Journal of Crop Health (2024) 76:783–792
https://doi.org/10.1007/s10343-024-01002-x

RESEARCH

Biocontrol of Bacterial Wilt Biotic Stress in Tomato Plants by Successful Host Root Colonization and Inducing Host Resistance

Xiang Li[1] · Saleh Alfarraj[2] · Mohammad Javed Ansari[3]

Received: 25 January 2024 / Accepted: 20 June 2024 / Published online: 25 June 2024

Abstract

Plant treatments with biocontrol agents to deal with biotic stress are widely reported, but the information regarding detailed action mechanisms of biocontrol and host response is rarely reported. This study investigated a biocontrol bacterial agent, *Bacillus cereus*, to manage tomato bacterial wilt (BW) disease. The *in vitro* antibacterial potential of *B. cereus* was assessed, followed by the ability of *B. cereus* to colonize tomato roots and induce host resistance. Additionally, we tested the application of *B. cereus* for managing tomato BW disease. *In vitro* investigations revealed the volatile mediated antibacterial activity of *B. cereus*, indicating that *B. cereus* produces antibacterial volatiles against *R. solanacearum*. The effectiveness of *B. cereus* in colonizing tomato roots was evaluated through its transgenic GFP-tagged strains and confirmed through qPCR analysis. It was found that the biocontrol bacterium successfully colonized the host root. The *B. cereus* concentration reached 9.37 × 107 at 48 h. The tomato plants under bacterial wilt stress, when treated with *B. cereus*, showed upregulation of genes linked to the plant defense system. The application of *B. cereus* to soil infested with *R. solanacearum* and planted with tomato plants reduced the pathogen population in the soil, resulting in a reduction in disease severity and improved plant growth. This study suggests the biocontrol potential of *B. cereus* to manage bacterial wilt disease.

Keywords Biocontrol · Action mechanism · Horticulture crop · Vegetable · Disease · Bacteria

Introduction

Among horticultural crops, tomato is one of the most consumed and widely cultivated crops, with 38.7 million tonnes of global production in 2021 (WPTC 2021). Tomatoes contain various minerals and micronutrients, making them a good choice for nutrient supplementation. It is a low-cost source of vitamins, niacin, and calcium. Tomato intake in daily nutrition can lower the risk of developing osteoporosis and heart disease (Salehi et al. 2019). Year-round high yield, low input cost, and short growing seasons attract farmers to cultivate tomatoes, especially in countries with warmer climates (Silva et al. 2017). China leads the world in tomato production, with an annual yield of 64.27 million tonnes (Costa and Heuvelink 2018). The cultivation of tomatoes is subject to various biotic and abiotic challenges. Concerning biotic challenges, tomatoes are vulnerable to different pathogens, including fungi, bacteria, and viruses, which seriously limit tomato quality and production.

Among bacterial pathogens, *R. solanacearum* causes bacterial wilt (BW) disease in solanaceous plants, a serious threat to the quality and quantity of tomatoes. Yield losses in tomatoes caused by *R. solanacearum* vary from 0–100% depending on the cultivar and strain of the pathogen, climate, and soil type (Nion and Toyota 2015). It has been reported that the bacterial wilt disease is responsible for a loss of 25% of the fresh fruit production of hybrid tomatoes, and with high disease incidence, yield losses might reach up to 93.51% (Wu et al. 2023). There are five biovars and five races of the pathogen (Alghuthaymi et al. 2016). The disease has caused a reduction in crop yield in seventy countries globally, which resulted in more than US $ 0.9 billion in annual losses (Yuliar and Toyota 2015). Bacteria initiate diseases through host roots, followed by blocking and colonization of xylem vessels, resulting in

✉ Xiang Li
dr.lixielab@gmail.com

[1] School of Biological Sciences and Engineering, North Minzu University, 750030 Yinchuan, China

[2] Zoology Department, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia

[3] Department of Botany, Hindu College Moradabad (Mahatma Jyotiba Phule Rohilkhand University Bareilly), 244001 Moradabad, India

disease symptoms such as wilting, stunting, and yellowing that eventually lead to plant death (Iraboneye et al. 2021). Because it causes considerable losses to economically important crops, bacterial wilt disease has been extensively investigated, and several management practices have been utilized to manage the pathogen. Several biocontrol agents were assessed to manage BW disease in tomato plants (Bing et al. 2024; Li et al. 2024). However, the desired control level of the disease is still awaited. High genetic variability of the pathogen, persistency in soil, and the ability to infect many hosts make it difficult to effectively control the disease (Nguyen and Ranamukhaarachchi 2010). Researchers in different countries are trying to develop proper management methods using chemical-free and eco-friendly alternatives to control BW disease effectively. Among several management practices, biocontrol is one of the environment-friendly, sustainable, and cost-effective methods of managing plant diseases (Collange et al. 2011).

Bacteria are one of the most prevalent soil-inhibiting microorganism groups in soil ecology, and some of them act as biological agents against plant diseases (Abo-Elyousr and Hassan 2021; Saputra et al. 2020). Several *Bacillus* spp. are reported as biocontrol bacteria that offer different effective strategies such as direct parasitism, production of antimicrobial secondary metabolites, and induction of plant resistance against pathogens (Tahir et al. 2017). Other species of *Bacillus* have been investigated against various plant diseases, but the effectiveness of this bacterium as a biocontrol agent against tomato BW has received little attention. In this study, we investigated the biocontrol potential of *B. cereus* against *R. solanacearum*. We assessed the ability of *B. cereus* to colonize tomato roots and induce host resistance at the molecular level. Additionally, we tested the application of *B. cereus* for managing tomato BW disease.

Materials and Methods

Bacterial Culturing

The pre-identified bio-control bacterium B. cereus 32i-B and pathogenic bacterium *R. solanacearum* (Race 1, Biovar-III) were routinely cultured on LB medium at 28 °C for 48 h. Based on experimental requirements, the desired concentration of the bacteria was obtained through dilution by sterilized LB medium.

In Vitro Antibacterial Test

The biocontrol bacterium *B. cereus* was tested for its antibacterial potential against *R. solanacearum* by disc diffusion method (Umar et al. 2024). The *R. solanacearum* suspension 5 mL was added to 150 mL of CPG medium (Casamino acid 1 g, Peptone 10 g, Glucose 5 g) and incubated at 28 °C in a shaking incubator (200 rpm) for 24 h. After 24 h, the suspension was poured into dishes and cooled. Four filter papers, each 5 mm in size, were positioned on the surface of the medium at four corners at equal distances from each other, and one filter paper was placed at the center. A fresh culture of *Bacillus* was dropped on two cornered filter paper at 10 µl each. The remaining two cornered filter papers were treated with 10 µl water as a negative control. The filter paper at the center was treated with 10 µl of standard antibiotic (streptomycin) as positive control after incubating the plates for 48 h at 28 °C. The antibacterial activity was calculated by calculating the diameter of the growth inhibition zone around the Bacillus-treated filter paper.

Volatile-Mediated Antibacterial Activity

The volatile-mediated antibacterial activity of *Bacillus* against *Ralstonia* was evaluated through a 1-plate system. The 1-plate system consisted of an 85 × 15 mm petri dish centrally partitioned into two compartments with no physical contact between the two microbes (*Bacillus* and *Ralstonia*) grown on either compartment. The *R. solanacearum* suspension 10 µl was poured into one compartment filled with CPG agar medium and incubated for 24 h, followed by pouring of *Bacillus* culture in another compartment of the same plate filled with minimal salt medium (MS) and incubated again for five days. For control, the *R. solanacearum* was incubated alone. The diameter of the pathogen colony was measured, and a 10-fold serial dilution method was used to count the viable cells of the pathogen. The test was conducted three times with three replicates.

Host Root Colonization

The GFP protein was used to evaluate the capacity of *Bacillus* to colonize host roots. To make GFP-tagged strains, the electroporation of pGFP4412 plasmid into *B. cereus* was performed at 1.7 kV. Using GFP-specific primers, the transgenic bacteria were identified and observed through a fluorescence microscope. The pattern of colonizing roots by biocontrol bacterium was evaluated through tomato plant seedlings treated with GFP-tagged strains or untreated seedlings used for control. With absorbent paper, the seedlings were dried and investigated with CLSM (Chi et al. 2005). To evaluate the bacterial quantity in plant roots, DNA was extracted from *B. cereus* labeled with GFP through the TIANamp Bacteria DNA Kit. The recombinant strain was verified by amplifying the GFP gene. The amplifying primers of the GFP gene are given in Table S1. RT-PCR SYBR Green I was used in a 20 µl reaction system (Table S2). At every 0.5 °C, the fluorescence signals were recorded between 70 and 90 °C generated after developing

a standard curve by plotting the Ct number and bacterial log concentration on the Y and X axes, respectively. In *B. cereus* suspension, seedling roots were dipped for 0, 1, 2, 4, 6, 12, 24 or 48 h. The roots were sampled at the given time points, paper dried, and mixed after crushing. At each time, DNA was extracted using 3 samples of 0.2 g each and amplified through RT-PCR following the above procedure. With the help of a regression equation and Ct value, the bacterium was quantified using roots.

B. cereus's Influence on *R. solanacearum* Infection to Tomato Roots

For treatment, the seedling roots were dipped in *B. cereus* suspension (1×10^7) for six hours while roots dipped in water were used as control. The treated and control seedling roots were then inoculated with pathogenic suspension and kept at 25 °C for 24 h. After 24 h, the pathogenic bacteria (*R. solanacearum*) were isolated from roots, and their quantity per gram of roots was calculated through a 10-fold serial dilution technique on *R. solanacearum*-specific nutrient media NATZC.

Evaluation of Induce Host Resistance

Split-Root Evaluation

Induction of host resistance by biocontrol bacterium against *R. solanacearum* was done by a split-root system. This system comprised a pot with two compartments, each filled with a culture medium (Martínez-Medina et al. 2017). The seedling roots were distributed so that each compartment received half of the tomato roots. The pots were divided into two treatment groups (T1 and T2). One compartment of all pots (in both groups) was infected with 5 ml of the *R. solanacearum* suspension. The other compartment in T1 was poured with 5 ml of the biocontrol bacterium suspension (1×10^7) and in T2 with sterilized water as control. After twenty-five days, the pathogenic bacteria (*R. solanacearum*) were isolated from the treated roots, and their quantity per gram of roots was measured as described above. Data were also recorded on plant growth parameters (root length, plant length, and plant fresh biomass) and the severity of the disease.

Expression Evaluation of Host Defense Genes

The tomato plants (20 days old) in pots were observed to evaluate host defense genes under four treatments. T1: Biocontrol bacterium (*B. cereus*) + pathogenic bacterium (*R. solanacearum*); T2: Only biocontrol bacterium; T3: Only pathogen; T4: Control. Twenty-day-old tomato plants were irrigated for the first treatment with a 5 mL cultural suspension of *B. cereus* for 24 h. Then, a 5 mL cultural suspension of *R. solanacearum* was applied. The plants under the second treatment received only 5 mL cultural suspension of *B. cereus*, and for the third treatment, plants were irrigated with 5 mL cultural suspension of *R. solanacearum* only. The control plants in the fourth group were treated with water. Total RNA was extracted from plants after 25 days using the RNeasy Plant Mini Kit (QIAGEN, Germany). Using the first strand cDNA synthesis kit and 2 µg of RNA, the cDNA was developed. For the PCR template, reverse-transcribed RNA was used with the primers of specific genes (PPO, PAL, LOX, and POX) (Table S3). In gene expression, the tomato 18S rRNA gene primer served as constitutive control (Chandrashekar and Umesha 2014). Each qPCR was performed in 20 µl reaction volume using a StepOnePlus™ Real-Time PCR machine (Applied Biosystems, USA). The composition of the reaction mixture and the details about the steps of qPCR are presented in Table S4. The transcripts in the control and treatment groups were normalized to 18S rRNA, and the difference in the 18S rRNA normalized cycle threshold value (CT) was used to calculate the fold change in gene expression in plants (Livak and Schmittgen 2001). Each experiment was performed in three replicates.

Pot Experiment

In the greenhouse, tomato seedlings (20 days old) were transplanted into plastic pots (one plant per pot) filled with 1 kg of soil. Plants were divided into four groups according to treatment. After two days of transplantation, each plant in the first, second, and third groups was irrigated with 15 mL water containing 3 mL, 6 mL, and 9 mL (10^8 cfu/ml) suspension of biocontrol bacterium, respectively. The plants in the fourth group received 15 mL of sterilized distilled water as a control. All the plants were infected with 9 mL of pathogen suspension (10^6 cfu/ml) after 24 h of treatment applications. The trial was conducted twice using CRD (completely randomized design) with ten replicates per treatment in each experiment. The experiment was terminated after 40 days, and data were taken on plant growth parameters (root length, shoot length, plant fresh biomass) and bacterial wilt disease severity. The soil bacterial population per gram of soil was also calculated using the serial dilution method and converted to $\log_{10}$ value. The bacterial population was counted twice, after 24 h of inoculation and at the end of the experiment. The difference between the two readings was calculated and expressed as a decrease in bacterial population.

Statistical Analysis

Data were analyzed using CRD design and one-way analysis of variance (ANOVA). Duncan's multiple range test ($P < 0.01$) was conducted to compare the significance of treatment means. Statistical software SPSS (ver. 21.0) was used for analysis.

Results

Antibacterial Evaluation of *B. cereus* Against *R. solanacearum*

Results showed that *B. cereus* significantly inhibited the *in vitro* growth of *R. solanacearum*. Interestingly, *B. cereus* produced an inhibition zone of 21.8 ± 1.3 mm, which is statistically similar to the inhibition zone of 22.4 ± 1.1 mm made by standard antibiotic streptomycin (Fig. 1a). No inhibition zone was recorded around the filter paper treated with water (negative control). The volatile mediated antibacterial evaluation test indicated that VCs emitted by biocontrol bacterium negatively affected the growth of *R. solanacearum*. Volatiles produced by *B. cereus* restricted the colony diameter of *R. solanacearum* to 0.97 ± 0.1 cm in diameter as compared to the control, where the colony of *R. solanacearum* grew to 1.82 ± 0.2 cm in diameter after 72 h of incubation (Fig. 1b). Volatiles also affected the viability of *R. solanacearum* cells. The number of viable cells was significantly lower, 7.3 ± 1.1 CFU/ml, under the influence of volatiles compared to the control, 49.2 ± 2.6 CFU/ml (Fig. 1c).

Host Root Colonization by *Bacillus*

The treated plant roots with unlabeled normal *B. cereus* and the *B. cereus* labeled with GFP were investigated under confocal laser scanning microscopy. It was observed that plant roots were colonized rapidly by *B. cereus*. The *B. cereus* labeled with GFP is evident as a sharp green. However, green fluorescence was absent in samples where *B. cereus* without GFP was applied (Fig. 2a). To investigate the concentration effect, the *B. cereus* DNA labeled with GFP was diluted in a 10-fold serial, and real-time PCR standards were developed by keeping DNA as a template from different dilutions. The results showed a linear association between the bacterial concentration ($y = -3.2489x + 28.878$; $R2 = 0.9799$) and the Ct value (Fig. 2b). A standard curve was made, and the regression equation was calculated using the Ct value (101 to 108 cfu/mL). The quantity of recombinant bacteria attached to the plant roots was measured through qPCR. The increase in time showed a reduction in Ct value, which indicates that the population *B. cereus* increased with time on tomato roots. Ct value greater than 30 at 0 h, suggesting that the GFP gene was barely detectable. The *B. cereus* concentration reached 6.82×10^5, 4.83×10^6, 7.83×10^6, 2.42×10^7, 6.42×10^7, and 9.37×10^7 at 6, 9, 12, 24, and 48 h, respectively (Fig. 2).

Effect of *B. cereus* on Root Invasion by Pathogen and Inducing Host Resistance

The root population of *R. solanacearum* was quantified after treatment with control (water) and biocontrol agent *B. cereus* and infected with *R. solanacearum*. The treated roots with *B. cereus* showed a significantly lower number of *R. solanacearum*, 0.587 cfu/g of the root, compared to the control, 3.482 cfu/g of the root (Fig. 3a). Results showed that *B. cereus* significantly influenced the access of *R. solanacearum* to tomato roots. The host resistance induction by *B. cereus* against *R. solanacearum* was analyzed using a roots split test and expression evaluation of genes

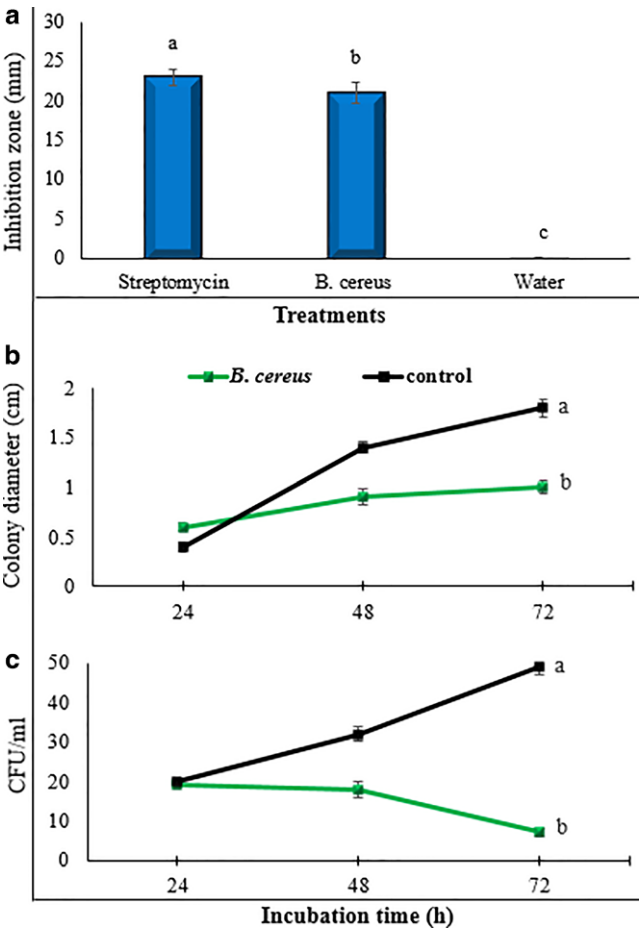

Fig. 1 Antibacterial activity of *B. cereus* against *R. solanacearum*. **a** Growth inhibition effect; **b** Volatile mediated effect; **c** Cell viability effect. Each value is a mean ± SE. Means with same letters are significantly different according to tukey's multiple range test ($P < 0.05$)

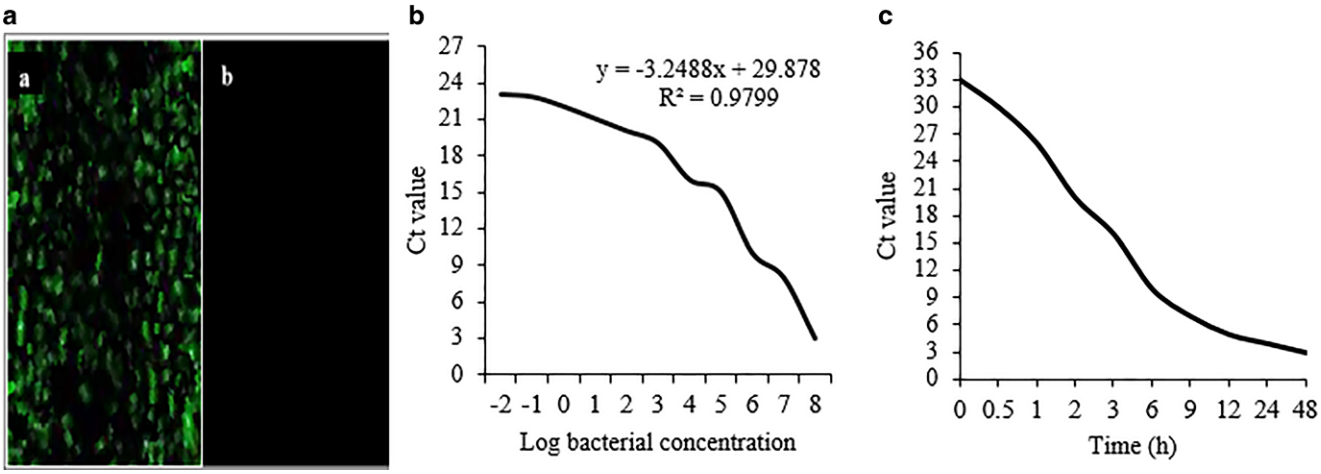

Fig. 2 Root colonizing ability of *B. cereus*. **a** Tomato roots with (*a*) GFP-tagged bacterium and (*b*) control. **b** Quantification of *B. cereus* in tomato roots. **b** standard curve of qPCR. **c** Ct value

linked to host resistance. In this test, roots were divided into two parts; one was treated with pathogenic bacterium *R. solanacearum*, while the other was treated with water (control) or biocontrol bacteria *B. cereus*. The plants treated with biocontrol bacteria exhibited more vigor growth (shoot length, fresh biomass, root length), lower root population of *R. solanacearum*, and less disease severity than control plants (Fig. 3b–f).

Analysis of Host Defense Genes

Results regarding qRT-PCR analysis of resistance genes showed that plants treated with only *B. cereus* exhibited 10-fold upregulation of PAL gene expression, while pathogen-inoculated plants showed upregulation of PAL to 8-fold and increased to 18-fold when *B. cereus* suspension was applied and inoculated with pathogen (Fig. 4). A similar upregulation effect was noted for *POX, PPO*, and *LOX* genes whose

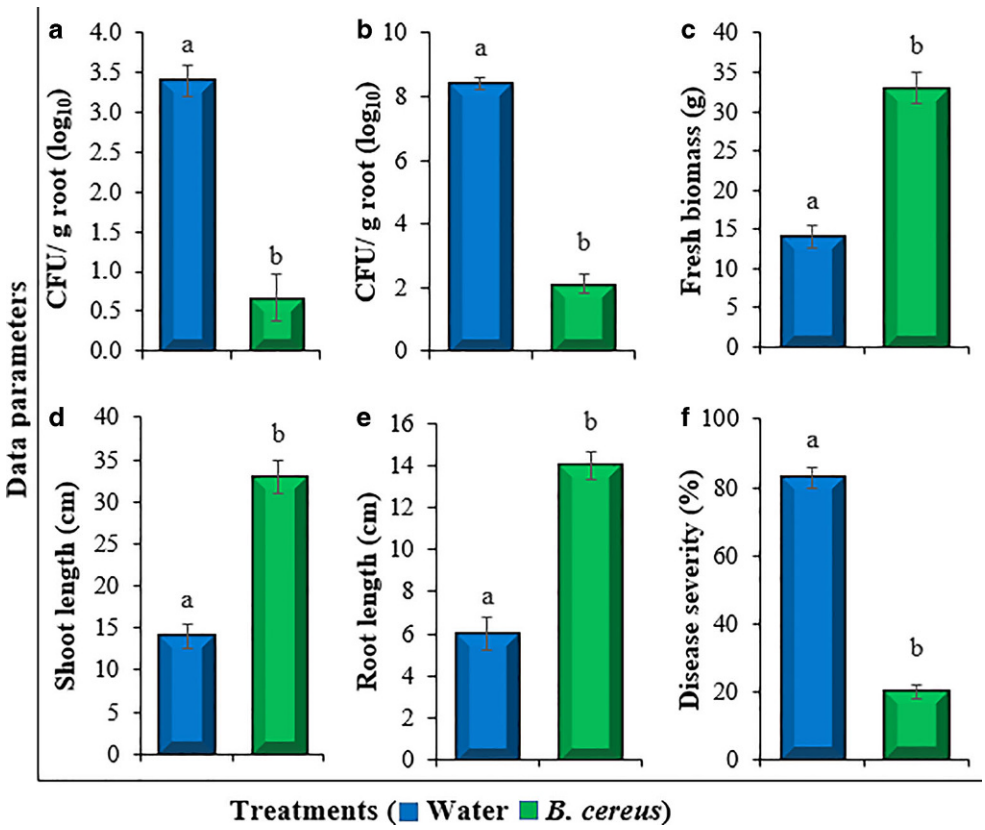

Fig. 3 Effect of *B. cereus* on root invasion by pathogen and inducing host resistance. **a** number of *R. solanacearum* cfu/g of root in root invision test, **b–f** (Split root test) **b** Root population of *R. solanacearum*, **c** Fresh biomass, **d** Shoot length, **e** Root length of tomato plants and **e** Bacterial wilt disease severity

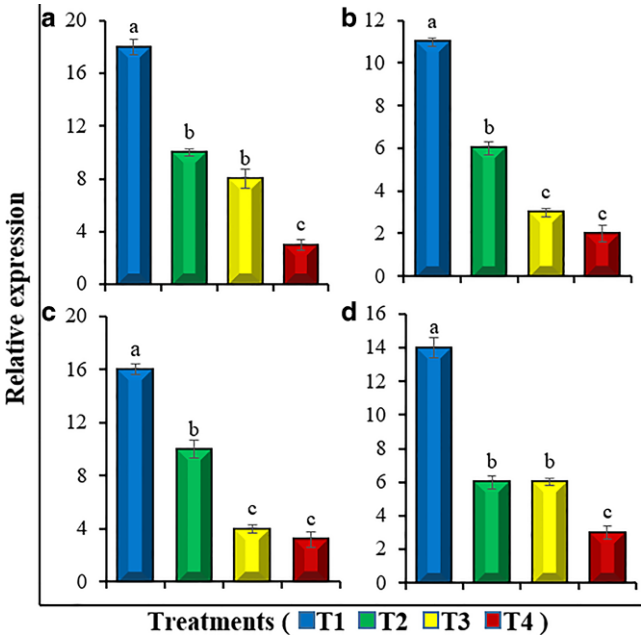

Fig. 4 Relative expression of resistance genes in tomato plants under different treatments. **a** *PAL*, **b** *POX*, **c** *PPO*, and **d** *LOX*. T1: *B. cereus* + *R. solanacearum*; T2: *B. cereus*; T3: *R. solanacearum*; T4: Control

expression was upregulated by 3, 4, and 6-fold, respectively, as compared to control, and the expression of these genes was increased significantly to 11, 16, and 14 fold, respectively, in *B. cereus* treated plants inoculated with pathogen.

Pot Experiment

Plant Growth

The biocontrol efficacy of controlling BW diseases was tested using the greenhouse test. The lowest concentration of biocontrol bacteria was not active; however, applying the highest two concentrations significantly improved plant growth (Table 1). Among all treatments, the maximum biomass of the plant (51.7 ± 3.5 g), root, and plant length (32.8 ± 3.2 cm, 48.3 ± 2.8 cm, respectively) were achieved when the highest concentration of *B. cereus* (9 mL) was applied. The untreated control plants and the application lowest concentration of *B. cereus* (3 mL) showed minimum biomass of the plant (23.6 ± 4.2 g), root, and plant length (14.7 ± 1.3 cm, 26.4 ± 1.8 cm, respectively).

Effect of *B. cereus* on Diseases Severity and *R. solanacearum* Population in Soil

Compared with the untreated control treatment, the higher two concentrations of *B. cereus* (6 and 9 ml) significantly suppressed the *R. solanacearum* population in soil and reduced the severity of the disease (Table 2; Fig. 5). The lowest concentration, however, was not very active and gave similar results as noted for control. At 45 days after pathogen inoculation, the lowest disease severity (16.4%) and maximum decrease in the *R. solanacearum* population (66.00%) was shown by a 9 mL concentration of *B. cereus* followed by 6 mL. The maximum disease severity (60.7%) and minimum decrease in the *R. solanacearum* population (6.6%) was recorded in the control treatment, followed by 3 mL of *B. cereus* concentration (8.3%) with no significant difference.

Discussion

Attention to biocontrol of plant diseases and pests has increased significantly in recent years, urged by the requirement for alternatives to synthetic chemicals that have frequently lost their effectiveness because of pathogen resistance. In this regard, biocontrol bacterial agents offer an environment-friendly and appropriate alternative for protecting plants from plant diseases. In this study, *B. cereus* was investigated for its potential to control one of the most devastating tomato diseases caused by *R. solanacearum* (Fig. 6). Results showed strong antibacterial action of *B. cereus* that considerably affected *in vitro* growth inhibition of *R. solanacearum*. The volatile-mediated antibacterial evaluation test indicated that volatile compounds produced by *B. cereus* inhibited the *R. solanacearum* growth and reduced cell viability. *B. cereus* was also found to colonize the host roots actively. The antibacterial action of *B. cereus* could be due to its ability to produce antibacterial volatile compounds. *B. cereus* was previously reported to produce antifungal and nematicidal

Table 1 Effect of different concentrations of biocontrol bacterium on growth of plants inoculated with bacterial wilt pathogen

Treatments *B. cereus* (mL)	Biomass (g)		Root length (cm)		Plant length (cm)	
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
Ck	24.3 ± 2.3 c	23.3 ± 1.6 c	12.3 ± 1.5 c	14.3 ± 1.7 c	23.3 ± 2.1 c	22.4 ± 1.6 c
3	23.6 ± 4.2 c	25.7 ± 2.6 c	14.7 ± 1.3 c	13.2 ± 1.1 c	26.4 ± 1.8 c	23.3 ± 2.3 c
6	42.2 ± 2.6 b	46.3 ± 3.1 b	25.3 ± 1.8 b	26.4 ± 2.3 b	40.8 ± 3.5 b	41.7 ± 2.8 b
9	51.7 ± 3.5 a	58.6 ± 4.7 a	32.8 ± 3.2 a	35.6 ± 2.6 a	48.3 ± 2.8 a	52.3 ± 4.7 a

Each value is a mean ± SE. Means with same letters in a column are significantly different according to Duncan's multiple range test ($P < 0.01$). Exp. I and II are the two repeated experiments with no modifications

Table 2 Effect of different concentrations of biocontrol bacterium on artificially infested soil bacterial population of R. solanacearum

Treatments		Exp. I		Exp. II	
		cfu/g of soil	% Decrease	cfu/g of soil	% Decrease
Ck	Pi	13.50	6.60 ± 1.3 c	11.30	11.50 ± 2.4 c
	Pf	12.60		10.00	
3	Pi	14.40	8.30 ± 1.8 c	11.80	13.50 ± 1.8 c
	Pf	13.20		10.20	
6	Pi	13.80	32.60 ± 3.4 b	12.50	33.60 ± 2.7 b
	Pf	9.30		8.30	
9	Pi	15.60	66.00 ± 3.5 a	11.10	69.30 ± 4.1 a
	Pf	5.30		3.40	

Each value is a mean ± SE. Means with same letters in a column are significantly different according to tukey's multiple range test ($P < 0.05$). Exp. I and II are the two repeated experiments with no modifications
Pi Initial population, *Pf* Final population, *Ck* Control

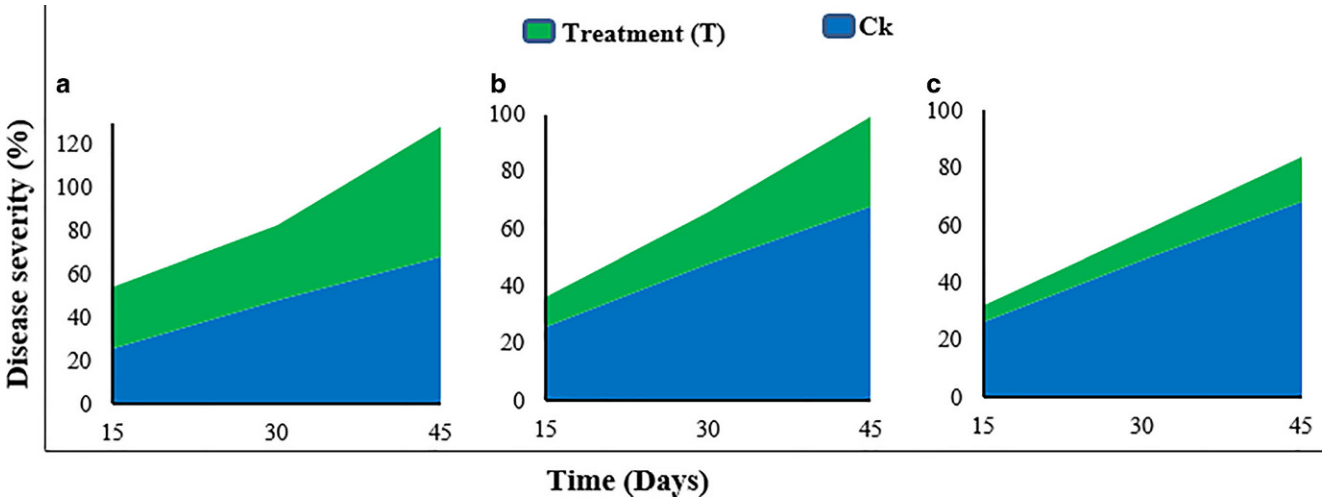

Fig. 5 Bacterial wilt disease severity on tomato plants inoculated with *R. solanacearum* under the influence of different concentrations of *B. cereus* application (**a** 3 mL, **b** 6 mL and **c** 9 mL)

volatile compounds (Weisskopf 2013; Caulier et al. 2019). An antibiotic, surfactin, from *Bacillus amyloliquefaciens*, is essential for biocontrol activity against *Xanthomonas axonopodis* (Preecha et al. 2010). In another study, this bacterium was reported to produce cyclic lipopeptides that have antifungal properties (Romano et al. 2013). Several other antagonistic bacteria were also reported to produce antibacterial volatiles against *R. solanacearum*. Recently, VOC produced by Pseudomonas fluorescens and *B. amyloliquefaciens* were shown to have a strong antibacterial potential against *R. solanacearum* (Raza et al. 2016; Chandrasekaran et al. 2016). Colonizing plant roots by biocontrol microbes is one mechanism that reduces pathogen infection in roots. It enhances the efficacy of the biocontrol agent and stabilizes its interaction with plants (Weng et al. 2013).

By directly influencing root secretions and metabolites, *B. cereus* root colonization reduces pathogen attack by enhancing the plant defense mechanism (Hashem and Abo-Elyousr 2011). After successfully colonizing host roots, some biocontrol agents also make biofilm on the root's surface (Davey and O'toole 2000). The root colonizing ability of *B. cereus* was evaluated at the molecular level through the development of GFP-tag *B. cereus*, and it was found that tomato plant roots were successfully colonized by *B. cereus*. Biocontrol microbes have received more attention during the past two decades for their eco-friendly and advantageous roles in agricultural production, such as induction of plant resistance against pest and diseases (Lee et al. 2012; Gu et al. 2007), plant growth enhancement (Song and Ryu 2013; Park et al. 2015) and biocontrol effectiveness against phytopathogenic fungi and parasitic nematodes (Guo et al. 2014; Weisskopf 2013).

The split root test confirmed the effectiveness of *B. cereus* in inducing host resistance against *R. solanacearum* and reducing root invasion by the pathogen. The regulation of host genes linked to the defense system was also assessed to explore further the process by which this biocontrol agent induces host resistance. The three key factors, phe-

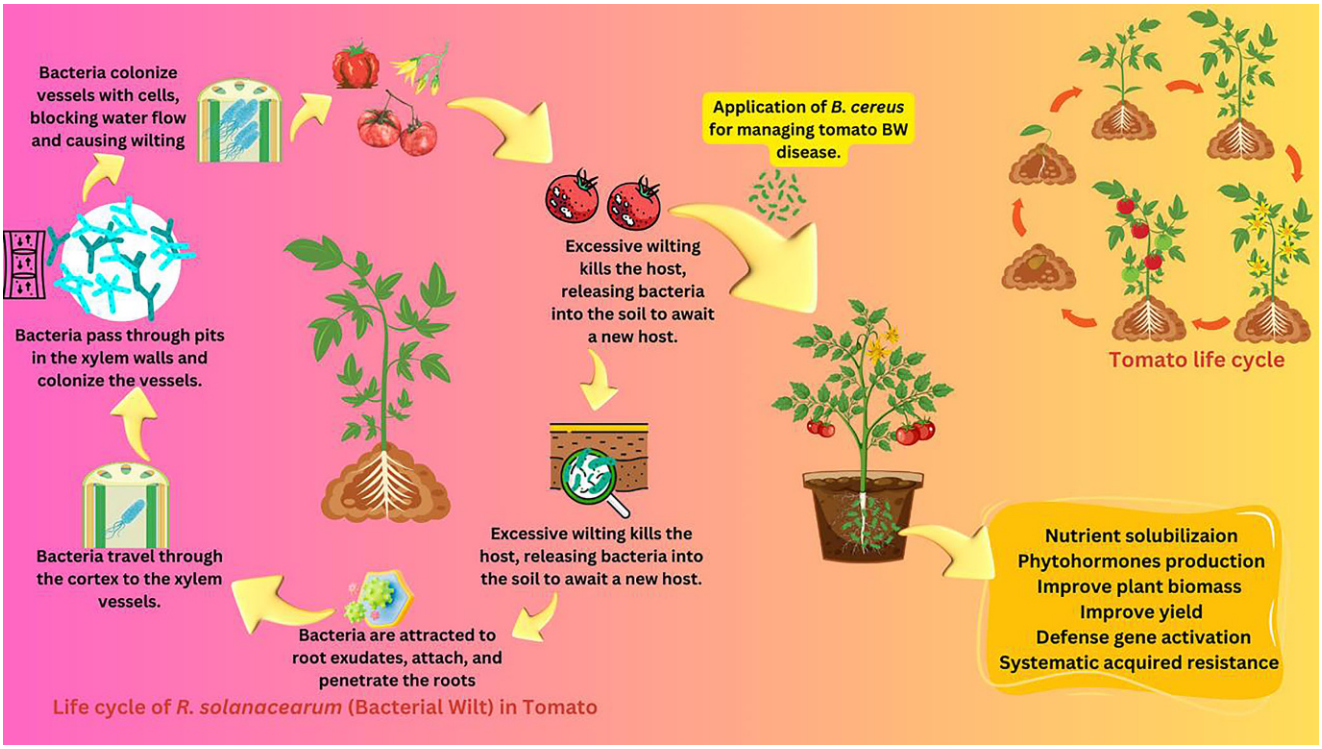

Fig. 6 Mechanism of bacterial wilt development in tomatoes and biocontrol management

nolics, phytoalexins, and lignin, are mainly responsible for plant disease resistance, and their biosynthesis is significantly driven by *PAL* and *POX* genes (Boulanger et al. 2000). Plant resistance to infections can be characterized by the early and increased expression of several host defense genes. Tomato plants infected with *F. oxysporum* showed enhanced expression of *PAL* and *POX* (Ramamoorthy et al. 2002). The involvement of defense-related genes during the *B. cereus*-mediated induction of tomato resistance against BW disease was examined for the first time in this work. Results revealed that *B. cereus*-treated plants exhibited higher expression of resistance genes than the control. According to previous reports, biocontrol bacteria may boost the activity of resistance-related enzymes and biomolecules in plants (Vos et al. 2013), Results obtained in this study indicated the host resistance ability of *B. cereus* against *R. solanacearum*. Results obtained in this study are also supported by Vanitha et al. (2009), who reported that tomato seedlings treated with a biocontrol strain of *P. fluorescens* and inoculated with BW pathogen exhibited high and quick induction of *POX* and *PAL*.

Soil application of *B. cereus* significantly reduced the severity of the disease on infected tomato plants, decreased pathogen count in infested soil, and improved the growth of tomato plants in pot experiments. In several studies, bacterial biocontrol agents have been reported to enhance plant growth and prevent microbial attack. Some *Bacillus* strains were also explored for their plant growth-promoting effect, ability to reduce disease severity, and host resistance induction ability against pathogens in several crops. Kloepper et al. (2004) reported that *B. velezensis, B. mycoides, B. pasteurii*, and *B. subtilis* can induce disease resistance in different host plants, reducing disease incidence and severity. Another study discovered that *B. firmus* causes host resistance against *Heterodera glycines* in a greenhouse and split root experiment. The reduction in disease severity in tomato plants and pathogen population in soil caused by the application of *B. cereus* is because of its direct antibacterial activity or indirect induction of host defense, which is evident in lab investigations. The findings of this study show the biocontrol potential of *B. cereus* against bacterial wilt disease in tomato crops.

Conclusion

The biocontrol bacterium *B. cereus* was assessed for antibacterial activity against *R. solanacearum*, and its application for controlling BW disease in tomatoes was explored. *B. cereus* showed a potent growth inhibition effect against *R. solanacearum* and affected cell viability by producing antibacterial volatiles. Applying *B. cereus* reduced bacterial wilt disease in tomato plants and the population of *R. solanacearum* in soil. The possible action mechanisms of *B. cereus* for managing BW disease in tomatoes confirmed in this study were host root colonization, inducing

host defense, and volatile mediated antibacterial activity against *R. solanacearum*.

Supplementary Information The online version of this article (https://doi.org/10.1007/s10343-024-01002-x) contains supplementary material, which is available to authorized users.

Acknowledgements This project was supported by Researchers Supporting Project Number (RSP2025R7) King Saud University, Riyadh, Saudi Arabia

Funding This project was supported by Researchers Supporting Project Number (RSP2025R7) King Saud University, Riyadh, Saudi Arabia

Author Contribution Data curation, XL, SA; Formal analysis, XL and MJA; Investigation, XL; Methodology, SA; Writing—review & editing, XL and MJA

Data Availability Not applicable

Conflict of interest X. Li, S. Alfarraj and M.J. Ansari declare that they have no competing interests.

References

Abo-Elyousr KA, Hassan SA (2021) Biological control of Ralstonia solanacearum (Smith), the causal pathogen of bacterial wilt disease by using Pantoea spp. Egypt J Biol Pest Cont 31:1–8. https://doi.org/10.1186/s41938-021-00460-z

Alghuthaymi MA, Ali AA, Hashim AF, Abd-Elsalam KA (2016) A rapid method for the detection of Ralstonia solanacearum by isolation DNA from infested potato tubers based on magnetic nanotools. Philip Agri Sci 99:113–118 (https://www.cabdirect.org/cabdirect/abstract/20163189342)

Bing H, Qi C, Gu J, Zhao T, Yu X, Cai Y, Zhang Y, Li A, Wang X, Zhao J, Xiang W (2024) Isolation and identification of NEAU-CP5: a seed-endophytic strain of B. velezensis that controls tomato bacterial wilt. Microb Pathog 141:104156. https://doi.org/10.1016/j.biocontrol.2019.104156

Boulanger R, El Hadrami I, Belanger RR (2000) Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of Reynoutria sachalinensis. J Chem Ecol 26:1579–1593. https://doi.org/10.1023/A:1005578510954

Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J (2019) Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group. Front Microbiol 10:435128

Chandrasekaran M, Subramanian D, Yoon E, Kwon T, Chun SC (2016) Meta-analysis reveals that the genus Pseudomonas can be a better choice of biological control agent against bacterial wilt disease caused by Ralstonia solanacearum. Plant Pathol J 32:216. https://doi.org/10.5423/PPJ.OA.11.2015.0235

Chandrashekar S, Umesha S (2014) 2, 6-Dichloroisonicotinic acid enhances the expression of defense genes in tomato seedlings against Xanthomonas perforans. Physiol Mol Plant Pathol 86: 49–56. https://doi.org/10.1016/j.pmpp.2014.03.003

Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microb 71:7271–7278. https://doi.org/10.1128/AEM.71.11.7271-7278.2005

Collange B, Navarrete M, Peyre G, Mateille T, Tchamitchian M (2011) Root-knot nematode (Meloidogyne) management in vegetable crop production: The challenge of an agronomic system analysis. Crop Prot 30:1251–1262. https://doi.org/10.1016/j.cropro.2011.04.016

Costa JM, Heuvelink EP (2018) The global tomato industry. Tomatoes 27:1–26

Davey ME, O'toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Res 64:847–867. https://doi.org/10.1128/mmbr.64.4.847-867.2000

Gu YQ, Mo MH, Zhou JP, Zou CS, Zhang KQ (2007) Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol Biochem 39:2567–2575. https://doi.org/10.1016/j.soilbio.2007.05.011

Guo Q, Li S, Lu X, Zhang X, Wang P, Ma P (2014) Complete genome sequence of Bacillus subtilis BAB-1, a biocontrol agent for suppression of tomato gray mold. Genome Announc 2:e744–14. https://doi.org/10.1128/genomea.00744-14

Hashem M, Abo-Elyousr KAM (2011) Management of the root-knot nematode Meloidogyne incognita on tomato with combinations of different biocontrol organisms. Crop Prot 30:285–292. https://doi.org/10.1016/j.cropro.2010.12.009

Iraboneye N, Charimbu MK, Mungai NW (2021) Effect of Canola and compound fertilizer on potato (Solanum tuberosum L.) bacterial wilt management. Eur J Agri Food Sci 3:28–38. https://doi.org/10.24018/ejfood.2021.3.1.130

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94:1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

Lee B, Farag MA, Park HB, Kloepper JW, Lee SH, Ryu CM (2012) Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by Paenibacillus polymyxa. Plos One 7:e48744. https://doi.org/10.1371/journal.pone.0048744

Li W, Sun L, Wu H, Gu W, Lu Y, Liu C, Zhang J, Li W, Zhou C, Geng H, Li Y (2024) Bacillus velezensis YXDHD1-7 prevents early blight disease by promoting growth and enhancing defense enzyme activities in tomato plants. Microorganisms 12:921

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2– ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Martínez-Medina A, Fernández I, Lok GB, Pozo MJ, Pieterse C, van Wees S (2017) Shifting from priming of salicylic acid- to jasmonic acid-regulated defenses by Trichoderma protects tomato against the root knot nematode Meloidogyne incognita. New Phytol 213:1363–1377. https://doi.org/10.1111/nph.14251

Nguyen MT, Ranamukhaarachchi SL (2010) Soil-borne antagonists for biological control of bacterial wilt disease caused by Ralstonia solanacearum in tomato and pepper. Eur J Plant Pathol 92(2):395–405 (https://www.jstor.org/stable/41998815)

Nion YA, Toyota K (2015) Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ. https://doi.org/10.1264/jsme2.ME14144

Park YS, Dutta S, Ann M, Raaijmakers JM, Park K (2015) Promotion of plant growth by Pseudomonas fluorescens strain SS101 via novel volatile organic compounds. Biochem Biophy Res Comm 461:361–365. https://doi.org/10.1016/j.bbrc.2015.04.039

Preecha C, Sadowsky MJ, Prathuangwong S (2010) Lipopeptide surfactin produced by Bacillus amyloliquefaciens KPS46 is required for biocontrol efficacy against Xanthomonas axonopodis pv. Glycines. Kasetsart J Nat Sci 44:84–99

Ramamoorthy V, Raguchander T, Samiyappan R (2002) Induction of defense-related proteins in tomato roots treated with Pseudomonas fluorescens pf1 and Fusarium oxysporum f. sp. lycopersici. Plant Soil 239:55–68. https://doi.org/10.1023/A:1014904815352

Raza W, Ling N, Yang L, Huang Q, Shen Q (2016) Response of tomato wilt pathogen Ralstonia solanacearum to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9. Sci Rep 6:1–13. https://doi.org/10.1038/srep24856

Romano A, Vitullo D, Senatore M, Lima G, Lanzotti V (2013) Antifungal cyclic lipopeptides from Bacillus amyloliquefaciens strain BO5A. J Nat Prod 76:2019–2025

Salehi B, Sharifi-Rad R, Sharopov F, Namiesnik J, Roointan A, Kamle M, Kumar P, Martins N, Sharifi-Rad J (2019) Beneficial effects and potential risks of tomato consumption for human health: an overview. Nutrition 62:201–208

Saputra R, Arwiyanto T, Wibowo A (2020) Biological control of Ralstonia solanacearum causes of bacterial wilt disease with Pseudomonas putida and Streptomyces spp. on some tomato varieties. IOP Conf Series: Earth Environ Sci 515(1):12007. https://doi.org/10.1088/1755-1315/515/1/012007

Silva RS, Kumar L, Shabani F, Picanço MC (2017) Assessing the impact of global warming on worldwide open field tomato cultivation through CSIRO-Mk3· 0 global climate model. J Agric Sci 155(3):407–420

Song GC, Ryu CM (2013) Two volatile organic compounds trigger plant self-defense against a bacterial pathogen and a sucking insect in cucumber under open field conditions. Intern J Mol Sci 14:9803–9819. https://doi.org/10.3390/ijms14059803

Tahir HAS, Gu Q, Wu H, Niu Y, Huo R, Gao X (2017) Bacillus volatiles adversely affect the physiology and ultra-structure of Ralstonia solanacearum and induce systemic resistance in tobacco against bacterial wilt. Sci Rep 7:1–15. https://doi.org/10.1038/srep40481

Umar FJ, Idris FT, Usman A, Balarabe FT, Adamu A (2024) Antibacterial activity of polyalthia longifolia leaf extracts against staphylococcus aureus and escherichia coli. UMYU J Microbiol Res: 8–12

Vanitha SC, Niranjan SR, Mortensen CN, Umesha S (2009) Bacterial wilt of tomato in Karnataka and its management by Pseudomonas fluorescens. BioControl 54:685–695. https://doi.org/10.1007/s10526-009-9217-x

Vos C, Schouteden N, van Tuinen D, Chatagnier O, Elsen A, De Waele D, Panis B, Gianinazzi-Pearson V (2013) Mycorrhiza-induced resistance against the root–knot nematode Meloidogyne incognita involves priming of defense gene responses in tomato. Soil Biol Biochem 60:45–54. https://doi.org/10.1016/j.soilbio.2013.01.013

Weisskopf L (2013) The potential of bacterial volatiles for crop protection against phytophathogenic fungi. In: Méndez-Vilas A (ed) Microbial pathogens a strategies for combating them: sci technol edu, pp 1352–1363

Weng J, Wang Y, Li J, Shen Q, Zhang R (2013) Enhanced root colonization and biocontrol activity of Bacillus amyloliquefaciens SQR9 by abrB gene disruption. Appl Microbiol Biot 97:8823–8830. https://doi.org/10.1007/s00253-012-4572-4

WPTC (2021) Tomato news. https://www.tomatonews.com/en. Accessed 28 Jan 2022

Wu S, Su H, Gao F, Yao H, Fan X, Zhao X, Li Y (2023) An insight into the prevention and control methods for bacterial wilt disease in tomato plants. Agronomy 13:3025

Yuliar NA, Toyota K (2015) Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ 30:1–11. https://doi.org/10.1264/jsme2.ME14144