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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