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SUPPRESSIVE ACTIVITY AND ECO-PHYSIOLOGICAL EFFECTS OF COMPOSTED OLIVE BY-PRODUCTS ON THE *VERTICILLIUMDAHLIAE-OLEAEUROPAEA*PATHOSYSTEM

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ABSTRACT: Olive oil by-products composted in a pilot scale in Molise (Italy) were assayed in vitro for their suppressivenessagainst various plant pathogens. Composts markedly reduced the growth of Verticilliumdahliae and other fungal pathogens and were further evaluated on various vegetal crops. On pot-grown olive plants various concentrations of mature cured compost were mixed with sterile orchard soil. Mixtures were artificially contaminated with various concentration of V. dahliaemicrosclerotia (MS). Periodically V. dahliae MS density in the rhizosphere, disease severity on plant canopy and plant eco-physiological response were assessed. In the rhizosphere of plants contaminated with 30 MS per g of soil 15% of the cured compost, alone or in combination with the antagonist fungus Trichodermaviridae, significantly reduced MS density; moreover, eco-physiological measurements showed no difference from control plants grown on sterile soil. Contents of compost higher than 30% showed some negative effects on plant growth, increasing also their mortality compared to plants grown on 15% compost and/or T. viridae or 100% sterile orchard soil. Elevated densities of V. dahliae MS in the rhizosphere affected gas exchange negatively. Overall, photochemical efficiency was not affected by treatments in any case. The results pointed out that composted olive by-products have a good potential for their re-use in organic and integrated agriculture systems as suppressive amenders if mixed with other substrates.

Keywords: fungal diseases, composted olive residues, organic and integrated agriculture, chlorophyll fluorescence, gas exchange.

INTRODUCTION

In recent years considerable research efforts have been paid to evaluate composts from various vegetal and agri-food by-products for their possible re-use as amenders and/or fertilizers. Olive (*Oleaeuropaea* L.) for oil production is one of the most widespread and economically important crops in the Mediterranean area. After oil extraction, large amounts of by-products consisting in olive husks and olive-mill wastewaters are produced. The disposal of these residues involves economical and environmental problems. However, if subjected to advanced bio-technologies of composting, crude by-products can be re-used in agriculture as eco-compatible and good quality organic amenders and fertilizers (Tomati*et al.* 1996; Ranalli*et al.*, 2002). In addition, composts from different vegetal origin, due to their beneficial effects, could also be used as natural pesticide to control plant pathogens (Lazarovits, 2001; El-Masry*et al.* 2002; Lima *et al.* 2004). The selection of composts with positive agronomic and suppressive characteristics requires interdisciplinary methods involving microbiological, phytopathological and eco-physiological competences.

The aim of this work was to evaluate the suppressive activity of some composts from olive oil by-products against *V. dahliae* on olive plants and to analyze the eco-physiological response of plants to these treatments.

MATERIAL AND METHODS

Composted olive oil by-products were obtained from an olive oil mill plant in Molise (Italy) by using olive humid husks produced by a two-phase oil mill plant. The following materials (w/w) were composted: pile 1= OL (olive leaves) 8%, OHH (olive humid husks) 92%; pile 2= OL 8%, OHH 67% and CHH (composted one year old humid husks) 25%; pile 3= OL 8%, OHH 76% and sheep manure (SM) 16%. Conditions of composting, compost quality determination (phytotoxicity, total polyphenols and absence of potential human pathogens), the main physical and chemical characteristics as well as antifungal activity *in vitro* of water extracts from composted materials against different fungal pathogens were previously reported (Ranalli*et al.* 2002; Lima *et al.* 2004).

The *in vivo* suppressive activity against *V. dahliae* was tested in two-year separate experiments in the soil on young plants of olive, cv. Leccino, from cuttings. The compost from pile 2 was used. The plants were grown on 2-liters plastic pots and kept in a screen-house under variable climatic conditions from April to September. The following treatments were tested: 1) 15% compost+85% sterilised orchard soil (SOS); 2) 30% compost+70% SOS; 3) 60% compost+70% SOS; 4) *Trichodermaviridae*biofungicide (strain TV1-Agribiotec, Italy); 5) 15% compost+85% SOS+TV1; 6) 100% SOS; 7) 100% SOS, uncontaminated with *V. dahliae*. At the beginning of each test, the substrate used in each treatment, except for treatment 7 (control), was artificially contaminated with 5, 30, 60 or 100 *V. dahliae*microsclerotia (MS) per gram. MS were produced according to Hawke and Lazarovits (1994). During the test, at 20-day intervals, soil samples from each pot were collected, air was dried for 30 days and the density of *V. dahliae* MS was quantified on semi-selective media as reported by Shetty*et al.* (2000).

Leaf photosynthesis and stomatal conductance were measured at different dates on fully expanded leaves, with a portable gas exchange system (Li-6400, Li-Cor, Lincoln, NE, USA). Gas exchange measurements were made at light intensity saturating photosynthesis (600 □mol m^{-2} s^{-1}, provided by a red-blue light diode source emitting at 670 nm), leaf temperature was set at 24-28°C, CO_2 concentration was maintained near ambient (about 380 □mol mol^{-1}), and vapour pressure deficit at 1-2 kPa; such conditions resembled those outside the cuvette. Contemporary to gas exchange determinations, chlorophyll fluorescence emissions in 30-min dark-adapted leaves were measured with a portable pulse amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany) to estimate the effect of water stress on the efficiency of PSII. The background fluorescence signal (F_o), the maximum fluorescence (F_m), and the potential quantum yield of PSII photochemistry [$F_v/F_m = (F_m - F_o)/F_m$] were determined. The steady state fluorescence signal (F_s) was then measured under sunlight conditions, and the obtained steady state maximum fluorescence yield (F_m') was used for the calculation of the actual quantum yield of PSII photochemistry (Genty*et al.* 1989): □$F/F_m' = (F_m'-F_s)/F_m'$.

RESULTS

On pot-grown olive plants, a single application of 15% compost or biofungicide containing *T. viride* TV1 as well as of 15 % compost and TV1 together, reduced the density of *V. dahliae* MS in the soil of olive plants compared to the untreated control. This was particularly obvious in the second year and up to 20 days after the application of treatments. In the uncontaminated soil MS were never found (Figure 1). The experiments also showed that contents of compost higher than 30% did not improve further suppressiveness of the substrate (Figure 1, first year) and even had negative effects on plant growth, increasing their mortality compared to plants grown on 15% compost and/or *T. viridae* or 100% SOS.

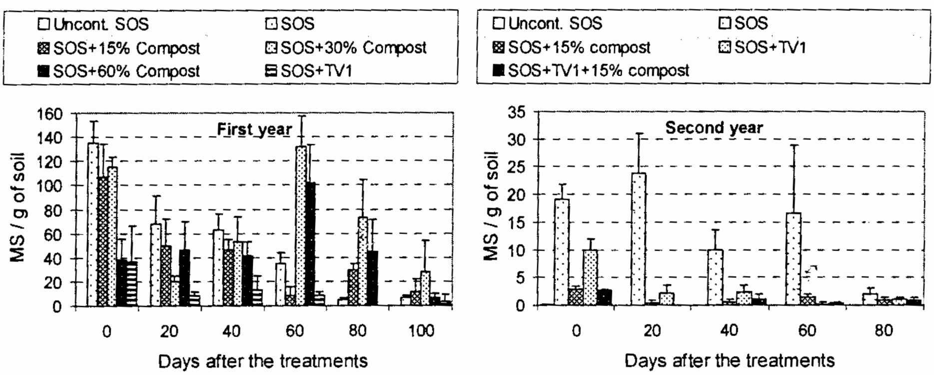

Figure 1.*V. dahliae*microsclerotia (MS) obtained from the rhizosphere of olive plant at different times of sampling from pots subjected to various treatments in two-year experiments. SOS= Sterilised Orchard Soil; TV1= *T. viridae*; Compost= compost from pile 2. Except for uncontaminated (Uncont.) control, the substrates were contaminated with 30 MS per gram. Bars represent SD from the mean.

In the second year, gas exchange measurements showed that the assimilation rate and the stomatal conductance were somewhat lower in treatments with 15% compost than in any other case (Figure 2). In the first year, gas exchange decreased progressively with increasing compost concentrations in the substrate (minimum values with 30-60% compost). Anyway, control plants showed a higher assimilation rate and stomatal conductance than all treatments, and trends in gas exchange were reflected in intercellular to ambient CO_2 ratio and instantaneous water use efficiency (not shown). Photochemical efficiency and quantum yield were never affected by treatments (Figure 2), regardless of the year of experiment.

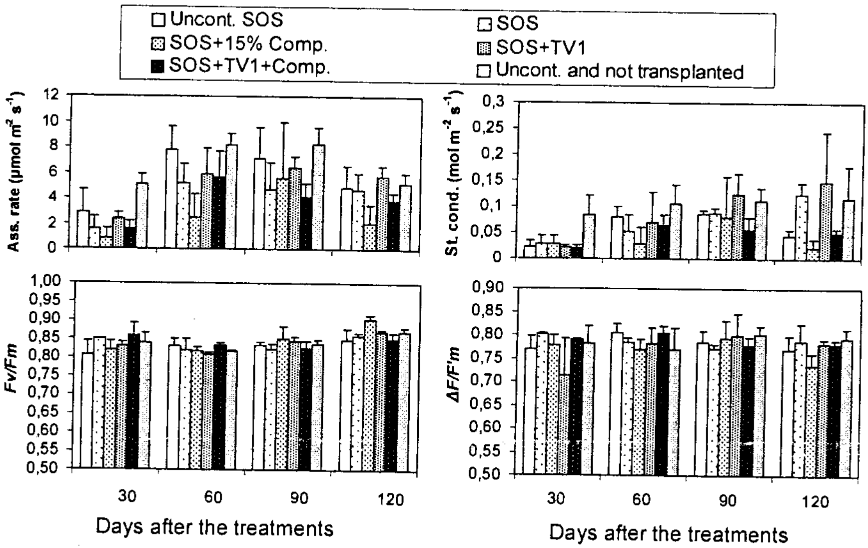

Figure 2. Gas exchange (A: CO_2 assimilation rate; B: Stomatal conductance to water vapour), and chlorophyll fluorescence (C: Fv/Fm= potential quantum yield; D: ΔF/F'm= actual quantum yield) in the leaves of olive plants subjected to different treatments in the second year of experiment. SOS= Sterilised Orchard Soil; TV1= *T. viridae*; Compost= compost from pile 2. Except for uncontaminated (Uncont.) controls, the other substrates were contaminated with 30 *V. dahliae*microsclerotia per gram. Bars represent SD from the mean.

DISCUSSION

On pot grown olive plants, the use of 15% (w/w) of the cured compost alone or in combination with biofungicide (TV1) significantly reduced the density of *V. dahliae* MS in the rhizosphere. According to our previous *in vitro* experiments results, where water extracts of this compost consistently reduced the growth of *V. dahliae* and other important fungal pathogens (Lima *et al.*, 2004), this suppressive activity could be mainly due to the beneficial residual microbial population contained in the cured compost. In particular, among the useful microorganisms isolated from composted olive by-product different species of bacteria, Actinomycetes and fungi (Lima *et al.*, 2004), were found.

Contents of compost higher than 30% had some negative effects on plant growth, even increasing their mortality. Elevated densities of *V. dahliae* MS in the rhizosphere negatively affected photosynthesis because of stomatal closure. Overall, the photochemical efficiency was not affected by treatments in any case. Indeed, photosystem II was never affected permanently by treatments, though reduced gas exchange rates could lead to decreased plant development.

The results of this investigation indicate that good quality composts for agricultural use can be obtained by composting, under suitable conditions, by-products remaining after olive oil extraction. The cured compost if used in mixture with other substrates and at a suitable concentration, shows a good potential for application in organic and integrated agriculture systems to control fungal pathogens of olive and other crops. Furthermore, economical assessments in comparison with standard commercial substrates will be performed in order to evaluate further convenience.

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النشاط التثبيطي والتأثيرات الفسيوبيئية لكمبوست نواتج الزيتون علي فطر فرتيسليم داليا

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الملخص العربي

أختبر التأثير التثبيطي للسماد العضوي (الكمبوست) الناتج عن عملية إستخلاص الزيت من الزيتون معمليا ضد مجموعات مختلفة من المسببات المرضية في منطقة موليزي – إيطاليا. وقد أظهرت النتائج حدوث نقص معنوي في نمو فطر الفرتيسليم داليا والمسببات المرضية الفطرية الأخري التي تصيب عدد كبير من الخضروات. في تجربة أصص لنبات الزيتون تم إضافة تركيزات مختلفة من الكمبوست الناضج الخام إلي تربة حقل معقمة ، ثم تم إجراء عدوي صناعية لذاك الخليط بتركيزات مختلفة من الأجسام الحجرية الصغيرة لفطر الفرتيسليم داليا، ثم قُدرت كثافة الاجسام الحجرية لفطر الفرتيسليم داليا في منطقة إنتشار الجذور، وشدة الإصابة علي النباتات بالإضافة إلي الإستجابات الفسيوبيئية للنباتات.

لقد وُجد أن النباتات الملوثة بـ 30 جسم حجري صغير للفرتيسليم/ جم تربة والمحتوية علي 15% كمبوست خام منفرداً أو مختلطاً بفطر التريكوديرما فيردي المضاد للفطريات يقل معنويا بمنطقة إنتشار الجذور إعداد الأجسام الحجرية الصغيرة للفرتيسليم، علاوة علي ذلك لم تكن هناك إختلافات فسيوبيئية ملموسة في نباتات الكنترول النامية بتربة معقمة. علي الجانب الآخر فالتركيزات المرتفعة من الكمبوست والتي تفوف 30% تسبب بعض التأثيرات السلبية علي نمو النبات، وتُزيد من نسبة الموت مقارنة بالنباتات النامية بتركيز 15% من الكمبوست أو النامية في وجود التريكوديرما فيردي أو النامية بتربة الحقل المعقمة.

عموماً، كفاءة التفاعلات الكيموضوئية لم تتأثر بالمعاملات المختلفة في كل الحالات. أوضحت النتائج أن الكمبوست يمكن إعادة إستخدامة في نظام الزراعة العضوية والمتكاملة ال كمثبط للفطريات الممرضة إذ تم خلطة مع مواد تفاعل أخري .