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Fertilization of bean plants with tomato plants hydrolysates. Effect on biomass production, chlorophyll content and N assimilation

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ABSTRACT

Humic-like substances obtained by alkaline hydrolysis of composted organic wastes are known to improve plant productivity. Little is known concerning the effect on plant growth of hydrolysates obtained by alkaline treatment of non-composted vegetal residues.

The aim of this study was to prepare, characterize, and apply in horticulture the soluble and the insoluble fractions obtained by alkaline hydrolysis of exhausted tomato plants. The hydrolysates were prepared in a pilot plant from the tomato plants residue in powder form and characterized by solid state ^{13}C NMR spectrometry, elemental analysis and potentiometric titration. The characterization of the soluble hydrolysate gave evidence of lignin and hemicellulose moieties, together with protein, peptide or amino acids while most of the cellulose was found in the insoluble fraction. Plant beans were grown on a peat and sand substrate fertilized with low application rate of the tomato plant powder and of the corresponding hydrolysates. The effect on bean plants was assessed by determination of plant growth, chlorophyll content, nitrate reductase, glutamine synthetase, glutamate synthase activities and soluble proteins.

The tomato plant powder had no effect on all parameters measured on the grown bean plants. On the contrary, the insoluble and soluble substances sourced by alkaline hydrolysis of the tomato plant powder exhibit strong effects, mainly the increase of nitrogen assimilation typical of biostimulants.

The results suggest that residual plant biomasses are source of efficient biostimolant and propose the hydrolysis of residual biomass as a viable profitable process to contribute important improvements also for waste management practices.

1. Introduction

It has been largely demonstrated that soil or plant treatments with humic substances (HS) consistently increase the plant growth. The effect of HS on plant growth depends on the source, concentration and molecular weight humic fraction (Nardi et al., 2002; El-Nemr et al., 2012). Humic-like substances obtained from composted organic wastes such as sewage sludges, animal and agricultural residues and household refuse have also been seen to improve plant productivity (Chen, 1992; Valdrighi et al., 1995, 1996). Recently, the alkaline hydrolysis of a composted mix of urban food and vegetable residues has been reported to yield water soluble and insoluble substances (Sortino et al., 2013). These substances were isolated and applied separately to a loamy–sandy soil for tomato (Sortino et al., 2012) and red pepper (Sortino et al., 2013) in greenhouse cultivation. The soluble substances were found to enhance leaf chlorophyll content, and to improve plant growth and fruit ripening rate and yield over the crop production cycle, significantly more than the sourcing compost and the co-produced insoluble residue. The most remarkable result was the observed maximum productivity for 140 kg ha^{-1} compost soluble substances

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dose. The increases amounted to 90% for the precocious crop yield, to 66% for the total crop production, and to 17% for the per fruit weight. Plant growth and productivity correlated with the enhancement of leaf chlorophyll content. The same soluble substances have also promoted the growth of several horticultural and floricultural species when used as substitute of peat in a commercial substrate (Negre et al., 2012). The promising results exhibited by the soluble substances were relevant from different points of view. In real agriculture practices, the use of biowaste derived soluble substances at low dose, in place of conventional mineral and organic N fertilizers, could allow promoting plant growth and crop production at low cost, while minimizing the risk of environmental impact. From the more general ecological point of view, this perspective implies also the fascinating scenario that bio-refuse sourced substances entered the natural carbon cycle and their use as plant growth promoters contributed to improved waste management practices.

The present paper reports the results of bean cultivation trials performed on peat and sand substrate fertilized with soluble and insoluble substances isolated from the alkaline hydrolysate of tomato plants collected at the end of the crop harvesting season. Product performance indicators were bean plant growth, leaf chlorophyll concentration, and plant nitrogen metabolism assessed through the determination of soluble proteins and of nitrate reductase, glutamine synthetase, and glutamate synthase activities. This approach intended to address some of the questions and perspectives posed by Sortino et al. (2012, 2013) studies on tomato and red pepper amended with a compost hydrolysate regarding the feasibility of using not composted plant materials are source of hydrolysates. Bean plants as target species to test were chosen to see if the effects observed with tomato plants were confirmed with other plant species and, thus, to add a valuable argument for using substances isolated from bio-residues matter in real agriculture practices.

2. Materials and methods

2.1. Tomato plant sourced soluble and insoluble substances

Tomato plants (*Lycopersicon esculentum* Cv. *Naomi F1*) were grown in greenhouse at the Angelo Zocco farm in Rosolini (SR), Italy, on a sandy soil amended with a mixed compost prepared from anaerobic sludges and lignocellulosic material in the conditions reported by Sortino et al. (2012). At the end of the crop harvesting season the exhausted plants were pulled out of the soil, roughly ground to 5–10 mm size on site and transported to the Studio Chiono pilot plant in Rivarolo Cavanese (TO), Italy. The material was further ground down to <0.5 mm particle size by use of Cimma, Pavia SF75 mill. The fine powder was further processed by alkaline hydrolysis and ultra filtration through a polysulphone membrane as previously reported (Sortino et al., 2013). The starting powder was reacted 4 h with KOH solution at pH 13, 60 °C and 4 (v/w) water/solid ratio. The liquid/solid hydrolysate mix was allowed to settle to separate the supernatant liquid phase containing the soluble substances from the insoluble substances. The recovered liquid phase was circulated at 40 L h^{-1} flow rate through the ultrafiltration membrane operating with tangential flow at 7 bar inlet and 4.5 bar outlet pressure to yield a retentate with 5–10% dry soluble substances content. The insoluble substances residue was washed once with fresh water at 4 (v/w) added water/solid ratio. The recovered ultrafiltration retentate and the insoluble substances residue were allowed to concentrate and/or dry in ventilated oven at 60 °C. The pristine tomato plant organic matter was recovered 30% as soluble substances and the rest as insoluble substances.

2.2. Chemical analyses

The tomato plant powder and its alkaline hydrolysis products, i.e. the soluble substances and the insoluble substances, were characterized for their content of C, N by elemental analysis and mineral elements by atomic absorption after nitric acid digestion. The pH was measured in a 1:5 (w/v) aqueous extract. The organic functional groups were determined by a previously published analytical protocol (Montoneri et al., 2010).

The tomato plant powder was also analyzed for its proximate content according to a previously procedure (Templeton and Ehrman, 1995) with the following modifications. The sample was treated in a Soxhlet extractor with 1:1 benzene/ethanol overnight to yield the benzene/ethanol extract and the insoluble residue. The benzene/ethanol insoluble residue was treated with refluxing 1 M HCl for 2 h and centrifuged to yield the 1 M HCl extract and the insoluble residue. The 1 M HCl insoluble residue was treated further with 12 M HCl at 4 °C for 24 h to yield the 12 M HCl extract and the insoluble residue. All extracts and the final insoluble residue were dried under vacuum at 40–60 °C and weighed.

Potentiometric titration of the soluble hydrolysate was performed according to a previously reported method (Montoneri et al., 2008) based on the procedure of Gran (1952).

2.3. Plant growth trials

The starting tomato plant powder and the soluble and insoluble substances obtained by hydrolysis were added to a substrate consisting of 1 L peat (230 g) and 1 L sand (1500 g) in pots with 14 cm × 14 cm × 15 cm size. The amount of added soluble substance was 4 g per pot. The amounts of applied tomato plant powder and insoluble substances were twice that of the soluble fraction in order to about equalize the nitrogen and carbon content. Four pregerminated seeds of bean (*Phaseolus vulgaris*) were placed in each pot and five replicates per trial were carried out. The pots were placed in a climatic cell at 25 ± 1 °C with a 16/8 h photoperiod. After 21 days, the shoots and roots were collected, weighted and stored at −80 °C.

2.4. Determination of nitrates and nitrogen mineralization

Nitrogen mineralization was determined by incubation of the substrates without plants and measurement of the nitrate concentration after 0, 7, 14, and 21 days incubation (Meli et al., 2006). Nitrates were determined by ionic chromatography, on the 1 N KCl extract using the Thermo Fisher Scientific Inc Dionex ICS-3000 system.

2.5. Determination of chlorophylls and carotenoids content

The determination of chlorophyll *a* and *b* and of carotenoids was performed on each plant by extraction of 300 mg fresh foliar tissue ground in liquid nitrogen with 10 mL 96% (v/v) ethanol. The samples were kept in the dark for 2 days at 4 °C, and the extracts were filtered and then analyzed by spectrophotometry using a Hitachi U-2000 spectrophotometer. The absorbance readings were performed at 665 nm for chlorophyll *a*, at 649 nm for chlorophyll *b*, and at 470 nm for total carotene. Chlorophyll *a*, *b* and total carotenoid concentrations were calculated according to Wellburn and Lichtenthaler (1984).

2.6. Enzyme extraction and assay conditions

Nitrogen reduction and assimilation enzymes were extracted by grinding plant tissues (1 g) in liquid nitrogen with 1 mL of a 1 mM disodium ethylenediaminetetraacetic acid, 25 mM KH_2PO_4, 10 mM