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ORIGINAL PAPER

Biostimulant derived from *Spirulina platensis* improves emergence and quality of melon seedlings

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Abstract: This study hypothesized that seed treatment with *Spirulina platensis* enhances melon seedling emergence and quality. The objective was to evaluate the effect of an *S. platensis*-based biostimulant applied via seed treatment on melon seedling development. The experiment followed a completely randomized design in a 2 × 3 + 2 factorial arrangement, with four replicates of 25 seedlings per treatment. The treatments consisted of two types of *S. platensis*-based biostimulants (filtrate and residue), each applied at three concentrations (5, 15, and 25%). Additionally, two controls were included: seeds immersed in distilled water and seeds sown under dry conditions (without immersion). Twenty-five days after emergence, the filtrate treatments positively influenced stem diameter, root fresh mass, and leaf greenness index (colorimetry), with the 25% concentration notably enhancing root system development. The biostimulant filtrate at a concentration of 25% resulted in a higher number of leaves and higher Dickson quality index values. The largest seedlings were obtained with the biostimulant residue at a concentration of 5%. Interestingly, the dry condition (no seed immersion) yielded the highest average values for both emergence percentage and emergence speed index. Conversely, immersion in water alone negatively affects these parameters. Biostimulant filtrate at concentrations ranging from 5 to 25% promotes superior emergence speed and overall seedling vigor, effectively optimizing biomass distribution. It thus represents an efficient and sustainable biotechnological tool for improving crop establishment.
Keywords: *Cucumis melo* L., microalgae, propagation, biotechnology.

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Introduction

Melon (*Cucumis melo* L.) is an economically important crop cultivated worldwide, appreciated for its unique flavor and high nutritional value. Optimizing the germination process to ensure rapid, uniform seedling emergence, which is essential for successful melon production (Zhang et al., 2020). Although commercial melon seeds typically do not exhibit primary dormancy, seed treatments can enhance physiological vigor and overcome potential mechanical resistance from the seed coat, leading to improved initial growth, especially under sub-optimal conditions (Fan et al., 2023).

Seed treatments with bactericidal agents, fertilizers, and growth regulators can optimize germination, ensuring rapid and uniform seedling emergence (Ma, 2019). However, when applied at high concentrations or used continuously, these conventional chemical inputs can cause phytotoxicity and long-term environmental impacts. Consequently, there is increasing demand for sustainable biotechnological alternatives, such as microalgae-based biostimulants, that can maintain crop productivity while reducing the ecological footprint (Han et al., 2021).

Over the past decade, efforts have intensified to identify sustainable biotechnological alternatives that enhance plant development while reducing dependence on synthetic inputs. In this context, the use of organic-based biostimulants, particularly those derived from microorganisms such as microalgae and cyanobacteria, has gained traction due to their proven efficacy in promoting plant growth and development (Alvarez et al., 2021; Gonçalves, 2021).

Microalgae are photosynthetic microorganisms that synthesize a wide variety of bioactive compounds using atmospheric CO_2 and inorganic nutrients (Mutale-Joan et al., 2023). Microalgae extracts have demonstrated the ability to stimulate germination rates, promote the emergence of green cotyledons, and increase plant biomass compared to the water control (Akgül, 2019; Supraja et al., 2020; Rupawalla et al., 2022). In addition, microalgae extracts have been reported to stimulate plant and seedling growth, improve root development, increase nutrient uptake, enhance photosynthetic efficiency, and increase resistance to biotic and abiotic stressors (Godlewska et al., 2019; Van Camp et al., 2022; Chaudhuri and Balasubramanian, 2025).

Among various microalgae species used as plant biostimulants, *Spirulina platensis* stands out due to its high concentration of protein-derived amino acids and phytohormones, which have been reported to enhance seedling vigor in several crops significantly (Guedes et al., 2018; Rupawalla et al., 2022; Van Camp et al., 2022; Chaudhuri and Balasubramanian, 2025). This blue-green alga, classified within the Cyanobacteria group (Order: Oscillatoriales; Family: Cyanophyceae), is unicellular and photosynthetic. Its biochemical composition includes approximately 51.82% protein, 14.20% carbohydrates, 6.90% lipids, 9.00% ash, 12.10% moisture, and trace amounts of essential minerals and amino acids, such as iron (Fe), nitrogen (N), potassium (K), phosphorus (P), manganese (Mn), leucine, and alanine, among others (Guedes et al., 2018). This rich composition enhances the properties of its biomass-derived extracts, making them promising biostimulants for agricultural applications.

Indeed, liquid extracts of microalgae have been shown to improve tomato plant growth by increasing chlorophyll content and improving the uptake of N, P, and K (Mutale-Joan et al., 2020). Such physiological enhancements are closely linked to seed vigor; more efficient nutrient uptake and pigment synthesis during the initial stages of development can accelerate the growth, thereby improving germination rates and uniformity of seedling emergence. For example, Akgül (2019) reported positive effects on wheat seed germination when a 25% *S. platensis* filtrate concentration was applied. Similarly, Supraja et al. (2020) observed accelerated germination in tomato seeds treated with 20-60% *S. platensis* extract within three days compared to untreated controls. Godlewska et al. (2019) found that immersing radish seeds in a 15% *S. platensis* filtrate significantly enhanced seedling growth. Barone et al. (2018) also noted that sugar beet seedlings treated with microalgae extracts showed improvements in root length, surface area, and leaf development.

Despite these promising results, the application of *S. platensis*-based formulations directly via seed treatment remains underexplored. Given its rich biochemical profile and previously observed benefits, this study hypothesized that seed treatment with *S. platensis* would improve melon seedling emergence and quality. Therefore, the objective was to evaluate the effect of a *Spirulina platensis*-based biostimulant applied to melon seeds on seedling performance and quality.

Materials and Methods

Characterization of the experimental area

The experiment was performed in a greenhouse located at the Center for Agrofood Science and Technology of the Federal University of Campina Grande (UFCG), Pombal, Paraíba, Brazil. The structure is a tunnel-type greenhouse with an arched roof, measuring 7.0 m in width, 18.0 m in length, and 3.0 m in height, covered with transparent low-density polyethylene film (0.15 mm thick). The frame is constructed from galvanized steel, and the lateral and front closures are composed of a 50% shade screen mesh.

Geographically, the experimental site is situated in the western region of Paraíba, at coordinates 6° 46’ 13” S and 37° 48’ 06” W, with an altitude of 175 m. According to the Köppen climate classification, the region is categorized as BSh, hot semi-arid, with an average annual rainfall of approximately 750 mm, a mean annual temperature of 27.4°C, and an average relative humidity of 68.9%. The climate is characterized by two distinct seasons: a prolonged dry period and a short, irregular wet season (Alvarez et al., 2013). During the experimental period, the average temperature (°C) and relative humidity (%) inside the greenhouse were recorded daily using a digital thermohygrometer (Pro-lab Materiais para Laboratorios Ltda., São Paulo, SP, Brazil), as shown in Figure 1.

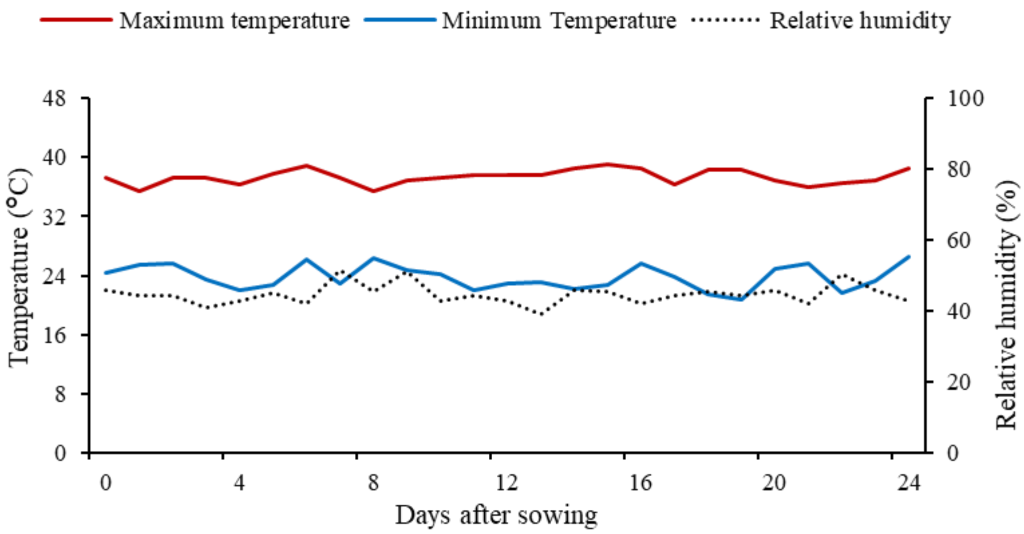


Figure 1. Temperature (maximum and minimum) and relative humidity during the experimental period in the greenhouse.

Experimental design

The experiment followed a completely randomized design in a 2 × 3 + 2 factorial arrangement, with four replicates of 25 seedlings per treatment. The treatments consisted of two types of *Spirulina platensis*-based biostimulants (filtrate – F and residue – R), each applied at three concentrations (5, 15, and 25%). Additionally, two controls were included: seeds immersed in distilled water and seeds sown under dry conditions (without immersion).

Preparation of the biostimulant

The biostimulant concentrations were prepared using *S. platensis* biomass, diluted in distilled water at a 1:10 (w/v) ratio. The suspension was stirred continuously at 40°C for 40 min using a heated magnetic stirrer model Ni 1103 (Nova Instruments Equipamentos para Laboratório Ltda., Piracicaba, SP, Brazil). Subsequently, the mixture was centrifuged at 3000 rpm for 40 min using a Excelsa II 206 benchtop centrifuge (Fanem®, São Paulo, SP, Brazil), resulting in two fractions: a supernatant (filtrate) and a solid residue.

Both fractions were then diluted to obtain the treatment concentrations of 5, 15, and 25%, which were used in the seed treatments. Prior to application, the electrical conductivity (EC) and pH of each prepared concentration were measured (Table 1), ensuring consistency and quality control of the biostimulant formulations (Godlewska et al., 2019).

Table 1. Characterization of electrical conductivity (EC) and pH of biostimulants based on *Spirulina platenses*.

Concentrations	Filtered		Residue	
	EC (dS m^{-1})	pH	EC (dS m^{-1})	pH
Distilled water	0.0044	8.78	0.0044	8.78
5%	0.0711	9.14	0.0840	7.89
15%	0.1894	8.70	0.2146	7.73
25%	0.3033	7.73	0.3314	7.36

Filtrate – solution based on the supernatant of the *S. platensis* solution; residue – solution based on the residue of the *S. platensis* concentrate.

Experiment setup and conduction

The experiment was conducted between November and December 2019, using 200-cell expanded polystyrene trays (43 × 335 × 664 mm) filled with previously sterilized sand as the substrate, and the trays were autoclaved (Prismatec Equipamentos, Itu, SP, Brazil) at 120°C and 1 atm for 60 min. The species used was *Cucumis melo* L. (‘Amarelo’ melon) (Feltrin® Sementes, Farroupilha, Brazil). Untreated seeds were specifically selected for this study to avoid any chemical interference with the biostimulant application. The *S. platensis* biomass (Tamanduá®, Patos, PB, Brazil) used in the treatments was acquired commercially in processed form.

For the filtrate (F) treatments, melon seeds were immersed in the prepared biostimulant solutions (at the three concentrations) for 12 h prior to sowing. For the residue (R) treatments, the concentrated biomass was used to coat the seeds under continuous agitation for 4 min, after which the seeds were sown.

The evaluation was divided into two stages. First, seedling emergence and vigor were assessed through daily counts until stabilization, allowing the calculation of the percentage of emergence (EP, %) and the emergence speed index (ESI) (Maguire, 1962). ESI was calculated using Equation 1.

$$\mathrm{ESI} = \frac{G1}{D1} + \frac{G2}{D2} + \frac{G3}{D3} \ldots + \frac{Gn}{Dn} \quad (1)$$

Where: G1, G2, G3 … Gn – number of seedlings germinated in each count; D1, D2, D3 … Dn – number of days from sowing to the 1st, 2nd, 3rd ... nth count.

At 25 days after emergence (DAE), the seedlings were harvested to evaluate initial growth and morphological characteristics. The following parameters were assessed: plant height (PH, cm), measured from the substrate level to the insertion of the last fully expanded leaf using a graduated ruler; number of leaves (LN), determined by manually counting fully expanded leaves; stem diameter (SD, mm), measured at the collar level using a digital caliper; and root length (RL, cm), determined by measuring the distance from the collar to the tip of the main root using a graduated ruler after careful washing of the root system. The seedlings were separated into shoots and roots to determine shoot fresh mass (SFM, g) and root fresh mass (RFM, g). The fresh material was placed in paper bags and dried in a forced-air oven at 65°C until constant mass was achieved to determine shoot dry mass (SDM, g) and root dry mass (RDM, g). The plant parts were weighed using a precision analytical balance (0.001 g accuracy).

The leaf greenness index (colorimetry) (GI, µg cm^{-2}) was determined through non-destructive chlorophyll quantification at six different points per leaf, using a CR-400 chroma meter (Konica Minolta, Ramsey, NJ, USA), calibrated with a standard white plate, and expressed as the ratio °h/(L* × C*). Where: L* represents lightness, C* represents chroma, and °h represents the hue angle (Amarante et al., 2008).

The Dickson quality index (DQI, Dickson et al., 1960) was determined using Equation 2.

$$DQI = \frac{TDM\ (g)}{\frac{PH\ (cm)}{SD\ (mm)} + \frac{SDM\ (g)}{RDM\ (g)}} \qquad (2)$$

Where: TDM – total dry mass (g), obtained by sum of SDM and RDM.

Statistical analysis

The data were analyzed using ANOVA with the F-test ($p \leq 0.05$). When significant effects were detected, the Tukey test ($p \leq 0.05$) was used to compare treatment means, and the Dunnett test ($p \leq 0.05$) was used to contrast treatments against the control group. These analyses were performed using the ExpDes.pt package (Ferreira et al., 2018) in R software version 4.3.1 (R Core Team, 2023).

For multivariate analysis, principal component analysis (PCA) was conducted using the prcomp function of the stats package. PCA plots were generated using the factoextra package (Kassambara and Mundt, 2020). Hierarchical clustering was performed and visualized as a heatmap using the pheatmap package (Alcântara and Porto, 2019).

Results and Discussion

The analysis of variance (ANOVA) revealed a significant interaction between the factors (*Spirulina platensis*-based biostimulant type and concentration) for the emergence percentage (EP) ($p < 0.01$), emergence speed index (ESI) ($p < 0.01$), plant height (PH) ($p < 0.05$), number of leaves (LN) ($p < 0.01$), and Dickson quality index (DQI) ($p < 0.05$) (Table 2). Conversely, stem diameter (SD), root fresh mass (RFM), and the leaf greenness index (GI) were significantly influenced ($p < 0.01$) only by the *S. platensis*-based biostimulant type. Root length (RL) was solely affected by the biostimulant concentrations ($p < 0.01$). Shoot fresh mass (SFM), shoot dry mass (SDM), and root dry mass (RDM) were not were not significantly affected ($p > 0.05$) by any of the factors evaluated.

The EP (Figure 2A) and ESI (Figure 2B) were not significantly influenced by biostimulant type or concentration. Nevertheless, a significant difference was observed regarding the additional treatments ($p < 0.01$). Specifically, the dry seed treatment (no prior imbibition) achieved the highest means for both EP and

ESI, surpassing the control treatment involving seed immersion in distilled water.

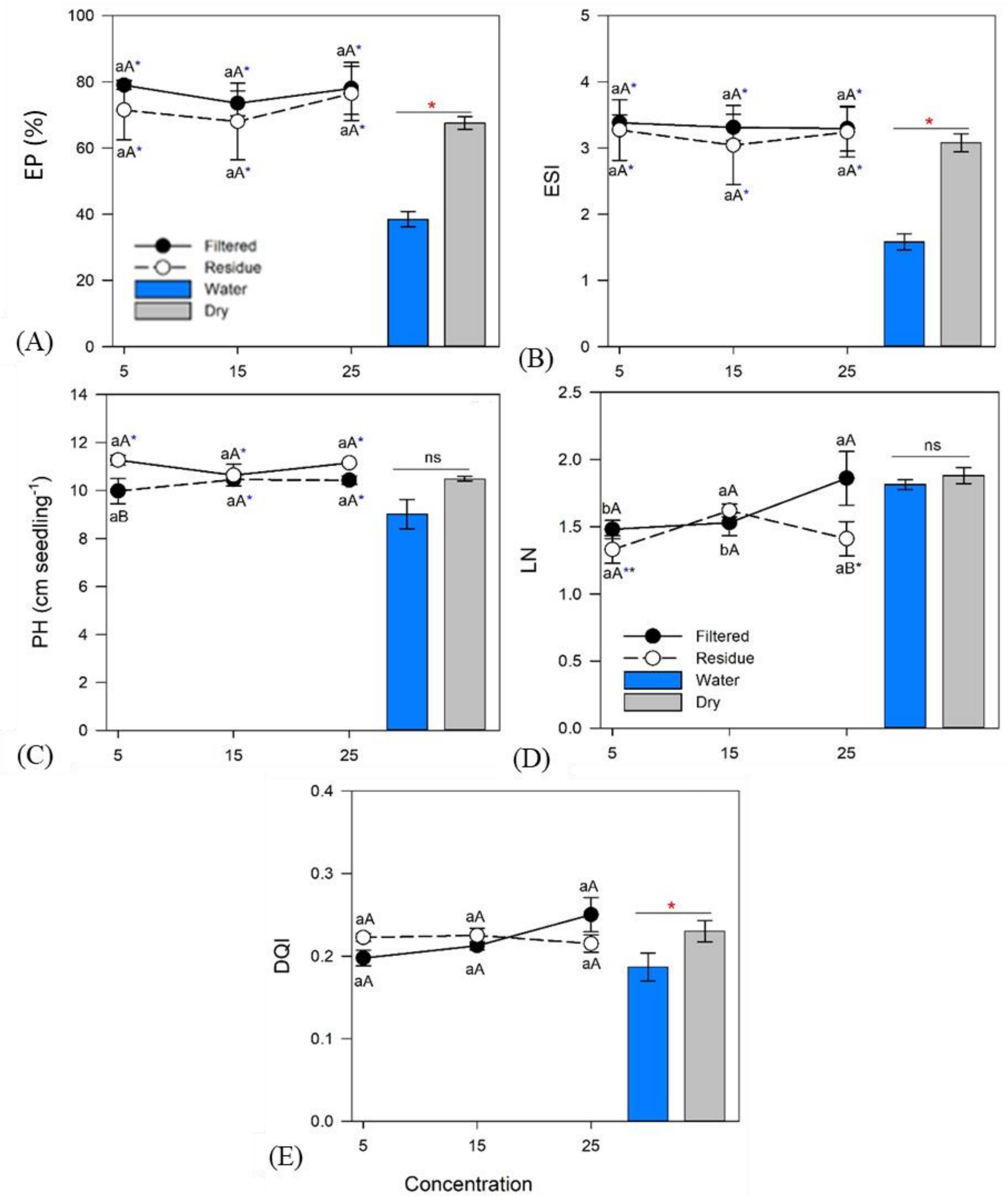
The high performance of the dry seed treatment suggests that the seeds reached their maximum germinative potential under the experimental conditions provided. While biostimulants are recognized for enhancing metabolic activation (Barone et al., 2018; Ma, 2019; Supraja et al., 2020), their effects are often less evident when seeds already possess high physiological quality and are germinated under non-stressful environments. Therefore, the substrate's natural hydration kinetics were sufficient for rapid and uniform seedling establishment, whereas prior immersion did not yield additional physiological benefits.

The residue-based biostimulant at 5% concentration produced seedlings with higher PH, equivalent to a 13% increase, outperforming the filtrate at the same concentration (Figure 2C). Interestingly, higher concentrations (15 and 25%) showed no significant differences between biostimulant types, suggesting a saturating effect or a specific sensitivity of melon seedlings to lower doses of the residue. This increased height growth at the 5% residue-based biostimulant potentially reflects the action of growth-promoting substances, such as gibberellins or their precursors, which are often found in microalgae biomass and are known to trigger cell elongation even at low concentrations (Thinh et al., 2021; Shedeed et al., 2022). As observed for other variables, the control treatments (distilled water and dry conditions) did not differ significantly.

Table 2. Summary of the analysis of variance for emergence percentage (EP), emergence speed index (ESI), plant height (PH), number of leaves (NL), stem diameter (SD), root length (RL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), leaf greenness index (GI), and Dickson quality index (DQI) in 'Amarelo' melon seedlings subjected to the biostimulant (filtrate and residue) based on *Spirulina platensis* (B) at different concentrations (C).

SV	DF	Mean squares					
		EP (%)	ESI	PH (cm)	LN	SD (mm)	RL (cm)
B	1	140.16^{ns}	0.1237^{ns}	3.1704*	0.1734^{ns}	0.3550**	0.5963^{ns}
C	2	88.666^{ns}	0.0455^{ns}	0.1202^{ns}	0.1138^{ns}	0.0395^{ns}	89.138**
B × C	2	18.666^{ns}	0.0554^{ns}	0.6130^{ns}	0.1464*	0.0189^{ns}	0.3539^{ns}
T	1	1682.0**	4.4760**	4.2865*	0.00911^{ns}	0.0191^{ns}	2.6353^{ns}
T × (B × C)	1	2752.0**	5.1601**	4.8920*	0.5688**	1.0891^{ns}	0.4879^{ns}
Residue	24	184.79	0.4576	0.5088	1.0542	0.026	3.1320
CV (%)		19.68	22.35	6.84	12.97	5.74	25.99
Mean		69.063	3.026	10.426	1.615	2.699	6.808
SV	DF	Mean squares					
		SFM (g)	RFM (g)	SDM (g)	RDM (g)	GI (µg cm^{-2})	DQI
B	1	0.0007^{ns}	0.2610**	0.00007^{ns}	0.00004^{ns}	0.00001**	0.0000^{ns}
C	2	0.0022^{ns}	0.0011^{ns}	0.0002^{ns}	0.0009^{ns}	0.000001^{ns}	0.00103^{ns}
B × C	2	0.0047^{ns}	0.0149^{ns}	0.00015^{ns}	0.0007^{ns}	0.000001^{ns}	0.0020*
T	1	0.0375^{ns}	0.1067^{ns}	0.00108^{ns}	0.0011^{ns}	0.000001^{ns}	0.0037**
T × (B × C)	1	0.0061^{ns}	0.0080^{ns}	0.00015^{ns}	0.00007^{ns}	0.000001^{ns}	0.00087^{ns}
Residue	24	0.011	0.0343	0.00028	0.00047	0.000001	0.0006
CV (%)		10.91	26.18	12.75	31.76	3.58	11.30
Mean		0.969	0.708	0.132	0.068	0.026	0.217

SV – source of variation; DF – degree of freedom; CV – coefficient of variation; ns, * and ** – not significant, significant at $p < 0.05$ and significant at $p < 0.01$, respectively, by F-test.



The bars refer to the standard error of the mean.

Figure 2. Emergence percentage (EP) (A), emergence speed index (ESI) (B), plant height (PH) (C), number of leaves (NL) (D), and Dickson quality index (DQI) (E) in 'Amarelo' melon seedlings subjected to the biostimulant (filtrate and residue) based on *Spirulina platensis* at different concentrations. Means followed by the same lowercase letters compare concentrations of the same type of biostimulant, and uppercase letters compare types of biostimulants (filtrate vs. residue) at the same concentration by Tukey's test ($p \leq 0.05$). Means followed by an asterisk (*) differ significantly from the control group, according to Dunnett's test ($p \leq 0.05$).

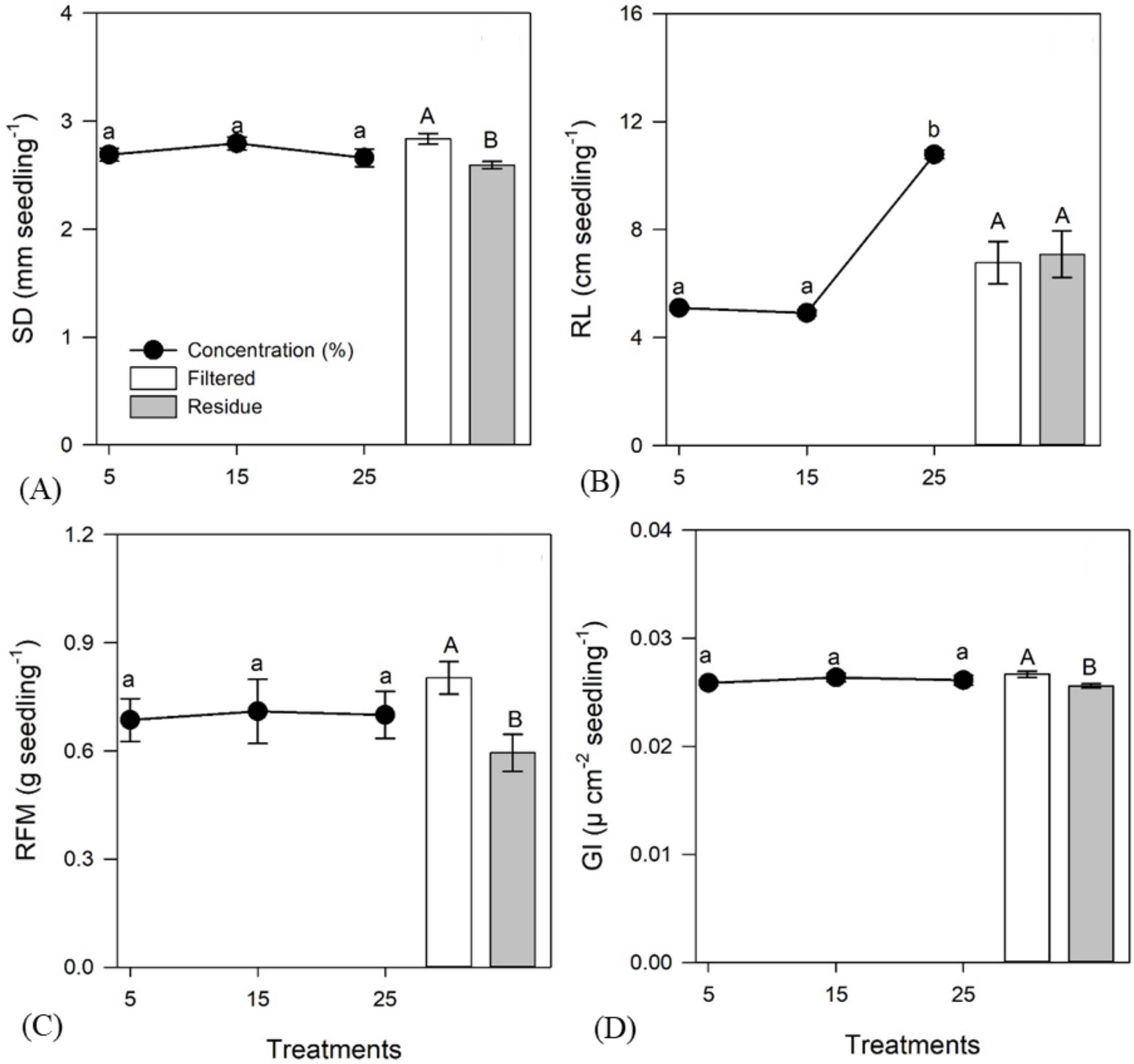
The filtrate-based biostimulant applied at 25% concentration (F25) produced the highest LN, outperforming all other biostimulant applications and concentrations. Specifically, F25 promoted a 22.22% increase in leaf production compared to the 25% residue-based biostimulant (R25) (Figure 2D). This enhanced vegetative development suggests that the higher concentration of soluble nutrients and signaling molecules in the filtrate effectively triggered primordia initiation and leaf expansion (Ertani et al., 2018; Bulgari et al., 2019). In contrast, the control treatments (distilled water and dry conditions) showed no significant differences, highlighting the biostimulatory effect of *S. platensis* on melon seedling vigor. In contrast, the residue-based treatments did not demonstrate comparable efficiency. This may be due to lower bioavailability or reduced concentrations of active components in the residue fraction, as previously reported by Guedes et al. (2018) and Alcântara and Porto (2019).

The F25 treatment produced the highest DQI, indicating superior robustness and biomass distribution in melon seedlings (Figure 2E). This index provides a holistic assessment of seedling vigor by incorporating morphological traits and biomass partitioning (Abreu et al., 2015). Notably, the dry condition outperformed seed immersion in water, although the *S. platensis* treatments significantly surpassed both. This suggests that while simple hydropriming may have induced imbibitional stress, potentially leading to the leaching of essential solutes (Vieira and Carvalho, 2023), the bioactive compounds in the F25 treatment provided a protective and stimulatory effect. The presence of diverse molecules, such as phenolic compounds, phytohormone mimics, and amino acids, in the algal extract likely optimized resource allocation between the shoot and root systems (Guedes et al., 2018; Ronga et al., 2019; Gonçalves, 2021), thereby ensuring greater resilience during post-transplant establishment.

The F25 treatment led to a greater number of leaves, which may partly explain the increased shoot dry mass and the higher DQI. Faster leaf emission contributes to earlier and more sustained photosynthetic activity, thereby enhancing the accumulation of carbohydrates and other photoassimilates in various plant organs. This outcome may be attributed to the biochemical richness of the filtered *S. platensis* biostimulant, which is recognized as a natural source of macro- and micronutrients, amino acids, and phytohormones such as cytokinins, auxins, and abscisic acid (Guedes et al., 2018; Alcântara and Porto, 2019). These compounds are known to stimulate cell division, support balanced mineral nutrition, and mitigate the effects of biotic and abiotic stresses.

The filtered *S. platensis*-based biostimulant promoted a higher SD compared to the residue-based treatment (Figure 3A). This enhanced stem thickening indicates that soluble bioactive compounds in the filtrate, such as arginine and tryptophan, are more readily available for uptake during early development. These amino acids serve as precursors to polyamines and auxins, respectively, which are essential for stimulating cell division and vascular expansion (Bulgari et al., 2019; Kapoore et al., 2021; Lee and Ryu, 2021; Shedeed et al., 2022). A robust stem diameter directly improves vascular transport and physical support, traits that are critical for preventing seedling lodging after transplanting and ensuring better establishment in the field.

Increasing concentrations of *S. platensis*-based biostimulants promoted an increase in the RL (Figure 3B). The 25% concentration produced the most extensive root systems, yielding substantial gains of 52.65 and 54.41% compared to the 5 and 15% concentrations, respectively. This marked root proliferation at higher doses suggests that the biostimulant enhanced the synthesis or activity of endogenous auxins, which are essential for cell elongation and branching in the radical system (Bulgari et al., 2019; Thinh et al., 2021). Such robust root architecture provides a critical advantage for melon seedlings, as it improves their capacity for water and nutrient uptake, considered a determinant factor for survival under the limiting conditions of semi-arid environments.



The bars refer to the standard error of the mean.

Figure 3. Stem diameter (SD) (A), root length (RL) (B), root fresh mass (RFM) (C), and leaf greenness index (GI) (D) in ‘Amarelo’ melon seedlings subjected to the biostimulant (filtrate and residue) based on *Spirulina platensis* at different concentrations. Means followed by the same lowercase letters compare concentrations of the same type of biostimulant, and uppercase letters compare types of biostimulants (filtrate vs. residue) at the same concentration by Tukey’s test ($p \leq 0.05$).

The filtered *S. platensis*-based biostimulant promoted significantly higher RFM (Figure 3C) and GI (Figure 3D) values compared to the residue-based treatment. This superior performance suggests that the filtrate formulation provides a more readily available pool of nutrients and signaling molecules, such as magnesium and nitrogen precursors, which directly enhance chlorophyll biosynthesis and root biomass accumulation. These improvements in stem diameter, height, and root development are largely attributed to the abundance of macro- and micronutrients and phytohormones in the extract (Shedeed et al., 2022; Ferreira et al., 2025). Indicating the high levels of arginine and tryptophan in the filtrate serve as precursors for polyamines and auxins, respectively, which are crucial for promoting cell division and elongation (Bulgari et al., 2019; Ferreira et al., 2025). Additionally, growth regulators such as gibberellic acids in the microalgae extract likely influenced seedling development from the earliest stages of seed treatment (Thinh et al., 2021; Ferreira et al., 2025), optimizing both resource acquisition and photosynthetic potential.

The relationship between the initial growth variables and the application of *S. platensis*-based biostimulants was explored using principal component analysis (PCA), as shown in Figure 4. The first two principal components (PC1 and PC2) accounted for 68.30% of the total variance among the variables, with PC1 explaining 43.8% and PC2 explaining 24.5%. These components adequately summarized the data's multidimensional structure, revealing treatment groupings and variable correlations.

PC2 was primarily associated with the variables NL, SD, GI, and RFM, all of which exhibited the highest loadings on this component. Notably, the filtrate-based biostimulant applied at 15% concentration (F15) also had the highest PC2 score, suggesting a strong positive correlation among these variables under this treatment. Conversely, F15 was associated with lower RL, reinforcing the trade-off observed between shoot development and root elongation under this condition (Figure 4).

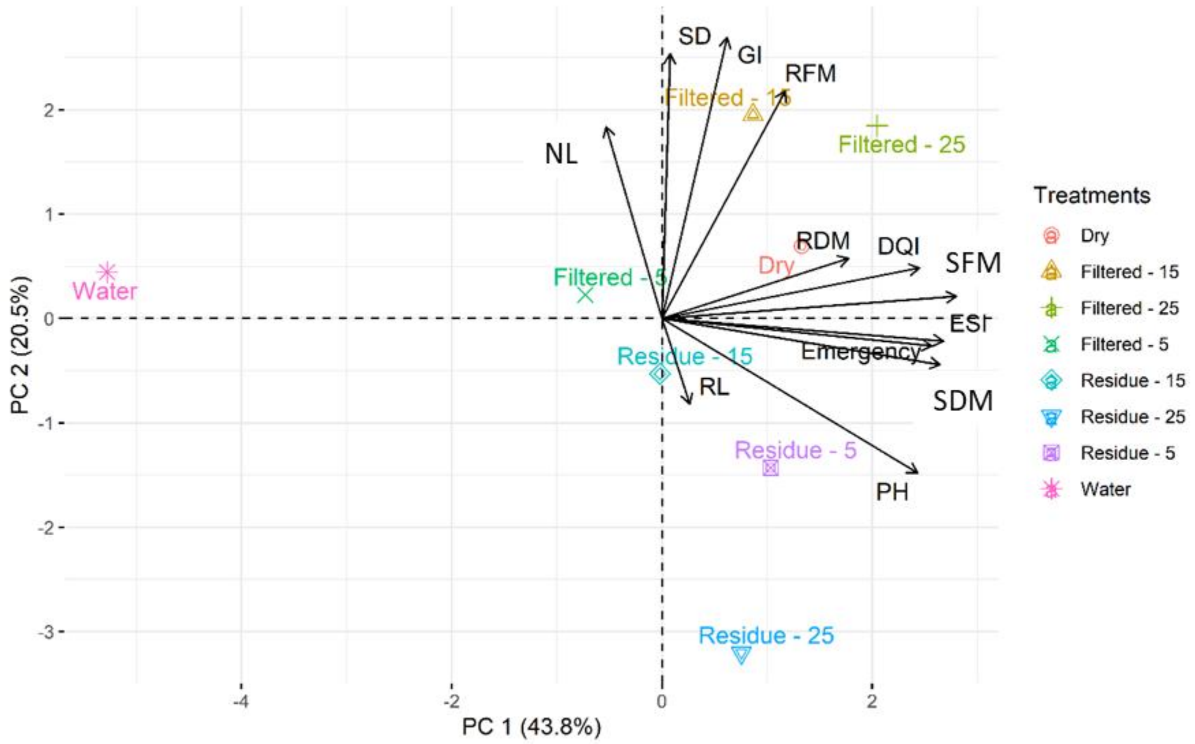


Figure 4. Principal component analysis (PCA) between the treatments and variables analyze of 'Amarelo' melon seedlings subjected to the biostimulant (filtrate and residue) based on *Spirulina platensis* at different concentrations. ESI – emergence speed index; PH – plant height; NL – number of leaves; SD – stem diameter; RL – root length; SFM – shoot fresh mass; RFM – root fresh mass; SDM – shoot dry mass; RDM – root dry mass; GI – greening index; DQI – Dickson quality index.

In contrast, PC1 was predominantly influenced by RDM, SDM, SFM, EP, ESI, and DQI, which showed the highest scores on this axis. These variables were also closely associated with the filtrate-based biostimulant applied at 25% concentration (F25), indicating that this concentration of the filtered biostimulant promoted superior performance in overall biomass accumulation and seedling quality (Figure 4).

Cluster analysis using a heatmap (Figure 5) further supported the PCA findings, revealing the degree of similarity among treatments and their effects on seedling growth variables. The hierarchical cluster analysis clearly segregated the treatments into three distinct functional groups based on the Euclidean distance and the distribution of color intensities, which represent the standardized variables (Z-score scale). Cluster 1 (filtrate-based biostimulant applied at 5, 15, and 25% concentrations, and along with dry seeds not immersed in water) displays a predominance of intense red tones, indicating that these treatments reached the highest values for critical growth and quality parameters. Specifically, the high color density for DQI, SDM, and ESI in this cluster highlights the effectiveness of the *S. platensis* filtrate in maximizing seedling vigor.

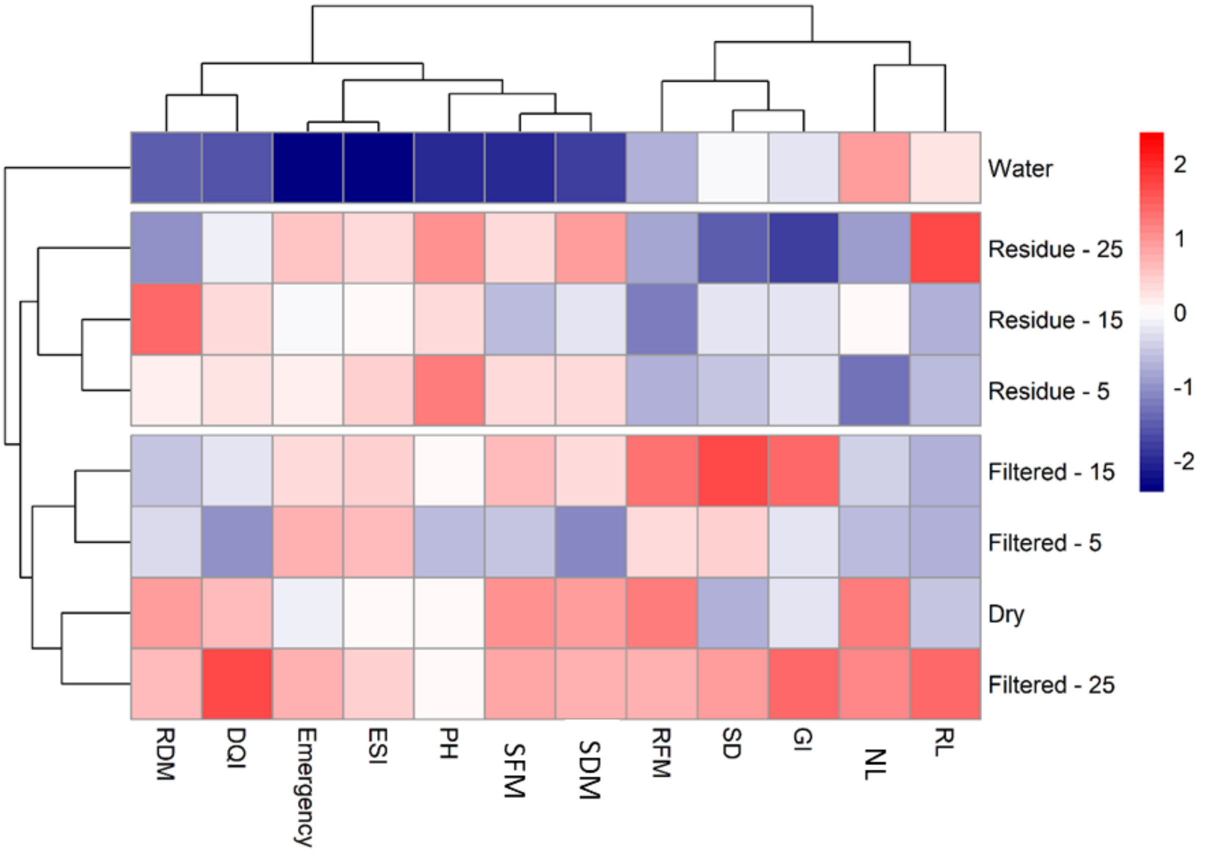


Figure 5. Heatmap cluster analysis in ‘Amarelo’ melon seedlings subjected to the biostimulant (filtrate and residue) based on *Spirulina platensis* at different concentrations. ESI – emergence speed index; PH – plant height; NL – number of leaves; SD – stem diameter; RL – root length; SFM – shoot fresh mass; RFM – root fresh mass; SDM – shoot dry mass; RDM – root dry mass; GI – greening index; DQI – Dickson quality index.

Cluster 2 contains the residue-based treatments (applied at 5, 15, and 25% concentrations), characterized by intermediate color shades (closer to white or light pink/red). This pattern reflects a moderate physiological response, where the residue-based formulation provided some growth stimulus but failed to reach the peak performance observed in the filtrate group. Negative correlations were observed for some variables in treatments involving *S. platensis* residues (applied at 5, 15, and 25% concentrations) (Figure 5), suggesting that the physical and chemical composition of the residue may limit its biostimulatory potential. These findings indicate that the efficacy of the biostimulant is strongly influenced by the fraction of biomass applied, with the filtrate showing superior effects compared to the residue.

In contrast, Cluster 3 consists exclusively of the distilled water control, which exhibits cooler/lighter tones across nearly all variables. This low color intensity indicates values below the overall mean, confirming that simple hydropriming without the microalga's biochemical components results in limited seedling development. Therefore, the transition from cool to intense colors across the heatmap validates the superior role of the filtered biostimulant in enhancing the metabolic and morphological traits of melon seedlings.

In our study, the seeds treated solely with distilled water formed a distinct cluster characterized by lower mean values across most evaluated variables (Figure 5). This pattern suggests that *C. melo* 'Amarelo' seeds may not tolerate prolonged water imbibition, as it negatively impacts their emergence and subsequent seedling development. As noted by Nalwa and Seth (2021), water uptake is essential for germination; however, it must occur in adequate and balanced amounts to avoid physiological stress or damage.

The results indicate that immersion in distilled water may have induced imbibitional stress. Although some studies report that soaking seeds prior to sowing can enhance germination parameters (Pinedo and Ferraz, 2008). In the present study, water soaking did not improve any of the evaluated variables. On the contrary, seeds subjected to the dry condition (i.e., without soaking) exhibited better performance in both germination and seedling emergence, underscoring that dry sowing may be more suitable for 'Amarelo' melon seeds under the tested conditions.

It is also important to consider that dicotyledonous seeds can absorb 35 to 40% of their weight in water within the first one to two hours of imbibition. However, prolonged exposure may lead to undesirable effects, including the leaching of essential compounds, such as gases, sugars, organic acids, amino acids, and potassium ions, which are essential for germination and early growth (Vieira and Carvalho, 2023). In our study, the 12-h immersion period likely exceeded the optimal window, causing stress that delayed or inhibited emergence.

The multivariate analysis confirms that higher seedling quality was closely associated with early melon seed emergence, which enabled longer biomass accumulation and increased seedling dry mass (Figures 4 and 5). Specifically, the filtrate-based biostimulant applied at 25% concentration (F25) promoted earlier emergence, granting the seedlings an extended period for physiological development and dry matter partitioning. This temporal advantage translated into superior quality compared to both the water control and residue-based treatments. According to the heatmap cluster analysis (Figure 5), the clear segregation of F25 into the high-performance group underscores the efficacy of using standardized matrix data to identify superior treatments (Gu et al., 2022). This pattern reinforces that the filtered *S. platensis* biostimulant, at its optimal concentration, acts as a robust tool for enhancing melon seedling production by synchronizing rapid germination with vigorous vegetative growth.

Therefore, the application of microalgae-derived cell extracts emerges as a promising, cost-effective, and environmentally sustainable strategy to support seedling development and promote more resilient and productive agricultural systems (Supraja et al., 2020).

Conclusions

The filtered *S. platensis* biostimulant significantly enhances the early development and quality of 'Amarelo' melon seedlings. The filtrate-based biostimulant applied at 5 and 25%

concentrations promoted faster emergence and improved overall seedling vigor, effectively optimizing biomass distribution and serving as an efficient, sustainable biotechnological tool for improving crop establishment.

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