



Effect of urban biowaste derived soluble substances on growth, photosynthesis and ornamental value of *Euphorbia x lomi*

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ARTICLE INFO

Article history:

Received 28 June 2015

Received in revised form 22 October 2015

Accepted 25 October 2015

Keywords:

Municipal biowastes recycling

Leaf chlorophyll content

CO₂ exchange rate

Plant photosynthetic activity

Plant growth

ABSTRACT

Soluble bio-based substances (SBS) isolated from municipal biowastes and a commercial Leonardite-based product were applied as substrate drench or as foliar spray to grow the ornamental hybrid *Euphorbia x lomi*. The SBS were found more powerful than the commercial Leonardite product in enhancing plant photosynthesis, growth and aesthetic effect, improving flower quality, and optimizing water use efficiency. Enhancement factors of plant performance indicators by SBS ranged from 1.3 to 8.6 relatively to the control plants, and from 1.2 to 4.5 relatively to plants treated with the commercial Leonardite product at equal applied dose. The environmental and economic implication of these results for agriculture, the management of urban wastes, and the chemical industry are discussed.

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1. Introduction

The management of urban wastes has become a priority environmental issue, due to increasing urbanization and human consumption habits. They represent a significant cost for society. Recent work has however shown that fermented urban biowastes are a viable source of soluble bio-based substances (SBS) which may perform as efficient eco-friendly chemical auxiliaries in diversified fields; e.g., in the formulation of detergents, textile dyeing baths, flocculants, dispersants and binding agents for ceramics manufacture (Montoneri et al., 2011), emulsifiers (Vargas et al., 2014), auxiliaries for soil/water remediation (Avetta et al., 2013; Gomis et al., 2014; Montoneri et al., 2014) and enhanced oil recovery (Baxter et al., 2014), nanostructured materials for chemical (Boffa et al., 2014; Deganello et al., 2015) and biochemical catalysis (Magnacca et al., 2012), plastic materials (Franzoso et al., 2015a,b,c), soil fertilizers and plant biostimulants for horticulture (Sortino et al., 2014) and animal feed supplements (Montoneri et al., 2013; Dinuccio et al., 2013). The SBS are obtained by alkaline hydrolysis of the urban biowastes previously fermented under anaerobic and aerobic conditions. They are mixtures of molecules with molec-

ular weight from 5 to several 100 kDa, comprising aliphatic and aromatic C atoms bonded to a variety of acid and basic functional groups. This is the likely reason of their multipurpose performance.

The above studies prospect the substitution of synthetic chemicals by SBS for many applications, and consequently the potential reduction of the depletion of fossil sources and of the added CO₂ to the environment contributed by synthetic chemicals at life end. Direct environmental implications of these substances have been described by Sortino et al. (2014) who have shown that SBS added to soil for horticulture enhance the plant photosynthetic activity, growth and productivity more than the sourcing fermented biowastes without any alkaline treatment. The same SBS are proven by Avetta et al. (2013) and Gomis et al. (2014) to enhance the photochemical degradation of organic pollutants in industrial effluents. These findings have suggested that SBS may promote either C fixation or mineralization, according to the different operational environments. In both cases it has been suggested that, by their capacity to complex Fe ions and keep them in solution at circum-neutral pH, the SBS may contribute to enhance a photo-Fenton process. These findings propose SBS as a friendly interface between plant and human activities.

In the present work we report the effects of three products containing organic and mineral matter on the photosynthetic activity, growth, aesthetic effect and biomass water use efficiency of ornamental hybrid *Euphorbia x lomi* Rauh (*Euphorbia lophogona*

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Lamarck \times *E. milii* Des Moulins). One product is a commercial formulation traded under the name of Enersoil, obtained by alkaline hydrolysis of Leonardite (Intrachem Bio Italia, 2008). The other two products are SBS isolated from the alkaline hydrolysate of two fermented biowaste materials. These are the digestate (DG) of the biogas production reactor fed with the organic humid refuse of separate source collection and the compost (CP) obtained from DG mixed with private and public gardening residues, and sewage sludge. The CP and DG were used in the previous work by Sortino et al. (2014) for tomato and pepper cultivation. The purpose of the present work was to assess whether the effects reported on horticultural species were confirmed also for ornamental plants. Consistently with their sources, the investigated products have different C, N and mineral composition, and therefore allow to evaluate the effects of the different nutrients content on the plant performance indicators. The hybrid *Euphorbia x lomi* Rauh plant was chosen as test plant, being perennial and so much different from many short-cycle crops as tomato and pepper. The former belongs to the Spurge family and is a succulent shrub with milky latex, long lanceolate leaves and large colored inflorescences, usually cultivated as potted flowering plant or as hedge plant for landscaping and xerogardening (Fascella and Zizzo, 2009) because of its low exigencies and tolerance to drought stress (Fascella et al., 2011). The results were expected to add further argument to the previous work on horticultural species (Sortino et al., 2014) supporting the role of SBS promoting a friendly C cycle in the ecosystems.

2. Materials and methods

2.1. SBS and Enersoil

The SBS were prepared and supplied by Studio Chiono ed Associati (SCA) in Rivarolo Canavese (TO), Italy. This company obtained the SBS by hydrolysis of two fermented urban biowaste materials according to a previously reported procedure (Sortino et al., 2014; Franzoso et al., 2015a,b,c). The first material was the anaerobic digestate (DG) of the organic humid fraction of urban waste from separate source collection. The second material was the compost (CP) obtained from a mix of DG, home gardening and park trimming residues and sewage sludge, at 3.5/5.5/1 respective weight ratio, which was aged under aerobic conditions for 110 days. The two fermented urban biowaste materials were further processed by SCA as follows. The DG or CP material, separately, was hydrolyzed with KOH alkaline water at pH 13 and 60 °C. The hydrolyzate was run through an ultra filtration polysulphone membrane with 5 kD cut off. The membrane retentate was dried at 60 °C to yield the final SBS product as black solid in a 15–20% yield, relative to the starting material. The CP and DG/SBS contained 91 and 99% dry matter, respectively. The commercial Enersoil product was supplied by Intrachem Bio in Grassobio (BG), Italy.

2.2. Greenhouse facilities and plant material

The plant growth trials were conducted in 2013 in an unheated (28 °C day/14 °C night) double-span East–West oriented greenhouse (34 \times 16 m) with steel structure and polyethylene cover (thickness 0.15 mm), located at the Research Unit for Mediterranean Flower Species near Palermo (38°5'N, –13°30'E, 23 m above sea level), on the North Western Sicily coastal area. Six months-old 8 cm-tall micropropagated plants of *Euphorbia x lomi* Rauh cv. 'Serena' were grown in plastic pots of 13 cm diameter (1 L capacity, 1 plant per pot) filled with a substrate of sphagnum peat (Dueemme marketing, Reggio Emilia, Italy) and perlite (Perlite Italiana, Milano, Italy) in 1:1 v/v ratio. Water, macro and micronutrients were supplied to plants through a drip fertigation system (1 dripper per

plant, 2 L h⁻¹) controlled by a computer. All plants were fed with the same nutrient solution which had the following composition (mg L⁻¹): 180 total N, 50 P, 200 K, 120 Ca, 30 Mg, 1.2 Fe, 0.2 Cu, 0.2 Zn, 0.3 Mn, 0.2 B. The pH and the electrical conductivity (EC) of the nutrient solution were maintained at 5.8 and 1.8 mS cm⁻¹, respectively. Irrigation scheduling was performed using electronic low-tension tensiometers connected to an electronic programmer, that controlled irrigation based on substrate matric potential. The SBS and Enersoil materials were dissolved or diluted in water to yield five solutions with the following dry matter g L⁻¹ concentrations: 45.5 CP, 31 and 15.5 DG, 18.7 and 9.4 Enersoil. An aliquot of 100 ml of each solution was applied as substrate drench or as foliar spray to each plant. Foliar sprays and drenches applications were provided to plants two times during the trial (120 days). The total applied amounts of dry matter g per plant were 4.6 for CP as substrate drench, 3.1 and 1.5 for DG as substrate drench and foliar spray, respectively, and 1.9 and 0.94 for Enersoil as substrate drench and foliar spray, respectively.

2.3. Plant growth measurements

Ten plants per treatment, randomly chosen from each replicate, were harvested every 30 days and separated into stems, leaves and roots for growth measurements (plant height, number of leaves per plant, number of flowers per plant). Plant height was determined as the distance from the surface of the substrate to the top of the plant. Dry weight of the biomass was determined after 72 h in a forced-air oven (at 100 °C) when harvested tissues reached a constant value. Shoot to root ratio (S/R) was calculated by dividing sum of leaf and stem dry weights by the root dry weight. Leaf area (LA) was measured using a digital area meter (WinDIAS 2; DELTA-T DEVICES Ltd., Cambridge, U.K.). Relative Growth Rate (RGR) was calculated according with the formula proposed by Hoffmann and Poorter (2002) using the following equation: $(\ln W_2 - \ln W_1)/(t_2 - t_1)$ where \ln = natural logarithm, W_1 = dry weight of plant at time one (in grams), W_2 = dry weight of plant at time two (in grams), t_1 = time one (in days), t_2 = time two (in days). Biomass Water Use Efficiency (WUE) was calculated as the ratio between total dry weight of plants and plants total water supply.

2.4. Leaf SPAD index, color and gas exchanges measurements

Leaf chlorophyll content (e.g., SPAD index) of three randomly selected leaves of all plants in each experimental unit was measured with a chlorophyll meter (SPAD 502, Konica Minolta Sensing, Inc., Osaka, Japan). Leaf color was determined with a shot in the middle of the blade on three leaves of all plants of each treatment with a colorimeter (Minolta CR10, Konica Minolta Sensing, Inc., Osaka, Japan) that calculated the color coordinates (CIELAB): lightness (CL), tone (CA) and saturation (CB); CL varies from 0 (completely opaque or black) to 100 (completely transparent or white); CA ranges from positive (redness) to negative (greenness) values, as well as CB (positive is yellowness, negative is blueness). Leaf gas exchanges (net assimilation CO₂ (A_{CO_2}) and stomatal conductance (g_s)) were also measured using a portable photosynthesis system (LI-6200; LI-COR Inc., Lincoln, NE, USA). Measurements were made on most recent fully expanded leaves between 10:00 and 13:00 h on sunny day, using five replicate leaves per treatment. The LI-6200 was equipped with a stirred leaf chamber with constant-area inserts and fitted with a variable intensity red source (leaf temperature chamber was 30 \pm 2 °C, leaf-air vapor pressure difference was 2.6 \pm 0.3 °C, and CO₂ concentration was 365 \pm 10 μ L L⁻¹).

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