoot dry

Table 4

Root dry weights (mg) of tomato (*Lycopersicon esculentum* L.) cultivated without and in the presence of 60 g kg⁻¹ of dry olive mill residue (DOR) incubated for different length of times with different saprobic fungi

| Saprobic fungi | Pots minus DOR | Time of incubation of DOR with saprobic fungi (weeks) | | | | | |
|--------------------------|----------------|---|-------|-------|--------|-------|-------|
| | | 0 | 2 | 5 | 10 | 15 | 20 |
| Not inoculated | 57b | 2.7a | 2.9a | 3.9a | 4.6a | 6.7a | 6.6a |
| <i>W. inflatus</i> | 61.4b | 3.4a | 2.5a | 2.7a | 2.6a | 4.4a | 4.4a |
| <i>T. koningii</i> | 62.2d | 3.6a | 3.9a | 10.1b | 10.2b | 10.1b | 20.2c |
| <i>T. harzianum</i> | 62d | 3.1a | 2.7a | 10.6b | 12.3b | 13.1b | 23.2c |
| <i>P. farinosus</i> | 60.1d | 4.6a | 5.2a | 6.3a | 16.1bc | 22.2c | 54.1d |
| <i>P. brevicompactum</i> | 60.3b | 2.9a | 2.4a | 3.4a | 3.5a | 3.6a | 3.4a |
| <i>P. chrysogenum</i> | 59c | 3.8a | 3.6ab | 10.9b | 11.7b | 13.1b | 13.3b |
| <i>P. ostreatus</i> | 62.1d | 5.6a | 6.1a | 13.3b | 14.1b | 16.2b | 30.1c |
| <i>F. oxysporum</i> 3 | 58b | 3.7a | 2.6a | 2.6a | 3.3a | 4.3a | 6.6a |
| <i>F. oxysporum</i> 738 | 59.2c | 5.2a | 6.3a | 7.1a | 21.6b | 26.3b | 60.2c |
| <i>F. graminearum</i> | 60e | 4.6a | 5.6a | 12.1b | 13.3b | 23.3c | 43.4d |
| <i>F. concolor</i> | 60d | 3.5a | 3.3a | 4.1a | 15.3b | 15.3b | 20.4c |
| <i>F. lateritum</i> | 57.5c | 2.8a | 3.6a | 7.1ab | 13.1b | 13.3b | 53.3c |

Row values followed by the same letter are not significantly different as determined by one way ANOVA followed by the LSD test ($P = 0.05$).

Table 5
Phenol content of olive mill dry residue (DOR) incubated for different length of time with different saprobic fungi (mg g^{-1})

| Saprobic fungi | Time of incubation of DOR with saprobic fungi (weeks) | | | | | |
|--------------------------|---|------|-------|-------|-------|------|
| | 0 | 2 | 5 | 10 | 15 | 20 |
| Not inoculated | 6.2a | 6.7a | 6.3a | 6.1a | 6.3a | 6.3a |
| <i>W. inflatus</i> | 6.1c | 3.9b | 3.7b | 3.2ab | 3.1ab | 2.4a |
| <i>T. koningii</i> | 6.2e | 2.5d | 1.9c | 1.5b | 1.3b | 0.5a |
| <i>T. harzianum</i> | 6.5e | 3.4d | 2.5c | 1.9b | 0.7a | 0.7a |
| <i>P. farinosus</i> | 6.5e | 5.1d | 4.1c | 2.8b | 2.1b | 1.1a |
| <i>P. brevicompactum</i> | 6.3c | 4.3b | 4.2b | 4.2b | 3.4a | 2.8a |
| <i>P. chrysogenum</i> | 6.6d | 2.8c | 2.3bc | 1.8b | 1.8b | 0.5a |
| <i>P. ostreatus</i> | 6.6d | 2.8c | 2.3bc | 1.5b | 1.5b | 0.6a |
| <i>F. oxysporum</i> 3 | 6.5c | 4.4b | 3.9b | 3.4ab | 3.6ab | 2.5a |
| <i>F. oxysporum</i> 738 | 6.7e | 4.4d | 3.3c | 1.5b | 1.1ab | 0.7a |
| <i>F. graminearum</i> | 6.2e | 3.1d | 2.4cd | 1.9bc | 1.1ab | 0.3a |
| <i>F. concolor</i> | 6.5e | 4.8d | 4.1cd | 3.1bc | 2.5b | 1.2a |
| <i>F. lateritum</i> | 6.1e | 4.2d | 2.9c | 1.6b | 1.5b | 0.6a |

Row values followed by the same letter are not significantly different as determined by one way ANOVA followed by the LSD test ($P = 0.05$).

weight of tomato compared with plants grown in the absence of DOR (Table 3). The phytotoxic effect of DOR on root dry weight of tomato (Table 4), was also reduced by inoculation with *T. koningii* ($F = 32.1$, $P = 0.0001$), *T. harzianum* ($F = 15.1$, $P = 0.002$), *P. farinosus* ($F = 41.3$, $P = 0.0007$), *P. chrysogenum* ($F = 8.4$, $P = 0.003$), *P. ostreatus* ($F = 79.2$, $P = 0.002$), *F. oxysporum* 738 ($F = 96.3$, $P = 0.005$), *F. graminearum* ($F = 14.4$, $P = 0.0001$), *F. concolor* ($F = 40.4$, $P = 0.005$) and *F. lateritum* ($F = 83.4$, $P = 0.0004$) after different periods of incubation. As Table 4 shows the phytotoxic effect of DOR on root dry weight of tomato was reduced when DOR was incubated for 5 weeks with *T. koningii*, *T. harzianum*, *P. chrysogenum*, *P. ostreatus* and *F. graminearum*, whereas the phytotoxic effect of DOR on root dry weight of tomato was decreased when DOR was incubated with *P. farinosus*, *F. oxysporum* 738, *F. concolor* and *F. lateritum* for 10 weeks. Incubation of DOR with *W. inflatus*, *P. brevicompactum* and *F. oxysporum* 3 did not decrease its toxicity to tomato roots (Table 4).

There were no significant differences in shoot and root dry weight of tomato inoculated or not inoculated with sterilized suspensions of saprobic fungi in the absence of DOR (Tables 3 and 4).

The total phenolic content of the DOR was 6 mg g^{-1} of phenolic compounds after water extraction. Incubation of DOR for 20 weeks at 28°C did not affect its phenolic content (Table 5). All saprobic fungi de-

creased the phenol content of DOR after 2 weeks of inoculation, and the phenol content diminish as the time of incubation increased.

The phenol content of DOR inoculated with saprobic fungi was strongly correlated with the shoot dry weight of soybean ($r = 0.75$, $P = 0.0001$) and tomato ($r = 0.73$, $P = 0.0005$). Less strong correlations between phenol content of inoculated DOR and the root dry weight of soybean ($r = 0.51$, $P = 0.001$) and tomato ($r = 0.56$, $P = 0.0001$) were found.

4. Discussion

Dry olive mill residue clearly has phytotoxic properties, as application of 60 g kg^{-1} of DOR to a soil sand mixture reduced the dry weight of tomato and soybean plants in the present study. Phenols are considered to be one of the main agencies responsible for the toxic effects of wastes on plant health (Fiestas Ros de Ursinos, 1986; Wang et al., 2002). It is possible that the phenolic content of DOR could be responsible for its phytotoxicity. Most phenolic acids begin to manifest their phytotoxicity at a concentration of 50 mg kg^{-1} (Wang et al., 1967). The DOR had 6 mg g^{-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 plant growth.

Microorganisms are able to decrease soil contamination caused by toxic residues from plants (Arfmann and Rainer, 1990; Moreno et al., 1990). One of the main mechanisms for the detoxification effects of microorganisms has been attributed to their capacity to metabolise phenolic compounds (Hamman et al., 1999; Wang et al., 2002). All saprobic fungi tested in our experiments were able to decrease the phenol content of DOR, and a correlation between the decrease in phenol content of DOR by saprobic fungi and the reduction of phytotoxicity of DOR on dry weight of tomato and soybean plants were found. These results suggest that the decrease in phenolic content of DOR caused by the saprobic fungi is important in bringing about the decrease in phytotoxicity. Most of the saprobic fungi tested decreased the phytotoxicity of DOR but after different times of incubation. These differences can be attributed to differences in the enzymatic machinery implicated in the degradation of phenolic compounds (Camarero et al., 1994; Giovannozzi Sermanni et al., 1994). However, the toxicity of DOR to tomato and soybean did not decrease in the same way in the presence of all saprobic fungi. For example, inoculation with *T. koningii* and *P. farinosus* diminished its inhibiting effect on tomato but not its phytotoxicity to soybean, whereas inoculation with *F. oxysporum* 3 and *P. brevicompactum* diminished its negative effect on soybean but not on tomato in spite of a decrease in phenolic compounds. It is known that some phenolic compounds have variable effects on the health of different plants (Wang et al., 1967). Therefore, there is the possibility that these saprobic fungi degrade certain phenols that are toxic to one of the test plant species but not to the other. Moreover, in certain circumstances when a phenolic compound present in complex mixtures is metabolised by a less adequate pathway, some toxic aromatic intermediate can be generated (Ramos and Timmis, 1987). Furthermore, the effect of phenolic substances upon plant growth would not be the same in different soils. The content of organic matter and pH of soils are important factors that influence the phytotoxicity of phenols (Wang et al., 1967).

Some of the saprobic fungi tested were capable of eliminating the toxicity of DOR. Among these *F. oxysporum* 738 and *F. lateritum* were capable of transforming DOR after incubation for 20 weeks, so that the tomato plants to which this transformed DOR was ap-

plied increased significantly in shoot dry weight compared with the plants of tomato that did not receive DOR. These results open the possibility of the use of DOR as an organic fertilizer.

Future studies will be carried out to optimise the transformation of DOR by saprobic fungi and their effects on plant growth will be tested in different soils in order to exploit the fertilizer value of this organic residue.

5. Conclusions

The dry olive mill residue (DOR) is toxic to soybean and tomato mainly because of its phenolic content. Almost all of the tested saprobic fungi were able to decrease the phenol content and the phytotoxicity of DOR. However, the phytotoxicity of DOR cannot be attributed solely to its phenolic content.

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