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Microbe-enriched mushroom compost supressing bacterial wilt caused by *Ralstonia solanacearum* and promoting tomato growth

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ABSTRACT

Ralstonia solanacearum is a phytopathogenic bacterium that causes bacterial wilt in tomato plants, often resulting in yield losses. To develop a novel bioproduct for managing the bacterial wilt in tomatoes, antagonistic bacteria and actinomycetes were isolated from mushroom media compost and subsequently applied individually or in combination to fortify organic fertilizers. This study aimed to identify effective antagonistic microorganisms and evaluate the efficacy of the compost enriched with these suppressive agents in controlling the pathogenicity of *R. solanacearum* as well as promoting tomato plant growth and yield. The experiment was conducted in the greenhouse. The treatments involved different planting media for the tomato cultivar 'Servo F1', including: soil without compost (K0), compost without suppressive microbes (K1), compost enriched with suppressive bacteria (KB), compost enriched with suppressive actinomycetes (KA), and compost enriched with a combination of suppressive bacteria and actinomycetes (KM). The measured parameters included the incidence and severity of bacterial wilt, shoot dry weight, fruit yield, and *R. solanacearum* population in the rhizosphere. The results demonstrated that the application of the compost enriched with suppressive microbes significantly reduced the severity of wilt disease up to 80% compared to the control. Furthermore, it significantly increased shoot dry weight by 99–134.4%, the number of fruits/plant by 130.33–150.15%, and fruit weight/plant by 258.80–331.81% compared to planting media without compost or with non-enriched compost.

Keywords: enriched compost, suppressive microbes, soil-borne pathogen, *Ralstonia solanacearum*, tomato.

INTRODUCTION

Ralstonia solanacearum is a predominant soil-borne pathogen in Indonesia, affecting a wide range of horticultural crops, including tomatoes. This bacterium can survive for several years in the absence of a host plant and may establish latent infections in weed species (Phiri et al., 2024). Moreover, *R. solanacearum* is highly adaptable, capable of surviving within a broad temperature range and under diverse environmental conditions (Stevens et al., 2018). Its transmission occurs through contaminated water, infested soil, and agricultural equipment (Álvarez et al., 2022).

Integrated management strategies, such as the cultivation of resistant tomato varieties, have been implemented. However, these approaches have not been fully effective in mitigating the risk of *R. solanacearum* infections (Chachar et al., 2025). A promising and environmentally friendly alternative is the application of suppressive compost, which can support the development of disease-suppressive planting media (Zhao et al., 2023). In addition, compost is known to improve soil fertility, thereby enhancing plant growth (Suvendran et al., 2025).

Compost, such as mushroom compost, is the product of microbial decomposition of organic matter and may exhibit suppressive activity against various soil-borne pathogens (Zhao et al., 2023). Mushroom compost is known to be rich in nutrients and microbes, including the microbes antagonistic to soil-borne pathogens. Therefore, compost has been used as an organic fertilizer and agricultural disease management (Mwangi et al., 2024). Enrichment of the compost with suppressive microbes, particularly antagonistic and synergetic groups, such as bacteria and actinomycetes, plays a central role in controlling pathogen. Therefore the greater the abundance of these suppressive and synergetic microbes in the compost, the higher the potential for pathogen suppression. Antagonistic microorganisms are capable of suppressing soil-borne pathogens through mechanisms such as antibiosis, competition, and the induction of plant resistance. Examples include *Bacillus velezensis*, *Trichoderma* sp., and *Pseudomonas fluorescens*, which have been shown to inhibit *Ralstonia solanacearum*, additionally, *P. fluorescens* enhances plant resistance by inducing defense-related enzymes (Pepoyan and Chikindas, 2025). Suppressive microbes, particularly antagonistic and synergistic groups such as bacteria and actinomycetes, play a central role in controlling pathogens. Therefore, the greater the abundance of these suppressive and synergistic microbes in the compost, the higher the potential for pathogen suppression.

However, previous studies have largely focused on the general suppressive effects of compost without specifically identifying, characterizing, and then reintroducing key antagonistic microbial consortia to enhance its efficacy against *Ralstonia solanacearum.* Therefore, this study bridged this gap by integrating the antagonistic bacteria and actinomycetes capable of suppressing soil-borne pathogens and evaluating the effectiveness of compost enriched with suppressive microbes in controlling the pathogenicity of *R. solanacearum* in tomato cultivation, as well as in promoting plant growth and yield.

MATERIALS AND METHODS

Pathogen and antagonistic microbes

A bacterial wilt pathogen, *R. solanacearum*, was obtained from the Laboratory of Plant Diseases at the Faculty of Agriculture, Universitas Gadjah Mada. The virulence of *R. solanacearum* was assessed using the streak plate method on Casamino Acids-Peptone-Glucose Triphenyl Tetrazolium Chloride (CPG-TZC) medium and incubated for 48 hours. The colonies exhibiting virulence were identified by their irregular shape, pink center, and fluidal texture (García et al., 2019)

Antagonistic bacteria and actinomycetes were isolated from champignon compost. Isolation was performed using nutrient agar (NA) for bacteria and starch nitrate agar (SNA) for actinomycetes. Individual colonies were purified and screened for antagonistic activity against soil-borne pathogens using the dual culture cross-streak technique, as described by (Nayak et al. (2012). The development of the interaction zone at the point of contact between compost isolates and the pathogen during incubation was used as the basis for selecting the isolates with antagonistic potential.

The interaction at the meeting point in the antagonistic assay may include colonization of the pathogen by the compost isolate, inhibition of the pathogen by the compost isolate, colonization of the compost isolate by the pathogen, inhibition of the compost isolate by the pathogen, or mutual coexistence where both organisms grow adjacently. The observed interactions between the pathogen and the isolates served as the reference for selection, and isolates demonstrating the strongest inhibitory interactions were selected.

DNA extraction

The selected antagonistic microbes, which exhibited strong antagonistic activity through competitive colonization, were identified based on the 16S rRNA gene. The actinomycetes and bacterial DNA were extracted using Geneaid Presto Mini gDNA Bacteria Kit with the manufacturer's guidelines (New Taipei City, Taiwan). The extracted genomic DNA templates were then used in PCR to amplify the 16S rRNA region.

PCR

MyTaq Redmix PCR kit was used to amplify using 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers. PCR was performed in a PCR Thermal Cycler using 50 µL reactions containing: 22 µL MyTaq Redmix PCR (Meridian, USA), 1 µL of each primer, 25 µL PCR-grade water, and 1 µL template DNA. PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 1 minute, followed by 35 cycles of denaturation at 95 °C for 15 seconds, 50 °C for 15 seconds for annealing, and extension at 72 °C for 10 seconds, with a final extension at 72 °C for 5 minutes. PCR products were visualized on 1.5% agarose gels and stained with Ethidium Bromide in 1x Tris-Borate-EDTA buffer for 60 minutes at 100 volts.

Sequencing

The PCR products amplified with 8F and 1492R were sequenced, and the sequences were deposited in GenBank. The sequences were assembled into contigs using the DNA Baser application. The resulting contigs were subsequently analyzed using the online bioinformatics tool Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the reference sequences with the highest similarity, which were used to infer the putative identity of the corresponding isolates. The Accession numbers for each isolate are: PZ213443 for sequence 9A; PZ213444 for sequence 15A; PZ213445 for sequence 14A, and PZ213446 for sequence 19A. Phylogenetic analysis was conducted by constructing a phylogenetic tree using the MEGA X software. The neighbor-joining method was employed for tree construction, with nucleotide sequences used as the evolutionary model, and bootstrap analysis was performed with 1.000 replicates. These selected microbes were subsequently used to fortify organic fertilizers.

Compost preparation

The organic fertilizer (OF) used in this study was a compost derived from the cultivation media of *Agaricus bisporus*, the same compost source used for the isolation of antagonistic microbes. The compost used was fully matured and sieved through a 2 mm mesh screen. Its chemical composition was as follows: 29.86% organic carbon (C-organic), 2.50% total nitrogen (N), 2.07% phosphorus pentoxide (P_2O_5), 3.50% potassium oxide (K_2O), and a C/N ratio of 11.94.

Antagonistic bacterial isolates were cultured individually in 500 mL Erlenmeyer flasks containing Nutrient Broth (NB), while actinomycetes were grown on Starch Nitrate Agar (SNA) plates. After incubation, bacterial suspensions were prepared in sterile distilled water and adjusted to 10^8 cells/mL (García et al., 2019).

Spores of actinomycetes were harvested from the SNA medium by adding a solution containing 0.2% Tween 80 and 0.85% NaCl. The spores were gently scraped using a sterile loop, transferred to Falcon tubes, and centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded, and the resulting pellet was resuspended in sterile distilled water. A 10 µL aliquot was then loaded onto a hemocytometer to determine spore density. Spore suspensions from the two selected Actinomycetes isolates were mixed in equal concentrations to achieve a final spore density of 10 spores/mL (Zhao et al., 2023).

The bacterial and actinomycete suspensions were blended with the organic fertilizer, either individually or in a combination (bacteria + actinomycetes, at the same ratio as the individual treatments), to produce enriched organic fertilizers containing approximately 10CFU/g of bacteria and 10^7 spores/g of actinomycetes in 500 grams of compost (García et al., 2019).

Test for suppression of bacterial wilt disease in tomato using enriched compost under greenhouse conditions

The experiment was conducted in the greenhouse of the Department of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta. During the experimental period, the average maximum and minimum temperatures were 32.5 °C and 19.9 °C, respectively—seeds of *Solanum lycopersicum* cv. Servo F1 was soaked in sterile distilled water for 60 minutes. Only the seeds that sank were selected for further use. The seeds were sown in seed trays filled with a 4:1 (v/v) mixture of regosol soil and rice husk, and covered with shade net (paranet) for 14 days. The seedlings were maintained in the greenhouse and irrigated daily in the afternoon with sterile water (García et al., 2019).

The air-dried regosol soil was amended with 20% (v/v) of each treatment: K1 (organic fertilizer only), KB (organic fertilizer enriched with bacteria), KA (organic fertilizer enriched with actinomycetes), KM (organic fertilizer enriched with both bacteria and actinomycetes), and unamended soil as the control (K0). A basal fertilizer (NPK Phonska 15-15-15) was applied to all treatments at a rate of 2 grams per polybag.

Fourteen days after germination, the seedlings were transplanted into 35 × 35 cm polybags filled with a growing medium composed of regosol soil, rice husk, and compost at a 3:1:1 (v/v) ratio. For the control treatment (K0), the medium consisted of regosol soil and rice husk at a 4:1 ratio (v/v). Each polybag contained 4.5 L of growing medium. Compost was applied at a rate of 500 g per polybag, as specified for each treatment.

At 26 days after transplanting (DAT), a 50 mL suspension of *R. solanacearum* (10^8 cells/mL) was applied directly to the root zone. The lateral roots were wounded using sterile scissors to facilitate infection. A total of 15 polybags (five treatments, 3 replicates each, 1 plant per polybag) were arranged in a completely randomized design on greenhouse benches. Each row contains all treatments in random positions. There were three replicate rows. One polybag per row was rotated every 2 days due to variations in the greenhouse conditions. Standard tomato cultivation practices were followed, including the use of plant stakes at 35 DAT and manual weeding every three days. The tomato plants were maintained for 54 days after transplanting.

Disease progression was monitored weekly following pathogen inoculation. Disease incidence (DI) and disease severity were assessed according to the method of Wamani et al. (2023), using the modification method of Kebei et al. (2020). DI was calculated using the following formula:

$$DI\ (\%) = \frac{\begin{matrix} Number\ of\ wilted \\ leaves\ observed \end{matrix}}{\begin{matrix} total\ number\ of \\ leaves\ per\ plant \end{matrix}} \times 100\% \tag{1}$$

Disease severity was rated using a 0–5 scale, 0= healthy leaves (no wilting symptoms), 1= ≤10% of leaf area affected (very mild), 2= >10% – ≤25% of leaf area affected (mild), 3 = >25% – ≤50% of leaf area affected (moderate), 4 = >50% – ≤75% of leaf area affected (severe), and 5 = >75% of leaf area affected. The overall disease severity index was calculated using the following formula:

$$Disease\ Severity\ (\%) =$$

$$= \frac{\Sigma \quad \left(\begin{matrix} Number\ of\ infected\ plants\ \times \\ disease\ score \end{matrix} \right)}{\begin{matrix} Maximum\ score\ \times \\ Total\ number\ of\ observed\ plants \end{matrix}} \times 100\% \tag{2}$$

To evaluate the impact of treatments on the *R. solanacearum* population density in soil, rhizosphere soil samples (three replicates per treatment) were collected at 0 days after inoculation (DAI) and at harvest. The population of *R. solanacearum* was quantified using the streak plate method on CPG-TZC medium supplemented with 25 ppm ampicillin. For 0 days after inoculation, 10^{-7} and 10^{-8} serial dilutions were used, and for the 28 days after inoculation 10^{-5} and 10^{-6} for K0 and 10^{-2} and 10^{-3} for other treatments were used. After incubation at 28 °C for two days, colonies characteristic of *R. solanacearum* were counted, and colony-forming units (CFU) were calculated per gram of soil (dry weight).

Plant growth parameters

At harvest, fresh and dry weights were determined according to Antoniu et al. (2017). Shoots were separated from roots by cutting at the stem base. Both shoot and root samples were placed in paper bags and dried in an oven at 65 °C until a constant weight was reached, after which the dry weight (in grams) was recorded (Hernández-Amador et al., 2024). Tomato fruits were harvested and evaluated by counting the number of fruits per plant, weighing the total fruit yield per plant, and calculating the average fruit weight.

Data analysis and interpretation

The data obtained from observations were analyzed using RStudio. Before statistical analysis, the normality and homoscedasticity assumptions were tested for variables such as dry weight and yield components. Homoscedasticity, or the homogeneity of variances, was assessed using the Breusch-Pagan test (Non-Constant Variance), while normality was evaluated using the Shapiro-Wilk test. The data that met the assumptions of normal distribution and homogeneous variance ($P \geq 0.05$) were subsequently analyzed using one-way analysis of variance (ANOVA). Post-hoc analysis was conducted using Duncan's multiple range test (DMRT) at a 5% significance level when ANOVA results indicated significant treatment effects ($P \leq 0.05$) (Apriyadi and Liestiany, 2019).

RESULTS

Characteristics of isolated bacteria and actinomycetes

A total of 28 bacterial isolates were obtained from the soil samples collected from champignon compost. Among them, isolates 9A and 15A exhibited the strongest antagonistic activity against *R. solanacearum* in vitro, particularly in terms of colonization capacity (Figure 1a). The observed colonization mechanism (Col) involved competition for nutrients and space, which is a common strategy used by biocontrol agents to suppress pathogen infection. This mode of action includes physical occupation of ecological niches and secretion of specific compounds that enhance micronutrient absorption by the biocontrol agent (Lahlali et al., 2022).

The 16S rRNA gene sequence analysis identified isolate 9A (accession number: PZ213443) as having similarity to *Bacillus subtilis,* and isolate 15A (accession number: PZ213444) showed similarity to *Bacillus inaquosorum* (Figure 2a).

From the same compost samples, 12 actinomycete isolates demonstrating antagonistic activity against soil-borne pathogens were isolated. Among these, two isolates, 14A and 19A were selected based on their superior antagonistic activity (Figure 1b). According to 16S rRNA gene sequence analysis, isolate 14A (accession number: PZ213445) as having similarity to *Streptomyces diastaticus* and isolate 19A (accession number: PZ213446) showed similarity to *Streptomyces thermocoprophilus* (Figure 2b).

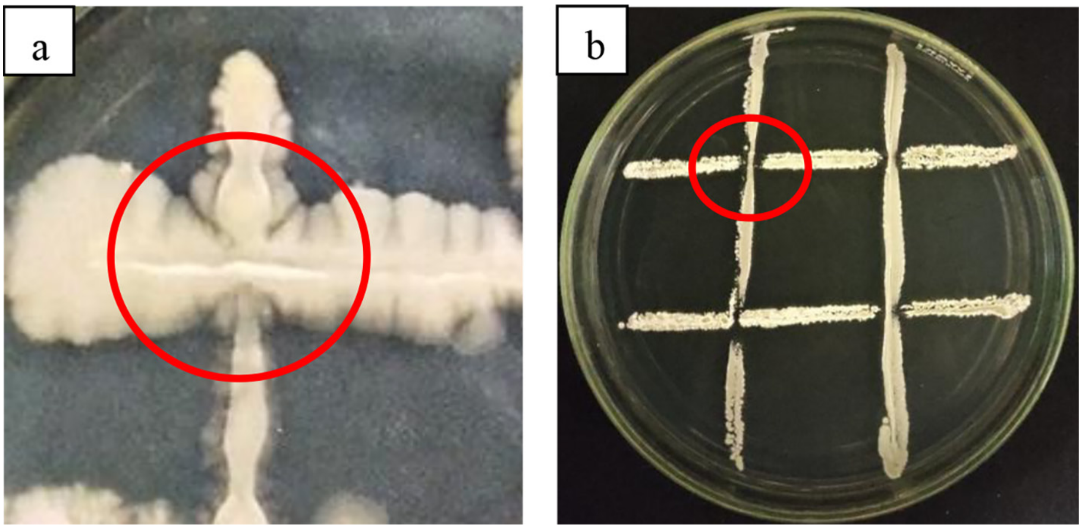

Figure 1. Antagonistic assay of actinomycete and bacterial isolates against soil-borne pathogens
Selected bacterial isolates (horizontal) colonizing pathogens (vertical) (a), while selected actinomycete isolates (vertical) suppress the pathogen (horizontal) through the formation of a clear inhibiting zone (b)

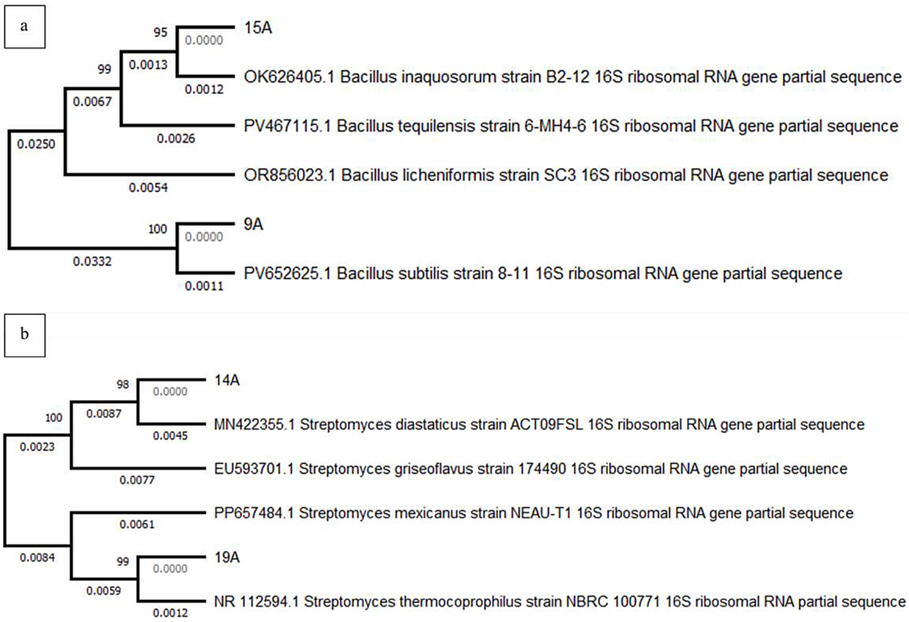

Figure 2. Phylogenetic tree constructed with the 16S rDNA sequences of isolate 9A and 15A (a), isolate 14A (b), and isolate 19A (c)

Streptomyces thermocoprophilus exhibited antagonism against soil-borne pathogens, as evidenced by clear inhibition zones (Figure 1b), indicating antibiotic production. These inhibitory effects are attributed to nutrient and spatial competition with the pathogen (Vos et al., 2017).

Suppression of BWT by enriched compost in the greenhouse

Leaf wilting progressed throughout the entire plant by 28 days after inoculation (DAI) in the untreated control without compost application (K0) (Figure 3). In addition, the cut stems from infected plants, when immersed in water, released a turbid exudate, indicating the presence of extracellular polysaccharides (EPS) produced by *R. solanacearum* (Figure 4).

Figure 3 shows that the application of champignon compost effectively suppressed disease incidence compared to the tomato plants grown without compost (K0). Among all treatments, the compost enriched with a combination of suppressive actinomycetes and bacteria (KM) resulted in the lowest disease incidence at 28 DAI. This finding is supported by the Total Plate Count of *R. solanacearum*, which showed a reduction in bacterial population at 28 DAI (Table 1).

Application of champignon compost reduced disease severity compared to the untreated control (K0) (Figure 5). Although no significant differences in wilting severity were observed among KA, KB, and KM treatments, all demonstrated greater effectiveness than K1 (compost only) and K0 (no compost).

Figure 5 indicates that compost treatments were effective in managing the bacterial wilt caused by *R. solanacearum*. Enriched compost significantly reduced the severity of wilt disease, with values as low as 20%, compared to 100% in the control treatment. This indicates that applying enriched compost reduced the severity of wilt disease by up to 80% compared with the control.

Plant growth and yield parameters

Compost use in tomato cultivation increases plant dry weight. As it is shown in Table 2, all compost-treated plants had higher total dry weight than those without compost. Notably, the compost enriched with a combination of suppressive microbes, regardless of pathogen inoculation, produced the highest total dry weight among all treatments.

The high-yielding tomato variety ’Servo F1’ is susceptible to *R. solanacearum* (Navitasari et al., 2021), as evidenced by high disease severity and reduced yields (Table 3). The analysis of yields under enriched compost treatments showed no significant differences among treatments in the number of fruits per plant or total fruit weight per plant. However, compared to the control treatment, the enriched compost treatments increased both the average number of fruits per plant and the average fruit weight per plant, with respective increases ranging from 130.33% to 150.15% and from 258.80% to 331.81%.

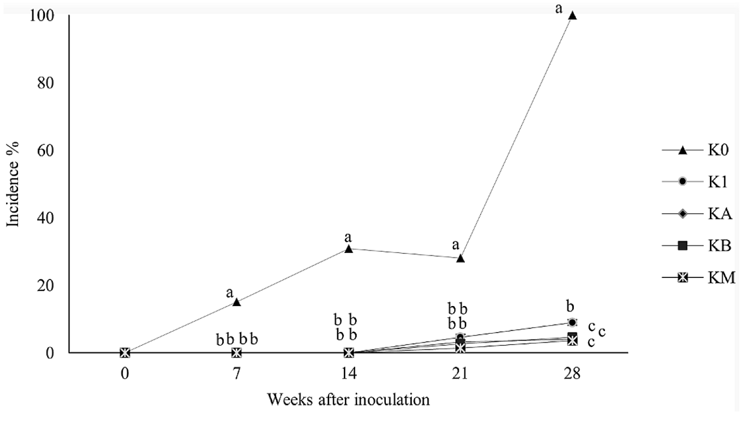

Figure 3. Disease incidence was recorded weekly over 28 days after inoculation (DAI)
Treatments include: no compost + inoculation (K0), compost + inoculation (K1), compost enriched with bacteria + inoculation (KB), compost enriched with actinomycetes + inoculation (KA), and compost enriched with a mixture of actinomycetes and bacteria + inoculation (KM)

Figure 4. Bacterial ooze. Cloudy white viscous exudate of *R. solanacearum* (bacterial ooze) emergin from the cut stem of an infected tomato plant

Table 1. Total plate count of R. *solanacearum*

Treatment	Total *R. solanacearum* count on the DAI (CFU/g)	
	0	28
K0	80.00×10^7	5.00×10^6
K1	78.00×10^7	9.00×10^3
KB	50.00×10^7	8.50×10^3
KA	52.00×10^7	8.00×10^3
KM	50.00×10^7	4.00×10^3

Note: Treatments include: no compost + inoculation (K0), compost + inoculation (K1), compost enriched with bacteria + inoculation (KB), compost enriched with actinomycetes + inoculation (KA), and compost enriched with a mixture of actinomycetes and bacteria + inoculation (KM).

DISCUSSION

The application of biological control agents offers a promising alternative strategy for managing bacterial wilt, with several advantages, including cost-effectiveness, environmental friendliness, and pathogen-specific targeting. According to Ling et al. (2020), traditional methods, such as plant breeding, soil sanitation, crop rotation, and bactericide application, have demonstrated limited success in controlling *R. solanacearum*. Biological control can be implemented by introducing beneficial microbes directly into soil or a field containing the target pathogen, for instance, through compost application (Antoniou et al., 2017). The authors also reported that the compost containing suppressive microbes effectively managed soil-borne pathogens that are otherwise difficult to control using bactericides or resistant cultivars.

Numerous studies have demonstrated the suppressive potential of compost. The research by He et al. (2020) and Ojo et al. (2021) found that the compost prepared from vinegar residue and cow dung reduced the incidence of the bacterial wilt caused by *R. solanacearum*. More significant disease suppression was achieved when the compost was inoculated with antagonistic bacteria, including *Bacillus amyloliquefaciens* (strains QL-5 and

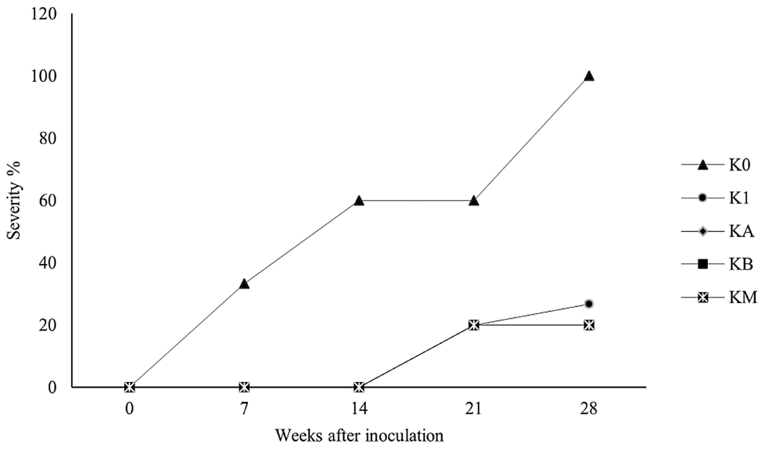

Figure 5. Wilt disease severity. Treatments include: no compost + inoculation (K0), compost + inoculation (K1), compost enriched with bacteria + inoculation (KB), compost enriched with actinomycetes + inoculation (KA), and compost enriched with a combination of actinomycetes and bacteria + inoculation (KM)

Table 2. Dry weight (DW) of tomato plants at 28 days after inoculation (DAI)

Treatment	Total DW (g)	Effectiveness (%)
K0	10.83±0.53^{d}	-
K1	17.98±0.45^{c}	66.02
KB	23.89±0.76^{a}	120.59
KA	21.56±0.17^{b}	99.08
KM	25.39±0.43^{a}	134.44

Note: Data are presented as mean ± standard error of the mean (n = 3). Different letters within the same column indicate statistically significant differences based on Duncan's Multiple Range Test (DMRT) at the 5% significance level. Treatments include: no compost + inoculation (K0), compost + inoculation (K1), compost enriched with bacteria + inoculation (KB), compost enriched with actinomycetes + inoculation (KA), and compost enriched with a mixture of actinomycetes and bacteria + inoculation (KM).

QL-18), *Streptomyces rochei* (L-9), and *Brevibacillus brevis* (L-25). Moreover, non-aerated compost teas (NCTs), characterized by high microbial diversity, have been shown to suppress bacterial wilt in potato (Mengesha et al., 2017). Secondary compost products (SCPs) have also demonstrated the ability to enhance soil suppressiveness against *R. solanacearum* and can be integrated with other disease management strategies (Liu et al., 2016).

A diverse range of antagonistic biocontrol agents from the domain *Bacteria* has been identified. Genera such as *Azotobacter*, *Bacillus*, *Pseudomonas*, *Paenibacillus*, and *Lysobacter* are frequently reported as phytostimulants or biocontrol agents (Dukare and Paul, 2021). Additionally, species from *Serratia* and *Pantoea* have shown antagonistic effects against plant pathogens (Dukare and Paul, 2021; Wang et al., 2018). *Chryseobacterium nankingense* sp. nov. WR21, reported by Huang et al. (2017), suppresses *R. solanacearum* through nutrient competition involving root exudates.

Members of the *Pseudomonadaceae* and *Bacillaceae* families, particularly *Bacillus* and *Pseudomonas*, exhibit antagonistic activity through competition and antibiosis (Dimkić et al., 2022; Hajek and Eilenberg, 2018). *Bacillus* spp. has been reported to produce several compounds and enzymes, such as bacteriocins and glucanases, to suppress plant pathogens (Nurcahyanti et al., 2023). *Bacillus thuringiensis* is also known to induce systemic defense genes in tomato plants against *R. solanacearum* (Hyakumachi et al., 2013). These bacterial taxa thus offer viable options for managing major soil-borne plant pathogens.

Beyond bacteria, actinomycetes, particularly those belonging to the genus *Streptomyces,* are strong candidates for biocontrol agent development. These microorganisms decompose organic materials, such as starch, cellulose, chitin, keratin, and lignin, thereby contributing to nutrient cycling (Krysenko and Wohlleben, 2024). Actinomycetes are also prolific producers of antibiotics, making them potent antagonists of soil-borne pathogens. They suppress pathogens by producing cell wall-degrading enzymes, antifungal compounds, and plant growth-promoting substances. *Streptomyces* spp. have been widely used as antagonistic microbes against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* (Khan et al., 2023; Perez et al., 2024). For instance, Chen et al. (2018) reported that the application of *Streptomyces* reduced the banana Fusarium wilt disease index from 70.45 (without inoculation) to 10.23 (with inoculation), increasing disease control efficacy from 10.45% to 83.12%. The antagonistic effects of actinomycetes are mediated through rhizosphere colonization, extracellular enzyme production, and antibiotic biosynthesis.

Table 3. Yield components of tomato plants

Treatment	Average number of fruits/plant	Effectiveness (%)	Average fruit weight in gram/plant	Effectiveness (%)
K0	3.33±0.33^{c}	-	21.41±1.74^{d}	-
K1	5.00±0.58^{b}	50.15	43.24±4.58^{c}	101.96
KB	8.33±0.33^{a}	150.15	92.45±3.86^{a}	331.81
KA	7.67±0.33^{a}	130.33	76.82±3.22^{b}	258.80
KM	8.00±0.58^{a}	140.24	84.12±4.30^{ab}	292.90

Note: Data are presented as mean ± standard error of the mean (n = 3). Different letters within the same column indicate statistically significant differences based on Duncan's multiple range test (DMRT) at the 5% significance level.

The common edible mushroom (*Agaricus bisporus*), the most widely cultivated mushroom species globally, produces large quantities of spent mushroom substrate (Okuda, 2022). Previous research has demonstrated the efficacy of champignon mushroom compost in reducing the pathogenicity of *R. solanacearum* in tomato (Jumas, 2022). The synergistic interaction between compost microbes and native soil microbiota forms a microbial consortium capable of suppressing pathogens. Suppressive compost is known to harbor the microorganisms that inhibit soil-borne pathogens through a such mechanisms as antagonism, competitive exclusion, and induction of plant resistance (Lutz et al., 2020). Singh et al. (2018) also emphasized that a diverse soil microbiome enhances plant health and indirectly reduces pathogen virulence. On the basis of this evidence, it was hypothesized that enriching mushroom compost with additional suppressive microbes could further enhance its disease-suppressive properties.

Jumas (2022) reported that incorporating champignon mushroom compost into the planting medium significantly reduced the pathogenicity of *R. solanacearum* in tomato compared with a soil-husk mixture alone. The current study confirmed that the bacteria isolated from this compost exhibit colonization capacity and antagonistic effects against *R. solanacearum*. Specifically, *Bacillus cereus* and *Bacillus subtilis* were identified as effective antagonists. According to Shafi et al. (2017), these species control plant pathogens through the production of lipopeptides, antibiotics, enzymes, and by inducing systemic resistance. Wang et al. (2018) further showed that *B. cereus* enhanced plant defense by stimulating root exudation.

Additionally, two antagonistic actinomycetes were successfully isolated from champignon mushroom compost: *Streptomyces diastaticus* and *Streptomyces thermocoprophilus*. These isolates demonstrated antagonistic activity through nutrient and spatial competition, as well as antibiotic production. *Streptomyces* spp. are known to suppress *R. solanacearum* through bacteriostatic mechanisms, including the production of antibacterials (He et al., 2024). Ling et al. (2020) reported that actinomycins could provide up to 100% control of bacterial wilt in tomato. Although 16S rRNA gene sequencing is widely used for bacterial identification, its resolution is often insufficient to distinguish closely related species. Therefore, taxonomic assignments at the species level in this study should be interpreted with caution, and future studies employing higher-resolution genomic approaches are needed to confirm species identity.

Recent studies have demonstrated the effectiveness of biological control strategies for managing bacterial wilt using antagonistic bacteria (Mekonnen et al., 2022; Sun et al., 2023). The introduction of one or more microbial strains, either native or genetically engineered, is referred to as bioaugmentation. Optimal microbial candidates are typically isolated from the same site and propagated under laboratory conditions (Tondera et al., 2021). Microbial performance is influenced by nutrient availability and environmental conditions. Ling et al. (2020) observed that high-density actinomycete application suppressed *R. solanacearum* more effectively than lower-density treatments. Therefore, increasing the population of antagonistic microbes is expected to enhance pathogen suppression. The conducted bioassay experiments demonstrated that enriched mushroom compost significantly reduced the pathogenicity of *R. solanacearum* in tomato, compared to the treatments without compost or with unenriched compost.

The obtained findings revealed that enriched compost reduced bacterial wilt disease severity in tomato by 80% compared with the control (Figure 5). Antoniou et al. (2017) also reported reduced disease development with suppressive compost. Sowmiya et al. (2025) explained that one mechanism of antagonism involves beneficial microbes outcompeting pathogens for root infection sites, thereby reducing *R. solanacearum* colonization. Additionally, nutrient availability influences soil microbial composition and contributes to disease suppression (Cao et al., 2024). An increased population of antagonistic microbes supports compost suppressiveness. Chachar et al. (2025) stated that plant defense responses to pathogen attacks involve the recruitment of beneficial microbes via root exudates, which, in turn, directly suppress pathogens.

Compost application also significantly affected the dry biomass of tomato plants. Several studies have shown that applying compost improves soil quality (Said et al., 2022), productivity, and sustainability (Shahadha et al., 2025). Zeleke et al. (2024) suggest that physiological and morphological tomato plant traits are more largely influenced by water and nutrient uptake. The tomato plants treated with enriched compost developed denser root systems compared to those in untreated controls. Although there were no statistically significant differences in fruit number or fruit weight per plant among enriched compost treatments (Table 3), the compost enriched with bacteria produced more fruits than the compost enriched with actinomycetes. This is likely due to nitrogen competition, as actinomycetes require nitrogen for both growth and antibiotic production, thereby reducing nitrogen availability for plant uptake (Zhang et al., 2025).

This study highlighted the critical role of compost in modulating the rhizosphere microbiome to suppress bacterial wilt. Disease-suppressive compost may protect plants through various mechanisms, including improved nutrient supply (Cao et al., 2024), pathogen inhibition through antibiotic production (De Corato, 2020), and activation of plant immune responses (He et al., 2020). The treatments involving bacterial, actinomycete, or combined microbial enrichment do not differ significantly in disease suppression, biomass accumulation, or yield enhancement, but all outperformed the non-enriched compost. However, potential synergistic or antagonistic interactions among the introduced microbes remain to be elucidated. Field conditions may influence the outcomes differently from controlled experiments due to variability in soil properties, climate, rainfall, crop cultivar, and temperature. Álvarez et al. (2022) noted that temperature fluctuations, particularly cold conditions, reduced *R. solanacearum* viability. Hence, further field-scale studies are needed to assess the effectiveness of enriched mushroom compost under diverse agricultural conditions. Factors such as environmental changes, soil homogeneity, and interactions with indigenous microbial communities may affect the effectiveness of the applied treatment. Therefore, field-based validation is necessary to understand and confirm the applicability and consistency of these findings under real farming conditions. Moreover, since no single method can fully control soil-borne pathogens, integrating mushroom compost application with other practices such as adjusted planting schedules, crop rotation, mechanical controls, and cultural methods could provide a more holistic and sustainable strategy for managing bacterial wilt.

CONCLUSIONS

The application of the compost enriched with suppressive microbes to the tomato plants exposed to R. solanacearum demonstrated significant effectiveness in suppressing pathogenicity by up to 80% compared to the treatments without compost or with unenriched compost. The enriched compost also significantly increased shoot dry weight by 99–134.4%, the number of fruits/plant by 130.3–150.2%, and fruit weight/plant by 258.8–331.8% compared to planting media without compost or with non-enriched compost.

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