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Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes

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ABSTRACT

The phytotoxic and antimicrobial properties of olive mill wastes have been widely investigated and demonstrated over the past decade. However, their potential utilization as biodegradable pesticides against plant pathogens is still poorly understood. In this study, a series of laboratory bioassays was designed to test the inhibitory effects of sterile water extracts of two-phase olive mill waste (TPOMW) and TPOMW composts with different degrees of stabilization on several different plant pathogens. Fungicidal properties of TPOMW extracts, assayed in a microwell assay format, showed that the growth of *Phytophthora capsici* was consistently and strongly inhibited by all TPOMW extracts diluted 1:10 (w:v). In contrast, suppression of *Pythium ultimum* and *Botrytis cinerea* by the extracts was not as strong and depended on the specific TPOMW sample. Mature compost inhibited *P. capsici* and *B. cinerea* at dilutions as great as 1:50, w:v. Neither TPOMW nor TPOMW compost extracts were able to inhibit the growth of the basidiomycete root rot agent *Rhizoctonia solani*. In addition, studies were conducted on the allelopathic effects of TPOMW extracts on seed germination of four highly invasive and globally distributed weeds (*Amaranthus retroflexus*, *Solanum nigrum*, *Chenopodium album* and *Sorghum halepense*). Both the TPOMW and immature TPOMW compost extracts substantially inhibited germination of *A. retroflexus* and *S. nigrum*, whereas mature composts extracts only partially reduced the germination of *S. nigrum*. Finally, TPOMW extracts strongly inhibited egg hatch and second-stage juvenile (J2) motility of the root-knot nematode *Meloidogyne incognita*. However, only higher concentrations of stage-one and stage-two TPOMW compost extracts exerted a suppressive effect on both J2 motility and on egg hatch. The study shows the high potential of naturally occurring chemicals present in TPOMW and TPOMW composts that should be further investigated as bio-pesticides for their use in sustainable agricultural systems.

1. Introduction

Olive mill wastes are known to contain a number of biologically active substances. The phytotoxic and antimicrobial properties of these residues have been extensively investigated and are associated with the presence of phenolic compounds and free fatty acids (Obied et al., 2005). Several investigators have reported on the inhibition of plant and

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microbial growth by low-molecular-weight phenols present in olive mill wastes (DellaGreca et al., 2001; Fiorentino et al., 2003; Isidori et al., 2005), although high-molecular-weight polyphenols such as oleuropein or lignin-like polymers have also shown toxic activity (Aziz et al., 1998; Bisignano et al., 1999).

Hydroxytyrosol has been identified as one of the major natural phenolics present in olive mill wastes (Lesage-Meessen et al., 2001; Romero et al., 2002; Fiorentino et al., 2003). However, many compounds remain unidentified and there is still controversy about the exact type and amounts of phytotoxic components in olive residues. For instance, Gonzalez et al. (1990) found that the antibacterial activity of phenolic acids (tested separately and in mixtures) did not coincide with the inhibitory effect of olive mill waste waters. Furthermore, some researchers have found toxicity even after total extraction of phenols, suggesting that other chemical products contribute to the overall toxicity (Capasso et al., 1992; Greco et al., 2006).

Phytotoxic and antimicrobial properties of olive mill wastes have been frequently approached as a negative attribute that limits the beneficial re-use of such materials. Thus, several methods have been developed in the last years to degrade phenols in liquid and solid olive oil residues (Martirani et al., 1996; Linares et al., 2003; Greco et al., 2006). However, the biocide capacity of olive mill wastes could be utilized to suppress plant pathogens, which would open new opportunities for the recycling of these unique bioactive by-products.

In recent years olive mill technology has been aimed at saving water during the extraction phase. Thus, a new centrifugation system was developed which reduced by 75% the olive mill wastes. This system was launched in Spain with the denomination of "two-phase", because it produces two fractions: a semisolid sludge (called by various names: two-phase olive mill waste (TPOMW), alperujo, olive wet husk, or wet pomace) and a liquid (olive oil) (Roig et al., 2006).

Recycling of TPOMW as a soil amendment, either unprocessed (Brunetti et al., 2005; López-Piñeiro et al., 2006) or composted (Cayuela et al., 2004; Hachicha et al., 2006), represents a promising agricultural practice in Mediterranean agro-ecosystems. The large proportion of organic matter and valuable nutrients, especially potassium, make TPOMW a valuable resource for beneficial utilization, particularly in degraded agricultural soils. TPOMW also has been successfully assayed as a foliar fertilizer (Tejada and Gonzalez, 2004) and as a soil-less substrate in combination with peat (García-Gómez et al., 2002). However, despite its potential agronomic value, the phytopathogen suppression capacity of olive mill waste and the compost derived from it has been barely investigated.

Pre-plant pest control of agricultural soils to control nematodes, soilborne pathogens and weeds has been a common practice in many horticultural crops for decades. At present, the severe limitations on the use of methyl bromide (MeBr) prior to its complete phase-out have stimulated research on a variety of alternatives. Use of natural substances as alternatives to synthetic chemical pesticides is attractive because they may be less persistent and have fewer non-target, toxic impacts than traditional agrochemicals. In the case of olive mill wastes, some investigators have evaluated the recovery of biological activity in soil after olive mill waste application. Piotrowska et al. (2006) observed a complete recovery of seed germination 42 days after olive mill wastewater had been applied at 40 m^3 ha^{-1}. This demonstrated that olive mill wastes could be used as pre-plant bio-pesticide or in crops on which they have no phytotoxic effect.

Our hypothesis was that there is a series of naturally occurring chemical compounds present in olive mill wastes that could act against various fungi, weeds and nematodes which have negative effects on crops. This mixture of chemicals can suppress some plant pathogens but not others, so our aim was to perform a series of laboratory bioassays to identify some important and widespread pathogens sensitive to these chemicals. This study focused on TPOMW, as it is the primary by-product in Spain, and the system that modern mills are most widely implementing.

2. Materials and methods

2.1. Materials

2.1.1. Two-phase olive mill waste (TPOMW) and TPOMW compost

Four samples of TPOMW (A, B, C and D) were collected from an olive mill in southern Spain. The sampling was made at four times during the whole olive oil extraction period in order to get the maximum heterogeneity depending on the maturation of the fruits and extraction yields. The main chemical characteristics are given in Table 1.

A composting pile (35 tons fresh weight) was prepared by mixing two-phase olive mill waste, sheep litter (SL) (TOC/TN: 14.0) and grape stalks (GS) (TOC/TN: 43.1) on fresh weight basis: 45% TPOMW+45% SL+10% GS (equivalent to 30:60:10, on dry weight basis). The pile was aerated by windrowing as previously described by Cayuela et al. (2006) and irrigated regularly to maintain moisture above 40%. Samples (500 g) were collected at four different stages in the composting process by mixing five sub-samples (100 g each) obtained from different locations in the pile: Stage I: initial non-decomposed mixture (week 1, day 1); Stage II: thermophilic phase (week 8, day 52); Stage III: thermophilic phase (week 18, day 126); and Stage IV: final compost (week 31, day 218). Table 1 shows their most important chemical characteristics.

2.1.2. Preparation of TPOMW and TPOMW water extracts

All samples were air-dried and ground to 0.5 mm. The TPOMW and TPOMW compost deionized, distilled water (DW)-extracts were prepared as 1:10, 1:20 and 1:50 (w:v) dilutions. They were rotated continuously end-over-end for 2 h at room temperature. After centrifugation (3000 rpm), the extraction supernatants were filtered through sterile 0.20 μm polyethersulphone filters (Whatman, Clifton, NJ, USA) to remove bacteria and fungi from the extracts. Extracts were prepared prior to utilization in the bioassays.

2.2. Bioassays

2.2.1. Fungi bioassays

Fungal isolates used in the bioassays included: *Pythium ultimum* Trow. USDA PUZC, *Phytophthora capsici* Leonian USDA R599, *Botrytis cinerea* Pers. USDA #5, and *Rhizoctonia solani* Kuhn USDA RS2A. All isolates were obtained from working

Table 1 – Main chemical characteristics of two-phase olive mill wastes (TPOMW) and compost samples

	pH	EC (dS m^{-1})	WSC (%)	TN (%)	WSC/TN	Toxicity (%)	Lipid (%)	Phenols (g l^{-1})
TPOMW samples								
A	5.23^{b}	4.97^{a}	n.d.	1.3^{a}	n.d.	28^{b}	16.0^{a}	1.29^{a}
B	5.28^{c}	5.31^{b}	n.d.	1.1^{b}	n.d.	25^{b}	20.8^{b}	1.50^{b}
C	5.53^{d}	5.40^{b}	n.d.	1.2^{b}	n.d.	34^{a}	31.3^{c}	1.18^{c}
D	4.99^{a}	5.75^{c}	n.d.	1.1^{b}	n.d.	19^{c}	18.6^{d}	1.24^{ac}
Compost samples								
Stage I	7.35^{A}	4.95^{A}	4.48^{A}	1.4^{A}	3.31^{A}	26^{D}	6.4^{A}	0.30^{A}
Stage II	7.69^{B}	3.03^{B}	3.14^{B}	1.3^{B}	2.52^{B}	45^{C}	2.7^{B}	0.19^{B}
Stage III	8.73^{C}	1.78^{C}	1.27^{C}	1.0^{C}	1.23^{C}	61^{B}	1.4^{C}	0.14^{C}
Stage IV	8.85^{D}	1.91^{C}	1.17^{D}	1.4^{A}	0.81^{D}	96^{A}	0.5^{D}	0.12^{D}

EC: electrical conductivity; WSC: water soluble carbon; TN: total nitrogen; nd: not determined. Toxicity is expressed as percentage of growth of the aquatic plant *Lemna gibba* in 1:10 sterile extracts with respect to water control. Higher values indicate lower toxicity (Cayuela et al., 2007). Results represent the mean of three replicates. pH, EC and phenols represent the values for 1:10 and WSC in 1:20 water extracts (w:v). Lipids and TN are expressed on dry weight basis.
In a column values followed by the same letter are not significantly different according to S-N-K test ($P<0.05$). TPOMW and composts were independently statistically analysed. Therefore, TPOMW treatments (lower case letters) are not comparable with compost treatments (upper case letters).

culture collections at USDA-ARS-Beltsville, MD; they were grown for 7 days on half-strength Potato Dextrose Agar (PDA; Difco, Detroit, MI) from stock cultures maintained on the same medium at 25 °C. Subsequently, discs (6 mm) of colonized agar were cut from actively growing edges of the colonies using a sterile, stainless-steel cork borer. Discs were placed centrally in each well of sterile 24-well culture plates (BD Falcon, New Jersey, USA), with each well containing 1.0 ml of the test solution. Five replicates were prepared for each treatment. Sterile DW was included as a control. The microwell plates were incubated at 25 °C for 3–4 days. After the incubation period, two perpendicular diameters of the fungi were linearly measured in mm and the mean diameter was calculated. The percentage of suppression was calculated as: diameter in the test filtrate/diameter in the control × 100. In the case of *B. cinerea*, a four-degree range from 0 (no growth) to 3 (maximum growth in the control) was used.

2.2.2. *Weed seed bioassays*

Seeds of *Amaranthus retroflexus* L., *Solanum nigrum* L., *Chenopodium album* L., and *Sorghum halepense* (L.) Pers. were supplied and identified by Dr. John Teasdale (USDA-ARS-BARC). Weed seeds were surface-disinfected to avoid fungal and bacterial contamination by shaking 5 min with a mixture of ethanol: DW (70:30) and subsequently rinsed with sterile DW. Fifty seeds of each species per assay were homogenously distributed on two layers of sterile Whatman filter paper in 10 cm diam. Petri dishes (Fisherbrand, Fisher Scientific, Waltham MA) and 2.0 ml of sterile TPOMW or TPOMW compost DW-extracts was added. In order to maintain adequate moisture, an additional 1.0 ml of sterile DW was added every 48 h. Seeds were incubated in a growth chamber at 27 °C for 8 days (*C. album* and *S. halepense*), 11 days (*A. retroflexus*), and 18 days (*S. nigrum*); and were considered germinated with the emergence of the radicle. Three replicates were used per treatment. Germination percentage *G* (%) was calculated relative to the control sample containing only sterile DW without OMW or compost extract.

2.2.3. *Root-knot nematode bioassay*

Microwell assays were performed to determine the activity of TPOMW and TPOMW compost extracts against egg hatch and second-stage juvenile (J2) motility of *Meloidogyne incognita* using procedures similar to those described by Meyer et al. (2004). Thus, *ca* 200 nematode eggs were placed per well into TPOMW, TPOMW compost filtrates or sterile DW in 24-well culture plates. Each of the two bioassay trials consisted of five replicate wells per treatment. Motility was determined by counting the spontaneously moving hatched J2 in each well 7 days after eggs were placed in the filtrates. In every well the percentage of hatched eggs was calculated as: number of hatched J2/number of eggs originally placed in the well × 100. Averages were calculated considering each well as a replicate. Percentage of hatched eggs for a treatment was calculated as: average % of hatched J2 in the filtrate/average % hatch in control with sterilised water × 100. The percent motile J2 was calculated as: average % motile J2 in test filtrate/average % motile J2 in control with sterilised water × 100.

2.3. *Chemical analysis*

Electrical conductivity (EC) and pH were determined in a 1:10 (w/v) water-soluble extract. Total water-soluble phenolic substances were determined in 1:10 (w:v) water extracts by a modified version of the Folin–Ciocalteau method (Cayuela et al., 2006). The total lipid content was determined using the traditional method of extraction in a Soxhlet with diethyl-ether and later weighing. Total nitrogen (TN) was determined by automatic elemental microanalysis (NA 1500 Carlo Erba Instruments, Milan, Italy). Water soluble carbon (WSC) was measured in a compost extract (1:20 w/v) using a Total Organic Carbon (TOC) analyzer (Formacs^{HT} Skalar analyzer,

Breda, The Netherlands). The toxicity was assessed by the *Lemna gibba* bioassay (Cayuela et al., 2007).

2.4. Statistical treatment of data

Since data had unequal variances, a non-parametric test (Kruskal–Wallis analysis of variance (ANOVA) on ranks) was used to compare the different treatments (statistical software Sigmastat 9). The Student Newman Keuls Method was used to isolate differences among treatments.

3. Results

3.1. Fungicidal potential of TPOMW and TPOMW compost

Table 2 shows the growth percentages of pathogenic fungi when the different treatments were applied. The diameter growth of *P. capsici* was significantly affected by all TPOMW extracts, whereas a sample-dependent effect was observed for *P. ultimum* and *B. cinerea*. Sample C was the least inhibitory in the case of *P capsici* and *P. ultimum*, and sample B for *B cinerea*.

In the case of compost, *B. cinerea* was the most vulnerable fungus and the inhibition was total at all stages and all dilutions. Stage I extracts, i.e., the undecomposed materials, were inhibitory against *P. capsici*, but the suppressiveness decreased with increasing dilution. The inhibition was more effective with mature compost (stage IV), very high even at 1:50 dilution. Only stage I extracts, at 1:10 dilution, exerted a significant suppressive effect on *P. ultimum*. However, this suppression disappeared in the presence of more dilute extracts.

R. solani growth was not inhibited by any TPOMW or compost extract (data not shown). This points to the fact that the chemical compounds present in TPOMW, or in any stage of compost produced with it and a mixture of SL and GS, are not inhibitory to this fungus.

3.2. Allelopathic potential of TOMW and TPOMW compost

Fig. 1 shows the percentage of germination with respect to the water control for the four weed species treated with TPOMW (A) or TPOMW compost (B) at different stages of maturity. The strongest inhibitory effect was found for *A. retroflexus* (pigweed), in which germination was reduced more than 90% with the different TPOMW treatments. Compost extracts exerted a significant ($P<0.05$) suppressive effect on *A. retroflexus* germination during compost process stages I, II and III (4, 14, 43% respectively). However, no germination inhibition effect was observed with the final mature compost extract. Germination of *S. nigrum* was inhibited to a lesser extent by TPOMW varying from 48% (TPOMW C) to 90% (TPOMW D), depending on the sample. Neither *C. album* nor *S. halepense* were influenced by TPOMW or compost extracts, showing percentages of germination not statistically different from the control. A stimulatory effect was found for TPOMW D on the germination of *S. halepense*.

Table 2 – Growth percentages of pathogenic fungi treated with different dilutions of two-phase olive mill wastes (TPOMW) and composts, calculated with respect to controls

Treatment	Sample	Dilution	*P. capsici*		*P. ultimum*		*B. cinerea*	
TPOMW	A	1:10	0	*a	0	*a	0	*a
		1:20	0	*a	40	b	60	*c
		1:50	22	*b	120	b	67	*c
	B	1:10	0	*a	0	*a	33	*b
		1:20	0	*a	40	b	100	d
		1:50	0	*a	120	b	100	d
	C	1:10	0	*a	100	b	67	*c
		1:20	37	*b	100	b	33	*b
		1:50	93	c	120	b	33	*b
	D	1:10	0	*a	0	*a	0	*a
		1:20	0	*a	43	b	20	*b
		1:50	0	*a	115	b	47	*c
Compost	Stage I	1:10	0	*A	23	*A	0	*A
		1:20	36	*C	100	B	0	*A
		1:50	62	*D	120	B	0	*A
	Stage II	1:10	72	*D	80	B	0	*A
		1:20	79	*D	100	B	0	*A
		1:50	102	E	120	B	0	*A
	Stage III	1:10	99	E	100	B	0	*A
		1:20	101	E	120	B	0	*A
		1:50	102	E	120	B	0	*A
	Stage IV	1:10	0	*A	100	B	0	*A
		1:20	6	*B	120	B	0	*A
		1:50	10	*B	100	B	0	*A

Values followed by * significantly differ from the control with water. In a column, values followed by the same letter do not differ according to the S-N-K test ($P<0.05$). TPOMW and composts were independently statistically analysed. Therefore, TPOMW treatments (lower case letters) are not comparable with compost treatments (upper case letters). *R. solani* growth was not affected by the different treatments (data not shown).

3.3. *Nematicidal potential of TPOMW and TPOMW compost*

All TPOMW samples strongly inhibited egg hatch and J2 motility (Table 3). The hatch inhibition was reduced with increasing dilution, however all TPOMW treatments differed significantly from the control even at 1:50 ratio. J2 motility was nearly completely inhibited (<5%) by TPOMW extracts at all dilutions. Fig. 2 shows healthy nematodes (2A,B) and changes that occurred in eggs and J2 immersed in one of the active treatments, TPOMW B dilution 1:10 (2C,D). The treatment resulted in degradation of the internal structures of a number of eggs and J2 (2C,D), with large vacuoles forming in some nematodes (2D).

With regard to the TPOMW compost, only concentrated extracts of stages I and II significantly inhibited egg hatch. The J2 motility was also slightly diminished by the least diluted compost treatment from stage III, but only stage I compost was able to inhibit nematode motility when diluted to the maximum amount tested in this study. Mature compost did not exert any stimulatory or inhibitory effect on hatch or motility.

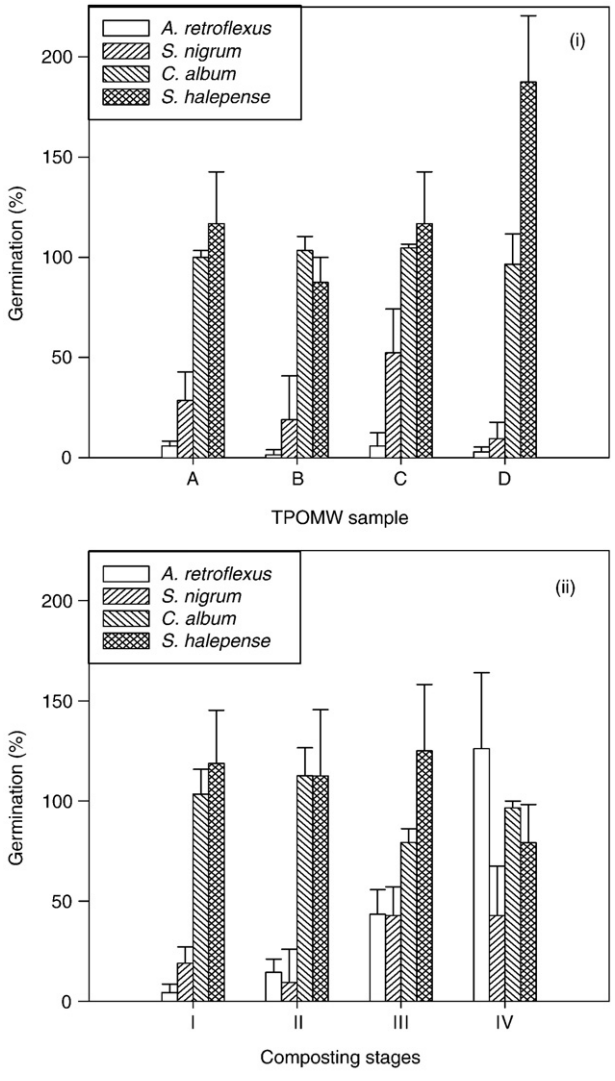

Fig. 1 – Germination percentages of different weed seeds treated with water extracts of two-phase olive mill wastes (i) and composts (ii), calculated with respect to water controls. Vertical bars represent standard deviations.

Table 3 – Percentages of egg hatch and second-stage juvenile (J2) motility of the root-knot nematode *Meloidogyne incognita* treated with different dilutions (dry weight to volume ratio) of water extracts from two-phase olive mill wastes (TPOMW) and composts, calculated with respect to water controls

Treatment	Sample	Dilution	Hatch (%)		Motility (%)	
TPOMW	A	1:10	11	*a	0	*a
		1:20	17	*b	1	*a
		1:50	34	*c	2	*a
	B	1:10	11	*a	0	*a
		1:20	25	*bc	0	*a
		1:50	47	*d	1	*a
	C	1:10	13	*a	0	*a
		1:20	40	*cd	1	*a
		1:50	65	*e	4	*a
	D	1:10	8	*a	0	*a
		1:20	10	*a	0	*a
		1:50	48	*d	2	*a
Compost	Stage I	1:10	44	*A	23	*A
		1:20	70	*B	30	*B
		1:50	92	C	41	*C
	Stage II	1:10	49	*A	37	*C
		1:20	90	C	53	*D
		1:50	116	C	90	E
	Stage III	1:10	60	C	53	*D
		1:20	127	C	84	E
		1:50	106	C	109	E
	Stage IV	1:10	94	C	86	E
		1:20	126	C	89	E
		1:50	128	C	86	E

Values followed by * significantly differ from the control with water. In a column, values followed by the same letter do not differ according to the S-N-K test ($P<0.05$). TPOMW and composts were independently statistically analysed. Therefore, TPOMW treatments (lower case letters) are not comparable with compost treatments (upper case letters).

4. Discussion

4.1. *Fungicidal potential of TPOMW and TPOMW compost*

P. capsici was the most vulnerable fungus to TPOMW water extracts. Only sample C, which showed the least toxicity according to the *Lemna gibba* test and the lowest concentration of phenols (Table 1) was unable to inhibit at high dilutions. In a recent study, Del Río et al. (2003) suggested that phenolic compounds were involved in the defence mechanisms of olive plants against *Phytophthora* sp., with tyrosol being the most active agent, followed by catechin and oleuropein and leading to greater suppression when they acted synergistically.

In the case of *P. ultimum* and *B. cinerea*, the TPOMW extracts showed variable results that were not correlated with any of the measured chemical characteristics of the residues and that might be related to specific compounds which had varying concentrations in the residues.

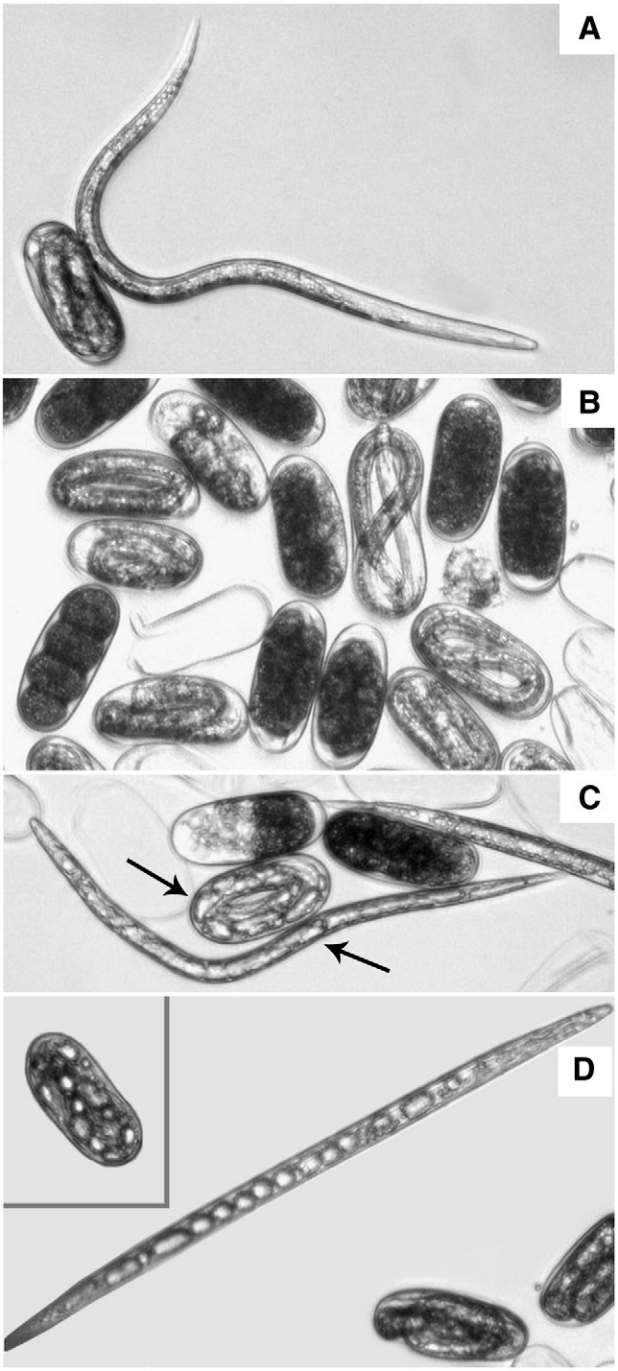

Fig. 2 – Eggs and second-stage juveniles (J2) of *Meloidogyne incognita* in water (A, B) and in a 1:10 two-phase olive mill waste (TPOMW):water extract (C, D). A healthy, active J2 (A) and viable eggs (A, B). Treated eggs and J2 with disrupted internal structures (C (arrows), and D); vacuolated J2 and egg (inset) (D). J2 and egg lengths ca. 395 μm and 94 μm, respectively, based on measurements of five J2 and eight egg specimens.

In our experiment, the inhibition of mature TPOMW compost extracts on the growth of *P. capsici* was noteworthy. Generally, the suppressive capacity of composts has been associated to several microbiologically-based phenomena such as competition for nutrients, parasitism and predation (Hoitink and Boehm, 1999). In our case, the higher inhibition capability of mature compost may be explained by antibiosis, i.e. the formation of toxic metabolites by some microbial communities that developed in the piles during the composting process. A clear example of this phenomenon is the case of *Pseudomonas* spp., which produces the antibiotic 2,4-diacetylphloroglucinol that is believed to be connected to suppression of several important plant pathogens (Weller et al., 2002).

Results with *B. cinerea* showed a trend opposite to that exhibited by the other fungi tested. While responses to the raw olive mill wastes varied with sample and/or dilution for *P. capsici and P. ultimum*, the TPOMW compost extracts were undoubtedly inhibitory to *B. cinerea* at all stages, even when diluted substantially. Experiments reported elsewhere recently have shown effective control of grey mould caused by *B. cinerea* by the use of compost extracts (Elad and Shtienberg, 1994; Welke, 2005). Thus, foliar application of TPOMW compost extracts might have some application with certain crops as both a biocontrol for *Botrytis* and as a foliar growth promoter.

No suppressive effect was observed for the basidiomycete root rot pathogen *R. solani*, which resists many biocontrol agents. However, other biotic mechanisms may explain the results found by Kotsou et al. (2004) who found that addition of olive mill wastewaters significantly suppressed growth of this root pathogen. The treatment of *R. solani*-infested soil with such wastewater is likely to have stimulated changes in the soil microbial populations favoring r-strategists that were hampering the growth and survival of *R. solani*.

The few studies in the bibliography that evaluated the fungicidal capacity of olive mill wastes showed, in many cases, contradictory results due to the large number of different factors that can influence the effectiveness of olive mill wastes as pathogen suppressors. In a recent study Bonanomi et al. (2006) demonstrated the phytotoxic effect of dry olive mill residue (DOR) on several crop species that in some cases led to an increase of fungal disease. It is important, therefore, to select non-sensitive crops to avoid a weakening of the plants as well as a selection of appropriate doses and time of application of the residue.

4.2. *Allelopathic potential of TOMW and TPOMW compost*

Recent studies indicated that phenols are one of the most important molecules accountable for phytotoxicity in olive mill wastes (DellaGreca et al., 2001; Fiorentino et al., 2003). Phenolic compounds influence physiological processes such as cellular expansion, membrane permeability, protein synthesis, respiration and enzymatic activity. In a recent study of the phytotoxicity of 15 phenolic compounds present in olive mill wastes, Isidori et al. (2005) showed that the most toxic compounds for one monocot and two dicots were catechol and hydroxytyrosol. However, olive mill wastes contain other organic compounds responsible for phytotoxicity, such as short chain fatty acids (C2–C8) and aldehydes which act as desiccants against some weed species (Coleman and Penner, 2006).

In our study, the most sensitive/vulnerable weed to olive mill waste was *A. retroflexus* (pigweed), which is considered one of the most persistent and noxious weeds to control.

Pigweed has an extensive range throughout the Mediterranean region because it easily adapts to a multitude of habitats, being able to grow on semi-arid conditions with resistance to both drought and salinity. The high germination inhibition produced by all TPOMW extracts was probably caused by phenolic compounds. Thus, Reigosa et al. (1999) reported a very strong (close to 100%) inhibition of *A. retroflexus* radicle growth at 10^{-2}M concentration of several phenols (coumaric, hydroxybenzoic, vanillic and ferulic acids) all present in olive mill wastes (Obied et al., 2005). They also reported a significant inhibition with diluted solutions (10^{-5} M). Composted olive mill waste extracts also were inhibitory to *A. retroflexus*, but only during early composting stages, when the concentration of phenols was still relatively high and the formation of intermediate decomposition compounds also contributed to high toxicity (Table 1).

The suppressive effect of olive mill waste on *S. nigrum* was also in agreement with the results of Reigosa et al. (1999) who found a strong inhibition against this weed for a variety of phenols at 10^{-2} M, but there were several cases of stimulatory response for lower concentrations. With respect to composted olive mill waste samples, it is notable that there was inhibition at all stages, even with the mature compost, which could be due to the high pH of the extracts.

In spite of the well-known phytotoxic properties of olive mill wastes, their agricultural utilization as weed suppressors has been rarely investigated. In general, the few laboratory or field experiments that estimate this prospective utilization have found positive results. For example, in a greenhouse experiment, Ghosheh et al. (1999) observed a complete inhibition of *Orobanche* spp. in bean, pea and tomato crops when olive husk was mixed with soil in potting media. In a field trial, Boz et al. (2003) studied the herbicidal effect of solid and liquid OMW against common weeds in wheat and maize crops, finding a high inhibition on the germination of *Portulaca oleracea* species.

Results in the present work showed that inhibition of seed germination was species-specific and therefore the toxic effect of these compounds should be thoroughly investigated for each crop-weed system.

4.3. *Nematicidal potential of TPOMW and TPOMW compost*

The significant hatch suppression exerted by all TPOMW samples applied to root-knot nematode eggs, even at high TPOMW dilutions, corroborates the existence of bioactive compounds able to pass through the nematode egg shell. However, the most remarkable effect was on J2 motility, which was inhibited more than 95% for all TPOMW samples at all dilutions. This intense antagonistic activity can play a key role in disease suppression, since application of TPOMW has the potential to suppress penetration of roots by J2, thereby disrupting the nematode life cycle. Chitwood (2002) reported that phenolics (pyrocatechol, caffeic acid, and vanillic acid) and fatty acid derivatives were among the phytochemical compounds with nematicidal capability. This fact seems to be corroborated in this study where sample C, which presented the lowest phenol concentration (Table 1), also produced the least inhibition.

TPOMW composting mixtures were able to inhibit egg hatch and J2 motility during compost stages I and II. However, the inhibition was reduced with increasing dilution. The non-inhibitory response of mature compost is in agreement with the results reported by Nico et al. (2004), who found limited nematotoxic activity of dry olive mark compost, suggesting that the decomposition process in compost eliminates the inhibitors. Thus, the mature product, while suitable for plants, is not capable of controlling root-knot nematodes in potting mixtures. They found different responses depending on the initial composting mixtures and suggested that the suppression was related to nematotoxic compounds (tannins or phenolic compounds) released from the composted material and was not due to the enhancement of microbial activity.

5. Conclusion and future research

The results from this laboratory bioassay study demonstrated the high potential of olive mill wastes as biobased pesticides against several species of fungi, weeds and nematodes. The high selectivity of the bioactive compounds in olive mill wastes demands specific research for each plant-pathogen system. Results reported here only show the abiotic capability produced by chemical compounds of TPOMW and composts for pathogen suppression, although the utilization of non-sterilised samples and compost teas would also imply biological mechanisms which could, in many cases, result in an increase in disease control.

Many compounds present in olive mill wastes could represent a promising option for the control of pests in the Mediterranean area, but field research is needed to evaluate effects on specific pest problems in particular cropping systems. In particular, the use of TPOMW extracts as pre-plant biobased pesticides or compost extracts as soil drenches may have applications in organic systems that rely on integrated and biologically based inputs.

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REFERENCES

Aziz NH, Farag SE, Mousa LAA, Abo-Zaid MA. Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios 1998;93:43–54.

Bisignano G, Tomaino A, Lo Cascio R, Crisafi G, Uccella N, Saija A. On the in vitro antimicrobial activity of oleuropein and hydroxytyrosol. J Pharm Pharmacol 1999;51:971–4.

Bonanomi G, Giorgi V, Giovanni DS, Neri D, Scala F. Olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogen. Agric Ecosyst Environ 2006;115:194–200.

Boz O, Dogan MN, Albay F. Olive processing wastes for weed control. Weed Res 2003;43:439–43.

Brunetti G, Plaza C, Senesi N. Olive pomace amendment in Mediterranean conditions: effect on soil and humic acid properties and wheat (*Triticum turgidum L.*) yield. J Agric Food Chem 2005;53:6730–7.

Capasso R, Cristinzio G, Evidente A, Scognamiglio F. Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable wastewaters. Phytochemistry 1992;31:4125–8.

Cayuela ML, Bernal MP, Roig A. Composting olive mill waste and sheep manure for orchard use. Compost Sci Util 2004;12:130–6.

Cayuela ML, Sánchez-Monedero MA, Roig A. Evaluation of two different aeration systems for composting two-phase olive mill wastes. Process Biochem 2006;41:616–23.

Cayuela ML, Millner P, Slovin J, Roig A. Duckweed (*Lemna gibba*) growth inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composting. Chemosphere 2007;68:1985–91.

Chitwood DJ. Phytochemical based strategies for nematode control. Annu Rev Phytopathol 2002;40:221–49.

Coleman R, Penner D. Desiccant activity of short chain fatty acids. Weed Technol 2006;20:410–5.

DellaGreca M, Monaco P, Pinto G, Pollio A, Previtera L, Temussi F. Phytotoxicity of low-molecular-weight phenols from olive mill wastewaters. Bull Environ Contam Toxicol 2001;67:352–9.

Del Río JA, Báidez AG, Botía JM, Ortuño A. Enhancement of phenolic compounds in olive plants (*Olea europaea* L.) and their influence on resistance against *Phytophthora* sp. Food Chem 2003;83:75–8.

Elad Y, Shtienberg D. Effect of compost water extracts on grey mould (*Botrytis cinerea*). Crop Prot 1994;13:109–14.

Fiorentino A, Gentili A, Isidori M, Monaco P, Nardelli A, Parrella A, et al. Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components. J Agric Food Chem 2003;51:1005–9.

García-Gómez A, Bernal MP, Roig A. Growth of ornamental plants in two composts prepared from agroindustrial wastes. Bioresour Technol 2002;83:81–7.

Ghosheh HZ, Hameed KM, Turk MA, Al-Jamali AF. Olive (*Olea europea*) jift suppresses broomrape (*Orobanche* spp.) infections in faba bean (*Vicia Faba*), pea (*Pisum sativum*) and tomato (*Lycopersicon esculentum*). Weed Technol 1999;13:457–60.

Gonzalez MD, Moreno E, Quevedo-Sarmiento J, Ramos-Cormenzana A. Studies on antibacterial activity of waste waters from olive oil mills (alpechin): inhibitory activity of phenolic and fatty acids. Chemosphere 1990;20:423–32.

Greco Jr G, Colarieti ML, Toscano G, Iamarino G, Rao MA, Gianfreda L. Mitigation of olive mill wastewater toxicity. J Agric Food Chem 2006;54:6776–82.

Hachicha S, Chtourou M, Medhioub K, Ammar E. Compost of poultry manure and olive mill wastes as an alternative fertilizer. Agron Sustain Dev 2006;26:135–42.

Hoitink HAJ, Boehm MJ. Biocontrol within the context of soil microbial communities: a substrate dependent phenomenon. Annu Rev Phytopathol 1999;37:427–46.

Isidori M, Lovorgna M, Nardelli A, Parrella A. Model study on the effect of 15 phenolic olive mill wastewater constituents on seed germination and *Vibrio fischeri* metabolism. J Agric Food Chem 2005;53:8414–7.

Kotsou M, Mari I, Lasaridi K, Chatzipavlidis I, Balis C, Kyriacou A. The effect of olive mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. Appl Soil Ecol 2004;26:113–21.

Lesage-Meessen L, Navarro D, Maunier S, Sigoillot JC, Lorquin J, Delattre M, et al. Simple phenolic content in olive oil residues as a function of extraction systems. Food Chem 2001;75:501–7.

Linares A, Cava JM, Ligero F, De la Rubia T, Martinez J. Detoxification of semisolid olive mill wastes and pine-chips mixtures using *Phanerochaete flavido-alba*. Chemosphere 2003;51:887–91.

López-Piñeiro A, Fernández J, Rato Nuñez JM, García-Navarro A. Response of soil and wheat crop to the application of two-phase olive mill waste to Mediterranean agricultural soils. Soil Sci 2006;171:728–36.

Martirani L, Giardina P, Marzulo L, Sannia G. Reduction of phenol content and toxicity in olive mill wastewaters with the lignolytic fungus *Pleurotus ostreatus*. Water Res 1996;30:1914–8.

Meyer SLF, Huettel RN, Liu XZ, Humber RA, Juba J, Nitao K. Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile motility. Nematology 2004;6:23–32.

Nico AI, Jimenez-Diaz RM, Castillo P. Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures. Crop Prot 2004;23:581–7.

Obied HK, Allen MS, Bedgood DR, Prenzler PD, Robards K, Stockmann R. Bioactivity and analysis of biophenols recovered from olive mill waste. J Agric Food Chem 2005;53:823–7.

Piotrowska A, Iamarino G, Rao MA, Gianfreda L. Short-term effects of olive mill waste water (OMW) on chemical and biochemical properties of a semiarid Mediterranean soil. Soil Biol Biochem 2006;38:600–10.

Reigosa MJ, Souto XC, Gonzalez L. Effect of phenolic compounds on the germination of six weeds species. Plant Growth Regulation 1999;28:83–9.

Roig A, Cayuela ML, Sánchez-Monedero MA. An overview on olive mill wastes and their valorisation methods. Waste Manag 2006;26:960–9.

Romero C, Brenes M, García P, Garrido A. Hydroxytyrosol 4-b-D-glucoside, an important phenolic compound in olive fruits and derived products. J Agric Food Chem 2002;50:3835–9.

Tejada M, Gonzalez JL. Effects of foliar application of a by-product of the two-step olive oil mill process on rice yield. Eur J Agron 2004;21:31–40.

Welke SE. The effect of compost extract on the yield of strawberries and the severity of *Botrytis cinerea*. J Sustain Agric 2005;25:57–68.

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Tomashow LS. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 2002;40:309–48.