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ORIGINAL RESEARCH

Chlorophyll performances as an indicator of compost quality: Effectiveness of liquid humic substances and compost tea

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Abstract

Purpose: This study was carried out with the aim of improving the use of compost products as compost tea and humic substances fertilizers, to study the evolution and the degree of compost maturity, and to use the chlorophyll as an indicator of compost quality.

Method: The compost tea and humic substances were prepared from different stages of a windrow compost (1/2 dewatered lagooning sludge + 1/2 green waste), the mineral content was investigated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis. The growth test was carried out in a greenhouse for two months on two plants: *Trigonella foenum-graecum* (Fenugreek) and *Lepidium sativum* (Cress). Plant observations including stem elongation, biomass, stomatal conductance, chlorophyll fluorescence and total chlorophyll content in the leaf were investigated at the end of the experiment.

Results: The findings revealed that compost tea and humic substances resulting from the initial stage and intermediate stage of composting present a lower stimulating effect on plants growth (biomass: 0.83-1.42 g/plant; total chlorophyll: 0.48-0.71 mg/plant). While those from the final stage showed significant effects on biometric and physiological properties (biomass: 1.399-2.396 g/plant; total chlorophyll: 0.74-0.83 mg/g). Furthermore, the chlorophyll performance, as a good indicator to determine plant stress and soil pollution, confirmed the maturity and the good quality of the final compost.

Conclusion: Compost tea and humic substances treatments approved their bioactive properties and beneficial effects on plant growth and their physiological properties.

Keywords: Compost tea, Humic substances, Chlorophyll, Compost maturity, *Trigonella foenum-graecum*, *Lepidium sativum*

Introduction

Organic food becomes more required worldwide, people tend to have healthier and environmentally friendly agriculture systems (Scotti et al. 2016). Nowadays, the land application of treated organic waste is the best way to avoid using chemical fertilizer and to minimize the higher quantity of organic waste, for greater agricultural and environmental sustainability. Composting is the most accessible and simplest technique for converting organic waste into an ecologically stable and

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agronomically beneficial product for the soil (Jouraiphy et al. 2008). Besides the efficiency of composting as a green technology for organic waste and sludge waste recycling, compost maturity should be investigated as it represents a crucial parameter of compost quality. Several techniques are used to determine the compost maturity, such as physico-chemical analysis, Fourier transform infrared spectroscopy (El Fels et al. 2016), thermogravimetric and differential thermal analysis (El Ouaqoudi et al. 2015), monitoring of phytotoxicity (Ait Baddi et al. 2004) and the cytotoxicity (El Fels et al. 2016). Nevertheless, in the rare presence of universal indices for determining the maturity of composts, it would therefore be necessary to merge several maturity indicators to confirm the good quality of compost. The selection of the appropriate indicators depends on the environmental conditions and technics available, which requires the search for new indicators of compost maturity.

Mature compost is known to have a beneficial effect on crop yields, improving plant growth and increasing photosynthetic pigments in the leaves (Libutti et al 2020). Mature compost releases a higher amount of soluble mineral nutrients and a lower concentration of phytotoxic elements and heavy metals than immature materials (Pant et al. 2012a; El Mezouari El Glauoi et al. 2018). For this reason, the age of the compost may influence the quality of compost by-products, such as compost tea (CT) and humic substances (HS) which are of increasing interest due to their usefulness as biofertilizers and plant growth regulators (Nardi et al. 2021; Scotti et al. 2016).

Compost tea is defined as a compost extract-based water, containing soluble mineral nutrients and beneficial microorganisms (Pant et al. 2012b). It can improve the quality and biomass of crops by increasing the photosynthetic activity, and reducing plant diseases due to the presence of beneficial bacteria and fungi (Sifatullah et al. 2011). Whereas, the humic substances are the humified fraction of organic matter. Humic acid and fulvic acid represent the majority of humic substances (Li et al. 2017).

Due to their direct interaction with plant physiological and metabolic processes and the increase in nutrient uptake and cell permeability, humic substances have beneficial impacts on soil and plant growth by stimulating germination and improving root, leaf and shoot growth (Eyheraguibel et al. 2008; Vaughan and Ord 1985). However, the determination of the most suitable compost extract for plants requires a comparative study of the effect of HS and CT based on the same compost.

Measurement of the functioning of the photosynthetic system and chlorophyll fluorescence of plants could highlight the stress conditions and can be used as biomarkers of soil pollution (Vernay et al. 2009).

In this context, this study has focused on the objective of using chlorophyll performances as an indicator of the safety and maturity of compost-based lagooning sludge and green waste. The aim of this research is also to evaluate the difference between the effect of humic substances and compost tea on the biometric and physiological parameters of *Trigonella foenum-graecum* and *Lepidium sativum* plants.

Material and methods

Experimental composting trials

Lagooning sewage sludge was collected from the natural lagooning wastewater treatment plant of Chichaoua city in Morocco (Fig. 1) (31°33'49.04"N; 8°43'55.65"w, altitude 644 m).

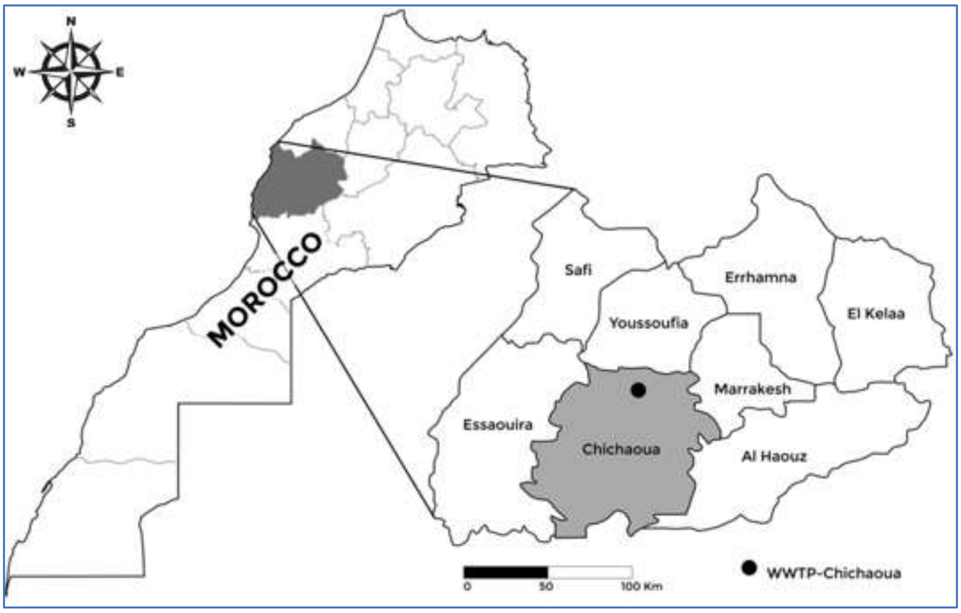

Fig. 1 Map of Morocco with study area localisation (31° 33' 49.04" N; -8° 43' 55.65" W)

The fresh sludge collected after plant cleaning has been dehydrated on the drying beds for three months. Dehydrated lagooning sludge (LS) and green waste (mixed blend of leaves, grass clippings, and shrub clipping) from the gardens of Chichaoua city were the two substrates used in composting. The main characteristics of the different composting substrates are presented in Table 1.

Table 1 Main characteristics of the substrates used for composting

Parameters	Green waste	Dehydrated sludge
Moisture (%) (FW)	35.23 ± 0.16	37 ± 0.4
TOC (%) (DW)	51.44 ± 0.35	22.06 ±0.18
KTN (%) (DW)	1.04 ±0.028	1.06 ±0.15
C/N	49.46	20.82

FW: Fresh weight; DW: Dry weight; TOC: Total organic carbon; KTN: Kjeldahl total nitrogen.

The composting trial was conducted in a windrow for 200 days, on a composting platform located in Chichaoua city (Morocco). The proportions of substrates in the windrows are as follows:

Composting mixture = ½ sludge + ½ green waste, (total volume of 4 m^3).

During the first 3 months of composting, regular brewing was achieved manually and weekly to ensure the aeration of windrows and to moderate the moisture by adding water to 60%. The temperature checking was carried out daily during the process of composting with special chips (PH0700115 Version 1.20 Ector Traceability) introduced inside the windrows. The moisture was determined by drying the compost at 105 °C for 48 h (AFNOR 2000). Samples were taken at T0 (first day of composting), Ti (T75 days) and Tf (T200 days) by collecting subsamples of 1 kg at different points along the length and height of the windrow. The carbon/nitrogen ratio (C/N) values were 29, 10.79 and 8.24 respectively for T0, Ti and Tf. Subsamples were combined, carefully mixed and homogenized by quartering. The composite samples were kept at -20°C until the analysis.

Solution treatment preparation

Treatment 1: Compost tea

The compost tea was obtained by soaking the compost mixture (T0, Ti and Tf) in tap water at a proportion of 10% (w/v). The contents were stirred and left to settle over 5 days at the temperature of 20 °C. Stirring was carried out 2 times during this period. After the absorption period of 5 days, the solution was filtered through a sieve (200 microns). The extracts obtained were kept in bottles in the refrigerator at a temperature of 4°C (Brinton and Droffner 1995).

Treatment 2: Liquid humic substances

The humic substances (HS) were extracted from 5 g of fresh sample by NaOH (0.1N), after being washed three times with 50 mL of distilled water to remove the water-soluble non-HS (sugars, proteins, etc). Humic substances extraction was repeated several times until obtaining a colourless extract. After HS extraction, the separation of humic acids (HA) from fulvic acids (FA) was carried out by the precipitation of HA using sulfuric acid (pH around 1) for 24 h at 4°C, the content of each fraction was determined by the permanganate oxidation method (Chaminade 1994; El Mezouari El Glauoi et al. 2018)

Humic solution extract was collected and adjusted to a pH 7 with nitric acid (1N) then purified and dialyzed against distilled water using cellulose ester membrane of 100 Da porosity (Spectra/Por CE®), to keep humic substance's molecules inside the membrane and eliminate the ions supplied to the solutions during the extraction process (Eyheraguibel et al. 2008). Dialysis is stopped when the conductivity of the dialysis water is constant. The dialyzed samples were stored at 4°C.

The pH and electrical conductivity of the two treatment solutions were measured using a pH meter and electrical conductivity meter (OAKTON PC 700), respectively.

Mineral elements analysis in compost tea and humic substances

To determine the content of the nitrogen (N), phosphorus (P), potassium (K), iron (Fe), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), and zinc (Zn), in the compost tea and humic substances extract, samples were filtered and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) on Agilent 5110 ICP-OES instrument (Model: GBO15A, Santa Clara, California, USA). This method was based on the thermal excitation of ions in the plasma and the analysis of the light emitted by the ions excited. The wavelengths emitted by each element are identified and their intensities were measured by a spectrometer. The final ion contents were expressed in mg/L.

Germination index

The phytotoxicity was evaluated based on the germination index, 20 seeds of *Trigonella foenum-graecum* and *Lepidium sativum* were used for the two treatment solutions (compost tea and humic substances) for each composting stage. The test was realized in three replicates in Petri dishes with filter paper soaked with the two solutions separately, in darkness at room temperature (25°C) for 72 h (El Fels et al. 2014; Zucconi et al. 1981). After monitoring the emergence of seeds, the germination index was calculated as the product of the percentage of viable seeds and the rootlets length using the following equation.

$$GI\ (\%) = \frac{(NGext \times LRext)}{(NGwater \times LRwater)} \times 100$$

NG: Number of seeds germinated in water-Soluble extracts (ext) and water,

LR: Length of rootlets in soluble extracts (ext) and water.

Plant growth test

In order to evaluate the effect of compost tea and liquid humic extract on plant growth and development, the culture of *Trigonella foenum-graecum* (Fenugreek) and *Lepidium sativum* (Cress) seedling was carried out. The experiment took place in a semi-controlled greenhouse at the faculty of sciences Semlalia

(Cadi Ayyad University, Marrakesh, Morocco) under ordinary light (Density of photon flux ranged from 500 to 750 µmol m^{-2} s^{-1}). The temperature was maintained between 21 and 23°C.

Pre-germinated seeds of uniform size, shape and healthy aspect were transported in pot culture with sand substrate. The plants were irrigated with the same volume of the two nutrient solutions separately during the treatment period. A negative control experiment without treatment was also carried out. After 2 months of application, plants were harvested and the lengths, roots and stems were measured. Plants were washed with tap water to remove any linked sand particles and were dried at room temperature to determine the fresh weight.

Stomatal conductance measurement

Stomatal conductance was determined using a portable porometer (Leaf Porometer L-1989, Decagon Device, Inc., USA). Measurements were released on the abaxial side of the leaf between 10 and 12:00 a.m. on a sunny day.

Chlorophyll fluorescence and chlorophyll content measurement

Parameters of chlorophyll fluorescence (Fo: minimum fluorescence, Fm: maximum fluorescence, Fv: variable fluorescence (Fv = Fm - Fo) and Fv/Fm: maximum quantum yield of Photosystem II), were measured by a fluorometer (Opti-sciences OSI 30p) for dark-adapted leaves during 20 minutes (Baker 2008).

Total chlorophyll content was determined by extraction of photosynthetic pigments from leaf samples in 80% acetone (Arnon 1949); centrifugation at 5000 g for 10 min, and measurement of the supernatant optical density at 645, and 663 nm using a UV–visible spectrophotometer. The total chlorophyll content was calculated according to Khaleghi et al. (2012) using the following equation :

$$Total\ chlorophyll\ (mg.g^{-1}) = \frac{[20.2(A645) + 8.02(A663)] \times V}{(1000\ \times W)}$$

With V: volume of solvent and W: fresh weight of tissues extracted.

Statistical analysis

All data were analysed statistically by comparison of the averages by ANOVA test (method test post hoc Tukey) with a confidence interval of 95% considering a significance level of PP 0.05, using SPSS Statistics software 24 Win version 10. The correlation between the different parameters and treatments was investigated using principal components analysis (PCA) by originPro 2019b software.

Results and discussion

Chemical characterization of compost tea and humic substances

The chemical properties of compost tea and humic substances extracted from different stages of composting are shown in Table 2. The two solution products have a brown color and neutral pH. The brown color of humic substances results from the oxidative polycondensation reactions that occur before total mineralization, and that can be influenced by the complexing power of metal ions (Tissaux 1996). Barancikova et al. (1997) mentioned different factors that act directly on the structure of humic substances such as the source and nature of organic matter, biological activities of microflora and environmental conditions.

Results of humic substances showed a significant decrease of fulvic acid content during composting from 33.9 mg/g in the initial stage of composting; 33.11 mg/g in the stage 75 days; to reach 30.9 mg/g in the final stage (200 days). On the other side humic acid showed an increase during composting the values were 24 mg/g in initial stage, 37.7 mg/g after 75 days of composting and 41.2 mg/g in the final stage of composting. This evolution is explained by the formation of complex molecules (humic acids), by the polymerization of simple molecules (fulvic acids) due to the humification process during composting (El Mezouari El Glauoi et al. 2018). Furthermore, the association of self-assembling heterogeneous and relatively small molecules resulting from the biodegradation of non-humic fraction and the formation of more polycondensed humic structures, indicate the composting evolution from immature substrate to mature product (El Hayany et al. 2020; Jouraiphy et al. 2005).

The pH of all treatments showed a value around neutrality between 6.95 and 7.48 due to the buffering capacity of humic substances formed during the process of composting.

The electrical conductivity (EC) values of humic substances (94.7 – 99.2 dS/m) are higher than those of compost tea (21.1 – 35.2 dS/m) which may be explained by the alkaline extraction method used. The EC increased from the initial to the final stage with an increasing rate of 4.5% for liquid humic extract and 21% for compost tea.

This is due to the richness of the final compost by mineral elements resulting from organic matter degradation and mineralisation during composting processes.

The mineral content in compost tea is characterized by higher amounts of nitrogen (1.5×10^{-3} %), potassium (3.4×10^{-3} %), calcium (7.3×10^{-2} %) and magnesium (1.03×10^{-2} %). Probably originating from the raw materials, mainly from sewage sludge which is known as a rich nitrogen, calcium, iron and phosphorus source (Watteau and Villemin 2011). A noticeable increase of Potassium and Ca^{2+} was shown from Ti to Tf of compost tea extract, which is probably originating from the degradation of nitrogenous organic matter and the release of calcium ions during the biodegradation process. On the other hand, the content on copper, manganese and zinc have remained stable during composting, which suggests that these elements are already in their mineralized form at the start of composting.

The analysis of humic solutions specifies the high concentration of phosphorus; iron and calcium with greater content in the final stage of composting (11.65; 10.75 and 18.61 mg/L respectively). The mineral element content in humic substances results from the humification process and the degradation of organic matter by microorganisms.

Phytotoxicity test

Germination index (GI) in both treatments showed that *Lepidium sativum* test is a rapid test, which gives a faster response compared to *Trigonella foenum-graecum* (Fig. 2). Humic substances arising from raw sludge showed lower values of GI compared to these of compost samples. Furthermore, a noticeable difference was observed between the different stages of composting. The GI of compost tea evolves from 39.63% and 215.38% in the initial stage to 101% and 237.9% in the final stage respectively for *Lepidium sativum* and *Trigonella foenum-graecum*. For the extracts of humic substances, the GI has increased with the compost age (Fig. 2), from 24.97% and 81.53% at the initial stage to 49.54% and 110.42% at the final stage respectively for *Lepidium sativum* and *Trigonella foenum-graecum*.

Table 2 Electrical conductivity, pH and mineral content of humic substances and compost tea solutions

		Compost Tea				Liquid Humic Extract				
		SS	T0	Ti	Tf	SS	T0	Ti	Tf	Control
Chemical parameters	pH	7.03	7.25	7.06	6.99	7.23	6.62	6.95	7.48	7.06
	Electrical Conductivity (dS/m)	35.2	21.1	22.4	26.6	95.5	94.7	97.7	99.2	0.74
Mineral content (%)	Nitrogen (N)	1.4×10^{-3}	1.1×10^{-3}	1.5×10^{-3}	1.5×10^{-3}	2×10^{-5}	10^{-4}	2×10^{-5}	2×10^{-5}	1×10^{-4}
	Phosphorus (P)	2×10^{-4}	1.6×10^{-4}	2.8×10^{-4}	3.4×10^{-4}	6×10^{-4}	6×10^{-4}	8.7×10^{-4}	1.1×10^{-3}	6×10^{-5}
	Potassium (K)	3.3×10^{-3}	1.9×10^{-3}	1.5×10^{-3}	3.4×10^{-3}	9×10^{-5}	5×10^{-5}	5×10^{-5}	2.3×10^{-4}	2×10^{-4}
	Iron (Fe)	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	10^{-5}	4×10^{-4}	3×10^{-4}	6×10^{-4}	10^{-3}	$<10^{-5}$
	Calcium (Ca)	6.7×10^{-2}	1.7×10^{-2}	2.4×10^{-2}	7.3×10^{-2}	1.3×10^{-3}	7×10^{-4}	8×10^{-4}	1.8×10^{-3}	8.5×10^{-3}
	Magnesium (Mg)	1.4×10^{-2}	5.6×10^{-3}	6×10^{-3}	1.03×10^{-2}	8×10^{-5}	6×10^{-5}	10^{-4}	2×10^{-4}	2.9×10^{-3}
	Copper (Cu)	2.5×10^{-5}	2×10^{-5}	2×10^{-5}	2×10^{-5}	9×10^{-5}	9×10^{-5}	9×10^{-5}	1.2×10^{-4}	$<10^{-5}$
	Manganese (Mn)	7×10^{-5}	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
	Zinc (Zn)	2×10^{-5}	2×10^{-5}	2×10^{-5}	2×10^{-5}	0.001	8×10^{-5}	10^{-4}	2×10^{-4}	$<10^{-5}$

(SS: dewatered sewage sludge; T0: initial stage of composting; Ti: stage 75 days of composting; Tf : stage 200 days of composting; Control: tap water)

The lower values of GI in the initial stage of composting (compost Ti), is mainly due to the phytotoxic elements. During the intermediate stage of composting the GI showed a slow increase for *Trigonella foenum-graecum* (0-1.07%), which can be due to the liberation of phytotoxic substances during the thermophilic phase. According to different authors, the intense degradation of the majority of organic matter during the maturation phase induces the production of toxic substances such as acetic acid and ammonia (El Fels et al. 2014; El Mezouari El Glauoi et al. 2018). Furthermore, a wide variety of substances have been determined by their significant inhibitory power on seed germination, such as, short-chain organic acids, phenols, alkaloids, aldehydes, ketones, and certain flavonoids and thus zinc; copper and boron and chromium

(El Fels et al. 2015). At the end of the composting process (maturation phase), the phytotoxicity decreases and the germination index increases to values that exceed 100% (Fig. 2), which justifies the reduction of phytotoxic compounds (El Fels et al. 2016; El Hayany et al. 2018), and consequently the maturity of the final stage of composting. According to El Fels et al. (2014) and El Mezouari El Glauoi et al. (2018) compost is considered non-phytotoxic if its germination index exceeds 80%, which confirms the safety of the final compost.

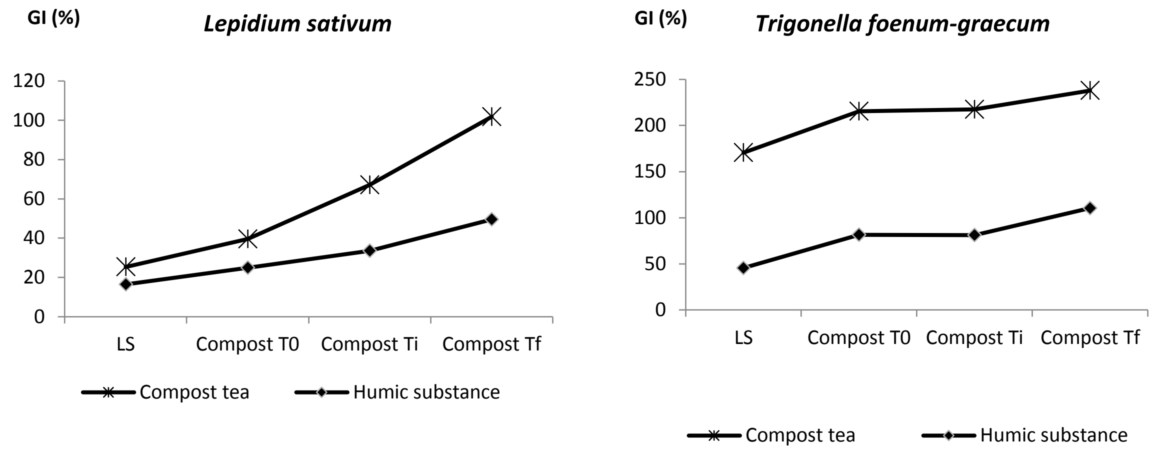

Fig. 2 Phytotoxicity evolution of humic substances and compost tea during composting LS (Lagooning Sludge); T0 (0 days); Compost Ti (75 days) Compost Tf (200 days)

Effect of compost tea and humic substances on biometric parameters

Fig. 3 presents the values of the shoot and root elongation measurements for both treatments. Results indicate that plants treated by CT and HS based final composting stage Tf (200 days) showed better growth than initial, intermediate stages and raw lagooning sludge which can contain phytotoxic elements. The values of shoot length for the two plants (17.5 – 38 cm) were fold higher than the control (16-30.5 cm) which was poor in mineral elements. This reveals a stimulating effect of CT and HS based final and mature compost (T200) on plants, the mature compost is characterised by a carbon/nitrogen ratio (C/N) of around 9, which is the required maturity value.

On the other hand, the length of plants showed a noticeable decrease during composting for *Trigonella foenum-graecum*, which may be due to its strong sensibility to the phytotoxic elements such as acetic acid, acetaldehyde and ethanol, released during the intermediate stage of composting through organic matter biodegradation (El Fels et al. 2014; El Mezouari El Glauoi et al. 2018). The final stage (Tf = 200 days) showed a good evolution of stems and roots, which can be explained by the reduction of the phytotoxic effect and the contribution of compost tea to better uptake.

Fig. 4 showed a noticeable effect of compost tea treatment on biomass evolution compared to humic substances. The positive effect of compost tea (Tf) was observed in the growth of all plants. CT exhibited a significant increase in total biomass from the initial stage (T0) to the final stage (Tf), with an increasing rate of about 63.2%; 40.53% respectively for *Lepidium sativum* and *Trigonella foenum-graecum.* According to other studies, the mature and hygienic compost does not induce inhibitory or phytotoxic effects on plants as the raw and immature materials do (El Mezouari El Glauoi et al. 2018; Masciandaro et al. 2002).

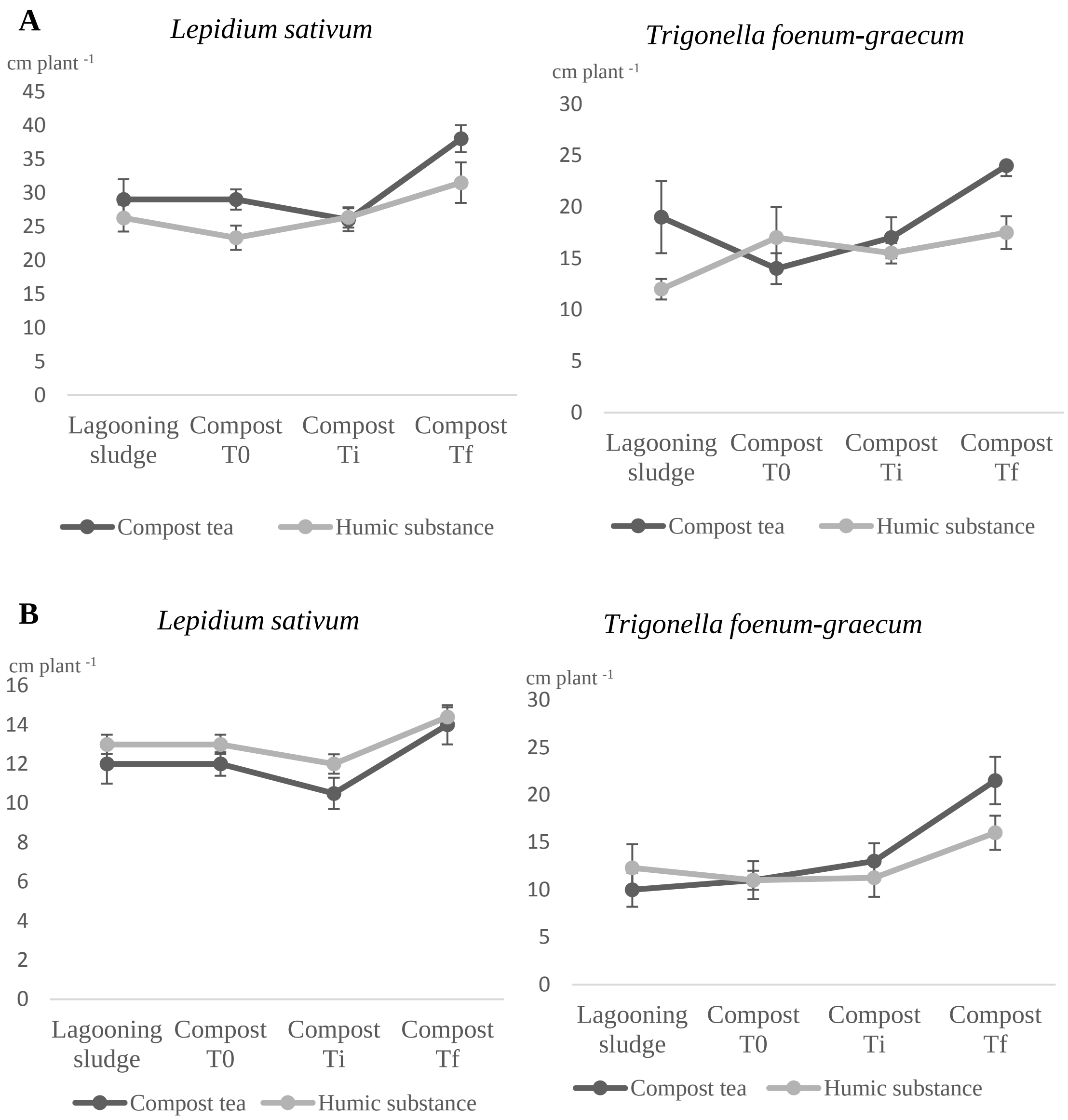

Fig. 3 Plant shoots (A) and roots (B) evolution during treatment by compost tea and humic substances: T0 (0 days); Compost Ti (75 days) Compost Tf (200 days)

Lepidium sativum control: Shoot (30.5±1.7cm) Roots (11±0.87cm); *Trigonella foenum-graecum control:* Shoot (16±1.36cm) Roots (13±0.6cm)

A significant difference was observed between the two treatments, the application of CT had increased shoot growth and plant weight, which may be due to the presence of available nutrients (N, P, K, Fe, Ca, Mg, Cu, Mn, Zn) at higher concentration in CT based final compost (Tf) (Table 2). On the other hand, the HS promotes root growth of *Lepidium sativum* due to its richness in phosphorus elements. That maybe due also to the great efficiency of HA-like substances from organic wastes in enhancing plant growth. This can be explained by the release of auxin-like plant growth promoters and the improvement of plant biochemical activities due to the conformational dynamics of humic hydrophobic associations in the rhizosphere by dint of conformational dynamics of humic hydrophobic associations in the rhizosphere (Jindo et al. 2012). In general, the noticeable positive effect of CT in total biomass indicates that CT is more suitable to be used as fertilizer.

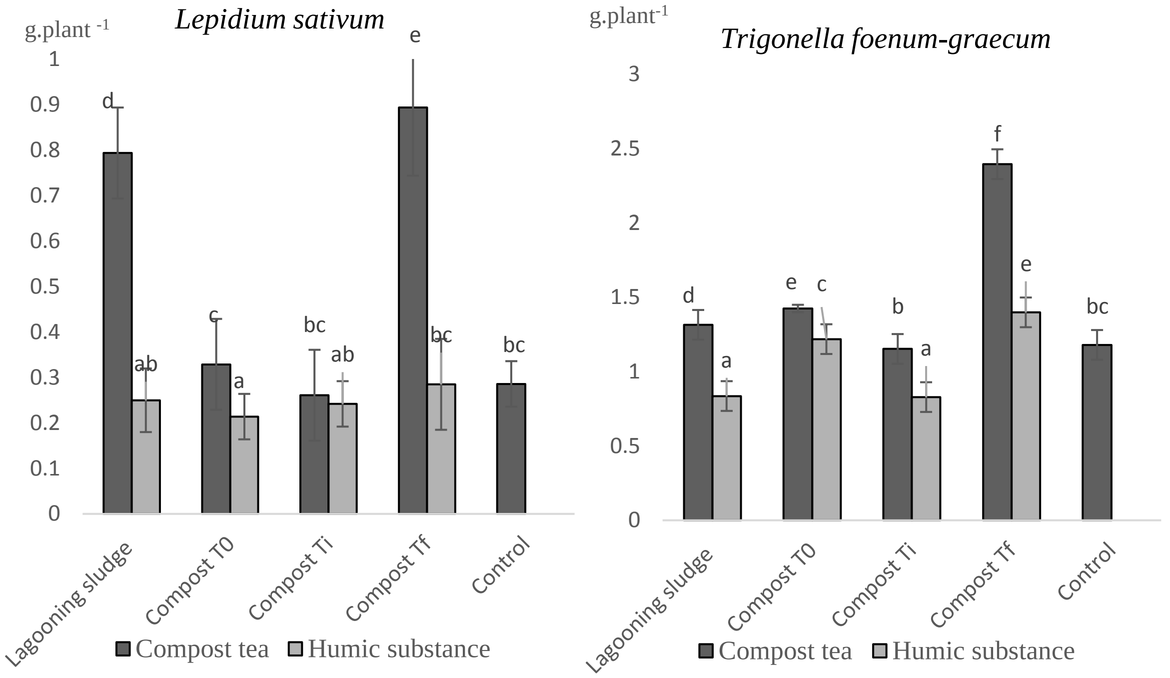

Fig. 4 Total biomass evolution during treatment by compost tea and humic substances: T0 (0 days); Compost Ti (75 days) Compost Tf (200 days)

Data followed by different letters above the bars mean a significant difference between treatments ($p \leq 0.05$; Tukey test)

The results of phenotypic characterization were consistent with previous studies of the CT effect. Pant et al. (2012b) indicated that the application of vermicompost tea increases the overall root development of *Brassica rapa* plants. Kim et al. (2015) studied the effect of compost tea on the growth promotion of lettuce, soybean, and sweet corn and indicated the beneficial effect of compost tea on plant evolution. Other studies showed the good effect of aerated and non-aerated compost tea on germination and biomass yield (Kasim et al. 2021; Sifatullah et al. 2011). The application of compost tea increases shoot and root growth and fresh weight of *pak choi* compared to the control with greater evolution in the case of the application of aged chicken manure-based vermicompost tea (Pant et al. 2012b). Similarly to CT, HS were declared in different studies as a stimulator of plant growth. Eyheraguibel et al. (2008) showed that humic-like substances have a good effect on the root proliferation of maize. Lee and Bartlett (1976) indicated the stimulant effect of HS from different sources of organic materials on the growth of corn seedlings and algae; Hartwigsen and Evans (2000) revealed the effectiveness of humic acid in increasing root fresh weight in cucumber, marigolds, and geranium.

Stomatal conductance and chlorophyll fluorescence parameters

The effect of the humic substances and compost tea from different composting stages on the physiological properties of *Trigonella foenum-graecum* and *Lepidium sativum* plants was investigated through chlorophyll fluorescence (CF), total chlorophyll content (TC) and stomatal conductance (SC) measurement. The CF, defined as the maximum quantum efficiency of PSII photochemistry (Fv/Fm), was chosen as the principal parameter to monitor the photosynthetic performance of plants as well as to describe if the plant is under stress or not (Khaleghi et al. 2012). Photosynthetic apparatus activity can be used as a bioindicator of soil pollution in combination with total chlorophyll content and stomatal conductance parameters (Vernay et al. 2009).

Results of chlorophyll fluorescence indicate that plants treated with compost tea (Tf), showed higher values of Fv/Fm of about 0.82±0.3 and 0.81±0.1 respectively for *Trigonella foenum-graecum* and *Lepidium sativum.* In contrast, the plant treated with CT of dewatered lagooning sludge (LS), initial stage and intermediate stage of composting showed Fv/Fm values less than 0.7. According to Murchie and Lawson (2013) and Roháček et al (2008) when Fv/Fm ratio is about 0.7 to 0.8, it means that no stress has been applied on the plant. Therefore, the values mentioned above (less than 0.7) show that plants treated by lagooning sludge and no mature compost were under a stress resulting from phytotoxic elements present in LS and in no mature composts. Plants treated with HS of the final compost showed a higher value of Fv/Fm ratio than the LS, initial and intermediate composting stages. The presented data demonstrate a significant increasing rate of chlorophyll fluorescence of 15.86% and 14.82% for CT; 3% and 6% for HS after 200 days of composting respectively for *Trigonella foenum-graecum* and *Lepidium sativum*.

The total chlorophyll content (TC) in plants treated with compost tea based final stage of composting is about 0.78 mg/g and 0.75 mg/g respectively for *Trigonella foenum-graecum* and *Lepidium sativum* (Table 3). The TC measurement indicates a significant increase during composting for the HS and CT treatment for both plants.

Stomatal conductance (SC) has increased during composting; the final values reached 37.20 mmol/m²s; 32.40 mmol/m²s for CT and 37.6 mmol/m²s; 37.80 mmol/m²s for HS respectively for *Trigonella foenum-graecum* and *Lepidium sativum*. The increase in SC from T0 to Tf can be attributed to the high phosphorus concentration in the leaves, the high relative water content and the considerable osmotic adjustment (Augé et al. 2015; Meddich et al. 2015). SC evolves in parallel with the evolution of CF and TC, which confirms that stomatal opening, induces greater efficiency of photosystem performance. In general, the results indicate that the two treatments prepared based on the final composting stage are beneficial for the production of chlorophyll in the leaf and for plant transpiration. The improvement of physiological properties of plants treated by final compost extract is due to the presence of nutrient elements, mainly potassium which contributes to photosynthesis, transport of carbohydrates, regulation of the stomatal opening and respiration (Santos et al. 2010). Our study showed that chlorophyll performance is a complex process that can be influenced by different factors such as the non-maturity of compost, the presence of contaminants and phytotoxic elements in sewage sludge. Our result is in line with the finding of Pant et al. (2012b), who revealed the positive influence of compost tea based aged compost on *pak choi* growth, and with that of Osama (2015) who demonstrated the positive effect of humic substances and compost tea on the growth and development of *faba bean*.

The significant increase of CF and TC during composting informs about the good quality of the final compost. This study takes advantage of the test growth on plants to suggest CF as a new indicator of the compost evolution process. It is a simple method that can investigate indirectly the compost quality and can be used as a potential bioindicator of compost maturity with the evolution of compost age.

Table 3 Effect of compost tea (CT) and humic substances (HS) on chlorophyll fluorescence; stomatal conductance and total chlorophyll of plants

		Chlorophyll fluorescence (Fv/Fm)		Stomatal conductance (mmol/m²s)		Total chlorophyll (mg/g)	
Plants	Treatment	CT	HS	CT	HS	CT	HS
Trigonella foenum-graecum	Dewatered lagooning sludge	0.67±0.2b	0.71±0.1b	31.50±3.5b	39.00±0.12d	0.52±0ab	0.65±0.2d
	Compost T0	0.69±0.1b	0.81±0.2c	34±0bc	34.00±0.4bc	0.55±0.1bc	0.71±0.2ef
	Compost Ti	0.61±0.2a	0.79±0.1c	19.90±0a	24.50±1.32a	0.49±0a	0.58±0.1c
	Compost Tf	0.82±0.1c	0.83±0.3c	37.20±0cd	37.50±1.21cd	0.78±0.2g	0.76±0.1fg
	Control	0.72±0.3b		30.40±0b		0.70±0.3cd	
Lepidium sativum	Dewatered lagooning sludge	0.66±0.1a	0,69±0.2ab	23.30±0.7ab	20.00±1.6a	0.48±0.1a	0.63±0.1b
	Compost T_0	0.69±0ab	0.78±0.1cde	22.50±0.2ab	23.00±0.5ab	0.48±0.1a	0.62±0.2b
	Compost T_i	0.70±0.1ab	0.76±0.3cd	26.00±0.3b	27.00±1.2b	0.60±0.2b	0.62±0b
	Compost T_f	0.81±0.2de	0.83±0.2e	32.40±2.3c	37.80±1.36d	0.75±0.1c	0.74±0.3c
	Control	0.73±0.6bc		26.30±0.2b		0.63±0.2b	

(Values in the same parameters and same plants followed by different letters indicate a significant difference between treatments at $p \leq 0.05$ by the Tukey test)

The difference between the effect of HS and CT on physiological parameters is not significant for both plants. Although CT was suggested to have a higher effect due to its richness in element content, the HS act through their chelating effect and their direct interactions with the physiological process of plants (Eyheraguibel et al. 2008; Vaughan and Ord 1985). On the other hand, regarding biometric parameters, CT was selected as an adequate treatment to improve plant performance. Furthermore, the application of aerated compost tea can influence plant health by inducing disease resistance through microbial communities (Kim et al. 2015; Verrillo et al. 2021). CT application is a good way to benefit from compost advantages, it increases the crop yield due to the beneficial effect of composted sewage sludge (Han et al. 2004) and plant hormones (Klimas et al. 2016). CT promote also the fruit quality by improving photosynthetic activity and increasing macronutrients in the leaf (Shrinivas et al. 2021), which increases crops yield (Sifatullah et al. 2011).

Statistical analysis

The results of the principal component analysis (PCA) of physiological and biometric parameters and type of treatments are reported in Fig. 5. The projection on the plane of these different variables in terms of two main components (I and II) shows the affinity of variables according to each PCA axis.

The first principal component (PC1) explained the majority of the variability: 56.20% and 55.92% % of the total variance, while the second component (PC2) explained 21.11% and 27.84% of the total variance, respectively for *Lepidium sativum* and *Trigonella foenum-graecum.*

Each variable surrounded in a circle shows a strong correlation between them. The plots of principal components are divided into four differently correlated domains, two domains are inversely correlated based on the axis of component I represented by the treatments (HS-Tf, CT-Tf) which are positively correlated, and by (CT-LS, CT-T0, CT-Ti, HS-LS, HS-T0, HS-Ti). The two other domains are inversely correlated based on the axis of component II, represented by biomass, shoot, and GI which are positively correlated, and by stomatal conductance, chlorophyll fluorescence and total chlorophyll.

In general, the plots of the principal component scores showed a strong correlation between the evolution of Shoots, roots, and germination index; and between stomatal conductance, chlorophyll fluorescence and total chlorophyll.

This correlation confirms that the reduction of phytotoxic compounds and the maturity of final products contribute to the *Lepidium sativum* and *Trigonella foenum-graecum* growth and the improvement of their physiological characteristics. The score plot showed that the treatment HS-Tf and CT-Tf, which have a better effect on both plants, were positively correlated with each other, while they are negatively correlated with the other treatment represented by the dewatered lagooning sludge (LS) treatment, stage T0 and intermediate stage of composting. The PCA analysis confirms the beneficial effect of treatment-based compost Tf as mature compost on the physiological and biometric parameters of *Lepidium sativum* and *Trigonella foenum-graecum.*

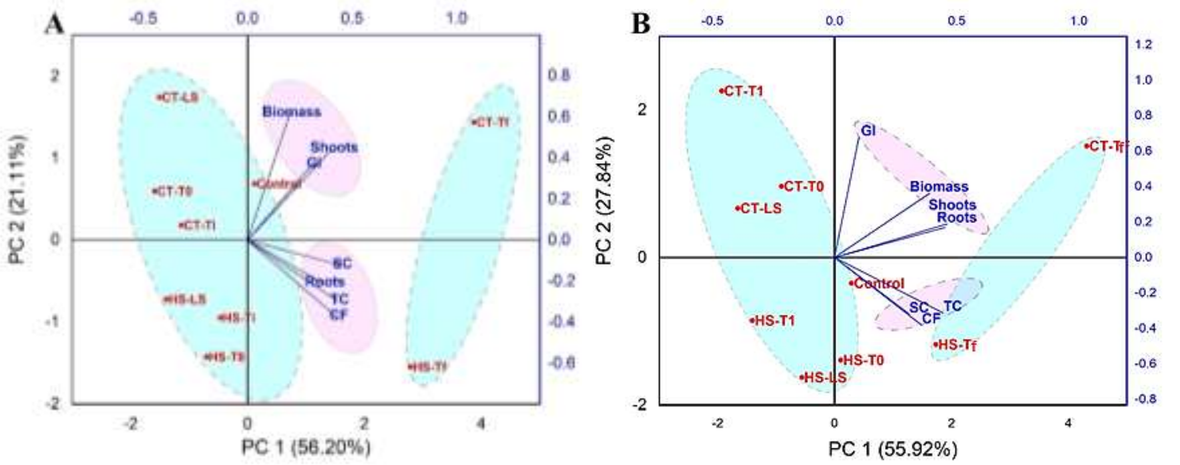

Fig. 5 PCA biplot (Score plot and Loading plot) showing the loading of each parameter (Biomass, Shoots, Roots, GI: Germination index, SC: Stomatal conductance, CF: Chlorophyll fluorescence, TC: Total chlorophyll) and the scores of each treatment (CT-Tf, CT-Ti, CT-T0, CT-LS, SH-Tf, SH-Ti, SH-T0 and SH-LS) for *Lepidium sativum* **(A)** *and Trigonella foenum-graecum* **(B)**

Conclusion

This study has investigated the use of compost (½ lagooning sludge + ½ green waste) as liquid soil fertilizer (Compost tea and humic liquid substances). It showed that the application of the compost tea extract has well improved the biometric parameters of *Lepidium sativum* (length up to 38 cm, biomass up to 0.89 g) and *Trigonella foenum-graecum* (length up to 24 cm, biomass up to 2.39 g), compared to humic substances (Length: 14.4-16 cm; Biomass: 0.28-1.39 g). In contrast, the effect of the two treatments on the physiological parameters did not show a significant difference. In general, the values depend on the composting stage and they ranged from 0.66 to 0.83, from 19.90 to 39 mmol/m²s and from 0.49 to 0.78 mg/g respectively for chlorophyll fluorescence, stomatal conductance, and total chlorophyll content.

The comparison between the different stages of composting showed that the treatments-based compost Tf (200 days) revealed a significant effect on the plant biomass, and on the elongation of roots and shoots. The positive effect of the compost Tf treatments was also well noticed on the physiological properties (Chlorophyll fluorescence, Stomatal conductance, and Total chlorophyll content) of both plants. Indeed, the good correlation between the growth parameters and germination index confirms that the final compost product has reached maturity.

This study drew two conclusions: (i) The compost tea and humic substances treatments approved their bioactive properties and beneficial effects on plant growth (ii) A new factor (chlorophyll fluorescence) may assess indirectly the compost maturity using photosynthesis and chlorophyll performance. Therefore, this study recommends compost tea application as an alternative to chemical fertilizers and pesticides.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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