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Vermicompost Tea in the Production, Gas Exchange and Quality of Strawberry Fruits

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Abstract

The water-soluble extract from vermicompost, also known as vermicompost tea (VT), has attracted interest in sustainable production research due to its potential to increase crop yields. However, information regarding the influence of this bioinput on strawberry cultivation remains limited. This study aimed to evaluate the effects of different VT solution concentrations on the mass fruit, physiology, and fruit quality of the hybrid strawberry cultivar 'Portola'. The experiment was conducted in a greenhouse, with foliar and substrate applications of VT solutions at varying concentrations (0%, 2%, 4%, 6% and 8%) over 150 days. Evaluations included the chemical composition of the VT, as well as the physiological and agronomic parameters of the strawberry plants, such as gas exchange, biometric data, the physicochemical quality of the fruit and the nutritional composition. Significant differences in gas exchange parameters, particularly intercellular CO_2 concentration and stomatal conductance, were observed at the final growth stage. Of the quality and compositional parameters of the strawberries, only the soluble solids/titratable acidity (SS/TA) ratio was affected. The various VT dilutions induced physiological alterations in the strawberry plants, with energy being allocated towards mass fruit at the expense of fruit quality, specifically in terms of the SS/TA ratio.

Keywords: gas exchange; bioinputs; plant stimulation; vegetable nutrition

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1. Introduction

Vermicomposting is a method of treating organic waste involving ingestion, fragmentation, and reduction in material volume through intense earthworm activity and the associated microbial community [1,2]. Several organic wastes produced by agricultural activities can be treated by vermicomposting, and cattle manure is notable for its nutrient and organic matter content, which is associated with the intensification of livestock production [3]. The final product, vermicompost, is a mixture of organic matter, nutrients, substances that promote plant growth and microorganisms. It is considered an excellent fertiliser that increases crop growth and yield [4,5].

The soluble substances and microorganisms present in vermicompost can be used directly on crops or produced as aqueous extracts, also known as vermicompost tea (VT) [6].

VT, which is derived from vermicompost through various methods, offers benefits associated with the individual, additive or synergistic effects of their various constituents such as trace nutrients, plant hormones, microorganisms, amino acids and humic acids [4,7,8]. Vermicompost and VT can contribute to reducing the impact of climate change on crops [9], particularly through their effects on managing pests, nematodes and phytopathogenic fungi, as well as reducing the occurrence of abiotic stresses such as water deficit, salt presence and nutritional imbalance [6].

In addition to influencing production parameters through the application of VT, recent studies have also reported enhancements in fruit quality [10,11]. Weekly applications of VT at concentrations ranging from 5% to 20% (v/v) have been shown to increase tomato productivity [7]. Spraying a solution of 5% VT on the leaves and fruits of pomegranate trees, and applying a solution of 20% VT around the trunk three times a year for two consecutive years, improved the quality of the fruit [10]. Strawberries are valued for their sweetness, colour and nutritional content, and they respond well to both organic and inorganic fertilisers [12]. However, information on the use of VT in strawberry production is still limited. Most existing studies have examined the impact of inorganic or organic fertilisers, including manure, vermicompost, vermicompost leachate and microbial consortia [12–14].

In a pioneering study, Hargreaves et al. [15] found that strawberries produced using composts and compost teas had an equal total antioxidant capacity and vitamin C content. A foliar spray of vermicompost leachate produced from vermicomposting cow dung, vegetable waste and a mixture of cow dung and vegetable waste (2 mL L^{-1}), applied monthly for a total of five sprays, improved leaf area by 10.1–18.9%, dry matter content by 13.9–27.2% and strawberry fruit yield by 9.8–13.9% for the 'Chandler' variety. 'Chandler' by 9.8–13.9%, which was significantly higher than the control group [16]. Strawberries obtained from plants exposed to a combined application of rhizosphere inoculation and foliar spraying with a microbial-enriched vermicompost tea had the highest concentrations of total sugars and total organic acids, which was considered a useful tool for organic strawberry cultivation [14].

The scarcity of research on the effects of VT on strawberries suggests there is untapped potential in influencing the number and mass of fruits, Brix and overall productivity [14]. This study aimed to evaluate the effects of applying different doses of vermicompost tea on the production, gas exchange and fruit quality of strawberry plants grown in a greenhouse. The hypothesis is that VT might influence plant growth and gas exchange, thereby affecting the quality of strawberry fruit.

2. Material and Methods

2.1. Experimental Characterisation

The experiment was carried out at the Biology Experimental Station (latitude: −15.736465°, longitude: −47.881203°) at the University of Brasília (UnB) in the Federal District of Brasília, Brazil.

The "Portola" variety of strawberry seedlings (*Fragaria* x *ananassa* Duch.) were imported from Spain. The seedlings were conditioned in Ziploc bags in a refrigerator at 5 °C for one day and then remained in a room at 23 °C for six hours. They were then pruned to a standard size of approximately 5 cm of roots and immediately planted in plastic trays containing 50 square cells (136 mL per cell) filled with Vivatto® substrate composed of sphagnum peat, expanded perlite, and calcium carbonate, with a density of 260 kg m^{-3} on a dry basis, a water retention capacity (WRC) of 200%. The chemical analysis revealed the following parameters: pH (H_2O) = 5.4; OM (%) = 12.3; Ca^{2+} ($cmol_c$ dm^{-3}) = 1.9; Mg^{2+} ($cmol_c$ dm^{-3}) = 2.2; K^+ ($cmol_c$ dm^{-3}) = 1.2; p (mg dm^{-3}) = 230; S (mg dm^{-3}) = 234.6; CEC

($cmol_c$ dm^{-3}) = 8.9; Mn (mg dm^{-3}) = 8.2; Fe (mg dm^{-3}) = 65.4; Cu (mg dm^{-3}) = 13.2; Zn (mg dm^{-3}) = 8.4; B (mg dm^{-3}) = 6.36.

The seedlings were acclimatised in a greenhouse for 14 days and then selected for uniform size before being transplanted (one seedling per 8 L plastic pot filled with the same substrate). Strawberry was cultivated in a greenhouse over a period of 24 weeks (June to November 2020). The experimental design consisted of five treatments based on the application of different vermicompost tea dilutions (0%, 2%, 4%, 6% and 8% v/v), replicated six times in a randomised complete block design (RCB).

2.2. General Description of Vermicomposting and Vermicompost Tea Production

Vermicomposting was carried out in 310 dm^3 polyethylene box using cattle manure and California red earthworms (*Eisenia fetida*). The process involved 30 days of initial composting, with the contents being turned and humidified weekly. A tap was installed to prevent liquid accumulation and allow for collection and recirculation. After this period, 80 adult earthworms were added per dm^3 of compost and vermicomposting continued for a further 90 days. VT was prepared using the method described by [17], with adaptations. In a plastic container, a suspension of vermicompost and chlorine-free water at a ratio of 1:10 (v/v) was kept aerated for 10 min every three hours for 48 h in total. After this, the suspension was filtered through cotton mesh to obtain concentrated vermicompost tea (VT). Samples (n = 3) were obtained and frozen at <4 °C for chemical analysis.

After obtaining the concentrated VT, it was diluted with distilled water to create solutions at 2%, 4%, 6% and 8% (v/v), which were used as treatments, as described in Section 2.1. The concentrated VT and the diluted VT solutions (2%, 4%, 6% and 8%) for application to the strawberry plants were prepared weekly. These solutions were then sprayed onto the leaves and around the crown of each plant, at a rate of 10 mL per plant. The control treatment received an equal volume of distilled water. This process began three days after the seedlings were transplanted into cultivation pots and continued weekly for four months, with a total of 300 mL applied to each plant. Prior to being transferred from the acclimated room to the greenhouse, the strawberry plants were given 40 mL of nutrient solution [18] at a quarter of the recommended strength on alternate days. After transplanting, the plants received 250 mL of the same nutrient solution daily at full strength.

2.3. Vermicompost Tea Analysis

To determine the pH and electrical conductivity (EC), a portable multi-parameter instrument (AK-88, AKSO®, São Leopoldo, Brazil) was used directly on the concentrated VT sample. For pH, the instrument was calibrated using standard buffer solutions at pH 4 and 7; for EC, a standard solution of 12.88 dS m^{-1} was used. The nutrients in the VT were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) after digestion using nitric and perchloric acids, as described in the manual of official analytical methods for fertilisers and correctives [19].

2.4. Biometric Analysis, Gas Exchange and the Quality of Strawberries

The strawberry fruits were harvested manually weekly once approximately 75% of their epidermis had turned a uniform red colour [20]. After harvesting, the fruit were weighed for fresh weight on a semi-analytical scale (Bel Engineering Precision Scale, 0.01 g, 1000 g). The width (transverse axis) and length (longitudinal axis) of each fruit were then measured using a digital calliper (Lee Tools 618250). The fruits were then frozen at −18 °C until the fruit quality analysis.

Gas exchange analyses in the plants were evaluated at 81 days after transplanting (DAT), 118 DAT and 138 DAT. Gas exchange analyses were performed on fully expanded leaves from the apex, between 10:00 and 12:00 a.m., with three measurements obtained for each leaf. The photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance to H_2O (gs, mol H_2O m^{-2} s^{-1}) and the intercellular concentration of CO_2 (Ci, μmol of CO_2 mol^{-1}) were measured using a portable infrared radiation photosynthesis analyser (IRGA-Li-6400XT, LICOR, Lincoln, United States). Water use efficiency was obtained from the relationship between the A and gs. The concentration of CO_2 artificially injected into the IRGA assimilation chamber was set at 400 μmol mol^{-1}. The average relative moisture content was 60.6% at 81 DAT, 61.8% at 118 DAT and 60.9% at 138 DAT. Evaluations were performed under a photosynthetically active flux density of 300 μmol m^{-1} s^{-1}. All measurements were performed under a saturating artificial light with a luminosity of 1000 μmol photons m^{-2} s^{-1} [21].

After the final harvest in the twenty-fourth week, the fruits from all previous harvests were mixed together in order to evaluate the following parameters: moisture content, protein content, lipid content, ash content, carbohydrate content, total titratable acidity (TA), pH value, total soluble solids (SS) content, and the SS/TA ratio. To analyse the quality of the fruit, all strawberries from the same plant were crushed together. Moisture content was determined using the gravimetric method according to the Adolfo Lutz Institute [22]. Protein content was determined using the Kjeldahl method as described in AOAC method 991.22 [23]. Lipid content was obtained using a fat extractor (Ankom® Model XT10, Macedon, United States) according to the American Oil Chemists' Society [24], while ash content was obtained by calcining at 600 °C in a muffle following method 945.45 of the [23]. Carbohydrate content was determined by subtracting 100 from the moisture, protein, lipid, and ash contents using method 986.25 of the [23]. The protein, lipid, ash and carbohydrate contents were expressed on a wet basis.

TA analysis was performed using a 0.5 mol L^{-1} sodium hydroxide (NaOH) solution and phenolphthalein as an indicator [22], and the results were expressed as a percentage of citric acid. Total soluble solids (SS) content was determined using an Atago digital refractometer (Model 1T, Ribeirão Preto, Brazil), with the results expressed in Brix [23].

2.5. Statistical Analyses

The Shapiro-Wilk test was used to assess normality of the data. For variables exhibiting normal distribution and homogeneity of variance, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test to compare the means of the treatments at a significance level of 5%. In addition to descriptive analyses and comparisons of means, a regression analysis was conducted to evaluate how production variables respond to different concentrations of VT. Linear and quadratic models were both tested and the model with the best statistical fit was selected based on the p-value and coefficient of determination (R^2). All analyses were performed using GraphPad Prism software (version 10.4.2).

3. Results and Discussion

3.1. Vermicompost Tea Chemical Characterisation

Analysis of the concentrated VT revealed EC values of 0.38 dS m^{-1} and a pH of 7.6. Higher EC values than those observed in this study were reported by [25] when they compared extracts of vermicompost from leaves, cattle manure, and a 1:1 mixture of leaves and cattle manure. They found EC and pH values, ranging from 1.8 dS m^{-1} and 7.2 (leaf vermicompost extract) to 2.76 dS m^{-1} and 7.1 (cattle manure vermicompost extract). Similar values were observed by [4], who obtained vermicompost extracts from kitchen waste with an EC of 0.5 dS m^{-1} and a pH of 6.5. While there are no regulations limiting the use of this type of bioinput based on EC values, applying materials with values greater than 4.0 dS m^{-1} may cause problems for plants, affecting seed germination and negatively impacting stomach opening and closure, thereby interfering with plant development [26].

In this sense, the VT used in the present study can be considered safe for direct application to plants. Furthermore, VT's pH close to neutrality does not pose a risk of nutritional imbalance in the soil.

Results revealed that concentration of N, P, S and Ca in the concentrated VT of 0.2; 0.1; 0.1 and 0.4 g kg^{-1}, respectively (Table 1). Absences of K and Mg were also observed. Low concentrations of N, P and K were also reported in similar materials published by [15,27,28], pointing to the need to supplement fertilization due to the limited supply of nutrients in this type of material. As for S, Ca and Mg, the levels observed were lower than those observed by [27,28]. The nutrition of the animals that generate the manure used in the vermicomposting process can alter the concentration of nutrients [29], influencing the final composition of the VT. In this sense, the use of VT seems more appropriate as a source of biomolecules capable of stimulating plant development (e.g., phytohormones, chelating agents, beneficial microorganisms) than as a nutritional supply [4,11,12,14].

Table 1. Chemical analysis of the vermicompost tea (VT) of the present work, compared with other authors (Busato et al. [27], Pereira et al. [28], Hargreaves et al. [15]).

Material	N	P	K	S	Ca	Mg	Zn	B	Cu	Fe	Mn
			(g/kg)						(mg/kg)		
VT	0.2	0.1	0.0	0.1	0.4	0.0	16.6	6.6	0.7	98.3	15.9
[27]	0.0	0.3	0.6	2.9	6.7	1.9	34.3	37.3	16.3	2.0	71.3
[28]	0.8	1.9	0.5	0.0	20.8	8.1	-	-	9.2	52.5	0.8
[15]	0.0	0.0	0.1	0.1	0.9	0.2	3.5	0.4	2.7	136.0	24.6
[15]	0.0	0.0	0.1	0.1	1.1	0.2	2.1	0.6	1.9	119.0	19.4

3.2. Biometric Analysis, Gas Exchange and the Quality of Strawberries

The number of fruits per plant showed no significant variation between the control and VT concentration treatments, according to the Tukey test at the 5% significance level (Figure 1A). However, the 4% VT treatment presented an average increase of around 54% compared to the control treatment, with an average of 15 fruits per plant. Similarly, the 4% VT treatment produced an average fruit mass that was 40.22% higher than the control treatment (Figure 1B). It is worth noting that the number of fruits per plant can vary between cultivars in conventional strawberry cultivation systems involving direct soil planting, ranging from 45 to 95 fruits per plant [30]. Although the number of fruits per plant in this study is lower, the variation in fruit mass observed is comparable to that reported by [30,31]. One possible explanation for the lower fruit production per plant is the growing conditions in pots and substrates, such as those used in this study.

The variation in mass observed per fruit in the present study was between 5.58 g and 19.42 g, with an average of 9.77 g per fruit (Figure 1B). An average value of 12.2 g per fruit is considered to be of excellent commercial quality, as highlighted by [32], who obtained similar average weights in their experiments. It was also observed an average weight of 12.2 g per fruit in the first cycle of the 'Portola' cultivar when evaluating the influence of successive cycles on the morphological and productive potential of strawberry cultivars [30]. Regression analyses of production variables (accumulated fruit mass per plant) were also carried out, revealing a quadratic adjustment ($y = 41.59 + 29.84x - 3.74x^2$) ($p$-value 0.099). Similar to the results for fruit mass per plant (Figure 1B), the maximum accumulated fruit mass was 101.36 g at a 4% VT concentration, representing a 51.91% increase in fruit mass compared to the control.

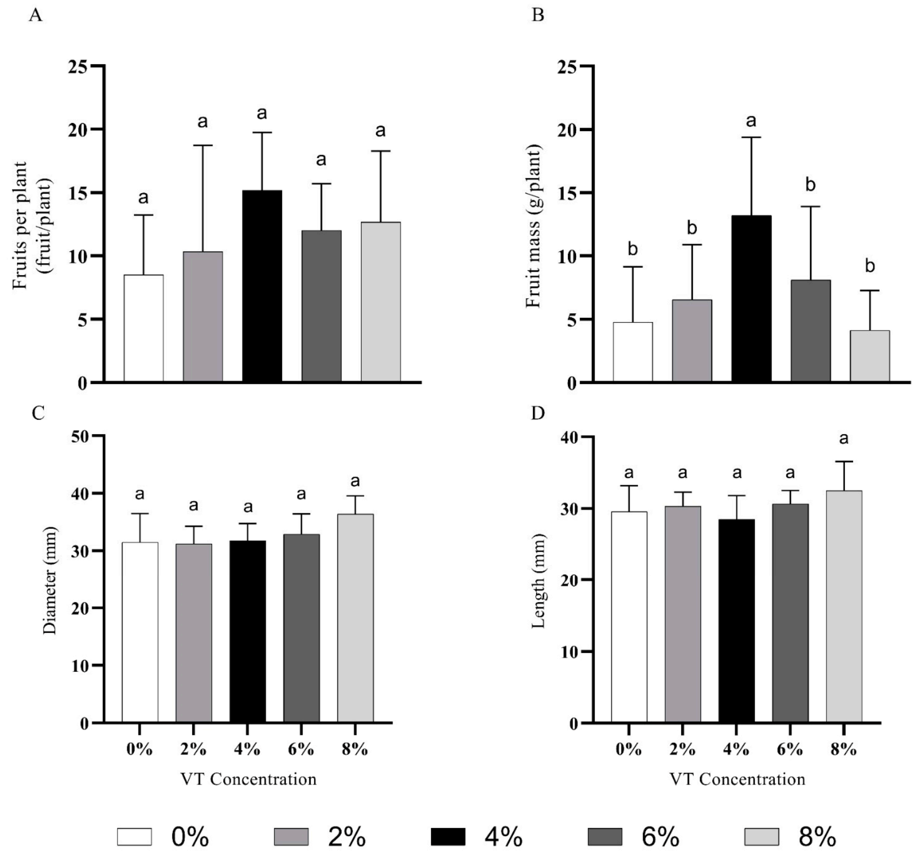

Figure 1. Influence of vermicompost tea at various concentrations on fruit weight and biometric parameters. Bars represent the standard deviation of the means (n = 6). Fruits per plant (**A**), fruit mass (**B**), diameter of the fruit (**C**) and length of the fruit (**D**). Error bars represent standard deviations. Significant differences were identified by small letters using Tukey's test at a 5% significance level.

The average fruit diameter was 32 mm across the VT concentrations (Figure 1C). However, considerable variation was observed between treatments: the 8% concentration presented an average fruit diameter that was 13.57% greater than the control treatment (36.41 and 31.47 mm per plant, respectively; Figure 1C). Similarly, the data showed no statistical difference in fruit length between the control and VT concentrations (Figure 1D), with an average length of 30 mm observed. These variations in diameter and length are consistent with the findings of [33], who investigated the responses of the 'Portola' strawberry variety to foliar biostimulant sprays.

The benefits of applying the VT to the strawberry crop, as observed with the 4% VT treatment in terms of the number of fruits per plant and the accumulated fruit mass, are consistent with studies on the application of biostimulants to strawberry [12,34] and on various crops, including broccoli [35], peppers and tomatoes [28], as well as lettuce [4]. These authors have explained this in terms not only of the supply of nutrients, but also of the presence of dissolved biomolecules in the VT that can increase nutritional efficiency and/or the quality characteristics, as well as tolerance to abiotic stress of the crop, regardless of its nutrient content. In an extensive review, du Jardin [36] highlights that these biomolecules include proteins, amino acids, hormones, organic acids, and biomolecule fragments agglomerated by hydrophobic interactions to form humic substances. According to the author, these agents can alter enzyme activity, membrane transporters, protein expression, photosynthetic activity and, also stimulate beneficial microbial activity in association with the crop and promote action similar to plant hormones. Taking these factors into account, the observed stimuli may be caused by the carboxylic, phenolic and amide groups present in the extract obtained from vermicompost rather than by the presence of nutrients.

Gas exchange in the strawberry crop was assessed at 81, 118 and 138 days after transplanting (DAT) of the plants. In the first two evaluations, at 81 and 118 DAT, no significant difference was observed between treatments (Figure 2A–D). The internal CO_2 concentrations in the first two evaluations averaged between 253 and 357 µmol CO_2 mol^{-1} (Figure 2A), while the Gs showed values between 0.30 and 0.50 mmol m^{-2} s^{-1} (Figure 2B), which is similar to that observed by [37] in their experiment with bovine biofertilisers applied to strawberries in a greenhouse. In the evaluation of seven strawberry cultivars treated or not with arbuscular mycorrhizal fungi, gas exchange was evaluated in the same period as the present study and Gs values were observed to vary between 0.60 and 0.81 mmol m^{-2} s^{-1} [38].

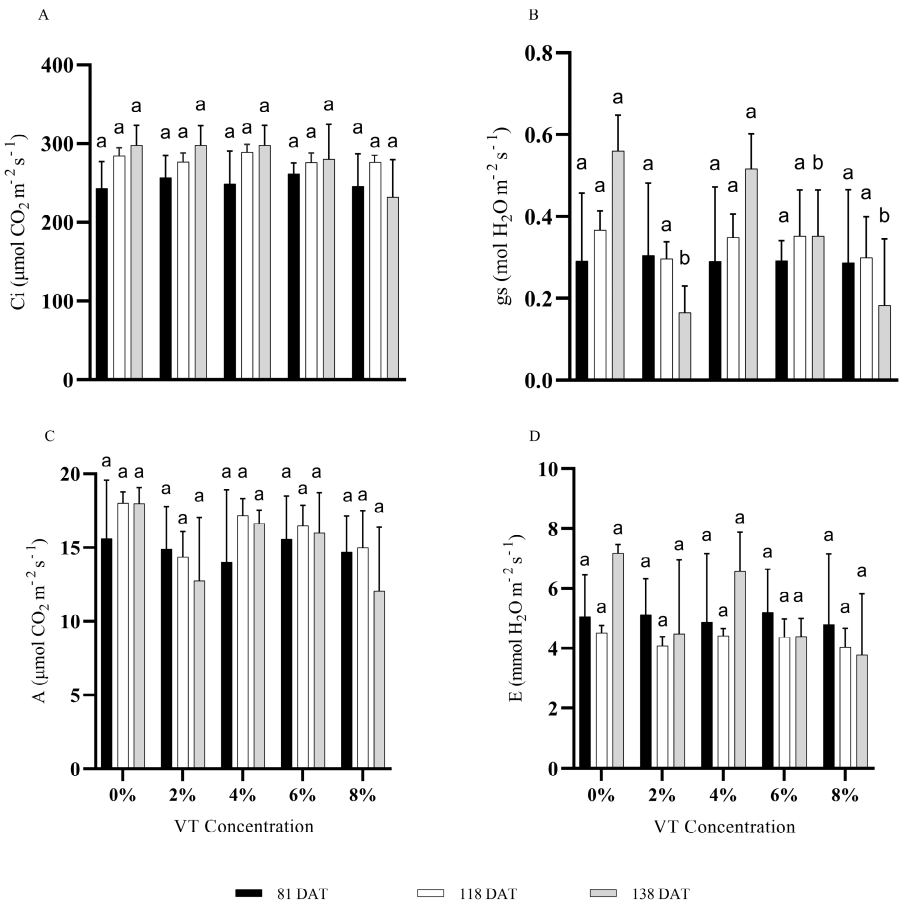

Figure 2. Evaluation of gas exchange of strawberries as a function of vermicompost tea (VT concentration). Bars represent the standard deviation of the means (n = 6). Internal CO_2 concentration (**A**), stomatal conductance (**B**), net CO_2 assimilation (**C**), transpiration rate (**D**). Error bars represent standard deviations. Significant differences in each evaluation period (81, 118 and 138 DAT) were identified by small letters using Tukey's test at a 5% significance level.

The average net CO_2 assimilation rate, considered the primary factor of crop productivity [7], was between 15 and 20 µmol CO_2 m^{-2} s^{-1} (Figure 2C), as observed in the experiment on the response of strawberries to biostimulants [39]. Transpiration rates varied from 4.04 to 5.31 mmol H_2O m^{-2} s^{-1} (Figure 2D) for the VT treatments, with an average of 4.90 mmol H_2O m^{-2} s^{-1} for the control treatment. Similarly, in a study evaluating the influence of three biostimulants on strawberry gas exchange, observed slight variations compared to other crops and attributed this effect to stability in strawberry gas exchange throughout its cycle, probably due to its root architecture [39].

The third evaluation, carried out at 138 DAT, revealed significant differences in the Gs (Figure 2B). It was found that the application of VT with the highest dose (8%) reduced Gs by 23.38%, compared to the control, and the same effect was observed for Ci. This suggests that higher concentrations of this material could negatively impact CO_2 assimilation in the stomatal chamber, potentially reducing photosynthesis. The effect on E was similar to that on Gs (Figure 2A,B,D).

In the control group, it was observed that all gas exchange parameters increased over time (Figure 2), which was different from the 8% VT treatment. For this treatment, in addition to a decrease in gas exchange over time, the lowest fruit mass was observed (Figure 1B). However, the 4% VT treatment produced a result very similar to the control and had much higher productivity (Figure 1A,B).

There was no statistical difference in the nutritional composition of strawberries for all treatments (Figure 3A–E). The mean values (0.43% ashes; 0.12% lipids; 92.1% moisture content; 8.9% carbohydrates; 0.57% proteins) are in agreement with the data reported by [40] for the 'Portola' cultivar. Therefore, regardless of the amount of VT applied to the strawberries, the nutritional composition of the fruit was kept the same, a positive characteristic of the VT.

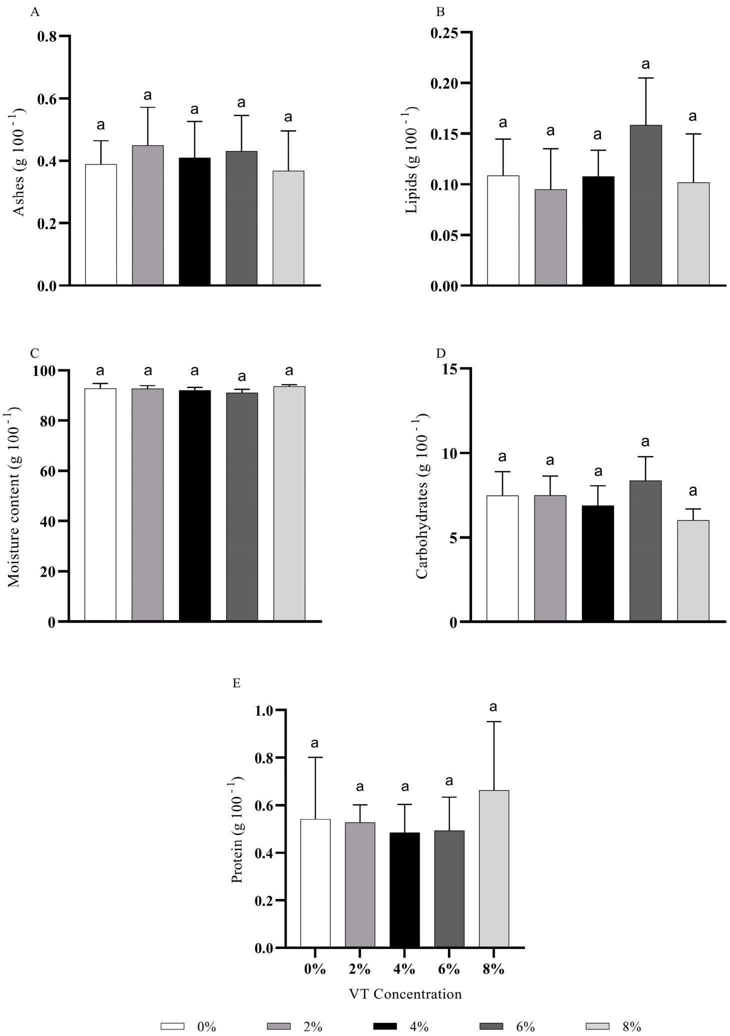

Figure 3. Evaluation of strawberry fruit quality as a function of vermicompost tea (VT concentration). Bars represent the standard deviation of the means (n = 6). Ashes (g 100^{-1}) (**A**), Lipids (g 100^{-1}) (**B**), moisture content (g 100^{-1}) (**C**), Carbohydrate (g 100^{-1}) (**D**) and Protein (g 100^{-1}) (**E**). Error bars represent standard deviations. Significant differences were identified by small letters using Tukey's test at a 5% significance level.

The weekly application of VT produced consistent outcomes across most strawberry pulp quality parameters (Figure 4A–C), with statistically significant differences observed only for the soluble solids to titratable acidity ratio (SS/AT) (Figure 4C).

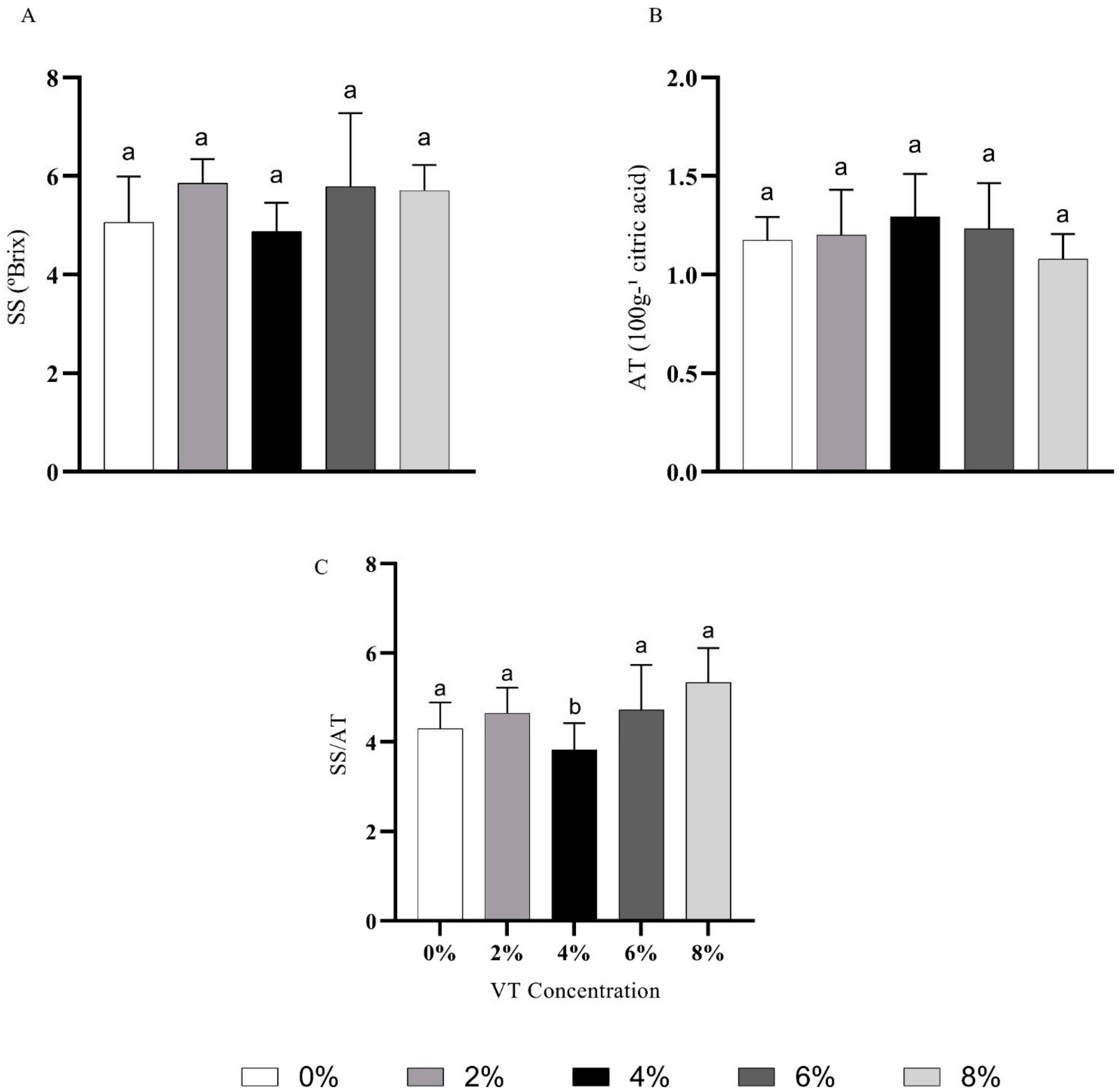

Figure 4. Evaluation of strawberry fruit quality as a function of vermicompost tea (VT concentration). Bars represent the standard deviation of the means (*n* = 6). Total soluble solids (SS) (**A**), total acidity (AT) (**B**), SS/AT ratio (**C**). Error bars represent standard deviations. Significant differences were identified by small letters using Tukey's test at a 5% significance level.

Considering the treatment with 4% of VT, which produced the highest average fruit mass per plant, the SS content (4.88 ± 0.58) was similar to that observed for the same cultivar in other studies [40,41]. However, the AT value (1.29 ± 0.22) was around twice that reported by [40] for the same cultivar (0.57 ± 0.02). This results in a lower SS/AT ratio (4.01 ± 0.61), giving the fruit a less attractive flavour for *in natura* consumption, which should be at least 8.75 [42].

According to [42] in his seminal work on the relationship between maturity, ripeness and fruit quality, the minimum recommended soluble solids (SS) value is 7° Brix. However, as in this study, other evaluations have reported lower SS values [43,44]. Strawberries produced with 6% and 8% AT had higher SS (5.80 ± 0.13) and SS/AT (5.34 ± 0.76) values, but lower fruit mass (Figure 1C). This may have influenced the accumulation of solids in the fruit, as this parameter directly affect it.

4. Conclusions

Applying VT at concentrations ranging from 2 to 8% (v/v) had a quadratic effect on the growth and fruit mass production of strawberry plants while maintaining the nutritional composition of the fruit. A concentration of 4% resulted in a 50% increase in the mass and number of strawberries compared to the control. VT concentrations ranging up to 6% influenced the gas exchange of strawberry plants in their advanced productive stage, particularly at the 8% concentration. This led to reductions in Ci and Gs. The nutritional composition of strawberries was not affected by VT and fruit quality was favoured by the application of up to 6% VT concentration. Vermicompost tea is a bio-input that has the potential to benefit strawberry production, showing promising results in increasing the mass of fresh fruit. Further studies in the field or commercial systems to evaluate the application of concentrations between 4 and 8% of VT on fruit production and quality will help consolidate the use of this bio-input in strawberry production.

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