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CC BY 4.0Çiçeklenme Döneminde Bakteriyel İnokülasyon Yoluyla Tohum Mikrobiyomunun Modifiye Edilmesinin ve Doğrudan Bakteriyel Tohum Kaplamanın Domates ve Patlıcan Fidelerinde *Fusarium* Kök ve Kökboğazı Çürüklüğüne EtkileriZühtü POLAT^{1*}, Didem CANİK OREL², Kutay Coşkun YILDIRIM³

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Fusarium kök ve kökboğazı çürüklüğü (*Fusarium oxysporum* f. sp. *radicis-lycopersici*, FORL), domates ve diğer *Solanaceae* türlerinde ciddi kayıplara yol açan toprak kökenli bir hastalıktır. Bu iki yıllık çalışmada, çiçeklenme döneminde endofitik bakteri inokülasyonu uygulanmış ana bitkilerden elde edilen tohumların domates ve patlıcan fidelerinde sağladığı koruma, aynı suşlarla doğrudan kaplanmış tohumlarıncı ile karşılaştırılmıştır. *Pseudomonas fluorescens* L5b veya *Pseudomonas gessardii* L13 ile inoküle edilen bitkilerden elde edilen tohumlar (L5B, L13) ile doğrudan kaplanan tohumlar (K-L5B, K-L13) değerlendirilmiştir. Kök uçları kesilen fideler FORL ile inoküle edilmiş ve altı hafta sonra hastalık şiddeti belirlenmiştir. Patlıcanda en yüksek etki oranı her iki yılda da K-L5B’de belirlenmiş (%19,44 ve %24,56); L13 ise ilk yıl koruma sağlamamıştır. Domateste K-L5B hastalık şiddetini %64,88’den %46,40’a düşürmüştür (etki oranı %28,48); bunu K-L13 (%24,49), L13 (%20,83) ve L5B (%18,02) izlemiştir. Bakteri uygulamaları FORL kaynaklı gelişme geriliğini kısmen hafifletmiş, özellikle klorofil SPAD değeri ve toprak üstü aksam yaş ağırlığında iyileşme kaydedilmiştir. Temel bileşen analizi toplam varyasyonun %84,3’ünü açıklamıştır. Çiçeklenme dönemi inokülasyonundan elde edilen tohumlar biyolojik açıdan anlamlı ancak değişken bir koruma sağlarken, şiddetli FORL baskısı altında en tutarlı koruma doğrudan tohum kaplama ile elde edilmiştir.

Effects of Modifying the Seed Microbiome via Bacterial Inoculation During the Flowering Stage and Direct Bacterial Seed Coating Against *Fusarium* Crown and Root Rot in Tomato and Eggplant Seedlings

Fusarium crown and root rot, caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), is a destructive soil-borne disease of tomato and other solanaceous crops. This two-year study evaluated the protection conferred by seeds obtained from parent plants inoculated at flowering with endophytic bacteria, in comparison with conventional direct seed coating. Seeds from plants inoculated with *Pseudomonas fluorescens* L5b or *Pseudomonas gessardii* L13 (L5B, L13) were compared with directly coated seeds (K-L5B, K-L13). Root-clipped seedlings were challenged with FORL and assessed six weeks later. In eggplant, K-L5B achieved the greatest disease reduction in both years (19.44% and 24.56%), whereas L13 gave no protection in the first year. In tomato, K-L5B reduced disease severity from 64.88% to 46.40% (28.48% reduction), followed by K-L13 (24.49%), L13 (20.83%) and L5B (18.02%). Bacterial treatments partially mitigated FORL-induced growth impairment, particularly for chlorophyll SPAD value and shoot fresh weight. Principal component analysis explained 84.3% of the total variation, with biomass traits driving treatment separation. Seeds derived from flowering-stage inoculation conferred biologically meaningful but variable protection, whereas direct bacterial seed coating provided the most consistent protection under severe FORL pressure.

INTRODUCTION

Fusarium crown and root rot, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), is one of the most important diseases affecting tomato production systems. The pathogen reduces seedling establishment, root function, plant growth and yield by colonizing root and crown tissues and impairing vascular function. The disease has a worldwide distribution and has been reported from many tomato-producing regions, including, but not limited to, Türkiye, Iran, Brazil and China (Can et al., 2004; McGovern, 2015; Manzo et al., 2016; Pirayesh et al., 2018; Cabral et al., 2020; Yang et al., 2024). Its persistence in soil and in infected plant residues, together with the difficulty of eradicating the pathogen once established, makes disease management particularly challenging.

Current management strategies for *Fusarium* crown and root rot rely mainly on resistant cultivars, cultural practices, soil disinfestation and chemical interventions. Although these approaches can reduce disease risk, they rarely provide complete or durable control. Their effectiveness may be limited by pathogen population variability, cultivar-dependent resistance responses, high implementation costs, restrictions on soil fumigants and fungicides, and environmental concerns associated with repeated chemical inputs, all of which have motivated the search for alternative control agents (Alabouvette et al., 1993; Nefzi et al., 2016; Ateş et al., 2019; Mohamed et al., 2021; Ramachela et al., 2021; Yang et al., 2024). These limitations have increased interest in biological control strategies that can be integrated into nursery and greenhouse production systems as more sustainable components of disease management.

Plant growth-promoting rhizobacteria and endophytic bacteria are among the most widely studied microbial resources for suppressing soil-borne *Fusarium* diseases. In particular, species of *Pseudomonas* have received considerable attention because of their rhizosphere competence, metabolic versatility and ability to interfere with pathogen development. Strains related to *Pseudomonas fluorescens*, *P. gessardii* and *P. chlororaphis* may suppress FORL through multiple and partly overlapping mechanisms,

including rapid colonization of root tips, competition for root exudates and infection sites, siderophore-mediated iron sequestration, production of antifungal metabolites such as phenazine derivatives and hydrogen cyanide, lytic enzyme activity, chemotaxis toward pathogen-associated compounds, direct colonization of fungal hyphae and induction of host defence responses (Chin-A-Woeng et al., 1998, 2000; Bolwerk et al., 2003; de Weert et al., 2004; Kamilova et al., 2005, 2008; Kamou et al., 2015). Endophytic bacteria recovered from tomato and from its wild relatives have likewise shown marked antifungal activity against *Fusarium oxysporum* (Nurlila et al., 2022; Saikia et al., 2025). Because these mechanisms depend strongly on early root colonization and on sufficient bacterial population density, the method used to deliver the antagonist to the seedling is a critical determinant of biocontrol performance.

Seed bacterization, or direct seed coating, is a practical delivery route because it positions bacterial cells directly on the seed surface and near the emerging radicle, where early interactions among the host, beneficial bacteria and soil-borne pathogens are initiated. This approach can promote root colonization at the earliest stages of seedling development and has been used for growth promotion and biological disease suppression in tomato and other crops (Sankar et al., 2017; Zhang et al., 2021). However, direct coating primarily establishes bacteria on the seed surface and may be affected by seed handling, storage, coating uniformity and survival of the applied cells before germination.

A newer and less explored strategy is the pre-harvest modification of the seed microbiome through inoculation of parent plants at the flowering stage. This approach was pioneered by Mitter et al. (2017), who introduced the endophyte *Paraburkholderia phytofirmans* PsJN into the flowers of parent plants and showed that it was incorporated into the seed microbiomes of the progeny in both monocot and dicot species, thereby establishing vertical inheritance and modifying seed microbiome composition and growth traits. In this transgenerational approach, beneficial endophytes are expected to colonize reproductive tissues and to become associated with developing seeds, thereby generating planting material that already carries selected

microorganisms before sowing. Tomato seeds have been shown to transmit plant-beneficial endophytes, and recent work demonstrated that seed microbiome modification with beneficial endophytic bacteria can improve seed quality and early plant growth (Bergna et al., 2018; Yildirim et al., 2025). Nevertheless, the functional value of this strategy under pathogen challenge remains insufficiently understood. In particular, it is not clear whether seeds obtained from flowering-stage-inoculated plants can provide disease protection comparable to that of direct seed coating when seedlings are exposed to severe FORL pressure.

Therefore, the present two-year study evaluated the protective efficacy of seed lots produced by endophytic bacteria-mediated seed microbiome modification against FORL in tomato (*Solanum lycopersicum* cv. Şencan 9) and eggplant (*Solanum melongena* cv. Pala 49). Seeds derived from parent plants inoculated at flowering with *P. fluorescens* L5b or *P. gessardii* L13 were compared with seeds directly coated with the same strains. Disease severity and plant growth responses were quantified after controlled root-dip inoculation with a virulent FORL isolate. The study specifically aimed to determine whether seed lots derived from flowering-stage inoculation can confer measurable protection against *Fusarium* crown and root rot and whether their performance approaches that of conventional direct bacterial seed coating under severe pathogen pressure.

MATERIALS AND METHODS

Plant Material, Bacterial Strains and Experimental Overview

Tomato (*Solanum lycopersicum* cv. Şencan 9) and eggplant (*Solanum melongena* cv. Pala 49) were used as host plants. Two endophytic bacterial strains, *Pseudomonas fluorescens* L5b and *Pseudomonas gessardii* L13, were evaluated. Both strains were originally isolated from the inner leaf tissues of lettuce (*Lactuca sativa*) and are maintained in the bacterial culture collection of the Department of Plant Protection, Faculty of Agriculture, Ankara University. They were identified by amplification and sequencing of the 16S rRNA gene (Yildirim et al., 2021), and the partial sequences are deposited in GenBank under accession numbers MW843845.1 (*P. fluorescens*

L5b) and MW843844.1 (*P. gessardii* L13). The strains were routinely maintained on King's B medium (King et al., 1954) and stored in 20% (v/v) glycerol at -80°C .

The two strains were delivered by two routes: seed microbiome modification through flowering-stage inoculation of parent plants, and direct bacterial seed coating. The two seed lots generated through flowering-stage inoculation were designated L5B and L13, and the two directly coated seed treatments were designated K-L5B and K-L13; the lower-case designations L5b and L13 always refer to the bacterial strains themselves. Two controls were included: a positive control consisting of FORL-inoculated seedlings without bacterial treatment, and a negative control consisting of non-inoculated healthy seedlings without bacterial treatment.

Production of Seed Lots Derived From Flowering-Stage Inoculation

The production of microbiome-modified seeds followed the procedure described by Yildirim et al. (2025). Briefly, *P. fluorescens* L5b or *P. gessardii* L13 was applied to flowering-stage parent plants to promote colonization of generative organs and subsequent seed-associated transmission. Seeds obtained from these plants constituted the L5B and L13 treatment groups.

The seed lots used in the present experiments are the same lots that were produced and characterized in that study; modification of the seed microbiome, including recovery of the inoculated strains from the harvested seeds, was therefore documented for these seed lots before the present disease assays were carried out. What was not repeated here is a contemporaneous re-quantification of the endophyte population at the time of sowing: the bacterial titre per seed, its uniformity among individual seeds and its survival over the storage interval between seed harvest and the challenge experiments were not re-measured. Accordingly, the present study does not add new evidence on the extent of seed colonization, but evaluates the disease-protection performance of seed lots whose microbiome modification had already been verified (Yildirim et al., 2025).

Direct Bacterial Seed Coating

For the direct-coating comparison, non-modified seeds were surface-disinfested in 2% sodium hypochlorite for 30 s, rinsed three times with sterile distilled water and dried overnight on sterile filter paper at room temperature. Each bacterial strain was suspended to $OD_{600} = 1.0$, corresponding to approximately 10^8 colony-forming units (CFU)·mL⁻¹. Carboxymethyl cellulose (CMC; 100 mg per 5 mL suspension) was used as an adhesive carrier. One gram of seeds was mixed with 5 mL of bacterial suspension and incubated on a shaker for 2 h to ensure uniform coating, following the principle of seed bacterization described by Sankar et al. (2017). Coated seeds were then transferred to sterile Petri dishes and dried overnight at room temperature; sowing was carried out the following day, so that the interval between coating and sowing was approximately 16–18 h for every seed lot. Seeds of the L5B, L13 and control groups were handled and sown on the same day and under the same conditions.

Seedling Production and FORL Inoculum Preparation

All seed lots were sown in plug trays filled with a peat: perlite substrate (3:1, v/v) and maintained at 25 °C under a 16:8 h light: dark photoperiod until seedlings reached the two- to three-true-leaf stage. A highly virulent isolate of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, designated FORL_ADN in the present study, was used as the challenge pathogen. The isolate was obtained from Hale Günaçtı (Phytopathology Laboratory, Biological Control Research Institute, Adana, Türkiye), where it had been identified as *F. oxysporum* f. sp. *radicis-lycopersici* by forma specialis-specific PCR and its high virulence had been determined in earlier pathogenicity tests (H. Günaçtı, personal communication). No sequence data were generated for the isolate and no GenBank accession number is available; the isolate is maintained in the culture collection of the authors' laboratory and is available from the corresponding author upon reasonable request. The isolate was cultured on potato dextrose agar (PDA) at 25 °C for 7 days. Mycelial and conidial growth was harvested by scraping colony surfaces into 50 mL sterile deionized water, and the suspension was filtered through two layers of

sterile cheesecloth to remove hyphal fragments. Conidial concentration was adjusted with a hemocytometer to 1×10^6 conidia·mL⁻¹.

For pathogen challenge, the root tips of tomato and eggplant seedlings were lightly clipped to facilitate infection, and seedlings were immersed in the FORL conidial suspension for 10 min. Inoculated seedlings were transplanted individually into 15 × 15 cm pots filled with sterilized peat: perlite: sand substrate (1:1:1, v/v/v). Negative-control plants were handled identically but dipped in sterile distilled water instead of the FORL suspension. Plants were maintained in a controlled growth room at 26 ± 2 °C, 60–70% relative humidity and a 16:8 h light: dark photoperiod for six weeks after inoculation.

Experimental Design

The experiment was arranged as a completely randomized design with six treatments: L5B, L13, K-L5B, K-L13, positive control and negative control. Each treatment included four replicates with five seedlings per replicate, giving 20 plants per treatment for each host species in each experimental year. The disease-severity experiment was conducted over two consecutive experimental years (2021 and 2022) using the same protocol. Growth and physiological measurements were recorded during the first experimental year to characterize the capacity of bacterial seed treatments to mitigate FORL-induced impairment of seedling performance.

Disease Assessment and Calculation of Disease Reduction

Six weeks after inoculation, plants were uprooted and root systems were washed to assess crown and root rot symptoms. Disease severity in tomato was assessed using a 0–3 scale adapted from Çolak & Biçici (2011): 0 = healthy plant with no visible symptoms; 1 = initial leaf wilting and mild to moderate necrotic lesions on primary and secondary roots and on the crown; 2 = moderate to severe wilting, plant stunting and extensive root decay; 3 = 50–100% internal vascular necrosis extending from the crown into the stem. Disease severity in eggplant was assessed using the 0–4 scale of Altınok et al. (2012): 0 = healthy; 1 = initial wilting and chlorotic discoloration in lower-leaf veins; 2 = approximately 50% canopy wilting,

reduced vegetative growth, chlorosis and localized necrosis; 3 = systemic wilting, leaf desiccation, abscission and apical dieback; 4 = complete plant desiccation and death.

Disease severity (%) was calculated using the Townsend–Heuberger formula (Townsend & Heuberger, 1943): $\text{disease severity} = [\Sigma(n \times v) / (Z \times N)] \times 100$, where n is the number of plants in a given disease class, v is the disease rating value, Z is the maximum rating of the scale and N is the total number of plants assessed. Disease reduction (%) relative to the positive control was calculated as $[(DS_{\text{positive control}} - DS_{\text{treatment}}) / DS_{\text{positive control}}] \times 100$, where DS is disease severity (%). Positive values indicate disease suppression, whereas negative values indicate disease severity greater than that of the positive control.

Growth and Physiological Measurements

At the end of the assessment period in the first experimental year, plant height (cm), stem diameter (mm), chlorophyll SPAD value, shoot fresh weight (g) and shoot dry weight (g) were measured. Plant height was measured from the substrate surface to the apical growing point. Stem diameter was measured at the stem base using a digital caliper. Chlorophyll SPAD readings were obtained from the third fully developed leaf using a SPAD-502 chlorophyll meter (Konica Minolta, Japan) and were reported as dimensionless relative chlorophyll indices. Shoot fresh weight was determined immediately after harvest, whereas shoot dry weight was measured after drying samples at 70 °C to constant weight.

Statistical Analysis

All statistical analyses were performed in R version 4.5.0 (R Core Team, 2025). Data were inspected for completeness, outliers and distributional properties. For disease severity, the tomato and eggplant datasets were analysed separately because the host species were biologically independent. Residual normality and variance homogeneity were assessed using the Shapiro–Wilk and Levene tests, respectively. Eggplant disease-severity data met parametric assumptions after square-root transformation; therefore, a two-way ANOVA was fitted with treatment, year and their interaction as fixed effects. Because the interaction was significant,

treatments were compared within each year using Tukey’s honestly significant difference (HSD) test.

Tomato disease-severity data did not adequately satisfy parametric assumptions after transformation; therefore, a Scheirer–Ray–Hare extension of the Kruskal–Wallis test was applied with treatment and year as factors. Because the treatment $\times$ year interaction was not significant, treatment effects were interpreted using pooled-year means, and post hoc pairwise comparisons were performed with Dunn’s test and Bonferroni correction. Growth and physiological parameters were analysed separately for each host species using one-way ANOVA followed by Tukey’s HSD test when parametric assumptions were satisfied, or Kruskal–Wallis and Dunn–Bonferroni tests otherwise. Principal component analysis (PCA) was performed on mean-centred and scaled growth and physiological variables. Statistical significance was set at $P < 0.05$.

RESULTS

Disease Severity

Disease severity was significantly affected by bacterial seed treatment in both eggplant and tomato seedlings challenged with FORL (Table 1; Figure 1). The response pattern, however, differed between the two host species. Eggplant exhibited a significant treatment $\times$ year interaction, indicating year-dependent efficacy of the bacterial treatments, whereas tomato showed a stable treatment ranking across years and was therefore interpreted using pooled-year means (Figure 2). Pathogen-inoculated positive control plants of both host species developed the characteristic dark brown crown and root lesions and vascular discoloration of *Fusarium* crown and root rot, whereas non-inoculated negative control plants remained symptomless, confirming successful infection by isolate FORL_ADN.

In eggplant, treatment ($F_{4,30} = 26.18$, $P < 0.001$), year ($F_{1,30} = 505.51$, $P < 0.001$) and the treatment $\times$ year interaction ($F_{4,30} = 2.88$, $P = 0.039$) were all significant. In the first year, disease severity in the positive control was $45.00 \pm 0.00\%$. Among the bacterial treatments, K-L5B produced the lowest disease severity ($36.25 \pm 1.25\%$) and the greatest disease reduction (19.44%). L5B and K-L13 each reduced disease severity to $41.25 \pm 1.25\%$, corresponding to an 8.33% reduction. In contrast,

L13 resulted in $48.75 \pm 1.25\%$ disease severity, a negative disease-reduction value (-8.33%) that indicates no protective effect in the first year.

In the second year, disease pressure in eggplant increased markedly and the positive control reached $71.25 \pm 2.39\%$. Under this higher pressure, K-L5B again provided the strongest suppression, reducing disease severity to $53.75 \pm 1.25\%$ (24.56% reduction). L5B and K-L13 each reduced disease severity to 62.50% (12.28% reduction), whereas L13 reduced it to 65.00% (8.77% reduction). K-L5B was thus the only treatment that maintained the lowest disease severity in eggplant in both years (Figure 3).

In tomato, the Scheirer–Ray–Hare analysis detected significant effects of treatment ($H(4) = 21.91$, $P < 0.001$) and year ($H(1) = 4.12$, $P = 0.043$), whereas the treatment $\times$ year interaction was not significant ($H(4) = 7.16$, $P = 0.128$). Across pooled years, the positive control had the highest disease severity ($64.88 \pm 1.07\%$). K-L5B was the most effective treatment, reducing severity to $46.40 \pm 1.74\%$ (28.48% reduction), followed by K-L13 ($48.99 \pm 2.52\%$; 24.49% reduction). The seed lots derived from flowering-stage inoculation also suppressed disease, with L13 reducing severity to $51.36 \pm 1.77\%$ (20.83% reduction) and L5B to $53.19 \pm 1.27\%$ (18.02% reduction).

Table 1. Effect of bacterial seed treatments on disease severity and disease reduction in eggplant and tomato seedlings under *Fusarium oxysporum* f. sp. *radicis-lycopersici* stress

Host	Year	Treatment	Disease severity (%)	Disease reduction (%)
Eggplant	2021	L13	48.75 ± 1.25 a	-8.33
Eggplant	2021	L5B	41.25 ± 1.25 b	8.33
Eggplant	2021	K-L13	41.25 ± 1.25 b	8.33
Eggplant	2021	K-L5B	36.25 ± 1.25 c	19.44
Eggplant	2021	Positive control	45.00 ± 0.00 ab	–
Eggplant	2022	L13	65.00 ± 0.00 ab	8.77
Eggplant	2022	L5B	62.50 ± 1.44 b	12.28
Eggplant	2022	K-L13	62.50 ± 2.50 b	12.28
Eggplant	2022	K-L5B	53.75 ± 1.25 c	24.56
Eggplant	2022	Positive control	71.25 ± 2.39 a	–
Tomato	Pooled	L13	51.36 ± 1.77 a	20.83
Tomato	Pooled	L5B	53.19 ± 1.27 ab	18.02
Tomato	Pooled	K-L13	48.99 ± 2.52 a	24.49
Tomato	Pooled	K-L5B	46.40 ± 1.74 a	28.48
Tomato	Pooled	Positive control	64.88 ± 1.07 b	–

Values are means $\pm$ standard errors ($n = 4$); a standard error of 0.00 indicates that the four replicate means were identical. Means followed by different lowercase letters within the relevant comparison set (within each year for eggplant, Tukey HSD; across pooled years for tomato, Dunn–Bonferroni) differ significantly at $P < 0.05$. Disease reduction was calculated relative to the pathogen-inoculated positive control. L5B and L13 = seeds derived from flowering-stage-inoculated parent plants; K-L5B and K-L13 = directly bacterial-coated seeds.

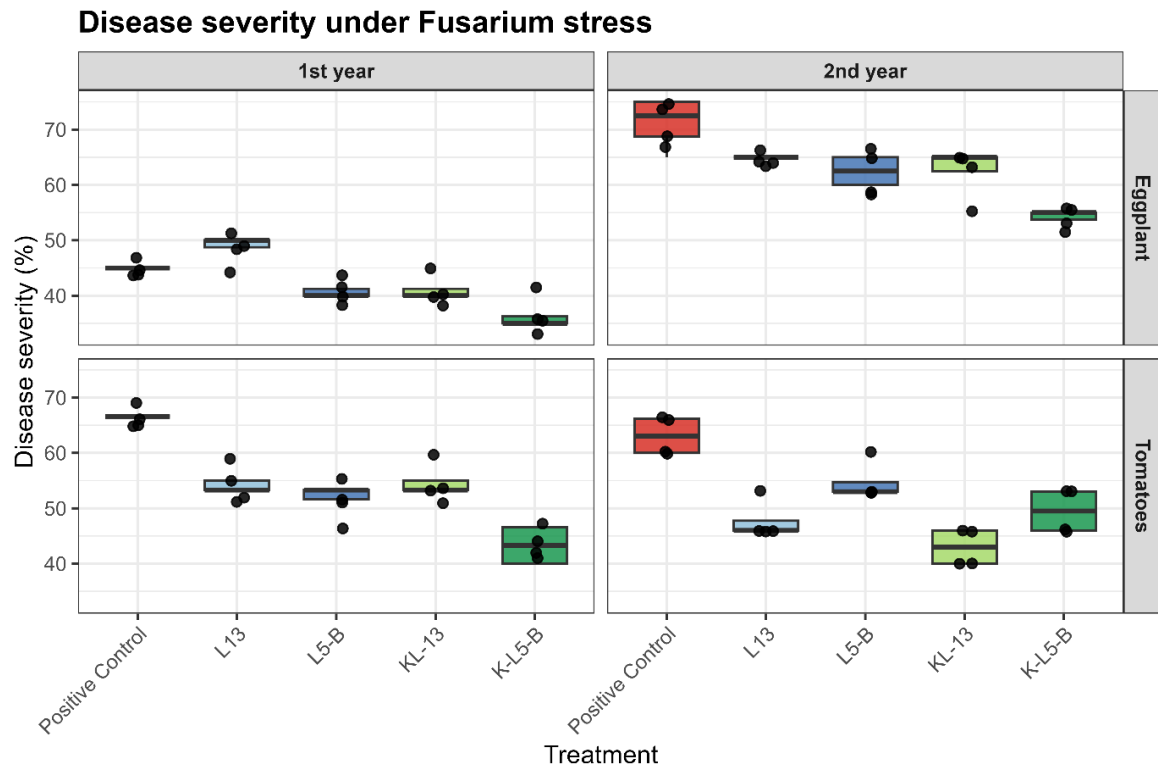


Figure 1. Disease severity (%) of eggplant and tomato seedlings subjected to different bacterial seed treatments under FORL stress. Boxes show interquartile ranges and horizontal lines show medians; overlaid points are the individual observations, jittered for visibility. A box reduced to a single horizontal line indicates that the quartiles coincided.

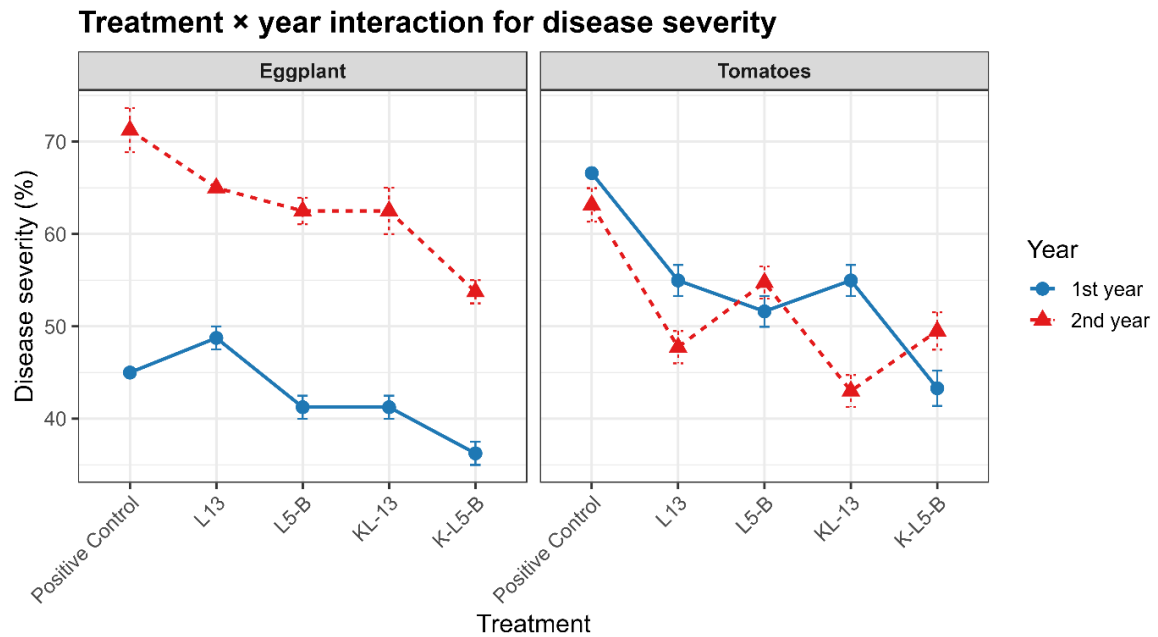


Figure 2. Treatment × year interaction for disease severity (%) in eggplant and tomato seedlings exposed to FORL. The interaction was significant in eggplant but not in tomato, which supports the pooled-year interpretation used for tomato.

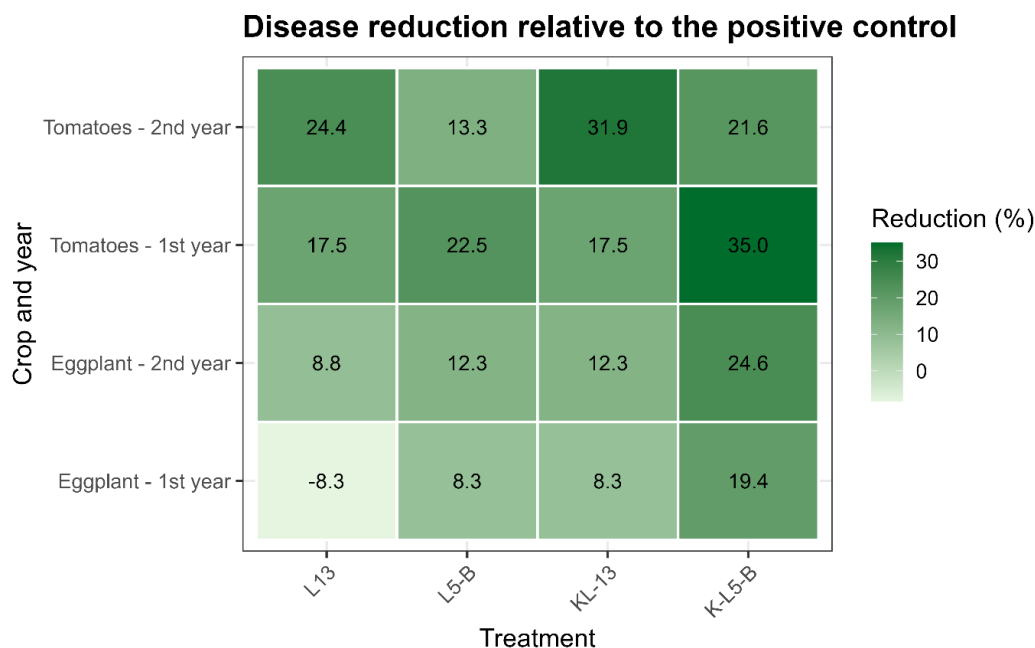


Figure 3. Heatmap of disease reduction (%) relative to the positive control, shown separately for each host and year. Darker green indicates greater suppression and pale shading indicates weak or negative suppression; the pooled-year tomato values given in Table 1 are the means of the two annual values shown here.

Growth and Physiological Responses

Bacterial seed treatments also modified the growth and physiological status of FORL-challenged seedlings, although these responses were trait-dependent and generally less pronounced than disease suppression (Table 2; Figure 4). In both hosts, FORL infection reduced plant performance relative to the healthy negative control.

In eggplant, treatment effects were significant for chlorophyll SPAD value ($P = 0.0076$), plant height ($P < 0.001$), stem diameter ($P = 0.0207$) and shoot fresh weight ($P = 0.0038$), whereas shoot dry weight was not significantly affected ($P = 0.257$). Plant height ranged from 11.85 ± 0.19 cm in K-L5B to 14.45 ± 0.54 cm in the negative control; the positive control reached 13.05 ± 0.42 cm and L13 produced 13.55 ± 0.22 cm. Chlorophyll SPAD values ranged from 13.16 ± 0.84 in K-L5B to 20.82 ± 0.54 in the negative control. Among the FORL-inoculated treatments, L13 had the highest SPAD value (18.16 ± 0.74), followed by K-L13 (16.50 ± 1.60) and L5B (15.90 ± 1.68), indicating partial maintenance of chlorophyll status under pathogen stress.

Eggplant stem diameter ranged from 5.47 ± 0.06 mm in L5B to 6.32 ± 0.04 mm in L13. Shoot fresh weight was highest in K-L13 (27.15 ± 0.79 g), exceeding the positive control (22.13 ± 0.95 g) and L5B (22.12 ± 1.49 g), and numerically the healthy negative control (26.19 ± 0.58 g). Shoot dry weight varied from 3.84 ± 0.38 g in the positive control to 4.55 ± 0.08 g in L13 but did not differ statistically among treatments.

In tomato, treatment effects were significant for chlorophyll SPAD value ($P = 0.022$), plant height ($P < 0.001$), stem diameter ($P = 0.021$) and shoot fresh weight ($P = 0.009$), whereas shoot dry weight remained non-significant ($P = 0.274$). The positive control had the lowest plant height (32.25 cm) and the negative control the highest (42.00 cm). Among the bacterial treatments, K-L13 produced the tallest plants (36.10 cm), the greatest shoot fresh weight among FORL-inoculated treatments (32.32 g) and the highest SPAD value (9.58). The positive-control SPAD value was 6.56, whereas the bacterial seed treatments reached 8.73–9.58, corresponding to a 33–46% relative improvement over the positive control. Tomato shoot fresh weight ranged from 27.83 g in K-L5B to 39.33 g in the negative

control, with K-L13 again ranking highest among the pathogen-challenged treatments.

Table 2. Growth and physiological responses of eggplant (*Solanum melongena* cv. Pala 49) seedlings to bacterial seed treatments under FORL stress during the first experimental year

Treatment	SPAD	Plant height (cm)	Stem diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)
L13	18.16 ± 0.74 ab	13.55 ± 0.22 ab	6.32 ± 0.04 a	25.81 ± 0.55 ab	4.55 ± 0.08 a
L5B	15.90 ± 1.68 ab	12.30 ± 0.17 bc	5.47 ± 0.06 b	22.12 ± 1.49 b	4.18 ± 0.32 a
K-L13	16.50 ± 1.60 ab	12.90 ± 0.26 bc	6.20 ± 0.06 ab	27.15 ± 0.79 a	4.40 ± 0.15 a
K-L5B	13.16 ± 0.84 b	11.85 ± 0.19 c	6.16 ± 0.07 ab	23.78 ± 0.97 ab	4.00 ± 0.11 a
Positive control	13.51 ± 2.03 b	13.05 ± 0.42 abc	5.94 ± 0.21 ab	22.13 ± 0.95 b	3.84 ± 0.38 a
Negative control	20.82 ± 0.54 a	14.45 ± 0.54 a	6.11 ± 0.05 ab	26.19 ± 0.58 ab	4.45 ± 0.16 a

Values are means ± standard errors (n = 4). Different lowercase letters within a column indicate significant differences among treatments (Tukey HSD, $P < 0.05$). SPAD values are dimensionless chlorophyll-meter readings. Positive control: FORL-inoculated untreated plants; negative control: healthy non-inoculated plants.

Principal Component Analysis of Growth and Physiological Traits

PCA provided a multivariate summary of treatment effects across plant height, stem diameter, chlorophyll SPAD value, shoot fresh weight and shoot dry weight (Figure 5). The first two principal components explained 84.3% of the total variation, with PC1 accounting for 66.7% and PC2 for 17.6%. PC1 was driven mainly by plant-size and biomass-related traits, including plant height, stem diameter, shoot fresh weight and shoot dry weight, whereas chlorophyll SPAD value showed an opposing loading pattern on this axis. PC2 was influenced most strongly by fresh- and dry-weight variation, reflecting differences in biomass accumulation and tissue water status.

The PCA ordination showed partial separation among healthy negative controls, diseased positive controls and bacterial treatments. Negative-control plants tended to occupy the region associated with higher biomass and plant stature, whereas positive controls clustered toward the lower-biomass portion of the

ordination. Bacterial treatments occupied intermediate positions, indicating partial but incomplete recovery from FORL-induced growth impairment. K-L13 was positioned closest to the healthy-control region for several growth attributes, supporting the univariate results showing improved shoot fresh weight and plant height.

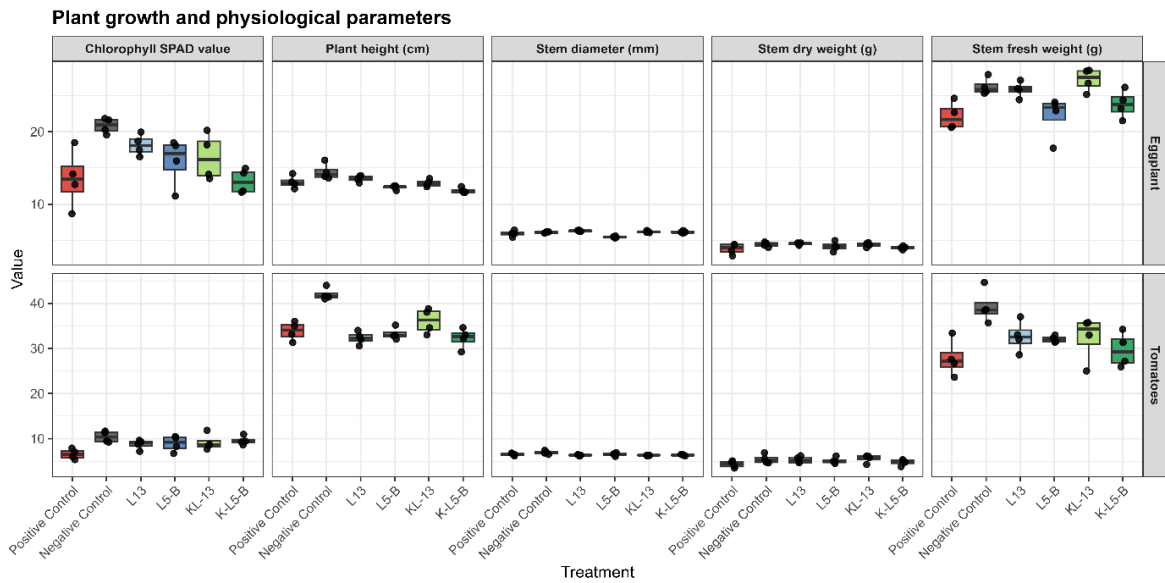


Figure 4. Plant growth and physiological parameters — chlorophyll SPAD value, plant height, stem diameter, shoot dry weight and shoot fresh weight — of eggplant and tomato seedlings under FORL stress.

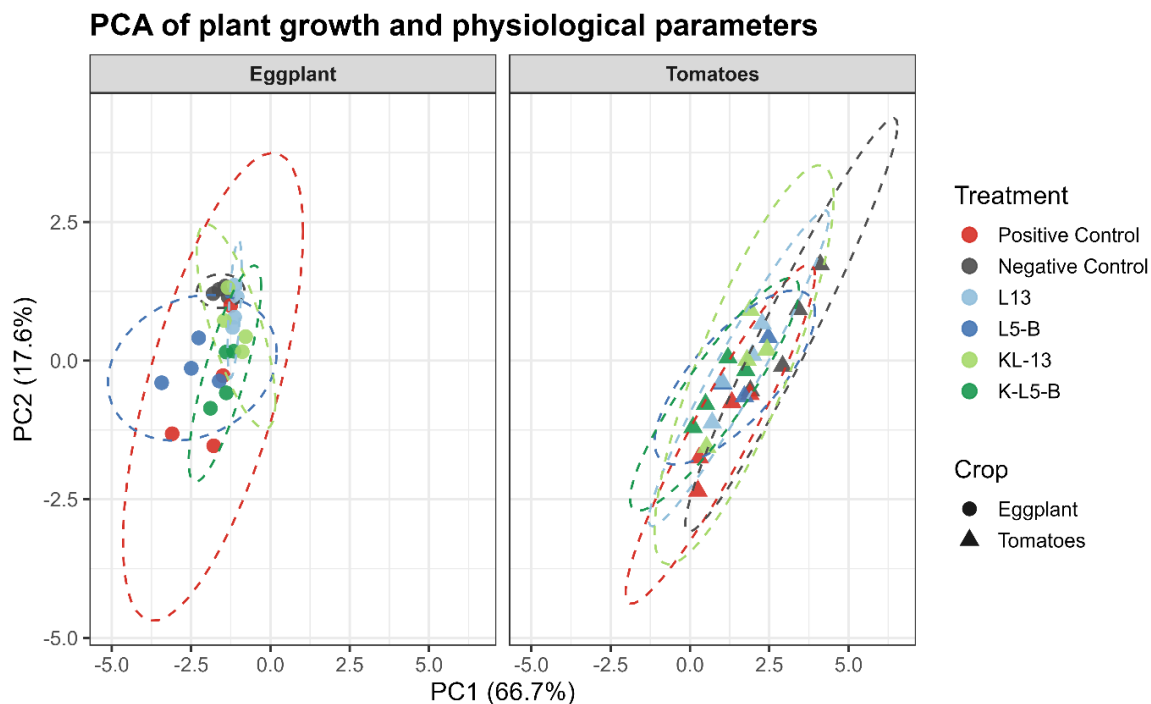


Figure 5. Principal component analysis (PCA) of the growth and physiological traits measured in eggplant and tomato seedlings under FORL stress. PC1 and PC2 explained 66.7% and 17.6% of the total variance, respectively.

DISCUSSION

This study compared two bacterial delivery strategies for suppressing *Fusarium* crown and root rot under controlled and deliberately severe pathogen pressure: the use of seed lots derived from flowering-stage inoculation of parent plants,

and conventional direct bacterial seed coating. Across the full disease dataset, direct seed coating generally provided higher and more stable disease suppression than the flowering-stage-derived seed lots. The strongest response in both host species was obtained with K-L5B, indicating that direct

coating with *P. fluorescens* L5b was the most consistent delivery route under severe root-wound FORL inoculation. Nevertheless, the measurable disease reductions obtained with the flowering-stage-derived L5B and L13 seed lots, particularly in tomato, show that this transgenerational route can confer biologically meaningful protection. Whereas Mitter et al. (2017) established the feasibility of this route by tracking a single endophyte into progeny seeds and measuring growth traits, the present work extends it to a disease-protection endpoint, testing whether seed lots produced in this way can restrict a virulent soil-borne pathogen in two *Solanaceae* hosts.

The stronger performance of direct seed coating is biologically plausible. Direct coating places a relatively high density of bacterial cells on the seed surface before germination, allowing the antagonist to colonize the emerging radicle and rhizosphere during the earliest stages of seedling establishment. This early colonization phase is critical in *Pseudomonas*-mediated biocontrol of FORL, because protection depends not only on the presence of the antagonist but also on its ability to reach infection courts before or simultaneously with the pathogen. Previous work has shown that effective root-tip colonization, competition for exudate-derived nutrients, chemotaxis toward pathogen-associated compounds, direct interaction with fungal hyphae and antifungal metabolite production all contribute to suppression of FORL by *Pseudomonas* strains (Chin-A-Woeng et al., 2000; Bolwerk et al., 2003; de Weert et al., 2004; Kamilova et al., 2005, 2006, 2008). The consistent ranking of K-L5B in the present study is therefore in line with the view that high initial cell density and rapid rhizosphere establishment are major determinants of reliable biological control (Validov et al., 2007; Kamou et al., 2015).

By contrast, the flowering-stage route relies on a more complex biological pathway. Bacterial cells must colonize floral or reproductive tissues, persist during seed development, survive seed maturation and storage and then re-establish from the seed into the germinating seedling. Previous studies have shown that tomato seeds can transmit plant-beneficial endophytes and that seed microbiome modification can improve seed quality and early plant growth (Bergna et al., 2018; Yildirim et al., 2025). The present disease-challenge

experiment extends this concept by showing that the L5B and L13 seed lots can reduce FORL severity, particularly in tomato. However, their lower and less stable efficacy compared with direct seed coating suggests that vertically transmitted bacterial populations may be smaller, less uniformly distributed among individual seeds or slower to colonize the rhizosphere after germination. Under the root-wound inoculation system used here, even a short delay in antagonist establishment may allow the pathogen to infect root and crown tissues before protective bacterial populations become fully functional. These population-level explanations remain hypotheses, however. Although colonization of the seed lots used here was established in the source study (Yildirim et al., 2025), the seed-borne and the coating-derived populations were not quantified side by side at sowing in the present work, so the difference in performance between the two delivery routes cannot yet be attributed directly to a difference in bacterial load or in its distribution among seeds.

The host-dependent pattern of response is another important outcome of this study. Tomato showed a stable treatment ranking across the two experimental years and all bacterial treatments reduced disease severity relative to the positive control. In eggplant, however, the significant treatment $\times$ year interaction indicated that treatment efficacy was more strongly shaped by interannual or host-associated variation. This difference may reflect host-specific root architecture, seedling vigour, exudate chemistry, susceptibility level or compatibility between each host and the introduced bacterial strains. Root exudates are known to influence both pathogen activity and bacterial colonization, and changes in organic acids and sugars can alter the competitive balance among the host, FORL and biocontrol bacteria (Kamilova et al., 2006; Zhang et al., 2021). The lower consistency observed in eggplant therefore does not necessarily indicate failure of the approach, but rather suggests that both seed microbiome modification and seed coating need to be optimized separately for each host species.

The disease-suppression values obtained in tomato were moderate rather than complete, with reductions ranging from 18.02% to 28.48%. This level of protection should be interpreted in relation to the stringency of the assay. Seedlings were root-

clipped and challenged by dipping in a 1×10^6 conidia·mL⁻¹ suspension of a virulent FORL isolate. This method creates immediate and uniform infection pressure and bypasses part of the natural infection process by providing wound sites for pathogen entry. Under such conditions, full disease control by a single bacterial strain is unlikely. Comparable biological control studies have reported variable levels of suppression depending on pathogen pressure, bacterial strain, host genotype, application method and production system (Abdallah et al., 2019; Nurlila et al., 2022; Ramírez et al., 2022; Saikia et al., 2025; Tunalı et al., 2018; Taiebikhah et al., 2026). The moderate but reproducible reductions observed here, especially for K-L5B and for the flowering-stage-derived tomato seed lots, therefore represent a meaningful protective effect under a highly challenging inoculation system.

The growth and physiological responses further indicate that disease suppression and plant-growth recovery were not always linked in the same way. K-L5B was the most consistent treatment for disease reduction, whereas K-L13 frequently ranked higher for some growth-related traits, particularly shoot fresh weight in eggplant and plant height, SPAD value and shoot fresh weight in tomato. This distinction suggests that antagonistic activity against FORL and growth-promoting capacity may be partially decoupled: one strain or delivery method may be more effective at restricting pathogen development, while another may better support plant physiological performance under infection. Such functional differentiation is compatible with the diverse mechanisms reported for endophytic and rhizosphere-associated bacteria, including phytohormone production, siderophore-mediated iron acquisition, nutrient mobilization, modulation of stress responses and induction of systemic resistance (Abdallah et al., 2019; Zhang et al., 2021).

The SPAD and biomass results also suggest that bacterial treatments partially mitigated FORL-associated stress rather than fully restoring healthy plant performance. In eggplant, K-L13 increased shoot fresh weight beyond the positive control and numerically approached the healthy control, while L13 maintained relatively high chlorophyll SPAD values under infection. In tomato, bacterial treatments improved SPAD values relative to the

diseased positive control, and K-L13 ranked highest among pathogen-challenged treatments for several growth traits. However, shoot dry weight was not significantly affected in either host. This pattern suggests that bacterial treatments may have helped to maintain tissue hydration, photosynthetic status or short-term physiological activity, whereas clear dry-biomass accumulation was less evident under the severe disease pressure imposed. This distinction is important, because fresh-weight recovery alone should not be interpreted as complete growth restoration.

The PCA supported this interpretation by showing that bacterial treatments shifted the multivariate growth profile away from the diseased positive controls but did not fully overlap with the healthy controls. Biomass-related traits contributed strongly to treatment separation, indicating that bacterial seed treatments influenced overall plant performance under FORL stress. Nevertheless, the incomplete overlap with the negative control suggests that the main practical benefit of these treatments was partial protection and stress mitigation rather than full restoration of normal seedling vigour. For nursery or greenhouse production this distinction is relevant, because moderate disease suppression may still be valuable if it improves transplant quality, reduces early seedling loss or delays disease development long enough for integrated management measures to act.

Some limitations should be borne in mind when these findings are generalized. Although the seed lots tested here are those whose microbiome modification was documented by Yıldırım et al. (2025), the endophyte population was not re-quantified at sowing, so the two delivery routes can be compared at the level of the seed lot but not at the level of the bacterial population. The experiments were also conducted in a controlled growth room with sterilized substrate and wound-assisted inoculation at a fixed inoculum density, which imposes a more severe and more uniform infection pressure than naturally infested soils; the relative ranking of the two routes could therefore differ under field conditions. Finally, only one FORL isolate, one cultivar per host species and two bacterial strains were tested — although virulence and the host defence response are known to differ among FORL strains (Çakır et al., 2014) — growth traits were recorded only in the first year, and the

assessment ended six weeks after inoculation at the seedling stage. Confirmation in naturally infested soil, under non-wounded infection and at nursery scale is therefore required before the approach can be generalized.

Taken together, the results support seed microbiome modification as a feasible transgenerational delivery strategy for protective endophytes, but they also show that direct seed coating remains the more dependable approach under acute FORL pressure. The value of flowering-stage inoculation lies in its potential to generate internally colonized seeds that carry beneficial microorganisms before sowing, whereas the value of direct coating lies in the ability to deliver a high and more uniform antagonist dose at germination. Future studies should quantify bacterial population densities in seeds, emerging radicles, roots and rhizosphere soil; determine whether vertically transmitted cells are uniformly distributed among individual seeds; evaluate the persistence of these bacteria after seed storage; and test whether strain mixtures can combine strong disease suppression with improved growth support.

CONCLUSION

Endophytic bacteria delivered through both flowering-stage seed microbiome modification and direct seed coating reduced *Fusarium* crown and root rot severity in tomato and eggplant seedlings. Direct coating with *P. fluorescens* L5b (K-L5B) provided the strongest and most consistent disease suppression, reaching a 28.48% reduction in tomato and 19.44–24.56% reductions in eggplant. Seed lots derived from flowering-stage inoculation also provided biologically meaningful protection, particularly in tomato, indicating that this transgenerational delivery route can contribute to disease tolerance. Efficacy was, however, host-dependent and less stable in eggplant, and the protection obtained was partial rather than complete under the severe inoculation pressure applied. Flowering-stage inoculation should therefore be regarded as a promising complement to — rather than a replacement for — direct seed coating. These conclusions apply to the conditions tested, namely a single FORL isolate, one cultivar per host species and wound-assisted root-dip inoculation in sterilized substrate; their transfer to naturally infested nursery and greenhouse soils remains to be verified.

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ETHICS STATEMENT

This study does not require ethics committee approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Abdallah, R. A. B., Jabnoun-Khiareddine, H., Nefzi, A., Ayed, F., & Daami-Remadi, M. (2019). Field suppression of *Fusarium* wilt and microbial population shifts in tomato rhizosphere following soil treatment with two selected endophytic bacteria. *Eurasian Journal of Soil Science*, 8(3), 208–220. <https://doi.org/10.18393/ejss.556780>
- Alabouvette, C., Lemanceau, P., & Steinberg, C. (1993). Recent advances in the biological control of *Fusarium* wilts. *Pesticide Science*, 37(4), 365–373. <https://doi.org/10.1002/ps.2780370409>
- Altınok, H. H., Can, C., Dikilitaş, M., & Çolak, H. (2012). Türkiye’de açık tarla patlıcan yetiştiriciliğinde *Fusarium* solgunluk hastalığının yaygınlığı ve izolatların virülenslikleri. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 16(3), 342–352.
- Ateş, C., Fidan, H., Karacaoğlu, M., & Daşgan, H. (2019). The identification of the resistance levels of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and Tomato yellow leaf curl viruses in different tomato genotypes with traditional and molecular methods. *Applied Ecology and Environmental Research*, 17(2), 2203–2218.

- https://doi.org/10.15666/aeer/1702_22032
218
- Bergna, A., Cernava, T., Rändler, M., Grosch, R., Zachow, C., & Berg, G. (2018). Tomato seeds preferably transmit plant beneficial endophytes. *Phytobiomes Journal*, 2(4), 183–193.
<https://doi.org/10.1094/PBIOMES-06-18-0029-R>
- Bolwerk, A., Lagopodi, A. L., Wijfjes, A. H. M., Lamers, G. E. M., Chin-A-Woeng, T. F. C., Lugtenberg, B. J. J., & Bloemberg, G. V. (2003). Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Molecular Plant-Microbe Interactions*, 16(11), 983–993.
<https://doi.org/10.1094/MPMI.2003.16.11.983>
- Cabral, C., Gonçalves, A., Fonseca, M., Urban, A., Costa, H., Lourenço, V., Boiteux, L., & Reis, A. (2020). First detection of *Fusarium oxysporum* f. sp. *radicis-lycopersici* across major tomato-producing regions in Brazil. *Phytoparasitica*, 48, 545–553.
<https://doi.org/10.1007/s12600-020-00824-5>
- Can, C., Yucel, S., Korolev, N., & Katan, T. (2004). First report of *Fusarium* crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Turkey. *Plant Pathology*, 53, 814.
<https://doi.org/10.1111/j.1365-3059.2004.01087.x>
- Chin-A-Woeng, T. F. C., Bloemberg, G. V., van der Bij, A. J., van der Drift, K. M. G. M., Schripsema, J., Kroon, B., Scheffer, R. J., Keel, C., Bakker, P. A. H. M., Tichy, H. V., de Bruijn, F. J., Thomas-Oates, J. E., & Lugtenberg, B. J. J. (1998). Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Molecular Plant-Microbe Interactions*, 11(11), 1069–1077.
<https://doi.org/10.1094/MPMI.1998.11.11.1069>
- Chin-A-Woeng, T. F. C., Bloemberg, G. V., Mulders, I. H. M., Dekkers, L. C., & Lugtenberg, B. J. J. (2000). Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. *Molecular Plant-Microbe Interactions*, 13(12), 1340–1345.
<https://doi.org/10.1094/MPMI.2000.13.12.1340>
- Çakır, B., Gül, A., Yolageldi, L., & Özaktan, H. (2014). Response to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato roots involves regulation of SA- and ET-responsive gene expressions. *European Journal of Plant Pathology*, 139, 379–391.
<https://doi.org/10.1007/s10658-014-0394-9>
- Çolak, A., & Biçici, M. (2011). Doğu Akdeniz Bölgesi örtü altı domates yetiştiriciliğinde *Fusarium oxysporum* spesiyal formlarının simptomatolojik ayrımı ile solgunluk ve kök-kök boğazı çürüklüğü hastalıklarının çıkış, şiddet ve yaygınlıklarının belirlenmesi. *Bitki Koruma Bülteni*, 51(4), 331–345.
- de Weert, S., Kuiper, I., Lagendijk, E. L., Lamers, G. E. M., & Lugtenberg, B. J. J. (2004). Role of chemotaxis toward fusaric acid in colonization of hyphae of *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Pseudomonas fluorescens* WCS365. *Molecular Plant-Microbe Interactions*, 17(11), 1185–1191.
<https://doi.org/10.1094/MPMI.2004.17.11.1185>
- Kamilova, F., Kravchenko, L. V., Shaposhnikov, A. I., Azarova, T., Makarova, N., & Lugtenberg, B. (2006). Effects of the tomato pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudate. *Molecular Plant-Microbe Interactions*, 19(10), 1121–1126.
<https://doi.org/10.1094/MPMI-19-1121>
- Kamilova, F., Lamers, G., & Lugtenberg, B. (2008). Biocontrol strain *Pseudomonas fluorescens* WCS365 inhibits germination of *Fusarium oxysporum* spores in tomato root exudate as well as subsequent formation of new spores. *Environmental Microbiology*, 10(9), 2455–2461.
<https://doi.org/10.1111/j.1462-2920.2008.01638.x>
- Kamilova, F., Validov, S., Azarova, T., Mulders, I., & Lugtenberg, B. (2005). Enrichment for

- enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology*, 7(11), 1809–1817. <https://doi.org/10.1111/j.1462-2920.2005.00889.x>
- Kamou, N. N., Karasali, H., Menexes, G., Kasiotis, K. M., Bon, M. C., Papadakis, E. N., Tzelepis, G. D., & Lagopodi, A. L. (2015). Isolation, screening and characterisation of local beneficial rhizobacteria based upon their ability to suppress *Fusarium oxysporum* f. sp. *radicis-lycopersici* and tomato foot and root rot. *Biocontrol Science and Technology*, 25(8), 928–949. <https://doi.org/10.1080/09583157.2015.1020762>
- King, E. O., Ward, M. K., & Raney, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine*, 44(2), 301–307.
- Manzo, D., Ferriello, F., Puopolo, G., Zoina, A., D'Esposito, D., Tardella, L., Ferrarini, A., & Ercolano, M. R. (2016). *Fusarium oxysporum* f. sp. *radicis-lycopersici* induces distinct transcriptome reprogramming in resistant and susceptible isogenic tomato lines. *BMC Plant Biology*, 16, Article 53. <https://doi.org/10.1186/s12870-016-0740-5>
- McGovern, R. J. (2015). Management of tomato diseases caused by *Fusarium oxysporum*. *Crop Protection*, 73, 78–92. <https://doi.org/10.1016/j.cropro.2015.02.021>
- Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Sheibani-Tezerji, R., von Maltzahn, G., & Sessitsch, A. (2017). A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology*, 8, Article 11. <https://doi.org/10.3389/fmicb.2017.00011>
- Mohamed, D., Gaston, K., Guy, B., Carine, N., Aicha, K., & Daouda, K. (2021). Efficacy of four essential oils on *Fusarium* head blight caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, a fungal telluric pathogen of tomato grown under cover. *International Journal of Advanced Research*, 9(11), 1018–1030. <https://doi.org/10.21474/ijar01/13835>
- Nefzi, A., Abdallah, R. A. B., Jabnoun-Khiareddine, H., Medimagh-Saidana, S., Haouala, R., & Daami-Remadi, M. (2016). Antifungal activity of aqueous and organic extracts from *Withania somnifera* L. against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Journal of Microbial & Biochemical Technology*, 8(3), 144–150. <https://doi.org/10.4172/1948-5948.1000277>
- Nurlila, R. U., La Fua, J., Abidin, M. S., Sulaeha, S., & Hadju, L. (2022). Effectiveness of endophytic bacteria from local tomato plants against wilt disease caused by *Fusarium oxysporum*. *Caraka Tani: Journal of Sustainable Agriculture*, 38(1), 53–64.
- Pirayesh, S., Zamanizadeh, H., & Morid, B. (2018). Molecular identification of physiological races of *Fusarium oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* causal agent of *Fusarium* wilt of tomato in Iran. *Journal of Agricultural Science and Technology*, 20, 193–202.
- R Core Team. (2025). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Ramachela, K., Mpaneng, M. A., Mathuthu, M., & Zeevaart, J. R. (2021). Responses of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) to $ZnNO_3$ under in vitro conditions. *Pakistan Journal of Phytopathology*, 33(1), 29–35.
- Ramírez, V., Martínez, J., Bustillos-Cristales, M. D. R., Castañeda-Antonio, D., Munive, J. A., & Baez, A. (2022). *Bacillus cereus* MH778713 elicits tomato plant protection against *Fusarium oxysporum*. *Journal of Applied Microbiology*, 132(1), 470–482. <https://doi.org/10.1111/jam.15179>
- Saikia, B., Bhattacharyya, A., Boro, R. C., & Savani, A. K. (2025). Bioprospecting of bacterial endophytes from *Solanum pimpinellifolium* with antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* causing vascular wilt. *Physiological and Molecular Plant Pathology*, 136, Article 102566. <https://doi.org/10.1016/j.pmpp.2025.102566>
- Sankar, P. M., Raguchander, T., & Shanmugabackiam, S. (2017). Seed

- bacterization: A novel prime tool for growth promotion in tomato (*Solanum lycopersicum* M.) under in vitro. *International Journal of Current Microbiology and Applied Sciences*, 6(11), 3076–3080.
- Taiebikhah, N., Mirtalebi, M., & Gharin, R. (2026). Antagonistic potential of arugula vermicompost-derived fungi for controlling tomato crown and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Physiological and Molecular Plant Pathology*, 141, Article 102982. <https://doi.org/10.1016/j.pmpp.2025.102982>
- Townsend, G. R., & Heuberger, J. W. (1943). Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter*, 27(17), 340–343.
- Tunalı, B., Tosun, C., Maldar, B. M., Meyva, G., & Kansu, B. (2018). Bazı antagonist *Trichoderma* ve endofitik *Acremonium* izolatlarının *Fusarium* kök ve kök-boğazı çürüklüğü hastalıklarına karşı etkinliklerinin belirlenmesi. *Türk Tarım-Gıda Bilim ve Teknoloji Dergisi*, 6(3), 266–272. <https://doi.org/10.24925/turjaf.v6i3.266-272.1613>
- Validov, S., Kamilova, F., Qi, S., Stephan, D., Wang, J. J., Makarova, N., & Lugtenberg, B. (2007). Selection of bacteria able to control *Fusarium oxysporum* f. sp. *radicis-lycopersici* in stonewool substrate. *Journal of Applied Microbiology*, 102(2), 461–471. <https://doi.org/10.1111/j.1365-2672.2006.03083.x>
- Yang, Y., Wang, Y., Gao, J., Shi, Z., Chen, W., Huangfu, H., Li, Z., & Liu, Y. (2024). Characterisation of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in infected tomatoes in Inner Mongolia, China. *Journal of Fungi*, 10(9), Article 622. <https://doi.org/10.3390/jof10090622>
- Yıldırım, K. C., Canik Orel, D., Okyay, H., Gürsan, M. M., & Demir, İ. (2021). Quality of immature and mature pepper (*Capsicum annuum* L.) seeds in relation to bio-priming with endophytic *Pseudomonas* and *Bacillus* spp. *Horticulturae*, 7(4), Article 75. <https://doi.org/10.3390/horticulturae7040075>
- Yıldırım, K. C., Canik Orel, D., Okyay, H., Gürsan, M. M., & Yıldız, G. (2025). Enhancing seed quality and plant growth through modification of seed microbiome with beneficial endophytic bacteria. *Scientia Horticulturae*, 354, Article 114531. <https://doi.org/10.1016/j.scienta.2025.114531>
- Zhang, Z., Li, J., Zhang, Z., Liu, Y., & Wei, Y. (2021). Tomato endophytic bacteria composition and mechanism of suppressiveness of wilt disease (*Fusarium oxysporum*). *Frontiers in Microbiology*, 12, Article 731764. <https://doi.org/10.3389/fmicb.2021.731764>