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Evaluation of Pathogen Characteristics and Management Strategies of Tomato Bacterial Stem Rot under Protected Cultivation in Sri Lanka

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Abstract - Tomato bacterial stem rot, caused by *Pectobacterium carotovorum* subsp. *carotovorum*, is a major constraint in protected tomato cultivation in Sri Lanka, particularly under humid polytunnel conditions. This study aimed to characterize the causal pathogen, evaluate resistance among tomato germplasm, and assess the efficacy of selected copper-based compounds for disease management. Five bacterial isolates were obtained from stem rot-infected tomato plants collected from polytunnels in the Kandy and Matale districts and cultured on Potato Dextrose Peptone Agar (PDPA). All isolates were identified as *P. carotovorum* subsp. *carotovorum* based on colony morphology, Gram reaction, catalase activity, pathogenicity on tomato plants, hypersensitivity reactions on tobacco leaves, and virulence on carrot slices. Greenhouse screening of 25 tomato germplasm lines revealed disease incidence ranging from 10-65%, with no fully resistant lines identified; however, AVTO 2117 showed the lowest disease incidence (10%) and was classified as moderately resistant. An *in vitro* poison food assay demonstrated complete inhibition of bacterial growth by copper hydroxide. These findings suggest that moderately resistant germplasm and copper hydroxide have potential as components of integrated management strategies for tomato bacterial stem rot; however, field-based validation is required to confirm their effectiveness under cultivation conditions.

Keywords **-** Bacterial stem rot, Copper hydroxide, Germplasm screening, *Pectobacterium carotovorum*

Recommended APA Citation

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Introduction

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop cultivated worldwide, both in open fields and under protected cultivation systems, due to its high nutritional and economic value (Akbar et al., 2015). It is a vital source of antioxidants such as lycopene, as well as essential nutrients including vitamins A and C, potassium, and dietary fiber (Akbar et al., 2015). In Sri Lanka, the demand for tomato continues to rise with its increasing use in local diets and the food processing industry. Consequently, commercial production has expanded into greenhouse-based systems, particularly polytunnels, to ensure year-round supply and higher productivity per unit area (Weerakkody & Peiris, 1998). Among the bacterial pathogens affecting tomato, *Pectobacterium carotovorum* subsp. *carotovorum* is recognized as a serious causal agent of stem rot diseases. This gram-negative, facultatively anaerobic bacterium belongs to the family Pectobacteriaceae and is capable of infecting a wide range of host plants, including solanaceous crops such as potatoes and other vegetables like cabbage and carrots (Sangeetha et al., 2020). It is particularly aggressive in warm and humid environments, where it secretes an array of plant cell wall-degrading enzymes, including pectinases, cellulases, and proteases, that promote rapid tissue maceration and rotting (Kolomiiets et al., 2020). The pathogen primarily enters the host through wounds or natural openings and colonizes vascular and parenchymal tissues, leading to systemic infection. In tomatoes, symptoms of bacterial stem rot often begin with small, water-soaked lesions at the stem base which progressively enlarge and cause softening, discoloration, and eventual collapse of the stem. As the infection spreads, plants exhibit leaf chlorosis, wilting, necrotic lesions, vascular browning, and fruit soft rot accompanied by a foul odor due to tissue decay (Sangeetha et al., 2020). In polytunnel environments common in the mid-country wet zone of Sri Lanka, particularly in Kandy and Matale districts, these symptoms can result in severe yield losses, especially during the fruiting stage when investments in labor, water, and nutrient inputs are highest (Weerakkody & Peiris, 1998).

The impact of bacterial diseases is further amplified by changing climate patterns. Elevated temperatures and humidity levels inside polytunnels intensify pathogen activity and disease incidence. It has been projected that a temperature increase of just 3-4 °C could double the rate of bacterial infections in controlled environments, a trend already observed under temperate greenhouse conditions (Kolomiiets et al., 2020). These conditions closely resemble the Sri Lankan polytunnel environments during the Yala and Maha seasons, suggesting an urgent need for effective disease management practices. The management of *P. carotovorum* infections is challenging due to its wide host range, rapid spread, and limited efficacy of traditional chemical controls. Moreover, excessive use of antibiotics and copper compounds has led to environmental concerns and resistance issues. Therefore, sustainable and integrated approaches, such as the use of resistant tomato germplasm and cost-effective chemical treatments, are increasingly being explored. Screening for resistance within local and improved tomato accessions offers a promising strategy for long-term control, especially when integrated with sound agronomic practices and sanitation measures (Lim et al., 2013).

Given this background, the present study was undertaken to isolate and identify the causal organism responsible for bacterial stem rot of tomato in Sri Lankan polytunnel systems, particularly in the Kandy and Matale districts. The study also aimed to assess the biological characteristics and virulence of the pathogen through morphological, biochemical, and pathogenicity tests, and to evaluate the resistance of selected tomato germplasm to the disease under greenhouse conditions. In addition, *in vitro* assays were conducted to determine the effectiveness of selected copper-based compounds in suppressing bacterial growth. The findings from this research are expected to contribute to the development of integrated disease management strategies for protected tomato cultivation in Sri Lanka.

Materials and Methods

Study Area

The present research was conducted at the Plant Pathology Division of the Horticultural Crops Research and Development Institute (HORDI) in Gannoruwa, Peradeniya. Laboratory and greenhouse experiments were performed within the institute's facilities. The site is located in the mid-country wet zone (Agro-Ecological Region: MW2) of Sri Lanka, at 484 meters above sea level. The region has a humid tropical climate, with a mean annual temperature of 24 °C and an annual rainfall of about 2,500 mm. These conditions are favorable for the development of bacterial diseases in tomato. The GPS coordinates of the experimental site are 7.2717° N, 80.5983° E.

Sample Collection

Tomato plants exhibiting characteristic symptoms of bacterial stem rot were collected from protected polytunnel cultivation systems in the Kandy and Matale districts. Selected plants displayed typical infection signs, including basal stem rot, water-soaked lesions, chlorosis, necrotic foliage, and soft tissue with a foul odor (Kolomiiets et al., 2020).

A total of five symptomatic samples were collected, with each sample representing a different polytunnel and labelled as A, B, C, D, and E. One representative plant was collected from each polytunnel to reflect differences in pathogen occurrence between locations.

All samples were placed in sterile polythene bags, transported to the laboratory, and stored under refrigerated conditions for further analysis. The locations and corresponding sample codes of the collected tomato plants are summarized in Table 1.

Table 1

Locations of collected tomato samples

No	Location	Sample Name
1	Rattota	A
2	Araththana	B
3	Pallegama	C
4	St. John, Araththana	D
5	Palapathwela	E

Pathogen Isolation

Infected plant samples were first washed under running tap water to remove soil and debris, then rinsed three times with sterile distilled water. The samples were surface-sterilized by immersing them in 70% (v/v) ethanol for one minute, followed by another three rinses with sterile distilled water. After drying on sterilized filter paper, the samples were aseptically handled within a laminar airflow cabinet to prevent contamination. Using sterile scalpel blades, small sections were excised from the margins of the infected tissues. These tissue sections were then inoculated onto PDPA plates and incubated at room temperature (approximately 28 ± 2°C) for 24 to 36 hours to allow bacterial growth (Jayasooriya et al., 2022).

Morphological and Biochemical Characterization

Emerging colonies were observed and sub-cultured to obtain pure cultures. The colony morphology was noted, including shape, color, elevation, and margin characteristics. Biochemical assays were conducted to confirm the identity of the bacterium. A 3% potassium hydroxide (KOH) test was performed to determine Gram reaction, where the formation of a viscous thread indicated a Gram-negative bacterium (Akbar et al., 2015). A catalase test was

conducted by adding 3% hydrogen peroxide (H_2O_2) to a fresh bacterial culture. The formation of effervescence indicated the presence of the catalase enzyme, a characteristic feature of *Pectobacterium spp*. Hypersensitivity reactions were tested by injecting bacterial suspensions (~10^7 CFU/mL) into the leaves of *Nicotiana tabacum* (tobacco) to evaluate pathogenicity on non-host plants, following the method described by Naligama and Halmillawewa (2022). Pathogenicity on tomato was assessed by inoculating healthy tomato plants through stem injection with the prepared bacterial suspension and monitoring for disease development under UV-protected greenhouse conditions.

The virulence of *Pectobacterium carotovorum* strains was evaluated using a carrot slice assay. Fresh, healthy carrots were washed, surface-sterilized with 70% ethanol, and sliced into 5 mm discs. Carrot slices were placed on moistened sterile tissue paper in Petri dishes. A single bacterial colony from a 24-hour-old PDPA culture was inoculated onto each slice, while uninoculated slices served as controls. Petri dishes were incubated at 28 ± 2 °C for 24-72 hours, and symptom development, including tissue maceration, was observed. Pathogenicity was confirmed by re-isolation of bacteria from symptomatic tissue (Hibar et al., 2007).

The characteristic colony morphology of *Pectobacterium carotovorum* subsp. *carotovorum* observed on PDPA is shown in Figure 1.

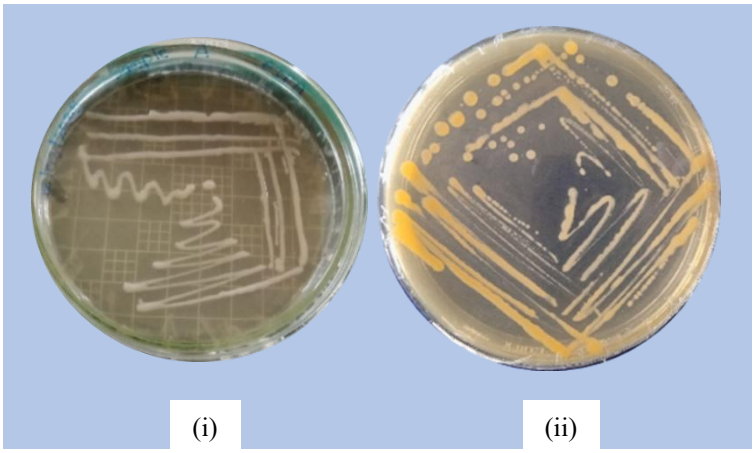

Figure 1. *Colony morphology of Pectobacterium carotovorum* subsp*. carotovorum on PDPA media: (i) top view (ii) bottom view.*

Screening of Tomato Germplasm for Resistance to *Pectobacterium carotovorum* subsp. *carotovorum*

Twenty-five tomato germplasm accessions were obtained from HORDI, Gannoruwa, Peradeniya, for evaluation. Seeds were sown in sterilized trays and maintained in a greenhouse at 25-28 °C and 70-80% RH. After 21 days, healthy seedlings were transplanted into polythene bags containing sterilized potting media (topsoil: compost: cattle manure, 2:1:1). Each accession was represented by 20 biological replicates. Routine agronomic practices were applied uniformly, and no bactericides were used.

A virulent isolate of *P. carotovorum* subsp. *carotovorum* was cultured in potato dextrose peptone broth at 28 ± 2 °C for 24 hours. The bacterial suspension was adjusted to 10^8 CFU/mL. One milliliter of the suspension was injected into the lower stem and root zone of three-month-old plants. Inoculated plants were maintained at high relative humidity (≥ 80%) and observed for symptom development over 7-14 days (Kolomiiets et al., 2020). The twenty-five tomato germplasm accessions evaluated in this study are listed in Table 2.

Table 2
List of evaluated germplasm

AVTO 1906, AVTO 1914, AVTO 1916, AVTO 2003, AVTO 2005, AVTO 2006, AVTO 2007, AVTO 2008, AVTO 2027, AVTO 2028, AVTO 2029, AVTO 2030, AVTO 2101, AVTO 2116, AVTO 2117, AVTO 2122, AVTO 2127, AVTO 2128, AVTO 2129, AVTO 2316, AVTO 2317, AVTO 2318, AVTO 2319, AVTO 2320, AVTO 2321

Disease Incidence (DI) was determined using the Formula 1;

$$\mathbf{DI}\% = \left(\frac{\textbf{Number of symptomatic plants}}{\textbf{Total number of plants}}\right)\textbf{x 100} \qquad \textbf{(1)}$$

Each germplasm line was evaluated and classified into resistance categories based on observed DI using the Asian Vegetable Research and Development Center (AVRDC)scale (Kolomiiets et al., 2020). The classification criteria are presented in Table 3.

Table 3
Rating scale for disease incidence

DI	Disease reaction
< 10%	Resistant
≥10% - <20%	Moderately resistant
≥20% - <40%	Moderately susceptible
≥ 40%	Susceptible

In vitro Bioassay of Copper-Based Compounds

The antibacterial activity of three copper-based compounds copper sulfate (T1), copper hydroxide (Champ®) (T2), and Bordeaux mixture (T3) was evaluated using a poison food plate assay on PDPA medium. All treatments, including the control (T4), were performed in four replicates. Each compound was incorporated into the medium at 1 g/L before solidification. After solidification, a loopful of 24-hour-old *Pectobacterium carotovorum* subsp. *carotovorum* culture was streaked across the center of each plate under aseptic conditions. Plates were incubated at 28 ± 2 °C, and bacterial growth along the streak was observed at 12, 24, 36, and 48 hour to assess the effect of each compound on pathogen development. (Aysan et al., 2003).

Data Analysis

The data collected from the poison food plate assay were analyzed using IBM SPSS software (Version 25.0). A one-way Analysis of Variance (ANOVA) was performed under a Completely Randomized Design (CRD) to compare bacterial growth among the four treatments, where differences were considered statistically significant at $p < 0.05$.

Results

Morphological and Biochemical Characterization of the Pathogen

All five bacterial isolates (A-E) consistently exhibited characteristics typical of *Pectobacterium carotovorum* subsp. *carotovorum*. On PDPA medium, the colonies appeared within 24-48 hours as circular, smooth, moist, cream-colored, and glistening, with entire margins. Biochemical characterization confirmed the identity of the isolates: the KOH solubility test revealed Gram-negative reactions, while catalase activity was positive, as indicated by bubble formation upon the addition of hydrogen peroxide.

The biochemical reactions and pathogenicity responses observed for all five isolates are summarized in Table 4.

Table 4

Biochemical and pathogenic characterization of Pectobacterium carotovorum subsp. carotovorum isolates

Isolate	KOH Test	Catalase Test (H_2O_2)	Pathogenicity on Tomato	Hypersensitivity on Tobacco	Virulence[1] on Carrot Slices
A	+	+	+	+	++
B	+	+	+	+	++
C	+	+	+	+	++
D	+	+	+	+	++
E	+	+	+	+	+++

Note. (+) Positive sign, (-) Negative sign

[1]Virulence was assessed by the extent of tissue maceration on carrot slices at 24 and 48 hours post-inoculation.

"+" = mild maceration, "++" = moderate maceration, "+++" = severe maceration.

Screening of Tomato Germplasm for Disease Resistance

Screening was carried out on 25 tomato germplasm lines (AVTO series) under UV-treated greenhouse conditions, with 20 plants per line, to assess resistance to stem rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum*. Disease incidence after inoculation varied widely, ranging from 10% to 65%, indicating considerable variation in susceptibility among the germplasm lines.

These results indicate substantial genetic variability among the AVTO lines in their response to bacterial stem rot, identifying several lines with useful partial resistance. Among them, AVTO 2117, which showed the lowest DI (10%), could serve as a valuable donor in breeding programs aimed at enhancing resistance to *P. carotovorum*. The variation in disease incidence among the 25 AVTO tomato germplasm lines is illustrated in Figure 2.

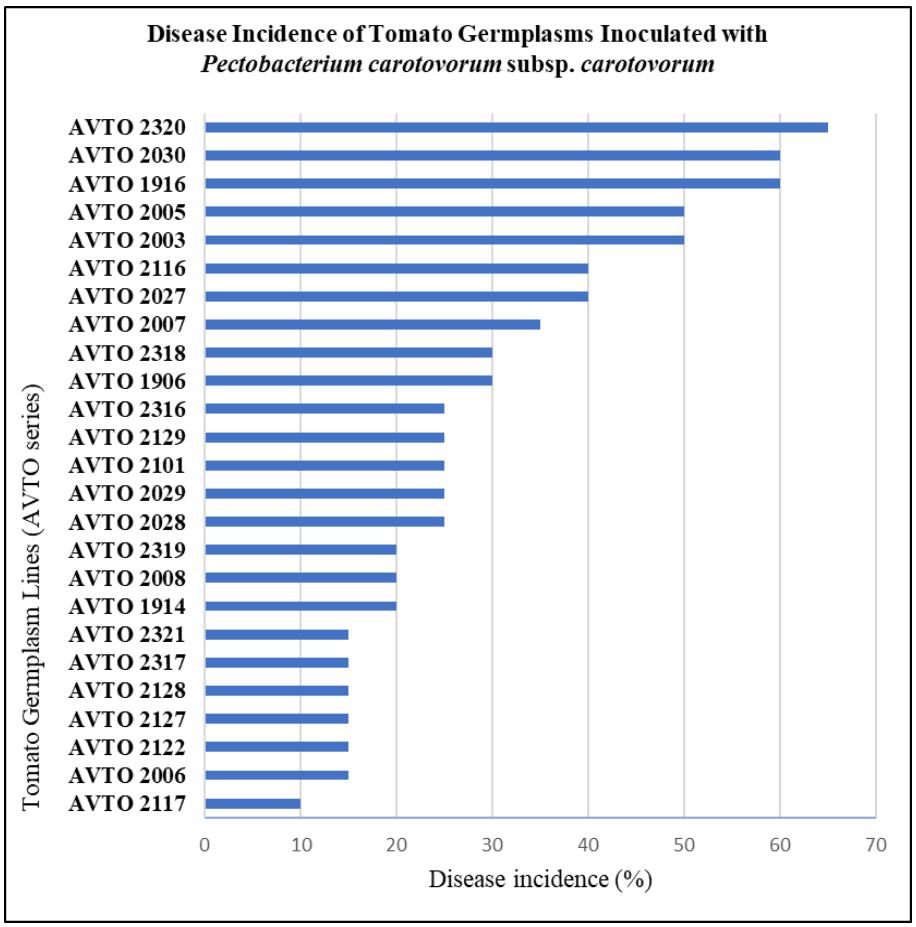

Figure 2. *DI (%) of 25 AVTO tomato germplasm lines inoculated with Pectobacterium carotovorum subsp. carotovorum under greenhouse conditions*

In Vitro Evaluation of Copper-Based Fungicides

The poisoned food plate bioassay revealed significant differences in antibacterial activity among the copper-based treatments (ANOVA, $p < 0.0001$ at all measured time intervals). Copper hydroxide (T2) exhibited complete inhibition of bacterial growth throughout the experiment, while copper sulfate (T1) also showed strong inhibition, reaching complete suppression by 48 hours. In contrast, Bordeaux mixture (T3) and the untreated control (T4) supported progressive bacterial growth, with mean colony diameters of 6.0 mm and 7.25 mm, respectively, at 48 hours.

The data were statistically analyzed using ANOVA, and the results are presented in Table 5, while the effects of copper-based fungicides on the in vitro growth of *Pectobacterium carotovorum* subsp. *carotovorum* on PDPA are illustrated in Figure 3.

Table 5
Effect of copper-based fungicides on the growth of Pectobacterium carotovorum subsp. carotovorum

Treatment	Growth Rate (Mean ± SE)			
	12 hours	**24 hours**	**36 hours**	**48 hours**
T1	0.5 ± 0.29^{b}	0.5 ± 0.29^{b}	0.25 ± 0.14^{b}	0 ± 0.00^{b}
T2	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}
T3	2.5 ± 0.29^{a}	3.5 ± 0.64^{a}	4.75 ± 0.63^{a}	6.0 ± 0.71^{a}
T4	3.5 ± 0.29^{a}	4.5 ± 0.64^{a}	5.5 ± 0.64^{a}	7.25 ± 0.63^{a}
f	43.67	21.36	40.50	66.49
df	3,12	3,12	3,12	3,12
p	0.0001	0.0001	0.0001	0.0001

Note: Values represent mean ± SE. T1 = Copper sulfate; T2 = Copper hydroxide; T3 = Bordeaux mixture; T4 = Control.

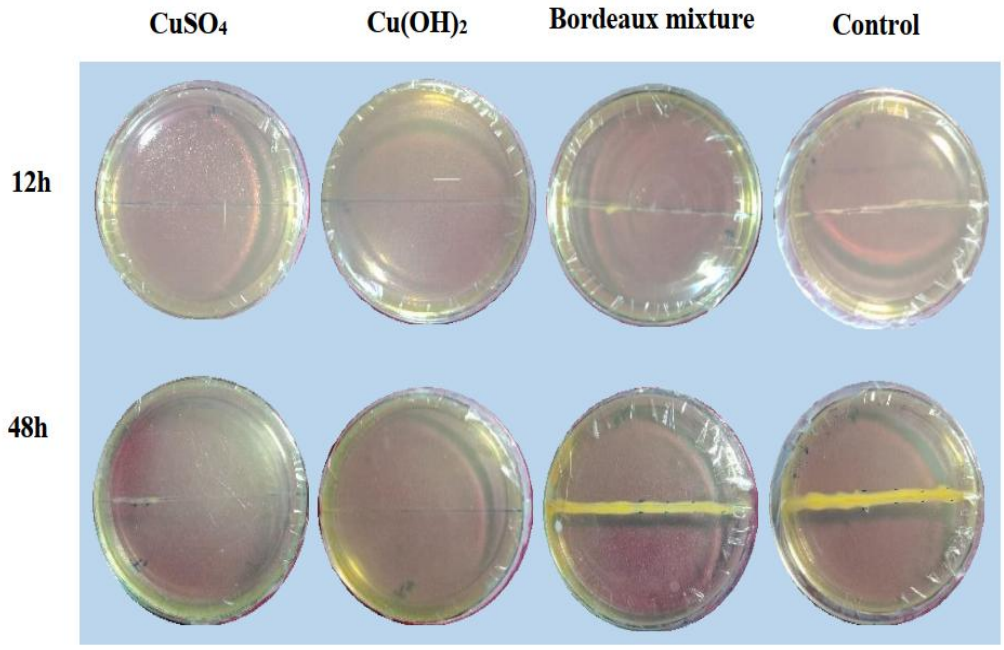

Figure 3. *In vitro growth of Pectobacterium carotovorum subsp. carotovorum on poisoned food plates at 12 and 48 h*

Discussion

The colonies grew rapidly and displayed a slightly mucoid texture due to extracellular polysaccharide secretion, a trait commonly linked to virulence in soft rot pathogens confirm that the pathogen and pathogenicity. (Jones et al., 1991; Toth et al., 2003). Pathogenicity tests on tomato plants showed that all isolates induced characteristic disease symptoms: water-soaked stem lesions, wilting, and tissue collapse within 72 hours post inoculation. In the hypersensitivity assay on tobacco (*Nicotiana tabacum*), necrotic lesions developed, indicating pathogenic potential. In the carrot slice assay, all isolates caused soft rot within 24 hours, with isolate E producing the most rapid and extensive maceration.

According to the AVRDC scale (Kolomiiets et al., 2020), lines AVTO 2117, AVTO 2006, AVTO 2122, AVTO 2127, AVTO 2128, AVTO 2317, and AVTO 2321 were classified as moderately resistant due to their low DI values (10 -20%). In contrast, AVTO 2320 recorded the highest disease incidence (65%), while AVTO 2030, AVTO 1916, AVTO 2005, AVTO 2003, AVTO 2116, and AVTO 2027 showed high susceptibility, placing them in the susceptible category (DI ≥ 40%). The remaining lines exhibited intermediate levels of infection, falling into the moderately susceptible category (DI 20 - > 40%).

These findings are consistent with prior research showing that tomato germplasm often exhibits a gradient of susceptibility rather than complete immunity (Jones et al., 1991; Lapidot & Friedmann, 2002). Future studies should validate these resistance ratings under field conditions and investigate the physiological or molecular mechanisms underlying resistance in the promising lines.

The poisoned food plate bioassay results confirm that copper hydroxide is the most effective copper-based treatment under in vitro conditions, followed by copper sulfate. The partial inhibition observed with Bordeaux mixture suggests lower bioavailability or reduced efficacy of copper ions in this formulation, consistent with earlier findings (Aysan et al., 2003).

Conclusion

This study confirmed *Pectobacterium carotovorum subsp. carotovorum* as the causal agent of bacterial stem rot in tomato through comprehensive morphological, biochemical, and pathogenicity analyses. All five isolates were pathogenic, with isolate E exhibiting the highest virulence, as evidenced by the rapid induction of soft rot in carrot slices. Greenhouse screening of 25 tomato germplasms revealed varying levels of susceptibility, and no accession showed complete resistance. However, seven accessions, including AVTO 2117, AVTO 2006, AVTO 2122, AVTO 2127, AVTO 2128, AVTO 2317, and AVTO 2321, exhibited moderate resistance (DI <20%), indicating their potential as valuable genetic resources for resistance breeding programs. In vitro evaluation of copper-based fungicides identified copper hydroxide (T2) as the most effective treatment, achieving complete inhibition of bacterial growth at all observation periods. Copper sulfate showed delayed but eventual complete inhibition, while Bordeaux mixture was significantly less effective.

Future research should focus on field-level evaluation of copper-based fungicides to validate their efficacy under natural conditions. In addition, molecular characterization of the bacterial isolates is also recommended to confirm species identity and assess genetic diversity. These approaches will improve pathogen diagnosis, support resistant variety development, and contribute to the formulation of effective and sustainable management strategies for bacterial stem rot in tomato.

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