Compost tea spraying increases yield performance of pepper (*Capsicum annuum* L.) grown in greenhouse under organic farming system

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Abstract

Compost tea (CT) is an organic liquid product derived from quality compost carrying useful microorganism and molecules capable to protect and stimulate growth of the plants. It is gaining a lot of interest for improving productivity of conventional and/or organic vegetable crops. In this research, the effects of an aerated water-extracted CT obtained from vegetable composts, applied as foliar spray on pepper plants, was evaluated for two years. In the first year, total production increased by 21.9% whereas, in the second year, it increased by 16.3%. The increment of the yields was related to an increase of the number of fruits *per* plant, whereas the weight of the single fruit was not affected by treatment. In both years, physiological and nutritional status of pepper plants were increased, as resulted by leaf-SPAD assessed during crop cycle. Findings indicate the effectiveness of CT application in improving significantly yield performances of vegetable crops under greenhouse organic farming system.

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Introduction

In the last years, the demand for most nutrient and taste, healthy and eco-friendly foods is increasing. For these reasons, vegetables coming from organic farming systems, in which operators apply natural cultivation methods and sustainable productive tools, are particularly appreciated by consumers. However, a general challenge of the modern agriculture is to increase the yield with the aim of the reduction of chemical pesticides and fertilizers.

The use of compost in organic agriculture is very important because it contributes to improve general soil fertility (Pane *et al*., 2013a; Scotti *et al*., 2016) and may be crucial for plant disease management by restoring soil suppressive properties (Pane *et al*., 2013b). Furthermore, another use of this organic amendment regards the production of compost teas (CTs), liquid extracts rich in useful microorganisms and organic and inorganic biomulecules (Ingham, 1999), that can be active in plant protection against phytopathogenic fungi and bacteria and in plant growth and yield promotion.

Aerated CTs are produced by a continuous aqueous extraction of compost in presence of oxygen for a time ranging from few hours to few days, with or without other organic additives such as molasses and fish meals (Zaccardelli *et al*., 2012). The use of CTs is spreading in organic farming worldwide (Litterick *et al*., 2004; Siddiqui *et al*., 2008; Hargreaves *et al*., 2009; Shaheen *et al*., 2013) because of benefits they provides as fertilizer, biostimulant or foliar spray against pathogens.

Literature survey shows many successful experiences with CTs to achieve productive biostimulation of different crops, including okra (Siddiqui *et al*., 2008, 2009), strawberry (Hargreaves *et al*., 2009), pak choi (Pant *et al*., 2012), tomato (Radin and Warman, 2011), *Centella asiatica* (Siddiqui *et al*., 2011), orange (Omar *et al*., 2012), lettuce and kohlrabi (Pane *et al*., 2014a), cowpea (Hegazi and Algharib, 2014), lettuce, soybean, and sweet corn (Kim *et al*., 2015). Mechanisms underlying these CT-based biostimulation functions are hypothesized to concern an enhanced plant physiological status due to carried nutrients (fertilization action) and/or dissolved organic moieties, humic substances and hormone-like molecules secreted by microbes (hormonal action) (Zaccardelli *et al*., 2012). Stimulation may also occur in combination with disease suppressiveness leading to additional yield increases as recently observed on processing tomato, where CT spray has been used in substitution of synthetic fungicides (Pane *et al*., 2016).

The fungicide-like effects of CTs was essentially due to the antagonistic activity of the overall native microbial community

(Pane *et al*., 2012, 2014b); but also abiotic antimicrobial factors and/or organic molecules can play a role in improving plant defences (Zaccardelli *et al*., 2012; Praveena Deepthi and Narayan Reddy, 2013). On the base of these perspectives, CTs may contribute to reduce the unsustainable use of chemical-based pesticides and fertilizers in agriculture, but insights regarding their spray application in conventional and organic systems are still necessary. The current research was carried out to investigate the effects of CT-spray treatments on the agronomic performances of pepper under greenhouse organic farming system. Two on-farm composts, obtained from residues of artichoke, fennel and escaroles, that are selected among different others because of their good endowment of macro and micro elements and of high disease suppressive bioactivity (Pane *et al*., 2013b), were used to produce CT formulation for these trials.

Materials and methods

On-farm compost tea production

Compost and compost tea were produced at CREA experimental farm of Battipaglia (Salerno district) by using the available on-farm composting plant and blowing extractor system. At first, two composting piles were obtained by mixing different vegetable materials as follows: one composting pile contained 78.0% artichoke, 20% woodchips and 2% mature compost used as starter; the second compost pile contained 43.5% artichoke, 23.5% fennel, 11% escaroles, 20% woodchips and 2% mature compost; all percentages are expressed as dry weigh. Initial C/N ratio of the two raw pile was about 30, in order to favour a good trend of composting process. The volume of each on-farm composting pile was about 6 m^3 in volume. Under each static pile, a forced aeration system was located to ensure forced aeration during the first 45 days, including thermophylic and mesophilic phases. In particular, mechanical aeration was provided by air injection through a net of tubes connected to a blower (0.75 KW) that was periodically activated (5 min every 3 h) with an electronic timer. Piles wetting was achieved through a PVC irrigation system, manually activated on demand when RH <50%. After forced aeration, a final curing period of about two months without aeration, was made to have mature compost from the piles. Composting temperatures were measured by thermo-sensors placed in the core of the pile at 15 cm from the pile bottom in order to follow the dynamic of the composting process. CTs were produced *on farm* using a compost extractor constituted by a 50-L polyethylene container connected to a forced air blowing system that, periodically (5 min every 3 h), injected air in 20 L of water in which a plastic bag of holes of 3 mm of diameter, contained 5 L of compost. Duration of fermentation process was one week and, at the end, the two CTs were filtered and mixed together in equal parts, so to have the compost tea mix (CTmix) to use in treatments of pepper plants for the two-year trial. CT mix was stored at 4°C.

Chemical and physico-chemical analyses of compost tea

All analyses were performed twice on CTmix at the end of extraction procedure. Total organic carbon (TOC) was determined as described in Pane *et al.* (2016), according to the Italian official method for compost analyses (ANPA, 2001). In particular, potassium dichromate ($K_2Cr_2O_2$) and concentrated H_2SO_4 are added to 10 mL of CTmix. After 10 min, distilled water was added to the solution to halt digestion. Barium diphenylamine sulfonate was added to the digestate and, then, the excess of $Cr_2O_7^{2-}$ was titrated using Möhr salt (ferrous ammonium sulfate).

Heavy metals (Cd, Cr, Cu, Mn, Pb, Zn), alkali metals (Na, K) and alkaline earth metals (Ca, Mg) were analysed as described in Pane *et al*. (2016). In particular, ten milliliters of such materials were previously subjected to an acid digestion at rising temperature steps using a microwave oven. Metal concentrations were determined in the extracts using an ICP-OES Spectrometer (iCAP 6000 Series - Thermo Scientific, Waltham, MA, USA).

Electrical conducivity (EC) and pH were measured at 25°C directly in a sample of CTmix using a Hanna Instruments pH-meter model 211 and a conducimetry-meter Hanna Instruments model 4321, respectively.

Microbiological analyses of compost tea

Microbiological groups determined were total bacteria, pseudomonads, spore-forming bacteria, total fungi, *Escherichia coli*, Enterobacteria and Clostridia. All these microbiological groups were encountered by three-replicated plating serial ten-fold dilutions on selective substrates. In particular, total bacteria were counted on selective medium (glucose 1 g L^{-1}, proteose peptone 3 g L^{-1}, yeast extract 1 g L^{-1}, K_2PO_4 1 g L^{-1}, agar 15 g L^{-1}) to which actidione (cycloheximide) 100 mg L^{-1}, was added. Pseudomonads were counted on selective agar medium without iron, to which actidione was added (Scher and Baker, 1982). Spore-forming bacteria were counted on Nutrient Agar (Sadfi *et al*., 2001) using CTmix preparation previously heated at 90°C for 10 min. Total fungi were counted on PDA (Oxoid) pH 6, to which 150 mg L^{-1} of nalidixic acid and 150 mg L^{-1} of streptomycin were added. *E. coli* and enterococci were counted in sample of the liquid tea (APAT, IRSA-CNR, 2003). The estimation of *E. coli* was performed using TBX medium (Oxoid); plates were incubated for 24 h at 44°C and blue colonies were counted as *E. coli*. Enterococci were enumerated on a Slanetz & Bartley medium (Oxoid); after plate incubation for 48 h at 37°C, red colonies were transferred on Bile Esculine Azide Agar (Merck, Germany) and incubated for 2 h at 44°C; when any blacking of the medium occurred, colonies were counted as Enterococci. Sulphite-reducing *Clostridium* spores were determined according to APAT, IRSA-CNR (2003) and APHA (1998) methods. In detail, compost-tea sample was pre-treated for 10 min at 80°C; spores were enumerated using SPS agar (Merck); plates were incubated for 24-48 h at 37°C in an anaerobic jar with the anaerobic atmosphere generating system Anaerogen (Oxoid); black colonies surrounded by a black zone were considered as sulphite-reducing *Clostridium* spores. Population densities of all detected microoganisms are reported as log c.f.u. mL^{-1} of CTmix.

Biostimulation activity of compost tea

Biostimulation activity of CTmix was determined twice on *Lepidium sativum* seeds. For each Petri dishes containing a disc of blotting paper, 20 seeds of *L. sativum* were put in, and serial ten-fold dilutions of CTmix were added (5 mL) in each plate. Petri dishes containing water were used as control. Plates were incubated in the dark at 25°C for 3 days and, thus, root elongation was measured and registered.

Greenhouse trial, compost tea treatments and relieves

Agronomic trials were carried out in ordinary conditions, in 2012 and 2013 seasons, under a greenhouse system in a loamy soil, at organic farm *IdeaNatura* located in Eboli (Salerno province, Campania region, Italy); experimental design was a randomized complete block with plot areas of 10.80 m^2 each, replicated three times. Plantlets of pepper cv. Scintilla were transplanted on March 19th 2012 and on April 8th 2013 in double rows, at distances of 0.40 m on each row, 0.90 m among the rows of each double rows and 1.5 m among each double rows, so to have a density of 33,000 plants ha^{-1}. CT mix, water diluted 10% vol., was weekly applied by spraying aerial parts on pepper until run-off. Plant's vegetative and phytosanitary status were monitored during crop cycles by direct observations, and physiologic-nutritional status of the plants was registered by clorophyll concentration in the first leaf completely developed, using SPAD-meter Minolta. Harvestings, performed on 10 plants (assay area 3.03 m^{-2}) for each replicate, occurred from June 20th to November 13th in 2012 (145 days, 23 harvesting) and from June 19th to October 17th in 2013 (121 days, 17 harvesting). For each assay area, total weight, number of the fruits collected and their longitudinal and equatorial measures, were registered (Figure 1).

Statistical analyses

In order to statistically evaluate the effects of compost tea treatments on total yield, number and weight of harvested fruits in and between years were registered; longitudinal and equatorial measures of single fruit for each year, were registered too. Data were submitted to Student's *t* test.

Results and discussion

Chemical and microbiological analyses of the CTmix provided precise indications about the quality of the produced tea. CTmix was characterized by the presence of plant nutritive macro and microelements, including potassium, calcium, magnesium and manganese and, on the contrary, by the absence or content of heavy metals lower than legal limits established for compost by Italian D.lgs. 75/2010 (Table 1). The microbiological quality of CTmix was high, due to the absence of potentially harmful bacteria such as *Escherichia coli*, Enterobacteria and Clostridia and larger concentration of beneficial PGPR/antagonistic bacteria such as *Pseudomonas* spp. and *Bacillus*-like spore-forming bacteria (Table 2). CTmix specific features make it potential for foliar application as an organic biofertilizer to sustain plant nutrition in all critical phases of the cycle, including growth, flowering and fruiting (Omar *et al*., 2012). On the other hand, beneficial effects of these organic treatments on growth, development and physiology of the

Figure 1. Comparison of pepper fruits collected from a plot treated with compost tea (on the right) respect to pepper fruits collected from a plot not treated with compost tea (control, on the left).

Table 1. Chemical characterization of the CTmix used. Legal limits are for compost.

Sample	pH	EC S cm^{-1}	Organic C (g L^{-1} or g kg^{-1} dry compost)	K	Ca	Mg	Na	Mn	Cd	CrVI	Cu	Pb	Zn
				(mg L^{-1} for CTmix and mg kg^{-1} for dry compost)									
CT	7.6±0.16	4778±500	1.06±0.05	1417.0±229	21.8±1.9	37.8±3.08	92.1±6.59	0.45±0.01	0.00±0.0	0.02±0.01	0.16±0.02	0.03±0.01	0.15±0.01
Legal limits	6.0-8.5	-	≥200	-	-	-	-	-	1.5	0.5	150	140	500

Table 2. Main microbiological populations in the CTmix used in this study.

Total bacteria	*Pseudomonas*	Spore-forming bacteria	Total fungi	*Escherichia coli*	Enterobacteria	Clostridia
Log CFU mL^{-1}						
6.47±0.00	5.57±0.23	5.23±0.34	2.15±0.13	-	-	-

Table 3. Effects of compost tea-spray treatments on the agronomic performances of pepper over two years of greenhouse trial. Values are the mean ±standard deviation of all data collected in the each harvesting season.

Treatments	Total yield (T ha^{-1})	Harvested fruits (N ha^{-1})	Single fruit Weight (g)	Single fruit Longitudinal f (cm)	Single fruit Equatorial f (cm)
2012					
CTRL	122.95±2.83	481.94±10.69	229.62±11.22	13.94±0.38	8.68±0.16
CTmix	149.88±6.30	611.81±52.76	246.61±4.08	14.04±0.06	8.56±0.04
Sign.	**	*	ns	ns	ns
2013					
CTRL	94.48±7.21	357.64±54.02	291.34±20.89	13.83±0.21	9.02±0.32
CTmix	109.93±5.26	415.28±18.20	300.21±14.02	13.91±1.04	8.96±0.47
Sign.	*	*	ns	ns	ns

* and ** indicate significance levels, P≤0.01 (**) and P≤0.05 (*), of differences among the values according to Student's t-test. ns, not significant.

plants, has been largely linked to the presence of hormone-like molecules, including gibberellins, indoleacetic acid and cytokinins (Bernal-Vicente *et al*., 2008; Pant *et al*., 2012; Ertani *et al*., 2013; Zhang *et al*., 2014) that were identified in highly bioactive compost teas and/or extracts.

Herein, findings of field trials showed that CTmix treatments enhanced agronomic performances of pepper greenhouse cultivation under organic management. Indeed, it improved significantly pepper production for both years. In detail, total yield of pepper in the treated plots were, on average, higher 21.9% and 16.3% than that of the reference control plots, respectively in 2012 and in 2013 seasons (Table 3). Analysing yields between years, no trends can be highlighted, since a general significant reduction of the production harvested in the 2013 was found (data not showed). Cumulative production graph shows an increasing gap throughout the harvesting season among yields of CTmix treated and untreated plants (Figure 2). Moreover, CTmix foliar-spraying increased the number of the harvested fruits, while it did not affected the weight and dimension of the single berry in comparison with the reference controls in both seasons (Table 3). However, the general trend of the weight of the single fruit throughout the crop cycle showed a decreasing tendency (Figure 3).

In the current study, data indicate the occurrence of general beneficial effects of CTmix treatment on the harvested production for the whole cropping cycle and an additional stimulation in its last part, due to source of organic substances used. Indeed, the dynamic of the yield let to hypothesize that CTmix promoted the longevity of the productive phase of pepper by stimulating plant's fruiting. The enhanced production registered for the pepper greenhouse system, under CTmix spray treatments, is in agreement with Radin and Waeman, (2011) who observed increases in tomato yield by spraying municipal solid waste compost tea very frequently during the growing cycle. On the other hand, Omar *et al*. (2012) reported similar effects on Washington navel orange, where production enhanced by rice straw compost tea foliar application induced large fruit weight, greater set of fruits and reduction of fruit drop. While, compost tea used in combination with NPK fertilizers, also incited significant increases in seed yield of cowpea (Hegazi and Algharib, 2014). Previous experiments carried-out with the use of CT to enhance sustainability of lettuce, kohlrabi and tomato systems, indicated that the organic-sourced product may act by physiological and/or nutritional biostimulation of the plants (Pane *et al*., 2014a, 2016).

In the current study, since diseases do not occurred in experimental trials, under natural pressure, CTmix disease suppressive mechanisms may be excluded from the hypothetical effectors underlying yield enhancement.

In our work, plants under CTmix treatments showed an enhanced global well-being, with improved physiological and nutritional status as indicated by SPAD temporal assessment. SPAD values linked to the chlorophyll foliar content, proved higher on plants treated with CTmix than non-treated ones in a large

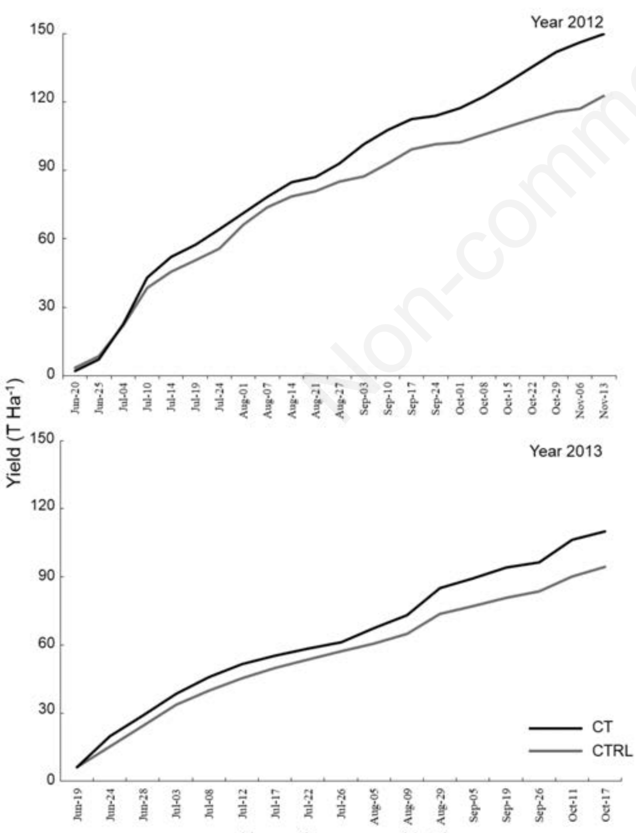

Figure 2. Effects of compost tea-spray treatments on cumulate total yield of pepper over cropping cycle in the two years.

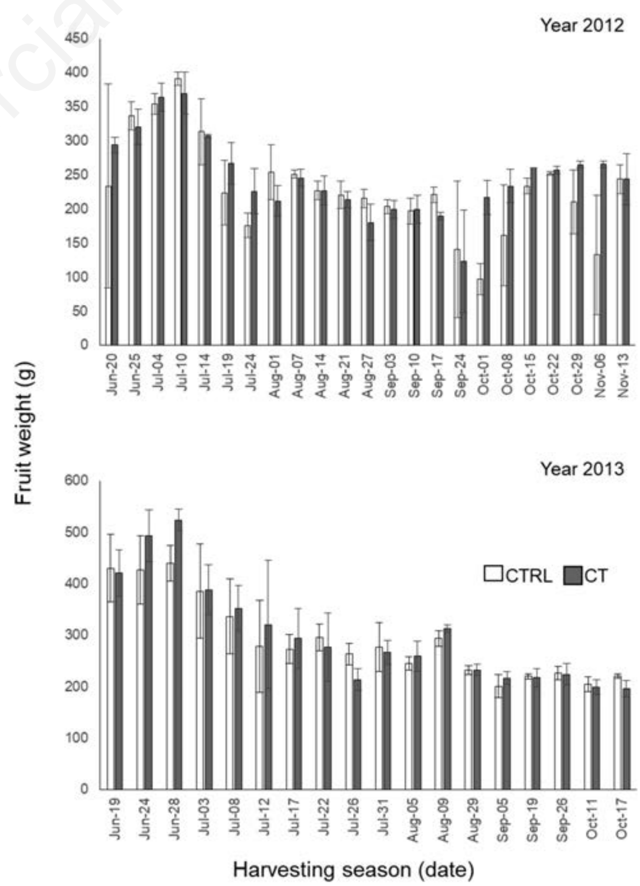

Figure 3. Weight of the single fruit, recorded in each harvesting date during the season, from plants cultivated under compost tea-spray treatments compared to non-treated control over two years.

part of cultivation cycle (Figure 4). Increases in chlorophyll content due to CT treatments was observed on muskmelon plants exhibiting stimulation of flowering, growth and yield (Naidu *et al.*, 2013) and on okra that showed an enhanced net photosynthesis rate (Siddiqui *et al*., 2008). Xu *et al.* (2012) also reported promotion of cucumber growth and increase of chlorophyll content in the leaves, after treatments of the plants with compost extracts.

The present research confirm that CTs from agricultural composts may be effective to biostimulate crop productivity, as recently reported by our research group on lettuce and kohlrabi, under greenhouse in organic system (Pane *et al*., 2014a), and on tomato grown in open field in conventional agrotechnics (Pane *et al.*, 2016). These results encourage the practical use of CTs in organic farming and in conventional farming system too. For these reason, other applicative experiments can be performed to get more knowledge about CTs use on other vegetable crops.

Conclusions

Researches on growth stimulation and productivity of the crops incited by compost teas and extracts, are receiving major attention in the last years. Field trials performed in our study, confirm the efficacy of CTs to induce biostimulant effects on the plants, so to improve efficiency use of the inputs and production. For this reason, the use of CTs can play a very crucial role on the development of sustainable agricultural systems focused on the reduction of fertilizers. Therefore, it is desirable to have a greater spread of the application of CTs in agricultural management with efforts of other further studies addressed to the fine individuation of the mechanisms of action, standardization and practical implementation works.

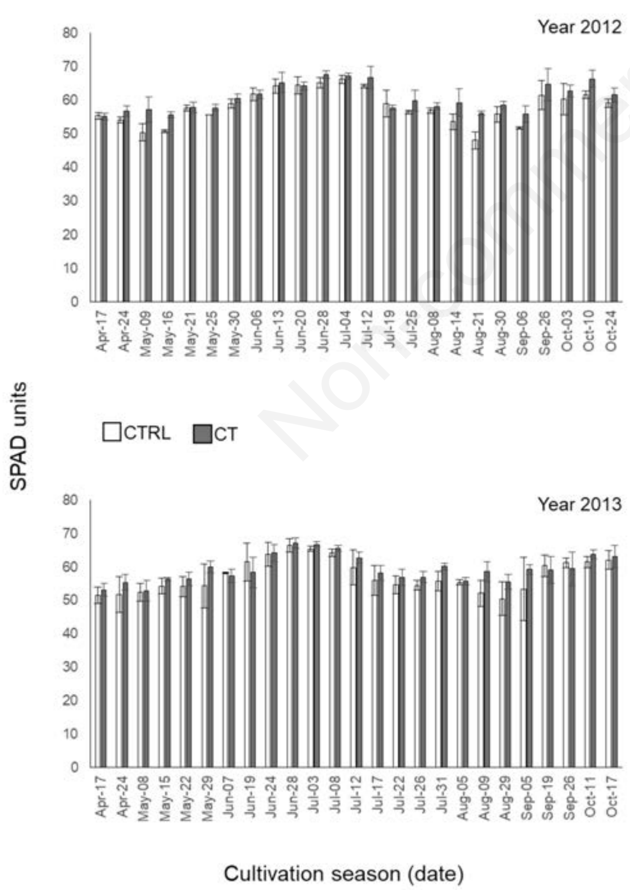

Figure 4. Effect of compost tea-spray treatments on physiological-nutritional status of the plants assessed as measure of SPAD units over the crop cycle in the two cropping seasons.

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