Journal of Agriculturl Science and Technology (JAST), 29(4)
In Press, Pre-Proof Version

Cultivar-Dependent Suppression of Tomato Leaf Curl Palampur Virus by Foliar-Applied *Trichoderma zelobreve* and *Bacillus subtilis* in Tomato

Roya Kazemi[1], Naser Safaie[1], Mohammad Reza Atighi[1], and Masoud Shams-Bakhsh*[1]

ABSTRACT

Tomato (*Solanum lycopersicum* L.) is a globally important vegetable crop whose quality and yield are affected by tomato leaf curl Palampur virus (ToLCPalV; *Begomovirus solanumpalampurense*). This study investigated the effectiveness of foliar applications of *Trichoderma zelobreve* T20 and *Bacillus subtilis* 6m, applied individually and in combination, in enhancing resistance of two tomato cultivars, Moneymaker and Tabas, to ToLCPalV under greenhouse conditions. Although responses varied considerably with cultivar and application timing, *B. subtilis* consistently reduced relative viral DNA accumulation in both cultivars. In Moneymaker, *T. zelobreve* was the most effective treatment, reducing ToLCPaLV DNA accumulation by up to 99.9%. By comparison, *B. subtilis* reduced viral levels by up to 89.2% in Tabas relative to virus-inoculated controls. The combined treatment did not improve protection in Moneymaker and offered only limited additional benefit in Tabas. Induced resistance was associated with increased antioxidant enzyme activity, including guaiacol peroxidase, polyphenol oxidase, and catalase, as well as increased proline accumulation, maintenance of chlorophyll content, and improved plant growth in plants treated with either *T. zelobreve* or *B. subtilis*, compared with infected controls. Re-isolation of *T. zelobreve* and *B. subtilis* from newly emerged upper leaves after spraying indicated their presence in distal tissues and suggested a possible contribution to systemic defense responses. These results highlight the potential of foliar microbial priming as a sustainable strategy for managing ToLCPalV, with efficacy dependent on host genotype and treatment timing.

Keywords: Antioxidant enzymes, Microbial priming, Symptom severity, Viral DNA accumulation.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide due to its nutritional value, economic importance, and extensive cultivation (Chethan *et al*., 2026). It is the second most widely cultivated vegetable after the potato (Panno *et al*., 2021). Among the viral diseases affecting tomato production, tomato leaf curl Palampur virus (ToLCPalV;

[1] Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University (TMU), Tehran, Islamic Republic of Iran.
*Corresponding author; e-mail: shamsbakhsh@modares.ac.ir

Begomovirus solanumpalampurense) is an important pathogen belonging to the genus *Begomovirus* within the family *Geminiviridae*. This bipartite begomovirus infects a wide range of plant hosts across diverse agroecological regions of Asia and is primarily transmitted by the whitefly vector *Bemisia tabaci*, resulting in substantial economic losses (Kumar et al., 2008; AlHudaib *et al*., 2022; Sattar *et al*., 2025).

Management of viral diseases largely depends on resistant cultivars and intensive insecticide applications against insect vectors. However, these approaches have limitations due to environmental risks, pesticide residues, and the development of resistance in pest populations (Abdelkhalek *et al*., 2021). Therefore, environmentally sustainable approaches, including the use of biological control agents (BCAs), have gained increasing attention as alternatives to chemical-based strategies (Alengebawy *et al*., 2021; Abdelkhalek *et al*., 2020a). Microbial BCAs suppress plant pathogens through both direct antagonistic activity and indirect mechanisms, including the induction of systemic resistance and the promotion of plant growth (Thambugala *et al*., 2020; Ayaz *et al*., 2023).

Species belonging to the genus *Bacillus* are among the most widely studied bacterial BCAs because of their environmental adaptability, spore-forming ability, and production of diverse antimicrobial compounds. These bacteria produce lipopeptides, antibiotics, and hydrolytic enzymes that inhibit pathogens while enhancing plant growth and defense responses (Jacobsen *et al*., 2004; Miljaković *et al*., 2020). Several *Bacillus* species have demonstrated effectiveness against tomato pathogens under greenhouse and field conditions, supporting their potential for sustainable disease management (Karačić *et al*., 2024). Similarly, *Trichoderma* species are beneficial fungi that promote plant health through multiple mechanisms, including mycoparasitism, competition, production of antimicrobial metabolites, and activation of plant defense pathways (Li *et al*., 2025). Their protective and growth-promoting effects have been reported in tomato production systems under different environmental conditions (Ivezić *et al*., 2025).

Foliar application provides an efficient strategy for delivering BCAs directly to aerial plant tissues and has shown promising effects against foliar-infecting viruses (Chien and Huang, 2020; Lee and Ryu, 2016). Previous studies have demonstrated that foliar application of *B. subtilis* reduced the severity of tomato yellow leaf curl virus (TYLCV) and tobacco mosaic virus (TMV) infections by enhancing host defense responses (Ghanem *et al*., 2022; Gendi *et al*., 2022). In addition, the

combined use of seed priming and foliar application of *T. viride* reduced the severity of Okra yellow vein mosaic virus and improved plant yield (Yadav *et al*., 2022). These findings underscore the promise of microbe-based strategies for enhancing antiviral resistance in crops.

Beneficial microorganisms can enhance antiviral defense by modulating hormone-associated pathways, including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling. Although *Bacillus* and *Trichoderma* species have been extensively investigated for their roles in plant growth promotion and induced resistance against various pathogens, their effects against specific begomovirus–tomato pathosystems remain insufficiently characterized. In particular, the responses of ToLCPalV-infected tomato plants to foliar application of *B. subtilis and T. zelobreve,* including their individual and combined effects on viral accumulation, physiological performance, and defense-related responses, have not been investigated.

Therefore, this study aimed to evaluate the effects of foliar-applied *B*. *subtilis* 6m and *T*. *zelobreve* T20, individually and in combination, on ToLCPalV infection in two tomato cultivars. The optimal timing of application for disease suppression was determined, and physiological responses, antioxidant enzyme activities, and defense mechanisms associated with microbial-mediated resistance were investigated. This study provides new insights into the interaction between beneficial microorganisms, viral infection dynamics, and host defense activation in tomato plants.

MATERIALS AND METHODS

Plant Material and Sources of Biocontrol Agents

Seeds of two tomato cultivars (*S. lycopersicum* L. cv. Moneymaker and Tabas) were obtained from the Field Crops Development Company (FCDCO, Iran). Plants were cultivated in sterile pots containing a 2:1 mixture of double-autoclaved soil and sand, and maintained in an insect-free greenhouse under controlled conditions (25 ± 2 °C, 16 h light/8 h dark photoperiod). The biocontrol agents *T. zelobreve* T20 (OP131226.1) and *B. subtilis* 6m (KU667092) KU667092 were kindly provided by Dr. Safaie (Tarbiat Modares University, Tehran, Iran).

Preparation of Biocontrol Agent Suspensions

T. zelobreve T20 was cultured on potato dextrose agar (PDA) at 27 °C for 4–5 days until sporulation. Spores were collected by gently scraping the culture surface, suspended in sterile distilled water, and centrifuged at 6,000 rpm for 15 min. The pellet was resuspended, and spore concentration was adjusted to 10^9 CFU mL^{-1} using a hemocytometer. *B. subtilis* 6m was grown in

Luria–Bertani (LB) broth at 28 °C for 48 h with continuous shaking. Bacterial cells were pelleted by centrifugation, washed with sterile distilled water, and diluted to a final concentration of 10^6 CFU mL^{-1} (OD_{600} = 0.35). For the combined treatment, individual suspensions were prepared as described and mixed to achieve the desired final concentrations in a total volume of 40 mL. All treatment suspensions were gently mixed on a shaker at 100 rpm for 1 h at room temperature prior to application. The conidial concentration was calculated based on the average number of conidia counted in the hemocytometer chambers using the following formula:

Conidial concentration (conidia mL^{-1}) = Average number of conidia counted × 50 × 1000. The suspension was then adjusted to 1×10^9conidia mL^{-1} before application.

Experimental Design

First Experiment

A factorial experiment was conducted based on a completely randomized design (CRD) with 12 biological replicates. Each plant grown in an independent pot was considered one experimental unit. The experiment included four factors: tomato cultivar (Moneymaker and Tabas), biological treatment, virus inoculation, and application timing. Biological treatments consisted of control (no biological treatment), foliar application of *T. zelobreve* (T; 5 mL at 10^9 CFU mL^{-1}), and foliar application of *B. subtilis* (B; 5 mL at 10^6 CFU mL^{-1}, OD_{600} = 0.35). Virus-inoculated treatments were indicated by adding the suffix "V" to treatment abbreviations (e.g., TV and BV), while treatments without the suffix "V" represented non-inoculated plants. In the first experiment, application timing was treated as an independent experimental factor. Foliar applications of the biological agents were made at four time points: 24 h before virus inoculation (24 hbi) and 24, 48, and 96 h post-inoculation (hpi). Each combination of biological treatment, virus status, and application timing included 12 biological replicates for each tomato cultivar.

Second Experiment

Based on the results of the first experiment, a second experiment was conducted using a CRD with four biological treatments and 12 biological replicates per treatment for each tomato cultivar. Treatments included untreated control plants, *T. zelobreve* T20 (T; 5 mL $plant^{-1}$ at 10^9 CFU mL^{-1}), *B. subtilis* 6m (B; 5 mL $plant^{-1}$ at 10^6 CFU mL^{-1}, OD_{600} = 0.35), and the combined application of both agents (BT; 5 mL $plant^{-1}$ at final concentrations of 10^6 and 10^9 CFU mL^{-1} for *B. subtilis* and *T. zelobreve*, respectively). Foliar applications were performed at 96 hpi, which was selected based

on the optimal disease suppression observed in the first experiment. Virus-inoculated treatments were indicated by adding the suffix "V" (e.g., TV, BV, and BTV).

Independent Replication and Data Pooling

Both experiments were independently repeated twice under identical greenhouse conditions. The resulting data were pooled after decomposition analysis in Minitab 18.1 to assess potential effects of experimental run and treatment × experiment interactions. Because no significant interaction was detected, the datasets were combined for the final statistical analysis and graphical presentation.

Virus Inoculation and Disease Severity Assessment

Infectious clones of ToLCPalV, comprising DNA-A (GenBank accession no. JQ825226) and DNA-B (GenBank accession no. FJ660423), were generously provided by Dr. Heydarnejad (Shahid Bahonar University of Kerman, Iran). These viral components were maintained separately in *Agrobacterium tumefaciens* strain C58 and subsequently used for agroinoculation. An infectious *Agrobacterium tumefaciens* C58 culture carrying ToLCPalV DNA-A and DNA-B was prepared by shaking at 28 °C for 24 h in Luria Broth containing 50 µg mL^{-1} each of kanamycin and rifampicin (Heydarnejad et al., 2013). The DNA-A and DNA-B bacterial cultures were adjusted separately to OD_{600} = 0.2, and equal volumes of the two suspensions were mixed immediately before inoculation. The mixed bacterial suspension was centrifuged at 6000 rpm for 10 min, and the resulting pellet was resuspended in sterile distilled water containing acetosyringone (50 mM). The prepared inoculum was incubated at room temperature on a shaker for 1 h and then transferred to the greenhouse on ice. A 100 µL volume of the inoculum was syringe-infiltrated into 30-day-old tomato seedlings at the two-true-leaf stage using an insulin syringe. Inoculation was performed at three sites on a single fully expanded leaf of each plant. Mock-inoculated plants were infiltrated with *A. tumefaciens* C58 carrying the empty vector without ToLCPalV DNA-A and DNA-B constructs. Following inoculation, plants were maintained in insect-free greenhouse conditions at 25 ± 2 °C.

Disease severity was rated on a 0–8 scale, where 0 = no visible symptoms and 8 = extreme leaf twisting, severe mosaic symptoms, marked leaf-area reduction, and severe stunting. Intermediate scores represented progressive increases in symptom severity. Disease symptoms were monitored during the infection period, and the final disease severity assessment was performed at 44 days

post-inoculation (dpi). The symptom severity index (SSI) was calculated from the recorded symptom scores at 44 dpi, following the method described by Kazemi et al. (2026). Because severity data are ordinal, they were rank-transformed and analyzed using the nonparametric methods described by Shah et al. (2004).

Assessment of Leaf Colonization by Biocontrol Agents

At the end of the experiment (40 days post-spray), upper leaves that had not been directly sprayed were collected to assess systemic colonization. Leaves were surface-sterilized by sequential immersion in 70% ethanol (1 min) and 96% ethanol (30 s), rinsed with sterile distilled water, cut into ten segments, and cultured on PDA and LB agar for recovery of *T. zelobreve* and *B. subtilis*, respectively.

Relative Quantification of Viral DNA by QPCR

Total genomic DNA was extracted from leaf tissues at 40 days post-spray using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Viral accumulation was quantified by real-time quantitative PCR (qPCR) using SYBR Green PCR Master Mix on an ABI StepOne System. Primers targeting a 122-bp region of the ToLCPalV DNA-B component were used (Sinaclon, Tehran, Iran). ToLCPalV is a bipartite begomovirus containing DNA-A and DNA-B components. In this study, a conserved region of DNA-B was selected as the target for qPCR analysis to evaluate systemic viral DNA accumulation in newly developed tissues distant from the inoculation site. DNA-B encodes movement-associated proteins essential for both cell-to-cell and systemic movement of begomoviruses. Therefore, the detection and quantification of DNA-B in newly emerged tissues indicate successful viral dissemination and accumulation throughout the plant. Each treatment included four independent biological replicates, each with two technical replicates. Relative viral DNA levels were calculated using the comparative ΔΔCt method, with the tomato *SlFRG27* gene (encoding ubiquitin-conjugating enzyme E2) as the internal reference. For each sample, ΔCt was calculated as the difference between the Ct value of the ToLCPalV DNA-B target and the Ct value of *SlFRG27*. The ΔΔCt value was determined by subtracting the mean ΔCt value of virus-inoculated untreated control plants (calibrator) from the ΔCt value of each sample. Finally, relative viral DNA accumulation was expressed as fold change using the equation $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). Primer sequences are provided in Kazemi *et al*., (2026).

Gene Expression Analysis

Total RNA was isolated using the Dena zist kit with DNase treatment. cDNA synthesis was performed with the Sinaclon (Sinaclon, Tehran, Iran). RT-qPCR quantified defense-related genes *SlPI-II* (Jasmonic acid marker)(Cheng *et al*., 2017), *SlPTI4* (Ethylene marker)(Di *et al*., 2017), and *SlWRKY70* (salicylic acid marker)(Azaryan *et al*., 2022), normalized to *SlFRG27*(Liu *et al*., 2019). Three biological replicates, each with two technical replicates, were analyzed at 48 hours post-spray. The list of primers is given in the Supplementary Table S1.

Quantitative PCR (qPCR) reactions were performed using RealQ SYBR Green PCR Master Mix (Amplicon A/S, Odense, Denmark) on an ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). RT-qPCR was performed in a 10 µL reaction mixture consisting of 5 µL RealQ SYBR Green PCR master mix (Amplicon A/S, Denmark), diluted cDNA template, and 1 µL (10 µM) of each gene-specific primer (Supplementary Table S1). Amplifications were performed using the following program: initial denaturation at 94 $^{\circ C}$ for 3 min, followed by 50 cycles of denaturation at 94 $^{\circ C}$ for 30 s, annealing at 58 $^{\circ C}$ for 25 s, and extension at 72 $^{\circ C}$ for 20 s. Melting curve analysis was performed from 65 $^{\circ C}$ to 95 °C with a ramp rate of 0.5 °C/s, with continuous fluorescence acquisition at each step.

Growth and Physiological Parameter Assessment

At 40 days post-spray, growth parameters were assessed from 12 plants per treatment across two independent experiments. Fresh weight, shoot length, and leaf area were measured, and dry biomass was determined after oven-drying samples at 70 °C for 48 h. In addition, leaf chlorophyll content was evaluated using a SPAD-502 chlorophyll meter at the same time point.

Antioxidant Enzyme Assays

Leaf samples were collected at 24, 48, and 72 h post-spray, and at 40 days post-spray. Activities of polyphenol oxidase (PPO), guaiacol peroxidase (GPX), and catalase (CAT) were assayed spectrophotometrically using a microplate reader (Epoch, BioTek, USA). PPO activity was measured at 420 nm (Siguemoto *et al*., 2017), GPX at 470 nm, and CAT at 240 nm (Maehly and Chance, 1954). For each assay, 100 mg of fresh leaf tissue was homogenized under standardized conditions. Four biological replicates were used, each with two technical replicates (Kazemi *et al*., 2026).

Statistical Analysis

All statistical analyses were conducted using SAS 9.2 software (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was performed using the GLM procedure, and normality of residuals was assessed with the UNIVARIATE procedure. SSI is an ordinal measure; therefore, disease severity data were rank-transformed prior to analysis. The rank-transformed SSI data were analyzed using the GLM procedure, and treatment means were compared using Fisher's least significant difference (LSD) test at $P \leq 0.05$. This procedure was used for the disease severity data presented in Figures 1 and 2. All graphs were generated using GraphPad Prism software (Version 10.0, GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Cultivar-Dependent Temporal Effects

Symptom severity was assessed at 24 h before inoculation (24 hbi) and at 24, 48, and 96 h post-inoculation (24, 48, and 96 hpi) to evaluate disease progression. Results revealed significant cultivar-dependent differences, with Moneymaker showing lower symptom severity and faster recovery compared to Tabas, which maintained higher severity under virus-only (V) treatment. Biocontrol treatments (BV and TV) significantly reduced symptom severity compared to V control. BV treatment showed biphasic dynamics in both cultivars, with an initial increase in severity followed by a significant reduction at 48 hpi in Moneymaker and at 96 hpi in Tabas, consistent with enhanced plant defense responses following microbial treatment. TV treatment consistently maintained lower severity across all time points (24, 48, and 96 hpi), consistent with a sustained reduction in symptom severity (Figure 1). Overall, SSI analysis showed that both BV and TV significantly reduced disease severity compared with infected controls ($P < 0.05$), suggesting a possible priming effect that varied according to cultivar and time of assessment. Visual assessments supported these results, showing typical ToLCPalV symptoms in infected plants, while primed plants exhibited milder symptoms and improved growth, particularly in Moneymaker compared to Tabas (Figure S4).

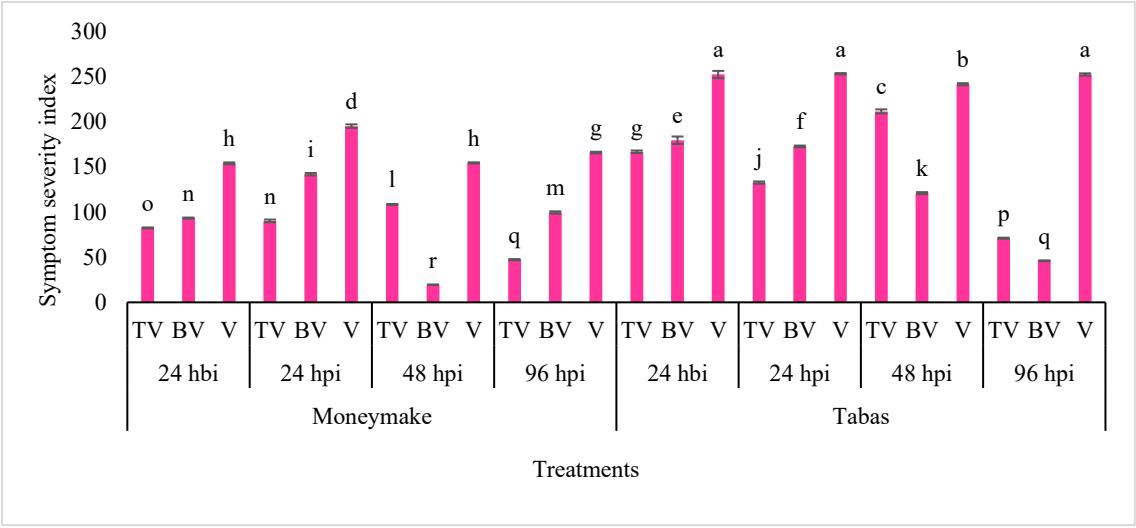

Figure 1. Symptom severity index (SSI) in tomato cultivars Moneymaker and Tabas at 44 dpi with ToLCPalV. Thirty-day-old plants (two-true-leaf stage) were agroinoculated with ToLCPalV. Treatments: V (virus-inoculated control); biocontrol treatments (foliar spray + ToLCPalV). Values represent means ± SE (n = 12). Different letters indicate significant differences (LSD test, $P < 0.05$).

Cultivar-Specific Biocontrol Efficacy

Second experiment: SSI was significantly affected by biocontrol treatments in both cultivars (Figure 2). In Moneymaker, *T. zelobreve* treatment (TV) resulted in the lowest SSI, followed by *B. subtilis* treatment (BV), and both treatments significantly reduced symptom severity compared with the virus-inoculated control (V). In contrast, the combined application (BTV) showed the highest SSI among microbial treatments and did not enhance disease suppression in this cultivar. In Tabas, the virus-inoculated control exhibited the highest SSI, whereas *B. subtilis* treatment (BV) resulted in the lowest SSI, followed by *T. zelobreve* treatment (TV). The combined application (BTV) showed an intermediate response but was less effective than individual microbial treatments. These findings demonstrate that the efficacy of biocontrol agents in reducing disease symptoms was cultivar-dependent. Symptom severity by individual and combined applications relative to viral control revealed that both the extent of symptom alleviation and biocontrol efficacy were significantly influenced by host cultivar, indicating genotype-specific interactions between plants and applied biostimulants. To facilitate interpretation of treatment efficacy, the percentage reduction in symptom severity relative to the virus-inoculated control was calculated for each treatment and displayed above the corresponding bars in the figure. These values indicate the relative decrease in disease severity achieved by each treatment in each tomato cultivar.

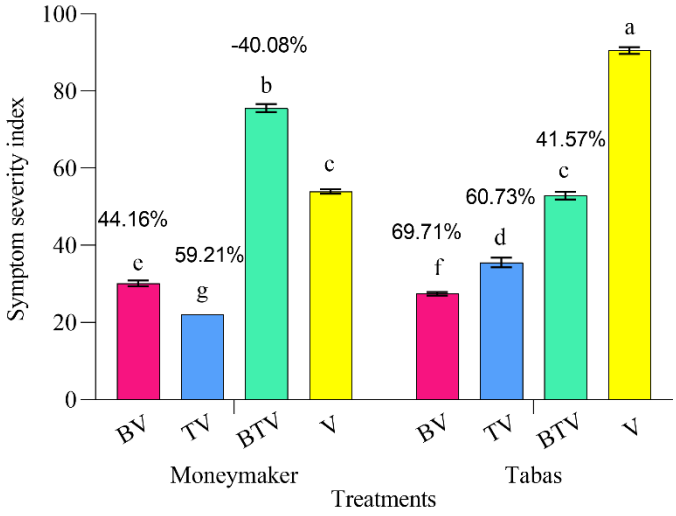

Figure 2. Symptom severity index (SSI) in tomato cultivars Moneymaker and Tabas at 44 dpi with ToLCPalV. Thirty-day-old plants (two-true-leaf stage) were agroinoculated with ToLCPalV. Biocontrol agents (*Bacillus subtilis*, BV; *Trichoderma zelobreve*, TV; and their combination, BTV) were applied as foliar spray at 96 h post-inoculation. Values represent means ± SE (n = 12). Different letters indicate significant differences (LSD test, $P < 0.05$).

Differential Viral Suppression

Quantitative PCR analysis revealed that *B. subtilis* treatment (BV) significantly reduced relative ToLCPalV DNA-B accumulation in both Moneymaker and Tabas compared to the virus-only control (V) ($P < 0.05$; Figure 3). *T. zelobreve* (TV) showed a stronger suppressive effect on ToLCPalV accumulation in Moneymaker than in Tabas, as indicated by the greater reduction in relative ToLCPalV DNA-B levels. Combined treatment (BTV) failed to enhance protection in Moneymaker, as relative ToLCPalV DNA-B accumulation remained comparable to that of the infected control. In Tabas, BTV was also less effective than either *B. subtilis* or *T. zelobreve* applied individually. In terms of relative viral DNA accumulation, *T. zelobreve* treatment reduced ToLCPalV DNA levels by up to 99.9% in Moneymaker, whereas *B. subtilis* showed the highest suppression in Tabas, reducing viral DNA accumulation by up to 89.2% compared with virus-inoculated untreated controls.

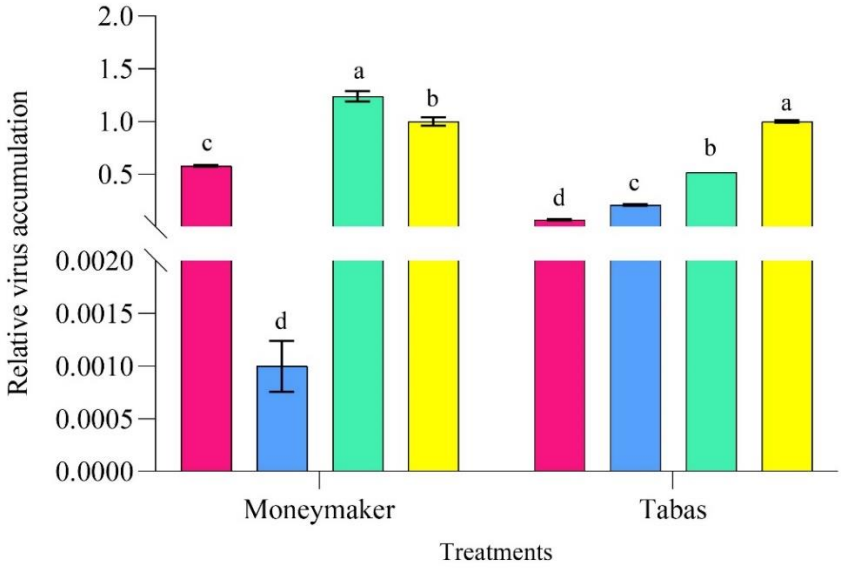

Figure 3. Relative accumulation of tomato leaf curl Palampur virus (ToLCPalV) in tomato plants under different treatments, quantified by real-time quantitative PCR (qPCR). Relative viral levels were calculated using the $2^{-\Delta\Delta CT}$ method. Data represent the mean of four biological replicates (each consisting of a pool of three individual plants), with two technical replicates per biological replicate. Means sharing the same letter are not significantly different according to the least significant difference (LSD) test at $P \leq 0.05$.

Chlorophyll Preservation and Growth Parameters

Biocontrol treatments significantly improved the physiological status of ToLCPalV-infected plants, as indicated by increased leaf greenness compared with virus-inoculated controls, particularly at 96 hpi (Figures S1 and S6). In Moneymaker, individual applications of B+V0 and T+V0 maintained SPAD values comparable to healthy controls, whereas BT+V0 exhibited moderate chlorophyll retention. Viral infection (V1) substantially diminished biocontrol efficacy, particularly in BT+V1 treatments. Tabas displayed greater resilience: T+V0 and BT+V0 treatments preserved chlorophyll content near control levels. The significant decline in SPAD values in virus-inoculated plants across both cultivars underscores severe chlorophyll degradation induced by ToLCPalV. Differential response patterns between cultivars suggest genotype-specific modulation of biocontrol-mediated physiological improvements.

Greenhouse experiments revealed that tomato plants inoculated with ToLCPaLV exhibited significant reductions in both fresh and dry weight compared with mock-treated plants. However, biocontrol treatments significantly improved these growth parameters in virus-infected plants at all evaluated time points, highlighting their potential to mitigate the detrimental effects of ToLCPalV infection (Figures S2 and S3). On the other hand, foliar application of biocontrol agents

at 96 hpi (BT, T, and B) significantly increased tomato fresh and dry weight compared with ToLCPalV-inoculated plants (Figure S7). The BT treatment in Moneymaker and the B treatment in Tabas were the most effective in increasing fresh and dry weights.

Genotype-Specific Proline Accumulation

Proline content was significantly influenced by treatment in both cultivars (Figure S9). In Moneymaker, the highest proline accumulation was observed in BT+V0, indicating that the combined application of biostimulants enhances the plant physiological response and stimulates proline biosynthesis. Treatments T+V1 and B+V1 also showed significantly higher proline levels compared to the control, suggesting that the interaction between biocontrol agents and virus inoculation is reflected in the modulation of plant stress responses. In Tabas, similar patterns emerged but with lower intensity. The highest proline level was recorded in B+V1, while BT+V1 and T+V1 showed intermediate levels. Virus-only treatment and control exhibited the lowest proline levels in Tabas, indicating reduced sensitivity. These data demonstrate: (1) combined biocontrol agents with virus inoculation exerted the most higher proline accumulation on proline increase (except in treatments showing higher symptom severity, e.g., BT+V1 in Moneymaker), likely reflecting activation of stress response and antioxidant pathways; (2) Moneymaker exhibited greater sensitivity to defense activation than Tabas, accumulating higher proline levels in response to combined treatments this appeared to correlate inversely with symptom severity; (3) virus alone, as a biotic stressor, reduced proline content compared to biocontrol-treated plants.

Antioxidant Enzyme Induction

Measurements of defense-related enzymes revealed higher guaiacol peroxidase (GPX) activity in primed, virus-infected plants compared to non-primed controls at 24, 48, and 72 h post-spray and at 40 d post-spray. Polyphenol oxidase (PPO) and catalase (CAT) activities were generally elevated in treated and infected plants relative to non-treated infected plants. These observations indicate that biocontrol agents support an association between microbial treatment and changes in antioxidant defense responses, which coincided with reduced viral DNA accumulation. In contrast, untreated Tabas exhibited persistently suppressed enzyme activity throughout the experiment, corresponding with the severe development of symptoms (Figures S9, S10, and S11).

Colonization of Leaf Tissues by Microbial Biocontrol Agents

Isolation assays indicated the presence and establishment of *B. subtilis* and *T.* zelobreve. Culturing of upper, non-sprayed leaves demonstrated that both fungal and bacterial agents, applied individually or in combination, could be recovered from tissues distant from the application site (Figure 4). Colony recovery from the upper leaves indicates that colonization extended beyond the application site. No growth was observed in the controls, confirming the effectiveness of surface sterilization and the specificity of colonization. No microbial growth was observed in control plants, supporting the effectiveness of surface sterilization and excluding external contamination. The identity of the microbial biocontrol agents used in this study has been previously verified through molecular characterization. *B. subtilis* and *T. zelobreve* strains were molecularly identified and validated in previous studies by Nourian et al. (2024) and Rosstamabadi (unpublished), respectively.

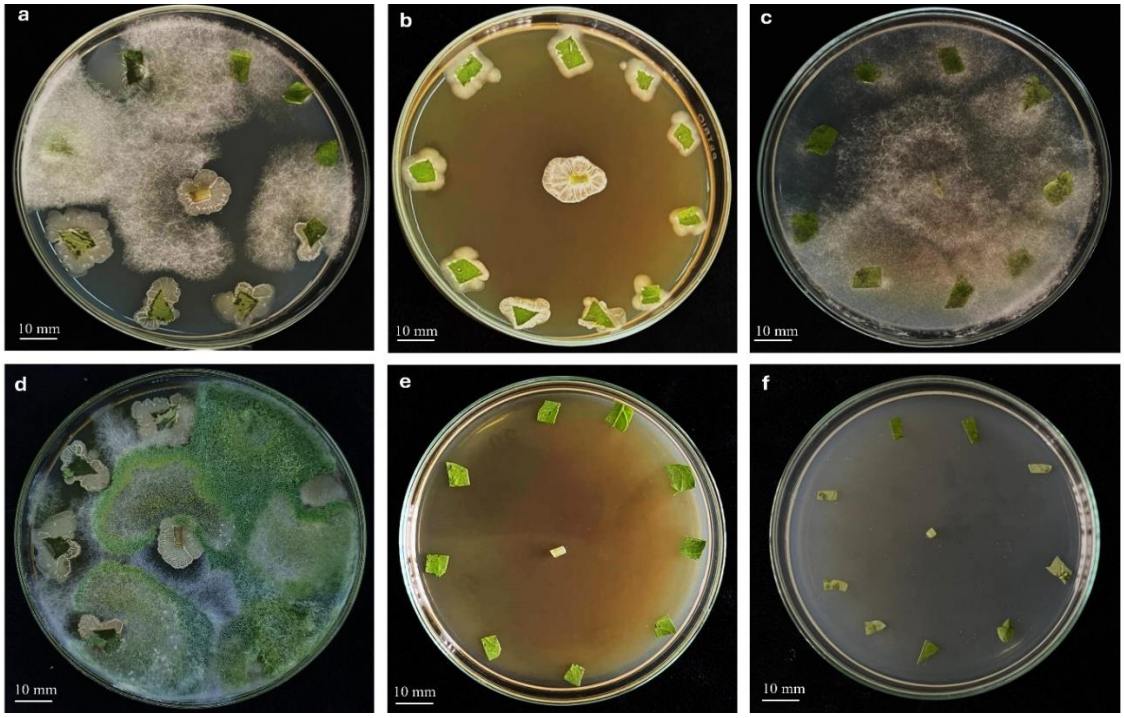

Figure 4. Assessment of microbial biocontrol agent colonization in leaf tissues following foliar application of biocontrol agents. Leaf segments were sampled at the end of the experiment from upper leaves positioned above the spray site. Treatments included (a, d) combined application (BT), (b) *Bacillus subtilis* (c) *Trichoderma zelobreve* (TZ), (e) bacterial control, and (f) fungal control. Colony outgrowth from upper leaves indicates colonization of tissues distal to the application site, whereas no microbial growth was detected in control samples, confirming the efficacy of surface sterilization. Scale bars = 10 mm.

Recovery of Biocontrol Agents from Upper Leaves

To evaluate the establishment of applied biocontrol agents, upper leaves located above the spray site were collected at the end of the experiment (40 days after foliar application). Viable colonies of the applied microorganisms were successfully recovered from all treated plants. As shown in Table S2, *Bacillus subtilis* 6m and *T. zelobreve* T20 were detected in 100% of the analyzed samples in both tomato cultivars (Moneymaker and Tabas), either when applied individually or in combination. These results indicate the persistence and establishment of the applied biocontrol agents in upper leaf tissues during the experimental period (Table S2).

Expression of Defense-Related Genes in Response to Microbial Treatments

The expression of defense-related marker genes *SlPI-II*, *SlWRKY70*, and *SlPTI4*, associated with jasmonic acid (JA)-, salicylic acid (SA)-, and ethylene (ET)-related signaling pathways, respectively, was evaluated by RT-qPCR at 48 h after treatment application. Significant differences in transcript accumulation were observed among treatments and between tomato cultivars, indicating genotype-dependent regulation of defense-related gene responses.

In Moneymaker, microbial treatments had differential effects on the expression of the defense-related genes analyzed (Fig. 5a–c). The JA-associated gene *SlPI-II* showed increased transcript accumulation in the TV treatment, while the BT treatment also exhibited elevated expression compared with non-induced treatments. The BV treatment resulted in an intermediate increase in *SlPI-II* expression, whereas the control, virus-only, and individual microbial treatments showed relatively lower transcript levels. The BTV treatment exhibited lower SlPI-II expression compared with other induced treatments.

The expression pattern of the SA-associated gene *SlWRKY70* differed among treatments. The highest transcript accumulation was detected in the BV treatment, and *B. subtilis* and *T. zelobreve* alone also enhanced *SlWRKY70* expression compared with non-treated plants. No significant difference was observed between the virus-only treatment and the control.

The ET-associated gene *SlPTI4* was strongly influenced by microbial treatments. The highest transcript levels were detected in the BTV and BT treatments, with no significant difference between these treatments. The TV0 treatment also significantly increased *SlPTI4* expression.

In Tabas, defense-related gene expression patterns differed from those observed in Moneymaker (Fig. 5 d–f). Virus inoculation alone did not result in a significant difference between the virus-

only and control treatments for *SlPI-II and SlWRKY70*. Microbial applications partially alleviated the reduction observed in *SlPI-II* expression. The BT treatment showed the highest *SlPI-II* transcript accumulation, while the BV and BTV treatments also showed increased transcript levels compared with virus-only plants.

For *SlWRKY70*, although the virus-only treatment showed the lowest transcript level, this reduction was not significantly different from the control treatment. The highest transcript accumulation was observed in the BV0 and TV1 treatments, while the BTV treatment also exhibited increased expression compared with virus-infected plants without microbial application. The ET-associated marker gene *SlPTI4* showed strong induction following microbial treatments, with the highest expression detected in the BTV treatment (Fig. 5d–f).

Overall, microbial treatments significantly modulated the expression of defense-related genes in Tabas, with the magnitude and pattern of responses varying according to the specific gene and treatment combination.

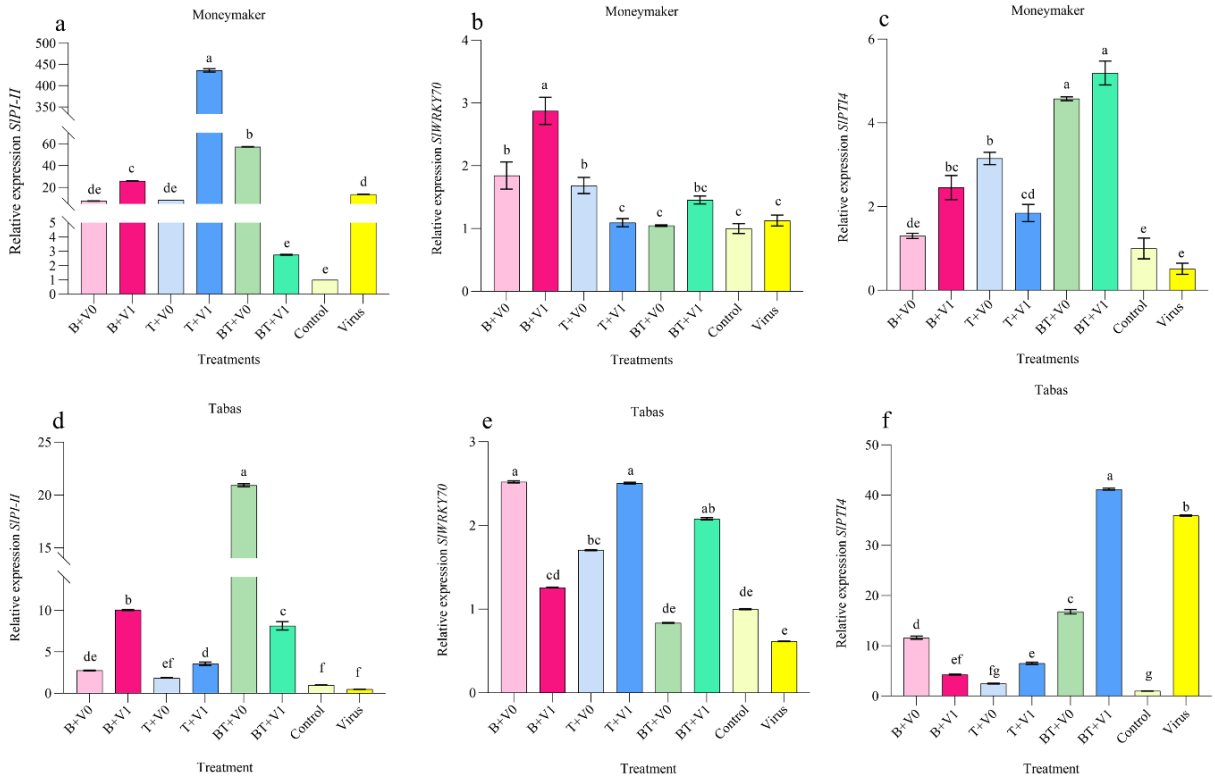

Figure 5. Results of changes in the expression of marker genes related to SA, JA, and ET phytohormones by RT-qPCR assay. a- c: in the Moneymaker cultivar, d-f: in the Tabas cultivar. Four-week-old tomato plants (at the two-true-leaf stage) were inoculated with ToLCPalV (OD600 = 0.2). Changes in the expression of defense-related genes at 48 hours post-spray with biocontrol

agents were monitored. Expression profiles of key genes, including *SlPTI4* (ET receptor gene), *SlWRKY70* (associated with the salicylic acid pathway), and *SlPI-II* (proteinase inhibitor-II (PI-II), jasmonic acid signaling pathway marker). The data were normalized using the internal control ubiquitin conjugating enzyme E2 gene. The data are presented as the mean; error bars= standard error of the mean (SEM). Three biological and two technical replicates for each treatment per time point were considered. Asterisks show a significant difference at $P < 0.05$. Treatments were designated as B = *Bacillus subtilis*, T = *Trichoderma zelobreve*, and BT = combined application. V0= non-inoculated with ToLCPalV, V1= inoculated with ToLCPalV.

DISCUSSION

This study demonstrates that foliar application of *B. subtilis* and *T. zelobreve* effectively reduces ToLCPalV accumulation in tomato and enhances plant physiological and biochemical performance, including antioxidant enzyme activity, proline accumulation, chlorophyll retention, and growth promotion. These responses, together with changes in defense-related gene expression, are consistent with enhanced plant defense responses and suggest the possible involvement of hormone-associated defense mechanisms. The reduced efficacy observed in some combined applications may not necessarily reflect antagonistic interactions between the two microorganisms. But may instead be associated with complex microbial interactions, possible competition for ecological niches, or genotype-dependent differences in plant–microbe responses. The increasing global impact of plant viral diseases highlights the need for sustainable biocontrol strategies as alternatives to chemical pesticides (Abdelkhalek *et al*., 2020b). Plant growth-promoting microorganisms are effective, environmentally friendly agents for the long-term management of plant diseases (Abo-Zaid *et al*., 2020). Consistently, previous studies have demonstrated that *Trichoderma* and *Bacillus* species activate systemic resistance, reduce viral accumulation, and enhance host defense responses under different application regimes (Abdelkhalek *et al*., 2021; Lee and Ryu,2016). Although DNA-B-based quantification provided valuable information on systemic viral DNA accumulation, ToLCPalV possesses a bipartite genome comprising DNA-A and DNA-B. Therefore, simultaneous quantification of both genomic components would provide a more comprehensive assessment of viral genome accumulation. The absence of DNA-A quantification is a limitation of the present study. Accordingly, the qPCR results should be interpreted specifically as relative changes in DNA-B accumulation in systemically developing tissues, rather than as a direct measure of viral replication or a complete assessment of total viral genome accumulation. Both *T. zelobreve* and *B. subtilis* significantly reduced symptom severity and relative ToLCPalV DNA-B accumulation in a cultivar-dependent manner, suggesting genotype-

related differences in the magnitude of plant defense responses (Van Loon, 2000; Kazemi *et al*., 2026). This aligns with previous evidence that beneficial microorganisms can reduce viral accumulation and restrict systemic virus movement through activation or modulation of host defense responses (Abdelkhalek *et al*., 2021). To further explore the molecular basis underlying these cultivar-dependent responses, the expression of defense-related marker genes associated with the jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) signaling pathways was evaluated. Microbial priming differentially modulated the expression of *SlPI-II***,** *SlWRKY70*, and *SlPTI4* in a genotype-dependent manner. Microbial treatments generally were associated with increased expression of the JA-responsive marker *SlPI-II*, although the extent of induction varied among treatments and cultivars. In Moneymaker, the strongest induction was observed following *T. zelobreve* treatment, whereas in Tabas the combined microbial treatment resulted in the highest transcript accumulation. Across treatments, higher *SlPI-II* expression generally coincided with lower disease severity, suggesting that JA-associated defense responses may be associated with the observed reduction in disease severity, consistent with previous reports highlighting the importance of JA signaling in antiviral defense (Pieterse *et al*., 2014; Martínez-Medina *et al*., 2013; Li *et al*., 2004).

In contrast, virus inoculation alone did not significantly affect *SlWRKY70* expression compared with the non-inoculated control in either cultivar, indicating that ToLCPalV infection alone was insufficient to induce this SA-associated marker at the sampling time. Nevertheless, several microbial treatments, particularly *B. subtilis*, enhanced *SlWRKY70* transcript accumulation, consistent with modulation of SA-associated defense responses following microbial treatment. Similar context-dependent regulation of SA-responsive genes has been reported previously (Pieterse *et al*., 2014).

The ET-responsive marker *SlPTI4* was also markedly affected by microbial treatments, with the highest transcript accumulation observed in the combined microbial treatments, particularly in virus-inoculated Tabas plants. Interestingly, increased *SlPTI4* expression generally coincided with treatments exhibiting relatively greater symptom severity, especially in the susceptible cultivar, suggesting that ET-associated signaling may reflect a stronger response to infection rather than being directly associated with effective resistance (Wu *et al*., 2002; Ohme-Takagi *et al*., 1995). Collectively, these findings suggest that microbial treatments are associated with differential regulation of phytohormone-associated defense responses depending on the host genotype.

However, because only marker gene expression was evaluated, these relationships remain correlative. They should not be interpreted as direct evidence for the causal involvement of JA-, SA-, or ET-mediated signaling pathways in resistance (Priya et al., 2023).

Enhanced activities of GPX, PPO, and CAT in treated plants further support the role of antioxidant metabolism in biocontrol-mediated resistance, consistent with earlier findings in *systems* treated with *Trichoderma and Bacillus* (Mafakheri *et al*., 2024). Proline metabolism was strongly influenced by both treatment and viral infection, with higher accumulation in primed plants and reduced levels under virus-only conditions, consistent with changes in osmoprotective and stress-related responses associated with ToLCPalV infection and microbial treatment. This supports the role of proline as a key metabolite in stress mitigation and defense activation (Singh *et al*., 2023; Kazemi *et al*., 2025). Improvements in chlorophyll content and plant growth parameters suggest enhanced physiological stability, likely due to reduced oxidative damage and microbial induction of growth-promoting pathways (Harman *et al*., 2004; Kazemi *et al*., 2026). Nevertheless, combined treatments did not always outperform single applications, indicating potential microbial competition or signaling interference. These responses are consistent with changes in JA-, SA-, and ET-associated defense responses. However, direct activation of induced resistance pathways, including ISR and SAR, requires further molecular validation.

CONCLUSIONS

Foliar application of *Trichoderma zelobreve* and *Bacillus subtilis* reduced disease severity and relative ToLCPalV DNA-B accumulation and improved physiological and biochemical responses in tomato, with efficacy varying between cultivars. Changes in defense-related gene expression were consistent with enhanced plant defense responses and suggested a possible priming effect; however, direct activation of ISR or SAR was not demonstrated. These findings highlight the potential of cultivar-specific application of beneficial microorganisms as a sustainable approach for managing ToLCPalV infection.

REFERENCES

1. Abdelkhalek, A., Al-Askar, A. A. and Behiry, S. I. 2020a. *Bacillus licheniformis* strain POT1 mediated polyphenol biosynthetic pathways genes activation and systemic resistance in potato plants against alfalfa mosaic virus. *Sci Rep.,* **10(2):**16120. https://doi.org/10.1038/s41598-020-73147-5.

2. Abdelkhalek, A., Behiry, S. I. and Al-Askar, A. A. 2020b. *Bacillus velezensis* PEA1 inhibits *Fusarium oxysporum* growth and induces systemic resistance to cucumber mosaic virus. *Agronomy*., **10(9):**1312. https://doi.org/10.3390/agronomy10091312.
3. Abdelkhalek, A., Al-Askar, A. A., Alsubaie, M. M. and Behiry, S. I. 2021. First report of protective activity of *Paronychia argentea* extract against tobacco mosaic virus infection. Plants., **10(11):**2435. https://doi.org/10.3390/plants10112435.
4. Abo-Zaid, G. A., Matar, S. M. and Abdelkhalek, A. 2020. Induction of plant resistance against tobacco mosaic virus using the biocontrol agent *Streptomyces cellulosae* isolate Actino 48. *Agronomy.,* **10(10):**1620. https://doi.org/10.3390/agronomy10101620.
5. Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R. and Wang, M. Q. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics., **9(3):**42. https://doi.org/10.3390/toxics9030042.
6. AlHudaib, K. A., Almaghasla, M. I., El-Ganainy, S. M., Arshad, M., Drou, N. and Sattar, M. N. 2022. High-throughput sequencing identified distinct bipartite and monopartite begomovirus variants associated with DNA-satellites from tomato and muskmelon plants in Saudi Arabia. *Plants*., **12(1):**6. https://doi.org/10.3390/plants12010006.
7. Ayaz, M., Li, C. H., Ali, Q., Zhao, W., Chi, Y. K., Shafiq, M., Ali, F., Yu, X. Y., Yu, Q., Zhao, J. T. and Yu, J. W. 2023. Bacterial and fungal biocontrol agents for plant disease protection: Journey from lab to field, current status, challenges, and global perspectives. *Molecules.*, **28(18):**6735. https://doi.org/10.3390/molecules28186735.
8. Azaryan, A., Atighi, M. R. and Shams-Bakhsh, M. 2024. Infection of tomato plants by tomato yellow leaf curl virus (TYLCV) potentiates the ethylene and salicylic acid pathways to fend off root-knot nematode (*Meloidogyne incognita*) parasitism. *Plant Physiol. Biochem.*, **217:** 109271. https://doi.org/10.1016/j.plaphy.2024.109271.
9. Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M. and Nicot, P. C. 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Front Plant Sci*., **6(1):**566. https://doi.org/10.3389/fpls.2015.00566
10. Chethan, D., Kavya, B. S., Arati, Chandana, R., Gowtham, H. P., Ashika, S., Chanchala, S., Nagaraju, N., Reddy, C. L., Kunjeti, S. G. and Ningaraju, T. M. 2026. Endophyte profiling of tomato leaf curl virus (ToLCV) resistant and susceptible tomato genotypes: Insights into

547 microbial diversity and growth promotion. *Sci Rep.,* **16(1):**5348.
548 https://doi.org/10.1038/s41598-026-37677-7.
549 11. Chien, Y. C. and Huang, C. H. 2020. Biocontrol of bacterial spot on tomato by foliar spray and
550 growth medium application of *Bacillus amyloliquefaciens* and *Trichoderma asperellum*. *Eur J*
551 *Plant Pathol*., **156(4):**995–1003. https://doi.org/10.1007/s10658-019-01861-2.
552 12. Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of
553 fresh leaf tissue. *Phytochem Bull*., **19(1):**11–15.
554 13. Ghanem, H. 2022. Induction systemic resistance in tomato plants against tomato yellow leaf
555 curl virus in protected cultivation using a local bacterial isolate, *Bacillus subtilis*. *Arab J Plant*
556 *Prot.,* **39:**289–295.
557 14. Gendi, H., Al-Askar, A. A., Király, L., Samy, M. A., Moawad, H. and Abdelkhalek, A. 2022.
558 Foliar applications of *Bacillus subtilis* HA1 culture filtrate enhance tomato growth and induce
559 systemic resistance against tobacco mosaic virus infection. *Horticulturae.*, **8(4):**301.
560 https://doi.org/10.3390/horticulturae8040301.
561 15. González-Miranda, M. D. C., Verastegui, P., Ogata-Gutiérrez, K. and Zúñiga-Dávila, D. 2026.
562 Effects of *Bacillus halotolerans* as a plant growth-promoting rhizobacterium and root
563 phytopathogen biocontrol agent in *Solanum lycopersicum* under field conditions. *Agronomy*.,
564 **16(4):**484. https://doi.org/10.3390/agronomy16040484.
565 16. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma*
566 species—Opportunistic, avirulent plant symbionts. *Nat Rev Microbiol*., **2(1):**43–56.
567 https://doi.org/10.1038/nrmicro797.
568 17. Heydarnejad, J., Hesari, M., Massumi, H. and Varsani, A. 2013. Incidence and natural hosts
569 of tomato leaf curl Palampur virus in Iran. *Australas Plant Pathol.*, **42(2):**195–203.
570 https://doi.org/10.1007/s13313-012-0164-0
571 18. Ivezić, A., Popović, T., Trudić, B., Krndija, J., Barošević, T., Sarajlić, A., Stojačić, I. and
572 Kuzmanović, B. 2025. Biological control agents in greenhouse tomato production (*Solanum*
573 *lycopersicum* L.): Possibilities, challenges and policy insights for Western Balkan region.
574 *Horticulturae*., **11(2):**155. https://doi.org/10.3390/horticulturae11020155.
575 19. Jacobsen, B. J., Zidack, N. K. and Larson, B. J. 2004. The role of *Bacillus*-based biological
576 control agents in integrated pest management systems: Plant diseases. *Phytopathology*.,
577 **94(11)**:1272–1275. https://doi.org/10.1094/PHYTO.2004.94.11.1272

20. Karačić, V., Miljaković, D., Marinković, J., Ignjatov, M., Milošević, D., Tamindžić, G. and Ivanović, M. 2024. *Bacillus* species: Excellent biocontrol agents against tomato diseases. *Microorganisms*., **12(3):**457. https://doi.org/10.3390/microorganisms12030457.
21. Kazemi, R., Safaie, N., Atighi, M. R. and Shams-Bakhsh, M. 2026. Biological and chemical inducers modulate growth and defense mechanisms of tomato against tomato leaf curl Palampur virus. *Physiol Mol Plant Pathol*., **141(1):**103043. https://doi.org/10.1016/j.pmpp.2025.103043.
22. Kazemi, R., Safaie, N., Pedram, M., Atighi, M. R. and Shams-Bakhsh, M. 2025. Reaction of commercial sugar-beet cultivars to beet curly top Iran virus (BCTIV). *J Crop Prot*., **14(1):**363–377. https://doi.org/10.48311/jcp.2025.76293.0.
23. Kumar, Y., Hallan, V. and Zaidi, A. A. 2008. Molecular characterization of a distinct bipartite begomovirus species infecting tomato in India. *Virus Genes*., **37(3):**425–431. https://doi.org/10.1007/s11262-008-0286-1.
24. Lee, G. H. and Ryu, C. M. 2016. Spraying of leaf-colonizing *Bacillus amyloliquefaciens* protects pepper from cucumber mosaic virus. *Plant Dis*., **100(10):**2099–2105. https://doi.org/10.1094/PDIS-02-16-0241-RE.
25. Li, J., Brader, G. and Palva, E. T. 2004. The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell*., **16(2):** 319–331. https://doi.org/10.1105/tpc.016980.
26. Li, X., Liao, Q., Zeng, S., Wang, Y. and Liu, J. 2025. The use of *Trichoderma* species for the biocontrol of postharvest fungal decay in fruits and vegetables: Challenges and opportunities. *Postharvest Biol Technol*., **219(1):**113236. https://doi.org/10.1016/j.postharvbio.2024.113236.
27. Liu, J., Wang, W., Wang, L., Sun, Y., Zhang, M., Li, Z. and Ren, X. 2019. Melatonin induces disease resistance to *Botrytis cinerea* in tomato fruit by activating jasmonic acid signaling pathway. *J. Agric. Food Chem.,* **67(21):** 6116–6124. https://doi.org/10.1021/acs.jafc.9b01234.
28. Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*., **25(4):**402–408. https://doi.org/10.1006/meth.2001.1262.
29. Maehly, A. and Chance, B. 1954. The assay of catalases and peroxidases. *Methods Biochem Anal.,* **1:**357–424. https://doi.org/10.1002/9780470110171.ch14.
30. Martínez-Medina, A., Fernández, I., Sánchez-Guzmán, M. J., Jung, S. C., Pascual, J. A. and Pozo, M. J. 2013. Deciphering the hormonal signalling network behind the systemic resistance

609 induced by *Trichoderma harzianum* in tomato. *Front. Plant Sci.,* **4:** 206.
610 https://doi.org/10.3389/fpls.2013.00206.
611 31. Mafakheri, K., Astaraki, S., Safaie, N. and Shams-bakhsh, M. 2024. Seed bio-/chemo-priming
612 affects the reaction of sugar beet plants to beet curly top virus-Svr. *Eur. J. Plant Pathol.*
613 **169:**473–482. https://doi.org/10.1007/s10658-024-02843-y.
614 32. Miljaković, D., Marinković, J. and Balešević-Tubić, S. 2020. The significance of *Bacillus* spp.
615 in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*.,
616 **8(1):**1037. https://doi.org/10.3390/microorganisms8101037
617 33. Nourian, A., Salehi, M., Safaie, N. and Khelghatibana, F. 2024. Biocontrol of *Diplodia*
618 *bulgarica*, the causal agent of apple canker, using *Trichoderma zelobreve*. *Arch Microbiol.,*
619 **206(3):**120. https://doi.org/10.1007/s00203-024-03852-5.
620 34. Ohme-Takagi, M. and Shinshi, H. 1995. Ethylene-inducible DNA binding proteins that interact
621 with an ethylene-responsive element. *Plant Cell.,* **7(2):** 173–182.
622 https://doi.org/10.1105/tpc.7.2.173.
623 35. Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., Noris, E. and
624 Matić, S. 2021. A review of the most common and economically important diseases
625 undermining tomato cultivation in the Mediterranean Basin. *Agronomy*., **11(1):**2188.
626 https://doi.org/10.3390/agronomy11112188.
627 36. Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M. and
628 Bakker, P. A. H. M. 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev.*
629 *Phytopathol.,* **52(1):** 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340
630 37. Priya, P., Chinnusamy, V., Ramu, V. S., Singh, P., Ramakrishnan, M., Sahu, P. P., Pandey, G.
631 K., Singh, A. K., Rao, V., Nataraja, K. N. and Udayakumar, M. 2023. Stress combinations and
632 their interactions in plants database: a one-stop resource on combined stress responses in plants.
633 *Plant J.,* **116(4):** 1097–1117. https://doi.org/10.1111/tpj.16539.
634 38. Sattar, M. N., Chellappan, B. V., ElGanainy, S. M., Almaghaslah, M. I., Al Hashedi, S. A. and
635 Al-Shoaibi, A. A. 2025. Genetic diversity, population structure, and cross-border dispersal
636 patterns of tomato leaf curl Palampur virus in South and West Asia. *Viruses*., **17(5):**678.
637 https://doi.org/10.3390/v17050678.
638 39. Shah, D. A. and Madden, L. V. 2004. Nonparametric analysis of ordinal data. *Phytopathology.,*
639 **94:**33–43. https://doi.org/10.1094/PHYTO.2004.94.1.33.

40. Siguemoto, E. S. and Gut, J. A. W. 2017. Validation of spectrophotometric microplate methods for polyphenol oxidase and peroxidase activities analysis in fruits and vegetables. *Food Sci Technol.,* **37(1):**148–153. https://doi.org/10.1590/1678-457X.23816.

41. Singh, P., Vaishnav, A., Liu, H., Xiong, C., Singh, H. B. and Singh, B. K. 2023. Seed biopriming for sustainable agriculture and ecosystem restoration. *Microb Biotechnol.,* **16(1):**2212–2222. https://doi.org/10.1111/1751-7915.14322.

42. Thambugala, K. M., Daranagama, D. A., Phillips, A. J. L., Kannangara, S. D. and Promputtha, I. 2020. Fungi vs. fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. *Front Cell Infect Microbiol*., **10(1):**604923.

43. Van Loon, L. C. 2000. Systemic induced resistance. In: Mechanisms of resistance to plant diseases, *Dordrecht: Springer Netherlands*., **38(1):** 521–574.

44. Wu, K., Tian, L., Hollingworth, J., Brown, D. C. W. and Miki, B. 2002. Functional analysis of tomato Pti4 in Arabidopsis. *Plant Physiol.,* **128(1):** 30–37. https://doi.org/10.1104/pp.010779.

45. Yadav, P., Malik, V. K., Kumar, R., Yadav, L., Kumar, S., Chauhan, R. S. and Singh, M. 2022. Use of agro-insecticide, plant extracts and resistance inducers to manage yellow vein mosaic of okra. *Int J Environ Clim Change.,* **12(11):**780–787. https://doi.org/10.9734/IJECC/2022/v12i1131038.

Journal of Agriculturl Science and Technology (JAST), 29(4)
In Press, Pre-Proof Version

مهار وابسته به رقم ویروس پالامپور پیچیدگی برگ گوجه‌فرنگی با استفاده از محلول‌پاشی برگی *Bacillus subtilis* و *Trichoderma zelobreve*

رویا کاظمی، ناصر صفایی، محمدرضا عتیقی، و مسعود شمس بخش

گوجه‌فرنگی (*Solanum lycopersicum* L.) یک محصول سبزی مهم در سطح جهانی است که کمیت و کیفیت آن تحت تأثیر ویروس پیچیدگی برگ گوجه‌فرنگی پالامپور (ToLCPalV ؛ *Begomovirus solanumpalampurense*) قرار می‌گیرد. در این پژوهش، اثر محلول‌پاشی برگی *Trichoderma zelobreve* T20 و *Bacillus subtilis* 6m، به‌صورت منفرد و ترکیبی، بر شدت بیماری و تجمع نسبی DNA ویروس در دو رقم گوجه‌فرنگی Moneymaker و طبس، تحت شرایط گلخانه بررسی شد. اگرچه پاسخ‌ها با توجه به رقم و زمان محلول‌پاشی متفاوت بود، *B. subtilis* تجمع نسبی DNA ویروسی را در هر دو رقم کاهش داد. در رقم Moneymaker، *T. zelobreve* مؤثرترین تیمار بود و تجمع نسبی DNA-B ویروس ToLCPalV را تا 99/9 درصد کاهش داد. در مقابل، *B. subtilis* تجمع نسبی DNA-B ویروس را تا 89/2 درصد در رقم طبس، در مقایسه با کنترل‌های مایه زنی شده با ویروس، کاهش داد. تیمار ترکیبی در رقم Moneymaker موجب بهبود بیشتر کنترل بیماری نشد و در رقم طبس نیز تنها مزیت محدودی نسبت به تیمارهای منفرد داشت. تیمارهای میکروبی با افزایش فعالیت آنزیم‌های آنتی‌اکسیدان (گایاکول پراکسیداز، پلی‌فنل اکسیداز و کاتالاز)، افزایش سطح پرولین، حفظ محتوای کلروفیل و بهبود رشد گیاه در مقایسه با کنترل‌های آلوده همراه بودند. جداسازی مجدد *T. zelobreve* و *B. subtilis* از برگ‌های بالایی تازه‌ظاهرشده پس از محلول‌پاشی، حضور آنها را در بافت‌های دور از محل کاربرد نشان داد و احتمال مشارکت آنها در پاسخ‌های دفاعی سیستمیک را مطرح کرد. این نتایج ظرفیت محلول‌پاشی میکروبی برگی را به‌عنوان یک راهبرد پایدار برای مدیریت ToLCPalV نشان می‌دهد و احتمال وجود اثر پرایمینگ را مطرح می‌کند، هرچند اثربخشی آن به ژنوتیپ میزبان و زمان تیمار وابسته بود.