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Article

Impact of microbial biofertilizers on the growth, yield, fruit quality, and biochemical characteristics of two sweet pepper (*Capsicum annuum* L.) cultivars

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Abstract:

Sweet pepper (*Capsicum annuum* L.) is a commercially essential vegetable crop globally, valued for its nutritional content and economic returns. Due to the environmental concerns associated with the excessive use of chemical fertilizers, there is a growing necessity for sustainable agricultural alternatives. This study evaluated the effects of three commercial biological formulations applied as foliar sprays: Bio-Arc (*Bacillus megaterium*), Bio-Nagy (*Trichoderma asperellum*), and Bio-Zied (*Trichoderma album*). The treatments were applied at concentrations of 1, 2, and 3 g L^{-1} on two sweet pepper cultivars ('Topy Star' and 'Madrid' hybrid) grown under field conditions across two consecutive seasons (2023 and 2024). The application of these biofertilizers significantly enhanced plant growth, fruit quality, and biochemical parameters compared to untreated control. Specifically, Bio-Nagy at 3 g L^{-1} produced the firmest fruits (8.19 and 8.10 kg cm^{-2} in 'Topy Star' and 'Madrid', respectively) and the highest total sugar content (35.34% and 37.44%). The 'Madrid' hybrid treated with Bio-Zied at 3 g L^{-1} recorded the highest total soluble solids (TSS) (8.07%) and vitamin C content (145.60 mg 100 g^{-1} FW). Furthermore, biochemical profiles, including chlorophyll and phenolic compounds, exhibited substantial increases under all biological treatments. Vegetative parameters such as fresh shoot weight and average fruit weight were also significantly improved, with the 'Madrid' hybrid reaching an average fruit weight of 12.70 g under the Bio-Zied (3 g L^{-1}) treatment. In conclusion, the foliar application of *T. asperellum*, *T. album*, and *B. megaterium* meaningfully improves the physiological performance, yield, and nutritional quality of sweet pepper. These findings strongly support the integration of microbial biofertilizers as a viable, eco-friendly alternative to chemical fertilizers in sustainable horticultural practices.

Keywords:

Sweet pepper, *Capsicum annuum*, biofertilizers, *Trichoderma* spp., *Bacillus megaterium*, fruit quality, sustainable agriculture.

1. Introduction

Sweet pepper (*Capsicum annuum* L.), a member of the *Solanaceae* family, is a globally prominent vegetable crop highly valued for its economic importance and nutritional benefits, including high levels of ascorbic acid and antioxidant compounds (Shahein et al., 2015). The increasing global demand for this crop has traditionally been met through the intensive application of chemical fertilizers. However, the excessive use of these agrochemicals has raised severe environmental and soil health concerns, necessitating a shift toward sustainable agricultural practices (Jarecki et al., 2025). Consequently, the integration of microbial biofertilizers has emerged as a promising, eco-friendly strategy to mitigate environmental damage while maintaining or enhancing crop productivity (Prisa et al., 2023). Biofertilizers, comprising live microorganisms such as beneficial fungi and bacteria, play a pivotal role in sustainable agriculture by improving nutrient availability and root zone dynamics. These microorganisms enhance plant nutrition through mechanisms such as nitrogen fixation, phosphorus solubilization, and the synthesis of phytohormones, which collectively improve soil structure and biological activity (Bai et al., 2015; Timofeeva et al., 2023). Among the widely studied fungal bio-agents, *Trichoderma* species are renowned for their ability to stimulate plant development and confer stress tolerance (Ahmad et al., 2015). Recent studies have demonstrated that *Trichoderma* inoculation can significantly enhance morphological traits, pigment content, and the accumulation of soluble sugars and proteins in various vegetable crops (Lian et al., 2023; Zhang et al., 2024). Furthermore, *Trichoderma*-based biofertilizers have been reported to increase the activity of beneficial enzymes, such as peroxidase and invertase, thereby improving soil nutrient supply and crop performance (Yan et al., 2020). Similarly, plant growth-promoting bacteria (PGPB), particularly the genus *Bacillus*, are extensively utilized in agricultural ecosystems (Sansinenea, 2019). *Bacillus* species improve physiological crop characteristics and yield through various mechanisms of action, potentially reducing the required dosage of chemical fertilizers by up to 50% without compromising plant performance (Sales & Rigobelo, 2024). Despite the proven individual benefits of *Trichoderma* and *Bacillus* species, comparative field studies evaluating their efficacy specifically as foliar bio-stimulants across different commercial sweet pepper cultivars remain limited. Therefore, the present study aims to evaluate and compare the effects of foliar applications of *Trichoderma asperellum*, *Trichoderma album*, and *Bacillus megaterium* on the vegetative growth, yield parameters, and biochemical profiles (including fruit quality, sugars, and antioxidant content) of two sweet pepper cultivars ('Topy Star' cv. and 'Madrid' hybrid) under field conditions.

2. Materials and Methods

2.1 Plant material and experimental site

Field experiments were conducted at the Experimental Farm of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt, during two consecutive growing seasons (2023 and 2024). This study evaluated the effects of selected microbial biostimulants on the vegetative growth, yield, fruit quality, and biochemical composition of sweet pepper (*Capsicum annuum* L.). Two commercial cultivars were used: 'Topy Star' cv. and 'Madrid' hybrid. Seeds of both cultivars were supplied by Kanza Group Company for Seeds, Fertilizers, and Chemical Fungicides, Giza, Egypt. The soil texture was clay loam, and the plants were irrigated regularly.

2.2 Experimental design and bio-treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates. Each replicate plot consisted of six plants. A total of 10 experimental treatments were evaluated, comprising an untreated control (sprayed with tap water) and three commercial biofertilizers applied at three different concentrations (1, 2, and 3 g L^{-1}). The biofertilizers used were:

- Bio-Arc: containing *Bacillus megaterium*.
- Bio-Nagy: containing *Trichoderma asperellum*.
- Bio-Zied: containing *Trichoderma album*.

All treatments were applied as foliar sprays. The first application was performed 30 days after transplanting, followed by two additional sprays at 15-day intervals.

2.3 Measurements and analysis

2.3.1 Vegetative growth and yield characteristics

Three plants from each plot were randomly sampled at 45, 60, and 75 days after transplanting to determine vegetative growth parameters.

- Plant height (cm): Measured from the soil surface to the plant apex.
- Stem diameter (cm): Measured using a digital vernier caliper.
- Plant fresh and dry weight (g): Roots, stems, and leaves were cleaned and weighed using an analytical balance to determine fresh weight. The plant samples were then oven-dried at 80°C until a constant weight was reached to record the dry weight.
- Yield parameters: Total yield per plant (g) and total number of fruits per plant were determined by cumulating the weekly harvested fruits. Average fruit weight (g) was calculated by dividing the total fruit weight by the total number of fruits per plant.

2.3.2 Fruit quality and biochemical characteristics

Pepper fruits were harvested every 15 days during the production season for the following analyses:

- Fruit firmness (kg cm^{-2}): Measured at four equatorial points using a handheld texture analyzer (Axis FB200, Poland).
- Total soluble solids (TSS, %): Determined using a digital refractometer (Milwaukee MA871, USA).
- Titratable acidity (TA, %): Determined by titrating the fruit juice filtrate with 0.1 N NaOH using phenolphthalein as an indicator, and expressed as malic acid percentage (La et al., 2021).
- Vitamin C (Ascorbic acid): Quantified using the 2,6-dichlorophenol indophenol dye titration method following homogenization in 3% metaphosphoric acid (AOAC, 1990).
- Chlorophyll content: Chlorophyll a and b were extracted using acetone, and optical density was measured at 663 and 645 nm using a spectrophotometer (Arnon, 1949; Holden, 1965).
- Phenolic compounds: Total and free phenols were determined calorimetrically using the Folin–Ciocalteu reagent (Snell & Snell, 1953). Conjugated phenols were calculated as the difference between total and free phenols.
- Sugars: Total and reducing sugars were quantified using the picric acid reduction method, with absorbance measured at 540 nm (Thomas and Dutcher, 1924). Non-reducing sugars were calculated as the difference between total and reducing sugars.

2.4 Statistical analysis

All data obtained from the field experiments were subjected to analysis of variance (ANOVA) appropriate for a Randomized Complete Block Design (RCBD). The statistical analysis was performed using CoStat software, version 6.4. Mean comparisons among treatments were performed using Fisher's Least Significant Difference (LSD) test at a probability level of $P \leq 0.05$. Results were expressed as means, and values sharing the same alphabetical letters within each column are not significantly different.

3. Results and Discussion

3.1 Fruit quality and ascorbic acid (Vitamin C) content

The data presented in Table (1) demonstrate that the foliar application of microbial biostimulants significantly ($P \leq 0.05$) enhanced fruit firmness, total soluble solids (TSS), titratable acidity, and vitamin C content in both sweet pepper cultivars compared to the untreated control. Regarding fruit firmness, the 'Madrid' hybrid and 'Topy Star' cultivar exhibited their maximum significant values when treated with Bio-Nagy (*Trichoderma asperellum*) at 3 g L^{-1}, recording 8.10 and 8.19 kg cm^{-2}, respectively, at 60 days post-transplanting. This enhancement in structural integrity is likely attributed to improved calcium uptake and the strengthening of cell wall polysaccharides induced by *Trichoderma* inoculation, which delays fruit softening (Harman et al., 2021; Kumar et al., 2022). Similarly, TSS and titratable acidity were positively modulated by the biological treatments. The 'Madrid' hybrid achieved its highest TSS peak (8.07%) with Bio-Zied (*Trichoderma album*) at 3 g L^{-1} (75 days), while 'Topy Star' responded earlier, peaking at 6.75% with Bio-Nagy at 2 g L^{-1} after 45 days. Ascorbic acid (Vitamin C) levels also exhibited a robust increase under microbial treatments. The Bio-Zied treatment (3 g L^{-1}) yielded the highest vitamin C content in the 'Madrid' hybrid (145.60 mg 100 g^{-1} FW), whereas 'Topy Star' responded optimally to Bio-Arc (*Bacillus megaterium*) at 3 g L^{-1} (102.34 mg 100 g^{-1} FW). These genotype-specific responses highlight the differential mechanisms of plant growth-promoting rhizobacteria (PGPR) and fungi in stimulating antioxidant defense pathways and carbohydrate partitioning, consistent with the findings of Su et al. (2024).

Table 1: Effect of foliar biofertilizers on fruit firmness, total soluble solids (TSS), titratable acidity, and vitamin C of two sweet pepper cultivars ('Topy Star' cv. and 'Madrid' hybrid) at 45, 60, and 75 days post-transplanting.

Bio-treatments	Conc. (g L^{-1})		Topy Star cv.				Madrid hybrid			
		Periods (days)	Firmness (kg cm^{-2})	TSS (%)	Titratable acidity (%)	Vitamin C (mg 100g^{-1})	Firmness (kg cm^{-2})	TSS (%)	Titratable acidity (%)	Vitamin C (mg 100g^{-1})
Bio-Arc	1	45	4.74	4.43	0.22	35.83	5.2	4.67	0.38	28.25
		60	5.29	4.78	0.2	39.39	4.79	5.63	0.9	37.15
		75	4.4	4.2	0.17	44.87	4.15	6.33	1.23	112.5
	2	45	5.89	3.48	0.26	60.37	5.65	5.57	1.3	44.45
		60	6.25	3.65	0.26	82.65	5.15	5.6	1.6	99.71
		75	4.5	3.55	0.13	94.59	4.73	6	1.1	127.54
	3	45	6.39	4.55	0.35	79.1	7.85	5.23	1.1	47.15
		60	6.48	5.08	0.28	89.11	7.53	5.36	0.73	95.07
		75	5.89	4.13	0.19	102.34	5.38	5.7	0.7	123.48
Bio-Nagy	1	45	4.78	5.05	0.28	48.1	4.53	4.7	0.97	72.27
		60	5.3	4.9	0.23	63.6	5.7	5.3	1.67	84.25
		75	4.36	4.18	0.17	72.64	5.36	5.87	2.03	90.44
	2	45	4.94	6.75	0.3	52.52	6.24	6.57	1.1	74.97
		60	6.18	4.48	0.25	67.15	6.7	6.83	0.8	78.84
		75	4.41	4.43	0.2	74.58	5.44	7.2	0.8	85.03
	3	45	5.68	4.05	0.33	68.44	8.1	4.63	1.57	73.81
		60	8.19	4.7	0.31	72.96	7.76	4.7	1.4	77.29
		75	5.44	3.55	0.28	76.51	6.25	4.9	1	78.26
Bio-Zied	1	45	4.28	5.95	0.22	41.97	3.64	5.4	1.4	61.84
		60	4.61	4.63	0.17	47.78	4.95	6.77	2.13	67.25
		75	4.14	4.15	0.16	51.98	3.56	7.57	2.6	87.35
	2	45	4.64	5.6	0.24	53.27	3.73	5.13	0.93	86.19
		60	4.61	4.3	0.19	68.77	5.79	6.8	1.63	92.36
		75	4.18	4.1	0.12	80.06	4.65	7.63	2.07	102.3
	3	45	5.23	5.05	0.29	79.1	4.23	4.57	0.93	123.67
		60	5.3	4.1	0.24	81.03	6.88	6.87	1.37	129.47
		75	4.73	3.45	0.16	84.26	4.75	8.07	1.97	145.6
Control	_	45	4.28	4.8	0.21	46.17	5.83	4.8	0.8	27.44
		60	4.29	4.8	0.19	51.65	5.3	5.5	0.93	46.77
		75	3.13	3.08	0.13	54.56	3.96	6.27	1.03	52.1

3.2 Sugar accumulation and phenolic profiles

As shown in Table (2), carbohydrate and phenolic compound profiles were significantly modified by the bio-treatments. Bio-Nagy (*T. asperellum*) at 3 g L^{-1} recorded the highest total sugar accumulation in both the 'Madrid' hybrid (37.44%) and the 'Topy Star' cultivar (35.34%) (Figure 1). Interestingly, the treatments exerted distinct effects on sugar fractions. The highest non-reducing sugar content in 'Madrid' was induced by Bio-Arc at 2 g L^{-1} (6.65%), whereas in 'Topy Star', it was maximized by Bio-Zied at 2 g L^{-1} (9.35%). This divergence indicates that specific microbial strains differentially regulate sucrose accumulation and hydrolysis pathways (Backer et al., 2018). Phenolic compounds, which are crucial for stress tolerance and nutritional value, increased significantly across all treatments. In the 'Madrid' hybrid, total phenols peaked with Bio-Arc at 3 g L^{-1} (6.25 mg g^{-1}), while Bio-Nagy at 3 g L^{-1} yielded the highest total phenols (5.25 mg g^{-1}) in 'Topy Star'. The observed shift between active (free) and reserve (conjugated) phenolic pools under different microbial applications suggests the activation of induced systemic resistance (ISR) pathways, an established plant response to beneficial *Trichoderma* colonization (Shoresh et al., 2010).

3.3 Photosynthetic pigments (Chlorophyll *a* and b)

Foliar bio-treatments significantly ($P \leq 0.05$) augmented chlorophyll accumulation in both pepper genotypes compared to the untreated control (Table 3). The 'Madrid' hybrid treated with Bio-Nagy at 3 g L^{-1} registered the maximum values for chlorophyll a, b, and total chlorophyll (2.89, 4.58, and 7.98 mg g^{-1} FW, respectively). Conversely, the 'Topy Star' cultivar demonstrated its highest total chlorophyll content (15.02 mg g^{-1} FW) under the Bio-Zied treatment at 3 g L^{-1}. The substantial increase in photosynthetic pigments can be mechanistically linked to the microbial enhancement of root architecture, which facilitates greater uptake of essential structural elements like nitrogen and magnesium. Furthermore, *Bacillus* and *Trichoderma* species are known to delay chlorophyll degradation by minimizing oxidative stress within chloroplasts (Harman et al., 2021).

Table 2: Effect of different bio-treatments on sugar content and phenolic compounds of sweet pepper ('Topy Star' cv. and 'Madrid' hybrid) under field conditions.

Bio-treatments	Conc. (g L^{-1})	'Topy Star' cv.						'Madrid' hybrid					
		Total Sugar (%)	Reducing Sugar	Non-reducing Sugar	Total Phenols (mg g^{-1})	Free Phenols	Conjugated Phenols	Total Sugar (%)	Reducing Sugar	Non-reducing Sugar	Total Phenols (mg g^{-1})	Free Phenols	Conjugated Phenols
Bio-Arc	1	11.24	7.33	3.91	3.25	2.75	0.5	14.1	12.8	1.3	3.85	3.15	0.7
	2	12.04	10.6	1.44	4.07	2.96	1.11	18.25	11.6	6.65	4.45	3.8	0.65
	3	16.86	15.18	1.68	4.44	3.11	1.33	22.3	16.65	5.65	6.25	5.1	1.15
Bio-Nagy	1	16.67	15.18	1.49	3.63	2.44	1.19	18.47	16.6	1.87	4.63	2.98	1.65
	2	23.3	21.68	1.62	4.1	2.67	1.43	24.2	22.33	1.87	4.88	3.09	1.79
	3	35.34	28.19	7.15	5.25	2.77	2.48	37.44	34.18	3.26	4.98	3.76	1.22
Bio-Zied	1	19.27	10.4	8.87	4.47	2.44	2.03	23.22	21.4	1.82	5.67	4.15	1.52
	2	24.09	14.74	9.35	4.68	3.7	0.98	27.1	24.74	2.36	5.74	4.55	1.19
	3	26.07	17.34	8.73	5.22	3.77	1.45	29.95	26.1	3.85	6.08	5.45	0.63
Control	—	15.09	11.25	3.84	3.35	2.6	1.25	16.7	12.8	3.9	4.4	3.11	1.29

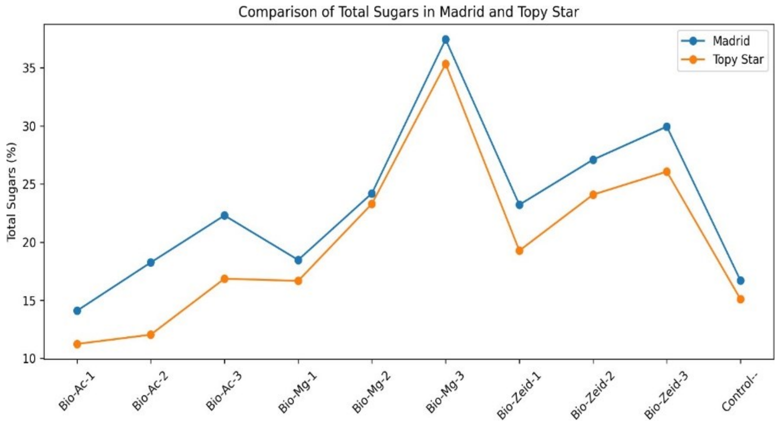

Figure 1: Comparative effect of different bio-treatments and concentrations on total sugar content (%) of Madrid hybrid and Topy star cultivar of sweet pepper under field conditions.

Table 3: Evaluation of different bio-treatments on photosynthetic pigments (chlorophyll a, b, and total) of sweet pepper ('Topy Star' cv. and 'Madrid' hybrid) at 60 and 75 days.

Bio-treatments	Conc. (g L^{-1})	'Topy Star' cv.			'Madrid' hybrid		
		Chl A	Chl B	Total (A+B)	Chl A	Chl B	Total (A+B)
Bio-Arc	1	3.00	6.13	9.37	4.41	3.36	7.94
	2	3.76	6.76	10.79	6.44	3.61	10.24
	3	4.46	6.94	11.69	6.95	3.84	11.00
Bio-Nagy	1	4.11	8.61	13.05	4.87	3.69	8.74
	2	4.26	8.63	13.22	7.34	4.00	11.65
	3	5.41	9.10	14.88	7.42	4.18	11.75
Bio-Zied	1	3.08	5.91	9.22	7.07	4.08	11.38
	2	3.27	6.36	9.88	7.55	4.11	11.88
	3	3.67	6.67	10.61	8.80	5.92	15.02
Control	—	7.58	11.05	19.09	4.16	2.53	6.83

3.4 Vegetative growth, root biomass, and yield characteristics

Data summarized in Tables (4 and 5) reveal that microbial inoculants significantly promoted plant vegetative vigor and overall yield compared to the control. The tallest plants in the 'Madrid' hybrid were observed under the Bio-Arc (*B. megaterium*) treatment at 3 g L^{-1} (80.67 cm), whereas Bio-Nagy (3 g L^{-1}) produced the tallest 'Topy Star' plants. Stem diameter followed a similar positive trend across both genotypes. Biomass accumulation (shoot and root fresh/dry weights) and yield traits were markedly superior under the bio-treatments. Notably, Bio-Zied (*T. album*) at 3 g L^{-1} recorded the highest fresh shoot weight (26.22 g) and average fruit weight (12.70 g) in the 'Madrid' hybrid. In 'Topy Star', the same treatment maximized fresh shoot weight (80.06 g) and average fruit weight (6.62 g). Meanwhile, root dry biomass was optimally enhanced by Bio-Nagy (3 g L^{-1}) in both genotypes.

Table 4: Effect of different bio-treatments on vegetative growth and yield characteristics of the sweet pepper Madrid hybrid under field conditions.

Bio-treatments	Conc. (g L^{-1})	Madrid hybrid						
		Plant Height (cm)	Stem Diameter (cm)	Fresh Shoot Weight (g)	Dry Shoot Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)	Average Fruit Weight (g)
Bio-Arc	1	67.33	0.97	14.33	8.92	9.77	6.17	8.56
	2	69.50	1.08	17.33	10.15	9.97	7.48	9.51
	3	80.67	1.45	20.17	10.64	10.91	7.32	10.25
Bio-Nagy	1	62.67	0.90	22.19	10.63	10.38	5.15	9.90
	2	67.00	1.15	23.83	12.82	10.93	5.60	10.15
	3	73.33	1.74	25.22	13.12	11.80	6.27	12.13
Bio-Zied	1	57.00	0.86	19.35	9.61	9.17	4.11	10.17
	2	62.83	1.10	21.55	10.68	10.98	4.70	11.05
	3	72.50	1.40	26.22	11.02	12.00	5.95	12.70
Control	—	51.67	0.93	19.15	7.35	10.83	3.18	8.07
LSD 5%	T	3.84	N.S	0.84	1.26	0.09	0.56	0.89
	C	4.66	0.11	0.70	0.76	0.42	0.59	0.63
	T × C	N.S	N.S	1.21	N.S	0.72	1.01	N.S

Table 5: Effect of different bio-treatments on vegetative growth and yield characteristics of the sweet pepper 'Topy Star' cultivar under field conditions.

Bio-treatments	Conc. (g L^{-1})	Topy Star cv.						
		Plant Height (cm)	Stem Diameter (cm)	Fresh Shoot Weight (g)	Dry Shoot Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)	Average Fruit Weight (g)
Bio-Arc	1	27.58	4.00	45.70	18.43	1.54	0.56	4.51
	2	31.50	4.35	49.89	19.11	1.74	0.81	4.63
	3	33.75	4.60	56.62	20.04	1.92	0.96	4.97
Bio-Nagy	1	31.33	4.77	58.38	24.52	2.63	1.42	4.47
	2	34.70	4.90	60.85	25.51	2.75	1.62	5.00
	3	37.33	5.27	73.01	25.76	2.86	1.69	5.23
Bio-Zied	1	28.75	4.63	66.06	20.67	2.28	0.67	4.65
	2	30.92	4.73	70.08	20.75	2.14	0.88	5.70
	3	33.17	5.35	80.06	27.74	2.41	1.04	6.62
Control	—	24.25	3.87	26.69	10.58	1.29	0.61	5.89
LSD 5%	T	N.S	0.16	7.26	1.59	0.395	0.06	N.S
	C	1.49	0.17	3.19	1.40	0.166	0.065	0.619
	T × C	N.S	0.30	5.53	2.42	0.287	0.112	N.S

These substantial increments in growth and yield parameters can be biologically attributed to the capacity of the applied bio-agents to synthesize phytohormones (such as auxins, gibberellins, and cytokinins) and to improve nutrient solubilization. Enhanced root growth, explicitly stimulated by these metabolites, improves water and nutrient acquisition efficiency, which directly translates into heightened photosynthetic activity, greater assimilate translocation to developing fruits, and ultimately, a significantly higher yield (Erturk et al., 2010; Olanrewaju et al., 2017).

4. Conclusion

The present study substantiates the efficacy of foliar-applied microbial biostimulants as a sustainable, eco-friendly alternative to conventional agrochemicals in sweet pepper cultivation. Our findings explicitly demonstrate that the foliar application of *Trichoderma asperellum* (Bio-Nagy), *Trichoderma album* (Bio-Zied), and *Bacillus megaterium* (Bio-Arc) significantly enhances vegetative vigor, overall yield, and critical fruit quality parameters, including ascorbic acid, total sugars, and phenolic compounds. Importantly, the study highlights distinct genotype-specific responses; the 'Madrid' hybrid exhibited optimal biomass and yield traits under the *T. album* treatment (3 g L^{-1}), whereas *T. asperellum* (3 g L^{-1}) maximized structural firmness and carbohydrate accumulation across both investigated cultivars. Integrating these specific microbial formulations into standard horticultural management practices offers a viable strategy to improve crop physiological performance while substantially mitigating the environmental footprint associated with excessive chemical fertilization. Future research should focus on unraveling the underlying molecular mechanisms driving these specific genotype-microbe interactions and evaluating the field efficacy of these biostimulants under varying abiotic stress conditions.

Declarations

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-assisted Technologies: The authors confirm that they have not used any artificial intelligence (AI) tools or technologies for the generation of text, images, or data in the preparation of this manuscript.

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