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Application of municipal biowaste derived products in *Hibiscus* cultivation: effect on leaf gaseous exchange activity, and plant biomass accumulation and quality

Daniele Massa[1*], Domenico Prisa[1], Enzo Montoneri[2], Daniele Battaglini[1], Marco Ginepro[3], Michele Negre[4], Gianluca Burchi[1].

[1] Council for Agricultural Research and Economics (CREA), Landscaping plants and nursery research unit, Via dei Fiori 8, 51012 Pescia (PT), Italy

[2]Biowaste Processing, Via XXIV Maggio 25, 37126 Verona, Italy

[3] Dipartimento di Chimica, Università di Torino, Via P. Giuria 7, 10125 Torino, Italy

[4] Dipartimento di Scienze Agrarie Forestali e Alimentari, Università di Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy

*Corrisponding author:

Daniele Massa

Tel +39 0572 451033

Fax +39 0572 453309

daniele.massa@entecra.it

Abstract

Two urban biowaste materials, fermented under anaerobic and aerobic conditions, their soluble and insoluble alkaline hydrolysates, and a commercial biostimulant product, were compared for their capacity to boost *Hibiscus* plants crop production and quality. Plants were grown in 4-litre pots, containing peat and pumice as substrate, under optimal nutrient. A randomized-block experimental design was adopted. Two types (main factors of variability) of treatments were applied, i.e. by blending the above products with the substrate at transplant, and by fertigation using the soluble hydrolysates only. Plant biomass characteristics and ecophysiological parameters were measured. Mean effect of factors and their interactions were assessed by two-way ANOVA. Principal component analysis was performed by using the different dependent variables to summarize the most relevant differences among treatments. Compared with the control (untreated plants), the applied treatments enhanced most of the investigated parameters. The most valuable effects were observed for total biomass accumulation (+25 %), plant height (+10 %), leaf chlorophyll SPAD index (+15 %) and net photosynthesis (+24 %). The hydrolysates performed better than the pristine materials. The former ones were comparable to the commercial biostimulant. The results confirmed the hypothesis that biowaste derived products induce biostimulant activity on *Hibiscus*; their application can improve cultivation sustainability.

Keywords

Biowaste, biostimulant, photosynthesis, substrate culture, *Hibiscus palustris*

Abbreviations

Acronym	Description	
D	Digestate obtained from organic humid fraction of urban waste	
DAT_1	Days after T_1	day
DW	Plant dry weight	$g\ m^{-2}$
E	Evapotranspiration rate	$mmol\ m^{-2}\ s^{-1}$
-F	Fertigation treatments	
FERT	Treatments applied during the cultivation through fertigation	
GC	Green compost obtained from urban gardening wastes	
Gs	Stomatal conductance	$mmol\ m^{-2}\ s^{-1}$
ID	Digestate insoluble alkaline hydrolysate	
IGC	Compost insoluble alkaline hydrolysate	
LAI	Leaf area index	$m^2\ m^{-2}$
NAR	Net assimilation rate	$g\ m^{-2}\ d^{-1}$
NO	Untreated	
NOVA	Commercial biostimulant	
OM	Organic matter	$kg\ m^3$
Pn	Net photosynthetic rate	$\mu mol\ m^{-2}\ s^{-1}$
RGR	Relative growth rate	$mg\ g^{-1}\ d^{-1}$
SD	Digestate soluble alkaline hydrolysate	
SGC	Compost soluble alkaline hydrolysate	
SUB	Treatments applied to the substrate as a powder blended with the substrate	
T_1	Beginning of the experimentation (30 days after transplant)	day

Introduction

Soluble biobased substances isolated from the alkaline hydrolysate of fermented urban biowastes have been proven to perform as efficient ecofriendly chemical auxiliaries in diversified fields of the chemical industry (Montoneri et al., 2011), agriculture (Sortino et al., 2014), and animal husbandry (Montoneri et al., 2013). The products are mixtures of molecules with molecular weight from 5 to several hundreds 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 (Montoneri et al., 2011). Particularly relevant for agriculture are the results published by Sortino et al. (2014) and Gomis et al. (2014). Sortino et al. (2014) have shown that the above soluble biobased substances added to soil increased tomato and red pepper plant photosynthetic activity, growth and productivity. The same substances enhanced the photochemical degradation of organic pollutants in industrial effluent (Gomis et al., 2014). These findings suggested that they might promote either C fixation or mineralization, according to the different operational environments. In both cases, it was suggested that, by their capacity to complex Fe ions and keep them in solution at slightly acidic or alkaline conditions, the above soluble biobased susbtances may contribute to enhance photo-Fenton processes. On this basis, the development of a self-sustainable ecosystem based on cycling renewable organic C between wastes and added value products appears a fascinating reachable goal, certainly worthwhile to pursuit. Generally, the environmental benefits of these substances lie in the fact that these products are potentially alternative to commercial synthetic chemicals. Thus, by their use, one may expect a reduction of fossil source depletion and of CO_2 emissions.

The realization of the above perspectives does not depend only on the product properties and performance, but also on sustainability of the production process. Rosso et al. (2015) have reported the hydrolysis of several materials recovered from different streams of a typical municipal waste treatment plant located in North West Italy. These materials were the digestate of the plant biogas reactor performing the anaerobic digestion of the organic humid fraction, and several composts obtained from mixtures of digestate, private and public gardening residues, and/or sewage sludge. The hydrolysis of these materials was carried out in water at pH 13 and 60 °C. In all cases, the soluble hydrolysate was obtained together with equal or higher amounts of insoluble hydrolysate. The published data showed that both the soluble and insoluble hydrolysates contain organic and mineral matter, but the organic/mineral ratio is higher in the former. The soluble hydrolysates contain the lowest relative amount of total mineral matter, compared with the insoluble hydrolysates and the pristine sourcing materials. Although this process, performed at low temperature and with complete solvent and reagents recycling, appears ecofriendly, a critical point remains the production of the insoluble hydrolysate. In the absence of demonstration of a promising use of this product, its disposal is an economic burden for the soluble hydrolysate production process. Yet, by its content of mineral elements, also the insoluble hydrolysate product might be useful for agriculture.

The possibility of boosting yield and quality of crops by using biostimulants has been largely investigated in the last years (e.g. Bulgari et al., 2015; Calvo et al., 2014; du Jardin, 2015). Many relationships between the application of biostimulants and crop performance have been assessed in several experimental trials. However, the mechanisms activated at plant level by these products are largely unknown and difficult to identify. Biostimulants have been found to enhance plant growth and/or production and quality (Calvo et al., 2014), nutrient uptake (Saa et al., 2015), photosynthetic activity (Castro et al., 2012), plant response to biotic and abiotic stresses (Petrozza et al., 2014), and several metabolic processes (Bulgari et al., 2015; Calvo et al., 2014). Most literature on biostimulants reports their use by foliar application (e.g. Saa et al., 2015). However, the possibility of supplying these substances directly to the root zone is valuable due to improved efficiency of distribution and dosage. This is especially true if fertigation is adopted for cover fertilization; obviously, this practice is restricted only to soluble compounds.

Much research activity on the use of biostimulants in agriculture has been carried out under soil conditions for extensive crops (Calvo et al., 2014; du Jardin, 2015), while scarce literature is available for substrate cultures (e.g. Bulgari et al., 2015). For the latter, root zone conditions are significantly different from soil in terms of bio-organic process (e.g. microbial activity), climate (e.g. temperature, humidity) and chemo-physical characteristics. In intensive agriculture, such as container-grown plant systems, the required agrochemicals, water, and energy inputs per unit area are significantly higher, compared with those needed for extensive crop systems. The former systems apply to ornamental plants, for which the high aesthetic quality is a critical marketability parameter. In this scenario, using natural products to boost plants high yield and quality could significantly improve the use efficiency of non-renewable resources (e.g. fresh water and chemical fertilizers), thus increasing environmental and economic sustainability. This poses worthwhile research scope on such products for understanding better their action on plant through the root zone and optimizing their direct administration to the substrate.

The present work was undertaken to address several questions posed in previous experiments on the performance of the above soluble biowaste hydrolysates as promoter of plant photosynthetic activity, growth and productivity, and on the sustainability of their production process. To this purpose, *Hibiscus* was chosen as test plant. The soluble hydrolysates were obtained, together with the corresponding insoluble hydrolysate, by alkaline hydrolysis of two different fermented municipal biowaste materials. The soluble and insoluble hydrolysates, and their sourcing materials, were distributed at transplant as powder blended with the substrate, and/or solubilised into the irrigation water and supplied through fertigation. A commercial product, claimed by the vendor to have biostimulant properties, was also tested by substrate application. This product, under the trade of NOVA@®GR, is produced and distributed by Biolchim Spa, Medicina (BO), Italy. The applied products were evaluated for their effects on several plant performance indicators related to plant biomass accumulation and quality, and to leaf gas exchange activity. Main goals of the experiment were: i) evaluation of the different urban biowaste sourced materials and/or their mixtures; ii) assessment of the products application methodology; iii) evaluation of the above urban biowaste sourced materials with respect to a commercial biostimulant.

Materials and Methods

Location, plant material and growing conditions

The experiment was carried out in the spring-summer of 2014, under typical Mediterranean climate conditions, in a open-air experimental nursery located in Pescia (Tuscany, Italy; latitude 43.54 °N, longitude 10.42 °E) at the Landscaping Plants and Nursery Research Unit of the Italian Council for Agricultural Research and Economics.

Hibiscus seedlings (*Hibiscus moscheutos* L. subsp. *Hibiscus palustris*) were transplanted on 10th of April into four-L black pots (Ø 18 cm) containing a base substrate (peat 50% and pumice 50%, V/V) adjusted to pH 6.

Plants were left 30 days under shade net (40% of shading) for acclimatisation to external (outdoor) conditions. Thirty days after transplant (T_1), pots were moved to the experimental site and arranged in a randomized-block experimental design, with three replicates per treatment (eight plants per replicate). Pots were positioned at 0.40 x 0.60 m each other, obtaining a crop density of 4.2 plants m^{-2}. Twenty days after T_1 (DAT_1), all plants were trimmed above the fourth true leaf to stimulate the emission of lateral shoots as recommended by the standard cultivation technique in commercial nursery production of *Hibiscus palustris*. The experiment was concluded at 110 DAT_1 with the destructive analysis of plant material performed roughly one month before the beginning of plant senescence (basing on local climate conditions).

Irrigation water was supplied through drip irrigation system (2 emitters per pot with a total flow rate of 7.5 L h^{-1}, on average), using a timer for trigging irrigation four times per day (on average, overall the cultivation period). Irrigation scheduling was adjusted weekly according to climate conditions and leaching fraction (the ratio between water drainage and water supply), which was calculated by measuring the volume of water drained out from a limited (three per block) number of pots. Irrigation was regulated to keep leaching fraction on a target value of roughly 10-15%. Irrigation water pH and electrical conductivity ranged 6.2-6.6 and 0.42-0.60 dS m^{-1}, respectively.

Prior to adding the biobased products under investigation, standard basic fertilization was applied to the cultivation substrate, based on plant nutrient uptake, and taking into account irrigation water characteristics and possible fertilizer losses due to leaching. However, the fertilization plan was disposed with the aim of preventing any nutritional stress. Macro and micro-nutrients were in part supplied at the transplant date, through controlled release fertilizers blended with the substrate (3 kg m^{-3} of Osmocote Pro® 3-4 months + 3 kg m^{-3} of Osmocote Pro® 5-6 month). An additional fertigation, between the exponential growth and incipient flowering phases, (from 40 to 50 DAT_1) was performed with water soluble salts for a total supply of 0.21 N, 0.28 P_2O_5 and 0.13 K_2O kg m^{-3} of substrate.

Climate conditions were monitored continuously (5-minute basis) during the experiment by recording radiation, relative humidity and temperature of the air through the on-site meteorological station (Dacagon Device, Pullman, WA 99163 USA). Minimum, mean and maximum daily average photosynthetic photon flux density values were 109.2, 568.3 and 750.5 µmol m^{-2} s^{-1}, respectively. Mean daily global radiation averaged 21.7 MJ m^{-2} d^{-1}. Minimum, mean and maximum daily average of air temperature values were 11.6, 20.5 and 22.3 °C, respectively. Mean daily relative humidity of air averaged 64.5 %.

Biobased materials under investigation

The soluble and insoluble hydrolysate products were produced and supplied by Studio Chiono ed Associati in Rivarolo Canavese, Torino, Italy. They were obtained as previously reported by Sortino et al. (2014). The pristine materials were the digestate recovered from the anaerobic fermentation of the organic humid fraction of municipal solid waste from separate source collection, and the green compost obtained by 180 days aerobic fermentation of private gardening and public park trimmings. The digestate and the compost materials were processed in a pilot production facility comprising a 500 litre capacity reactor. The solid material was treated under stirring with alkaline water at 4 ml g^{-1} liquid/solid and 0.02 w/w KOH/solid ratios at 60 °C for 4 h. The reaction mix was allowed settling to separate the supernatant liquid containing the soluble hydrolysate fraction from the solid insoluble hydrolysate residue. The latter residue was washed once with fresh water at 4 ml g^{-1} added water/insoluble hydrolysate ratio. The total collected liquid phase was centrifuged to separate fine solid particles, and run through a 5 kDa cut off ultrafitration polysulphone membrane. The membrane retentate containing the soluble hydrolysate, and the solid insoluble hydrolysate were dried in ventilated oven at 60 °C. The final dried products were analyzed according to previously reported methods (Montoneri et al., 2011) and yielded the analytical values reported in Table 1 for the sourcing digestate (D) and green compost (GC) materials, and for the respective hydrolysates, i.e. the soluble (SD) and insoluble (ID) digestate hydrolysates, and the soluble (SGC) and insoluble (IGC) compost hydrolysates.

Treatments

Treatments were arranged based on the following criteria and/or objectives: i) to supply amounts of treatments' products per unit area or plant, which were negligible, compared with the applied doses of most biowaste sourced materials (such as compost), which are reported in literature (Dorais et al., 2007); ii) to supply amounts of macronutrients contributed by the treatments' products, which were negligible, compared with the normally applied amounts of chemical fertilizers; iii) to account for effects of treatments' products as a function of their formulation and distribution mode; iv) to study possible interactions between products obtained by different sourcing materials.

Two different types (factors of variability) of treatments were applied (Table 2): i) the substrate treatments (SUB) comprising 11 treatments and the control, and the fertigation (FERT) treatments comprising 2 treatments and the control. In the first case, the substrate at seedlings transplant was blended with the different materials, which are listed in Table 2. These are the commercial NOVA biostimulant, the green compost (GC), the insoluble compost hydrolysate (IGC), the soluble compost hydrolysate (SGC), the compost mixed with its soluble hydrolysate (GC/S), the insoluble compost hydrolysate mixed with the compost soluble hydrolysate (IGC/S), the digestate (D), the insoluble digestate hydrolysate (ID), the soluble digestaste hydrolysate (SD), the digestate mixed with the soluble digestate hydrolysate (D/S), the insoluble digestate hydrolysate mixed with the digestate soluble hydrolysate (ID/S). In the fertigation experiments, only the soluble compost (SGC-F) and digestate (SD-F) hydrolysates versus the untreated control were tested. In this case, the total dose of each soluble hydrolysate, which is reported in Table 2, was delivered through the irrigation system in four weekly irrigations, starting at 50 DAT_1. The NOVA product was not included in the fertigation experiments, as no claim for this application mode was reported in the vendor's product description (Biolchim, 2015). The day before treatments, irrigation was interrupted, which allowed a loss of water up to the same quantity of easily available water (roughly 400 ml pot^{-1}) in the pot volume. This quantity was then recovered by supplying the same amount of water with the dispensed soluble product solution. This expedient allowed preventing reduction of the applied dose per plant per week, due to possible products' leaching.

Table 2 reports also dry matter, and organic matter (OM) and carbon (C) applied doses. These were calculated basing on previous studies carried out with similar products in tomato and green pepper cultivation trials (Sortino et al., 2013 and 2014). Based on the chemical composition of the applied products (Table 1), dry matter doses were arranged in order to apply the same amount of OM with each product. The only exception was the commercial NOVA product, which was inevitably applied at the dose suggested by the producer. The products' mixtures for treatment 6, 7, 11 and 12 (Table 2) were made at 4/1 OM w/w ratio of the respective ingredients.

The above experimental plan, containing the two SUB and FERT factors of variability (Table 2), allowed analysing 36 SUBxFERT combinations. In this fashion, three types of effects could be investigated: i) effects due to the nature of the applied product; ii) effects related to the product application mode; iii) effects from the possible interaction of the product nature and application mode. In addition to what reported in Table 2, six more treatments were also carried out. In this case, the control substrate and soluble hydrolysates in fertigation treatments were replicated but with a total supply of nutrients equal to roughly 1/3 the dose reported in Table 2. These treatments were excluded from statistical analyses, except for the linear regression analysis of photosynthetic rate and leaf tissue characteristics. To this purpose, the additional treatments were necessary to have a wider range of values, especially below optimal growing conditions (i.e. limited nutrient availability).

Plant biomass analyses

Plant growth was monitored measuring leaf area index (LAI), by portable ceptometer (AccuPAR LP-80, Decagon Devices Inc, Pullman WA 99163 USA), and plant height, twice per month. Two destructive plant analyses, at 50 (just before the beginning of fertigation treatments) and 110 DAT_1 (at the end of the experiment) were performed to quantify the effect of treatments on physiological and biometric parameters, biomass accumulation and partitioning, and tissue mineral content of the plants. Before destructive analysis, SPAD index was measured through a portable SPAD-502 (Konica Minolta Optics, 2970 Ishikawa-machi, Hachioji, Tokyo, Japan) on six healthy leaves per plant, from the bottom to the top, and then averaging a total of 18 measurements per replicate. Plant height, mean diameter (of the canopy projected to the soil) and number of flowers and stems were measured. Plan height (cm pt^{-1}) and mean diameter (cm pt^{-1}) were used to calculate plant ellipsoid volume (cm^3 pt^{-1}) and the plant shape index (as the plant height to diameter ratio). Afterward, the plant material was separated into flowers, stems and leaves, and weighted to assess biomass fresh weight. A portion (roughly 150 g) of leaves was used for measuring unit leaf area through a leaf area meter (WinDIAS Image Analysis System, Delta-T Devices, U.K.). Unit leaf area was used to calculate leaf area index (LAI, m^2 m^{-2}). Plant fresh material was dried in a forced-air oven (80°C for 72 h) and weighed for assessing plant dry matter partitioning.

Leaf tissues were analyzed for the organic nitrogen content (N) by Kjeldhal method. Leaf dry matter (0.5 g) was weighted, put into pyrex tubes with a selenium catalyser (potassium sulphate 4.63 g, copper sulphate 0.28 g and selenium 0.09 g) and digested with 12 ml of phosphosulfuric acid at 370 °C for 40’ using the VELP-K20 apparatus by VELP Scientifica, Usmate MB, Italy. At the end of the digestion, the samples were distilled using the VELP-UDK127 apparatus (VELP Scientifica, Usmate MB, Italy) after adding 40 ml of NaOH (40 % w/V). The distillate was collected in a conical flask containing boric acid (4 % w/V) and bromocresol green methyl red colour indicator. Finally, the content of N was determined by titration with 0.1 N HCl.

Leaf gas exchange measurements

Leaf gas exchange measurements were performed in two different periods, during the experiment, at 45 DAT_1, just before the first destructive plant analysis, or 105 DAT_1, at the end of the experiment. A portable gas analyzer (Portable Photosynthesis System Ciras-2, PPSystems, Amesbury, MA 01913 USA) was used to perform onsite measurements during the morning (between 9.00 and 12.00 am). During measurements, to maintain comparable analytical conditions, the chamber was set at a constant value of light saturating photosynthesis (PPFD = 1000 µmol m^{-2} s^{-1}, primarily determined through photosynthesis light-response curves), CO_2 (400 g m^{-3}), vapour pressure deficit (VPD = 1.0 ±0.2 kPa) and temperature (27.5 ±1 °C). The temperature value was the average of climate data recorded with a datalogger, in the same daily period of measurements, over the three days prior to measurement start up. Two mature and healthy leaves (second and fourth leaf, completely unfolded above the apex of the main stem) were chosen for gas exchange analysis. For these measurements, two plants per replicate were used. A total of twelve measurements per treatment combination at each stage was carried out. This procedure provided acquisition of values for current net photosynthetic (carbon assimilation) rate (Pn, µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (Gs, mmol m^{-2} s^{-1}). The net photosynthetic rate values were finally used for the computation of water use efficiency (Pn E^{-1}, µmolCO_2 mmol^{-1}H_2O).

Statistics

Collected data were analysed by ANOVA two-ways variance analysis, in order to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by Duncan's multiple range test (P = 0.05). Data analysis included also multiple-variable analysis (correlation analysis), variables' relationships by linear regressions and principal component analysis

(PCA). Statistics and graphics were supported by the programs Statgraphics Centurion XV (Stat Point, Inc., Herndon, VA, USA) and Prism 5 (GraphPad Software, Inc., La Jolla, California USA).

Results

Chemical composition of biowaste and commercial products.

Table 1 reports the chemical composition of the municipal biowaste and the commercial products tested for their effects in the cultivation of *Hibiscus*. All contain organic and mineral matter. NOVA is clearly distinguished for the highest content of OM, the relatively lowest organic C and highest N contents. Indeed, the NOVA OM, C/N and OM/(C+N) values are 73.8 %, 4.5 and 2.6, respectively, while the values for the biowaste products range from 50.7 to 66.4 % OM, from 6 to 22.1 C/N and from 1.4 to 1.8 OM/(C+N). These features reflect the different nature of the products' sources. In essence, the municipal biowaste products are derived from mixtures of unsorted kitchen wastes and gardening residues, pre-treated by anaerobic and/or aerobic fermentation followed by chemical hydrolysis. The commercial NOVA product (Biolchim, 2015) is described by the vendor as a proprietary mix of plant extract rich in fulvic acids, humic acids, amino acids and glycine betaine as biostimulants. Also, the biowaste products present differences, one from the other. Compared with the pristine digestate (D) and compost (GC) materials, and with the insoluble digestate (ID) and compost (IGC) hydrolysates, the soluble digestate (SD) and compost (SGC) hydrolysates have the highest content of organic matter, C and N. Compared with the soluble digestate hydrolysate (SD), the soluble compost hydrolysate (SGC) has higher content of all mineral elements, except for P and N, which are higher in the soluble digestate hydrolysate (SD). It is also worthwhile to observe that, compared with all other products in Table 1, the insoluble compost hydrolysate (IGC) has the higest content of Si and Fe, followed by the pristine compost (GC) in the order of decreasing Si and Fe.

Biomass accumulation and biometric parameters

Data collected during the first destructive analysis, carried out in the middle of the experimental period, before the initiation of fertigation treatments (50 DAT_1), did not show any significant effect on biomass accumulation and other biometric parameters by the substrate treatments (data not shown). On the contrary, the data (Table 3) collected by destructive analysis, performed at the end of the experiment (110 DAT_1), demonstrated significant effects on dry biomass accumulation and leaf area index (LAI). Table 3 shows that all substrate treatments, except treatment 7 by the mix of insoluble and soluble compost hydrolysates, increased significantly the leaf dry weight (DW), compared with the control no treatment 1. Significant increases in leaf DW were also observed by the fertigation treatments 14 (SGC-F) and 15 (SD-F), compared with the control plants. Similar results were found for LAI (Table 3). A significant positive correlation between LAI and leaf DW (R = 0.90 and $P < 0.001$, data not shown) was found. In particular, in the fertigation treatments 14 (SGC-F) and 15 (SD-F), the soluble compost and soluble digestate hydrolysates, respectively, caused 30.2 % higher LAI, compared with the control plants in the no treatment 13 (NO-F).

The stem and flower DW, and the total shoot DW (Table 3) showed higher selectivity in establishing the ranking order of the treatments effects. Shoot DW correlated better with stem and flower DW (R = 0.99, $P < 0.001$) than with the leaf DW (R = 0.92, $P < 0.001$). In the substrate treatments, the highest increase (25-26 %) was caused by the soluble digestate hydrolysate (SD) treatment 10 only, compared with the control no treatment 1. For the stem and flower DW, the other treatments did not appear to cause significantly different effects, compared with the control no treatment 1. For the total shoot DW, only the commercial NOVA, and the insoluble (ID) and soluble (SD) digestate hydrolysates caused significant increase, compared with the control no treatment 1. In the fertigation treatments 14 (SGC-F) and 15 (SD-F) by the soluble compost and soluble digestate hydrolysates caused significant 24.2 % stem and flower DW, and 22.4 % total shoot DW increase, compared with the control no treatment 13 (NO-F).

Unlike the significant differences found in DW accumulation, the different treatments did not affect at all neither biomass partitioning nor the percentage of DW in plant tissues (data not shown). The latter was 17.2 % for flowers, 27.3 % for leaves, and 29.8 % for stems, calculated as the average of all treatment combinations.

Neither the substrate nor the fertigation treatments influenced significantly the main biometric parameters, which were measured during the first destructive analysis (50 DAT_1). In the substrate treatments, the only statistically significant proven effect was the plant volume increase, which was caused by the commercial NOVA product in treatment 2 at the end of the cultivation cycle (Table 3). On the other hand, the soluble compost and soluble digestate hydrolysates, which were supplied through fertigation treatments 14 (SGC-F) and 15 (SD-F), increased significantly plant height and volume (Table 3), compared with the control no treatment 13 (NO-F).

Table 3 shows also that the plant shape index was reduced by the fertigation treatments 14 (SGC-F) and 15 (SD-F), performed with the soluble compost and soluble digestate hydrolysates, compared with the control no treatment 13 (NO-F). Indeed, the control plants showed larger and shorter vegetative habitus, compared with the plants treated by fertigation.

Leaf analysis and gas exchange activity

The experimental data collected for leaf chlorophyll and N content, and for leaf gas exchange activity are reported in Table 4 and 5, respectively. Chlorophyll (represented in this work by the SPAD index) and N content in leaf samples, did not show significant differences among all treatments in the first destructive plant analysis at 50 DAT_1. However, significant differences were observed later during the last period of analyses (Table 4). In the substrate treatments, the insoluble compost hydrolysate (IGC) treatment 4, the mix of the insoluble soluble hydrolysate (ID/S), and the soluble compost hydrolysate (SGC) treatments 12 and 5, respectively, caused the highest SPAD value compared with the control no treatment 1. For leaf N, only the insoluble compost hydrolysate (IGC) treatment 4, the mix of the insoluble and soluble digestate hydrolysates (ID/S), and the commercial

NOVA product treatments 12 and 2, respectively, were significantly higher than the control no treatment 1. In the fertigation treatments, the soluble compost hydrolysate treatment 14 (SGC-F) gave the highest SPAD value. This was 4.4 % and 10.8 % higher than the respective values, which were recorded for the soluble digestate hydrolysate treatment 15 (SD-F) and the control no treatment 13. For leaf N, no fertigation treatment gave significantly different values from the value that was recorded for the control no treatment 13 (NO-F). The data in Table 4 also show that an interaction was found between the main experimental factors for chlorophyll SPAD index; such an interaction was due to the significantly higher response of the soluble compost (treatment SGC-F) and the soluble digestate (treatment SD-F) hydrolysates, when combined with the insoluble digestate hydrolysate (ID). A heterogeneous response was observed for the other combinations (data not shown).

Leaf N concentration was highly correlated with SPAD (R = 0.85, P < 0.001) while a poor, although significant, correlation was found with leaf DW (R = 0.46, P = 0.002) and total shoot DW (R = 0.59, P < 0.001) as can be also deduced by Fig. 1. The relationship between SPAD and N was significantly (P < 0.001) represented by a linear equation (SPAD = 13.09N + 0.72) with a determination coefficient explaining 73 % of the experimental variability (Fig. 1).

Gas exchange activity measured at 45 DAT_1 did not show any significant difference among treatments for the measured photosynthetic rate (Pn), stomatal conductance (Gs), and evapotranspiration (E). However, the measurements at 105 DAT_1 (Table 5) showed significant treatment effects. Substrate treatments caused significant increases of the photosynthetic activity, compared with the control no treatment 1. Photosynthetic rate of treated plant averaged 20.4 μmol m^{-2} s^{-1}, compared to 17.4 μmol m^{-2} s^{-1} for untreated plants. The compost (GC) treatment 3 gave the highest 24.3 % Pn increase, compared with the control no treatment 1. In the fertigation treatments, the soluble compost and soluble digestate treatments 14 (SGC-F) and 15 (SD-F) gave significantly higher average 22.7 % Pn increase, compared with the control no treatment 13 (NO-F). A heterogeneous plant response to the different treatment combinations was highlighted by a significant interaction of the main experimental factors. In this case, the general tendency of soluble compost and soluble digestate fertigation treatments 14 (SGC-F) and 15 (SD-F) to increase Pn was significantly more pronounced for the following combinations: treatments 1x14, 5x14, 8x14, 11x14 and 9x15 (see Table 2 for combinations) with respect to untreated plants (data not shown). These results were generally denoting that: i) soluble compost had a major effect on Pn with respect to soluble digestate; ii) combinations of products (between substrate and fertigation treatments) derived from the same organic matrixes had major effects respect to their mixtures. The collected data showed a strong correlation between Pn and SPAD (R = 0.87, P < 0.001) as confirmed by data analysis reported in Fig. 1, and a poorer although significant correlation with total shoot DW accumulated at the end of the cultivation cycle (R = 0.65, P < 0.001).

Stomatal conductance (Table 5) was significantly increased, by 45 % on average, only by the insoluble compost hydrolysate (IGC) and the GC treatments 4 and 3, respectively, compared with the control no treatment 1. In the fertigation treatments, the soluble compost hydrolysate treatment 14 (SGC-F) increased Gs by 25.7 % and 33.5 %, compared with the soluble digestate hydrolysate treatment 15 (SD-F) and to the control no treatment 13 (NO-F), respectively. A roughly similar plant response to the different treatments was observed for the evapotranspiration rate (E) reported in Table 7. In the fertigation treatments, the soluble compost hydrolysate treatment 14 (SGC-F) increased the evapotranspiration rate by 28.2 and 44.4 %, compared with the soluble digestate hydrolysate treatment 15 (SD-F) and the control no treatment 13 (NO-F), respectively. The evapotranspiration rate and the stomatal conductance were found significantly correlated (R = 0.61, P < 0.001; data not shown).

Finally, in the substrate treatments, the crop water use efficiency values (Table 5) showed no significant difference among most of treatments. The only exception was treatment 12 by the mix of the insoluble and soluble digestate hydrolysates (ID/S), which caused a 27.1 % significantly higher water use efficiency value, compared with the control no treatment 1. In the fertigation treatments, the soluble digestate hydrolysate (SD) treatment 15 (SD-F) caused 16.9 % significantly higher water use efficiency value compared with the control no treatment 13 (NO-F). On the contrary, the soluble compost hydrolysate treatment 14 (SGC-F) caused 20.1 % significant decrease of water use efficiency, compared with the control no treatment 13 (NO-F).

Treatments' ranking order and principal component analysis

Summarizing the data reported in Table 3, 4 and 5, it may be observed that, for substrate treatments, the biowaste sourced products rank significantly first in most cases. Exceptions are the plant volume, for which the commercial NOVA biostimulant ranks first, and the plant height and shape index, which showed no treatment effects. The soluble (SD) and/or insoluble (ID) digestate hydrolysates rank significantly first for their effects on four indicators connected to plant biomass accumulation and biometric parameters (Table 3). These are the leaf, stem and flowers, and total shoot DW, and LAI. The insoluble compost hydrolysate (IGC) and/or the compost (GC) rank significantly first for the effects connected to the Pn, leaf N and SPAD index (Table 4 and 5). The ranking order of the fertigation treatments summarizes the results described in the above subsections. In essence, the soluble digestate and compost hydrolysates treatments 14 (SGC-F) and 15 (SD-F), compared with the control no treatment 13 (NO-F), showed significant effects on all indicators. The only exception was the leaf N content, for which no significant difference occurred among treatments 14 and 15, and the no treatment 13 (Table 4). Treatment 15 was better than treatment 14, for the effect on plant height and volume. On the contrary, for the effects on Gs, E and SPAD index, the soluble compost hydrolysate in treatment 14 (SGC-F) performed better than the soluble digestate hydrolysate in treatment 15 (SD-F).

The above-summarised results were used as a base for the interpretation of relationships among treatments, between treatments and nature of the pristine materials, and between treatments and investigated variables. To this end, principal component analysis (PCA) was performed by using the investigated parameters to summarise main differences among treatments and sourcing materials under investigation (Fig. 1). As reported in Fig. 1, the first principal component 1 (PC1) separates treated and untreated plants. Such a separation is more marked for fertigation treatments that are grouped in three main areas related to: i) no treatment 13 (100 % of its combinations); ii) soluble green compost hydrolysate treatment 14 (92 % of its combinations); iii) soluble digestate hydrolysate treatment 15 (83 % of its combinations). The latter two treatments were then separated by the second principal component 2 (PC2). According to the loadings (Fig. 1), treatment combinations with soluble green compost hydrolysate (treatment 14) were more correlated with ecophysiological (Pn, E and Gs) parameters and tissue characteristics (leaf N content and SPAD) than combinations treatments obtained by soluble digestate treatment 15. However, by PC1 it was clear that both treatments were, in general, more correlated with biomass accumulation, plant height and volume, SPAD, leaf N content, and photosynthesis than untreated plants (no treatment 13). Within the three main clusters, treatment combinations with NOVA and hydrolysate products were more correlated with the above parameters than untreated plants (control treatment 1), which performed at the lowest level. More in details, hydrolysates products (treatment 4, 5, 9 and 10) were, on average, more correlated with the above parameters than pristine materials (treatment 3 and 8) and/or mixed treatments (treatment 6, 7, 11 and 12) that instead gave heterogeneous responses.

Discussion

To avoid any nutritional plant stress, the crop received an optimal amount of nutrients by standard chemical fertilization (see Materials and Methods, growing conditions section). The applied products under investigation (Table 1) contained a quantity of potential plant nutrients (for instance roughly 2-6 % N on dry matter basis) that varied, as a function of product chemical nature. As well known, any increase in nutrient supply, above an optimal threshold, does not produce any significant effect on plant yield and dry biomass accumulation (Massa et al., 2009; Reid 2002; Silberbush et al., 2005). Therefore, the amounts of potential additional nutrients, which were contributed by the added experimental biowaste and commercial NOVA products (Table 1 and 2), were deemed negligible (2-5 %) compared with the amounts of nutrients, which were available to the crop by the standard chemical fertilization. This consideration appears highly relevant for the following discussion.

The data reported in Table 3-7 show that the plant response to the applied treatments was generally positive, showing higher accumulation of fresh (data not shown) and dry biomass and photosynthetic activity, compared with untreated plants. Considering the relatively low amount (Table 2) of products blended with the substrate (in the substrate treatments), and/or delivered through the fertigation system (in the fertigation treatments), the increase in most of the measured plant parameters (Tables 3-5) was remarkable. In fact, the total quantity of products used was in the order of 5-15 % of the minimum dose for common organic fertilizers and/or generic amendments, which are normally applied in agriculture (Dorais, 2007).

In recent on-field red pepper and tomato cultivation trials, Sortino et al. (2013 and 2014) found that the application of 700 kg ha^{-1} of urban biowastes' hydrolysates did not alter significantly the soil chemical composition. Yet, plant photosyntethic activity, growth, and productivity were enhanced significantly by the added soluble hydrolysates. The authors concluded that the tested products enhanced the plant photosyntethic activity and that, in turn, the increase of photosynthetic activity was the main factor increasing plant growth and productivity. More recently (Fascella et al., 2015), this hypothesis was confirmed by applying the same soluble hydrolysates for the cultivation of Euphorbia in pots. The data collected on *Hibiscus* seem to support the role of the tested products as promoters of the plant photosynthetic activity (Table 5). In relation to these previous works, and to the present work, there are two key issues deserving further discussion. These are (i) the relationship between leaf chemical features, gas exchange activity, and biomass accumulation, and (ii) the relationship of the observed effects with the applied products chemical nature and/or composition.

Several authors (Bulgari et al., 2015; Calvo et al., 2014; du Jardin, 2015; Ertani et al., 2013a) have associated the increase in dry biomass of agricultural crops, treated with products derived from organic matrixes, to the presence of organic molecules that stimulate plants. More generally, biostimulant substances augment biomass accumulation and yield, improve nutrient uptake and enhance metabolic functions. Also, many biostimulant organic substances, such as algae and/or seaweed extract, soil and water humic and fulvic acid, have been found to improve photosynthetic capacity in several different plant species, which are grown either under optimal conditions (Castro et al., 2012; Jannin et al., 2012) or in presence of abiotic stress (Anjum et al., 2011; Ertani et al., 2013b). Humic-like substances, obtained from different organic wastes, fall in this category, either for their chemical nature or for their biostimulant activity (Eyheraguibel et al., 2008; Morard et al., 2011). The biowaste derived products tested in this work belong to the category of "complex organic material" (du Jardin, 2015) and bear structural similarities with natural humic substances (Montoneri et al., 2011). They are rich in molecules, which are typically found in products claimed to have biostimulant activity (e.g. Biolchim, 2015).

For the indicators connected to *Hibiscus* biomass accumulation in the substrate treatments, the products' ranking in order of decreasing effect changes, depends on the plant parameter and organ (Table 3). By comparison, in the fertigation treatments, the soluble products (SGC-F and SD-F) improved most of the above indicators, compared with the control (NO-F). However, it should be highlighted that fertigation treatments practically received a quantity of products higher than substrate treatments (Table 2), which could raise the hypothesis of an additive and/or dose response effect of the tested products as observed for example by Sortino et al. (2013) with soluble hydrolysates. As matter of fact, plant response to fertigation treatments was more pronounced and defined than for substrate treatments (Tables 3-5 and Fig. 1).

Attempts to correlate the observed effect ranking order with the products' chemical characteristics (Table 1) did not allow assessing definite clear product-properties relationships. It is true that the investigated products differ for the concentration of macro- and micro-nutrients. However, when the products are supplied to the plants at the doses reported in Table 2, the mineral composition differences are likely to be levelled out by the higher relatively amount of nutrients supplied by the conventional chemical fertilizers. It was worth to determine the amount of beneficial elements such as Se and Si among the oligoelements in the texted products. Selenium can have a growth-promoting effect for many plant species (Pilon-Smits et al., 2009), for example by increasing N reductase activity (Nowak et al., 2004). It naturally occurs as trace element in most soils, at typical levels below 1 mg kg^{-1} (Pilon-Smits et al., 2009). Selenium in the investigated products (Table 1) has been found slightly above this level, only in the soluble digestate hydrolysate (SD) and the commercial product (NOVA). However, neither any significant variation of Se nor correlation with N content was found in leaf tissues (data not shown). These findings did not help to explain the products' performance ranking order and were in agreement with previous works (Hawrylak-Nowak et al., 2015; Rios et al., 2013).

Leaf N correlated (Fig. 1) well with SPAD, but less well with Pn, and biomass accumulation parameters. Weak correlations between Pn and leaf N were observed by other authors (e.g. Kenzo et al., 2015). In the present experiment, it is likely that the optimal nutrient availability in the root allowed plants taking up the needed quantity of nutrients to support the higher growth rate induced in treated plants, without affecting leaf N content (Table 4). The high correlation between Pn and SPAD, as observed in the present work (Fig. 1), is usual. Very often, the increase in Pn, in biostimulated plants, is coupled to higher chlorophyll and nutrient content in plant tissues (Bulgari et al., 2014; Calvo et al., 2014). To this purpose, most of literature is based on the study of extensive crops, cultivated on soil and treated through foliar application (e.g. Anjum er al. 2011; Jannin et al., 2013). By comparison, very little is known on potted ornamental plants cultivated in substrate and treated directly in the root zone.

The positive influence of tested products on chlorophyll content and Pn (Table 4 and 5) is in agreement with previous works conducted on plant biostimulation obtained with various substances and products (e.g. Amanda et al., 2009; Anjum er al. 2012; Ertani et al., 2013b; Fascella et al., 2015; Jannin et al., 2013). Furthermore, the relationship between SPAD index and leaf colour (e.g. Papasavvas et al., 2008; Shibayama et al., 2012) helps to assess a relevant commercial parameter for ornamental plants such as leaf greenness. To this purpose, Loh et al. (2002) proposed SPAD meter as effective tool for the evaluation of plant quality in ornamental crops.

The data collected on tissue characteristics and leaf gas exchanges (Table 4 and 5) support the intriguing role of the biowaste derived products as photosensitizers (Bianco Prevot et al., 2011, and

Gomis et al., 2014), promoters of photosynthetic activity, in agreement with Sortino et al. (2013 and 2014) and Fascella et al. (2015). This hypothesis is consistently with similar findings for humic acids (Bulgari et al., 2015; Calvo et al., 2014), bearing structural similarities with the above biowaste product (Montoneri et al., 2011).

The compost (GC) and the insoluble compost hydrolysate (IGC) rank first for the effects on Pn in the substrate treatments (Table 5). These data were consistent with PCA shown in Fig. 1. Table 1 shows that these products contain the highest amounts of Fe and Si, compared with all other tested products. The contribution of the compost (GC) and insoluble compost hydrolysate (IGC) to the total amount of Fe and Si was roughly 64 % and 177 %, respectively, higher than the average value contributed by the other applied products listed in Table 1. Both Si (Houben et al., 2013) and Fe (Miller et al., 1995) are known to have important role in photosynthesis. Gomis et al. (2014) have proposed that Fe in the soluble compost hydrolysate is responsible of the photosensitizing properties of this material, which were demonstrated for the remediation of chemical industrial waste waters containing organic pollutants. Other works (du Jardin, 2015; Li et al., 2015; Pilon-Smits et al 2009) report that Si is a beneficial element for plants, inasmuch as it plays a key role against several biotic and abiotic stress, and promotes plant growth and development, chlorophyll concentration and photosynthetic activity. The data collected in the present work seem consistent with the above literature findings. However, considering all treatments, a definite correlation of photosynthetic activity and/or SPAD index vs. the amounts of applied Si and Fe amounts could not be established. Very likely, the effects of the above mineral elements do not rely to the applied amounts, but depend also on the nature and solubility of the organic matter to which they are bonded.

The confirmation that the biowaste-derived products, which were tested in the present work, promote plant photosynthetic activity is certainly relevant. Nevertheless, under the adopted experimental conditions, many other factors could contribute to the observed plant growth and development. The weak, although significant, correlation found between Pn and the total shoot DW (R = 0.65, P < 0.001; see also Fig. 1) leads to suppose that not all applied products in the present work were able to induce a real improved efficiency in the conversion of carbon assimilates in plant biomass. As matter of fact, only the soluble (SD) and insoluble (ID) digestate hydrolysates, and the commercial NOVA biostimulant, in the substrate treatments, and the soluble compost (SGC) and soluble digestate (SD), in fertigation treatments, caused a significant higher accumulation in total shoot DW (Table 3). This could also imply likely further positive effects by the above mentioned treatments on different aspects of plant primary and secondary metabolism, other than photosynthesis. For example, other complex organic materials have been found improving nutrient uptake (e.g. Ertani et al., 2013b; Morard et al., 2011) and other metabolic processes (du Jardin, 2015; Ertani et al., 2013a). On the other hand, a high carbon intake does not necessarily results in high long-term carbon storage. Indeed, not all assimilated CO_2 is converted in structural biomass due to different respiration efficiency during dark hours and carbon allocation to the secondary metabolism, as for example related to volatile organic compounds (Herms and Mattson 1992). However, the use of products stimulating photosynthetic activity appears of great interest to ornamental cultivation for boosting high yield and quality, and for improving the efficiency of the production process. Photosynthesis is a process very sensitive to biotic and abiotic stresses (Ashraf and Harris, 2013). The application of the tested products could be a practice for overcoming and/or containing moderate stressful conditions, which affect photosynthesis (e.g. Flexas et al., 2004; Lieth and Pasian 1991; Yamori et al., 2005), with positive effects on production sustainability and market competition (e.g. shorter cultivation cycle).

In the present work, together with the highest enhancement of Pn, the compost-derived products caused the highest E and Gs values compared to untreated plants, thus decreasing crop water use efficiency (Tables 5). Digestate-derived products resulted more efficient in enhancing the crop water use efficiency. Generally speaking, the supply of water can not be a limiting factor in container-grown plants. This is especially the case of ornamentals where the aesthetic value of the plant is the fundamental market parameter. However, water use efficiency must be highly considered in agriculture for its environmental implications.

The collected data allow drawing some conclusions on some important issues, which have both economic and environmental relevance. These are related not only to agriculture, but also to urban biowastes processes and product development. One main issue is the value of the biowaste hydrolysates, compared with their sourcing materials. The data reported in Tables 3-5 and Fig. 1 indicate that the soluble and/or insoluble hydrolysates appear more efficient than the sourcing digestate or compost materials. Undoubtedly, the most remarkable and defined effects were obtained with soluble hydrolysates applied by fertigation (Fig. 1); this effect was also due to a likely higher bioavailability of biostimulant substances. As matter of fact, PCA (Fig. 1) highlighted the presence of three main clusters, related to fertigation treatments; treated plants were more correlated with biomass accumulation, plant height and volume, SPAD, leaf N content, and photosynthesis than control treatment 13. Within these macro-clusters, hydrolysate products were more correlated with the above parameters than untreated plants and/or plants treated only with the pristine materials. The evidence that both the soluble and insoluble hydrolysates are valuable products, more than their sourcing biowaste materials, offers positive prospects for their production process.

A further issue addressed by the present work is how general the effects of the biowaste-derived products are for different plant species (ornamentals), considering previous experiments reported on other plant species with the same or similar fermented biowastes and hydrolysates. Fascella et al. (2015) compared the same soluble digestate hydrolysates (SD) with the soluble hydrolysate obtained from a composted mix of the above digestate, and urban gardening wastes and sewage sludge. They showed that, in the case of Euphorbia cultivation, the soluble hydrolysate obtained from the compost was more efficient than the soluble digestate hydrolysate (SD). Sortino et al. (2013 and 2014) compared the same above soluble digestate (SD) and soluble compost (SGC) hydrolysates for greenhouse tomato cultivation. They found that the soluble compost hydrolysate (SGC) as well or better than the soluble digestate hydrolysate (SD). Rovero et al. (2015) compared the composted mix of the above digestate, and urban gardening wastes and sewage sludge, and its soluble and insoluble hydrolysates, in maize cultivation trials performed in open field. No added benefits were shown from the use of the soluble and insoluble compost hydrolysates, compared with the source compost. The results of the above reported studies, and the results of the present work, demonstrate that product performance depends much on the cultivated plant and on the boundary (environmental) conditions. However, different products may be obtained from a variety of different municipal biowastes sourced from different locations and pre-treated by anaerobic and aerobic fermentation (Rosso et al., 2015). This offers the prospect to obtain a wide variety of products, which can be used *ad hoc* for different cultivations. It seems therefore that the results and conclusions of the present work offer worthwhile scope for further research aiming to develop biowaste-sourced products tailored for specific plants' studies and cultivation. To this purpose, it should be underlined that many commercial biostimulant products offer scarce and often insufficient and or vague information on product dosage and distribution. One of the reasons for selecting the NOVA product, as reference commercial product in the present work, was the detailed information reported by the producer about the dosage for substrate cultivations. The comparative data reported in this work, for the biowaste-sourced products and for the commercial NOVA biostimulant, prove that the biowaste-derived products have great potential in intensive agriculture.

Conclusions

The results obtained in the present work assess the effect of biowaste-sourced products on container-grown *Hibiscus*. All treatments have been found to enhance most of the investigated plant parameters, at different degree and depending on their nature, compared to untreated plants. The most valuable effects were observed on biomass accumulation, relative growth rate, net assimilation rate, SPAD index and gas exchange activity. The comparison of the above biowaste-sourced products with the commercial NOVA biostimulant demonstrates that the biowaste-sourced products have a commercially exploitable potential. The comparison of the results obtained in the present work, with those previously reported on the performance of similar products in the cultivation of other ornamental and food plants, shows that a wide variety of biowaste-sourced products is potentially obtainable, which may be targeted to the cultivation of different plant species. This work offers scope for further worthwhile investigation on other plant species, using other different biowastes, in order to assess the full potential of municipal biowaste as source of added value products for use in agriculture. This would also involve a better understanding of the biomolecular mechanisms ruling the effects of these products.

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Figure captions

Fig. 1 Variable loadings (Fig. 1a) and data scores (Fig. 1b) obtained by principal component analysis. Acronyms (Fig 1a) represent leaf stomatal conductance (Gs), transpiration (E) and photosynthesis (Pn), water use efficiency (Pn/E), leaf chlorophyll (SPAD) and nitrogen (N) content, plant volume (V), height (H) and shape index (SI), and leaf (LeD), stem (StD) and total shoot (stems, leaves and flowers) dry weight (TD). Points and numbers (Fig. 1b) represent treatment combinations (see Table 2): i) green numbers show combinations with control treatment 1; ii) red numbers show combinations with NOVA treatment 2; iii) blue numbers show combinations with non-mixed hydrolysate products (treatment 4, 5 9, and 10). Dashed lines group all combinations with fertigation treatments: i) dashed circle for control treatment 13; ii) top dashed rectangle for soluble green compost hydrolysate treatment 14; iii) bottom dashed rectangle for soluble digestate hydrolysate treatment 15.