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Chlorophyll fluorescence and leaf nutritional status of passion fruit (*Passiflora edulis f. flavicarpa*) seedlings grown in different organic substrates

Fluorescencia de la clorofila y estado nutricional foliar de plántulas de maracuyá (*Passiflora edulis f. flavicarpa*) cultivadas en distintos sustratos orgánicos

Fluorescência da clorofila e estado nutricional foliar de mudas de maracujá (*Passiflora edulis f. flavicarpa*) cultivadas em diferentes substratos orgânicos

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ABSTRACT

Passion fruit (*Passiflora edulis* f. *flavicarpa*) has high economic and nutritional value, and the nursery stage is crucial for its early development. The objective of the present study was to evaluate chlorophyll fluorescence, foliar nutritional status, and growth of passion fruit seedlings in response to four organic substrates: S1 (sand + biochar + organic matter), S2 (sand + compost + organic matter), S3 (sand + compost + agricultural soil), and S4 (agricultural soil), under a completely randomized design. The physiological variables F_v/F_m, Φ(II), and ETR were measured, as well as the foliar concentration of macro and micronutrients and growth variables, whose data were analyzed using ANOVA and Tukey's test (5 %). Plants grown in substrate S2 showed the highest photochemical efficiency ($F_v/F_m \approx 0.79$; Φ(II) ≈ 0.44; ETR ≈ 160), whereas those in substrate S1 exhibited the highest foliar concentrations of K, Fe, and Zn. The principal component analysis explained 71 % of the total variability, associating substrate S2 with better physiological yield and growth. It can be concluded that substrates containing compost enhance PSII photochemical efficiency, while those including biochar are associated with greater foliar mineral accumulation.

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Resumen

El maracuyá (*Passiflora edulis* f. *flavicarpa*) presenta un alto valor económico y nutricional, y la fase de vivero es determinante para su desarrollo inicial. El presente trabajo tuvo como objetivo evaluar la fluorescencia de la clorofila, el estado nutricional foliar y el crecimiento de plántulas de maracuyá en función de cuatro sustratos orgánicos: S1 (arena + biocarbón + materia orgánica), S2 (arena + compost + materia orgánica), S3 (arena + compost + tierra agrícola) y S4 (tierra agrícola), bajo un diseño completamente al azar. Se midieron las variables fisiológicas Fv/Fm, Φ(II) y ETR, así como la concentración foliar de macro y micronutrientes y variables de crecimiento, cuyos datos fueron analizados mediante ANOVA y prueba de Tukey (5 %). Las plantas cultivadas en el sustrato S2 presentaron la mayor eficiencia fotoquímica ($F_v/F_m \approx 0,79$; $Φ(II) \approx 0,44$; $ETR \approx 160$), mientras que las cultivadas en el sustrato S1 mostraron las mayores concentraciones foliares de K, Fe y Zn. El análisis de componentes principales explicó el 71 % de la variabilidad total, asociando el sustrato S2 con un mejor desempeño fisiológico y de crecimiento. Se puede concluir que los sustratos con compost favorecen la eficiencia fotoquímica del PSII, mientras que aquellos que incluyen biocarbón se asocian con una mayor acumulación foliar de minerales.

Palabras clave: biocarbon, compost, fluorescencia de clorofila, nutrición mineral, materia orgánica.

Resumo

O maracujá (*Passiflora edulis* f. *flavicarpa*) apresenta elevado valor econômico e nutricional, e a fase de viveiro é determinante para o seu desenvolvimento inicial. O objetivo deste estudo foi avaliar a fluorescência da clorofila, o estado nutricional foliar e o crescimento de mudas de maracujá em função de quatro substratos orgânicos: S1 (areia + biocarvão + matéria orgânica), S2 (areia + composto + matéria orgânica), S3 (areia + composto + solo agrícola) e S4 (solo agrícola), sob um delineamento inteiramente casualizado. Foram avaliadas as variáveis fisiológicas Fv/Fm, Φ(II) e ETR, bem como as concentrações foliares de macro e micronutrientes e variáveis de crescimento, cujos dados foram analisados por ANOVA e teste de Tukey (5 %). As mudas cultivadas em substratos S2 apresentaram a maior eficiência fotoquímica ($Fv/Fm \approx 0,79$; $Φ(II) \approx 0,44$; $ETR \approx 160$), enquanto substratos S1 apresentou as maiores concentrações foliares de K, Fe e Zn. A análise de componentes principais explicou 71 % da variabilidade total, associando o substrato S2 a melhor desempenho fisiológico e de crescimento. Conclui-se que substratos contendo composto favorecem a eficiência fotoquímica do PSII, enquanto aqueles que incluem biocarvão estão associados a maior acúmulo foliar de minerais

Palavras-chave: biocarvão, composto, fluorescência da clorofila, nutrição mineral, matéria orgânica.

Introduction

In Ecuador, passion fruit has high economic relevance, with an annual production of approximately 48,000 tons according to official reports from the National Institute of Statistics and Censuses (INEC, 2023), and constitutes one of the main fruits destined for export (MAG, 2020). The quality of the plant material, as well as the vigor and yield in the field, are directly associated with nursery management (Santos *et al*., 2020).

Substrate selection is decisive for the initial growth and quality of seedlings (Bonifácio *et al*., 2025; Lessa *et al*., 2023). Substrates with low fertility reduce nutrient availability and root expansion, affecting the initial vigor (Paixão *et al*., 2021). On the contrary, the incorporation of compost and biochar improves porosity, water holding capacity, and nutrient availability, favoring early development in the nursery (Yang *et al*., 2023; Wu *et al*., 2023). From the physiological approach, the photosynthetic efficiency evaluated through the maximum quantum efficiency of photosystem II (F_v/Fm), maximum quantum yield of photosystem II (Φ[II]), electron transport rate (ETR), and chlorophyll index, is a key indicator of plant yield (Ni *et al*., 2020). Organic substrates such as compost and biochar increase these parameters by improving light use and energy generation, favoring the growth and productivity of passion fruit (Silva *et al*., 2020). Chlorophyll concentration is directly related to photosynthetic efficiency and potential yield of seedlings, making it a key physiological indicator in the nursery stage (Sun *et al*., 2025; Su *et al*., 2024). The application of organic matter and renewable substrates has been shown to increase chlorophyll, PSII efficiency, and leaf expansion in Passifloraceae (Da Silva *et al*., 2023; Bonifácio *et al*., 2025). Despite these advances, it is unknown how the combinations of organic substrates used with locally available material and accessible to producers modify the photochemical responses of chlorophyll fluorescence and foliar nutritional concentration of *P. edulis*. The objective of the study was to evaluate chlorophyll fluorescence, nutritional status, and the growth of *Passiflora edulis* seedlings grown in different organic substrates during the nursery stage.

Materials and methods

The research was carried out in the agro-industrial crop nursery of the Faculty of Agronomic Engineering of the Technical University of Manabí, located in the canton of Santa Ana, Manabí, Ecuador. The site is located at an altitude of 60 m.a.s.l, with an average annual temperature of 26 °C, a rainfall of 625 mm, and a relative humidity that varies between 74.8 mm (dry season) and 82.48 mm (rainy season). The experiment was developed under a Completely Randomized Design (CRD) with four types of substrates as treatments (detailed in Table 1) and four replications, each consisting of 25 plastic bags per experimental unit. For the test, 400 black polyethylene plastic bags of 6 × 8 inches, with an approximate volume of 2.36 L, were used. The substrates were formulated using a mixture of agricultural soil, washed river sand, compost, and organic matter in a ratio of 1:1:1:1.5, to which biochar from *Ceratonia siligua* was added in a ratio of 1:0.25.

Table 1. Formulation of substrates based on combinations of organic materials.

Treatments	Composition
Substrate 1	Sand + Biochar + Organic matter
Substrate 2	Sand + Compost + Organic matter
Substrate 3	Sand + Compost + Agricultural soil
Substrate 4	Agricultural soil

The physicochemical characteristics of the substrates are presented in Tables 2 and 3. The materials were pre-composted for three months before sowing. Two certified passion fruit seeds, *Passiflora edulis f. flavicarpa* (INIAP-2009), treated with Thiodicarb + Imidacloprid (systemic insecticides; i.a) at a dose of 25 $mL.kg^{-1}$ seed, were placed in bags with substrate previously treated with fungicide Captan 80 WP (180 $g.100\ L^{-1}$). After 15 days, thinning was performed, leaving one seedling per experimental unit. Irrigation was applied through micro-sprinklers with an 8 mm depth twice a week. Agronomic management included manual weeding and preventive applications of an insecticide, fosetyl-aluminum (2 $g.L^{-1}$) and a fungicide, Spinetoram (0.12 $mL.L^{-1}$).

Photochemical evaluations, chlorophyll index, and growth variables (plant height, stem diameter, and leaf area) were performed eight weeks after sowing. Chlorophyll fluorescence (F_v/F_m, Φ(II), and ETR) and chlorophyll index were determined in adult leaves exposed to full luminosity, using an OS-1p+ fluorometer (Opti-Sciences) and a SPAD 502 chlorophyll meter, respectively. For the determination of F_v/F_m, the leaves were previously adapted to the dark for 30 min. Plant height was measured with a flexometer from the base of the stem to the insertion of the last fully expanded leaf. The diameter of the stem was determined with a manual (Vernier) caliper, and the leaf area was estimated using the gravimetric method. For this purpose, ten leaf discs of a known area were collected using a punch; these were then dried to a constant weight and used as a reference to calculate the total leaf area per plant, with values expressed in cm² per plant. The foliar concentration of macro and micronutrients was quantified in samples of 200 g per experimental unit, following INIAP protocols.

Table 2. Chemical composition of carob biochar, washed river sand, and compost.

Chemical Composition					
Carob biochar	**%**	**River sand**	**%**	**Compost**	**%**
pH	8.7	Silica (SiO_2/quartz)	90	pH	7.37
EC ($dS.m^{-1}$)	1.49	Metal oxides (Fe_2O_3, Al_2O_3, etc.)	2	EC ($dS.m^{-1}$)	3.5
CEC $cmol^{+}.kg$	15.7			Humidity	34.45
H/C	0.11	Other minerals (Feldspar, calcite, clay, etc.)	5	Organic Matter	53.65
O/C	0.13			C/N ratio	12.97
C	71.17			Ashes	18.75
H	2.96			Total Nitrogen	1.2
O	23.86			Total Phosphorus	0.3
N	1.3			Total Potassium	0.79
S	0.37				
Humidity	2.7				
Ashes	0.32				
Fixed carbon	29.64				
Volatile matter	67.47				

Table 3. Physical-chemical composition of agricultural soil and organic matter used in the mixture of substrates.

Soil chemical composition	Agricultural soil	Organic matter
Organic Matter (%)	4.2	32.5
Texture (%)	Clay loam – silty	-
Base saturation (%)	74	62
CIC (meq.100g)	28.9	45.3
EC (mS.cm)	0.15	1.82
pH (H_2O)	6.6	7.1
Nitrate (NO_3-N) ($mg.kg^{-1}$)	7.4	215
Ammonium (NH_4-N) ($mg.kg^{-1}$)	2.1	96
Total N (NO_3+NH_4) ($mg.kg^{-1}$)	9.5	311
Phosphorus (P) ($mg.kg^{-1}$)	38.8	412
Potassium (K) ($mg.kg^{-1}$)	228	1450
Magnesium (Mg) ($mg.kg^{-1}$)	259	520
Calcium (Ca) ($mg.kg^{-1}$)	845	1820
Sulfur (S) ($mg.kg^{-1}$)	8.3	112
Iron (Fe) ($mg.kg^{-1}$)	22.2	310
Manganese (Mn) ($mg.kg^{-1}$)	12.8	145
Copper (Cu) ($mg.kg^{-1}$)	3.3	21
Zinc (Zn) ($mg.kg^{-1}$)	2.3	38
Boron (B) ($mg.kg^{-1}$)	0.28	1.9
Sodium (Na) ($mg.kg^{-1}$)	12	89
Chloride (Cl) ($mg.kg^{-1}$)	9	165

The data were subjected to analysis of variance (ANOVA; α = 0.05) and comparison of means through Tukey's test (5 %), using the InfoStat 8.0 software (2018). Additionally, a principal component analysis (PCA) was performed to identify multivariate patterns between physiological, nutritional, and growth variables.

Results and discussion

Nutrient concentration in different substrates

The foliar concentration of N was higher in substrates S1 and S3, while substrate S2 showed the lowest accumulation. This variation is explained by the ability of biochar to retain N in substrate S1 and by the gradual release of organic N from the compost in substrate S3, which coincides with studies that describe the importance of biochar in ion retention and the tendency of *Passiflora* to present suboptimal foliar levels of N (Antunes *et al*., 2022; Hauer-Jákli & Tränkner, 2022; de Lima *et al*., 2023). Substrates S1 and S2 showed the highest concentrations of P, mainly associated with the contribution of organic matter, which has higher concentrations of phosphorus and a higher fraction available for absorption by the plant, compared to agricultural soil. This trend is consistent with studies that point to an increase in P availability and acid phosphatase activity in substrates enriched with organic materials (Antunes *et al*., 2022; Yang *et al*., 2023).

K reached its maximum in substrate S1, which is consistent with the compost-biochar synergy that improves monovalent cation retention and reduces leaching; in addition, *P. edulis* has a high demand for this nutrient (Antunes *et al*., 2022; de Lima *et al*., 2023). Finally, the accumulation of Ca and Mg was higher in substrates with agricultural soil and compost (S3 and S4), in accordance with reports that highlight that organic matter increases the solubility and availability of these divalent cations in tropical soils (Paixão *et al*., 2021).

The growth assessment showed that substrate S2 (sand + compost + agricultural soil) generated the most vigorous seedlings (higher height, diameter, and leaf area), as the compost optimized the nutrition and physical structure of the substrate (Figure 1), a result that is consistent with recent studies in *P. edulis* (Da Silva *et al*., 2023; Bonifácio *et al*., 2025). Substrates S1 and S3 offered intermediate improvements, while substrate S4 (unamended soil) resulted in the poorest growth due to its nutritional and physical limitations (Paixão *et al*., 2021). The best foliar development in substrate S2 aligns with the reported relationship between organic matter and improved photosynthetic efficiency in Passifloraceae (Sun *et al.*, 2025; Su *et al*., 2024), making the use of compost key to improving the quality of *P. edulis* seedlings in the nursery stage.

On the other hand, sulfur (S) (Figure 2E) presented its highest concentration in substrate S1 (≈ 490 mg.100 kg^{-1}), attributed to carob biochar, which contains 0.37 % S and volatile compounds that act as a slow-release reservoir. Unlike soluble sulfate forms, biochar improves the retention and availability of the element, showing how organic amendments modify the dynamics of S and its interaction with other nutrients (Freire *et al*., 2022; Antunes *et al*., 2022).

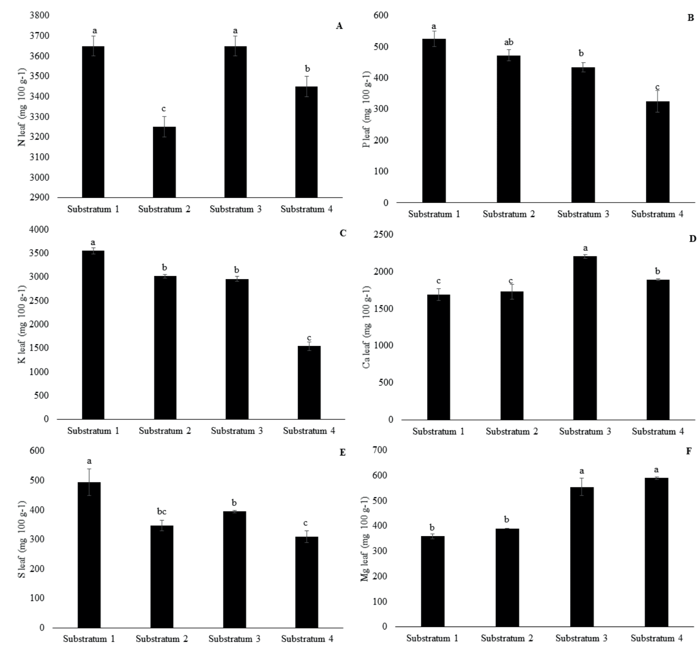
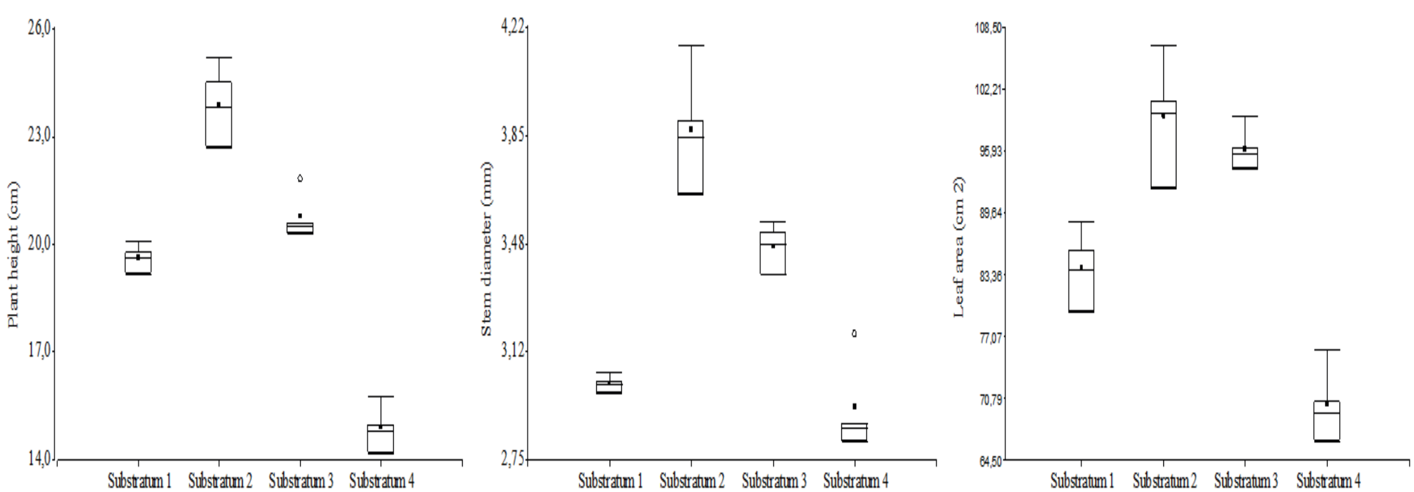


Figure 2. Foliar nutrient concentration of *Passiflora edulis f. flavicarpa* grown in four substrates. S1 = biochar + river sand; S2 = river sand + agricultural soil; S3 = compost + agricultural soil; S4 = biochar + compost; where. A) Nitrogen, B) Phosphorus, C) Potassium, D) Calcium, E) Sulfur, and F) Magnesium. Different letters in the variables differ statistically according to Tukey's test (p≤0.05).

Figure 1. Growth variables of passion fruit seedlings (*Passiflora edulis f. flavicarpa*) grown in four organic substrates during the nursery stage. A) Seedling height (cm); B) Stem diameter (mm); C) Leaf area (cm²). Values represent means ± standard deviation.



Foliar micronutrient concentration varied significantly between treatments (Table 4). Substrate S1 presented the highest values of Zn (0.53 $mg.g^{-1}$), Cu (0.13 $mg.g^{-1}$), and Fe (2.48 $mg.g^{-1}$), reflecting a greater availability and absorption of these elements, favored by the combined action of biochar and compost. Similar results were reported by Da Silva *et al*. (2023) in *Passiflora* and other horticultural crops. Instead, Mn reached its maximum concentration in substrate S4 (0, $mg.g^{-1}$), possibly due to the higher acidity of the medium. Boron (B) showed no significant differences between treatments (~0.04 $mg.g^{-1}$), in agreement with Silva *et al*. (2020), who highlighted the stability of this element in substrates with high organic content.

The micronutrient analysis (Table 4) showed a clear influence of the type of substrate. The substrate with carob biochar (S1) recorded the highest concentrations of Zn (≈0.53 $mg.g^{-1}$) and Fe (≈2.48 $mg.g^{-1}$), significantly exceeding S4 (Zn ≈0.29 $mg.g^{-1}$). This behavior is related to the higher cation exchange and ion retention capacity generated by biochar in organic mixtures (Yang *et al*., 2023).

The rest of the micronutrients presented differentiated responses: Mn reached its highest value in substrate S4 (≈0.28 $mg.g^{-1}$), while Cu showed its highest accumulation in substrate S1 (≈0.13 $mg.g^{-1}$). The lower availability of Mn in biochar-based substrates is consistent with studies reporting immobilization of Mn and Cu by pH increases and surface adsorption of biochar (Zhou *et al* 2023). Regarding B, no differences were detected between treatments, indicating that the effect of biochar is not uniform between micronutrients.

The fluorescence of chlorophyll (Figure 3) showed clear differences in the photochemical efficiency of PSII in the plants grown in the different substrates evaluated. Plants grown on substrate S2 showed the highest values of F_v/Fm (~0.79), Φ(II) (~0.44), and ETR (~160), indicating a stable PSII, with efficient electron transport and low photoinhibition, in response to the physical and nutritional conditions of the substrate (Da Silva *et al*., 2023). In contrast, plants grown in S4 had the lowest values (F_v/Fm ~0.70; Φ(II) ~0.23; ETR < 100), reflecting marked physiological stress associated with reduced aeration or water imbalance. The F_v/Fm values < 0.75 are associated with damage to reaction centers and reduced energy dissipation (Faria *et al*., 2020).

Substrates S1 and S3 promoted intermediate responses, evidencing adequate photochemical efficiency. Together, these results confirm that the chemical and physical characteristics of the substrate modulate the photosynthetic efficiency of plants**.** Previous studies in passion fruit agree that substrates with higher organic matter content and better water holding capacity favor fluorescence and photochemical efficiency (Sun *et al*., 2025), while abiotic stress conditions, such as water deficit or salinity, significantly reduce Φ(II) and ETR (Tomaškinová *et al*., 2025).

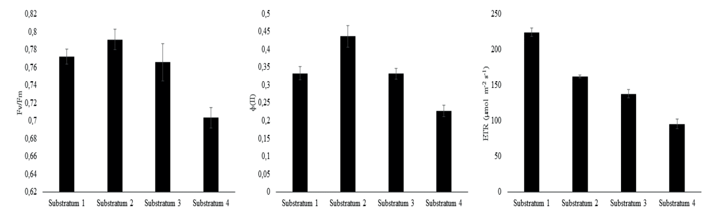


Figure 3. Chlorophyll fluorescence parameters in *Passiflora edulis f. flavicarpa* seedlings grown in four substrates. S1 = biochar + river sand; S2 = river sand + agricultural soil; S3 = compost + agricultural soil; S4 = biochar + compost, where Fv/Fm = maximum PSII efficiency, Φ(II) = effective quantum yield of PSII, and ETR = electron transport rate**.**

Principal component analysis (PCA) explained 71 % of the total variability (PC1 = 51.06 %; PC2 = 20.39 %), clearly differentiating the substrates (Figure 4). Substrate S2 was associated with higher values of F_v/F_m, Φ(II), and ETR, reflecting high photosynthetic efficiency and stable electron transport, in agreement with Zhang *et al.* (2023), who reported that adequate nutrition and light management increase photosynthetic yield in *P. edulis* (Figure 4). In contrast, substrate S4 was located at the negative end of PC1, linked to nutritional deficiencies; Zn or P deficiency reduces F_v/F_m and ETR (Zhang *et al.*, 2023), and the low availability of N under salinity limits pigments and physiological efficiency (Tomaškinová *et al*., 2025). Substrate S3 was related to Ca, Mg, and Mn, essential elements for thylakoid stability and enzyme activity, as observed by Lessa *et al.* (2023) in passion fruit grown in substrates with carbonized rice husk. Substrate S1, located in the positive quadrant of PC1, showed a yield conditioned by its physical properties. Under abiotic stress, Φ(II) and ETR decrease before F_v/F_m in triploid *P. edulis* (Su *et al.*, 2024), which confirms that an adequate water and nutritional balance, as in substrate S2, optimizes photosynthetic efficiency.

Correlation analysis (Figure 5) revealed that N, P, and K were positively associated with photochemical yield, particularly with ETR, reflecting their essential role in the synthesis of PSII components, energy flow, and stomatal regulation (Zhou *et al*., 2023). In contrast, Mg and Mn showed weak or negative correlations: Mg could generate cationic interferences without reaching toxic levels (Hauer-Jákli & Tränkner, 2019), while high Mn values are related to oxidative stress and PSII impairment (Millaleo *et al*., 2020). Among micronutrients, Fe, Zn, and S showed positive correlations with photochemical parameters, consistent with their role in electron transport, enzyme stability, and maintenance of redox homeostasis (Saleem *et al*., 2022).

Table 4. Foliar concentration of zinc, copper, iron, manganese, and boron in *Passiflora edulis f. flavicarpa*, under the effect of organic substrates with different physical mixtures.

Substrates	Zn	Cu	Fe	Mn	B
	mg.g-1				
	M* D.E**	M D.E	M D.E	M D.E	M D.E
Substrate 1	0.53 ± 0.02a	0.13 ± 0.005a	2.48 ± 0.18a	0.23 ± 0.005b	0.36 ± 0.042ns
Substrate 2	0.35 ± 0.04b	0.09 ± 0.01b	1.95 ± 0.11bc	0.23 ± 0.025b	0.38 ± 0.062
Substrate 3	0.33 ± 0.005b	0.11 ± 0.005b	1.78 ± 0.13c	0.25 ± 0.015ab	0.42 ±.047
Substrate 4	0.29 ± 0.009b	0.11 ± 0.005b	2.28 ± 0.01ab	0.28 ± 0.044a	0.43 ±0.02

*Means; ** Standard deviation

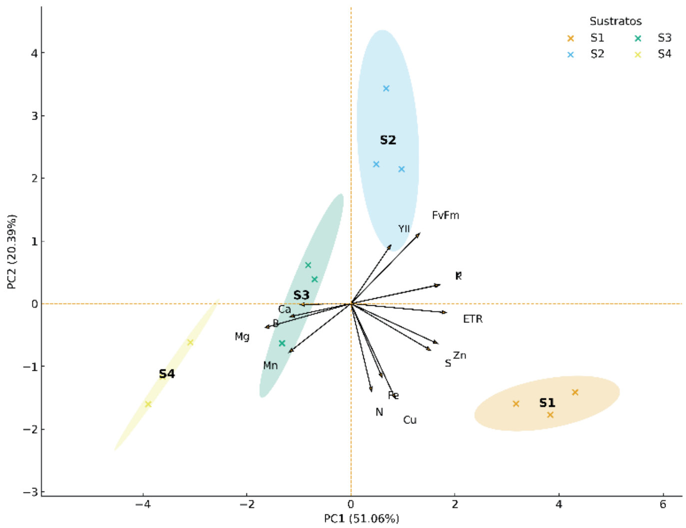


Figure 4. Principal component analysis (PCA) of physiological and nutritional variables of *Passiflora edulis f. flavicarpa* seedlings grown in four substrates. S1 = biochar + river sand; S2 = river sand + agricultural soil; S3 = compost + agricultural soil; S4 = biochar + compost—showing the grouping and relationships among the evaluated variables.

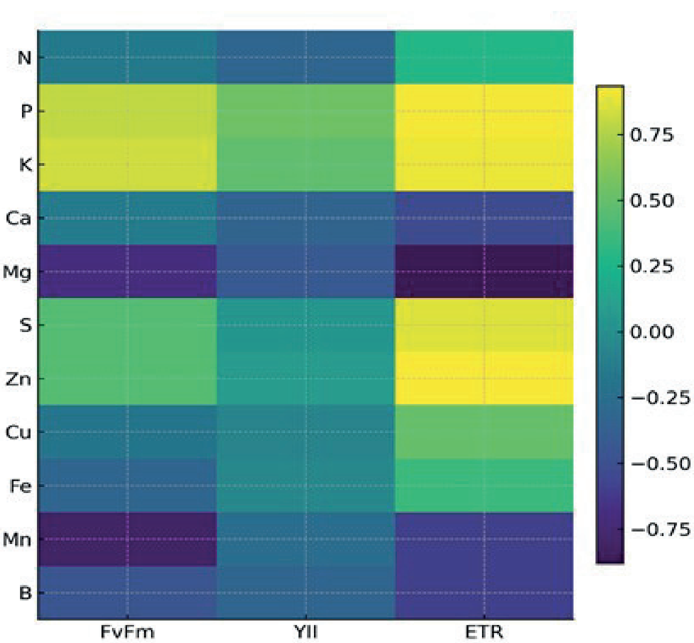


Figure 5. Correlation matrix between foliar nutrients (N, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn, and B) and chlorophyll fluorescence parameters (F_v/F_m, Φ(II), and ETR) in *Passiflora edulis* seedlings grown in different organic substrates.

Conclusions

The physiological response of *Passiflora edulis* depended on the nutritional balance provided by the substrates. High contents of N, P, and K in the substrate improved photochemical efficiency and PSII functionality, whereas Mg and Mn imbalances reduced F_v/F_m, Φ(II), and ETR in seedlings. Overall, the results show that balanced nutritional management in organic substrates is essential for maintaining seedlings with high photochemical efficiency and promoting more sustainable passion fruit production systems.

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