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

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

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

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1 Introduction

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

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

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

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

2 Materials and methods

2.1 Starting materials and chemical characteristics.

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

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

2.2 Set up of cultivation trials.

The red pepper cultivation trials were carried out in the Piombo farm located along the sea shore in Punta Secca in the province of Ragusa, Italy, where the previously reported tomato cultivation trials were also carried out (Sortino et al. 2012). The farm soil was classified loamy-sandy, based on its texture % w/w data: 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 completely randomized design with 3 replications in a greenhouse fabricated with 0.15 mm thick polyethylene film supported by cement and wood. The greenhouse soil was divided into 21 parcels, each covering 30 m^2 soil surface. Three control parcels had no added soluble substance. The other 18 parcels were divided in 6 groups of three parcels per groups. The six parcel groups were treated with 7, 35, 70, 140, 350 e 700 soluble substances kg ha^{-1} respectively. The soluble substances were applied to the soil on November 12, 2010 as aqueous solution at pH 10.4 containing 14 % dry matter. These, by their chemical composition and applied doses, were expected to contribute to soil organic carbon, organicnitrogen, and mineral elements in 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 0.05-5.0, Fe 0.069-6.9, and P 0.026-2.6. Four days later, the test plant seedlings were transplanted in all parcels to yield three sets of double rows per parcel, with distance of 120 cm between sets, 80 cm between rows in each set and 30 cm between plants in each row. This design yielded 3.3 plants per square meter density. After transplanting the soil was covered with white polyethylene film equipped for underneath drip irrigation. All other cultivation details were the same for all parcels and carried out according to the protocol adopted by the hosting farm in its normal cultivation activity (Sortino et al. 2012). The experimental plan was carried out over 7 months from seedling transplantation to crop harvesting.

2.3 Soil, plant and harvest analyses and measurements.

Soil samples were taken at 0-30 cm depth. Four samples per parcel were taken and homogenized. The homogenized sample was analysed in triplicates according to the official methods for soil analysis issued by the Italian Ministry of Agriculture (Ministero per le Politiche Agricole 1997 and 1999). The pH and electrical conductivity were determined in water at 1:2.5 solid/water ratio. Microanalyses for carbon and nitrogen content were performed on 0.5 mm sieved samples. Analyses were performed for exchangeable cations, held on negatively charged soil sites, and assimilable nutrients, i.e. those which may be absorbed by the roots. The assimilable P concentration was determined colorimetrically (phosphomolybdic complex), after $NaHCO_3$ extraction. The assimilable Na, Mg and Fe concentrations were measured by atomic absorption spectrophotometer after ammonium acetate/ethylenediaminetetraacetic acid extraction. The exchangeable K, Ca and Mg concentrations were determined by atomic absorption spectrophotometer after $BaCl_2$ extraction. Plant and harvest measurements were performed on four plants sampled in the center row of each soil plot. Considering the three replicates per treatment, values for each treatment reported hereinafter are averages of measurements performed over 12 plants (3 replicate plots and 4 plants per replicate), unless otherwise indicated. Four fruit clusters per plant were sampled. The plant leaf chlorophyll content was measured by means of a portable spad-502 Minolta chlorophyll meter. Through optical analysis this instrument yields an indirect estimate of the chlorophyll content of plant leaves without damaging the leaves during the plant growth and production cycle. It measures the absorbance at one wavelength falling within the chlorophyll absorbance range and calculates a chlorophyll concentration index value that is proportional to the amount of chlorophyll in the sample. Absolute chlorophyll content per unit area was not computed. Plant height, diameter at branches' forking and leaf chlorophyll content were measured at different dates from the moment of seedlings transplantation until April. Plant productivity was determined by measuring number, weight and size of fruits starting from May 2 to production end.

2.4 Statistical treatment of data

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

3 Results and discussion

Table 1 reports the chemical composition and physico-chemical features of the untreated control soil and the soil treated with the highest dose of the soluble substances investigated in this work. Based on the chemical composition data of the soluble substances (see section 2.1), the kg ha^{-1} amounts of carbon, nitrogen and mineral elements contributed to the soil by the highest 700 kg ha^{-1} soluble substances applied dose were 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 statistical analysis of the soil data collected at two different dates over the crop production cycle, even the highest 700 kg ha^{-1} dose did not alter the chemical composition and physico-chemical features of the treated soil, compared to the untreated control soil. No significant changes from February to April occur in the measured soil physico-chemical parameter, except for Ca and Mg being significantly lower in April. However, at each measurement date, no significant differences are proven between the control and the treated soil.

The data in Table 2-4 prove however that strong effects by the dose treatments occur on the plant chlorophyll content, growth and productivity. Specifically, Table 3 shows that the effect of the soluble substances on the plant leaf chlorophyll content is evident already during the first month of the experiment. At each measurement date the leaf chlorophyll content increases to the 64-67 spad unit maximum values upon increasing the soluble substances' dose up to 140 kg ha^{-1}and then apparently tends to decrease for higher dose. However, the superior effect of the 140 kg ha^{-1} dose treatment is proven statistically significant only in February. In March and April, and to some extent December also, the difference between the 140 kg ha^{-1} is non-significant compared to 35, 70, 350 and 700 kg ha^{-1} dose treatments. These findings show that the 35 or 70 kg ha^{-1} doses are also very effective in increasing chlorophyll contents and the similar kind of role has also been observed at 350 or 700 kg ha^{-1}. For tomato cultivation, the same soluble substances applied to the same soil at 1.55 ton ha^{-1} dose were found to enhance significantly leaves chlorophyll content by 13 % relatively to the control and by 8-13 % relatively to the sourcing compost (Sortino et al. 2012). In this previous study, over five determinations performed from January through May 2010, 54 and 50 Spad unit peak levels were measured in April for plant leaves grown in soil treated with the soluble substances and their sourcing compost respectively. The results reported now for red pepper show that the same chlorophyll enhancement relative values may be obtained even at much lower doses, down to 2 % of those applied in the tomato cultivation trials. The relatively lack of sensitivity of the chlorophyll content versus the soluble substances dose increase above 35 kg ha^{-1} reproduces the dose-effect relationship reported for compost derived soluble substances tested as photosensitizer for the abatement of organic pollutants in solution (Avetta et al. 2012): i.e. the rate of the probe molecule photodegradation increases upon increasing the soluble substances' concentration to a maximum value; higher concentration causes no enhancement of the probe molecule photodegradation rate.

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

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

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

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

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

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

The most relevant features of the experimental data collected in this work are three: i.e. (i) the no effect on soil characteristics by the soluble substances, (ii) the magnitude of effects on the plant leaves, crop and fruit parameters in relation to the applied soluble substances' doses and (iii) the intriguing pattern of the dependence of plant leaf chlorophyll content, growth and productivity upon the applied soluble substances' dose. The no effect on soil chemical data was expected on basis of the quite high carbon and nitrogen content of the control soil (Table 1) and the relatively low applied doses of soluble substances. For instance, considering the N content of the control soil 0.15 % and assuming a bulk density of 1.4 and a soil layer of 30 cm the calculated N content per soil ha is 6200 kg. Therefore the 36 kg ha^{-1} maximum value of added N by the highest dose of the soluble substances applied to the soil corresponds to an increase of 0.58% relatively to the control soil N amount. Based on Table 1 statistics, this change would result not significant. In this work, no measurements were made on leaves or crop uptake of nutrients since in the previous tomato cultivation study, performed on the same soil with the same soluble substances applied at higher doses, no significant differences were found either in the soil physico-chemical features and in the leaves and crop uptake of nutrients due to the treatment relatively to the control experiment (Sortino et al, 2012).

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

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The complex processes of photosynthesis and chlorophyll formation are known to be regulated by the availability of enzymes and light (Whitmarsh and Govindjee 1999). The light dependency of chlorophyll formation is particularly true for the protochlorophyllide to chlorophyllide conversion step in angiosperms (Von Wettstein et al. 1995) to which the red pepper test plant belongs. This work does not provide any data to support any action of the investigated soluble substances as regulator of the natural enzymatic pool involved in vegetable matter biosynthesis. Certainly, the similarity of the dose-effect relationship pattern observed in this work with that observed for the photodegradation of organic pollutants, performed with the same soluble substances in the absence of any possible biochemical reaction (Avetta et al. 2012), may suggest the fascinating hypothesis that these substances perform a photosensitizing activity also in photosynthesis. Understanding the mode of action of the soluble substances under the experimental conditions reported for the photodegradation of organic pollutants is simpler than for the present work. In the former case, these substances were tested in homogeneous solution containing the probe substrate, in order to function as photosensitizers. Under the experimental conditions of the present case study, the soluble substances must undergo a number of mass transfer processes from the soil to the plant in order to perform their action. In these processes, the soluble substances may undergo several chemical and biochemical reactions in the soil and in the plant. Few data are presented in this work to confirm that the hypothesis of the role of the soluble substances as photosensitizers in this work is true and that other types of interactions of these substances in the biochemical processes responsible of plant growth can be excluded. Within its experimental constraints, the present work has merit for two reasons. It provides new important information to guide the use of these refuse derived substances for optimum performance in agriculture. This information is of primary interest to farmers. To scientists, this work offers unexpected intriguing scope for further research to assess the role in agriculture for the herewith reported soluble substances and for other soluble substances that may be isolated from different biomass residues (Montoneri et al. 2011).

4 Conclusion

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

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

	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 (μS cm^{-1})	846	759	476	376	4.63	0.037
Salinity (μeq 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 (μeq g^{-1})	2.7	2.7	4.7	4.7	2.18	0.168
Ca (μeq g^{-1})	234 a	236 a	93.3 b	95.3 b	434	0.000
Mg (μeq 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

[a] Soil K, Ca, Mg as exchangeable ions and other elements as assimilable ions, unless otherwise indicated

[b] Average 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

[c] Assimilable ion.

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 susbtances'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

[a] F and Pr values

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

[c] Average 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 susbtances'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

[a] F and Pr values

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

[c] Average 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 susbtances'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

[a] F and Pr values

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

[c] Average values calculated over 4 plants per parcel in the three replicates per dose treatment

Table 5 Values of costants and standard error, and of regression coefficient, for Table 4 crop production and fruit parameters dependence upon Table 3 April leaf chlorophyll content according to the empirical equation $y = a_2 + (a_1-a_2)/[1 + e^{(X-x_0)/dx}]$ 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 lenght	0.92	12.7±0.2	14.4±0.3	61.8± 0.3	0.25± 0.39

[a]Standard error $< 10^{-4}$

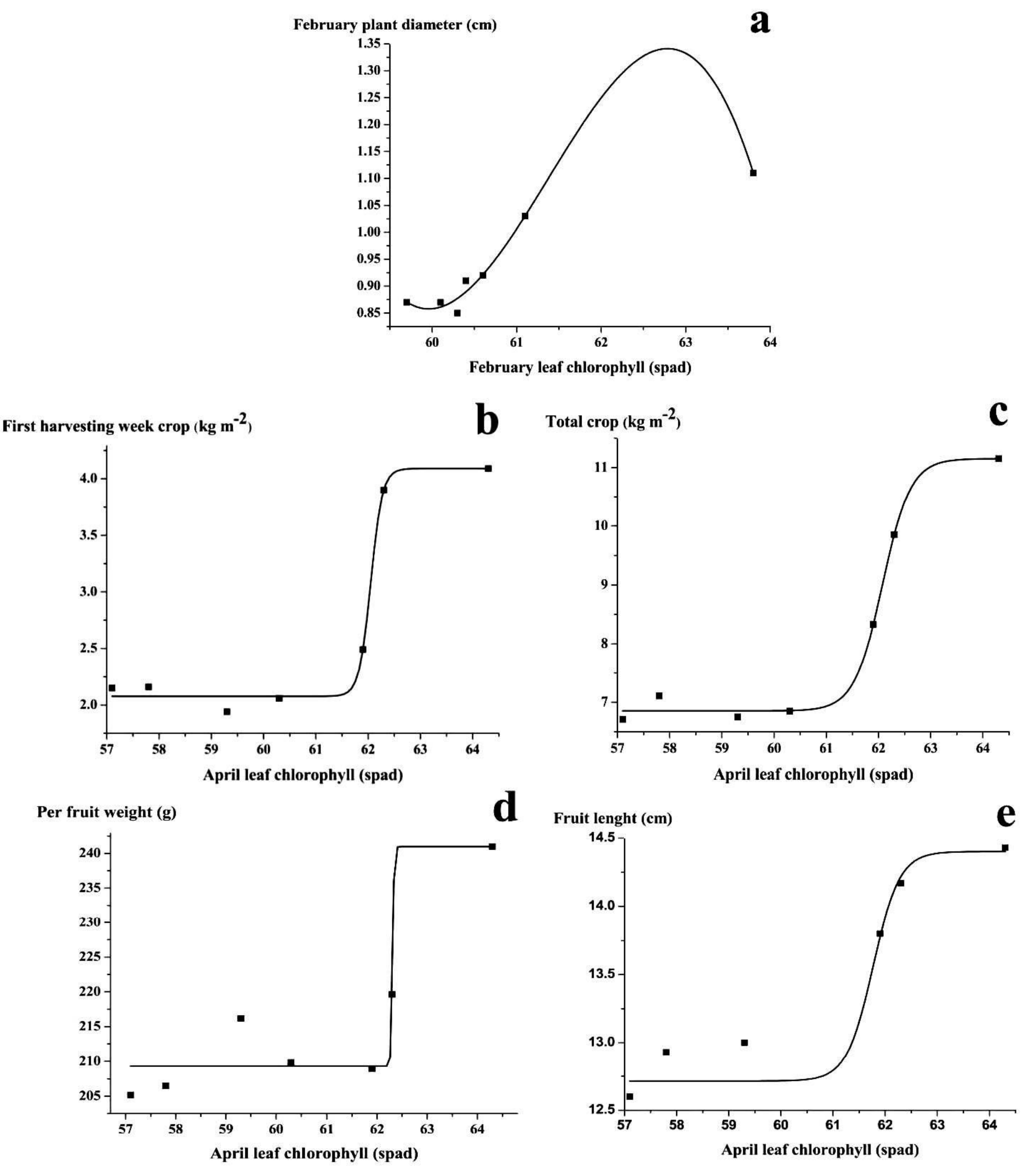

Fig. 1 Plot of plant growth and production data versus leaf chlorophyll content: **a** plant diameter versus leaf chlorophyll, both measured in February; **b, c, d, e** crop and fruit data versus April leaf chlorophyll content. Solid lines in the graph obtained according to the following regression equations:

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.