

Article

Beyond Conventional Fertilizer: Tannin–*Chlorella vulgaris* Blends as Biostimulants for Growth and Yield Enhancement of Strawberry (*Fragaria x ananassa* Duch)

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Abstract

The increasing demand for sustainable agricultural practices has led to the exploration of natural biostimulants. This study investigates the effects of tannin extracts obtained via hydrodynamic cavitation and *Chlorella vulgaris* microalgae on the growth and physiological performance of strawberry (*Fragaria x ananassa* Duch) plants. A preliminary phytotoxicity test using *Lepidium sativum* L. confirmed the safety of the tannin water extract. Subsequently, two main experiments were conducted: the first identified the optimal tannin concentration, while the second assessed the individual and combined effects of tannins and *C. vulgaris* on strawberry plants. The results show that tannin water extract at double concentration of the commercial tannin (54% T.E.) significantly increased leaf dry biomass by 75% and doubled the number of main roots compared to the control. In the second experiment, *C. vulgaris* at 50% concentration (C1) enhanced fresh leaf biomass by 14% and fresh roots by 20%, while tannin extract (T) showed a declining effect on plant biomass as compared to the control. Positive effects were also observed for root growth in the combined treatment T+C1, with 32% fresh root biomass more than in the control. Regarding fruit, C1 maintained high fruit yield from the beginning of the experiment until September, while T+C1 showed a marked rising trend, reaching a comparable number of fruits to C1, about twofold more than the control. A chemical analysis of the main micro- and macro-elements in roots and leaves resulted in T+C1 having the highest content of Zn and Fe and C1 having the highest content of Fe and K (the latter only in the leaves) as compared to other treatments. In contrast, T+C1 showed about 50% less P and K in the leaves than in *C. vulgaris* treatments. In addition, in the tannin treatment, microelements such as Fe and Zn accumulated in the roots, evidencing absorption from the soil, but low translocation to the leaves. However, all treatments showed similar photosynthetic performance in terms of leaf gas exchange and chlorophyll fluorescence. These findings suggest that extracts of *C. vulgaris* and tannins or their blends represent a promising strategy for improving crop productivity and resilience in a sustainable manner.

Keywords: biostimulant; *Chlorella vulgaris*; fluorescence; morphological and physiological parameters; sustainable agriculture; yield



Academic Editor: Luciano Kayser Vargas

Received: 17 October 2025

Revised: 17 November 2025

Accepted: 24 November 2025

Published: 27 November 2025

Citation: Giordano, C.; Ugolini, F.; Faraloni, C.; Dal Prà, A.; Sabatini, F.; Meneguzzo, F.; Petruccielli, R. Beyond Conventional Fertilizer: Tannin–*Chlorella vulgaris* Blends as Biostimulants for Growth and Yield Enhancement of Strawberry (*Fragaria x ananassa* Duch). *Agriculture* **2025**, *15*, 2459. <https://doi.org/10.3390/agriculture15232459>

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1. Introduction

Strawberry (*Fragaria x ananassa* Duch) is a perennial herbaceous plant belonging to the Rosaceae family. It produces aggregate fruits, commonly called “false fruit” or “pseudo-fruit”, as they develop from an enlargement of the receptacle. Strawberry is one of the most popular and widely consumed crops worldwide, appreciated for its sweet taste, characteristic aroma, and nutritional properties. It contains high levels of bioactive compounds such as vitamins, polyphenols, fibers, fructose, micronutrients such as vitamin C and folate, and it is considered a highly nutritious fruit [1,2]. Strawberry fruits are consumed both fresh and processed into a variety of products such as jams, juices, and ice cream. They represent one of the most important fruit crops, with global production exceeding 10 million tons per year [3]. The significant development of its cultivation/production is also due to the introduction of innovative agricultural practices and advanced management techniques. However, strawberries require special care, as they are susceptible to pest attacks, nutrient deficiencies, and adverse weather conditions, which compromise the quality and yield at the harvest [4,5]. These vulnerabilities have led to the adoption of agricultural practices that involve the heavy use of chemical inputs, including pesticides, synthetic fertilizers, and growth regulators to ensure high yields and quality standards [6,7]. However, such intensive agricultural practices lead to disastrous long-term effects on terrestrial and aquatic ecosystems, animals and plants, as well as on human health [8–10]. For these reasons, it is increasingly urgent to adopt alternative cultivation approaches to reduce dependence on chemical inputs without compromising productivity [11], such as the use of biostimulants and precision farming techniques. The definition of a biostimulant proposed by Du Jardin is “any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms” [12]. Regulation (EU) 2019/1009 on fertilizing products defines a plant biostimulant as a product whose function is to stimulate “plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, and availability of confined nutrients in soil or rhizosphere” [13]. In recent years, national and international political organizations have focused particular attention on the technological development of new biostimulants and their marketability [14]. Tannins and microalgae promote plant growth and tolerance to environmental stress [15,16]. Tannins are polyphenolic compounds found throughout almost the entire plant kingdom, where they play a key role in protecting plants from predators and may also be involved in growth-regulation mechanisms. “Tannin” is a term that defines a class of oligomeric water-soluble polyphenolic compounds obtained mainly from wood. Chemical classification distinguishes between hydrolysable and condensed tannins. Condensed tannins are flavonoid polymers (flavan 3-ol or flavan 3,4-diol) without a central sugar. In contrast, the structure of hydrolysable tannins is based on a carbohydrate nucleus (typically glucose) linked to gallic or ellagic acid units [17]. Their traditional applications, such as leather tanning and winemaking, are linked to their ability to complex and precipitate proteins and their antioxidant properties. In the last two decades, new and unexpected applications emerged for tannins obtained from chestnut wood, which are hydrolysable tannins, initially in animal nutrition and subsequently in agriculture as biopesticides and biostimulants [18].

Tannin extracts are known for their ability to improve soil health, promote root growth and architecture, and improve plant resistance to biotic and abiotic stress [15].

Their action mechanisms are linked to the ability to complex, precipitate, and inactivate the hydrolytic enzymes (such as pectinases and cellulases) secreted by fungi. Tannins

also act as natural chelating agents, sequestering micronutrients essential for the growth and metabolism of fungi and bacteria while making them unavailable to pathogens. In addition, tannin astringency and bitter taste deter phytophagous and nematodes from feeding on the treated plant [19,20]. Although the action of tannins in agriculture has been sparsely investigated, their positive effects have been found in crops such as tomatoes [15], vines [18], and tobacco [21]. Microalgae are a vast family of mostly photosynthetic organisms [22]. *Arthrospira* spp., *Chaetoceros* spp., *Chlorella* spp., *Dunaliella* spp., and *Isochrysis* spp. are commercially available microalgal species. Among these, *Arthrospira* spp. and *Chlorella* spp. are the most cultivated and utilized in industry [23]. Numerous studies have emphasized the enormous potential of *Chlorella* as biostimulant and as biological control agent against fungi and pathogenic microorganisms in a range of agricultural crops, including kale, lettuce, beets, and strawberries [24]. Indeed, its biochemical composition includes a wide range of advantageous beneficial components such as proteins, minerals, dietary fibers, amino acids, bioactive compounds, phenols, tocopherols, chlorophylls, antioxidants, phytohormones [25,26], and polysaccharides, which are able to increase the expressions of genes encoding antioxidant enzymes that can bind to cell walls of various phytopathogenic fungi [27]. Because of its high nutritional value, *C. vulgaris*-derived biochemical components are used in medicine, pharmaceuticals, cosmetics, aquaculture, biofuel production, the food industry, and agriculture [22]. Specifically, recent studies have shown how the application of *C. vulgaris* extracts improves seed germination, stimulates root growth, and increases agricultural yield [28]. Considering the beneficial effects of tannins and *Chlorella*, we aimed to contribute to the ongoing challenge of identifying new sustainable biostimulants to enhance strawberry production. In line with the principles of the circular economy and waste recycling, the tannins used in this study were extracted from sawdust from a local sawmill through a green technological process (hydrodynamic cavitation). In this context, the aim of the present study was to evaluate, for the first time, the effects of tannin and *C. vulgaris* on the growth, yield, and physiological performance of *Fragaria x ananassa* to identify the best biostimulant and contribute to sustainable agronomic practices.

2. Materials and Methods

This research followed a stepwise approach comprising three experiments: A preliminary experiment aimed to assess the toxicity of tannin water extract produced via hydrodynamic cavitation. A second experiment aimed to identify the most effective concentration of tannin water extract (Exp. 1) to be used in the third experiment (Exp. 2). Eventually, Exp. 2 aimed to assess the effects of tannin and *C. vulgaris* microalgae on the growth, yield, and physiological performance of the strawberry plants, as described in the following sections.

2.1. Production and Test of the Alternative Fertilizers (Tannins and *C. vulgaris* Extracts)

2.1.1. Tannin Water Extract and Phytotoxicity Test

The tannin water extract was obtained from hydrodynamic cavitation, which is a phenomenon that occurs when a liquid passes through a device that induces a flow restriction, increasing its velocity and kinetic energy, resulting in significant pressures and extreme heat over a short time. This causes cell enlargement or destruction, releasing the cell metabolites (plant bioactives, enzymes, proteins, etc.) in the liquid, forming a nanoemulsion [29,30]. Chestnut wood sawdust collected at a local sawmill (Segheria Tani, Borgo San Lorenzo, Firenze, Italy) was manually sieved with a 1 mm mesh, then mixed with water at a concentration of 1 kg in 16 L, and it underwent the extraction process for 237 min and 30 min up to 100 °C temperature [31]. This process provided a water extract with 13.75 g of tannins per liter.

The phytotoxicity test on *Lepidium sativum* L. was conducted to evaluate the effects of the tannin water extract on seed germination as a new non-commercial substance. We followed the instructions provided by the official method [32], which assesses the germination of the test species under different concentrations of the new substance. Pure tannin water extract (100% concentration, pH: 5.9) was compared to dilutions at two concentration levels (75% and 50%) and to distilled water as control. Five Petri dishes (Ø 9 cm) for each treatment (100%, 75%, and 50%) and control (CTRL) were prepared with filter paper on the bottom of the dish and placing 10 seeds of *Lepidium sativum* L. previously soaked in distilled water for one hour. All dishes were watered with 5 mL of the respective solution and incubated at 22° C for 24 h. Then, the number of germinated seeds was taken to calculate the germination rate (G, %) and to measure the radicle length (mm). The germination index (Ig, %) was calculated as

$$Ig(\%) = (Gc \times Lc) / (Gt \times Lt) \times 100 \quad (1)$$

where Gc and Gt are the average number of germinated seeds in the control and in the treatment, respectively, and Lc and Lt are the average radicle length of control and treatment, respectively. Eventually, the final germination rate (Gfin, %) was calculated after three days, when most Petri dishes showed all seeds germinated.

2.1.2. *Chlorella vulgaris* Growth and Extract Preparation

The green microalga *Chlorella vulgaris* G-120 (registered as *Chlorella vulgaris* BEIJ., 1996/H 14, CICALA 30001, Culture Collection of Autotrophic Organisms, Institute of Botany, Trebon, Czech Republic) is a natural, non-GMO strain. The strain was maintained in 100 mL of stock liquid cultures in BG11 medium (Table S1) [33] in an Erlenmeyer flask of working volume of 500 mL on an orbital shaker (100 rpm) under controlled temperature conditions of 28 °C, exposed to the light intensity of a cool white fluorescent lamp 50 µmol photons m⁻² s⁻¹, refreshing the culture medium weekly, avoiding reaching the steady state. For the production of the biomass to be used in this work, the cells were transferred and cultivated in tubular glass tubes, with a working volume of 0.5 L, bubbled with a filtered mixture of air/CO₂, 97/3%, v/v, in BG11 medium at 28 °C, exposed to a cool white fluorescent lamp at the intensity of 70 µmol photons m⁻² s⁻¹ on two sides. After a week of permanence under these conditions, cells were used as inoculum and transferred into new tubular glass tubes, diluted in fresh BG11, to monitor the growth of the cultures and obtaining the growth curve (Figure S1) as total chlorophyll and biomass accumulation. The growth rate (measured according to [34]) indicated the efficient light utilization by the photosynthetic apparatus for the biomass accumulation under the adopted culture conditions. The growth curve was important to monitor the proper growth of the cultures and additionally indicated the phase of growth of the culture, which was necessary to know when it was time to collect the cells. The cells were collected at the end of the exponential growth phase, corresponding to a cell density of $1.55 \times 10^8 \pm 0.35$ cells mL⁻¹. The cell density of the cultures of *C. vulgaris* was evaluated by means of counting the cell numbers with the Neubauer Hemocytometer [35]. The counting was repeated three times. The cells were harvested by means of centrifugation and washed in fresh distilled water two times to remove all of the possible nutrients and released compounds from the culture medium. In this way, the unique effect of the cells' content could be monitored in the following treatment with the cell extracts. The cells were then frozen at -20 °C before their utilization. The extract for the strawberry leaf treatment was obtained using the following procedure: the cells were resuspended in fresh distilled water at the concentration of 1.31×10^7 cell mL⁻¹ and sonicated, maintained on ice, at 70% amplification (two cycles, pulse 1 s On, 0.5 s Off, for 7 min). Subsequently, the extract solution was used as a foliar

spray at 50× and 100× dilutions to wet the adaxial and abaxial sides of the leaves once a week. The control plants were sprayed with distilled water. This procedure, and the relative microalgal cell concentration, were adopted according to previous findings on *C. vulgaris* leaf application on *Brassica napus* [36].

2.2. Plant Material and Setup of Experiment 1 and 2

2.2.1. Experimental Setup of Experiment 1: Tannin Concentration Determination

The experiment aimed to identify the concentration of tannin water extract providing the best plant growth. Bare-root plants of *Fragaria x ananassa* ‘Charlotte’ remontant (strawberry) were purchased from commercial nurseries in March 2023. Sixty uniform plants were selected: the plants had an average fresh weight of 6.2 ± 1.04 g (dry weight of 2.2 ± 0.27 g). On 20 March, the strawberries were planted in 1.4 L pots ($9.5 \times 9.5 \times 20$ cm) filled with commercial coarse sand. Pots were organized in a randomized block design with three blocks per treatment and four replicates (pots) per block, for a total of twelve plants per treatment.

The treatments included a commercial powder extract of chestnut tannins (COM) (Cifo, San Giorgio di Piano, Bologna, Italy) with 75% tannin content at a dilution of 5 g per liter according to the manufacturer’s instructions (resulting in 3.75 g of tannins per liter). In addition, three dilutions of chestnut tannin water extract produced by hydrodynamic cavitation in water (subsequently referred to as T.E.) were tested: 27% T.E. (corresponding to the same concentration of tannins as the commercial dilution, 13.5% T.E. (half the tannin concentration), and 54% T.E. (double the tannin concentration). These treatments were compared to irrigation water as control (CNT).

The pots were treated approximately every 2 weeks, from 20 March (T0) with 150 mL of water (control) or 150 mL of the relative tannin dilution in the treatments until the end of the experiment (Tf) on 26 April (three treatments in total).

In addition, each plant received 100 mL of Greenleaf® 20-20-20 (Biolchim SPA, Medicina, Bologna, Italy) at a concentration of 0.1 g/L applied after 3 and 10 days (24 and 31 March). The nutrient solution was prepared to achieve the following macro-/micronutrient concentrations: N = 0.2 mg/L, P₂O₅ = 0.2 mg/L, K₂O = 0.2 mg/L, MgO = 0.2 mg/L, Fe = 10 µg/L, Mn = 5 µg/L, Zn = 2 µg/L, Cu = 1 µg/L, B = 2 µg/L, and Mo = 1 µg/L. The solution is consistent with a maintenance fertilization regime for strawberries, suitable for experiments involving tannin–microalgae blends.

At Tf, biomass analyses were performed by measuring the fresh weight (FW) and dry weight (DW) of the aerial parts (leaves and stem) and roots after drying in an oven at 75 °C until a constant weight was reached. Leaf pigments were also measured using a non-destructive method on 6 fully developed leaves per treatment with a Dualex sensor (FORCE-A, Orsay, France), which measures chlorophyll, flavonols, and anthocyanins contents by analyzing the light transmitted through the leaf at specific wavelengths. The content of chlorophyll is measured as absolute values (µg cm^{−2}), while flavonols and anthocyanins are measured in relative absorbance units.

2.2.2. Experimental Setup of Experiment 2: Effects of Tannin and *C. vulgaris* Microalgae on Plant Performance and Growth

On March 2023, strawberry plants (*Fragaria x ananassa* ANIA®CIRVH612) were purchased at the nursery. In total, 105 homogeneous plants with an average of three leaves and fresh weight of 8.1 g were transplanted individually into pots of 3.6–3.8 L volume. The pots were filled with a mix of commercial substrate (Doroter, Vigorplant, FombioLodi, Italy) and pine bark (Vigor Plant Verde, Fombio, Italy) (pH = electric conductivity = dS m^{−1}; total porosity = % v v^{−1}). Then the pots were placed under a waterproof shading fabric in the nursery area of the Italian National Research Council in Central Italy (Sesto Fiorentino; lat.

43°49'04.2" N, 11°12'06.6" E; 55 m a.s.l.). Climate conditions were continuously monitored by a weather station (Decagon Em50; Decagon Devices Inc., Pullman, WA 99163, USA). The average minimum and maximum temperatures recorded in the period (23 March–31 October) were 16.6 °C and 26.9 °C, respectively, although April recorded an exceptional cold period with a minimum absolute temperature of 2 °C. In addition, three heatwaves occurred during the summer for a total of 30 days with maximum temperature over 34 °C. The pots were irrigated every day with 400 mL of water to maintain the substrate water content near the field capacity until mid-September.

In total, 100 mL of Greenleaf® 20-20-20 (Biolchim SPA, Medicina, Bologna, Italy) nutrient solution at a concentration of 0.1 g/L was applied to the plants every two weeks. The nutrient solution was prepared to achieve the following macro-/micronutrient concentrations: N = 0.2 mg/L, P₂O₅ = 0.2 mg/L, K₂O = 0.2 mg/L, MgO = 0.2 mg/L, Fe = 10 µg/L, Mn = 5 µg/L, Zn = 2 µg/L, Cu = 1 µg/L, B = 2 µg/L, and Mo = 1 µg/L. The nutrient solution was supplied to the strawberries adopting proper dilution rates according to the plant growing stage (50% concentration at seedling establishment stage, 1–30 days after transplant, 100% at vegetative–early flowering stage, 30–90 days after transplant) before the application of the tannin–microalgae blends and treatments.

After three-month adaptation (on 27 June, T₀, Table 1), treatments began. The selected tannin water extract dilution [54%] and *C. vulgaris* algae at two different concentrations [50× dilution] and [100× dilution] were applied as follows:

- CNT = Control (irrigation with water);
- T = tannin water extract irrigation treatment [54% T.E.];
- C1 = *C. vulgaris* algae spray treatment [50× dilution];
- C2 = *C. vulgaris* algae spray treatment [100× dilution];
- T+C1 = tannin water extract [54% T.E.] irrigation + *C. vulgaris* algae [50× dilution] spray treatment.

Table 1. Date, time (T), experimental activities, and analyses performed in Experiment 2.

Date	T	Experiment Activity	Analysis
28 April		Fertilization with nutrient solution	
13 June		Fertilization with nutrient solution	
27 June	T ₀	Start of <i>C.v</i> and tannin treatments	C.f.
4 July		<i>C.v</i> and tannin treatment	
11 July		<i>C.v</i> and tannin treatment	
18 July		<i>C.v</i> and tannin treatment	
19 July	T ₁	Performance and production evaluation	FFP; C.f.
26 July	T ₂	<i>C.v</i> and tannin treatment	
1 August	T ₃	Production evaluation	FFP
2 August		<i>C.v</i> and tannin treatment	
10 August		<i>C.v</i> and tannin treatment	
22 August	T ₄	Performance and production evaluation	FFP; C.f.; LGE; Pigm
29 August		<i>C.v</i> and tannin treatment	
12 September	T ₅	End of <i>C.v</i> and tannin treatments	Bio; C.f.; FFP; M.E.
31 October	T ₆	End of experiment	Bio; C.f.; LGE; Pigm

Abbreviations: Bio: biomass; *C.v.*: *Chlorella vulgaris*; C.f.: chlorophyll fluorescence; FFP: fruit and flower production; LGE: Leaf gas exchanges; M.E.: mineral elements; Pigm: pigments.

As we hypothesized greater effects on plants from more concentrated microalgal treatment, *C. vulgaris* 50× dilution (C1), which corresponded to the highest sonicated cell density, was chosen in combination with T (T+C1) as a first attempt to study the effect of the combined application of algae and tannins. When plants in the treatments T and T+C1 were irrigated fortnightly with 100 mL of tannin solution (instead of water), the other

treatments received 100 mL of water in addition to the regular irrigation. Plants in the treatments with *C. vulgaris* (C1 and C2) were sprayed once a week on the leaves.

On 12 September (T5, Table 1), treatments with tannins and *C. vulgaris* were suspended, and from September to the end of October (T5–T6, Table 1), the plants were kept untreated to evaluate the residual effect of the two stimulants. The times and dates of experimental activities and analysis and dates of treatments are shown in Table 1.

2.3. Biometric Parameters: Plant Growth

Destructive evaluations were performed for Experiment 1 at T6 and for Experiment 2 at T5 and T6. The following biometric parameters were assessed: fresh weight (FW) of leaves, stem, and root; dry weight (DW) of leaves, stem, and root. All the dry weights occurred after oven-drying at 75 °C until a constant weight was reached.

2.4. Leaf Gas Exchange and Leaf Pigments Measurements

Leaf gas exchange parameters such as stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$), net photosynthesis rate (NP, $\mu\text{mol m}^{-2} \text{s}^{-1}$), and leaf evapotranspiration (T, $\text{mmol m}^{-2} \text{s}^{-1}$) were measured using a CIRAS2 gas analyzer (PPSystem, Hitchin, UK) under saturating PAR (photosynthetic active radiation at $1000 \text{ mol m}^{-2} \text{s}^{-1}$) on four fully expanded leaves per treatment. Then, intrinsic Water-Use Efficiency (WUE), the ratio between the photosynthesis rate and stomatal conductance, was calculated on the sample of data. Measurements were performed around 11 a.m. on sunny days, on 22 August (T4), and at the end of the experiment on 10 October (T6). Leaf pigments were measured on the same dates by a non-destructive method using a DualEx sensor (see above) on 3 leaves per plant in four pots per treatment.

2.5. Chlorophyll *a* Fluorescence Measurement

Chlorophyll *a* fluorescence transients were measured using a Handy-PEA (Hansatech Instruments Limited, Norfolk, UK) on five fully developed leaves that were previously dark-adapted for 30 min. Then, the leaves were exposed to saturating light (650 nm peak wavelength, $3500 \text{ mol photons m}^{-2} \text{s}^{-1}$) from light-emitting diodes (LEDs) to track the plants' photosynthetic activity. The Biolyzer HP 3 software version 3.03 (Bioenergetics Laboratory, University of Geneva, Geneva, Switzerland) was used to examine each Chlorophyll *a* fluorescence induction curve according to the JIP-test [37]. The following parameters were calculated from the fluorescence measurements, $V_j = (F_j - F_0)/(F_m - F_0)$, indicating the level of Q_A reduction, and the parameters describing the flux ratio, calculated according to Appenroth et al. Ref. [38], are as follows: F_v/F_m the maximum quantum yield of PSII for primary photochemistry, and $\phi E_o = F_v/F_m \cdot Y_o$, the quantum yield of electron transport.

2.6. Mineral Analysis

Three dry leaf and root samples per treatment were ground for the determination of the total organic carbon (TC) and nitrogen (TN). These were evaluated through dry combustion using a FlashSmart elemental analyzer (Thermo Fisher Scientific, Milan, Italy). Micro- and macronutrients (P, K, Ca, Cu, Fe, Mg, Mn, Zn) were measured using an inductively coupled plasma–optical emission spectrometer (ICP–OES 5900, Agilent, Santa Clara, CA, USA) after acid digestion with $\text{H}_2\text{O}_2/\text{HNO}_3$ 1:3 v/v (EPA 3051A, 6010C). For the determination of P, the samples underwent acid digestion in a microwave (Ethos 1; Milestone S.r.l, Bergamo, Italy) with hydrogen peroxide–nitric acid $\text{H}_2\text{O}_2/\text{HNO}_3$ 1:3 v/v. Then, the content of P was determined by the colorimetric method described by [39], which uses a phosphate-containing water with a single-reagent solution of acidified ammonium molybdate containing ascorbic acid and a small amount of antimony for and spectrophotometric determination of phosphate using the Thermo Spectronic Unicam UV (Milan, Italy).

2.7. Statistical Analysis

The normality of quantitative data (biomass and yields, leaf gas exchange, chlorophyll fluorescence and chemical analysis) in Experiments 1 and 2 was checked using the Shapiro–Wilk’s test; homogeneity of variance was tested using the Levene test. When the assumptions were met, one-way analysis of variance (ANOVA) was applied, followed by the Tukey or Duncan test as a post hoc comparison of the means between treatments at a 95% confidence level. When the assumptions were not met, the Kruskal–Wallis nonparametric test was applied, followed by a multiple-comparison test. A summary of the variances for all measurements and the coefficient of variation in the groups are reported in the Supplementary Materials (Table S2). In addition, the *t*-test for independent samples at $p < 0.05$ was applied to compare the leaf pigment content at T4 and T6 within each treatment.

The data are reported as the mean and Standard Deviation (SD). Statistical analysis was carried out using Statistica Statgraphics Centurion XV (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Phytotoxicity Test on the Tannin Water Extract Produced via Hydrodynamic Cavitation

The germination test on *Lepidium sativum* L. (Table 2) after 24 h resulted in $90 \pm 12\%$ seed germination in the pure tannin water extract, $88 \pm 13\%$ in 75% dilution, $76 \pm 21\%$ in 50% dilution, and $92 \pm 18\%$ in distilled water. Radicle lengths were 3.7 ± 1 mm in the pure tannin water extract, 3.4 ± 0.9 and 2.9 ± 1.3 in 75% and 50% dilution, respectively, and 5 ± 1.6 mm in control, without significant differences among the treatments. Calculated Ig resulted in 71% in the pure tannin water extract, 66% Ig in the 75% dilution, and 48% in the 50% dilution. These values indicate the pure tannin extract produced by hydrodynamic cavitation as a non-phytotoxic product.

Table 2. Seed germination after 24 h of incubation (Germination—G (%); Radicle length (mm); Germination Index—Ig (%); final germination rate at the end of the experiment—Gfin (%)).

	G (%)	Radicle Length (mm)	Ig (%)	Gfin (%)
CTRL	$92\% \pm 18\%$	5.00 ± 1.58		$100\% \pm 0\%$
50% T	$76\% \pm 21\%$	2.92 ± 0.91	48%	$96\% \pm 9\%$
75% T	$88\% \pm 13\%$	3.44 ± 1.28	66%	$98\% \pm 4\%$
100% T	$90\% \pm 12\%$	3.65 ± 0.95	71%	$96\% \pm 5\%$

Each value represents the mean $\pm$ Standard Deviation (SD). CTRL = control, distilled water; 100% T = pure tannin water extract produced via hydrodynamic cavitation; 75% T = 75% dilution in water of the tannin water extract; 50% T = 50% dilution in water of the tannin water extract. ANOVA turned non-significant (no letters are reported).

3.2. Experiment 1: Tannin Treatments at Three Different Concentrations on Strawberry Plants

In Exp. 1, strawberry plants were treated with three different concentrations of tannin water extract obtained from hydrodynamic cavitation and compared to commercial tannin (COM) and water as control (CNT).

In total, 54% T.E. treatment produced plants with greatest fresh and dry leaf mass (0.86 and 0.21 g, respectively), while stems and root biomass were similar in all treatments (Table 3). This induced a higher ratio between the aerial part and root (0.40), although it was only statistically different from COM. Under the same treatment, plants showed the highest number of main roots (117.2 ± 55) and secondary roots (49.8 ± 16.5) and the longest secondary roots (64.1 ± 25 mm), although the high variability within the samples did not result in a statistical difference between the treatments.

Table 3. Experiment E1: strawberry plants treated with different concentrations of tannin at Tf. FW: fresh weight; DW: dry weight; L: length; N number.

	CNT	COM	13% T.E.	27% T.E.	54% T.E.
Leaves FW (g)	0.62 ± 0.19 bc	0.68 ± 0.05 b	0.55 ± 0.04 c	0.69 ± 0.24 bc	0.86 ± 0.07 a
Stem FW (g)	0.64 ± 0.11 ns	0.72 ± 0.13 ns	0.64 ± 0.19 ns	0.63 ± 0.18 ns	0.81 ± 0.35 ns
Root FW (g)	5.01 ± 0.65 ns	5.7 ± 0.62 ns	4.84 ± 1.20 ns	4.89 ± 1.53 ns	4.22 ± 0.33 ns
Total FW (g)	6.26 ± 0.70 ns	6.35 ± 0.15 ns	6.03 ± 1.30 ns	6.22 ± 1.8 ns	5.80 ± 0.81 ns
Leaves DW (g)	0.12 ± 0.04 b	0.14 ± 0.00 b	0.11 ± 0.04 b	0.15 ± 0.07 b	0.21 ± 0.05 a
Stem DW (g)	0.09 ± 0.04 ns	0.07 ± 0.03 ns	0.08 ± 0.03 ns	0.08 ± 0.04 ns	0.10 ± 0.03 ns
Root DW (g)	1.11 ± 0.21 ns	1.26 ± 0.24 ns	1.00 ± 0.30 ns	1.15 ± 0.50 ns	1.13 ± 0.12 ns
Total DW (g)	1.19 ± 0.20 ns	1.32 ± 0.5 ns	1.07 ± 0.30 ns	1.23 ± 0.50 ns	1.23 ± 0.10 ns
Aerial part/root (FW)	0.25 ± 0.05 ab	0.22 ± 0.05 b	0.26 ± 0.07 ab	0.28 ± 0.07 ab	0.40 ± 0.09 a
Aerial part/root (DW)	0.19 ± 0.09 ns	0.17 ± 0.06 ns	0.20 ± 0.08 ns	0.21 ± 0.05 ns	0.28 ± 0.09 ns
N main roots/plant	48.8 ± 9.2 b	128.8 ± 57.5 a	94.2 ± 50 a	121.5 ± 59.3 a	117.2 ± 55 a
N sec roots/plant	34.8 ± 25.3 ab	27.5 ± 7.7 ab	13.6 ± 18 b	43 ± 27 a	49.8 ± 16.5 a
Main root L (mm)	125.1 ± 48.7 ns	103.9 ± 24.8 ns	102.1 ± 27.3 ns	100.6 ± 22.8 ns	119.2 ± 12 ns
Sec root L (mm)	40.5 ± 11 ns	48.1 ± 8.3 ns	35.2 ± 11.4 ns	43.1 ± 10.5 ns	64.1 ± 25.1 ns
Chlorophyll (µg cm ⁻²)	30.96 ± 1.95 a	29.56 ± 2.23 bc	28.85 ± 2.56 c	31.25 ± 1.55 a	30.62 ± 2.45 ab
Flavonols	0.03 ± 0.02 ns	0.08 ± 0.08 ns	0.07 ± 0.06 ns	0.02 ± 0.02 ns	0.11 ± 0.01 ns
Anthocyanins	0.02 ± 0.014 ns	0.01 ± 0.01 ns	0.01 ± 0.01 ns	--	0.01 ± 0.01 ns

Each value represents the mean ± Standard Deviation (SD); different letters between treatments indicate a significant difference ($p < 0.05$) according to Duncan test after ANOVA and multiple-comparison test after Kruskal–Wallis (N main roots/plant); ns: non-significant; --: not measured.

The increase in leaf and root growth in rooting plants due to tannin treatment is consistent with previous studies [18,40]. Regarding the pigments, only chlorophyll showed a statistical difference among treatments, with COM and 13% T.E. recording the lowest values.

Although the differences between commercial tannin and the tannin water extract produced by hydrodynamic cavitation at 54% T.E. concentration were not striking, as the latter showed slightly higher values in some growth parameters (leaves and roots), it was selected for Exp. 2.

3.3. Experiment 2: Effect of Tannin and Chlorella Treatments on Strawberry Plants

In Exp. 2, a different concentration of microalgae *C. vulgaris* (C1 and C2), the tannin water extract selected from Exp. 1 (in this experiment called T), and T together with *C. vulgaris* (T+C1) were administered to strawberry plants (Figure 1A).

3.3.1. Biomass

Table 4 shows the biomass of the plants at the end of the treatment period (T5). C1 plants recorded the highest leaf fresh weight, especially as compared to T and T+C1 (15.72 ± 0.05 g vs. 11.16 ± 0.08 and 10.15 ± 2.77 g, respectively). In addition, C1 recorded a greater fresh root biomass than T ($p < 0.05$) and was like other treatments, although the greatest dry root mass was found in T+C1 (7.22 ± 0.03 g). For this reason, this treatment showed the lowest ratio between the aerial part and roots, indicating a prevalent biomass allocation below-ground (Table 4; Figure 1B–F).

Table 4. Experiment 2: fresh weight (FW) and dry weight (g) (DW) of leaf, stem, and root of strawberry plants treated with different concentrations of microalgae *C. vulgaris* (C1 and C2) and tannin water extract (T) at the end of the treatments (T5).

	CNT	C1	C2	T	T+C1
FW Leaf (g)	13.82 ± 1.96 ab	15.72 ± 0.05 a	12.51 ± 1.69 ab	11.16 ± 0.08 b	10.15 ± 2.77 b
DW Leaf (g)	3.10 ± 0.45 ns	3.34 ± 0.63 ns	2.89 ± 0.27 ns	3.08 ± 0.97 ns	2.37 ± 0.53 ns
FW Root (g)	30.12 ± 3.89 bc	36.22 ± 3.1 ab	31.04 ± 1.94 abc	24.55 ± 4.76 c	39.75 ± 5.67 a
DW Root (g)	5.12 ± 0.07 b	5.88 ± 0.03 b	4.90 ± 0.1 b	5.11 ± 0.07 b	7.22 ± 0.03 a
FW Stem (g)	3.18 ± 1.17 ns	1.53 ± 0.20 ns	2.25 ± 0.68 ns	2.12 ± 0.69 ns	1.25 ± 0.41 ns
DW Stem (g)	0.40 ± 0.17 ns	0.26 ± 0.05 ns	0.38 ± 0.15 ns	0.24 ± 0.02 ns	0.22 ± 0.09 ns
FW Aerial Part (g)	16.45 ± 3.03 a	16.57 ± 2.12 a	14.20 ± 0.09 ab	13.40 ± 1.59 ab	11.69 ± 2.58 b
DW Aerial part (g)	3.4 ± 0.64 ns	3.28 ± 0.42 ns	2.59 ± 0.61 ns	3.26 ± 0.91 ns	3.6 ± 0.61 ns
Aerial Part/Root (FW)	0.58 ± 0.16 ns	0.48 ± 0.04 ns	0.48 ± 0.10 ns	0.55 ± 0.07 ns	0.29 ± 0.09 ns
Aerial Part/Root (DW)	0.70 ± 0.26 ns	0.61 ± 0.19 ns	0.67 ± 0.14 ns	0.64 ± 0.32 ns	0.35 ± 0.13 ns

Each value represents the mean ± Standard Deviation (SD); different letters among treatments indicate significant difference ($p < 0.05$) according to Tukey's test after ANOVA or multiple-comparison test after Kruskal–Wallis (FW leaf), ns: non-significant.



Figure 1. Strawberry plants treated during Experiment 2. (A) Strawberry plants in the nursery area at T1; (B–F) plants at the end of treatment (T5): (B) control; (C) treatment with microalgae *Chlorella vulgaris* at 50× dilution (C1); (D) treatment with *Chlorella vulgaris* at 100× dilution (C2); (E) treatment with tannin water extract (T); (F) treatment with tannins and *C. vulgaris* (T+C1).

The results shown in Table 4 indicate that *C. vulgaris* acted as a biostimulant for leaf and root biomass, and the combination of tannins and *C. vulgaris* further promoted root development. Such results are in line with those reported in previous works, where the foliar application of *Chlorella vulgaris* extracts positively influenced plant growth (leaves and root) in terms of the fresh and dry weight of bean [41] and lettuce plants [42].

Seven weeks after the end of the treatments (T6), plants treated with tannins showed higher fresh root weights than control and *C. vulgaris* treatments (Figure 2). In particular, T and T+C1 showed the highest fresh root weight (28.66 and 25.30 g, respectively) and treatment T showed the highest dry root weight (10.5 g). In contrast, in the same treatment T, plants showed a lower aerial part than C2 ($p = 0.04$), but similar to the other treatments (Figure 2).

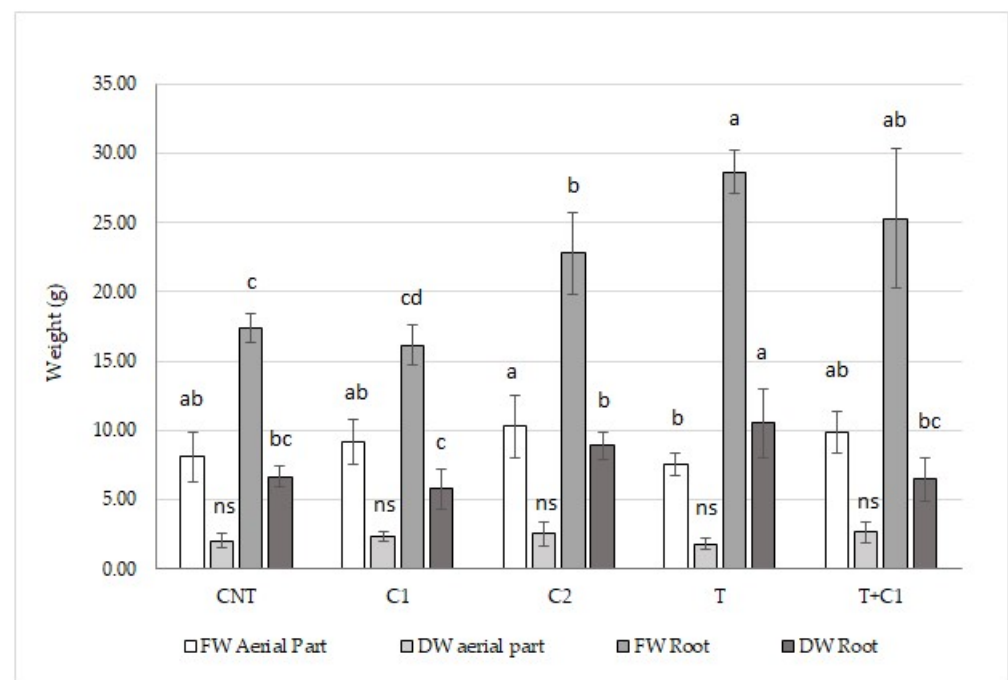


Figure 2. Experiment 2: fresh and dry weight (FW; DW, g) of the aerial part (leaf and stem) and root of strawberry plants in the different treatments at the end of the experiment (T6). Mean values and Standard Deviation bars are shown. Different letters above each column indicate the significant differences among the treatments (Tukey's test $p < 0.05$ after ANOVA or multiple-comparison after Kruskal–Wallis (DW roots); ns = non-significant).

Comparing the biomass between T5 and T6, there was a marked decrease in the fresh weight of the aerial part in CNT and C1 (−8.9 and −8.1 g, respectively), while the least decrease was observed in T+C1 (−1.5 g). These treatments also showed a marked decrease in root fresh weight (−12.7, −20.1, and −14.5 g, respectively), while T showed a slight increase (+4.1 g). In our experiment, the effect of *Chlorella* as a biostimulant for aerial growth did not persist over time. Tannin, on the other hand, seemed to maintain its effect even several weeks after the last treatment, probably due to the mode of application to the substrate. The stimulatory effect of tannin on root growth has already been observed by other authors in tomato and grapevine plants [15,18,40]. For instance, a study on tomato [15] revealed that a tannin-based biostimulant upregulated genes correlated to root development and involved in nutrient uptake, contributing to adequate plant nourishment [15]. The study by [18] confirms the development of fine roots, and indicates an increase in the total above-ground mass in grapevine.

3.3.2. Production of Flowers and Fruits

Figure 3 shows the trend in flower (Figure 3a) and fruit yield (Figure 3b) in the five treatments. At T1, there was a flowering peak (Figure 3a), with C1 recording more fruits than CNT and T+C1 ($p < 0.05$) (Figure 3b). The most striking finding was the decline in the number of flowers in all treatments after time T1. Indeed, after T1, the flower production decreased in all treatments and remained stable until the end of the experiment (Figure 3a), probably due to the high summer temperatures. Regarding the fruit yield, C1 and T+C1 counted the highest number of fruits at T3, significantly higher than CNT ($p < 0.05$), although the peak of fruit occurred at the end of August (T4) in all treatments (Figure 3b). Subsequently, in September (T5), fruit production declined in all treatments, reaching values similar to those recorded in July (T1) and at the beginning of August (T3). This reflected the normal cycle of fruit ripening and harvesting. Remontant strawberries produce flowers and fruit in a staggered and continuous manner from late spring to late autumn, with peaks alternating with periods of lower activity.

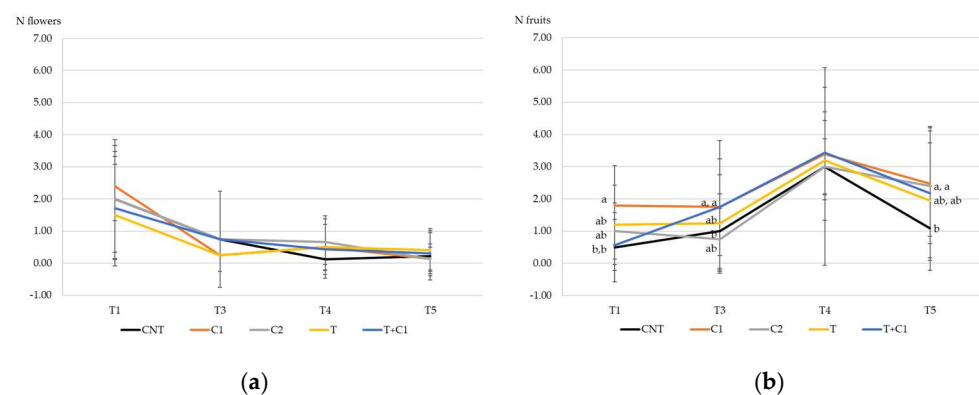


Figure 3. Average number of flowers (a) and fruits (b) and Standard Deviation bars at times T1, T3, T4, and T5. Treatments with different concentrations of microalgae *C. vulgaris* (C1 and C2), tannin (T), and tannin together with *C. vulgaris* (T+C1). Different letters indicate significant differences between treatments at $p < 0.05$ by Duncan test.

The application of high-dose *C. vulgaris* (C1) alone or in combination with T proved to be an effective strategy to increase plant productivity, enhancing both flowering and final fruiting. C1 and C2 also showed slighter decline, suggesting that the positive effect on production was mainly due to the microalgae in the long term. *C. vulgaris*, particularly at a high concentration (C1), may have provided the right nutritional/hormonal ‘boost’ to maximize ongoing flowering. It has been demonstrated that foliar application of *C. vulgaris*, in addition to stimulating vegetative growth on different crops [43–45], also promotes flower and fruit productions in strawberries [45]. Indeed, some of the literature evidences the production of a wide range of bioactive compounds, including growth regulators such as auxins and cytokinins, by microalgae such as *C. vulgaris* [46], which are likely the main contributors to plant biostimulation. Moreover, foliar application is supported by literature, showing that spraying is the most effective method for the absorption of plant growth promoters through stomata.

3.3.3. Leaf Gas Exchange and Leaf Pigments

No statistically significant differences were found between the treatments in leaf gas exchange parameters at T4 (Figure 4), although plants under T (tannin treatment) showed slightly lower values for evapotranspiration and stomatal conductance and similar net photosynthesis values, resulting in higher WUEi. At the end of the experiment, at T6, the trend was confirmed.

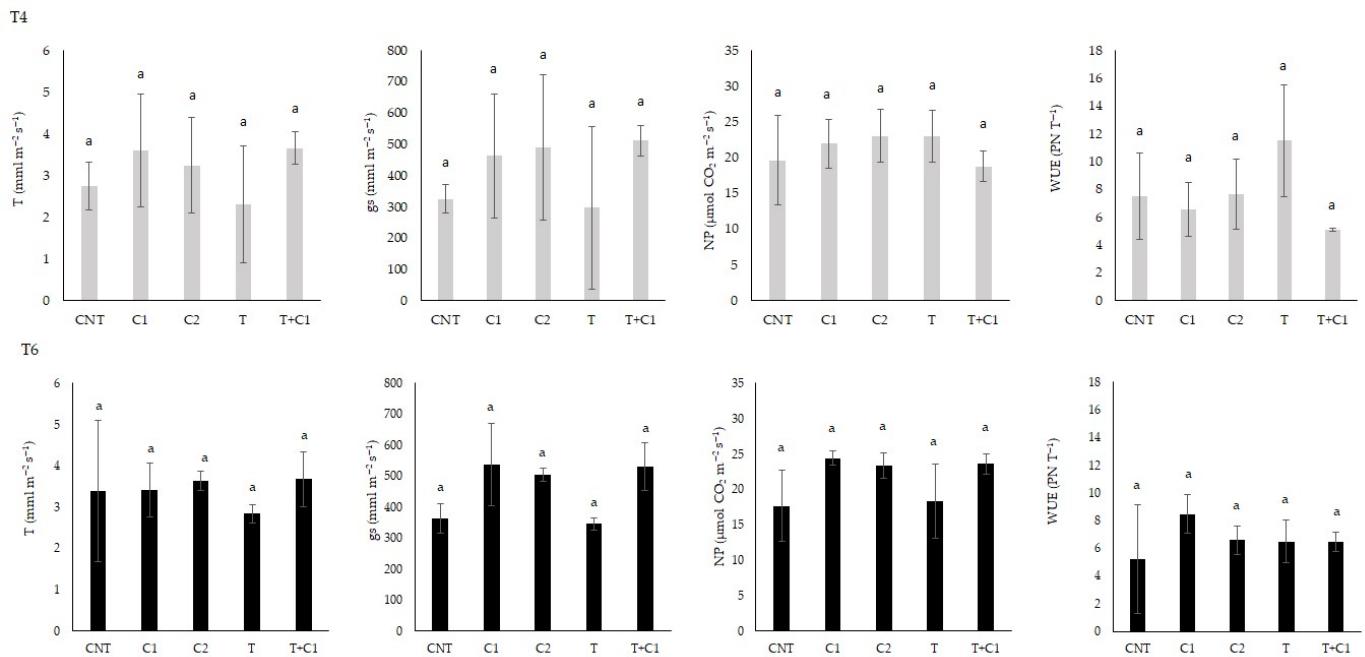


Figure 4. Leaf gas exchanges on fully developed leaves at T4 (upper graphs) and at T6 (below graphs). Same letters above the bars indicate no statistical difference between treatments by Tukey's test at $p < 0.05$. Error bars represent the Standard Deviation (SD).

No significant differences in pigment content (chlorophyll, flavonols, and anthocyanins) were found among the treatments, either at T4 or T6 (Table 5). Differences were found in chlorophyll contents between T4 and T6 in CNT ($p = 0.006$) and C1 ($p < 0.01$) and for anthocyanins in T+C1, with higher values in T4 ($p = 0.02$). This is in line with studies reporting the possible direct and indirect mechanisms of microalgae (such as *Chlorella* spp.)-based biostimulants on the upregulation of chlorophyll biosynthesis and delayed leaf senescence [36].

Table 5. Leaf pigments contents (chlorophyll, flavonols, anthocyanins ($\mu\text{g cm}^{-2}$) of fully developed leaves measured by non-destructive sampling using Dualex.

	Chlorophyll			Flavonols			Anthocyanins		
	T4	T6	T4 vs. T6	T4	T6	T4 vs. T6	T4	T6	T4 vs. T6
CNT	25.1 ± 2.5 ns	32.7 ± 2.6 ns	**	1.2 ± 0.1 ns	1.1 ± 0.4 ns	ns	0.03 ± 0.01 ns	0.013 ± 0.00 ns	n.a.
C1	26.4 ± 1 ns	29.8 ± 1.8 ns	*	1.2 ± 0.1 ns	1.3 ± 0.1 ns	ns	0.022 ± 0.00 ns	0.013 ± 0.01 ns	n.a.
C2	27.3 ± 3.5 ns	29.2 ± 2 ns	ns	1.2 ± 0.2 ns	1.2 ± 0.1 ns	ns	0.033 ± 0.01 ns	0.015 ± 0.01 ns	n.a.
T	26.2 ± 2.1 ns	27.9 ± 4.2 ns	ns	1.2 ± 0.2 ns	1.1 ± 0.5 ns	ns	0.026 ± 0.01 ns	0.02 ± 0.01 ns	n.a.
T+C1	25.6 ± 1.3 ns	28.4 ± 2.4 ns	ns	1.4 ± 0 ns	1.2 ± 0.1 ns	ns	0.036 ± 0.01 ns	0.014 ± 0.00 ns	n.a.

Each value represents the mean ± Standard Deviation (SD). '**', '**' indicate a significant difference ($p < 0.05$, $p < 0.01$) between T4 and T6 according to the *t*-test for independent samples within the treatment, ('ns' non-significant). 'ns' near the values indicate non-significant differences between treatments. 'n.a.' not available.

3.3.4. Chlorophyll Fluorescence Measurements

Table 6 shows the data of the JIP test parameters. At each time, the photosynthetic activity, measured as F_v/F_m , did not show significant differences among the treatments, despite recording slightly higher values in T and lower in C2 with respect to other treatments. The average F_v/F_m during the experiment was 0.746, indicating photosynthetic efficiency in all plants, although it decreased at T6 as compared to T5 in CNT ($p < 0.01$), C1 ($p < 0.01$), and T+C1 ($p < 0.05$).

Table 6. Changes in JIP test parameters during the growth with the different treatments.

Time	Treatment	V_J	F_v/F_m	ϕEo
T0		0.468 ± 0.059	0.721 ± 0.041	0.385 ± 0.061
T1	CNT	0.426 ± 0.038	0.768 ± 0.020 AB	0.441 ± 0.029
	C1	0.435 ± 0.044	0.764 ± 0.024 AB	0.431 ± 0.035
	C2	0.465 ± 0.080	0.746 ± 0.054	0.402 ± 0.080
	T	0.464 ± 0.045	0.782 ± 0.005	0.419 ± 0.034
	T+C1	0.426 ± 0.065	0.758 ± 0.035 AB	0.435 ± 0.049
T4	CNT	0.424 ± 0.001	0.781 ± 0.003 AB	0.439 ± 0.008
	C1	0.441 ± 0.001	0.781 ± 0.003 AB	0.440 ± 0.004
	C2	0.453 ± 0.002	0.774 ± 0.007	0.429 ± 0.008
	T	0.454 ± 0.002	0.782 ± 0.005	0.431 ± 0.002
	T+C1	0.436 ± 0.001	0.775 ± 0.006 A	0.434 ± 0.001
T5	CNT	0.423 ± 0.025	0.800 ± 0.022 A	0.462 ± 0.031
	C1	0.446 ± 0.029	0.797 ± 0.026 A	0.441 ± 0.029
	C2	0.441 ± 0.048	0.782 ± 0.024	0.437 ± 0.036
	T	0.446 ± 0.031	0.797 ± 0.018	0.441 ± 0.033
	T+C1	0.445 ± 0.057	0.786 ± 0.023 A	0.436 ± 0.044
T6	CNT	0.681 ± 0.062	0.622 ± 0.043 B	0.203 ± 0.017
	C1	0.336 ± 0.056	0.648 ± 0.011 B	0.432 ± 0.205
	C2	0.448 ± 0.030	0.667 ± 0.096	0.339 ± 0.003
	T	0.421 ± 0.019	0.687 ± 0.013	0.350 ± 0.002
	T+C1	0.451 ± 0.064	0.658 ± 0.083 B	0.337 ± 0.022

Each value represents the mean $\pm$ Standard Deviation (SD). There were non-significant differences among the treatments within the sampling time (no letters are shown). Different capital letters indicate significant differences at $p < 0.05$ between sampling times within the treatment according to the multiple-comparisons test after Kruskal–Wallis.

The V_J is related to the accumulation of electrons at the plastoquinone level and is associated with a reduction in photosynthetic activity. Similarly to F_v/F_m , V_J was similar in all treatments, being, on average, 0.449. V_J did not change significantly during the experiment in any treatment.

The electron transport rate, indicated by ϕEo , was, on average, 0.408, without significant differences among the treatments or dates. It also showed low values at T6 without statistically significant differences between treatments.

These results indicate that the different treatments did not induce any reduction in the efficiency of the photosynthetic apparatus, as the photosynthetic activity and the electron transport rate were maintained at the same level in all the plants. Moreover, electron accumulation was not detected, avoiding the possibility of damage at the photosynthetic apparatus level. Indeed, the decrease of F_v/F_m value, associated with increase of V_J and decrease in ϕEo values, are considered to be a signal of physiological stress and may be concomitant to the induction of the photoprotection mechanism and the reduction in plant production [37].

Since no differences were recorded in any of the analyzed physiological parameters (leaf gas exchange, pigments, chlorophyll fluorescence), we can conclude that, under our experimental conditions, the plants performed optimally.

3.3.5. Mineral Composition

There were no differences between treatments for nitrogen and carbon contents in roots and or in leaves (Figure 5). All macro elements (P, K, Ca, Mg) showed higher values in control leaves and lower values in treatment T+C1. P ranged from 4574 to 1587 mg/kg (Figure 4). These values fell within the appropriate P levels, as reported in the study by [46]. In the roots, there were no statistically significant differences in the concentrations of TC, TN, and P. The remaining elements recorded the highest concentration in the tannin

treatment (20.6 mg/kg for K, 10.7 mg/kg for Ca, and 2.7 mg/kg for Mg). The concentration of microelements, particularly Fe and Zn, was higher in T and T+C1 leaves, while Cu ranged from 19 mg/kg in T to 11.8 mg/kg in C1 in the leaves. Mn had the highest value in CNT (128.9 mg/kg) and the lowest value in C1 (30.8 mg/kg) leaves. The values recorded in the leaves were within the appropriate microelement ranges for this species, except for Fe and Zn in T and T+C1 [47]. Unlike the leaves, the roots recorded the lowest levels of Fe and Zn in treatment T (56.3 mg/kg and 61.5 mg/kg, respectively; Figure 5).

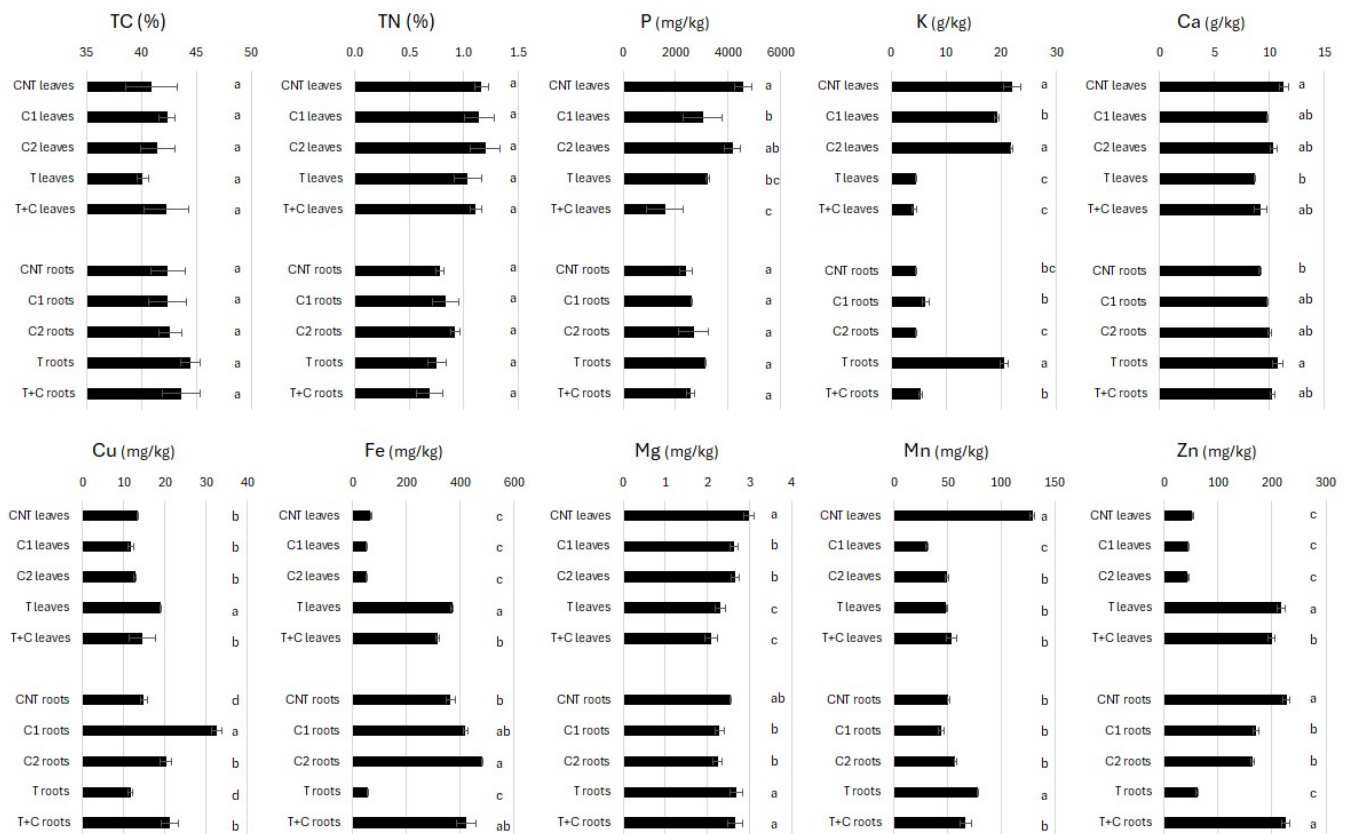


Figure 5. Macro- and micronutrients in leaves and roots of strawberry plants in Experiment 2. Means and Standard Deviation bars are shown. Small different letters indicate statistical differences between treatments for leaves and roots by Tukey's test as post hoc comparisons of means after ANOVA or by the multiple-comparisons test after Kruskal–Wallis.

Despite the differences observed in the various treatments in terms of the concentration of different macro- and microelements, the plants did not show any symptoms of nutritional imbalance. It is therefore possible to hypothesize that the addition of *C. vulgaris* and tannins stimulated nutrient uptake efficiency. Similar results were observed by Kim et al., who treated strawberry plants with *Chlorella fusca* [48].

There is no direct relationship between tannins and chlorella on plant growth reported in the literature. However, based on our results, we can hypothesize a synergistic effect of the two compounds. Under our experimental conditions, the positive effect of tannins on root structure was confirmed, but at the same time, an average increase in root biomass of about 10% was observed in plants treated with tannins and *Chlorella*. The presence of phytohormones (auxins, cytokinins, abscisic acid, gibberellins, polyamines, and brassinosteroids) in *Chlorella* could increase the stimulating action of tannins at the root level. It is also possible to hypothesize a positive action of the two compounds on the absorption of mineral elements. In fact, the physiological action of the major and minor nutrients that *Chlorella* is rich in and a more efficient root system favored by tannins could improve

nutrient absorption and utilization by the plant. In addition, the combined action of tannins and *Chlorella* on plant health could also increase the action of the active molecules present in microalgae.

4. Conclusions

Under our experimental conditions, the application of *Chlorella vulgaris* microalgae to strawberry plants influenced the growth of vegetative and generative organs. *C. vulgaris* extract lost its effectiveness at the end of treatment. At the time of the final measurements, *C. vulgaris* did not appear to have any influence on growth. This suggests that it is likely that the treatments should be prolonged throughout the entire vegetative cycle to maintain an influence on strawberries. Future studies are also needed to define the optimal period and the frequency of application of the microalgae, as well as to further understand the most effective type of application, such as comparing aerial spraying with addition to soil, or the combination of both, which could make the treatment more effective over time.

Tannin acted as a stimulant on the strawberry root system, especially in combination with *C. vulgaris*. This result is particularly interesting because a stronger root system enhances the plant's resistance to environmental stress (e.g., water and nutrient deficiencies) and to certain pathogenic attacks (e.g., microbes and nematodes present in the soil). However, there are few studies using the tannin extract produced via hydrodynamic cavitation, so further research is needed to define the optimal species-specific concentration of tannin, assessing any negative effects it may have on plant performance.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture15232459/s1>. Figure S1: Growth curve, as increment of both total chlorophyll (triangles) and dry weight (bars) (biomass accumulation). The grow rate is also reported, as text; Table S1: Composition of BG11 culture medium used for the cultivation of *C. vulgaris*; Table S2: Descriptive statistics of treatments with coefficient of variations and ANOVA results for all tables and figures inside the manuscript.

Author Contributions: Conceptualization, C.G. and R.P.; methodology, C.G., R.P., C.F., F.U. and A.D.P.; data curation: C.G., C.F., F.U. and R.P.; technical support, F.S. and F.M.; supervision: C.G. and R.P.; writing—original draft preparation, C.G. and R.P.; writing—review and editing, C.G., R.P., C.F., F.U. and A.D.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The research was carried out with the support of the CNR-IBE technician Mario Lanini for help and assistance during the setup of the experiment.

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

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