

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

90% yield increase of red pepper with unexpectedly low doses of compost soluble substances

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/118882> since 2016-10-04T12:25:35Z

Published version:

DOI:10.1007/s13593-012-0117-6

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

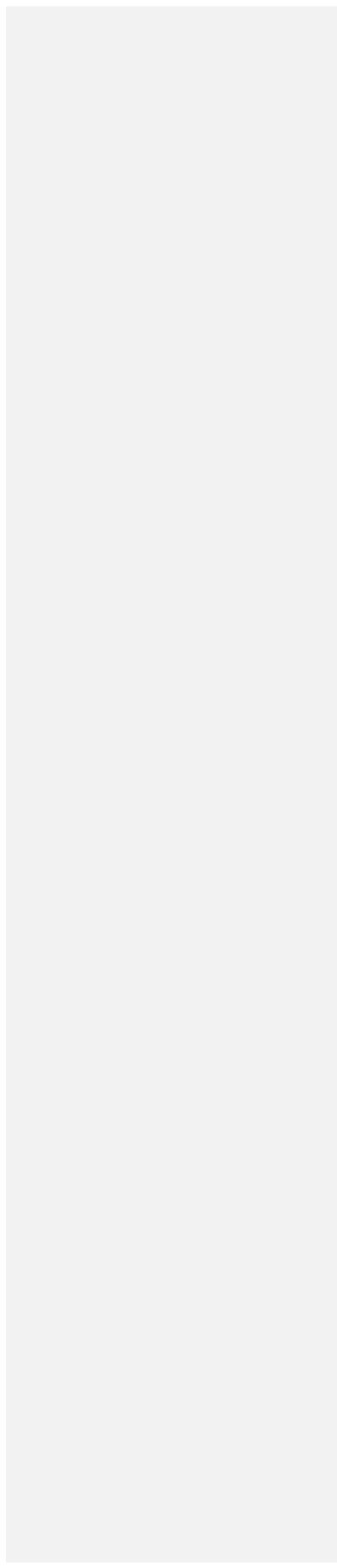
(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

1
2
3
4
5
6

The final publication is available at Springer via <http://dx.doi.org/10.1007/s13593-012-0117-6>



7 **"90% yield increase of red pepper with unexpectedly low doses of compost soluble**
8 **substances"**

10 Orazio Sortino • Mauro Dipasquale • Enzo Montoneri • Lorenzo Tomasso • Paola Avetta • Alessandra
11 Bianco Prevot.

13 **Abstract** To ensure sustainable agriculture productivity and environmental quality, composts are
14 considered for the substitution of chemical fertilizers. These materials are applied to soil at 20-30 dry matter
15 ton ha⁻¹ year⁻¹ doses to contribute organic carbon and nitrogen and minerals to compensate soil nutrient
16 depletion. Recently, soluble substances isolated from urban compost, applied to soil at 1.55 dry matter ton
17 ha⁻¹ dose, have been reported to enhance tomato plant growth and productivity more than the sourcing
18 compost. To realize the full potential of these substances for developing ecofriendly agriculture, proving the
19 above effects for other plant species and studying dose-effect relationships seemed necessary. In the present
20 study, an experimental plan for the cultivation of red pepper was undertaken. The soil and the compost
21 derived soluble substances were the same as in the previous tomato cultivation study. In this study, however,
22 the soluble substances were applied to the soil at 0-700 dry matter kg ha⁻¹ doses. The reported results show
23 that the soluble substances did not yield detectable soil chemical composition changes relatively to the
24 control soil. However, the plant leaf chlorophyll content, growth and productivity increased to maximum
25 values upon increasing the soil treatment dose up to 35-140 kg ha⁻¹. The most remarkable results were the
26 maximum productivity increases observed for the 140 kg ha⁻¹ treatment dose compared to the control soil.
27 The increases amounted to 90 % for the precocious crop yield, to 66 % for the total crop production and to
28 17 % for the per fruit weight. The discovery that the highest effects occur at such low treatment dose
29 prospects using the above soluble substances to enhance plant growth and productivity, while minimizing the
30 potential environmental impact of conventional fertilizers. The results offer worthwhile research scope to
31 explain the observed dose-effect pattern.

34 Orazio Sortino • Mauro Dipasquale Dipartimento di Scienze Agronomiche Agrochimiche e delle Produzioni
35 Animali, Università degli Studi di Catania, Via Valdisavioia 5, 95123 Catania, Italy

37 Enzo Montoneri(*) • Lorenzo Tomasso • Paola Avetta • Alessandra Bianco Prevot.
38 Dipartimento di Chimica, Università di Torino, Via P. Giuria 7, 10125 Torino, Italy

39 (*)e-mail: enzo.montoneri@unito.it

41 **Keywords:** biophotosensitizers • Biorefuse • Chlorophyll • Photosynthesis • Plant growth-productivity •
42 Renewable carbon cycle

44 **1 Introduction**

45 Eco-friendly agriculture is a new trend not only to ensure sustainable productivity but also to conserve
46 environmental quality of soil and water, reduce pollution, recycle organic resources, and produce safe foods

Formattato: Inglese (Stati Uniti)

47 (Dorais 2007; Giola et al. 2012). Indeed, synthetic chemicals have been dumped continuously over the years
48 making the land infertile and leading to yield losses. Biofertilizers have become an ideal substitute for
49 chemical fertilizers for conditioning the soil fertility and for maintaining the agro-ecosystem. There is a
50 common belief that biowastes can promote plant growth by supplying nutrients to soil. Therefore a diffuse
51 practice is to apply doses at 20-30 dry matter ton ha⁻¹ level over several years to contribute 1300-2400
52 organic carbon kg ha⁻¹ and 90-130 nitrogen kg ha⁻¹ (Dorais 2007; Haber 2008). Such nutrient amounts are
53 intended to make up for soil nutrient depletion due to repeated use of soil for plant cultivation. In some cases,
54 the effects of biowastes on plant production are expected to become evident not before several years from
55 their application to soil. However the effects of organic fertilizers depend on soil physico-chemical features.
56 In sandy aerated soil the organic carbon mineralization can be very fast and thus added organic fertilizers can
57 support crop nutrition (Fagnano et al. 2011).

58 Composted urban biowastes as biofertilizers are interesting for two reasons. They contribute to the
59 development of eco-friendly agriculture and at the same time alleviate the economic burden and
60 environmental impact of the increasing waste production by recycling to agriculture. Urban wastes' compost
61 has been reported to bring about significant changes of the physico-chemical parameters of soil, such as
62 cation exchange capacity and nitrogen and organic carbon content, which may improve soil fertility (Furrer
63 and Gupta 1983; Haber 2008). Very recently soluble substances isolated from a composted mix of food and
64 vegetable residues and applied as organic fertilizers to loamy-sandy soil for tomato greenhouse cultivation
65 have been reported to enhance leaf chlorophyll content, and to improve plant growth and fruit ripening rate
66 and yield over the crop production cycle, significantly more than the sourcing compost matter (Sortino et al.
67 2012). In separate studies the compost derived soluble substances have been found also to promote the
68 photochemical transformation of organic pollutants in aqueous solution (Avetta et al 2012). These facts
69 proposed for the above compost derived substances a possible link of solubility and photosensitizing
70 properties with the enhancement of leaf chlorophyll content and of plant and crop production in the above
71 tomato cultivation study (Sortino et al. 2012).

72 Although the perspective of using photosensitizers to promote photosynthesis is rather intriguing, one
73 experiment is certainly not sufficient to prove this new role of the above compost soluble substances in
74 agriculture. In addition, there are a number of other reasons for the observed performance of these substances
75 as plant growth promoter. They have been reported to contain 29 % minerals together with organic matter
76 (Sortino et al. 2012). They could therefore add soluble mineral plant nutrients to soil, in addition to organic
77 matter. These substances could also act as bio-effectors. They might stimulate the uptake from roots of soil
78 nutrients with a hormone-like effect and/or plant growth by promoting rhizobacteria. In any case, the results
79 of the above tomato cultivation study (Sortino et al. 2012) offer worthwhile scope for further investigation.
80 Proving the observed phenomena general for other plant species would add a valuable argument for use of
81 compost derived soluble matter to enhance plant productivity. For this reason, the present paper reports the
82 results obtained in the cultivation of red pepper. To guarantee similar experimental conditions as in the
83 previous tomato cultivation study, the same soil and the same compost derived soluble matter was used for

84 the reported red pepper cultivation study. Also, for the proposed biofertilizer, the present study includes the
 85 investigation of dose-effect relationships which were not comprised in the previous study.

86

87 **2 Materials and methods**

88

89 2.1 Starting materials and chemical characteristics.

90 The investigated soluble substances were obtained from compost supplied by Acea Pinerolese Industriale
 91 SpA, Pinerolo (TO), Italy in October 2009. The company has an urban waste treatment plant performing
 92 anaerobic digestion of the organic humid fraction of urban refuse for 14 days. The refuse, obtained by
 93 separate source collection practice, yields biogas and digestate containing residual lignocelluloses' material.

94 The following scheme summarizes the four main process steps:

digestate + fresh vegetable matter → compost (1)

compost + water (pH 13) → hydrolyzate + insoluble residue (2)

hydrolyzate ultrafiltration → retentate + permeate (3)

retentate drying at 60 °C → solid soluble substances (4).

95 In step 1 the digestate was mixed in 1:2 w/w ratio with fresh vegetable matter constituted by gardening and
 96 park trimming residues and composted for 110 days. Composting took place in 1.5 m high piles laid over a
 97 3x70 m area. During the first 21 days the pile was turned once a week and reached temperatures of up to 70
 98 °C. Afterwards, the solid residue was aged for 90 days and turned once on the 75th day. The compost was
 99 then further processed in a pilot plant made available by Studio Chiono e Associati in Rivarolo Canavese,
 100 Italy. The plant comprises an electrically heated mechanically stirred 500 L reactor, a 102 cm long x 10.1
 101 cm diameter polysulfone ultrafiltration membrane with 5 kD molecular weight cut-off supplied by Idea
 102 Engineering s.r.l. from Lessona (Bi), Italy, and a forced ventilation drying oven. According to the operating
 103 experimental conditions, in step 2 the compost was mixed with an aqueous solution of NaOH at pH 13 in a
 104 4:1 V/w water/solid ratio and treated 4 h at 60 °C. The liquid/solid mix was allowed to settle to separate the
 105 top liquid phase containing the soluble compost hydrolyzate from the insoluble residue. In step 3 the
 106 recovered liquid phase was circulated at 40 L h⁻¹ flow rate through the ultrafiltration membrane operating
 107 with tangential flow at 7 bar inlet and 4.5 bar outlet pressure to yield a retentate with 5-10 % dry matter
 108 content. In step 4 the concentrated retentate was finally dried at 60 °C to 10 % moisture content. The final
 109 product constituted by the soluble substances was isolated as a black solid in 10 % w/w yield, relatively to
 110 the starting compost dry matter. The product was characterized for its content of organic carbon and nitrogen
 111 and of mineral elements: i.e. C 40.8, N 5.1, Na 8.4, K 1.2, Ca 2.6, Mg 0.71, Fe 0.98, and P 0.37 % w/w
 112 referred to dry matter. These data were obtained by the same analytical methods reported below for soil
 113 analyses (see section 2.3). The red pepper plant seedlings (*Capsicum annum*, F1 barocco) produced in Chile
 114 in 2010 were supplied by Clause, France.

115 2.2 Set up of cultivation trials.

116 The red pepper cultivation trials were carried out in the Piombo farm located along the sea shore in Punta
117 Secca in the province of Ragusa, Italy, where the previously reported tomato cultivation trials were also
118 carried out (Sortino et al. 2012). The farm soil was classified loamy-sandy, based on its texture % w/w data:
119 sand 79.9 ± 2.5 , fine sand 5.3 ± 0.7 , silt 10.6 ± 1.8 , clay 4.2 ± 0.4 . The experiment was set up as a
120 completely randomized design with 3 replications in a greenhouse fabricated with 0.15 mm thick
121 polyethylene film supported by cement and wood. The greenhouse soil was divided into 21 parcels, each
122 covering 30 m^2 soil surface. Three control parcels had no added soluble substance. The other 18 parcels were
123 divided in 6 groups of three parcels per groups. The six parcel groups were treated with 7, 35, 70, 140, 350 e
124 700 soluble substances kg ha^{-1} respectively. The soluble substances were applied to the soil on November 12,
125 2010 as aqueous solution at pH 10.4 containing 14 % dry matter. These, by their chemical composition and
126 applied doses, were expected to contribute to soil organic carbon, organicnitrogen, and mineral elements in
127 the following kg ha^{-1} amount ranges: C 2.86-286, N 0.36-36, Na 0.59-59, K 0.081-8.1, Ca 0.18-18, Mg 0.05-
128 5.0, Fe 0.069-6.9, and P 0.026-2.6. Four days later, the test plant seedlings were transplanted in all parcels to
129 yield three sets of double rows per parcel, with distance of 120 cm between sets, 80 cm between rows in each
130 set and 30 cm between plants in each row. This design yielded 3.3 plants per square meter density. After
131 transplanting the soil was covered with white polyethylene film equipped for underneath drip irrigation. All
132 other cultivation details were the same for all parcels and carried out according to the protocol adopted by
133 the hosting farm in its normal cultivation activity (Sortino et al. 2012). The experimental plan was carried
134 out over 7 months from seedling transplantation to crop harvesting.

135 2.3 Soil, plant and harvest analyses and measurements.

136 Soil samples were taken at 0-30 cm depth. Four samples per parcel were taken and homogenized. The
137 homogenized sample was analysed in triplicates according to the official methods for soil analysis issued by
138 the Italian Ministry of Agriculture (Ministero per le Politiche Agricole 1997 and 1999). The pH and
139 electrical conductivity were determined in water at 1:2.5 solid/water ratio. Microanalyses for carbon and
140 nitrogen content were performed on 0.5 mm sieved samples. Analyses were performed for exchangeable
141 cations, held on negatively charged soil sites, and assimilable nutrients, i.e. those which may be absorbed by
142 the roots. The assimilable P concentration was determined colorimetrically (phosphomolybdic complex),
143 after NaHCO_3 extraction. The assimilable Na, Mg and Fe concentrations were measured by atomic
144 absorption spectrophotometer after ammonium acetate/ethylenediaminetetraacetic acid extraction. The
145 exchangeable K, Ca and Mg concentrations were determined by atomic absorption spectrophotometer after
146 BaCl_2 extraction. Plant and harvest measurements were performed on four plants sampled in the center row
147 of each soil plot. Considering the three replicates per treatment, values for each treatment reported
148 hereinafter are averages of measurements performed over 12 plants (3 replicate plots and 4 plants per
149 replicate), unless otherwise indicated. Four fruit clusters per plant were sampled. The plant leaf chlorophyll
150 content was measured by means of a portable spad-502 Minolta chlorophyll meter. Through optical analysis
151 this instrument yields an indirect estimate of the chlorophyll content of plant leaves without damaging the
152 leaves during the plant growth and production cycle. It measures the absorbance at one wavelength falling

153 within the chlorophyll absorbance range and calculates a chlorophyll concentration index value that is
154 proportional to the amount of chlorophyll in the sample. Absolute chlorophyll content per unit area was not
155 computed. Plant height, diameter at branches' forking and leaf chlorophyll content were measured at
156 different dates from the moment of seedlings transplantation until April. Plant productivity was determined
157 by measuring number, weight and size of fruits starting from May 2 to production end.

158 2.4 Statistical treatment of data

159 Dose treatments were compared for their average values by Anova analysis of variance and multiple
160 comparison post-hoc testing using Addinsoft XLSTAT software dated 2009.4.07.

161

162 **3 Results and discussion**

163

164 Table 1 reports the chemical composition and physico-chemical features of the untreated control soil and the
165 soil treated with the highest dose of the soluble substances investigated in this work. Based on the chemical
166 composition data of the soluble substances (see section 2.1), the kg ha^{-1} amounts of carbon, nitrogen and
167 mineral elements contributed to the soil by the highest 700 kg ha^{-1} soluble substances applied dose were
168 estimated as follows: C 286, N 36, K 8.1, Ca 18, Mg 5.0, Na 59, Fe 6.9, and P 2.6. As shown by the
169 statistical analysis of the soil data collected at two different dates over the crop production cycle, even the
170 highest 700 kg ha^{-1} dose did not alter the chemical composition and physico-chemical features of the treated
171 soil, compared to the untreated control soil. No significant changes from February to April occur in the
172 measured soil physico-chemical parameter, except for Ca and Mg being significantly lower in April.
173 However, at each measurement date, no significant differences are proven between the control and the
174 treated soil.

175 The data in Table 2-4 prove however that strong effects by the dose treatments occur on the plant
176 chlorophyll content, growth and productivity. Specifically, Table 3 shows that the effect of the soluble
177 substances on the plant leaf chlorophyll content is evident already during the first month of the experiment.
178 At each measurement date the leaf chlorophyll content increases to the 64-67 spad unit maximum values
179 upon increasing the soluble substances' dose up to 140 kg ha^{-1} and then apparently tends to decrease for
180 higher dose. However, the superior effect of the 140 kg ha^{-1} dose treatment is proven statistically significant
181 only in February. In March and April, and to some extent December also, the difference between the 140 kg
182 ha^{-1} is non-significant compared to 35, 70, 350 and 700 kg ha^{-1} dose treatments. These findings show that the
183 35 or 70 kg ha^{-1} doses are also very effective in increasing chlorophyll contents and the similar kind of role
184 has also been observed at 350 or 700 kg ha^{-1} . For tomato cultivation, the same soluble substances applied to
185 the same soil at 1.55 ton ha^{-1} dose were found to enhance significantly leaves chlorophyll content by 13 %
186 relatively to the control and by 8-13 % relatively to the sourcing compost (Sortino et al. 2012). In this
187 previous study, over five determinations performed from January through May 2010, 54 and 50 Spad unit
188 peak levels were measured in April for plant leaves grown in soil treated with the soluble substances and
189 their sourcing compost respectively. The results reported now for red pepper show that the same chlorophyll

190 enhancement relative values may be obtained even at much lower doses, down to 2 % of those applied in the
 191 tomato cultivation trials. The relatively lack of sensitivity of the chlorophyll content versus the soluble
 192 substances dose increase above 35 kg ha⁻¹ reproduces the dose-effect relationship reported for compost
 193 derived soluble substances tested as photosensitizer for the abatement of organic pollutants in solution
 194 (Avetta et al. 2012): i.e. the rate of the probe molecule photodegradation increases upon increasing the
 195 soluble substances' concentration to a maximum value; higher concentration causes no enhancement of the
 196 probe molecule photodegradation rate.

197 A similar trend is true for the plant biometric data (Table 2) and for the plant crop productivity and per
 198 fruit weight (Table 4). As the February leaf chlorophyll content, also the plant height and diameter measured
 199 in February increase upon increasing the soluble substance dose applied to the soil. The plant height reaches
 200 a plateau level at 35 kg ha⁻¹. The plant diameter increases to a statistically significant peak value at 140-350
 201 kg ha⁻¹ soluble substances' dose, and then decreases for the higher soluble substances dose. The plot of the
 202 February plant diameter versus the February leaf chlorophyll content (Fig. 1) is well fit by the empirical
 203 equation (5),

$$204 \quad \Phi = (9870 \pm 2678) - (482.9 \pm 130.8) X + (7.873 \pm 2.127) X^2 - (0.0428 \pm 0.0115) X^3 \quad (5),$$

205 where Φ is the plant diameter and X is the leaf chlorophyll content, both measured in February. Fitting
 206 equation 5 to the data shown in Fig. 1a yields a regression coefficient of 0.98. The other data in Table 2
 207 result poorly correlated with the leaf chlorophyll content (Table 3) at the same sampling data.

208 As the February leaf chlorophyll content and plant diameter, also the total crop production reaches a
 209 statistically significant peak value for the 140 kg ha⁻¹ dose treatment. All Table 4 crop production and fruit
 210 parameters, except the fruit width, plotted against the April leaf chlorophyll content yield highly significant
 211 sigmoid trends (Fig. 1). Table 5 reports the results of Fig. 1 data regression according to the Boltzmann
 212 sigmoidal model equation (6),

$$213 \quad y = a_2 + (a_1 - a_2) / [1 + e^{-(X - x_0)/dx}] \quad (6).$$

214 In this equation, y is the crop production or fruit parameter reported in Table 4 and X is the leaf chlorophyll
 215 content measured in April reported in Table 3.

216 The most relevant features of the experimental data collected in this work are three: i.e. (i) the no effect
 217 on soil characteristics by the soluble substances, (ii) the magnitude of effects on the plant leaves, crop and
 218 fruit parameters in relation to the applied soluble substances' doses and (iii) the intriguing pattern of the
 219 dependence of plant leaf chlorophyll content, growth and productivity upon the applied soluble substances'
 220 dose. The no effect on soil chemical data was expected on basis of the quite high carbon and nitrogen content
 221 of the control soil (Table 1) and the relatively low applied doses of soluble substances. For instance,
 222 considering the N content of the control soil 0.15 % and assuming a bulk density of 1.4 and a soil layer of 30
 223 cm the calculated N content per soil ha is 6200 kg. Therefore the 36 kg ha⁻¹ maximum value of added N by
 224 the highest dose of the soluble substances applied to the soil corresponds to an increase of 0.58% relatively
 225 to the control soil N amount. Based on Table 1 statistics, this change would result not significant. In this
 226 work, no measurements were made on leaves or crop uptake of nutrients since in the previous tomato

227 cultivation study, performed on the same soil with the same soluble substances applied at higher doses, no
 228 significant differences were found either in the soil physico-chemical features and in the leaves and crop
 229 uptake of nutrients due to the treatment relatively to the control experiment (Sortino et al, 2012).

230 Considering the low soluble substances dose level applied to soil, the strong effects on plant growth and
 231 productivity shown in Table 2 and 4 are quite remarkable. In this study, the carbon and nitrogen amounts
 232 supplied to soil by the addition of the soluble substances were equivalent from 0.2 to 22 % of the minimum
 233 dose normally applied by compost addition to soil (Dorais 2007; Haber 2008; Fagnano et al. 2011).
 234 Nevertheless, as shown by Table 2-4 data, the plant growth and productivity were rapidly and strongly
 235 affected by the application of the above soluble substances to soil. Particularly, Table 4 shows that the peak
 236 of the plant productivity occurs in the soil treated with the 140 kg ha⁻¹ dose. This dose contributes carbon and
 237 nitrogen amounts equivalent to 4-8 % of the minimum doses reported in the above cited studies (Dorais
 238 2007; Haber 2010; Fagnano et al. 2011). The most striking result is the relative magnitude of the peak total
 239 crop production (Table 4) attained for the 140 kg ha⁻¹ dose of soluble substances. This production reaches
 240 11.1 kg m⁻², corresponding to 66 % increase relatively to the control crop production. From the commercial
 241 point of view, the crop production in the first harvesting week and the per fruit weight are also highly
 242 important. The former is an index of fruit maturity precocity which allows the farmer to obtain the highest
 243 fruit sale prices of the earlier production. The latter is related to product quality which also has an economic
 244 impact in the farm revenue. In the soil treated with the 140 kg ha⁻¹ dose of soluble substances, these plant
 245 production indicators result increased by 90 and 17 % relatively to the control soil. These results appear even
 246 more surprising when compared with those obtained in the previous tomato cultivation experimental plan
 247 carried out with the same soluble substances applied to the same soil at over 10x higher dose (Sortino et al.
 248 2012). In the case of tomato, the total crop production in the soil treated with 1.55 dry matter ton ha⁻¹ soluble
 249 substances dose was only 5.5 % higher than in the control soil, while no significant change was measured in
 250 the per fruit weight. The comparison between tomato and red pepper yields is consistent with the peculiar
 251 soluble substance dose-effect pattern evidenced by the Table 3 February chlorophyll content and Table 4
 252 total crop production data; i.e an optimum peak performance reached by the soluble substances at relatively
 253 low dose and lower performance at higher doses. There is plenty of literature on the relationship between
 254 leaf chlorophyll content measurements and plant growth and yield (Dowdell and Dodge 1970; Enriquez et
 255 al. 2005; Ciganda et al. 2009). The data in Table 5 show that indeed in this study the plant growth and
 256 productivity are highly correlated with the plant leaf chlorophyll content. This confirms that chlorophyll
 257 formation and photosynthesis are intimately related.

258 The complex processes of photosynthesis and chlorophyll formation are known to be regulated by the
 259 availability of enzymes and light (Whitmarsh and Govindjee 1999). The light dependency of chlorophyll
 260 formation is particularly true for the protochlorophyllide to chlorophyllide conversion step in angiosperms
 261 (Von Wettstein et al. 1995) to which the red pepper test plant belongs. This work does not provide any data
 262 to support any action of the investigated soluble substances as regulator of the natural enzymatic pool
 263 involved in vegetable matter biosynthesis. Certainly, the similarity of the dose-effect relationship pattern

Codice campo modificato

Codice campo modificato

264 observed in this work with that observed for the photodegradation of organic pollutants, performed with the
265 same soluble substances in the absence of any possible biochemical reaction (Avetta et al. 2012), may
266 suggest the fascinating hypothesis that these substances perform a photosensitizing activity also in
267 photosynthesis. Understanding the mode of action of the soluble substances under the experimental
268 conditions reported for the photodegradation of organic pollutants is simpler than for the present work. In the
269 former case, these substances were tested in homogeneous solution containing the probe substrate, in order to
270 function as photosensitizers. Under the experimental conditions of the present case study, the soluble
271 substances must undergo a number of mass transfer processes from the soil to the plant in order to perform
272 their action. In these processes, the soluble substances may undergo several chemical and biochemical
273 reactions in the soil and in the plant. Few data are presented in this work to confirm that the hypothesis of the
274 role of the soluble substances as photosensitizers in this work is true and that other types of interactions of
275 these substances in the biochemical processes responsible of plant growth can be excluded. Within its
276 experimental constraints, the present work has merit for two reasons. It provides new important information
277 to guide the use of these refuse derived substances for optimum performance in agriculture. This information
278 is of primary interest to farmers. To scientists, this work offers unexpected intriguing scope for further
279 research to assess the role in agriculture for the herewith reported soluble substances and for other soluble
280 substances that may be isolated from different biomass residues (Montoneri et al. 2011).

281

282 **4 Conclusion**

283

284 The capacity of the investigated refuse derived soluble substances to enhance plant growth and
285 productivity at relatively low concentration has strong practical beneficial implications. The use of these
286 substances at low dose level allows promoting plant growth and crop production, while minimizing the risk
287 of environmental impact following application of conventional mineral and organic N fertilizers. From the
288 more general ecological point of view, the results of this work, together with the results of previous work
289 (Avetta et al. 2012), propose the fascinating hypothesis that bio-refuse sourced soluble substances may enter
290 the natural carbon cycle and be used to enhance vegetable matter growth or mineralization of organic matter
291 from anthropogenic origin, depending upon the conditions in which they are used.

292

293 **Acknowledgements** This work was carried partly with Regione Piemonte Cipe 2006 funds within the Biochemenergy
294 project and partly with Ministero delle Politiche Agricole funds within the Agrienergia project. The authors are grateful
295 to the following private and/or public Italian institutions: (i) the Piombo farm by Giovanni Gambuzza for making
296 available the greenhouse installation located along the sea shore in Punta Secca in the province of Ragusa; (ii) Acea
297 Pinerolese Spa in Pinerolo (TO) for supplying the soluble substances's sourcing compost; (iii) Studio Chiono ed
298 Associati in Rivarolo Canavese (TO) for making available pilot equipment and services for the production of the
299 soluble substances.

300

301 **References**

- 302 Avetta P, Bianco Prevot A, Fabbri D, Montoneri E, Tomasso L (2012) Photodegradation of
 303 naphthalenesulfonic compounds in the presence of a bio-waste derived sensitizer. *Chemical Engineering*
 304 *Journal* 197, 193-198, and references therein . doi:10.1016/j.cej.2012.04.086
- 305 Ciganda V., Gitelson N, Schepers J (2009) Non-destructive determination of maize leaf and canopy
 306 chlorophyll content. *Journal of Plant Physiology* 166, 157-167, and references therein.
 307 doi:10.1016/j.jplph.2008.03.004
- 308 Dorais M (2007). Organic production of vegetables: state of the art and challenges. *Can. J. Plant Sci.* 87,
 309 1055–1066, and references therein. doi:10.4141/CJPS07160
- 310 Dowdell R J, Dodge A D (1970) The photosynthetic capacity of pea leaves with a controlled chlorophyll
 311 formation. *Planta* 94, 282-290, and reference therein. doi:10.1073/pnas.0914299107.
- 312 Enriquez S, Marbà N, Cebrian J, Duarte MC (2004) Annual variation in leaf photosynthesis and leaf nutrient
 313 content of four Mediterranean seagrasses. *Botanica Marina* 47, 295-306.
- 314 Fagnano M, Adamo P, Zampella MV, Fiorentino N (2011) Environmental and agronomic impact of
 315 fertilization with composted organic fraction from municipal solid waste. *Agriculture Ecosystem*
 316 *Environment*. 141, 100-107, and references therein.
- 317 Furrer OJ, Gupta SK (1983). Effects of agricultural use of municipal composts. *Plant Foods for*
 318 *Human Nutrition* 33, 251-259.
- 319 Giola P, Basso B, Pruneddua G, Giunta F, Jones JW (2012) Impact of manure and slurry applications on soil
 320 nitrate in a maize–triticale rotation: Field study and long term simulation analysis. *Europ. J. Agronomy*
 321 38, 43– 53. doi:10.1016/j.eja.2011.12.001.
- 322 Haber N. (2008) Sustainable Compost Application in Agriculture. ECN INFO PAPER, 02, 1-36. European
 323 Compost Network ECN e.V. LTZ. Center for Agricultural Technology Augustenberg Karlsruhe,
 324 Germany.
- 325 Ministero per le Politiche Agricole (1997) Metodi ufficiali di analisi fisica del suolo. D.M. del 1° agosto
 326 1997, *Gazzetta Ufficiale* n. **204** del 2.09.97.
 327 <http://www.consiglionazionalegeologi.it/cngwww/AODocumento.asp?iddoc=2842andidcat=7>. Accessed
 328 27 December 2011.
- 329 Ministero per le Politiche Agricole (1999) Metodi ufficiali di analisi chimica del suolo. D.M. del 13/09/99,
 330 *Gazzetta Ufficiale* n. **248** del 21.10.99. <http://www.crcnet.it/DOCS/DM-13-09-1999.pdf>. Accessed 27
 331 December 2011.
- 332 Montoneri E, Mainero D, Boffa V, Perrone DG, Montoneri C (2011) Biochemenergy: a project to turn a
 333 urban wastes treatment plant into biorefinery for the production of energy, chemicals and consumer's
 334 products with friendly environmental impact. *Int. J. Global Environmental Issues* 11, 170-196. doi:
 335 10.1504/IJGENVI.2011.043508.

Codice campo modificato

- 336 Sortino O., Dipasquale M, Montoneri E., Tomasso L, Perrone DG, Vindrola D, Negre M, Piccone G (2012).
337 Refuse derived soluble bio-organics enhancing tomato plant growth and productivity. *Waste Management*
338 32, 1788-1797. doi:10.1016/j.wasman.2012.04.020.
- 339 Von Wettstein D, Gough S, Kannangara GC (1995) Chlorophyll biosynthesis. *The Plant Cell* 7, 1039-1057.
340 doi:10.1105/tpc.7.7.1039.
- 341 Whitmarsh J, Govindjee G (1999). The photosynthetic process. In: Singhal GS, Renger G, Sopory SK,
342 Irrgang KD, Govindjee G (eds). *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*.
343 Narosa Publishers, New Delhi and Kluwer Academic, Dordrecht, pp. 11-51.

344 **Table 1** Analytical data^a for February and April samples of the control untreated soil and of the soil treated with the
 345 highest 700 kg ha⁻¹ soluble substance dose: average value and statistical parameters (F and Pr) obtained by ANOVA
 346 analysis of variance and multiple comparison Newman-Keuls Studentized test at significance level < 0.05^b
 347

	February 2011		April 2011		F	Pr
	Treated soil	Control soil	Treated soil	Control soil		
pH	8.03 a	8.03 a	7.23 b	7.37 b	34.5	0.000
Electrical conductivity ($\mu\text{S cm}^{-1}$)	846	759	476	376	4.63	0.037
Salinity ($\mu\text{eq g}^{-1}$)	113 a	102 ab	59.5 ab	47.0b	5.51	0.024
Total C (% w/w)	4.79	4.46	4.62	4.66	0.237	0.868
Total N (% w/w)	0.18	0.19	0.15	0.14	2.52	0.132
C/N	26.7 b	28.5 ab	31.1 ab	34.1 a	4.35	0.043
P (mg kg^{-1})	177	182	172	172	3.26	0.081
K ($\mu\text{eq g}^{-1}$)	2.7	2.7	4.7	4.7	2.18	0.168
Ca ($\mu\text{eq g}^{-1}$)	234 a	236 a	93.3 b	95.3 b	434	0.000
Mg ($\mu\text{eq g}^{-1}$)	4.3	4.7	4.0	4.3	0.444	0.728
Na (% w/w)	0.56	0.51	0.56	0.55	3.53	0.068
Mg ^c (mg kg^{-1})	469 ab	542 a	382 bc	332 c	20.2	0.000
Fe (mg kg^{-1})	308 a	368 a	184 b	182 b	17.0	0.001

^aSoil K, Ca, Mg as exchangeable ions and other elements as assimilable ions, unless otherwise indicated

^bAverage values calculated over three soil samples taken from the control or treated parcel replicates; within each row, values with no letter in common differ significantly in the order a > b

^cAssimilable ion.

348
 349
 350
 351
 352

Table 2 Plant height and diameter at branches'fork after soluble substances' application to soil: average and standard deviation values, and statistical parameters obtained by ANOVA analysis of variance^a and multiple comparisons Newman-Keuls Studentized test^b at significance level ≤ 0.05

Soluble substances' dose (kg h ⁻¹)	December 2010		January 2011		February 2011	
	Height ^c (cm)	Diameter ^c (cm)	Height ^c (cm)	Diameter ^c (cm)	Height ^c (cm)	Diameter ^c (cm)
none	24.0	0.47	37.2 d	0.75	45.7 b	0.87 b
7	24.2	0.45	37.0 d	0.78	45.1 b	0.85 b
35	24.4	0.47	40.8 b	0.78	46.9 ab	0.87 b
70	23.5	0.47	38.4 c	0.77	49.7 ab	0.91 b
140	24.5	0.42	41.2 b	0.80	52.5 ab	1.11 a
350	25.8	0.43	42.8 a	0.81	53.9 a	1.03 a
700	25.3	0.46	43.4 a	0.79	54.2 a	0.92 b
F	1.909	1.318	60.86	0.515	4.308	8.706
Pr	0.155	0.316	< 0.0001	0.787	0.013	0.001

^aF and Pr values

^bWithin each column and for the same stage, values with no letter in common differ significantly: a > b > c > d

^cAverage values calculated over 4 plants per parcel in the three replicates per dose treatment

Table 3 Leaf chlorophyll content after soluble substances' application to soil: average values and statistical parameters obtained by ANOVA analysis of variance^a and multiple comparisons Newman-Keuls Studentized test^b at significance level ≤ 0.05

Soluble substances' dose (kg h ⁻¹)	Leaf chlorophyll content (spad Unit)			
	December 2010 ^c	February 2011 ^c	March 2011 ^c	April 2011 ^c
none	51.1 c	59.7 b	59.5 b	57.1 c
7	52.5 bc	60.3 b	59.8 b	57.8 bc
35	53.9 abc	60.1 b	61.5 ab	59.3 bc
70	53.3 bc	60.4 b	62.6 ab	60.3 abc
140	56.3 a	63.8 a	66.8 a	64.3 a
350	54.0 abc	61.1 b	64.1 ab	62.3 ab
700	55.0 ab	60.6 b	63.3 ab	61.9 ab
F	6.024	2.837	4.054	5.648
Pr	0.003	0.054	0.016	0.004

^aF and Pr values

^bWithin each column and for the same stage, values with no letter in common differ significantly: a > b > c > d

^cAverage values calculated over 4 plants per parcel in the three replicates per dose treatment

Table 4 Fruit production and characteristics after soluble substances' application to soil: average and standard deviation values, and statistical parameters obtained by ANOVA analysis of variance^a and multiple comparison Newman-Keuls Studentized test^b at significance level ≤ 0.05

Soluble substances' dose (kg h ⁻¹)	Fruit production		Fruit characteristics		
	First harvesting week crop (kg m ⁻²)	Total crop ^c (kg m ⁻²)	Per fruit weight ^c (g)	Length ^c (cm)	Width ^c (cm)
none	2.15 bc	6.71 d	205.18 b	12.60	8.01
7	2.16 bc	7.11 d	206.47 b	12.93	8.52
35	1.94 c	6.75 d	216.17 ab	13.00	8.56
70	2.06bc	6.85d	209.84 b	12.33	8.31
140	4.09 a	11.15 a	240.98 a	14.43	8.94
350	3.90 a	9.85 b	219.62 ab	14.17	8.99
700	2.49 b	8.33 c	208.93 b	13.80	8.67
F	66.11	88.25	3.279	1.360	1.051
Pr	<0.0001	<0.0001	0.034	0.300	0.438

^aF and Pr values

^bWithin each column and for the same stage, values with no letter in common differ significantly: a > b > c > d

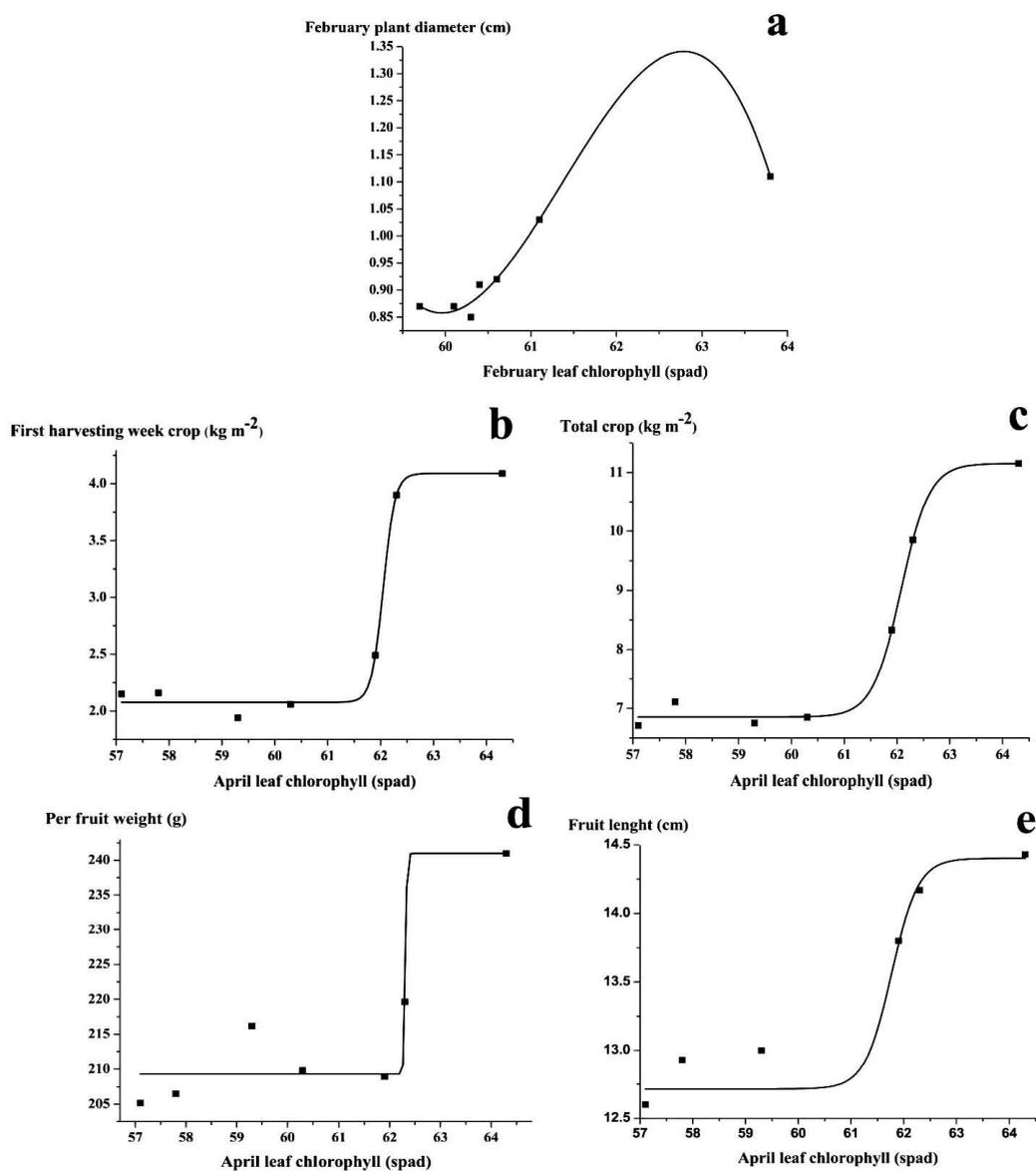
^cAverage values calculated over 4 plants per parcel in the three replicates per dose treatment

353 **Table 5** Values of constants and standard error, and of regression coefficient, for Table 4 crop production and fruit
 354 parameters dependence upon Table 3 April leaf chlorophyll content according to the empirical equation
 355 $y = a_2 + (a_1 - a_2) / [1 + e^{-(X - x_0)/dx}]$ where y = crop production or fruit parameter, X = April leaf chlorophyll content

y	Regression coefficient	a_1	a_2	x_0	dx
First harvesting week crop	0.99	2.08±0.05	4.09±0.10	62.0±0.0	0.11±0.03
Total crop	0.99	6.85± 0.09	11.1±0.2	62.1±0.0	0.27±0.05
Per fruit weight	0.92	209.32 ^a	241±5	62.31 ^a	0.01 ^a
Per fruit length	0.92	12.7±0.2	14.4±0.3	61.8± 0.3	0.25± 0.39

^aStandard error < 10⁻⁴

356



357

358

359

360

361

362

363

364

Fig. 1 Plot of plant growth and production data versus leaf chlorophyll content: **a** plant diameter versus leaf chlorophyll, both measured in February; **b, c, d, e** crop and fruit data versus April leaf chlorophyll content. Solid lines in the graph obtained according to the following regression equations:
a $\Phi = (9870 \pm 2678) - (482.9 \pm 130.8) X + (7.873 \pm 2.127) X^2 - (0.0428 \pm 0.0115) X^3$, for which Φ = plant diameter, X = February leaf chlorophyll content, regression coefficient = 0.98; **b, c, d, e** $y = a_2 + (a_1 - a_2) / [1 + e^{-(X - x_0)/dx}]$, for which y = first harvesting crop, total crop, per fruit weight or fruit length, X = April leaf chlorophyll content, equation parameters and regression coefficient as in Table 5.