agronomy

Application of Directly Brewed Compost Extract Improves Yield and Quality in Baby Leaf Lettuce Grown Hydroponically

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Abstract: The aim of this work was to study whether the application of a directly brewed compost extract (added in the nutrient solution or by microsprinkler) could be used to improve the yield and quality of baby leaf red lettuce growing in a floating system, and to control the incidence of *Pythium irregulare*. Its effect on the quality of fresh-cut red lettuce was also studied. For this, two experiments were carried out over two growing cycles (winter–spring and autumn). The results showed that the use of compost extract added to the nutrient solution improved baby leaf lettuce growth and quality, reducing the nitrate content and enhancing the content of potentially health-promoting compounds such as phenols and flavonoids and the antioxidant capacity. Microbial quality was maintained during storage and the compost extract had no negative effect on the microbial load of the final product. In addition, application of the compost decreased the population of *P. irregulare* in the water. It is concluded that the application of directly brewed compost extract is of potential use in a sustainable soilless production system for baby leaf red lettuce, since it improves the yield and quality of the product and is able to control the incidence of *P. irregulare.*

1. Introduction

The use of compost in agriculture is gradually gaining in popularity, mainly due to its environmental and agronomic benefits. In addition, compost can be considered as a key element in the circular economy, allowing a more sustainable production system [1]. More particularly, composts from the fruit and vegetable processing industry are used as a nutrient-rich fertilizer or soil amendment to improve crop production and quality [2–4]. Furthermore, such composts present a lower risk of containing pathogens, heavy metals or pharmaceuticals as components [5], and have interesting biological activities [6]. Some composts have shown an ability to suppress soil-borne diseases [2,7], in which the microbial activity of the compost plays a major role [8,9]. However, some abiotic properties have also been suggested to be associated with suppression activity [10]. It has been demonstrated that the suppressive effect of composts depends on the raw material origin, the pathogen to be controlled and the plant being cultivated [7].

The suitability of using compost in the soilless culture of horticultural crops has also been confirmed in several studies, particularly when it is used as a substrate for the nursery production of vegetable crops [4,11]. However, few studies have been carried out on the use of compost in crops

grown hydroponically. Recent studies have demonstrated that an agroindustrial compost can be used as an alternative to peat organic substrate in a floating system, since it is not only able to control *Pythium irregulare*, but also to improve the yield and quality of the crop [12,13].

Compost-derived products, such as compost extract (CE), are used as a source of nutrients to improve crop production, and as an inducer of systemic acquired resistance against soil-borne diseases [14–17], allowing a more sustainable production system. Depending on the origin of the compost and the way in which the CE is obtained, CEs have different compositions, although they are mainly composts of a mixture of humic and fulvic acids, organic molecules and soluble inorganic substances carried in suspension [18,19]. It has been demonstrated that this type of compound has a direct effect on some metabolic processes [20–23]. The biostimulant capacity of a CE is exercised through direct and/or indirect effects on nutrition, leading to hormone-like activity that influences the photosynthetic capacity of the plant [21,22]. Arancon et al. 2012 [20] suggested that analogs of hormones contained in vermicompost extracts were responsible for aiding plant growth and increasing yield.

As is the case with most composts, the potential mechanism of suppression of CE is often, or predominantly, biological, although chemical and physical factors have also been implicated [24]. The application of CE has been shown to significantly suppress several pathogens such as bacterial spot on tomato [25], *Botrytis cinerea* on strawberry [26] and foliar fungal pathogen on tomato [27]. CEs have normally been used as a soil drench or directly sprayed on plants [28–31], but, to our knowledge, their use in hydroponic systems has not been studied in depth before. The use of directly brewed CE has been studied even less despite the fact that they could be a valuable tool in hydroponic management [20]. Our hypothesis was that a CE obtained by passing aerated water through a fine mesh bag containing compost in a floating bed or inside an irrigation tank for subsequent spraying by microsprinkler would stimulate plant growth and help control diseases. For this, we studied the effect of two different ways of applying a directly brewed compost extract added in the nutrient solution (CENS) or by microsprinkler (CEMP) on the yield and quality of baby leaf red lettuce growing in a floating system, and the effect on the incidence of *Pythium irregulare*. In addition, the quality of fresh-cut red lettuce produced in this way was studied.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiments were conducted in the Agricultural Experimental Field Station of the Technical University of Cartagena (UPCT; lat. $37°41'$ N; long. $0°57'$ W). A cultivar of red baby leaf lettuce (*Lactuca sativa* L.), "Antoria" (Rijk Zwaan, De Lier, The Netherlands), was cultivated in a floating system in an unheated greenhouse covered with thermal polyethylene. Two crop cycles were carried out with sowings on 27 February 2017 (winter–spring cycle) and 3 October 2017 (autumn cycle) in styrofloat trays measuring 60×40 cm [12], which were filled with a commercial peat substrate (Pindstrup Blond Gold). After sowing, the trays were transferred to flotation beds ($1.35 \times 1.25 \times 0.2$ m), floating on tap water with an electrical conductivity (EC) of 1.1 dS m^{-1} and pH 7.8. Aeration was provided using a blow pump connected to a perforated pipe trellis positioned at the bottom of each flotation bed.

A week after sowing, the lettuce plants were thinned, leaving 8 plants per cell (1600 plants m^{-2}). At the same time, the tap water in the beds was replaced with a nutrient solution (8 mM NO$_3^-$, 2 mM NH$_4^+$, 2mM H$_2$PO$_4^-$, 2.6 mM Ca^{2+}, 4.65 mM K$^+$ and 1.12 mM Mg^{2+}, plus a commercial solution of microelement Nutromix 10, Biagro (2 mg L^{-1}) and Sequestrene (an Iron chelate) (1.5 mg L^{-1})) [32]. The nutrient solution was adjusted to EC 2.5 dS m^{-1} and pH 5.8. The EC and temperature of the nutrient solution and the oxygen concentration were monitored throughout the growing cycles using sensors located in each flotation bed. The dissolved oxygen concentration, EC and temperature ranged from 6.5 to 8.7 mg L^{-1}, from 2.5 to 3.6 dS m^{-2} and from 12 to 28 °C, respectively, for the winter–spring cycle, and, from 5.5 to 8.6 mg L^{-1}, from 2.5 to 3.4 dS m^{-2} and from 16 to 27 °C, respectively for the autumn cycle. The light conditions and temperature during the experiments were an average daily

light integral (DLI) of 7.42 mol m^{-2} d^{-1}, and 6.39 °C, 38.72 °C and 19.56 °C (minimum, maximum and average air temperature) in the winter–spring cycle; and an average DLI of 4.27 mol m^{-2} d^{-1}, and 12.61 °C, 40.01 °C and 23.21 °C (minimum, maximum and average air temperature) in the autumn cycle.

Harvesting was carried out at the same phenological stage for both cycles, when the plants had four to five leaves. This occurred 30 days after sowing in the winter–spring and after 25 days in the autumn cycle. Seventy-two plants from three randomly chosen cells from each tray were harvested for each treatment for postharvest analysis. Water from floating bed samples was collected and stored at −20 °C to measure pathogen concentrations.

2.2. Compost Extract Characteristics and Application

The compost used to produce the CE was provided by CEBAS-CSIC. The raw materials for composting expressed as dry weight, were tomato (71%), onion (17%) and vineyard residues (12%). Composting was carried out in open-air piles, with a bioxidative and maturation phases of 75 and 42 days, respectively. The piles were turned periodically to ensure aeration, and to control the temperature. Once the composting process had finished (120 days), the compost was milled and passed through a 2-cm sieve. The main characteristics of the compost are shown in Giménez et al. 2019 [12].

The CE was obtained by passing aerated water through a fine mesh bag containing 150 g compost. The bags were placed in the nutrient solution contained in each flotation beds (CENS) or inside the irrigation tank used to apply the CE by microsprinkler (CEMP). Microsprinkler irrigation was scheduled three days per week for 3–5 min, morning and afternoon. The bags containing the compost were placed in the above-mentioned water deposits a week after sowing and kept in situ until harvesting. The control treatment (C) did not contain CE.

The ion content of the CE was analyzed and quantified by ion chromatography [33] in the water emitted by the microsprinklers and in the water contained in the flotation beds before adding the nutrient solution (Table 1).

Table 1. Chemical characteristics of compost extract for the two ways of application (CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler) recorded 7 days after sowing.

	NO$_3$$^-$ (mg L^{-1})	SO$_4$$^{2-}$ (mg L^{-1})	Cl$^-$ (mg L^{-1})	Na$^+$ (mg L^{-1})	K$^+$ (mg L^{-1})	Mg^{2+} (mg L^{-1})	Ca^{2+} (mg L^{-1})
CENS	13.6	143.2	170.6	109.4	26.9	30.3	77.2
CEMP	13.4	143.5	176.1	115.6	21.9	30.0	77.0

2.3. Pathogen and Inoculation

To evaluate the effectiveness of the CE for the biological control of *Pythium irregulare*, the pathogen was added to the flotation beds 5 days after sowing. The pathogen solution was prepared by blending a 7-day old *P. irregulare* culture grown on potato dextrose agar (PDA) in 100 mL of distilled water for one minute. The dose added to the flotation beds was 100 mL in 200 L water, the equivalent to 2.6×10^3 copies ITS per mL. The abundance of *P. irregulare* in the inoculated substrates was measured as described by Giménez et al. 2019 [13].

2.4. Analysis at Harvesting Time

At harvesting time, the following parameters were analyzed: biomass production (yield), calculated as g plant^{-1}; dry matter content (%) of shoots; specific leaf area (SLA); root growth; number of adventitious roots. The dry matter contents were determined by drying in an oven at 50 °C until a constant weight. The leaf area was measured with a leaf area meter (LICOR-3100 C; LICOR Biosciences Inc., Lincoln, NE, USA). Total root length, the length of 0−0.5 mm diameter root and root diameter per plant were determined using Winrhizo LA 1600 root counter (Regent Inc., Quebec City, QC, Canada).

The nitrate content in leaves and in the water of the irrigation systems was analyzed in triplicate using 0.2 g of dry leaf samples per treatment and quantified by ion chromatography [33]. The total phenolic content was determined by the Folin–Ciocalteu colorimetric method [34]. The antioxidant capacity was evaluated in terms of their free radical-scavenging capacity [35]. The total flavonoid content was determined as described by [36].

2.5. Postharvest Product Management and Analysis

The postharvest analysis was only performed in non-inoculated plants. Harvested leaves were placed in plastic bags and immediately transported 6 km in a box with ice to the Instituto de Biotecnología Vegetal of the UPCT where they were kept for 4 h at 5 °C. The leaves were disinfected, washed and packed following Niñirola et al. 2014 [37]. Then, 20 g of leaves were placed in polypropylene (PP) baskets of 1 L capacity, the tops of which were thermosealed with a 34-mm thick film composed of polyethylene terephthalate (PET) + oriented polypropylene (OPP) and stored at 5 °C for 7 days.

Microbial growth, for both mesophilic and psychrophilic microorganisms, was assessed following Niñirola et al. 2014 [37] after processing and after 7 d of storage. The nitrate, total phenolic, total flavonoid contents and antioxidant capacity were measured as described above after 7 days of storage.

2.6. Experimental Design and Statistical Analysis

A randomized complete block design with three replicates per way of CE application was used in the greenhouse in both growing seasons. Each bed had three floating trays. Data were analyzed using Statgraphics Plus. An analysis of variance of agronomical and biochemical parameters (two-way ANOVA) was performed, in which the CE application (Control, CENS and CEMP) and inoculation (non-inoculation and inoculation with *P. irregulare*) were included for each crop cycle. Furthermore, an analysis of variance was performed for the biochemical parameters and microbial content for each crop cycle in the postharvest assay CE application (Control, CENS and CEMP) and storage time (0 and 7 days). When interactions were significant, they were included in the ANOVA, and a least significant difference test was performed to compare ways of application, inoculation and storage time.

3. Results

3.1. Growth and Yield of Lettuce at Harvesting Time

The fresh biomass (yield) of lettuce was affected by the way of CE application in both growing cycles, while yield was only affected by pathogen inoculation in the winter–spring cycle, reducing it by ca. 8% (Tables 2 and 3). The highest yield was recorded in plants grown with CENS. In the winter–spring cycle, there was a statistically significant interaction between pathogen inoculation and way of CE application in terms of percentage of dry matter and SLA. The higher values of dry matter were obtained in control inoculated plants and those treated by CENS. Inoculation decreased the SLA values in every combination of factors. In the autumn cycle, there were no significant differences in dry matter for inoculation treatment, way of CE application or their interaction (Table 3). In regards to SLA, there was a statistically significant interaction between pathogen inoculation and way of CE application, the highest values being obtained in inoculated plants. As regards root growth (total root length, length of 0 to 0.5 mm diameter roots (fine roots) and root diameter), there was a significant interaction between both factors in both growing cycles, except the length of fine roots in the autumn cycle. The greatest total root length and fine roots were achieved with CENS in both growing cycles. The greatest length of fine roots was also obtained with the CENS application. In addition, the inoculation with *P. irregulare* decreased the total length of roots and fine roots in both growing cycles. Inoculation increased the root diameter in every factor combination.

Table 2. Influence of inoculation with *Pythium irregulare* (NI: non-inoculated, I: inoculated) and way of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler) on growth parameters (fresh biomass, leaf area, total root length, root diameter and length of 0−0.5 mm diameter root) at harvest, in baby leaf red lettuce cultivated in the winter–spring cycle in a floating system.

	Fresh Biomass (g Plant^{-1})	Dry Matter (%)	SLA (m^2 kg^{-1})	Total Root Length (cm)	Root Diameter (mm)	Length of 0 to 0.5 mm Diam. Root (cm)
Inoculation (A)						
NI	2.16 ± 0.04 b	3.76 ± 0.07 b	81.08 ± 0.73 b	307.61 ± 5.30 b	0.54 ± 0.01 a	248.59 ± 4.22 b
I	1.98 ± 0.04 a	4.19 ± 0.08 a	75.06 ± 0.82 a	176.57 ± 2.97 a	0.64 ± 0.01 b	137.73 ± 2.77 a
Application (B)						
C	2.00 ± 0.05 a	4.14 ± 0.10 b	80.27 ± 1.40 b	232.28 ± 8.09 b	0.69 ± 0.01 b	186.19 ± 6.65 b
CENS	2.22 ± 0.05 b	4.04 ± 0.10 b	75.07 ± 0.61 a	289.65 ± 4.31 c	0.56 ± 0.01 a	239.43 ± 6.65 c
CEMP	2.00 ± 0.04 a	3.76 ± 0.08 a	78.87 ± 0.72ab	204.36 ± 8.41 a	0.54 ± 0.01 a	153.86 ± 3.54 a
A × B						
NI × C	2.05 ± 0.07	3.60 ± 0.13 a	90.36 ± 1.26 d	311.96 ± 7.08 d	0.66 ± 0.01 d	256.73 ± 3.61 c
NI × CENS	2.32 ± 0.07	3.62 ± 0.10 a	80.34 ± 0.70 b	368.81 ± 7.85 e	0.46 ± 0.02 a	300.65 ± 6.32 d
NI × CEMP	2.12 ± 0.06	3.45 ± 0.10 a	85.18 ± 0.71 c	242.06 ± 3.94 c	0.53 ± 0.01 b	188.40 ± 5.37 b
I × C	1.96 ± 0.07	4.68 ± 0.14 c	72.55 ± 0.56 a	152.59 ± 3.64 a	0.72 ± 0.02 e	115.65 ± 2.68 a
I × CENS	2.12 ± 0.08	4.46 ± 0.15 c	70.19 ± 1.76 a	211.49 ± 4.98 b	0.64 ± 0.01 d	178.21 ± 4.37 b
I × CEMP	1.88 ± 0.05	4.07 ± 0.12 b	69.79 ± 0.34 a	166.65 ± 3.76 a	0.59 ± 0.01 c	119.31 ± 2.21 a
Significance						
Inoculation (A)	**	***	***	***	***	***
Application (B)	**	***	***	***	***	***
A × B	n.s.	**	***	***	***	***

Asterisk indicates significances at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s: non-significant. Different letters indicate significant differences. Values are the mean ± SD ($n = 20$).

Table 3. Influence of inoculation with *Pythium irregulare* (NI: non-inoculated, I: inoculated) and way of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler) on growth parameters (fresh biomass, leaf area, total root length, root diameter and length of 0−0.5 mm diameter root) at harvest, in baby leaf red lettuce cultivated in autumn cycle in a floating system.

	Fresh Biomass (g Plant^{-1})	% Dry Matter	SLA (m^2 kg^{-1})	Total Root Length (cm)	Root Diameter (mm)	Length of 0 to 0.5 mm Diam. Root (cm)
Inoculation (A)						
NI	1.49 ± 0.02	4.43 ± 0.07	76.40 ± 0.51	164.24 ± 2.74 b	0.29 ± 0.01 a	138.79 ± 1.92 b
I	1.45 ± 0.03	4.41 ± 0.08	76.20 ± 0.66	148.62 ± 2.13 a	0.32 ± 0.01 b	132.01 ± 1.85 a
Application (B)						
C	1.49 ± 0.03 b	4.30 ± 0.10	79.05 ± 1.66 b	156.07 ± 2.70 b	0.28 ± 0.01 a	136.40 ± 2.31 b
CENS	1.52 ± 0.03 b	4.45 ± 0.10	74.94 ± 1.79 a	176.13 ± 3.21 c	0.31 ± 0.01 b	148.58 ± 2.04 c
CEMP	1.39 ± 0.03 a	4.49 ± 0.08	74.91 ± 1.07 a	138.60 ± 2.37 a	0.32 ± 0.01 b	121.22 ± 1.97 a
A × B						
NI × C	1.52 ± 0.04	4.26 ± 0.13	79.40 ± 0.82 b	164.66 ± 4.01 c	0.24 ± 0.01 a	143.58 ± 3.30
NI × CENS	1.55 ± 0.05	4.39 ± 0.13	76.31 ± 0.99 ab	190.63 ± 4.51 d	0.32 ± 0.01 bc	149.20 ± 2.85
NI × CEMP	1.40 ± 0.04	4.62 ± 0.13	76.33 ± 0.53 b	140.44 ± 3.48 ab	0.30 ± 0.01 b	123.60 ± 2.97
I × C	1.47 ± 0.05	4.34 ± 0.15	78.71 ± 2.76 b	147.48 ± 3.30 b	0.31 ± 0.01 bc	129.23 ± 2.45

Table 3. *Cont.*

	Fresh Biomass (g Plant^{-1})	% Dry Matter	SLA (m^2 kg^{-1})	Total Root Length (cm)	Root Diameter (mm)	Length of 0 to 0.5 mm Diam. Root (cm)
I × CENS	1.50 ± 0.05	4.51 ± 0.15	73.57 ± 1.54 a	161.62 ± 3.83 c	0.33 ± 0.01 c	147.97 ± 2.74
I × CEMP	1.39 ± 0.04	4.37 ± 0.11	73.48 ± 0.68 a	136.77 ± 3.27 a	0.33 ± 0.01 c	118.83 ± 2.79
Significance						
Inoculation (A)	n.s.	n.s.	n.s.	***	***	**
Application (B)	**	n.s.	**	***	**	***
A × B	n.s.	n.s.	**	***	***	n.s.

Asterisk indicates significances at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s: non-significant. Different letters indicate significant differences. Values are the mean ± SD ($n = 20$).

The content of *P. irregulare* in the nutrient solution was significantly reduced by both CE treatments compared with the control, with no significant difference between them (Figure 1). There were no significant differences between the pathogen content of spring–winter and autumn growth cycles.
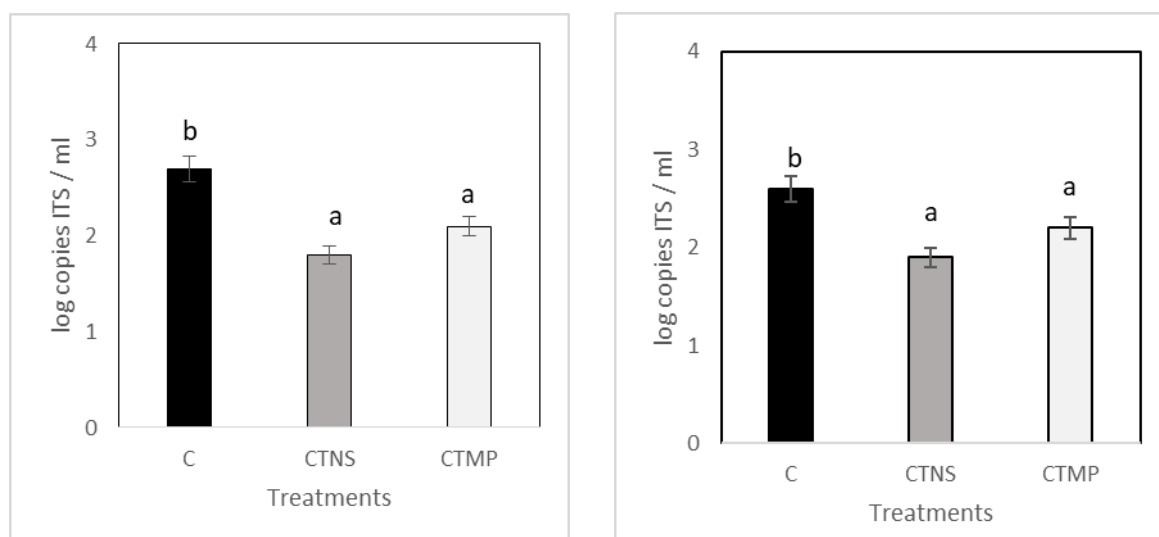
Figure 1. *Pythium irregulare* in the water at harvesting time in the winter–spring (**left**) and autumn (**right**) cycles according to the different ways of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler). Values are the mean ± SD (n = 4). Different letters indicate significant differences.

3.2. Nutritional and Microbiological Quality of Fresh-Cut Product

In the winter–spring cycle, the nitrate content was affected by the way of CE application and storage (Table 4). The leaves of plants grown with CEMP had the lowest content. There was a significant interaction between the way of CE application and storage in the autumn cycle (Table 5), the lowest value is found with the CEMP application. After 7 days of storage at 5 °C, the nitrate content had significantly decreased (31% and 58% in winter–spring and autumn, respectively); once again the CEMP treatment leading to the lowest nitrate content.

Table 4. Influence of way of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler) and storage (0 and 7 days) at 5 °C on the quality (nitrate, total phenolics, total flavonoids and antioxidant capacity) and microbial load (mesophilic and psychrophilic microorganisms) of baby leaf red lettuce cultivated in winter–spring cycle in a floating system.

	Nitrate (mg kg^{-1} FW)	Total Phenolics (mg GA kg^{-1} FW)	Total Flavonoids (mg Rutin kg^{-1} FW)	Antioxidant Capacity (mg DPPH$_{reduced}$ kg^{-1} FW)	Mesophilic Microorganisms (Log CFU g^{-1})	Psychrophilic Microorganisms (Log CFU g^{-1})
Application (A)						
C	1379.76 ± 74.91 b	1044.02 ± 151.38 a	1912.56 ± 162.07 a	105.32 ± 9.57 a	3.57 ± 0.24	3.74 ± 0.38 b
CENS	1290.69 ± 71.72 b	1168.74 ± 137.57 b	2691.93 ± 92.71 b	120.84 ± 7.49 b	3.48 ± 0.25	3.59 ± 0.64 a
CEMP	1079.91 ± 61.10 a	1280.77 ± 131.99 c	2917.70 ± 80.25 b	141.93 ± 12.74 c	3.59 ± 0.27	3.99 ± 0.37 c
Storage (B)						
0 days	1491.93 ± 39.58 b	1727.93 ± 41.73 b	2872.28 ± 77.43 b	159.37 ± 6.39 b	2.74 ± 0.05 a	2.23 ± 0.13 a
7 days	1007.27 ± 38.22 a	604.09 ± 25.58 a	2142.51 ± 126.60 a	86.02 ± 12.88 a	4.35 ± 0.09 b	5.32 ± 0.07 b
A × B						
C × 0 days	1636.46 ± 49.72	1636.45 ± 98.01	2550.72 ± 98.89 bc	141.22 ± 7.71 c	2.88 ± 0.06	2.41 ± 0.02 b
CENS × 0 days	1582.35 ± 58.61	1717.41 ± 67.29	3009.85 ± 88.43 de	148.76 ± 6.47 c	2.67 ± 0.12	1.47 ± 0.003 a
CEMP × 0 days	1300.96 ± 43.94	1820.92 ± 26.62	3056.26 ± 149.35 e	188.14 ± 11.73 d	2.68 ± 0.03	2.76 ± 0.02 c
C × 7 days	1123.06 ± 70.01	451.60 ± 6.63	1274.39 ± 31.76 a	69.42 ± 2.84 a	4.25 ± 0.26	5.03 ± 0.005 d
CENS × 7 days	1043.04 ± 55.61	620.06 ± 25.37	2374.01 ± 58.67 b	92.93 ± 1.46 b	4.29 ± 0.07	5.71 ± 0.03 f
CEMP × 7 days	855.70 ± 39.27	740.62 ± 20.03	2779.13 ± 16.20 cd	95.73 ± 4.34 b	4.51 ± 0.06	5.21 ± 0.04 e
Significance						
Application (A)	***	***	***	***	n.s.	**
Storage (B)	***	***	***	***	***	***
A × B	n.s.	n.s.	***	*	n.s.	***

Asterisk indicates significances at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s: non-significant. Different letters indicate significant differences. Values are the mean ± SD ($n = 9$).

Table 5. Influence of way of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler) and storage (0 and 7 days) at 5 °C on the quality (nitrate, total phenolics, total flavonoids and antioxidant capacity) and microbial load (mesophilic and psychrophilic microorganisms) of baby leaf red lettuce cultivated in autumn cycle in a floating system.

	Nitrate (mg kg^{-1} FW)	Total Phenolics (mg GA kg^{-1} FW)	Total Flavonoids (mg Rutin kg^{-1} FW)	Antioxidant Capacity (mg DPPH$_{reduced}$ kg^{-1} FW)	Mesophilic Microorganisms (Log CFU g^{-1})	Psychrophilic Microorganisms (Log CFU g^{-1})
Application (A)						
C	2015.92 ± 135.74 c	597.96 ± 32.85 a	1316.82 ± 26.75 a	90.95 ± 2.83 a	3.56 ± 0.35 b	3.22 ± 0.52 a
CENS	1812.72 ± 130.42 b	841.05 ± 78.45 b	1496.79 ± 22.36 b	117.31 ± 3.20 b	3.24 ± 0.13 a	2.96 ± 0.33 a
CEMP	1463.84 ± 106.93 a	1005.97 ± 98.33 c	1594.37 ± 13.94 c	138.27 ± 2.98 c	3.32 ± 0.18 ab	4.08 ± 0.49 b
Storage (B)						
0 days	2331.93 ± 90.41 b	1093.75 ± 56.79 b	1514.17 ± 26.52 b	126.26 ± 4.08 b	2.71 ± 0.08 a	2.08 ± 0.10 a
7 days	1196.39 ± 26.52 a	536.23 ± 18.02 a	1424.48 ± 27.99 a	104.75 ± 3.88 a	4.04 ± 0.14 b	4.76 ± 0.27 b
A × B						
C × 0 days	2807.25 ± 103.84 e	727.74 ± 11.56 c	1372.11 ± 20.45	100.43 ± 2.83	2.39 ± 0.02 a	1.85 ± 0.11
CENS × 0 days	2337.72 ± 37.09 d	1156.81 ± 17.69 d	1535.90 ± 40.69	130.28 ± 0.71	2.94 ± 0.14 b	1.94 ± 0.20
CEMP × 0 days	1850.82 ± 101.76 c	1396.71 ± 47.84 e	1634.51 ± 17.01	148.10 ± 3.06	2.80 ± 0.09 b	2.45 ± 0.02
C × 7 days	1224.58 ± 29.40 ab	468.17 ± 15.56 a	1261.53 ± 43.12	81.46 ± 1.85	4.72 ± 0.14 d	4.59 ± 0.64
CENS × 7 days	1287.71 ± 44.81 b	525.29 ± 30.28 a	1457.68 ± 9.31	104.36 ± 1.11	3.55 ± 0.12 c	3.99 ± 0.13
CEMP × 7 days	1076.87 ± 28.29 a	615.22 ± 25.15 b	1554.24 ± 11.56	128.44 ± 2.07	3.85 ± 0.16 c	5.71 ± 0.03
Significance						
Application (A)	***	***	***	***	*	**
Storage (B)	***	***	***	***	***	***
A × B	***	***	n.s.	n.s.	***	n.s.

Asterisk indicates significances at * $p < 0.05$; ** p < 0.01; *** $p < 0.001$; n.s: non-significant. Different letters indicate significant differences. Values are the mean ± SD ($n = 9$).

The total phenolics content was affected by the way of CE application and storage in the winter–spring cycle (Table 4). The leaves of plants grown with CEMP had the highest content. In the autumn cycle, there was a significant interaction between both factors, the highest value is reached with the CEMP application at harvesting (Table 5). Storage decreased the phenolics content in every CE treatment in both cycles.

There was a significant interaction between both factors for the total flavonoids content and antioxidant capacity in the winter–spring cycle, the highest values for total flavonoids obtained with CEMP and CENS and the highest antioxidant capacity with CEMP. Total flavonoids had decreased in all the CE treatments (50%, 21% and 22% for control, CENS and CEMP, respectively) after 7 days of storage. The antioxidant capacity also decreased for each treatment after 7 days of storage, but with only slight differences between them. In the autumn cycle, the total flavonoids content and antioxidant capacity were affected by both factors. Both values were higher when CE was applied, particularly with the CEMP application. Storage decreased both parameters in both cycles.

The microbial load for mesophilic microorganisms was only affected by storage in the winter–spring cycle, whereas there was an interaction between both factors in the autumn cycle, the highest value being found at day 7 in the leaves from the control plants. There was a significant interaction between both factors for psychrophilic microorganisms in the winter–spring cycle, in this case, the highest value is found at day 7 in the leaves from plants grown in CENS. However, the microbial load for psychrophilic microorganisms was affected by both factors in the autumn cycle, the highest load is found in the leaves of plants grown with CEMP application.

4. Discussion

The effect of CE on growth differed depending on the way it was applied. CENS application provided the highest yield (Tables 1 and 2). The nitrate concentration of the irrigation water, which increased weekly due to its gradual release from CE in the CENS application (data not shown), may have influenced the increase in lettuce yield. The use of CE in the nutrient solution could provide benefits both as fertilizer and biostimulant [30]. The application of CENS also reduced the incidence of the pathogen added to the water (Figure 1), due to the suppressive effect of the compost, again increasing the crop yield. Giménez et al. 2019 [13] demonstrated that the same compost lowered the incidence of *P. irregulare*, producing higher yields in lettuce when the substrate was infected by the pathogen. Conversely, the CEMP application did not increase yield in spite of the additional 13.4 mg/L of nitrate, on average, in each microsprinkler irrigation event (Table 1). In addition, the Na^+ and Cl^- concentration could have had a higher negative influence in the growth when CE was applied by microsprinkler than directly to the nutrient solution, although the plants did not show symptoms of phytotoxicity. However, this way of CE application also reduced the concentration of *P. irregulare* in the water (Figure 1). A decrease in SLA is related to an increase in leaf thickness; in our experiment, CE application decreased SLA in inoculation conditions, particularly in the autumn cycle. Panova et al. 2011 [38] found a decrease in SLA values in cucumber plants inoculated with a moderate concentration of *Pythium aphanidermatum*, although SLA increased with a high concentration of the pathogen. The treatments with CE could have induced a greater resistance to the fungus, as manifested by the greater thickness of the leaves. In addition, the total root length and fine roots were significantly higher in CENS, confirming the importance of a healthy root system for the plants to use the water and nutrients in the most efficient way, and thus provide high yields. This beneficial effect of CE, whose application was more efficient when directly applied to the nutrient solution (CENS), on yield and/or root length could be due to the production of auxin or auxin-like components from humic substances [39], which would promote radicular growth. Inoculation with *P. irregulare* reduced root length and increased the root diameter in every treatment (Tables 1 and 2). Our results agree with those of Schwarz and Grosch 2003 [40], who found that after the inoculation of tomato with *P. aphanidermatum* the number and length of roots were significantly reduced, the strongest effect being on young and fine roots, while an increase in root diameter was also detected. In general, *Pythium* spp. causes a reduction

in root length [41], while root diameter is generally the most responsive plant trait associated with inoculation by root rot pathogens [42].

The reduction of *P. irregulare* in the water to CE had been applied (Figure 1) could be attributed to antibiotic-like compounds or microorganisms [2,7] from the original compost [12]. This demonstrates that the suppressiveness observed in compost is probably transferred and maintained in its water extract, reducing pathogen incidence as a direct effect [30,43]. Indirectly, the presence of compounds or microorganisms capable of triggering plant resistance would provoke an over-reduction of the pathogen in the water solution by excreting antimicrobial compounds through the root system [43–45].

The nitrate content is an important quality characteristic of vegetables [46], and the EU encourages good agricultural practices to reduce the presence of nitrates in lettuce by imposing a limit for its sale [47]. Our data suggest that the use of compost extract, both as CENS and CEMP, reduced the amount of nitrate compared with the control, increasing the quality of the baby leaf red lettuce. This might be the result of the biostimulant effect of the compost, inducing changes in the expression of nitrate transporter genes, as well as in several metabolic pathways involved in N metabolism (nitrate and nitrite reductase, glutamate synthase and glutamine synthetase activities), leading to a more efficient assimilation of nitrates into amino acids [48,49]. Of note was the capacity of the compost to reduce this content when plants were grown with the CEMP application. Possibly, the above-mentioned increase on the nitrate concentration of the nutrient solution due to its gradual release from the CENS application would be responsible for the nitrate level in leaves being slightly higher in CENS than in the CEMP. The nitrate concentrations were higher in the autumn cycle, as was expected since DLI was lower in the autumn than in the winter–spring cycle. This is because light conditions influence nitrate reductase activity and decrease the conversion rate of nitrate to amino acids, leading to a higher concentration of nitrates [50,51]. Moreover, nitrate concentrations did not exceed the maximum level allowed by the EU for this type of lettuce and way of cultivation. After 7 days of storage at 5 °C, the nitrate concentration in leaves had been reduced. Our findings agree with the results of Gomez et al. 2003 [52] in celery and Miceli et al. 2019 [53] in rocket leaves, where a general decrease in the nitrate content of leaves was observed after storage at 4 °C. By contrast, Konstantopoulo et al. 2010 [54] and Miceli et al. 2019 [53] showed that the nitrate content remained constant in different types of green lettuce during storage at 5 and 10 °C and at 4 °C, respectively. As the nitrate content of leaves and changes in the same during cold storage are species-dependent, red lettuce might have a different nitrate accumulation pattern from green lettuce [55,56], its content during cold storage falling as a consequence.

The postharvest quality of fresh vegetables is generally influenced by several preharvest factors and environmental conditions [57]. Hence, the CE application used could have resulted in the induction and activation of the plant secondary metabolism, increasing the content of total phenolics and flavonoids and the antioxidant capacity compared with the control plants at harvest (Tables 3 and 4). As regards phenolic compounds, these results agree with those of Kołton and Baran, 2008 [58], who found that compost application significantly increased the phenol content in corn salad compared with mineral fertilization. Also, the fruit of pepper plants grown with carrot compost had a high phenol content [59]. Nevertheless, in pak choi total phenolics were lower in vermicompost tea-treated plants than in plants treated with only mineral nutrient solution and those from the water-only control [60]. Regarding total flavonoids at harvest, our results agree with those of Khalid et al. 2006 [45] and Ezz El-Din et al. [43], who showed that the concentration of flavonoids increased significantly following compost tea treatments. Likewise, higher flavonoid contents were observed in *Moringa oleifera* plants treated with NPK + compost [61]. Similarly, the antioxidant capacity at harvest was higher in CE treated plants, (Tables 2 and 3) as reported in other studies where compost was applied to lettuce, spinach [62,63] and pak choi [60]. Lettuce leaves accumulated significantly more phenolic compounds and flavonoids in the autumn than in the winter–spring cycle at harvest probably due to the difference in temperature and light between cycles, as suggested by Marin et al. 2015 [64] in red oak lettuce, who found a positive correlation between the content of phenolic acids and flavonoids and

cold temperatures. Among environmental factors, light/radiation and temperature are the two most influential climatic variables for the biosynthesis of phenolics in red lettuce [65]. Furthermore, other authors have suggested that there is competition between the flavonoid and phenolic acids pathways, the flavonoids route being favored in conditions of high light intensity [66], since they can act as photoprotectors as occurred in the winter–spring cycle of our experiment (Table 4), which had a higher DLI than the autumn cycle. In the winter–spring cycle, too, there was a positive correlation between phenolic compounds and antioxidant capacity (r = 0.891, $p \leq 0.01$; r = 0.942, $p \leq 0.01$ and r = 0.846, $p \leq 0.01$ for control, CENS and CEMP treatment, respectively) and also between flavonoids and the antioxidant capacity (r = 0.941, $p \leq 0.01$, r = 0.917, $p \leq 0.01$, r = 0.463, $p \geq 0.5$ for control, CENS and CEMP treatment, respectively). However, in the autumn cycle this positive relation was only evident between total phenolics and the antioxidant capacity (r = 0.817 $p \leq 0.01$, r = 0.966, $p \leq 0.01$, r = 0.770, $p \leq 0.01$, for control, CENS and CEMP, respectively) and there was no correlation with flavonoids. These data indicate that flavonoids could have an important role in radical-scavenging [67], as was seen in the winter–spring cycle when the amount of them was higher.

After 7 days of storage, the total phenolics and flavonoids and the antioxidant capacity were significantly lower than at harvest time, as was observed by Kalt et al. 1999 [68], Serafini et al. 2002 [69] and Ninfali and Bacchiocca 2005 [70]. The decrease in total phenolics that occurs during storage may be due to increased antioxidant enzymatic activities [71]. However, other authors found that total flavonoids remained quite constant in spinach [72] and the phenolic contents increased in lettuce [73]. DuPont et al. 2000 [74] reported the loss of flavonol glycosides in lettuce stored at 1 °C for 7 d, confirming that significant changes in the relative concentration of individual phenolics that may occur during storage are sometimes cultivar-dependent.

Since lettuce is consumed raw, information on possible microbial contamination is of great importance. As expected, mesophilic and psychrophilic populations increased significantly during the storage period, but were below 6 log units at the end of product shelf-life (Tables 3 and 4). These values were lower than those reported in previous studies in red lettuce [75]. Our results demonstrated that microbial quality was maintained during storage and that CE treatment had no negative effect on the microbial load of the product, with values typical for fresh-cut lettuce ready for marketing.

5. Conclusions

Directly brewed compost extract added to the nutrient solution improves baby leaf lettuce growth and quality (reducing the nitrate content and enhancing the content of potentially health-promoting compounds such as total phenolics and flavonoids as well as the antioxidant capacity). The application of compost extract by microsprinkler slightly reduces plant growth, but notably increases the quality of the product. The microbial quality is maintained during shelf-life and compost extract has no negative effect on the microbial load of the final product. In addition, both ways of compost extract application decrease the population of *Pythium irregulare* in the water. However, further studies are needed on the application of compost extract in order to develop sustainable agricultural production based on the reduced use of fertilizers.

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