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Solid-state fermentation of green waste for the production of biostimulants to enhance lettuce (*Lactuca sativa L.*) cultivation under water stress: Closing the organic waste cycle

Roberto Carlos Solano Porras [a], Golafarin Ghoreishi [a], Antoni Sánchez [a], Raquel Barrena [a], Xavier Font [a], Cindy Ballardo [b], Adriana Artola [a,*]

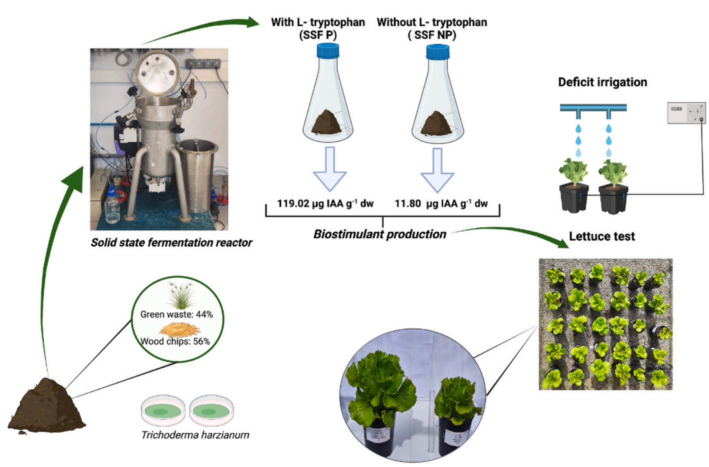
[a] *Composting Research Group (GICOM), Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain*

[b] *Solid Waste Research Centre (CIRSO), Universidad Nacional del Centro del Perú, 12006, El Tambo, Huancayo, Peru*

HIGHLIGHTS

- Solid-state fermentation is a sustainable way to produce biostimulants from green waste.
- Green waste and wood chips are used as substrate for *Trichoderma harzianum* growth.
- The application of the fermented solid with biostimulants improved lettuce growth.
- An increase in total phenol content, carotenoids, and antioxidant activity was observed.
- A valuable bioproduct was obtained and applied following circular economy principles.

GRAPHICAL ABSTRACT



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ABSTRACT

Food production faces important challenges such as water scarcity and the overall need of novel sustainable strategies. This study assesses the effect of the biostimulant produced through solid-state fermentation (SSF) of green waste (wood chips and grass residues) inoculated with *Trichoderma harzianum* with and without L-tryptophan as a precursor for indole-3-acetic acid (IAA) production, a well-known plant hormone. The fermented solid demonstrated significant positive effects on the growth of lettuce (*Lactuca sativa* L.) under different irrigation conditions. Substantial enhancements were observed in growth parameters such as fresh weight, plant height, leaf area and leaf quantity, along with chemical parameters including total phenol content, chlorophylls, carotenoids, and antioxidant activity (DPPH). The results also showed a positive impact on the nutritional quality

* Corresponding author. Department of Chemical, Biological and Environmental Engineering, Edifici Q. Carrer de les Sitges s/n. Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain.

E-mail address: adriana.artola@uab.cat (A. Artola).

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of lettuce, particularly under normal irrigation conditions. In conclusion, this study highlights the biostimulant potential to improve the yield and nutritional quality of lettuce crops by reusing plant residues. Additionally, it poses the relevance of applying circular economy principles in sustainable agriculture and organic waste management.

1. Introduction

Agriculture is facing the crucial challenge of incorporating more sustainable methods to follow the principles of the circular bioeconomy (Velasco-Muñoz et al., 2022). The application of these principles aims to improve and optimize resource use, to minimize waste and promote its reuse throughout the food chain (Borrello et al., 2017). However, the successful integration of these wastes will depend on the availability of solid technologies as proper management of these residues is essential for achieving more efficient and sustainable agriculture (Haque et al., 2023; Koul et al., 2022). Specifically, the reuse of this waste in the production of biostimulant materials not only addresses the waste issue but also contributes to closing the loop of an integrated management system (Ashokkumar et al., 2022; Tolisano and Del Buono, 2023).

In this sense, Solid-State Fermentation (SSF) has become a promising technique to produce different bioproducts from organic waste. This is the case of biostimulants (Solano Porras et al., 2023). In this process, a combination of organic solid substrate and fungi, including beneficial fungi like *Trichoderma harzianum,* has been used, allowing not only the valorisation of organic waste but also to obtain a product with biostimulant properties containing indole-3-acetic acid (IAA) (Ghoreishi et al., 2023). IAA is a key phytohormone, derived from the physiological precursor of auxins, L-Tryptophan (Naveed et al., 2015), that regulates various growth processes and the development of plants (Bunsangiam et al., 2021; Gören-Sağlam et al., 2020). Besides *Trichoderma* sp., other fungal species, such as *Aspergillus flavipes* have demonstrated the ability to synthesize IAA (do Prado et al., 2019). The addition of L-Tryptophan as a precursor in IAA production by SSF plays a crucial role in the significant increase of the final IAA concentration (Ghoreishi et al., 2023). Furthermore, fungi as *Trichoderma* sp. present biostimulant effects by themselves (Illescas et al., 2021). Similarly, the production and use of *Arthrospira platensis* as a protein source in food has been reported under a zero-waste approach, where the waste biomass remaining after protein extraction was evaluated as plant biostimulant, resulting in the increase of root growth, cotyledon weight, total biomass, and chlorophyll content in wheat cultivation (Villaró et al., 2023). Additionally, the biostimulant potential derived from plant biomass of various species not commonly used for this purpose has been highlighted, showing an increase in shoots, roots, and photosynthetic pigments in cabbage cultivation (Godlewska et al., 2019). Therefore, the production of biostimulants from green or agricultural waste not only promotes circularity, but its use can also represent a step towards more environmentally friendly agriculture.

Lettuce (*Lactuca sativa* L.*)* is a worldwide widely consumed vegetable valued for its nutritional importance and versatility in the kitchen (Medina-Lozano et al., 2021). It has become an essential ingredient in salads and numerous culinary dishes due to its fresh flavour and crisp texture (Kim et al., 2016). It belongs to the *Asteraceae* family (Choi et al., 2020). Its high content of dietary fibre, vitamins and folic acid is appreciated for human health, benefiting digestion and contributing to disease prevention (Shi et al., 2022). However, lettuce cultivation faces constant threats because of various limiting factors. Pests and diseases, such as aphids, thrips and mildew can reduce production posing a constant challenge for growers (Barriere et al., 2015). Additionally, abiotic factors, such as adverse weather conditions and prolonged droughts, also harm production (Francini and Sebastiani, 2019). Lettuce is particularly vulnerable to drought, that can have a significant impact on food security and the economic stability of farmers (Stephan et al., 2023; He et al., 2019). In this context, deficit irrigation emerges as a potential strategy for the efficient use of water in agriculture (Chaves et al., 2010). This practice, which involves the administration of an amount of water below the necessary to fully meet the water demand of plants (Chai et al., 2015). However, its proper application is crucial to maximize crop yield and minimize water supply wastage (Zhang et al., 2006).

Considering the principles of circular economy, the aim of this research was to assess the potential of the biostimulant produced through SSF of green waste (using *T. harzianum* with L-Tryptophan as IAA precursor) in lettuce cultivation. To accomplish this objective, SSF of green waste was developed at pilot scale and the resulting products tested in the growth of lettuce under greenhouse conditions. Growth and chemical parameters were measured under different irrigation conditions during lettuce cultivation. This study highlights the importance of valorising organic waste by promoting SSF as an innovative and sustainable strategy for producing high-value-added agricultural products.

2. Materials and methods

2.1. Production of biostimulant through SSF

2.1.1. SSF conditions and set-up

SSF to produce biostimulant involved the utilization of lawn grass clipping (from Universitat Autònoma de Barcelona Campus) and wood chips as bulking agent. *Trichoderma harzianum* served as the microorganism in this process. SSF was developed in a 22-L packed bed reactor consisting of a stainless-steel cylinder and a removable basket. This reactor was equipped with inlet and outlet valves, where a constant air flow (1000 ml/min) passed through the material inside the reactor for the prevalence of aerobic conditions during SSF. The oxygen percentage in the outlet air was measured using an oxygen sensor (Alphasense, UK) and the specific Oxygen Uptake Rate (sOUR) was calculated by a self-made software based on an Arduino system.

Two SSF experiments were developed with and without L-Tryptophan in the initial fermentation media (Table 1) obtaining SSF products with L-Tryptophan as precursor (SSF P) and without precursor (SSF NP). Temperature was monitored using button temperature sensors (Standard Thermochron iButton device, Maxim Integrated, U.S) through the entire fermentation process. After the SSF process was completed, the resulting material (fermented solid) was stored in a freezer until its application in lettuce cultivation (less than one week) without any further treatment. Optimum moisture content, temperature, and SSF time process were previously determined by Ghoreishi et al. (2023), where other details of the IAA production process through SSF can be found.

2.1.2. Analytical methods for the characterization of the fermented solid

To prepare the suspension for the analysis of IAA concentration and counting *Trichoderma harzianum* spores, 20 g of the fermented solid were taken from different points of the SSF reactors and mixed with 100 ml of ultrapure water. The mixture was stirred in an incubator at 180 rpm for 20 min at room temperature. Spore count was undertaken in a Neubauer chamber (Brand™ 717805). To analyse IAA, the suspension was centrifuged at 10,000 rpm for 15 min at 4 °C, the supernatant was collected and filtered through a 0.22 μm membrane. The evaluation and quantification of IAA in this study were performed using a Dionex Ultimate 3000 HPLC system, which includes an Ultimate 3000 autosampler and an Ultimate 3000 matrix detector. An LC Kinetex 5 μm EVO C18 100 A chromatographic column (250 × 4.6 mm) was used, with

Chromeleon software. The mobile phase A was a 2.5% acetic acid solution in milli-Q water, and mobile phase B was 80% acetonitrile in milli-Q water. The detection wavelength was set at 280 nm, the column temperature was maintained at 30 °C, and the flow rate was 0.7 mL min^{-1}. The total run time was 15 min, with the IAA peak observed at 11 min. Additionally, a standard curve was prepared using pure IAA (98%) from Merck Sigma-Aldrich (CAS 87-51-4) at different concentrations. A 5 ppm IAA aliquot was added to all samples to improve analysis and reduce matrix effects. The analysis of IAA was conducted in triplicate for the extract of each fermented solid. This method was based on that used by Ghoreishi et al. (2023), with some modifications.

The elemental characterization and determination of components in the SSF P were conducted through an external chemical analysis service (Scientifico-technical services of the Universitat Autònoma de Barcelona, Spain). To carry out this characterization, the material was dried and crushed beforehand. The following parameters were determined as weight percentages through elemental analysis: carbon (C), nitrogen (N), sulfur (S), and organic carbon (OC). Measurements were performed using the combustion method at 1200 °C in an oxygen atmosphere. Quantification was conducted via gas chromatography using a Thermo Scientific Flash 2000 CHNS Elemental Analyzer. The content of macronutrients (in mg/g, dry weight) was also determined, including magnesium (Mg), potassium (K), calcium (Ca), and iron (Fe). Additionally, the concentrations of trace micronutrients—copper (Cu), zinc (Zn), and molybdenum (Mo)—were quantified (in μg/g, dry weight). The analysis was performed using an Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS), These data are presented in Table 2.

Table 2
Elemental characterization (macro and micronutrients) of the fermented solid.

Parameter	Concentration
Elemental characterization (%)	
C	**40.3**
N	**2.01**
S	**0.17**
Organic carbon (OC)	**39.4**
Macronutrients (mg/g dw)	
Mg	**1.8**
K	**5.0**
Ca	**12.2**
Fe	**1.2**
Micronutrients (μg/g dw)	
Cu	**8.6**
Zn	**37.4**
Mo	**1.7**

2.2. Experimental conditions for fermented solid application in lettuce cultivation

2.2.1. Soil and cultivation conditions

The experiment took place at the Agricultural Experimental Station of the Universitat Autònoma de Barcelona, located in Cerdanyola del Vallès (Barcelona, Spain). Lettuce seedlings (*Lactuca Sativa* L.) of the "wonder variety" were transplanted into pots and placed in a greenhouse in August 2023. These seedlings, 25 days old, were purchased from a nearby commercial nursery (Planter Faura, Castellbisbal, Barcelona, Spain). The agricultural soil used in the experiment was collected from the Agricultural Experimental Station at a depth of 0–30 cm. Soil samples were air-dried, gently crushed and sieved through a 2 mm diameter sieve. To assess nutrient availability in the soil and determine fertilizer requirements, an analysis of the soil's physical and chemical properties was conducted, including an evaluation of water retention capacity. Additionally, the soil's physicochemical properties were characterized as follows: USDA soil textural class was determined using the Bouyoucos method; bulk density (kg/m^3) was measured by internal gravimetric method; cation exchange capacity (mEq/100 g dw) was determined by internal UV-VIS spectrometry; electrical conductivity (EC) at 25 °C was measured with a 1:5 water extract using conductimetry; organic carbon content (%) was determined by potentiometric titration; organic matter content (%) was also determined by potentiometric titration; nitrate nitrogen (mg/kg dw) was analyzed using UV-VIS spectrophotometry; phosphorus (mg/kg dw) was measured by the Olsen method with UV-VIS spectrophotometry; and potassium (mg/kg dw) was analyzed with an ammonium acetate extract using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Subsequently, seedlings were transplanted into pots filled with this agricultural soil.

NPK fertilizers equivalent to 100/50/160 kg/ha were applied following the practical guide for rational fertilization of crops in Spain, Royal Decree 47/2022 (MAFF, 2010). The maximum and minimum temperature in the greenhouse during the lettuce growing period was 52.9 ± 11.8 °C.

2.2.2. Application of fermented solid and establishment of irrigation deficit

SSF P or SSF NP were applied to the top 5 cm of agricultural soil and thoroughly mixed with soil in a percentage of 5%, relative to the weight of agricultural soil used in each pot (w/w) (Soussani et al., 2023) involving 165 g (SSF P or SSF NP) in 3300 g of agricultural soil per pot. Immediately, lettuce seedlings were transplanted.

Two factors were implemented in a completely randomized design: fermented solid application and irrigation deficit. Soil treatments were: (1) control (CT: no fermented solid added), (2) SSF P (fermented solid with precursor) and (3) SSF NP (fermented solid without precursor). Irrigation treatments included: (a) normal irrigation at 100-70% of field capacity (W+) and (b) deficit irrigation at 50% of field capacity (W-) (Petropoulos et al., 2020). Five replicates were set for each treatment.

For irrigation deficit management, soil moisture sensors (TEROS 10 model from Meter Group, USA) were used, configured with a ZL6 datalogger and monitored in real-time through the network via the Zentra Cloud program to track soil water content during the vegetative period (Ali Sarıdaş et al., 2021). Moisture sensors were placed in the planter at a depth of 12 cm.

To determine the irrigation water demand, all pots were saturated with water, waiting until complete drainage occurred. This process was conducted in triplicate, and the field capacity was determined using soil moisture sensors. Six standard soil water content curves (m^3/m^3) were constructed using six sensors, and the most representative curve was selected. Soil water content readings are expressed as a percentage of soil water content from field capacity (100%) to dry soil (0%). The irrigation frequency was adjusted based on soil water content sensor readings and the established standard curve. Collection of data of soil moisture were configured to occur every 5 min throughout the lettuce cultivation cycle, with data stored for real-time monitoring. Irrigation

Table 1
Process characteristics of SSF with *Trichoderma harzianum*, in SSF P (with precursor), SSF NP (without precursor). Substrate: wood chips (56%) and grass (44%) (w:w). IAA: indole-3-acetic acid, dw: dry weight. EC: electrical conductivity.

Fermented solid	L-Tryptophan	Final IAA concentration	SSF conditions		Final properties	
	(%)	(μg IAA g^{-1} dw)	Initial moisture (%)	Temperature range (°C)	pH	EC (mS/cm)
SSF P	0.43	119.02	74	20–27	7.78	1.23
SSF NP	0	11.80		22–29.5	7.10	1.65

was manually applied per plant. The total irrigation water amount was 5.4 L per plant for normal irrigation treatments (W+) and 2.7 L per plant for deficit irrigation treatments (W-). Measurements of physical and chemical parameters were conducted up to 45 days after transplanting when the lettuces were harvested. Crop management followed the guidelines of good agricultural practices for lettuce cultivation.

2.3. Determination of lettuce growth and chemical parameters

All lettuce plants were harvested at 45 days after transplanting. Immediately after harvesting, the following parameters were measured: fresh weight per plant (Fw), plant height, and leaf number. Subsequently, the samples were transferred to a cold room at 4 °C for preservation. To determine dry plant matter, the samples were dried in an oven at 80 °C for two days until constant weight was achieved. The plants were ground using a mortar and pestle and vacuum-sealed using a FoodSaver vacuum sealer, then stored in a freezer at −18 °C for further analysis. Fresh leaf material was utilized to determine chlorophyll content, carotenoid content, total phenolic content and antioxidant capacity.

2.3.1. Leaf area

The rosette leaf area was determined by image digitalization. All leaves from each replicate were separated, photographed using a digital camera, and the images processed with the ImageJ software 1.54 developed by Wayne Rasband (NIH), to measure the rosette leaf area for each repetition. The results were expressed in cm^2.

2.3.2. Chlorophylls and carotenoids

For the extraction of chlorophylls and carotenoids, a 0.50 g sample of crushed fresh leaves in 80% methanol was used. Afterwards, it was agitated at 120 rpm for 5 min and centrifuged at 6000 rpm at 15 °C. The determination of chlorophyll *a* and *b* content was performed by spectrophotometry (Hach lange DR 3900) measuring the absorbance of the supernatant at 666 and 653 nm (Wellburn, 1994). Carotenoid content was determined by measuring the absorbance of the supernatant at 470 nm. Total pigments were calculated by adding chlorophyll *a* and *b* amounts. The results were expressed in mg per gram of fresh weight (mg/g fw).

2.3.3. Total phenolic content and antioxidant activity

The determination of total phenolic content (TP) and antioxidant activity (DPPH) in fresh leaf samples was carried out according to the procedures previously described (Cruzado et al., 2013).

The TP content was determined using the Folin Ciocalteu reagent, with gallic acid as a standard. The absorbance of the blue-coloured solution was measured at 750 nm using a spectrophotometer (Hach Lange DR 3900). Extracts were obtained from lettuce leaves, and measurements were performed in triplicate for each repetition. The results were expressed as milligrams of gallic acid equivalents (AGE) per gram of dry matter (mg AGE g^{-1} of dry weight).

The antioxidant activity (DPPH) was determined using the 2,2-diphenyl-1-picrylhydrazyl radical assay with some modifications. A solution of DPPH in 80% methanol was prepared and poured into Eppendorf tubes previously lined with aluminium foil to protect them from light. 100 μL of the extract from each repetition was used, and this procedure was performed in triplicate. After the preparation of the solution, 2900 μL of the DPPH solution were added, followed by 1 min of agitation. Subsequently, it was allowed to stand for 30 min at room temperature. After this period, the absorbance of each sample was measured at a wavelength of 517 nm. The results were expressed in micromoles of Trolox equivalent (TE) per gram of dry matter (μmol TE g^{-1} dw), as Trolox is used as a reference standard to compare antioxidant capacities.

2.4. Statistic analysis

Analysis of variance (ANOVA) was conducted using MINITAB 19 software (InfoStat, 2020; Origin, 2022). In cases where the data did not meet the normality test, Kruskal-Wallis test, suitable for non-parametric data, were applied. For parametric data, differences among means were evaluated using a post hoc LSD test ($p \leq 0.05$). Additionally, Principal Component Analysis (PCA) was performed on growth and chemical parameters. Finally, hierarchical cluster analysis (HCA) was also conducted for growth and chemical parameters.

3. Results and discussion

3.1. Production of biostimulants from green waste through SSF

The results related to the production of biostimulant through SSF are presented in Fig. 1, including the concentration of IAA (μg IAA g^{-1} dw), *Trichoderma harzianum* spores (spore g^{-1} dw) and the specific oxygen consumption rate during the process (sOUR).

In Fig. 1A it can be observed that the presence L-Tryptophan as precursor (SSF P) resulted in a substantial increase in IAA levels, averaging 119.2 ± 15.8 μg IAA g^{-1} dw, whereas its absence (SSF NP) yielded a significantly lower level of 11.8 ± 7.3 μg IAA g^{-1} dw. Bader et al. (2020) who developed research on the effect of different *Trichoderma* isolates on tomato plant growth, reached concentrations of 13.38–21.14 μg IAA/mL after 5 days of inoculation. The positive effect of the addition of L-tryptophan as a precursor for IAA synthesis was also reported by Chagas et al. (2016) for different *Trichoderma* isolates. Although all isolates produced IAA in the culture conditions, in the cases where

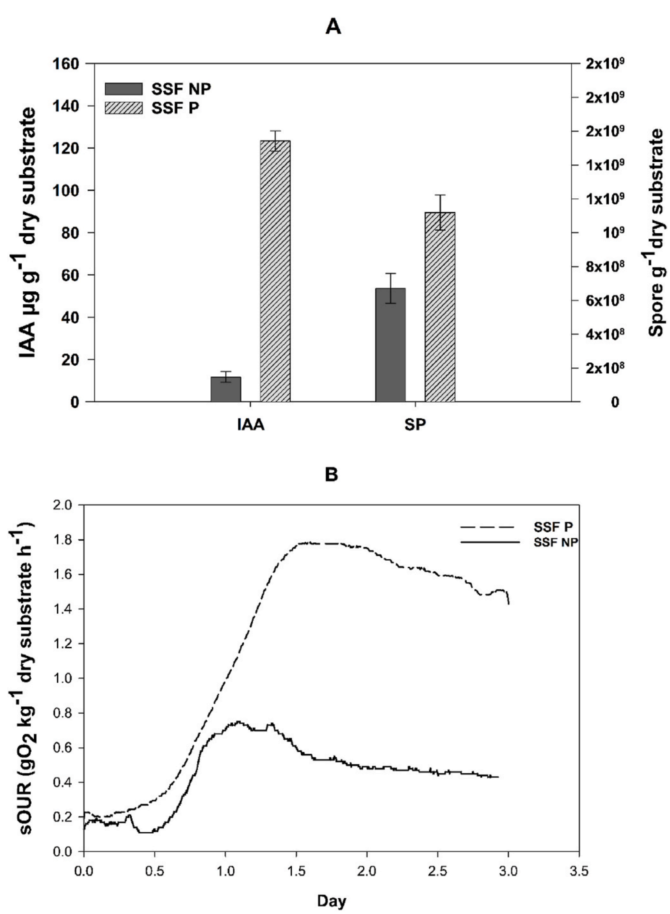

Fig. 1. SSF performance with precursor (SSF P) and without precursor (SSF NP): IAA and spores (SP) produced (A) and profile of sOUR obtained (B) in. IAA and spores are represented as average ± standard deviation.

L-tryptophan was added (100 mg/L) the increment in IAA concentration ranged from 48 to 76% after 8 days of inoculation. These findings are consistent with our research. The addition of L-tryptophan as a precursor can be considered a significant variable to optimize biostimulant production by *Trichoderma harzianum* through SSF using green waste and wood chips.

Additionally, SSF P revealed notable differences in the count of *Trichoderma harzianum* spores and the oxygen consumption curve depending on the presence or absence of L-Tryptophan. Regarding the spore count, the addition of the precursor also increased sporulation, with spore counts of $1.1 \times 10^9 \pm 3.6 \times 10^8$ and $6.7 \times 10^8 \pm 2.5 \times 10^8$ spores g^{-1} dw, in the treatments SSF P and SSF NP, respectively. A higher number of spores could enable a broader distribution and more effective interaction of *Trichoderma* with plant roots, which is crucial for activating resistance mechanisms against environmental stresses like drought and salinity, and for enhancing plant growth (López-Bucio et al., 2015).

The curve of oxygen consumption for the two fermentations is presented in Fig. 1B. The maximum sOUR for SSF P was 1.8 g O_2 kg^{-1} dw h^{-1} while this value for SSF NP was 0.7 g O_2 kg^{-1} dw h^{-1}, which is again substantially lower.

Therefore, this study revealed that using green waste and wood chips could facilitate the production of biostimulants by SSF. This could be considered environmentally sustainable and cost-effective by utilizing waste (do Prado et al., 2019). Furthermore, this approach aligns with the principles of waste management and the circular economy, offering an innovative strategy for developing more sustainable products which not only help mitigate environmental impact but also enable these new technologies to increase profitability for self-sustaining farms under a circular model (Bandh et al., 2023). However, further research is needed to assess the sustainability of these biostimulants.

3.2. *Characterization of the fermented solid and application in lettuce cultivation*

3.2.1. *Characterization of the fermented solid*

The results of the elemental characterization, macronutrients, and micronutrients of the fermented solid SSF P are presented in Table 2.

The application of 5% fermented solid (w/w), equivalent to 165 g of SSF P and SSF NP in 3300 g of agricultural soil, is similar to compost application as reported by Soussani et al. (2023). This applied amount ensured sufficient concentrations of IAA and *Trichoderma harzianum* spores in the root zone at the time of lettuce seedling transplantation. This application method could improve root access to the fermented solid, promoting better IAA absorption. IAA facilitates root elongation when applied in adequate amounts; for example, 0.4 mmol/L was shown to promote root length elongation in tea cultivation (Gao et al., 2022). Additionally, the presence of *Trichoderma harzianum* spores ($1.1 \times 10^9 \pm 3.6 \times 10^8$ and $6.7 \times 10^8 \pm 2.5 \times 10^8$ spores g^{-1} dw for SSF P and SSF NP, respectively) would facilitate the colonization of lettuce roots, potentially improving the plant's tolerance to water stress. As evidenced in previous studies, the application of 10 g of wheat grains inoculated with 10 mL of a 10^6 Trichoderma spores/mL suspension in pots containing 2000 g of soil for tomatoes cultivation improved water deficit stress tolerance (Khoshmanzar et al., 2020).

3.2.2. *Characterization of agricultural soil*

The results were as follows: the soil exhibited a loamy texture (41.1% sand, 34.4% silt, 24.5% clay); bulk density was 1082 kg/m^3; cation exchange capacity was determined as 14.0 mEq/100 g dw; EC recorded a value of 0.17 dS/m; organic matter content was found to be 2.56% (dw); nitric nitrogen content was 30 mg/kg (dw); available phosphorus was 9.79 mg/kg (dw) and K_2O was quantified at 289 mg/kg (dw).

3.2.3. *Influence in lettuce growth parameters*

The results for growth parameters, including fresh weight (Fw), plant height, number of leaves per plant, leaf area and dry matter percentage are provided in Table 3.

Regarding the main effects, SSF P treatment on fresh weight was statistically superior by 35.6%, and SSF NP by 24% compared to the control (CT). The SSF NP treatment (W-) showed a 30% statistical superiority over the CT (W-) (Table 3). Conversely, no significant statistical differences were observed in plant height, number of leaves, and dry weight. Considering irrigation factors (W+), plant height, number of leaves, dry weight, and leaf area were statistically superior by 9.5%, 16.6%, 10%, and 13.16% respectively compared to (W-) (Table 3).

In addition, we can highlight the interaction effects of SSF P, SSF NP, and CT with (W+) and (W-) that showed statistically significant differences. Treatments SSF P (W+) and SSF NP (W-) were statistically superior by 24% and 30.4% respectively in the fresh weight of lettuce compared to CT (W+) and CT (W-) respectively. For plant height, number of leaves, and leaf area, there were no statistical differences in the comparison of means (Table 2).

In this sense, it is worthwhile to mention that although biostimulants

Table 3
Effects of deficit irrigation and fermented solid application on fresh weight, plant height, number of leaves, % dry matter and leaf area.

Treatment	Fresh weight (g)	Plant height (cm)	Number of leaves (n)	dry material (%)	leaf area (cm^2)
Factor A (Fermented solid containing biostimulant)					
SSF P	148 ± 46 a	22 ± 3	17 ± 2	5.1 ± 0.6	933 ± 143 a
SSF NP	125 ± 26 ab	20 ± 2	17 ± 1	5.2 ± 0.4	886 ± 143 a
Control					
CT	95 ± 28 b	20 ± 1	16 ± 1	5.4 ± 0.3	656 ± 119 b
Factor B (Irrigation)					
W+	140 ± 37 a	21 ± 2 a	18.0 ± 0.9 a	5.5 ± 0.4 a	883 ± 200 a
W-	101 ± 22 b	19.0 ± 0.9 b	15.0 ± 0.6 b	5.0 ± 0.3 b	767 ± 140 b
Interaction of factors A x B:					
SSF P (W+)	182 ± 17 a	25.0 ± 0.8 a	18.6 ± 0.6 a	4.8 ± 0.1 b	1041 ± 99
SSF NP (W+)	136 ± 25 b	21.0 ± 1.4 a	17.6 ± 0.6 a	4.8 ± 0.07 b	898 ± 202
CT (W+)	138 ± 24 b	20.2 ± 0.5 ab	17.0 ± 0.7 ab	5.3 ± 0.1 a	711 ± 143
SSF P (W-)	101 ± 7 bc	18.6 ± 1.1 c	15.2 ± 0.8 c	5.6 ± 0.2 a	825 ± 84
SSF NP (W-)	114 ± 28 b	18.8 ± 0.2 bc	15.4 ± 0.6 bc	5.5 ± 0.07 a	874 ± 68
CT (W-)	79 ± 10 c	18.8 ± 1.1 bc	15.2 ± 0.5 c	5.4 ± 0.2 a	601 ± 63
Significance					
Fermented solid containing biostimulant	**	ns	ns	ns	**
Irrigation (W)	**	**	**	**	**
Fermented solid containing biostimulant x W	**	**	**	**	ns

SSF P (with precursor), SSF NP (without precursor), CT (control), normal irrigation at 100-70% of field capacity (W+), irrigation deficit at 50% of field capacity (W-), SSF P (W+) (Interaction with precursor + normal irrigation), SSF NP (Interaction without precursor + normal irrigation), CT (W+) (control interaction + normal irrigation), SSF P (W-) (Interaction with precursor + deficit irrigation), SSF NP (W-) (Interaction without precursor + deficit irrigation), CT (W-) (Control interaction + deficit irrigation). The results represent the median ± standard deviation. The letters (a, ab, bc, b, c) in each column indicate significant differences based on the post hoc test ($p > 0.05$). The symbol "**" denotes statistically significant differences, while "ns" indicates no significant differences were found. Results without letters (a, ab, bc, b, c) indicate that no significant differences were found.

have been reported to enhance lettuce crop performance, including growth parameters (Malécange et al., 2022), these effects can vary depending on the type of biostimulant used and the specific conditions of each crop (Clément et al., 2023). In this study, a significant increase was observed in the growth parameters of lettuce cultivation, (Table 3), including fresh weight, plant height, and leaf number, in response to the application of fermented solid containing biostimulants produced by *Trichoderma harzianum.* As stated above, IAA promotes cell division and elongation (Campanoni and Nick, 2005), which could explain the effect observed in the increase in fresh weight of lettuce cultivation in treatments. Different works reported the effect of *Trichoderma harzianum* and IAA in crops. Moreira et al. (2022) observed that lettuce fresh leaf yield increased by 24% with *Trichoderma harzianum* inoculation, while Eslahi et al. (2022) attributed the increase in fresh weight and root length in bean seeds specifically to the effect of IAA produced by *Trichoderma harzianum***.** Also, Illescas et al. (2021) found that the application of *Trichoderma harzianum* in wheat, combined with IAA, resulted in higher weights compared to the control. These results are in accordance with those found in this study; however, to our knowledge, this is the first time when biostimulant produced by the SSF of green waste and wood chips has been used to promote lettuce cultivation in a circular approach.

It is also crucial to highlight the effect of irrigation as a key factor in plant growth (Seidel et al., 2017). On one hand, water is essential as it influences crop yield reflected in increased lettuce development and weight (Table 3). Furthermore, it was evidenced that SSF P (W+) and SSF NP (W-) treatments were superior in certain growth parameters such as FW and number of leaves compared to CT (W+) and CT (W-) respectively. This indicates a positive tolerance to the effect of fermented solid containing biostimulant under different irrigation conditions in lettuce cultivation. It is important to highlight the role of water in nutrient absorption (Scharwies and Dinneny, 2019) and in maintaining cellular turgor (Illescas et al., 2021). Irrigation deficit, applied to the control treatment CT (W-), limited the amount of water available for lettuce cultivation, resulting in lower values for growth parameters, specifically in reduced fresh weight, number of leaves, plant height, leaf area, and dry weight (Table 3). This underscores the importance of providing an irrigation plan management to achieve an optimal lettuce yield.

The activity of biostimulants produced by *Trichoderma harzianum* was limited under 50% irrigation conditions in fresh shoot and root weight in wheat cultivation, as reported by other authors (Silletti et al., 2021). This aligns with these results, (Table 3), emphasizing the significant effect of irrigation. However, the dry matter percentage in the treatments SSF P (W-) and SSF NP (W-) resulted in higher values compared to the treatments SSF P (W+) and SSF NP (W+) by 15.6% and 12.9% respectively (Table 3). These results suggest a potential adaptation of this lettuce variety to deficit irrigation conditions (W-) by producing a higher amount of dry matter. These findings are consistent with the increase in dry matter content under a deficit irrigation regime with the application of biostimulants found by Chaski and Petropoulos (2022). Some plants can activate defence and adaptation in response to stress, which may involve an increase in the production of secondary compounds like metabolites to protect against water stress (Isah, 2019). Dry matter content in this context could be attributed to a high production of these metabolites, such as polysaccharides, sugars, or phenolic compounds, which help in water retention and protect the plant against oxidative damage (Keunen et al., 2013). These compounds may contribute to a higher dry matter percentage without a substantial increase in total biomass under deficit irrigation treatments (W-).

Additionally, the application of biostimulants at the beginning of lettuce cultivation transplantation emerges as a key factor in the observed positive effects on growth parameters (Chaski and Petropoulos, 2022). At this application stage, tied to a critical period in plant development, early stimulation can trigger beneficial effects throughout the vegetative period. In this study, the SSF P treatment, with 119.02 μg IAA g^{-1} dw, had a positive effect by increasing the weight of the lettuce crop by 35.6%, while SSF NP, with 11.80 μg IAA g^{-1} dw, showed a 24% increase compared to CT (Table 3). IAA at doses of 50 and 150 ppm demonstrated higher effects on bulb fresh weight in onion cultivation (Solano et al., 2023). In chickpeas, the application of 61.53 μg/ml of IAA produced by *Enterobacter hormaechei* and *Brevundimonas naejangsanensis* improved plant attributes, grain yield, and nutritional content (Mukherjee et al., 2022). This suggests that applying biostimulants at the time of lettuce cultivation transplantation can maximize its effectiveness in promoting growth parameters, which is a crucial information for sustainable agriculture and crop production improvement. In future research, it would be valuable to explore the specific mechanisms behind the improvement in growth parameters in other crops induced by biostimulants.

3.2.4. Influence on lettuce chemical characteristics

The chemical parameter results, including chlorophyll *a* and *b*, carotenoids, total pigments, total phenols, and DPPH, are detailed in Table 4. For the main effects, SSF P significantly increased chlorophyll *a*, total pigments, and DPPH by 27.3%, 16.6%, and 42.5% respectively compared to CT. SSF NP significantly enhanced carotenoid content by 19.4% compared to CT. However, chlorophyll *b* and total phenols showed no significant difference. Under main irrigation factors (W+), chlorophyll *a*, *b*, carotenoids, total pigments, and phenols were significantly higher by 28.3%, 25.9%, 22.9%, 27.3%, and 22.6% respectively compared to (W-), (Table 4).

In the interaction of factors SSF NP and CT (W-), statistically significant differences were observed in the SSF NP (W-) treatments, which were superior by 40.2% and 25.3% in carotenoid content and chlorophyll *b* respectively compared to CT (W-). For chlorophyll *a*, total pigments, total phenols, and DPPH, no significant statistical differences were found. These observations highlight the effect on photosynthetic and antioxidant parameters, emphasizing their effectiveness in improving the physiological response of plants due to the effect of fermented solids with biostimulant properties.

Chlorophylls play a crucial role in photosynthesis (Mandal and Dutta, 2020), suggesting a positive impact on photosynthesis due to the application of the fermented solid. These results imply that the application of this fermented solid can influence total pigment content, enhancing photosynthetic efficiency and thereby contributing to the reported increase in growth parameters in this study.

These results have been confirmed by other authors using IAA. The application of IAA produced by *Trichoderma harzianum* had a positive effect on total pigment content and growth rate in *Vigna unguiculata* (Mendes et al., 2020). It increased chlorophyll content in Arabidopsis roots and had multivariate effects on growth and chemical parameters (Nieto-Jacobo et al., 2017). Likewise, carotenoid content has protective functions and serves as potential antioxidant in plant stress (Rey et al., 2020).

These results may support the hypothesis of the potential effect of fermented solids with biostimulant properties from plant residues, leveraging nutrients from grass waste (Verduzco-Oliva and Gutierrez-Uribe, 2020).

The limited water supply inhibits plant photosynthesis, causing changes in chlorophyll content and components and damage to the photosynthetic apparatus (Waraich et al., 2011). These findings can be related to the role of IAA in cell growth and shoot elongation (Tank et al., 2015; Zhang et al., 2006). Possible physiological mechanisms could involve the regulation of plant growth facilitated by the presence of IAA (Nieto-Jacobo et al., 2017). The interaction of fermented solid and irrigation conditions highlights the importance of understanding how they interact with environmental factors to maximize their benefits in sustainable agriculture (Rouphael and Colla, 2020). Therefore, the application of these fermented solids derived from plant residues and *Trichoderma harzianum* emerges as a promising strategy to enhance these photosynthetic pigments, thereby improving crop yield and quality.

Table 4
Effects of deficit irrigation and fermented solid application on the levels of chlorophyll *a*, chlorophyll *b*, carotenoids, total pigments total phenol content (gallic acid equivalents) and antioxidant activity (DPPH) in lettuce cultivation.

Treatments	Chlorophyll *a* (mg g^{-1} fw)	Chlorophyll *b* (mg g^{-1} fw)	Carotenoids (mg g^{-1} fw)	Total pigments (mg g^{-1} fw)	Total phenol (mg AGE g^{-1} dw)	DPPH (μmol TE g^{-1} dw)
Factor A (Fermented solid containing biostimulant)						
SSF P	16.5 ± 2.2 a	10.2 ± 2.5	4.7 ± 1.4 ab	24.8 ± 3.5 a	4.9 ± 1.0	150 ± 26 a
SSF NP	14.6 ± 3.8 a	11.1 ± 3.6	5.6 ± 1.1 a	27.6 ± 7.1 a	4.3 ± 1.1	137 ± 41 a
Control						
CT	12 ± 4 b	8.6 ± 2.5	4.5 ± 1.3 b	20.7 ± 6 b	4.0 ± 1.3	86 ± 30 b
Factor B Irrigation						
W+	16.7 ± 2.8 a	11.5 ± 2.7 a	5.6 ± 1.1 a	28.± 5 a	4.9 ± 0.9 a	126 ± 52
W-	12 ± 3 b	8.5 ± 2.5 b	4.3 ± 1.2 b	20.5 ± 4.9 b	3.9 ± 1.1 b	122.5 ± 32.6
Interaction of factors A x B:						
SSF P (W+)	16.3 ± 1.5	9.9 ± 0.7 bc	5.8 ± 1.2 a	26.3 ± 2.4	5.5 ± 0.8	163.8 ± 24.2
SSF NP (W+)	18.7 ± 3.7	13.6 ± 1.5 a	5.4 ± 1.4 a	32.3 ± 6.5	4.9 ± 0.8	147.8 ± 41.9
CT (W+)	15.00 ± 1.6	10.9 ± 0.5 ab	5.5 ± 0.9 a	26. ± 3	4.5 ± 1.3	66.3 ± 21.3
SSF P (W-)	12.9 ± 1.2	10.38 ± 1.78 bc	3.7 ± 0.7 b	23.3 ± 4.2	4.3 ± 0.4	136 ± 21
SSF NP (W-)	14.3 ± 2.5	8.7 ± 0.6 c	5.8 ± 0.4 a	23 ± 4	3.7 ± 1.2	126 ± 43
CT (W-)	8.8 ± 0.72	6.5 ± 0.3 d	3.4 ± 0.5 b	15.3 ± 1.3	3.6 ± 1.2	105.8 ± 24.7
Significance						
Fermented solid containing biostimulant	**	*ns*	**	**	ns	**
Irrigation (W)	**	**	**	**	**	ns
Fermented solid containing biostimulant x W	*ns*	**	**	*ns*	ns	ns

SSF P (with precursor), SSF NP (without precursor), CT (control), normal irrigation at 100-70% of field capacity (W+), irrigation deficit at 50% of field capacity (W-), SSF P (W+) (Interaction with precursor + normal irrigation), SSF NP (Interaction without precursor + normal irrigation), CT (W+) (control interaction + normal irrigation), SSF P (W-) (Interaction with precursor + deficit irrigation), SSF NP (W-) (Interaction without precursor + deficit irrigation), CT (W-) (Control interaction + deficit irrigation).The results are expressed as mean ± standard deviation. The letters (a, ab, bc, c, d) in each column indicate significant differences according to the Fisher LSD post hoc test ($p > 0.05$). The symbol "**" denotes statistically significant differences, while "ns" indicates no significant differences were found. Results without letters (a, ab, bc, b, c) indicate that no significant differences were found.

The total phenolic content and antioxidant activity (DPPH) were also evaluated. Other studies have highlighted the biostimulant effect based on *Trichoderma* sp. Increased levels of polyphenols, flavonoids and DPPH in *Passiflora caerulea* (Şesan et al., 2020). Fertilizer applications with *Trichoderma* sp. can enhance yield, producing a higher-quality product with increased antioxidant content in horticultural crops (Lanzuise et al., 2022). The application of *Trichoderma harzianum* can also enhance the antioxidant mechanism under abiotic and biotic stress conditions (Mastouri et al., 2012). Our results showed a significant increase in chlorophyll *a*, *b*, total pigments, and total phenols due to irrigation (W+) (Table 4). Additionally, an increase in DPPH was observed, especially with the application of fermented solids with biostimulant properties (SSF P and SSF NP), as noted in Table 4. This indicates that SSF products not only stimulate growth parameters, as discussed earlier, but also play a key role in enhancing plants antioxidant response to various limiting factors, such as biotic and abiotic stress. The application of IAA produced by *Trichoderma harzianum* can activate the antioxidant response metabolism in plants, protecting them from stress (Illescas et al., 2021).

Beyond the specific effects on phenolic content and DPPH, it is essential to consider nutritional quality. The high presence of phenolic content suggests not only a higher antioxidant capacity but also a potential increase in these phytochemical compounds beneficial for human health (González-Burgos and Gómez-Serranillos, 2021). This aspect is important in the context of food production, focusing not only on performance bulk indicators but also on nutritional quality and its benefits for end consumers. Overall, these results related to the improvement of total phenol content and DPPH reinforce that the effect of applying fermented solids with biostimulant properties and the optimized type of irrigation can be an effective strategy to improve both the production and nutritional quality of crops. These findings also pave the way for future research on the specific interaction between fermented solid containing biostimulant and the phytochemical composition of plants in different agricultural contexts.

3.3. Analysis of principal components and clustering

3.3.1. Principal Component Analysis

The Principal Component Analysis (PCA), depicted in Fig. 2, offers a comprehensive overview of the effect of the fermented solid with biostimulant properties derived from green waste under various irrigation regimes, assessing the interplay between growth parameters and chemical responses in lettuce cultivation. The analysis yielded two primary components accounting for 68.81% of total variance. The first principal component (PC1), elucidating 53.8% of variability, correlates with growth metrics and crop development, as indicated by fresh weight, plant height, leaf number, and leaf area. Moreover, a strong

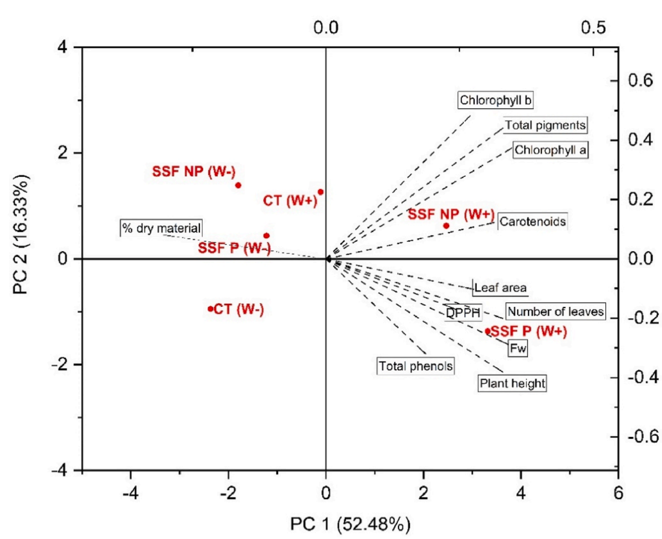

Fig. 2. Principal Component Analysis (PCA) of growth and chemical parameters under different treatments of SSF P and SSF NP application, under normal irrigation (W+) and deficit irrigation conditions (W-) in lettuce cultivation.

affiliation of DPPH with PC1 intimates a potential linkage with lettuce growth, denoting a beneficial impact of biostimulant application on both plant development and enhancement of antioxidant mechanisms (Desoky et al., 2021). The action mode of SSF P and SSF NP might be tied to auxin production, particularly IAA. This aligns with findings that biostimulants, abundant in organic compounds and microbes, positively influence biomass and stress resilience (Islam et al., 2023; Saia et al., 2019). The second component (PC2), embodying 16.33% of the variability, is associated with dry matter content. An uptick in dry matter percentage in SSF NP (W-) treatment, as reflected in PC2, underscores a potential adaptative response to hydric stress from deficit irrigation (W-), which is consequential for crops facing climate change challenges. It also underscores the imperative to reduce and optimize water allocation amid scarcity (Playán and Mateos, 2006). Treatments SSF P (W+) and SSF NP (W+) gravitate towards the apex of positive values on PC1, suggesting an enhanced physical parameter performance and elevated DPPH levels compared to the control (CT). Fw displays a negative association with PC1, predominantly aligning with SSF NP (W-) and SSF P (W-), indicating lower Fw values for these treatments. PCA implies that fermented solid application under well-watered conditions (W+) enhances several physical parameters, supporting the theory that optimal irrigation strengthens biostimulant efficacy in fostering plant growth (Petropoulos et al., 2020). Conversely, SSF P (W-) and SSF NP (W-) treatments, situated at the negative end of PC1, may reflect lower scores in the physicochemical parameters examined, interpreting these treatments as less effective compared to those with standard irrigation. This underscores the significance of irrigation management as a critical determinant shaping crop response to biostimulants (Franzoni et al., 2022). Despite biostimulants propensity to augment water stress tolerance, our findings indicate that deficit irrigation (W-) might curtail the benefits of their application (Turan et al., 2023). The CT (W+) and CT (W-) treatments, located nearer to the origin, may represent an intermediate influence or a non-significant effect of the fermented solid compared to other treatments concerning the parameters assessed in PC1. Chemical variables, such as chlorophyll *a*, *b*, and total pigments, with vectors directed towards the SSF NP (W+) treatment, suggest a favourable impact on these parameters. The aligned orientation of these photosynthetic pigment-associated variables underlines the notion that biostimulants might enhance plant growth and quality via photosynthesis enhancement, potentially leading to increased chlorophyll content and photochemical efficiency (Michalak et al., 2021) as well as enhanced uptake of essential nutrients, directly implicating crop production where augmented photosynthetic pigments could significantly increase biostimulant use (El-Nakhel et al., 2022).

3.3.2. Cluster analysis for growth and chemical parameters

The Hierarchical Cluster Analysis (HCA), depicted in the dendrogram (Fig. 3), utilized squared Euclidean distance as the dissimilarity metric between treatments, grouping them into two clusters based on their responses in growth parameters and chemical properties.

The first group, comprising SSF P (W+) and SSF NP (W+), aligned with standard irrigation conditions (W+), displays a high degree of similarity in both growth parameters and chemical properties. These results emphasize that the application of fermented solids with biostimulants properties, in conjunction with regular watering (W+), results in a tighter clustering, potentially having a positive impact on the growth development of lettuce crops. The second group, including CT (W+), CT (W-), SSF P (W-), and SSF NP (W-), exhibits significant differences in growth parameters and chemical properties, demonstrating no significant effect. This indicates that these treatments do not respond in a similar manner to the application of SSF P and SSF NP. However, it is also clear that water stress imposes growth restrictions, as evidenced by the separate clustering of the treatments under deficit irrigation (W-), which suggests that their effect on crop enhancement might be limited compared to the first group. This could be attributed to the presence of adequate moisture facilitating the absorption of nutrients and active

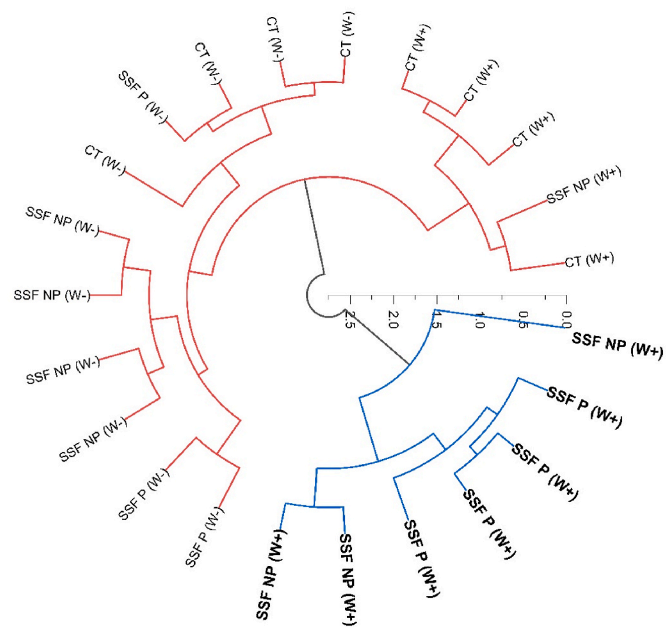

Fig. 3. Cluster dendrogram for growth and chemical parameters in SSF P, SSF NP with application of (W+) and (W-).

components, such as the utilization of IAA from the fermented solids. These outcomes underline the potential effect of applying SSF P and SSF NP, as well as the (W+) irrigation conditions, on the results in improving optimal plant growth These results emphasize the need to develop new management strategies that not only focus on biostimulant application but also on optimal irrigation management to promote the growth and development of crops in the face of climate variability (Deligios et al., 2019). In addition, the results highlight the possibility of adopting circular economy principles in the recycling and reuse of green waste, developing biostimulants from green waste and wood chips, thus adding added value and accompanied by appropriate irrigation management.

4. Conclusions

This study emphasizes sustainable agricultural practices in the framework of the circular economy, highlighting the transformative potential of green waste and wood chips as a significant resource to produce an organic amendment with biostimulant properties. The application of the fermented solids obtained from green waste with *Trichoderma harzianum* with (SSF P) and without (SSF NP) L-Tryptophan as a precursor for IAA production were found to positively influence fresh weight, leaf area, and overall biomass of lettuce, outperforming control treatments (CT), particularly under normal irrigation conditions (W+). Additional growth parameters such as plant height and leaf count also exhibited higher values, especially with adequate watering (W+). The application of SSF NP demonstrated a pronounced effect on dry matter content under deficit irrigation (W-), linking improved performance directly to the biostimulants capacity to enhance plant development, suggesting their potential as crop growth enhancers. Chemical parameters like chlorophyll *a*, carotenoids, total pigments, and DPPH were significantly elevated with the application of SSF P and SSF NP. Additionally, total phenolic content particularly increased under normal irrigation (W+), while the SSF NP (W-) interaction resulted in higher carotenoid and chlorophyll *b* levels compared to the CT (W-). In conclusion, this study offers evidence that SSF P and SSF NP can be effective tools for boosting the growth and development of lettuce crops under standard irrigation conditions (W+). These findings contribute to the advancement of knowledge in the field of the circular economy in agriculture, emphasizing the importance of integrating innovative approaches to ensure higher agricultural yields alongside food safety and quality.

CRediT authorship contribution statement

Roberto Carlos Solano Porras: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Golafarin Ghoreishi:** Writing – original draft, Methodology, Investigation. **Antoni Sánchez:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Raquel Barrena:** Writing – review & editing, Supervision, Conceptualization. **Xavier Font:** Writing – review & editing, Supervision, Conceptualization. **Cindy Ballardo:** Supervision, Conceptualization. **Adriana Artola:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antoni Sanchez reports financial support was provided by Spain Ministry of Science and Innovation. Roberto Carlos Solano-Porras reports financial support was provided by PRONABEC-Perú. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143919.

Data availability

Data will be made available on request.

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