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Research Article

UNVEILING THE ANTIBACTERIAL POTENTIAL OF ORGANIC ACIDS AGAINST TOMATO BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM*

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ABSTRACT

Bacterial wilt, caused by *Ralstonia solanacearum*, is a major threat to tomato production worldwide, leading to significant yield losses. With increasing concerns over chemical pesticide use, there is growing interest in exploring eco-friendly alternatives such as organic acids for disease management. The main objective of this study was to evaluate the antibacterial potential of organic acids against *R. solanacearum*, the causal agent of bacterial wilt in tomatoes. The pathogen was confirmed through biochemical and morphological characterization under *in vitro* conditions. Biochemical analysis showed negative results for gram staining and indole production tests, while catalase, hydrogen sulfide production, and oxidase tests were positive. Morphologically, *R. solanacearum* appeared rod-shaped, forming dirty white colonies with an irregular growth pattern on nutrient agar medium. Organic acids were tested under *in vitro* and greenhouse conditions using a Completely Randomized Design, while *in vivo* experiments were conducted using a Randomized Complete Block Design. Among the tested organic acids, citric acid exhibited the largest inhibition zone (12.085 mm), followed by lactic acid (9.1041 mm) and ascorbic acid (7.2196 mm), as compared to the control. Under greenhouse and *in vivo* conditions, citric acid resulted in the lowest disease incidences of 26.330% and 29.591%, respectively. This study confirms that citric acid is an effective organic solution for managing bacterial wilt in tomatoes. For growers, integrating citric acid into disease management strategies can help reduce crop losses, minimize chemical usage, and promote sustainable tomato production.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is a major vegetable crop after potato (Bashir et al., 2020). Solanaceous crops are also known as 'nightshades' due to the presence of some toxic alkaloid-containing species such as belladonna, mandrake, henbane, Jimson weed, climbing nightshade, and the widely used

tobacco (Gebhardt, 2016). Tomatoes are rich in health-promoting compounds and can be easily incorporated into a balanced diet as a nutritious component (Marti et al., 2016). They are an excellent source of essential nutrients like vitamin C, β -carotene, and lycopene, all of which offer significant health benefits (Bergougnoux, 2014). Tomato seeds and peels, common by-products of the tomato

processing industry, are also rich in bioactive compounds such as carotenoids (Coelho et al., 2019). The major tomato-producing countries include China, the European Union, the USA, and Turkey (FAOSTAT, 2019), with China leading global production, contributing approximately 35% in 2020 (FAOSTAT, 2021).

Ralstonia solanacearum is a soil-borne pathogenic bacterium responsible for bacterial wilt in tomatoes, particularly in hot and humid tropical regions, where it can cause yield losses ranging from 35% to 90%. The pathogen is a gram-negative, rod-shaped, motile, aerobic, and non-spore-forming bacterium (Aslam and Mukhtar, 2023a, b; 2024). It affects more than 200 plant and crop species across 54 different families (Chen et al., 2020) and can persist in the soil for up to eight years (Zhang et al., 2020). The severity of bacterial wilt is significantly influenced by environmental factors such as temperature, soil moisture, and soil pH. The development of bacterial wilt in tomatoes typically occurs at temperatures of 28-29.5°C, with 71.59% relative humidity and 71.86 mm of rainfall (Begum et al., 2012; Shahbaz et al., 2015). Symptoms of the disease include wilting, drooping of leaves, yellowing of foliage, stem discoloration, and vascular blockage (Oussou et al., 2020).

Management of bacterial wilt includes various strategies such as the use of chemical treatments, biocontrol agents, soil solarization, and resistant varieties to minimize the impact of the disease (Aslam et al., 2017a, b, 2019; Asghar et al., 2020; Yaseen et al., 2025). Due to their ease of application, chemical treatments are often the first choice of farmers. However, prolonged use of certain chemicals can pose health risks to humans and livestock (Rani et al., 2021). Biocontrol agents offer an environmentally friendly alternative and are effective in managing soil-borne plant diseases. Nonetheless, environmental factors such as temperature, soil pH, and moisture can negatively impact the survival, reproduction, and efficacy of these agents (Abd-Elgawad, 2020).

There is an increasing trend toward reducing chemical inputs in agriculture and adopting environmentally sustainable alternatives for disease management. Among these, the use of organic acids has emerged as a promising strategy due to their low toxicity and cost-effectiveness (Morgunov et al., 2017). Organic acids are weak carboxylic acids (R-COOH) naturally present in all plants. They serve as metabolic building blocks and play crucial roles in plant physiology (Shahidi et al., 2014; Vallarino and Osorio, 2019; Hadavi and Ghazijahani, 2022). Furthermore, they contribute to soil formation, enhance phosphorus

availability, improve nutrient uptake, and mitigate heavy metal toxicity in plants (Ash et al., 2016). Organic acids not only inhibit bacterial and fungal growth but also enhance disease resistance by lowering the intracellular pH of pathogens, thereby reducing disease incidence in fruits and vegetables (Patrignani et al., 2015). Considering the severity of the issue, the present study was conducted to evaluate the efficacy of organic acids against *R. solanacearum*, the causal agent of bacterial wilt in tomatoes.

MATERIALS AND METHODS

Collection of symptomatic specimens

Symptomatic leaf samples were collected from various areas of district Faisalabad, including Samundari, Jaranwala, and Jhumra (Figure 1). The samples were wrapped in brown paper bags (12" × 8.5") and labeled with the name, date, and collection site. These samples were transported to the Citrus Pathology Laboratory, Department of Plant Pathology, University of Agriculture Faisalabad (UAF), and stored in a refrigerator (MR-FS45ES) at 4°C for short-term preservation.

Isolation and purification of *R. solanacearum*

Bacterial isolation and purification were carried out using Nutrient Agar (NA) medium following the method described by Atiq et al. (2023). To prepare the medium, 29 g of synthetic NA powder was dissolved in distilled water to make a total volume of 1000 ml. The medium was sterilized at 121°C for 15 min at 15 psi in an autoclave (JSAT-45). After sterilization, Nilstat was added to the lukewarm medium to prevent fungal contamination. The medium was then poured into 90 mm autoclaved Petri dishes and allowed to solidify.

Diseased leaf samples were washed with water and cut into small sections (3-4 mm) including both diseased and adjacent healthy tissue using a sterilized scissor (Haq et al., 2021). These sections were surface-sterilized with 1% sodium hypochlorite (NaOCl), followed by drying with sterile filter paper (Ghaffar et al., 2022). Using sterile forceps, the treated sections were transferred onto NA plates. Petri dishes were sealed with paraffin tape and incubated at 25°C for 24 h in an incubator (MTBI) to observe bacterial growth. All work was performed under a Laminar Air Flow cabinet (BLHZ-201) to maintain aseptic conditions.

To obtain a pure culture, a single colony was picked and streaked onto fresh NA plates using a sterile inoculating loop (Ranjan et al., 2021). Plates were sealed with paraffin tape and incubated at 25°C for further bacterial purification.

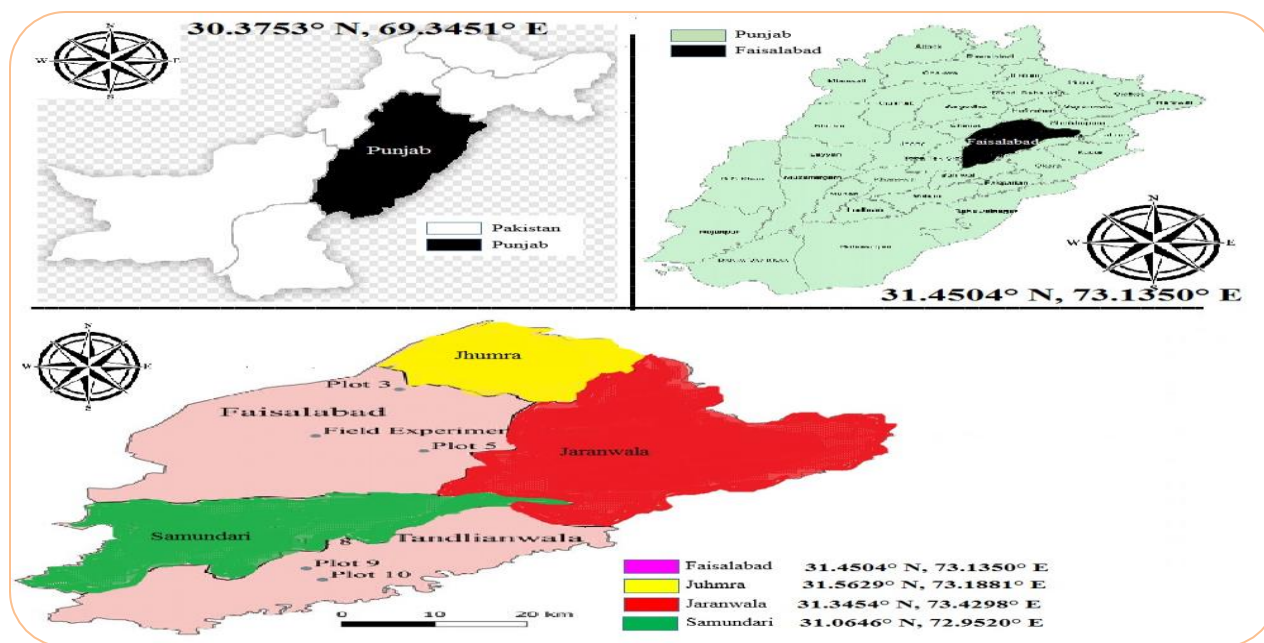


Figure 1. Survey map of Faisalabad district showing the locations of diseased sample collection.

Identification and preservation of the pathogen

The pathogen responsible for bacterial wilt of tomato was identified based on morphological characteristics (e.g., colony shape, color, and growth pattern) and biochemical tests including gram staining, KOH solubility, hydrogen sulfide production, catalase, and oxidase activity (Sharma and Singh, 2019).

Pure cultures were preserved in sterilized test tubes. Bacterial suspensions were incubated at 28°C for 24 h. In 2 ml Eppendorf tubes, 1 ml of the culture was mixed with 1 ml of sterile glycerol. Tubes were labeled and stored in a deep freezer (HDSSP-2S-47-12-S4A) at -18°C for long-term preservation.

Pathogenicity test

A pathogenicity test was conducted according to Koch's postulates to confirm the causal organism of bacterial wilt. A bacterial suspension was prepared at a concentration of 1×10^8 CFU ml⁻¹ using a spectrophotometer (Hitachi U-2001, Model 121003) (Agarwal et al., 2020). Tomato seeds of a susceptible variety (Rocky) were sown in peat moss in seedling trays (200 holes). After 40-45 days, seedlings were transplanted into sterilized pots (28×14 cm) containing formalin-treated soil.

Once the plants were established, the bacterial suspension was applied using the soil drenching method (Planas-Marquès et al., 2018). Symptoms of wilt appeared within 6-7 days. The pathogen was re-isolated from diseased plants and compared with the original

isolate to fulfill Koch's postulates (Sharif et al., 2021).

Collection and preparation of organic acid concentrations

Three organic acids (citric, lactic, and ascorbic acids) were tested at concentrations of 0.25%, 0.35%, and 0.45%. For each concentration, 0.25 g, 0.35 g, and 0.45 g of the respective organic acid were dissolved in 100 ml of distilled water in 200 ml conical flasks. Prepared solutions were stored at 25°C for subsequent evaluation under *in vitro*, greenhouse, and field conditions.

In vitro evaluation of organic acids against *R. solanacearum*

The antibacterial activity of organic acids was assessed using the inhibition zone technique. Nutrient Agar medium was prepared and poured into 90 mm Petri dishes. Once solidified, bacterial culture was uniformly spread using a sterile cotton swab under laminar airflow (BLHZ-201). Sterile 1 cm filter paper discs were soaked in prepared organic acid solutions (0.25%, 0.35%, and 0.45%) and placed at the center of the agar plates. Filter paper soaked in distilled water served as a control. Plates were sealed with paraffin tape and incubated at 25°C for 2-3 days in an incubator (MTBI).

Each treatment was replicated three times in a Completely Randomized Design (CRD), and the experiment was repeated twice. Zones of inhibition were measured at 24-h intervals using a vernier caliper (Model: Cinema 4D).

Evaluation of antibacterial efficacy under greenhouse conditions

Tomato plants of the moderately susceptible variety 'Nagina' were grown in peat moss in pots (28×14 cm). After 40 days, inoculum was prepared from a 2-day-old culture of *R. solanacearum* adjusted to 1×10^8 CFU ml⁻¹ using a spectrophotometer (Hitachi U-2001). Inoculum was applied via soil drenching early in the morning (Planas-Marquès et al., 2018).

After 5-7 days, characteristic wilt symptoms appeared. The most effective concentration from *in vitro* trials was evaluated. The experiment followed a CRD with three replications per treatment and distilled water as control. Trials were conducted over two years. Disease incidence (DI) was recorded weekly over three intervals using the following formula:

$$DI (\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

In vivo evaluation of organic acids against *R. solanacearum*

Tomato seeds (Nagina) were sown in peat moss-filled seedling trays (200 holes). After 40 days, seedlings of the susceptible variety 'Nagina' were transplanted into field plots at the Department of Plant Pathology, UAF, maintaining plant spacing of 1.5-2 feet and row spacing of 2-3 feet.

Inoculum (1×10^8 CFU ml⁻¹) prepared from a 2-day-old culture was applied by soil drenching early in the morning (Planas-Marquès et al., 2018). Symptoms developed within 5-7 days, and the most effective concentration from earlier trials was evaluated. Distilled water was used as a control. The experiment was conducted under a Randomized Complete Block Design (RCBD) with three replications and repeated over two years. Disease incidence was recorded after 1, 2, and 3 weeks using the formula above.

Data analysis

Laboratory and greenhouse trials were conducted using CRD, while the field experiment followed RCBD. Data were analyzed using analysis of variance (ANOVA), and treatment means were separated using the LSD test at a 5% significance level (Steel et al., 1997).

RESULTS

Morphological and biochemical identification of *R. solanacearum*

Morphological characterization of *R. solanacearum* under *in vitro* conditions revealed rod-shaped cells with

dirty white colonies exhibiting circular and irregular growth patterns (Table 1). Biochemical tests confirmed the identity of the pathogen, showing negative results for gram staining and indole production, while tests for hydrogen sulfide production, catalase, and oxidase were positive (Table 2).

Table 1. Morphological features for the identification of *R. solanacearum* under *in vitro* conditions.

Morphological Characterization	Morphological results observed
Shape	Rod shaped
Colour	Dirty white colour
Growth	Circular and irregular growth

Table 2. Biochemical characterization for the identification of *R. solanacearum* under *in vitro* conditions.

Biochemical Tests	Response
Gram staining test	-ve
Indole production test	-ve
Hydrogen sulphide production test	+ve
Catalase test	+ve
Oxidase test	+ve

Pathogenicity tests

Pathogenicity tests confirmed *R. solanacearum* as the causal agent of bacterial wilt in tomato. Typical wilt symptoms appeared 6-7 days after soil drenching with the bacterial suspension (Figure 2). The pathogen was successfully re-isolated from infected plants, and its characteristics matched the original isolate, thereby fulfilling Koch's postulates.



Figure 2. Comparison between healthy (a) and diseased tomato plants (b).

Assessment of different organic acids against *R. solanacearum* under *in vitro* conditions

Among all treatments, the highest zone of inhibition was observed with citric acid (12.085 mm), followed by lactic acid (9.1041 mm) and ascorbic acid (7.2196 mm), compared to the control (Figure 3). The interaction between treatments and concentrations (T×C) revealed that the lowest zones of inhibition were recorded for ascorbic acid at 0.25%, 0.35%, and 0.45% (9.8444 mm, 7.3167 mm, and 4.4978 mm, respectively), followed by lactic acid (11.134 mm, 8.731 mm, and 7.446 mm), and citric acid (13.838 mm, 12.114 mm, and 10.302 mm) at the same concentrations, in comparison to the control (Table 3).

Similarly, the interaction between treatments and durations (T×D) indicated that the maximum zones of inhibition were produced by citric acid (9.6467 mm, 12.061 mm, and 14.547 mm), followed by lactic acid (7.4722 mm, 8.8222 mm, and 11.018 mm), and ascorbic acid (5.9678 mm, 6.8711 mm, and 8.8200 mm), as compared to the control treatment (Table 4).

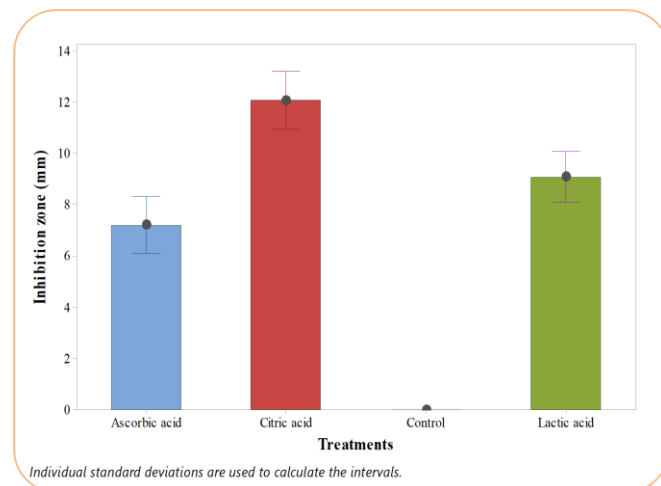


Figure 3. *In vitro* evaluation of different organic acids against *R. solanacearum*.

Table 3. *Interaction effect of treatments and concentrations on the inhibition zone of R. solanacearum.*

Treatments	Inhibition zone (mm) at concentration of		
	0.25%	0.35%	0.45%
Citric acid	10.302 d	12.114 b	13.838 a
Lactic acid	7.4467 f	8.731 e	11.134 c
Ascorbic acid	4.4978 g	7.3167 f	9.8444 d
Control	0.0000 h	0.0000 h	0.0000 h
LSD	0.6177		

Mean values in the column followed by the same letter are not significantly different, as determined by the LSD test ($P \leq 0.05$).

Table 4. *Interaction effect of treatments and exposure duration on the inhibition zone of R. solanacearum.*

Treatments	Inhibition zone (mm) after		
	24 h	48 h	72 h
Citric acid	9.6467 d	12.061 b	14.547 a
Lactic acid	7.4722 f	8.8222 e	11.018 c
Ascorbic acid	5.9678 g	6.8711 f	8.8200 e
Control	0.0000 h	0.0000 h	0.0000h
LSD	0.6177		

Mean values in the column followed by the same letter are not significantly different, as determined by the LSD test ($P \leq 0.05$).

Assessment of different organic acids against bacterial wilt of tomato under greenhouse conditions

Among all the treatments, the lowest disease incidence was observed with citric acid (26.33%), followed by lactic acid (29.71%) and ascorbic acid (32.78%), compared to the control (Figure 4). The treatment × duration (T×D) interaction revealed that the highest DI was recorded with ascorbic acid (39.25%, 31.42%, and 27.66%) after 7, 14, and 21 days, respectively, followed by lactic acid (35.68%, 28.47%, and 24.97%) and citric acid (31.12%, 25.20%, and 22.67%) over the same intervals, as compared to the control treatment (Table 5).

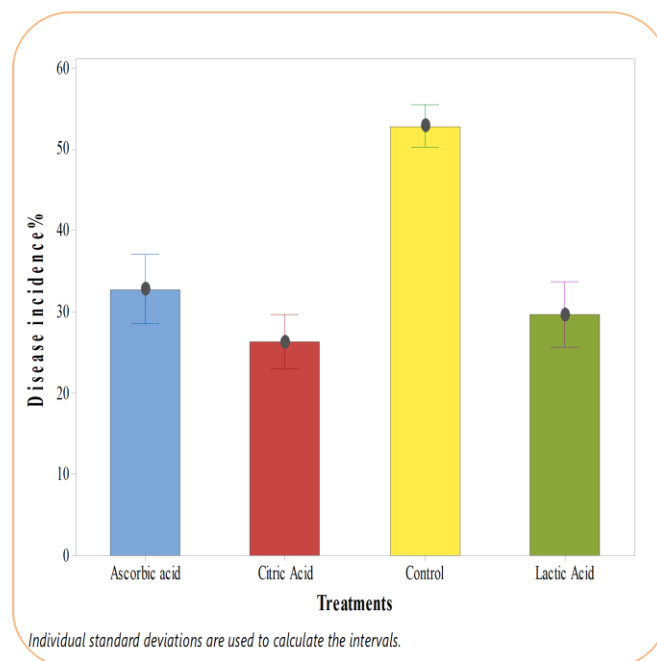


Figure 4. Evaluation of different organic acids against bacterial wilt of tomato.

Table 5. Interaction effect of treatments and days on the disease incidence of bacterial wilt in tomato.

Treatments	Disease incidence (%) after		
	7 days	14 days	21 days
Citric acid	31.120e	25.203fg	22.667g
Lactic acid	35.677cd	28.473ef	24.967fg
Ascorbic acid	39.250c	31.417de	27.660ef
Control	50.043b	52.987ab	55.600a
LSD	4.3275		

Mean values in the column followed by the same letter are not significantly different, as determined by the LSD test ($P \leq 0.05$).

Assessment of different organic acids against bacterial wilt of tomato under field conditions

Among all the treatments, citric acid showed the lowest disease incidence (29.591%), followed by lactic acid (33.223%) and ascorbic acid (36.526%), compared to the control (Figure 5). The interaction between treatments and days ($T \times D$) indicated that the highest DI was observed in the ascorbic acid treatment, 43.113%, 35.630%, and 30.833% at 7, 14, and 21 days, respectively, followed by lactic acid (39.213%, 32.077%, and 28.380%) and citric acid (34.497%, 28.483%, and 25.793%) over the same time intervals, as compared to the control (Table 6).

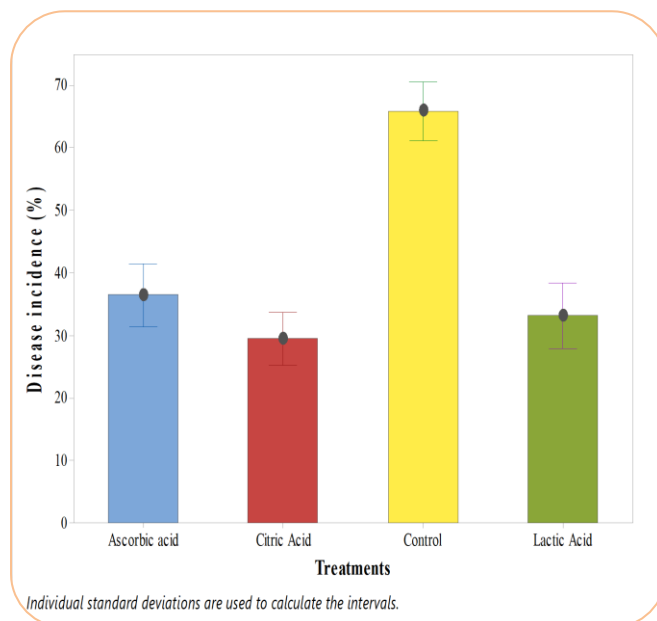


Figure 5. *In vivo* assessment of different organic acids against bacterial wilt of tomato.

Table 6. Interaction effect of treatments and days on the incidence of bacterial wilt in tomato.

Treatments	Disease incidence (%) after		
	7 days	14 days	21 days
Citric acid	34.49de	28.48ef	25.79f
Lactic acid	39.21cd	32.07def	28.38ef
Ascorbic acid	43.11c	35.63cde	30.83ef
Control	59.81b	65.99ab	71.98a
LSD	7.7751		

Mean values in the column followed by the same letter are not significantly different, as determined by the LSD test ($P \leq 0.05$).

DISCUSSION

Morphological and biochemical characterization are fundamental approaches for the detection and identification of plant pathogens (Wu et al., 2025). In this study, *R. solanacearum* exhibited distinct morphological traits, appearing as rod-shaped cells forming smooth, irregular colonies with a dirty white coloration. Biochemical analyses further confirmed its identity, showing negative results for gram staining and indole production tests, while catalase, hydrogen sulfide production, and oxidase tests were positive. These findings are consistent with the observations of Sharma and Singh (2019), reinforcing the established characteristics of *R. solanacearum*.

Various tools have been employed to combat *R. solanacearum*, the causative agent of bacterial wilt in tomato. However, the pathogen is soil-borne in nature, making its eradication from the field particularly challenging. The most commonly used method for plant disease management is the application of chemical pesticides. Nevertheless, prolonged exposure to these chemicals poses significant health risks to humans and animals (Rani et al., 2021). Therefore, scientists have increasingly focused on eco-friendly strategies, such as the use of organic acids.

In the current study, the efficacy of citric, lactic, and ascorbic acids was evaluated under laboratory, greenhouse, and field conditions against *R. solanacearum*. Among all treatments, citric acid exhibited the largest inhibition zone *in vitro* and resulted in the lowest disease incidence under both greenhouse and *in vivo* conditions. These findings align with those of Braun and Hildebrand (2013), who reported the antimicrobial activity of citric, ascorbic, and benzoic

acids in suppressing *Xanthomonas fragariae*. Similarly, Abdalla et al. (2019) demonstrated the antibacterial potential of citric and acetic acids against *Erwinia carotovora* subsp. *carotovora*. The results are further supported by Hasegawa et al. (2023), who documented that citric, malic, and lactic acids inhibited the growth of *R. pseudosolanacearum*. Ozdemir (2009) also confirmed the antibacterial potential of citric acid against *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato*.

Citric acid is a weak organic acid that releases hydrogen ions upon dissolution in water, creating an acidic environment. Since bacteria require specific pH ranges for growth, exposure to low pH conditions disrupts their cellular processes (Morgunov et al., 2017). *R. solanacearum* prefers a neutral to slightly acidic pH; thus, an acidic environment inhibits its growth and reproduction (Ji et al., 2005). Lactic acid, a naturally occurring organic acid, inhibits bacterial growth through various mechanisms. It disrupts the bacterial cell membrane by lowering the pH and compromising membrane integrity, which leads to electrolyte leakage (Zhang et al., 2021). Ascorbic acid, also known as vitamin C, inhibits key enzymatic activities essential for bacterial metabolism. The reduction in pH due to ascorbic acid can denature or inactivate enzymes and disrupt essential cellular functions (Mousavi et al., 2019).

This study tested a limited number of organic acids and used a single strain of *R. solanacearum*, which may not represent the genetic diversity of field populations. Although a general mechanism of citric acid action was described, further exploration is required to elucidate its detailed antibacterial mechanisms.

CONCLUSIONS

Under laboratory conditions, citric acid exhibited the largest inhibition zone, while under greenhouse and field conditions, it resulted in the lowest disease incidence, followed by lactic and ascorbic acids. These findings suggest that citric acid is a promising organic treatment for managing bacterial wilt in tomatoes. Given its efficacy and eco-friendly nature, citric acid can be recommended as part of an integrated and sustainable disease management strategy for tomato cultivation. Future research should focus on optimizing application methods, concentrations, and frequency to maximize its effectiveness under diverse field conditions.

AUTHORS CONTRIBUTIONS

MJM and MA designed, formulated and laid out the study; NAR, AS, and MA conducted the experiments; MJM, MA and NRK collected, arranged and analysed the data; MA, and I provided technical assistance; MA, FA and AS supervised the work; MJM, ZA, and MFK wrote the manuscript; MJM, MA and NAR proofread the paper.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 13: Climate Action

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