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Physico-Chemical, Microbiological, Agronomical, and Phytopathological Aspects in the Recycling of Olive Waste Composted Residues

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ABSTRACT

Olive mill wastes might impact plants, soil, microbial population, aquatic ecosystems and air media unfavourably. In the present work, olive waste composting confirmed to be a suitable microbial biotechnology for transformation of these by-products into organic fertilizers (cured composted residues) with no phytotoxicity, free of pathogens and able to improve soil fertility and plant production. In our experimental conditions, physico-chemical, biochemical, phytotoxicity and microbiological analysis confirmed the negligible agronomical qualitative characteristics of the cured composted residues. Moreover, olive mill residues after the composting process showed positive properties which suppressed soil-borne plant pathogens. Composted olive waste evidenced good suppressive activity against microsclerotia of *V. dahliae* and other fungal plant pathogens. Agronomical trials on tomato and sunflower crops showed that seed and fruit production was not decreased by the exclusive addition of compost. Compost amendment caused an increase in soil organic matter, although crop yield and plant growth increased only after mineral fertilization. Furthermore, olive yield and olive oil quality were not negatively affected by compost distribution. These results are encouraging for the agronomic use of composted residues in addition to mineral fertilizers. This may represent an initial phase to increase organic matter in the soil and possibly to reduce mineral fertilization. This process may be considered a new environmental opportunity for greater agriculture sustainability. All these aspects take into consideration that the application of good quality cured composts with high agronomic, microbiological and suppressive characteristics seem to be a very promising strategy for organic and integrated agriculture systems and for organic matter re-integration of soil.

Keywords: agri-food by-products, compost amendment of soil, disease suppressive effect, olive oil, yield and crop quality
Abbreviations: CAWE, compost A water extract; **CBWE**, compost B water extract; **MS**, microsclerotia; **OH**, olive husks; **OL**, olive leaves; **SGS**, standard growth substrate

INTRODUCTION

Conventional agriculture is characterized by using a great amount of chemical fertilizers, synthetic pesticides, and growth regulators, etc., resulting in heavy reliance on non-renewable resources, chemical residues in food, soil degradation, and health risks to farm workers handling pesticides, all of which bring into question the sustainability of the conventional farming system (Matson *et al.* 1997; Drinkwater *et al.* 1998; Tillman 1999; Zhu *et al.* 2000; Reganold *et al.* 2001; Xie *et al*. 2003). Chemical pesticides may provide highly effective pest control, but may also be harmful to the environment (García *et al*. 2004). The repeated use of chemicals encourages the development of resistance in pathogens and has negative effects on beneficial organisms (Goldman *et al*. 1994; Relyea and Hoverman 2006). Furthermore, some pesticides (e.g., metalaxyl) may enter groundwater supplies. Some chemicals, too, may predispose plants to greater damage from root pathogens (Cook *et al*. 1996). It is clear, then, that alternatives to these chemicals would be advantageous. Moreover, the ban of methyl bromide, the most effective and world-wide used pesticide for soil disinfestation, has stimulated research on the use of composts as alternative tools to control soil-borne plant pathogens which represent the major factor limiting the productivity of vegetal crops (Martin 2003; Lima *et al*. 2008a).

Biological control, the use of microorganisms to prevent plant diseases, offers an attractive alternative without the negative impact of chemical control. Therefore, biological control tactics have become an important approach to facilitating sustainable agriculture (Wang *et al*. 2002; Chae *et al*. 2006).

Olive tree (*Olea europea* L.) is one of the main crop in the Mediterranean basin with a cultivated area of about 8.2 Mha (IOOC 2009). The majority of the olive grove soils are also characterized by presenting very low levels of organic matter, and are exposed to progressive degradation processes. Organic matter application is therefore required to compensate this C deficiency and to improve soil characteristics. Thus industrial waste rich in C, such as olive mill residues may represent an interesting solution to this kind of soil degradation (Brunetti *et al*. 2005; Alburquerque *et al*. 2006; Roig *et al*. 2006; López-Piñeiro *et al*. 2008) and could play a fundamental role in the maintenance of the olive tree ecosystem, contributing to plant nutrition and/or soil conditioning (Kavdir and Killi 2008). Also for organic farming systems application to soils of organic amendments to improve the physicochemical and biological properties and thus their productiveness and natural fertility (Pascual *et al*. 1997), represents an interesting option, closing the cycle of residue-resource (Roig *et al*. 2006).

Due to its high carbon and nitrogen contents, olive mill wastes can be used as a soil conditioner/ fertilizers, amendment. Both the three-phase and the two-phase olive husks contains more than 94% organic matter and has the potential to increase organic matter contents of soils (Abu-Zreig and Al-Widyan 2002).

Even though the direct application of olive mill wastes

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on soil can be an approach of recycling nutrients and organic matter, it might cause unfavourable impact on plants (Capasso *et al*. 1992; Kachouri and Hamdi 2004; Niaounakis and Halvadakis 2006; Kavdir and Killi 2008), soil microbial population and activity (Paredes *et al*. 1987; González 1990; Riffaldi *et al*. 1993; Alburquerque *et al*. 2004; Kachouri and Hamdi 2004; Komilis *et al*. 2005), aquatic ecosystems (Della Greca *et al*. 2001) and even in air media (Rana *et al*. 2003) because of phenolic, fatty acid and mineral salts contents and high COD and BOD_5 (Martin *et al*. 2002; Alburquerque *et al*. 2004; Vlyssides *et al*. 2004; Roig *et al*. 2006; Boubaker and Ridha 2007; Kavdir and Killi 2008).

Composting of solid and liquid olive residues has been extensively examined as a reliable bioremediation treatment which allows the transformation of these by-products separately or mixed with agricultural, urban, zootechnical wastes into organic fertilizers (composts) with no phytotoxicity to improve soil fertility and plant production (Vlyssides *et al*. 1999; Paredes *et al*. 2000, 2001; Ranalli *et al*. 2001; Paredes *et al*. 2002; Ranalli *et al*. 2002; Principi *et al*. 2003; Klammer *et al*. 2005; Cayuela *et al*. 2006; Roig *et al*. 2006; Alfano *et al*. 2008). Additionally there is evidence in the literature which show that composts possess plant growth regulators and properties which suppress soilborne plant pathogens (Lumsden *et al*. 1986; Hoitink *et al*. 1997; Hoitink and Boehm 1999; Boulter *et al.* 2000; Abbasi *et al*. 2002).

Before the application of compost in the field, its nutritional value and possible negative effects need to be assessed through microbiologic and agronomic testing (Zhang *et al*. 2006). Soumaré *et al*. (2003) recommended the preventive characterization of composts before their use in agriculture, in particular regarding agronomic value (availability of elements) and contaminant content (heavy metals).

Tomato and sunflower production in Molise (south-central Italy), is largely based on chemical sources to supply nutrients for crop needs, and extensive tillage for land preparation prior to seeding.

Aim of the present work was: i) to submit olive mill and agricultural by-products at composting process; ii) to assess chemical and microbiological quality of residues composted (cured composts); iii) to assess *in vitro* and *in vivo* composts disease suppressive effect of *Verticillium dahliae* and other fungal plant pathogens; iv) to evaluate compost amendment effect on the fertility of soils cultivated with olive trees, sunflowers and tomatoes; v) to identify and compare the effects of compost combinations with inorganic fertilizers on maximizing tomato and sunflower yield in full field (a production area of south-central Italy), and, finally, vi) to assess the compost amendments effect on olive yield and olive oil quality.

MATERIALS AND METHODS

Composting trials

Two composting experimentations were carried out on olive mill residues:

Compost A, on small scale plant the composting trials were carried out in an olive mill farm located in Mafalda (Campobasso, Italy) during the olive oil campaign 2003/2004, on olive humid husks, olive leaves (OL), and other agricultural wastes as reported in Alfano *et al*. (2008). The composting experimentation was repeated in the following two olive seasons, with the same methodology cited.

Compost B, on semi-industrial scale plant the composting experimentation was carried out in the olive season 2006/2007 in an Agricultural farm located in S. Martino in Pensilis (Campobasso, Italy). The farm covers an area of 320 ha mostly cultivated with cereals (wheat, barley and corn), sunflower, beets, vegetables. Part of the area is used to produce forage and alfalfa necessary for a 1,000 Comisana sheep breeding. Seventy hectares are devoted to high density olive tree growing with oil cultivars (Gentile di Larino, Rosciola, Leccino, Peranzana). The farm also owns a continuous three phase olive mill.

A composting pile (35 m in length, 1.7 m in width, 1.1 m in height) was realized by mixing 31.5 tons (63.6% w/w) of olive husks to 6 tons of OL (12.1% w/w), 2.5 tons of wheat straw (5.1% w/w), 8 tons of liquid sheep slurry (16.2% w/w) and 1.5 tons of sheep manure (3% w/w) to facilitate the start up of the composting process. The process was carried out in a pilot scale plant on a concrete slab in a confined environment which used to be a sheep shelter. The composting process lasted 90 days, 60-days of forced bio-oxidation through pile mechanical turnover every 2-3 days, followed by a curing phase in static piles. Pile turning over operations were carried out with a forage mixer/grinding machine (Seko, mod. Samurai Double Mix, Curtarolo, Italy) that was useful in the farm, because used in the past for preparation of cattle forage. The machine is constituted by two endless screws with knives turning in opposite direction allowing forage grinding and mixing. The machine was loaded with the selected agricultural wastes with a front end loader.

Physical and chemical analyses of composts and soils

To obtain homogeneous samples (3.0 kg), one kg of cured compost was collected from three different depths (bottom, middle, top) of the pile and mixed. Soil samples (0-30 cm), before compost and fertilizer applications, were taken from each block. The samples were stored in polyethylene zipper bags in a cooled box (< 5°C) for transport, and the analyses were carried out within 24 h. Cured composts A and B were analysed for pH, moisture, organic matter (OM), electrical conductivity (EC), total organic carbon (TOC), humic and fulvic acids (HFA), humification rate (HR), total Kjeldahl nitrogen (TK-N), organic nitrogen (ON), organic C/total N ratio (C/N), NO_3-N, NH_4-N, P_2O_5, K_2O, Ca, Mg, Na, Pb, Cd, Ni, Zn, Cu, Hg, Cr according to DI.VAPRA-IPLA (1992). Soil samples were analyzed for pH, OM, EC, cation exchange capacity (CEC), TK-N, C/N, NO_3-N, NH_4-N, according to standard procedures (SISS 2000). Environmental and composting pile temperatures were measured and recorded by specific probes Testostor 179 mod. (Testo, Milano, Italy) placed under the covering roof and within the pile.

Microbiological analyses

Cured compost samples were analysed for total aerobic viable bacteria (TAB) on Standard Plate Count Agar (Difco), at 28 and 55°C for 48 h; eumycetes (EU) on Malt Agar (Difco) + rose bengal 33 mg/l and tetracycline 100 ml/l, at 28°C for 72 h; actinomycetes (ACT) on Actinomyces Agar (Difco), at 28 and 55°C for 48-72 h; aerobic spore-forming bacteria (SP) on Nutrient Agar (Difco), inoculation of sample after treatment for 10 min at 80°C, incubation at 28 and 55°C for 24-48 h; total coliform bacteria count (TC) on Bacto Desoxicolate Lactose Agar (Oxoid), at 37°C for 24 h; faecal coliform bacteria (*E. coli*) count (FC) on Bacto m-Fc Agar (Oxoid), at 45°C for 24 h; *Salmonella* spp. on Bacto Selenite Broth, (Oxoid) and SS Agar (Difco), at 37°C for 18 h and 24 h, respectively, following the method described by Ranalli *et al*. (2001).

Chitinolytic aerobic microorganisms (CHIT) were analyzed using mineral salt medium (MSM), (K_2HPO_4 2.5 g/l, KH_2PO_4 2.5 g/l, $(NH_4)_2HPO_4$ 1.0 g/l, $MgSO_47H_2O$ 0.2 g/l, $FeSO_47H_2O$ 0.01 g/l, $MnSO_4H_2O$ 0.0043 g/l, Agar 16 g/l, pH 7.0-7.4) added with 0.5% (w/v) chitin (Sigma-Aldrich Inc., St. Louis, MO, USA) as sole carbon source. The agarized MSM was sterilized, plated, inoculated and incubated for 5 days at 28°C.

Cellulolytic aerobic microorganisms (CELL) were analyzed using liquid MSM poured into bacteriological test tubes and using 0.5x8.0 cm strips of Whatman no. 1 filter paper as carbon source. Tubes were sterilized, inoculated and incubated for 15 days at 37°C. Tubes with visible dissolution of the macerated filter paper strips were assumed positive and the population was assessed through the Most Probable Number (MPN) according to Harrigan and McCance (1976). Analyses were carried out in triplicate and quantitative determinations were made on the basis of Colony Forming Units (CFU) in agarised media and according to the Most Probable Number (MPN) in liquid media. 95% confidence limit was calculated through standard deviation for plate counts and for

MPNs (Harrigan and McCance 1976). All results are expressed on a dry weight (dw) basis, after drying aliquots of the samples at 105°C for 48 h. Population of CELL are expressed as log/MPN/g dw, while all the others as log cfu/g dw.

ATP, enzymatic assays and germination index

ATP assay was performed using a specific enzymatic kit modified by Jago *et al*. (1989) (NMR/Lumit-Qm, code 9332-1; Lumac B.V., Landgraaf, The Netherlands) (Ranalli *et al*. 2002). A Biocounter 1550 P luminometer (Lumac B.V.) equipped with a photomultiplier tube ser at 7200 RLU with 200 pg ATP in 100 µl of Lumit-QM reagent was used (Ranalli *et al*. 1996, 1998).

Levels of enzymatic activity of the compost samples were determined using the "Api Zym" system (BioMerieux SA Marci l'Etoile, France) based in a colorimetric reaction that allow the conversion to amount of hydrolyzed substrates, as per manufacturer instruction. The test allows to obtain the rapid semi quantitative evaluations of 19 hydrolytic activity enzymes (Ranalli *et al*. 2002; Principi *et al*. 2003).Germination assay of selected seed (*Lepidium sativum*) in water solutions (50 and 75%) from sample waster extracts was measured (Zucconi *et al*. 1981; Ranalli *et al*. 2001).

In vitro disease suppressive effect of cured compost

In vitro disease suppressive effect of compost A was assayed on against seven fungal plant pathogens *Fusarium oxysporum*, *Phytophthora infestans*, *Pyrenochaeta lycopersici*, *Phytium debarianum*, *Pythium ultimum*, *Verticillium albo-atrum*, *Verticillium dahliae*, using PDA (Oxoid) amended with autoclaved or unautoclaved compost A water extracts (CAWE) according to Lima *et al*. (2008b).

Furthermore, *in vitro* antifungal effect was also assessed in liquid water-glucose (1% w/v of glucose) media, amended with 10% (v/v) of compost B water extract (CBWE). Two treatments were tested: T1 (media not amended with CBWE, control); T2 (media amended with CBWE) and flasks (4 replicates for each treatment) were inoculated with 10^5 conidia/ml of the fungal pathogen *Fusarium solani* (strain FP 96 from plant pathology mycological collection) incubated on a rotary shaker (120 rpm) at 23°C for 48 h. The suppressive effect CBWE was evaluated by microscope observations aimed at assessing the both percentage of fungal spore germination and germ tube elongation by counting a quantity of 100 conidia with a graduated micrometer (mod. BH2 Olympus, IT) as reported by Lima *et al*. (2006).

In vivo disease suppressive effect of cured compost

The suppressive effect of cured compost B was assessed in pot experiments against the fungal plant pathogen *Verticillium dahliae*. Experiments were carried out either in growth chamber and nursery (Vivai Verde Molise, Termoli, Italy), using a commercial standard growing substrate (SGS) mixed (v/v) with the experimental compost. The following three treatments were tested: 1) SGS 85% + Compost B 15% (SGS-CB); 2) SGS 100% Contaminated Control (SGS-CC); 3) SGS 100% Uncontaminated Control (SGS-UC). Treatment 1 and 2 were contaminated with 50 microsclerotia (MS) of *V. dahliae* g^{-1} of dry weight substrate. *V. dahliae* MS were obtained on agarized media according to Hawke and Lazarovits (1994). A completely randomized block design was used. Each treatment included a total 24 pots (3.5 liters) with 4 replications (blocks) of 6 pots per treatment. Trials were performed on potted mixtures without plant kept in growth chamber for 90 days under controlled conditions (12,000 lux, T 25°C, 70-80% RH). Nursery experiments were carried out using pot grown olive (*Olea europaea*) plants, cv. 'Leccino', from 2-years rooted cuttings. Immediately before planting into plastic pots, plants were wounded by removing few mm of the apical roots. To reduce stress of transplanting, plants were kept for 3 weeks in shadowed glass-house (RH ranging from 80-95% and T from 22-26°C) and then transferred outside in a nursery section under variable climatic conditions. Trials were conducted from May to August and climatic conditions were constantly monitored.

During both the growth chamber and nursery experiments the compost suppressive activity was evaluated by monitoring the density of *V. dahliae* MS in the substrate. To this aim, at 30 days intervals, soil sample from each pot was collected, air dried for 30 days and MS were assessed using a semi-selective media (Shetty *et al*. 2000). Moreover, periodically assessment were also performed to evaluate the *V. dahliae* symptoms on the canopy of olive plants by using an empirical scale with 4 degree of disease (data not show).

Agronomical trials

Two sets of agricultural trials were carried out. The first one was realized using Compost A, while the second used Compost B. The first set of agronomical trials was carried out in Mafalda (CB), Italy, in order to assess compost amending and fertilizing effect on olive grove soil properties and on olive yield and olive oil quality.

Within the property of the olive mill farm, about 4,000 m^2 flat land devoted to not-irrigated olive cropping was selected for the agronomical trials. Olive trees cv 'Leccino' and 'Moraiola', were cultivated at distance 8x6 metres. Within this area, two sub-areas of 30 × 60 m, separated by a 15 m-wide lane, were prepared. The first sub-area was amended with 10 t ha^{-1} of compost A, while the second served as control and was not subjected to fertilization nor amendment treatments. On April 2004, and in the following two productive seasons, 10 tons/ha of compost A produced in each olive season from olive mill and agricultural by-products (Alfano *et al*. 2008) was spread using a manure spreader machine. Immediately after compost distribution soils were ploughed. After 60 days from compost distribution, soils were sampled for the analysis of chemical parameters.

The second set of agricultural trials was carried out in the 2007-2008 growing season in two experimental field sites in the Molise Region (south-central Italy): Montorio nei Frentani (site 1, for sunflower) and Colletorto (site 2, for tomato). Colletorto and Montorio nei Frentani are positioned on the eastern side of the Apennines watershed, and have a typical mountainous Mediterranean climate of interior lands in south-central Italy.

The experiment was arranged in a randomized complete block design with four replications at each site and for each species. The three common treatments for the two species were: treatment 1 (an unfertilized and unamended control); treatment 2 (a crop amended with 3 t ha^{-1} of compost B) and treatment 3 (a crop amended with 10 t ha^{-1} of compost B). For sunflower the others treatments were: treatment 4 (only fertilized with inorganic N (90 kg ha^{-1}) and P (90 kg ha^{-1}), and unamended); treatment 5 (fertilized with inorganic N (90 kg ha^{-1}) and P (90 kg ha^{-1}), and amended with 3 t ha^{-1} of compost B) and treatment 6 (fertilized with inorganic N (90 kg ha^{-1}) and P (90 kg ha^{-1}), and amended with 10 t ha^{-1} of compost B); and for tomato the others treatments were: treatment 4 (only fertilized with inorganic N (270 kg ha^{-1}), P (170 kg ha^{-1}) and K (200 kg ha^{-1}), unamended); treatment 5 (fertilized with inorganic N (270 kg ha^{-1}), P (170 Kg ha-1)and K (200 kg ha^{-1}), and amended with 3 t ha^{-1} of compost B) and treatment 6 (fertilized with inorganic N (270 kg ha^{-1}), P (170 Kg ha^{-1}) and K (200 kg ha^{-1}), and amended with 10 t ha^{-1} of compost B). For tomato, inorganic manure was distributed by fertigation, while sunflower was a rainfed crop. The used varieties of tomato and sunflower (Early Magnum and Heroic, respectively) were selected and certified.

The soil texture was characterized as clay at site 2, and as clay-loam at site 1. In general, organic matter contents at all sites averaged 1.0%, being relatively lower at site 1 and higher at site 2. Total N content followed the ranking position observed for organic matter content. The soil profile was overall uniform, containing good amounts of available P (phosphorous, overall mean 25.5 µg g^{-1}) and medium quantities of exchangeable K (potassium, overall mean 133 µg g^{-1}). Soils had very low active $CaCO_3$, and pH was neutral to sub-alkaline (Colletorto-site 2, and Montorio nei Frentani site 1, in ascending order); salinity was low.

At site 1, each plot consisted of 6 rows of sunflower 4.44 m long spaced 75 cm apart (20 m^2). At site 2, each plot consisted of 6 rows of tomato 4.16 m long spaced 120 cm apart (30 m^2). Durum wheat (*Triticum durum* L.) was the previous crop in both cases. After ploughing (30 cm depth), 90 kg P ha^{-1} was applied during

land preparation. Planting of sunflower and tomato was done at 5 and 3 plants m^{-2}, respectively. Each field was surrounded by a buffer strip to allow for uniform growing conditions. Weeds were manually controlled.

Yield values were based on a hand made harvesting.

Assessment of olive oil quality

From the areas subjected to amendments with composted olive mill residues and from the control, mid ripened olives where hand harvested on November 2004 and in the two following olive seasons. Olives were worked and milled within 24 h using a two-phase extraction plant (Rapanelli, Foligno, Italy). Olive oils from the two theses were stored in 5 litres stainless steel tanks and analyzed for the main quality parameters: acidity, peroxides, spectrophotometrical indexes according to Commission Regulation EEC 2568/91 and its subsequent amendments and additions, and total polyphenols content according to (Owen *et al*. 2000).

Plant performance and grain yield

At sunflower floral emergence and at tomato harvest time, representative plants were sampled in each of the four replicate plots. On leaves of ten plants selected from the representative sample, the leaf area was measured with a planimeter (LI-3100, LiCor Inc., Lincoln, USA) and the whole plant dry weight obtained after oven drying at 75°C for 72 h.

At floral emergence (July), gas exchange was measured in the field with a portable gas analysis system (LI-6400, LiCor Inc., Lincoln, USA) equipped with a 6-cm^2 cuvette (Delfine *et al*. 2001). Photosynthetic rate (P_n) and stomatal conductance (g_s) were measured on fully expanded leaves, exposed to the same actual incident radiation and with the same surface temperature (across midday, at saturating PAR of about 1600 μmol m^{-2} s^{-1}). Each measurement was repeated on 7 plants for each replicate (n = 4); plants were randomly sampled between treatments to avoid diurnal effects on PSII and experiencing similar field conditions of light and vapor pressure deficit. At physiological maturity, the number of fruit $plant^{-1}$ was determined for 10 tomato plants in each plot. Sunflower was harvested by hand (seeds) on each of the four replicate plots; the thousand seeds weight was determined by means of handing procedure.

Statistical analysis

All data concerning microbial counts and physical-chemical responses were submitted for statistical analyses using the SAS statistical software package (SAS Institute 1997). Agricultural data were analyzed by ANOVA. Statistical significance was defined as $P<0.01$.

RESULTS AND DISCUSSION

Composting trials and composts characterization

All the composting pile developed temperatures higher than 55°C for more than three days according to the Italian Law (LD n. 22 of 5 February 1997).

Table 1 shows the results of microbiological analysis carried out on cured composts after 90 days of process. Values for compost A refer to the average of three composts obtained in three years of experimentation. Population of CELL are expressed as log/MPN/g dw, while all the others as log cfu/g dw.

Microbial population in cured composts seems to be quite similar for compost A and B.

The results show a relatively high microbial population of total viable bacteria 8.04 ± 0.80 and 8.93 ± 0.02 for compost A and B, respectively. Both composts show the presence of microbial groups that may be involved in antagonistic mechanisms (ACT 4.69 ± 1.2, SP 4.40 ± 1.1, CHIT 2.12 ± 0.4, CELL 2.21 ± 0.6) (El-Masry *et al*. 2002). Furthermore, TC, FC, SAL were not detected in both the composts due to the high temperatures developed during the processes which ensure matrixes hygienic safety.

Table 1 Microbial population in cured composts.

Microbial Groups	Mean values ± SD, n=3	
	Compost residues	
	A (small scale)	B (medium scale)
TAB	8.04 ± 0.80	8.93 ± 0.02
EU	6.69 ± 1.24	5.96 ± 0.05
ACT	6.95 ± 0.82	7.55 ± 0.30
SP	5.50 ± 1.28	4.94 ± 0.04
CHIT	4.14 ± 1.08	8.18 ± 0.05
CELL	4.29 ± 1.12	2.36 ± 0.51
TC	1.54 ± 0.4	2.36 ± 0.15
FC	0.00 ± 0.00	0.00 ± 0.00
SAL	0.00 ± 0.00	0.00 ± 0.00

Legend: total aerobic viable bacteria (TAB), eumycetes (EU), actinomycetes (ACT), aerobic spore-forming bacteria (SP), chitinolytic aerobic microorganisms (CHIT), cellulolytic aerobic microorganisms (CELL), total coliform bacteria count (TC), faecal coliform bacteria (*E. coli*) count (FC), *Salmonella* spp. (SAL).

Table 2 ATP content, enzymatic activity and Germination index of the cured composts after 90 days process.

Parameter	Mean values ± SD, *n*=3	
	Compost A	Compost B
ATP (ng/g dw)	10.02 ± 0.65	8.82 ± 0.10
Enzymatic activity (nmol hydrolyzed substrate)		
Alkaline phosphatase	233.25 ± 55.70	150.25 ± 25.50
Acid phosphatase	150.50 ± 50.20	250 ± 25.30
Phosphoamidase	175.25 ± 25.50	100 ± 25.10
Esterase-lipase (C8)	50.00 ± 25.00	50 ± 25.50
Leucine arylamidase	100.25 ± 25.75	200 ± 25.75
β-Glucosidase	200.75 ± 50.00	250 ± 25.89
Gi (%)	84.67 ± 9.45	97.43 ± 6.22

ATP content (**Table 2**) for both compost A and B (10.02 ± 0.65 and 8.82 ± 0.10 nmol hydrolyzed substrate, respectively) after 90 days are ten times lower than the values reached in the thermophilic phase of the composting process (data not shown). This could be probably due to the decreased microbial activity after 90 days of composting. Among the 19 tested enzymes, the highest activity (**Table 2**) was found for the phospho-hydrolases (alkaline and acid phosphatase), for phosphoamidase, esterase-lipase, arylamidase and β-glucosidase. Instead not found were the lipases, catalysts of long chain hydrolisis, valine arylamidase, cistine arylamidase, trypsin or chemotrypsin enzymes, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. Enzymatic activity decreased after the thermophilic phase of the process showing similar trends as ATP (data not shown). Final values of germination index (**Table 2**) show absence of residual phytotoxicity as both the composts show Gi higher than 75% (Principi *et al*. 2003).

Chemical characterization of composts

Chemical parameters of Composts are reported in **Table 3**. Composts show a neutral pH and a low EC. TOC is relatively high. HFA and HR show optimal values but lower than 50%. Values around 50% indicate good stabilization and curing of the composts. Furthermore heavy metals were not detected or detected in trace.

In vitro disease suppressive effect of cured compost

In vitro growth on solid media of seven fungal pathogens was consistently inhibited particularly by the un-autoclaved composts (**Table 4**) as previously evidenced by El-Masry *et al*. (2002). These seem due to the antagonistic activity exerted by the microflora developed on solid media amended with un-autoclaved CAWEs. Growth of *Pyrenochaeta lycopersici*, *Verticillium albo-atrum*, *Verticillium dahliae* (-39.0 ± 2.6%, -47.0 ± 2.8% and -46.0 ± 2.6%, respectively) was also inhibited by autoclaved CAWEs, this positive effect

Table 3 Chemical comparison between the cured composts.

Parameters	Compost A	Compost B	Italian DL 29/04/2006 n. 217
pH (1:10)	7.6 ± 0.3	7.8 ± 0.4	6-8.5
Moisture (%)	40.0 ± 5.3	36 ± 2.3	≤50%
OM (%)	5.3 ± 2.1	6.4 ± 0.3	--
EC (mS/cm)	5.7 ± 0.9	6.5 ± 0.7	n.a.
TOC (% d.w.)	55.5 ± 3.4	46.3 ± 5.5	≥30%
HFA (% d.w.)	11.02 ± 0.9	9.8 ± 0.3	≥2.5%
HR	0.34 ± 0.1	0.35 ± 0.3	n.a.
TK- N (% d.w.)	1.5 ± 0.4	2.8 ± 0.1	n.a.
ON (% d.w.)	1.31 ± 0.2	2.8 ± 0.2	≥80% of TKN
C/N	37.2 ± 8.4	16.5 ± 2.3	<50
NO_3-N (mg/kg)	25 ± 2.3	33 ± 3.3	n.a.
NH_4-N (mg/kg)	20 ± 1.3	17 ± 1.3	n.a.
P_2O_5 (g/kg)	6.0 ± 1.0	1.4 ± 0.8	n.a.
K_2O (g/kg)	13.0 ± 1.0	15.0 ± 2.4	n.a.
Ca (g/kg)	9.50 ± 1.4	11.2 ± 1.3	n.a.
Mg (g/kg)	0.67 ± 0.1	1.8 ± 0.1	n.a.
Na (g/kg)	2.1 ± 0.1	1.6 ± 0.1	n.a.
Pb (mg/kg)	4.2 ± 0.2	6.0 ± 1.3	≤140
Cd (mg/kg)	<0.1 ± 0.3	0.8 ± 0.1	≤1.5
Ni (mg/kg)	3.4 ± 0.0	2.5 ± 0.0	≤100
Zn (mg/kg)	6.8 ± 0.6	73.0 ± 0.3	≤500
Cu (mg/kg)	2.1 ± 0.3	43.0 ± 0.3	≤230
Hg (mg/kg)	<0.1 ± 0.0	<0.1 ± 0.0	n.a.
Cr (mg/kg)	<0.1 ± 0.0	<0.1 ± 0.0	n.a.

Table 4 Percentage of inhibition (-) or stimulation (+) of the *in vitro* growth of seven common fungal plant pathogens by water extracts prepared from three different composts from olive oil by-products.

Fungal pathogen	Autoclaved	Unautoclaved
Fusarium oxysporum	+7.0 ± 0.9	-68.0 ± 1.1
Phytophthora infestans	+12.0 ± 0.1	-63.0 ± 1.5
Pyrenochaeta lycopersici	-39.0 ± 2.6	-61.0 ± 0.6
Pythium debarianum	-6.0 ± 1.7	-55.0 ± 0.9
Pythium ultimum	0.0 ± 0.0	-67.0 ± 0.9
Verticillium albo-atrum	-47.0 ± 2.8	-68.0 ± 4.6
Verticillium dahliae	-46.0 ± 2.6	-68.0 ± 0.6

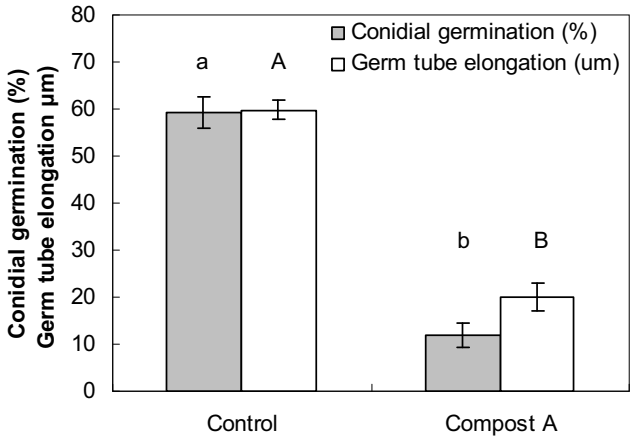

Fig. 1 Activity of CAWEs against *Fusarium solani* conidia germination and germ tube elongation in liquid media, after 48 h of treatment. Results are expressed as the mean of four replications ± standard deviation. Histograms surmounted by the same letter (a, b or A, B) do not significantly differ (P<0.01).

could probably due to the residual presence of microbial chemical antifungal compounds. Positive results were also obtained on liquid media. In the experiments against *F. solani*, CAWEs after 48 h induced the reduction of both percentage of conidia germination and germ tube elongation (**Figs. 1, 2**).

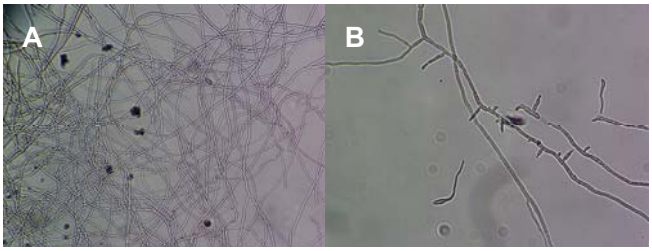

Fig. 2 Inhibition of germination and germ tube elongation of *F. solani* conidia. (**A**) Conidia grown on water-glucose liquid medium (control). (**B**) Conidia grown on water-glucose liquid media containing 10% of compost A (400X).

In vivo disease suppressive effect of cured compost

In vivo tests for compost disease suppressive were assessed either in climatic chamber (**Fig. 3**) and nursery (**Fig. 4**). Results of compost *in vivo* suppressive effect carried out in climatic chamber are reported in **Fig. 3**. After 90 days the number of viable MS of *V. dahliae* decreased by 83.6 and 18.8% for SGS amended with compost B (SGS-CB) and the contaminated control (SGS-CC), respectively.

Results of compost *in vivo* suppressive effect carried out in nursery on 2-years old olive tree potted in SGS amen-

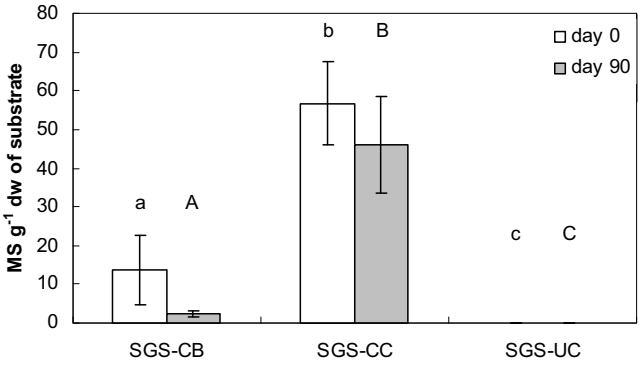

Fig. 3 Reduction of the number of viable *V. dahliae* MS using substrate (SGS) amended or not with 15% (v/v) of compost B after 90 days in climatic chamber. SGS-CB: Standard Growth Substrate + Compost B, SGS-CC: Standard Growth Substrate - Contaminated Control, SGS-UC: Standard Growth Substrate - Uncontaminated Control. Results are expressed as the mean of three replications ± standard deviation. Histograms surmounted by the same letter (a, b, c or A, B, C) do not significantly differ (P<0.01).

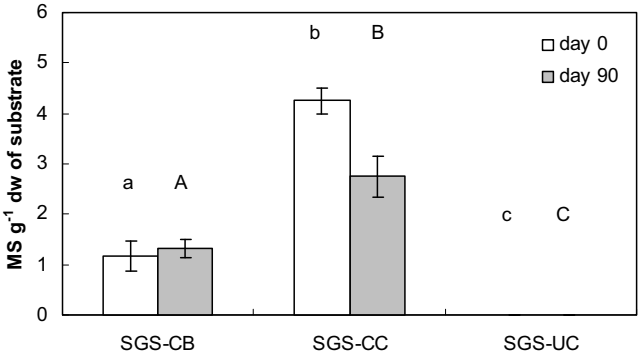

Fig. 4 Reduction of the number of viable *V. dahliae* MS using substrate (SGS) amended or not with 15% (v/v) of compost B after 90 days nursery trials. SGS-CB: Standard Growth Substrate + Compost B, SGS-CC: Standard Growth Substrate - Contaminated Control, SGS-UC: Standard Growth Substrate - Uncontaminated Control. Results are expressed as the mean of three replications ± standard deviation. Histograms surmounted by the same letter (a, b, c or A, B, C) do not significantly differ (P<0.01).

Table 5 Chemical characterization of soils after three years of amendments with composts.

Parameters	Trial 1 (2004-2006)		Trial 2 (2007-2008)		
	Soil A (control)	Soil A + Compost A (10 t/ha)	Soil B (control)	Soil B + Compost B (3 t/ha)	Soil B + Compost B (10 t/ha)
pH (1:10)	8.50 ± 0.7	8.43 ± 0.09	8.4 ± 0.2	8.5 ± 0.7	8.3 ± 0.8
OM (%)	2.50 ± 0.3	3.02 ± 2.14	1.1 ± 0.1	1.17 ± 0.5	1.7 ± 0.6
EC (mS/cm)	0.18 ± 0.11	0.31 ± 0.09	0.11 ± 0.02	0.15 ± 0.09	0.19 ± 0.03
CEC (meq/100 g)	16.80 ± 0.1	17.73 ± 1.6	200.3 ± 12.2	190 ± 11.2	220 ± 8.2
TKN (% d.w.)	0.34 ± 0.15	0.52 ± 0.57	0.92 ± 0.1	1.06 ± 0.3	1.21 ± 0.1
C/N	4.32 ± 1.5	4.60 ± 2.26	2.2 ± 0.9	2.60 ± 0.6	2.90 ± 1.6
NO_3-N (mg/kg)	54.2 ± 0.2	69.4 ± 0.4	60.3 ± 2.2	73 ± 3.2	95 ± 2.2
NH_4-N (mg/kg)	22.4 ± 0.5	25.8 ± 1.1	32 ± 3.2	33.3 ± 1.2	26.3 ± 0.9

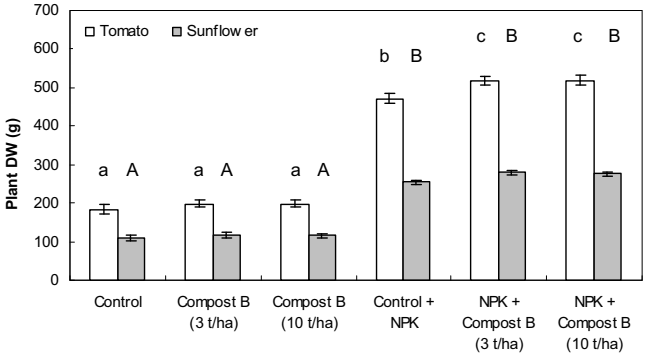

Fig. 5 Plant DW as affected by composting and fertilization treatments. NPK: mineral fertilization. Results are expressed as the mean of three replications ± standard deviation. Histograms surmounted by the same letter (a, b, c or A, B, C) do not significantly differ (P<0.01).

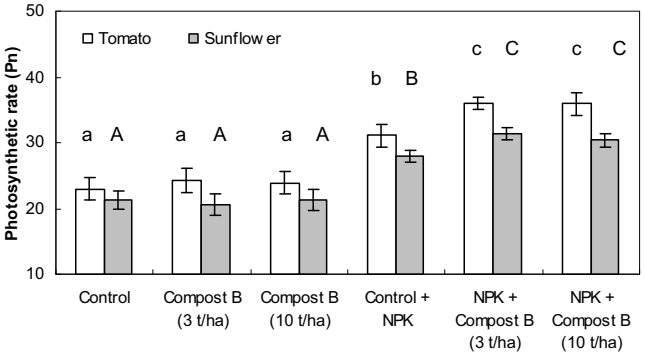

Fig. 6 Plant photosynthetic rate (Pn) as affected by composting and fertilization treatments. NPK: mineral fertilization. Results are expressed as the mean of three replications ± standard deviation. Histograms surmounted by the same letter (a, b, c or A, B, C) do not significantly differ (P<0.01).

ded or not with compost B are reported in **Fig. 4**. After 90 days the number of viable MS of *V. dahliae* decreased by 83.6 and 18.8% for SGS amended with compost B (SGS-CB) and the contaminated control (SGS-CC), respectively.

Agronomical trials

In **Table 5** are reported the results of the chemical analyses carried out on soils subjected to compost amendments. Trial 1 evidenced no negative effect on soil and the same results were obtained after compost distribution on soil B.

Weather conditions

Overall, weather conditions reflected the specific orographic position (distance from the sea, Eat-West appearance, elevation above the sea level) of each experimental site. Total amount of precipitation was similar in the two experimental fields of Montorio nei Frentani and Colletorto (about 400 mm throughout the whole experimental period), while in Mafalda precipitation averaged 1100 mm throughout three years. Montorio nei Frentani and Colletorto showed a mean temperature during the crop cycle of 15.3 and 15.1°C, respectively, while Mafalda showed a mean temperature of 12.2°C during the three years. Averaged maximum temperature for the whole crop cycle also showed little separation of the two locations (26.1 and 25.7°C, respectively), while for olive grove trials averaged 15.7°C for three years.

Plant traits

At physiological maturity, the whole plant dry biomass was significantly and positively affected only by treatment obtained combining organic and inorganic manure (treatments 5 and 6), similarly in both species (**Fig. 5**). In both species, the total plant dry biomass accumulation increased significantly (P < 0.01), on average 10% in treatments 5 and 6, compared with unamended control (treatments 4). Plants of treatments 2 and 3 did not show an increase in plant dry mass compared with unamended and unfertilized control (treatment 1).

Tomato and sunflower plants of treatment 5 and 6 grew bigger (higher accumulated biomass), which may be ascribed to the positive effect of organic matter, characterizing the compost B, on plants nutrition. The interaction between organic and inorganic manure was overall significant.

The physiological parameters (**Fig. 6**) (P_n and g_s) revealed significant differences (P < 0.05) between treatments 5 and 6 compared with control (treatment 4). These results suggested a significant reduction of seasonal stress when compost B was added to soil, probably due to increasing water availability and plant nutrition, as revealed by gas exchange for rainfed sunflower. In tomato, the addition of compost B probably increased the level of nutrient availability reducing nitrogen deficiency. In both species, a positive effect was observed in treatments 5 and 6 compared with treatment 4. This positive effect on photosynthetic metabolism was reflected in a significant increase of plant growth and yield, only in treatments 5 and 6.

Differences between the amounts of compost B applied (3 and 10 t ha^{-1}) were never significant.

Yield components

The effect of combined treatments (compost B and inorganic nutrient) was reflected in the values of yield for the two species (**Fig. 7**).

Compared to the control (treatment 4), seed (sunflower) and fruit (tomato) yield was 13.3 and 11.1% higher, in treatments 5 and 6, respectively. Differences between treatment 5 and 6, amended with two levels of compost B (3 and 10 t ha^{-1}), were never significant.

Accordingly, the thousand seeds weight and the number of tomatoes per plant was 6.4 and 11.9% higher in treatment 5 and 6, respectively, compared to the control (treatment 4). Crop yield increased with the main yield traits, similarly in tomato and sunflower. As for plants traits, this behavior may be the positive consequence of a concomitant presence of organic matter and mineral nutrition, increasing soil fertility, during crop cycle.

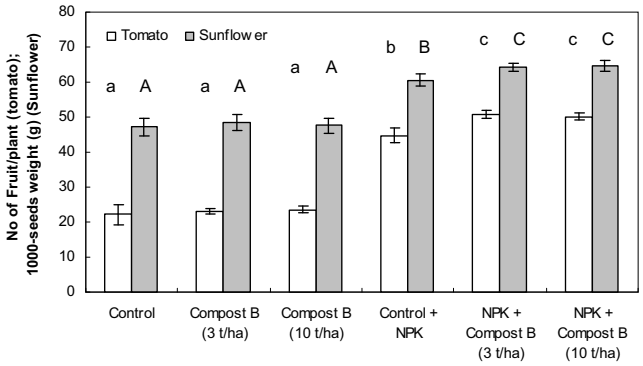

Fig. 7 Tomato number of fruits per plant; Sunflower 1000-seed weight (g) as affected by composting and fertilization treatments. NPK: mineral fertilization. Results are expressed as the mean of three replications ± standard deviation. Histograms surmounted by the same letter (a, b, c or A, B, C) do not significantly differ (P<0.01).

Table 6 Quality classification of the olive oil samples obtained from olive harvested in the control area on in the compost amended. Results are expressed as average of olive oils values obtained in three years.

Parameters	Control	Amended area	EC Reg. 2568/91
Acidity (% oleic acid)	0.51 ± 0.02	0.29 ± 0.1	≤0.8
Peroxide (meq O_2/kg)	10.60 ± 1.9	8.25 ± 1.78	≤20
K_{232}	1.867 ± 0.14	1.659 ± 0.02	≤2.4
K_{270}	0.185 ± 0.02	0.150 ± 0.01	≤0.20
Δ K	0.004 ± 0.0	0.007 ± 0.0	≤0.01
Total Polyphenols (ppm Caffeic acid)	166.0 ± 2.3	246.5 ± 28.8	--

Effect on olive oil quality

Olive yield did not show significant differences between control area and the compost A amended area. **Table 6** shows the results of olive oil quality assessment. In particular olive oils from the area amended with composted olive mill residues, show a lower acidity and peroxide and higher total polyphenols content. However, all the olive oils produced can be classified as "Extra Virgin Olive Oil", according to the EC Reg. 2568/91 and subsequent amendments.

CONCLUSIONS

Olive oil extraction generates large amounts of olive residues that, depending on the extraction method present very large variability in amount and composition, are generally recognised as greatly polluting effluents with strong impact on soil, plants, water and soil microbial population.

The composting process proved to be a reliable method for bioremediation and recycling of solid and sludge residues from olive oil mills. In our experimental condition it allowed the transformation of these by-products mixed with agricultural and zootechnical wastes into high quality organic fertilizers (composts). Physico-chemical, biochemical, phytotoxicity and microbiological analysis confirmed the negligible agronomical qualitative characteristics of both the cured compost A and B. Composts proved to be free of potentially harmful microrganisms (*Salmonellae* spp. and faecal coliforms) and with no phytotoxic effect. Olive mill waste compost also proved to enhance soil organic matter content, structure and fertility. Furthermore, cured composts showed suppressive activity against *V. dahliae* and other fungal plant pathogens both *in vitro* and *in vivo* tests. In climatic chamber and olive nursery trials, compost addition resulted in a significant reduction of viable *V. dahliae* MS. Crop yield of tomato and sunflower were positively related to the simultaneous presence of compost residues and mineral manure. This effect was greater than that observed when only mineral fertilization was applied. The latter treatment affected, by the way, positively plant growth and crop yield. Seed and fruit production was not decremented by the addition of compost exclusively. The organic matter present in the soil after the amendment probably increased crop yield and plant growth, only after the application mineral fertilizer. Furthermore, also olive yield and olive oil quality seems not to be negatively affected by compost distribution to olive groves.

Future scenarios for Mediterranean-type agro-ecosystems, which predict harsher environmental conditions, need to test alternative agronomic strategies, including the use of compost in semi-arid agro-ecosystems. Results of this first experiment appear encouraging for the combination of compost distribution and mineral fertilization. This arrangement may represent a sustainable solution for combining the addition of organic matter to soils and the increase in soil moisture content.

All these aspects bring to the consideration that the application of good quality cured composts with high agronomical, microbiological and suppressive characteristics seem to be a very promising strategy for organic and integrated agriculture systems. In particular, the elevated suppressiveness showed by composts suggest the potential of these products to be used in eco-compatible agriculture.

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