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Biostimulants Alleviate Heat Stress in Organic Hydroponic Tomato Cultivation: A Sustainable Approach

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Abstract. **Heat stresses significantly reduce crop yield by 50% to 70%, irrespective of stress exposure time, plant species, or cultivars, posing substantial challenges for organically growing hydroponic tomatoes in controlled environment conditions. Low productivity in tomatoes is attributed to the inadequate stress tolerance of existing cultivars, which hinders their ability to optimize fruit set and yield. In this study, three biostimulants—Liquid Seaweeds, MycoApply®, and MycoLife—were applied to the leaf surface of tomato plants at 7-day intervals starting 4 weeks after planting. Their effects on plant growth, physiology, phenology, fruit yield, and quality were assessed. Each biostimulant was applied at intervals of 4, 8, 12, and 16 weeks to optimize application frequency. The experimental design employed a strip plot, with the biostimulants (Liquid Seaweeds, MycoApply®, MycoLife) as the main plot treatments, and application frequency (4, 8, 12, 16 weeks) randomly assigned to the subplots. We observed that the foliar application of biostimulants promoted plant vigor to varying degrees under heat stress conditions compared with the control. Specifically, MycoApply® applied for 12 weeks enhanced chlorophyll synthesis and photosynthesis rates, thereby boosting plant productivity. Tomato ('Valdeon RZ') plants treated with MycoApply® demonstrated an increased net assimilation rate (20%), and stomatal conductance (40%), along with reduced transpiration loss (28%) and electrolyte leakage (31%), while maintaining intercellular CO_2 concentrations. Flowering occurred 5 days earlier in tomato plants treated with MycoApply® compared with untreated plants. MycoApply®-treated tomato plants also exhibited superior fruit set (19%), pollen viability (37%), and fewer incidences of flower drop (10%) compared with the control. Among the application frequencies, MycoApply® applied for 12 weeks exhibited superior plant growth and tomato productivity compared with the control. MycoApply® treated for 12 weeks outperformed the control in terms of marketable fruit yields, with a significantly higher yield (30%). In addition, 'Valdeon RZ' tomato plants treated with MycoApply® demonstrated superior postharvest quality, including firmness, soluble solids, acidity, and color dynamics. Correlogram, heat map, and cluster analysis further confirmed that under heat stress, biostimulants had various promotional effects on tomato growth and productivity. Therefore, MycoApply® emerged as a promising biostimulant with strong heat stress tolerance capacity in organic hydroponic systems.**

Climate change exacerbates abiotic and biotic stresses that harm crop growth and development, putting additional pressure on resources needed to feed people. Abiotic stresses such as heat, drought, salinity, hypoxia, and nutrient deficiency limit plant growth and often degrade product quality (Mariani and Ferrante 2017). Heat stress is a major issue affecting crop productivity, plant growth, physiology, phenology, fruit quality, and tomato yield (Dash et al. 2023). These stresses vary in intensity and nature based on the growing season and environmental conditions (Ro et al. 2021). In Qatar, high temperatures are prevalent, leading to significant heat stress that limits crop productivity and necessitates substantial produce imports. The region's drive for food security research is fueled by increasing demand due to population growth (van Dijk et al. 2021) and geopolitical factors [Qatar National Food Security Strategy 2018–2023 (QNFSS) 2020]. Vegetable supply is crucial for food security, with tomatoes being among the most valuable fresh market commodities (QNFSS 2020). Qatar's agricultural sector has grown considerably due to population growth, increased food demand, and rapid economic expansion. Therefore, effective management strategies are essential for achieving food security in Qatar.

Qatar's average maximum outdoor temperature often exceeds 38 °C, particularly during the tomato growing season in October (Weather Atlas 2024). The Intergovernmental Panel on Climate Change (IPCC) has warned that global warming will raise temperatures in Asia by 1.5 °C by 2050 (IPCC 2022), posing a significant threat to crop production. Research shows high temperatures severely impact plant physiological traits (Rajametov et al. 2021). Combined heat and drought stress reduce tomato CO_2 assimilation rates, disrupt the leaf photosynthetic apparatus, and induce photo-oxidative stress through excessive reactive oxygen species (ROS) production (Li et al. 2015). This process damages proteins, chlorophylls, membrane lipids, and nucleic acids, lowering photosystem II efficiency and affecting water status. Elevated air temperatures also increase leaf temperature and transpiration, causing fluctuations in stomatal conductance, which disrupts the electron transport chain and photosynthesis (Moore et al. 2021). Furthermore, heat stress can reduce tomato pollen viability and cause anther deformities (Muller and Rieu 2016), ultimately decreasing fruit production and yields. Under greenhouse conditions, extreme heat stress (24 to 43 °C) results in significant tomato yield losses, ranging from 37% to 98% (Ro et al. 2021). These results indicate that the intricate interaction between environmental conditions and management strategies can significantly affect crop physiology, leading to substantial yield variations (Potgieter et al. 2021). Thus, choosing the best management practices is essential for attaining high yields and addressing the increasing food demand (Aldubai et al. 2022).

Applying biostimulants to plant foliage could effectively promote robust growth, ensure plants adhere to their optimal growing schedule, and mitigate adverse environmental conditions without compromising yield potential. Plant growth modulation using biostimulants has gained popularity due to its positive effects on the growth and development of diverse crops (Garza-Alonso et al. 2022). Plant biostimulants can enhance a plant's nutritional efficiency and tolerance to abiotic stress or improve crop quality (du Jardin 2015). They include humic substances, protein hydrolysates, seaweed and botanical extracts, chitosan, other biopolymers, beneficial elements (such as Si, Se, and I), beneficial fungi (*Trichoderma* spp.), and beneficial bacteria (Rhizobacteria). One of the primary mechanisms of biostimulation

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involves changes in the redox balance of cells, where the ratio of reactive oxygen, hydrogen, and sulfur species to antioxidants is increased (Kapoor et al. 2015). Plants respond to environmental stimuli through membrane fluidity and structure changes, similar to their response to stress conditions like salinity or drought (Rawat et al. 2021; Rouphael et al. 2022). Some biostimulants, such as seaweed extract, have been used to enhance plant vigor and productivity in strawberries (Righini et al. 2018) and mitigate the adverse effects of salinity stresses in tomatoes (Gedeon et al. 2022). Different biostimulants act through various mechanisms. For example, *Ascophyllum nodosum* seaweed extract, a highly bioactive liquid, acts as a plant stimulant and anti-stress agent, promoting root growth and increasing nutrient uptake efficiency from the soil (Ali et al. 2021). In addition, MycoApply® contains *arbuscular mycorrhizal* fungi and is designed for organic greenhouse production. *Arbuscular mycorrhizal* fungi have been linked to several benefits, including enhanced macro- and micronutrient uptake, improved water absorption, salinity and drought stress suppression, trace metal detoxification, and protection against pathogens and pests (Makarov 2019). The advantages of various biostimulants have been attributed to vigorous root architecture, improved water and nutrient uptake efficiency, a stronger antioxidant defense system, and increased synthesis of endogenous hormones, alleviating the negative effects of abiotic stresses (Hasanuzzaman et al. 2021).

In Qatar, tomatoes are cultivated in both open fields and greenhouses. Greenhouse cultivation is especially beneficial in harsh climatic conditions, frequently leading to higher yields than open-field farming (Shamshiri et al. 2018). The popularity of controlled environment vegetable production is on the rise due to its numerous benefits, such as decreased risk of pests and diseases and enhanced management of abiotic stresses. Research has demonstrated that biostimulants enhance tolerance to abiotic stresses like extreme temperatures, drought, and salinity, aiding horticultural crops in recovering from stress damage (Bulgari et al. 2019). The synergy of improved nutrient absorption and increased resistance to stress factors, achieved through biostimulant application, can boost both the yield and quality of the produce (Kocira et al. 2020). However, no research has yet demonstrated the effects of different types of biostimulants (Liquid Seaweeds, MycoApply®, and MycoLife) on improving tomato plant growth and development in an organic hydroponic system. This study aims to assess the heat stress tolerance and growth-enhancing potential of tomatoes using various biostimulants (Liquid Seaweeds, MycoApply®, and MycoLife) and optimize application frequency within an organic hydroponic system under controlled environmental conditions.

Materials and Methods

Plant material and growth conditions. The organic hybrid 'Valdeon RZ' tomato seeds (Fitoagricola, Avenida Benicasim, Castellon, Spain) were sown in polystyrene trays containing 50 cells, each with dimensions of 4.8 × 3.8 × 5.8 cm and a cell volume of 80 cm^3 (XQ50; Wilson Garden Co. Ltd., Zhengzhou, China). These trays were filled with a growing medium made up of 90% cocopeat and 10% compost (LivePlant Biotec; Hortalan Group, LivePlant Biotec, Almeria, Spain). Trays were irrigated and incubated at 24 °C and 80% relative humidity (RH) for 72 h in an insulated cold room. After this period, the trays were transferred to a propagation unit, where the seedlings were nurtured for 33 d before being transplanted into the greenhouse. During this time, the seedlings were fertilized every 3 d with organic nitrogen (N), phosphorus (P), and potassium (K) fertilizers (N20–P10–K30) at a concentration of 200 $mg \cdot L^{-1}$ of N according to Lee et al. (2023). In addition, they received trace elements (Fe, Zn, Br, Mb, Cu, Mn) at a concentration of 10 $mg \cdot L^{-1}$ (Yara; Hortalan Group, Madrid, Spain), starting 24 d after seedling emergence.

The experimental design used a strip plot arrangement, with the biostimulants (Liquid Seaweeds, MycoApply®, MycoLife) designated as the main plot treatments, and the application frequencies (4, 8, 12, and 16 weeks) were randomly assigned to the subplots. Beginning 21 d after planting, foliar treatments were applied at 7-d intervals. Different groups received treatments for varying durations: one group for 4 weeks, another for 8 weeks, another for 12 weeks, and the final group for a maximum of 16 weeks. Untreated plants served as controls. Each treatment combination was replicated four times, with each replication comprising 12 plants. Liquid Seaweeds contain 0.3% total nitrogen (N), 0.2% available phosphorus (P_2O_5), and 1.0% soluble potash (K_2O), all derived from *Ascophyllum nodosum* seaweed (Blue Planet Nutrients, Katy, TX, USA). In a similar vein, MycoApply® offers 6.0% soluble potash (K_2O) sourced from kelp extract (*Ascophyllum nodosum* and potassium hydroxide) and potassium humates (Mycorrhizal Applications LLC., Grants Pass, OR, USA). Likewise, MycoLife includes 0.03% soluble potash (K_2O), 0.004% calcium (Ca), 0.004% magnesium (Mg), and 2.0% humic acid (San Jacinto Environmental Supplies, Houston, TX, USA). Liquid Seaweeds was applied at a concentration of 3 $mL \cdot L^{-1}$ using a knapsack sprayer at 7-d intervals, starting 21 d after planting. Likewise, MycoApply® was administered at 0.6 $g \cdot L^{-1}$ water, and MycoLife at 0.4 $mL \cdot L^{-1}$ water, both at 7-d intervals. Biostimulants were selected based on a comprehensive literature review of previous studies. For instance, seaweed extracts have been shown to positively impact growth, yield, and the chemical composition of cucumbers (Hassan et al. 2021). In addition, a study on grapevines revealed that arbuscular mycorrhizal fungi (AMF)-based biostimulants, such as 'MycoApply DR' sustain photosynthetic and physiological activities while modulating fruit quality under unfavorable conditions (Ganugi et al. 2023). The plants were cultivated at a density of 3.5 plants/m^2 within a large commercial hydroponic substrate growing system employing grow bags (1.0 × 0.2 × 0.1 m) where solid media filled with a cocopeat (90%) and compost (10%) mix (Polydime; Kirulapone, Colombo, Sri Lanka) were used to support the plants and facilitate efficient nutrient distribution at AGRICO Organic Farm in Al-Khore, Qatar (lat. 25°41′N, long. 51°30′E). The grow bags were positioned on metal benches with a center-to-center spacing of 1.2 m and an interbag spacing of 0.2 m, each equipped with a drip tube hose to supply irrigation and nutrients. Greenhouse air temperature (°C) and RH (%) were continuously monitored using an Ambient weather monitoring system (WS80BN; Chandler, AZ, USA) (Fig. 1). In addition, data loggers recorded growing media temperatures and water content ($m^3 \cdot m^{-3}$) at a depth of 2 cm (HOBO® MX2307; ONSET®, Bourne, MA, USA) (Fig. 2). The electrical conductivity of the growing media was tracked at a depth of 2 cm using a digital EC meter (EC-1385; ServoVendi Sl., Malaga, Spain) (Fig. 3). Irrigation was provided daily from 0800 to 1600 HR via a drip irrigation system with emitters delivering a flow rate of 0.3 L per hour. Weekly fertilization with N20–P10–K30 fertilizer (200 $mg \cdot L^{-1}$ of N) was continued until 22 May 2024.

Growth and physiological measurements. Beginning 45 d after planting, the canopy area (cm^2) was measured weekly by capturing images from the top of the plants using a Nikon AF-S DX Nikkor camera (D5500 DSLR; Bangkok, Thailand). Image analysis was conducted with ImageJ software (Version 1.53e), according to the methodology outlined by Martin et al. (2020). Chlorophyll levels (SPAD values) were recorded using a portable chlorophyll meter (SPAD-502 Plus; Konica Minolta, Tokyo, Japan) on the third fully expanded leaf from the top. Gas exchange variables, including leaf transpiration rate (E), leaf assimilation rate (A), intercellular CO_2 concentration (Ci), and stomatal conductance to water vapor (gs), were measured on the same leaf using a portable photosynthesis system (LI-6800; LI-COR Inc., Lincoln, NE, USA) between 1000 and 1300 HR. The system was set with a flow rate of 500 $\mu mol \cdot s^{-1}$, reference CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$, fan speed at 10,000 rpm, fluorometer set point at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and an aperture size of 6 cm^2. These gas exchange measurements began 45 d after planting and were conducted at 21-d intervals for a total duration of 129 d. Electrolyte leakage (EL) was calculated following the method described by Dash et al. (2023) using the following formula:

$$\mathrm{EL}\,(\%) = \frac{E1}{E2} \times 100$$

EC1 was recorded using a conductivity meter (Cond 6+ Conductivity meter; Oakton Instruments, Bunker Court, Vernon Hills, IL, USA) after six leaf discs were placed in deionized water for 20 h. Subsequently, the leaf samples were boiled for 15 min, cooled, and EC2 readings were recorded.

Phenological measurements. The flowering characteristics, such as days to first

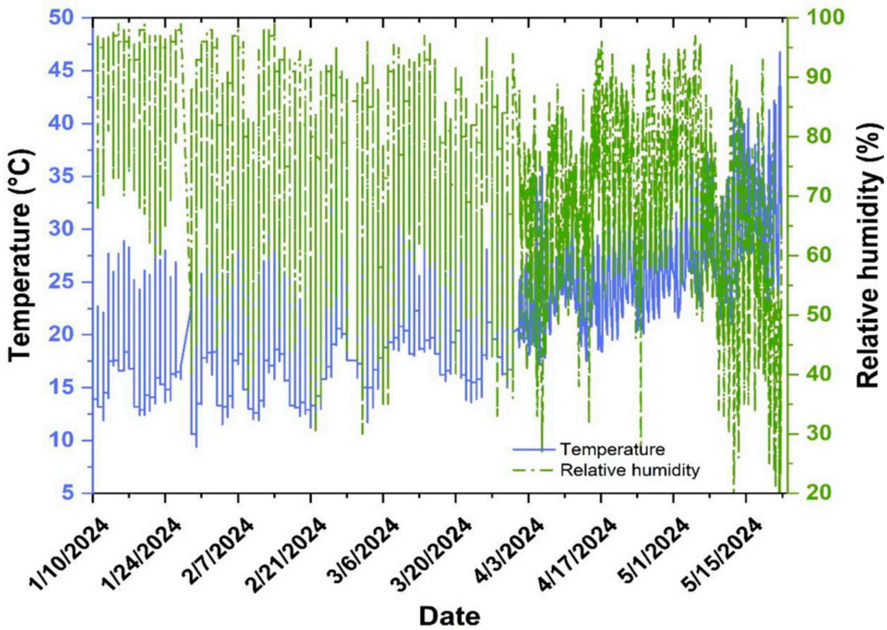

Fig. 1. Real-time air temperature and relative humidity inside the greenhouse at AGRIOCO organic farm, Al-Khore, Qatar.

flowering (D1F) and days to 50% flowering (D50F), were tracked by recording the number of days from planting to the appearance of the first flowers and the point at which 50% of the flowers had bloomed in each experimental unit. To evaluate flower drop (FD) and fruit set (FS) performance under abiotic stress conditions, five mature flower clusters (each containing 5 to 12 flowers) were tagged in each plot, and data on flower drop and fruit set were collected at regular intervals. Pollen viability (PV) was determined using the method described by Dash et al. (2023) involving an IKI (iodine potassium iodide) staining test. This procedure prepared an IKI solution by dissolving 1 g of potassium iodide and 0.5 g of iodine in 100 mL of distilled water. PV was assessed by observing pollen grains 5 min after placing them in the IKI solution. Pollen grains stained dark red, or brown were considered viable, and further examination was conducted using a microscope (DM 2700M; Leica Microsystems Inc., Deerfield, IL, USA).

Yield and postharvest quality evaluation. Fully ripe marketable fruits were harvested every other day throughout 54 harvests, starting on 9 Jan 2024, and ending on 30 May 2024. The total yield was calculated from these harvests. Various postharvest quality attributes, including fruit weight, firmness, color dynamics, acidity, and soluble solids, were evaluated for the stored fruits. The harvested tomatoes were promptly stored in the laboratory of the Mechanical Engineering Program at Texas A&M University in Qatar. These fruits were kept under ambient conditions at 23 °C and 75% RH to assess their postharvest quality. Fruit firmness was measured using a digital force gauge (Chatillon force measurement; Ametek®; DFS3, Largo, FL, USA) equipped with a 2-mm probe, with the force applied calculated in N/cm^2. The color traits of the stored fruits, including L* (lightness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma), and °h (hue angle), were recorded using a portable Chromo Meter (CR 410; Konica Minolta, Inc., Chiyoda City, Tokyo, Japan). The soluble solids and acidity percentage in tomato juice, following a dilution ratio of 1:50, were determined using a pocket Brix-Acidity meter (PAL-BXIACID3; Atago Co. Ltd., Shiba-Koen, Minato-ku, Tokyo, Japan).

Statistical analysis. A two-factor strip plot design was used for this experiment. The collected data were analyzed using Origin 2023 (Version 9.6.5; OriginLab Corporation, Northampton, MA, USA). A normality test was conducted to evaluate the standard normal distribution of the data using the Shapiro-Wilk test at a significance level of $P < 0.05$. A two-way analysis of variance (ANOVA) was performed to identify statistical differences among the treatments, and pairwise mean comparisons were conducted using Tukey's honestly significant difference test at $P < 0.05$. In addition, correlation analysis among variables was carried out to explore the relationships between variables and treatments and to visually represent trends and patterns in the data. A heatmap was generated using the scaled values of each parameter, and the group average method of hierarchical clustering algorithm analysis was performed using the Pearson correlation distance.

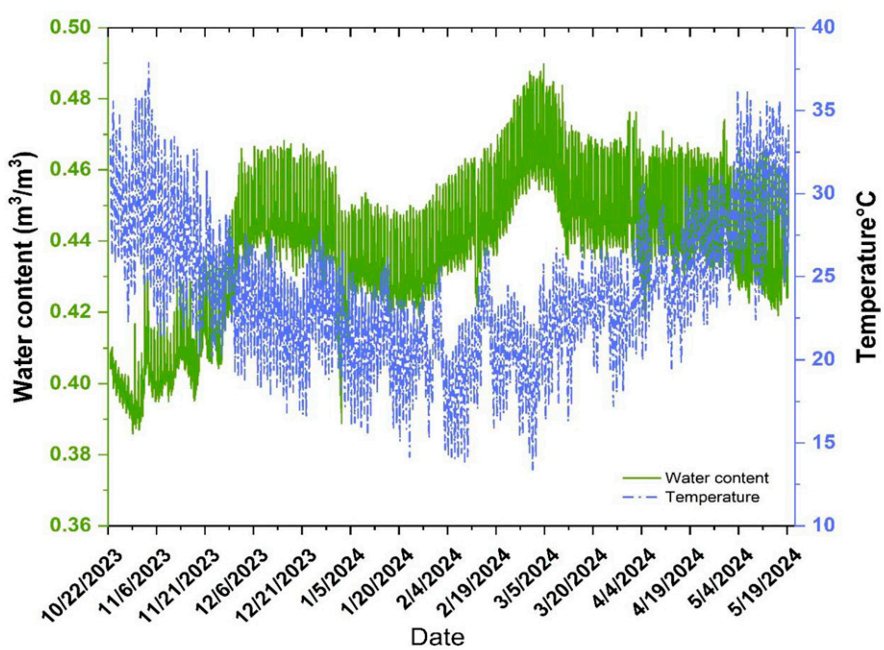

Fig. 2. Real-time growing media temperature and water content at 2 cm depth at AGRIOCO organic farm, Al-Khore, Qatar.

Results

Growth and physiological assessment. The hybrid 'Valdeon RZ' tomato plants exhibited superior vegetative growth. The ANOVA revealed significant interactions between biostimulant types and application frequency in the canopy area. Notably, significant differences ($P < 0.05$) in canopy growth were observed among the treatments (data not shown). Across all measured treatments, the MycoApply® treatment for 12 consecutive weeks consistently outperformed the nontreated control plants in maintaining canopy growth. For example, at 15 weeks after transplanting (WAT), the MycoApply®-treated plants exhibited 18% higher canopy growth than the control plants. In addition, tomato plants treated with MycoApply® for 12 consecutive weeks had significantly higher SPAD (chlorophyll index) values over time compared with the nontreated control plants (Fig. 4). There were significant interactions between biostimulant

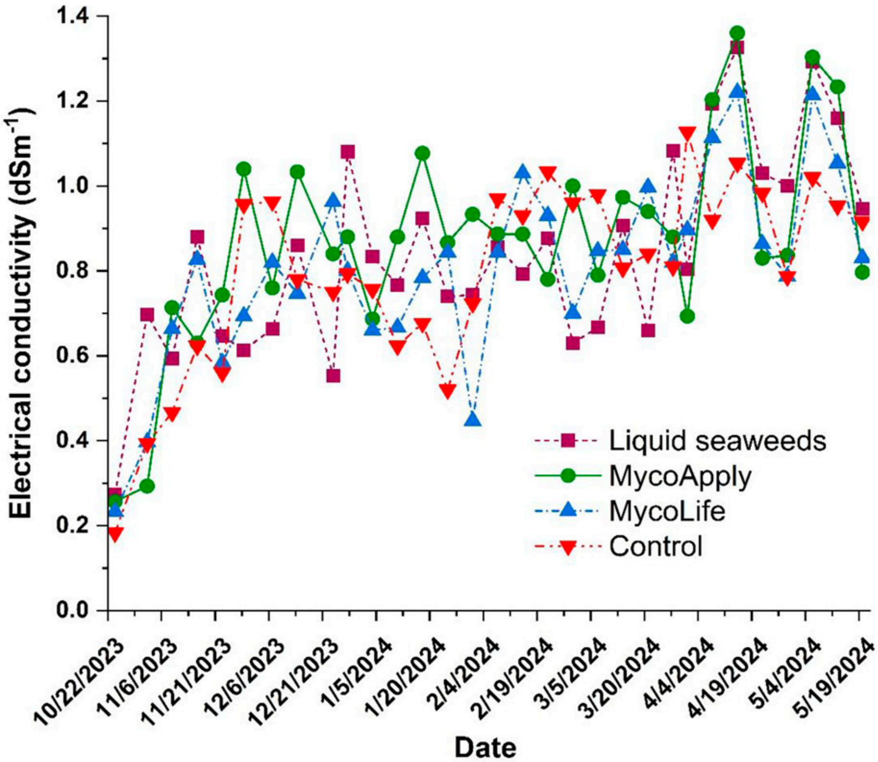

Fig. 3. Electrical conductivity of growing media over time at AGRIOCO organic farm, Al-Khore, Qatar.

types and application frequency, with MycoApply®-treated plants showing a 16% greater increase in SPAD value compared with the control plants.

The leaf assimilation rate was significantly higher in tomato plants treated with MycoApply® for 12 consecutive weeks compared with the nontreated control plants, as depicted in Fig. 5A. For instance, at 15 WAT, the MycoApply®-treated plants showed a 20% increase in leaf assimilation rates compared with the control plants. In addition, the leaf transpiration rate was significantly reduced in tomato plants treated with MycoApply® for 12 consecutive weeks compared with the nontreated control, as shown in Fig. 5B. Transpiration rates decreased by 28% in plants treated with MycoApply® for 12 consecutive weeks compared with the control plants at 15 WAT. Likewise, stomatal conductance was significantly higher in tomato plants treated with MycoApply® for 12 consecutive weeks than in the control plants (Fig. 5C). This treatment combination resulted in a 40% higher stomatal conductance compared with the nontreated control plants at 15 WAT. Furthermore, tomato plants treated with MycoApply® for 12 consecutive weeks exhibited a 31% reduction in electrolyte leakage compared with the control plants at 15 WAT, as shown in Fig. 5D. Similarly, tomato plants treated with MycoApply® for 12 and 16 consecutive weeks maintained higher intercellular CO_2 concentration (Ci) than other genotypes (data not shown).

Phenological assessments. There were significant interactions between biostimulant types and application frequency on phenological attributes, such as the days required for the first flower, which varied among the treatment combinations. Flowering occurred 3 d earlier in tomato plants treated with MycoApply® for 12 consecutive weeks compared with the control plants (data not shown). A similar trend was observed for 50% flowering, with the nontreated control plants flowering 5 d later than the plants treated with MycoApply® for 12 consecutive weeks, as shown in Fig. 6A. In addition, there was a 10% reduction in flower drops in the tomato plants treated with MycoApply® for 12 consecutive weeks compared with the control plants. Furthermore, tomato plants treated with MycoApply® for 12 consecutive weeks exhibited a 19% higher fruit set compared with the control plants, as shown in Fig. 6B. The same treatment also resulted in a 37% increase in PV compared with the control plants.

Yield and postharvest quality measurements. Marketable fruit yield was significantly influenced by the interaction between biostimulant types and application frequency ($P < 0.05$). Tomato plants treated with MycoApply® for 12 consecutive weeks produced a 30% higher marketable fruit yield compared with the control plants, as shown in Fig. 7. Similarly, plants treated with MycoApply® for 16 consecutive weeks exhibited a 28% higher fruit yield compared with the control plants. Generally, fruits collected from various treatment combinations maintained postharvest quality over a 12-d storage period, as shown in Table 1. However, fruit weight decreased significantly across all treatment combinations during storage. By the end of the storage period, fruits from biostimulant-treated plants experienced an average weight loss of 11%, whereas fruits from control plants had a 16% weight loss. Fruit firmness, a crucial indicator of tomato quality during storage, deteriorated more rapidly in control plants, with a 26% loss in firmness, while still retaining sweetness, as indicated by soluble solids values and acidity, compared with other treatment combinations. At the end of the storage period, fruits from biostimulant-treated plants exhibited higher surface color, with an 11% increase in L* compared with the control. However, the results indicated that biostimulants and application frequency did not significantly impact the postharvest quality attributes of stored tomatoes. The average values recorded for the stored tomato fruits were as follows: soluble solids content (3.5%), acidity (1.0%), and color characteristics including a* (21.9), b* (23.7), C* (32.8), and °h (47.5).

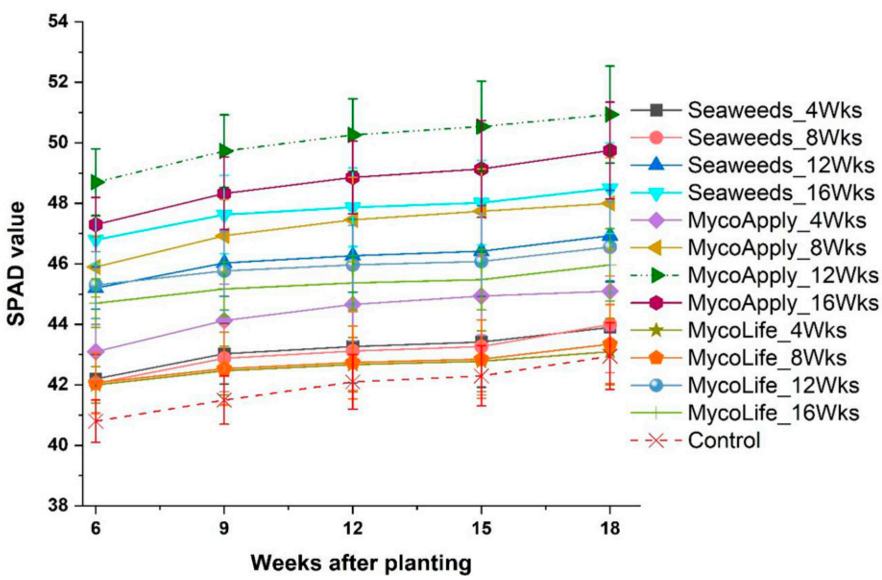

Fig. 4. Effect of biostimulant types and application frequency on SPAD value of tomato. Vertical bars represent standard error, n = 12 plants.

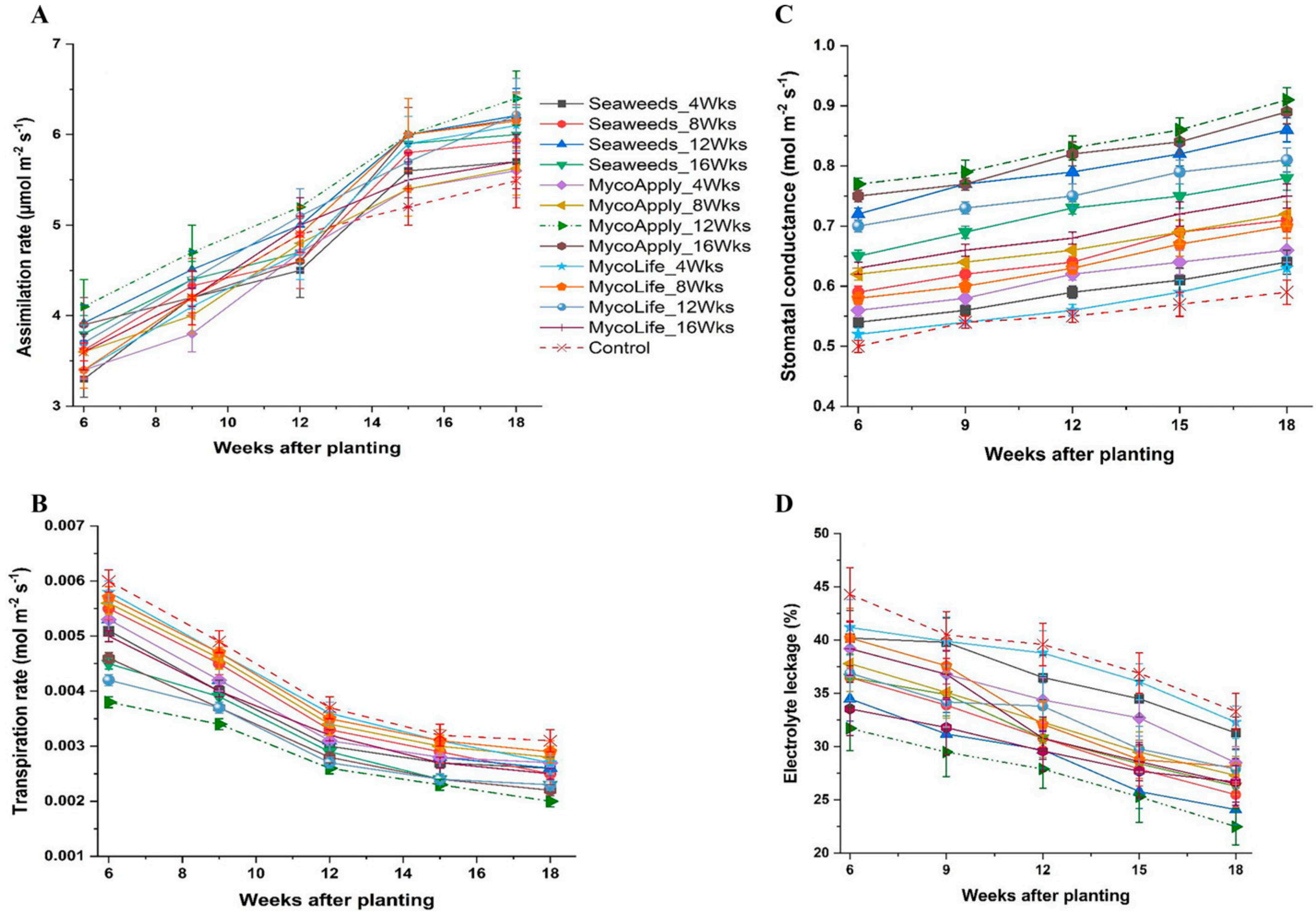

Fig. 5. Effect of biostimulant types and application frequency on leaf assimilation rate (**A**), transpiration rate (**B**), stomatal conductance (**C**), and electrolyte leakage (**D**) of tomato. The vertical bars represent standard error, n = 12 plants.

Correlogram, heat map, and clustering analysis evaluation. Figure 8 presents a correlogram illustrating the correlations among growth, physiological, phenological, yield, and postharvest quality variables in biostimulant-treated tomato plants under greenhouse conditions. The canopy area shows strong positive correlations with SPAD value (r = 0.81), leaf assimilation rate (r = 0.88), stomatal conductance (r = 0.98), FS (r = 0.93), PV (r = 0.96), fruit yield (r = 0.95), shelf life (r = 0.94), fruit weight (r = 0.92), firmness (r = 0.85), and lightness (r = 0.85). It has significant negative correlations with transpiration (r = −0.78), electrolyte leakage (r = −0.84), days to first flower (r = −0.84), days to 50% flower (r = −0.92), and FD (r = −0.92). SPAD value (Chlorophyll Index) positively correlates with assimilation rate (r = 0.65), stomatal conductance (r = 0.88), FS (r = 0.87), PV (r = 0.88), fruit yield (r = 0.91), shelf life (r = 0.78), fruit weight (r = 0.91), firmness (r = 0.85), and lightness (r = 0.82). Transpiration rate shows positive correlations with electrolyte leakage (r = 0.68), days to first flower (r = 0.68), days to 50% flower (r = 0.89), and FD (r = 0.86). Assimilation rate positively correlates with stomatal conductance (r = 0.82), FS (r = 0.76), PV (r = 0.84), fruit yield (r = 0.82), shelf life (r = 0.82), fruit weight (r = 0.78), firmness (r = 0.75), and lightness (r = 0.78). Stomatal conductance exhibits strong positive correlations with FS (r = 0.95), PV (r = 0.96), fruit yield (r = 0.98), shelf life (r = 0.94), fruit weight (r = 0.95), firmness (r = 0.90), and lightness (r = 0.90). Electrolyte leakage positively correlated with days to first flower (r = 0.91), days to 50% flower (r = 0.82), and FD (r = 0.88), and negatively correlated with FS (r = −0.88), PV (r = −0.87), fruit yield (r = −0.88), shelf life (r = −0.84), fruit weight (r = −0.90), firmness (r = −0.92), and lightness (r = −0.89). Fruit yield displays significant negative correlations with transpiration (r = −0.76), electrolyte leakage (r = −0.88), days to first flower (r = −0.82), days to 50% flower (r = −0.92), and FD (r = −0.97). Positive correlations are observed with canopy area (r = 0.95), SPAD value (r = 0.91), assimilation rate (r = 0.82), stomatal conductance (r = 0.98), FS (r = 0.95), PV (r = 0.97), shelf life (r = 0.92), fruit weight (r = 0.95), firmness (r = 0.91), and lightness (r = 0.90). In summary, canopy area, stomatal conductance, and fruit yield exhibit strong interrelations with several physiological and quality variables, highlighting their importance in the overall performance of biostimulant-treated tomato plants.

A heatmap, as shown in Fig. 9, was generated to illustrate the relationships between variables among different biostimulants and their application frequencies, and to cluster these variables based on their responses. The heatmap revealed distinct clusters that categorized the biostimulants and their application frequencies as highly tolerant, sensitive, or moderately tolerant to abiotic stresses. These clusters were clearly distinguished based on variables such as yield, canopy area, assimilation rate, PV, FS, FD, transpiration, electrolyte leakage, days to first flower, and days to 50% flowering. The highly abiotic stress-tolerant treatment group included tomato plants treated with MycoApply® for 12 consecutive weeks. This treatment combination exhibited resilience to abiotic stress conditions in organic hydroponic greenhouse systems, primarily characterized by their high fruit yield and canopy area. The highly stress-tolerant group displayed low transpiration, electrolyte leakage, and FD (indicated by blue color), and high yield and canopy area (indicated by brown color). Conversely, the sensitive group, consisting of the control, Liquid Seaweeds, and MycoLife treatments, exhibited high electrolyte leakage, increased FD, delayed flowering, and low fruit yield.

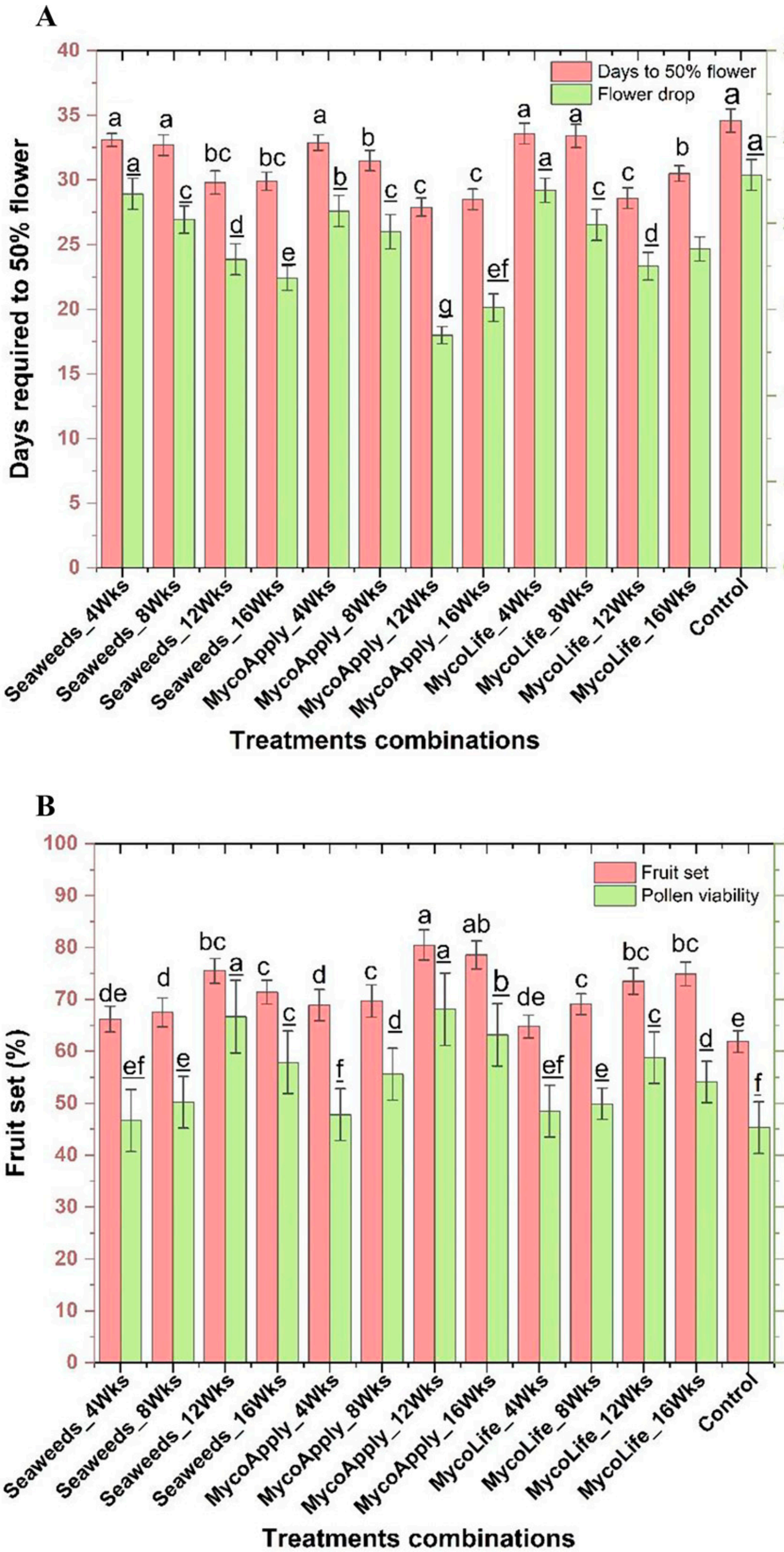

Fig. 6. Effect of biostimulant types and application frequency on days required to 50% flowering and flower drop (**A**), fruit set, and pollen viability (**B**) of tomato. The vertical bars represent standard error; n = 12 plants. Bar graphs having dissimilar letters are statistically different, whereas bars sharing the same letter are statistically similar as per Tukey's honestly significant difference test at $P < 0.05$.

Discussion

This research provides new insights into resilient biostimulants and their optimal application frequency for organic hydroponic systems under heat stress environments. Although previous studies have focused on biostimulants for conventionally grown tomatoes in greenhouses (Gedeon et al. 2022), few have explored their application in organic hydroponic systems under heat stress conditions. Our study found significant differences between biostimulant-treated and untreated plants in growth, physiological traits, phenological characteristics, yield, and postharvest quality. Notably, we observed substantial phenotypic variability in canopy areas, photosynthetic rates, and phenological attributes among treated plants, highlighting the potential for selecting suitable biostimulants and application frequencies for hydroponic substrate growing systems using grow bags. Hydroponics (soilless grow bag cultivation systems) allows precise nutrient and irrigation management, enhancing fruit yields (Urrestarazu 2013), and previous research confirms the consistency of tomato yields in protected environments (Nordey et al. 2017).

Biostimulant-treated tomato plants are hypothesized to resist heat stress by modulating microclimates, enabling them to thrive in challenging conditions. Under stress, plants experience reduced intercellular CO_2 concentration, stomatal conductance, and transpiration rate. Adverse conditions lead to increased production of ROS, causing severe cellular damage through lipid peroxides and disrupting physiological processes (Soares et al. 2019; Wakeel et al. 2020). Recent estimates suggest that heat stresses could lead to reductions of up to 50% or more in global agricultural productivity, varying by region (Kumar and Verma 2018). Ganugi et al. (2023) highlighted the crucial role of AMF in sustaining photosynthesis and physiological functions, as well as improving fruit quality in grapevines under abiotic stress. Extensive research over recent years has shown that AMF symbiosis reduces photoinhibition in plants under abiotic stress (He et al. 2017; Zai et al. 2021). A meta-analysis by Chandrasekaran et al. (2019) demonstrated that AMF positively affects photosynthetic rates and stomatal conductance in salt-stressed C_3 and C_4 plants. Specifically, Nicolás et al. (2015) reported higher photosynthetic rates in AMF-inoculated Crimson grapevines, with significant improvements in net carbon assimilation, water use efficiency, and stomatal conductance. Similarly, Torres et al. (2021) found that AMF enhanced the photosynthetic performance of young Merlot grapevines, leading to increased net carbon assimilation and water use efficiency. Our study shows that different biostimulants and their application frequencies result in varied physiological responses, such as changes in transpiration rate, assimilation rate, intercellular CO_2 concentration, and stomatal conductance, highlighting the role of biostimulants in mitigating heat stress in tomato plants.

In our study, tomato plants treated with MycoApply® for 12 weeks showed accelerated flowering, likely due to the biostimulants mitigating transplant shock and establishing strong root systems early, enhancing nutrient uptake compared with untreated control plants. Drought stresses are known to reduce PV, impact pollination, fertilization, and fruit development

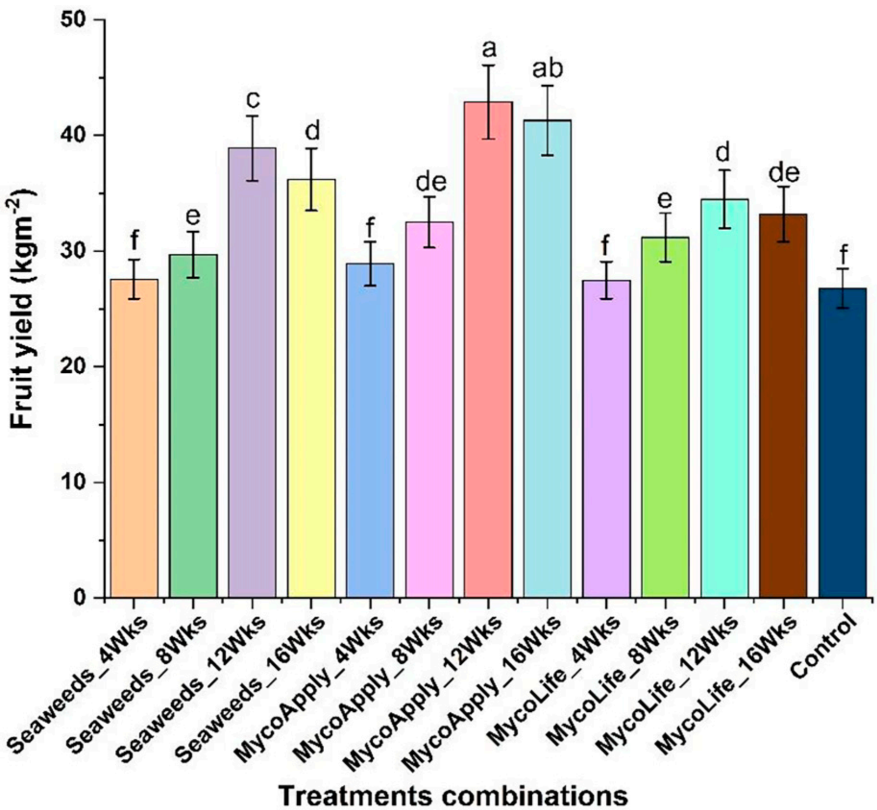

Fig. 7. Effect of biostimulant types and application frequency on marketable fruit yield of tomato. The vertical bar represents standard error; bar graphs having dissimilar letters are statistically different, whereas bars sharing the same letter are statistically similar as per Tukey's honestly significant difference test at $P < 0.05$.

(Halo et al. 2023). Heat stress negatively affects anthers and pollen, significantly reducing FS (Borghi and Fernie 2020). High temperatures also decrease PV, disrupt physiological processes, and lead to FD and reduced yield (Alsamir et al. 2021; Muller and Rieu 2016; Osei-Bonsu et al. 2022). The improved flower and FS, lower FD, and higher PV in plants treated with MycoApply® for 12 weeks indicate that this biostimulant effectively alleviates heat stresses, promoting proper growth and development in hydroponic systems.

Our study found that tomato plants treated with MycoApply® for 12 weeks consistently yielded significantly more than those treated for 16 weeks, outperforming all other treatment combinations. While we assessed various aspects of tomato growth, yield, and postharvest performance, further evaluation of the 'Valdeon RZ' genotype with a 12-week MycoApply® treatment is needed, including traits like cell membrane stability. Investigating molecular traits related to mutations (Ayenan et al. 2019) could identify valuable genes for breeding programs (Shaheen et al. 2016). Effective management techniques, such as using biostimulants, are crucial for enhancing tomato yield in organic hydroponic systems under heat stress. In addition, 'Valdeon RZ' tomatoes treated with MycoApply® showed superior postharvest quality, including firmness, soluble solids, acidity, and color dynamics.

The correlogram, heatmap, and cluster analysis have effectively highlighted the key variables influencing tomato growth and yield traits. The heatmap and dendrogram complement by grouping variables into distinct clusters based on similarity. Variables such as FD, transpiration rate, electrolyte leakage, and flowering times contrast with those related to FS, PV, stomatal conductance, leaf assimilation rate, SPAD value, canopy area, and fruit yield. This study on the optimization of biostimulant types and application frequency for the 'Valdeon RZ' tomato has revealed valuable insights into the complex relationships among these variables, demonstrating their impact on yield and postharvest quality. These findings are clearly illustrated in the heatmap clustering analyses.

Biostimulants have become a revolutionary tool, embraced by farmers, researchers, and policymakers alike. When properly formulated and applied, they offer a sustainable approach to agricultural practices, reducing the reliance on chemical fertilizers and enhancing crop resilience (Drobek et al. 2019). Their ability to improve nutrient efficiency and reduce the need for synthetic pesticides aligns perfectly with the principles of sustainable agriculture, contributing to a more food-secure and ecologically balanced world. These products are in harmony with the United Nations Sustainable Development Goals, particularly Goals 2 (Zero Hunger) and 15 (Life on Land) (Cowell et al. 2022). In addition, they align with the European Union's Green Deal and Farm to Fork strategy, both of which emphasize the importance of sustainable agricultural practices (Bazzan et al. 2023). We are on the brink of a new era in which innovative biostimulants (Seaweeds, AMF) promise to transform how we cultivate crops and sustain our planet.

Organic hydroponic tomato production, particularly using the 'Valdeon RZ' genotype and the MycoApply® biostimulant, offers a promising solution to enhance food security and safety in arid climates. With careful management, these systems can significantly boost agricultural productivity, addressing the rising global food demand and

Table 1. Effect of biostimulant types and application frequency on postharvest quality of tomato (12-d storage).

Treatments	Shelf life (day)	Fruit wt (g)	Firmness (N)	L*
Seaweeds_4Wks	9.0 b	152.2 bc	7.5 c	36.2 b
Seaweeds_8Wks	10.0 ab	155.3 b	8.6 b	38.7 ab
Seaweeds_12Wks	12.0 a	163.4 ab	9.3 a	39.2 a
Seaweeds_16Wks	11.0 a	162.5 ab	9.4 a	39.2 a
MycoApply_4Wks	9.0 b	154.6 b	7.7 c	37.0 b
MycoApply_8Wks	10.0 ab	156.4 b	8.7 b	38.9 ab
MycoApply_12Wks	12.0 a	167.5 a	9.7 a	41.2 a
MycoApply_16Wks	12.0 a	165.2 a	9.6 a	40.5 a
MycoLife_4Wks	9.0 b	151.3 bc	7.5 c	36.8 b
MycoLife_8Wks	10.0 ab	153.0 b	8.4 b	38.6 ab
MycoLife_12Wks	12.0 a	161.7 ab	9.2 a	39.7 a
MycoLife_16Wks	11.0 a	161.1 ab	9.2 a	39.8 a
Control	9.0 b	147.9 c	7.1 c	36.5 b
Significance				
Biostimulant types (A)	*	*	*	*
Application frequency (B)	NS	*	*	NS
A × B	*	*	**	*

All-pairwise comparisons by Tukey's honestly significant difference test at $P < 0.05$; columns having dissimilar letters are statistically different, whereas columns sharing the same letter are statistically similar; $^{**}P < 0.01$, $^{*}P < 0.05$, NS = nonsignificant, L* = lightness, Wks = weeks.

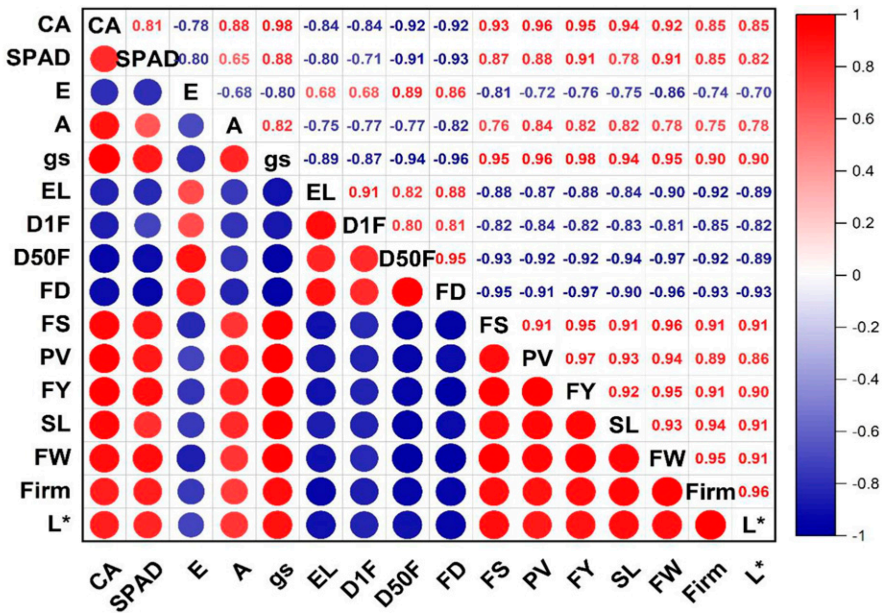

Fig. 8. The correlogram illustrating the relationship between average values of the variable in greenhouse conditions is presented. The color intensity and circle size increase with greater correlation significance. The cell values display the correlation coefficients.

contributing to Qatar's gross domestic product. The combination of a 12-week application of MycoApply® and the 'Valdeon RZ' tomato shows great potential for organic hydroponics, emphasizing the importance of breeding programs focused on increasing yields and resistance to heat stress. This approach presents a valuable opportunity for the organic produce sector to support nations facing similar challenges. To gain a comprehensive understanding of the effectiveness of MycoApply® biostimulant in mitigating heat stress and enhancing tomato yield in organic hydroponic systems, further evaluation with a wider range of tomato cultivars is necessary.

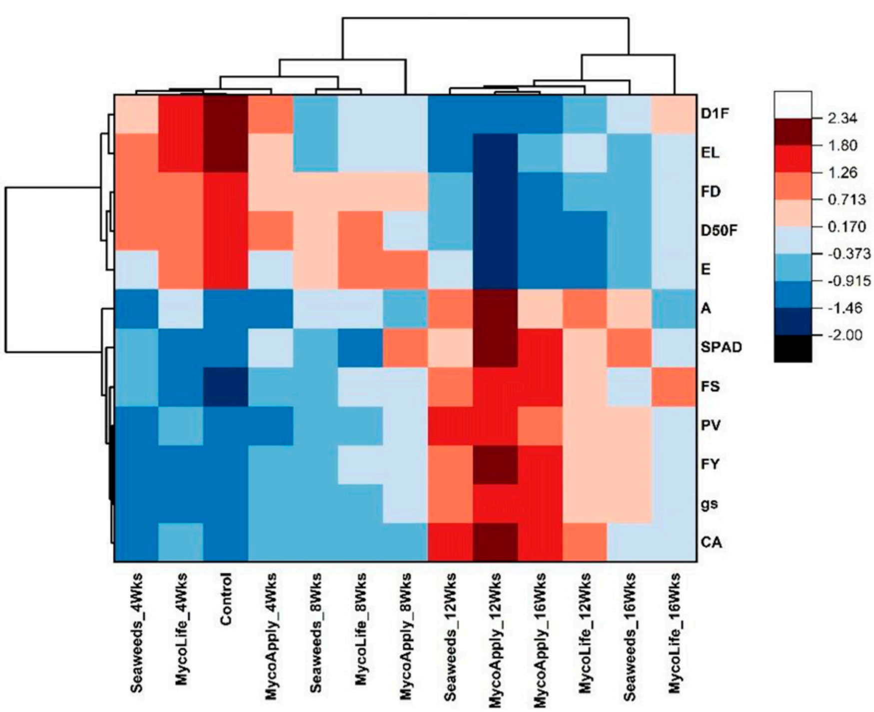

Fig. 9. Heatmap and clustering of biostimulant types and their application frequency based on the scaled values of the measured variables attained under greenhouse conditions. Each column represents a treatment combination, and each row indicates a measured parameter. Treatment combinations are clustered based on their measured variables and variable groups are clustered based on their correlation. The variables that are clustered together have a high positive correlation.

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