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Integration of microbiological and molecular approaches in phytopathogen management to enhance disease resistance and agrobiological traits of vegetable crops

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ABSTRACT

Background: Modern food production is increasingly focused on developing functional food products with pronounced biologically active properties. Tomatoes (*Solanum lycopersicum* L.) and sweet peppers (*Capsicum annuum* L.) are valuable vegetable crops rich in vitamins, antioxidants, and other phytonutrients. However, they are susceptible to fungal diseases, especially under greenhouse conditions.

Objective: To study the effectiveness of microbiological complexes based on *Bacillus subtilis* and *Trichoderma viride* as plant growth stimulators and biological control agents for tomatoes and sweet peppers.

Methods: The study was conducted in a greenhouse at the Scientific Centre of Vegetable and Industrial Crops, Ministry of Economy of the Republic of Armenia, during the spring and summer-autumn seasons of 2024–2025. Microbiological complexes based on *B. subtilis* and *T. viride* were applied to the soil via root treatment, both individually and in combination. Phenological parameters, yield, and biochemical characteristics of the fruits were assessed: the contents of ascorbic acid, total phenolic compounds, carotenoids, and sugars were measured spectrophotometrically; dry matter content- refractometrically; and nitrate levels- calorimetrically. A phytosanitary evaluation of the plants was also performed. The pathogens *Fusarium oxysporum* and *Verticillium dahliae* were identified using qPCR, followed by determination of the infection rate. Data were analyzed using ANOVA and the LSD test.

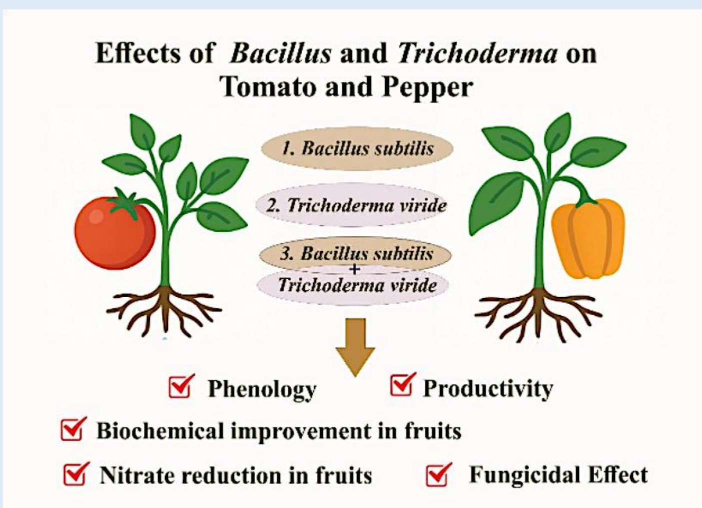
Results: The conducted research demonstrated that the application of microbiological formulations had a positive effect on all stages of ontogenesis in tomato and sweet pepper, especially when *B. subtilis* and *T. viride* were used in combination, compared to both the control and the individual treatments. The formulations contributed to the acceleration of phenological phases, an extended fruiting period, and an increase in overall yield. Biochemical analysis of the fruits showed an increase in the content of vitamin C, total phenolic compounds, and carotenoids, indicating enhanced antioxidant capacity, as well as higher levels of sugars and dry matter. A reduction in nitrate content was also recorded, confirming an improvement in both nutritional and environmental value of the produce.

Phytosanitary monitoring revealed a consistent decrease in the incidence of Fusarium wilt *(F. oxysporum*) and Verticillium wilt (*V. dahliae*). The formulations exhibited pronounced antagonistic activity against phytopathogens, particularly under combined application. Moreover, a cumulative effect observed in the second year of use resulted in further reduction of diseased plants, underscoring the formulations' potential as effective tools for long-term biological protection.

Novelty: For the first time in Armenia, a comprehensive study was conducted on the synergistic action of microbial complexes based on PGPR (*Bacillus* spp.) and PGPF (*Trichoderma* spp.) when applied together on tomato and sweet pepper. A strong bioprotective and growth-stimulating effect was established, accompanied by improvements in agrobiological and biochemical characteristics. The results lay the groundwork for the implementation of environmentally safe bioprotection strategies in greenhouse vegetable production in Armenia and neighboring regions.

Conclusion: Microbiological complexes based on *Bacillus subtilis* and *Trichoderma viride* are effective plant growth biostimulants and bioprotective agents, contributing to the production of environmentally friendly and functionally enriched tomatoes and peppers, making them promising for sustainable agriculture.

Keywords: *Bacillus subtilis, Trichoderma viride*, biostimulants, tomato, sweet pepper, microbial complex, phytosanitary status, crop yield, antioxidant compounds, fruit quality



Graphical Abstract: Integration of microbiological and molecular approaches in phytopathogen management

INTRODUCTION

Modern food industry development focuses not only on providing the population with high-quality and safe products but also on creating functional foods with pronounced biological activity [1-6]. Against the backdrop of increasing chronic diseases, declining immune status among the population, and ecosystem degradation, approaches based on the use of environmentally friendly biotechnologies that enhance the nutritional value and safety of products are becoming increasingly relevant [7].

Functional foods play a critical role in the prevention of chronic diseases and the maintenance of health through the consumption of products rich in bioactive compounds [8–11]. Among such foods, tomatoes (*Solanum lycopersicum* L.) and sweet/hot peppers (*Capsicum annuum* L.)- important representatives of the Solanaceae family- are widely used in the human diet. These crops not only contribute to the sensory and nutritional qualities of foods but also exert significant beneficial effects on human health due to their high content of vitamins, minerals, antioxidants, and other physiologically active compounds [12–13].

Tomatoes and peppers exhibit antioxidant [14–15], anti-inflammatory [16], anticancer [17], and cardioprotective properties [18–19], as well as contribute to immune system support and cognitive function improvement [20]. Consumption of these vegetables, both fresh and processed, enriches the diet with essential nutrients, promoting human health. The bioactive compounds present in these crops encompass a broad spectrum of substances, including carotenoids [21–23], flavonoids [24–26], organic acids [27], phenolic compounds [28–29], vitamins [30–32], mineral elements [33–34], and dietary fibers [35]. Despite the high potential of tomatoes and peppers as sources of functional food, these crops remain vulnerable to fungal diseases, especially under protected cultivation conditions. Due to the need to reduce chemical pesticide use, there is growing interest in microbiological plant protection agents as an environmentally safe and sustainable alternative.

In recent years, considerable attention has been focused on the use of rhizosphere microorganisms that promote plant growth, including both rhizobacteria (PGPR, Plant Growth-Promoting Rhizobacteria) and symbiotic fungi (PGPF, Plant Growth-Promoting Fungi). Among these, representatives of the *Bacillus* spp. (PGPR) and *Trichoderma* spp. (PGPF) are of particular value due to their pronounced biostimulatory and protective properties [36–37].

Bacillus subtilis is an aerobic Gram-positive bacterium known for its ability to produce a wide range of antibiotics, enzymes, and phytohormones that stimulate plant growth and suppress phytopathogens [38]. *Trichoderma viride* is a fungus with strong antagonistic activity capable of inhibiting the development of pathogenic fungi by competing for nutrients and producing enzymes that degrade the cell walls of pathogens. The use of these microorganisms in bioproducts contributes not only to plant disease protection but also to improving their physiological condition, which is essential for obtaining environmentally safe and functionally valuable produce [39–41].

The main biological effects of *B. subtilis* and *T. viride* are presented in Table 1, which summarizes their key functions and modes of action:

Table 1. Key Effects of Soil Microbial Agents

Biological basis of the complex	Key effects
Bacillus subtilis	Fungicidal activity (production of lipopeptides, antibiotics); induction of systemic resistance (ISR); stimulation of root formation and shoot growth; synthesis of phytohormones (auxins, gibberellins); ability to solubilize insoluble phosphorus compounds in soil, making them available to plants; nitrogen fixation; humus formation and improvement of soil structure; antagonism against pathogens in the rhizosphere.
Trichoderma viride	Suppression of fungal pathogens (mycoparasitism, antagonism); production of lytic enzymes (cellulases, chitinases); mineralization of organic residues; synthesis of phytohormones (IAA, GA3, cytokinins); stimulation of root system growth and development; enhancement of local and systemic plant immunity; increased stress tolerance (salinity, temperature, drought); degradation of pesticides and heavy metals.

The integration of such microbial-based preparations into protected cultivation technologies offers the potential to reduce the use of chemical plant protection agents and to maintain a stable phytosanitary status of greenhouse crops [36].

The present study aimed to evaluate the efficacy of microbial complexes based on *Bacillus subtilis* and *Trichoderma viride* as natural plant growth stimulators and biological control agents for tomato and sweet pepper cultivation, with a view toward the subsequent use of the harvested produce in the development of environmentally friendly functional food products.

MATERIAL AND METHODS

The study was conducted at the Scientific Centre of Vegetable and Industrial Crops of the Ministry of Economy of the Republic of Armenia (SCVIC, MEofRA).

Research material: Within the framework of the study, two soil-applied microbial formulations were investigated: one based on *Bacillus subtilis* and the other on *Trichoderma viride* (commercial name.

Both microbiological complexes were applied as root treatments at a rate of 5 g per 100 m² (equivalent to 0.5 kg/ha). The study was conducted over two years (2024–2025) under greenhouse conditions during both the spring and summer-autumn growing seasons. The test crops were tomato F1 Lusarpi and sweet pepper cv. Loshtak.

The experiment included four treatment variants:

- N1 (Control): without application of microbiological complexes;
- N2: application of the *Bacillus subtilis*-based complex;
- N3: application of the *Trichoderma viride*-based complex;
- N4: combined application of both *B. subtilis*+ *T. viride* complexes.

In all treatments, including the control, a uniform mineral nutrition system based on an NPK complex was used. The absence of microbiological products in the control variant ensured an objective assessment of their effectiveness against the background of identical mineral supply.

Greenhouse conditions: The experiments were conducted in a glass-covered greenhouse of the Scientific Center located in Darakert village, Ararat Province, Armenia (coordinates: 40.115018° N, 44.417768° E). Soil electrical conductivity (EC) ranged from 1.2 to 2.5 dS·m^{-1}, with a pH level between 7.0 and 7.5. Mineral nutrition was applied based on the agrochemical characteristics of the soil and the developmental stage of the crops. The temperature regime in the greenhouse was maintained at 25–28 °C during the day and 16–20 °C at night.

Seedling transplantation was carried out on March 5–6 for the spring season and on May 19–20 for the summer-autumn season. The experiment was laid out in a randomized block design with three replications. The planting density for tomatoes is 2.5 plants per 1 m^2, and for peppers - 4 plants per 1 m^2.

Phenological observations and yield assessment: Phenological observations were conducted throughout the entire growing season, with the registration of key developmental stages: from mass seedling emergence to the onset of flowering (50%), from emergence to the stage of biological ripeness (BR, 50%) for both crops, and additionally to the stage of technical ripeness (TR, 50%) for sweet pepper. Yield assessment was carried out during all stages of fruiting and included the measurement of early yield (during the first 15 days of fruiting) as well as total yield accumulated over the entire harvesting period.

Fruit quality parameters: Biochemical analysis of tomato and pepper fruits was carried out at the Laboratory of Plant Biotechnology, Phytopathology, and Biochemistry of the SCVIC. The evaluation was performed in three replicates at the stage of biological ripeness of the fruits.

Ascorbic acid content was determined using a spectrophotometric method with a Carry 60 UV-Vis spectrophotometer (Agilent Technologies, USA), following a standard protocol involving 2,4-dinitrophenylhydrazine and measuring absorbance at λ = 520 nm. Calibration solutions were prepared using L-ascorbic acid [13].

For total carotenoid extraction, a mixture of hexane and ethanol in a 3:1 (v/v) ratio was used at a volume of 10 mL per 1 g of ground sample. The extraction was carried out at room temperature for 30 minutes. The quantitative determination of total carotenoid content was performed spectrophotometrically at a wavelength of 450 nm, and the results were expressed in micrograms of beta-carotene equivalents per 100 g of fresh weight (FW) of the sample [13].

The total phenolic content was determined using a spectrophotometric method with the Folin–Ciocalteu reagent, measuring absorbance at λ = 765 nm. Calibration solutions were prepared using gallic acid, and the results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight [42].

Total sugar content was measured spectrophotometrically by recording the absorbance at λ = 490 nm. Calibration solutions were prepared using glucose as the standard [3]. Dry matter content was determined using a refractometer.

The determination of nitrate content in vegetable fruits is carried out using the colorimetric method with hydrazine reduction (SM 4500-NO3-H) on the automatic analyzer Gallery Aqua Master Discrete Analyzer (Thermo Fisher Scientific) in accordance with ISO 14001:2015. During the analysis, nitrates are reduced by hydrazine to nitrites, which react with Griess reagents to form a colored azo dye. The intensity of the coloration is measured photometrically at a wavelength of 520–540 nm. The nitrate concentration is determined based on a calibration curve of standard solutions.

Phytosanitary assessment: Molecular identification of *Fusarium oxysporum* and *Verticillium dahliae* was performed using quantitative real-time PCR (qPCR) at the Laboratory of the SCVIC. Samples included infected stems, roots, and vascular tissues of tomato and pepper plants exhibiting wilting symptoms. Sample collection was carried out under natural infection conditions.

All stages of molecular analysis—including DNA extraction, preparation of the reaction mixture, and amplification- were conducted in accordance with the protocol provided by the manufacturer of the Genetic PCR kits (Spain).

DNA was extracted using the CTAB method with MiniSpin spin columns, ensuring high-purity DNA. The 20 µL reaction mixture consisted of 10 µL DNase/RNase-free water, 4 µL MixStable qPCR.5x, 1 µL species-specific primer mix (TargetSpecies), and 5 µL DNA template. Amplification was performed on a LightCycler 96 system (Roche, Germany) using 6-FAM as the fluorescent dye. The amplification protocol included: 94 °C for 5 minutes (1 cycle), followed by 40 cycles of 94 °C for 30 seconds, 72 °C for 10 seconds, and 62 °C for 10 seconds.

The percentage of infected plants was calculated based on the number of positive samples, allowing for an objective assessment of the phytosanitary status and pathogen prevalence.

Statistical Analysis: Experimental data were statistically processed using analysis of variance (ANOVA) at a significance level of $p \leq 0.05$. The least significant difference (LSD) test was applied to compare mean yield values. Biochemical analysis results are presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

Phenological development, productivity, and fruit quality: In tomato F1 Lusarpi, treatment with the preparations reduced the period from mass emergence to the onset of fruiting by 2–3 days compared to the control, while the harvesting period was extended by 2–3 days. This effect was observed both with individual applications and with their combined use (*B. subtilis + T. viride*). In sweet pepper cv. 'Loshtak', accelerated fruiting was also noted, most prominently under combined treatment.

The positive trend was consistent during both the spring and summer-autumn growing seasons, indicating the stability of the microbiological complexes' effectiveness under different seasonal conditions. Data on the duration of phenological phases are presented in Table 2.

Table 2. Duration of phenological phases of vegetable crops depending on the treatment and vegetation period

N	Treatment	Days from mass emergence to				Duration of harvest, days
		flowering *a/b	fruiting a/ b	first harvest a/ b	last harvest a/ b	a/ b
Tomato "Lusarpi"						
1	Control	66ᵃ/ 64ᵇ	74ᵃ / 73ᵇ	112ᵃ / 109ᵇ	188ᵃ / 180ᵇ	76ᵃ / 71ᵇ
2	*B. subtilis*	66ᵃ / 64ᵇ	72ᵃ / 70ᵇ	109ᵃ / 106ᵇ	188ᵃ / 180ᵇ	79ᵃ / 74ᵇ
3	*T. viride*	66ᵃ / 64ᵇ	72ᵃ / 70ᵇ	110ᵃ / 106ᵇ	188ᵃ / 180ᵇ	78ᵃ / 74ᵇ
4	*B. subtilis + T. viride*	66ᵃ / 64ᵇ	72ᵃ / 70ᵇ	109ᵃ / 106ᵇ	188ᵃ / 180ᵇ	78ᵃ / 74ᵇ
Sweet pepper "Loshtak"						
1	Control	81ᵃ / 79ᵇ	77ᵃ / 74ᵇ	113ᵃ / 111ᵇ	199ᵃ / 196ᵇ	86ᵃ / 85ᵇ
2	*B. subtilis*	77ᵃ / 70ᵇ	75ᵃ / 73ᵇ	113ᵃ / 110ᵇ	200ᵃ / 198ᵇ	87ᵃ / 88ᵇ
3	*T. viride*	76ᵃ / 70ᵇ	74ᵃ / 72ᵇ	111ᵃ / 110ᵇ	205ᵃ / 200ᵇ	94ᵃ / 90ᵇ
4	*B. subtilis + T. viride*	77ᵃ / 70ᵇ	74ᵃ / 72ᵇ	109ᵃ / 107ᵇ	208ᵃ / 205ᵇ	99ᵃ / 98ᵇ

*a – spring / b – summer-autumn vegetation periods

In addition to accelerating plant development, the microbiological complexes significantly enhanced yield and improved fruit quality across different growing seasons.

For tomato, treatment with *Bacillus subtilis* resulted in an increase in early yield by 7.0–8.0%, while total yield rose by 12.0–12.2%, depending on the season. The average fruit weight ranged from 225.5 to 230.0 g. Treatment with *Trichoderma viride* was even more effective, increasing early yield by 7.7–8.0%, total yield by 13.1–13.3%, and average fruit weight reached 228.5–233.7 g. The greatest effect was observed with the combined application of *B. subtilis+T. viride*, where early yield increased by 7.7–8.7%, and total yield by 15.0–15.4%, mainly due to an increase in average fruit weight.

A similar positive trend was noted in sweet pepper. The combined use of microbiological preparations led to an increase in early yield by 6.5–7.1% and total yield by 14.6–14.7%. The average fruit weight increased to 144.6–147.3 g (Table 3).

Table 3. Yield parameters of vegetable crops depending on the tested treatment and vegetation period

N	Treatment	Early yield, kg/m² * a/ b	Early yield increase, % a/ b	Total yield, kg/m² a/ b	Total yield increase, % a/ b	Average fruit weight, g (±SD) a/ b
Tomato "Lusarpi"						
1	Control	1.50ᵃ / 1.42ᵇ	-	18.0ᵃ / 17.5ᵇ	-	222.4±4.2ᵃ / 218.1±3.0ᵇ
2	*B. subtilis*	1.60ᵃ / 1.52ᵇ	8.0ᵃ / 7.0ᵇ	20.2ᵃ / 19.6ᵇ	12.2ᵃ / 12.0ᵇ	230.0±4.6ᵃ / 225.5±3.7ᵇ
3	*T. viride*	1.60ᵃ / 1.53ᵇ	8.0ᵃ / 7.7ᵇ	20.4ᵃ / 19.8ᵇ	13.3ᵃ / 13.1ᵇ	238.7±5.1ᵃ / 228.5±3.5ᵇ
4	*B. subtilis + T. viride*	1.63ᵃ / 1.53ᵇ	8.7ᵃ / 7.7ᵇ	20.7ᵃ / 20.2ᵇ	15.0ᵃ / 15.4ᵇ	240.2±4.5ᵃ / 236.0±3.1ᵇ
	LSD_{05}	0.31ᵃ / 0.30ᵇ		1.35ᵃ / 1.28ᵇ		
Sweet pepper "Loshtak"						
1	Control	1.27ᵃ / 1.24ᵇ	-	14.4ᵃ / 14.3ᵇ	-	136.4±3.2ᵃ / 132.5±3.0ᵇ
2	*B. subtilis*	1.35ᵃ / 1.32ᵇ	6.3ᵃ / 6.5ᵇ	16.1ᵃ / 16.0ᵇ	11.8ᵃ / 11.9ᵇ	138.1±3.7ᵃ / 135.1±3.4ᵇ
3	*T. viride*	1.36ᵃ / 1.32ᵇ	7.1ᵃ / 6.5ᵇ	16.3ᵃ / 16.4ᵇ	13.2ᵃ / 13.3ᵇ	143.4±4.0ᵃ / 138.7±3.5ᵇ
4	*B. subtilis + T. viride*	1.36ᵃ / 1.32ᵇ	7.1ᵃ / 6.5ᵇ	16.5ᵃ / 16.4ᵇ	14.6ᵃ / 14.7ᵇ	147.5±4.2ᵃ / 144.6±3.5ᵇ
	LSD_{05}	0.54ᵃ / 0.44ᵇ		1.55ᵃ / 1.50ᵇ		

*a – spring / b – summer-autumn vegetation periods

Biochemical analysis of fruits demonstrated a positive effect of microbiological preparations on the quality of tomatoes and sweet peppers. All treatment variants showed an increase in the content of dry matter, sugars, ascorbic acid, total phenolic compounds, and carotenoids compared to the control (Table 4, Figure 1).

The most pronounced effect was observed with the combined treatment of *B. subtilis +T. viride*, which resulted in the highest values of all measured parameters. In tomatoes, dry matter content increased to 6.35–6.45%, sugars to 3.26–3.28%, ascorbic acid to 21.60–22.76 mg/100 g, total phenolics to 38.1–45.4 mg/100 g, and carotenoids to 3.72–3.82 mg/100 g.

A similar positive trend was recorded in sweet peppers, with dry matter content reaching 4.86–5.01%, sugars 3.58–3.64%, ascorbic acid 203.25–207.74 mg/100 g, total phenolics 70.1–76.3 mg/100 g, and carotenoids 1.57–1.60 mg/100 g.

This increasing trend was consistent across both spring and summer-autumn vegetation periods, confirming the stable efficacy of the biopreparations under varying cultivation conditions.

Table 4. Quality parameters of vegetable fruits depending on treatment and vegetation period (p≤0.05)

N	Treatment	Dry matter, % (±SD) a/ b	Sugars, % (±SD) a/ b	Ascorbic acid, mg/% (±SD) a/ b	Total phenols, mg/100g (±SD) a/ b
Tomato "Lusarpi"					
1	Control	6.12±0.14[a]/ 6.26±0.13[b]	3.04±0.08 [a] / 3.12±0.05 [b]	20.09±0.23 [a] / 19.77±0.22 [b]	35.5±1.5 [a] / 28.4±.1.2 [b]
2	*B. subtilis*	6.31±0.12 [a] / 6.46±0.09 [b]	3.25±0,05 [a] / 3.31±0,04 [b]	21.81±0.17 [a] / 20.59±0.18 [b]	39.8±1.5. [a] / 32.3±.1.3 [b]
3	*T. viride*	6.23±0,15 [a] / 6.32±0,14 [b]	3.09±0,03 [a] / 3.19±0,02 [b]	21.56±0,21 [a] / 20.58±0,20 [b]	38.6±1.3 [a] / 33.3±1.3 [b]
4	*B. subtilis + T. viride*	6.35±0.13 [a] / 6.45±0.09 [b]	3.26±0,06 [a] / 3.28±0,04 [b]	22.76±0,16 [a] / 21.60±0,18 [b]	45.4±1.5[a] / 38.1±1.4 [b]
Sweet pepper "Loshtak"					
1	Control	4.48±0.22 [a] / 4.62±0.19 [b]	3.39±0.32 [a] / 3.54±0.35 [b]	189.05±2.90 [a] / 185.20±3.05 [b]	60.7±2.4 [a] / 56.3±2.2 [b]
2	*B. subtilis*	4.51±0.16 [a] / 4.63±0.15 [b]	3.55±0.25 [a] / 3.52±0.33 [b]	191.81±0.17 [a] / 190.30±2.65 [b]	65.8±2.1 [a] / 58.7±1.9 [b]
3	*T. viride*	4.82±0.20 [a] / 4.93±0.18 [b]	3.52±0.32 [a] / 2.58±0.30 [b]	196.30±3.60 [a] / 192.15±3.10 [b]	72.6±2.2 [a] / 65.8±2.0 [b]
4	*B. subtilis + T. viride*	4.86±0.18[a] / 5.01±0.18 [b]	3.58±0.24 [a] / 3.64±0.35 [b]	207.74±0.16 [a] / 203.25±2.55 [b]	76.3±2.4 [a] / 70.1±2.5 [b]

*a – spring / b – summer-autumn vegetation periods

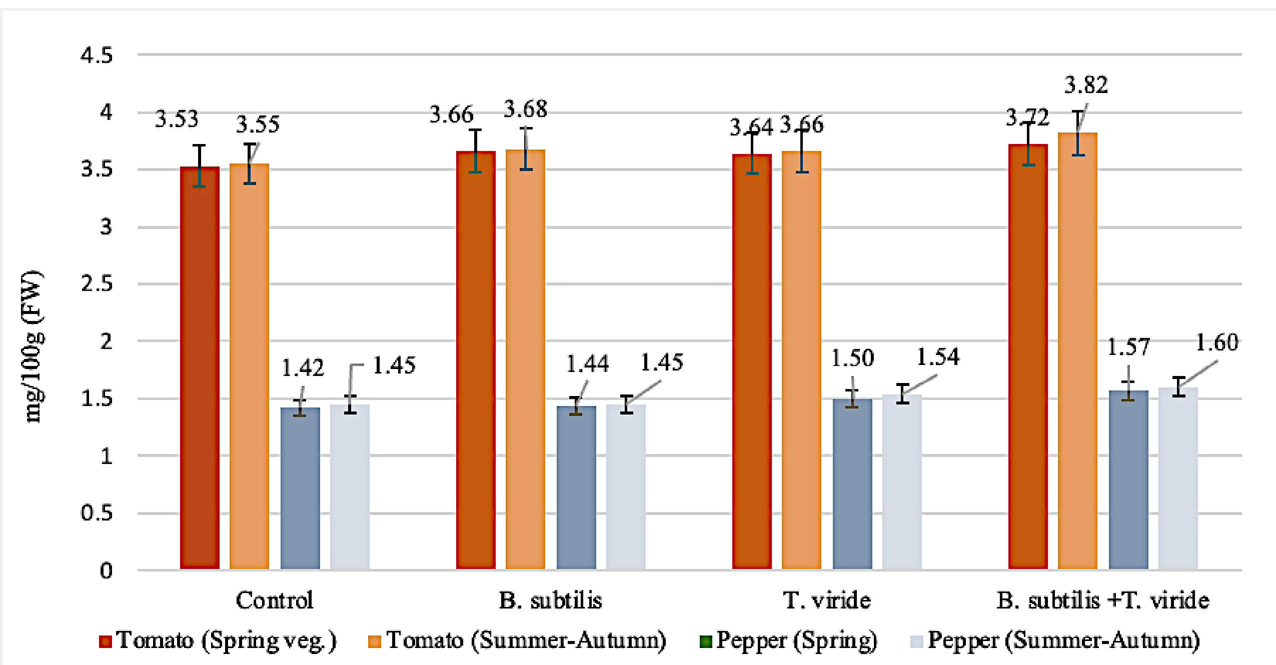


Figure 1. Total carotenoid content in tomato and sweet pepper fruits depending on the applied treatment and vegetation period (p ≤ 0.05)

Our results are consistent with current literature data on the high effectiveness of microorganisms from the genera *Bacillus* and *Trichoderma* in promoting plant growth and overall development.

Several authors have reported that *Bacillus* spp. effectively colonize the rhizosphere by transitioning from spores to an active vegetative form and exert a positive influence on plants [37–38]. These aerobic, Gram-positive bacteria employ multiple plant growth-promoting mechanisms, including the production of siderophores that facilitate iron acquisition and mobilization, solubilization of poorly available nutrient forms (such as nitrogen and phosphorus), synthesis of phytohormones and volatile organic compounds, biofilm formation, and the production of compounds that protect plants against abiotic stress. Collectively, these processes contribute to enhanced root system development, improved nutrient uptake, and activation of metabolic functions, ultimately resulting in accelerated plant growth and increased productivity [36, 43].

Fungi of the genus *Trichoderma* are free-living microorganisms widely distributed in the soil and on plant roots, where they can act as both symbionts and mycoparasites. *Trichoderma* spp. actively promotes plant growth and seed germination through the production of phytohormones and enzymes such as indole-3-acetic acid (IAA), gibberellin (GA_3), and ACC deaminase. These compounds stimulate root system development, increase the absorptive surface area of roots, and thereby improve plant nutrient uptake.

In addition, *Trichoderma* produces a range of secondary metabolites, including harzianic acid, which has iron-chelating properties that enhance iron availability and assimilation by plants. These mechanisms contribute to enhanced plant growth, development, and productivity, while also increasing plant tolerance to environmental stresses [43].

Particular attention in the present study should be given to the positive impact of microbiological treatments on the biochemical composition of fruits, especially in terms of enhancing their antioxidant activity. Our results clearly demonstrate that the application of *B. subtilis* and *T. viride* significantly increases the content of antioxidant compounds.

The most pronounced improvements were observed in the combined treatment with *B. subtilis + T. viride*. In tomatoes, the content of ascorbic acid increased by 9.3–13.3%, total phenolic compounds by 27.9–34.2%, and carotenoids by 5.4–7.6%, depending on the growing season. In sweet pepper, phenolic compounds increased by 24.5–25.7%, vitamin C by 9.7–9.9%, and carotenoids by 10.3–10.6%.

The obtained results confirm the antioxidant effect of microbial preparations, which not only improves the nutritional value of the produce but may also enhance plant resistance to various stress factors. The synergistic effect observed under combined inoculation is likely associated with the induction of secondary metabolite synthesis, including phenolics, ascorbate, and carotenoids.

Similar mechanisms have been previously described by Patloková (2024) and Adedayo (2023), who emphasized the important role of microbial phytohormones and secondary metabolites in activating phenolic biosynthetic pathways [43–44]. Moreover, according to Zheng (2024), *Trichoderma* spp. exhibit not only antagonistic activity against phytopathogens but also induce systemic resistance in plants, including the activation of the antioxidant defense system [24].

Nitrate content and phytosanitary assessment: The application of microbiological preparations had a statistically significant effect ($p \leq 0.05$) on reducing nitrate content in tomato and sweet pepper fruits compared to the control (Figure 2).

Elevated nitrate content was recorded in the control variants and ranged from 128.9 to 130.7 mg/kg for tomatoes and from 159.3 to 170.7 mg/kg for sweet peppers, depending on the growing season. The lowest nitrate levels were observed with the combined application of *B. subtilis*+ *T. viride*, reaching 54.3–74.6 mg/kg in tomatoes and 109.1–125.1 mg/kg in peppers, indicating a pronounced synergistic effect of the microbial treatment.

All obtained values were within the established sanitary and hygienic standards, confirming the safety of the produce for consumption.

The reduction in nitrate accumulation is likely due to improved mineral nutrition and enhanced nitrogen uptake efficiency, attributed to active microbial transformation of nitrogen forms in the rhizosphere. In addition, these effects may be associated with the induction of plant anti-stress mechanisms, leading to metabolic optimization and decreased nitrate residues [36].

Alongside improvements in biochemical parameters and reduced nitrate accumulation, microbial treatments also had a positive impact on the phytosanitary condition of plants. In particular, under natural infection conditions, a reduction in tomato and pepper infestation by major soil-borne pathogens - *Fusarium oxysporum* and *Verticillium dahlia* - was observed (Table 5).

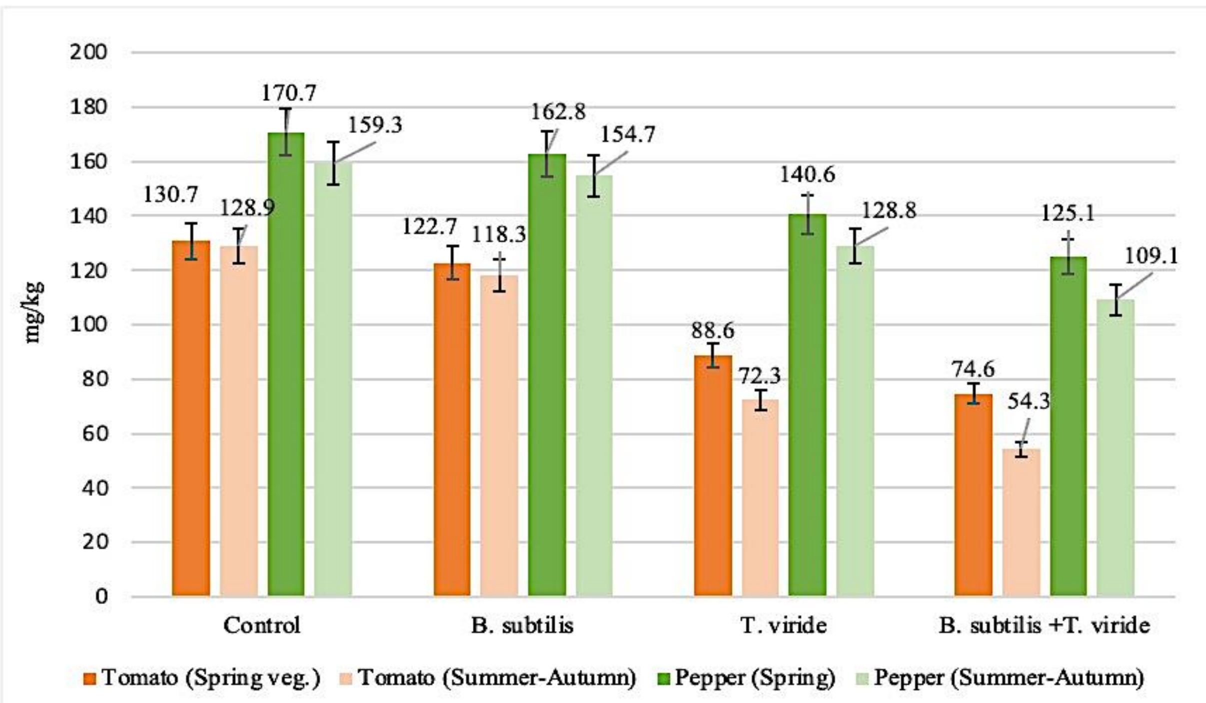


Figure 2. Nitrate content in tomato and sweet pepper fruits (p ≤ 0.05)

Table 5. Phytosanitary assessment of plants depending on the applied treatment

N	Treatment	Percentage of infected plants							
		2024		2025		2024		2025	
		Verticillium wilt (*V. dahliae*)				Fusarium wilt (*F. oxysporum*)			
		Tomato	Pepper	Tomato	Pepper	Tomato	Pepper	Tomato	Pepper
1	Control	12.9	17.5	18.4	20.5	22.4	0	28.2	0
2	*B. subtilis*	10.8	13.0	6.1	8.2	17.7	0	10.6	0
3	*T. viride*	8.1	12.3	3.7	7.5	15.2	0	9.4	0
4	*B. subtilis + T. viride*	7.0	9.3	2.5	4.4	10.5	0	5.3	0

Verticillium wilt incidence in tomatoes decreased from 12.9% in the control (2024) to 10.8% in 2024 and further to 6.1% in 2025 with *B. subtilis* treatment, demonstrating a clear positive effect over time. Similarly, Fusarium wilt incidence decreased from 22.4% in the control to 17.7% in 2024 and 10.6% in 2025.

Treatment with *T. viride* was even more effective, reducing Verticillium wilt incidence from 12.9% (control, 2024) to 8.1% in 2024 and 3.7% in 2025. Fusarium wilt rates dropped from 22.4% to 15.2% in 2024 and 9.4% in 2025. These results suggest that *T. viride* may be more effective than B. subtilis when applied individually.

The most pronounced disease reduction was observed with the combined application of *B. subtilis+T. viride*, where Verticillium wilt incidence decreased from 12.9% (control, 2024) to 7.0% in 2024 and 2.5% in 2025. Fusarium wilt declined from 22.4% to 10.5% in 2024 and 5.3% in 2025. This synergistic effect highlights the benefit of using a combined microbial approach for improved phytopathogen management.

In sweet pepper plants, Verticillium wilt infection was 17.5% in the control group in 2024 and increased slightly to 20.5% in 2025. Application of *B. subtilis* lowered infection levels to 13.0% and 8.2%, respectively, while *T. viride* further reduced disease incidence to 12.3% in 2024 and 7.5% in 2025. The greatest improvement was again achieved with the combined treatment, where the incidence dropped from 17.5% (control, 2024) to 9.3% in 2024 and from 20.5% (control, 2025) to 4.4% in 2025.

Notably, no cases of Fusarium wilt were detected in sweet pepper plants under any treatment or in the control throughout the experiment. This may reflect the inherent resistance of the pepper cultivar, as well as the protective influence of the microbiological treatments.

To enhance the accuracy of phytosanitary diagnostics, molecular identification of phytopathogens was performed using qPCR with species-specific primers. Analysis of plant tissue samples allowed for the detection of *V. dahliae* and *F. oxysporum* DNA, thereby eliminating the possibility of false-negative results.

The use of qPCR enabled the detection of pathogens at early infection stages, including latent infections that do not exhibit external symptoms, thus providing a more accurate and timely phytosanitary assessment. The high sensitivity of the method (up to 10^2 DNA copies per gram of soil or plant material) allowed for the detection of even minimal pathogenic loads in the rhizosphere.

In our study, the high efficiency of the qPCR method for detecting *V. dahliae* and *F. oxysporum* in samples was confirmed. For *V. dahliae*, the amplification (E) was 98.7%, the slope of the standard curve was –3.514, and the coefficient of determination (R^2) was 0.996. For *F. oxysporum*, the corresponding values were: efficiency E- 98.4%, slope –3.510, and R^2 - 0.994. These parameters fall within the accepted normative ranges, indicating optimal reaction conditions and high method reliability. The obtained data are consistent with other studies demonstrating that qPCR exhibits high sensitivity and specificity in detecting phytopathogens in plant and soil samples [45].

The results obtained in our study are in full agreement with previously published findings confirming the bioprotective and growth-promoting activity of microbial agents. Trichoderma spp. are well known as powerful inducers of systemic resistance and plant growth stimulators due to the synthesis of phytohormones and secondary metabolites [44]. *Bacillus subtilis*, in turn, contributes to biocontrol through the production of lipopeptides, antibiotics, and signaling molecules [46], which aligns with the observed reduction in Fusarium and Verticillium infections in our experiments.

Earlier, Choudhary and Johri (2009) emphasized that successful colonization of plant roots by probiotic microorganisms is a prerequisite for both biocontrol efficacy and plant growth promotion. He noted that *Bacillus* spp. not only actively colonize the rhizosphere and form microcolonies in zones of root exudation, but also play a crucial role in the activation of induced systemic resistance (ISR), providing protection against a wide range of pathogens, including fungal, bacterial, and viral diseases. Furthermore, the author highlighted the potential of *Bacillus* spp. to protect tomato and pepper crops from root rots, blossom-end rot, nematodes, and foliar phytopathogens, which fully corresponds with our findings [47].

Recent studies by Wu et al. (2024) demonstrated that *B. subtilis* induces the formation of polyphenolic metabolites with fungicidal properties [48], while Harish et al. (2023), using KEGG pathway mapping, confirmed the activation of antifungal metabolic pathways [49].

Research by Harman et al. (2004) significantly expanded our understanding of the mechanisms underlying the action of *Trichoderma* spp. as symbiotic rather than merely antagonistic organisms. The author showed that specific strains of *Trichoderma* can trigger both local and systemic immune responses in plants, analogous to systemic acquired resistance (SAR) and rhizobacteria-induced systemic resistance (ISR). These effects are mediated by signaling peptides, proteins, and low-molecular-weight compounds that activate plant defense gene expression. Furthermore, *Trichoderma* spp. are capable of forming stable symbiotic associations with plant roots, penetrating surface root cells, and inducing complex metabolic changes that promote plant growth, productivity, and tolerance to abiotic stresses [50].

The study by Behiry (2023) highlights the high efficacy of *Trichoderma* against *Rhizoctonia solani*, one of the main pathogens responsible for tomato root rot, along with *Fusarium oxysporum* and *Verticillium dahliae*. According to the author, the disease index in Trichoderma-treated plants was only 16.0%, compared to 78.7% in the untreated control, indicating a pronounced protective effect of the biopreparation [51]. These findings are consistent with our results, where treatment with *T. viride* reduced tomato infection by *F. oxysporum* from 22.4% (control) to 9.4% and by *V. dahliae* from 12.9% to 3.7%, corresponding to a decrease of 58.0% and 71.3%, respectively.

It is also worth emphasizing the potential of *Trichoderma viride* in the control of viral plant diseases. According to Aseel, D.G. (2023), foliar application of this strain on potato plants led to significant improvement in morphophysiological parameters and a substantial reduction in the concentration of potato virus Y in plant tissues [52].

Although numerous studies have examined the individual use of *Bacillus subtilis* and *Trichoderma viride*, research on their combined application under greenhouse conditions for crops like tomato and sweet pepper remains limited. This study demonstrates that dual treatment improves yield, reduces nitrate accumulation, and enhances the phytosanitary condition of plants, offering a novel and sustainable microbiological approach. It is the first study conducted in Armenia to investigate the combined use of PGPR and PGPF locally.

The unique integration of molecular, biochemical, and phenological data provides a comprehensive evaluation of treatment efficacy. Thus, the collective data obtained in this study indicate the high efficacy and promising potential of *B. subtilis* and *T. viride* both as individual strains and in combination. The observed synergistic effect from their joint application underscores the rationale for developing integrated biological agents aimed at plant protection and growth, aligned with the principles of sustainable agriculture.

CONCLUSION

The application of microbial complexes based on *Bacillus subtilis* and *Trichoderma viride* demonstrated a comprehensive positive effect on the growth, development, and productivity of tomatoes and sweet peppers under protected cultivation conditions. Accelerated phenological phases, extended fruiting periods, increased early and total yields, as well as improved fruit quality were observed. The use of these biopreparations also contributed to a reduction in nitrate content in the harvest, enhancing its nutritional and ecological value. A significant decrease in plant infection by *Fusarium oxysporum* and *Verticillium dahliae* indicates the strong antagonistic activity of the treatments and their potential for use in biological crop protection.

These findings confirm the promising potential of PGPR/PGPF approaches as sustainable alternatives to chemical agents in both intensive and organic farming systems. Nevertheless, to fully unlock the capabilities of microbial agents, further field trials and technology adaptations to diverse agroclimatic conditions are required.

List of abbreviations: Scientific Centre of Vegetable and Industrial Crops - SCVIC, MEofRA – Ministry Economy of the Republic of Armenia**,** PGPR**-** Plant Growth-Promoting Rhizobacteria**,** PGPF- Plant Growth-Promoting Fungi, TR - technical ripening, BR- biological ripening, g- gram, mg- milligram, FW- fresh weight.

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Authors' contributions: IV and GS conceived and designed the study. IV, HM, and ZH performed the biochemical analyses. IV and ZH interpreted the biochemical and statistical results. AS and GD conducted the literature review and references. AP, MZ and MD were responsible for the preparation of tables, graphical materials. GS and AV edited the manuscript. DS carried out ANOVA using statistical software. All authors read and approved the final version of the manuscript.

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