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MODULATION OF GROWTH AND STRESS-RELATED PHYSIOLOGY IN ROUND PEPPER (*CAPSICUM ANNUUM* L.) SEEDLINGS VIA OPTIMIZED FERTILIZATION

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Abstract

The present study investigates the effects of some fertilization treatments on the growth and physiological attributes of `Creolica` round pepper (Capsicum annuum L.) seedlings cultivated under controlled conditions at the Vegetable Research and Development Station Bacău. Seven fertilization variants were assessed using distinct commercial products at different concentrations: V1 (Albit 0.1% + Turboroot 0.25%), V2 (Albit 0.01% + Turboroot 0.375%), V3 (Albit 0.01% + Turboroot 0.5%), V4 (Turboroot 0.25%), V5 (Turboroot 0.375%), V6 (Turboroot 0.5%), and V7 (Control - untreated). Key morphological and physiological parameters were evaluated, including stem height, stem weight and diameter, root length and weight, number of leaves per seedling, leaf length and width, leaf surface area, leaf shape (expressed as the ratio of leaf width to leaf length), chlorophyll content, anthocyanin concentration, dry matter content, and water content. The findings of this study provide valuable insights into the potential of Albit and Turboroot combinations to enhance seedling quality and vigor in round pepper cultivation.

Key words*: seedling vigor, biostimulant-mediated regulation, controlled-environment, root morphogenesis, chlorophyll and anthocyanin dynamics.*

INTRODUCTION

The efficient cultivation of horticultural crops in controlled environments is vital to addressing the global demand for high-quality produce while ensuring resource sustainability (Ahmed et al., 2024; Jain et al., 2023). *Capsicum annuum* L. var. Grossum, commonly known as round pepper, stands out among the diverse array of cultivated vegetable species due to its substantial economic importance, as well as its notable nutritional and medicinal attributes (da Veiga Jr. et al., 2022). The round pepper variety, characterized by its robust growth habit and high yield potential, holds particular promise in sustainable vegetable growing systems (Crosby, 2008). However, achieving optimal growth and physiological performance in greenhouse-grown seedlings requires precise management of nutritional and biostimulatory inputs (Zapata-García et al., 2024).

Biostimulants have emerged as innovative tools in modern horticulture, offering an environmentally friendly alternative to conventional fertilizers (Bell et al., 2022). These bioactive formulations often comprise plant extracts, microbial agents, and signaling molecules designed to enhance plant vigor by improving nutrient assimilation, hormonal balance, and stress tolerance (Nephali et al., 2020; Aamir et al., 2020). Unlike traditional fertilizers, biostimulants operate through complex biochemical and physiological mechanisms, influencing processes such as root architecture development, photosynthetic efficiency, and stress resilience (Johnson et al., 2024). Given the high-value nature of pepper crops and the growing emphasis on sustainable agriculture, understanding the impact of biostimulant-based fertilization regimes on seedling development is of paramount importance (Farouk, 2023).

Seedlings represent a critical phase in the life cycle of plants, serving as the foundation for subsequent growth stages (Leck et al., 2008). Ensuring robust seedling development is particularly essential in controlled greenhouse systems, where environmental variables such as light, temperature, and humidity are carefully managed to maximize productivity (Paradiso & Proietti, 2022). The application of biostimulants during this stage has the potential to mitigate

environmental stressors and optimize morphological and physiological traits, including stem and root development, leaf expansion, photosynthetic pigment synthesis, and water-use efficiency (Melo-Sabogal & Contreras-Medina, 2024). Despite these advantages, the outcomes of biostimulant applications often vary depending on their composition, concentration, and frequency of use, requiring comprehensive evaluations tailored to specific crops and growing conditions (Parađiković et al., 2019).

Limited research has explored the synergistic effects of several biostimulants when applied concurrently or at varying concentrations, particularly in the context of round pepper seedlings. Such insights are crucial for determining optimal fertilization regimes that maximize the benefits of biostimulants while avoiding potential trade-offs, such as over-fertilization or imbalanced nutrient allocation.

A key objective of modern horticultural practices is the optimization of vegetative and physiological traits that underpin plant productivity (Langensiepen et al., 2020). Morphological characteristics, such as stem length, collar diameter, root architecture, and leaf area, are pivotal for resource acquisition and structural integrity (Zhang et al., 2024). Concurrently, physiological parameters, including chlorophyll and anthocyanin content, provide insights into the plant's photosynthetic capacity and stress response (Yan et al., 2022). Evaluating these parameters under varying fertilization regimes can elucidate the mechanisms by which biostimulants influence plant performance, offering a basis for tailored nutrient management strategies (Dalal et al., 2019).

The significance of this study extends beyond its immediate application to round pepper seedlings, contributing to the broader discourse on sustainable vegetable growing practices. As the global agricultural sector faces mounting challenges related to climate change, resource scarcity, and soil degradation (Hossain et al., 2020), the adoption of biostimulant-based fertilization regimes offers a viable pathway toward resilient and resource-efficient crop production systems (Pinto et al., 2023). Furthermore, the findings have potential implications for other high-value crops grown in greenhouse settings, reinforcing the importance of evidence-based approaches to biostimulant application.

By leveraging controlled greenhouse conditions, this research provides a comprehensive evaluation of the interplay between biostimulant treatments and plant performance, thereby advancing our understanding of sustainable fertilization practices in vegetable growing.

MATERIALS AND METHODS

This research was conducted in 2024 and 2025 growing seasons at the Vegetable Research and Development Station situated in the northeastern region of Bacău city, under controlled conditions, in an Almeria-style heated greenhouse to ensure optimal environmental requirements for seedling development. Regarding the biological material, it encompassed `Creolica` round pepper – an early semi-variety developed at the Vegetable Research and Development Station Bacău, characterized by a vegetation period of 125 days, round-flattened fruits with a smooth surface and slight ribbing, an average fruit weight of 180-220 g, and a yield capacity of 40-45 t/ha;

To produce the round pepper seedlings, seeds were sown in mid-February in trays specifically designed for this purpose, using Rekyva Remix 1 horticultural peat as a nutritive substrate. This substrate is characterized by small and/or medium fractions, with the following properties: pH 5.5-6.5, electrical conductivity of 0.5-1 mS/cm, and NPK (chlorine-free) $\leq$ 1 kg/m³. Subsequently, after full germination, the seedlings were transplanted into 70-cell alveolar trays (at the end of February), using Rekyva Remix 2 peat, which differs from the sowing variant by containing medium and/or large fractions, an electrical conductivity of 1-2 mS/cm, and NPK (chlorine-free) $\leq$ 2 kg/m³.

Regarding the two products used as fertilizers and/or biostimulants in the experiment, Albit is a poly-beta-hydroxybutyric acid (a natural biopolymer derived from beneficial soil bacteria: *Bacillus megaterium* and *Pseudomonas aureofaciens*), supplemented with a set of potentiating substances, including magnesium sulfate, potassium phosphate, potassium nitrate, and urea. On the other hand,

Turboroot is a comprehensive rooting and growth stimulator containing humic acids, L-alpha free amino acids, NPK, and micronutrients chelated with EDTA.
On the description of the experimental variants, the concentrations applied, as well as the number of treatments and the time intervals between them, the details are presented in Table 1.

Table 1. Fertilization Variants, Composition and Application Intervals

No.	Description of variants (commercial product and applied concentration)	Treatment application/interval between treatments
V1	Albit 0.01% + Turboroot 0.25%	I - immediately after transplanting; II - two weeks after the first treatment; III - two weeks after the second treatment.
V2	Albit 0.01% + Turboroot 0.375%	
V3	Albit 0.01% + Turboroot 0.5%	
V4	Turboroot 0.25%	
V5	Turboroot 0.375%	
V6	Turboroot 0.5%	
V7	Control (untreated)	-

Thus, the experiment included six fertilization treatments (Figure 1), along with a control variant; each consisted of 20 seedlings in three replications, resulting in a total of 60 plants per treatment.

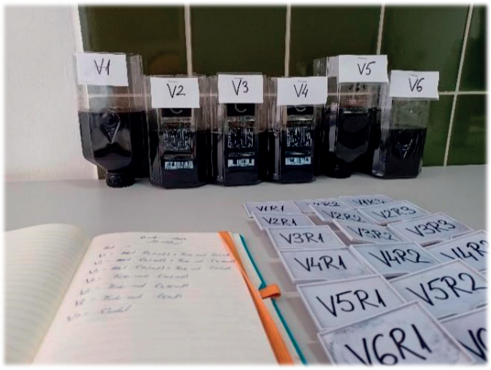

Figure 1. Methodological framework for the design and implementation of fertilization treatments

All three fertilization applications were carried out using a fine mist sprayer, ensuring uniform application across the entire leaf surface of the seedlings (Figure 2).
Furthermore, the experimental protocol adhered strictly to the prescribed conditions, including a controlled temperature range of 22-24°C, supplemental lighting using violet LED lights, and irrigation in accordance with the applicable standards.



Figure 2. Different fertilization schemes applied using a fine mist sprayer

Quantitative and qualitative assessment of round pepper seedlings

Biometric assessments were performed using direct measurement and weighing techniques to evaluate the growth parameters of seedlings at their optimal age for transplantation (45-50 days). Measurements included seedling height, root length, collar stem diameter, and the number of leaves per seedling, while biomass assessments comprised stem and root weights (Figure 3).



Figure 3. Aspects concerning quantitative determinations in round pepper seedlings

The tools employed for these procedures consisted of a precision ruler for linear measurements, a caliper for diameter determinations, and a highly accurate electronic balance for mass quantification. This methodology ensured

precise and reliable data acquisition for the characterization of seedling morphological and physiological attributes.

Leaf width, leaf length, and leaf area were quantified using the Area Meter AM 350, a precision instrument manufactured by ADC BioScientific Ltd (Figure 4).

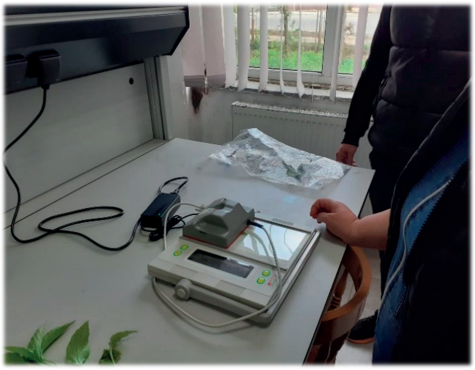

Figure 4. Determination of leaf area in round pepper seedlings

The measurements of leaf width and length were recorded in millimeters (mm), while the leaf area was calculated and expressed in square millimeters (mm^2).

The quantification of both chlorophyll pigment and anthocyanin content was conducted using two comparable instruments, the CCM 200 Plus and the ACM 200 Plus, manufactured by Optisciences, by positioning the sensor on the seedling's leaf blade and recording the measured values (Figure 5).



Figure 5. The evaluation of chlorophyll pigment and anthocyanin content in round pepper seedlings

These readings were conducted under optimal conditions, with clear skies and a temperature of approximately 25°C. The data obtained from these measurements were expressed through specific indices, namely the Chlorophyll Concentration Index and the Anthocyanin Content Index, which provide a precise representation of the total concentration of chlorophyll pigments and anthocyanins, respectively. These indices ensure accurate and reliable assessment of pigment levels, facilitating a comprehensive analysis of the plant's biochemical composition.

Dry Matter Content (DM): Fresh, homogenized samples (Figure 6) were subjected to drying in a forced-air oven (Biobase) at a controlled temperature of 103 ± 2°C for 24 hours, until a stable weight was achieved, in accordance with the AOAC (2000) guidelines.



Figure 6. Sample preparation prior to forced-air oven placement for the determination of dry matter content

The dry matter content was then expressed as a percentage (%).

Water Content (W): The water content was determined by subtracting the dry matter percentage from 100%, using the formula:

$$W\% = 100\% - DM\%.$$

Statistical Analysis - The variations in the analyzed parameters were assessed using the ANOVA test, which revealed statistically significant differences among the various fertilization treatments. When significant differences were detected, multiple comparison tests based on Duncan's test ($p < 0.05$) were applied. All statistical analyses were conducted using SPSS version 21 (IBM® Corp., Armonk, NY, USA).

RESULTS AND DISCUSSIONS

This section evaluates the impact of different fertilization treatments (V1-V7) on various vegetative and physiological parameters of the Creolica round pepper seedlings under controlled greenhouse conditions. Treatments include combinations of Albit and Turboroot applications at varying concentrations and frequencies, with V7 serving as the untreated control. Parameters assessed include stem and root length, stem and root weight, collar diameter, leaf count, leaf dimensions, foliar surface area, dry and water content percentages, anthocyanin content index (ACI) and chlorophyll content index (CCI).

Vegetative Growth: Stem and Root Metrics

Stem length varied significantly across treatments, with V5 (Turboroot at 3.75 ml) yielding the highest stem length at 25.11 cm, indicating a 28.6% increase over the control (V7). This outcome suggests a robust positive effect of intermediate Turboroot concentrations on stem elongation, potentially enhancing light capture and photosynthetic efficiency. Similarly, root length was most pronounced in V5, recording an 8.79 cm length, which represented a 30.6% improvement over the untreated plants. This pronounced root growth under V5 may indicate enhanced nutrient and water uptake capabilities, essential for optimizing plant vigor in greenhouse environments (Ahmed et al., 2024).

In terms of biomass accumulation, V5 continued to show superior performance, with the highest stem weight at 6.32 g and root weight at 4.15 g, representing increases of approximately 17.5% and 29.7%, respectively, over V7. This enhancement in biomass suggests that Turboroot at 3.75 ml offers optimal conditions for root and stem tissue development, likely due to improved nutrient assimilation and cellular expansion facilitated by the bio-stimulant components in Turboroot.

The findings underscore the profound impact of biostimulant application on vegetative growth parameters (Johnson et al., 2024; Rajesaheb et al., 2024), emphasizing the efficacy of intermediate Turboroot concentrations (3.75 ml, V5). The significant elongation of stems and roots highlights the role of optimized biostimulant concentrations in augmenting structural growth (Rani et al., 2024; Baltazar et al., 2021). These enhancements likely result from synergistic effects of nutrient mobilization and phytohormonal regulation (Savarese et al., 2022) induced by Turboroot.

Furthermore, the increases in biomass, sustain the biostimulant's capacity to enhance metabolic activity and cellular proliferation (Gatti et al., 2024; Lephatsi et al., 2022). Such improvements in structural integrity and resource acquisition suggest a potential for significant agronomic benefits, particularly in controlled environments where resource efficiency is paramount (Nephali et al., 2020; Sible et al., 2021). These results advocate for the strategic use of Turboroot at intermediate dosages to maximize vegetative growth and overall plant vigor, paving the way for further exploration into biostimulant-mediated growth optimization.

Collar Diameter and Leaf Count

Collar diameter, an indicator of stem robustness, showed the greatest values in V7 and V1, at 4.30 mm and 4.31 mm, respectively. This suggests that while fertilization treatments generally enhance vegetative growth, collar thickness may not directly correlate with the application of Albit or Turboroot, as the untreated control performed comparably well.

Maximum leaf count was observed in V1, with an average of 11 leaves per plant, indicating that the combined application of Albit and Turboroot effectively promotes leaf proliferation, thereby enhancing the photosynthetic surface and potentially increasing overall productivity. Conversely, leaf production was lowest in V5, with an average of 10.67 leaves per plant, highlighting potential constraints such as over-fertilization or nutrient allocation dynamics that may influence foliar development (Table 2).

Thus, collar diameter exhibited minimal variation across treatments. This suggests that collar thickness may not be significantly influenced by Albit or Turboroot, potentially indicating a limited role of these biostimulants in fortifying structural stability (Parađiković et al., 2019). compared to untreated control.

Similarly, leaf production exhibited minimal variation, indicating that while the combined application of Albit and Turboroot may support enhanced foliar proliferation (Şesan et al., 2020; Kisvarga et al., 2022) - potentially mediated by improved hormonal equilibrium and nutrient

assimilation mechanisms to maximize photosynthetic capacity (Rathor et al., 2023; Martínez-Lorente et al., 2024) -evidence of over-fertilization effects or resource allocation trade-offs was also apparent. These trade-offs suggest a potential shift in nutrient allocation dynamics, prioritizing root development at the expense of foliage growth under specific treatment conditions (Monson et al., 2022). The outcomes emphasize the paramount significance of optimizing dosage in biostimulant application strategies to achieve balanced and targeted enhancement of plant growth parameters (Trejo-Téllez et al., 2023).

Table 2. Morphological and Biomass Parameters of Round Pepper Seedlings Under Different Fertilization Treatments

Variant	Seedling Height (cm)	Root length (cm)	Collar Stem Diameter (mm)	Stem Weight (g)	Root Weight (g)	Number of Leaves per Seedling
V1	23.38±1.55bc	7.43±1.08a	4.32±0.38a	6.13±0.77ab	6.02±1.88b	11.00±1.66a
V2	24.51±0.86ab	7.27±0.54a	4.18±0.23a	6.36±0.87a	7.73±1.61a	10.78±0.83a
V3	22.85±0.43c	7.08±1.03a	4.15±0.45a	6.11±0.90ab	4.47±1.75c	10.61±1.19a
V4	23.71±0.85abc	7.09±0.60a	4.01±0.26a	5.61±0.71ab	3.94±1.08c	10.00±0.82a
V5	25.11±2.03a	7.39±0.67a	4.13±0.38a	6.32±1.26a	4.15±1.11c	10.67±0.50a
V6	25.11±1.13a	7.10±0.57a	4.00±0.25a	6.07±0.77ab	3.70±0.70c	10.56±1.24a
V7	19.51±1.61d	6.73±1.13a	4.30±0.39a	5.38±0.92b	5.70±1.69b	10.56±0.88a

Within each column and within each experimental factor, different letters mean fertilization treatments differ significantly according to Duncan's test at $p \leq 0.05$

Leaf Morphology and Foliar Surface Area

Leaf morphology, assessed through leaf length and width, showed that V1 produced the largest leaves, with a length of 92.14 mm and a width of 8.51 mm, leading to the highest foliar area of 2258.56 mm². This result implies that the V1 treatment, combining Albit and Turboroot at lower concentrations, effectively promotes leaf expansion, which can enhance the plant's photosynthetic potential (Table 3).

This pronounced leaf expansion suggests an enhanced physiological response likely driven by improved hormonal modulation and nutrient efficiency (Paponov et al., 2021; Zhang et al., 2023). The resulting increase in photosynthetic surface area underscores the critical role of balanced biostimulant application in optimizing leaf growth dynamics (Namratha et al., 2024). to maximize photosynthetic capacity (Vitale et al., 2021) and overall plant productivity (Li et al., 2022). Furthermore, the formation of more elongated leaves highlights a characteristic that enhances light capture and facilitates gas exchange (Zhang et al., 2022; Xie et al., 2020). Such morphological adaptations underline the potential of V1 fertilization scheme to improve resource acquisition efficiency in controlled greenhouse systems.

Ratio Analysis: Length-to-Width and Width-to-Length

The analysis of leaf length-to-width and width-to-length ratios reveals significant insights into the morphological responses of plants to different fertilization treatments. V1 and V5 exhibited the highest length-to-width ratios (2.24 and 2.20, respectively), indicating a shift towards more elongated leaves relative to the control, which maintained a more balanced ratio. This elongation likely confers an adaptive advantage in controlled environments (Steed et al., 2021), enhancing the plant's capacity for efficient light interception (Williams et al., 2021; Slattery & Ort, 2021). In contrast, width-to-length ratios remained relatively stable across all treatments, suggesting that while fertilization influences leaf size, it does not substantially alter the fundamental leaf shape.

Table 3. Leaf Morphological Parameters of Round Pepper Seedlings Under Different Fertilization Treatments

Variant	Leaf width (mm)	Leaf length (mm)	Length/Width Ratio	Width/Length Ratio	Leaf Area (mm^2)
V1	41.28±4.46a	92.14±8.51a	2.24±0.20a	0.45±0.04a	2258.56±316.86a
V2	41.19±4.00a	81.78±6.62a	1.99±0.14a	0.50±0.03a	2132.89±395.51a
V3	40.43±4.66a	83.15±4.83a	2.08±0.23a	0.49±0.05a	2094.28±307.83a
V4	40.44±2.50a	87.62±5.45a	2.17±0.12a	0.46±0.03a	2103.78±246.41a
V5	40.30±5.99a	86.89±8.90a	2.20±0.39a	0.47±0.06a	2156.89±344.39a
V6	42.89±3.60a	91.76±10.01a	2.14±0.23a	0.47±0.05a	2383.00±431.34a
V7	39.21±2.50a	81.57±6.34a	2.08±0.11a	0.48±0.03a	1987.67±196.21a

Within each column and within each experimental factor, different letters mean fertilization treatments differ significantly according to Duncan's test at $p \leq 0.05$.

Anthocyanin Content Index (ACI) and Chlorophyll Content Index (CCI)

The ACI values, reflecting pigment concentration associated with stress response and antioxidant activity, were highest in the untreated control (5.08) and lowest in V4 (4.12), suggesting that fertilization treatments might reduce the anthocyanin production by minimizing stress-related responses in the plants (Figure 7). Treatments V1 and V2 also exhibited elevated ACI values at 4.58 and 4.34, respectively, possibly due to mild stress induced by Albit and Turboroot at these concentrations, which may stimulate pigment synthesis without compromising growth.

CCI values, indicating chlorophyll concentration, were most pronounced in V1 (16.14) and V7 (15.31), suggesting that Albit and Turboroot can marginally improve chlorophyll synthesis, thereby enhancing photosynthetic capacity. CCI was lowest in V4 (12.86), possibly due to lower nutrient availability, which could impair chlorophyll synthesis pathways.

The observed variations in the Anthocyanin Content Index (ACI) and Chlorophyll Content Index (CCI) reflect complex physiological responses to different fertilization treatments. The high ACI in the untreated control suggests that plants may produce more anthocyanins in response to stress, likely as part of an antioxidant defense mechanism (Li & Ahammed, 2023; Kaur et al., 2023). In contrast, the lower ACI in fertilized treatments, particularly V4, indicates that nutrient supplementation may reduce stress-induced pigment production by optimizing plant health and minimizing stress responses (Kumari et al., 2022). This suggests that fertilization may help mitigate stress-related pathways that typically stimulate anthocyanin synthesis (Mannino et al., 2021).

Regarding chlorophyll content, the highest CCI values in V1 and V7 highlight the potential of Albit and Turboroot, particularly at lower concentrations, to enhance chlorophyll synthesis. This enhancement in chlorophyll content may improve the plants' photosynthetic capacity and overall productivity (Simkin et al., 2022). The lowest CCI in V4 suggests that insufficient nutrient availability could impair chlorophyll biosynthesis, thereby limiting photosynthetic efficiency (Morales et al., 2020; Muhammad et al., 2021). These findings underline the importance of optimizing fertilization strategies to balance nutrient availability and metabolic responses (Barłóg et al., 2022), ultimately improving plant growth and stress resilience.

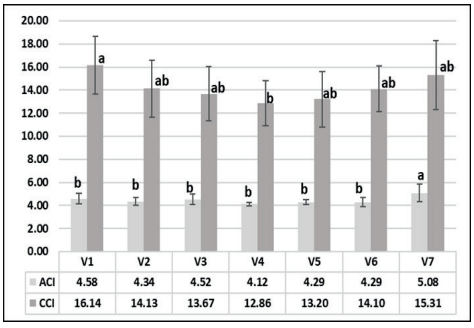

Figure 7. Chlorophyll Concentration Index (CCI) and Anthocyanin Content Index (ACI) in Round Pepper Seedlings Across Different Fertilization Treatments

Note: Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; p≤ 0.05)

Water Content and Dry Matter Analysis

The analysis of water content and dry matter in seedlings provides valuable insights into their physiological responses to fertilization treatments. Higher dry matter percentages in V4 and V3 (10.12% and 9.77%, respectively) suggest enhanced seedling vigor, indicative of more efficient nutrient and biomass accumulation (Cui et al., 2024), which typically correlates with better plant quality and improved resilience (Penella & Calatayud, 2018). In contrast, V5 and V6 exhibited lower dry matter content (7.8% and 6.6%) and higher water retention, suggesting that these treatments promote greater hydration in seedling tissues (Figure 8). While this may support early growth, excessive water retention could compromise long-term stability and drought resistance (Shoaib et al., 2022). The inverse relationship between water content and dry matter, with V7 showing the highest water content (94.4%), emphasizes the role of bio-stimulants in regulating osmotic balance within seedlings. These findings highlight the potential of specific treatments to optimize water use efficiency and stress tolerance (Anli et al., 2020), ultimately enhancing seedling establishment and survival in controlled environments (He et al., 2020).

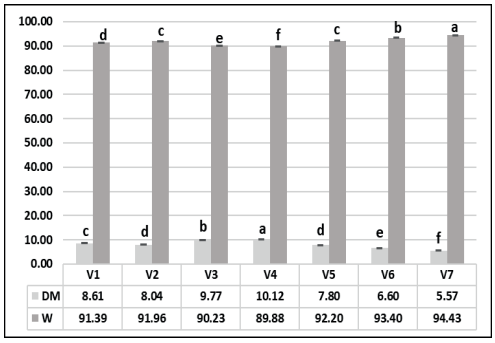

Figure 8. Dry Matter (DM) and Water Content (W) in Round Pepper Seedlings Across Different Fertilization Treatments

(Note: Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$)

CONCLUSIONS

The results demonstrate the pivotal influence of biostimulant treatments on the morphological and physiological performance of *Capsicum annuum* L. seedlings cultivated under controlled conditions. Intermediate dosages - particularly V5 (Turboroot 3.75 ml) - significantly improved key growth parameters, including stem height, root development and biomass accumulation, confirming their effectiveness in promoting vigorous vegetative growth. Notable synergistic effects between Albit and Turboroot in V1 further enhanced leaf morphology, foliar area and chlorophyll content, thereby increasing photosynthetic capacity and overall seedling quality.

The observed variation in dry matter and water content reflects treatment-dependent adjustments in osmotic balance, while differences in chlorophyll and anthocyanin concentrations indicate a dynamic interplay between growth promotion and stress-related metabolic responses. Collectively, these findings emphasize the necessity of precise dosage calibration in biostimulant application to optimize seedling performance, providing essential guidance for optimizing resource-efficient and sustainable horticultural practices.

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