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SUSTAINABLE PRODUCTION OF BABY LEAF LETTUCE AND SPINACH THROUGH THE USE OF COMPOST AND ITS EXTRACTS

Doctorado en Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario

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Dra. Catalina Egea Gilabert

Cartagena, 2020

PhD Thesis

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D. JUAN ANTONIO FERNÁNDEZ HERNÁNDEZ, D. JOSÉ ANTONIO PASCUAL VALERO y Dª. CATALINA EGEA GILABERT Director y codirectores de la Tesis doctoral "SUSTAINABLE PRODUCTION OF BABY LEAF LETTUCE AND SPINACH THROUGH THE USE OF COMPOST AND ITS EXTRACTS".

INFORMA:

Que la referida Tesis Doctoral, ha sido realizada por Dª. ALMUDENA GIMÉNEZ MARTÍNEZ, dentro del Programa de Doctorado TÉCNICAS AVANZADAS EN INVESTIGACIÓN Y DESARROLLO AGRARIO Y ALIMENTARIO, dando mi conformidad para que sea presentada ante el Comité de Dirección de la Escuela Internacional de Doctorado para ser autorizado su depósito.

X Informe positivo sobre el plan de investigación y documento de actividades del doctorando/a emitido por el Director/ Tutor (**RAPI**).

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En Cartagena, a 18 de Septiembre de 2020

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Sra. Dña. Almudena Giménez Martínez

Visto el informe favorable del Director de Tesis y el Vº Bº de la Comisión Académica del Programa de Doctorado "Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario" para la presentación de la Tesis Doctoral titulada: **"Sustainable production of baby leaf lettuce and spinach through the use of compost and its extracts"** solicitada por DÑA. ALMUDENA GIMÉNEZ MARTÍNEZ, el Comité de Dirección de la Escuela Internacional de Doctorado de la Universidad Politécnica de Cartagena, en reunión celebrada el 29 de septiembre de 2020, considerando lo dispuesto en el artículo 23 del Reglamento de Estudios Oficiales de Doctorado de la UPCT, aprobado en Consejo de Gobierno el 17 de diciembre de 2015,

ACUERDA

Autorizar la presentación de la Tesis Doctoral a Dña. Almudena Giménez Martínez en la modalidad de "compendio de publicaciones".

Contra el presente acuerdo, que no agota la vía administrativa, podrá formular recurso de alzada ante el Sr. Rector-Magnífico de la Universidad Politécnica de Cartagena, en el plazo de un mes a partir de la notificación de la presente.

Cartagena, 29 de septiembre de 2020

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This thesis is a compendium of papers previously published or accepted for publication. It consists of the following articles:

- **Giménez, A.,** Fernández, J.A., Pascual, J.A., Ros, M., Lopéz-Serrano, M. and Egea-Gilabert, C. 2019. An agroindustrial compost as alternative to peat for production of baby leaf red lettuce in a floating. **Scientia Horticulturae**. 246, 907-915. http://doi.org/10.1016/j.scienta.2018.11.080
- **Giménez, A.,** Fernández, J.A., Pascual, J.A., Ros, M. and Egea-Gilabert, C. 2020. Application of directly brewed compost extract improves yield and quality in baby leaf lettuce grown hydroponically. **Agronomy**. 10, 270. http://doi.10.3390/agronomy10030370
- Ros, M., Hurtado-Navarro, M., **Giménez, A.**, Fernández, J.A., Egea-Gilabert, C., Lozano-Pastor, P. and Pascual, J.A. 2020. Spraying Agro-industrial compost tea on baby spinach crops: evaluation of yield, plant quality and soil health in field experiments. **Agronomy**. 10, 440. http://doi.10.3390/agronomy10030440

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Resumen

El incremento de residuos orgánicos y subproductos procedentes de la industria agroalimentaria genera problemas medioambientales y económicos. Una alternativa para su uso es el compostaje, mediante el cual se generan compost y sus extractos biológicos que pueden ser empleados, entre otros usos, como fuente de nutrientes para las plantas, mejorando la productividad de las cosechas. Además, los compost pueden contribuir a mejorar la calidad de los cultivos y a favorecer el control biológico de patógenos, debido a la capacidad de supresión que tienen algunos de ellos. Todo ello contribuye a la reducción del uso de fertilizantes y plaguicidas químicos, haciendo la agricultura más sostenible y promocionando la economía circular.

En la actualidad, las hortalizas mínimamente procesadas son un grupo de alimentos que genera un gran interés por parte del consumidor, propiciando que exista una gran demanda en el mercado. Dentro de este grupo se encuentran la lechuga y la espinaca de hoja pequeña (*baby leaf*). Su producción se ha realizado tradicionalmente en suelo, pero en los últimos años se están cultivando en sistemas de cultivo sin suelo, fundamentalmente hidropónicos. El cultivo hidropónico permite un mayor control de la planta durante todo su ciclo, propiciando una mayor automatización de los cultivos. De esta forma se logra una uniformidad en el crecimiento, un acortamiento de los ciclos, un uso eficiente de los fertilizantes, y un mayor rendimiento y calidad del producto final. Entre los sistemas de cultivo hidropónico destaca el sistema de cultivo en bandejas flotantes (*floating system*), que permite corregir y controlar parámetros importantes como son los compuestos nutricionales (vitaminas, fenoles y antioxidantes) y anti-nutricionales (nitratos y oxalatos). Las bandejas en este sistema se rellenan con turba, que es un material

que aumenta la susceptibilidad de la planta a algunas enfermedades causadas por hongos u oomicetos como *Pythium* spp., provocando pérdidas de producción. Adicionalmente, la demanda de turba ha ido creciendo en los últimos años, por lo que ha aumentado su extracción excesivamente, provocando un efecto negativo en el medio ambiente. Para contrarrestar este efecto se están estudiando sustratos alternativos a la turba, como los compost y sus extractos biológicos, que pueden favorecer no solo la productividad de los cultivos sino también su calidad de una forma respetuosa con el medio ambiente.

El objetivo principal de esta tesis doctoral fue demostrar que ciertos tipos de composts podrían ser utilizados como sustratos alternativos a la turba en *floating system*, así como enmiendas orgánicas en cultivo en suelo de hortalizas de hoja pequeña. Para ello, se estudió si el compost y sus extractos biológicos pueden ser considerados productos agrícolas de valor añadido, minimizando y/o limitando la incidencia de patógenos y mejorando la acumulación de compuestos nutricionales en lechuga y espinaca *baby leaf*.

Para alcanzar este objetivo en primer lugar, se estudió un compost agroindustrial, compuesto por tomate (71%), cebolla (17%) y restos de viñedo (12%), durante dos ciclos de cultivo de lechuga roja *baby leaf* cultivada en *floating system*. Se llevaron a cabo dos experimentos simultáneos: i) se evaluó la influencia de dos sustratos orgánicos (turba y compost) sobre la germinación, crecimiento y calidad nutricional de la lechuga, ii) se realizó un bioensayo de supresión utilizando los dos sustratos inoculados con *Pythium irregulare*. Los resultados mostraron que el compost mejoró el porcentaje de semillas germinadas y aumentó la calidad nutricional de la lechuga en comparación con la turba. Así mismo, las plantas cultivadas con compost inoculado con *P. irregulare* mostraron una mayor supervivencia y un mayor rendimiento del cultivo.

En segundo lugar, se evaluó el modo de aplicación de un extracto de compost, añadido a la solución nutritiva directamente (CENS) o mediante microaspersión (CEMP), durante dos ciclos de cultivo de lechuga roja *baby leaf* cultivada en floating system, sobre la producción y calidad de la lechuga y sobre la incidencia de *P. irregulare*. El tratamiento CENS mostró un efecto positivo sobre el crecimiento de las plantas tanto en condiciones inoculadas como no inoculadas. Además, el tratamiento CEMP en condiciones de no inoculación redujo considerablemente el contenido de nitratos y mostró un efecto positivo sobre los fenoles totales, los flavonoides y la capacidad antioxidante. Por último, el tratamiento CENS redujo notablemente la población de *P. irregulare* en el agua y no tuvo ningún efecto negativo sobre la carga microbiana del producto final.

Finalmente, se estudió el posible efecto beneficioso de la aplicación de un té de compost producido a partir de cebolla y restos de viñedo, solo (CT) o en combinación con el microorganismo beneficioso *Trichoderma harzianum* T78 (CT+Th), sobre la calidad y el rendimiento de espinaca baby leaf, durante dos ciclos de cultivo en suelo. Los tratamientos CT y CT+Th mostraron un mayor rendimiento del cultivo que el control, pero no produjeron un aumento de la actividad de la deshidrogenasa del suelo. Además, el tratamiento CT+Th mejoró la calidad del producto final, mostrando un aumento de fenoles totales, flavonoides y capacidad antioxidante.

Estos resultados nos muestran que el compost y el extracto de compost obtenidos a partir de residuos agroindustriales pueden ser una alternativa prometedora a la turba para su uso como sustrato orgánico en un sistema de producción sostenible sin suelo en lechuga roja *baby leaf*, ya que no sólo es capaz de controlar el efecto del patógeno *P. irregulare*, sino también de mejorar el rendimiento y la calidad del producto. Además, el té de compost enriquecido con *T. harzianum* se muestra como una alternativa sostenible para mejorar el rendimiento y la calidad de la espinaca *baby leaf* producida en suelo.

Abstract

The increase in organic waste and by-products from the agro-food industry generates environmental and economic problems. An alternative for its use is composting, which is a natural biological process of decomposing organic waste material. Compost is the product of the controlled biological decomposition of the above materials. Compost and its biological extracts can be used, among other uses, as a source of nutrients for plants, improving crop productivity. In addition, compost can contribute to improve the quality of crops and favour the biological control of pathogens, due to the suppression capacity that some of them have. All the above contributes to the reduction of the use of chemical fertilizers and pesticides, making agriculture more sustainable and promoting the circular economy.

At present, minimally processed vegetables are a group of foods that generate a great interest by the consumers, leading to a great demand in the market. Within this group, baby leaf lettuce and spinach are important vegetable products in the industry. Their production has been traditionally carried out in soil, but in recent years they are being grown in soilless cultivation systems, mainly hydroponics. Hydroponic cultivation allows a better control of the plant growth during its entire cycle, leading to greater automation of crops. In addition, growth uniformity, shortening of cycles, efficient use of fertilizers, and higher yield and quality of the final product are achieved. Among the hydroponic cultivation systems, the floating system allows correcting and controlling important parameters in plant, such as nutritional compounds (vitamins, phenols and antioxidants) and anti-nutritional compounds (nitrates and oxalates). The trays in this system are normally filled with peat, which is a material that increases the plant susceptibility to

some diseases caused by fungi or oomycetes such as *Pythium* spp. causing production losses. Furthermore, the demand for peat has been growing in recent years, increasing its extraction excessively and causing a negative effect on the environment. To counteract this effect, alternative substrates to peat are being studied, such as compost, which can favour not only the productivity of crops but also their quality in an environmentally friendly way.

The main objective of this thesis was to demonstrate that certain types of composts could be used as alternative substrates to peat in floating system, as well as organic amendments in cultivation of baby leaf vegetables in soil. For this purpose, it was studied whether compost and its biological extracts can be considered added value agricultural products, minimizing and/or limiting the incidence of pathogens and improving the accumulation of nutritional compounds in baby leaf lettuce and spinach.

In order to achieve this objective, first an agroindustrial compost, composed of tomato (71%), onion (17%) and vineyard residues (12%), was studied during two cycles of red baby leaf lettuce cultivated in floating system. Two simultaneous experiments were carried out: i) the influence of two organic substrates (peat and compost) on the germination, growth and nutritional quality of lettuce was evaluated, ii) a suppression bioassay was carried out using the two above substrates inoculated with *Pythium irregulare*. The results showed that compost improved the percentage of germinated seeds and increased the nutritional quality of the lettuce compared to peat. Likewise, plants grown with compost inoculated with *P. irregulare* showed a higher survival rate and a higher crop yield.

Secondly, the way of application of a compost extract, added to the nutritive solution directly (CENS) or by microsprinkler (CEMP), was evaluated on the yield and quality of the lettuce and on the incidence of *P. irregulare* during two growing cycles of baby leaf red lettuce grown in floating system. The CENS treatment showed a positive effect on plant growth under both inoculated and non-inoculated conditions. In addition, the CEMP treatment in non-inoculation conditions considerably reduced the nitrate content and showed a positive effect on total phenols, flavonoids and antioxidant capacity. Finally, the CENS treatment significantly reduced the population of *P. irregulare* in the water and had no negative effect on the microbial load of the final product

Finally, it was studied the possible beneficial effect of the application of a compost tea produced from onion and vineyard residues, alone (CT) or in combination with the beneficial microorganism *Trichoderma harzianum* T78 (CT+Th), on the quality and yield of baby leaf spinach, during two cycles of cultivation in soil. The CT and CT+Th treatments showed a higher crop yield than the control but did not produce an increase in soil of dehydrogenase activity. Moreover, CT+Th treatment improved the quality of the crop, showing an increase of total phenols, flavonoids and antioxidant capacity.

The results of this investigation show that compost and compost extract obtained from agroindustrial wastes can be a promising alternative to peat in a sustainable soil-less production system for baby leaf red lettuce, as they are not only able to control the effect of the pathogen *P. irregulare*, but also to improve the yield and quality of the product. In addition, compost tea enriched with *T. harzianum* is shown to be a sustainable alternative for improving the yield and quality of soil-produced baby leaf spinach.

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Introduction

1.1. Production systems for baby leaf vegetables: lettuce and spinach

1.1.1. Characteristics of lettuce plant (*Lactuca sativa* L.)

Lettuce (*Lactuca sativa* L.) is a crop that originated in the Mediterranean basin, with traces dating back to 2500 b.C. in the form of paintings in the tombs of ancient Egypt. The Romans introduced lettuce to Europe, and around 600 a.C. it reached China. Lettuce is a plant belonging to the *Asteraceae* family, also called *Compositae*. The stem is usually short in most varieties, where the leaves are attached. Depending on the botanical variety and the cultivar the leaves can be arranged to form heads or not. Currently, the following types of lettuce can be distinguished according to some agronomic characteristics, such as the ability to form heads, the consistency of the leaves or adaptation to a particular season: iceberg, romaine, batavia, lollo, oak leaf, multileaf and baby leaf (Figure 1.1). This great variety in the types of lettuce is largely due to the demand of processing industry, which is looking for different shapes, textures, colours, due to consumer interest in healthier foods and the changes that have been taking place in recent years in consumption habits.

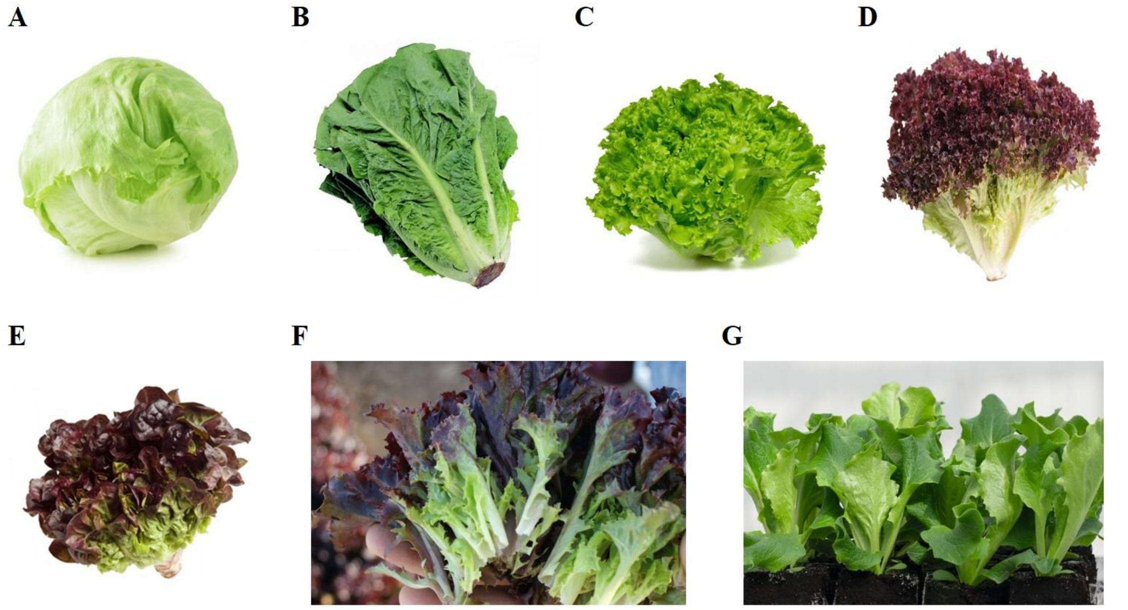

Figure 1.1: Lettuce iceberg (A), romaine (B), Batavia (C), lollo (D), oak leaf (E), multileaf (F) and baby leaf (G).

One of the products that is attracting a great deal of interest is the baby leaf lettuce. Baby leaf type lettuces have tender leaves, 8-12 cm long, with small petioles, therefore with less oxidation surface, wich lengthens their post-harvest life (González et al., 2004). These characteristics provide a higher percentage of useful product, simple and quick processing, and a very attractive presentation (Martinez-Sanchez et al., 2012). Up to 98% of production of baby leaf is destined for the fresh-cut industry. In general, the production cycles are short, 30-35 days, shortening to 25 days in greenhouse cultivation in summer.

Lettuce, as other leafy vegetables, is an important source of antioxidant compounds, such as phenols, carotenoids and vitamins, (Tomas-Barberán et al., 1997). The nutritional quality of lettuce, particularly the red leaf cultivar, is notable for its potassium content (187 mg/100 g fw), iron (1.2 mg/100 g fw), and also for its vitamin C content (3.7 mg/100 g fw) (Table 1.1). Besides being more attractive to eat, red leaf lettuce has a higher content of phytochemicals (García-Macías et al. 2007), contains more phenolic compounds and carotenoids (Llorach et al., 2008), and its antioxidant activities are stronger under the same growing conditions than other cultivars (Liu et al., 2007).

Table 1.1: Nutritional content of lettuce.

Nutrient	Unit	Red leaf 100 g	Green leaf 100 g
Water	g	95.64	94.98
Energy	Kcal	16	15
Protein	g	1.33	1.36
Total lipid (fat)	g	0.22	0.15
Carbohydrate, by difference	g	2.26	2.87
Fiber, total dietary	g	0.9	1.3
Sugars, total	g	0.48	0.78
Minerals			
Ca	mg	33	36
Fe	mg	1.2	0.86
Mg	mg	12	13
P	mg	28	29
K	mg	187	194
Na	mg	25	28
Zn	mg	0.2	0.18
Vitamins			
Vitamin C, total ascorbic acid	mg	3.7	9.2
Thiamin	mg	0.064	0.07
Riboflavin	mg	0.077	0.08
Niacin	mg	0.321	0.375
Vitamin B6	mg	0.1	0.09
Vitamin A, RAE	µg	375	370
Vitamin A, IU	IU	7492	7405
Vitamin E (alpha-tocopherol)	mg	0.15	0.22
Vitamin K (phylloquinone)	µg	140.3	126.13
Folate, DFE	µg	0	38
Lipids			
Fatty acids, total saturated	g	no data	0.02
Fatty acids, total saturated	g	no data	0.02
Fatty acids, total monounsaturated	g	no data	0.006
Fatty acids, total polyunsaturated	g	no data	0.082

(Source: USDA)

The nutritional quality of lettuce can be affected by several factors such as cultivation system, harvesting system or pre-harvest and post-harvest processes (Kader, 2008). Irrigation and fertilization also influence the biosynthesis and accumulation of bioactive compounds. A fundamental characteristic in leaf vegetables and specifically in baby leaf lettuces is the nitrate content (Santamaria, 2006), regulated by Regulation CE 1258/2011. For lettuce, limits are set between 2,000 mg/kg fresh weight for 'iceberg' lettuce grown in the open field cropping and 5,000 mg/kg fresh weight for lettuce grown under greenhouse cropping for harvesting between 1 October and 31 March. In addition, leaf nitrate concentration can be influenced by environmental factors such as temperature and photoperiod (Burns et al., 2011).

Lettuce production can be affected by diseases altering growth and quality of the crop, leading to losses of significant commercial value. Root disorder diseases can be caused by plant pathogens mainly oomycetes (*Phytium* spp.), nematodes (*Pratylenchus* spp.) and viruses (big vein). Damage to leaves can be also frequently occurred by foliar pathogens such as *Pseudomonas cichorii*, or *Erysiphe cichoracearum* causing powdery mildew, or *Bremia lactucae* producing spots (downy mildew). In addition, *Sclerotinia sclerotiorum* and *S. minor* produce another disease that causes white and neck rot. Grey rot also occurs, triggered by the fungus *Botrytis cinerea*, causing problems in post-harvest, which shows the symptoms after harvest. Also, lettuce can be affected by physiological disorders such as Tip burn, producing a necrosis in the outer leaves, Brown stain characterized by brown colorations produced during the conservation at low temperatures and Pink rib, in which the nerves of the basal leaves acquire a pink color, due to intense rains and cloudy periods, thus shortening the shelf life of the lettuce.

Spain produces 934,670 t of lettuce (FAOSTAT, 2018), which is 3.43% of world production. At European level, Spain is the first country in lettuce production with 31.36% of total production. It is estimated that 75% of the lettuce is destined for fresh-cut salads. By Autonomous Community, the first one in terms of volume of tonnes produced is the Region of Murcia with 430,459 t in 2019 (MAPA, 2019) (Figure 1.2).

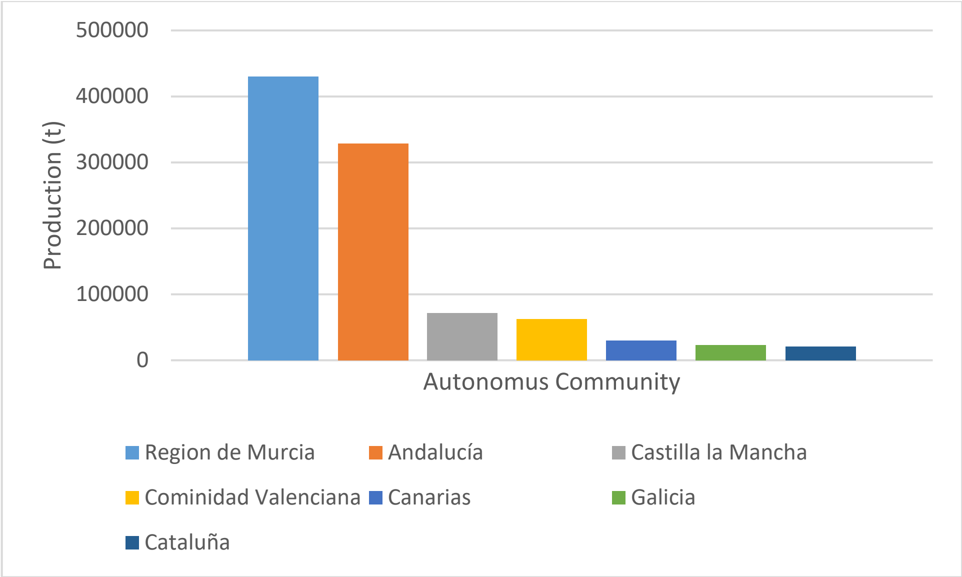

Figure 1.2: Lettuce production (t) by Autonomous Community (MAPA, 2019).

1.1.2. Characteristics of spinach plant (*Spinacia oleracea* L.)

The spinach is native to Southeast Asian (Boswell, 1949). The spinach was consumed as medicine by Greeks and Romans and highly appreciated by Arabs. The Arabs introduced spinach in Spain in the XI century and later was consumed and cultivated in the rest of Europe. (Marhuenda and García-Vergara, 2017a).

Spinach (*Spinacia oleracea* L.) belongs to the *Chenopodiaceae* family and is a dioecious plant. It has a pivoting root, little branched and superficial, forming a rosette of petiole leaves. The leaves of baby spinach are alternate, dark green, 9-12 cm long and tender and sweeter than mature spinach. Its optimal growth temperature is 10-25 °C (MAPA, 2019). Depending on location and time of year the growth cycle of baby spinach ranges from 4 to 9 weeks. Spinach requires soils with good structure, rich in organic matter, and with good drainage, since it does not support an excess of moisture in the soil, preventing a good development of the plant and producing yellowing of the leaves.

Like lettuce, spinach is an important source of bioactive compounds and provides a large amount of vitamin C, vitamin A and polyphenols (Xie et al., 2013). Therefore, it has a very high nutritional and gastronomic value (Fernandez, et al., 2015). It has a high content of β-carotene (0.32 mg/100 g fw), which has antioxidant and immune system-stimulating capabilities. Spinach is a good source of folic acid (194 µg/100 g fw), minerals such as calcium, magnesium and iron and a great source of fiber (Table 1.2).

Table 1.2: Nutritional content of spinach.

Nutrients	Units	Spinach 100 g
Water	g	91.4
Energy	Kcal	23
Protein	g	2.9
Total lipid	g	0.39
Fiber	g	2.2
Carbohydrate	g	3.6
Minerals		
Ca	mg	99
Fe	mg	2.71
Mg	mg	79
P	mg	49
K	mg	558
Na	mg	79
Zn	mg	0.53
Mn	mg	0.9
Se	µg	0.1
Vitamins		
Retinol (vit. A)	µg	469
β-carotene Thiamine (vit. B1)	mg	0.078
Riboflavin (vit. B2)	mg	0.189
Niacin (vit. B3)	mg	0.724
Vitamin C	mg	28.1
Vitamin E	mg	2
Vitamin K	µg	483
Vitamin B6	mg	0.195
Lipids		
Folic acid (vit. B9)	mg	28.1
Pantothenic acid (vit. B5)	mg	0.065

(Source: USDA)

Spinach, as baby leaf lettuce, is also a species which accumulates nitrates in its leaves. The maximum limit for the nitrate content in fresh spinach is regulated by Regulation CE 1258/2011, which sets a limit of 3,500 mg/kg of fresh weight. In addition, spinach is also characterized by accumulating oxalates in its leaves. The content of oxalate in spinach oscillates between 7 and 13 mg/g fresh weight (Koh et al., 2012). The accumulation of nitrate and oxalate is affected by several factors, such as variety, grown season, light intensity and high input of nitrogen fertilisers (Kawazu et al, 2003; Proietti et al, 2004; Elia et al., 1998).

In spinach his most common disease is spinach mildew generated by *Peronospora farinosa* f. sp. *spinaciae*, which affects plants in any stage of development and can cause the loss of product quality and the rejection by the food industry (Gilardi et al., 2018). The symptoms produced by *Peronospora farinosa* f. sp. *spinaciae* are the presence of greyish mold and chlorotic spots on the underside of the leaves, causing a rapid proliferation in young leaves. This problem can be solved with the use of disease-resistant varieties, which are adapted to environmental conditions and have a more efficient use of water and nitrogen.

Currently, the demand for spinach has increased its consumption, favoured by the salad processing industry, used alone or as part of mixtures with other baby leaf vegetables. Spinach cultivation in Spain reaches 4,299 ha with a total production of 79,216 t (MAPA, 2019), which represents 0.3% of worldwide production. By Autonomous Community, the first Spanish producer is the Region of Murcia with 22,074 t (MAPA, 2019) (Figure 1.3).

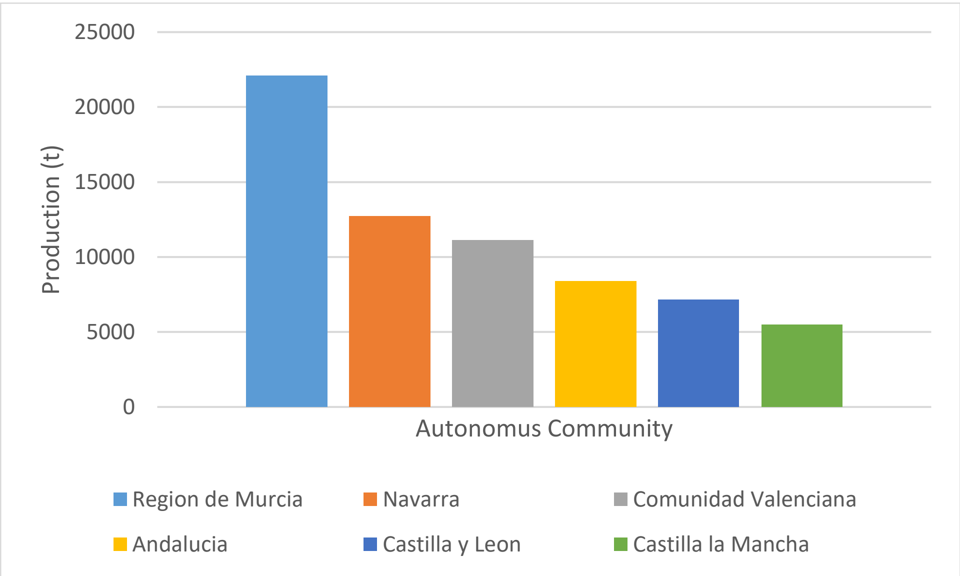

Figure 1.3: Spinach production (t) by Autonomous Community (MAPA, 2019).

1.1.3. Production of baby leaf vegetables in soil

The production of baby leaf vegetables in soil, where the plants are sown and harvested mechanically, can be done outdoors or under protected cultivation, mainly in the colder seasons. Growing leafy vegetables requires that the planting beds are well prepared for a right sowing and therefore a good rate of germination. These are crops with high planting densities, where uniform germination is important for an optimum yield. In addition, it requires the optimization of the use of fertilizers, because it is very important to improve their quality and productivity.

In the case of baby leaf lettuce, it requires soils with good structure and is advisable to add organic matter (1-5 kg/m^2) in the top of 25 cm of the soil, prior to planting (Marhuenda and García-Vergara, 2017b). The cultivation of baby leaf lettuce is carried out on beds of 1.8 and 2 m wide, in which 30 lines of seeds are sown, reaching densities of 12,000,000 seeds/ha (Figure 1.4).

Figure 1.4: High densities of baby leaf lettuce in soil (Source: FreshPlaza).

For a spinach crop, also a good soil structure and good drainage are required. It is a crop that needs soils rich in organic matter, so it is recommend the use of organic fertilizer in the background, like manure, well composted once a year, with doses between 2-4 kg/m^2. The cultivation of baby leaf spinach, like baby leaf lettuce, is done in beds of 1.8 and 2 m wide, with a useful beds of 1.4 to 1.6 m (Figure 1.5). Baby leaf spinach is sown in 30-36 lines per beds, with planting densities of 7,000,000 seeds/ha and it reaches a yield of

7,000 to 9,000 kg/ha. The irrigation system is normally by sprinkler of low flow (550-650 l/hour). Since spinach is a species susceptible to accumulate nitrates in the leaves, fertilization should be done with slow release or nitrification inhibitors fertilizers.

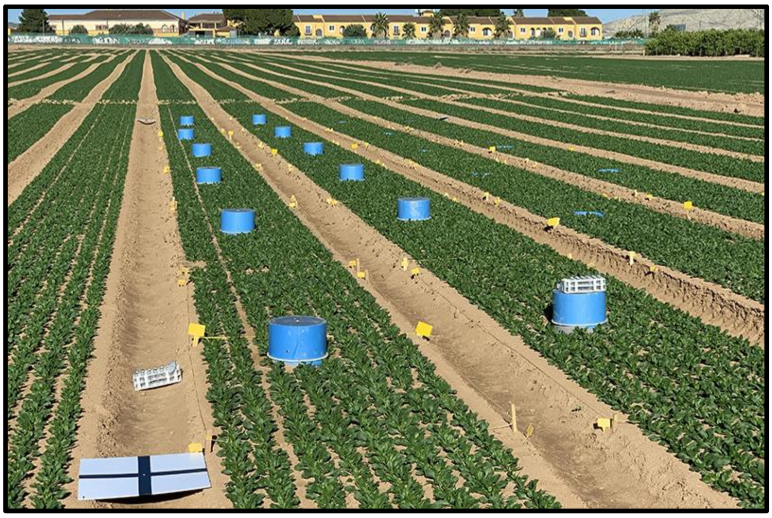

Figure 1.5: Growing baby leaf spinach in open soil (Source: UMH).

In a traditional crop in the soil, there are a number of disadvantages such as soil impoverishment, contamination of the product by soil pathogens, which reduce the yield, quality and shelf life of vegetables such as baby leaf lettuce and spinach. Thus, new cultivation systems are being developed to improve the production aspects of this type of small leafy vegetables, such as soilless cultivation systems like the floating system.

1.1.4. Production of baby leaf vegetables in soilless culture (floating system)

Due to the problems of soil-based agriculture, new cultivation techniques have been developed, which are based on a soilless agriculture, in which the use of the soil or a substrate is raised to a minimum. The plants can be cultivated in a nutrient solution or any other substrate with a supply of nutrient solution (hydroponic cultivation system). According to Carrasco and Izquierdo (2005), hydroponics is defined as the cultivation technique that allows a plant to develop outside the soil throughout its cycle.

Soilless production systems are widespread in South-East Spain (Murcia and Almeria) since it has the best climatic conditions (temperatures and solar radiation) in Europe for optimum vegetable development. At present, there are more than 5,000 hectares dedicated to the soilless cultivation of vegetables.

Among the soilless systems, the floating tray system is a variant of the Hydroponic Deep Water Cultivation Systems that allows a quick and clean harvest (Tomasi et al., 2015). This consists of polyethylene trays that float in permanent contact with the nutrient solution (Cros et al., 2003), where the plants grow with their roots submerged in it (Figure 1.6). It is a system that makes efficient use of water and space within a greenhouse (Galloway et al., 2000). The floating tray system allows the mechanization and robotization of production (Benoit and Ceustermans, 1994), with greater efficiency. According to Santamaria et al. (1997), it is a system that allows a high density of plants and therefore a high yield. One of the main advantages is that it can positively influence the nutritional status of the plants, making an efficient use of fertilizers. In addition, it is possible to establish a control of parameters such as nitrates and oxalates that accumulate in some small leafy species.

Figure 1.6: Floating system in baby leaf lettuce crop.

With a soilless culture, it is possible to obtain a greater number of harvests per year than if the plants are cultivated in the soil (Urrestarazu, 2004). This depends on the species and the time of year. In winter, the cultivation cycles in floating system are longer than in summer, obtaining at that time of year the production of purslane in 13-15 days (Kaskar et al., 2008; Lara et al., 2011) and baby leaf lettuce in 22 days (Giménez et al., 2019a). Therefore, it is an adequate system for shortening cultivation cycles using high planting density, as is the case of baby leaf vegetables.

The main elements of the floating tray system are polystyrene trays (styrofloat) formed by low-volume truncated cone cracks with little amount of substrate, which guarantees germination and growth (Figure 1.7). In addition, the beds containing the water with the nutritive solution must be deep enough to place an aeration system, which produces oxygen to the roots. The concentrations of the nutrients in the nutritive solution vary according to the nutritional needs of each species. According to Favela et al. (2006) the pH, temperature, electrical conductivity, ion competition and oxygen solution are the fundamental parameters to consider in the nutrient solution.

Figure 1.7: The main elements of the floating tray system

Regarding substrates, they are used to act as a support for the plant, since they have a limited physical volume. The substrates used keep this way an adequate relationship between air and nutritive solution to provide the root with the necessary oxygen and nutrients. They must have favorable properties for the growth of the plants such as greater porosity, easy to drain and allow the roots accessibility to the nutritive solution. The most used substrate in this system is peat. Cros et al. (2007) demonstrated that a peat-based floating cultivation system can be considered the most suitable growing medium to grow purslane because of its ideal physical and chemical characteristics. Nicola et al. (2015) also recommended a peat-based horticultural medium for baby leaf vegetables grown in floating system. However, in recent years, there have been increasing environmental and ecological concerns about the use of peat as a growing medium, because its collection is jeopardizing endangered worldwide wetland ecosystems (Zaller, 2007). Likewise, peat utilization contradicts the basic principles of the organic agriculture method as defined in Regulation 834/2007 (EC 2007). For this reason, the Expert Group Report for Technical advice on Organic Production (EGTOP) proposed that the amount of peat to be used should be a maximum of 80% by volume of culture media.

Increasing demand and rising costs for peat as a growing media in horticulture have led to a search for high quality, low-cost substrates and readily available to replace peat moss. The rate of peat consumption compared to other alternative materials has started to decrease, but not as growing media in nurseries where it shows no sign of losing dominance (Altmann, 2008). One of these types of substrates are compost and its biologics extract, which can improve soilless growing media (Limpens et al., 2011; Morales et al., 2016). In addition, they provide beneficial effects (biofertilizers, biostimulants and biopesticides) by reducing the use of chemical fertilizers and pesticides (Colla et al., 2015).

On the other hand, one of the inconvenient of soilless, like floating system is the plants can be affected by diseases that affect the growth and quality of the crop. Even if they are less exposed than a soiled crop, proliferation is much easier due to the typology of the system. The most frequent diseases are those caused by oomycetes and fungi such as *Pythium* spp., *Peronospora farinosa* f. sp. betae, *Rhizoctonia* sp., *Alternaria* spp., *Cercospora* sp., *Botrytis* sp., both in the root and the aerial part.

1.1.5. Preharvest handled affecting post-harvest characteristics of baby leaf vegetables

Post-harvest quality depends on previous handling, nutrition management and the technology used, among other factors, to maintain the properties of the plant as fresh cut. According to Crisosto and Mitchelle (2002), there are two types of factors that affect pre-harvest: factors that do not depend on plant material, which are those related to the environment (temperature, humidity, radiation) and cultivation practices (mineral nutrition, irrigation, attack by microorganisms), and factors that depend on plant material (species, variety, state of maturity). Both dependent and not on plant material are really correlated themselves being difficult to separate one from each other (Burns et al., 2012). In summary, the quality of baby leaf vegetables will be influenced by the net balance of energy, water and nutrient flows to the interior and exterior of the plant. This balance will be modified by pre-harvest factors such as environmental, including soil characteristics or soilless used for cropping. These factors are difficult to control in the open-field, but not in the greenhouse.

One of these preharvest factors is the contribution of organic amendments to the soil and the use of organic substrates in soilless cultivation. In this context, compost made from agro-food industry residues are highlighted for its high benefits by improving soil quality and thus improving crop quality, increasing crop yields (Ros et al., 2007). Likewise, they can be also used as soilless culture substrates alternative to non-renewable substrates such as peat. Also they could add some values such as suppression of pathogens (Hadar and Papadopoulou, 2012) and biofertilizing and biostimulating effects (Colla et al., 2015). It has been also demonstrate their capacity to increase antioxidant compounds and vitamin compounds in the produced crops, which are beneficial to human health (Santos et al., 2016).

After the processes carried out in pre-harvest, the time comes for harvesting, in which must be considered the use of a harvesting system that does less damage to the product, the selection of the cultivar and the optimal state of maturity. Vegetables minimally processed for preparation as pre-prepared products must undergo a series of post-harvest operations (transport, precooling and storage, selection and classification, washing and disinfection, drying, weight and packaging, quality control and cold storage and cold transportation and distribution).

1.2. Compost and compost tea in the production and quality of horticultural crops

1.2.1. Importance of organic matter

In order to increase yields and feed more people in today's world, modern agriculture has provided the means for faster production that had not been possible before. Conventional agriculture has been accelerated by using chemical fertilizers to increase crop yields and chemical pesticides to reduce the growing number of pests and diseases, while reducing the input of organic matter into the soil. This led to the deterioration of the environment surrounding the crop being less stable. One of the wrong considerations is that soil is only the physical substrate for maintaining plant roots, forgetting important soil functions such as maintaining the balance between inputs and outputs. Traditional agriculture kept this under the current umbrella of the circular economy, one of the pillars being the contributions of organic matter to the soil as organic amendments. These traditional inputs of organic matter came from animal husbandry such as manure.

As has been established so far, soilless culture does not take into account the substrate that supports the plant (peat, coconut fiber, vermiculite, perlite, etc.), and there is a special attention on their capacity to be as much inert as possible, since it is believed that plant nutrition is managed by supplying the chemical nutrients in the appropriate dose. However, there are sectors that indicate, that crop production is related to the complex relationships that are maintained in the rhizosphere where not only the nutrients themselves play, but also the microorganisms that are related to organic matter. The microorganisms play important functions such as biostimulation, biopesticide effect, or biofertilization, favoring the absorption of nutrients from the plants. Their establishment in the rhizosphere depends on the environment that surrounds them and their interactions, and can be provided by organic matter.

1.2.2. Compost as soil organic amendments and peat alternative

1.2.2.1. Composting process of organic residues

Compost is the product obtained after a composting process defined as the aerobic decomposition and biological stability of organic wastes, under conditions that allow the development of thermophilic temperatures as a result of the heat produced biologically, to produce a stable product, free of pathogens and weed seeds and that can be used in a beneficial way for the soil and plant growth (Costa et al., 1991). The degradation process of composting has been known and used for centuries. Its original objective was to obtain a fertilizer product by transforming organic waste under controlled conditions.

The composting process degrades the heterogeneous organic matter in solid state by bio-oxidation, like that naturally occurs in the soil (Ahmed and Varshney 2011; Deepesh et al. 2016). Organic matter is decomposed by the action of microorganisms, which passes through a thermophilic and temporary phase, releasing phytotoxins, and generating the biodegradation of CO_2 and H_2O minerals and stabilized matter.

The main factors that control the process of composting organic waste are temperature, moisture content, pH, aeration, and those related to the nature of the substrate such as the C/N ratio, particle size, nitrogen content (Kumar et al., 2010), C/P ratio, nutrient and organic matter content and electrical conductivity (Bueno Márquez et al., 2008). The values of these parameters and their optimal ranges are influenced by the environmental conditions, the type of waste and the composting system, which must be controlled during the whole process (Table 1.3).

Table 1.3: Reasonable and optimal intervals during the composting process (Fermor, 1993; Rynk et al., 1992).

Parameter	Reasonable interval	Optimal interval
C/N	20:1-40:1	25:1-30:1
Moist content	40-65 %	50-60 %
Oxygen concentration	< 5 %	<< 5 %
pH	5.5-9	6.5-8
Temperature	45-66 °C	55-60

The composting process is divided into two phases: (*i*) the bio-oxidative phase, in which there is a high availability of nutrients and a high growth of microorganisms; and (*ii*) the maturation phase, in which nutrients are limited and there is less microbial activity (Figure 1.8). Each one of these phases is carried out by means of different transformations, depending on the stage in which the composting is found, being the fluctuation of the temperature during the process the one that marks the different stages and causes the succession in the time of different groups of microorganisms.

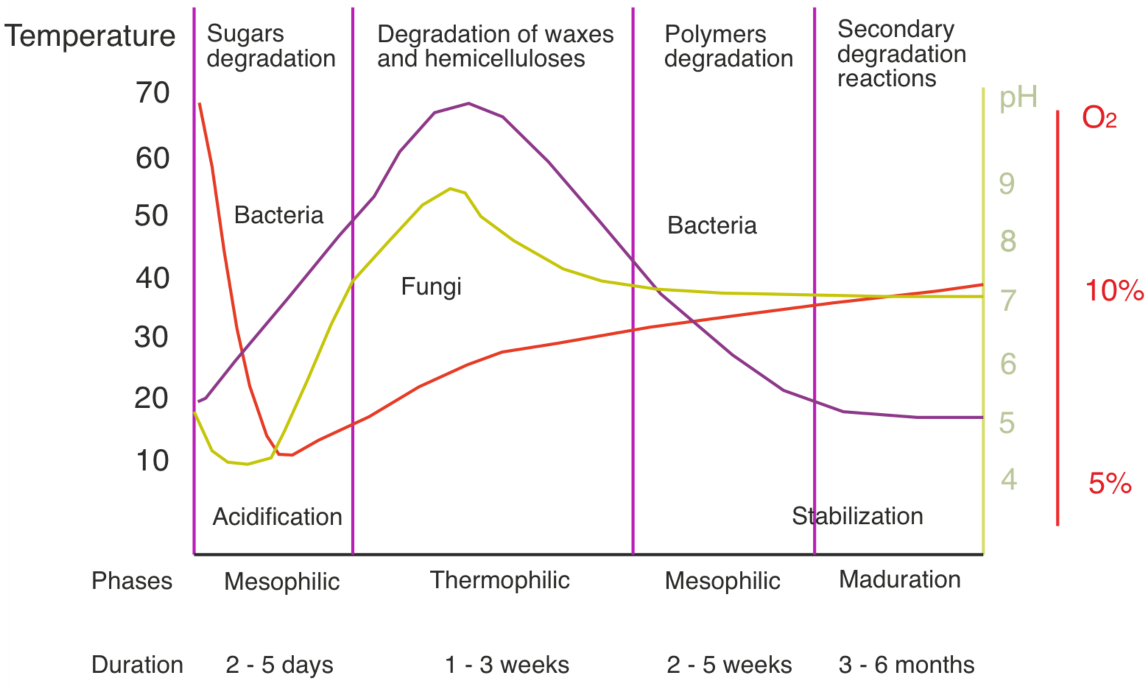

Figure 1.8: Phases of the composting process, environmental succession and associated microbial populations (Source: P. Roman).

The composting process can be carried out using different systems that are classified according to their level of complexity, degree of process control, method of ventilation used and whether they are open or closed. Composting systems are grouped into two groups: open systems and closed systems. Open systems consist in allocate material into piles, which can be mobile or static. In closed systems, the material will not be in contact with the outside, therefore, there will be a greater control of the process conditions. It is carried out in bioreactor systems (Kumar et al., 2010) where the whole composting process is carried out in a shorter time.

The nature of the starting materials significantly determines the composition and fertilizing capacity of the compost. In order to obtain an adequate compost, the first thing to do is to choose the materials to be composted, considering their characteristics (Zhang and He, 2006). Also, the availability of by-products is subject to the seasonality and availability of them. Therefore, it is important to characterize the available by products to evaluate their possible agricultural application and study their possible use as a substrate (Abad et al., 2001).

In general, in Europe, compost is used primarily to feed soil as organic amendment, and only 15% of the compost produced is used as a base material for the formulation of commercial substrates for cultivation in containers (Rynk and Richard, 2001; Raviv, 2009).

1.2.2.2. Agro-food industry wastes as candidates to be used as feedstock or raw material to obtain high quality compost

The largest source of organic waste generated in Spain comes from primary sector activities, mainly livestock and agriculture, representing 61% of the total mass generated, followed by waste from the food and agriculture industry (del Val, 2011). The production of fruit and vegetables in Europe in 2017 was 105 million tons, with Spain being the largest producer at European level with 25% of production (EUROSTAT, 2017). In Spain, the agro-food industry is a very important economic sector, being the main industrial branch in our country. Within the agro-food industry, there is the vegetable processing industry, as manufacture of canned fruits and vegetables and juice production and frozen vegetable. The Region of Murcia produced more than 3 million t of fruit and vegetables in 2019 (MAPA, 2019), the highest production being broccoli, lettuce, artichoke, melon, watermelon, tomato and pepper crops. According to Saval (2012), the agroindustrial wastes are materials in solid or liquid state that are produced from the consumption of primary products, being susceptible to use, transforming it to generate a product with a commercial and/or social interest. The waste obtained in the agro-food industry is used as a starting material to produce compost (Ros et al., 2012; Arvanitoyannis and Varzakas, 2009).

On the other hand, there are other residues (woody agricultural bio-waste) that come mainly from the pruning of olive trees, vineyards and fruit trees. These organic residues can represent an important source of nutrients and microorganisms for plants, and therefore improve crop production (Hernández et al., 2014). The use of pruning remains presents a series of problems derived from the infrastructures and machinery necessary for the process to be economically viable.

1.2.2.3. The compost extracts (CEs) or compost tea (CTs) used in horticulture

They are obtained through a liquid extraction of a mature compost, conformed by organic, inorganic molecules and microorganisms that are water soluble (Ingham, 1999; Arancon et al., 2007). They can be used as organic amendments in baby leaf crops, in both soil and soilless agriculture management, resulting their beneficial properties for the plants (Arancon et al., 2007; Pant et al., 2012). According to Zaccardelli et al. (2012), the nutritional elements present in compost tea (CT), as well as humic substances and hormone molecules secreted by microbes, can act positively on the biostimulation of plants by improving their physiological state. It is therefore an innovative organic product in crop management (Deepthi and Reddy, 2013). The effectiveness of compost teas (CTs) may vary depending on the type of compost, the handling of the compost and the procedures used for its preparation (Pant et al., 2012).

The composting system of a CT can be aerobic (aerated tea) or anaerobic (non-aerated tea) (Brinton et al., 2004). Aerated compost tea consists of introducing a previously selected, mature compost into a permeable container (bag or net) in a container with water (Figure 1.9). The water is bubbled by means of pressurized air, considering the extraction ratio (Kg compost/ L H_2O) being the most common 1:10-1:20 with room temperature between 20-25 °C (Tortosa, 2017).

Figure 1.9: System for making compost tea.

1.2.3. Added value of compost and its biological extract for controlling plant diseases. Biofertilization and biostimulation effect

An important aspect to consider is the use of compost and compost tea (CT) as substrates and extracts with suppressive character for the biological control of some of the main diseases affecting horticultural crops as *Phytium* spp., *Rhizoctonia solani*, *Phytophthora* spp. and *Fusarium* spp. (Hadar and Papadopoulou, 2012; Bonanomi et al., 2010; Litterick et al., 2004; Dianez et al., 2006), thus helping to reduce the use of chemical pesticides. The natural suppression of compost was suggested by Hoitink et al. (1975), revised and further developed by different authors (Hoitink and Boehm, 1999; Noble and Coventry, 2005; Avilés et al., 2011; Hadar and Papadopoulou, 2012), thus demonstrating the reduction of plant diseases.

For a compost to be suppressive it will depend on certain characteristics such as the activities of the antagonistic microorganisms, the host plant, the pathogenic species involved and the characteristics of the fertilizer (Fuch, 2002; Bonanomi et al., 2006). Other aspects to consider are particle size, nitrogen content, cellulose and lignin content, electrical conductivity, pH, inhibitors released by the fertilizer, and the microbiota of the fertilizer can also affect the incidence of plant pathogens in the soil (Hoitink and Boehm, 1999).

Compost maturity is also another important factor to attend depending on the pathogen to be controlled (Bonanomi et al., 2010; Hadar and Papadoulou, 2012). For example, young compost is more effective in suppressing *Pythium*, while mature compost is recommended against *Rhizoctonia* (Harman et al., 2004; Bernal-Vicente et al., 2008, 2012). However, extremely stable composts do not support microbiological activity, so the potential for biological suppression potential is lost (Widmer et al., 1998). Next to the production processes, storage of compost also affects the activity of a compost (van Rijn et al., 2007).

The suppressive effect in composts has been classified as either general or specific. General suppression is induced by a large metabolically active microbial community, while specific suppression is attributed to specific microbial agents that proliferate in the presence of compost, and affect pathogen growth or infection through a particular biological control mechanism (i.e. competition, antibiosis, parasitism, induced plant resistance or a combination of these mechanisms) (Hadar and Papadopoulou, 2012).

A more recent approach to compost suppressiveness has been the enrichment of compost with specific strains of biocontrol agents (Hadar and Papadopoulou, 2012). Good results have been achieved with the introduction of species of genera *Acremonium*, *Chaetomium*, *Gliocadium*, *Trichoderma* and *Zygorrhynun* spp. (Hadar and Papadopoulou, 2012). It is also important taking into account the inoculation time (after the heat peak of the composting process) and obtaining a critical concentration of the biocontrol agent (Bernal-Vicente et al., 2012). The use of certain *Trichoderma* spp. has been proposed because of their ability to rapidly colonize the rhizosphere, control pathogenic and competitive microbiota, improving plant health and root growth (López-Mondejar et al., 2010; Bernal-Vicente et al., 2012).

Some microbial species promote plant growth through nitrogen fixation, phosphate solubilization, production of phytohormones like auxin and cytokinin, and production of volatile growth stimulants such as ethylene and 2,3-butanediol (Ryu et al., 2003; Vessey, 2003; Castro et al., 2009). The nitrogen fixation by microorganisms such as *Azoarcus* sp., *Beijerinckia* sp., *Klebsiella pneumoniae*, *Pantoea agglomerans*, and *Rhizobium* sp. which a fix atmospheric N_2 (Riggs et al., 2001; Vessey, 2003) are a clear example of biofertilization.

One of the most common disease, *Pythium* spp. causes damping-off and withering in several horticultural species, producing significant production losses (Limpens et al., 2008). *Pythium* spp. affects seedling roots and the base of the stem, slowing their growth and eventually producing irreversible withering. However, several studies have shown that the contribution of compost and its extracts in both soil and soilless crops (floating system) reduced the effects of this pathogen, in pea plants (*Pisum sativum* L.) and baby leaf lettuce (*Lactuca sativa* L.), respectively (Pascual et al., 2002; Giménez et al., 2019ab).

1.2.3.1. Soil fertility and compost

The natural fertility of a soil is largely determined by the presence of organic matter, which is not stable but constantly renewed. Soil organic matter is the sum of organic waste, of plant and animal origin, more or less decomposed and in evolution (Moreno et al., 1997). Organic matter has a positive influence on soil fertility, increasing the effectiveness of mineral fertilizers and increasing crop yields. This has beneficial effects on the chemical, physical and biological characteristics of the soil (Liu et al., 2006).

The sources of organic matter are very varied, with the highest percentage of contribution (> 95%) coming from livestock waste such as manure, slurry and compost. The improper use of manure can produce negative effects on the environment, harming the water, air and soil (Pinos et al., 2012). Therefore, the use of manure as a fertilizer must comply with the limitations required by current regulations, minimizing the possible environmental risks, due to the excess of nutrients, such as nitrogen and phosphorus, which in surface water causes eutrophication, and the former, which in the form of nitrates, affects the quality of groundwater. Currently, the use of these organic fertilizers is being reduced in Spain, and it is researching on new an alternative to these manures that are added to the soils.

Intensive agriculture has profound effects on the environment (Tilman et al., 2002), which is endangering the future of human feed. The negative effects are extensive due to the use of mineral fertilizers and pesticides, producing a negative effect on soils, eliminating beneficial insects, generating resistance and contaminating groundwater. According to Kanter (2018), the excessive application of chemical fertilizers or the inefficient use of the main nutrients (N and P) in fertilizers, contribute to the associated problems in agriculture, as in the productivity and quality of fruit and vegetables (Swietlik, 1992).

The accumulation of contaminants in agricultural soils can be problematic. There is a need to reduce levels of contaminants by applying measures that reduce or prevent damage, seeking alternatives to the contribution of organic fertilization in soils. Therefore, due to the increase in agricultural activity, new sources of organic matter are being sought. In this sense, one type of waste that presents attractive management opportunities is the organic waste generated by agricultural activities and the agro-food industry, which increases production yields, both by providing nutrients since its use increases the content of humic substances (Urbano, 2002) and by improving the physical, chemical and biological fertility of soils (Hernandez et al., 2016). The use of these wastes as a contribution of organic matter to the soil, in the form of compost, could mitigate the animal manure scarcity and it could also provide a sustainable outlet for the great amount of this type of organic waste, as well as producing a number of environmental benefits and impacts on the soil (Table 1.4). In addition, its application as a soil amendment decreases the use of inorganic fertilizers and chemical pesticides (Colla et al., 2015). The application of this type of organic matter to the soil must be carried out in an adequate manner, to improve the properties of soil and plants, improving their quality and having a bio-pesticidal value to combat microorganisms that may exist in the soil (Bello et al., 2013). The use of compost provides the soil with beneficial effects (biofertilizer, biostimulant and biopesticide), favouring a more sustainable agriculture.

Table 1.4: Benefits of the use of compost on the physical, chemical and biological properties of the soil.

Compost Benefits	
Physical properties	• Provide good soil structure, bind clusters of soil particles, the aggregates. • In sandy soil retain water and nutrients. • In clay or silt soil, loosens tightly bound particles so roots can spread, water drain, and air penetrate. • Alters soil structure, for less erosion. • Increases soil's ability to retain water and decreases runoff.
Chemical properties	• Contains macro and micronutrients. • Releases nutrients slowly—over months or years. • An enriched soil retains fertilizers better. • Its input buffers the soil, neutralizing acid and alkaline soils, bringing pH levels to the optimum range for nutrient availability to plants. • Can reduce or eliminate use of synthetic fertilizers. • Can reduce chemical pesticides since it contains beneficial microorganisms that may protect plants from diseases and pests.
Biological properties	• Feeds diverse life in the soil, where the bacteria, fungi, insects and worms helper support healthy plant growth. • The organisms containing keep soil well aerated. • Their used may suppress diseases and pests. • Encourages healthy root systems.

(Source: Washington State University)

1.2.3.2. Soilless horticulture and compost

Peat has been the most common constituent of growing medium (Bunt, 1988) because it increases the water-holding capacity, has a good cation exchange capacity, does not contain phytotoxic substances and has a low bulk density. This is an issue that must be addressed, since almost 80% of growing media used in Europe is constituted of peat materials (Apodaca, 2016; Gruda et al., 2019). The amount of blond peat consumed annually has been estimated as approximately 30 million m^3, half of it is used for producing growing media for commercial horticulture (Altmann, 2008). The use of peat and the expanding growing media industry in the European Union is estimated to be worth €13,000 million and generates approximately 11,000 jobs (EPAGMA, 2012).

There is a wide variety of peat on the market with different botanical composition, conditions of formation and degree of decomposition, particle size and degree of fertilization (Urrestarazu et al., 2004). Within the variety, the most used peats in agriculture are blond and black peats (Table 1.5).

Table 1.5: Properties blond and black peat.

Properties	Blonde Peat	Black Peat
Bulk density (g/cm^3)	0.06-0.1	0.3-0.5
Density (g/cm^3)	1.35	1.65-1.85
Porosity (%)	≥ 94	80-84
Gravimetric water content (g/100 g dry matter)	1.049	287
Air (% vol)	29	7,6
Accessible water reserve (% vol)	33.5	24
Water reserve (% vol)	6.5	4.7
Non-accessible water (% vol)	25.3	47.7
Cation exchange capacity (meq/100g organic matter)	110-130	≥ 250

(Source: Fernández et al. (1998))

Likewise, the inclusion of compost in soilless culture media can improve the physical and chemical properties of the growing media and increases the availability of macro-, micronutrients as well as plant growth regulators (Abdallah et al., 2000; Ozores-Hampton et al., 2001). Klock and Fitzpatrick (1997) pointed out that composts may be used alone as growing media where these criteria are met: porosity in the range 50-80%, water holding capacity between 25 and 60%, bulk density from 0.30-0.75 g cm-3, initial pH 5.5-6.5, initial soluble salts concentration from 0.33-0.51 dS m-1, and a C/N ratio in the range of 15-20. The main constraints in the use of compost in growing media formulation are the high electrical conductivity, the slightly alkaline pH (Verdonck et al., 1983) and the low water-holding capacity (Abad et al., 2001). Soluble salt levels in compost depend on feedstock and processing. It has been proved that composts with lower salt levels supported growth better than those with higher levels (Garcia-Gomez, 2002). Compost for use as a constituent of growing media must be stable, with relatively low salinity, low concentration of phytotoxic ions and molecules and free of phytopathogenic organisms (Raviv et al., 2002).

The combination of peat and compost in growing media is synergistic. Peat often enhances aeration and water retention, while compost or other additives improve the fertilizing capacity of a substrate (Jayasinghe, 2012). In addition, specific by-products and composts tend to have porosity and aeration properties comparable to those of bark or peat and, as such, are ideal substitutes in propagating media (Chong, 2005). The greatest plant growth responses and largest yields have usually occurred when composts constituted only a relatively low proportion (25–50%) of the volume of the nursery container medium mixture (Pinamonti and Sicher, 1997; Atiyeh et al., 2001; Garcia-Gomez et al., 2002; Papafotiou et al., 2004; Perez-Murcia et al., 2006). Likewise, compost is used as a cultivation substrate in baby leaf cultivation systems without soil, such as floating systems, and can be used as substitutes for peat (Lopez-Mondejar et al., 2010; Blaya et al., 2015, Giménez et al., 2019a), providing interesting results to go ahead.

1.2.4. Regulations to use compost in agriculture

Compost can be used both as organic soil amendments and as horticultural substrates for plant cultivation. Depending on the use that is given to the compost, this must be regulated, if it is as an organic amendment must take into account the soil and thus avoid possible harmful effects on water, flora, fauna and humans. In the case of being used as a growing medium to replace peat, rules should be established to regulate its composition and not to create health risks.

There are some regulations at EU level, national and regional level. In the RD 506/2013, of 28 June, on fertilizer products, certain parameters and heavy metals are legislated, classifying them by categories (A, B and C) according to the content of heavy metals. It also indicates the raw materials that can produce organic fertilizers and organic amendments (Table 1.6).

Table 1.6: Minimum requirements for a product to be considered as compost according to RD 506/2013.

Parameter		RD 506/2013	
Total organic matter (%)		35	
Maximum humidity		40	
C/N		< 20	
Maximum inorganic (% N total)		15	
Heavy metals (mg/kg d.w.)	Class A	Class B	Class C
Cd	0.7	2	3
Cr (total)	70	250	300
Cr (VI)	Nd	Nd	Nd
Cu	70	300	400
Hg	0.4	1.5	2.5
Ni	25	90	100
Pb	45	150	200
Zn	200	500	1000
Organic contaminants			
Polyphenols (%, p/p)		0.8	
Furfural (%, p/p)		0.05	
Microorganisms			
Salmonella spp.		Absent in 25 g compost	
Escherichia coli		< 1000 MPN/g	
Weed seeds			
Particles (%)		90 (25mm)	
Impurities (%)		Cannot contain	
Gravel and stones (%)		Cannot contain	

nd: not detectable according to official method; MPN: most probable number.

Another of the uses of compost, as cultivation substrates, is regulated in Spain by Real Decreto 865/2010, modified by RD 1039/2012, which guarantees that these substrates are agronomic ally effective for use in agriculture, gardening and landscaping and are not harmful to water, soil, flora, fauna or human beings (Table 1.7).

Table 1.7: Characteristics of compost as a growing medium or growing medium component, according to RD 865/2010.

Designation type of product	Description	Specifications	Mandatory declarations	Optional declarations
Compost	Sanitized and sterilized product obtained by aerobic biological decomposition (including thermophilic phase) of biodegradable organic materials of Annex V under controlled conditions	Organic matter on dry matter > 20% (m/m)	-Main components more than 10% (v/v) ordered in decreasing order of percentage - Organic matter on dry matter -Electrical conductivity, CE. -pH -Quantity in volume	-Dry bulk density -Air volume -Water volume 1, 5 and 10 KPa -Dry matter -Total pore space -Granulometry

1.2.4.1. Circular Economy Strategy 2030

The intensive use of natural resources worldwide is generating high pressure on the environment. Until today, high volumes of waste, soil and water pollution. Therefore, an ecological deterioration is taking place at a global level. In Spain in particular, a Circular Economy Strategy 2030 has been drawn up, approved on 2nd June 2020, which aims to develop a new model of production and consumption, in which waste generation is reduced to a minimum and those that cannot be avoided are taken advantage. One of the objectives of the Strategy is to reduce the generation of food waste in all food chains: 50% reduction per capita at household level and retail consumption and 20% in production and supply chains from this year, thus contributing to the Sustainable Development Goals (SDA). Within the 20% reduction in production chains are organic waste from the agro-food industries, which cannot be avoided and must be given an outlet as sustainable as possible.

In the case of these organic agricultural and agro-food industry wastes, there is a lack of environmental awareness in their management, aggravated by the fact that there is a lack of technological capacity and economic resources necessary to give them an appropriate final destination. For this reason, there is a law that proposes the development of waste management plans and programs at the national, regional and local levels (Law 22/2011). At the national level, there is the Integrated National Waste Plan 2008-2015 (PNIR), where selective collection aims to increase the amount of organic fraction to be used in composting. At the level of the Region of Murcia there is the Waste Plan of the Region of Murcia 2016-2020. These organic wastes from the agro-food industries, through composting processes, obtain organic amendments that improve soil fertility, crop quality and reduce the use of chemical products. Therefore, it contributes to a basic principle of the Circular Economy Strategy, which is to reuse and reduce the waste originated.

Chapter 2

Objectives

This PhD Thesis studies the use of diverse compost, obtained from agro-industry and primary agriculture wastes origin, and its biological extracts to grow baby leaf lettuce and spinach in a sustainable way both in soil in open field conditions and in floating system in protected cultivation. The main objective is to show whether compost can be used as an alternative to organic amendments in soil cultivation and as an alternative to peat as substrate in the floating system. In addition, if the composts and their biological extracts can be considered as value-added agricultural products, limiting plant pathogen affection and improving healthy compound accumulation in baby leaf lettuce and spinach

The general objective can be achieved through the following specific objectives:

1. To study whether the use of compost as an alternative to pea in floating system affects the yield and nutritional quality of fresh-cut red baby leaf lettuce and reduces the incidence of *Pythium irregulare* on the crop.
2. To study if a directly brewed compost extract added in the nutrient solution or by microsprinkler influences on the yield and quality of baby leaf red lettuce growing in floating system and on the incidence of *Pythium irregulare*.
3. To assess whether the compost extracts supplemented with a beneficial microorganism affects baby spinach yield in open field conditions, as well as its

nutritional quality, and whether any residual affects the quality of the soil at the end of the cultivation cycle, studying soil microbial activity, nutrient content and soil-borne fungal pathogens.

Chapter 3

An agroindustrial compost as alternative to peat for production of baby leaf red lettuce in a floating system

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Abstract

Peat is the usual substrate used to fill the holes in the trays used for growing baby leaf vegetables in floating systems. Nevertheless, peat increases susceptibility to some diseases, such as damping off, which is caused by fungi or oomycetes like *Pythium* spp., which can lead to significant production losses. Therefore, alternatives to peat are being developed using organic waste from agroindustry, the so-called agroindustrial compost. This type of compost can have a suppressive effect to control these pathogens and also have a biofertilizing effect. In this study it was analyzed if an agroindustrial compost could be used as a substitute for peat in a floating system for the cultivation of red lettuce. For this purpose, two different experiments were carried out in two cultivation cycles (summer and autumn) in the same greenhouse and at the same time. The first experiment compared the two organic substrates (peat and fertilizer) and the second involved a suppression bioassay using both substrates inoculated with *Pythium irregulare*. The agroindustrial compost was composed of tomato (71%), onion (17%) and vineyard residues (12%) expressed in dry weight, and the peat used was a commercial blond peat (Pindstrup Blond Gold). For the suppression bioassay, the substrates (compost and peat) were inoculated with *P. irregulare* at 1 10^5 cfu g^{-1} of organic substrate, adding 0.3 mL g^{-1} of the pathogen solution. In the first experiment, the results showed that the compost increased the percentage of seed germination and the quality of the red baby leaf lettuce by reducing the accumulation of nitrates and increasing the antioxidant capacity and the vitamin C content of the leaves compared to peat. In addition, in the second experiment, under pressure from *P. irregulare* the plants grown in the compost showed a lower incidence of disease (a higher percentage of plant survival) and produced higher yields. These results suggest that the agroindustrial compost used in this study is promising as an alternative to peat for use as an organic substrate in a sustainable soilless production system for baby leaf red lettuce, since it is not only able to control the effect of *P. irregulare*, but also to improve the yield and quality of the product.

An agroindustrial compost as alternative to peat for production of baby leaf red lettuce in a floating system

Abstract

Peat alternatives are being developed using materials such as agroindustry compost. The aim of this work was to study whether an agroindustrial compost could be used as substitute for peat in a floating system for cultivating baby leaf red lettuce. For this, two different experiments were carried out over two growing cycles (summer and autumn) in the same greenhouse at the same time. The first experiment compared the two organic substrates (peat and compost) and the second involved a suppressive bioassay using both substrates inoculated with *Pythium irregulare*. The results showed that compost increased the percentage of seed germination and the quality of baby leaf red lettuce by reducing nitrate accumulation and increasing the antioxidant capacity and vitamin C content of leaves compared with peat. Furthermore, under *P. irregulare* pressure plants grown in compost showed less incidence of the disease (a higher percentage of plant survival) and produced higher yields. It is concluded that the agroindustrial compost used in this study shows promise as an alternative to peat for use as organic substrate in a sustainable soilless production system for baby leaf red lettuce, since it is not only able to control the effect of *P. irregulare*, but also improve the yield and quality of the product.

Keywords: *Lactuca sativa* L., hydroponic, suppressiveness, *Phytium irregulare*, nitrate

3.1. Introduction

In recent years, the use of soilless cultivation systems has grown in importance in vegetable production. Among the reasons, floating systems allow clean and safe leafy vegetables to be obtained for the processing industry and reduce crop cycle duration with respect to soil culture (Cros et al., 2007).

Floating systems enable efficient fertilizer use, the rapid correction of nutritional deficiencies and the control of important anti-nutritional compounds, such as nitrates, which tend to accumulate in some species (Santamaria, 2006). A floating system is particularly appropriate for baby leaf vegetable production like lettuce, which is primarily consumed fresh or in salad mixes. Among lettuces types, baby leaf red lettuce has gradually gained in popularity, due to its attractive colour and its high content of phytochemicals with healthy effects (Mampholo et al., 2016).

Peat is usually used as a substrate for growing baby leaf vegetables in floating systems (Pane et al., 2011). However, its use raises environmental concerns and increases the susceptibility to some diseases, such as damping off, which is caused by fungi or oomycetes such as *Pythium* spp., which can lead to significant production losses (Limpens et al., 2008).

Composts from the fruit and vegetable processing industry can be used as organic substrate or organic amendment to improve crop production and quality (Benitez et al., 2003; Hernández et al., 2014; Blaya et al., 2015); and their use is regarded as an environmental friendly practice in the circular economy. One of the particular properties of some composts is their suppressive ability against pathogens (Bonanomi et al., 2010), which has been attributed to abiotic and/or biotic factors although it is considered that the latter are mainly responsible (Noble and Coventry, 2005). The mechanisms involved in this activity are based on competition, antibiosis, hyperparasitism, and the induction of systemic resistance in host plants (Hoitink et al., 1996; Zhang et al., 1996). In addition, abiotic properties such as nitrogen content, pH, C/N ratio, heat, moisture, raw materials or degree of compost maturity have been suggested as being associated with suppression activity (Hoitink and Grebus, 1997).

Our hypothesis was that agroindustrial compost from organic residues could be an appropriate as substitute of peat in a floating cultivation system, increasing crop and yield quality and reducing the incidence of *Pytium irregulare* in baby leaf red lettuce. For this, two baby leaf red lettuce growing cycles in a floating system were investigated. In addition, the effect on the quality of fresh-cut red lettuce was also studied.

3.2. Material and methods

3.2.1. Plant material and growing conditions

The experiments were conducted at the "Tomás Ferro" Experimental Agro-Food Station of Technical University of Cartagena (UPCT; lat. 37 41´N; long. 0 57´W). A cultivar of baby leaf red lettuce (*Lactuca sativa* L.), ´Antoria´ (Rijk Zwaan, De Lier, Netherlands), was cultivated in floating system in an unheated greenhouse covered with thermal polyethylene. Two crop cycles were carried out, with manual sowings on 5 July 2016 (summer) and 23 November 2016 (autumn).

Seeds were sown in styrofloat trays filled with organic substrates. These trays had pyramidal-trunk 172-mm long -cells 20 mm apart and grouped in three for a total of 42 cells per tray; cells measure 10 mm on the top and 2.5 mm on the bottom, leading to a volume of 32.4 cm^3 per cell. After sowing, the trays were placed in a climatic chamber at 18 °C and 90% relative humidity and left in the dark for 48 hours to improve germination. After seedling emergence, the trays were transferred to flotation beds and maintained floating on 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 nutrient solution was applied to the water (Egea-Gilabert et al., 2009). The nutrient solution was adjusted to EC 2.5 dS m^{-1} and the pH to 5.8. The EC and temperature of the nutrient solution were monitored throughout the growing cycles using Campbell CS547 sensors (Campbell Scientific Inc., Logan, UT) and the oxygen concentrations were monitored using Campbell CS512 sensors located in each flotation bed, reaching a mean value of 7.5 + 0.5 and 8.1 + 0.6 mg L^{-1} in summer and autumn cycles, respectively.

In the greenhouse, the temperature and light conditions during the experiments were 18.81 °C, 42.81 °C, 28.43 °C minimum, maximum and average air temperature, respectively, and an average daily light integral (DLI) of 18.96 mol $m^{-2}d^{-1}$ in the summer cycle; 5.67 °C, 37.74 °C, 16.14 °C minimum, maximum and average air temperature, respectively, and an average daily light integral (DLI) of 10.22 mol $m^{-2}d^{-1}$ in the autumn cycle.

Harvesting was carried out at the same phenological stage during both cycles, when the plants had four to five leaves. The plants were harvested at 22 and 35 days after sowing in summer and in autumn, respectively. Thirty-two plants from four cells randomly chosen from each tray were harvested for each treatment and stored at -80 ºC for later analysis. Substrate samples were collected and stored at -20 ºC for pathogen concentration measurement.

Two different experiments were carried out in the two growing cycles in the same greenhouse at the same time. The first dealt was a comparison of two organic substrates (peat and compost) and the second was a suppressive bioassay against *Pythium irregulare*, both in floating system conditions.

3.2.2. Experiment 1. Growing of baby leaf lettuce on different substrates

Two types of substrate were assayed to fill the cells of the styrofloat trays: a commercial peat and an agroindustrial compost, which was provided by CEBAS-CSIC, Murcia. The raw materials for composting expressed on a dry weight basis were tomato (71%), onion (17%) and vineyard residues (12%). Composting was in open-air piles, with a bioxidative phase of 75 days and maturation phase of 42 days. The piles were turned periodically to ensure aeration, and to control the temperature (Figure 3.1). Once the composting process had finished (120 days), the compost was milled and passed through a 2-cm sieve. A commercial blonde peat substrate (Pindstrup Blond Gold), produced from block and mill harvested peat was used as control. The main chemical characteristics of the compost and the commercial peat are described in Table 3.1.

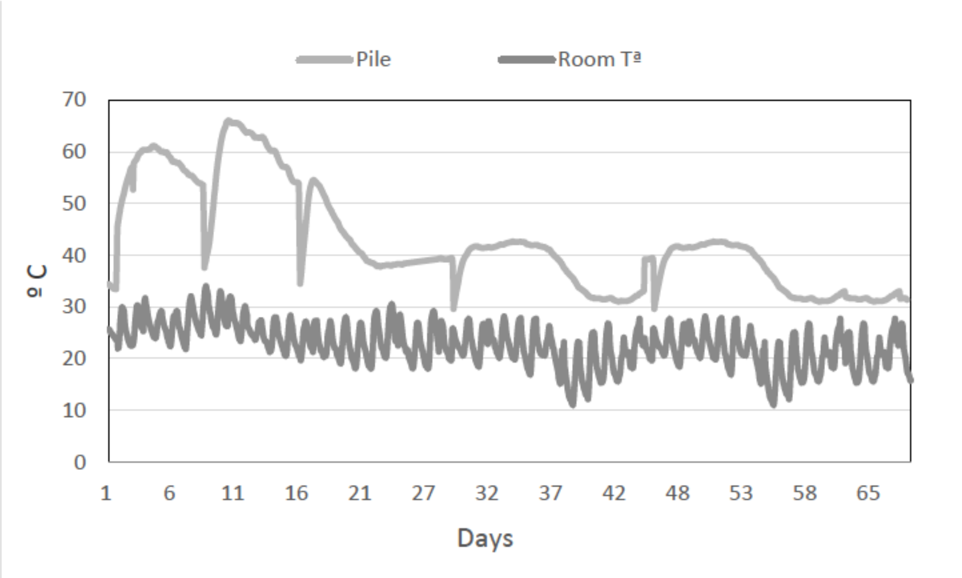

Figure 3.1: Temperature profile of bioxidative phase of composting process.

Table 3.1: Main chemical characteristics of the substrates used (compost and peat).

	Compost	Peat
pH	8.9	5.5
Electrical conductivity (mS cm^{-1})	3.1	2.0
Total organic C (g kg^{-1})	482	450
Total N (g kg^{-1})	26	13
P (g kg^{-1})	4.9	0.3
K (g kg^{-1})	15.4	0.6

Nine trays were used to calculate the germination percentage: three trays per substrate randomly placed on three stainless steel beds located in the same greenhouse described above. Ten seeds of baby leaf lettuce were sown in each cell of the tray, with sixteen cells per replication. Trays were placed for two days in a germination chamber at 18 ºC and 90% relative humidity and then transferred to flotation beds with tap water for five days with temperature condition of 21.18 °C, 42.81 °C, 29.69 °C minimum, maximum and average air temperature, respectively, in the summer cycle and 5.67 °C, 35.40 °C, 16.00 °C minimum, maximum and average air temperature, respectively, in the autumn cycle. The percentage of seed germination was measured 7 days after sowing (das) and calculated as the ratio between the number of germinated seeds and the total number of seeds sown, multiplied by 100.

Another nine trays were used to determinate the quality parameters and the yield, as described in section 2.4.

3.2.3. Experiment 2. Suppressive bioassay against *Phytium irregulare*

A suppressive bioassay against *P. irregulare* was set up to evaluate the effectiveness of the agroindustrial compost and the peat for the biological control of this pathogen. The trays were filled with substrate (compost and peat) inoculated with the pathogen *P. irregulare* at 1 10^5 cfu g^{-1} organic substrate, by adding 0.3 mL g^{-1} of the pathogen solution. The pathogen solution was prepared by blending a 7 days *P. irregulare* culture grown on potato dextrose agar (PDA) in 100 mL of distilled water for one minute.

Nine trays were used to calculate the percentage of plant survival, three trays per substrate inoculated on three stainless steel beds, floating on fresh tap water located in the above-mentioned greenhouse. The procedure was carried out in the same way as for calculating the percentage of germination on flotation beds for thirteen days with temperature condition of 18.81 °C, 42.81 °C, 28.26 °C minimum, maximum and average air temperature, respectively, in the summer cycle; 5.67 °C, 37.74 °C, 17.04 °C minimum, maximum and average air temperature, respectively, in the autumn cycle. The percentage of seedling survival was measured 15 days after sowing (das) and calculated as the ratio between the number of seedlings surviving and the total number of seeds sown, multiplied by 100.

Another nine trays were used to determine the quality parameters and the yield as described in section 2.4.

The abundance of *P. irregulare* in the inoculated substrates was measured in four samples per treatment at the beginning of the experiment and at harvesting time in a 7500 Fast Real-time PCR system (Applied Biosystems, Waltham, MA, USA), with Microamp1 Fast Optical 96-Well Reaction plate with barcode (Life Technologies, Carlsbad, California). The real-time PCR mixture in a final volume of 15 µl contained a final concentration of 15 µM of each primer (Roche Diagnostics, Germany), Pir-F2N-CTTTCCACGTGAACTGTCGTTATT; Pir-R2N CACACAGCAACACACGACCTT 5µM probe Pir-Pr TGCGTGTTGGTAGCATGCGTGTTTG, 0.3 mg mL^{-1} BSA, 1x

Premix Ex Taq (Probe Qpcr 2X), 0.5X Rox reference Dye II 50X and 3 µl of DNA sample. The thermocycling conditions for the pathogen were 95ºC for 1 min, followed by 40 cycles of 95ºC for 10 s and 60ºC for 40 s and a final step 50ºC 2 min. An internal positive control (IPC), from *Phocine Herpesvirus* were included in all reactions PhHV-267s:5'-GGGCGAATCACAGATTGAATC-3'; PhHV-337as:5'-GCGGTTCCAAACGTACCAA-3'; PhHv-305tq: CY5-5'-TTTTTATGTGTCCGCCACCATCTGGAT-3'-BHQ. Each run contained a negative (bdW) and a DNA positive control.

3.2.4. Agronomical and biochemical parameters

At harvesting time, the following agronomical parameters were analysed in 32 plants in both experiments: biomass production (yield), calculated as g plant^{-1}; total root length and the length of 0-0.5 mm diameter roots per plant were determined using a Winrhizo LA 1600 root counter (Regent Inc., Quebec, Canada).

Nitrate was extracted in triplicate using 0.2 g of dry leaf samples (the dry matter content was determined by drying in an oven at 60 °C until constant weight) for each treatment and quantified by ion chromatography (Lara et al., 2011). The total phenolic content was determined by the Folin-Ciocalteu colorimetric method (Everette et al., 2010). The antioxidant capacity was evaluated in terms of free radical-scavenging capacity (Brand-Williams et al., 1995). The total flavonoid content was determined as described by Meda et al. (2005). The content of vitamin C, measured as ascorbic acid (AA) and dehydroascorbic acid, was measured in shoots using high-performance liquid chromatography (Shimadzu Corporation, Canby, OR) equipped with a degasser, DGU-20A, autosampler SIL-30AC, column oven CTO-10AS, communications module CMB-20A, and diode array detector SPDM-20 (Rodríguez-Hidalgo et al., 2010).

3.2.5. Experimental design and data analysis

A randomized complete block design with three replicates per level of treatment was used in the greenhouse in each experiment in both growing seasons. Each level of treatment was carried out in 135 x 125 x 20 cm beds randomly located in three places inside the greenhouse used for all the experiments. Each bed had three floating trays. Data were analysed using Statgraphics Plus. An analysis of variance (two-way ANOVA) was performed with type of substrate (peat, compost) and growing seasons (summer and autumn) as factors in experiment 1 and type of inoculated substrate (peat or compost) and growing seasons (summer and autumn) as factors in experiment 2. When the interaction between factors was significant, ANOVA was carried out for each factor independently.

3.3. Results

3.3.1. Comparing physicochemical properties and growing in organic substrates

Both the pH and EC values were higher for the compost than peat (Table 3.1). The C/N ratio ranged between 18.5 for the compost and 34.6 for the peat; total N, total P and total K were higher in compost than in peat (Table 3.1). The levels of microorganisms considered to be pathogenic for humans were below the maximum limit for the use of composts as fertilizers, according to Spanish legislation (Real Decreto 506/2013;19 US EPA regulations20) and the European guidelines, indicated in the Working Document on Biological Treatment of Biowaste21 (data not shown).

The percentage of seed germination was significantly higher in plants grown in compost than in peat and was also higher in summer than in autumn (Figure 3.2). The two-way ANOVA showed a significant interaction between the substrate and cycle for all the agronomic parameters measured (Table 3.2). There were no differences in terms of yield (kg m^{-2}) between plants grown in peat or compost in the summer cycle, but production in autumn was higher in plants grown in compost (Figure 3.3A). Total root length and length of 0 to 0.5 mm diameter roots were significantly longer in plants grown in compost in summer; both substrates showed the longest length in the autumn cycle but with no significant difference between them (Figures 3.3B and 3.3C).

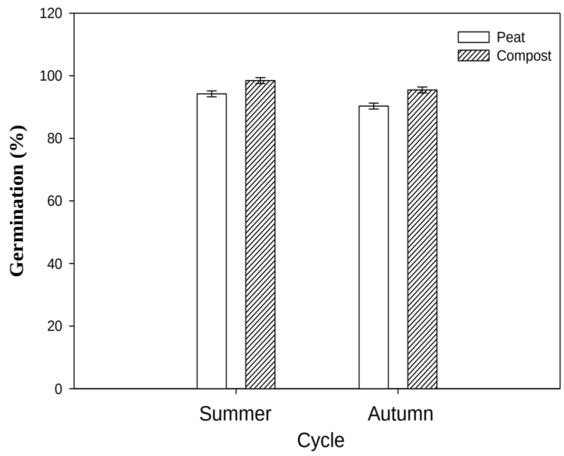

Figure 3.2: Effect of substrate (peat or compost) on germination percentage in baby leaf red lettuce cultivated in summer and autumn cycles in a floating system. Values are the mean ± SD (n=32). Different letters indicate significant differences ($P < 0.05$).

Table 3.2: Influence of substrate (peat or compost) on yield, total root length and length of 0 to 0.5 diameter roots of baby leaf red lettuce cultivated in summer and autumn cycles in a floating system.

Parameters	Statistical signification		
	Cycle	Substrate	Interaction
Yield (kg m^{-2})	***	n.s.	***
Total root length (cm)	***	n.s.	***
Length of 0-0.5 diam. root (mm)	***	n.s.	***

Asterisk indicates significances at * p<0.05; ** p<0.01; *** p<0.001; n.s: non-significant.

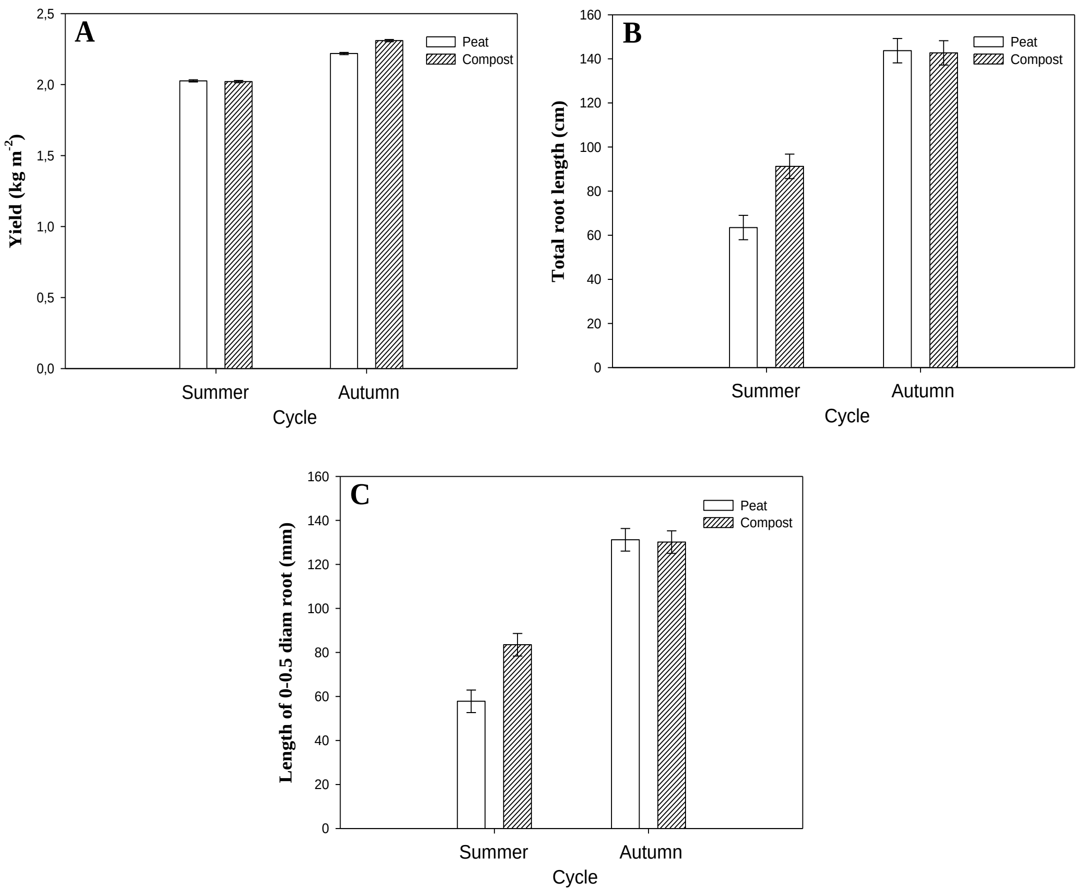

Figure 3.3: Effect of substrate (peat or compost) on yield (A), total root length (B) and length of 0 to 0.5 diameter root (C) in baby leaf red lettuce cultivated in summer and autumn cycles in a floating system. Values are the mean ± SD (n=32). Different letters indicate significant differences ($P < 0.05$).

Regarding lettuce quality, the two-way ANOVA pointed to a significant interaction between the substrate and cycle for all the biochemical parameters measured, except the total phenolic content (Table 3.3). As regards the total flavonoid content, the cycle had a significant effect, being significantly higher in the summer cycle than in autumn (Figure 3.4A). The antioxidant capacity was 1.5 fold higher in the leaves of plants grown on compost in the autumn cycle than in summer (Figure 3.4B). The vitamin C content was 1.43 fold greater in the leaves of plants grown in compost in the summer cycle than in autumn, but there were no differences between the substrates in the autumn cycle (Figure 3.4C). The nitrate content was significantly lower in both cycles in the plants grown in compost than in those grown in peat (5.78 and 1.62-fold lower in summer and autumn cycle, respectively) (Figure 3.4D).

Table 3.3: Influence of substrate (peat or compost) on the biochemical parameters total phenolic and, flavonoid contents, antioxidant capacity, vitamin C and nitrate contents of baby leaf red lettuce cultivated in summer and autumn cycles in floating system.

Parameters	Statistical signification		
	Cycle	Substrate	Interaction
Total phenolic (mg GA kg^{-1} FW)	n.s.	n.s.	n.s.
Total flavonoid (mg Rutin kg^{-1} FW)	***	n.s.	***
Antioxidant capacity (mg $DPPH_{reduced}$ kg^{-1} FW)	**	n.s.	***
Vitamin C (mg kg^{-1} FW)	**	n.s.	***
Nitrate (mg kg^{-1} FW)	***	***	***

Asterisk indicates significances at * p<0.05; ** p<0.01; *** p<0.001; n.s: non-significant.
GA: galic acid equivalent; FW: fresh weight; DPPH: 2.2-diphenyl-1-picrythydrazyl.

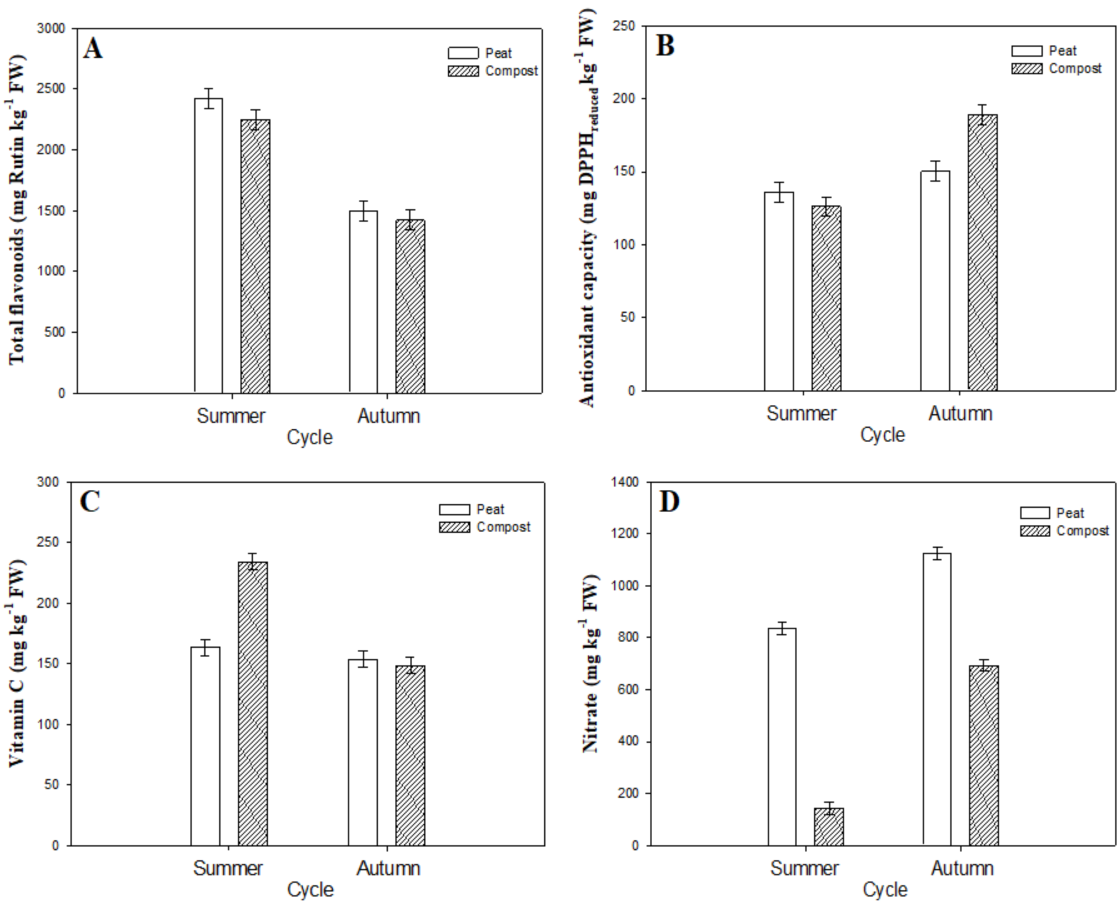

Figure 3.4: Effect of substrate (peat or compost) on total flavonoid (A), antioxidant capacity (B), vitamin C (C), nitrate content (D), in baby leaf red lettuce cultivated in summer and autumn cycles in a floating system. Values are the mean ± SD (n=9). Different letters indicate significant differences (P < 0.05).

3.3.2. Suppressive effect against *P. irregulare*

The suppressive effect of the agroindustry compost against *P. irregulare* was evaluated as a percentage of dead seedlings and yield compared to plants grown in peat. Two-way ANOVA showed a significant interaction between the inoculated substrate and cycle for all the agronomical parameters (Table 3.4). The amount of pathogens (log ITS copies g^{-1}) at harvesting time in peat and compost did not differ significantly (Figure 3.5). The percentage of dead seedlings was significantly higher in the inoculated peat (17% and 16% in summer and winter cycle, respectively) than in inoculated compost (5% and 6% in summer and autumn cycle, respectively) (Figure 3.6). Yield was significantly higher in plants grown in inoculated compost in both cycles (Figure 3.7A). Total root length and the length of 0 to 0.5 mm diameter roots were significantly longer in plants grown in inoculated compost in both cycles, reaching the longest length in the autumn cycle for both substrates (Figures 3.7 B and 3.7 C).

Table 3.4: Influence in the biological control of *P. irregulare* of inoculated substrate (peat or compost) yield, total root length and length of 0 to 0.5 diameter root of baby leaf red lettuce cultivated in summer and autumn cycles in floating system.

Parameters	Statistical signification		
	Cycle	Substrate	Interaction
Yield (kg m^{-2})	***	***	***
Total root length (cm)	***	n.s.	***
Length of 0-0.5 diam. root (mm)	***	n.s.	***

Asterisk indicates significances at * p<0.05; ** p<0.01; *** p<0.001; n.s: non-significant.

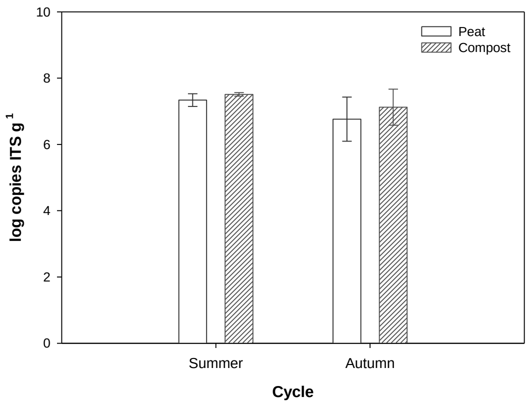

Figure 3.5: Development of *P. irregulare* in the inoculated substrate (peat or compost) used to grown baby leaf red lettuce in summer and autumn cycles in a floating system, measured at harvesting time. Values are the mean ± SD (n=4).

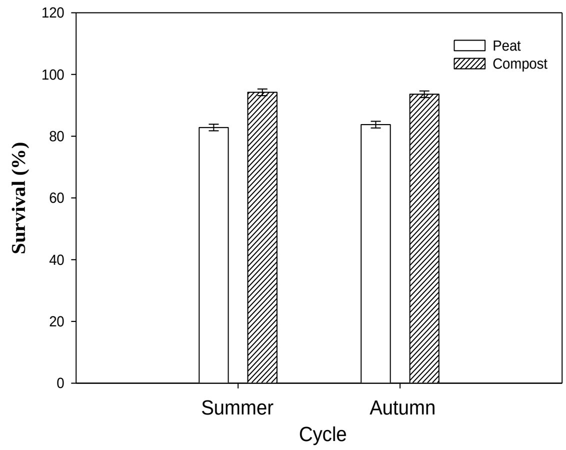

Figure 3.6: Effect of substrate (peat or compost) inoculated for the biological control of *P. irregulare* on survival in baby leaf red lettuce cultivated in summer and autumn cycles in a floating system. Values are the mean ± SD (n=64).Different letters indicate significant differences (P < 0.05).

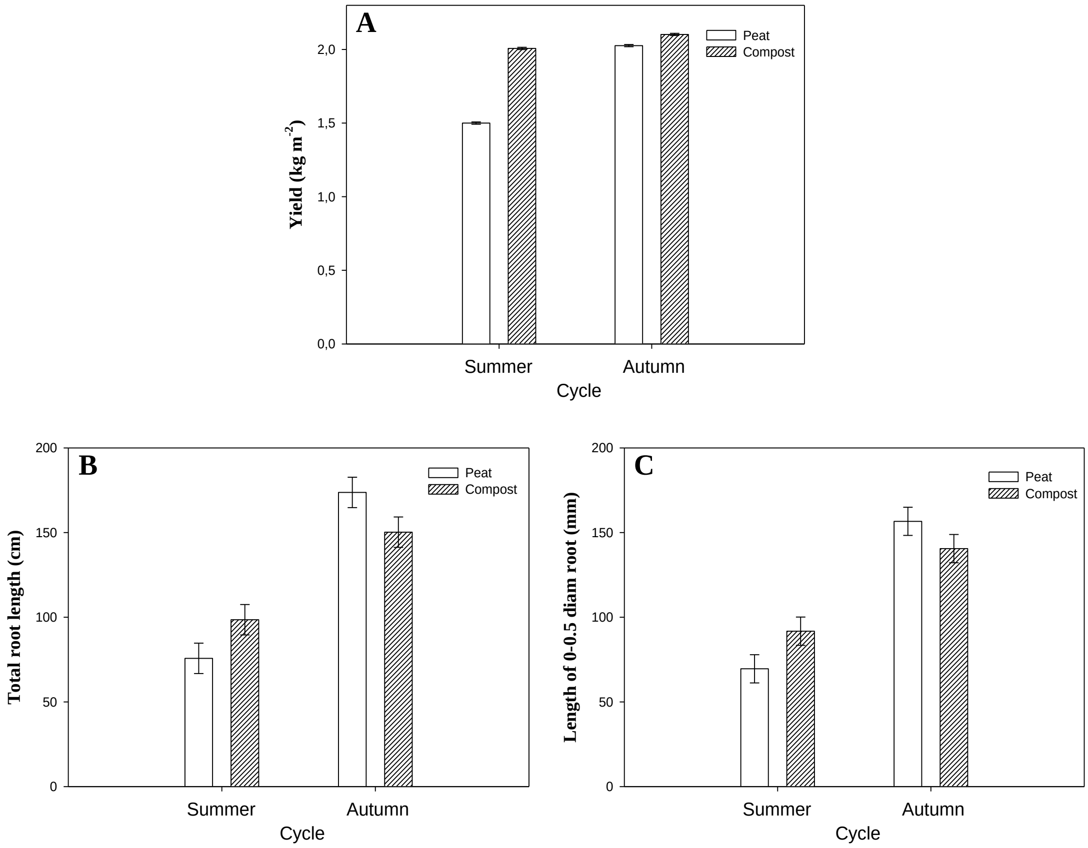

Figure 3.7: Effect of substrate(peat or compost) inoculated for the biological control of *P. irregulare* on yield (A), total root length (B) and length of 0 to 0.5 diameter roots (C) in baby leaf red lettuce cultivated in summer and autumn cycles in a floating system. Values are the mean ± SD (n=32).Different letters indicate significant differences (P < 0.05).

As regards the quality of the lettuce, two-way ANOVA showed a significant interaction between the inoculated substrate and cycle for all the biochemical parameters measured, except antioxidant capacity (Table 3.5). The total phenolic content of leaves of plants grown on inoculated compost was lower in the autumn cycle but there were no differences between substrates in the summer cycle (Figure 3.8A). The total flavonoid content of leaves of plants grown on inoculated compost was lower in both crop cycles (Figure 3.8B). The vitamin C content was 1.34 fold higher in the leaves of plants grown on inoculated compost in the summer cycle but its concentration was lower in autumn cycle (Figure 3.8C). Again, the nitrate content was significantly lower in both cycles in the plants grown on inoculated compost (1.53 and 1.12 fold lower than on inoculated peat in summer and autumn, respectively) (Figure 3.8D).

Table 3.5: Influence in the biological control of *P.irregulare* of inoculated substrate (peat or compost) on the biochemical parameters total phenolic and flavonoid contents, antioxidant capacity, vitamin C and nitrate content of baby leaf red lettuce cultivated in summer and autumn cycles in floating system.

Parameters	Statistical signification		
	Cycle	Substrate	Interaction
Total phenolic (mg GA kg^{-1} FW)	n.s.	*	**
Total flavonoid (mg Rutin kg^{-1} FW)	**	*	***
Antioxidant capacity (mg $DPPH_{reduced}$ kg^{-1} FW)	n.s.	n.s.	n.s.
Vitamin C ($mg.kg^{-1}$ FW)	n.s.	n.s.	***
Nitrate ($mg.kg^{-1}$ FW)	***	n.s.	***

Asterisk indicates significances at * p<0.05; ** p<0.01; *** p<0.001; n.s: non-significant.

GA: galic acid equivalent; FW: fresh weight; DPPH: 2.2-diphenyl-1-picrythydrazyl.

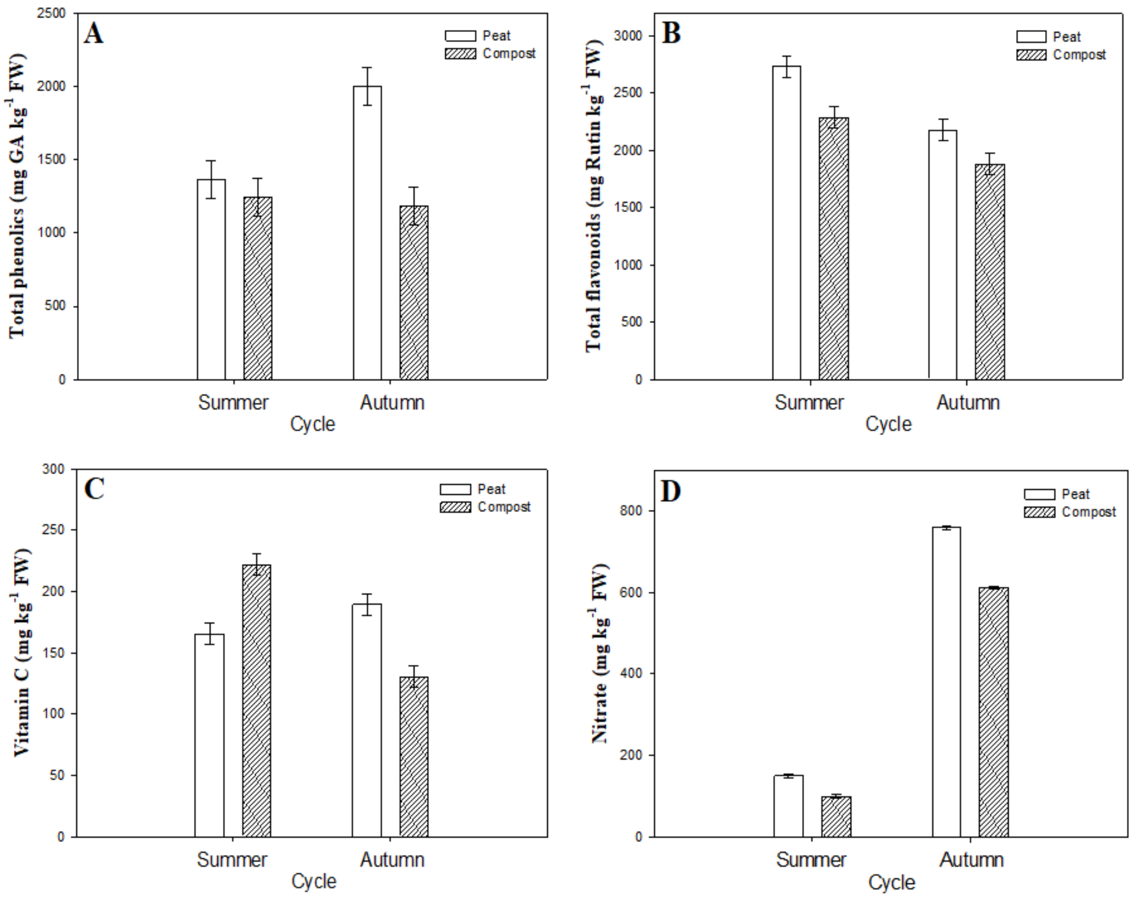

Figure 3.8: Effect of inoculated substrate (peat or compost) for the biological control of P. *irregulare* on total phenolic content (A), total flavonoid content(B), vitamin C (C), nitrate content (D), in baby leaf red lettuce cultivated in summer and autumn cycles in a floating system. Values are the mean ± SD (n=9). Different letters indicate significant differences ($P < 0.05$).

3.4. Discussion

The suitability of the growing medium used in styrofloat trays for fresh leafy vegetables such as baby leaf red lettuce is largely dependent on its capacity to support seedling emergence and growth vigour. In addition, there is a growing demand for more sustainable organic substrates as alternatives to peat. Specific composts could be used for this purpose, especially if they can also contribute new properties that help suppress phytopathogenic microorganisms (Blaya et al., 2016).

The agroindustrial compost used in our assay should not present any risk regarding human pathogens, and it can be considered a Class A biosolid according to the US EPA regulations (EPA, 2003). Compost pH and EC are parameters of great importance in germination and seedling quality. Although pH values in the range 5.2-7.0 are recommended (Bunt, 1988), the pH of the assayed compost (8.9) may also be considered adequate for use in agriculture (Hogg et al., 2002). The compost EC was ≤3.5 dS m^{-1}, which is the limit indicated by Lemaire et al. (1985) for seedlings to grow vigorously in a growing medium. The percentage of germination obtained in experiment 1, where seeds of red lettuce grown in compost showed a greater percentage of germination than in peat (4.1% and 3.1% more in summer and autumn cycles, respectively) (Figure 3.2), confirms the suitability of this agroindustrial compost for this type of cultivation. Furthermore, it should be pointed out that the compost also provided compounds similar to phytohormones that stimulated the germination process (Arancon et al., 2012; Scaglia et al., 2015).

The compost did not have any effect on normal plant development because the yield was not reduced (Figure 3.3A), in agreement with the results of Aleandri et al. (2015), who used green compost from residues of the pruning of woody plants and mowed grass to replace peat in the composition of nursery potting mixes, and with those of García-Gomez et al. (2002) who assayed used two composts prepared from agroindustrial wastes as composts. In addition, the total root length and length of 0 to 0.5 mm diameter roots of red lettuce grown in compost were significantly higher in autumn (Figures 3.3B and 3.3C), just the same to that which occurred with plant yield. This beneficial effect of compost on yield or root length could be due to the production of auxin or auxin-like components from humic substances (Trevisan et al., 2010), principally during germination, which would promote radicular growth. Also, the total porosity of this compost was higher than that of peat (data not shown), and it has been demonstrated that this trait promotes root growth (Arancon et al., 2012; Scaglia et al., 2015). It should be noted that in this type of hydroponic system the lettuce seeds germinate in both compost and peat, and after 5-7 days the main rhizosphere system is no longer in contact with the substrate and starts to grow into the nutrient solution. The seasonal differences in the effect of the compost on plant yield or root length could be attributed to the microbial metabolism of the compost, which is influenced by climatic conditions.

The ability of some composts to control pathogens has made them very interesting for use as alternative substrates in the search for a more sustainable agriculture. More specifically, in fresh-cut vegetable crops, some diseases caused by soil-borne and foliar pathogens have been observed as a consequence of use of intensive cultivation techniques (Gilardi et al., 2015)., The incidence of *Pythium* spp. in hydroponics crops particularly is hazardous because pathogens may spread very quickly in the growing system and remain in the hydroponic growing facilities through their long-term surviving structures (Kanjanamaneesathian et al., 2014). The compost inoculated with *P. irregulare* diminished the percentage of dead seedlings and also increased yield compared with inoculated peat (Figure 3.6 and Figure 3.7A). Both observations mean that the agroindustrial compost showed a suppressive effect in red lettuce, unlike other researchers who only found a reduction in the percentage of dead seedlings (Stanghellini and Kronland, 1986; Raudales and McGehee, 2016). The suppressiveness of composts has been studied in depth, and it can be concluded that the raw materials from which the compost is prepared are crucial to the development of a suppressive microbiota in it (Castaño et al., 2011). Several mechanisms may explain compost suppressiveness: general suppression, specific suppression and induced resistance in plants. These mechanisms vary depending on the compost and the pathogen, and it is not always clear which is more relevant. It has been suggested that a combination of general and specific suppressive potentials is active against *Pythium ultimun* in substrates formulated with composts, but specific suppression is only expressed when the compost is colonized by a specific antagonist during composting (Hoitink et al., 2001; McKellar and Nelson, 2003; Vallance et al., 2011). The abundance of *P. irregulare* in compost did not differ with respect to peat (Figure 3.5), which might mean that no specific suppression occurred, which means that the compost does not target directly the pathogen. In this case the compost could enhance the defence capacity of the plant as it has been suggested by other authors Sang and Kim (2011). So the compost would induce a primed phase on the plant that could display faster and stronger activation of the various cellular defence responses (Conrath et al., 2006), resulting in a higher survival percentage of plant compared with those grown on peat.

As regards the phytochemical compounds that are considered to act as health-promoters, the use of compost did not affect the phenolic and flavonoid contents, but increased the vitamin C content and the antioxidant capacity, with differences between both cycles (Figure 3.4), probably because plant secondary metabolites may be generated in response to environmental stress (Yang et al., 2018). The flavonoid levels were about 60% higher in summer than in autumn (Figure 3.4A). Such an increase in flavonoids with light intensity is well documented in a great variety of plants, and the role of flavonoids in photoprotection seems to be clear (Agati et al., 2013). The accumulation of flavonoids in red lettuce under high light conditions has also been described (Pérez-López et al., 2018), and positive correlations between radiation and temperature with the phenolic and flavonoid contents in pigmented baby leaf lettuce has been established as the season progresses (Marín et al., 2015).

The phenolic and flavonoid contents in the presence of the pathogen (Table 3.5) were higher than those observed in its absence (basal level in each condition assayed) (Table 3.3). The increase of phenolic and flavonoid contents as a response against biotic (and abiotic) stress have been well documented over many decades (Saddique et al., 2018), and may explain the results obtained in this study. However, there is an apparent contradiction when the phenolic and flavonoid levels obtained in both cycles in the absence of *P. irregular* (Table 3.3) are compared with those obtained in its presence (Table 3.5). In this case, the phenol content in autumn was about 22% greater than in summer, while the flavonoid content in summer was about 24% greater than in autumn. When these values of phenolics and flavonoids are compared with the values obtained in the absence of pathogen, the increases were not high (18% phenolics and 7% flavonoids in the presence of pathogen in summer). But in autumn, in the pathogen inoculation treatment, the increases were higher, about 43% in the case of phenolics and 39 % for flavonoids. These results suggest that in summer the plants are stressed by the high light intensity (abiotic stress), especially the flavonoid content. An additional stress (the presence of pathogen, a biotic stress) slightly increased the values of these parameters because the response of the plant with the synthesis-accumulation would be saturated. The greater increases of the flavonoid and phenol contents in autumn suggests differences in the activity of the pathogen in different environmental condition, more active in autumn and less active in summer. The activity of the pathogen would also explain the values obtained in the two different substrates, the slight increases in the phenolic and flavonoid contents in compost (10% and 13%, respectively) and the moderate-high increase in peat (50% and 25%, respectively) suggest that the pathogen is more active in peat, and would explain why the phenolic content in peat was 39% higher than in compost and the flavonoid content 18% higher. To throw further light on the action mechanism of pathogen and its interaction with the roots in the two substrates, more studies are needed at biochemical and molecular levels.

An important quality trait for leafy vegetables is the nitrates they accumulate (Chiesa et al., 2009). High nitrate concentrations may accumulate in the edible parts of some vegetables, particularly if excessive N fertilizer has been applied (Liu et al., 2014). Our data suggested that the use of compost increased the quality of the baby lead red lettuce since the amount of nitrate was significantly lower than when peat was used (Figure 2.4D). Of note was the capacity of the compost to reduce this content, particularly taking into account that the roots only grow in the substrate during germination and a few days following germination, and then grow in the nutrient solution, which had the same composition whether the trays were filled with compost or peat, thus minimising the potential effect of substrates on them. So it is possible that the agroindustry compost transferred its biostimulant effect to roots. A reduction in nitrate accumulation has been reported in several leafy vegetables, including lettuce, rocket, Swiss card, spinach and packchoi, when biostimulants were applied as substrates (Liu and Lee, 2012; Tsouvaltzis et al., 2014, Wang et al., 2007; Colla et al., 2018). This might be the result of the biostimulants, as well as the compost, inducing changes in the expression of nitrate transporter genes, as well as in the several metabolic pathways involved in N metabolism (nitrate and nitrite reductase, glutamate synthase and glutamine synthetase activities (Colla et al., 2015). The nitrate concentrations were higher in the autumn cycle (Figure 2.4D) as was expected, since DLI was lower in autumn than in summer., 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 (Tamme et al., 2009; Burns et al., 2010; Lillo and Appenroth, 2001).

There was also a reduction in the nitrate concentration in plants growing in pathogen-inoculated substrates, especially in the case of plants grown on compost (Figure 3.8D). This reduction could be due to part of the nitrogen from the nutrient solution being required by the plant for the biosynthesis of proteins related to pathogenesis as well as for other components of the plant defence against attack by Oomycetes (Schultz et al., 2013).

Chapter 4

Application of directly brewed compost extract improves yield and quality in baby leaf lettuce grown hrydroponically

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Abstract

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. CE is normally used as a soil drench or directly sprayed on plants but, to our knowledge, their use in hydroponic systems has not been studied in depth before. The aim of this work was to study if the application of a compost extract (CE) elaborated directly (added in the nutrient solution (CENS) or by microsprinklers (CEMP)) could be used to improve the yield and quality of the baby leaf red lettuce growing in a floating system, and to control the incidence of *Pythium irregulare*. In addition, the quality of fresh-cut red lettuce produced in this way was studied. Two experiments were conducted in two growth cycles (winter-spring and autumn). The compost used to produce the compost extract (CE), was composed of tomato (71%), onion (17%) and vineyard residues (12%) expressed in dry weight. Fine mesh bags containing 150 g of compost were placed in the nutrient solution (CENS) and in the irrigation tank for the treatment of mircrosprinkler (CEMP), thus obtaining a compost extract produced directly. To evaluate the effectiveness of the compost extract in the biological control of *P. irregulare*, 100 mL in 200 L of water (2.6 10^3 ITS copies per mL) was added to the flotation beds. The results showed that the use of compost extract added to the nutrient solution improved the growth and quality of the baby leaf lettuce, reducing the nitrate content and increasing 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, the application of the compost reduced the population of *P. irregulare* in the water. In conclusion, the application of the directly produced compost extract is of potential use in a sustainable, soilless production system for small leaf red lettuce, as it improves yield and product quality and is able to control the incidence of *P. irregulare*.

Application of directly brewed compost extract improves yield and quality in baby leaf lettuce grown hydroponically

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.*

Keywords: *Lactuca sativa*, *Pythium irregular*, nitrate, postharvest, antioxidant capacity, total phenolics

4.1. 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 (Razza et al., 2018). 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 (Bernal-Vicente et al., 2008; Ronga et al., 2019). Furthermore, such composts present a lower risk of containing pathogens, heavy metals or pharmaceuticals as components (Moretti et al., 2015), and have interesting biological activities (Ros et al., 2015). Some composts have shown an ability to suppress soil-borne diseases (Bernal-Vicente et al., 2008; Pascual et al., 2002), in which the microbial activity of the compost plays a major role (Hoitink and Fahy, 1986; Noble and Coventry, 2005). However, some abiotic properties have also been suggested to be associated with suppression activity (Hoitink and Grebus, 1997). 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 (Pascual et al., 2002).

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 (Ronga et al., 2016; Ronga et al., 2019). 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 (Giménez et al., 2019ab).

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 (Zhang et al., 1998; Litterick et al., 2004; Dafermos et al, 2012; Giotis et al., 2012), 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 (Diver et al., 2001; Eyheraguibel and Morard, 2008). It has been demonstrated that this type of compound has a direct effect on some metabolic processes (Arancon et al., 2012; Khaled and Fawy, 2011; Morard et al., 2011; Nardi et al., 2002). 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 (Khaled and Fawy, 2011; Morard et al., 2011). Arancon et al. (2012) suggested that analogues of hormones contained in vermicompost extracts were responsible of 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 (St. Martin and Brathwaite, 2012). The application of CE has been shown to significantly suppress several pathogens such as bacterial spot on tomato (Al-Dahmani et al., 2003), *Botrytis cinerea* on strawberry (Welke, 2005), and foliar fungal pathogen on tomato (Koné et al., 2010). CEs have normally been used as a soil drench or directly sprayed on plants (Arancon, 2011; Kim et al., 2015; Zaccardelli et al., 2018; Radovich and Arancon, 2011), but, to our knowledge, their use in hydroponic systems has not been studied in depth before. Also, the use of directly brewed CE has been studied even less despite the fact that, they could be a valuable tool in hydroponic management (Arancon et al., 2012). 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.

4.2. Materials and methos

4.2.1. 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, Netherlands), was cultivated in 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 x 40 cm (Giménez et al.;, 2019a), which were filled with a commercial peat substrate (Pindstrup Blond Gold). After sowing, the trays were transferred to flotation beds (1.35 × 1.25 × 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 $H_2PO_4^-$, 2.6 mM Ca^{2+}, 4.65 mM K^+, 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}) (Egea-Gilabert et al., 2009). 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 28ºC, 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 27ºC, 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 were collected and stored at -20 ºC to measure pathogen concentrations.

4.2.2. 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. 2019a.

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 analysed and quantified by ion chromatography (Lara et al., 2011) in the water emitted by the microsprinklers and in the water contained in the flotation beds before adding the nutrient solution (Table 4.1).

Table 4.1: Chemical characteristics of compost extract for the two ways of application (CENS: compost extract in the nutrient solution, CTMP: 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

4.2.3. 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 x 10^3 copies ITS P per ml. The abundance of *P. irregulare* in the inoculated substrates was measured as described by Giménez et al. 2019b. .

4.2.4. Analysis at harvesting time

At harvesting time, the following parameters were analysed: biomass production (yield), calculated as g plant^{-1}; dry matter content (%) of shoots; specific leaf area (SLA); root growth, and number of adventitious roots. The dry matter contents were determined by drying in an oven at 50 ºC until 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, Canada).

The nitrate content in leaves and in the water of the irrigation systems was analysed in triplicate using 0.2 g of dry leaf samples per treatment and quantified by ion chromatography (Lara et al., 2011). The total phenolic content was determined by the Folin-Ciocalteu colorimetric method (Evertte et al., 2010). The antioxidant capacity was evaluated in terms of their free radical-scavenging capacity (Brand-Williams et al., 1995). The total flavonoid content was determined as described by (Meda et al., 2005).

4.2.5. 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). 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 5ºC for 7 days.

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

4.2.6. 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 analysed 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.

4.3. Results

4.3.1 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 4.2 and 4.3). 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 contrrol inoculated plants and those treated by CENS. Inoculation decreased the SLA values in every combination of factor. In the autumn cycle, there were no significant differences in dry matter for inoculation treatment, way of CE application or their interaction (Table 4.3). As regards 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 4.2: 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 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 x B						
NI x 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 x 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 x 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 x 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 x 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 x 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 x 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 4.3: 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
CTNS	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
CTMP	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 x B						
NI x 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 x CTNS	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 x CTMP	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 x 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
I x CTNS	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 x CTMP	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 x 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 4.1). There were no significant differences between the pathogen content of spring winter and autumn growth cycles.

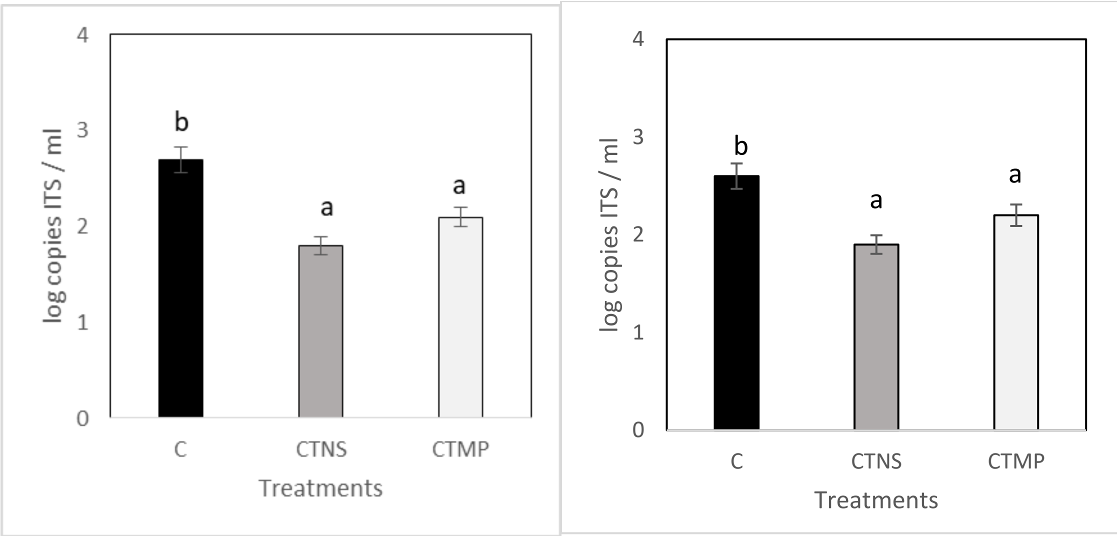

Figure 4.1: *Pythium irregulare* in the water at harvesting time in the winter-spring (left) and autumn (right) cycles according to the different ways of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler). Values are the mean ± SD (n = 4). Different letters indicate significant differences.

4.3.2 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 4.4). The leaves of plants grown with CEMP had the lowest content. There was a significant interaction between way of CE application and storage in the autumn cycle (Table 4.5), the lowest value being found with the CEMP application. After 7 days of storage at 5ºC, 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.

The total phenolics content was affected by the way of CE application and storage in the winter-spring cycle (Table 4.4). 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 being reached with the CEMP application at harvesting (Table 4.5). 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 being 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 being 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 being found in the leaves of plants grown with CEMP application.

Table 4.4: 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 5ºC on the quality (nitrate, total phenolics, total flavonoids, 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 x B						
C x 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 x 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 x 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 x 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 x 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 x 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 x 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 4.5: 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 5ºC on the quality (nitrate, total phenolics, total flavonoids, 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 x B						
C x 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 x 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 x 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 x 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 x 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 x 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 x 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).

4.4. Discussion

The effect of CE on growth differed with the way of its application. CENS application provided the highest yield (Tables 4.1 and 4.2). 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 (Zaccardelli et al 2018). The application of CENS also reduced the incidence of the pathogen added to the water (Figure 4.1), due to the suppresive effect of the compost, again increasing the crop yield. Giménez et al. (2019b) 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 the additional 13.4 mg/L of nitrate, on average, in each microsprinkler irrigation event (Table 4.1). 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 4.1). A decrease in SLA is related with an increase in leaf thickness; in our experiment, CE application decreased SLA in inoculation conditions, particularly in the autumn cycle. Panova et al. (2011) found a decrease in SLA valus in cucumber plants inoculated with a moderate concentration of *Pythium aphanidermatum,* although SLA increased with 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 (Trevisan et al., 2010), which would promote radicular growth. Inoculation with *P. irregulare* reduced root length and increased the root diameter in every treatment (Tables 4.1 and 4.2). Our results agree with those of Schwarz and Grosch (2003), who found that after 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. cause a reduction in root length (Wulff et al., 1998), while root diameter is generally the most responsive plant trait associated with inoculation by root rot pathogens (Biernackiand Bruton, 2000).

The reduction of *P. irregulare* in the water to CE had been applied (Figure 4.1) could be attributed to antibiotic-like compounds or microorganisms (Bernal-Vicente et al., 2008; Pascual et al., 2002) from the original compost (Giménez et al., 2019a). This demonstrates that the suppressiveness observed in compost is probably transferred and maintained in its water extract, reducing pathogen incidence as a direct effect (Zaccardelli et al., 2018; Ezz El-Din and Hendawy, 2010). 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 (Ezz El-Din and Hendawy, 2010; Keeling et al., 2003; Khalid et al., 2006).

The nitrate content is an important quality characteristic of vegetables (Santamaria, 2006), and EU encourages good agricultural practices to reduce the presence of nitrates in lettuce by imposing a limit for its sale (European Union, 2011). 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 (Tsouvaltzis et al., 2014; Colla et al., 2018). 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 (Tamme et al., 2009; Burns et al., 2010). Moreover, nitrate concentrations did not exceed the maximum level allowed by EU for this type of lettuce and way of cultivation. After 7 days of storage at 5ºC, the nitrate concentration in leaves had been reduced. Our findings agree with the results of Gomez et al. (2003) in celery and Miceli et al. (2019) in rocket leaves, where a general decrease in the nitrate content of leaves was observed after storage at 4ºC. By contrast, Konstantopoulo et al. (2010) and Miceli et al. (2019) showed that the nitrate content remained constant in different types of green lettuce during storage at 5 and 10ºC and at 4ºC, 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 (Virsile et al., 2019; Yahia et al., 2019), 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 (Yahia et al., 2019). 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 4.3, 4.4). As regards phenolic compounds, these results agree with those of Kołton and Baran, 2008, 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 (Fiascorano et al., 2019). 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 (Pant et al., 2011). Regarding total flavonoids at harvest, our results agree with those of Khalid et al. (2006) and Ezz El-Din et al. (2010), 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 (Sarwar et al., 2019). Similarly, the antioxidant capacity at harvest was higher in CE treated plants, (Tables 4.2 and 4.3) as reported in other studies where compost was applied to lettuce, spinach (Haghighi, 2011; Tavarinia et al., 2011) and pack choi (Pant et al., 2011). 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) 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 (Oh et al., 2009). Furthermore, other authors have suggested that there is competition between the flavonoid and phenolic acids pathways, the flavonoids route being favoured in conditions of high light intensity (Pérez-López et al., 2018), since they can act as photoprotectors as occurred in the winter-spring cycle of our experiment (Table 4.4), 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≤0.01; r=0.942, P≤0.01 and r=0.846, P≤0.01 for control, CENS and CEMP treatment, respectively) and also between flavonoids and the antioxidant capacity (r=0.941, P≤0.01, r=0.917, P≤0.01, r=0.463, P≥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≤0.01, r=0.966, P≤0.01, r=0.770, P≤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 (Ouzounis et al., 2015), 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), Serafini et al. (2002) and Ninfali and Bacchiocca (2005). The decrease in total phenolics that occurs during storage may be due to increased antioxidant enzymatic activities (Islam et al., 2019). However, other authors found that total flavonoids remained quite constant in spinach (Gil et al., 1999) and the phenolic contents increased in lettuce (Zhao et al., 2007). DuPont et al. 2000 reported the loss of flavonol glycosides in lettuce stored at 1ºC 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 4.3 and 4.4). These values were lower than those reported in previous studies in red lettuce (Selma et al., 2012). 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.

Chapter 5

Spraying agro-industrial compost tea on baby spinach crops: Evaluation on yield, plant quality and soil health in field experiments

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Abstract

Spinach is one of the healthiest vegetables in the human diet due to its high concentration of phytonutrients and health-promoting compounds. In addition, it is a good source of vitamins and mineral elements, such as calcium, iron, phosphorus, sodium and potassium. Compost tea is a liquid fraction extracted from compost, whose use is of great interest because it reduces the use of fertilizers and chemical pesticides in a sustainable agriculture. In this study, we evaluated the potential beneficial effect of foliar application of a compost tea made from onion (67.6%) and vineyard compost (32.4%), alone (CT) or implemented with the beneficial microorganism *Trichoderma harzianum* T78 (TC + Th) on the "healthy quality" and yield of baby spinach during two cycles of spinach cultivation in soil. The results showed that both CT and CT+Th treatments produced higher spinach yield than the control, but these treatments did not result in increased soil dehydrogenase (DHA) activity or soil nutrient content. In addition, the CT + Th treatment showed the highest yield, phenolic content, antioxidant capacity and flavonoid levels. Nitrate contents were significantly lower ($p<0.05$) in the CT and CT+Th treatments than in the control, being their content below the legal amounts. These results suggest that compost tea extracts from onion residues and vineyard manure and/or enriched with *T. harzianum* can be used in sustainable agriculture to increase the yield and quality of baby spinach.

Spraying agro-industrial compost tea on baby spinach crops: evaluation of yield, plant quality and soil health in field experiments

Abstract

Compost tea is a liquid fraction extracted from composts, and it is of great interest in sustainable agriculture because it reduces the unsustainable use of chemical-based pesticides and fertilizers. In this study, during two spinach field cycles, we evaluated the potential beneficial effect of the foliar application of a compost tea made from onion and vineyard composts either by itself (CT) or implemented with the beneficial microorganism *Trichoderma harzianum* T78 (CT+Th) on the "healthy quality" and yield of baby spinach. Results showed that both the CT and CT+Th treatments produced a higher spinach yield than the control, but these treatments did not result in an increase in soil dehydrogenase activity (DHA) or soil nutrient content. Furthermore, CT+Th treatment showed the highest yield, phenolic content, antioxidant capacity and flavonoid levels. Nitrate levels were below legal amounts, and they were significantly ($P \leq 0.05$) lower in the CT and CT+Th treatments than in the control. Data suggest that compost tea extracts from onion waste and vineyard compost and/or enriched with *T. harzianum* can be used in a sustainable agriculture to increase yield and quality of baby spinach.

Keywords: *Trichoderma harzianum,* soil-borne pathogens, antioxidant capacity, Phenolic acid, nitrates, spinach crops

5.1. Introduction

Spinach is one of the healthiest vegetables in the human diet due to its high concentration of phytonutrients and health-promoting compounds (Morelock et al., 2008). It is a good source of vitamins and mineral elements, such as calcium, iron, phosphorus, sodium and potassium (Avsar, 2011). Baby spinach is characterised by its tiny and perfectly tender leaves, which are smaller (between 8 and 12 cm) and tenderer than the larger, mature spinach leaves. The production cycles are very fast, and the spinach can be harvested between 45-60 days after sowing in the Mediterranean region.

Composts teas (CTs) are oxygenated compost water extracts obtained through a suitable liquid-phase blowing process. The application of CTs benefits plant yields, mainly by improving the physiological status of the plant and/or enhancing protection from pathogens (Siddiqui et al., 2008). CTs have been found to stimulate root and vegetative growth (Shaheen et al., 2013), showing behaviour close to that of biostimulants (Piccolo et al., 1992). This behaviour is related to the, the microorganisms within the compost tea, which lead to pest suppression and enhancement of microbial communities improving nutrient uptake or production of bioactive compounds (Parr et al., 2002). The potential of CTs for supplementing or substituting other types of fertilisers is also promising (Morales-Corts et al., 2018). It is well documented, however, that effects of a given CT depend on the source of the cultures and raw materials used for its production (Parr et al., 2002; Morale-Corts et al., 2018; Moretti et al., 2015). Composts from agro-industrial waste are considered more advantageous than other kinds of organic wastes (Ros et al., 2005), because they present a lower risk for pathogens, heavy metals and pharmaceuticals (Moretti et al., 2015). Furthermore, the efficiency of CTs can be increased if they are supplemented with beneficial microorganisms like *Trichoderma sp.*, which can promote seedling establishment and enhance plant growth and plant defence reactions in some vegetable crops (Celar and Valic, 2005; Rabeendran et al., 2000; Hoyos-Carvajal et al., 2000; López-Monddejar et al., 2010).

The objective of this work was to assess the effects of a compost tea made from onion and vineyard composts, both on its own (CT) and supplemented with the beneficial microorganism *T. harzianum* T78 (CT+Th) in comparison with untreated soil (control) on baby spinach yield in open field conditions in two crop experiments. We also tested the effects of these treatments on nutraceutics concentration in the spinach leaves, such as phenolic and flavonoid content and antioxidant capacity. We also studied the leaf nitrate concentrations as a limiting parameter for marketing and anti-nutritional compound. Finally, we determined the effect of these treatments on soil microbial activity, nutrient content and soil-borne fungal pathogens after the crop was finished, in order to assess whether any residual effect could affect soil quality at the end of the experiments.

5.2. Materials and methods

5.2.1. Compost Tea production

An onion waste (67.6%) and a vineyard residue (32.4%) compost was produced on a dry-weight basis at the University Miguel Hernandez (UMH) composting site. The composting process (15 Tn) was carried out using open-air piles (15 Tn) with a biooxidative phase of 75 days and a maturation phase of 40 days. The piles were turned periodically to ensure aeration and to control the temperature. Once the composting process was finished (120 days), the compost was milled and passed through a 2-cm sieve and stored at 4 ºC until use. The compost tea (CT) was prepared weekly by mixing compost with distillate water in the ratio 1:100 w/v by a continuous forced air blowing system at 25±2 ºC for 24 h, just before foliar application. The mix was filtered through cheesecloth and stored at 4 ºC until use. After that, the mixture was diluted (1:9; v/v) to obtain the CT used to spray the experimental spinach fields as suggested by Pane et al. (2012). An aliquot of every CT used for spraying was sampled in each experiment and freeze-dried for chemical analyses. The main compost and CT chemical characteristics are shown in Table 5.1.

Table 5.1: Main characteristics of compost and compost tea (CT) used in the experiments (EW: early winter; LW: late winter). Values are the mean± SD (n=8).

	Parameters								
	pH	EC (dS/m)	Ct (%)	Nt (%)	C/N	P (%)	K (%)	Ca (%)	Na (%)
Compost	7.25±0.20	7.52±0.25	36.50±1.00	2.00±1.50	18	2.68±0.30	1.58±1.40	7.23±2.00	1.07±0.70
CT	pH	EC (dS/m)	Ct (mg L^{-1})	Nt (mg L^{-1})	C/N	P (mg L^{-1})	K (mg L^{-1})	Ca (mg L^{-1})	Na (mg L^{-1})
EW	8.33±0.05	0.45±0.20	69.06±1.25	18.1±1.00	3.8	5.8±0.40	99.8±1.50	23.6±2.80	8.02±0.60
LW	8.93±0.30	0.45±0.24	57.85±2.85	15.61±3.30	3.7	6.02±0.40	91.6±8.30	26.1±1.20	9.26±0.60

5.2.2. Plant material, experimental set-up and design

The field experiments were conducted in the "Campo de Cartagena" (Cartagena, Murcia, Spain) in two close plots with the following soil characteristics: TOC 36.54±0.37 g kg^{-1}; Total N 5.27±0.12 g kg^{-1}; Total P 0.39±0.03 g kg^{-1}; Total K 7.97±0.47 g kg^{-1}. A cultivar of baby spinach (*Spinacia oleracea*), "Maya" (Enza Zaden), was cultivated. The first experiment, "early winter", was conducted from 7 December 2017 to 22 February 2018, with a duration of 77 days (11 weeks); the second experiment, "late winter", was conducted from 12 February 2018 to 2 April 2018, with a shorter cropping duration of 49 days (7 weeks). The average temperature and radiation for the early winter was 11.35±0.66 ºC and 125.77±21.37 w/m^2, respectively. The average temperature and radiation for the late winter was 13.88±2.98 ºC and 201.44±52.49 w/m^2, respectively (SIAM, IMIDA).

The experimental design was a randomised complete block design with three replicates per treatment. Each replicate was carried out in 5x2 m plots randomly located. The spinach seeds were sown at a ratio of 700-900 seeds per m^{-2} (according to the company protocol for this crop). The treatments were sprayed over each spinach plot once a week, beginning one week after sowing for a total of 8 times in the early winter and 6 times in the late winter, with the following equivalents: a) CT at 12 m^3 per ha (CT); b) CT at 12 m^3 plus *T. harzianum* T78, (Ttichosym Bio, Symborg S.L) to 5 10^8 cfu mL^{-1} per ha (CT+Th); and c) the Control, which consisted of spraying the same volume of distilled water at 12 m^3 per ha (Control).

5.2.3. Harvesting

Harvest was carried out when the leaves reached the commercial value (8-12 cm in length). The spinach harvested from each plot was weighed for yield (g m^{-2}) determination. Furthermore, 20 spinach leaves for each replicate were used to measure a) the foliar area by WinRhizo (Regents Instruments Inc.) (cm^2) and b) the nitrate content, phenolic and flavonoid content and antioxidant capacity. The nitrate content was analysed by ion chromatography on fresh samples water extracted (1:10) (Lara et al., 2011). The total phenolic content was determined by the Folin-Ciocalteu colorimetric method (Everette et al., 2010). The total flavonoid content was determined as described by Meda et al. (2005). The antioxidant capacity was evaluated in terms of the free radical scavenging capacity (Brand-Williams et al., 1995).

5.2.4. Soil chemical and biochemical parameters

The soil was sampled at both field experiments after spinach harvesting to a depth of 10.20 cm. Five soil cores were taken in a W-pattern from each replicate and mixed thoroughly. The following parameters were measured: total organic carbon (Total C) and nitrogen (Total N) using a LECO TruSpec C/N, Elemental Analyzer; total P, Na, K and Ca using inductively coupled plasma–mass spectrometry (ICP-MS; ICAP 6500 DUO); and dehydrogenase activity (DHA), using the reduction of 2-p-iodo-3-nitrophenyl-5-phenyl tetrazolium chloride to iodonitrophenyl formazan method (García, et al., 1993).

5.2.5. Soil-borne pathogens

Total DNA was extracted from the soil samples (500 mg) using the DNeasy PowerSoil Kit (Qiagen) following the modification described by Taskin et al. (2011).

Fungal pathogen detection and quantification was performed using the Vegalert qPCR quantitative kit for cucurbits (Microgaia Biotech S.L, Murcia, Spain), following the PCR conditions described by Santisima-Trinidad et al. (2018). Real-time PCR was performed using a 7500 Fast Real-Time PCR system (Applied Biosystems).

T. harzianum T-78 quantification was estimated in soils by quantitative real-time PCR (qPCR) from soil DNA (Lopez-Mondejar et al., 2010), in a total volume of 15 µL, using a 7500 Fast Real-Time PCR system (Applied Biosystems) with the same PCR conditions used by Santisima-Trinidad et al. (2018). The quantification was performed in triplicate.

5.2.6. Statistical analysis

The parameters adjusted to a normal distribution were subjected to multivariate analysis of variance (MANOVA). Only if there was a significant difference between groups, post hoc tests (Tukey’s) were performed.

5.3. Results

5.3.1. Effects of CT on baby-leaf spinach growth and quality

The spinach yield was significantly higher in both CT treatments (CT and CT+Th) than in the control in both experiments (early and late winter) (Figure 5.1a, Table 5.2). The CT+Th treatment showed on average 51% more yield than the control and 15% more than CT. No significant differences were observed between the experiments (Table 5.2). The foliar area in the CT was significantly higher than in the control and CT+Th (Figure 5.1, Table 5.2). Furthermore, the foliar area in late winter was on average 66% lower than in early winter (Figure 5.1b).

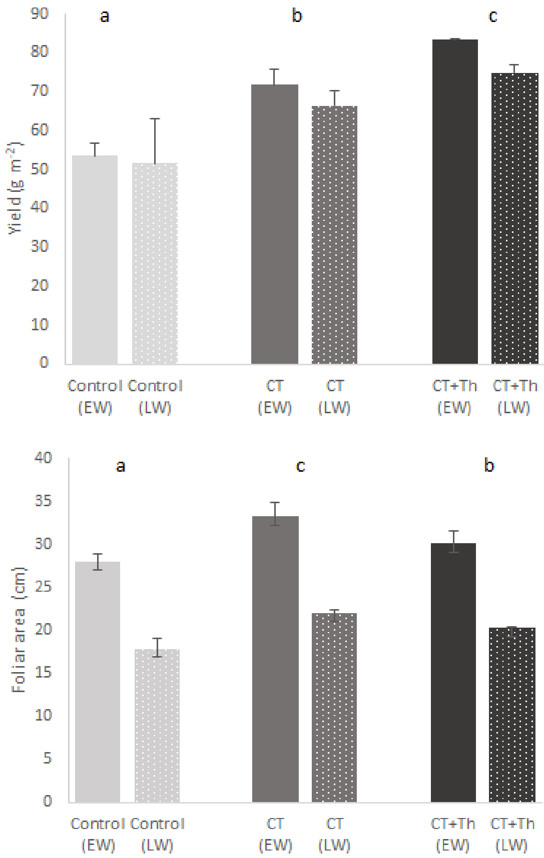


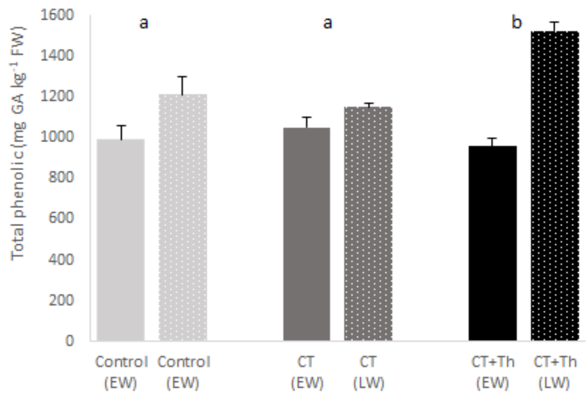
Figure 5.1: Effect of different compost tea (CT) treatments on yield (a) and Foliar area (b) of baby spinach in both experiments. Bars are mean values n=3. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. Different lowercase letters about bars indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters about bars indicate differences between treatments ($p \leq 0.05$) in LW.

Table 5.2: Influence of different CT treatments on yield, foliar area, total phenolic, total flavonoids, antioxidant capacity and nitrate content in both experiments (early winter and late winter).

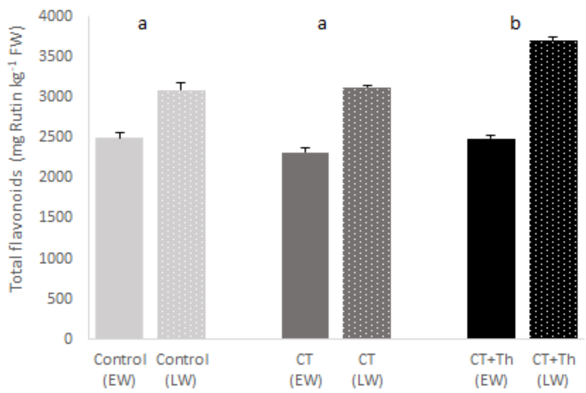
		F	P
Treatment	Yield (g m^{-2})	35.137	0.000
	Foliar área (cm)	31.972	0.000
	Total phenolic (mg GA Kg^{-1} FW)	6.761	0.009
	Total flavonoids (mg Rutin Kg^{-1} FW)	9.272	0.003
	Antioxidant capacity (mg DPPH reduced Kg^{-1} FW)	16.068	0.000
	Nitrate (mg Kg^{-1} FW)	68.236	0.000
Experiment	Yield (g m^{-2})	3.676	0.076
	Foliar area (cm)	392.073	0.000
	Total phenolic (mg GA Kg^{-1} FW)	69.912	0.000
	Total flavonoids (mg Rutin Kg^{-1} FW)	145.327	0.000
	Antioxidant capacity (mg DPPH reduced Kg^{-1} FW)	56.433	0.000
	Nitrate (mg Kg^{-1} FW)	2929.441	0.000
Interaction	Yield (g m^{-2})	0.505	0.614
	Foliar area (cm)	2.441	0.123
	Total phenolic (mg GA Kg^{-1} FW)	14.320	0.000
	Total flavonoids (mg Rutin Kg^{-1} FW)	6.436	0.010
	Antioxidant capacity (mg DPPH reduced Kg^{-1} FW)	13.463	0.001
	Nitrate (mg Kg^{-1} FW)	7.675	0.007

GA: gallic acid; FW: fresh weight; DPPH: 2,2-dephenyl-1-picrylhydrazyl free radical

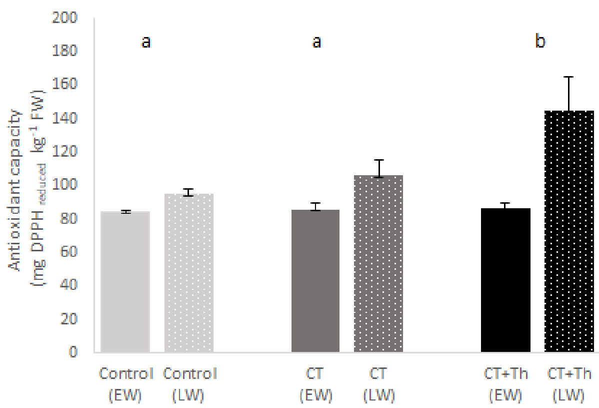
The quality of the baby-leaf spinach as measured by total flavonoids, total phenols and the antioxidant capacity did show significant interaction between treatment and crop cycle (Table 5.2**).** In terms of quality, on average, the CT+Th showed 13% higher total flavonoids and total phenolic content, and 25% higher content of antioxidant capacity than the CT and control (Figure 5.2). Moreover, flavonoids and antioxidant capacity values were respectively 74% higher in late winter than in early winter, while for phenols were on average 77% higher. On the other hand, we also observed a significant difference in the interaction between treatment and experiment for nitrate content (Table 5.2). The spinach nitrate content showed lower values in the CT and CT+Th than in the control in early winter, while in late winter it was only CT+Th. The spinach nitrate content was lower in early winter than in late winter (Figure 5.3).



(a)



(b)



(c)

Figure 5.2: Effect of different compost tea (CT) treatments on total phenolic (a) total flavonoids (b) and antioxidant capacity (c) of baby spinach in both experiments. Bars are mean values n=3. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. Different lowercase letters about bars indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters about bars indicate differences between treatments ($p \leq 0.05$) in LW.

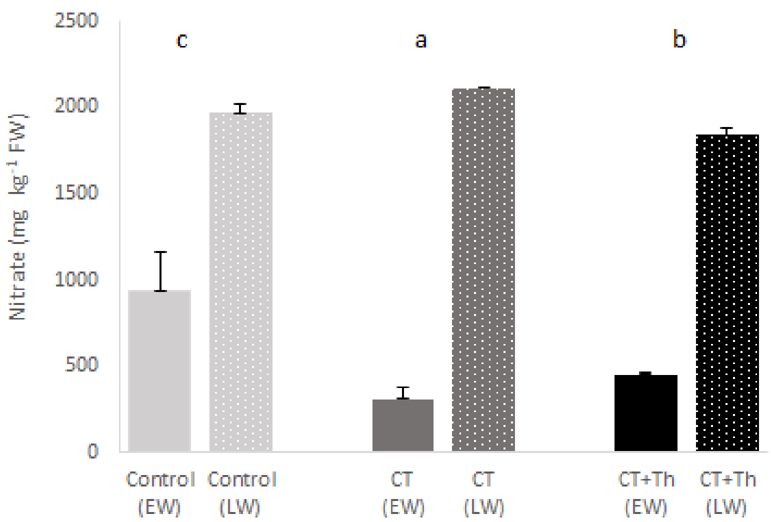


Figure 5.3: Effect of different compost tea (CT) treatments on nitrate content of baby spinach in both experiments. Bars are mean values n=3. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. Different lowercase letters about bars indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters about bars indicate differences between treatments ($p \leq 0.05$) in LW.

CT treatments (CT and CT+Th) significantly increased the nutrient content of the spinach leaves (P and Ca) compared to the control treatment in early winter (Table 5.3). Moreover, the nutrient content of the baby spinach was significantly different according to the experiment.

Table 5.3: Influence of different compost tea (CT) treatments on baby spinach tissue nutrient content in both experiments. Values are the mean ± standard deviation; n=3). Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter; LW: late winter. Different lowercase letters indicate differences between treatments (p≤0.05) in EW and different uppercase letters indicate differences between treatments (p≤0.05) in LW.

Treatment	P (g kg^{-1})		K (g kg^{-1})		Ca (g kg^{-1})	
	EW	LW	EW	LW	EW	LW
Control	1.49±0.19 b	0.41±0.04 A	6.22±0.19 a	7.70±0.45 A	0.78±0.06 b	0.90±0.06 A
CT	2.47±0.30 a	0.41±0.03 A	6.28±0.08 a	8.14±0.36 A	0.86±0.04 a	0.62±0.04 B
CT+Th	2.34±0.30 a	0.40±0.03 A	6.34±0.06 a	7.99±0.49 A	0.88±0.06 a	0.57±0.04 B

Parameters	Multivariate analysis of variance					
	Treatment		Experiment		Interaction	
	F	P	F	P	F	P
P (g kg^{-1})	7.388	0.005	208.560	0.000	7.583	0.004
K (g kg^{-1})	2.822	0.087	142.904	0.000	1.551	0.241
Ca (g kg^{-1})	2.847	0.086	131.732	0.000	1.908	0.179

5.3.2. Effects of CT on soil chemical properties and soil microbial activity

After harvesting, we analysed the soil chemical parameters (Table 5.3). We only found significant differences between treatments in the total organic C content, which was significantly higher in CT and CT+Th than in the control (Table 5.3). We also observed significant differences between crop cycles (Table 5.3): total organic C was higher in early winter, while Total N, P and K were higher in late winter (Table 5.3). Dehydrogenase activity (DHA) showed significant differences between both crop cycles, but not between treatments (Figure 5.4).

Table 5.4: Influence of different compost tea (CT) treatments on soil chemical properties and soil microbial activity in both experiments after harvesting. Values are the mean ± standard deviation; n=3). Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter; LW: late winter. Different lowercase letters indicate differences between treatments (p≤0.05) in EW and different uppercase letters indicate differences between treatments (p≤0.05) in LW.

Treatments	Total C (g kg^{-1})		Total N (g kg^{-1})		Total P (g kg^{-1})		Total K (g kg^{-1})	
	EW	LW	EW	LW	EW	LW	EW	LW
Control	29.35±1.07 a	29.35±1.07 a	29.35±1.07 a	29.35±1.07 a	29.35±1.07 a	29.35±1.07 a	29.35±1.07 a	29.35±1.07 a
CT	30.23±0.75 a	30.23±0.75 a	30.23±0.75 a	30.23±0.75 a	30.23±0.75 a	30.23±0.75 a	30.23±0.75 a	30.23±0.75 a
CT+Th	30.60±1.90 a	30.60±1.90 a	30.60±1.90 a	30.60±1.90 a	30.60±1.90 a	30.60±1.90 a	30.60±1.90 a	30.60±1.90 a

	Treatment		Cycle		Interaction	
	F	P	F	P	F	SP
Total C (g kg^{-1})	12.39	0.01	0.846	0.373	7.140	0.005
Total N (g kg^{-1})	1.122	0.353	19.873	0.001	1.366	0.287
Total P (g kg^{-1})	1,599	0.237	98.897	0.000	2.192	0.149
Total K (g kg^{-1})	1.272	0.311	6.229	0.026	3.012	0.082

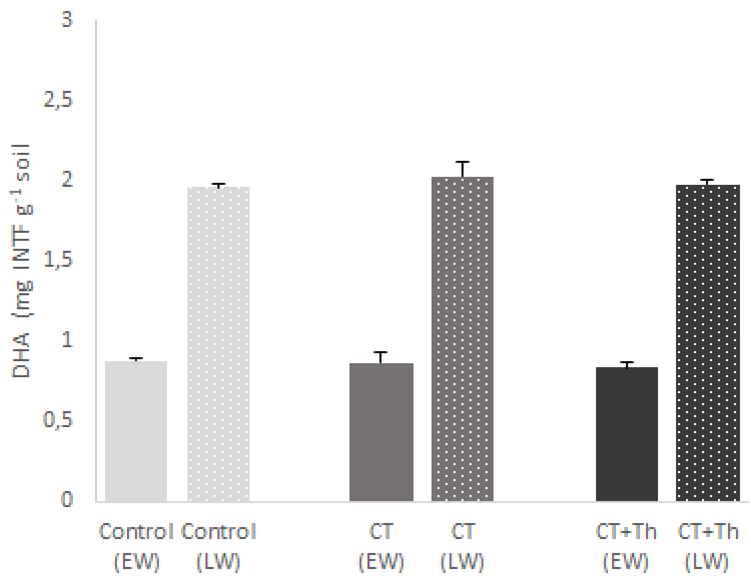


Figure 5.4: Effect of different compost tea (CT) treatments on soil Dehydrogenase activity (DHA) (mg INTF g^{-1} soil) Bars are mean values n=3. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. (Treatment: F=2.145; p=0.160; Cycle: F=5604.522; p=0.000; Interaction: F=2.167; p=0.157). Different lowercase letters indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters indicate differences between treatments ($p \leq 0.05$) in LW.

5.3.3. Effects of CT on soil-borne fungal pathogen abundance and disease incidence

Among the different fungal pathogens analysed in soils by Vegalert qPCR, only *Alternaria sp.*, *Fusarium oxysporum, Fusarium solani* and *Stemphylium botryosum* were detected in both experiments (Table 5.5). Furthermore, significant differences were only observed between crop cycles: the abundance of fungal pathogens was significantly higher in late winter than in early winter (Table 5.5). In spite of the presence of the above-mentioned pathogens in the soils studied, the baby spinach did not show any disease incidence.

Table 5.5: Influence of different compost tea (CT) treatments on abundance (log copies gen g^{-1} soil) of fungal pathogen in soil after harvesting in both experiments. Values are the mean ± standard deviation; n=3). Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT+Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5 10^8 cfu mL^{-1} per ha). EW: early winter; LW: late winter.

Fungal pathogens	Treatments	EW	LW
Alternaria spp.	Control	4.64±0.44	5.95±0.92
	CT	4.88±0.35	5.79±0.20
	CT+Th	5.17±0.09	6.19±0.19
F. oxysporum	Control	4.32±0.39	5.62±0.07
	CT	4.23±0.13	5.95±0.21
	CT+Th	3.87±0.31	6.12±0.01
F. solani	Control	4.99±0.05	6.47±0.07
	CT	4.88±0.32	6.76±0.12
	CT+Th	4.81±0.47	6.82±0.03
Stemphylium botryosum	Control	4.00±0.15	4.74±0.21
	CT	3.95±0.12	4.97±0.22
	CT+Th	3.85±0.16	5.13±0.14

	Treatment		Experiment		Interaction	
	F	P	F	P	F	P
Alternaria spp.	1.165	0.340	22.772	0.000	0.291	0.752
F. oxysporum	0.517	0.607	269.759	0.000	7.011	0.008
F. solani	0.383	0.689	317.683	0.000	2.748	0.098
Stemphylium botryosum	286.72	0.000	967.035	0.000	308.609	0.000

5.4. Discussion

The compost showed similar nutrient contents, pH and EC values to those of other agro-industrial composts (Blaya et al., 2015; Morales et al., 2017). The germination index of the compost (88%) demonstrated no negative germination effect (Luo et al., 2018) considering that compost reached the stable and mature stage validating its suitability for using in compost tea preparation (Bernal-Vicente et al., 2008; Shaban and Fazeli-nasab 2015; Arancon et al., 2007).

5.4.1. Effects of CT on baby spinach growth and quality

Applying CT produced a higher spinach yield and foliar area than the control treatment. This improvement corroborates previous studies, such as those conducted by Hargreaves et al. (2009), Marin et al. (2014) and Bernal-Vicente et al. (2008). This fact could be attributed to a combination of factors, including beneficial plant microorganisms like biostimulants, biofertilisers, biopesticide microorganisms and/or growth promoter compounds like phytohormones that can be found in compost teas (Edwards et al., 2006; Zamora-Nahum et al., 2008). In our experiment, in general, no nutrient differences were observed in the spinach tissue in the CT treatments, except in the case of Ca and P. The amount of nutrients applied through the CT treatment should be minimal in comparison to the amount of nutrients applied via chemical fertilizers in commercial crops. However, Micheal (2001) pointed out that nutrients from compost tea could be also responsible for yield increase, demonstrating that as soon as one hour after application, some nutrients were in the plant rhizosphere available to be taken in. From our results, it therefore seems more plausible that a kind of biostimulation could be responsible for the yield increase. Furthermore, Morales-Corts et al. (2018) indicated that CT from green waste materials usually shows the presence of indole 3-acetic-acid (IAA) and salicylic acid-like compounds (Contreras-Cornejo et al., 2016) that could positively affect spinach growth.

The incorporation of *T. harzianum* into the compost tea (CT+Th) also increased the spinach yield. It could be due to the secondary metabolites released by *T. harzianum* that might influence plant growth, mainly through signalling hormone-like compounds, most notably auxins (Contreras-Cornejo et al., 2016). The positive effect of *T.* on plant growth is equally remarkable and has been recognised as an ability independent to its antifungal ability (Harman et al., 2004; Haggag and Abo-Sedera 2005; Nahar et al., 2012), because an increase in growth has been observed in the absence of any detectable diseases and in sterile soil (Topolovec-Pintaric 2019).

Bioactive compounds (flavonoids, phenolic acids, and tannins, among others) are extra nutritional constituents that naturally occur in small quantities in plant and food products, and they are considered human health-promoters (Kris-Etherton et al., 2002; Gimenez et al., 2019a). The incorporation of *T. harzianum* into the compost tea (CT+Th) also increased the antioxidant capacity and phenolic compounds by 42% and 29%, respectively, compared to the control and CT treatments in the late winter but not in early winter, probably due to the higher amount of *T. harzianum* in the soil. Hua-Bin et al. (2008) found a strong correlation between antioxidant activity and the total phenolic and flavonoid content in plants suggesting that phenolic compounds could be the major contributor to antioxidant capacity, thus activating or priming induced systemic resistance mechanisms (Pascale et al., 2017) Similar results were observed by Yedidia et al. (2003) in cucumber plants treated with *Trichoderma asperellum* and by Pascale et al. (2017) in grapes treated with two *Trichoderma* strains.

Nitrate concentrations accumulated in the edible parts of the spinach leaves must be within the legal EU limits (<3500 mg kg^{-1} FW) (Commission regulation Nº1258/2011). The nitrate values of both commercial trials in this study were under the maximum allowed. The most notable result in terms of nitrate content in our assay is the fact that the spinach tissue in the CT treatment showed lower nitrate levels than in the other treatments in early winter. The reduced nitrate level in different vegetables like lettuce when is treated with compost and compost tea is something to take into account for human health and has been previously investigated (Giménez et al., 2019a; Hassan et al., 2013) The most plausible explanation for this decrease is related to the potential inhibition of nitrifying bacteria due the potential organic mineralization rate from the incorporated CT, which would produce nitrates reducing nitrification processes (Jensen 1950; Rittenberg 1969; Smith and Hoare 1977). The obligate chemolitho autotrophs *Nitrosomonas spp.* and *Nitrospira spp*. are particularly inhibited by the presence of organic compounds (Krummel and Harms 1982; Takahashi et al., 1992; Stutte 1996; Xu et al., 2000). The increase in nitrates in the CT+Th treatment, on the other hand, is probably due to the fact that *T. harzianum* can act as a root nitrate uptake helper (Harman 2000; Lynch et al., 1991).

It is notable that the nitrate accumulation in late winter showed values similar to those found by Manojlovic et al. (2017), in the range of 1,000-2,300 mg kg^{-1} FW, while the early winter values were below 500 mg kg^{-1} FW, similar to the pattern observed by Kapoulas et al. (2017). This is because low light conditions influence nitrate reductase activity and decrease the conversion of nitrate into amino acids, leading to a higher concentration of nitrates (Burns et al., 2010).

5.4.2. Effects of CT on soil chemical properties and soil microbial activity

The application of different CTs did not increase the soil microbial activity, although we did observe improvements in baby spinach yield and quality. It is possible that the CT dose was not enough to enhance soil microbial activity after cropping (Parr et al., 2002; Morales-Corts et al., 2018) but it was sufficient to contribute supplementary beneficial substances for plant growth that were produced by microorganisms during tea production process or contained within the original material. We likely observed an increase in total carbon in CT treatments due to the higher root exudates and plant remains directly related to the higher yield observed (Pascual et al., 2000; Ros et al., 2003).

The qPCR analysis indicated an abundance of different soil fungal pathogens (*Alternaria sp., Fusarium oxysporum, Fusarium solani* and *Stemphylium botryosum*) in all treatments after harvesting, although no symptoms were observed in the spinach crop. It is possible that the amount and type of pathogens in conjunction with the environmental factors were not enough to affect spinach crops (Santisima-Trinidad et al., 2018), or that the spinach plants are "asymptomatic hosts".

Chapter 6

Conclusions

- The agroindustrial compost used as an organic substrate in the sustainable production of red baby leaf lettuce in a floating system, whose composition is: tomato (71%), onion (17%) and vineyard waste (12%), is presented as an alternative to the use of peat, since it presents a higher yield of the crop in this system and favors the decrease of the content of nitrates in the leaf. The inoculated compost showed a better control of the disease caused by *Phytium irregulare* than peat, producing higher yields and increasing the nutritional quality of the lettuce, by reducing the accumulation of nitrates and increasing the antioxidant capacity and vitamin C in these conditions.

- The use of compost extract (CE) made directly from the nutrient solution (CENS) in the floating system, improves the growth of baby leaf lettuce both in the absence and in the presence of the pathogen *P. irregulare* (inoculated and non-inoculated). The application of CE by means of microsprinkler (CEMP) improves the quality of the lettuce, reducing the content of nitrates and increasing the content of compounds such as total phenols, flavonoids and antioxidant capacity, which are beneficial to health. Its application decreases the population of *P. irregulare* in the water regardless of how it is applied.

- Foliar application of a compost tea (CT), whose composition is onion waste (67.6%) and vineyard waste (32.4%) to a small leaf spinach crop in the soil increases yield and improves nutritional quality. The contribution of *Trichoderma harzianum* (Th) to CT increases its effectiveness and improves the quality of baby leaf spinach, since it increases its content in total phenols, flavonoids and antioxidant capacity. Its application implies a lower content of nitrates in the leaf, both alone and in association CT + Th at the beginning of the winter.

- We conclude that the application of compost on baby leaf lettuce in a floating system or by applying compost tea (CTNS, CTMP), in substitution of the widely used peat, improves both the nutritional quality and the yield of the crops. In the same way, the application of a compost tea associated with *T. harzianum* as a biological control agent (CT + Th) in the cultivation of baby leaf spinach improves the productivity and quality of the crop, as well as the quality of the soil. However, further studies on compost and compost extracts, as well as on the contribution of biological control agents, are needed in order to develop more sustainable farming techniques and healthier vegetables.

Chapter 7

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APPENDIX 2. ORIGINAL ARTICLES

Giménez, A., Fernández, J.A., Pascual, J.A., Ros, M., Lopéz-Serrano, M. and Egea-Gilabert, C. 2019. An agroindustrial compost as alternative to peat for production of baby leaf red lettuce in a floating. **Scientia Horticulturae**. 246, 907-915. http://doi.org/10.1016/j.scienta.2018.11.080

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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*.

Keywords: *Lactuca sativa*; *Pythium irregulare*; nitrate; postharvest; antioxidant capacity; total phenolics

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 × 1.25 × 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 $H_2PO_4^-$, 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.

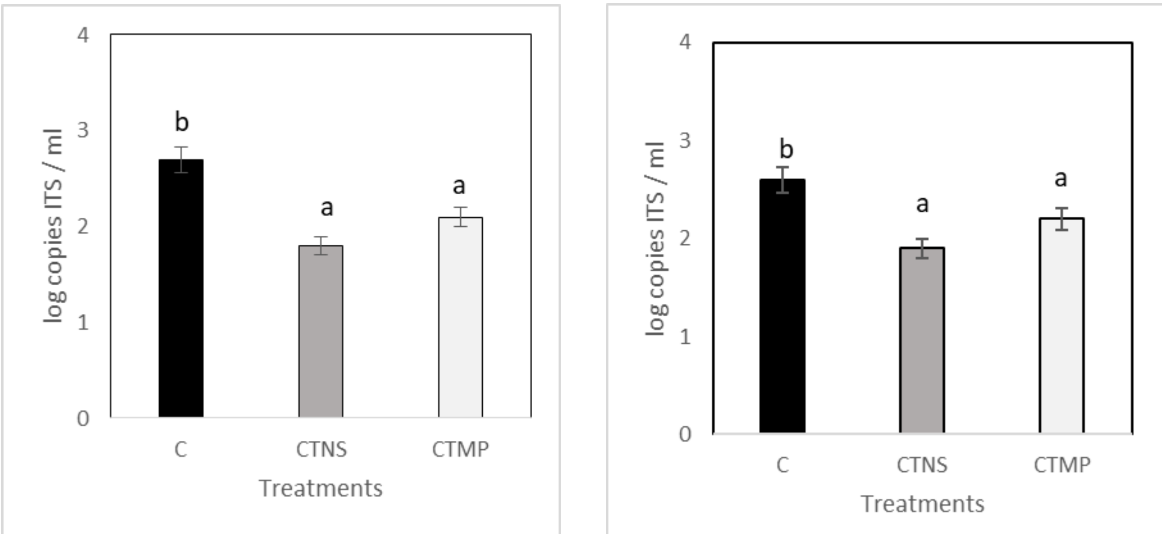

Figure 1. *Pythium irregulare* in the water at harvesting time in the winter–spring (**left**) and autumn (**right**) cycles according to the different ways of compost extract application (C: control, CENS: compost extract in the nutrient solution, CEMP: compost extract delivered by microsprinkler). 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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agronomy

Article

Spraying Agro-Industrial Compost Tea on Baby Spinach Crops: Evaluation of Yield, Plant Quality and Soil Health in Field Experiments

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Abstract: Compost tea is a liquid fraction extracted from composts, and it is of great interest in sustainable agriculture because it reduces the unsustainable use of chemical-based pesticides and fertilizers. In this study, during two spinach field cycles, we evaluated the potential beneficial effect of the foliar application of a compost tea made from onion and vineyard composts either by itself (CT) or implemented with the beneficial microorganism *Trichoderma harzianum* T78 (CT + Th) on the "healthy quality" and yield of baby spinach. Results showed that both the CT and CT + Th treatments produced a higher spinach yield than the control, but these treatments did not result in an increase in soil dehydrogenase activity (DHA) or soil nutrient content. Furthermore, CT + Th treatment showed the highest yield, phenolic content, antioxidant capacity and flavonoid levels. Nitrate levels were below legal amounts, and they were significantly ($p \leq 0.05$) lower in the CT and CT + Th treatments than in the control. Data suggest that compost tea extracts from onion waste and vineyard compost and/or enriched with *T. harzianum* can be used in a sustainable agriculture to increase yield and quality of baby spinach.

Keywords: *Trichoderma harzianum*; soil-borne pathogens; antioxidant capacity; phenolic acid; nitrates; spinach crops

1. Introduction

Spinach is one of the healthiest vegetables in the human diet due to its high concentration of phytonutrients and health-promoting compounds [1]. It is a good source of vitamins and mineral elements, such as calcium, iron, phosphorus, sodium and potassium [2]. Baby spinach is characterized by its tiny and perfectly tender leaves, which are smaller (between 8 and 12 cm) and tenderer than the larger, mature spinach leaves. The production cycles are very fast, and the spinach can be harvested between 45–60 days after sowing in the Mediterranean region.

Composts teas (CTs) are oxygenated compost water extracts obtained through a suitable liquid-phase blowing process. The application of CTs benefits plant yields, mainly by improving the physiological status of the plant and/or enhancing protection from pathogens [3]. CTs have been found to stimulate root and vegetative growth [4], showing behavior close to that of biostimulants [5]. This behavior is related to the, the microorganisms within the compost tea, which lead to pest suppression and enhancement of microbial communities improving nutrient uptake or production of bioactive compounds [6]. The potential of CTs for supplementing or substituting other types of fertilizers is also promising [7]. It is well documented, however, that effects of a given CT depend on the source of the cultures and raw materials used for its production [6–8]. Composts from agro-industrial waste are considered more advantageous than other kinds of organic wastes [9], because they present a lower risk for pathogens, heavy metals and pharmaceuticals [8]. Furthermore, the efficiency of CTs can be increased if they are supplemented with beneficial microorganisms like *Trichoderma* sp., which can promote seedling establishment and enhance plant growth and plant defense reactions in some vegetable crops [10–13].

The objective of this work was to assess the effects of a compost tea made from onion and vineyard composts, both on its own (CT) and supplemented with the beneficial microorganism *T. harzianum* T78 (CT + Th) in comparison with untreated soil (control) on baby spinach yield in open field conditions in two crop experiments. We also tested the effects of these treatments on nutraceutics concentration in the spinach leaves, such as phenolic and flavonoid content and antioxidant capacity. We also studied the leaf nitrate concentrations as a limiting parameter for marketing and anti-nutritional compound. Finally, we determined the effect of these treatments on soil microbial activity, nutrient content and soil-borne fungal pathogens after the crop was finished, in order to assess whether any residual effect could affect soil quality at the end of the experiments.

2. Materials and Methods

2.1. Compost Tea Production

An onion waste (67.6%) and a vineyard residue (32.4%) compost was produced on a dry-weight basis at the University Miguel Hernandez (UMH) composting site. The composting process (15 Tn) was carried out using open-air piles (15 Tn) with a bio-oxidative phase of 75 days and a maturation phase of 40 days. The piles were turned periodically to ensure aeration and to control the temperature. Once the composting process was finished (120 days), the compost was milled and passed through a 2 cm sieve and stored at 4 °C until use. The compost tea (CT) was prepared weekly by mixing compost with distillate water in the ratio 1:100 w/v by a continuous forced air blowing system at 25 ± 2 °C for 24 h, just before foliar application. The mix was filtered through cheesecloth and stored at 4 °C until use. After that, the mixture was diluted (1:9; v/v) to obtain the CT used to spray the experimental spinach fields as suggested by Pane et al. [14]. An aliquot of every CT used for spraying was sampled in each experiment and freeze-dried for chemical analyses. The main compost and CT chemical characteristics are shown in Table 1.

Table 1. Main characteristics of compost and compost tea (CT) used in the experiments (EW: early winter; LW: late winter). Values are the mean ± SD (*n* = 8).

	Parameters								
	pH	**EC (dS/m)**	**Ct (%)**	**Nt (%)**	**C/N**	**P (%)**	**K (%)**	**Ca (%)**	**Na (%)**
Compost	7.25 ± 0.20	7.52 ± 0.25	36.50 ± 1.00	2.00 ± 1.50	18	2.68 ± 0.30	1.58 ± 1.40	7.23 ± 2.00	1.07 ± 0.70
CT	**pH**	**EC (dS/m)**	**Ct (mg L^{-1})**	**Nt (mg L^{-1})**	**C/N**	**P (mg L^{-1})**	**K (mg L^{-1})**	**Ca (mg L^{-1})**	**Na (mg L^{-1})**
EW	8.33 ± 0.05	0.45 ± 0.20	69.06 ± 1.25	18.1 ± 1.00	3.8	5.8 ± 0.40	99.8 ± 1.50	23.6 ± 2.80	8.02 ± 0.60
LW	8.93 ± 0.30	0.45 ± 0.24	57.85 ± 2.85	15.61 ± 3.30	3.7	6.02 ± 0.40	91.6 ± 8.30	26.1 ± 1.20	9.26 ± 0.60

2.2. Plant Material, Experimental Set-Up and Design

The field experiments were conducted in the "Campo de Cartagena" (Cartagena, Murcia, Spain) in two close plots with the following soil characteristics: TOC 36.54 ± 0.37 g kg^{-1}; Total N 5.27 ± 0.12 g kg^{-1}; Total P 0.39 ± 0.03 g kg^{-1}; Total K 7.97 ± 0.47 g kg^{-1}. A cultivar of baby spinach (*Spinacia oleracea*), "Maya" (Enza Zaden), was cultivated. The first experiment, "early winter", was conducted from 7 December 2017 to 22 February 2018, with a duration of 77 days (11 weeks); the second experiment, "late winter", was conducted from 12 February 2018 to 2 April 2018, with a shorter cropping duration of 49 days (7 weeks). The average temperature and radiation for the early winter was 11.35–10.66 °C and 125.77–121.37 w/m^2, respectively. The average temperature and radiation for the late winter was 13.88–12.98 °C and 201.44–152.49 w/m^2, respectively (SIAM, IMIDA).

The experimental design was a randomized complete block design with three replicates per treatment. Each replicate was carried out in 5 × 2 m plots randomly located. The spinach seeds were sown at a ratio of 700–900 seeds per m^{-2} (according to the company protocol for this crop). The treatments were sprayed over each spinach plot once a week, beginning one week after sowing for a total of 8 times in the early winter and 6 times in the late winter, with the following equivalents: (a) CT at 12 m^3 per ha (CT); (b) CT at 12 m^3 plus *T. harzianum* T78, (Trichosym Bio, Symborg S.L, Murcia, Spain) to 5×10^8 cfu mL^{-1} per ha (CT + Th); and (c) the Control, which consisted of spraying the same volume of distilled water at 12 m^3 per ha (Control).

2.3. Harvesting

Harvest was carried out when the leaves reached the commercial value (8–12 cm in length). The spinach harvested from each plot was weighed for yield (g m^{-2}) determination. Furthermore, 20 spinach leaves for each replicate were used to measure (a) the foliar area by WinRhizo (Regents Instruments Inc., Quebec City, Quebec, Canada) (cm^2) and (b) the nitrate content, phenolic and flavonoid content and antioxidant capacity. The nitrate content was analyzed by ion chromatography on fresh samples water extracted (1:10) [15]. The total phenolic content was determined by the Folin-Ciocalteu colorimetric method [16]. The total flavonoid content was determined as described by Meda et al. [17]. The antioxidant capacity was evaluated in terms of the free radical scavenging capacity [18].

2.4. Soil Chemical and Biochemical Parameters

The soil was sampled at both field experiments after spinach harvesting to a depth of 10.20 cm. Five soil cores were taken in a W-pattern from each replicate and mixed thoroughly. The following parameters were measured: total organic carbon (Total C) and nitrogen (Total N) using a LECO TruSpec C/N (LECO Corporation, St Joseph, Michigan, USA), Elemental Analyzer; total P, Na, K and Ca using inductively coupled plasma-mass spectrometry (ICP-MS; ICAP 6500 DUO, Thermo Fisher Scientific, Hayward, California, USA); and dehydrogenase activity (DHA), using the reduction of 2-p-iodo-3-nitrophenyl-5-phenyl tetrazolium chloride to iodonitrophenyl formazan method [19].

2.5. Soil-Borne Pathogens

Total DNA was extracted from the soil samples (500 mg) using the DNeasy PowerSoil Kit (Qiagen, Germantown, Maryland, USA) following the modification described by Taskin et al. [20].

Fungal pathogen detection and quantification was performed using the Vegalert qPCR quantitative kit for cucurbits (Micrograia Biotech S.L, Murcia, Spain), following the PCR conditions described by Santisima-Trinidad et al. [21]. Real-time PCR was performed using a 7500 Fast Real-Time PCR system (Applied Biosystems).

T. harzianum T-78 quantification was estimated in soils by quantitative real-time PCR (qPCR) from soil DNA [13], in a total volume of 15 µL, using a 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA) with the same PCR conditions used by Santisima-Trinidad et al. [21]. The quantification was performed in triplicate.

2.6. Statistical Analysis

The parameters adjusted to a normal distribution were subjected to multivariate analysis of variance (MANOVA). Only if there was a significant difference between groups, post hoc tests (Tukey's) were performed.

3. Results

3.1. Effects of CT on Baby-Leaf Spinach Growth and Quality

The spinach yield was significantly higher in both CT treatments (CT and CT + Th) than in the control in both experiments (early and late winter) (Figure 1a, Table 2). The CT + Th treatment showed on average 51% more yield than the control and 15% more than CT. No significant differences were observed between the experiments (Table 2). The foliar area in the CT was significantly higher than in the control and CT + Th (Figure 1, Table 2). Furthermore, the foliar area in late winter was on average 66% lower than in early winter (Figure 1b).

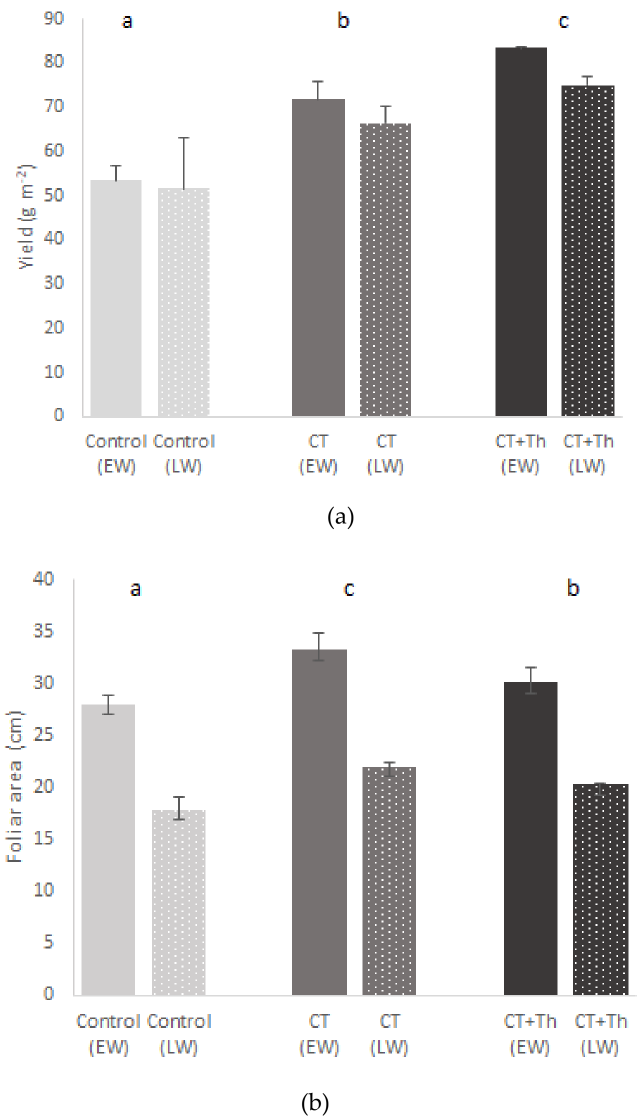


Figure 1. Effect of different compost tea (CT) treatments on yield (**a**) and foliar area (**b**) of baby spinach

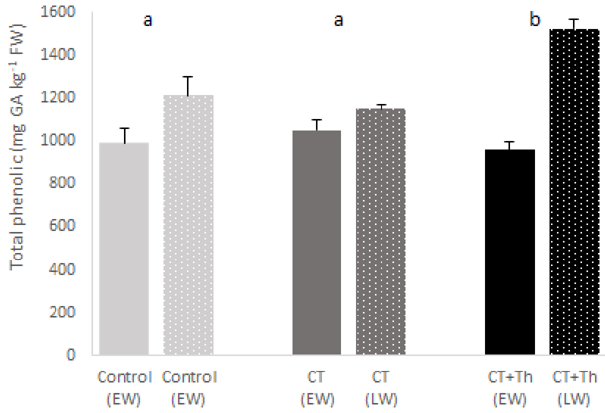
in both experiments. Bars are mean values $n = 3$. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. Different lowercase letters about bars indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters about bars indicate differences between treatments ($p \leq 0.05$) in LW.

Table 2. Influence of different CT treatments on yield, foliar area, total phenolic, total flavonoids, antioxidant capacity and nitrate content in both experiments (early winter and late winter).

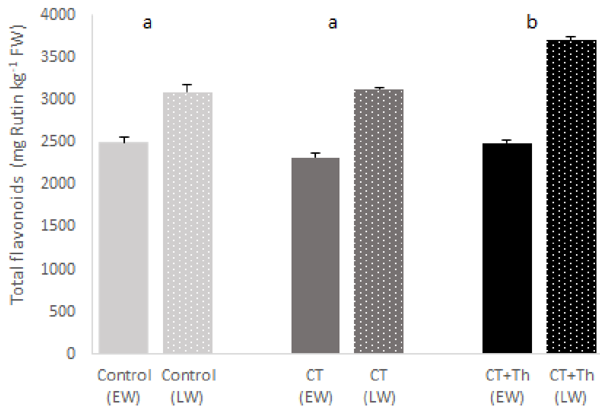
		F	P
Treatment	Yield (g m^{-2})	35.137	0.000
	Foliar área (cm)	31.972	0.000
	Total phenolic (mg GA Kg^{-1} FW)	6.761	0.009
	Total flavonoids (mg Rutin kg^{-1} FW)	9.272	0.003
	Antioxidant capacity (mg DPPH reduced kg^{-1} FW)	16.068	0.000
	Nitrate (mg kg^{-1} FW)	68.236	0.000
Experiment	Yield (g m^{-2})	3.676	0.076
	Foliar area (cm)	392.073	0.000
	Total phenolic (mg GA kg^{-1} FW)	69.912	0.000
	Total flavonoids (mg Rutin kg^{-1} FW)	145.327	0.000
	Antioxidant capacity (mg DPPH reduced kg^{-1} FW)	56.433	0.000
	Nitrate (mg kg^{-1} FW)	2929.441	0.000
Interaction	Yield (g m^{-2})	0.505	0.614
	Foliar area (cm)	2.441	0.123
	Total phenolic (mg GA kg^{-1} FW)	14.320	0.000
	Total flavonoids (mg Rutin kg^{-1} FW)	6.436	0.010
	Antioxidant capacity (mg DPPH reduced kg^{-1} FW)	13.463	0.001
	Nitrate (mg kg^{-1} FW)	7.675	0.007

GA: gallic acid; FW: fresh weight; DPPH: 2,2-diphenyl-1-picrylhydrazyl free radical.

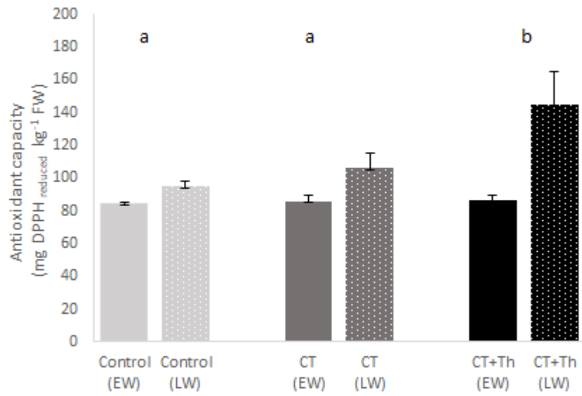
The quality of the baby-leaf spinach as measured by total flavonoids, total phenols and the antioxidant capacity did show significant interaction between treatment and crop cycle (Table 2). In terms of quality, on average, the CT + Th showed 13% higher total flavonoids and total phenolic content, and 25% higher content of antioxidant capacity than the CT and control (Figure 2). Moreover, flavonoids and antioxidant capacity values were respectively 74% higher in late winter than in early winter, while for phenols were on average 77% higher. On the other hand, we also observed a significant difference in the interaction between treatment and experiment for nitrate content (Table 2). The spinach nitrate content showed lower values in the CT and CT + Th than in the control in early winter, while in late winter it was only CT + Th. The spinach nitrate content was lower in early winter than in late winter (Figure 3).



(a)



(b)



(c)

Figure 2. Effect of different compost tea (CT) treatments on total phenolic (**a**) total flavonoids (**b**) and antioxidant capacity (**c**) of baby spinach in both experiments. Bars are mean values $n = 3$. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. Different lowercase letters about bars indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters about bars indicate differences between treatments ($p \leq 0.05$) in LW.

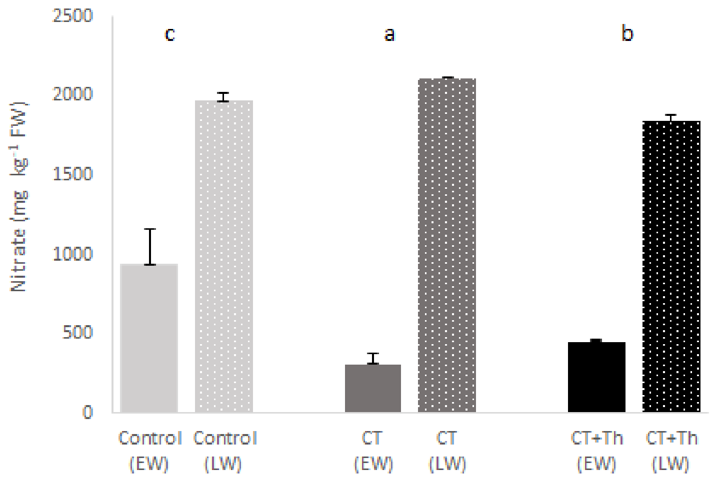


Figure 3. Effect of different compost tea (CT) treatments on nitrate content of baby spinach in both experiments. Bars are mean values $n = 3$. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. Different lowercase letters about bars indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters about bars indicate differences between treatments ($p \leq 0.05$) in LW.

CT treatments (CT and CT + Th) significantly increased the nutrient content of the spinach leaves (P and Ca) compared to the control treatment in early winter (Table 3). Moreover, the nutrient content of the baby spinach was significantly different according to the experiment.

Table 3. Influence of different compost tea (CT) treatments on baby spinach tissue nutrient content in both experiments. Values are the mean + SD; $n = 3$). Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter; LW: late winter. Different lowercase letters indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters indicate differences between treatments ($p \leq 0.05$) in LW.

Treatment	**P (g kg^{-1})**		**K (g kg^{-1})**		**Ca (g kg^{-1})**	
	EW	**LW**	**EW**	**LW**	**EW**	**LW**
Control	1.49–10.19 b	0.41–10.04 A	6.22–10.19 a	7.70–10.45 A	0.78–10.06 b	0.90–10.06 A
CT	2.47–10.30 a	0.41–10.03 A	6.28–10.08 a	8.14–10.36 A	0.86–10.04 a	0.62–10.04 B
CT + Th	2.34–10.30 a	0.40–10.03 A	6.34–10.06 a	7.99–10.49 A	0.88–10.06 a	0.57–10.04 B
Parameters	**Multivariate Analysis of Variance**					
	Treatment		**Experiment**		**Interaction**	
	F	**P**	**F**	**P**	**F**	**P**
P (g kg^{-1})	7.388	0.005	208.560	0.000	7.583	0.004
K (g kg^{-1})	2.822	0.087	142.904	0.000	1.551	0.241
Ca (g kg^{-1})	2.847	0.086	131.732	0.000	1.908	0.179

3.2. Effects of CT on Soil Chemical Properties and Soil Microbial Activity

After harvesting, we analyzed the soil chemical parameters (Table 4). We only found significant differences between treatments in the total organic C content, which was significantly higher in CT and CT + Th than in the control (Table 4). We also observed significant differences between crop cycles: total organic C was higher in early winter, while Total N, P and K were higher in late winter (Table 3). Dehydrogenase activity (DHA) showed significant differences between both crop cycles, but not between treatments (Figure 4).

Table 4. Influence of different compost tea (CT) treatments on soil chemical properties and soil microbial activity in both experiments after harvesting. Values are the mean + SD; *n* = 3). Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter; LW: late winter. Different lowercase letters indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters indicate differences between treatments ($p \leq 0.05$) in LW.

Treatment		**Total C (g kg^{-1})**	**Total N (g kg^{-1})**		**Total P (g kg^{-1})**		**Total K (g kg^{-1})**	
	EW	**LW**	**EW**	**LW**	**EW**	**LW**	**EW**	**LW**
Control	29.35–11.07 a	29.35–11.07 a	29.35–11.07 a	29.35–11.07 a	29.35–11.07 a	29.35–11.07 a	29.35–11.07 a	29.35–11.07 a
CT	30.23–10.75 a	30.23–10.75 a	30.23–10.75 a	30.23–10.75 a	30.23–10.75 a	30.23–10.75 a	30.23–10.75 a	30.23–10.75 a
CT + Th	30.60–11.90 a	30.60–11.90 a	30.60–11.90 a	30.60–11.90 a	30.60–11.90 a	30.60–11.90 a	30.60–11.90 a	30.60–11.90 a

	Treatment		**Cycle**		**Interaction**	
	F	**P**	**F**	**P**	**F**	**SP**
Total C (g kg^{-1})	12.39	0.01	0.846	0.373	7.140	0.005
Total N (g kg^{-1})	1.122	0.353	19.873	0.001	1.366	0.287
Total P (g kg^{-1})	1,599	0.237	98.897	0.000	2.192	0.149
Total K (g kg^{-1})	1.272	0.311	6.229	0.026	3.012	0.082

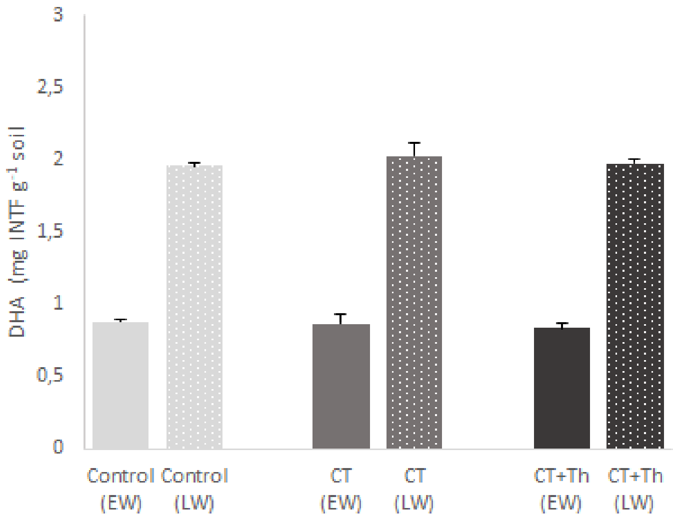


Figure 4. Effect of different compost tea (CT) treatments on soil dehydrogenase activity (DHA) (mg INTF g^{-1} soil) Bars are mean values n = 3. Error bars indicate standard deviation. Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter and bars without points; LW: late winter and bars with points. (Treatment: $F = 2.145$; $p = 0.160$; Cycle: $F = 5604.522$; $p = 0.000$; Interaction: $F = 2.167$; $p = 0.157$). Different lowercase letters indicate differences between treatments ($p \leq 0.05$) in EW and different uppercase letters indicate differences between treatments ($p \leq 0.05$) in LW.

3.3. Effects of CT on Soil-Borne Fungal Pathogen Abundance and Disease Incidence

Among the different fungal pathogens analyzed in soils by Vegalert qPCR, only *Alternaria sp.*, *Fusarium oxysporum*, *Fusarium solani* and *Stemphylium botryosum* were detected in both experiments (Table 5). Furthermore, significant differences were only observed between crop cycles: the abundance of fungal pathogens was significantly higher in late winter than in early winter (Table 5). In spite of the presence of the above-mentioned pathogens in the soils studied, the baby spinach did not show any disease incidence.

Table 5. Influence of different compost tea (CT) treatments on abundance (log-copies gen g^{-1} soil) of fungal pathogen in soil after harvesting in both experiments. Values are the mean + SD; $n = 3$). Control: sprayed distilled water (12 m^3 per ha); CT: sprayed compost tea (12 m^3 per ha); CT + Th: sprayed compost tea (12 m^3 per ha) and *T. harzianum T-78* (5×10^8 cfu mL^{-1} per ha). EW: early winter; LW: late winter.

Fungal Pathogens	Treatment	EW	LW
Alternaria spp.	Control	4.64–10.44	5.95–10.92
	CT	4.88–10.35	5.79–10.20
	CT + Th	5.17–10.09	6.19–10.19
F. oxysporum	Control	4.32–10.39	5.62–10.07
	CT	4.23–10.13	5.95–10.21
	CT + Th	3.87–10.31	6.12–10.01
F. solani	Control	4.99–10.05	6.47–10.07
	CT	4.88–10.32	6.76–10.12
	CT + Th	4.81–10.47	6.82–10.03
Stemphylium botryosum	Control	4.00–10.15	4.74–10.21
	CT	3.95–10.12	4.97–10.22
	CT + Th	3.85–10.16	5.13–10.14

Table 5. *Cont.*

	Treatment		Experiment		Interaction	
	F	**P**	**F**	**P**	**F**	**P**
Alternaria spp.	1.165	0.340	22.772	0.000	0.291	0.752
F. oxysporum	0.517	0.607	269.759	0.000	7.011	0.008
F. solani	0.383	0.689	317.683	0.000	2.748	0.098
Stemphylium botryosum	286.72	0.000	967.035	0.000	308.609	0.000

4. Discussion

The compost showed similar nutrient contents, pH and EC values to those of other agro-industrial composts [22,23]. The germination index of the compost (88%) demonstrated no negative germination effect [24] considering that compost reached the stable and mature stage validating its suitability for using in compost tea preparation [25–27].

4.1. Effects of CT on Baby Spinach Growth and Quality

Applying CT produced a higher spinach yield and foliar area than the control treatment. This improvement corroborates previous studies, such as those conducted by Hargreaves et al. [28], Marin et al. [29] and Bernal-Vicente et al. [26]. This fact could be attributed to a combination of factors, including beneficial plant microorganisms like biostimulants, biofertilisers, biopesticide microorganisms and/or growth promoter compounds like phytohormones that can be found in compost teas [30,31]. In our experiment, in general, no nutrient differences were observed in the spinach tissue in the CT treatments, except in the case of Ca and P. The amount of nutrients applied through the CT treatment should be minimal in comparison to the amount of nutrients applied via chemical fertilizers in commercial crops. However, Micheal [32] pointed out that nutrients from compost tea could be also responsible for yield increase, demonstrating that as soon as one hour after application, some nutrients were in the plant rhizosphere available to be taken in. From our results, it therefore seems more plausible that a kind of biostimulation could be responsible for the yield increase. Furthermore, Morales-Corts et al. [7] indicated that CT from green waste materials usually shows the presence of indole 3-acetic-acid (IAA) and salicylic acid-like compounds [33] that could positively affect spinach growth.

The incorporation of *T. harzianum* into the compost tea (CT + Th) also increased the spinach yield. It could be due to the secondary metabolites released by *T. harzianum* that might influence plant growth, mainly through signaling hormone-like compounds, most notably auxins [33]. The positive effect of *T.* on plant growth is equally remarkable and has been recognized as an ability independent to its antifungal ability [34–36], because an increase in growth has been observed in the absence of any detectable diseases and in sterile soil [37].

Bioactive compounds (flavonoids, phenolic acids, and tannins, among others) are extra nutritional constituents that naturally occur in small quantities in plant and food products, and they are considered human health-promoters [38,39]. The incorporation of *T. harzianum* into the compost tea (CT + Th) also increased the antioxidant capacity and phenolic compounds by 42% and 29%, respectively, compared to the control and CT treatments in the late winter but not in early winter, probably due to the higher amount of *T. harzianum* in the soil. Hua-Bin et al. [40] found a strong correlation between antioxidant activity and the total phenolic and flavonoid content in plants suggesting that phenolic compounds could be the major contributor to antioxidant capacity, thus activating or priming induced systemic resistance mechanisms [41] Similar results were observed by Yedidia et al. [42] in cucumber plants treated with *Trichoderma asperellum* and by Pascale et al. [41] in grapes treated with two *Trichoderma* strains.

Nitrate concentrations accumulated in the edible parts of the spinach leaves must be within the legal EU limits (<3500 mg kg^{-1} FW) (Commission regulation No1258/2011). The nitrate values of both commercial trials in this study were under the maximum allowed. The most notable result in terms of nitrate content in our assay is the fact that the spinach tissue in the CT treatment showed lower nitrate levels than in the other treatments in early winter. The reduced nitrate level in different vegetables like lettuce when is treated with compost and compost tea is something to take into account for human health and has been previously investigated [39,43] The most plausible explanation for this decrease is related to the potential inhibition of nitrifying bacteria due the potential organic mineralization rate from the incorporated CT, which would produce nitrates reducing nitrification processes [44–46]. The obligate chemolitho autotrophs *Nitrosomonas spp.* and *Nitrospira spp*. are particularly inhibited by the presence of organic compounds [47–50]. The increase in nitrates in the CT + Th treatment, on the other hand, is probably due to the fact that *T. harzianum* can act as a root nitrate uptake helper [51,52].

It is notable that the nitrate accumulation in late winter showed values similar to those found by Manojlovic et al. [53], in the range of 1000–2300 mg kg^{-1} FW, while the early winter values were below 500 mg kg^{-1} FW, similar to the pattern observed by Kapoulas et al. [54]. This is because low light conditions influence nitrate reductase activity and decrease the conversion of nitrate into amino acids, leading to a higher concentration of nitrates [55].

4.2. *Effects of CT on Soil Chemical Properties and Soil Microbial Activity*

The application of different CTs did not increase the soil microbial activity, although we did observe improvements in baby spinach yield and quality. It is possible that the CT dose was not enough to enhance soil microbial activity after cropping [6,7] but it was sufficient to contribute supplementary beneficial substances for plant growth that were produced by microorganisms during tea production process or contained within the original material. We likely observed an increase in total carbon in CT treatments due to the higher root exudates and plant remains directly related to the higher yield observed [56,57].

The qPCR analysis indicated an abundance of different soil fungal pathogens (*Alternaria sp., Fusarium oxysporum*, *Fusarium solani* and *Stemphylium botryosum*) in all treatments after harvesting, although no symptoms were observed in the spinach crop. It is possible that the amount and type of pathogens in conjunction with the environmental factors were not enough to affect spinach crops [21], or that the spinach plants are "asymptomatic hosts".

5. Conclusions

Our results indicate that the foliar application of compost tea, from mature onion-vineyard compost, increases the yield and improves the quality of the baby spinach, which is richer in phenolic content, antioxidant capacity and flavonoids and has lower nitrate content. The efficiency of compost tea is increased when it is supplemented with *Trichoderma harzianum*, a biological control agent. We believe that this is the first work that shows the effectiveness of the application of this combination. However, further studies are needed on the mechanisms by which these benefits are obtained, particularly those relate to plant metabolomic and resistance induction. In addition, further research into this aspect could help to develop sustainable agricultural techniques based on the reduced use of fertilizers and pesticides. Overall, our findings suggest that the application of compost tea plus *T. harzianum* can be a sustainable practice in intensive cropping systems to enhance crop productivity and quality.

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Conflicts of Interest: The authors declare no conflict of interest.

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